

## Effects of presowing gamma irradiation on the photosynthetic pigments, sugar content and carbon gain of *Cullen corylifolium* (L.) Medik.

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To determine the effects of gamma radiation on the photosynthetic pigments, sugar content and total carbon gain, seeds of *Cullen corylifolium* (L.) Medik. were irradiated with variable doses (0, 2.5, 5, 10, 15, and 20 kGy) at the rate of 1.65 kGy h<sup>-1</sup> from <sup>60</sup>Co gamma source. *Cullen corylifolium* represents an important Chinese medicine with adequate levels of secondary metabolites, thus we hypothesized that gamma irradiation could modulate primary metabolites which could supplement secondary metabolite levels. The seeds were then transferred to field for biochemical analysis at different developmental stages; pre-flowering, flowering and post-flowering. Gamma dosage at 10 kGy resulted in a significant increase in concentration of chlorophyll *a* (61.17%), chlorophyll *b* (93.18%) and total chlorophyll (71.66%), suggesting that low doses of radiation could activate photosynthetic pigment system while at 15 and 20 kGy dose resulted in depletion of such parameters. Sugar and total C analysis of plants irradiated at 10 kGy demonstrated significantly maximum (216.01%) sugar content in leaves at all developmental stages and significantly minimum (46.13%) and (57.81%) in plants raised from seeds irradiated at 15 and 20 kGy respectively. Effective stimulatory dose for *C. corylifolium* '11062' is 10 kGy. In contrast, the carotenoid content of the plants exposed to 15 and 20 kGy was maximum than control. Significance of such stimulation correlated with increasing C mass of the plant concerned is discussed in the light of newer aspects in research.

**Key words:** Carbon gain, carotenoid, chlorophyll a, chlorophyll b, gamma rays, sugar.

### INTRODUCTION

Gamma rays are imperative in development of mutant varieties and increase of genetic variability (Jan et al., 2011a). Further ever, gamma irradiation has been suggested for quarantine treatment and as valid method of decontamination of medicinal herbs (Variyar et al., 1998; Maity et al., 2009). Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues (Gunkel and Sparrow, 1961; Jan et al., 2010). Research on the effects of gamma rays on food sources and animal cells has been done, with little information to date on medicinal plants (Sanada, 1986). Several reports on the stimulatory effects on plant growth using radioactive rays at low doses are available. Such radiation practices, although within range of low doses (5-500 Gy) can enhance the physiological activities of cells

in plants and photosynthetic microbes by ameliorating germination and growth rates (Thiede et al., 1995; Al-Safadi and Simon, 1996; Lee et al., 1998; Thapa, 2004; Puzon, 2005; Melki and Sallami, 2008; Melki and Marouni, 2009; Jan et al., 2010), increase stress resistance (Zaka et al., 2002; Lee et al., 2002a; 2002b; 2003) and/or improving crop yields (Wiendl et al., 1995; Kim et al., 1998; Al-Safadi et al., 2000). On the erstwhile hand, high dose of gamma ray (2-20 kGy), applied to the seed before sowing, disturbs the protein synthesis (Xiuzher, 1994), results in improper hormone levels (Dwelle, 1975; Rabie et al., 1996), altered enzyme activity (Al-Rumaih and Al-Rumaih, 2008; Vandenhove et al., 2009; Stajner et al., 2009; Jan et al., 2011b), impaired leaf gas and water exchange (Stoeva and Bineva, 2001).

*Cullen corylifolium* (L.) Medik., a member of *Fabaceae* family, is significant endangered medicinal plant found in tropical and sub-tropical region of the world (Jain, 1994). The valuable components of this herb are coumarins, psoralen and isopsoralen. Pharmacological tests revealed that they have anti-tumor, anti-oxidative, anti-microbial, anti-inflammatory, anti-mutagenic, and insect hormonal activities (Haraguchi et al., 2002; Khatune et al., 2004; Bapat et al., 2005). In spite of being such an important medicinal plant in Indian and traditional Chinese medicine, there is scarce research documenting the effects of the various environmental stresses on growth, development and metabolism in *C. corylifolium*. Abiotic

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and biotic stresses cause alterations in the physiological processes of plants and decrease their productivity.

