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Effect of Gamma Rays on the Induction and Growth of *Portulaca Olereacea* Callus and it's Relationship with the Content of Active Compounds in it

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ABSTRACT

The current study showed the efficiency of gamma rays on the seeds and callus of *Portulaca oleracea*, where samples exposed to radiation with an energy of 662 MeV and an activity of 1µci for Cs-137 source, with exposure time (0.0, 60, 120 and 180) minutes. Effect of gamma was studied on: Seed germination, shoot and root system, callus induction, callus fresh weight, protein, in addition to estimating of active compounds. The overall results showed that highest percentage for seed germination when exposed for (180 minutes), which reached (95%). Callus results confirm that stem and node parts were more responsive to callus induction than root parts, the best result for the fresh weight of callus was at 180 minutes for stem callus which reached (8.0 and 9) g, and for the nodes it reached (8.2 and 8.8) g. protein obtained the best result at 180 min for the callus of stems, where it reached (808.0) µg/ml, and for the nodes it reached (825.14) µg/ml. As for the effect of gamma rays on callus content of active compound, it was found that gamma rays have an effect on stimulating construction of active phenolic compounds, as the percentage of accumulation of active compound was very low, but gamma rays stimulated it and increased its construction, the best result for accumulation of (2-6 dimethoxyphenol) was at 60 min of exposure to the rays with an accumulation percentage of stem callus is (1.043 and 0.231) µg/ml and for nodes callus (0.671and 0.009) µg/ml in general the accumulation of the compound increased in all exposure time periods. Gamma rays at high times stimulated vital signs, primary metabolism and protein accumulation with better results than at low times. As for secondary metabolism compounds, short periods of gamma rays had a better effect. It is concluded from this study that low doses of gamma rays have a stimulating, effective and positive effect on the *Portulaca oleracea* plant.

Keywords: Active compounds, gamma rays, HPLC technique, callus induction, planet tissue culture.

INTRODUCTION

Portulaca olereacea plant is one of the succulent plants belonging to the family of *Portulacaceae*, a seasonal plant up to 40 cm high grown in large quantities in China and growth is in warm climates (Huxley, 1992; Joy *et al*., 1998). The term *Porulaca* is derived from two Latin words, where the word porto means (to carry) and the word lac means (milk), meaning the presence of milky liquid in the plant (Elkhayat *et al*., 2008; Chugh *et al*., 2019). *P. olereacea* plant is known as purslanein Australia and America, the name of pigweed in England, also known as Rigla in Egypt, in China, its name is Ma_chi_Xian, and name is Pourpeir in France (Elkhayat *et al*., 2008). *Portulaca olereacea* have smooth, reddish stem. Leaves are flat, fleshy, of various shapes, oblique, green or green with a red margin, which may be alternate or opposite, and clustered at the joints and ends of the stems. Flowers appear at any time of the year, and arise singly or in groups of two to five at the tips of the stems. The flowers are small, orange-yellow, purple or pinkish-white, fruit consists of capsules that are almost round and egg-shaped. The seeds are formed in small chambers that open when the seeds mature. It has a taproot with secondary roots. The taproot is able to tolerate weak, compacted and dry soils. It is one of the herbal plants used in many countries of the world as food and is included in folk medicine as a medicine, where it has a wide range of pharmacological qualities such as neuroprotective processes, antimicrobial, antioxidant, anti-inflammatory, antidiabetic, anti-ulceration and anti-cancer as well (Zhou *et al*., 2015). This plant contains fatty acids, vitamins, minerals, terpenoids and flavonoids (Ghorani *et al*., 2023). *P. olereacea* contains mucous that is of medical importance and is a rich source of potassium, followed by magnesium and calcium, which can be used as a plant source of omega-3 fatty acid, which is one of the most effective and important sources of alpha-linolenic and gamma-linolenic acid compared to any other leafy vegetables (Kamal Uddin *et al*., 2020). Among the different tissue culture systems, callus culture is part of the bioremediation steps (Ali and Abbasi., 2013; Abbasi *et al*., 2007; Ahmad *et al*., 2013a). Growth organizations, the addition of other substances, the use of catalysts and radiation are among the most important steps that promote bioactive compounds and biomass (Ahmad *et al*., 2013b). Plant tissue culture is an isolation technique for the cell, tissue or organ within a range free of pathogens, where plant parts or cells are sterilized and cultured in sterile industrial media (Mohammad and Omer, 1990). Tissue culture has an important and effective role in the production of genetically modified molecules and the production of secondary metabolic compounds that help in one way or another in the manufacture of cosmetic and medical drugs (Huxley *et al*., 1992) Gamma radiation is electromagnetic radiation (EM) that prevents or stimulates the differentiation and growth of various plant cells or organs (Zhao and Verpoorte, 2007). Gamma radiation is one of the physical mutations and is widely used (Hasbullah *et al*., 2012).

The aim of this work is to evaluate of the effect of gamma rays on cultures of *P. olereacea* plant by observing its impact on seed viability, seedling growth, and its effect on the shoot and root system, proving the effect of radiation and the speed of response to callus induction, measuring the vital weight of irradiated samples, protein levels in callus of *P. olereacea* plant and to study the effect of these rays on the active compound and its concentration.

MATERIALS AND METHODS

This study began in November 1/1/2023 with 5 replicates for each sample for vitality and appearance tests such as seedling germination, total shooting and rooting lengths, as well as protein accumulation and secondary metabolites and it conducted in the University of Mosul/ Plant Tissue Culture Laboratory/ College of Agriculture and Forestry/ Department of Horticulture and the Nuclear Laboratory/ Department of Physics/ College of Science. All tissue culture experiments were conducted inside the laminar airflow table (Hepier). Diagram (1) illustrates the practical steps of this research.

1. Plant material

P. oleracea seeds were obtained from local markets in Mosul city.

2. Seed vitality test

The vitality of the seeds was tested seeds were placed on a filter paper inside a Petri dish (15 seeds), covered with filter paper, sprayed with dipped water, and kept in dark conditions and temperature 22±1C° until germination was observed, taking into account spraying and moistening seeds regularly for 2 days.

