



## Studies on effect of induced physical mutagenesis on seed germination, seedling survival and lethal dosage of little millet (*Panicum sumatrense* Roth ex Roemer & Schults)

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### Abstract

In the present study was the induced on physical mutagenesis of gamma rays the seed germination, (LD<sub>50</sub>) value and morphological character of little millet. (*Panicum sumatrense* Roth ex Roemer & Schults) The crop has low genetic variability for different essential traits and improvement of this crop through conventional breeding. Little millet production and productivity is very low. It is a Red list of books. The seeds were irradiated with different dose of gamma rays (5KR to 50KR). The normal seeds were taken as control. The physical mutagen gamma rays treatment in seed germination and seedling survival percentage were calculated. In morphological parameter was analysed in above 100days. The germination percentage was calculated on the 15<sup>th</sup> day and seedling survival proportion of 30<sup>th</sup> day. The 50% Reduction of seed germination was observed as LD<sub>50</sub> value (Lethal dose) the Maximum 50per cent reduction of seed germination in 20KR of gamma rays treatment. The seeding survival also increased with decreased below 50 percent observed in 20KR gamma rays treatment. The Little Millet (*Panicum sumatrense*) morphological parameters days to first flower, plant height (cm) Numbers of branches present, Numbers of berries per plant, seed yield per plant (gm) and fresh weight per plant were analysed in all the different dose of gamma rays treatment.

**Keywords:** 20 KR, gamma rays, germination, LD<sub>50</sub>, little millet, morphology, mutation

### Introduction

Little Millet (*Panicum sumatrense*. Roth ex Roemer & Schults) is belonging to the family (Poaceae) English Name: Barefoot panicgrass, Tamil Name: Samai) is a herb. Millets are suitable crops for cultivation in arid and semiarid regions. Indian Little millet is having a short crop cycle. It is listed in the Red Data book. Mutation breeding is one of conventional breeding methods in various fields like morphology and morphological characters (Gustafsson 1947) <sup>[1]</sup>. It is relevant with various fields like morphology, Cytogenetics, Biotechnology, molecular biology, etc. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement (Acharya *et al.*, 2007) <sup>[2]</sup>. Studies indicate a wide variability in the requirement of these components for the best growth and development of seedlings (Zamith and Scarano, 2004) <sup>[3]</sup>. Identification of the wild ancestors of crop is essential for understanding their evolutionary history during and after domestication changes. (Hammer. 1984; Vaighan *et al.*, 2007) <sup>[6]</sup>. Some seed treatments enhance seed germination, seed lot emergence uniformity, seeding stand and the ability of the seedlings to survive unfavourable field conditions. (Olsen *et al.* 2011) <sup>[8]</sup>.

Hugo de Vries advanced the concept of utilizing induced mutations in breeding new forms for the first time (1901). Various experimental and philosophical works in the field added exhaustive information that has been generated on the role and application of induced mutations in several crop plants and were taken up by many breeders all around the world (Goyal and Khan, 2010; Nakagawa *et al.*, 2011) <sup>[9]</sup>. In the beginning, mutation breeding was based primarily upon x-rays, gamma rays, thermal neutrons and radioisotopes of certain heavy elements (Donini and Sonnino, 1998) <sup>[11]</sup>. In

addition to several ionizing radiations a number of chemical mutagens induce mutations in plants, when applied singly or combined with other chemicals and successively or simultaneously with physical mutagens (Encheva, *et al* 2009) <sup>[12]</sup>. Many workers feel that a dose close to lethal dose 50(LD<sub>50</sub>) is optimal. It is that dose of the Mutagen that would kill 50% of the treated individuals. (Solanki and waldia, *et al* 1997) <sup>[13]</sup> are of the opinion that on optimum dose is the one that produces maximum frequency of mutations and causes minimum killings.

