The Pharma Innovation Journal 2022; 11(3): 1117-1121

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(3): 1117-1121

www.thepharmajournal.com
Received: 18-01-2022
Accepted: 24-02-2022

Jyothsna J
Ph.D., Research Scholar, Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, Madhya Pradesh, India

Dr. Reena Nair
Assistant Professor, Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, Madhya Pradesh, India

Dr. SK Pandey
Professor and Head, Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, Madhya Pradesh, India

Dr. AK Mehta
Principal Scientist and Head, Department of Plant Breeding and Genetics, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, Madhya Pradesh, India

Corresponding Author:
Jyothsna J
Ph.D., Research Scholar, Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, Madhya Pradesh, India

Assessment of biological response and semi-lethal dose of ems for fenugreek CV. RMT-1

Jyothsna J, Dr. Reena Nair, Dr. SK Pandey and Dr. AK Mehta

Abstract
The fenugreek, being an in breeder is short of genetic variability due to which the scope for improvement is also narrowed down. The induced mutagenesis is one of the effective ways to generate genetic variability in the crop. The alkylating agent, Ethylene Methyl Sulphonate (EMS) is the most common chemical mutagen used in mutagenesis. Since the overdose of mutagen would kill the seedlings while the under-dose would be insignificant, the assessment of semi-lethal dose (LD_{50}) is highly essential to initiate a mutation breeding work on any crop. Since there is no information on the semi-lethal dose of EMS in fenugreek, the experiment was set up to assess the same. Through this investigation, it was found that the semi-lethal dose of EMS for fenugreek cv. RMt-1 be EMS 0.30 per cent at eight hours. A steady decrease in the germination, growth and vigor of the seedlings was observed as the dose of the mutagen increased. The information generated through this investigation would facilitate further mutagenesis-related research in fenugreek.

Keywords: Induced mutation, EMS, Fenugreek, biological response, LD_{50}, GR_{50}

Introduction
The breeding perspective towards the improvement of any crop relies on the genetic variability available within the species. However, the genetic polymorphism in most of the crops is deflated by natural selection and slow-paced conventional breeding methods. The mutations are the intrinsic origin of genetic variations within a crop species (Kharkwal, 2012) [12]. The consequential variation offers the primal matter for natural selection and evolution. Spontaneous mutations are seldom and random in nature, which disable their usage in the crop improvement plan (Lonnig, 2005) [13]
The modern plant breeding industry is centered on mutation breeding, recombinant DNA and transgene technologies. Mutagenesis involves sudden heritable alterations in the genetic information of an organism that is derived from biological, chemical, or physical agents, but not by genetic segregation (Roychowdhury and Tah, 2013) [19]. Mutation breeding fends for new cultivars by creating mutations that potentially desirable for all desirable traits (Lonnig, 2005) [13]. The induced mutagenesis can remediate the sparse genetic variability from the scares spontaneous mutations in nature and thus aid to break and expand the yield plateau.
Chemical mutagens tend to induce physiological damage, macro- and micro-mutations in the gene, and chromosomal aberrations in M_1 generation. Various chemical mutagens are being employed to generate the variability. Among the chemical mutagens, the alkylating agent EMS is the widely employed mutagen favored for its high-frequency nucleotide substitutions (point mutations) (Yadav *et al.*, 2016) [25] and it also and generates desirable gene mutations and chromosomal aberrations at higher and lower frequencies, respectively (Bashir *et al*., 2013).
Fenugreek (*Trigonella foenum-graecum* L.) is a multipurpose leafy vegetable packed with various nutraceutical phytochemicals and an important traditional ingredient of the Indian subcontinent and South Asian cuisines. Its emergence into the Americas and Europe as a new spice (Zandi *et al*., 2017) [26] demand its improvement for serving better purposes. However, owing to its inbreeding nature and traditional selection-based breeding methods applied in the previous decades for variety development have concentrated the available genetic diversity. So, the breeding methods are restricted to extract the desirable traits from the available narrow range of germplasms. Moreover, despite the impressive productivity across the country, further improvement of productivity and quality is a forceful target. Mutation breeding could serve as a better way to ascend the genetic variability within the cultivated gene pool. Many successful mutations with desirableness in plant architecture, yield and quality traits have been produced and utilized the crop improvement (Acharya *et al.*, 2006; Basu *et al.*, 2008;

Giridhar *et al.*, 2016; Rajoria *et al.*, 2016) [1, 3, 7, 17]

The key step in mutation breeding is the determination of the semilethal dose (LD_{50}) of the mutagen, besides the dose at which maximum frequency of point mutations with minimum damage to the treated material. Semi-lethal dose or LD_{50} is the dose wherein only 50 per cent of the seed germination is observed or the dose that is required to kill the 50 per cent of the plant population under test (Sikder *et al.*, 2013) [20]. There are no reports on LD_{50} assessment in fenugreek and thus the experiment was set up to determine the semi-lethal dose of EMS in fenugreek cv. RMt-1.

