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Impact of EMS Induction on Morphological, Anatomical and Physiological Traits of Bhindi *Abelmoschus Esculentus* (L.) Moench

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Abstract: **Ethyl methanesulfonate (EMS) is a common, powerful and one of the most effective chemical mutagen, to induce a large number of functional variations in crops. Present study is to analyse the mutagenic effect of EMS in M_1 generation of Bhindi (*Abelmoscus esculanthus* L. Moench. va Arka anamika). Seeds were treated with different doses (0.5%, 1%, 2% and 3%) of mutagen for 4 hrs and grown in gunny bags along with control. Morphological, anatomical and physiological traits of Bhindi were analyzed for 50 days at definite intervals. All parameters decreased with increase in doses of EMS. Strong deleterious effect on the germination percentage was seen in 3% of EMS. There was a negative correlation in length of root, shoot length, numbers of secondary roots and fresh weight with EMS percentage. Values of growth coefficient (GC), relative growth rate (RGR), tolerance index (TI) and net productivity were gradually decreasing with increasing doses of EMS. Anatomical parameters also showed marked decrease in root and shoot. Leaf area and chlorophyll content were lowest in 3% EMS.**

Keywords**: Anatomy, Chlorophyll, EMS, Growth Coefficient, Leaf Area, Net Productivity, Relative Growth Rate, Tolerance Index.**

1. INTRODUCTION

Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars in cereals, fruits and other crops. Ethyl methanesulfonate (EMS) is a common, powerful and one of the most effective chemical mutagen, to induce a large number of functional variations in crops, especially recommended when mutation is introduced to the seed materials [1]. An important advantage of using EMS is its utility in forward genetic screens in a variety of organisms. In plants, EMS usually causes point mutations; on the other hand, loss of a chromosome segment or deletion can also occur in lesser extent [2]. Since EMS produces a large number (genome-wide) of non-lethal point mutations, a relatively small mutant population (approximately 10,000) is sufficient to saturate the genome with mutations. EMS is also able to produce significant levels of alkylation at oxygen such as the O-6 of guanine and in the DNA phosphate groups. An often cited hypothesis is that DNA bases ethylated by EMS (mostly the N-7 position of guanine) gradually hydrolyse from the deoxyribose on the DNA backbone leaving behind a purinic (or possibly an a pyrimidinic) site that is unstable and can lead to single-stranded breakage of the DNA [3]

There are ample references for the mutagenic effect of EMS on plants such as hexaploid wheat [4]; mulberry [5]; *Vernonia galamensis* [6]; *Abelmoschus esculentus* [7]; *Pisum sativum* [8] etc.

The present study is to assess the mutagenic effect of EMS on seed germination, morphological, physiological, anatomical and growth traits of *Abelmoschus esculentus* (Bhindi). In particular, very little research on mutagenicity has been studied in Bhindi, one commonly used vegetable crop. This high fiber vegetable is known for its high soluble and insoluble fiber content. It is enriched with folates and vitamin C, A, E and K. It can prevent diabetes, constipation and colon cancer. It controls asthma, obesity and cholesterol level.

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2. MATERIALS AND METHODS

Healthy seeds were treated with freshly prepared solutions of Ethyl methanesulfonate for 4h with intermittent shaking. The different treatments were 0.5%, 1%, 2% and 3%. Untreated seeds were taken as control. After treatment, seeds were thoroughly washed in running water for 4h to leach out the residual of chemicals.

For the studies, potting mixture was prepared according to the recommendation given by the Kerala Agriculture University and filled in gunny bags. 10 sets of gunny bags were arranged in each concentration. Percentage germination was studied at 24th, 48th, 72thand 96th hr. Plant analysis was carried out at definite intervals i.e., on 4th, 6th, 8th, 10th, 20th, 30th, 40th and 50th day.

3. RESULTS AND DISCUSSION

Results implied that EMS adversely influenced the seed germination in $M_1$from very low doses itself. The highest percentage germination was observed in the control. Considerable reduction in the germination percentage was witnessed in 3% of EMS treatment in all days of observation. Eighty three and 74% reduction in germination was seen in 3% EMS treatments after 48 and 72 hours (Table 1). Similar results have been reported in *Jatropha curcas* [9] and Malaysian rice [10]. Severe reduction in germination is an indication of effective mutagenesis [11]. Reduced seed germination may be due to chromosomal damages or damage of meristematic tissues of the seed [12].

