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EFFECT OF GAMMA IRRADIATION ON MORPHOLOGICAL CHARACTERISTICS AND ISOLATION OF CURCUMINOIDS AND OLEORESINS OF *CURCUMA LONGA* L.

S. Ilyas and S. Naz

Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan
Corresponding authors E-mail: drsnaz31@hotmail.com;saiqailyas@gmail.com

ABSTRACT

The present investigation was carried out to enhance the plant growth and production of curcuminoids and essential oils from turmeric (*Curcuma longa)* by gamma rays treatments. The plant samples of turmeric were collected from different regions of Kasur, Bannu and Faisalabad, Pakistan. The rhizomes were irradiated with 00 as control, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100Gy by cobalt 60 source. The germination response and percentage, plant height, average length, number and width of leaves, average root length and number, fresh and dry weights, rhizome weight, yields of essential oil and curcuminoids were affected with different levels of doses. Germination response was delayed at higher doses. Germination percentage was not significantly affected by increasing doses of gamma rays. The maximum plant height (70.8 cm) was attained at 60 Gy in plants collected from Kasur while higher doses showed reduction in heights. Lower doses have stimulatory effects on length, number and width of leaves where as higher doses had inhibitory effects. Maximum leaf length (47.6 cm) with 40 Gy and leaf number (8.8) at 70 Gy were recorded in samples of Kasur while highest leaf width was 14.6 cm at 50 Gy from plants of Faisalabad area. The rooting pattern of length showed increase gradually upto 60 Gy in Kasur plants and 70 Gy in Faisalabad while plants from Bannu gave maximum number of roots (6.2) at 70 Gy dose of gamma rays. Fresh and dry weights of plants were 75.0g and 53.0g respectively at 50 Gy with plants of Kasur. Rhizomes of plants from Kasur gave 0.73% yield of essential oil at 50 Gy as compared to non-irradiated (0.31%). Similarly yield of curcuminoids were also increased (12.4%) at 60 Gy in plants collected from Kasur.

Key words: Turmeric rhizome, curcuminoids, gamma rays, germination percentage, essential oil and *Curcuma longa*.

INTRODUCTION

Irradiation is one of the physical modification methods (Relleve *et al*., 2005). In comparison with other physical modification methods, such as microwave, UV, ultrahigh hydrostatic pressure and hydrothermal treatment, irradiation treatment is rapid, convenient and more extensive because ionizing energy penetrates through the polysaccharide granule rapidly (Bao *et al*., 2005). This process is beneficial in solving various agricultural problems such as reduction of post-harvest losses through suppressing sprouting and contamination, eradication or control of insect pests, reduction of food-borne diseases, extension in shelf life and disease resistant varieties (Emovon, 1996). Gamma radiation induces several cytological, genetic (Haris and Jusoff, 2013), morphogenetic (El-Sherif *et al*., 2011), biochemical (Chandrashekar *et al*., 2013) and physiological alteration in cell and tissues of plants (Jan *et al*., 2012; Rahimi and Bahrani, 2011). The gamma ray treatments to plants with high doses disturb the leaf gas-exchange, hormone balance, water exchange and enzyme activities (Kiong *et al*., 2008). These effects include changes in the plant cellular structure and cell metabolism such as alteration in photosynthesis, dilation of membranes of thylakoids, modulation of the antioxidant systems and accumulation of phenolic compounds.

Mutagenesis has already been used for the improvement of many important characters such as size of plants, time of flowering, colors of fruits, ripening of fruits, self compatibility, self thinning and resistance to pathogenic organisms. Nowadays, the number of cultivars improved through mutation techniques increases rapidly (Majeed *et al*., 2010; Maluszynski, 1995; Hearn, 2001).

Curcuma longa L., the member of family *Zingiberaceae*, is significant for its medicinal and pharmacological potentials. The rhizomes of *C. longa* (turmeric) contain volatile and non-volatile compounds. The volatiles are oleoresins (essential oil) and non-volatiles are curcuminoids. The chemical constituents of essential oil are many monoterpenes and sesquiterpenes such as ar-turmerone, turmerone, curlone and zingiberene (Apisariyakul*et al*., 1995). The curcuminoids contain curcumin, demethoxycurcumin and bisdemethoxycurcumin (Jayaprakasha *et al*., 2005) and act as an inhibitor of the Human Immunodeficiency Virus HIV-1 (Mazumder *et al*., 1995). The essential oils from rhizomes of turmeric have biological activities such as antibacterial (Naz *et al*., 2010; Naz *et al*., 2011), antifungal, carminative and also act as an antiplatelet agent (Lee, 2006). Turmeric also possesses antitumor, anti-inflammatory, hepatoprotective, antiviral (Ammon and Wahl, 1991) and anticancer activities (Polasa *et al*., 1991).

The objectives of the current study were to investigate the effects of different doses of gamma irradiation on morphological characteristics and to find out the effect of optimum dose yielding high quantity of curcuminoids and oleoresins present in turmeric.