3. Preparation of medium and sterilization of seeds for germination

For seed germination, the procedure described by (Murashige and Skoog, 1962) was followed to prepare MS basal medium free of plant growth regulators and the PH value adjusted to (5.8-6). Sterilization of seeds was according (Mousa *et al*., 2022), the seeds were washed with plain water for 5 minutes to remove dust and stuck dirt and remove floating seeds the seeds were immersed in ethanol (70%) for 3 minutes, the seeds were immersed in a 10% sodium hypochlorite solution for 25 minutes, followed by five rinses with sterile distilled water. The seeds were immersed for 60 seconds in distilled water with constant stirring to remove traces of disinfectant materials. After that seeds were cultured on sterilized MS basal medium under aseptic conditions in the laminar airflow for the germination of seed. Then incubated under controlled conditions (6 h light and 8 h dark) photoperiod at 25 C° in the growth champer (Al-Bakr, 2018).

4. Germination and growth

Signs of growth appeared on the seeds that were planted after 12 hours and after 24 hours.

5. Preparation the medium for the induction of callus

The medium is prepared from M and S-Sucrose-Agare Agare-sterile distilled water and (2 $mg.l^{-1}$) from (BA, NAA) according to (Al-Bakr, 2018).

6. Callus cultures induction

After age of the seedlings reached 30 days, the plant parts were cut, the root, stem (middle part of plant) and nodes (upper part) were cut, these parts were planted in the media then samples were incubated (photoperiod at 25 C°) in the growth chamber.

7. Gamma irradiation

Seeds grown on MS media were exposed to gamma rays after seedlings when samples age was 30 days were cut using the tools for this operation. Different parts of the root, stem and nodes were used and then the height of the MS medium containing (2 $mg.l^{-1}$) of (BA, NAA) was planted. The glass decanters containing the plant pieces were transferred to the development incubator, after 24 hours, the samples were transferred to the gamma irradiation laboratory, where the samples were irradiated with Cs-137 source of activity 1µci at (0.0, 60, 120 and 180) minute different periods of time.

8. Fresh weight

The fresh weight of callus was measured at 30 and 60 days of age.

9. Protein measurement

The Lowry method was adopted to measure protein using Folin reagent (Lowery *et al.*, 1951) duplicates of samples treated with gamma rays (0.0, 60, 120 and 180) minutes of the node and stem callus, a spectrophotometer was used to measure the absorbance at a wavelength of 750 nm. The standard curve prepared from bovine serum protein, whose concentrations ranged between (100-900) µg/ml, was adopted.

10. Measurement of active compound accumulation

The concentration of active compounds was examined on induced callus cultures (nodes, stems) at the age of 50 days using High-performance Liquid Chromatography (HPLC) techniques, the mobilephace of 2-6 Dimethoxyphenol was (35 water :65 CAN) (0.1 phosphoric acida) (Silec technology-2024) with wavelength 250.

11. Statistical analysis

The presence or absence of statistical differences in shoot and root system and fresh weight of the callus of the *Portulaca oleracea*, was recorded for two ages, 30 days and 60 days. The average was calculated using ANOVA and the Duncan test was used using the SPSS program. The presence

of differences was considered statistically significant when a=0.05. The measurements were conducted in five replicates.

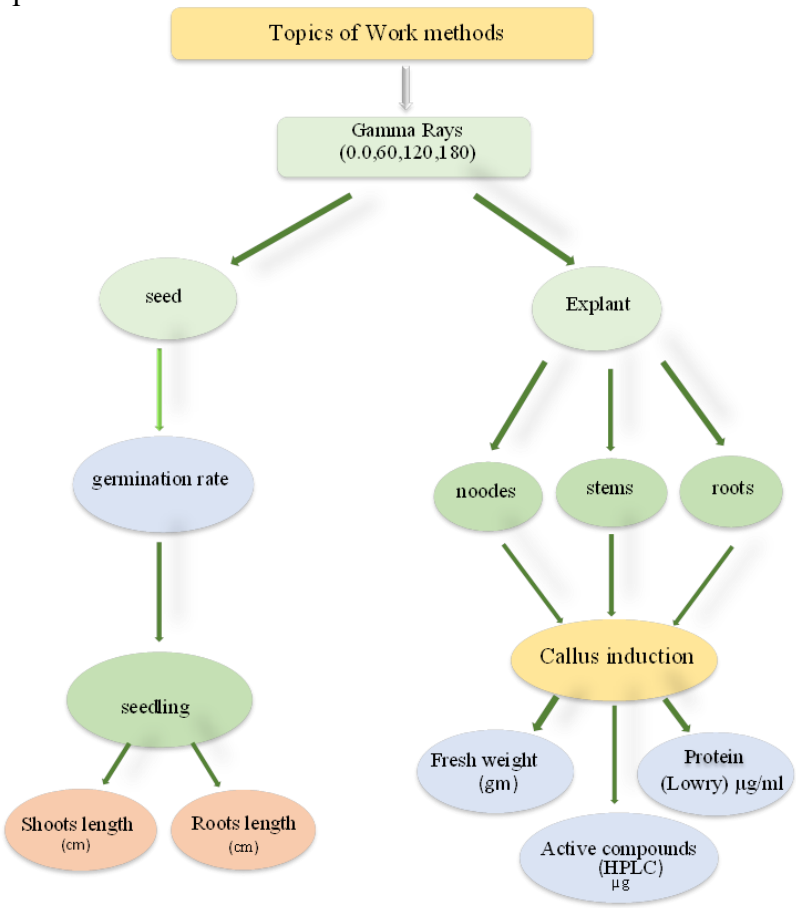

Diagram 1: The practical steps of the methods.