TABLE 1: Impact of EMS on Percentage germination in M_1 generation

Treatments	% germination				% reduction		
	24 hrs	48 hrs	72 hrs	96 hrs	48 hrs	72 hrs	96 hrs
Control	0	89.8	96.93	100			
0.50%	0	66.7	77.38	95.2	25.72	20.17	4.77
1%	0	57.14	69.04	85.7	36.37	28.77	14.29
2%	0	42.85	53.57	77.4	52.28	44.73	22.60
3%	0	15.5	25	57.1	82.74	74.21	42.86

Mutagenic treatment also affected the morphological parameters of Bhindi in M_1.There was a negative correlation between length of root and EMS percentage. Maximum reduction was seen in 3% EMS. In 3% EMS, 76%, 77%, 77%, 78%, 80%, 75% and 75% shrinkage in root length of M_1 plants on 4th, 6th, 8th, 10th, 30th and 40th day respectively (Table 2). Length and number of secondary roots also showed a decreasing pattern when compared with that of control (Table 3).The reduction in root length with increasing EMS concentration has been reported in chick pea [13] and *Coix lacryma-jobi* [14]. The high dose treatment of EMS causing growth inhibition has been ascribed to the cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome [15].

TABLE 2: Impact of EMS on Root length in M_1 generation

Treatments	4th day	6th day	8th day	10th day	30th day	40th day
Control	6.84 ± 1.53	7.3 ± 0.25	8.28 ± 0.41	8.93 ± 0.26	11.18 ± 1.19	11.96 ± 0.02
0.50%	3.84 ± 0.48	3.96 ± 0.36	4.14 ± 0.28	4.76 ± 0.27	6.27 ± 0.85	6.83± 0.7
1%	2.68 ± 0.28	1.7 ± 0.43	2.84 ± 0.2	2.97 ± 0.72	3.42± 0.36	3.92 ± 0.15
2%	6.56 ± 1.14	2.76 ± 0.34	2.79 ± 0.64	2.81 ± 0.33	3.16 ± 0.59	3.69 ± 0.37
3%	1.68 ± 0.39	3.56 ± 0.77	1.94 ± 0.83	2 ± 0.87	2.34 ± 0.34	2.94 ± 0.28

To identify the biological influences chemical mutagens in M1, shoot length is mostly utilized as an index [2]. It has been shown that a negative linear relation exists between plant height and the dosage of EMS. In all stages, peak shoot length

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was measured in control. More pronounced effect was viewed in 3% in all days, with 80%, 69%, 70%, 67%, 30%, 26% and 53% reduction in length of shoot in 4th, 6th, 8th, 10th, 30th and 40th days respectively (Table-3). Such a reduction in length of shoot arising out of mutagenic treatments was previously reported in chickpea [14], durum and bread wheat [16] and crop plants [17]. The reduction in length of shoot was attributed to the effects of mutagens on the physiological system [18]

TABLE 3: Mean value of secondary roots, nodes and internodes following EMS mutagenesis on 40th day.

Treatments	Length of sec. roots	Number of sec. roots	No of nodes	No of Internodes
Control	15.23 ± 2.7	50.33±2.51	6.33 ± 0.57	5.33 ± 0.57
0.50%	11.77±1.15	44.33±5.68	6.33 ± 0.57	4.33± 0.57
1%	8.43 ± 1.28	23.64±3.51	4± 0	3 ± 0
2%	4 ± 0.1	15.67±2.08	3 ± 0	2± 0
3%	3.27 ± 0.3	12.67±3.05	2.67± 0.57	1.6 ± 0.57

Number of nodes and internodes of 3% EMS treated Bhindi showed 50% and 70% reduction on 40th day (Table- 3). The 3% EMS treated plants also recorded minimum leaves in M_1. Higher doses of EMS might have stopped the enzymes necessary for leaves initiation. The higher doses might have damaged the genetic material and also blocked cell division by decreasing the rate of physiological processes [19].