### Material and Methods

Little millet (*Panicum sumatrense* Roth ex Roemer & Schults) variety of Samai CO-4 Seeds were Collected from TNAU (Tamil Nadu Agricultural University) Coimbatore. 5gm of well matured seeds were taken from Zip Cover For irradiation. The mutagen of gamma Irradiation was given at Indian Gandhi Centre for Atomic Research (IGCAR) Kalpakkam, a dose rate of 234KR/h. Ten sets of 5gm seed were taken as irradiated with different dosage of gamma rays, (5KR to 50KR) were given inside the gamma chamber in source of cobalt 60. The gamma ray irradiated seeds were sown in the pot culture in the botanical garden. Department of Botany. Faculty of Science, Annamalai University Annamalai Nagar, The assessment of seed germination percentage and seedling survival percentage of treated and control seed of *Panicum sumatrense* were recorded in laboratory at room temperature (24±2) Growth of the radical following bursting of the seed coat was taken as an index for germination in petriplates. The extent of injury was assessed by determining the relative reduction in growth in the treated samples under controlled condition as compared to control. A gradual reduction of seed germination and

seedling survival in *Panicum* (Little Millet Seeds under similar condition in moist filter paper kept on petri plates, because the gamma irradiation affects the seed germination and seedling survival as the consequence of mutagenic effect on growth phenomenon. Lethality was determined from reduction in germination with respect to control. Seed germination was recorded at 15<sup>th</sup> day after sowing seedling survival percentage was determined from 30<sup>th</sup> day after sowing.

**Results and Discussion**

The reduction in germination and survival may be due to absorption of ionizing radiation in biological materials, acting directly on critical targets in the cell. (kovacs and keresztes, 2002) [14]. The decrease in seed germination induced by mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (khan and Goyal 2009) [19]. That irradiation with lower doses of gamma rays significantly improved vegetative traits while higher doses proved depressing for same parameters. (Shah *et al.*, 2008) [16]. In wheat gamma radiations improved germination plant height, seed per plant and seed yield per plant (Koen *et al.*, (2007) [17]. Exposure to gamma radiations is known to produce morphological mutants, physiological mutants and biochemical mutants (Songsri *et al.*, 2011) [18]. LD<sub>50</sub> value for germination ranged between 5KR, 10KR, 15KR, 20KR, 25KR, 30KR, 35KR, 40KR, 45KR and 50 KR and also Cowpea plant germination. The LD 50 value in cowpea plant was in 25KR of gamma rays.

The seed germination, seedling survival days to first flower, plant height, numbers of branches per plant, number of leaves per plant, number of berries per plant, seed yield per plant(gm) and root yield per plant fresh weight (gm) were gradually decreased with increase in various dosage of gamma rays. Similar results in morphological parameters were analysed in cowpea (Gnanamurthy *et al* 2013) [19]. Ashwagandha seed reduction in seed germination percentage and gradually increase in the production of active radicals responsible for seedling survival (Lethality) and increasing dosage gamma rays was immediately damage the physiological activities of Seeds. (Bharathi. T *et al.* 2013) [20]. Mutagenic treatments revealed a gradual decreasing trend in germination from lower to higher doses (Sunil *et al.*, 2011) [21]. The reduction in plant survival is a scribed to damage cause at cytological and physiological levels (Srivastava *et al.*, 2011) [22]. Suggested that the reduction in seedling survival may be to the hindrance caused by the mutagen on different metabolic pathways of the cells. In an another report (Sarada *et al.*, 2015) [23]. Increasing frequency of chromosomal harm with increasing radiation dose may be responsible for reduction in plant survival (Talebi *et al.*, 2012) [24]. Reduction in seed germination may be due to the effect of mutagen on meristamatic tissues of radical plumule (Deepika *et al.*, 2016) [25].

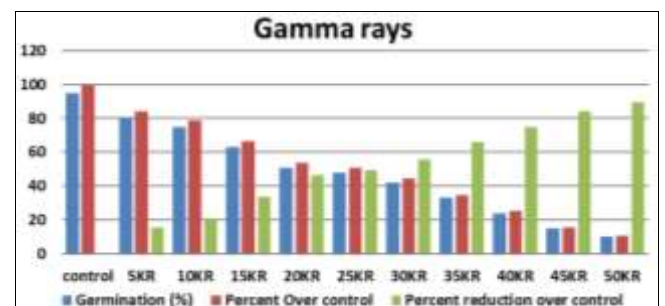
The seed germination percentage and per cent survival decreased with an increase in dose /concentration of the

mutagens, they calculated that lower treatment of mutagens have influenced less biological damage and would be suitable for inducing desirable mutations. The present findings are in agreement with above mentioned reports. Similar finding were also reported wherein. Higher doses of gamma radiation reduced germination percentage and survival in fennel (Varma *et al.*, 2017).

