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Research Article

Studies on the Effect of Maleic Hydrazide on Root Tip Cells and Pollen Fertility in *Trigonella foenum-graecum* L.

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Abstract: In the present investigation, the effect of maleic hydrazide (MH) has been studied on seed germination, mitosis, and pollen fertility of *Trigonella foenum-graecum* L. to test its cytotoxicity. Six different concentrations (0.01%, 0.02%, 0.03% 0.04%, 0.05%, and 0.06%) of maleic hydrazide (MH) were given at seed level. The abnormalities noticed were both physiological and clastogenic. Fragments, stickiness of chromosomes, univalents, multivalents, and anaphase bridges were the most frequent abnormalities. The decrease in seed germination and pollen fertility was found significant at 0.05 level. Mitotic index reduced gradually as the concentrations of Maleic Hydrazide increased and it was found significant at 0.01 and 0.05 levels.

Key Words: Cytotoxicity, Fenugreek, Maleic Hydrazide (MH), Mitotic Index (MI)

Introduction

Fenugreek (*Trigonella foenum-graecum L.*), an annual legume cash crop of India (Fazli & Hardman, 1978), commonly known as "methi", is cosmopolitan in distribution, believed to be native to the Mediterranean region, and now widely cultivated in most parts of the world (Acharya et al., 2006). It can also be efficiently incorporated into short-term crop rotations in western Canada (Moyer et al., 2003). Fenugreek has been reported to generate a high yield of good quality forage and hay or silage (Mir et al., 1998). In addition, the small inconspicuous flowers from fenugreek had a 4% success rate when crossed (Choudhury & Singh, 2001), making hybridization a less attractive route for crop improvement. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement (Acharya et al., 2007). Some legume crops that have been improved through mutation breeding are soybeans, string beans and French beans (Sigurbjornsson & Micke, 1974); navy pea beans and haricot beans (Sigurbjornsson, 1983); peas and fenugreek (Petropoulos, 1973, 2002); and lupines (Gaul, 1961). A majority of induced mutations in these plants are recessive, and can be observed to segregate in a 3:1 ratio in diploid crops like fenugreek (Gaul, 1961; Petropoulos, 1973; Singh & Singh, 1974).

Maleic hydrazide (1, 2-dihydro-3, 6-pyridazinedione) has been introduced into agriculture as a major commercial herbicide and a depressant of plant growth. The extensive use of herbicides in agriculture and their potential carcinogenicity strongly suggest the need to extend the cytotoxic evaluation of these compounds by using different methods. Here we report a cytotoxic evaluation of the herbicide maleic hydrazide in root tip cells and on pollen fertility.

Mode of action of maleic hydrazide presumably involved interference with sulphydryl groups which reduced the synthesis of those enzymes in which SH-groups were required. Therefore, this chemical affects SH-bond in some way as a consequence of which the proteins necessary for the formation of spindle apparatus are also affected (Khan, 1997). Chromosome breakage effects of MH in plants were first described by Darlington and McLeish in 1951. MH also resulted in the inhibition of spindle formation and chromosome breakage during

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mitosis in root tips of onion and barley (Kaul & Choudhary, 1975).

Maleic hydrazide is a pyridazine that inhibits the synthesis of nucleic acids and proteins (De Marco et al., 1992). It is a well-known clastogenic agent in plants (Swietlinska & Zuk, 1978) that induces chromosomal aberrations and sister-chromatid exchanges (SCE) in root-tip cells of *V. faba* (Evans & Scott, 1964; Kihlman, 1975).

The present work was designed to study the cytotoxic effect of the maleic hydrazide (MH) herbicide using *Trigonella foenum-graecum L.* as a biological system.

Materials and Methods

Dry and healthy seeds of *Trigonella foenum-graecum* were pre-soaked in distilled water for 12 h, treated with Maleic Hydrazide (MH) at 6 different concentrations (0.01%-0.06%), and prepared in sodium phosphate buffer with 7.0 pH for 8 h with constant intermittent shaking. The treated seeds were washed in running tap water 2 to 3 times to remove the residual effect of the mutagen sticking to the seed coat. One set of the seeds was kept untreated to act as control for comparison. Hundred seeds were used from each dose and control. In order to find out the effect of MH on seed germination and root tip collection, 50 seeds were spread over moist cotton in petri plates and kept in a B.O.D. incubator at 24±2 °C temperature. Other sets of 50 seeds of control and 6 treatments of maleic hydrazide were sown separately in a randomized pot design with 3 replicates for the study of pollen fertility in control and treated populations in M_1 generation.