TABLE 4: Impact of EMS on Shoot length of Bhindi in M_1 generation

Treatments	4th day	6th day	8th day	10th day	30th day	40th day
Control	14.5 ± 1.23	19.08 ± 1.09	20.1 ± 0.8	21.72 ± 0.42	25.7 ± 1.03	25.37 ± 0.66
0.50%	9.75 ± 4.4	16.58 ± 0.45	17.84 ± 0.74	18.24 ± 1.13	21.43 ± 0.53	22.6± 0.52
1%	10.12 ± 1.32	15.86 ± 0.82	17.28 ± 0.69	17.86 ± 1.81	19.76 ± 1.5	21.3 ± 1.2
2%	2.4 ± 0.39	12.52 ± 1.73	13.08 ± 1.48	14.96 ± 3.13	18.98 ± 0.33	20.43 ± 1.25
3%	2.76 ± 0.49	6.06 ± 1.85	5.98 ± 0.88	7 ± 3.66	17.98 ± 0.77	18.76 ± 0.81

Fresh weight of whole plant becomes decreased gradually from control to 3% in all intervals. During 10th day, 23% to 58% loss in weight was recorded in various treatments. Up to 35% and 20% depletion was noticed in 3% on 30th day and 40th day (Table 5).

TABLE 5: Impact of EMS on Fresh weight of Bhindi

Treatments	10th day	30th day	40th day
Control	0.59 ± 0.18	2.06 ± 0.68	5.03 ± 0.7
0.50%	0.396 ± 0.11	1.86 ± 0.46	4.67 ± 0.84
1%	0.42± 0.09	1.76 ± 0.64	4.45 ± 0.34
2%	0.45 ± 0.13	1.7 ± 0	4.23 ± 0.6
3%	029 ± 0.06	1.35 ± 0.38	4.01 ± 0.23

Shoot growth coefficient (GC) in length in all days of experimental period showed a negative trend. At the early phases of 4th, 6th and 8th day, control has the maximum value (Fig 1, 2 and 3). Maximum retardation in growth was viewed in plants treated with 3% EMS, with 80%, 51% and 71% decrease in their growth in those days. Suppression of 17 to 30% growth between 10th and 30thdays and10 to 26% growth between 30th to 40th days was registered in 3% EMS (Fig 4, 5 and 6). Growth coefficient (GC) in length of root also decreased from that of the control to 3% of EMS. Seventy five percentage, 69%, 76%, 77%, 43% and 75% reduction were calculated in 3% of EMS in 4th, 6th, 8th, 10th, 30thand 40thday respectively (Fig 1-6).

Chemical mutagens generally produce induced mutations, which lead to base pair substitution especially GC to AT resulting in amino acid changes, which changes the function of proteins, but do not abolish their functions. These chemo mutagens also induce a broad variation of morphological and yield structure change in comparison to normal plants [3].

Tolerance Index is an integrated calculation of particular parameters, and make for a summary assessment of effect of stress factor on plant growth and development. Index values of tolerance were gradually decreasing with increasing doses

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of EMS (Table 6). Present investigation also established the inhibition of relative growth rate. Maximum inhibition in growth rate was recorded in 3% (Fig 7). From lower concentration to higher concentrations, net productivity (Fig N) showed a linear negative trend. Previous studies in finger millet [20] were in unison with present investigation. Significant decrease in these parameters of mutant plants compared with that of the control was reported in wheat [21] and banana [22].

