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Effect of Ethyl Methane Sulphonate on Induced Mutagenesis in Fenugreek (*Trigonella foenum-graecum* L.)

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Abstract: Mutations are the tools used to study the nature and function of genes which are the building blocks and basis of plant growth and development, there by producing new materials for genetic improvement of economic groups. The present investigation was conducted to study the mutagenic effect of EMS Ethyl Methane Sulfonate [EMS (CH_3 OSO_2 C_2H_5)] in the local variety of fenugreek (Trigonella foenum-graecum L.). The seeds of fenugreek were treated with different treatment of chemical mutagen. Two sets containing 400 healthy seeds were selected for treatment. To determine the LD50 value, fenugreek seeds were pre soaked in double distilled water for 6 hours followed by EMS 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0% concentrations, respectively. The main objectives of this study were; to determine the optimum dose of LD_{50} value of EMS in Fenugreek, to study the effect of EMS mutagenic treatments on various biological parameters, to observe the chromosome behavior of treated plants with respect to controls, isolate chlorophyll mutants based on changes in phenotypic traits and assess the effectiveness and efficiency of EMS mutagen.

Key words: Lethality · Ems · Mutagens · Trigonella Foenum-Graecum

INTRODUCTION

Trigonella foenum-graecum L. (2n= 16) commonly known as fenugreek (methi), an annual dicotyledonous herbaceous plant belongs to the family Leguminosae with branched stems, trifoliate ovate-orbicular leaves, roots bearing nodules, white flowers, paplionaceous corolla, stamens diadelphous [1+(9)], ovary superior, ovules many, pods bearing golden yellow seeds. Seeds vary from rectangular to round in outline with a deep groove between the radical and cotyledons. The young plants serve as vegetable for human consumption seeds as a spice or as herbal medicine [1]. Fenugreek leaves and seeds have been used extensively to extracts and powders for medicinal uses [2]. Fenugreek is reported to have anti-diabetic, anti-fertility, anti-cancer, antimicrobial, anti-parasitic and hypocholesterolemic effects [3].

In India, Fenugreek is used as a stimulant [4]. Fenugreek seed in powdered or germinated form exhibits anti-diabetic properties [5], hypocholesterolemic effects, anti cancer effects and effect on thyroxine induced hyperglycemia. Other pharmacological properties of Fenugreek include anti-inflammatory, antiulcer, sexual stimulant and antioxidant. The biological and pharmacological properties of fenugreek are attributed to the variety of its constituents, namely; steroids, N - compounds, polyphenolic substances, volatile constituents, amino acids etc. [6].

Fenugreek seed contains 45-60% carbohydrates, mainly fibre (galactomannans), 20-30% proteins high in lysine and Tryptophan, 5-10% fixed oils (lipids), Pyridine alkaloids, mainly trigonelline (0.2 0.38%), choline (0.5%), free amino acids, such as 4 -hydroxyisoleucine (0.09%), arginine, histidine and lysine, calcium and iron, saponins (0.6 1.7%), glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin), cholesterol sitosterol, vitamin A, B1, C and nicotinic acid [7]. Fenugreek is an important cultivated crop in parts of Europe, Northern Africa, West and South America and Australia [8]. Major fenugreek producing countries are Russia, India, Pakistan, Germany, Argentina, Egypt, Canada, Iran, Canada, USA and China [9]. India is the largest producer of fenugreek in the world where Rajasthan, Gujarat, Uttaranchal, Uttar Pradesh, Madhya Pradesh, Maharashtra, Haryana and Punjab are the major fenugreek producing states [10]. Rajasthan produces the lion's share of India's production, accounting for over 80% of the nation's total fenugreek output.

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Mutagens: Plant breeding is often regarded as an important one of the branches among applied genetics. It forms the most important breakthroughs in the history of genetics that led to the discovery of experimental mutagenesis in the early 21st century. Mutation techniques can generate genetic variation and increase the desired characters significantly in plants of new cultivars [11]. The application of mutagenesis in agriculture for improving the crop plants presented a new departure from the conventional breeding methods. In conventional breeding methods, the store of natural variability present either in the base population initially or introduced through hybridization, is subjected to recombination and selection so as to increase the frequency of favourable combinations of genes in the selected line.