Keeping this background, we used variable doses of gamma rays to study effects of seed irradiation on developing seedlings of *C. corylifolium*. Our objective was to analyze the changes in photosynthetic pigment concentration, C gain, and sugar content. To do so, we measured and compared photosynthetic pigments, C, and sugar content in control and treated plants at different developmental stages.

## MATERIAL AND METHODS

### Procurement of seeds and irradiation

The seeds of babchi (*Cullen corylifolium* [L.] Medik.), belong to family *Fabaceae*, were procured from National Bureau of Plant Genetic Resources (NBPGR), Indian Agricultural Research Institute (IARI), New Delhi, India. Each sample of 25 g seeds was packed in polythene and were irradiated with different doses of gamma rays ( $T_1$ : 2.5,  $T_2$ : 5,  $T_3$ : 10,  $T_4$ : 15, and  $T_5$ : 20 kGy) at dose rate of 1.65 kGy h<sup>-1</sup> (Fricke and Hart, 1996) at room temperature (25 ± 1 °C) from <sup>60</sup>Co gamma irradiator (Gamma chamber GC-5000, BRIT, India) at Institute of Nuclear Medicine and Allied Sciences (INMAS), New Delhi.

Field experiments were repeated three times with 10 replicates. All the seeds were planted in an open atmosphere of Hamdard University, New Delhi (28°38' N, 77°11' E; 228 m a.s.l.), India, about 70-80% relative humidity, 35 °C temperature, sandy-loam soil (pH 7.3). Levels of available N and S in soil were 51 and 7.9 mg kg<sup>-1</sup>, respectively. Seeds were sown the first week of April 2009. Each sample was planted in six rows, 4 m long and 0.6 m wide, making an area of 14.4 m<sup>2</sup>. Hills were 30 cm apart; five seeds per hill to avoid root disturbance as *Cullen* plants are very sensitive in the sense that they cannot endure any disturbance of the root. Other agricultural practices such as irrigation and weeding were carried out as required. The planted seeds were observed daily until germination commenced. Dates of commencement and termination of germination as well as the number of seeds that germinated for each day were noted for each of the samples. Leaves from developing seedlings were collected at three developmental stages: pre-flowering (45 d after sowing, DAS), flowering (90 DAS) and post-flowering (135 DAS), to analyze the effects of the gamma irradiation on photosynthetic pigment contents, C, and sugar content in control and treated plants.

### Soluble sugar content determination

Soluble sugar was estimated by the method of Dey (1990). Half gram of fresh leaf material was kept in 10 mL of 90% ethanol for 1 h at 60 °C in incubator. The extract was then decanted into a 25 mL volumetric flask and the residue was re-extracted. Final volume was made up to 25 mL by adding 90% ethanol. An aliquot (1 mL)

was then transferred to a thick walled test tube and 1.0 mL of 5% phenol was added and mixed thoroughly and 5 mL of analytical grade sulfuric acid (97-99%) was then added and mixed thoroughly by vertical agitation with a glass rod. For exothermic reaction, the test tube was cooled in the air. Absorbance was recorded at 485 nm on UV-Vis spectrophotometer (model DU 640 Beckman Coulter, Brea, California, USA). The corresponding concentration was determined against a standard curve prepared by using a glucose solution. The amount of sugar was expressed as mg g<sup>-1</sup> fresh weight.

### Photosynthetic pigments and carotenoid content

Total chlorophyll and carotenoid were measured from the fresh leaf by the method of Hiscox and Israelstam (1979).

**Extraction.** The method involves the estimation of plant pigments without maceration. Leaves, kept on a moist filter paper in an icebox, were washed with cold distilled water. Leaf discs were taken from either side of the midrib at the intraveinal region for the determination of chlorophyll and carotenoid content. Chopped leaf material (100 mg) was taken in vials in triplicates containing 7 mL of dimethyl sulfoxide (DMSO). The vials were then kept in an oven at 65 °C for 1 h for complete leaching of the pigments. Thereafter, the volume of DMSO was made up to 10 mL. The chlorophyll content was then measured immediately.