TABLE 6: Impact of EMS on Tolerance Index of Bhindi

Treatments	10th day	30th day	40th day
Control	100 ± 0	100 ± 0	100 ± 0
0.50%	73.68 ± 0.63	71.42 ± 0.67	58.22 ± 0.57
1%	80 ± 0.57	50.69 ± 0.46	50.05 ± 0.82
2%	42.1 ± 0.82	25.59 ± 0.36	33.9 ± 0.57
3%	26.31± 0.33	11.9 ±0.19	9.7 ± 0.8

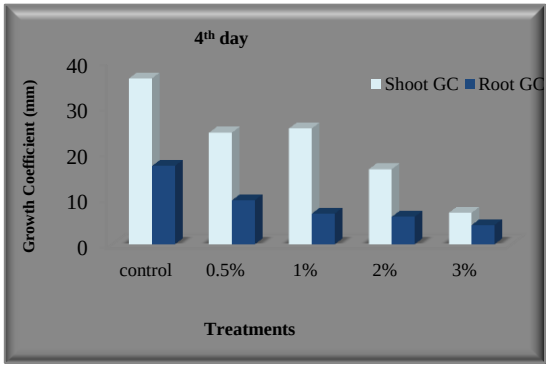

Fig 1:Impact of EMS on GC (4th day)

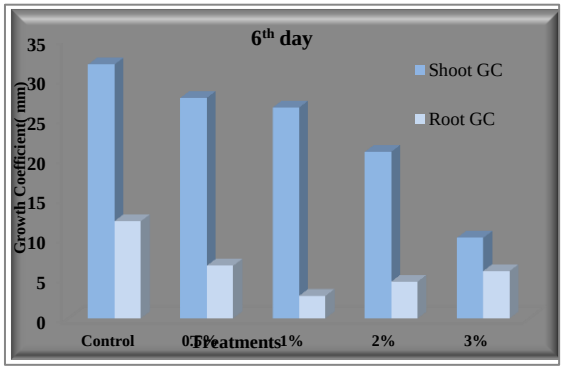

Fig 2: Impact of EMS on GC (6th day)

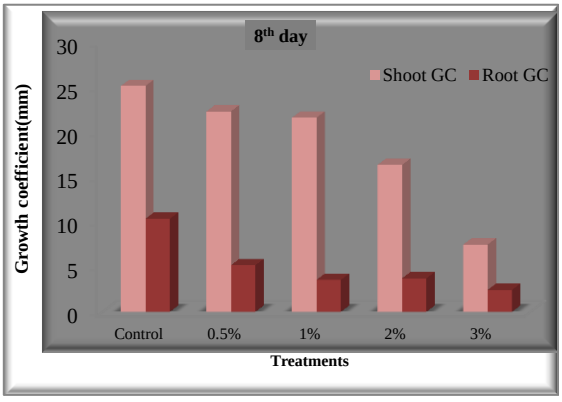

Fig 3: Impact of EMS on GC (8th day)

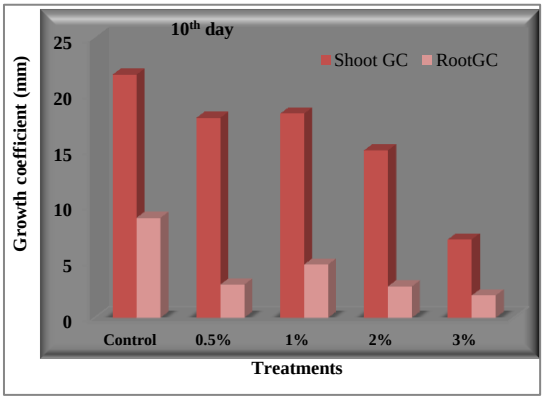

Fig 4: Impact of EMS on GC (10th day)

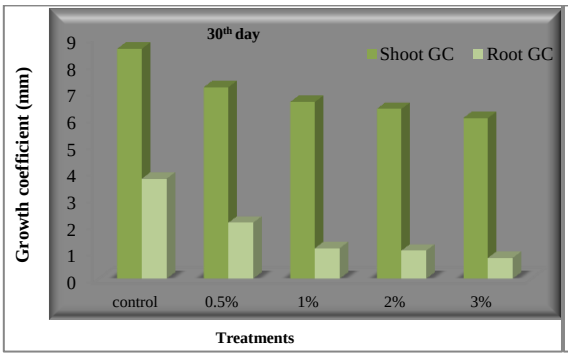

Fig 5: Impact of EMS on GC (30th day)