MATERIALS AND METHODS

Plant materials: Different genotypes of *C. longa* were collected from Kasur, Bannu and Faisalabad, different ecological zones of Pakistan.

Irradiation treatments: The rhizomes of all samples were irradiated with 00, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100Gy gamma rays from a source of cobalt - 60 at PARAS (Pakistan Radiation Services) in Pakistan. All samples collected from different regions were coded according to the given doses of gamma irradiations presented in Table 1.

The experiment was conducted in two consecutive seasons of 2011 and 2012 in field of Bagh-e-Jinnah, Lahore. Treated rhizomes were sown in normal soil in the month of April in both years. Treatments were replicated in duplicates.

Table 1: Collection and genotypic codes used for different doses of gamma irradiation.

Collection Area	Irradiation doses	genotype codes
Kasur	00 - 100Gy	$K_1 – K_{11}$
Bannu	00 - 100Gy	B_1 - B_{11}
Faisalabad	00 - 100Gy	F_1 - F_{11}

Vegetative growth characters: Following morphological parameters were recorded; germination response after sowing (days), germination percentage (%), plant height (cm), average leaf number, average leaf length (cm) and average leaf width (cm). After harvesting, average length of roots (cm), average number of roots, fresh weight (g), dry weight (g), rhizome weight (g) yield of essential (volatile) oils (%) and yield of curcuminoids (%) were also determined.

Estimation of essential oils: The volatile (essential) oils were estimated through hydro-distillation using dean stark apparatus. The rhizomes of all samples were weighed and crushed separately. Crushed materials were added to reaction vessel and attached to steam generator and a water cool condenser. Steam (produced through steam generator) passed through the sample condensed and collected with oils. If the oils contain water particles, then anhydrous sodium sulphate was used to absorb all water drops. The oil was stored in a sealed vial at 4ºC for further use. The oil yield was calculated on the basis of fresh weight of samples. The amount of oil was determined volumetrically and oil percentage was calculated as follows:

$$\text{Percentage of volatile oil} = \frac{\text{Amount of oil in mL}}{\text{Total weight of material in Kg (rhizomes)}} \times 100$$

Estimation of curcuminoids: The curcuminoids were extracted by using Soxhlet apparatus. The plant rhizomes were partially crushed in pestle mortar and their weighed amount was placed in the thimble fitted in extraction chamber. A condenser was attached above the extraction chamber and flask containing the solvent called collecting flask was attached below to extraction chamber. The flask was heated and the solvent get evaporated and moved above into the condenser. The condenser converted these vapours into a liquid that trickled into the extraction chamber. In extraction chamber when the solvent surrounding the sample exceeded acertain level it overflowed and moved back down into the collecting flask. The extract mixed with solvent present in flask was removed after specific interval of time and solvent was separated from extract through rotary evaporator to obtain crude form of curcuminoids. The weight of the extracts was measured and then percentage yields for each sample were calculated.

Statistical analysis: For the experiments, a completely randomized design with at least three replicates was used. The data were analyzed through analysis of variance (ANOVA) and the treatments' means were compared for significance by Duncan's New Multiple Range (DMR) test at 0.05% P using the COSTAT V-63.

RESULTS AND DISCUSSION

Germination response and germination percentage: Germination responses and percentages of all turmeric samples were observed and presented in Table 2. After sowing, the germination was initiated within ten days in control plants (non-irradiated) of turmeric collected from area of Kasur and Faisalabad while non-irradiated plants collected from Bannu showed 11.0 days to start initiation. All samples of *C. longa* were showed significant delay in days to initiation of germination by higher doses of gamma irradiation (Fig. 6A-C). Germination time was decreased with increasing doses of gamma rays. Maximum delay of 35.6 days was observed with dose of 100Gy in plant samples collected from Bannu.

The germination percentage was 100.0 % in all control plants and was not much affected with the increase in doses of gamma rays. Higher doses of gamma rays like at 100Gy minimum germination percentage (85.23%) was recorded. Chaudhuri (2002) reported similar results that in lower dose i.e., at 0.1 kGy the germination percentage was not significantly different from non-irradiated whileon higher radiation doses, germination percentage was reduced and root and shoot length were also reduced. Higher doses of gamma rays may cause damage (Mehetre *et al*., 1994) and usually have inhibitory effects (Thapa, 1999; Akhaury and Singh, 1993). Melki and Marouani (2009) worked on seedlings of hard wheat and revealed that gamma rays treatments had no significant effect on germination response and percentage. According to Koing *et al*., (2008) the nature and extent of chromosomal damage are important factors forsurvival of plants.

The stimulating effects of gamma rays treatments ongermination percentage might be due to the activation of synthesis of RNA (Kuzin *et al*., 1975) on caster bean, or synthesis of protein (Kuzin *et al*., 1976) when seeds were irradiated with 4.0 Krad. Gruner *et al*., (1992) stated that it could be because of the elimination of bacterial populations, mould fungi, bacterial and fungal spores. These results are similar to the results of Grover and Dhanju, (1979) who worked on *Papaver somniferum*. Habba (1989) reported that germination percentage was gradually increased and then decreased with increased doses of gamma rays up to 100.0Gy in *Hyoscyamus muticus*. Hell *et al*., (1974) stated that germination percentage was reduced when seeds of *Phaseolus vulgaris* treated with high doses of gamma radiation.