Plants are living organisms and they respond to growth challenges in a systematic manner. Growth challenges may be indigenous or exogenous in nature. They are supposed to show better growth and vigor if they possess genetic traits capable of coping with environmental stresses and other growth challenges. Several mechanisms govern the growth and developmental phases of plants and their adaptation to imposed challenges which depend on proper mobilization of growth hormones, regulation of cell cycle, activation of concerned enzymes, appropriate functioning of metabolic machinery and timely expression of genes which regulate growth phenomena (Gray, 2004; Santiago *et al.*, 2013; Salehin *et al.*, 2015) [27, 28, 29]. Under the work it was found that the little millet was treated with in 5KR, 10KR, 15KR, 20KR, 25KR, 30KR, 35KR, 40KR, 45KR and 50KR in doses of Gamma rays. The percentage of Seed germination was increasing in the LD<sub>50</sub> value of 20KR (51.00). After that step by step decreased in the seed germination level, the germination % was Maximum 5KR (80.00) and minimum 50KR (10.00) of the Gamma rays table (1). Thereafter it was Seedling survival % and mean value of Shoot length (cm), Root length(cm), Leaf length(cm) and Leaf Breath(mm) Table (2).

**Table 1:** LD<sub>50</sub> value of gamma rays.

| S.no | Dose/Gamma rays | Germination (%) | Percent Over control | Percent reduction over control |
|------|-----------------|-----------------|----------------------|--------------------------------|
| 1.   | Control         | 95              | 100                  | 0                              |
| 2.   | 5KR             | 80              | 84.21                | 15.78                          |
| 3.   | 10KR            | 75              | 78.94                | 21.05                          |
| 4.   | 15KR            | 63              | 66.31                | 33.68                          |
| 5.   | 20KR            | 51              | 53.68                | 46.31                          |
| 6.   | 25KR            | 48              | 50.92                | 49.47                          |
| 7.   | 30KR            | 42              | 44.21                | 55.78                          |
| 8.   | 35KR            | 33              | 34.73                | 65.78                          |
| 9.   | 40KR            | 24              | 25.26                | 74.73                          |
| 10.  | 45KR            | 15              | 15.78                | 84.21                          |
| 11.  | 50KR            | 10              | 10.52                | 89.47                          |



**Graph 1:** Effect of gamma rays on Germination %, Percent over control, Percent reduction over control.

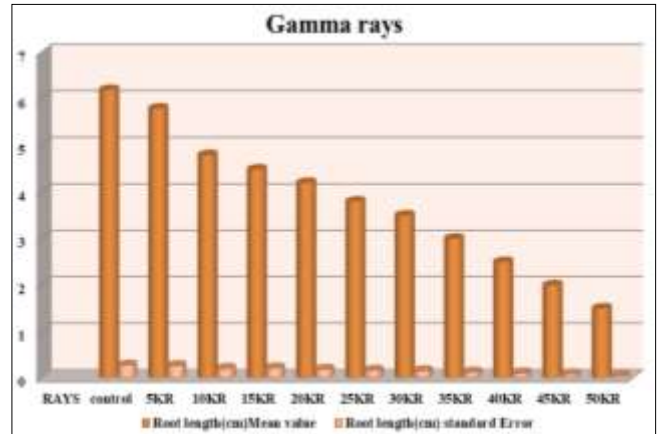
**Table 2:** Effect of gamma rays on Mean value of Shoot length, Root length, Leaf length, Leaf Breath