Mutation breeding helps in greater magnitude of variability in various plant traits in a comparatively shorter time. Mutation is a sudden heritable change in an organism and generally a structural change in genes. Mutation produced by changes in the base sequences of genes (as a result of base pair transition or transversion, deletion, duplication or inversion etc.) are known as gene or point mutations. Some mutations produced by change in chromosome structure, or even in chromosome number are known as chromosomal mutations. The induced mutations are caused artificially by mutagenic factors. The agents that induce mutations are called mutagens and mutagens mainly consist of two different kinds; radiation (physical) and certain chemical mutagens. Mutagens are not only beneficial to create genetic variability in a crop species, but also useful for the effective control of pests during post-harvest storage [12]. Practicing of induced mutation for crop improvement is known as mutation breeding. Mutation breeding has been widely used for the improvement of plant characters in various crops. It is a powerful and effective tool in the hands of plant breeders especially for autogamous crops having narrow genetic base .Mutation induction offers significant increase in crop production and the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evaluation. Treatments with mutagens alter genes or break the chromosomes. Gene mutations occur naturally as errors in DNA replication. Most of these errors are repaired but some may pass on to the next cell division to become established in the plant offspring as spontaneous mutations. Gene mutations without phenotypic expressions are usually not recognized. Consequently, genetic variation appears rather limited and breeders have to resort to mutation induction [13]. Mutagenic agents have been used to induce useful phenotypic variations in plants for more than seventy decades [14].

Ethyl Methane Sulphonate (EMS): Ethyl methane sulphonate (EMS) is a chemical mutagen of the alkylating group and has been commonly used in plant breeding because it can cause high frequency of gene mutations and low frequency of chromosome aberration [15]. EMS alkylates guanine residues, producing O6-ethyl guanine, which pairs with T but not with C [16]. As a result, replication of un-repaired alkylation damage will effectively replace the G/C base pair with an A/T. This mechanism predicts a strong G/C to A/T bian in EMS induced mutations, as observed in numerous mutagenic studies. In fenugreek, several have tried for artificial induction of mutations through the use of mutagens [9]. Despite the release of different cultivars, fenugreek production has not increased to any noticeable extent over the last decades. The present work is therefore, designed to evaluate the morphological and cytological effects of chemical mutagens in fenugreek with the main objective of inducing changes in the genotype to enhance genetic variability in this plant as to broaden its genetic base for selection of desirable genotypes for commercial cultivation [16].

MATERIALS AND METHODS

The dry and dormant seeds of the fenugreek (*Trigonella foenum-graecum*) local variety were treated with EMS treatments were used in the present study. The present study was carried out in Pachaiyappa College Botanical Gardern.

Mutagen Used: The seeds of fenugreek were treated with different treatment of chemical mutagen. The chemical mutagens used were Ethyl Methane Sulfonate [EMS (CH_3 OSO_2 C_2H_5)]. The chemical was obtained from HI-MEDIA laboratories, Mumbai, having a half life period of 30 hours with a molecular weight of 124.16 and density of 1.20.

Two sets containing 400 healthy seeds were selected for treatment. To determine the LD50 value, fenugreek seeds were pre soaked in double distilled water for 6 hours followed by EMS 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0% concentrations freshly prepared solution for 3 hours. After the EMS treatment, the treated seeds were washed thoroughly in running tap water to terminate the residual effect of the mutagenic

chemicals. After the completion of the treatment the treated seeds were sown immediately in the field along with their respective controls to raise the M_1 generation in a randomized block design with three replications. The seedling height reduction (I) in different M1 generation was studied following Nilan *et al.* [17] and Velu *et al.* [18]. The plant survival (L) was computed as the percentage of plants surviving till maturity. The biological damage (lethality/ injury) was computed as the reduction in plant survival and plant height. The respective control and treatment progenies were screened several times for morphological mutations throughout the crop duration. Different kinds of chlorophyll mutants (Albino, Xantha, Chlorina,Viridis) were scored from emergence till the age of four week in M1 generation by using modified classification of Kharkwal [19]. Mutation frequency was calculated as percentage of mutated M1 progenies for both chlorophyll and morphological mutations in each treatment. The Mutagenic effectiveness and efficiency were calculated on the basis of formulae suggested by Konzak *et al.* [20].