Plant height: The data related to plant height was recorded and showed that gamma irradiation treatments significantly increased the plant height in all samples of *C. longa* as compared to control plants as shown in Table 2. The stimulating effect of lower doses of gamma irradiations on growth of plants might be because of stimulation of cell division, alteration of metabolic processes that affect synthesis of nucleic acids (Pitirmovae, 1979).

It is evident from Table 2 that dose of 60Gy gave highest plant height 70.8 cm, 62.2 cm and 66.7 cm in turmeric samples collected from Kasur, Bannu and Faisalabad respectively. At higher doses of gamma rays, all plants started reduction in heights (Fig. 6 E & F). Higher dose of 100Gy showed 25.9 cm plant height in Bannu turmeric plants. Khalil *et al.*, (1986) reported that it might be due to reduced mitotic division in meristematic tissues and reduced moisture content. Similarly Norfadzrin *et al.,* 2007 noticed that 600 and 800 Gy doses of gamma rays had negative results on the morphology of tomato and okra seedlings. Reduced plant height in many plants after treating with higher gamma ray doses had already been reported by Kon *et al*., 2007; Yaqoob and Ahmad, 2003; Al-Salhi *et al*., 2004; Token *et al*., 2005. Banerji and Datta, 1992) reported a reduction in plant height of *Chrysanthemum* when irradiated with gamma rays doses of 20 or 25 krad.

Average leaf length, leaf number and leaf width: Gamma irradiation had significant effect on average length, number and width of leaves as mentioned in Table 3. The control plants showed average length of leaves ranging from 30.1-34.0 cm. Irradiated plants collected from Kasur gave maximum (47.6 cm) length of leaves at 60Gy while higher doses showed decrease in average length of leaves (Fig. 6D). Bannu turmeric plants had highest length of leaves (40.5 cm) with 60Gy and above doses had negative effect on length of leaves. Maximum length was 37.5cm with lower dose of 30Gy in plants collected from Faisalabad while higher doses had inhibitory effect on length of leaves i.e.5.3 cm at 100Gy.

The average number of leaves gradually increased with increasing doses of gamma rays. In comparison of *C. longa* plants collected from different regions, maximum number of leaves was 8.8 with 70Gy as compared to control (6.5) in Kasur plants. Ramachandran and Goud, (1983) worked on safflowers and they reported that number of leaves were decreased on higher doses of gamma irradiations.

Lower doses of gamma rays had stimulatory effects on average width of leaves while higher doses had inhibitory effects on gamma rays treatments (Fig. 6E). Non-irradiated plants showed 11.0 cm width of leaves. Maximum width of leaves was 14.6 cm with gamma rays dose of 50Gy in plants collected from Faisalabad while minimum width of leaves was recorded as 5.3cm with dose of 100Gy. Kiong *et al*., (2008) reported that plant sensitivity is increased after gamma irradiations and it might be because of reduced level of endogenous growth hormones, such as cytokinins, as a result of breakdown or lack of synthesis.

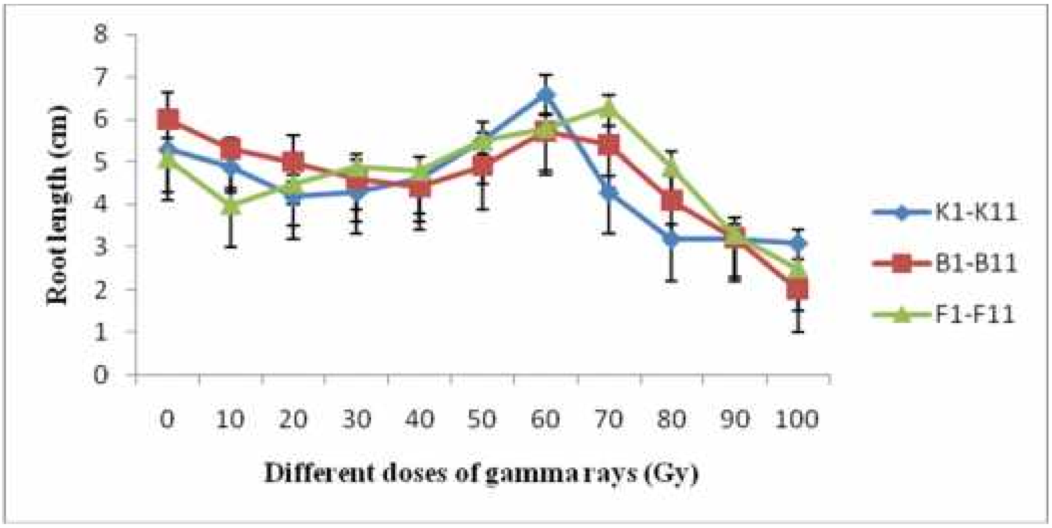
Length of roots and number of roots: The observed highest root length was 6.6 cm at 60Gy and 6.3 cm at 70Gy in *C. longa* of Kasur and Faisalabad respectively (Fig. 1A). Further increase in irradiation doses to 80, 90 and 100Gy, the plants showed decline in root length.