| S. No | Gamma rays | Seed germination% | Seedling survival% | Mean value of Shoot length(cm) | Mean value of Root length(cm) | Mean value of Leaf length(cm) | Mean value of Leaf Breath(mm) |
|-------|------------|-------------------|--------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 1.    | control    | 95.00             | 90.77              | 6.48±0.31                      | 6.20±0.28                     | 7.50±0.35                     | 4.50±0.20                     |
| 2.    | 5KR        | 80.00             | 75.18              | 5.50±0.25                      | 5.80±0.27                     | 6.00±0.28                     | 4.00±0.18                     |

|     |      |       |       |           |           |           |           |
|-----|------|-------|-------|-----------|-----------|-----------|-----------|
| 3.  | 10KR | 75.00 | 72.58 | 5.00±0.24 | 4.80±0.21 | 5.00±0.23 | 3.80±0.17 |
| 4.  | 15KR | 63.00 | 60.58 | 4.50±0.21 | 4.49±0.22 | 4.00±0.18 | 3.60±0.17 |
| 5.  | 20KR | 51.00 | 48.05 | 4.00±0.18 | 4.20±0.19 | 3.80±0.17 | 3.47±0.22 |
| 6.  | 25KR | 48.00 | 45.03 | 3.30±0.09 | 3.80±0.17 | 3.50±0.16 | 3.20±0.15 |
| 7.  | 30KR | 42.00 | 40.12 | 3.00±0.15 | 3.50±0.16 | 3.30±0.15 | 3.10±0.14 |
| 8.  | 35KR | 33.00 | 31.03 | 2.70±0.14 | 3.00±0.13 | 3.20±0.15 | 3.00±0.13 |
| 9.  | 40KR | 24.00 | 21.72 | 2.50±0.12 | 2.50±0.11 | 2.55±0.12 | 2.90±0.13 |
| 10. | 45KR | 15.00 | 13.66 | 2.20±0.10 | 2.00±0.09 | 2.44±0.18 | 2.70±0.12 |
| 11. | 50KR | 10.00 | 7.33  | 1.78±0.07 | 1.50±0.06 | 1.73±0.19 | 2.20±0.10 |

**Table 3:** Effect of Gamma rays on Shoot length mean value and Standard error

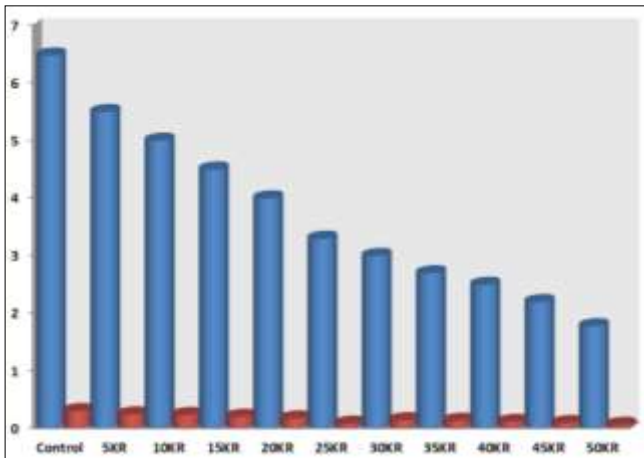
| S. no | Dose/Gamma rays | Shoot length (cm) Mean | Shoot length (cm) Standard Error |
|-------|-----------------|------------------------|----------------------------------|
| 1.    | Control         | 6.48                   | 0.31                             |
| 2.    | 5KR             | 5.50                   | 0.25                             |
| 3.    | 10KR            | 5.00                   | 0.24                             |
| 4.    | 15KR            | 4.50                   | 0.21                             |
| 5.    | 20KR            | 4.00                   | 0.18                             |
| 6.    | 25KR            | 3.30                   | 0.09                             |
| 7.    | 30KR            | 3.00                   | 0.15                             |
| 8.    | 35KR            | 2.70                   | 0.14                             |
| 9.    | 40KR            | 2.50                   | 0.12                             |
| 10.   | 45KR            | 2.20                   | 0.10                             |
| 11.   | 50KR            | 1.78                   | 0.07                             |