Mutagenic effectiveness (Chemical mutagens) = Mf×100/c×t.

- Mutation frequency = Chlorophyll / Viable mutants per100 M1 plants.
- C=Concentration of mutagen in mM.
- t=Period of treatment with chemical mutagen in hours.
- L=Percentage of lethality (or) survival reduction.
- I=Percentage of injury (or) reduction in seedling size.

Cytological Observation: The root tips collected from control and treated seedlings were fixed in 1:3 acetic ethanol. The root tip squashes were made by using Iron alum Haematoxylin squash technique [21].

The root tips were hydrolyzed in 0.1N HCl for 5 to 10 minutes at 60°C and then they were thoroughly washed in distilled water and transferred to 4% iron alum for 3 minutes. The root tips were then washed in distilled water and transferred to ripened dilute haematoxylin stain and kept for 3 hours. The root tips were thoroughly washed in distilled water and then they were treated in 45% acetic acid for 1 minute to soften the tissues. Acetic acid being a de-staining agent, the time of study in haematoxylin had to be adjusted to the time required for softening in acetic acid. One or two root tips were placed on a clean slide and squashed by using a cover slip and the slide was sealed and mounted in DPX solution and then examined. The normal and abnormal mitotic stages were photographed.

Evaluation of M_1 generation

Seed Germination: The data on seed germination was recorded right from the emergence of first shoot in each treatment including control. After recording the data, percentage of seed germination was calculated by using the formula,

Germination (%) = No. of seeds germination ÷ total No. of seeds × 100

Seedling Height (cm): Seedling height was estimated on 20th day of germination by measuring root and shoot lengths of 15 randomly selected seedlings from each treatment as well as control. Seedling injuries as measured by the reduction in root and shoot length and calculated in terms of percentage of root and shoot injury.

Seedling Injury % = control ÷ treated plants × 100

Plant Survival: The surviving plants in different treatments were counted at the time of maturity and the survival percentage and percent lethality were calculated by the following formula.

Survival (%) =Number of plants at maturity x100 ÷ Number of seed.

RESULTS

The present investigation was undertaken in order to study the artificial inducement of mutation in fenugreek local by using EMS mutagens through the biological changes in M_1 generation. This was aimed to find out the economic potentialities of the viable mutant and the nature of induced variability in the qualitative and quantitative traits in all generation.

M_1 Generation

LD_{50} for Gamma Rays and EMS: Data on the effect of mutagens on germination, expressed as per cent control and LD_{50} is presented in Table 1. The untreated seeds of genotype had 100 per cent germination. The germination percentage decreased with increase in the dose/conc. of the treatment. Fenugreek seeds were treated with EMS,

Plate 1: Seed Germination on 7th day

Table 1: Determination of LD_{50} value for Ethyl methane sulphonate in fenugreek

EMS treatment (Conc. mM)	Seed Germination (%)	Percent of over control
Control	95%	_
0.1 %	93%	97.8%
0.2 %	72%	75.7%
0.3 %	64%	67.3%
0.4 %	48%	50.5%
0.5 %	42%	44.2%
0.6 %	38%	40%
0.7 %	25%	26.3%
0.8 %	17%	17.8%
0.9%	9%	9.4%
1.0%	2%	2.1%

showed reduction in germination at higher concentration. Lowest germination percentages (2.1. %) were observed at 1.0% of EMS on 7th day after the germination. Based on the germination studies, 50% lethality was observed at 0.4% of EMS (Table 1), (Plate 1)

Cytological Studies: Cytological analysis with respect to their mitotic behavior is considered to be one of the most dependable indices to estimate the potency of mutagen. Cytological studies provide information regarding the response of Fenugreek genotypes to a particular mutagen and provide greater chances for the selection of desired characters. Root mitotic studies revealed a wide range of chromosomal aberration such as nullisome, anaphasic bridge with laggard, anaphasic multiple bridges and laggards, anaphasic bridge, late anaphase, clumping of chromosome and precocious movement of chromosomes. Chromosome laggards were observed for all mutagenic treatments. In the present study, the aberrations caused by mutagens are due to partial or complete failure of spindle mechanism. Maximum chromosome aberrations were observed in 0.7% of EMS when compared to control (Plate-2-4). (Table-2).

