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Gamma irradiation as activator of antioxidant activity and essential oil contents in lavender (*Lavandula multifida*) plantlets

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ABSTRACT

This study was conducted to evaluate the stimulation effect of γ-irradiation on the chemical composition of essential oils, total phenolic compounds, flavonoid contents and antioxidant activities in lavender plantlets (*Lavandula multifida*) at three multiplication stages. Lavender plantlets were irradiated using different γ-irradiation dose levels (0.0, 5, 15, 30, 45, 60 and 75 Gy). After irradiation; plantlets were sectioned to start the multiplication stage (three subcultures). Increasing irradiation dose levels at multiplication stages significantly increased the total phenolic content and reached to the maximum increment at the dose level of 75 Gy (26.88 g/100g DW) in zero time stage in comparison with the untreated plantlets (7.250 g/100g DW). The highest content of flavonoids (21.50 g/100g DW) was detected at dose level of 75 Gy at zero time stage (M0). The highest applied irradiation dose of 75 Gy gave the highest reducing power activity compared with control at zero time stage (M0). Scavenging activity by DPPH was increased gradually by increasing irradiation dose levels in all multiplication stages until the high dose of 75 Gy which gave the maximum scavenging activity (91.05%) in zero time stage. Also, there was a significant increase in antioxidant activity on linoleic acid system with increasing the dose of γ-irradiation level. The application of γ-irradiation at dose level of 15 Gy and M3 stage produced the highest value of essential oil content (0.12%), followed by 5 Gy treatments (0.082%). The most increased volatile oil compounds by γ-irradiation were; limonene which increased from 4.87% to 5.37% at 0.0 and 5

Gy, respectively and linalool increased from 86.07% to 91.5% at 0.0 and 15 Gy respectively. The present study suggests that γ-irradiation led to increase antioxidant activities of lavender plantlets by increasing the availability of free polyphenolic compounds and also the content of volatile oil. This shows that lavender plants may be potent sources of natural antioxidants and these natural antioxidants could be stimulated by application of low doses of γ-irradiation.

Keywords: Antioxidant activity, γ-irradiation, essential oil composition, Lavandula multifida, phenolic compounds.

INTRODUCTION

Antioxidant components, which inhibits oxidation, or free radicals induced oxidative damage and therefore are potential quenchers of oxidative stress induced lipid peroxidiation. Antioxidants effectively prevent free radical induced cellular and tissue damage which are solely responsible for several pathogenic condition including cancer [1, 2]. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytolune (BHT), have restricted use in foods as these synthetic antioxidants are suspected to be carcinogenic [3]. Therefore, recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their potential health risks and toxicity [1, 4]. Antioxidants are naturally present in biological systems such as plant and plant derived products. Among the various natural antioxidants, phenolic compounds are reported to have the character of quenching oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical [5]. There are several usages of nuclear techniques in agriculture; in plant improvement, irradiation of seeds may cause genetic, variability that enable plant breeders to select new genotypes with improved characteristics such as precocity, salinity tolerance, grain yield and quality [6].

The biological effect of gamma-rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals [7]. These radicals can modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation dose [6]. These effects include changes in the metabolism, alteration in photosynthesis, modulation of the anti-oxidative system, and accumulation of phenolic compounds [8]. Radiation processing could cause an increase in most of the active aroma components of

coffee beans by releasing phenolic acids from their glycosidic bonds [9]. Several studies have been carried out to elucidate the effect of low doses of gamma rays on some aromatic plants such as chamomile [10], lemongrass [11], *Mathiola incana* and *Delphinium ajacis* [12]. The previous authors reported that low doses of gamma rays stimulated seed germination, plant growth and volatile oil production. In the search for alternatives to production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites [13].

Plants are sources of many bioactive compounds such as phenolic acids, and flavonoids which all have significant antioxidative effects [14]. Lavandula species are important medicinal plants because of its delightful odour, lavender is one of the most useful medicinal plant and has found wide application in perfumes and cosmetics, colognes, skin lotions and other cosmetics [15]. It has medicinal properties such as sedative, diuretic, diaphoretic, antiseptic carminative, spasmolytic, antidepressent and anti-rheumatic properties [16]. In food manufacturing, lavender essential oil is employed in flavoring beverages, ice-cream, candy, baked goods, and chewing gums [17]. Lavender oil, obtained from lavandula, is composed mainly of linalyl acetate, linalool, lavandulol, 1,8-cineole, lavandulyl acetate and camphor [18].

The propagation of lavender by seeds is slow and also exhibits variation in growth rate, growth by stem is not only slow but its rooting ability is also poor and due to repeated vegetative propagation it leads to modification of the morphological and chemical characteristics [19]. Thus, micropropagation through axillary buds, leaves and apical buds has been proposed as a useful technique and alternative method to cope up with this problem [20]. To the best of our knowledge there are no reports on the antioxidant activity and oil content of *in vitro* propagation on this plant under γ-irradiation stress. Therefore the main objectives of the present study were the evaluation of the regenerated plantlets for three subcultures of *Lavandula. multifida* to analyze their phenolic compounds, antioxidant activity and composition of essential oils by using low doses of γ-irradiation as activator.