Materials and methods

The experiment to ascertain the semi-lethal dose of EMS was carried out in the Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, Madhya Pradesh. Seeds of one of the common cultivars in Madhya Pradesh, cv. RMt-1 were subjected to mutagenic treatments. The mutagenic treatment comprised various concentrations of EMS with different treatment duration. The treatment details are furnished in Table 1. The experiment was laid on a completely randomized design.

The semi-lethal dose is considered to be the most suitable dose for high-frequency mutations (Anbarasan *et al*., 2013) [2]. LD_{50} is the dose wherein only 50 per cent of the seed germination is observed or the dose that is required to kill the 50 per cent of the plant population under test (Sikder *et al.*, 2013) [20]. The seed germination is used to calculate the LD_{50}. Besides the data on seed germination, the survival of seedlings (as percentage) adds up to the reliability of the determination of LD_{50} (Tiwari *et al*., 2020). Thus, the germination (per cent) and survival (per cent) would act as better predictors of the mutagen dosage at which maximum point mutations are likely to occur.

The germination percentage, survival as percentage of control at 20 days after sowing (DAS) (as $GR_{50(20)}$) and 30 DAS are derived through the below formulae (Tiwari *et al.*, 2020).

$$\text{Germination percentage} = \frac{\text{No. of seeds germination at 30 DAS}}{\text{No. of seeds sown}} \times 100$$

$$\text{Survival percentage} = \frac{\text{No. of survived plants at 30 DAS}}{\text{No. of seeds sown}} \times 100$$

$$GR_{50\,(20)} = \frac{\text{No. of seeds germination at 20 DAS}}{\text{No. of germinated plants}} \times 100$$

The shoot length corresponds to the length of the aerial portion of the seedling on 30 DAS and root length to the length of the root portion expressed as centimeter (cm). The seedling height is the sum of the shoot and root length. The vigor index is a measure of the seedling vigor as affected by the various mutagenic treatments. The vigor index is calculated as below (Kandasamy *et al.*, 2020) [10].

Vigor index = Germination (percentage) X seedling height

Results and Discussion

The germination percentage as affected by the mutagenic treatments was found to be significant. The maximum germination of 100 per cent was observed with EMS 0.10 per cent at 2 hours, EMS 0.10 per cent at 4 hours, EMS 0.15 per cent at 2 hours, EMS 0.15 per cent at 4 hours and control, while the least germination was observed with EMS 0.30 per cent at 8 hours (47.62 per cent) (Fig. 1) (Table 2). The germination percentage reduced as the dose of EMS increased. This is in line with the report of Ramandeep *et al.*, (2019) [18] in French bean. The seedlings that emerged with delay showed abnormalities and couldn't recover to normal, which may due to the lethal effects of the EMS at higher doses. A similar trend was reported by Yadav *et al.*, (2016) [25] in rape mustard. The reduction in seed germination by the EMS might be due to the damage in cell constituents and altered enzyme activity of the cell (Khan and Goyal, 2009). Micco *et al.* (2011) [11, 15] associated the reduced germination with anomalies in the mitotic cycles and metabolic system of the plant cells.

The survival percentage on 20 DAS and 30 DAS was found insignificant. However, numerically maximum survival percentage (100 per cent) was found in control, EMS 0.10 per cent at 2 hours, EMS 0.10 per cent at 4 hours, EMS 0.15 per cent at 2 hours and control (Fig. 1) (Table 2). The decrease in survival of the seedlings might be attributed to the mutagenic effect on various cellular metabolic pathways (Srivastava *et al*., 2011) [23], cytological damages and physiological hindrances (Sato and Gaul, 1967) [21].

