Effect of Gamma Irradiation on Production and Chemical Composition of Some Medicinal and Aromatic Plants in KSA Using Tissue Culture Technique

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Abstract: This investigation was carried out on four species of medicinal and aromatic plants (Lavender, Chamomile, Sweet basil and Visnage), seeds of plants were treated with different Clorox concentrations (10%Cl, 20%Cl, 25%Cl and 30%Cl) for15 min. Growing explants of all species cultured on MS medium sterilized with 25%Cl gave uncontaminated lavender plants, also highest survival percentage and low contaminated for other species plants. Seeds cultured on solid MS medium supplemented with 0.0, 1.0, 2.0 or 3.0 mg/l of BA. After two months the optimal concentration of BA was chosen and explants were sub-cultured on shooting medium (MS) enriched with best BA and were exposed to gamma rays at 10, 20, 30, 40 and 50 Gy, and then plantlets were cultured on MS medium without or with NAA at the rate of 0.0, 1.0 and 2.0 mg/l. The results indicated that maximum number of shoots of lavender plants was produced on MS medium enriched with 1.0 and 2.0 mg/l of BA, whereas the maximum number of shoots of sweet basil and visnage plants were obtained on medium enriched with 1.0 BA alone. The plants of chamomile formed significantly number of shoots with MS medium without BA. The plants of lavender, sweet basil and visnage plants were formed significantly length of shoots with MS medium with 1 mg/l BA. The growing of the explants from chamomile on MS control medium gave the longest shoots. The highest survival percentage (98.6%) was recorded with the control of sweet basil plants. The addition of BA at 1.0, 2.0 or 3.0 mg/l to MS medium gave a significant decreasing of the survival percentage of all species,except1 mg/l BA of lavender explant. The low dose of gamma rays (20 Gy), significantly increased the average number of roots and root length on lavender plants with or without NAA. Most high irradiation treatments without or with NAA decreased the number and the length of roots. Gamma rays induced changes on the chemical composition in species under this study.

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1.Introduction

Medicinal and aromatic plants have been used for a wide variety of purposes such as food preservation, pharmaceutical, alternative medicine, and natural therapies for many thousands of years. It is generally considered that compounds produced naturally, rather than synthetically.

Lavender plants (*Lavandula officinalis*): Fam. Labiatae, is an important aromatic plants. It grows well in North Africa, Europe and Asia. Tissue culture technique is also very often a model system, which allows one to investigate physiological, biochemical, genetic and structural problems related to plants **(Biondi and Thorpe, 1981)**.

Bondarenko and Murin (1981) studied lavandula species and obtained morphological mutation with gamma irradiation at 1 k-rad dose. In recent years, *Lavandula officinalis* flowers exhibit such various biological and pharmacological activities as anti-tumor, anti-inflammatory, antihistaminic, antidiabetic, and antimicrobial activity and modulating the central nervous system **(Munchid *et al.,* 2005,Tahraoui *et al.,* 2007 and Tawaha *et al.,* 2007)**.

The fruits of *Ammi visnaga* L. (Apiaceae; "Khella") have traditionally been used to relieve pain of kidney stone passage by drinking a tea prepared from the crushed or powdered fruits of Khella **(Khan *et al.,* 2001, Gunaydin and Beyazit, 2004)**, investigated the effects of an *Ammi visnaga* extract in an animal model of nephrolithiasis and found that the plant extract significantly reduced the incidence of calcium oxalate deposition in the rat kidneys. However, the extract in that study was not further phytochemically characterized leaving the question regarding possible active compounds open. We recently showed that an aqueous extract of *Ammi visnaga* L., as well as the main compounds khellin and visnagin could prevent cell damage caused by oxalate (Ox) or calcium oxalate monohydrate (COM) crystals in renal epithelial cells **(Vanachayangkul *et al.,* 2010)**.

Chamomile (*Matricaria chamomil* L.) is one of the oldest and agronomical most important medicinal plant species in Europe. It originates from southeastern Europe and western Asia, but is nowadays cultivated throughout the world.

The essential oil of chamomile flowers has significant anti-phlogistic **(Jakovlev *et al.,* 1983)**, spasmolytic **(Maschi *et al.,* 2008)** and antimicrobial activity **(Lis-Balchin *et al.,* 1998)** and is therefore used for several pharmaceutical, nutritional and cosmetic applications. The pharmaceutically active components of the flower oil are chamazulene, a degradation product spontaneously formed during steam distillation from the sesquiterpene lactone matricine, several bisabolol-type sesquiterpenes [(−)-α-bisabolol, bisabolol oxides], flavonoids and two en-in-dicycloethers **(Repcak *et al.,* 1980; Reichling *et al.,* 1984 and Kumar *et al.,* 2001)** with chamazulene and the bisabolols being the main active constituents **(Schilcher *et al.,* 2005)**.

Ocimum basilicum is one of the more than 60 Ocimum species. These Ocimum plants, plant parts, extracts and essential oils are used as spices and flavors for various food products as well as effective drugs for many applications in folk medicine especially in Africa and Asia.