MATERIALS AND METHODS

Plant materials:

Seeds of lavender plants (*Lavandula Mulifida*) were obtained from Experimental Farm, Faculty of Agriculture, Cairo University. The experiments of this investigation were carried out in the tissue culture laboratory of Natural Products Dept. National Centre for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo-Egypt and the tissue culture laboratory of Strawberry and Non-Traditional Crops Improvement Centre, Faculty of Agriculture, Ain Shams University, Kalubia-Egypt.

Culture establishment:

Seeds of lavender were washed thoroughly with tap water under aseptic conditions, sterilized using 70% ethanol (30 sec.) followed by 25% Clorox (15 min.) and then rinsed several times using sterile distilled water. The seeds were cultured on MS medium [21]. Culture jars were maintained in the growth chamber at 25±2ºC under 16-hrs/day photoperiod (1000 lux) and 8 hrs dark. The produced plantlets were used for the irradiation treatments.

Irradiation treatments:

Four weeks after establishment, culture jars were exposed to seven different dose levels (0.0, 5, 15, 30, 45, 60, and 75 Gy) in a ^{60}Co gamma radiation chamber (10400 CI [26/3/1988] ^{60}Co with a dose rate of 0.7 Gy/min) at the National Centre of Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo-Egypt. Irradiated plantlets (M0 stage) were immediately aseptically transferred into sterile fresh MS medium, placed in a growth chamber and subculture has been done every four weeks for three times after irradiation (M1, M2 and M3) whereas, M1, M2 and M3 stages were the first, the second and the third subculture, respectively. Plantlets samples were taken from M0, M1, M2 and M3 stages for the following determinations:

Plant extraction:

Fresh plantlets (0.5 g) were macerated in 10-20 ml 80% ethanol for 24 hrs at room temperature. The ethanolic extract was clarified and the remained tissue re-extracted with 10-20 ml 80% ethanol for three times. At the end clarified extract was completed to 100 ml using 80% ethanol. This extraction will be used for determining the total phenolic compounds, flavonoids content, and antioxidant activities by using three assays such as reducing power, scavenging effect by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) and antioxidant activity by a linoleic acid system.

Total phenolic content:

The phenolic contents were determined according to the method of Shahidi and Naczk [22] using the Folin-Ciocalteu reagent. Aliquots of 500 µl of each ethanolic extract were used for the measurements. Phenolic contents of the samples were calculated on the basis of the standard curve of gallic acid (GA). The results were expressed as g/100g of GA equivalent of plantlets dry weight.

Identification of phenolic compounds:

The extract was filtered through a whatman filter paper No. 1 and the solvent was evaporated under vacuum. The dried residues containing phenolic compounds were dissolved for HPLC analysis in a solution consists of methanol: water: acetic acid (40: 59.3: 0.7, v: v: v) and stored in vials as suggested by Christine *et al*. [23]. The separation and determination were performed on C18 column (150 x 4.6mm). The mobile phase yielded results of methanol: water: acetic acid, (40: 59.3: 0.7). The wave length of UV detector was 254 nm and the total run time for the separation was approximately 25 min at a flow rate of one ml/min. Identification of phenolic compounds was carried out by comparing retention times and spectral data with those of authentic standards (acceptable matches were 90–100%). Quantification was done by an external standard method.

Determination of total flavonoids content:

Aluminum chloride colorimetric method was used for flavonoids determination according to Marinova *et al.* [24]. The absorbance was measured against prepared reagent blank at 510 nm. Total flavonoids were expressed as g Quercetin equivalent / 100g plantlets dry weight.

Determination of antioxidant activities:

1. Determination of reducing power:

Reducing antioxidant power of plant ethanolic extracts were determined by the method of Oyaizu [25]. The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer. The increase in absorbance of the reaction indicated the reducing power of the samples.

2. Scavenging activity in 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical:

The free radical scavenging activity of ethanol extracts was measured by DPPH method proposed by Gulluce *et al*. [26]. Briefly, a 2.0 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 0.5 ml of samples. After 30 min, the absorbance (A) was measured at 517 nm. The DPPH radical-scavenging activity was calculated according to the following equation:

Scavenging activity (%) = $[A_0 - A_S / A_0] \times 100$.

Whereas A_0 and A_S are the absorbance at 517 nm of the control and sample solution, respectively.

