International Journal of Minor Fruits, Medicinal and Aromatic Plants. Vol. 8 (1) : 40-46, June 2022

Effect of gamma irradiation on seed germination, survivability and growth performances of *Calotropis gigantea* (Vara)

Nelka S.A.P[1*], NP Vidanapathirana[1], N Dahanayake[2], S Subasinghe[2], TD Silva[3], S Weerasinghe[1], Rifnas L.M.[1], Rohanadeera H.[1], Madushanka W.C.M.S.[1] and Dushane S.[1]

[1] *Institute for Agro technology and Rural Sciences (UCIARS), University of Colombo, Weligatta, Sri Lanka*

[2] *Faculty of Agriculture, University of Ruhuna, Sri Lanka*

[3] *Department of Plant Science, Faculty of Science, University of Colombo, Sri Lanka*

**Email: nelka@uciars.cmb.ac.lk*

Received : 01.02.2022 ; Revised : 26.04.2022 ; Accepted : 28.04.2022

DOI : 10.53552/ijmfmap.8.1.2022.40-46

ABSTRACT

Physical mutagenesis is an effective mutational breeding method for improving various morphological characteristics of agricultural and medicinal crops. The objective of the conducted experiment was determining the effective dose of gamma radiation to induce mutations in Calotropis gigantea. Well matured seeds of C. gigantea were exposed to Gamma irradiation using "Gamma chamber 1200 Cobalt-60" research irradiator and these treatments were carried out at the Horticultural Crop Research and Developmental Institute at Gannoruwa, Sri Lanka. Mature seeds were subjected to seven treatments as 150Gy, 300Gy, 450Gy, 600Gy, 750Gy, 900Gy and non-irradiation (0 Gy) as a control to study their effect on germination percentage, survival rate and growth performances of C. gigantea. Treated seeds were arranged under shade house condition in Completely Randomized Design with four replications and each replication contained twenty-four seeds. The mutagenic treatments were tested for lethal dose of 50% and the dose at which 50% of the survival at four months was considered as LD50 value. Data were analyzed by ANOVA using Minitab 17 software and compared the treatment means using Dunnett's test at 0.05 significant level. The study revealed that the higher dosages of gamma irradiation was significantly decreased the germination percentage, survivability and growth performances ($P<0.05$). Plant survivability, number of leaves and plant height were significantly ($P<0.05$) reduced with the higher dosages of 450 Gy to 900 Gy. Lethality level of gamma irradiation for Calotropis gigantea was found as 395 Gy at maturity stage and it could be concluded that the radiation below 395 Gy should be imposed to induce mutations in Calotropis gigantea.

***Keywords*: *Calotropis gigantea*, dosage, gamma irradiation, germination, survival**

INTRODUCTION

Calotropis gigantea is a shrub which belongs to family Apocynaceae, commonly known as "milk weed" or "crown flower" in English, *Wara* or *Hela-wara* in Sinhala and *Mannakkovi* in Tamil (Abeysinghe, 2018). The shrub has a branched woody stem covered with a corky bark (Sharma *et al.*, 2011). Thick, waxy and grey green leaves show ovate shaped and simple, opposite leaf arrangement. Flower colour is white or lavender and having waxy appearance (Ganeshan *et al.*, 2018). The fruit is green in color, spongy simple and follicle. It is identified as a diploid (2n=22) outcrossing plant and reproduction success mostly depends on hymenopteran insect pollination. This plant is most diverse in Asian and South East Asian countries such as Sri Lanka, India, Cambodia, Bangladesh, Indonesia, Malaysia, Pakistan and Philippines where tropical and subtropical climate prevails (Ganeshan *et al.*, 2018). There are several medicinal values in *C. gigantea*. Indonesians use the roots of *C. gigantea* as an antidote for snake bite (Kitagawa *et al.*, 1992). Leaves of *C. gigantea* are used to treat ailments such as skin and liver diseases, leprosy, earaches and worms (Rajakaruna *et al.*, 2002). Its latex is reported to possess wound healing ability due to the presence of proteases such as Calotropins DI (Urs *et al.*, 2017). Several studies reported that antimalarial and anticancer effects of *C. gigantea* (Wong *et al.*, 2011).