**Graph 3:** Effect of Gamma rays on Root length mean value and Standard error

**Table 5:** Effect of Gamma rays on Leaf length mean value and Standard Error

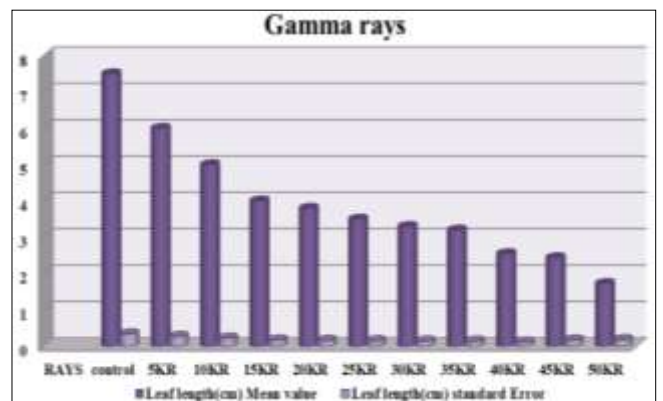
| S.NO | GAMMA RAYS | Leaf length(cm) Mean value | Leaf length (cm) standard Error |
|------|------------|----------------------------|---------------------------------|
| 1.   | control    | 7.50                       | 0.35                            |
| 2.   | 5KR        | 6.00                       | 0.28                            |
| 3.   | 10KR       | 5.00                       | 0.23                            |
| 4.   | 15KR       | 4.00                       | 0.18                            |
| 5.   | 20KR       | 3.80                       | 0.17                            |
| 6.   | 25KR       | 3.50                       | 0.16                            |
| 7.   | 30KR       | 3.30                       | 0.15                            |
| 8.   | 35KR       | 3.20                       | 0.15                            |
| 9.   | 40KR       | 2.55                       | 0.12                            |
| 10.  | 45KR       | 2.44                       | 0.18                            |
| 11.  | 50KR       | 1.73                       | 0.19                            |



**Graph 2:** Effect of Gamma rays on Shoot length mean value and Standard error

**Table 4:** Effect of Gamma rays on Root length mean value and Standard error

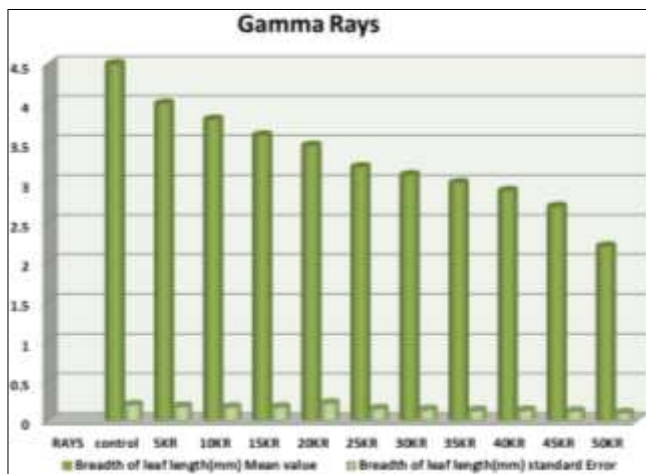
| S.NO | GAMMA RAYS | Root length(cm) Mean value | Root length (cm) standard Error |
|------|------------|----------------------------|---------------------------------|
| 1.   | Control    | 6.20                       | 0.28                            |
| 2.   | 5KR        | 5.80                       | 0.27                            |
| 3.   | 10KR       | 4.80                       | 0.21                            |
| 4.   | 15KR       | 4.49                       | 0.22                            |
| 5.   | 20KR       | 4.20                       | 0.19                            |
| 6.   | 25KR       | 3.80                       | 0.17                            |
| 7.   | 30KR       | 3.50                       | 0.16                            |
| 8.   | 35KR       | 3.00                       | 0.13                            |
| 9.   | 40KR       | 2.50                       | 0.11                            |
| 10.  | 45KR       | 2.00                       | 0.09                            |
| 11.  | 50KR       | 1.50                       | 0.06                            |