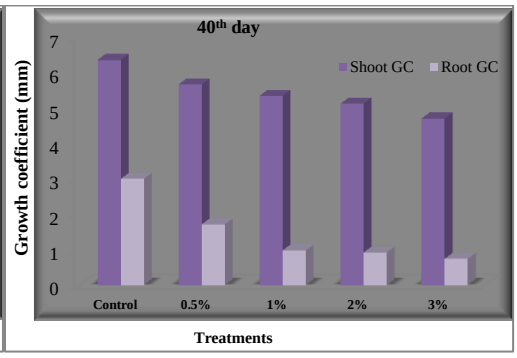

Fig 6: Impact of EMS on GC (40th day)

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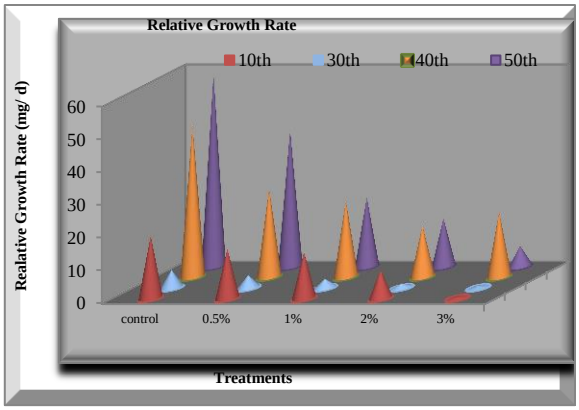


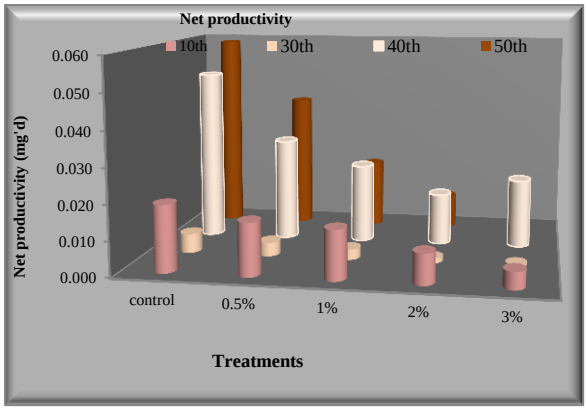


Fig 7: Impact of EMS on RGR

Fig N: Impact of EMS on Net Productivity

Anatomical parameters registered a marked diminution along with increasing dose of treatment in root and shoot on 20th and 50th days of analysis (Plate :1). Changes mainly occur in the number and dimensions of secondary xylem, secondary phloem, medullary rays, periderm and pith. In 3% EMS, the amount of lignified cells like secondary xylem vessels, secondary xylem trachieds were greatly shrinked. Secondary phloem region, secondary phloem fibres, number of medullary rays, length and width of medullary rays and periderm formation compressed considerably. The application of EMS dramatically shrank secondary xylem and secondary phloem region. EMS induces the formation of soft parenchyma cells.

Plate 1: Impact of EMS on Anatomy of Bhindi Stem (20th Day)