Table 2: The effect of different doses of gamma irradiations on the germination response, germination percentage and plant height of various samples of *C. longa* L.

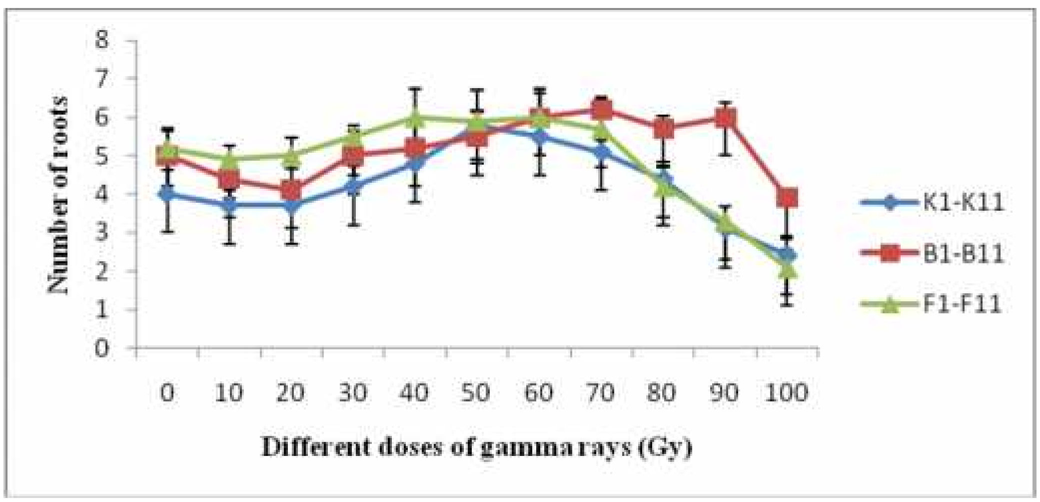
Treatments	K_1 - K_{11}			B_1 – B_{11}			F_1 - F_{11}		
Gamma rays (Gy)	Germination Response (days)	Germination percentage (%)	Plant height (cm)	Germination response (days)	Germination percentage (%)	Plant height (cm)	Germination response (days)	Germination percentage (%)	Plant height (cm)
00 (control)	$10.2^{f}\pm0.76$	100.0	$50.2^{e}\pm1.03$	$11.0^{f}\pm1.41$	100.0	48.8 ± 0.81	$10.5^{g}\pm0.63$	100.0	$51.3^{e}\pm0.33$
10	$10.3^{f}\pm0.55$	98.75	$49.2^{e}\pm0.86$	$11.3^{f}\pm0.89$	99.01	45.5 ± 0.44	$11.5^{g}\pm0.77$	99.01	$48.5^{f}\pm0.44$
20	$12.8^{ef}\pm0.76$	98.25	$51.4^{e}\pm0.72$	$11.9^{f}\pm0.57$	99.51	46.4 ± 0.45	$11.7^{fg}\pm0.76$	99.5	$49.9^{ef}\pm0.60$
30	$12.9^{ef}\pm1.003$	95.33	$55.7^{d}\pm0.84$	$13.2^{ef}\pm1.24$	96.24	50.2 ± 0.52	$14.2^{ef}\pm0.76$	98.2	$50.8^{e}\pm0.33$
40	$15.6^{de}\pm0.67$	96.11	$60.7^{c}\pm1.58$	$15.9^{de}\pm0.89$	94.63	55.6 ± 0.45	$15.7^{de}\pm0.95$	95.5	$53.3^{d}\pm0.52$
50	$16.7^{d}\pm0.71$	95.67	$66.3^{b}\pm0.89$	$15.5^{de}\pm0.77$	95.22	60.2 ± 0.52	$15.1^{de}\pm0.96$	96.4	$62.4^{c}\pm0.45$
60	$20.3^{c}\pm0.89$	96.88	$70.8^{a}\pm0.76$	$17.7^{cd}\pm0.86$	94.37	62.2 ± 0.52	$17.3^{cd}\pm0.86$	94.1	$66.7^{a}\pm0.38$
70	$22.7^{bc}\pm1.59$	93.25	$70.0^{a}\pm0.63$	$17.2^{d}\pm0.65$	94.19	58.8 ± 0.65	$19.0^{c}\pm0.63$	93.3	$64.2^{b}\pm0.52$
80	$25.1^{b}\pm1.02$	90.66	$40.2^{f}\pm0.91$	$20.4^{c}\pm0.96$	94.65	48.4 ± 0.60	$19.9^{c}\pm0.60$	90.4	$53.5^{d}\pm0.4$
90	$31.2^{a}\pm1.36$	88.33	$32.3^{g}\pm0.48$	$26.5^{b}\pm0.87$	90.21	33.7 ± 0.38	$24.8^{b}\pm0.91$	89.7	$44.4^{g}\pm0.45$
100	$32.6^{a}\pm1.003$	87.91	$30.0^{g}\pm0.63$	$35.6^{a}\pm0.77$	85.23	25.9 ± 0.35	$28.7^{a}\pm0.97$	89.2	$31.3^{h}\pm0.52$

Table 3:The effects of different doses of gamma rays on the length of leaves, number of leaves and width of leaves of various samples of *C. longa* L.