**Graph 4:** Effect of Gamma rays on Leaf length mean value and Standard Error

**Table 6:** Effect of Gamma rays on Breadth of leaf length mean value and Standard error

| S.NO | GAMMA RAYS | Breadth of leaf length (mm) Mean value | Breadth of leaf length (mm) standard Error |
|------|------------|--|--|
| 1.   | control    | 4.50                                   | 0.20                                       |
| 2.   | 5KR        | 4.00                                   | 0.18                                       |
| 3.   | 10KR       | 3.80                                   | 0.17                                       |
| 4.   | 15KR       | 3.60                                   | 0.17                                       |
| 5.   | 20KR       | 3.47                                   | 0.22                                       |
| 6.   | 25KR       | 3.20                                   | 0.15                                       |
| 7.   | 30KR       | 3.10                                   | 0.14                                       |
| 8.   | 35KR       | 3.00                                   | 0.13                                       |
| 9.   | 40KR       | 2.90                                   | 0.13                                       |
| 10.  | 45KR       | 2.70                                   | 0.12                                       |
| 11.  | 50KR       | 2.20                                   | 0.10                                       |

**Graph 5:** Effect of Gamma rays on Breadth of leaf length mean value and Standard error

### Conclusion

The present investigation, different dose of gamma irradiation induced mutation in  $R_1$  generation inhibit seedling growth and other growth parameters in field condition. The inhibitory effects were due to physiological disturbances on Little Millet by gamma irradiation especially with imbalance of growth hormones. From the results LD<sub>50</sub> value was fixed at 20KR gamma rays.

### Reference

- Gustafsson A, Hagberg A, Lundqvist U, Persson G, A proposed system of symbols for the collection of barley mutants at Svalöv, *Hereditas*. 62(3):409-414.
- Acharya SN, Thomas JE, Basu SK. Improvement in the medicinal and nutritional properties of fenugreek (*Trigonella enum-graecum* L.). In: Acharya SN, Thomas JE (eds) *Advances in medicinal plant research*, Research Signpost, Trivandrum, Kerala, India, 2007.
- Zamith LR, Scarano FR, Produção de mudas de espécies das restingas do município do Rio de Janeiro, RJ, Brasil. *Acta Botanica Brasilica*. 2004; 18:161-176.
- Clayton WD, Renvoize SA. *Genera Graminum*. Kew Bulletin Adicional Series XIII, London, 1986, 389p
- Sandra S. Aliscioni liliana m. Giussani, fernando o. Zuloaga, and elizabeth a. Kellogg, a molecular phylogeny of panicum (poaceae: paniceae): tests of monophyly and phylogenetic placement within the panicoideae, *American Journal of Botany*. 2003; 90(5):796-821.

