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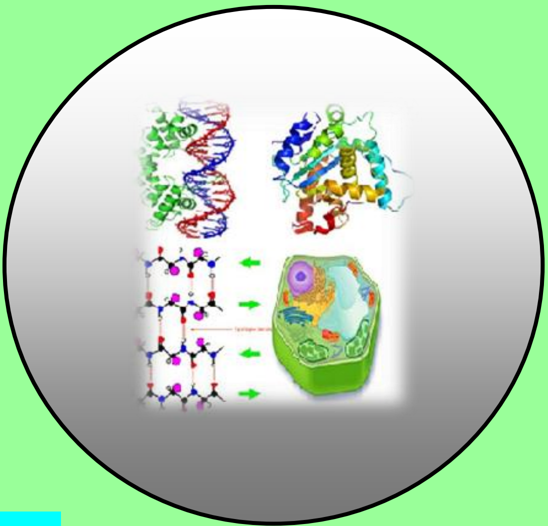

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RESEARCH PAPER

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Performance of *Catharanthus roseus* Plants in Response to Gamma Irradiation

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ABSTRACT

This experiment was conducted at the Experimental Farm of Taif University, Faculty of science to study the effect of three doses of gamma rays; 10, 30 and 50 Gy on growth of Catharanthus roseus plants. The obtained results indicated that the highest survival percentage of Catharanthus roseus seeds resulted from control plants. Meanwhile, Catharanthus roseus seeds which irradiated with 30 Gy gammas produced the maximum number of shoots and shoot length, also give the thickness stems after four months from culturing the seeds. Exposing the seeds of the Catharanthus roseus to gamma irradiation at high dose decreased the average shoot number and shoot length. The longest leaf area and the biggest fresh weight were resulted with Catharanthus roseus seeds irradiated with 30 Gy after 4 months compared with the other treatments. The high dose of gamma rays (50 Gy) significantly decreased the fresh weight, leaf size and number of leaves per plant compared with other treatments. on the other hand, the Catharanthums roseus untreated plants produced increase in number of roots and root length compared with other treatments ,also gave the highest total in doles 0.844 mg/100 g. Mostly, the irradiation treatments decreased the total indoles mg/100 g for all studied plants. Otherwise, the highest total phenols 28.12 and 33.78 mg/100 g were recorded with the irradiated plants with 30 and 50 Gy, respectively.

The most effective bands were bands number (2 and13) with molecular weights 72.25 and 20.21 Kda were present and absent this bands with control, for that these bands considered a negative marker associated with gamma rays. ***Keywords: Catharanthus roseus, Taif Region, Gamma Irradiation, In doles, Phenols, Growth and SDS protein electrophoresis.***

INTRODUCTION

Herbs play an important role in maintaining human health and their extracted essential oils, which have been of great interest as they have been the sources of natural products and are well known for their antioxidant capacities, are used for many medical products.

Catharanthus roseus, is an important floral species in horticulture and is one of the few pharmacological plants that have a long history. This plant still plays a considerable role today in herbal and traditional medicine for treatment of various diseases. The therapeutic properties were ascribed to a number of chemicals in the alkaloid class sometime in the mid-1950s (Faheem, et al., 2011 and Koehn and Carter, 2005).

Catharanthus roseus (L.) known as Madagascar periwinkle (MP) is a legendary medicinal plant mostly because of possessing two invaluable antitumor terpenoid, indole, alkaloids, vincristine and vinblastine (Naghmeh, et al., 2005).

MP is a plant species native to Western Indian Ocean's large island of Madagascar next to Africa. It has been introduced as a popular ornamental plant in many tropical and subtropical regions worldwide. This herb is widely cultivated commercially in Spain, United States, China, Africa, Australia, India, and Southern Europe for its medicinal uses. The drugs derived from this plant find major markets in USA, Germany, Italy, and UK (Joy, et al., 1998 and Lata, 2007).

MP is a tolerant plant against abiotic stresses such as drought and salinity, which can survive in a variety of habitats such as sandy soils, shrubs lands, grasslands, inland river banks, dunes in savannas, dry wastelands, houses, roadsides, and even beaches and limestone rocks all due to its hardiness (Plaizier, 1981, van Bergen, 1996 and Thomas and Latimer, 1996).