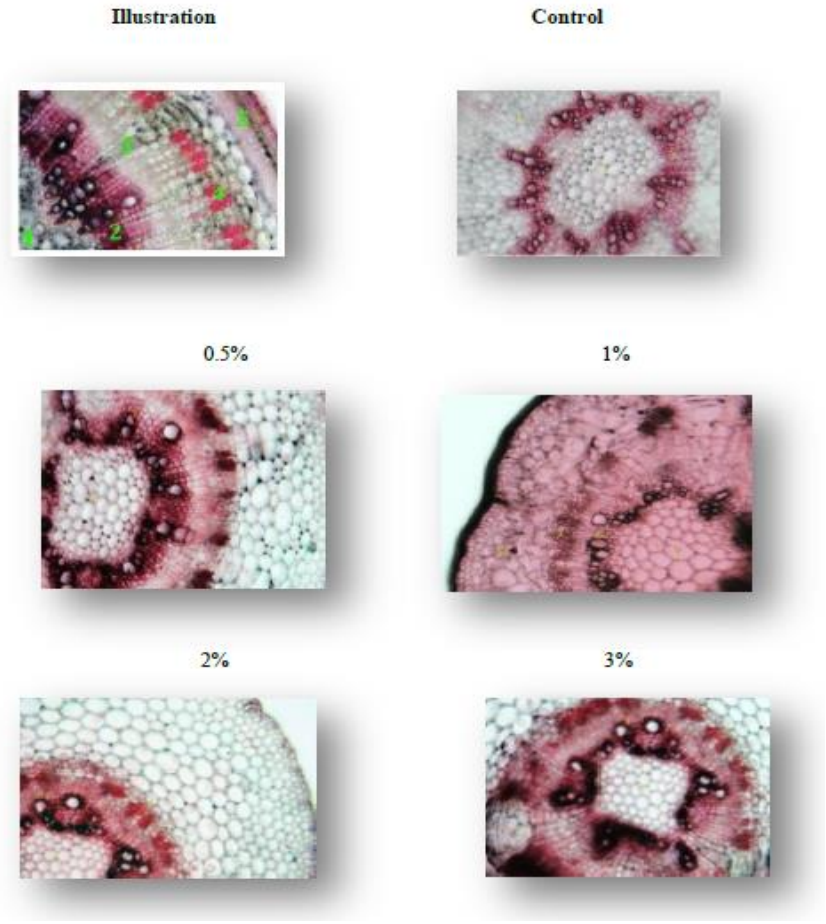
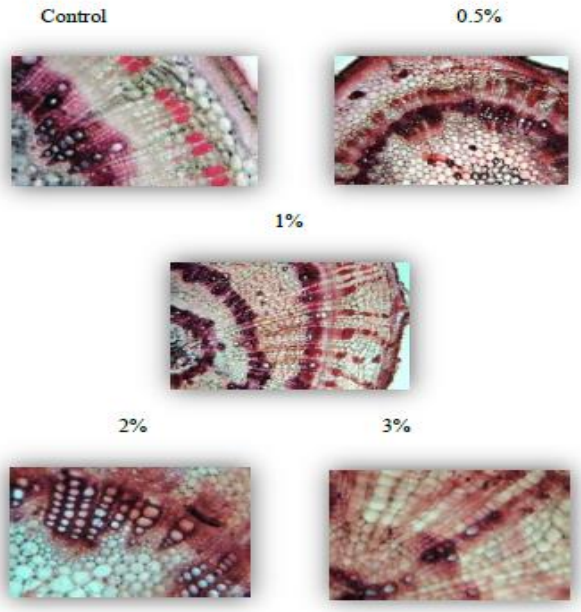


Illustration: - 1) pith 2) secondary xylem 3) medullary ray 4) secondary phloem 5) periderm

Plate 2: Impact of EMS on Anatomy of Bhindi Stem (50th Day)



Leaf area was observed maximum in the control plants and it progressively decreased in treatment categories (Table 7). This result corroborated with the findings in *Capsicum annuum* [23] and *Avena sativa* [24]. Mutagen inhibits the leaf area [25]. It is obvious from the current findings that with an increase in the chemical concentration, there was also an increase in the rate of mutation leading to deleterious effects. Leaf abnormalities were attributed to the chromosomal breakage, disturbed auxin synthesis, disruption of mineral metabolism and accumulation of free amino acids [26].

Chlorophyll studies provide one of the most dependable indices of mutagenic treatments. Chlorophyll a, b and total chlorophyll diminished proportionately with increasing doses of EMS (Table 7). EMS treatment diminished the total chlorophyll content as reported in safflower [27].

Plate 3: Impact of EMS on Anatomy of Bhindi Root (50th Day)