RESULTS AND DISCUSSION

Seed vitality

The seed vitality test showed that the seeds were very high and fast germination rate, as the seeds grew within 2 days by 99% as shown in Fig. (1).

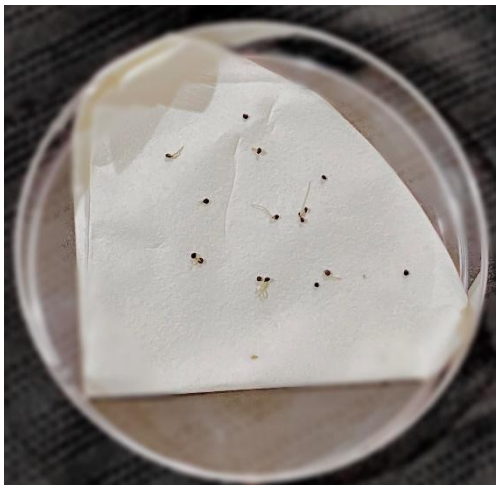

Fig. 1: Seed vitality test experience.

Seed sterilization

The sterilization results that were adopted in the materials and methods of work, showed that the sterilization efficiency was 95% and with very low pollution.

Effect of gamma rays on seed germination

P. oleracea seeds were exposed to gamma rays for different time periods (0.0, 60, 120 and 180) minute and (Table 1) showed that the germination rates of *P. oleracea* seeds showed clear differences. The highest germination rate was at the 180-minute time period, reaching 95% compared to the standard sample not exposed to radiation, which had a germination rate of 91%, the 60 minute irradiation period showed a tendency to decrease the germination rate, as the germination rates in this period reached 88%, while the 120-minute time period increased the germination rate and reached 92% as shown in Fig. (2). The results in (Table 1) have proved that exposing sample to gamma rays for a long period of time to a significant and positive difference in germination rate in the period of 180 minutes is the best result for germination rate 95% while it was slowdown in period 60 the decrease or slowdown that occurred during the 60 minute exposure period of gamma rays may be attributed to the fact that low gamma radiation leads to the synthesis of auxins and DNA, which in turn leads to a defect in the mechanism of mitotic division of meristems atic cells, leading to a decrease in germination and plant growth (Jan *et al*., 2012; Caplin and willey, 2018), or the reason of the slow growth may be due to the cells being exposed to shock from the effects of gamma rays during the division period. (Preuss and Britt, 2003; Von Well *et al*., 2018).

Although there are no specific explanations for the stimulatory effects of low doses of intramuscular gamma radiation yet, but the results obtained by (Wi *et al*., 2007) suggest that there is a control hypothesis that low dose will affect growth stimulation by changing the biochemistry in the network. In plant cells or by increasing their ability to resist oxidation, cells easily overcome daily stress such as light and temperature fluctuations in the home growing condition, and (Kiong *et al*., 2008) showed that the survival of plants until the senescence stage depends on the nature and extent of chromosomal damage.

Table 1: Effect of gamma rays on seed germination rate after 2 days of irradiation

Duration of exposure to gamma rays (minutes)	Germination rate after 2 days (%)
0.0	91
60	88
120	92
180	95

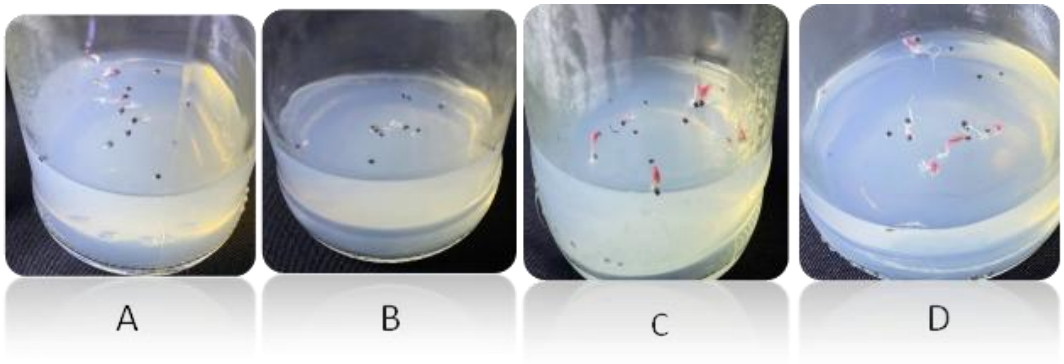

Fig. 2: Effect of gamma rays on seed germination.
A: Standard sample not exposed to gamma radiation.
B: 60 minutes, (exposure time).
C: 120 minutes, (exposure time).
D: 180 minutes, (exposure time).

Effect of gamma rays on the seedling growth of *p. oleracea*

The results in (Table 2) shown gamma rays effect of the total shoot and roots lengths of *P. oleracea* seedlings after 30 days of planting them on solid MS media and exposing them to gamma rays for different time periods (0.0, 60, 120 and 180) minute. The results confirmed that the exposure period of 180 minutes was the best and caused a noticeable and positive increase in the total shoot and roots lengths. The total shoot length was (8.140) cm and the roots length were (2.595) cm. Compared to the standard sample; we find that the total shoot length was (6.980) cm and the roots length was (1.980) cm. The results showed that the exposure of 60 minutes for gamma rays caused a decrease in the total lengths, the shoot length was (5.860) cm and the roots length was (1.800) cm, indicating that growth inhibition occurred. With increasing the exposure period to 120 minutes, there was a noticeable and different increase in the total lengths, and the roots length was (2.760) cm and the shoot length was (7.080) cm Fig. (3) shows difference in lengths of seedlings exposed to gamma rays the. These results may be an important indication that there is a close relationship between the growth rate and the irradiation period. The lengths of the shoot and roots systems is an important indicator of biometric indicators and the impact of transactions in these seedlings. Gamma rays showed a clear effect on the roots and the shoot systems of *P. oleracea* seedlings, where the length of the shoot systems reached (8.140) cm in the time period 180 minutes, and the length of the roots systems reached (2.595) cm compared to the standard sample 0.0 where the length of the shoot systems reached (6.980) cm and the roots (1.980) cm. These results were similar to the results of the study (Borzouei *et al*., 2010), which found that the effect of gamma rays was significant on the shoot and roots systems at low doses of radiation. (Kiong *et al*., 2008) was found that radiation increases the sensitivity of the plant to gamma rays. This may be due to a decrease in the amount of endogenous growth regulators, especially cytokinin, as a result of breakdown or lack of synthesis due to radiation. This result is considered one of the reasons why seedlings were gradually affected by gamma rays over different time periods, as the length of the shoot and roots systems during the period 60 minutes decreased in length compared to the standard sample. As for the time period 120 minutes, the results were close to the standard sample, so this he symptoms frequently observed in the low or high dose irradiated plants are enhancement or inhibition of, seedling growth and other biological responses (Kim *et al*., 2000; Wi *et al*., 2005).