Two commercially and pharmacologically important cytotoxic dimeric alkaloids of MP, vinblastine and vincristine, have been widely used for cancer chemotherapy, which are only present in extremely low yields in the leaves (Heijden et al., 2004).

In addition to alkaloids and phenolics (as the most important compounds of MP), the presence of different chemical groups such as polyphenols, alkaloids, steroids, flavonoid glycosides, anthocyanins, and iridoidglucosides has been confirmed in different parts of MP (10).

Phenolic compounds are a group of metabolites available in all plant species. These compounds can range from simple compounds bearing just one phenolic hydroxyl to some more complex ones, like flavonoids, which are often polyphenols. Besides alkaloids, MP produces a wide spectrum of phenolic compounds with radical scavenging ability, including C6C1 compounds such as 2, 3-dihydroxybenzoic acid, as well as phenylpropanoids such as cinnamic acid derivatives, flavonoids, and anthocyanins (Naghmeh et al., 2015, Heijden et al., 2004 and Mustafa and Verpoorte et al., 2007).

Gamma rays are a type of electromagnetic radiation which originates within the nucleus of a radioisotope.

Irradiation facilities generally use the radionuclide cobalt-60, instead of cesium-137, as the source of gamma rays because it is easier to obtain, has a lower environmental risk and emits four times more energy per disintegration, respectively. The energy threshold for inducing radioactivity in herbs is 5 MeV for gamma sources (WHO, 1999). Therefore, the energy of gamma rays from cobalt-60 (1.17 MeV and 1.33 MeV) is not sufficient to generate radioactive substances in medicinal plants. Gamma irradiation has been the most popular method used in commercial preparations for medicinal plants (Fang and Wu, 1998) due to its efficiency and high penetration. Additionally, it can be used for products in final packaging (IAEA, 2008). The amount of radiation energy that a material absorbs is measured in SI unit's called-gray (Gy). One gray is equivalent to one joule per Kg (IAEA 1990). The Gray replaced the earlier unit, the rad (1 Gy = 100 rad).irradiation is an effective method to decontaminate medicinal plants; however, it may affect active components of the plants (Thongphasuk and Thongphasuk, 2012).

Proven that cymbidium plants exposition to dose 0.8 kr stimulate the growth and development of protocorms, the dose 20.0 kr totally inhibited the growth (Kozlowska, 1994). Effect of low gamma irradiation dose on growth may be due to the increase of cell length or cell number and size, (Pitirmovae, 1979). They found increase in chamomile plant high, with gamma irradiation at 1Krad (Youssef and Moussa, 1998).

On sunflower found a significant increase in protein and DNA with increasing doses of irradiation. For biochemical genetic markers SDS Protein electrophoresis (Omar et al., 1999) (Kocsy, 1991). Investigate sodium dedecyl sulphate polyacrylamid gel electrophoresis (SDS-PAGE) to analyze separate proteins with certain molecular weight in order to establish and evaluate genetic relationships. (Komov et al., 1996) On Rauwolfia serpentina, they showed radiation activated biosynthetic processess, the protein content was unaffected but the rats of protein synthesis and degredation increased. (Kim et al., 1999). On Lilium hansonii they showed quantitative difference in molecular weight of 66 KDa between dormant and non dormant bulb lets regenerated in vitro.

Studies regarding the impact of gamma irradiation on medicinal plants are very scare and limited. To the best of our knowledge, no more studies has been conducted concerning growth and biochemical changes in medicinal plants seeds, under seed radiation. So, the present work aims to propagate Catharanthus roseus under different gamma rays mutations and determine the best dose of gamma irradiation that induces the highest genetic variabilities and mutations.

MATERIALS AND METHODS

Plant material

This investigation was carried out in Biotechnology Department, Faculty of Science, Taif Univ., during 2014. Irradiated seeds Catharanthus roseus seeds, were cultured on pots containing sand and peat moss (1:1) in Faculty of Science -Taif university nursery.