The shoot length of seedlings across the treatments pronounced significance. Maximum shoot length (18.07 cm) was registered with EMS 0.15 per cent at 2 hours, while minimum shoot length was noted with EMS 0.30 per cent at 8 hours (11.50cm) (Table 2). The effect on various EMS concentrations and treatment duration was found significant for root length, having maximum and minimum root length noted in the EMS 0.10 per cent at 2 hours (5.43 cm) and EMS 0.30 per cent at 8 hours (2.13 cm), respectively. The total seedling height also found significant with maximum seedling height (23.77 cm) in EMS 0.15 per cent at 2 hours and minimum seedling height (13.36 cm) in EMS 0.30 per cent at 8 hours (Table 2). These results are in similarity with that of Mahajan *et al.* (2015) [14]. The major reasons for the descent of seedling height are chromosomal damage and cell division inhibition (Evans and Sparrow, 1961) [6]. The decline in seedling height might be brought in by reduction in endogenous auxin level, alterations in the activity of specific enzymes and physiological damage in the cells of the seedlings (Goud and Nayar, 1968; Cherry *et al.* 1962; Ignacimuthu and Babu, 1988). Blixt (1970) [8, 5, 9, 4] suggested that the gross injury at the molecular level or the acute chromosomal aberrations might have resulted in the reduction in seedling height. The vigor index was found significant across the treatments. The highest vigor index (429.38) was recorded in EMS 0.15 per cent at 2 hours whereas, the lowest vigor index (156.78) was with EMS 0.30 per cent at 8 hours (Table 2) (Fig. 2). Therefore, reduction in germination, survival and seedling growth conditions the sensitivity of fenugreek seedlings and the prevalence of chromosomal aberrations and physiological injuries at higher doses at the higher doses of the EMS.

The germination percentage and survival percentage (on 20 and 30 DAS) were plotted against the different doses of EMS and treatment duration to determine the LD_{50} of EMS for fenugreek in Fig. 1. It is obvious from the graph that the LD_{50} corresponds to EMS 0.30 per cent at 8 hours. The scrutiny on both germination percentage and $GR_{50\ (20)}$ (survival percentage on 20 DAS) indicates EMS 0.30 per cent at 4, 6 and 8 hours as the safer doses for a maximum frequency of gene mutations (Fig.1). Nilan (1981) [16] stated that EMS could induce a higher proportion of chromosomal aberrations along with high frequencies of base-pair substitutions, particularly transition mutations at G_C site and low-level lethality.

Table 1: Mutagenic treatment details

Treatments	EMS concentration (%)	Treatment duration (hours)	Treatments	EMS concentration (%)	Treatment duration (hours)
T_1	0.10	2	T_{12}	0.20	8
T_2	0.10	4	T_{13}	0.25	2
T_3	0.10	6	T_{14}	0.25	4
T_4	0.10	8	T_{15}	0.25	6
T_5	0.15	2	T_{16}	0.25	8
T_6	0.15	4	T_{17}	0.30	2
T_7	0.15	6	T_{18}	0.30	4
T_8	0.15	8	T_{19}	0.30	6
T_9	0.20	2	T_{20}	0.30	8
T_{10}	0.20	4	T_{21}	Control	
T_{11}	0.20	6			

Table 2: Effect of EMS on germination, seedling growth and survival of fenugreek cv. RMt-1

Treatment	Germination (%)	Survival (%)	GR_{50} (%)	Shoot length (cm)	Root length (cm)	Total seedling height (cm)	Vigor index
0.10% + 2 h	100.00	100.00	100.00	17.63	5.43	23.07	406.74
0.10% + 4 h	100.00	100.00	92.86	17.60	4.80	22.40	394.24
0.10% + 6 h	95.24	93.33	93.33	16.47	4.67	21.13	348.00
0.10% + 8 h	90.48	80.00	80.00	16.03	4.33	20.37	326.55
0.15% + 2 h	100.00	100.00	100.00	18.07	5.70	23.77	429.38
0.15% + 4 h	100.00	93.33	73.33	17.70	5.07	22.77	402.97
0.15% + 6 h	95.00	85.71	78.57	16.70	4.30	21.00	350.70
0.15% + 8 h	90.48	86.67	73.33	16.23	4.20	20.43	331.70
0.20% + 2 h	94.74	92.31	76.92	16.93	4.80	21.73	368.02
0.20% + 4 h	94.44	91.67	91.67	16.90	4.67	21.57	364.48
0.20% + 6 h	89.47	92.31	76.92	16.40	4.37	20.77	340.57
0.20% + 8 h	80.95	86.67	66.67	15.87	4.40	20.27	321.56
0.25% + 2 h	85.00	85.71	78.57	15.20	4.23	19.43	295.39
0.25% + 4 h	80.00	78.57	64.29	15.17	4.03	19.20	291.20
0.25% + 6 h	76.19	66.67	60.00	14.90	3.93	18.83	280.62
0.25% + 8 h	66.67	66.67	60.00	14.47	3.50	17.97	259.92
0.30% + 2 h	75.00	64.29	57.14	14.53	3.83	18.37	266.93
0.30% + 4 h	65.00	64.29	50.00	13.50	3.10	16.60	224.10
0.30% + 6 h	57.14	53.33	40.00	12.70	2.77	15.47	196.43
0.30% + 8 h	47.62	46.67	33.33	11.50	2.13	13.63	156.78
Control	100.00	100.00	100.00	15.80	4.87	20.67	326.53
CD (0.05)	2.265	NS	NS	1.261	1.001	1.532	23.858