Table (2): Effect of gamma rays on the length of the shoot and roots systems of *p.oleracea* plant after 30 days of planting and irradiating the seeds.

Vegetative part / GR exposure time(min)	Length shoot systems (cm)	Length roots systems (cm)
0.0	6.980±0.1304 (a)	1.980±0.2168 (a)
60	5.860±0.1140 (b)	1.800±0.3240 (a)
120	7.080±0.1924 (b)	2.760±0.2302 (b)
180	8.140±0.2881 (c)	2.595±0.8575 (c)

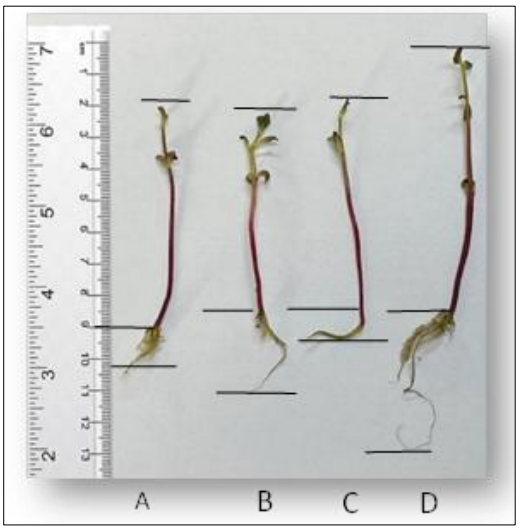

Fig. 3: Shoot and roots systems of seedlings exposed to gamma rays.
A: Standard sample not exposed to gamma radiation.
B: 60 minutes (expose time).
C: 120 minutes (expose time).
D:180 minutes (expose time).

Effect of gamma radiation in callus induction of *P. olereacea*

The explant *P. oleracea* of seedling (nodes, stems and roots) exposed to gamma rays for different periods times showed a positive difference and clear variation compared to the standard sample (non-irradiated) according to the results shown in (Table 3). It was noted that the best result for inducing callus from the different plant parts was at the time period 180 minutes and the nodes were the most responsive and affected by gamma rays of these parts and the lowest induction rate was for the roots explant. The response of the nodes explant to the 180 minute exposure period began during the first five days, and this period was the best as a result of callus induction, as the induction rate reached 100% after 30 days, as all explants turned into callus compared to the sample not exposed to gamma rays, in which the callus induction rate reached 80% and the callus induction rate was at the 60 minute time period 60% as this result is considered the lowest percentage, and the 120 minute time period, in which the induction rate reached after 30 days 100%, as callus induction began in the first five days, and the result of this period is similar to the result of the 180 minute time period. For the stems explant, the best result was at the 180-minute time period, as the induction rate reached 100% compared to the 120-minute time period, in which the induction rate was also 100%, and the standard sample reached 66% induction rates, and the lowest induction rate was at the 60-minute time period. The callus induction from roots explant induction rates were low compared to the rest of explant, but there were also positive differences between the induction rates for different times (0.0, 60, 120 and 180) minutes. The best result was at the 180-minute time period with an induction rate of 66% compared to the standard sample, which had an induction rate of 27%. The exposure rate was 10% at the 60-minute time period, and at the 120-minute time period, the induction rate was 50%, Fig. (4) shows cultures of different age calluses the above results and data in (Table 3) proved that gamma rays at low doses and specific time periods affect the stimulation and growth of callus. This study confirmed the effect of gamma rays on the induction of callus from different plant parts in a positive way. The results were consistent with the study conducted by (Venkateshwarlu, 2008) where he reported that low doses of gamma rays stimulate and grow while high doses inhibit the process of stimulation and growth of callus cultures. The results varied among themselves in response to irradiation. Times (120 and 180) minute were positively affected by the rays, while the exposure period of 60 minutes in each of the parts (nodes,

stems and roots) was negatively affected by gamma rays. The reason may be attributed to the sensitivity of the plant material, which depends on the: Genetic composition, the radiation dose used, the amount of DNA, the time of replication in the initial stages, the moisture content, the development stage, and the genotype (Deshpande *et al*., 2010).

Table (3): Effects of gamma ray efficiency in callus induction from *P. olereacea* seedlings explant cultivation on solid MS media equipped with 2mg.l^{-1} (BA, NAA).