Treatments	K_1– K_{11}			B_1 – B_{11}			F_1– F_{11}		
Gamma rays (Gy)	Average length of leaves (cm)	Average no. of leaves	Average width of leaves (cm)	Average length of leaves (cm)	Average no. of leaves	Average width of leaves (cm)	Average length of leaves (cm)	Average no. of leaves	Average width of leaves (cm)
Control	$33.0^{de}\pm0.63$	$6.5^{bc}\pm0.48$	$11.5^{cd}\pm0.44$	$30.1^{e}\pm0.57$	$6.6^{ab}\pm0.45$	$10.2^{c}\pm0.71$	$34.0^{bc}\pm0.63$	$6.5^{abc}\pm0.4$	$11.0^{c}\pm0.4$
10	$34.4^{cd}\pm0.45$	$7.5^{b}\pm0.48$	$11.3^{cd}\pm0.68$	$31.7^{d}\pm0.43$	$6.0^{ab}\pm0.63$	$10.5^{c}\pm0.44$	$35.5^{b}\pm0.4$	$6.0^{bc}\pm0.63$	$11.5^{c}\pm0.48$
20	$34.0^{cd}\pm0.63$	$7.5^{b}\pm0.49$	$12.0^{bcd}\pm0.93$	$33.6^{c}\pm0.53$	$6.3^{ab}\pm0.48$	$11.1^{bc}\pm0.35$	$32.8^{c}\pm0.59$	$6.7^{abc}\pm0.33$	$12.5^{bc}\pm0.66$
30	$35.2^{bc}\pm0.59$	$6.6^{bc}\pm0.21$	$13.1^{abc}\pm0.57$	$38.5^{b}\pm0.52$	$6.5^{ab}\pm0.48$	$12.5^{ab}\pm0.77$	$37.5^{a}\pm0.44$	$6.0^{bc}\pm0.63$	$13.7^{ab}\pm0.26$
40	$47.6^{a}\pm0.45$	$6.2^{cd}\pm0.33$	$14.2^{a}\pm0.59$	$30.1^{e}\pm0.57$	$6.6^{ab}\pm0.53$	$13.9^{a}\pm0.29$	$35.5^{b}\pm0.4$	$5.6^{cd}\pm0.21$	$13.9^{ab}\pm0.49$
50	$35.7^{bc}\pm0.33$	$7.0^{bc}\pm0.4$	$14.4^{a}\pm0.66$	$26.4^{g}\pm0.45$	$6.9^{ab}\pm0.29$	$14.2^{a}\pm0.52$	$33.3^{c}\pm0.33$	$6.7^{abc}\pm 0.48$	$14.6^{a}\pm0.45$
60	$36.3^{b}\pm0.48$	$7.5^{b}\pm0.2$	$14.1^{ab}\pm0.60$	$40.5^{a}\pm0.4$	$7.0^{a}\pm0.63$	$13.4^{a}\pm0.45$	$35.5^{b}\pm0.4$	$7.2^{ab}\pm0.33$	$14.1^{ab}\pm0.85$
70	$30.5^{f}\pm0.49$	$8.8^{a}\pm0.33$	$13.7^{ab}\pm0.38$	$26.2^{g}\pm0.33$	$7.3^{a}\pm0.48$	$9.9^{c}\pm0.60$	$28.5^{d}\pm0.48$	$7.6^{a}\pm0.45$	$12.4^{bc}\pm0.45$
80	$32.1^{ef}\pm0.57$	$6.7^{bc}\pm0.26$	$10.4^{de}\pm0.72$	$28.5^{f}\pm0.2$	$5.7^{ab}\pm0.33$	$7.7^{d}\pm0.43$	$21.4^{f}\pm0.45$	$6.6^{abc}\pm0.21$	$10.6^{c}\pm0.45$
90	$27.2^{g}\pm0.52$	$5.2^{de}\pm0.23$	$8.6^{e}\pm0.45$	$21.5^{h}\pm0.44$	$5.2^{b}\pm0.33$	$5.9^{e}\pm0.40$	$28.2^{d}\pm0.52$	$5.2^{cd}\pm0.33$	5.0d±0.63
100	$26.3^{g}\pm0.48$	$4.5^{e}\pm0.2$	$6.6^{f}\pm0.45$	$17.5^{i}\pm0.56$	$3.3^{c}\pm0.38$	$6.3^{de}\pm0.38$	$25.5^{e}\pm0.4$	$4.2^{d}\pm0.33$	$5.3^{d}\pm0.59$