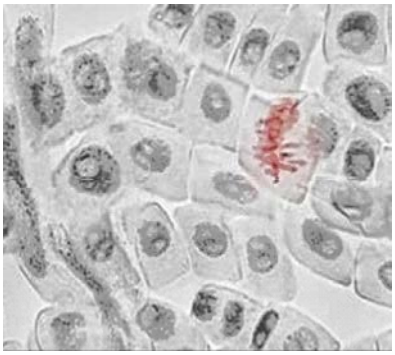

Plate 2: Anaphasic laggards;

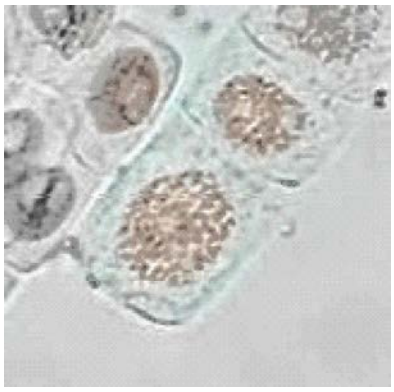

Plate 3: Clumping of Chromosome

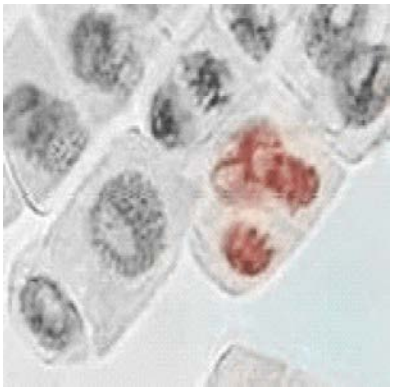

Plate 4: Anaphase clumping

Chemical Mutagen (EMS): Root tip squash was carried out in the different concentration of 0.1%, 0.2%, 0.3%, 0.4%, 0.5% 0.6% 0.7% of EMS treated seedlings. In the present study, some of the cytological behavior likes normal metaphase, anaphasic laggards, anaphasic bridge and stickiness with precocious movement of chromosome, anaphasic and nullisomic chromosomes were also observed (Plate-5 and 6).

Field Studies

Seed Germination on 15th day: A general reduction in seed germination was recorded due to all mutagenic

Table 2: Effect of Ethyl methane sulphonate on mitotic cell division of fenugreek

Control	Number of cell observed	Number of abnormal cell			Total number of abnormal cell	% of abnormal cell frequency
		Bridge	Laggard	Stickiness		
0.1 %	70	8	6	4	18	25.7
0.2 %	65	7	5	5	17	26.1
0.3 %	68	12	8	10	30	44.1
0.4 %	73	16	8	14	38	50.0
0.5 %	76	17	12	11	40	66.0
0.6 %	61	20	17	13	55	70.5
0.7 %	78	22	18	20	60	76.9

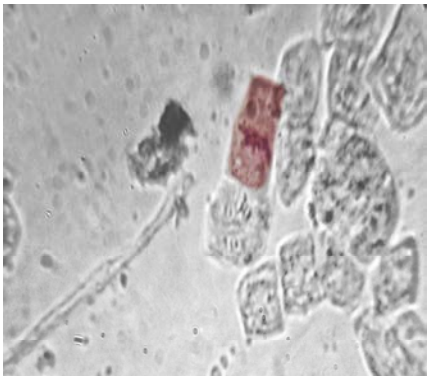

Plate 4: Anaphasic bridge

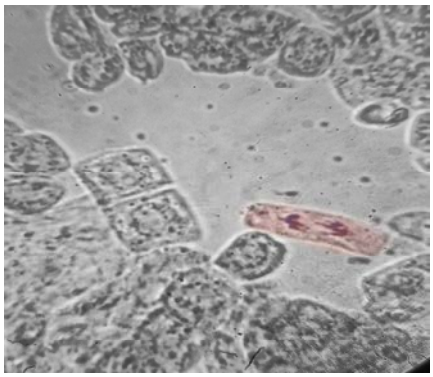

Plate 5. Spindle fibre failure

Table 3: Effect of Ethyl methane sulphonate on seed germination in M_1 generation of fenugreek