Changes in the plant's genetic structure can result in more physiologically and chemically efficient plant varieties with higher secondary metabolite synthesis. Mutation is a genetic difference caused by a rapid change in the gene. Gamma radiation is a mutagenic agent that is commonly used to induce mutations (Rifnas *et al.*, 2020). *C. gigantea* is still an undomesticated plant with uncertain economic returns. There is a need of facilitating for breeding purpose and improvement of growth characters of this plant to domesticate as miniature plant due to its medicinal and aesthetic value. Therefore, this experiment was carried out to find out the effect of gamma irradiation on survivability and growth performances of *C. gigantea.* Further, this will provide a baseline for exploration of physical mutagenesis by gamma irradiation on germination, survival and growth performances of *C. gigantea* for researchers.

MATERIALS AND METHODS

The experiment was performed at the University of Colombo's Institute for Agro Technology and Rural Sciences at Weligatta, which is located in Sri Lanka's Dry Zone, where the average annual rainfall is 1250 mm-1500 mm and the average annual temperature is 29 - 33^{0}C. Soil types of the area are reddish brown earth and low humic gley soil. Healthy and mature *C. gigantea* seeds collected from different areas in the dry zone were used as experimental material. At the Horticultural Crops Research and Development Institute in Gannoruwa, Sri Lanka, seeds of *C. gigantea* were irradiated with gamma rays from a Cobalt 60 research irradiator. The seedlings were treated with seven different treatments: 150 Gy, 300 Gy, 450 Gy, 600 Gy, 750 Gy, 900 Gy, and no irradiation (0 Gy). The treatment dosages were determined based on the previous experiments conducted on the related plant families. Treated seeds were planted in poly bag containers (250 gauge, 12.5 cm height ×10 cm width) at 0.5 cm depth in sand: compost 1:1 media. The poly bags were kept in a net house and watered daily to maintain adequate moisture. The experimental design consisted of four replicates and 24 seeds per replicate under the Completely Randomized Design (CRD). Radical emergence was taken as the indicator for seed germination. Plants were transferred to 30 cm diameter pots containing sand: compost: top soil 1:1:1 media, 8 weeks after germination.

Measurement of traits

Data on seed germination were recorded starting from the first day of emergence of the radicals of the plant from the growth medium. Cumulative number of germinated seeds was recorded until eleventh day from germination where plant germination was observed to be constant. The germination percentage was calculated as follows:

$$\text{Cumulative germination percentage (\%)} = \frac{No.of\ seeds\ germinated\ at\ final\ count}{No.of\ seeds\ planted} \times 100$$

Plant growth parameters such as cumulative leaf number and height of seedlings (measured from the surface of the planting medium to the tip of the longest branch in cm) were recorded from plants growing in the net house at 2 weeks after planting by using ruler and steel tape. Survival rate of seedling plants was calculated after 6 months from planting as the ratio of number of living (survived) plants to the total number of seedlings planted as follows;

$$Survival\ rate\ of\ seedling\ plant\ (\%) = \frac{No.\ of\ survived\ plants}{No.\ of\ seedlings\ plant} \times 100$$

The percentage inhibition or stimulation over control which is lethality over control (LOC) was calculated as follows;

$$LOC = \frac{(Survival\ in\ control\text{-}Survival\ in\ treatments)}{Survival\ in\ control} \times 100$$

Data analysis

Minitab was used to statistically analyse the collected data using Analysis of Variance (ANOVA), and Dunnets's test was used to separate the means at the 0.05 significant level.