3. An antioxidant activity in a linoleic acid system:

The total antioxidant activities of the samples were carried out by using of a linoleic acid system [27]. The peroxide value was determined by reading the absorbance at 500 nm on a spectrophotometer. The inhibition of lipid peroxidation was calculated as follows:

Inhibition of lipid peroxidation (%) = $100 - [(Abs_{sample} / Abs_{control}) \times 100]$

Essential oil content (%):

Oil percentage was determined using the method described by British Pharmacopoeia [28] using Clavenger's apparatus for determination of oil content. A known weight (500 gm) of fresh plantlets from each treatment was replaced in a flask of 1000 ml capacity for distillation. The distillation continued for three hours until no further increase in the oil was observed. The percentage of volatile oil was determined for the fresh plantlets of the different treatments with dose levels (0.0, 5, 15 and 30 Gy) from M3 stage. The oil quantity was determined volumetrically and its percentage was estimated (v/w).

GLC identification of essential oil constituents:

The volatile oil obtained from the fresh plantlets was analyzed using DsChrom 6200 Gas Chromatograph (At Medicinal and Aromatic Plants Institute, Agricultural Research Centre, Giza-Egypt) equipped with a flam ionization detector for separation of volatile oil constituent. Obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main components of volatile oil [29]. The products were quantified (mg/100g fresh tissue) by comparison of detector response with that of the internal standards, assuming equal response factors. Also, percentages of compounds were determined from their peak areas.

Statistical analysis:

Data were subjected to statistical analysis using the analysis of variance method and the means of treatments were compared by using the least significant difference (LSD) at 0.05 level of probability according to Duncan [30] multiple range test using two factors randomized complete block design (RCBD).

RESULTS AND DISCUSSION

Total phenolic content of plantlets:

Phenolic compounds are known to act as antioxidants not only because of their ability to donate hydrogen atoms or electrons but also because of their stable radical intermediates, which prevent the oxidation of various food ingredients, particularly fatty acids and oils [31]. The obtained results (Figure 1) indicated that the phenolic content increased gradually by increasing irradiation dose levels in all multiplication stages till the highest dose level of 75 Gy which gave the highest phnolic content (26.88 g/100g DW) at zero time stage (M0) in comparison with the untreated plantlets (7.250 g/100g DW). However, the total phenolic values at dose level of 60 Gy in the three multiplication stages, (M1, M2 and M3) were 21.58, 9.98 and 6.28 g/100g DW, respectively. While untreated plantlet still has the lowest content (9.27, 3.15 and 2.24 g/100g DW) of phenolic content as compared with treated plantlets, respectively.

The ethanolic extracts of phenolic compounds from lavender plantlets were identified by comparison of their retention times and UV spectra with those of known standards. The contents of these phenolic compounds were shown in (Tables 1, 2 and 3). Among thirteen identified phenolic compounds; catechein, pyrogallol, chlorogenic, *P*-OH-benzoic, catechol, caffeic, vanillic, syringic, ferullic, caffeine, *P*-coumaric, salicylic and cinnamic. Irradiation stimulated the biosynthesis of some phenolic compounds such as, the value of pyrogallol stimulated from 30.0 mg/100g FW at control to 47.2 mg/100g FW at dose level of 30 Gy at M0 stage (Table 1). On the other hand, in same stage catechein is not detected in treated or untreated plantlets. Meanwhile, at M1 stage (Table 2) catechein detected in irradiated plantlets only and increased by increasing irradiation dose level and reached to the maximum increase at dose level of 45 Gy (17.61 mg/100g FW). Also, there were some other phenolic compounds which had more abundant in irradiated treatments than in unirradiated one such as *P*-OH-benzoic which increased from 11.87 mg/100g FW at control treatment to 53.41 mg/100g FW at dose level of 60 Gy and ferulic was increased from 2.90 at control to 17.57 mg/100g FW at the same dose level. Data in Table (3) showed that the maximum increase for catechein, catechol and caffeic was appeared at dose level of 45 Gy. But for pyrogallol, *P*-OH-benzoic and ferulic their maximum increase was observed at dose level of 60 Gy. These results are in agreement with those of Aly [32] on *Eryngium foetidum*, El-Hady *et al*. [33] on

strawberry and Hussain, *et al*. [34] on peach fruit. Phenolic acids are a major class of phenolic compounds, widely occurring in the plant kingdom especially in fruits and vegetables. Moreover, the increase in total phenolic in irradiated fruits has also been reported by Lee *et al*. [35]. Mean while Oufedjikh *et al*. [36] indicated that γ-irradiation was known to stimulate the activity of phenylalanine ammonia lyase, which is responsible for the synthesis of polyphenolic acids. Such increase in total plenolic is due to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by γ-irradiation as suggested by Harrison and Were [37]. Also the increased amount of phenolic compounds was detected after γ-irradiation, which was attributed to the degradation of tannins and consequently, higher extractability of phenolic acids [9]. In addition Fan *et al*. [38] reported that the free radicals generated in plants during irradiation may act as stress signals and may trigger stress responses in plants, resulting in increasing polyphenolic acid synthesis which had notable antioxidative.