- Hammer K. Das Domestikationssyndrom, *Kulturpflanze*, 1984, 32:11-34.
- Vaughan DA, Balázs E, Heslop-Harrison JS. From Crop Domestication to Super-domestication, *Annals of Botany*. 2007; 100(5):893-901.
- Olsen M, Nischwitz C, Norton R. Effects of seed Treatment Fungicides and Seed Vigor on Cotton Seedling Stand and Yield in Southeastern Arizona in 2009 and 2010. *Arizona Cotton Report (P-161)* August, 2011.
- Goyal S, Khan S. Induced mutagenesis in urdbean (*Vigna mungo* L. Hepper): A review *International Journal of Botany*. 2010a; 6(3):194-206.
- Nakagawa Y, Fujiwara-Fukuta S, Yorimitsu T, Tanaka S, Minami R, Shimooka L, *et al.* Spatial and temporal requirement of Defective proventriculus activity during *Drosophila* midgut development. *Mech. Dev*. 2011; 128(5-6):258-267.
- Donini P, Sonnino A. Induced Mutation in Plant Breeding: Current Status and Future Outlook. In: Jain S.M., Brar D.S., Ahloowalia B.S. (eds) *Somaclonal Variation and Induced Mutations in Crop Improvement*. Current Plant Science and Biotechnology in Agriculture, vol 32. Springer, Dordrecht, 1998.
- Encheva J. Creating sunflower mutant lines (*Helianthus annuus* L.) using induced mutagenesis. *Bulg. J Agric. Sci*. 2009; 15:109-118.
- Solanki IS, Waldia RS. Mutagenesis techniques, pp. 271. In: R.L. Kapoor and M.L. Saini, (Eds.), *Plant Breeding and Crop Improvement-II*. CBS Publishers and Distributors, New Delhi, India, 1997.
- Kovacs E, Keresztes A. Effect of gamma and UV-B/C radiation on plant cells. *Micron*. 2002; 33(2):199-210.
- Khan S, Goyal S. Improvement of mungbean varieties through induced mutations. *Afr. J Plant Sci*. 2009; 3:174-180.
- Shah TM, Mirza JI, Haq MA, Atta BM. Induced genetic variability in chickpea (*Cicer arietinum*(L.) II. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. *Pak. J Bot*. 2008; 40(2):605-613.
- Koen EOH, Ahmed SS, Majid NMA. Gamma radio sensitively study on long bean (*Vignasesquipedalis*). *Amer. J App. Sci*. 2007; 4:1090-1093
- Songsri P, Suriharn B, Sanitchon J, Srisawangwong S, Kesmla T. Effects of Gamma Radiation on Germination and Growth Characteristics of Physic Nut (*Jatropha curcas* L.), *Journal of Biological Sciences*. 2011; 11(3):268-274.
- Gnanamurthy S, Dhanavel D, Girija M. Effect of gamma radiation on the morphological characters of cowpea [*Vigna unguiculata* (L.) Walp.]. *International Journal of Current Trends in Research*. 2013; 2(1):38-43.
- Bharathi T, Gnanamurthy S, Dhanavel D, Murugan S, Ariraman M. Induced Physical mutagenesis on seed germination, lethal dosage and morphological mutants of Ashwagandha (*Withania somnifera* (L.) Dunal). *International Journal of Advanced Research*. 2013; 1(5):136-141.
- Sunil M Sangle, Swapnil E Mahamune, Sopon N Kharat, Kothekar VS. Effect of mutagenesis on

- germination and pollen sterility in pigeonpea. *Bio science discovery*, 2011; 102(1).
22. Srivastava P, Marker S, Pandey P, Tiwari DK. Mutagenic effects of sodium azide on the growth and yield characteristics in wheat (*Triticumaestivum* L. em. Thell.). *Asian Journal of Plant Science*. 2011; 10:190-201.
  23. Sarada C, Jyothi KUV, Rao S, Reddy PV. Mutagenic sensitivity of gamma rays, EMS and their combinations on germination and seedling vigour in coriander (*Coriandrum sativum* L). *International Journal of Advances in Pharmacy, Biology and Chemistry*. 2015; 4(2):430-438.
  24. Talebi AB, Talebi AB, Shahrokhifar B. Ethyl Methane Sulphonate (EMS) Induced Mutagenesis in Malaysian Rice (cv. MR219) for Lethal Dose Determination. *American Journal of Plant Sciences*. 2012; 3:1661-1665.
  25. Deepika Minakshi pal, Pahuja SK. Morphological variations induced by Ethyl Methane Sulphonate in Cluster bean (*Cyamopsistetragonoloba* (L.) Taub.). *Forage Res*. 2016; 41:218-221.
  26. Verma AK, Sharma S, Kakani RK, Meena RD, Choudhary S. Gamma radiation effects seedgermination, plant growth and yield attributing characters of fennel (*Foeniculum vulgare* Mill.). *International Journal of Current Microbiology and Applied*. 2017; 6:2448-2458.
  27. Gray WM. Hormonal regulation of plant growth and development. *PLoS Biol*. 2004; 2(9):e311.
  28. Santiago J, Henzler C, Hothorn M. Molecular mechanism for plant steroid receptor activation by somatic embryogenesis co-receptor kinases. *Sci*. 2013; 341(6148):889-892.
  29. Salehin M, Bagchi R, Estelle M. SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. *The Plant Cell*. 2015; 27(1):9-19.