RESULTS AND DISCUSSION

Effects of gamma irradiation on seed germination

The cumulative germination (CG) percentages of *C. gigantea* seeds treated with 6 doses of gamma rays and the control are shows the increasing pattern of their germination (Figure 1). According to the results, it was found that, there were significant ($P>0.05$) differences between the treatments over control on seed germination. The highest cumulative germination was observed in control (96.25%) where seeds were not exposed to any irradiation doses and the lowest (32.5%) was observed where seeds treated with the highest dosage (900 Gy). As in many other studies an inverse relationship between the gamma dosages and germination percentage was observed. Several studies of gamma radiation on different crops are on par with present results. According to Aynehband *et al*. (2012), gamma radiation ranging from 0 Gy-250 Gy was significantly decreased the percentage of Amaranth seeds germination. The highest percentage of germination (54.4%) was reported at zero and the lowest germination percentage (51.1%) was reported at the highest dose of gamma radiation at 250 Gy in Amaranth seeds. As mentioned by Rifnas *et al.* (2019), there was a progressive reduction in seed germination of sunflower was observed while exposing the seeds to increasing gamma radiation doses ranging 0-125 Gy. According to Kon *et al.* (2007), when maize seeds were exposed to gamma irradiation at dosages ranging from 0.1-1 kGy, the highest inhibition of the germination process (51%) was reported at 1 kGy. Therefore, it is clear that when increasing the radiation dosage, seed germination is decreased as experienced in present experiment as well. When increased the radiation dosage, seeds may have been damaged and this may have prevented them from germinating well.

Seedling survival

Survival of seedlings, 1- 4 months after germination of seeds treated with different gamma radiation dosages are given in Table 1. Seedling survival rate has shown significant difference when compared to the control and the other treatments in each month. Survival rate of seedlings was 100% up to 450 Gy and a significant reduction of survival rate of seedlings (>46%) was observed in treatments over 600 Gy after one month from germination. Seedling survival percentage was significantly reduced over 450 Gy and no plants were able to survive in treatments of 750 Gy and 900 Gy after two months from germination. The result shows that the survivability of all plants gradually declined after four months from germination, even 0 Gy – 300 Gy treatments had 100% survival rate at 2 months after germination. Further, survival of plants was poor in higher dosages of this study such as 600Gy- 900Gy treatments throughout, and after 4 months more than 90% of the plants died. The plants germinated in 750 Gy and 900 Gy treatments were weak and they appeared with pale yellow color cotyledons. After 1 month from germination, all the plants germinated were died in above treatments. There are many studies on different plants reported the effects of higher irradiation doses on seedling survival.

In *Alamanda cathartica*, Rifnas *et al.* (2020) found that as the dose of gamma radiation increased (0 Gy to 21 Gy), survival of plants dropped. Regardless of the method of irradiation, the effect of gamma rays on plant viability was gradual, depending on the degree of exposure (Sawangmee *et al.*, 2011). The rationale for the decreased survival may be due to the damage to cells and cellular components while another factor behind mortality is the rupture of cellular organelles (Navabi *et al.*, 2016). Plants were started to die slowly during the early months of treatment and later they recovered. However, the survival rate has been significantly reduced over time. Therefore, it could be stated that gamma ray doses below 450 Gy should be applied to recover useful induced mutations in *C. gigantea*. The LD50 (50% lethal dose) value obtained by plotting survival data after 4^{th} months against the applied dose rate is shown in Figure 2. The half lethal dose obtained from the figure is 395 Gy. Therefore, exposing below 395 Gy gamma ray dosages are suitable for mutation induction of *C. gigantea* with minimizing the lethality effect.