Sweet basil (*Ocimum basilicum*) is a culinary herb belonging to the mint family that also serves as a model system to investigate specialized metabolism in specific cell types in plants. The volatile compounds defining the flavor of basil are produced by the secretory cells and then stored in the oil cavity under the elevated cuticle of the peltate glandular trichomes on the leaf surface **(Werker *et al.,* 1993 and Gang *et al.,* 2001)**. Basil also produces PMFs, whose occurrence and distribution in a number of basil cultivars have been studied in detail **(Vieira *et al.,* 2003)**. The production of “secondary” or specialized metabolites is part of the chemical defense, ecological adaptation, and signaling mechanisms of plants **(Wink, 2008 and Pichersky and Lewinsohn, 2011)**.

In vitro propagation has been successfully applied for the conservation of a large number of medicinal and aromatic plant species that have limited reproductive capacity and exist in threatened habitats **(George *et al.,* 2008 and Pence, 2010)**. Recently, there have been a number of established protocols for *in vitro* propagation of rare and threatened medicinal plants such as *Dysosma versipellis* **(Jiang *et al.,* 2012)**, *Polygonatum verticillatum* (**Bisht *et al.,* 2012**), *Pseudarthria viscida* **(Cheruvathur and Thomas, 2011)**, and *Thymus lotocephalus* **(Coelho *et al.,* 2012)**.

Many workers pointed out the effect of cytokinins, auxins as well as gamma irradiation on shoot formation and rooting of many medicinal and aromatic plants, **(Witomska and Gadamska, 1995)** on *Limonium caspium* obtained best shoot production with low level of NAA. **(Ilahi *et al.,* 1995)** obtained good shoot proliferation with 1.0, 2.0 and 3.0 mg/l BA. On Anthurium plants, **(Somaya, 1998)** found that MS medium enriched with 1 mg/l BA +0.25 mg/l NAA increased the number of roots. Adding, 0.25 mg/l NAA to medium increased the number of roots.

Radiation effect was found on plant height, size of leaf and flower. **(Kozlowska, 1994)** on cymbidium plants found that the exposition dose 0.8 kr proved to stimulate the growth and development of protocorms, the dose 20.0 k-rad totally inhibited the growth.

Effect of low gamma irradiation dose on growth may be due to the increase of cell length or cell number and size, **(Pitirmovae, 1979)**. **Youssef and Moussa, (1998)** in their study on chamomile, they found increase in plant high with gamma irradiation at 1Krad. **(Nagata *et al.,* 1999)** used gamma irradiation at dose 1-3 KGy to induced mutation in Arabidopsis, this result suggested that radiation induced trichome formation. **Badawy *et al.,* (2003)** reported that gamma irradiation inhibited formation of shoots of *Lavendula officinalis* cultured in vitro but exposing shoots to 10 Gray resulted in the highest essential oil yield.

Concerning the effect of irradiation, **(Venkatachalam *et al.,* 1999)** on ground nut, found that exposing the calli to 0 to 250 Gy gamma rays stimulated the shoot formation and plant regeneration capacity increased with increasing gamma rays dose. **(Omer *et al.,* 1999)** on sunflower found a significant increase in protein, carbohydrates, DNA, and reduction in callus weight with increased doses of irradiation. **Predieri and Edoardo, (2000)** on plum reported that micro cuttings originated from shoots irradiated with 30 and 40 Gy showed a reduced rooting capacity. **(Datta *et al.,* (2005)** on *Chrysanthemum morifolium* cvs. Flirt, Puja, Maghi and Sunil, treated the explants with 500 and1000 rad gamma rays and cultured on MS medium supplemented with different concentrations and combinations of growth regulators. They found that the frequency of direct shoot regeneration decreased in gamma-ray-treated florets. Radiation effect was found on plant height, size of leaf and flower.

Youssef and Moussa (1998) on *Melaeuca armillaris*, they reported increase in chemical composition with increase doses of gamma rays.

This study was conducted to determine the effects of BA as well as NAA at different concentrations as well as gamma rays treatments on in-vitro propagation and chemical composition of four species of medicinal and aromatic KSA plants.

2.Material and Methods

This investigation was carried out in Plant Tissue Culture Lab of Biotechnology Department, Faculty of Sci., Taif Univ., during 2013. Seeds of four species of medicinal and aromatic plants of Taif region in KSA plants namely (*Lavendula officinallis*, *Matricaria chamomil*, *Ocimum basilicum* and *Ammi visnage*) were washed in soap water for 30 min. and rinsed with running tap water for two hours and sterilized by 10, 20, 25 and 30% Clorox (sodium hypochlorite). The seeds were cultured on jars containing solid Murashige and Skoog (MS) medium at full strength, supplemented with agar (7.0 gm /l) and sucrose (30 gm/l and supplemented with 6-benzyladenine (BA) at 0.0, 1.0, 2.0 and 3.0 mg/l. The jars were sterilized at 121°C for 20 min., and Temp:24±2°Cday/night. Photoperiod: 16hrslight/8hrs'darkness, Illumination intensity: 3000 lux.