Explant MS (2 mg.l^{-1} BAA, NAA)	Gamma exposure time (minute)	Treatment time						Callus initiation %
		5	10	15	20	25	30	
Nodes	0.0	-	+	+	++	+++	+++	80
	60	+	++	++	+++	+++	++++	66
	120	+	++	++	+++	+++	++++	100
	180	+	++	++	+++	++++	++++	100
Stems	0.0	-	+	+	++	++	+++	66
	60	-	+	++	++	+++	+++	60
	120	+	++	+++	+++	++++	++++	100
	180	-	+	++	+++	++++	++++	100
Roots	0.0	-	-	-	-	+	+	10
	60	-	-	-	+	+	+	27
	120	-	-	+	+	++	++	50
	180	-	+	++	++	++	++	66

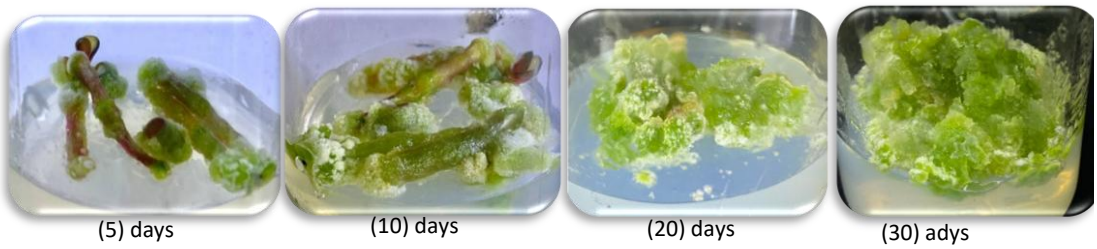

Fig. 4: Cultures of different age calluses produced from different plant parts exposed to radiation.

Estimate of callus fresh weight

After callus induction, the fresh weight was measured at two ages (30 and 60) days as in (Table 4), where the results showed good growth of the callus culture, especially at the time period of 180 minutes for callus induced from (nodes, stems and roots) explant. The best result for callus induced from nodes was at 180 minutes with a weight of (8.280 and 9.540) g for the two ages (30 and 60) day compared to the standard sample not exposed to radiation, where the fresh weight reached (7.240 and 8.080) g for the two ages (30 and 60) day the fresh weight of the irradiated callus decreased in the time period (60) minutes (7.040 and 8.760) g at ages (30 and 60) day and it was found that the period of 120 minutes increased the fresh weight of the two ages (7.680 and 9.000) g where it reached (30 and 60) day. As for the callus induction from the stems, its best result was at the time period 180 minutes with a fresh weight rate (8.020 and 9.020) g compared to the non-irradiated sample where reached (5.720 and 6.720) g for age (30 and 60) day, the fresh weight for the irradiation period 60 minutes also decreased at a fresh weight rate where it reached (6.720 and 7.280) g, and at the period 120 minutes the fresh weight of the callus reached (7.680 and 8.300)

g for the two ages (30 and 60) day. These results show that increasing the irradiation period with constant irradiation power increases the fresh weight of the callus.

The best fresh weight of callus induction from roots was for the time period of 180 minutes (5.040 and 5.860) g for the two ages (30 and 60) day and the lowest fresh weight was for the standard sample (4.100 and 4.260) g for the two ages. The fresh weight increased slightly from the standard sample at the time period 60 minutes, the weight was (4.300 and 4.660) g for the two ages and at the exposure period of 120 minutes the fresh weight was (4.640 and 5.080) g for the two ages. These results indicate a difference between the fresh weight of the (nodes, roots and stems) where the highest weight was in the callus developed from the nodes. It can be concluded that the increase or decrease in fresh weight during periods of callus growth and using different time periods or doses is linked to a certain extent to an increase and decrease in the basic cell contents because the process of cell division and growth of the callus depends on the activities of the enzymes that participate in the process of biosynthesis of the cellular compounds that make up the callus cell (Aboud *et al*., 2007). There is a possibility that the decrease in wet weight occurred at the 60-day exposure period. The possible reason in gradual decline in callus fresh weight may be due to the effect of radiation on endogenous growth regulators that stimulate cell division, (Bajaj *et al*., 1970) observed significant reduction in callus growth of *Gerbera jamesonii* when exposed to 20-50 Gy doses.

Table (4): Fresh weight of callus introduced from explant exposed to gamma rays after (30/60) days of planting on MS media.

Explant	Gm exposure time (minute)	F. W (g)	
		After 30 days	After 60 days
Nodes	0.0	7.240±0.5941 (a)	8.080±0.7662 (a)
	60	7.040±0.1342 (a)	8.760±0.2881 (ab)
	120	7.680±1.0872 (a)	9.000±0.5568 (bc)
	180	8.280±1.2256 (a)	9.540±0.3847 (c)
Stems	0.0	5.720±0.5450 (a)	6.720±0.3701 (a)
	60	6.720±0.4764 (b)	7.280±0.8075 (a)
	120	7.680±0.7950 (c)	8.300±1.1247 (b)
	180	8.020±0.3962 (c)	9.020±0.1304 (b)
Roots	0.0	4.100±0.5874 (a)	4.260±0.2881 (a)
	60	4.300±0.6442 (ab)	4.660±0.2074 (ab)
	120	4.640±0.5595 (ab)	5.080±0.4494 (b)
	180	5.040±0.2408 (b)	5.860±0.2966 (c)

Protein determination in callus of *P. olereacea* grown on MS media supplemented with growth regulators (NAA, BA).

The protein concentration of callus generated from (nodes, stems and roots) and exposed to gamma rays was measured according to the Lowry method and using the standard protein curve after 30 days of generation. It was found that the highest value of protein concentration was where the callus exposed to gamma rays for 180 minutes, stems and roots. The data showed a clear difference in the protein ratios between the different parts from which callus was generated, it was higher in the nodes than in the stems, stems than in the roots. The concentration (825.14) µg/ml at the 180 minute time period was the best result in the nodes callus compared to the standard sample (0.0) where the protein concentration in it was (753.7) µg/ml, and the 60 minute time period the protein concentration in it was (772.2) µg/ml and at the 120 minute exposure period the protein percentage was (808.0) µg/ml, and the stems callus had the highest protein concentration in it (702.2) µg/ml at the 180 minute period compared to the standard sample where the protein concentration was (672.2) µg/ml and the protein concentration percentages gradually