Effect of gamma radiation on different growth parameters

Plant height

Plant height of *C. gigantea* as affected by different doses of gamma radiation is given in Table 2. Different treatments showed differences in plant height, and height of plants in higher doses (>600 Gy) was significantly different to height in lower doses. When the lower doses of gamma radiations were considered (<450 Gy) it was found that there was no significant difference in the height of the seedlings compared to the control in every week. Extreme reduction in the plant height was observed with the higher dosages of 450 Gy to 900 Gy. It is evident from the data presented in Table 2 that the

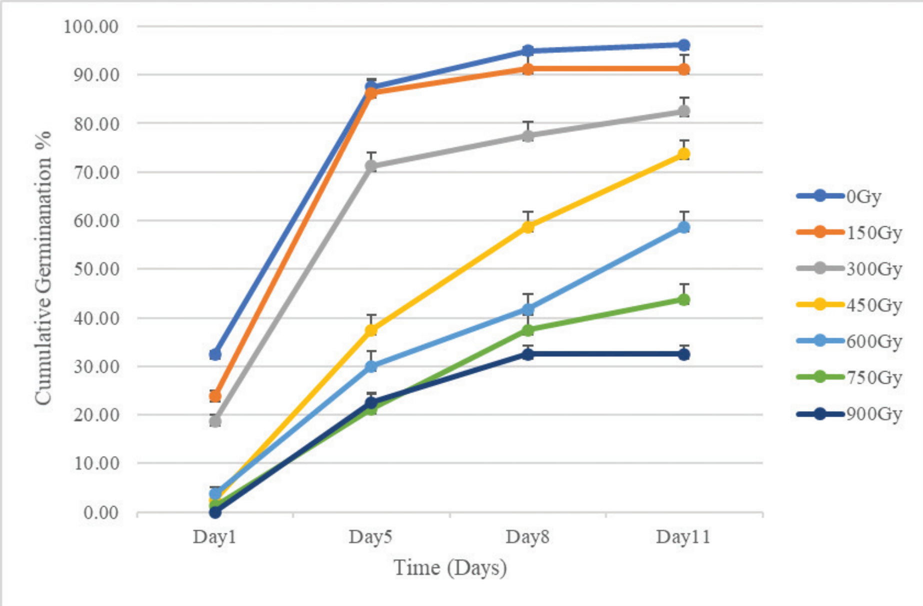

Figure 1: Cumulative Germination percentage of *C. gigantea* as affected by various dosages of gamma rays

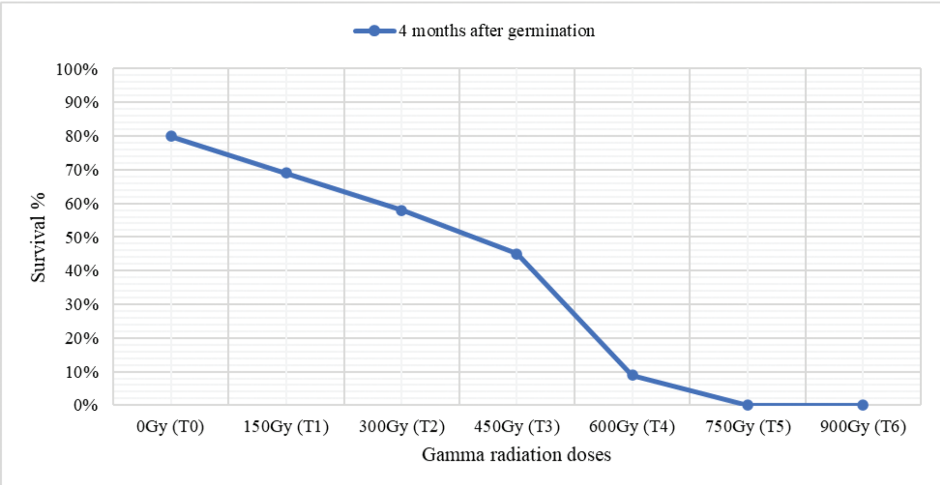

Figure 2: Survival percentage of *C. gigantea* seedlings with different doses of gamma radiation and the LD50 level

highest plant height was recorded from plants grown from non- irradiated seeds followed by seeds from 150 Gy of gamma dosage at every week after planting when compared to other treatments. A similar correlation *i.e.* "low dose-high growth" was observed in *Molluccella lavis* by Minisi *et al.* (2013), in chick pea by Hameed *et al.* (2008) and in amaranth by Aynehband *et al.* (2012). A reducing trend in plant height of *Allamanda cathartica* was observed by Rifnas *et al.* (2020) when exposing the plants to increasing doses of gamma radiation.