The root tips were excised and pre-treated with 0.02% Para-dichlorobenzene for 3 h, washed with distilled water, fixed in glacial acetic acid: ethanol (3:1) for 24 h, again washed with distilled water and stored in 70% alcohol. For cytological observations, the fixed roots were hydrolyzed at 60 °C for 15 min. in 1/10 N HCl and stained in Leuco Basic Fuchsin for 10-20 min. (Darlington and La Cour, 1976). The deeply stained region of root tips was squashed in 1% acetocarmine. Photomicrographs were taken from both temporary as well as permanent slides.

Mitotic index (MI), relative division rate (RDR) and relative abnormality rate (RAR) were calculated by

$$MI = \frac{\text{Total number of dividing cells}}{\text{Total number of cells examined}} \times 100$$

$$RDR = \frac{\text{Percentage of dividing cells in treated root-percentage of dividing cells in control root tips}}{\text{100-Percentage of dividing cells in control seedlings}} \times 100$$

$$RAR = \frac{\text{Percentage of dividing cells in root tips-percentage of dividing cells in control tips}}{\text{100-Percentage of dividing cells in control root tip cells}} \times 100$$

Lethal Dose-50 (LD50)

LD_{50} (dose reducing germination up to 50%) was determined for maleic hydrazide on the basis of the total number of seed germinated in a treatment. Seeds giving rise to both radicle and plumule were considered as germinated. For each treatment and control, the percentage of seed germination was calculated by the following formula:

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds placed for germination}} \times 100$$

After having calculated the germination percentage, LD_{50} dose was determined by the graph plotting the germination percentage along Y-axis against the concentration of the maleic hydrazide along X-axis, and the value of the LD_{50} dose was worked out with the help of the germination curve.

Statistical Analysis

The experimental data were statistically analyzed using the analysis of variance techniques according to Gomez and Gomez (1984). In applying the F test, the error due to replicates was also determined. When the F value was found to be significant at 1% and 5% level of probability, the least significant difference (LSD) was calculated.

Observations

Maximum germination was recorded in control (98%), whereas it decreased in treated populations from 92%-60% in (0.01%-0.06%) maleic hydrazide. The LD_{50} dose was 0.078% for maleic hydrazide treatments. Pollen fertility also decreased with the increasing concentrations of the mutagen. The mitotic index in control was observed to be maximum (19.30%), but decreased from 17.90%-5.84% in 0.01%-0.06% maleic hydrazide concentrations (Table 1). The relative divisional rate (RDR) following cytotoxic effect of mutagen came down from 1.74% to 16.68% in 0.01% to 0.06% maleic hydrazide. Consequently, the relative abnormality rate (RAR) out of the total number of cells increased as the concentrations of the mutagen increased but it did not show any linear dependence and the relative abnormality rate out of the total number of dividing cells increased significantly from 4.17% to 71.43% in 0.01%-0.06% MH (Table 1).

The behaviour of chromosomes at prophase and metaphase and their separation at anaphase and telophase was normal in control (Figure 1). As a result of MH treatment, multivalent formation at metaphase in 0.02% concentration (Figure 2), stickiness and precocious separation in 0.04% concentration (Figure 3), clumping of chromosomes at metaphase in 0.06% concentration (Figure 4), fragments in 0.03% concentration (Figure 5), and single & double bridges at anaphase in 0.04% and 0.05% concentrations (Figures 5 and 6, respectively) occurred in different frequencies and increased with increasing concentrations of mutagen (Figures 1-6).

Discussion

The reduced germination due to inhibitory effect of chemical mutagens as observed in *Trigonella foenum-graecum* L. has also been reported in same plant by Laxmi and Gupta (1983), Jain and Agarwal (1993), Siddiqui and Hasan (2003), Siddiqui et al. (2007) and Siddiqui et al. (2008), and also in *Vicia faba* L. by Agarwal and Ansari (2001).