increased with the increase in the irradiation period and reached (682.2) µg/ml at the 60 minute period and (696.2) µg/ml at the 120 minute period. Roots callus highest value was at period 180 with a concentration of (670.8) µg/ml and the lowest concentration was in the standard sample (581.2) µg/ml. At exposure period 60, the average protein concentration was (611.8) µg/ml and the concentration increased with increasing the irradiation period to 120 minutes and was (649.1) µg/ml, in (Table 5) the increase in protein concentration with increasing irradiation period may be attributed to the fact that gamma rays have the ability to interact with the internal materials of cells and release free radicals. These free radicals either negatively affect or modify the morphological differentiation process according to the applied doses. The biological effect of gamma rays depends on the interaction of molecules in the plant cell, especially with water molecules, which produce free radicals in the cells. These free radicals can have a negative or positive effect they damage or modify important components in the cells. It has been proven that they have a different effect on the shape, anatomy, biochemistry and physiology of plants depending on the level of radiation. The rays affect protein synthesis, hormonal balance, gas exchange in leaves, water exchange and enzymatic activity (Jankuloski and Forster, 2018). Also, the reasons that stimulate gamma rays to germinate may be the activation or synthesis of protein, which occurred during the early stage of germination after seed irradiation (Abdel-Hady *et al*., 2008). The increase in the protein concentrations of *P. oleracea* callus (nodes, stems and roots) according to the results of (Table 5) is the opposite of the results reported by (El-Beltagi *et al*., 2011) for rosemary callus culture due to the high dose with which the samples were treated, gamma rays at high doses led to enhancing the production of antioxidants and reduced the production of sugars, protein and amino acids. According to the study conducted by (Esfandiari *et al*., 2008) that exposure to radiation at high doses causes a disruption and disturbance in protein synthesis and hormonal balance.

The results of this study proved that the protein concentration at low doses synthesis rates is high compared to high doses.

Table 5: Protein concentration of *P. olereacea* callus grown on MS media supplemented with 2 mg.l^{-1}(BA, NAA) after 30 day.

GR exposure time (minute)	Protein concentration (µg/ml)		
	Nodes callus	Stems callus	Roots callus
(0.0)	753.7	672.2	581.2
(60)	772.2	682.2	611.8
(120)	808.0	696.2	649.1
(180)	825.14	702.2	670.8

Effect of gamma rays on compound accumulation

The results shown in (Table 6) demonstrated the efficiency of the effect of gamma rays on the concentration of active compounds in the extract of the callus of the nodes and the stem of the *P. oleracea* plant at the age of 50 days so these results generally indicate that there is stimulation of the compound's formation. The results proved a significant increase in the compound's accumulation during the 60-minute period, followed by a decrease in the accumulation at the 120-minute period, followed by a slight increase at the 180-minute period. The results confirmed that the 60-minute exposure period is the best. These results confirmed that there was a positive increase in phenolic compounds in the extracts of the callus of the nodes. The accumulation of the compound 2-6 dimethoxyphenol was measured at time periods of exposure to gamma rays (0.0, 60, 120 and 180) minute, and the best result was at the time period of 180 minutes. The results were as follows: In the callus of the stem callus extracts also had the best result at 60 minutes, where the accumulation of the active compound was (1.043 and 0.231) µg/ml and the accumulation in the control sample was (0.053) µg/ml , the concentration at 120 minutes was (0.104) µg/ml and the

concentration at minute 180 was (0.170 and 0.660) µg/ml, in the nodes callus, the highest concentration of the active compound was (0.671 and 0.009) µg/ml at the time period of 60 minutes, compared to the control sample, where the concentration was (0.077) µg/ml, and at the time period of 120, the concentration was (0.158 and 0.055) µg/ml, and the concentration at the exposure period of 180 minutes was (0291, 0.094) µg/ml. The above results show the presence of phenolic compounds in the callus extracts of the *P. oleracea* plant and that low-dose gamma rays have an effect on the accumulation of the compound 2-6 dimethoxyphenol. These results are consistent with the results of the study (Azeez *et al*., 2017), which showed that gamma rays have a stimulating effect on the active compounds and that low-dose irradiated callus cultures are considered a suitable system for the production of active compounds for pharmaceutical purposes. There are other studies that showed changes in the accumulation of active plant compounds after irradiation (Stajner *et al*., 2007) and according to (Mustapha *et al*., 2014) the degradation resulting from irradiation releases some natural chemical components from their precursors, which may provide an important explanation for the high amount of some components also, under specific conditions of exposure to radiation sources and temperatures, whether very high or low, and also storage conditions at different temperatures, an increase in the concentration of plant photochemicals occurs, (Khawory *et al.*, 2023) showed in their study that plant extract samples exposed to gamma rays showed slight increases in the accumulation of active compounds. (Jan *et al*., 2012) showed that ionizing radiation has the ability to change the concentrations and levels of active plant compounds. The study of (Khalaf *et al*., 2018) showed that low radiation doses led to a decrease in the accumulation of phenolic compounds in the leaf and root extract, while high doses led to an increase in the accumulation of phenols in the plant extract. The results of (Lee *et al*., 2018) confirmed that high radiation doses increase the accumulation of phenolic compounds. Fig (5 and 6) Retention time of 2-6 dimethoxyphenol compounds Fig. (6 and 7) shows the retention time of phenolic compounds.

Table 6: Effect of gamma rays in 2-6 dimethoxyphenol content in *P. oleracea* callus.