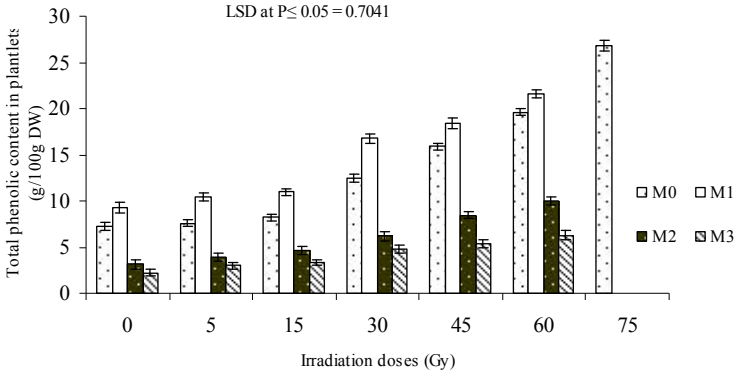

Figure (1): Total phenolic content of lavender plantlets (*Lavandula multifida*) during different multiplication stages for irradiated and unirradiated plantlets. Each value is expressed as mean ± SE (n = 3). The vertical bars represent the ±SE for each data point.

Table (1): Phenolic composition of lavender plantlets (*Lavandula multifida*) at M0 stage of irradiated and unirradiated plantlets.

Phenolic compounds	Irradiation treatment (Gy) (mg /100g FW)						
	0.0	**5**	**15**	**30**	**45**	**60**	**75**
Catechein	ND	ND	ND	ND	ND	ND	ND
Pyrogallol	30.0	46.3	42.2	47.2	46.4	46.2	38.9
Chlorogenic	5.94	2.22	2.8	3.6	3.25	3.96	1.2
***P*-OH-benzoic**	6.59	2.34	3.81	1.98	2.74	2.78	1.87
Catechol	ND	6.9	5.24	3.32	6.75	6.35	3.31
Caffeic	7.52	1.68	2.3	2.74	2.91	1.77	2.04
Vanillic	0.61	1.37	0.844	0.87	ND	1.16	1.27
Syringic	2.36	2.04	4.1	1.52	1.39	1.37	1.62
Ferullic	0.283	0.11	ND	0.233	0.17	0.08	0.36
Caffeine	1.74	2.16	1.91	2.99	2.86	2.46	1.63
***P*-Coumaric**	1.65	0.65	ND	0.99	1.49	0.583	2.63
Salycilic	0.64	0.204	0.54	0.192	0.16	0.09	0.38
Cinnamic	0.4	0.902	0.224	0.802	1.45	0.841	0.87

ND: None detected

Table (2): Phenolic composition of lavender plantlets (*Lavandula multifida*) at M1 stage of irradiated and unirradiated plantlets

Phenolic compounds	Irradiation treatment (Gy) (mg /100g FW)					
	0.0	**5**	**15**	**30**	**45**	**60**
Catechein	ND	10.88	11.84	11.64	17.61	14.84
Pyrogallol	23.5	11.33	13.00	12.90	12.75	10.80
Chlorogenic	18.06	14.20	12.10	11.56	7.00	3.46
***P*-OH-benzoic**	11.87	49.83	9.35	09.6	15.75	53.41
Catechol	17.28	14.50	13.07	6.93	19.25	22.66
Caffeic	8.32	10.2	3.77	5.90	7.94	15.64
Vanillic	4.79	6.18	03.5	7.00	010.4	06.7
Syringic	4.51	6.50	9.27	8.00	7.50	7.05
Ferullic	2.90	7.04	1.80	0.83	7.05	17.57
Caffeine	9.50	1.06	1.33	4.57	3.50	02.1
***P*-Coumaric**	5.00	5.52	3.00	0.168	1.90	3.02
Salycilic	3.5	01.7	1.61	0.64	0.50	0.16
Cinnamic	1.00	0.90	0.70	0.40	0.25	ND

ND: None detected

Table (3): Phenolic composition of lavender plantlets (*Lavandula multifida*) at M2 stage of irradiated and unirradiated plantlets

Phenolic compounds	Irradiation treatment (Gy) (mg /100g FW)					
	0.0	**5**	**15**	**30**	**45**	**60**
Catechein	3.94	28.02	26.67	31.96	32.79	15.85
Pyrogallol	2.83	4.02	5.10	16.01	5.10	18.95
Chlorogenic	13.00	11.50	10.00	8.90	8.00	5.36
P-OH-benzoic	9.01	25.51	12.02	14.26	14.44	47.35
Catechol	10.90	12.09	12.82	12.87	16.82	12.72
Caffeic	4.15	5.00	5.92	8.86	8.96	7.38
Vanillic	5.90	5.03	8.08	7.82	7.60	7.03
Syringic	4.00	6.00	8.18	9.50	8.50	7.11
Ferullic	1.58	5.45	6.10	2.85	1.72	11.71
Caffeine	4.00	3.84	3.00	2.78	2.28	1.62
P-Coumaric	2.50	1.90	1.20	6.90	6.40	ND
Salycilic	2.00	1.70	1.40	0.90	0.20	ND
Cinnamic	0.90	0.70	0.60	0.30	ND	ND