A



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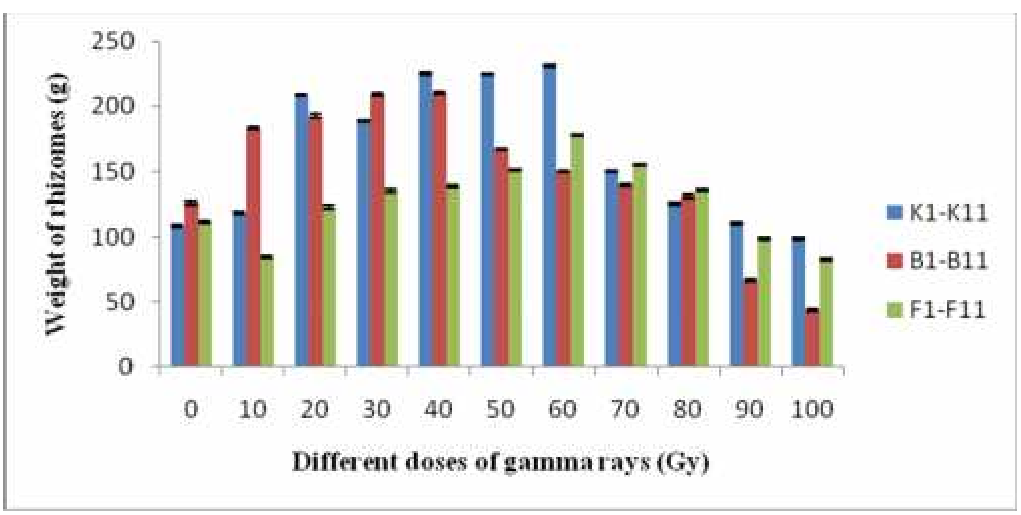

C

Fig. 1 (A-C): Effect of different doses of gamma irradiation on length of roots (A), number of roots (B) and weight of rhizomes (C) of different samples of *C. longa* L.

The irradiated plants showed symptoms of enhancement or inhibition in germination of seedlings and their biological responses (Kim *et al.*, 2000).

In case of number of roots (Fig. 1B), the control plants gave 4-5.2 average number of roots. Turmeric plants showed maximum number of roots (6.2) at 70 Gy in plants of Bannu and minimum number of roots (2.1) at 100 Gy in Faisalabad plants. Melki & Marouani (2009) also reported that in hard wheat with dose of 20 Gy, 18% improvement was observed in root number and 32% in root length.

Weight of rhizomes: After harvesting, the weight of rhizomes was calculated and presented in Fig. 1C. Generally, weight of rhizomes increased gradually with increasing doses of gamma rays. Non-irradiated plants had 100-126g weight of rhizomes. Maximum increase of 231.0 g was measured at 60 Gy in plants of Kasur area and 210.0 g was calculated at 40 Gy in plants of Bannu area. The plants of Faisalabad area did not show large increase in weight of harvested rhizomes i.e. maximum obtained value was 178 g at 60 Gy. Maximum decrease (44 g) was measured at 100 Gy in plants of Bannu.

Fresh and dry weight of plants: The present results also showed the measured data about fresh and dry weight of plants (Fig. 2 & Fig. 3). Lower doses promoted fresh and dry weights of plants while higher doses inhibited. Fresh and dry weights of plants were maximum (75.0g and 53.6g respectively) at 50 Gy in plants of Kasur then gradually decreased with increased radiation doses.