Stem callus extraction			
Exposure time (minute)	**Ret. Tim (ml/min)**	**Peak area**	**2-6 Dimethoxyphenol content (µg/ml)**
Stander 2-6 dimethoxyphenol	6.855	109968112	50
0.0	6.233	116627	0.053
60	6.425 6.853	2295347 508845	1.043 0.231
120	6.244	230420	0.104
180	6.302 6.782	37546 1452203	0.170 0.660
Nodes callus extraction			
Exposure time (minute)	**Ret. Tim (ml/min)**	**Peak area**	**2-6 Dimethoxyphenol content (µg/ml)**
Stander 2-6 dimethoxyphenol	6.855	109968112	50
0.0	6.233	170251	0.077
60	6.383 6.799	1476996 204479	0.671 0.009
120	6.169 7.159	348516 122479	0.158 0.055
180	6.162 7.246	640391 207346	0.291 0.094

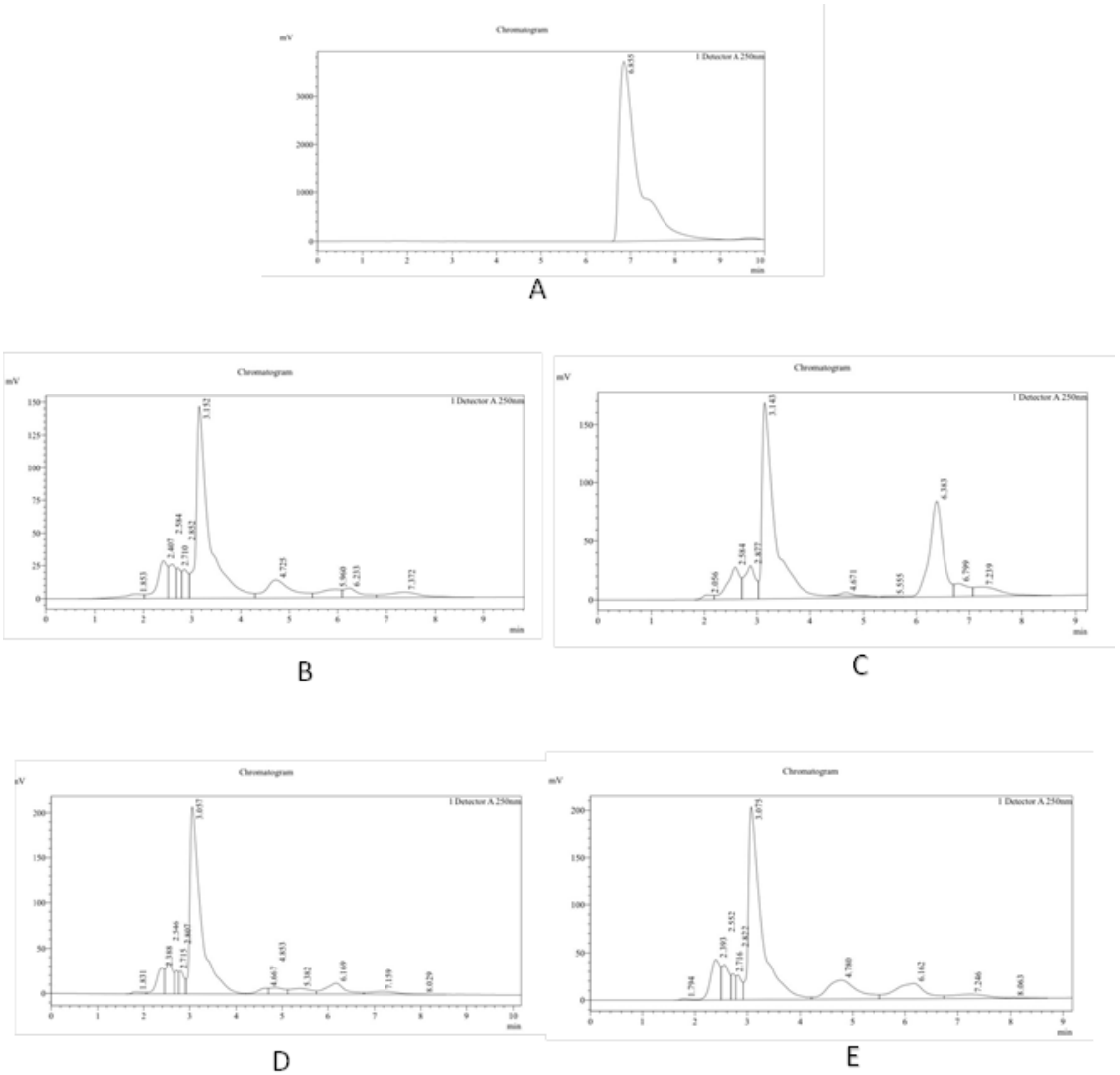

Fig. 6: Retention time of phenolic compounds isolated from stem callus extracts of *P. oleracea* that exposed to gamma rays at different time (0.0, 60, 120, 180) by HPLC technique.

A. Standard sample. B. Control sample. C. 60 minutes (exposure time).
D. 120 minutes (exposure time). E. 180 minutes, (exposure time).