**Estimation.** A volume of 2 mL extract was transferred to a cuvette and the absorbance was read at 480, 510, 645 and 663 nm using a Beckman spectrophotometer (model DU 640, Fullerton, California, USA) against DMSO as a blank. Values of optical densities (ODs) were used to compute the Chl *a*, Chl *b*, total Chl, and carotenoid contents using the following formula:

$$\text{Chl } a \text{ (mg g}^{-1} \text{ fw)} = \{[12.3(OD_{663}) - 0.86(OD_{645})]/(D \times 1000 \times W)\} \times V \quad (\text{Maclachlan and Zalik, 1963})$$

$$\text{Chl } b \text{ (mg g}^{-1} \text{ fw)} = \{[19.3(OD_{645}) - 3.60(OD_{663})]/(D \times 1000 \times W)\} \times V \quad (\text{Duxbury and Yentsch, 1956})$$

$$\text{Total Chl (mg g}^{-1} \text{ fw)} = \{[20.2(OD_{645}) + 8.02(OD_{663})]/(D \times 1000 \times W)\} \times V \quad (\text{Arnon, 1949})$$

$$\text{Carotenoids (mg g}^{-1} \text{ fw)} = \{[7.6(OD_{480}) - 1.49(OD_{510})]/(D \times 1000 \times W)\} \times V \quad (\text{Barnes et al., 1992})$$

where, *fw* is fresh weight, *D* is distance travelled by the light path; *W* is weight of the leaf material taken; *V* is volume of the extract; *OD* is optical density.

### Elemental composition and estimation

Plant parts were analyzed at different samplings for assessing C status. Five plants were collected from each treatment and were wiped free of any adhering dust. Sampling was first washed in running tap water for 1 min followed by 1 min in distilled water. Each sample was dried for 48 h in hot air oven at 65 °C. Dried samples were fine powdered and passed through a 72 mm mesh screen.

The powder was stored in polyethylene bags labeled and used for analysis later.

Carbon content (% dry weight) was analyzed by packing the known weight of plant sample-powder in tin boats with the help of Elementar System (CHNS Analyse, vario EL, Hanau, Germany).

## RESULTS AND DISCUSSION

Gamma rays belong to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. Photosynthetic pigments can be destroyed by high doses of gamma irradiation, with concomitant loss of photosynthetic capacity (Strid et al., 1990). Photosynthetic apparatus seems to be most sensitive target in plants (Kulandaivelu and Noorudeen, 1983). Gamma irradiation of seeds to improve photosynthesis by modulating pigment system has been reported (Kim et al., 2004; Hegazi and Hamideldin, 2010). The chlorophyll *a*, Chl *b*, and total chlorophyll content in leaves enhanced significantly ( $P < 0.05$ ) with plant age up to flowering stage and thereafter declined steadily. Chl *a*, Chl *b*, and total chlorophyll increased 61.17%, 93.18%, and 71.66%, respectively, with low dose 10 kGy at flowering stage (Tables 1-2-3). Two wheat (*Triticum aestivum* L.) genotypes ('Roshan' and 'T-65-58-8') raised from seeds irradiated with 100, 200, 300, and 400 Gy exhibited 64.5% increment in total chlorophyll in both genotypes seedlings that were irradiated at 100 Gy followed by gradual decrease at 200 Gy dose (Borzouei et al., 2010). The evident rise of chlorophyll concentration in plants exposed to low dose radiation could be a consequence of plant recuperation after exposure. Irradiation of corn seeds increased synthesis of chlorophyll and carotene in seedlings (Vlasyuk and Marina 1970). However, Fan et al. (2003) observed contradictory results since no irradiation-induced increase in chlorophyll or carotenoid was observed probably due to differences in species and/or growing

**Table 1. Variation in chlorophyll *a* content (mg g<sup>-1</sup> fw) at various growth stages of *Cullen corylifolium* exposed to different doses of gamma rays.**

Treatments	Developmental stages		
	Pre-flowering	Flowering	Post-flowering
Control	0.73a ± 0.002	0.85e ± 0.005	0.78h ± 0.029
2.5 kGy	0.86 ± 0.002 (17.80)b	1.07 ± 0.001 (25.88)e	0.97 ± 0.031 (24.35)h
5 kGy	0.98 ± 0.003 (34.24)c	1.21 ± 0.001 (42.35)f	1.08 ± 0.036 (38.46)i
10 kGy	1.07 ± 0.006 (46.57)d	1.37 ± 0.001 (61.17)f	1.28 ± 0.027 (64.10)j
15 kGy	0.63 ± 0.001 (13.69)d	0.74 ± 0.001 (12.94)g	0.70 ± 0.015 (10.25)j
20 kGy	0.39 ± 0.001 (46.57)d	0.53 ± 0.005 (37.64)h	0.51 ± 0.019 (34.61)k

Values in parenthesis represent percent variation. Values with different letters are significantly different from each other according to Duncan's multiple range test ( $P < 0.05$ ); values represent mean ± SE (n = 10). Critical difference at  $P > 0.05$ : Treatments: 0.019; Developmental stages: 0.014; Treatment developmental stages: 0.044.