EMS treatment (Conc. mM)	Seed Germination (15th day)		
	Total no of seeds	Mean	% over control
Control	20	18	-
0.1 %	20	17	94.4%
0.2 %	20	16	88.8%
0.3 %	20	15	83.3%
0.4 %	20	10	50.0%
0.5 %	20	8	44.4%
0.6 %	20	6	30%
0.7 %	20	4	22.2%

treatments as shown in Table 3. The reduction in seed germination ranged from 22.20 to 94.4 per cent in 0.7% of EMS (Table 3).

Seedling Survival on 20th day: There was a general reduction in the seedling survival in all mutagenic treatments as shown in table-4. The maximum per cent of seedling survival was recorded at 0.7% of EMS was respectively (Table 4).

Plant Height Reduction on 20th day: Among the different mutagens, 20th day plant height was gradually decreased with increased in dose/concentration. 0.7% of EMS (4.12cm) was showed more declination than 0.1% of EMS (22.5cm) and control (24cm) (Table 5 and Plate-6).

Table 4: Effect of Ethyl methane sulphonate on seedling survival in M_1 generation of fenugreek

EMS treatment (Conc. mM)	Seedling survival (20th day)		
	Range	Mean	Percent over control
Control	20	18	100%
0.1 %	20	15	83.3%
0.2 %	20	13	72.2%
0.3 %	20	11	61.6%
0.4 %	20	9	50.2%
0.5 %	20	7	38.2%
0.6 %	20	5	27.1%
0.7 %	20	2	11.1%

Table 5: Effect of Ethyl methane sulphonate on plant height in M_1 generation of fenugreek

EMS treatment (Conc. mM)	Plant height (20th day)		
	Range	Mean (cm)	Percent over control
Control	23-25	24.0	100%
0.1 %	21-24	22.5	93.7%
0.2 %	20-23	20.5	85.5%
0.3 %	18-21	18.6	77.8%
0.4 %	16-19	14.2	59.2%
0.5 %	15-18	11.3	47.2%
0.6 %	12-17	8.65	36.4%
0.7 %	10-15	4.12	17.16%

Plate 6: Plant height observed in fenugreek on 20th day.

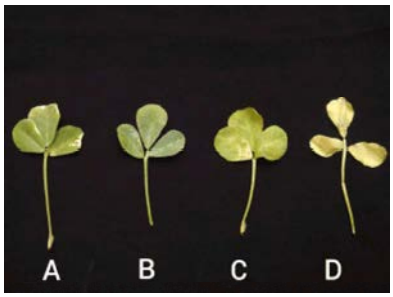

Plate 7: A- Albino, B- Xantha, C- Viridis, D- Chlorina

Table 6: Effect of EMS on frequency of chlorophyll mutants in M1 generation of fenugreek

EMS Treatment	Total No of Plants Studied	Spectrum of Chlorophyll mutants				Total number of Chlorophyll mutants	% of mutants frequency
		Albino	Xantha	Viridis	Chlorina		
0.1 %	15	-	-	-	-	0	0
0.2 %	13	-	-	-	-	0	0
0.3 %	12	-	-	1	-	1	8.33
0.4 %	9	1`	-	1	1	3	33.3
0.5 %	7	1	-	1	-	2	28.57
0.6 %	5	1	-	-	-	1	20.1
0.7 %	2	-	-	-	-	0	0

Field Observation: The M_1 seedlings were examined for identification of chlorophyll mutants (up to 20th day) which was helpful to analyze the frequency, effectiveness and efficiency of mutagens.

Observation of Chlorophyll mutants (M_1 and M_2 generations)

The chlorophyll mutants were scored on 20th day after sowing. Four types of chlorophyll mutants were observed in treated plants (Plate- 7 and Table-6).

Albino: Albino-white, lethal, no chlorophyll (or) carotenoids are formed. *Xantha* Carotenoid pigment predominantly found but chlorophylls are not formed. *Chlorina* Light green to flush yellow green, mostly viable seedlings. They change to normal condition. *Viridis*- Light yellow patches, along with green following either regular or irregular pattern and viable.