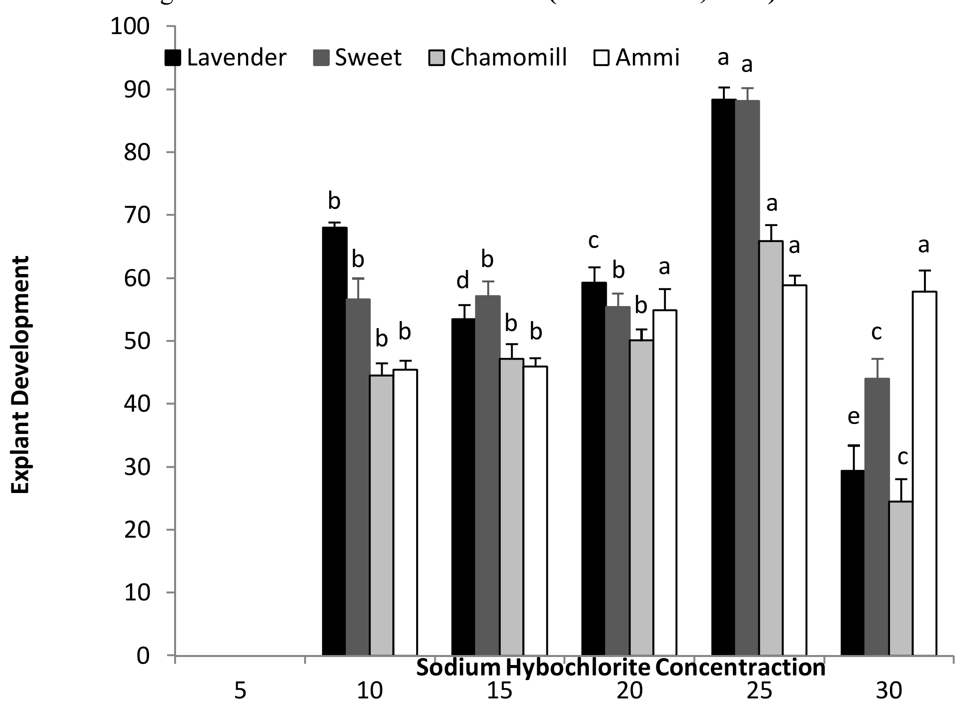
After two months the explants were transferred into shooting medium (MS) enriched with the optimal concentration of BA. In relation to the irradiation studies, the explants in the jars were received five doses of gamma rays: 10, 20, 30, 40 and 50 Gy. emitted from cobalt 60 source from unit gamma chamber 4000, after four weeks the jars containing MS medium supplemented with a-naphthalene acetic acid (NAA) at 0.0, 1.0 and 2.0 mg/l., were placed in the growth chamber at the following conditions: Temp: 24±2°C day/night.Photoperiod:16 hrs light/8hrs' darkness fluorescent tubes, using Gro-Lux 20 Wm-2 as a source of light. Regenerated plants were transferred to green house to acclimatization to study the effect of different concentration of in vitro plantlets treatments behavior and other effects on chemical composition after exposing to gamma doses. Every treatment consisted of 6 replicates.

Data recorded: number of shoots, shoot length, survival percentage, number of roots and root length. Statistical analysis: All results were subjected to one-way ANOVA and the means were compared according to the Student–Newman–Keuls (SNK) multiple range test (P≤0.05).

3.Results And Discussion

Effect of Sterilization Treatments on Explant Growth Of Lavender, Sweet Basil, Chamomile And Visnage Seeds.

As shown in Fig, (1) regardless sterilizations levels, the survival% and contamination % of Lavender,Sweet basil and Chamomile seeds were decreased to lowest values with increasing Cl concentrations to maximum rate 30 % except the survival % of Visnage seeds comparing with other treatments, and sterilized the all seeds by 25% Cl was more effective in increasing survival% to highest level (88.33,88.22, 66.33, and 59.44 %) for Lavender, Sweet basil, Chamomile and Visnage seeds, respectively. These results are in agreement with that of **(Montes *et al.*, 1997)**.



Fig,(1): Different letters on the same column are differ significantly at $p \leq 0.05$.

Effect of BA concenterations on number of shoots and shoot length of lavender,sweet basil, chamomile and visnage explants.

As shown in Table (1), regardless media composition, the lavender seeds formed number of shoots more significantly with BA at different concentrations compared with control and BA at 1.0 or 2.0mg/l produced the highest number of shoots/explants (3.3 and 4.2 shoots, respectively).

The results, also shown in Table (1) indicated that there was a significant difference of the shoot length. The lavender seeds formed significantly average of shoot length more with BA at 1 or 2 mg/l than the control or 3 BA, the shoot lengths were 4.1and 4.0 cm, respectively.

Similar findings were reported by **(Ilahi *et al.*, 1995)** obtained good shoot proliferation with 1, 2 and 3 mg/l BA.

As shown in Table (1), regardless media composition, the sweet basil seeds formed number of shoots more significantly with 1 mg/l BA compared with control and BA at different concentrations and produced the highest number of shoots/explants (5.3 shoots/plant).