**Table 2. Variation in chlorophyll *b* content (mg g<sup>-1</sup> fw) at various growth stages of *Cullen corylifolium* exposed to different doses of gamma rays.**

Treatments	Developmental stages		
	Pre-flowering	Flowering	Post-flowering
Control	0.34a ± 0.008	0.44ab ± 0.007	0.42b ± 0.002
2.5 kGy	0.40 ± 0.006 (17.64)a	0.55 ± 0.008 (25)b	0.48 ± 0.004 (14.28)b
5 kGy	0.47 ± 0.013 (38.23)a	0.67 ± 0.008 (52.27)b	0.58 ± 0.013 (38.09)b
10 kGy	0.66 ± 0.011 (94.11)a	0.85 ± 0.011 (93.18)b	0.74 ± 0.013 (76.19)b
15 kGy	0.27 ± 0.012 (20.58)a	0.37 ± 0.010 (15.90)b	0.29 ± 0.008 (30.92)b
20 kGy	0.23 ± 0.013 (32.35)a	0.35 ± 0.020 (20.45)b	0.26 ± 0.474 (38.09)b

Values in parenthesis represent percent variation. Values with different letters are significantly different from each other according to Duncan's multiple range test ( $P < 0.05$ ); values represent mean ± SE (n = 10).

Critical difference at  $P > 0.05$ : Treatments: 0.161; Developmental stages: 0.114; Treatment developmental stages: 0.361.

**Table 3. Variation in the total chlorophyll (mg g<sup>-1</sup> fw) at various growth stages of *Cullen corylifolium* exposed to different doses of gamma rays.**

Treatments	Developmental stages		
	Pre-flowering	Flowering	Post-flowering
Control	1.14 ± 0.09	1.20 ± 0.05	1.24 ± 0.04
2.5 kGy	1.37 ± 0.15 (20.17)a	1.57 ± 0.03 (30.83)b	1.45 ± 0.13 (16.93)b
5 kGy	1.6 ± 0.15 (40.35)a	1.78 ± 0.04 (40.27) b	1.68 ± 0.11 (35.48)b
10 kGy	1.89 ± 0.15 (65.78)a	2.06 ± 0.11 (71.66)b	1.98 ± 0.11 (59.67)b
15 kGy	0.96 ± 0.07 (15.78)a	1.066 ± 0.02 (11.16)b	1.04 ± 0.06 (16.12) b
20 kGy	0.69 ± 0.09 (39.47)a	0.81 ± 0.01 (32.50)b	0.73 ± 0.08 (41.12)b

Values in parenthesis represent percent variation. Values with different letters are significantly different from each other according to Duncan's multiple range test ( $P < 0.05$ ); values represent mean ± SE (n = 10).

Critical difference at  $P > 0.05$ : Treatments: 0.141; Developmental stages: 0.124; Treatment developmental stages: 0.381.

conditions. Maize (*Zea mays* L.) dry seeds exposed to gamma doses ranging from 0.1 to 1 kGy exhibited similar biochemical differences based on photosynthetic pigment (Chl *a*, Chl *b*, Car) content displaying an inversely proportional relationship to exposure doses. Furthermore, the concentration of Chl *a* was higher than Chl *b* in both irradiated and non-irradiated seedlings (Marcu et al., 2013a). Similarly lettuce (*Lactuca sativa* var. *capitata*) dry seeds exposed to gamma doses ranging from 2-70 Gy indicated that seeds irradiated at doses ranging from 2-30 Gy enhanced the photosynthetic pigments (Chl *a*, Chl *b*, Car) content, while at higher doses (70 Gy) resulted in decline of the assimilatory pigments (Marcu et al., 2013b).