Mutagenic Effectiveness and Efficiency: Estimates of mutagenic effectiveness and efficiency for different mutagenic treatments based on the mutation frequency on M_1 plant basis are presented in (Table 7). The effectiveness of mutagens indicated that 0.5% of EMS treatment was the most effective mutagen for production of morphological mutations. The maximum mutagenic effectiveness was observed at 0.4% of EMS (0.496), while the minimum mutagenic effectiveness was observed at 0.1% of EMS treatment (0.124). The mutagenic efficiency was worked out based on injury and lethality. On the basis of lethality, the highest mutagenic efficiency was recorded at 1.0% of EMS (1.24), while the lowest mutagenic efficiency was observed at 0.1% of EMS (0.124). In general the mutagenic treatment 0.5% of EMS treatment was found to be highly efficient to induced chlorophyll and viable mutants. On the basis of injury, the maximum mutagenic

Table 7: Effectiveness and Efficiency of fenugreek in M1 Generation

EMS treatment	Survival Reduction Leathality (L)%	High Reduction Injury (I)%	Mutation frequency	Effectiveness Mf×100/C×T	Efficiency Mf ×100/L	Efficiency Mf×100/I
0.1	83.33	93.75	0	-	-	-
0.2	72.22	85.54	0	-	-	-
0.3	61.11	77.83	8.33	15.42	13.63	10.70
0.4	50	59.25	28.57	39.68	73.48	60.46
0.5	38.88	47.25	20.0	18.51	72.02	55.3
0.6	27.77	36.16	0	-	-	-
0.7	11.11	17.16	0	_	_	_

efficiency was observed at 1.0% of EMS treatment (1.24). The minimum mutagenic efficiency was observed at 0.1% of EMS (0.124).

DISCUSSION

Mutation (a change in genetic material of an organism) induced both in seeds and vegetatively propagated crops are of scientific and commercial interest to improve both the growth and yield parameters of economic plants. It provides raw materials for the genetic improvement of economic crops. It facilitates the isolation, identification and cloning of genes which would ultimately help in designing crops with improved yield, increased stressed tolerance and longer life span and reduced agronomic in-puts [22]. Induced mutations increase genetic variability and are used in plant breeding [23]. The increase of the plant variability is cross combination in hybridization, spontaneous mutation and induced mutations. The rate of spontaneous mutations in nature is too low for plant breeding. Therefore, physical and chemical mutagens can be used for inducing mutation in cultivated plants. It can be possible to increase the genetic variability by inducing many mutations in plants [24].

Mutation breeding in crop plants is an effective tool in hands of plant breeders especially in crops having narrow genetic base. Many mutants have been identified as donors of desirable traits in breeding program. Mutation breeding has contributed significantly to plant improvement, resulting in release of at least 2250 varieties of different crops. In India, at least 300 cultivars have been developed in at least 55 plant species [25]. By mutagen treatment which breaks the nuclear DNA during the process of DNA repair mechanism, new mutations are induced randomly. The changes can occur also in cytoplasmic organelles and also results in chromosomal or genomic mutations and that enable plant breeders to select useful mutants such as flower colour, flower shape, disease resistance and early flowering types [26].

The low level of genetic diversity in black gram, mutation induction constitutes a valuable strategy to create genetic variability, which in turn reduces the time required to breed new varieties compared with traditional methods [27].

M_1 Generation (Laboratory Studies)

LD_{50} Value: The availability of efficient seed germination system after the mutagenic treatment is crucial in achieving successful mutagenesis. The higher exposure of gamma rays may cause injury in seeds and usually show inhibitory effects on seeds of Angiosperms and Gymnosperms. Compared to physical mutagen, the germination of seeds reduced more under chemical mutagen that they damage the biological material as reflected in the quantitative parameters [27].

In the present study to find out optimum dose/conc. of the mutagens, germination percentage of the seeds was calculated with effect of chemical mutagens of fenugreek. Among the mutagenic doses/conc., of the LD_{50} (optimum) value was recorded at 0.5% of EMS (51.43%, 51.05%) and the maximum reduction of germination percentage were noted at 0.8% of EMS showed more lethal effect of fenugreek (Table 1). Similar results were noted in mungbean [28] sesame [29] and *Lepidium sativum* [30].