The results,also shown in Table (1) indicated that the highest shoot length was produced with 1 mg/l BA and control (4.6 cm) The sweet basil seeds formed significantly average of lowest shoot length with BA at 2 or 3 mg/l than the control or 1 BA, the shoot lengths were 3.6 and 3 cm. Similar findings were reported by **(El-Sawy and Bekheet, 1999)** on *Dieffenbachia picta* cv. Tropica. Found that BA at 1.0 mg/l was more effective for increasing the number of shoots. Also, **(El-Sawy *et al.*, 2000)** on *Dracaena* cv. Tricolour, obtained the largest number of shoots/explant with adding BA.

As shown in Table (1), regardless media composition, the chamomile seeds formed number of shoots more significantly with control compared with BA at different concentrations and produced the highest number of shoots/explants (3.7 shoots/plant).

The results, also shown in Table (1) indicated that the highest shoot length was produced with control MS medium (4.6 cm). The chamomile seeds formed significantly average of lowest shoot length with BA at different concentrations. A similar findings were reported by **(El-Sawy *et al.*, 2000).**

As shown in Table (1), regardless media composition, the Visnage seeds formed number of shoots more significantly with BA at 1 mg / l compared with control and BA at 2.0 or 3.0mg/l, and produced the highest number of shoots/explants (1.7 shoots).

The results, also shown in Table (1) indicated that there was a significant difference of the shoot length between the control and BA treatments. The Visnage seeds formed more average of shoot length with BA at 1 mg/l than the control or other BA concentrations, the shoot lengths were 2 cm.

A similar findings were reported by **(Ilahi *et al.*, 1995)**, also with **(El-Sawy and Bekheet, 1999)** on *Dieffenbachia picta* cv. Tropica. Found that BA at 1.0 or 2.0 mg/l was more effective for increasing the number of shoots.

Table (1): Effect of BA concentration on number of shoot and shoot length of Lavender, Sweet basil, Chamomile and Ammi explants.

		No. of shoots					Shoot length			
		Lavender	Sweet	Chamomill	Ammi		Lavender	Sweet	Chamomill	Ammi
Control		2.3^{c}	4.1^{b}	3.7^{a}	1.3^{b}		2.9^{b}	4.6^{a}	4.6^{a}	1.3^{a}
1 mg		3.3^{b}	5.3^{a}	3.0^{b}	1.7^{a}		4.1^{a}	4.6^{a}	3.7^{b}	2.0^{a}
2 mg		4.2^{a}	4.1^{b}	3.4^{ab}	1.0^{c}		4.0^{a}	3.6^{b}	3.7^{b}	1.7^{a}
3 mg		2.7^{b}	4.3^{b}	3.3^{ab}	0.9^{c}		3.0^{b}	3.0^{b}	3.6^{b}	1.3^{a}

Different letters on the same column are differ significantly at $p \leq 0.05$.

Effect of BA concenterations on number of leaves and survival % of lavender,sweet basil, chamomile and visnage explants.

As shown in Table (2),the addition of BA at 2 or 3 mg/l to MS medium had a significant effect on the number of leaves of lavender plants than other treatments and produced (4.0 and 4.3 leaves/plant, respectively).

Concerning the response of survival percentage to media composition (Table 2), the results clearly indicated that the highest survival percentage (95.4%) was recorded with the MS medium containing 1 mg/l BA. Similar findings were reported by **Sachez and Calvo (1996)** who found similar results with *Lavandula latifolia* plants.

The addition of BA at 2 mg/l to MS medium had a significant effect on the number of leaves of sweet basil than other treatments and produced (5.6 leaves/ plant). The addition of BA on MS medium containing 3.0 mg/l gave the lowest number of shoots (3.1 leaves/plant).

Concerning the response of survival percentage to media composition (Table 2), the results clearly indicated that the highest survival percentage in sweet basil (98.6%) was recorded with the control medium without BA.

The addition of BA at 1.0, 2.0 or 3.0 mg/l on the MS medium were the most effective treatments in decreasing the survival percentage in sweet basil

A similar findings were reported by (**El-Sawy *et al.*, 2000** and **Badawy *et al.*, 2003**).

Data also reported that the highest number of leaves on chamomile plants was produced with MS medium containing 1 mg/ BA, and produced (5.0 leaves/plant).

Concerning the response of survival percentage to media composition (Table 2), the results clearly indicated that the highest survival percentage (84.6%) was recorded with the control medium without BA. The addition of BA at 1.0, 2.0 or 3.0 mg/l on the MS medium were the most effective treatments in decreasing the survival percentage in chamomile. A similar findings were reported by (**El-Sawy *et al.*, 2000** and **Badawy *et al.*, 2003**).

Data in Table (2),declared that the control of visnage explants gave the highest number of leaves (1.7 leaves/plant).

Concerning the response of survival percentage to media composition (Table 2), the results clearly indicated a significant increase in highest survival percentage visnage (59.6%) was recorded with the control than other treatments.

The addition of BA at 1.0, 2.0 or 3.0 mg/l on the MS medium was the most effective treatments in decreasing the survival percentage Visnage. A similar findings were reported by (**Badawy *et al.*, 2003**).

Table (2): Effect of BA concentration on number of leaves and survival percentage of Lavender, Sweet basil, Chamomile and Ammi explants.