Bold values indicate the highest mean value: Underlined values indicate the least mean value

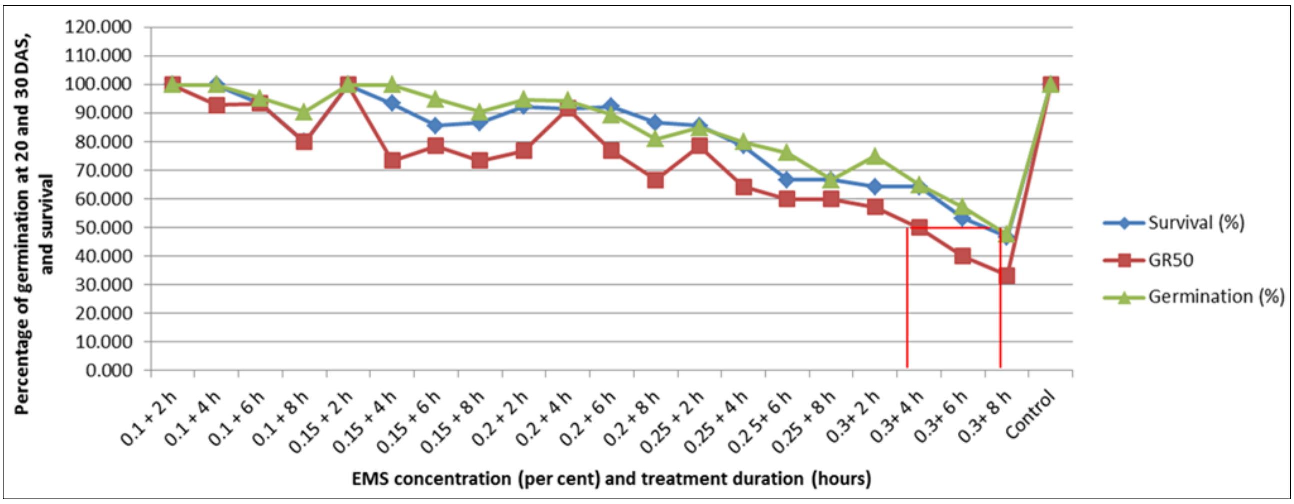

Fig 1: Effect of EMS on germination and survival of Fenugreek cv. RMt-1

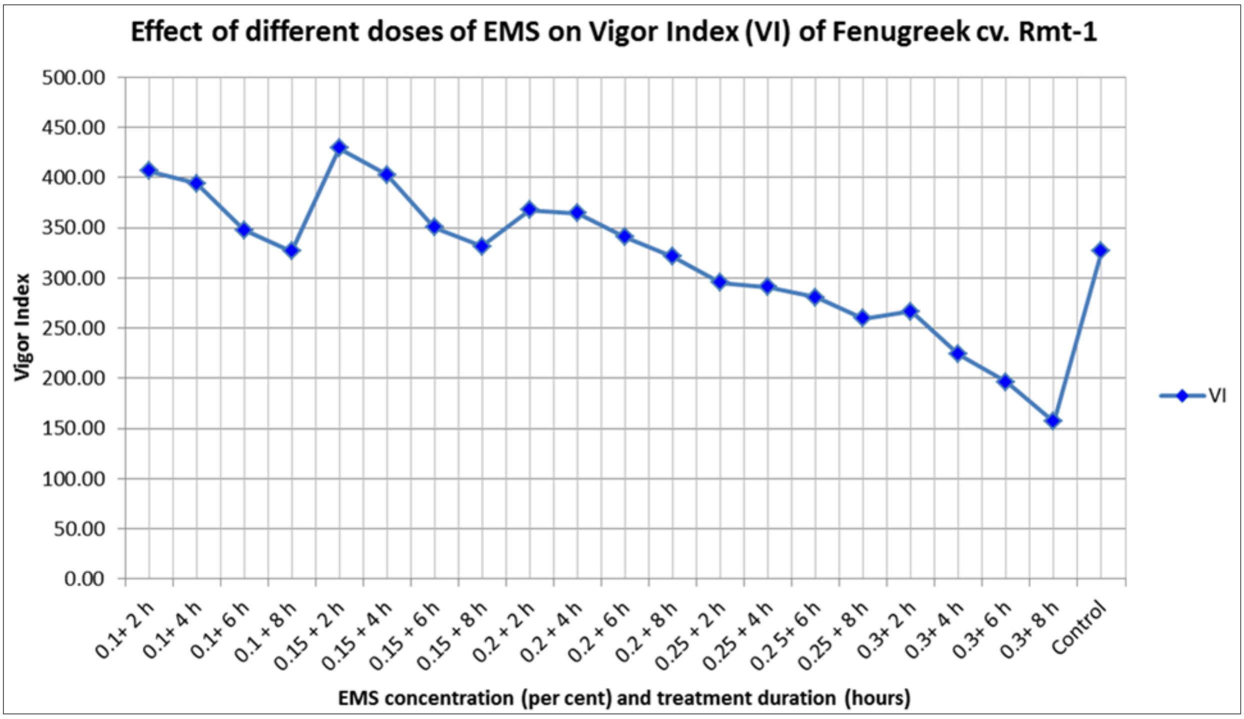

Fig 2: Effect of EMS on vigor index of Fenugreek cv. RMt-1

Conclusion

Generally, the mutagens are more likely to reduce seed germination, seedling growth and vigor. In contrast, the mutagenic treatment EMS 0.15 per cent at 2 hours produced superior results when compared to the control, indicating its potential to induce desirable polymorphisms and point mutations. At higher doses, there was substantial damage and killing of plants with reduced survivability. Thus, neither the overdose nor the underdose of the mutagens, but the LD_{50} yields the desirable and optimal mutagenic effects. The semi-lethal dose of EMS for fenugreek cv. RMt-1 was found to be EMS 0.30 per cent at 8 hours. Thus, we recommend that relatively lower doses of EMS 0.30 per cent at 8 hours could be tried in other cultivars to generate useful variability for crop improvement. Moreover, EMS 0.30 per cent at 4 and 6 hours may be safer with increased seedling survival. Therefore, the information generated through this investigation on the safer and suitable dose of EMS towards higher useful mutations in fenugreek cv. RMt-1 would facilitate further research on mutation breeding in this crop.

References

1. Acharya S, Srichamroen A, Basu S, Ooraikul B, Basu T. Improvement in the nutraceutical properties of fenugreek (*Trigonella foenum-graecum* L.) Songklanakarin J. Sci. Technol. 2006; 28(1):1-9.
2. Anbarasan K, Sivalingam D, Rajendran R, Anbazhagan M, Chidambaram AIA. Studies on the mutagenic effect of EMS on seed germination and seedling characters of Sesame (*Sesamum indicum* L.) Var. T MV3. Int J Res Biol Sci. 2013; 3:68-70.