In this study, plant height of all the plants above 450 Gy gamma dosages was showing a significant reduction in their height. In mutagenic treatments, this may result in auxin degradation, suppression of auxin production (Gordon, 1954), and chromosomal abnormality (Gunckel and Sparrow, 1961). An experiment in *Moluccella laevis* by Minisi *et al.* (2013) proved that increase in gamma radiation dose decreased the height of *Moluccella laevis* plant. They also stated that higher dosage irradiation induced growth retardation, which they attributed to cell cycle arrest at G2/M phase during

Table 1: Survival percentage of *C. gigantea* seedlings after gamma irradiation treatments

Seedling Survival						
Treatments	**1 month after germination (%)**	**SE±**	**2 months after germination (%)**	**SE±**	**4 months after germination (%)**	**SE±**
T_0 - 0Gy	100[a]	±0.0	100[a]	±0.0	80[a]	±0.82
T_1 - 150Gy	100[a]	±0.0	100[a]	±0.0	69[b]	±1.68
T_2 - 300Gy	100[a]	±0.0	100[a]	±0.0	58[b]	±1.08
T_3 - 450Gy	100[a]	±0.0	86[b]	±0.91	45[b]	±1.08
T_4 - 600Gy	30[b]	±1.41	12.7[b]	±1.04	9[b]	±0.41
T_5 - 750Gy	46[b]	±0.91	0[b]	±0.0	0[b]	±0.0
T_6 - 900Gy	27[b]	±0.58	0[b]	±0.0	0[b]	±0.0

Means labelled with the same letter are not significantly different at p< 0.05

Table 2: Plant height of *C. gigantea* with different doses of gamma irradiation

Plant height (cm)						
Treatments	**2nd Week**	**4th week**	**6th week**	**8th week**	**12th week**	**16th week**
0 Gy	3.72[a]	4.59[a]	10.03[a]	11.5[a]	25.9[a]	69.75[a]
150 Gy	3.62[a]	4.58[a]	8.37[a]	10.15[a]	21.5[a]	64.60[a]
300 Gy	2.85[a]	3.73[a]	7.87[a]	10.12[a]	14.17[b]	52.60[b]
450 Gy	1.20[b]	2.02[b]	4.50[b]	7.37[a]	9.80[b]	41.30[b]
600 Gy	0.70[b]	1.18[b]	1.37[b]	2.47 [b]	7.00[b]	26.40[b]
750 Gy	0.68[b]	0.70[b]	-	-	-	-
900 Gy	0.50[b]	0.70[b]	-	-	-	-

Means labelled with the same letter are not significantly different at p< 0.05.

Table 3: Number of leaves of *C. gigantea* as affected by different dosages of gamma irradiation

Mean No. of leaves					
Treatments	**4th Week**	**6th Week**	**8th Week**	**10th Week**	**12th Week**
0 Gy	5.04[a]	5.41[a]	6.54[a]	7.60[a]	12.57[a]
150 Gy	5.08[a]	6.00[a]	6.58[a]	7.41[a]	9.70[a]
300 Gy	5.25[a]	6.62[a]	6.10[a]	6.83[a]	9.40[a]
450 Gy	3.68[b]	5.00[a]	5.75[a]	5.85[a]	6.01[b]
600 Gy	1.83[b]	2.06[b]	2.90[b]	4.20[b]	6.05[b]
750 Gy	1.75[b]	-	-	-	-
900 Gy	0.72[b]	-	-	-	-

Means labelled with the same letter are not significantly different at p< 0.05.