Irradiation Treatments

After two months the explants were transferred to other pots. In relation to the irradiation studies, the seedlings in the pots were received three doses of gamma rays: 10, 30, and 50 Gy., emitted from cobalt 60 source from unit gamma chamber 4000, after four months regenerated shoots were separated from the explants and transferred into new pots containing sand and peat moss (1:1). Each treatment consisted of 6 replicates.

Growth characters

The growth characters taken in this experiment were shoot length (cm), survival percentage, number of roots; number of main branches/plant as well as stems diameter and fresh weights/plant (g).

Biochemical genetic studies

SDS- Polyacrylamide gel electrophoresis (SDS- PAGE) was performed according to the method of (Laemmli, 1970) as modified by (Studier, 1973). The leaves samples were takes from Catharanthus roseus before rooting stage under gamma irradiation and control.

Statistical Analysis

The experiment was performed twice each of them had 6 replicates. The two experiments had qualitative and quantitative similar trend. Where indicated, the results are expressed as means. The results of two experiments were pooled and the analysis of variance (ANOVA) was performed using MSTAT program, USA. Means were separated using Duncan's multiple range tests (Mital et al., 1972) at a significance level of 0.05.

RESULTS AND DISCUSSION

Effect of gamma irradiation on Catharanthus roseus seeds Germination

Concerning the response of survival percentage to gamma irradiation (Fig, 1), the results clearly indicated that the highest survival percentage (97 %) was recorded with the untreated Catharanthus roseus plants.

Irradiated Catharanthus roseus seeds by gamma irradiation at different doses were most effective treatments in decreasing the survival percentage compared with control. Exposing the Catharanthu sroseus explants to highest gamma dose reduced the survival percentage to the minimum values.

Similar findings were reported by (Benega et al., 1996) on pineapple, they reported that growth and viability of pollens in vitro were decreased with increasing gamma dose.

Number of shoots/plant

The results on shoot number per plant of Catharanthus roseus as affected by different gamma radiation treatments are presented in Table (1). Clearly, gamma radiation reduced the number of shoots compared with control plants except the irradiated plants with 30 Gy. The averages shoots number were 3.80 shoots for control and 3.60, 4.40, and 2.60 for gamma radiation at 10, 30 and 50 Gy, respectively,. These are in agreement with (Kozlowska, 1994) on cymbidium plants who found that the exposition of low gamma radiation proved to stimulate the growth and development of protectors, while the high dose of gamma rays 20.0 kr inhibited the growth. In addition, the simulative effect of low gamma irradiation dose on growth may be due to the increase of cell length or cell number and size, (Pitirmovae, 1979).

Shoot Height

The results shown in Table (1) indicated that there was a significant difference of the shoot length among Catharanthus roseus plants treatments. The plant formed significantly average of shoot length, the shoot lengths were 3.10, 3.00, 3.90 and 2.30 cm, for the control, 10, 30 and 50 Gy of gamma ray doses, respectively after four months from culturing.

Gamma irradiation effect revealed that growing the Catharanthus roseus plants treated with 50 Gy gave the lowest shoots (2.30 cm), whereas the longest shoots (3.90 cm) after four months from culturing of seeds was recorded with gamma ray at 30 Gy. These results with the findings with (Youssef and Moussa, 1998), on Chamomile, found increase in plant high with gamma irradiation at 1 Krad (Omer et al., 1997), while on sunflower found a reduction in growth with increased doses of irradiation (Kozlowska, 1994) on cymbidium plants found that the exposition dose 20.0 k rad totally inhibited the growth.

Stem Diameter

As shown in Table (1) the results clearly indicated that there was a significant difference in the stem diameter among Catharanthus roseus plants. The plants irradiated with 30 Gy resulted the thickness stems than the other studied plants. On the contrary, gamma irradiation significantly reduced stem diameter in other studied plants compared with irradiated plants with 30 Gy after four months from planting.

The low dose of gamma irradiation showed insignificant effect on shoot elongation in comparison with the other gamma doses. Similar finding was reported by (Debasis, 1999) on some Chrysanthemum cvs. Stated that plantlets exposed to low gamma irradiation dose grew vigorously.