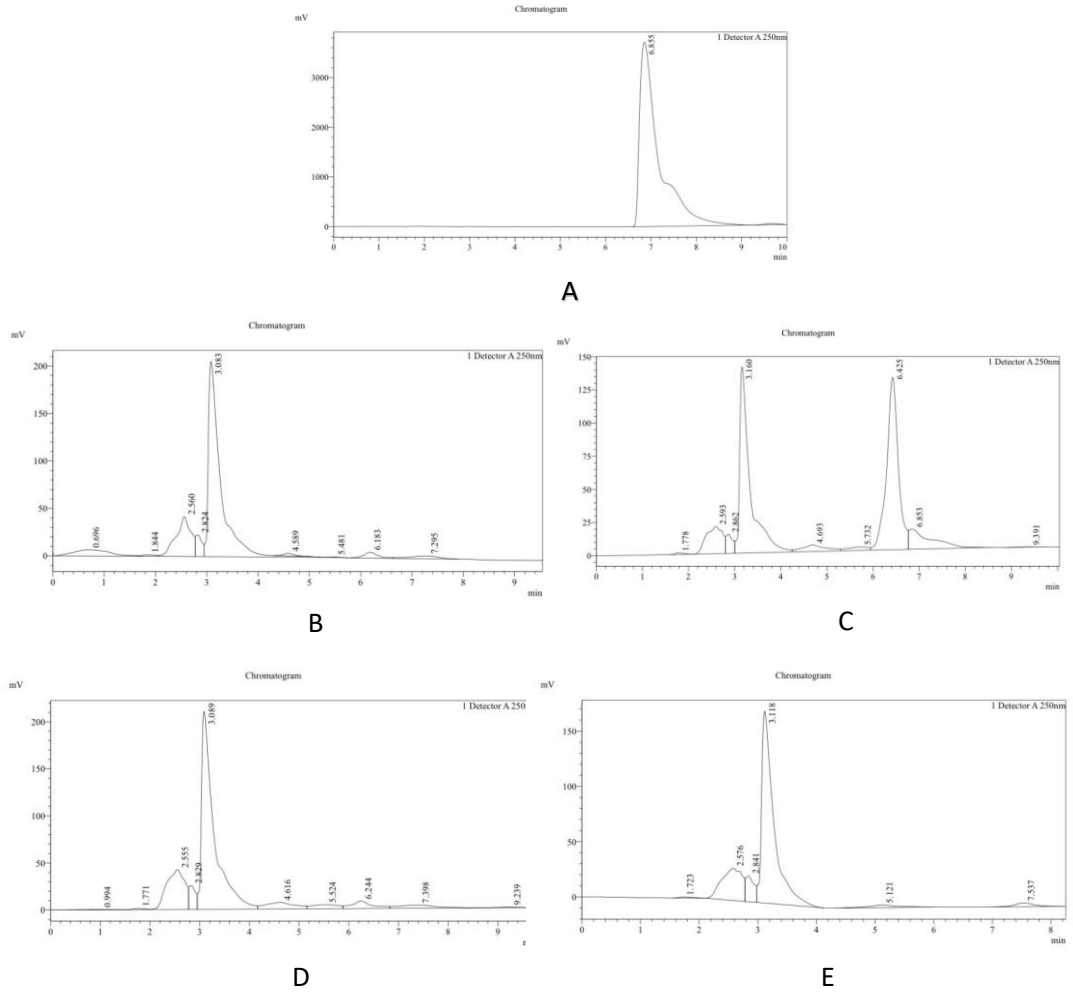

Fig. 7: Retention time of phenolic compounds isolated from nodes callus extracts of *P. oleracea* that exposed to gamma rays at different time (0.0, 60, 120 and 180) by HPLC technique.
A. Standard sample. B. Control sample. C. 60 minutes (exposure time).
D. 120 minutes (exposure time). E. 180 minutes (exposure time).

CONCLUSIONS

The current study confirmed that gamma rays at different time periods and constant intensity had a clear effect on the germination of purslane seeds, as the percentages reached (95%) at the time period of 180 minutes, which is considered the best result. The study also showed that gamma rays had an important and statistically significant effect on the induction of callus from the purslane stem, as the percentages at the time period of 180 minutes reached 100%. The results obtained in measuring protein ratios confirmed that protein ratios increased at the time period of 180 minutes and were the best due to the effect of gamma rays on stimulating protein synthesis. Gamma rays also stimulated the synthesis of the phenolic compound 2-6-dimethoxyphenol, and the best result was at the time period of 60 minutes. In general, the results of this study confirmed that gamma rays during the 180-minute period had a direct effect on the products of primary and secondary metabolism in the plant cell.

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تأثير أشعة كاما على استحثاث ونمو الكالس *Portulaca Olereacea* وعلاقته بمحتوى المركبات النشطة فيه

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الملخص

أظهرت الدراسة الحالية كفاءة أشعة كاما على بذور وكالس نبات *Portulaca oleracea*، تعرضت العينات للإشعاع بطاقة 662 مليون إلكترون فولت ونشاط 1 ميكروكوري لمصدر السيزيوم−137، وبفترات تعرض زمنية (0.0، 60، 120 و180) دقيقة. وتم دراسة تأثير أشعة جاما على: إنبات البذور، المجموع الخضري والجذري، استحثاث الكالس، الوزن الطازج للكالس، البروتين، بالإضافة إلى تقدير المركبات الفعالة. وأظهرت النتائج الإجمالية أعلى نسبة لإنبات البذور عند التعرض لمدة (180 دقيقة) حيث بلغت (95%). وتؤكد نتائج الكالس أن أجزاء الساق والعقد كانت أكثر استجابة لتحريض الكالس من أجزاء الجذر، وكانت أفضل نتيجة للوزن الطازج للكالس عند 180 دقيقة لكالس الساق حيث بلغ (8.0 و9) غم، وللعقد بلغ (8.2 و8.8) غم. وحصل البروتين على أفضل نتيجة عند 180 دقيقة لكالس السيقان حيث بلغ (808.0) ميكروغرام/مل، وللعقد بلغ (825.14) ميكروغرام/مل. أما بالنسبة لتأثير أشعة جاما على محتوى الكالس من المركب الفعال فقد وجد أن لأشعة جاما تأثير في تحفيز بناء المركبات الفينولية الفعالة حيث كانت نسبة تراكم المركب الفعال قليلة جداً إلا أن أشعة جاما حفزته وزادت من بنائه وكانت أفضل نتيجة لتراكم (2−6 دايميثوكسيفينول) عند 60 دقيقة من التعرض للأشعة حيث بلغت نسبة تراكم الكالس الساقي (1.043 و0.231) ميكروجرام/مل وللكالس العقدي (0.671، 0.009) ميكروجرام/مل وبشكل عام زاد تراكم المركب في جميع فترات التعرض كما حفزت أشعة كاما في الأوقات العالية العلامات الحيوية والتمثيل الغذائي الأولي وتراكم البروتين بنتائج أفضل من الأوقات المنخفضة أما بالنسبة للمركبات الأيضية الثانوية فقد كان لفترات قصيرة من أشعة جاما تأثير أفضل ونستنتج من هذه الدراسة أن الجرعات المنخفضة من أشعة كاما لها تأثير محفز وفعال وإيجابي على نبات البورتولاكا أوليراسيا.

الكلمات الدالة: *Portulaca olereacea*، أشعة كاما، المركبات النشطة، استحثاث الكالس، تقنية HPLC.