Table 4: Germination % and LOC % of *C. gigantea* with different dosages of gamma rays

Treatments	Germination % (After 11 days)	Lethality Over Control (LOC)%
0 Gy	96.25	0
150 Gy	91.25	5.44
300 Gy	82.5	14.51
450 Gy	73.75	23.58
600 Gy	58.75	39.12
750 Gy	43.75	54.66
900 Gy	32.5	66.32

somatic cell division and/or different genomic damages.

Number of leaves

Number of leaves of *C. gigantea* as affected by different doses of gamma radiation is given in Table 3. Every treatment showed a significant difference on mean number of leaves when compared to the control (P<0.05). The results revealed that there is a significant reduction of number of leaves always above 450 Gy dosages in every week. When the gamma irradiation dosage is higher, it was significantly affected for initiation of number of leaves per plant and it drastically reduced. According to Yadav (2016), larger doses of gamma radiation reduced the number of leaves in *Canscora decurrens* Dalz. According to Asare *et al.* (2017), the average number of leaves produced by okra reduced dramatically as gamma irradiation dosages were raised. Plants exposed to 400 Gy had the largest average number of leaves, whereas plants exposed to 1000 Gy had the lowest. The production of growth regulators and kinetin may have been blocked in this study, which may have resulted in an increase in the number of leaves.

Lethality Over Control (LOC%)

Mortality of the seedlings becomes evident with increased dosage of gamma treatment in *C. gigantea*. The plant survival to which the controls were adjusted 100 percent as LOC (Table 4). Seedlings from irradiated seeds have not been kept their survival capacity compared to the control. Germinability, plant development, and ultimately survival are all dependent on increased chromosomal damage when the radiation dose is increased. The results of previous work are aligned with our findings where *Centella asiatica* plantlets survival kept decreasing with the increased dose for three weeks after the irradiation (Moghaddam *et al.,* 2011).

CONCLUSION

Calotropis gigantea seeds exposed to greater doses of gamma rays (^{60}CO) revealed a declining trend of seed germination, survival rate, plant height, and leaf number as the irradiation dose was increased. Plants developed from seeds exposed to a dosage of more than 750 Gy were died. Further, the plants showed better results with the optimum survival rate below 395 Gy of gamma irradiation dose. Hence it could be concluded that the gamma irradiation below 395 Gy can be suggested to induce mutations in *Calotropis gigantea* with a minimum mortality rate.

ACKNOWLEDGEMENT

This study was supported by the World Bank-funded Accelerating Higher Education Expansion and Development (AHEAD) project, which is operated by the Ministry of Higher Education in Sri Lanka.

REFERENCES :

Abeysinghe, P.D. 2018. Review article on *Calotropis* (Wara): Is it a miracle shrub or just a plant? *Journal of the University of Ruhuna*, **6**(2):55-60.

Asare, A.T., Mensah, F., Acheampong, S., Asare-Bediako, E. and Armah, J. 2017. Effects of gamma irradiation on agromorphological characteristics of okra (*Abelmoschus esculentus* L. Moench.). *Advances in Agriculture*, pp. 1-7. 10.1155/2017/2385106

Aynehband, A. and Afsharinafar, K. 2012. Effect of gamma irradiation on germination characters of amaranth seeds. *European J. Exp. Biol*, **2**(4): 995-999.

Ganeshan, P., Nagaraja Ganesh, B., Ramshankar, P. and Raja, K. 2018. *Calotropis gigantea* fibers: A potential reinforcement for polymer matrices. *International Journal of Polymer Analysis and Characterization*, **23**(3):271-277.

Gordon, S.A. 1954. Occurrence, formation and inactivation of auxins. *Annual Review of Plant Physiology,* **5**:341-378.