Number of leaves

As shown in Table (2), the results clearly indicated regardless the gamma irradiation treatments, there was a significant difference in the formation of leaves among the Catharanthus roseus plants, the untreated plants formed more leaves / explant.

On the other hand, exposing the Catharanthus roseus explants to gamma irradiation at the different doses decreased the number of leaves compared with control but the decrease was in proportional with rate of dose. Gamma irradiation at the dose of 50 Gy reduced the number of leaves /explant to lowest value (2.60 leaves/ plant) against for the control which reached the highest value (3.80 leaves/plant). These results are in agreement with that of (Badawy, et al., 2003).

Leaf area

Data in Tables (2) revealed that the leaf size of Catharanthus roseus plants was changed by gamma irradiation at dose 30 Gy which reached more increasing (1.46 cm) compared with control or other gamma rays treatments. These result are in agreement with Nagat et al (Studier, 1973), who stated that radiation can induce mutation that affected in number of trichomes and its size also., by increasing the rate of leaves growth Lamiaceae species characterized by the relation between leaves growth and number of Trichomes and its filling by essential oil (Turner, 2000) .

Leaves fresh weight

Data presented in Tables (2) show the effect of different dose of gamma irritation treatments on herb fresh weights (g/plant) for Catharanthus roseus plants.

As shown in Table (2), the irradiated Cathranthus roseus explants by gamma irradiation at dose 30 Gy were more significant heavier plants average (2.52 gm) than the other treatments. On the other hand, all irradiation treatment decreased the fresh weight as compared with control.

After four months data revealed the effect of gamma rays at dose 50 Gy gave the lowest average of fresh weight (1.08 gm). In this regard, (Nagata et al., 1999) on Mint plants, which reported that gamma irradiation at 2 Krad was more effective in growth than high doses.

Root number

As shown in Table(3), Concerning the effect of gamma irradiation on the root number average, the results clearly indicated that, the gamma rays treatments reduced the root number, and the reduction was more pronounced with the high dose of gamma rays., that there was no significant difference in the formation of roots among the Catharanthus roseus treatments. On the other hand, irradiated explants by 30 Gyand control were slight increased number of roots.

Table 1. Effect of gamma irradiation on number of shoots, length of shoot and stem diameter of *Catharanthus roseus* seeds.

Treat.	Shoot high (cm)	No. branches/ plant	Stem diameter (cm)
0	3.10	3.80	0.20
10	3.00	3.60	0.14
30	3.90	4.40	0.24
50	2.30	2.60	0.14
$LSD_{0.05}$	1.07	N.S	0.11

Table 2. Effect of gamma irradiation on number of leaves, leaf area and leaves fresh weight of *Catharanthus roseus* seeds.

Treat.	No. of Leaves/ plant	leaf area (cm^2)	leaves fresh weight(gm)
0	3.80	1.12	2.28
10	3.40	1.06	1.98
30	3.00	1.46	2.52
50	2.60	0.92	1.08
$LSD_{0.05}$	N.S	0.14	0.28

Table 3. Effect of gamma irradiation on number of roots, length of root and root fresh weight of *Catharanthus roseus* seeds.

Treat.	No. of root/ plant	Root length (cm)/ plant	Root fresh weight(gm)
0	2.20	1.08	0.49
10	1.60	0.92	0.30
30	2.20	0.98	0.50
50	1.40	0.80	0.14
$LSD_{0.05}$	N.S	N.S	0.12

In this regard , (Kozlowska, 1994) on cymbidium plants found that the exposition dose 0.8 krad proved to stimulate the growth and development of protocorms, the dose 20.0 krad totally inhibited the growth. On plum reported that micro-cuttings originated from shoots irradiated with 30 and 40 Gy showed a reduced rooting capacity.

Root length

Concerning the effect of gamma on the root length average, the results (Table 3), clearly indicated that, all treatments of gamma rays reduced the root length, and the reduction was more pronounced with the high dose of gamma irradiation.

Table 4. Effect of gamma irradiation on chemical composition of *Catharanthus roseus* seeds.

Treat.	Total chemicals composition	
	Total Phenols(mg/100gm)	Total Indoles (mg/100gm)
0	14.367	0.844
10	7.497	0.399
30	28.128	0.297
50	33.782	0.293

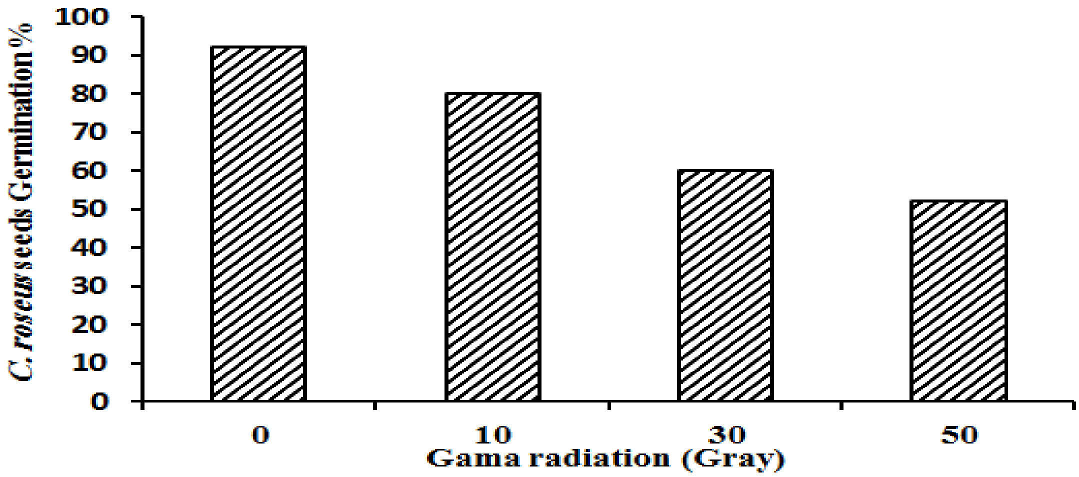
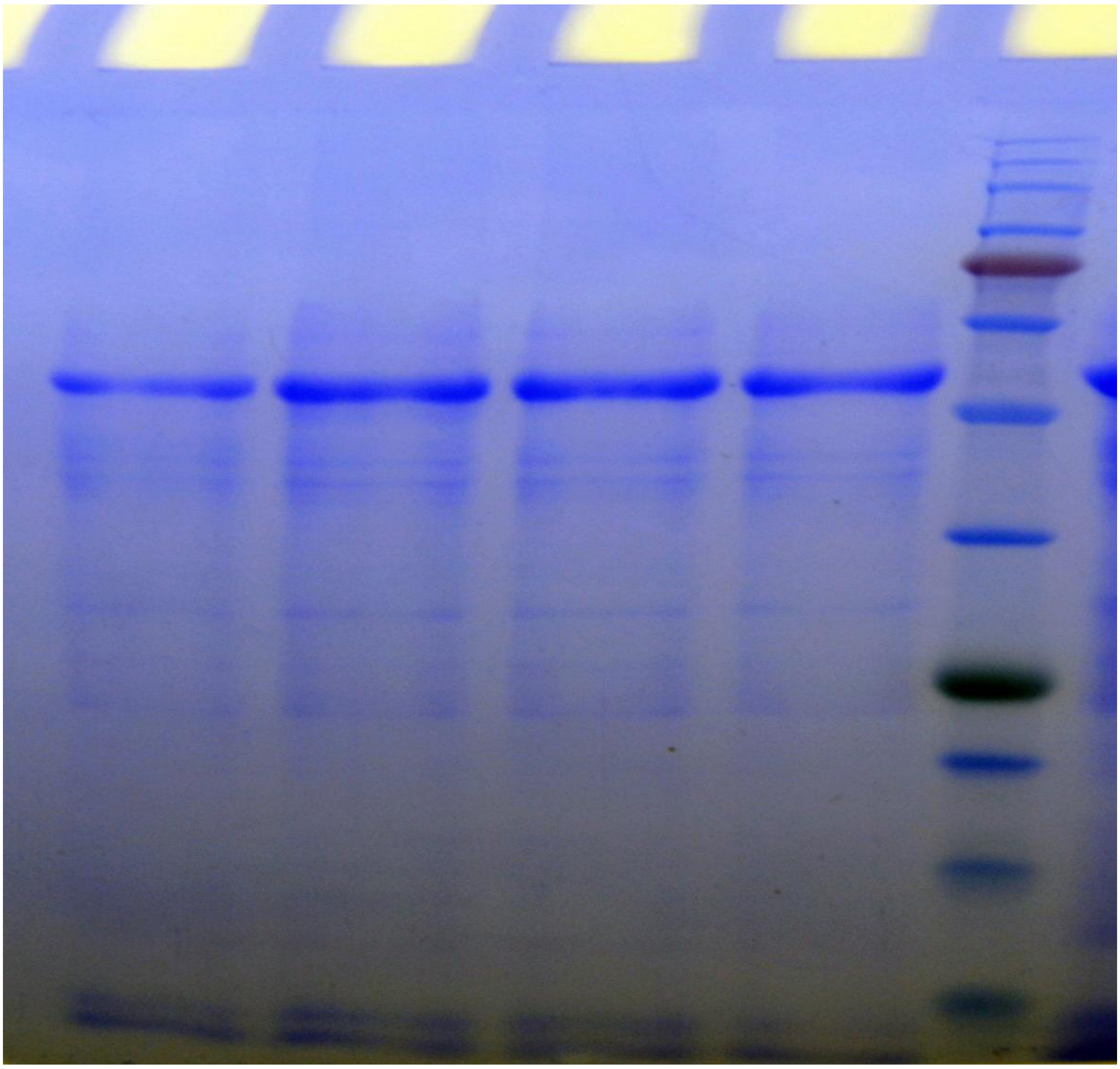