Several other mutagenic agents and heavy metals have been shown to inhibit the seed germination (Seregin & Kozhevnikova, 2006). Several factors such as oxygen concentration, light, moisture level, and incubation temperature are known to have an influence on seed germination (Isabelle et al., 2000).

Griffiths and Johnson (1962) and Srivastava (1979) considered that reduction in germination percentage was due to weakening and disturbance of growth processes

Table 1. Germination percentage, pollen fertility, and mitotic studies in Maleic Hydrazide treated *Trigonella foenum-graecum* L. (Mean of replicates with standard deviation).

Treatments (%)	Seed germination (%)	Percentage Inhibition	Pollen fertility (%)	Relative Red. in Pollen fertility	Mitotic Index (%)	Relative Division Rate (RDR)	Relative abnormality rate (RAR) as compared to total number of cells observed	Relative abnormality rate (RAR) as compared to total number of dividing cells
Control	98±2.45	–	99.2±2.48	–	19.30±0.51	–	–	–
0.01%	92±2.30	6.13**±0.15	97.4±2.44	1.82**±0.05	17.90*±0.48	-1.74*±0.04	0.75**±0.02	4.17*±0.10
0.02%	80**±2.00	18.37**±0.46	91.7±2.92	7.56**±0.19	16.50**±0.45	-13.47**±0.34	1.73**±0.04	10.52*±0.26
0.03%	76**±1.90	22.45**±0.56	88.9**±2.22	10.38**±0.26	13.30**±0.41	-7.44**±0.19	3.34**±0.08	25.00**±0.63
0.04%	74**±1.85	24.49**±0.61	82.7**±2.07	16.64**±0.42	7.70**±0.33	-14.38**±0.36	5.17**±0.13	66.67**±1.67
0.05%	65**±1.63	33.67**±0.84	78.9**±1.97	20.46**±0.51	6.70**±0.19	-15.65**±0.39	4.45**±0.11	66.67**±1.67
0.06%	60**±1.50	38.78**±0.97	71.1**±1.78	28.32**±0.71	5.84**±0.17	-16.68**±0.42	4.16**±0.10	71.43**±1.79
LSD at 5% (*)	7.13	2.09	7.89	1.32	1.26	0.99	0.28	3.90
LSD at 1% (**)	8.77	2.57	9.71	1.62	1.54	1.22	0.35	4.80

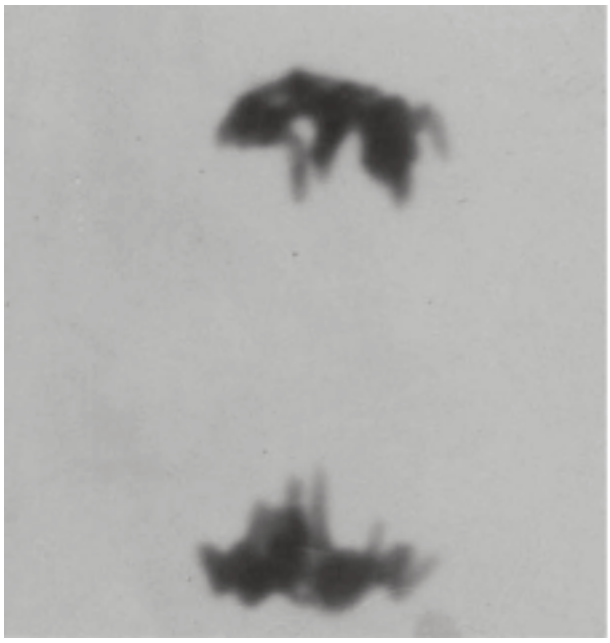

Figure 1. Root tip cell at normal anaphase with 8 chromosomes at each pole (control).

Figure 2. Root tip cell at metaphase showing 2-IV and 8-I formation (0.02%).