Gunckel, J.E. and Sparrow, A.H. 1961. Ionising radiations: biochemical, physiological and morphological aspects of their effects on plants. *Encyclopedia of Plant Physiol*ogy, Ruhland, W. (ed.). Berlin, Springer-Verlag, **16**: 555-617.

Hameed, A., Shah, T.M., Atta, B.M., Haq, M.A. and Sayed, H.I.N.A. 2008. Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in desi and kabuli chickpea. *Pakistan Journal of Botany*, **40**(3): 1033-1041.

Kitagawa, I., Zhang, R., Park, J.D., Baek, N.I., Takeda, Y. and Yoshikawa, M. 1992. Indonesian medicinal plants. I. Chemical structures of calotroposides A and B, two new oxypregnane-oligoglycosides from the root of *Calotropis gigantea* (Asclepiadaceae). *Chem Pharm Bull*., **40**:2007"13.

Minisi, F.A., El-mahrouk ME, El-Din M Rida and Nasr FN. 2013. Effects of gamma radiation on germination, growth characteristics and morphological variations of *Moluccella laevis* L. *American-Eurasian J Agric & Environ Sci*., **13**(5):696-704.

Moghaddam, S.S., Jaafar, H., Ibrahim, R., A. Rahmat, A., Aziz, M.A., and Philip, E., 2011. Effects of acute gamma irradiation on physiological traits and flavonoid accumulation of *Centella asiatica Molecules*, **16**(6): 4994-5007.

Navabi, Y., Norouzi, M., Arab, M. and Daylami, S.D. 2016. Mutagenesis via exposure to gamma-rays in tuberose (*Polianthes tuberosa*). *Electronic Journal of Biology*, **12**(2):168-172.

Rajakaruna, N., Harris, C.S. and Towers, G.H.N. 2002. Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. *Pharmacol Bio*., **40**:235"44.

Rifnas, L. M., Vidanapathirana N. P., Silva T. D., Dahanayake, N., Subasinghe, S. and Madushani, W. G. C. 2019. Effects of different doses of gamma radiation on germination of sunflower (*Helianthus annuus*) seeds. Book of abstracts of the National Symposium on Floriculture Research. Department of National Botanic Gardens, Sri Lanka, 29th November 2019. Pp. 17.

Rifnas, L.M., Vidanapathirana, N.P., Silva, T.D., Dahanayake, N., Weerasinghe, S.S. and Subasinghe, S. 2020. Effect of gamma radiation on survival rate of *Allamanda cathartica*-an indigenous medicinal plant. International *Journal of Minor Fruits, Medicinal and Aromatic Plants*, **6**(1): 50-53.

Sawangmee, W., Taychasinpitak, T., Jompuk, P. and Kikuchi, S. 2011. Effects of gamma-ray irradiation in plant morphology of interspecific hybrids between *Torenia fournieri* and *Torenia baillonii. Kasetsart J. Nat. Sci*, **45:**803-810.

Sharma, A.K., Kharb, R. and Kaur, R. 2011. Pharmacognostical aspects of *Calotropis procera* (Ait.) R. Br. *International Journal of Pharma and Bio Sciences*, **2**(3): 480-487.

Urs, A.P., Manjuprasanna, V.N., Rudresha, G.V., Yariswamy, M. and Vishwanath, B.S. 2017. Plant Latex Proteases: Natural Wound Healers. In: *Proteases in Physiology and Pathology* (pp. 297-323). Springer, Singapore.

Wong, S.K., Lim, Y.Y., Abdullah, N.R. and Nordin, F.J. 2011. Assessment of antiproliferative and antiplasmodial activities of five selected Apocynaceae species. *BMC complementary and alternative medicine*, **11**: 3

Yadav, V. 2016. Effect of gamma radiation on various growth parameters and biomass of *Canscorad ecurrens Dalz. Int. J. Herb. Med*, **4**(5): 109-115.