ND: None detected

Flavonoids content in plantlets:

Data recorded in Figure (2) showed that flavonoids content in lavender plantlets were increased in response to γ-irradiation treatments at different multiplication stages. The results showed that, all irradiation dose levels caused significant increases in flavonoids content through all the multiplication stages (M0, M1, M2 and M3). The highest content of flavonoids (21.50 g/100g DW) was detected under the influence of irradiation dose level of 75 Gy at zero time stage (M0), while in the other multiplication stages (M1, M2 and M3) the values were (19.49, 7.017 and 4.187 g/100g DW), respectively. Generally, it could be concluded that γ-irradiation treatments followed by multiplication stages increased gradually the flavonoids content. Whereas, the flavonoids content in the unirradiated treatment were 4.067 g/100g DW at zero time stage (M0) and (6.497, 1.557 and 1.370 g/100g DW) at M1, M2 and M3 stages, respectively. These results are in concomitant with those reported by Antognoni, *et al.* [39] on *Passiflora* and Aly [32] on culantro plantlets. The variation in total flavonoids content at different dose levels of γ-irradiation may be due to the induces of γ-irradiation on oxidative stress and de novo synthesis of flavonoids by increasing phenylalanine ammonia lyase (PAL) activity which is a crucial enzyme of flavonoids and tannins biosynthesis [36]. In addition, such increases in total

phenolic and total flavonoids is due to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by gamma irradiation as suggested by Harrison and Were [37]. Irradiation exerts its effects as radiolysis of water results in the production of free radicals such as hydroxyl radicals, hydroperoxide radicals and hydrated electrons. These radicals may break the glycosidic bonds of procyanidin trimer, tetramer and hexamer that are present in fruits, leading to the formation of procyanidin monomers, which increase the total phenolic content in irradiated fruits [35].

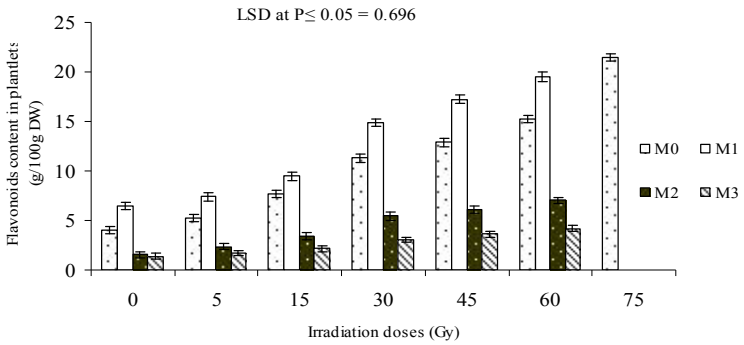

Figure (2): Flavonoids content of lavender plantlets (*Lavandula multifida*) during different multiplication stages for irradiated and unirradiated plantlets. Each value is expressed as mean ± SE (n = 3). The vertical bars represent the ±SE for each data point.

Antioxidant activity:

1. Reducing power:

The measurement ferric-reducing antioxidant power (FRAP) based on compounds' ability to reduce Fe^{3+} to Fe^{2+}. Therefore, the increase in values may be due to the change in the redox state of metal ions, formation of reductants, and/or formation of new antioxidants [41]. The reducing power of ethanolic extracts of lavender plantlets in response to gamma irradiations treatment followed by different multiplication stages recorded in Figure (3). Generally results indicated that there was a slight increase in reducing power with increasing irradiation dose level. The highest applied irradiation dose (75 Gy) gave the highest absorbance for the reducing power (0.420) compared with the control (0.291) at zero time stage (M0) while at 60 Gy the absorbance for the reducing power were (0.4630, 0.365 and 0.328) in the three multiplication stages (M1, M2 and M3), respectively, compared with their controls (0.253, 0.313 and 0.307, respectively). These results are in agreement with those of Fan and Thayer, [40] on apple juice and Suhaj and

Horváthová, [41] on clove (*Syzygium aromaticum*) and ginger (*Zingiber officinale*). During irradiation, reductants and novel new antioxidants may be formed [42]. The antioxidant activity of putative antioxidants have been attributed to various mechanisms, such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging [43]. Worth mention that, the redox state of ions and compounds may also be changed by irradiation. For example, the hydrated electrons (aqueous) formed from water radiolysis can react strongly with metal ions (such as Fe^{3+}) and reduce them to lower redox states (Fe^{2+}).