Cytological Studies: The mitotic behavior is considered to be one of the most dependable indices to estimate the potency of mutagen. It provides useful information regarding the response of various genotypes to particular mutagens and give greater chances for the selection of desired characters. Cytological aberrations in plants serve as an excellent monitoring system for the detection of environmental chemicals that may pose a genetic hazard. In general, chromosome aberrations can provide both quantitative and qualitative data on the effect of exposure to a mutagen [31].

In the present study, fenugreek contains 8 bivalents (2n = 16) which are small in size and recognizable at higher magnification (10x, 100x). Lower concentration of mutagens like 0.2% of EMS revealed more or less normal

pairing like that of control and mutagenic treated seeds. However, a consistent increase in the frequency of various types of chromosomal abnormalities was observed with increasing concentration of mutagens at 0.6% of EMS. Chromosomal abnormalities included the formation of anaphase bridges, laggards; multiple bridges, late anaphase and precocious movement of chromosomes, unequal separation of chromosomes, clumping of chromosomes were also observed. Among the 0.6% of EMS, the maximum abnormalities, both structural and behavioral were induced in both the varieties. Dose dependent increase in frequency of different chromosomal aberrations has also been reported in Cowpea [32].

Low frequency of anaphasic bridges was observed with 0.2% of EMS in fenugreek. They were produced due to the sub-chromatid exchanges, unequal exchange or by formation of dicentric chromosomes. The occurrence of breaks at the same locus and their lateral fusion leads to the formation of dicentric chromosome which is pulled equally to both the poles forming a bridge. The precocious separation of chromosomes at metaphase was observed at higher concentration at 0.6% of EMS only. It might have resulted due to the disturbed homology for chromosome pairing or disturbed spindle mechanism. Besides the precocious separation of univalents, the bivalents were also observed to move ahead and seemed as stray chromosome, this may move to one pole resulting into unequal distribution of chromosome or loss of a complete bivalent at metaphase stage [33].

Laggard chromosome was commonly observed at anaphase and telophase stages at 0.6% of EMS in present study. The occurrence of lagging chromosomes may be due to abnormal spindle formation and as a result of spindle fibers failed to carry the respective chromosomes to the Polar Regions and resultantly lagging chromosome appeared. Similar aberrations were reported by many workers in *Cicer arietinum* [34] and *Vicia faba* [35]. The formation of chromosome fragments might be due to the stickiness of chromosomes and consequent failure of separation. Breakage and reunion of the broken ends cause the chromosome bridges and these types of bridges continue its existence up to early telophase. It was found to bring about condensation and stickiness of chromosomes ultimately resulting in the formation of ring chromosomes. The probable cause of condensed and sticky chromosomes might be due to the fact that the chromosomes start contraction at metaphase/anaphase stages while as a result of any toxic material the chromosomes can not reach the poles and remain scattered in the cytoplasm. However, chromosome stickiness arises due to improper folding of chromosome fibres into single chromatid and chromosome.

A plausive explanation of the multipolar and unequal separation of chromosomes might be due to the formation of more than two poles followed by the development of more than one spindle. The multipolar condition is determined by position and number of poles [36].

The number of poles in a cell depends on the position of the assemblage of RNA and polysaccharides which remain disturbed in the from of sol or gel. The present findings suggest that seed aging has effects similar to those of ionizing radiation and chemical mutagens. Furthermore, it might be suspected that the mitotic inhibition caused by seed aging in this study as well as that of ionizing radiation and chemical mutagens is related to the induction of chromosomal aberrations, because all these treatments have the effect of inducing chromosomal aberrations.

The inhibition of seedling growth seemed to be well correlated with the amount of chromosomal damage. The EMS was found to react with the genetic material by alkylating DNA bases and phosphate groups [37]. The radio sensitivity was related to nuclear volume and interphase chromosome volume [38]. Chromosomal bridge may be formed due to the breakage and fusion of chromosomes. The bridge formation can be due to the general stickiness of chromosome of metaphase stage [39].