3. Basu SK, Acharya SN, Thomas JE. Genetic improvement of fenugreek (*Trigonella foenum-graecum* L.) through EMS induced mutation breeding for higher seed yield under western Canada prairie conditions. Euphytica. 2008; 160:249–258. https://doi.org/10.1007/s10681-007-9545-9
4. Blixt S. Studies of induced mutations in peas. Genetically Controlled Differences in Radiation Sensitivity. Agri. Hort. Genet. 2008; 28:55-116.
5. Cherry JH, Hageman RH, Hanson JB. Effect of X-Irradiation on Nucleic Acids in Zea mays: II. On the Level of Ribonuclease Activity in Growing Seedlings. Radiation Research. 1962; 17:740-751.
6. Evans HJ, Sparrow AH. Nuclear Factors Affecting Radiosensitivity. In: Fundamental Aspects of Radiosensitivity. Brookhaven Symposia in Biology. 1961; 14:101-124.
7. Giridhar K, Kumari SS, Rajani A, Sarada C, Naidu LN. Identification of potential genotypes of fenugreek in rainfed vertisols for yield and diosgenin content. Indian J. Agric. Res. 2016; 50(4):311-317. https://doi.org/10.18805/ijare.v0iOF.8603
8. Goud JV, Nayar KMD. Effect of Irradiation on Seedlings of Methi. Mysore J. Agric. Sci. 1968; 11:53-55.
9. Ignacimuthu S, Babu CR. Radio Sensitivity of the Wild and Cultivated Urd and Mung Beans. Indian J. Genet. Plant Breed. 1988; 48(3):331-342.
10. Kandasamy S, Weerasuriya N, Gritsiouk D, Patterson G, Saldias S, Ali S *et al*. Size Variability in Seed Lot Impact Seed Nutritional Balance, Seedling Vigor, Microbial Composition and Plant Performance of Common Corn Hybrids, Agronomy. 2020; 10:157. https://doi.org/10.3390/agronomy10020157
11. Khan S, Goyal S. Improvement of Mungbean Varieties Through Induced Mutations. Afr. J. Plant Sci. 2009; 3:174-180
12. Kharkwal MC. A brief history of plant mutagenesis. In: Shu QY, Forster BP, Nakagawa H, editors. Plant mutation breeding and biotechnology. Wallingford: CABI, 2012, 2130.
13. Lonnig WE. Mutation breeding, evolution, and the law of recurrent variation. Recent Res Dev Genetics Breeding. 2005; 2:45-70.