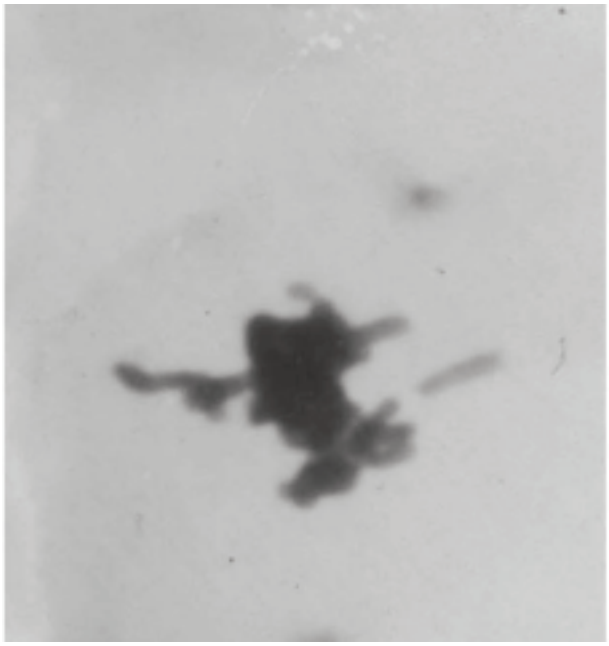

Figure 3. Root tip cell at metaphase showing stickiness and precocious separation of chromosomes (0.04%).

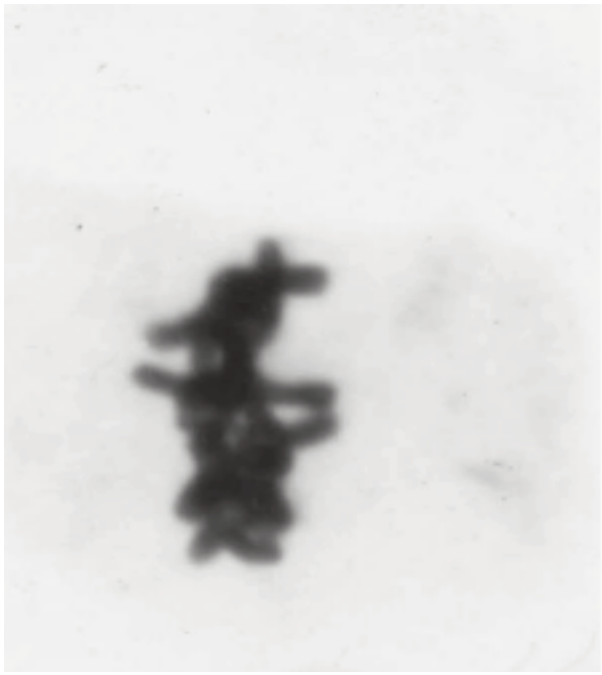

Figure 4. Root tip cell at metaphase showing clumping of chromosomes (0.06%).

Figure 5. Root tip cell at anaphase showing bridge, fragment and stray chromosome formation (0.04%).

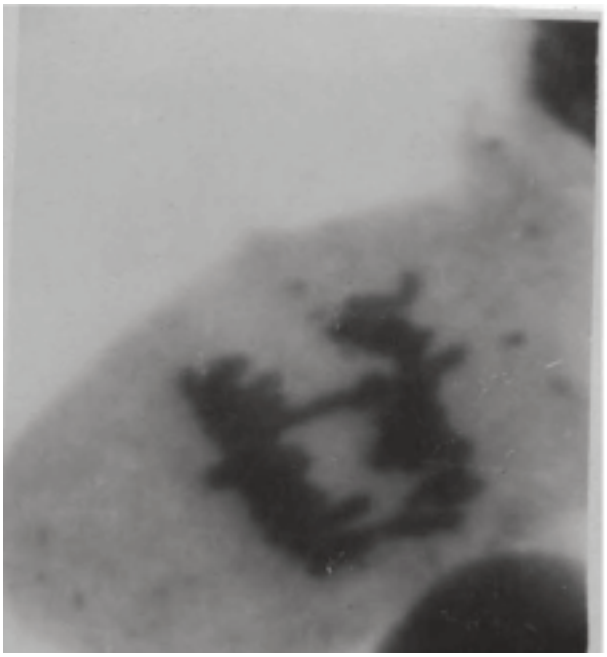

Figure 6. Root tip cell at anaphase showing double bridge formation (0.05%).

resulting in early elimination of seedlings. The inhibition of growth regulators and metabolic disturbances during germination may also be one of the reasons (Sideris et al., 1971).

The pollen fertility decreased significantly as seen in mitotic index (MI) with the increasing concentrations of maleic hydrazide. