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Effect of Gamma Irradiation and Phosphorus on Growth and Oil Production of Chamomile (*Chamomilla recutita* L. Rauschert)

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ABSTRACT

The effect of gamma rays irradiation and phosphorus on chamomile growth and oil production was studied. Chamomile seeds were pre-sowing irradiated with 0, 2, 4, 6, 8 or 10 k-rad gamma rays. Phosphorus was soil added at concentrations of 10 or 20 kg/ feddan as calcium super-phosphate. Gamma irradiation enhanced plant height, branching capacity and shoot and root fresh and dry weights compared with plants produced from non-irradiated seeds. The combination of gamma irradiation and phosphorus had a stimulating effect on the growth parameters measured. Gamma rays irradiation and phosphorus had also pronounced effect on carbohydrates, soluble sugars, mineral contents and on the percentage of oil production from chamomile flowers compared with the control.

Key Words: Chamomile; Gamma rays; Phosphorus; Oil

INTRODUCTION

Chamomile (*Chamomilla recutita* L. Rauschert) is a member of the family Asteraceae. It is an annual herbaceous plant that flowers from May to August. The original area of distribution was southern and eastern Europe and the Near East. From there, the chamomile has spread through most of Europe and has extended further to Iran, Afghanistan, Pakistan, India, Egypt and East Africa (Reichling & Beiderbeck, 1991).

Drug derived of chamomile is applied externally, in various types of preparations, to inflammations of the skin, as well as the mucous membranes as well as to bacterial infection of the oral cavity and the gums and diseases of the respiratory tract. Internally, chamomile extracts and infusions are applied to inflammatory diseases and spasms of the gastrointestinal tract (Carle & Isaac, 1985; Schilcher, 1987).

Pre-sowing seed irradiation is one of the most effective methods to improve plant production, yield components and chemical composition (Khan, 1970; Selenia & Stepanenko, 1979). Studies have been carried out to elucidate the effect of gamma rays on some aromatic plants such as chamomile (Selenia & Stepanenko, 1979; Youssef & Moussa, 1998), lemongrass (Deaf, 2000), *Mathiola incana* and *Delphinium ajacis* (Mahmoud, 2002) and peppermint (Zheljazkov *et al*., 1996). The previous studies report that low doses of gamma rays stimulated seed germination, plant growth and oil production.

Application of phosphorus was also found to increase plant height, number of branches, fresh and dry weight as well as essential oil content of black cumin (Das *et al*., 1991) and coriander (Ughreja & Chundawat, 1992). In addition, phosphorus application tends to increase N and P contents in *Atropa belladonna* (El-Deeb, 1982).

The aim of the present work was to investigate the effect of pre-sowing gamma irradiation of seeds and soil added phosphorus on growth, carbohydrates and soluble sugars, nutrient content as well as oil production of chamomile.

MATERIALS AND METHODS

Plant material. Seeds of *Chamomilla recutita* (L. Rauschert), were obtained from the Farm of Faculty of Pharmacy, Cairo University, Giza, Egypt.

Treatments. Seeds were irradiated with 0, 2, 4, 6, 8 or 10 k-rad gamma rays using Canady cell (10 min/k-rad dose rate) at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt.

Phosphorus (0, 10 or 20 kg/Fed.) was applied, as calcium super-phosphate, to the soil twice: first, 15 days before transplanting, second, 15 days after transplanting. The seeds were sown immediately after irradiation. All plants received the usual agricultural practices. All treatments are replicated three times.

At full flowering stage, some growth parameters (plant height and number of branches) were recorded. Plants were then harvested and fresh weight and dry weight were determined.

Estimation of soluble sugars and carbohydrates. Total soluble sugars were determined according to Tanaka *et al*. (1975). Total carbohydrates were determined using the method of Herbert *et al*. (1971).

Nutrient content (NPK). Nitrogen was estimated according to micro-Kjeldahl method (A.O.A.C., 1990) using nitrogen distillation instrument model Buchi 323. Phosphorus was colorimetrically determined by Vanadate-Molybdate-Yellow method (Chapman & Pratt, 1961). Potassium was determined according to Jackson (1960) using flame photometer (Jenway model PFP7).

Estimation of chamomile oil. The chamomile oil content was determined in air dried flowers according to British Pharmacopoeia method (1963) using Clavenger's apparatus for determination of oil lighter than water as follow: 20 g air dried chamomile heads were put in a distillation flask with 400 mL water. The distillation process continued for 2.5 – 3.0 h until no further increase in the oil reading was observed. At the end of distillation, the apparatus was allowed to cool to obtain a good separation. The oil quantity was determined volumetrically and its percentage was estimated (v/w) as follows:

$$Oil\,\% = \frac{Oil\ quantity\,(reading\ of\ the\ apparatus)}{Weight\ of\ air-dried\ head\ sample\,(20\,g)} \times 100$$

Statistical analysis. Data obtained were subjected to analysis of variance. The values of LSD were obtained from F values at 5% level (Snedecor & Cochran, 1967).

RESULTS AND DISCUSSION

Effect of gamma irradiation and phosphorus on growth. Irradiating chamomile seeds with different doses of gamma rays (0, 2, 4, 6, 8 or 10 k-rad) before sowing in the absence of phosphorus (P_0) or in the presence of 10 (P_{10}) or 20 (P_{20}) kg/ feddan phosphorus changed the plant growth parameters tested (Tables I, II). The plant height and the number of branches were significantly increased with increasing the dose of gamma irradiation (G.I.) or the application of phosphorus (P_{10} or P_{20}) (Table I). The effect was more pronounced at 8 k-rad, except the number of branches at P_0 which had a peak at 10 k-rad. Fresh weight of the shoot was increased gradually with increasing the dose of irradiation (Table II), and more so at 8 k-rad. However, the increase in dry weight in response to G.I. was obtained at 10 k-rad. The application of phosphorus in combination with G.I. resulted in a further increase in fresh and dry weights of the shoot and the root. The combination of G.I. and P_{10} resulted in increase in the shoot fresh and dry weights and the root fresh weight at only lower doses of irradiation reaching a maximum at 4 k-rad. A decline was then noticed at higher doses. The combination of G.I. and P_{20} proved to be the best in stimulating shoot fresh weight (maximum value at 10 k-rad) and the shoot dry weight and the root fresh and dry weights with a maximum at 8 k-rad.

Table I. Effect of gamma irradiation and phosphorus on plant height and number of branches at full flowering stage. Each value is the mean of three replicates

Phosphorus (kg/feddan) Dose (k-rad)	Plant Height (cm) P_0	P_{10}	P_{20}	nber of Branches P_0	P_{10}	P_{20}
0	67.33	71.67	71.67	13.3	13.0	13.0
2	75.00	66.67	75.00	14.3	13.6	18.3
4	69.00	75.00	75.00	18.3	17.0	16.3
6	76.67	74.33	77.67	16.6	16.0	18.0
8	86.67	86.67	90.00	20.3	22.3	26.6
10	80.00	83.33	85.67	22.3	19.0	23.3
LSD 5%	*9.98*	*8.99*	*9.12*	*2.85*	*2.10*	*2.75*

Table II. Effect of gamma irradiation and phosphorus on the fresh weight and dry weight of chamomile shoots and roots at full flowering stage. Each value is the mean of three replicates

Dose (k-rad)	Phosphorus (kg/feddan)	Shoot Fresh wt. (g)	Shoot Dry wt. (g)	Root Fresh wt. (g)	Root Dry wt. (g)
0.0	P_0	363.60	73.37	16.60	6.73
	P_{10}	476.50	73.11	16.49	6.03
	P_{20}	677.39	107.30	25.98	11.15
2	P_0	598.16	128.07	27.37	11.96
	P_{10}	671.21	103.53	27.27	12.81
	P_{20}	845.34	148.67	35.33	13.90
4	P_0	533.48	101.80	19.90	8.84
	P_{10}	905.59	163.90	55.07	13.48
	P_{20}	519.65	94.60	41.68	15.80
6	P_0	596.74	110.50	25.51	11.70
	P_{10}	635.72	110.86	35.80	11.31
	P_{20}	667.11	116.25	39.53	11.76
8	P_0	803.47	136.23	46.50	15.51
	P_{10}	607.82	125.53	48.80	14.77
	P_{20}	800.64	179.00	51.55	18.64
10	P_0	783.11	138.40	48.60	16.26
	P_{10}	869.94	146.57	46.32	15.00
	P_{20}	1074.15	166.97	50.83	13.72
	LSD 5%	*95.23*	*109.50*	*2.78*	*2.98*

The stimulative effect of gamma rays irradiation on growth, especially at low doses was reported in several investigations. When Khan (1970) exposed seeds of *Cicer arietinum* to gamma rays at doses ranging from 5 to 15 K-rad, a stimulation of branching capacity, fresh weight and dry weight was reported. Similar results were observed by Kaul and Bradu (1972) in *Atropa belladonna*, Suhas *et al*. (1976) in *Cassia angustifalia*, Selenina and Stepanenko (1979) in *Matricaria recutita* and Youssef *et al*. (2000) in geranium. On the contrary, Banerji and Datta (1992), and Shukla and Datta (1993) found a reduction in plant height, branches and leaves number when root cuttings of *Chrysanthemum* were irradiated with low doses of gamma rays (1.5, 2 & 2.5 k-rad). In addition, high doses of gamma irradiation (over 15 k-rad) were reported to be harmful in several studies. Ramachandran and Goud (1983) reported that higher doses of G.I. (40-120 k-rad) reduced plant height, number of leaves and branching capacity of safflower. A reduction in plant height, branches number, leaves number and size was found when root cuttings of *Chrysanthemum* were irradiated with 20 or 25 k-rad gamma rays (Banerji & Datta, 1992).

The stimulating effect of low doses of gamma rays irradiation on plant growth may be due to stimulation of cell division or cell elongation, alteration of metabolic processes that affect synthesis of phytohormones or nucleic acids (Pitirmovae, 1979).

The effect of phosphorus on growth was reported in several investigations. Munshi *et al*. (1990) reported an increase in plant height and number of branches of *Carum carvi* grown from root tubers, when phosphorus was applied in a concentration of 40 kg/ ha. Das *et al*. (1991) found an increase in black cumin (*Nigella sativa*) height, number of braches and fresh weight and dry weight of shoots and roots with increasing phosphorus concentration from 20 to 40 kg/ ha. El-Khateeb *et al.* (1994) reported that phosphorus fertilization (20-40 kg/ ha.) increased plant height, fresh and dry weights of caraway and *Ruta graveolens*, respectively. Contradictory results were reported, however, by Johri (1991) and Rubio (1992) who found that phosphorus did not increase the biomass yield, nor bring any benefit to chamomile plants.

Effect of Gamma Irradiation and Phosphorus on Nutrient Content

Nitrogen. Pre-sowing gamma irradiation of seeds in presence or absence of phosphorus had clear effect on nitrogen level of chamomile shoots and flowers (Table III). The highest percentage of nitrogen with P_0 and G.I. was recorded at 6 k-rad in the case of shoots and 4 k-rad in case of flowers. The combination of G.I. and P_{10} showed a maximum increase in the percentage of nitrogen at 6 and 2 k-rad for shoots and flowers, respectively. Meanwhile, the maximum level of nitrogen with P_{20} was recorded at 8 k-rad for shoots and 6 k-rad for flowers (Table III).

Phosphorus. G.I. alone increased phosphorus level in the shoots at 6 k-rad. Application of phosphorus (P_{10}) also showed a maximum level of phosphorus in shoots at 6 k-rad. The combination of G.I. and P_{20} had higher phosphorus content than P_0 or P_{10}, with a maximum value at 10 k-rad. In flower, phosphorus level increased linearly in response to irradiation alone (P_0) or in presence of P_{10} or P_{20}, maximum values in all treatments were obtained at 10 k-rad. (Table III).

Potassium. Plants grown from non-irradiated seeds and without the application of phosphorus (control) had the highest percentage of potassium in the shoots, while in flowers the maximum potassium content was recorded with P_{10} in non-irradiated plants (Table III). P_{20}, however, showed high potassium level in the shoots and the flowers at 2 and 4 k-rad, respectively.

Gamma irradiation was reported to affect the mineral content of several plants. An increase in nitrogen content

Table III. Effect of gamma irradiation and phosphorus on the percentage of nitrogen, phosphorus and potassium in chamomile shoots and flowers at full flowering stage. Each value is the mean of three replicates

Phosphorus (kg/feddan)		(Shoot)			(Flower)		
Dose(k-rad)	%	P_0	P_{10}	P_{20}	P_0	P_{10}	P_{20}
0.0	N	4.67	6.01	7.17	16.68	17.35	18.18
	P	0.11	0.14	0.18	0.16	0.17	0.19
	K	2.83	2.36	2.07	4.17	4.28	3.93
2	N	5.17	5.34	5.84	17.35	18.52	18.18
	P	0.11	0.17	0.20	0.17	0.19	0.25
	K	2.43	2.23	2.48	4.19	3.99	3.70
4	N	5.00	3.84	5.84	19.85	17.85	17.02
	P	0.12	0.19	0.22	0.18	0.20	0.26
	K	2.03	2.18	2.39	4.15	4.10	4.23
6	N	6.67	7.34	6.01	18.35	17.18	18.52
	P	0.17	0.21	0.25	0.19	0.23	0.27
	K	2.41	2.45	1.99	3.95	3.79	4.06
8	N	4.67	5.51	8.17	18.35	18.19	12.51
	P	0.12	0.18	0.18	0.18	0.20	0.22
	K	2.24	2.14	2.36	3.83	4.04	3.76
10	N	6.51	7.17	5.17	14.51	13.85	17.02
	P	0.15	0.19	0.28	0.20	0.25	0.29
	K	2.75	2.12	2.08	3.89	3.9	3.86
LSD 5 %		*0.41*	*0.19*	*0.27*	*0.34*	*0.35*	*0.54*

was found by Maltseva and Kuzin (1975) when *Vicia faba* seeds were irradiated with 0.1 and 10 k-rad of gamma rays. Nitrogen and phosphorus contents of cabbage, onion and carrot were also increased by 0.1 to 1.25 k-rad G.I. (Rennie & Nelson, 1975). Habba (1989) exposed seeds of *Hyoscyamus* and *Atropa* spp. to 1-2.5 k-rad G.I. and also reported an increase in nitrogen percentage. Deaf (2000) reported an increase in nitrogen, phosphorus and potassium contents of lemongrass when seeds exposed to 1-4 k-rad G.I. Mahmoud (2002) indicated that G.I. increased phosphorus and potassium content of delphinium plants. Contradictory results were reported: a decrease in nitrogen content was found in *Capsicum annum* (Iqbal, 1976), and in *Datura innoxia* (Tikhonov *et al.,* 1980) when their seeds were exposed to G.I. In addition, Deaf (2000) found a decrease in phosphorus percentage of lemongrass. Zham and Voloozh (1976), however, reported that irradiating tomato seeds with 2.5 k-rad gamma rays did not affect the concentration of nitrogen or phosphorus.

El-Deeb (1982) stated that *Atropa belladonna* plants received phosphorus application showed an increased accumulation of nitrogen and phosphorus in different tissues. Raising phosphorus level increases nitrogen and phosphorus contents and decreases potassium percentage content in coriander (Tiwari & Banafar, 1995)

Effect of gamma irradiation and phosphorus on total carbohydrates and total soluble sugars. The pre-sowing irradiation of chamomile seeds showed gradual increase in total carbohydrates and total soluble sugars with increasing the dose of gamma irradiation from 0.0 to 10 k-rad (Table IV). Applying phosphorus at both concentrations tested in this study (P_{10} and P_{20}) resulted in an increase in carbohydrate and sugar concentrations, and more so at 8 K-

Table 1V. Effect of gamma irradiation and phosphorus on the percentage of oil production, total carbohydrates and soluble sugars in chamomile flowers at full flowering stage. Each value is the mean of three replicates

Phosphorus(kg/feddan)	% Essential oil			Total Carbohydrates (mg/g d.wt.)			Soluble sugars (mg/g d.wt.)		
Dose (k-rad)	P_0	P_{10}	P_{20}	P_0	P_{10}	P_{20}	P_0	P_{10}	P_{20}
0.0	1.01	1.10	1.16	465.9	475.8	492.9	88.2	90.64	96.8
2	1.30	1.38	1.48	508.4	512.8	526.5	104.4	106.8	108.7
4	1.55	1.67	1.73	531.2	542.2	560.4	111.4	113.2	115.5
6	1.70	1.85	2.00	570.1	580.1	581.7	117.8	121.9	122.9
8	1.34	1.52	1.22	596.4	601.7	602.2	126.4	129.8	131.8
10	1.43	1.62	1.84	622.9	598.7	574.6	139.2	123.1	117.3
LSD 5%	*0.12*	*0.09*	*0.15*	*98.64*	*101.12*	*93.24*	*11.98*	*13.09*	*11.79*

rad. Our results are in agreement with those of Frank and Lendvi (1971) and Mahmoud (2002) who report an increase in carbohydrates and soluble sugars in response to seed irradiation. However, a decrease in carbohydrate content was reported in other studies (Tselishev *et al*., 1970; Jiracek *et al.*, 1970). Inoue *et al.* (1980) demonstrated that irradiation of rice and maize seeds had no significant effect on soluble sugar content in the produced plants.

Effect of gamma irradiation and phosphorus on the production of oil. Table IV showed an increase in the percentage of oil with increasing gamma irradiation dose in presence or absence of phosphorus. The highest oil production from chamomile flowers was recorded at 6 K-rad for all treatments.

Our results are supported by previous published studies that report an increase in oil production by G.I. in several plant species (Verma-Sheela *et al.*, 1980; Shylaraj & Thomas, 1989). However, high dose of G.I. decreases the oil production in other studies (Gupta & Raj, 1980, Francis *et al.*, 1983, Zheljazkov *et al.*, 1996). Phosphorus was also found to increase oil production in plants (Ughreja & Chundawat, 1992; Tiwari & Banafar, 1995). Rao *et al.* (1983), however, found that application of phosphorus to *Coriandrum sativum* did not influence oil yield.

The results obtained in the present study, clearly showed the stimulative effect of gamma irradiation and phosphorus on growth, biochemical characteristics and oil production of chamomile plant. Current experiments are in progress to investigate the factors affecting the oil production *in vitro* from callus culture initiated from irradiated seeds of chamomile. A comparison between chemical composition of oil produced *in vitro* and *in vivo* is also under study.

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