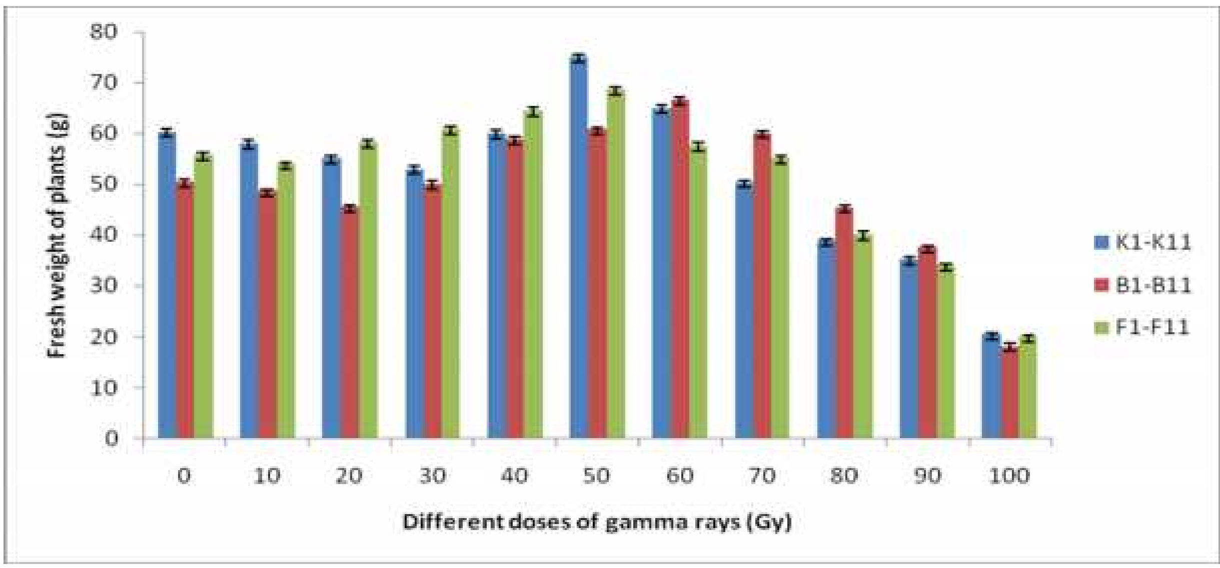

Fig. 2: Effect of different doses of gamma irradiation on fresh weight of plants of different samples of *C. longa* L.

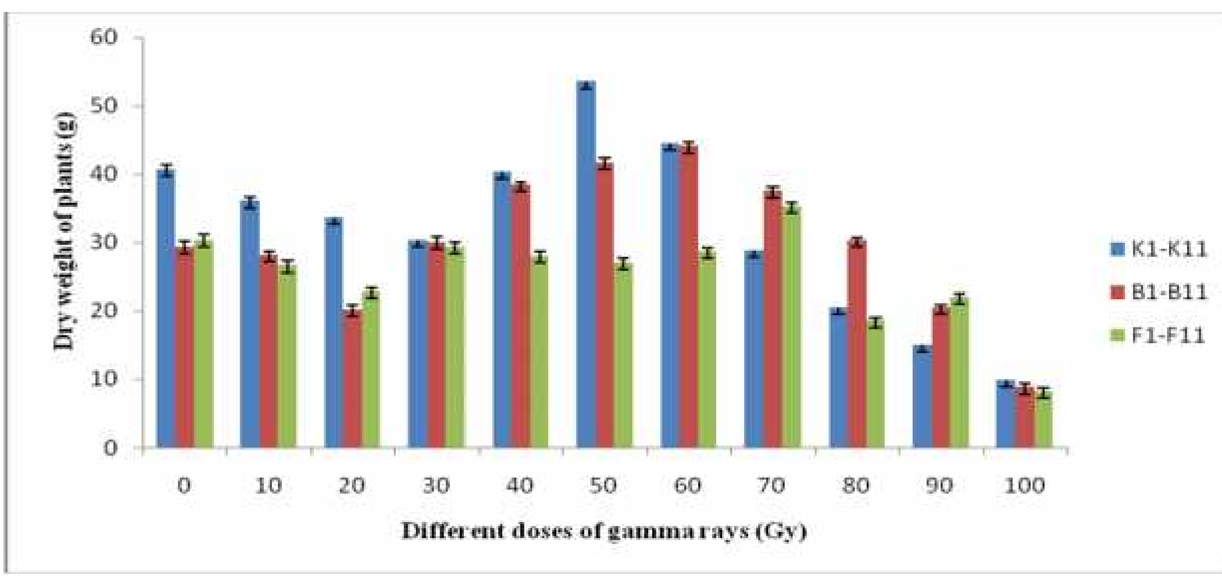

Fig. 3: Effect of different doses of gamma irradiation on dry weight of plants of different samples of *C. longa* L.