		Leave No.					Survival (%)			
		Lavender	Sweet	Chamomill	Ammi		Lavender	Sweet	Chamomill	Ammi
Control		2.3^{c}	4.2^{b}	4.0^{b}	1.7^{a}		91.8^{a}	98.6^{a}	84.6^{a}	59.6^{a}
1 mg		3.3^{b}	4.3^{b}	5.0^{a}	1.3^{a}		95.4^{a}	95.2^{b}	81.5^{a}	54.6^{b}
2 mg		4.0^{a}	5.6^{a}	3.4^{b}	1.4^{a}		90.0^{a}	94.9^{b}	80.5^{a}	50.0^{c}
3 mg		4.3^{a}	3.1^{c}	3.7^{b}	1.0^{b}		91.6^{a}	95.0^{b}	81.4^{a}	50.2^{c}

Different letters on the same column are differ significantly at $p \leq 0.05$.

Effect of gamma irradiation and NAA on number of roots of lavender,sweet basil, chamomile and visnage explants

This experiment (Table 3) was done to study the effect of gamma irradiation and the effect of NAA added to MS medium on root formation of the irradiated explants.

As shown in Table (3), the results clearly indicated regardless the gamma irradiation treatments, there was a significant difference in the formation of roots among the lavender explants exposing to gamma irradiation at the different doses. The root number was decreased with all gamma irradiation doses except 20 Gy compared with control under MS medium without NAA, but the decrease was in proportional with rate of dose. Gamma irradiation at the dose of 50 Gy reduced the number of roots/explant to 1.0 against 2.7 for the 20 Gy.

The interaction effect of gamma irradiation and the NAA on root formation of lavender explants culturing on MS medium containing 1 mg/l NAA indicated that exposing the explants of the lavender to gamma irradiation with 20 Gy of gamma dose increased the root number compared with other treatments and the decrease was in proportional with rate of dose, whereas the highest number of roots was recorded with irradiated lavender explants to 20 Gy of gamma dose and cultured on MS medium supplemented with NAA at 2 mg/l (3.7 roots/plant).

As shown in Table (3), the results clearly indicated regardless the gamma irradiation treatments, there was a significant difference in the formation of roots among the sweet basil explants exposing to gamma irradiation at the different doses without adding NAA to MS medium. The root number was increased with gamma irradiation doses 10, 20 and 30 Gy compared with control under MS medium without NAA. Gamma irradiation at the dose of 50 Gy reduced the number of roots/explant to 1.1 against 3.0 for the 20 Gy.

The interaction effect of gamma irradiation and the NAA on root formation of sweet basil explants culturing on MS medium containing 1 mg/l NAA, indicated that exposing the explants of the sweet basil to gamma irradiation with 10 Gy of gamma doses increased the root number to 3.4 roots/plant compared with control, and other gamma treatments and the decrease was in proportional with rate of dose, whereas the highest number of roots was recorded with irradiated sweet basil explants to 10 and 20 Gy of gamma dose and cultured on MS medium supplemented with NAA at 2 mg/l (3.5 and 3.3 roots/plant, respectively).

In this regard, **(Debasis *et al.*, 1999)** on 16 Chrysanthemum cvs stated that plantlets exposed to gamma irradiation at 1.5 and 2 k-rad grew vigorously, **(Venkatachalam *et al.*, 1999)** on groundnut, found that exposing the calli to 0 to 25 Gy gamma rays stimulated the shoot formation.

As shown in Table (3), the results clearly indicated regardless the gamma irradiation treatments, there was a significant difference in the formation of roots among the chamomile explants exposing to gamma irradiation at the different doses except 50 Gy without adding NAA to MS medium. The root number was increased with all gamma irradiation doses 10, 20, 30 and 40 Gy compared with control under MS medium without NAA. Gamma irradiation at the dose of 50 Gy reduced the number of roots/explant to 1.3 against 2.3 for the 30 Gy.

The interaction effect of gamma irradiation and the NAA on root formation of chamomile explants culturing on MS medium containing 1 mg/l NAA, indicated that exposing the explants of the chamomile to gamma irradiation with 40 or 50 Gy of gamma doses decreased the root number to 1.3 and 1.0 roots/plant compared with control, and other gamma treatments.

In this regard, **(Predieri and Edoardo, 2000)** on 16 Chrysanthemum cvs stated that plantlets exposed to gamma irradiation at 1.5 and 2 krad grew vigorously.

This experiment (Tables 3) was done to study the effect of gamma irradiation and the effect of NAA added to MS medium on root formation of the irradiated Visnage explants.

As shown in Table (3), the results clearly indicated regardless the gamma irradiation treatments, there was no significant decrease in the formation of roots among the Visnage explants exposing to gamma irradiation at the different doses without adding NAA to MS medium than control. Gamma irradiation at the dose of 20, 30,40 and 50 Gy reduced the number of roots/explant to 1.0 against 1.3 for the 10 Gy.

The interaction effect of gamma irradiation and the NAA on root formation of Visnage explants culturing on MS medium containing 1 mg/l NAA, or 2 mg/l NAA indicated that exposing the explants of the Visnage to all gamma irradiation doses decreased the root number compared with control. In this regard, **(Debasis *et al.,* 1999)** on 16 Chrysanthemum cvs stated that plantlets exposed to gamma irradiation at 1.5 and 2 krad grew vigorously **(Venkatachalam *et al.,* 1999)** on groundnut, found that exposing the calli to 0 to 25 Gy gamma rays stimulated the root formation. **(Omer *et al.,* 1999)** on sunflower found an attributed the reduction in shoot and callus weight with increased doses of irradiation to the reduced amount of endogenous growth regulators, especially the cytokines.