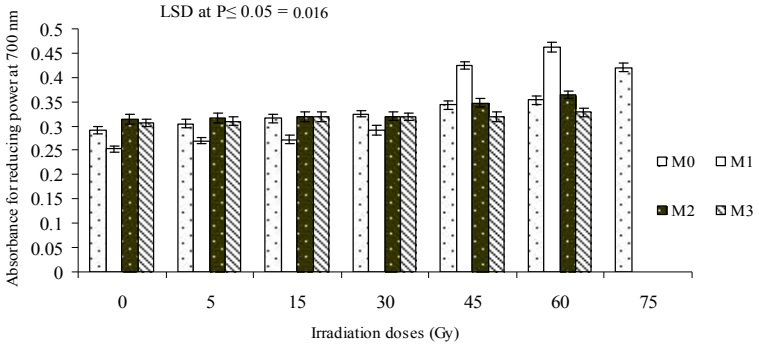

Figure (3): Reducing power (Absorbance at 700 nm) of ethanolic extracts of lavender plantlets (*Lavandula multifida*) during different multiplication stages for irradiated and unirradiate plantlets. Each value is expressed as mean ± SE (n = 3). The vertical bars represent the ± SE for each data point.

2. Scavenging activity on DPPH radical:

Radical scavengers were evaluated by their reactivity toward a stable-free radical (DPPH). The effect of gamma irradiation on DPPH radical scavenging activity (RSA) of lavender extracts are shown in Figure (4). The obtained results indicated that the scavenging activity increased gradually by increasing irradiation dose levels in all multiplication stages. The highest dose of 75 Gy gave the highest scavenging activity (91.05%) at zero time stage (M0) while, the values in the three multiplication stages at dose level 60 Gy were 92.90, 85.49 and 71.91%, respectively. Moreover, untreated plantlets still has the lowest contents (81.17, 87.35, 45.68 and 31.48%), respectively of scavenging activity compared with treated plantlets in all the multiplication stages (M0, M1, M2 and M3, respectively). Regarding to the relation between scavenging activity of lavender plantlets (Figure 4) and total phenolic (Figure 1), flavonoids content (Figure 2), it could be noticed that scavenging activity increased with increasing

total phenolic and flavonoid contents in lavender plantlets. These results are in agreement with those of Vicente *et al.* [44] on peppers and Kim *et al.* [45] on *Citrus unshiu* pomaces and Khattak and Simpson [46] on *Glycyrrhiza glabra*. DPPH is a stable free radical capable to accept an electron from reactive radicals, thus behaving as a radical scavenger. Also, Mohamed [47] reported that γ-irradiation at dose level of 40 Gy significantly increased the DPPH radical-scavenging activity in culantro plantlets. Additionally, DPPH acts as an acceptor of electrons from antioxidants; several electron transfer reactions of DPPH with phenols, amines and other compounds were described in literature [48, 49]. Higher levels of DPPH antiradical activity were in correlation with plant polyphenols [50]. The phenolic compounds are commonly contained at least one hydroxyl substituted aromatic ring system, that can easily oxidized, as well as serving as important units for donating electrons. Plant extracts which show the potent proton-donating ability on DPPH to produce DPPHH, considered as an important mechanism of antioxidants. In addition, low irradiation dose level results in the intracellular generation of reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2) in plant tissues which may alter their phytochemical antioxidant content [7].

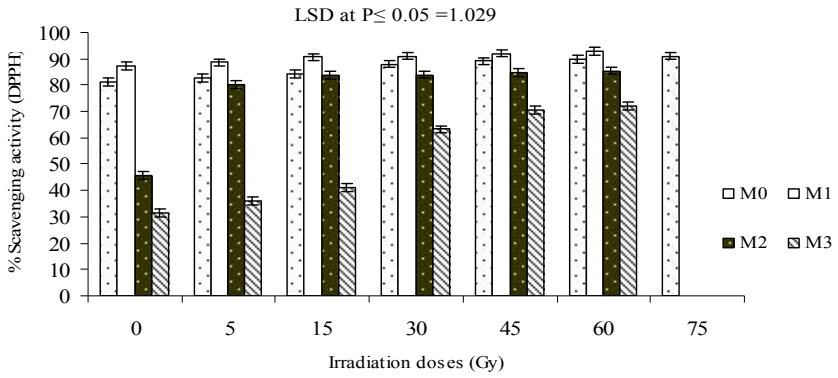

Figure (4): Antioxidant activity % on DPPH of ethanolic extracts of lavender plantlets (*Lavandula multifida*) during different multiplication stages for irradiated and unirradiated plantlets. Each value is expressed as mean ± SE (n = 3). The vertical bars represent the ±SE for each data point.

3. Antioxidant activity on a linoleic acid system:

Data recorded in Figure (5) showed the, percentage of the antioxidant activity of lavender plantlets on linoleic acid system. The obtained results indicated that there was significant increase in antioxidant activity on linoleic