Seedling Survival Percentage: Gradual reduction of seedling survival percentage and plant height was observed in different doses/concentrations on 20^{th} day (field condition) by the effect of EMS mutagen. This mutagen was effective in reducing the survival percentage and plant height of M_1 plants. The phenotypic response varied with respect to mutagenic treatments. 0.6% of EMS recorded highest reduction in survival percentage and plant height reduction in fenugreek respectively (Table 6). This probability reflects the organ specific action of the mutagens. The gamma rays may be affecting shoot initials more than the root initial [40]. Similar results were reported in mungbean [41] and cowpea [32] and Millet [42].

Chlorophyll Mutation: Leaf color mutations are one kind of most frequently observed mutation in both spontaneous and induced mutant populations and often used as an indicator of mutagenic effects and efficiency of various mutagens**.** Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromere and proximal segment of chromosomes [43].

In the present investigation, different chlorotic abnormalities were scored infenugreek plants. The chlorophyll mutants were observed in different doses/concentrations of gamma rays. They were albino, chlorina, viridis and xantha. 0.6% of EMS was higher frequency of chlorophyll mutations local fenugreek varieties. The green-revertible albino mutation was observed in 0.6% of EMS regarded as a better mutant because it seldom affects the growth of such lines at late stage and is more visibly different from green ones when compared with chlorina mutants. The seedlings appeared white were much dependent on the growth temperature, the higher temperature shorter the time it took for the leaves to turn into green in rice [44]. Chlorina mutation was observed in 0.6% of EMS respectively. For the chlorina mutation, as there were colour level differences among both mutant and normal seedlings. It became particularly worse when seedling growth was subjected to environmental stress, such as low nitrogen level and extremely low/high temperatures [45]. The xantha mutations were noted in 0.6% of EMS of fenugreek. It is expressed uniformly in all tissues of the plant, e.g., leaves and sheath, during whole growth duration, which made it readily distinguishable from green seedlings. The mutation apparently affected the content of all pigments chl a, chl b and carotene content at different levels [45]. Viridis mutation seedlings are uniform light yellow green color leaves it's a viable mutant observed at 0.6% of EMS of fenugreek varieties. The viridis types were predominant than albino, xantha and chlorina types, irrespective of the cultivar in rice as reported by Prakash and Khanure [46]. The appearance of greater number of viridis after xantha may be attributed to involvement of polygenes in chlorophyll formation [47]. These types of mutations were observed in mungbean [48], chickpea [49] and in grasspea [50].

Mutation Frequency: The mutation frequency was calculated on the M_1 plant basis showed a dose dependable measure of genetic effects in mutagens [51]. In the present study the spectrum of morphological mutations in two varieties induced included for chlorophyll mutants (albino, chlorina, viridis, xantha).

Effectiveness and Efficiency: Mutagenic effectiveness means the frequency of mutations induced by unit dose/conc. of a mutagen, while efficiency means undesirable biological effects like lethality and sterility caused by the mutagen [20]. The utility of a particular mutagen depends not only on its effectiveness and inducing mutation but also on its efficiency. The efficiency of a mutagenic agent is of complex nature, as it does not only depend on the reactivity of the agent with the material and on its applicability to the biological system but also to the degree to which physiological damage, chromosomal aberrations and sterility are induced in addition to mutations. Higher efficiency at lower concentration of the mutagen appears mainly due to the fact that injury, lethality and sterility increases with an increase in the mutagen concentration [19]. Such difference in the effects of mutagens on different material might be due to the seed metabolism and onset of DNA synthesis [36]. In the present study the treatment with low concentrations of mutagens were found to be more effective and efficient as measured on the basis of lethality and injury than treatments with higher concentrations. The maximum effectiveness and efficiency was observed at 0.6% of EMS of fenugreek. Similar results were reported in cowpea [32],Grass pea [50] and Cluster bean [18].

CONCLUSION

The results of the experiment indicated that increasing doses of EMS caused severe effects on the plant development. In general, according to the results of the present work, the best treatment was the application of 0.4% of EMS dose was stimulate plant growth and increase its active substances. The crucial aim of a mutagenic treatment is to induce mutations leading to genetic improvement of a specific trait and selection of economically important mutants. For breeding purposes mutagenic treatments with low physiological effects and strong genetic effects are desirable.

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