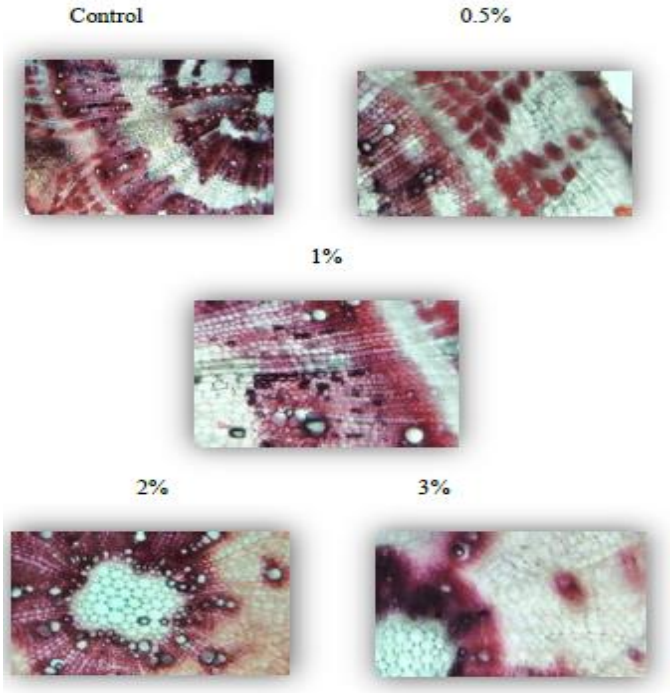


Table 7: Impact of EMS on Leaf area and Chlorophyll content of Bhindi

	Leaf area in mm^2		Chlorophyll content on 40^{th} day		
Treatments	10^{th} day	40^{th} day	Chlorophyll a mg/g (fw)	Chlorophyll b mg/g (fw)	Total chlorophyll mg/g (fw)
Control	78.44±14.43	327.03±17.87	0.896	0.827	1.723
0.50%	40.4 ± 8.65	292.8 ± 7.45	0.85	0.819	1.669
1%	62.86 ± 7.95	197.5± 13.29	0.781	0.785	1.566
2%	42.3 ± 4.82	84.03 ± 1.71	0.765	0.775	1.54
3%	39 ± 2.62	56.66 ± 2.3	0.761	0.715	1.476

The mutagenic action of EMS results from its reaction with DNA by alkylating the phosphate groups [28]. Alkylation of a phosphate can cause breakage of the linkage between deoxyribose and phosphate. EMS shows more specification to guanine and cytosine and specifically N-7 position of guanine is highly susceptible to alkylation by the mutagen [29]. Incorporation of alkyl group into a base may result in the formation of a gap in the DNA template [30] and subsequent replication defects leading to mutations.

4. CONCLUSION

Results revealed even lower concentration can induce mutation in Bhindi. The efficiency of EMS was found to depend upon its concentration and it was deleterious in all parameters studied even at 1% EMS treated for four hours. No beneficial mutant was obtained from this study.

REFERENCES

[1] Okagaki RJ, Neuffer MG and Wessler SR (1991). A deletion common to two independently derived waxy mutations of maize. Genetics 425-431.

[2] Bhat R, Upadhyaya N , Chaudhury A, Raghavan C, Qiu F, Wang H, Wu J, McNally K, Leung H and Till B (2007). Chemical and irradiation induced mutants and tilling. In: N. M. Upadhyaya, Ed. Rice functional genomics: challenges progress and prospects. pp 148-180.

[3] Greene EA (2003). Spectrum of chemically induced mutations from a large scale reverse genetic screen in *Arabidopsis.* Genetics 164(2): 731-740.

[4] Washington WJ and Sears ER (1970). Ethyl methanesulfonate induced chlorophyll mutations in *Triticum aestivum.* Can J Gen Cyto 12(4): 851-859.

[5] Yogananda Murthy VN, Ramesh HL and Munirajappa RK (2007). Ethyl methane sulphonate induced morphological variations in mulberry (*Morus*) variety M5. Int J Plant Sci 2 (2): 219-220.

[6] Sandile TH (2012). Ethyl methanesulfonate mutagenesis in selected *Vernonia galamensis* variety *ethiopica* lines. Dissertation submitted in fulfillment of the requirements for the degree of Master of Science in agriculture (crop science). South Africa.

[7] Jagajanantham N, Dhanavel D, Gnanamurthy S and Pavadai P (2013). Induced on chemical mutagens in Bhindi [*Abelmoschus esculentus* l. moench]. Int J Curr Sci 5: 133-137.

[8] Dhulgande GS, Ghogare DS and Dhale (2015). Mutagenic effect on seed germination, seedling growth and seedling survival of pea (*Pisum sativum* L.). Int J Curr Res Biosci Plant Biol 2(4): 59-64.