acid system with increasing the dose level of γ-irradiation. In the case of zero time stage (M0), there was significant increase in the percentage of antioxidant activity on linoleic acid system from 16.22% to 22.62% with increasing γ-irradiation dose level from 5 to 75 Gy, respectively in comparison with the untreated sample (11.31%). Moreover, there were significant increases in the percentage of the antioxidant activity on linoleic acid system for the other multiplication stages of lavender plantlets in M1 from 55.19% to 62.39% with increasing γ-irradiation dose level from 5 to 60 Gy, respectively, in M2 from 12.66% to 21.18% and in M3 from 13.31% to 22.41% in comparison with the controls (54.56, 9.99 and 12.04%, respectively). The results indicated that there was positive relationship between total phenolic and flavonoids contents and the percentage of the antioxidant activity on linoleic acid system. These results are in agreement with those of Huang *et al.* [51] on Brazilian mushroom (*Agaricus blazei*). Also, Dixit *et al* [52] observed that soybean genotypes showed an increase in antioxidant constituents and antioxidative properties at lower doses of gamma irradiation 0.5 and 2.0 kGy. Antioxidant activity of *Lavandula multifida* is a result of phenolic compounds, in the same respect, Aly and Mohamed [53] cited that natural antioxidant compounds such as phenolic compounds and glutathione can be induced and increased by using low doses of gamma irradiation in maize callus tissues.

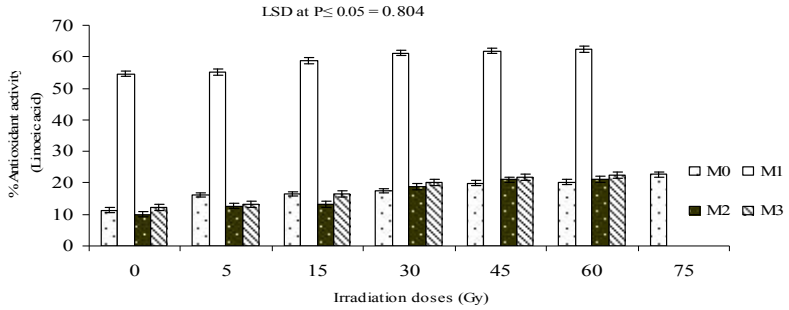

Figure (5): Antioxidant activity (%) on a linoleic acid system of ethanolic extracts of lavender plantlets (*Lavandula multifida*) during different multiplication stages for irradiated and unirradiated plantlets. Each value is expressed as mean ± SE (n = 3). The vertical bars represent the ± SE for each data point

Essential oil content:

The data presented in Table (4) showed the effect of γ-irradiation on the percentage of essential oil content in lavender plantlets. The data indicated that the application of γ-irradiation at dose level of 15 Gy produced the highest value of essential oil content (0.12%), followed by 5 Gy treatment (0.082%). A clear decline was observed in the essential oil content by increasing the irradiation dose at 30 Gy which gave the lowest value of essential oil content (0.036%) in comparison with the control (0.079%). The enhancement of γ-irradiation to the essential oil may be due to its stimulative effect on fresh mass, as well as the activation of enzymes involved in the metabolism of essential oil formation. The essential oil extracted from the plantlets exposed to γ-irradiation dose levels 5, 15 and 30 Gy were analyzed using GLC in combarison with control as shown in Table (5). The major principal components of *Lavandula multifida* oil were α-pinene, camphene, myrcene, α-cymene, limonene, camphor, linalool, β-caryophellen and the most predominant one in all samples was linalool since its percentage was between 86.07 – 91.50% at 0.0 and 15 Gy, respectively. Limonene also showed a relatively high percentage since its values was between 4.87 to 5.37 at 0.0 and 5 Gy, respectively. The results of the present study are supported by previous published studies that report, an increase in oil production by gamma irradiation in several plant species [54, 55]. Also, Zeid *et al*. [56] indicated that exposing the fennel seeds to pre sowing γ-irradiation was beneficial at dose level of 20 Gy, which markedly increased essential oil percentage and enhanced yield of essential oil. In the same respect, percent of essential oil content of coriander seedlings increased at the range between 40 to 80 Gy in comparison with control samples [57]. However, Srivastava and Tyagi [58] observed a significant increase in palmarosa (*Cymbopogon martinii*) essential oil production at 100 and 150 Gy of γ-irradiation. On the other hand, high dose of γ-irradiation decreased the oil production in other studies [59, 60].

Table (4): Essential oil content of lavender plantlets (*Lavandula multifida* in the end of the third multiplication stage (M3) for γ-irradiation dose levels of 0.0, 5, 15 and 30 Gy.

Irradiation doses (Gy)	Essential oil (%)
Control.	0.079
5	0.082
15	0.120
30	0.036

Table (5): GLC analysis of major compounds of *Lavandula multifida* oil in

the end of the third multiplication stage (M3) for γ-irradiation dose levels 0.0, 5, 15 and 30 Gy

Compound name	Irradiation doses (Gy)				
	Rt	**0.0**	**5**	**15**	**30**
α-Pinene	3.546	1.21	0.84	ND	0.38
Camphene	3.871	0.57	ND	ND	0.17
Myrcene	4.150	3.07	2.49	0.16	1.49
α-Cymene	4.921	1.35	1.54	ND	0.53
Limonene	5.689	4.87	5.37	4.26	5.35
Camphor	7.187	0.66	0.74	0.30	0.68
Linalool	8.872	86.07	87.33	91.50	89.00
β-Caryophellen	10.907	1.61	1.68	1.87	1.76
Unknown	-	-	-	1.73	0.44