Figure 1. Effect of gamma irradiation on *Catharanthus roseus* seeds Germination

Table 5. Records of protein SDS- PAGE showing band numbers and presence or absence for plants treated with gamma rays.

Band No.	M	1	2	3	4
1	109.626	-	+	-	+
2	73.252	-	+	+	+
3	69.232	+	+	+	+
4	64.213	+	+	+	+
5	59.079	+	+	+	+
6	48.164	+	+	+	+
7	44.913	+	+	+	+
8	43.722	+	+	+	+
9	42.562	+	+	+	+
10	31.498	+	+	+	+
11	27.612	+	+	+	+
12	23.817	+	+	+	+
13	20.216	-	+	+	+
14	14.292	+	+	+	+
15	12.529	+	+	+	+
	lortnoc	Irradiation 1K	Irradiation 3K	Irradiation 5K	



The untreated Catharanthus roseus explants revealed that there were slight increases in the root length average and reached (1.08 cm) than other treatments. In this regard on plum reported that shoots irradiated with 30 and 40 Gy showed a reduced rooting capacity.

Fresh weight of roots

As shown in Table (3), the irradiated explants by 30 Gy of gamma rays formed slight significant heavier root average (0.50 gm) than the other two gamma rays treatments; the averages root weight were 0.49, 0.30and 0.14 gm, for control ,10 and 50 Gy respectively.

These results are in agreement with,

Total phenols and total indoles contents.

The data presented in Table (4) revealed the percentage of total phenols and total indoles for plantlets irradiated with different gamma doses and incubated compared with untreated plants, data showed that increasing in total phenols with increasing gamma doses to 30 or 50 Gy compared with control treatment. On the other hand exposing Catharanthus mroseus explants to gamma rays at dose 10 Gy reached lowest value (7.497g/100g) as compared with 50 Gy which produced the highest value (33.782 g/100g).Data also showed that total indoles were increased with control plants to highest value (0.844 mg/100g) and then decreased with increasing gamma doses to the lowest value (0.293/100g) with 50Gy.

SDS- Protein electrophoresis

SDS- polyacrylamide gel electrophoresis used in this study to investigate the effect of different treatments of gamma irradiation on the gene expression of Catharanthus roseus using three different gamma irradiation (1, 3 or 5 k rad) compared with control .The data showed that some bands were present and absent on some treatment For all gamma rays treatment a bands number (2 or 13) with molecular weight 72.25 or 20.21 Kda were present and absent this bands with control. These bands could be considered a negative molecular weight associated with gamma rays in this study.

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