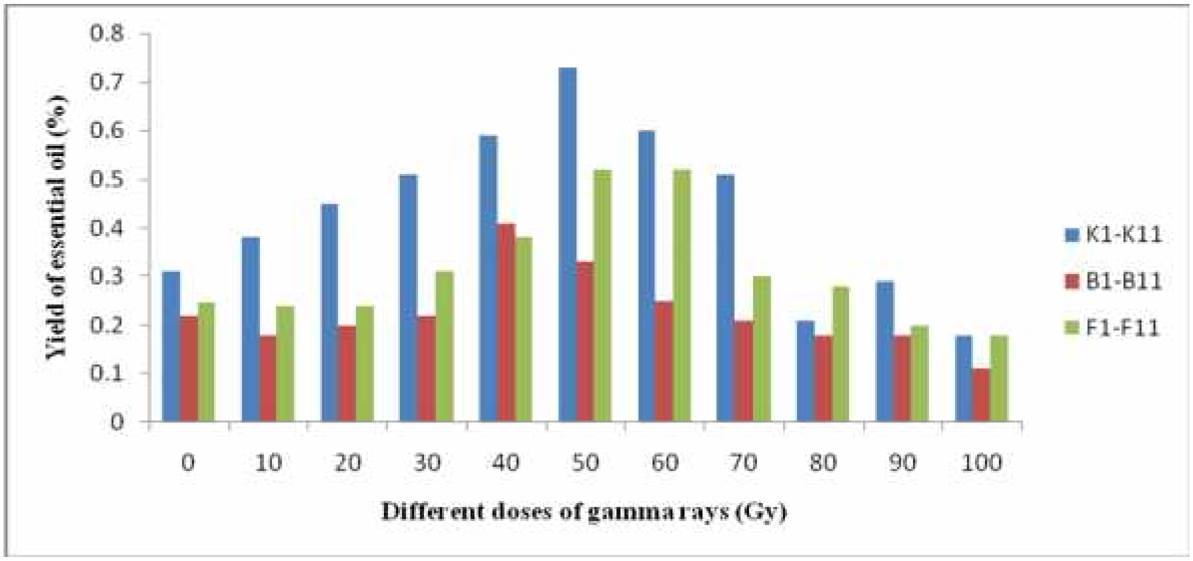

Fig. 4: Effect of different doses of gamma irradiation on percentage yield of volatile oil extracted from different samples of *C. longa* L.

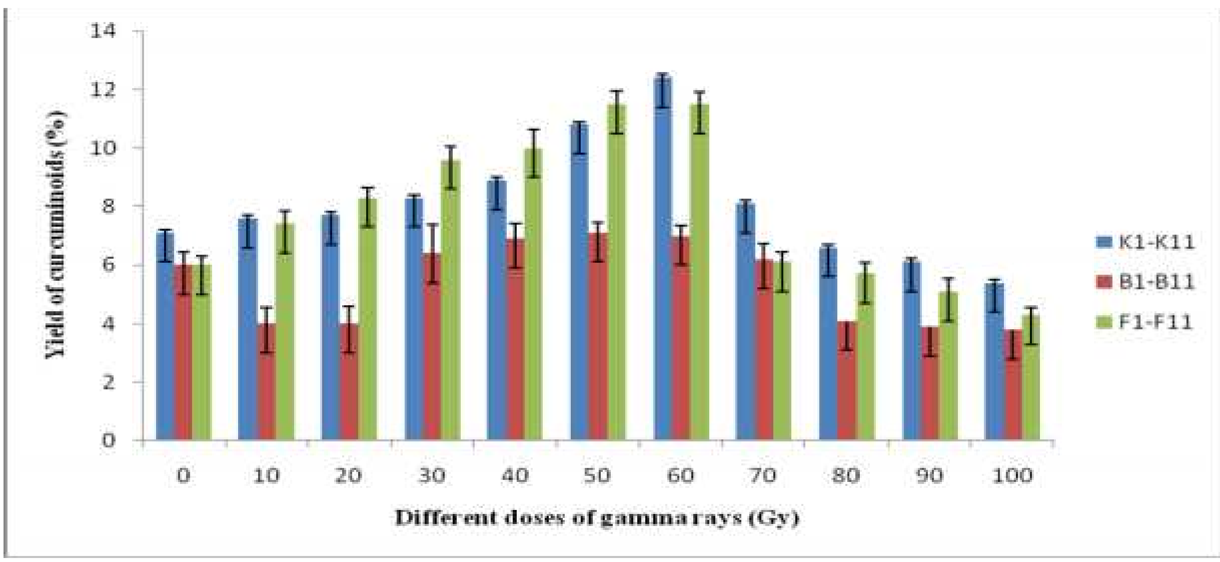

Fig. 5: Effect of different doses of gamma irradiationon percentage yield of curcuminoids extracted from rhizomes of different samples of *C. longa* L.

These results are in good agreement with the results of Melki and Salami (2008). They observed that gamma radiation treatment at 15 Gy exhibited a significant improvement in dry weight of chickpea plants which was 20.0% higher as compared to control plants (0 Gy). At lower doses, an increase was measured in fresh weight of shoots of winged beans while decreased on higher doses of gamma radiation (Veeresh *et al*., 1995).

Yields of essential oils and curcuminoids: Gamma radiation had highly significant effects on the yields of essential oils and curcuminoids extracted from *C. longa*. It is illustrated from Fig. 4 that maximum yield of essential oil was 0.73% at 50 Gy in plants of Kasur area whereas control yielded 0.31%. The plants from Faisalabad and Bannu also showed 0.52% and 0.41% at 50 Gy and 40 Gy respectively. Higher doses yielded reduced amount of oil. Nassar *et al*., (2004) reported that essential oil production from *Chamomilla recutita* L. was increased by gamma rays and phosphorus addition. In many plant species, an increase in essential oil production through gamma irradiation was reported by many researchers {Youssef *et al.*, (2000); Sattar *et al*., (1976)} while higher doses of gamma irradiation decreased the oil production (Zheljazkov *et al.*, 1996; Francis *et al.*, 1983; Gupta & Raj, 1980). Similarly curcuminoids also yielded maximum increase (12.4%) at 60 Gy as compared to control (7.1%) plants from Kasur as presented in Fig. 5.

Maximum decrease (3.8%) was also measured at higher dose of 100 Gy in plants of Bannu.

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