ND: None detected; **Rt:** retention time

CONCLUSION

In vitro mutagenesis through gamma radiation can be employed to create economically superior mutants. Gamma irradiations are often applied on plants for developing varieties which are economically important and comprise high productivity and efficiency potential. The results indicated that the irradiated plantlets of lavender displayed higher total phenolic and flavonoid contents than the non-irradiated ones. Moreover, the irradiated plants were found to have the highest antioxidants activity. It is noteworthy in this study that under low doses of gamma irradiation the contents of essential oil were increased. In the future prospects, the in vitro propagation of *Lavandula multifida* as well as the use of gamma irradiation as elicitor could enhance some valuable secondary metabolites such as phenolic and flavonoids which possesses important medicinal properties and are extensively used as a food supplements in many countries. Further investigation on the effects of gamma irradiation on *Lavandula multifida* could be conducted to facilitate the creation of a mutant with superior physiological, agronomical and biochemical qualities for commercial use.

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مجلة البحوث الإشعاعية والعلوم التطبيقية

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التشعيع الجامي كمحفز للمركبات المضادة للأكسدة ومحتويات الزيت العطري فى نبيتات اللافندر

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أجريت هذه الدراسة لتقييم التأثير المحفز لأشعة جاما خلال ثلاثة مراحل من التكاثر الخضري عن طريق زراعة الأنسجة لنبتات اللافندر(*Lavandula multifida* L) علي التركيب الكيميائي للزيوت العطرية، الفينولات الكلية، محتوي الفلافونويدات حيث تم تعريض نبيتات اللافندر الي أشعة مستويات مختلفة من الجرعات صفر، 5 ، 15، 30 ، 45 ، 60 ، 75 جراي (M0) ثم بعد ذلك تم إجراء التكاثر الخضري لثلاث مراحل متتالية M1, M2, M3.

وجد انه زاد محتوي النبتات من الفينولات الكلية بزيادة الجرعة الإشعاعية خلال مراحل الإكثار الثلاثة و وصلت الي الحد الأقصى للزيادة عند مستوى الجرعة 75.0 جراي (26.88 جرام/100 جرام وزن جاف) في مرحلة M0 مقارنة بنبيتات الجرعة الضابطة (الكنترول) حيث كانت (7.250جرام/100 جرام وزن جاف). كذلك تم الحصول علي أعلى محتوي من الفلافونويدات (21.50جرام/100جرام وزن جاف) عند مستوى الجرعة 75.0 جراي في مرحلة M0. بينما كان محتوي الفلافونيدات عند مستوي الجرعة 60 جراي لمراحل الإكثار الثلاثة M1، M2، M3 هي 19.49، 7.017 ، جرام/100 جرام وزن جاف 4.187 علي التوالي. أعطت نشاط قوة الاختزال اعلي معدل لها عندي مستوي جرعة 75.0 جراى مقرنة بالجرعة الضابطة في مرحلة (M0). مضادات الاكسدة (DPPH) زادت بزيادة الجرعة الإشعاعية المستخدمة الي ان وصلت الي اقصي حد للزيادة عند الجرعة 75،0 جراي حيث كانت (91.05٪) في مرحلة (M0). لوحظ ان النشاط المضاد للأكسدة زاد بزيادة محتوي كلا من الفينولات والفلافونيدات. كذلك وجد أيضا انه كانت هناك زيادة معنوية في النشاط المضادة للأكسدة في النظام حمض اللينوليك مع زيادة مستوي الجرعة الإشعاعية.

وجد ان الجرعة المنخفضة (15 جراى) أعطت أعلى محتوى للزيت العطرى 0.12 % بينما قل محتوى الزيت بزيادة مستوي الجرعة الإشعاعية المستخدمة. اما بالنسبة للتركيب الكميائي للزيت العطري تحت تأثير الإشعاع الجامي وجد ان أكثر المركبات التي تم زادت تحت تأثير أشعة جاما هي ليمونين (lemonene) زاد من 4.87 % للجرعة الضابطة الي 5.37 % عند مستوي الجرعة الإشعاعية 5 جراي وايضا الليناليول (linalool) زاد من 86.07 % في الجرعة الضابطة الي 91.5 % عند مستوي جرعة 15 جراي.

أوضحت تلك الدراسة إن المستويات المنخفضة من الإشعاع الجامي يزيد من محتوي مضادة الأكسدة للمركبات الثانوية للافندر وذلك عن طريق زيادة المركبات الفينولية الكلية وكذلك مكونات الزيوت العطرية. وهذا يشير الي ان نبات اللافندر قد يكون مصدر قوي من مصادر مضادات الأكسدة الطبيعية التي يمكن زيادة محتواها عن طريق تعرض النبيتات الناتجة من زراعة الأنسجة الي الجرعات المنخفضة من أشعة جاما.