Table (3): Effect of NAA and gamma irradiation on number of roots of Lavender, Sweet basil, Matricaria and Ammi explants.

	Lavender			Sweet			Matricaria			Ammi		
	0.0	1 mg	2 mg	0.0	1 mg	2 mg	0.0	1 mg	2 mg	0.0	1 mg	2 mg
Control	1.7 b	3.0 ab	3.2 b	1.3 b	3.1 ab	2.7 a	1.3 c	2.3 a	2.0 ab	1.3 a	2.3 a	2.3 a
10	1.4 b	3.0 ab	3.0 b	3.0 a	3.4 a	3.5 a	1.7 bc	2.3 a	2.1 ab	1.3 a	2.0 a	2.3 a
20	2.7 a	3.3 a	3.7 a	2.8 a	2.7 ab	3.3 a	2.0 ab	2.3 a	2.3 a	1.0 a	1.7 b	2.0
30	1.3 b	3.0 ab	2.8 b	2.3 a	3.0 ab	2.9 a	2.3 a	2.0 a	2.5 a	1.0 a	1.3 b	1.7 a
40	1.3 b	3.0 ab	2.7 b	1.3 b	1.3 b	1.8 b	1.7 bc	1.3 b	1.9 ab	1.0 a	1.0 b	1.9 a
50	1.0 b	1.5 b	1.5 b	1.1 b	1.1 b	1.5 b	1.3 c	1.0 c	1.6 b	1.0 a	1.0 b	2.0 a

Different letters on the same column are differ significantly at $p \leq 0.05$.

Effect of gamma irradiation and NAA on root length of lavender, sweet basil, chamomile and visnage explants

Concerning the effect of lavender plants on root length, regardless media composition, the results shown in Table (4), clearly indicated that there was a significant difference in the root length between lavender plants and NAA concentrations', the root length average of this MS medium without NAA reached 2.1 cm against 3.6 and 3.0 cm for MS medium containing 1 and 2 mg/l NAA, respectively. On the other hand, regardless the effect of explants, exposing the lavender explants to gamma irradiation at low dose (20 Gy), increased insignificantly the root length compared with control and the other doses reduced it.

The interaction effect between gamma irradiation and NAA,indicate that exposing the lavender explants to the different doses of gamma irradiation decreased the root length compared with control and the decrease reached the level of significance with the doses of 30 and 50 Gy with 2mg/l NAA , in case of lavender explants, there was a significant increase in the root length with the treatment of 20 Gy (3.3) and significant decrease in the shoot length with the treatment of 50 Gy as compared with control (3 roots/explant). A similar finding was reported by **(Debasis *et al.,* 1999)** on some Chrysanthemum cvs. stated that plantlets exposed to low gamma irradiation dose grew vigorously. **Omer *et al.,* (1999)** on sunflower found a reduction in growth with increased doses of irradiation.

Concerning the effect of gamma and NAA on the root length average, the results (Table 4), clearly indicated that, high treatments of gamma rays 40 and 50 Gy reduced the root length of sweet basil, and the reduction was more pronounced with the high doses.

The interaction effect revealed that there were slight increases in the root length average of sweet basil explants which may due to the application of 2 mg/l NAA +10 Gy than the control and other treatments. In this regard, **(Predieri and Edoardo 2000)** on plum reported that shoots irradiated with 30 and 40 Gy showed a reduced rooting capacity.

Concerning the effect of gamma and NAA on the root length average, the results (Table 4), clearly indicated that, high treatments of gamma rays 40 Gy alone reduced the chamomile explants root length, but incrase the size of roots.

The interaction effect revealed that there were slight increases in the root length average of chamomile explants which may due to the application of 2 mg/l NAA +20 gamma treatment than the control. In this regard, **(Predieri and Edoardo, 2000)** on plum reported that shoots irradiated with 30 and 40 Gy showed a reduced rooting capacity.

Concerning the effect of gamma and NAA on the root length average, the results (Table 4), clearly indicated that, high treatments of gamma rays 30 and 40 Gy without NAA reduced the root length of Visnage explants.

The interaction effect revealed that there was decreasing in the root length average of Visnage explants with all gamma doses than the control media with or without NAA. In this regard, **Predieri and Edoardo (2000)** on plum reported that shoots irradiated with 30 and 40 Gy showed a reduced rooting capacity.

Table (4): Effect of NAA and gamma irradiation on root length of Lavender, Sweet basil, Matricaria and Ammi explants.