[9] Dhakshanamoorthy D, Selvaraj R and Chidambaram A (2010). Physical and chemical mutagenesis in *Jatropha curcas* to induce variability in seed germination, growth and yield traits. Rom J Biol Plant Biol 55: 113–125.

[10] Ali BT, Amin BT and Behzad S (2012). Ethyl methanesulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination. Ame J Plant Sci 3:1661-1665.

[11] Rupinder S and Kole C (2005). Effect of mutagenic treatment with EMS on germination and some seedling parameters in mung bean. Crop Res 30(2): 236-240.

[12] Kumar G and Yadav RS (2010). EMS Induced genomic disorders in Sesame (*Sesamum indicum* L.). Rom J Biol Plant Biol 55(2): 97–104.

[13] Haq MA, Singh KB, Abidin Z and Ahmad MS (1992). Mutation studies in chickpea (*Cicer arietinum* L.). Mutagen sensitivity. Pak J Agri Sci 29(4): 429-438.

[14] Ya Ping Y (1996). Studies on damage effects of EMS in *Coix lacryma-jobi* L. J Jilin Agri Uni 18(1):18-20.

[15] Borzouei M and Kafi H (2010). Effects of gamma radiation on germination and physiological aspects of wheat (*Triticum aestivum* L.) Seedlings. Pak J Bot 42(4): 2281-2290.

[16] Kalia CS, Kharakwal MC, Singh MC and Vari AK (2001). Mutagenic effects of environmental industrial chemical agents in inducing cytogenetical changes in wheat. Ind J Genet 6(3): 203-208.

[17] Amernath S and Prasad AB (1998). Induced variability in homozygous and heterozygous genotypes of tobacco. Ind J Gen58 (1): 69-77.

[18] Gaul H (1977). Mutagen effects observable in the first generation, I. plant injury and lethality, II. Cytological effects, I1I sterility In: Manual on mutation breeding (second edition) IAEA technical report series 119: 85-99.

[19] Neary GJ, Evans HJ and Tonkinson SJ (1957). Mitotic delay induced by gamma radiation in Broad Bean root meristems. Nature 179: 917-918.

[20] Eswari K, Gogulan G, Hari Prathab KA and Sakila M (2014). Development of early maturing mutants in finger millet. Res J Agri Fors Sci 2(6):1-9.

[21] Bilge B and Mahinur SA (2009). Effects of EMS treatment on the seed germination in wheat. J Appl Biol Sci 3(1): 59-64.

[22] Siamak SB, Sariah M, Zakaria W, Sreeramanan SM and Maziah M (2012). *In vitro* selection and characterization of water stress tolerant lines among Ethyl methane sulphonate (EMS) induced variants of banana (*Musa* spp) with AAA genome. AJCS 6(3): 567-575.

[23] Patil AN, Meshram LD and Nandanwar RS (1997). Induced quantitative variation in economic characters by chemical mutagens in chilli. J Soils and Crop 7: 15–8.

[24] Krishna Murthy CS and Vasudevan K (1984). Induced polygenic variation following single and combination treatment with Azide, EMS and Gamma rays in oats. Crop Imp 11: 128–31.

[25]] Nusrat S and Bushra M (2002). Ethyl methanesulfonate induced genetic variability in *Lycopersicon esculentum.* Int J Agri Biol 04(1): 89–92.

[26] Gunkel JK and Sparrow AH (1961). Ionizing radiations: biochemical, physiological and morphological aspects of their effect on plants. In: W. Rubland (ed.). Encyclopedia of plant physiology, Springer, Berlin 16: 555-611.

[27] Preeti S and Jitendra P (2012). LICF Spectrum as a fast dectector of chlorophyll damage in safflower growing under mutagenic stress. World J Agri Sci 8(3): 322-325.

[28] Heslot H (1965). The nature of mutations. Rad Bot 5: 3-45.

[29] Bautz E and Freese E (1960). On the mutagenic effects of alkylating agents. Proc Nat Acad Sci 46: 1585 -1597.

[30] Kreig DR (1963). EMS induced reversion of bacteriophage T4 rII mutants. Gen 48: 561- 570.