Carotenoid pigment plays an important role in radiation damage and free radical scavenging (Fukuzawa et al., 1998). Age and dose dependent significant ( $P < 0.05$ ) increase was observed in Car content in leaves of *C. corylifolium* being maximum at pre-flowering (75%) at 10 kGy followed by flowering (68.08%) and post-flowering (59.61%) stages (Table 4). However, Kim et al. (2004) contradicted this statement by proposing that,

**Table 4. Variation in carotenoid content (mg g<sup>-1</sup> fw) at various growth stages of *Cullen corylifolium* exposed to different doses of gamma rays.**

Treatments	Developmental stages		
	Pre-flowering	Flowering	Post-flowering
Control	0.441a ± 0.015	0.471e ± 0.013	0.523i ± 0.001
2.5 kGy	0.552 ± 0.007 (25)b	0.565 ± 0.013 (19.14)f	0.657 ± 0.001 (25.62)ij
5 kGy	0.703 ± 0.023 (59.09)bc	0.680 ± 0.008 (44.68)fg	0.745 ± 0.001 (42.30)j
10 kGy	0.771 ± 0.013 (75)c	0.790 ± 0.014 (68.08)g	0.837 ± 0.002 (59.61)k
15 kGy	0.337 ± 0.013 (25)d	0.369h ± 0.009 (23.40)h	0.393 ± 0.004 (25)kl
20 kGy	0.223 ± 0.015 (50)de	0.196 ± 0.006 (59.57)h	0.247l ± 0.002 (53.84)l

Values in parenthesis represent percent variation. Values with different letters are significantly different ( $P < 0.05$ ) different from each other (Duncan's multiple range test).

Values represent mean ± SE (n = 10).

Critical difference at  $P > 0.05$ : Treatments: 0.0159; Developmental stages: 0.0112; Treatment developmental stages: 0.0355.

chlorophylls are practically insensitive to low dosage gamma irradiation whereas the levels of carotenoid increase in a dosage dependent manner at the same degree of irradiation. Furthermore, plants adapt to environment more widely through high photon energy use efficiency by increasing the proportion of Chl *b* in reaction centers involved in photosynthesis. These findings are consistent with our results showing positive correlation with growth attributes of *C. corylifolium*, considering the fact that plant biomass and nitrogen content was also increased at flowering following low doses gamma rays (Jan, 2011).

The sugar content of leaves enhanced significantly ( $P < 0.05$ ) with plant age being maximum (216.01%) at 10 kGy dose of gamma irradiation while minimum (57.81%) with 20 kGy at pre-flowering stage (Table 5). Such a decline in sugar content at post-flowering stage following higher gamma dosage is correlated with observed suppression in pigments. Increased soluble sugar content at flowering stage may be associated with increased photosynthetic activity. Sucrose content of both potato (*Solanum tuberosum* L.) tubers and sweet potato roots

**Table 5. Variation in sugar content (mg g<sup>-1</sup> fw) at various growth stages of *Cullen corylifolium* exposed to different doses of gamma rays.**

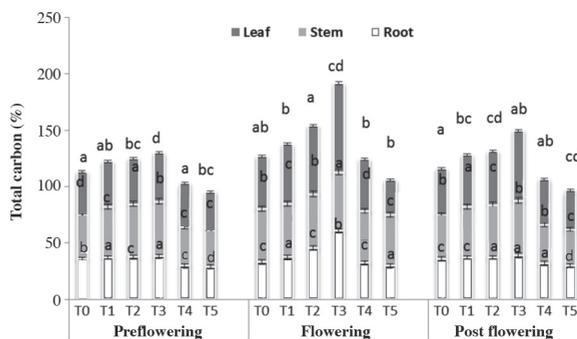
Treatments	Developmental stages		
	Pre-flowering	Flowering	Post-flowering
Control	2.56a ± 0.06	3.49 ± 0.062	2.76g ± 0.01
2.5 kGy	3.40b ± 0.03 (32.81)b	4.56 ± 0.08 (30.65)m	3.96 ± 0.02 (43.47)h
5 kGy	5.61 ± 0.02 (119.1)c	6.81n ± 0.04 (95.12)n	6.17 ± 0.01 (123.55)i
10 kGy	8.09 ± 0.01 (216.01)d	9.04 ± 0.003 (159.02)o	8.64 ± 0.06 (213.04)i
15 kGy	1.47 ± 0.02 (42.57)e	1.88 ± 0.01 (46.13)p	1.78 ± 0.04 (35.50)j
20 kGy	1.08 ± 0.002 (57.81)f	1.62 ± 0.02 (53.58)q	1.33 ± 0.01 (51.81)k

Values in parenthesis represent percent variation. Values with different letters are significantly different from each other according to Duncan's multiple range test ( $P < 0.05$ ); values represent mean ± SE (n = 10).