	Lavender			Sweet			Matrecaria			Ammi		
	0.0	1 mg	2 mg	0.0	1 mg	2 mg	0.0	1 mg	2 mg	0. 0	1 mg	2 mg
Control	2.1 ^{b}	3.6 ^{a}	3.0 ^{a}	1.2 ^{c}	2.6 ^{a}	2.5 ^{b}	1.8 ^{c}	1.9 ^{b}	2.7 ^{a}	.1 ^{a}	1.9 ^{a}	1.8 ^{a}
10	2.1 ^{b}	3.2 ^{a}	2.8 ^{ab}	2.2 ^{a}	2.5 ^{a}	2.8 ^{a}	2.3 ^{a}	1.3 ^{d}	2.4 ^{a}	1.0 ^{a}	1.8 ^{a}	1.4 ^{a}
20	3.1 ^{a}	2.7 ^{a}	3.3 ^{a}	2.1 ^{a}	2.1 ^{a}	2.4 ^{b}	1.7 ^{c}	2.7 ^{a}	2.9 ^{a}	1.0 ^{a}	0.7 ^{b}	1.1 ^{b}
30	2.1 ^{b}	2.7 ^{a}	2.6 ^{a}	1.7 ^{b}	2.4 ^{a}	2.3 ^{b}	2.0 ^{b}	2.3 ^{a}	2.8 ^{a}	0.7 ^{b}	0.7 ^{b}	1.0 ^{b}
40	2.1 ^{b}	3.2 ^{a}	2.7 ^{ab}	1.0 ^{d}	1.2 ^{b}	2.0 ^{c}	1.6 ^{c}	1.6 ^{c}	2.5 ^{a}	0.3 ^{c}	0.8 ^{b}	0.3 ^{c}
50	2.2 ^{b}	2.2 ^{a}	1.9 ^{c}	0.7 ^{e}	0.6 ^{c}	1.5 ^{d}	1.7 ^{c}	1.3 ^{d}	2.7 ^{a}	1.1 ^{a}	1.2 ^{a}	0.2 ^{c}

Different letters on the same column are differ significantly at $p \leq 0.05$.

Effect of gamma irradiation and NAA on number of leaves of lavender,sweet basil, chamomile and visnage explants

As shown in Table (5), the results clearly indicated regardless media composition irradiation treatments, that there was a significant difference in the formation of leaves of exposing lavender explants to 20 Gy cultured on MS medium containing 0 NAA and formed significantly more leaves / explant (3.1 leaves/plant). On the other hand, all irradiation treatment without NAA decreased the roots number compared with control.

The interaction effect between gamma irradiation and NAA, indicated significantly increased the number of leaves with 30 and 40 Gy with MS medium containing NAA at 1 and mg/l,also stimulated leaf shape and size. The highest number of leaves/explant of lavender explants was recorded with MS medium containing NAA at 2 mg/l and exposed to the 20 Gy dose of gamma rays. In this regard, **(Somaya, 1998)** found that the addition of low NAA increased the number of roots.

As shown in Table (5), the results clearly indicated regardless media composition irradiation treatments, that there was a significant difference in the formation of leaves of exposing sweet basil explants to 20 Gy cultured on MS medium containing 0 NAA and formed significantly more leaves / explant (2.3 leaves/plant).

The interaction effect between gamma irradiation and NAA, indicated significantly increased the number of leaves with all gamma treatments with MS medium containing NAA at 1 mg/l. The highest number of leaves/explant of sweet basil explants was recorded with MS medium containing NAA at 1 mg/l and exposed to the 10 Gy doses of gamma rays. In this regard, **(Predieri and Edoardo, 2000)** on plum reported that micro cuttings originated from shoots irradiated with 30 and 40 Gy showed a reduced rooting capacity.

As shown in Table (5), the results clearly indicated regardless media composition irradiation treatments, that there was a significant difference in the formation of leaves of exposing chamomile explants to all gamma doses cultured on MS medium containing 0 NAA and formed significantly more leaves / explants than control.

The interaction effect between gamma irradiation and NAA, indicated significantly decreased the number of leaves with all gamma treatments with MS medium containing NAA at 2 mg/l than control.

As shown in Table (5), the results clearly indicated regardless media composition irradiation treatments, that there was a significant difference in the formation of leaves of exposing Visnage explants to all gamma dose on MS medium with or without NAA.

The highest number of leaves/ explants of Visnage explants was recorded with MS medium containing NAA at 0 mg/l and without exposing to gamma rays and reached (1.3 leaves /plant).

Table (5): Effect of NAA and gamma irradiation on number of leaves of Lavender, Sweet basil, Matricaria and Ammi explants.

	Lavender			Sweet			Matrecaria			Ammi		
	0.0	1 mg	2 mg	0.0	1 mg	2 mg	0.0	1 mg	2 mg	0.0	1mg	2 mg
control	2.8^{ab}	1.5^{a}	1.8^{a}	1.3^{c}	1.0^{b}	3.1^{a}	1.0^{a}	1.4^{b}	2.3^{a}	1.3^{a}	0.5^{a}	1.0^{a}
10	2.5^{bc}	1.8^{a}	1.9^{a}	1.2^{b}	3.3^{a}	2.8^{b}	1.5^{a}	1.3^{b}	1.8^{b}	1.0^{b}	0.5^{a}	0.3^{d}
20	3.1^{a}	1.3^{a}	2.2^{a}	2.3^{a}	3.3^{a}	2.0^{d}	1.3^{a}	1.5^{b}	1.3^{c}	1.0^{b}	0.5^{a}	1.0^{a}
30	2.3^{c}	2.1^{a}	1.9^{a}	1.0^{c}	2.3^{a}	2.1^{c}	1.5^{a}	1.9^{a}	1.0^{c}	1.0^{b}	0.3^{a}	0.8^{c}
40	1.8^{d}	2.0^{a}	1.8^{a}	1.3^{c}	2.8^{a}	2.3^{c}	1.3^{a}	1.3^{b}	0.8^{d}	0.8^{c}	0.3^{a}	0.5^{d}
50	1.8^{d}	1.3^{a}	1.3^{b}	1.3^{c}	1.3^{b}	1.8^{e}	1.3^{a}	1.3^{b}	0.9^{cd}	0.3^{d}	0.8^{a}	0.3^{d}