14. Mahajan V, Devi A, Khar A, Lawande KE. Studies on mutagenesis in garlic using chemical mutagens to determine lethal dose (LD_{50}) and create variability. Indian J. Hort. 2015; 72(2):289-292. https://doi.org/10.5958/0974-0112.2015.00055.9
15. Micco VD, Arena C, Pignalosa D, Durante M. Effects of Sparsely and Densely Ionizing Radiation on Plants. Radiat. Environ. Biophys. 2011; 50:1-19.
16. Nilan RA. Induced gene and chromosome mutants. Philos. Trans. Royal Soc. London. 1981; 292:457-466.
17. Rajoria CM, Ahmad R, Rawat RS, Jat BL. Studies on induction of mutation in fenugreek (*Trigonella foenum-graecum*). International Journal for Research in Applied Science & Engineering Technology, 2016; 4(10):333-373.
18. Ramandeep, Dhillon TS, Dhall RK, BS Gill. Effect of mutagen-ethyl methane sulphonate on yield increasing parameters of French bean (*Phaseolus vulgaris*), Genetika. 2018; 50(1):199-207. https://doi.org/0.2298/GENSR1801199R
19. Roychowdhury R, Tah J. Mutagenesisa potential approach for crop improvement. In: Hakeem KR, Ahmad P, Ozturk M, editors. Crop improvement: new approaches and modern techniques. New York (NY): Springer, 2013, 149187.
20. Sikder S, Biswas P, Hazra P, Akhtar S, Chattopadhyay A, Badigannavar AM, D'Souza SF. Induction of mutation in tomato (*Solanum lycopersicum* L.) by gamma irradiation and EMS. Indian J Genet. 2013; 73(4):392-399. https://doi.org/10.5958/j.0975-6906.73.4.059
21. Sato M, Gaul H. Effect of EMS on Fertility in Barley. Rad. Bot. 1960; 7:7-10.
22. Shagufta B, Wani AA, Nawchoo IA. Mutagenic sensitivity of Gamma rays, EMS and Sodium azide in *Trigonella foenum-graecum* L. Sci. Res. Rept. 2013; 3(1):20-26.
23. Srivastava P, Marker S, Pandey P, Tiwari DK. Mutagenic Effects of Sodium Azide on the Growth and Yield Characteristics in Wheat (*Triticum aestivum* L. Em. Thell.). Asian J. Plant Sci. 2011; 10:190-201.
24. Tiwari JK, Sharma D, Kumar V, Das BK. Effects of gamma radiation on germination and growth characteristics of spine gourd (*Momordica dioica* Roxb.), Vegetable Science. 2019; 46(1):120-122
25. Yadav P, Meena HS, Meena PD, Kumar A, Gupta R, Jambhulkar S *et al*. Determination of LD_{50} of ethyl methanesulfonate (EMS) for induction of mutations in rapeseed-mustard. Journal of Oilseed Brassica. 2016; 7(1):77-82.
26. Zandi P, Basu SK, Cetzal-Ix W, Kordrostami M, Chalaras SK, Khatibai LB. Fenugreek (*Trigonella foenum-graecum* L.): An Important Medicinal and Aromatic Crop, Intech open science, 2017. http://dx.doi.org/10.5772/66506