Critical difference at  $P > 0.05$ : Treatments: 0.044; Developmental stages: 0.031; Treatment developmental stages: 0.099.

was increased at irradiation dose of 3 to 4 kGy and 0.8 to 2 kGy, respectively (Ezekiel et al., 2007). Further, free sugar, glucose, sucrose, and maltose were significantly increased by 7.5 and 10 kGy gamma irradiation in fruits (Byun et al., 1997). The increase in sugar content supports some changes in grain starch granules which have been reported earlier (Błaszczak et al., 2002; Gralik and Warchalewski, 2006), where ionizing radiation caused visible changes in the microstructure of kernel starchy-endosperm at 10 kGy. Increment and decrease in soluble sugar content following low and high gamma doses respectively has been reported in *Eruca vesicaria* subsp. *sativa* (Moussa, 2006). The decrease in sugar contents of plants under maturation stage can be due to two factors, the leaf senescence induced by the gamma radiation, because the reserves as N compounds and sugars are transported to the sink. Other explanation is the photosynthetic reduction through stomatal closure and consequent lower entrance of CO<sub>2</sub> in leaf tissue, as well as degradation of photosynthetic pigments promoted by oxidative stress during irradiation (Jan et al., 2011b).

Maximum increase in root (72.23%), stem (40.57%) and leaf C (69.92%) was recorded with 10 kGy at flowering stage where as maximum decline of root (18.84%), stem (21.49%) and leaf C (34.17%) was recorded with 20 kGy (Figure 1). Carbon content in root and aerial parts of *C. corylifolium* plants was recorded highest during flowering stage following low doses of gamma rays but declined thereafter. This decline coincided with plants irradiated with 15 and 20 kGy. The maximum N content was recorded in stem and minimum in roots at post-flowering stage. Results of our experiment indicate that the photosynthesis and sugar content was increased in plants raised from seeds irradiated with low doses of gamma rays. These results were almost in agreement with those of Cailian et al. (1993); El-Sayed et al. (1994); Al-Kobaisi et al. (1997); Gautam et al. (1998); Rascio et al. (2001) and Osama (2002), who have reported the improvement



Bars represents the mean ± SE (n = 10). Values with different letters are significantly different from each other according to Duncan's multiple range test ( $P < 0.05$ ). T<sub>1</sub>: 2.5, T<sub>2</sub>: 5, T<sub>3</sub>: 10, T<sub>4</sub>: 15 and T<sub>5</sub>: 20 kGy.

**Figure 1. Variation in total C (%) content per mg at various developmental stages of *Cullen corylifolium* exposed to different doses of gamma rays.**

of yield components and C containing compounds (chlorophyll parameters and carotenoid) in various plants such as tomato, maize, rice and wheat following gamma irradiation. Reduced C gain was also supported by Ursino et al. (1974), who found that photosynthesis was reduced by half in *Pinus strobes* exposed to 10 Gy. Higher doses of gamma rays were not lethal and they caused very little visual damage to the plants. Presence of significant differences between total biomass would suggest change in the rate of fixed C. Our study exhibited that radiation resulted in decrease in leaf area at higher doses of gamma rays, while showing negative relationship to photosynthesis and a strong positive relationship to leaf density. Similar results have been observed in sunflower plants exposed to variable doses of gamma rays (Thiede et al., 1995).

## CONCLUSION

Our results presented above are concerned with persistence of changes prevalent from seed to seed cycle, where the inhibition in photosynthetic, accessory pigments, sugar content and C mass were more prevalent over doses of 15 and 20 kGy. Low doses of ionizing radiations have modulatory role in the metabolic and biochemical processes of seedling. Effective stimulatory dose for plant development is 10 kGy for *Cullen corylifolium*, while the dosage of 20 kGy can prove detrimental for the concerned plant.

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