Different letters on the same column are differ significantly at $p \leq 0.05$.

Effect of gamma irradiation on chemical composition.

Table (6) pointed out that control lavender plants was decreased nitrogen, Phosphorus and potassium % to (1.351, 0.061 and 0.44%, respectively) and increased the total carbohydrate and total phenols % (19.03 and 0.83 %) than lavender plants exposed to 20 Gy of gamma irradiation which reached nitrogen, Phosphorus and potassium % (1.7, 0.069 and 0.60%, respectively) by 26,13 and 36% than control plants, also reached total carbohydrate and total phenols %(13.77 and 0.71 %).

Data also showed that the control plants of sweet basil was decreased nitrogen, potassium and total phenols% than sweet basil plants irradiated by 20Gy and reached (2.08, 0.56 and 0.134 %, respectively) and increased the Phosphorus and total carbohydrate % (0.078 and 10.30 %) than sweet basil plants exposed to 20 Gy of gamma irradiation which reached nitrogen, Phosphorus and potassium % (2.81, 0.065 and 0.66% respectively), also reached total carbohydrate and total phenols % (6.59 and 0.357%).

Data on Table (7), also showed that the control plants of chamomile plants was decreased nitrogen and total phenols % and reached (0.92 and 0.456 % respectively) than irradiated plants by 10 or 20 Gy and increased the phosphorus, potassium and total carbohydrate % (0.09, 0.62 and 8.1% respectively) than chamomile plants exposed to 30 Gy of gamma irradiation.

Data in Table (7),also showed that the control plants of Visnage plants was decreased nitrogen, phosphorus, potassium and total carbohydrate % and reached (2.4, 0.05, 0.51 and 6.11 % respectively) and increased the total phenols % (0. 30 %) than Visnage plants exposed to 10 or 30Gy of gamma irradiation which reached total carbohydrate % (12.67 %) more 100% than control plants. A similar finding was reported by Youssef and Mousa (1998) and Omar et al (1999).

The above results summarized that gamma irradiation is useful and improved the parameters of tissue culture and chemical composition.

Table (6): Effect of gamma irradiation on nitrogen, Phosphorus, potassium, total carbohydrates and total phenols content on Lavender and Sweet basil explants.

Treatment	Lavender					Sweet				
	N_2	P	K	Total Carbohydrates	Total phenols	N_2	P	K	Total Carbohydrates	Total phenols
Control	1.35^{b}	0.061^{b}	0.44^{b}	19.03^{a}	0.83^{a}	2.08^{b}	0.078^{a}	0.56^{a}	10.30^{a}	0.134^{b}
10	-	-	-	-	-	-	-	-	-	-
20	1.7^{a}	0.069^{a}	0.60^{a}	13.77^{b}	0.71^{b}	2.81^{a}	0.065^{b}	0.66^{a}	6.59^{a}	0.357^{a}
30	-	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-	-

Different letters on the same column are differ significantly at $p \leq 0.05$. - Not tested.

Table (7): Effect of gamma irradiation on nitrogen, Phosphorus, potassium, total carbohydrates and total phenols content on Chamomile and Ammi explants.

Treatment	Chamomill					Ammi				
	N_2	P	K	Total Carbohydrates	Total phenols	N_2	P	K	Total Carbohydrates	Total phenols
Control	0.92^{c}	0.09^{b}	0.62^{a}	8.1^{b}	0.46^{b}	2.4^{a}	0.05^{b}	0.51^{a}	6.11^{c}	0.30^{a}
10	1.81^{b}	0.14^{a}	0.61^{a}	12.6^{a}	0.63^{a}	2.7^{a}	0.11^{a}	0.55^{a}	8.41^{b}	0.28^{a}
20	-	-	-	-	-	-	-	-	-	-
30	2.73^{a}	0.06^{c}	0.48^{b}	7.8^{b}	0.48^{b}	2.6^{a}	0.05^{b}	0.56^{a}	12.67^{a}	0.26^{a}
40	-	-	-	-	-	-	-	-	-	-

Different letters on the same column are differ significantly at $p \leq 0.05$. -Not tested.

Conclusion

According to the results obtained in the present study,it could be concluded that exposing the explants of species under this study to low doses of gamma irradiation caused changes in the growth parameters and chemical composition,also using gamma irradiation at the level of 20 or 30 Gy affected either leaf shape and size of stem and roots and found increase in important chemical contents.

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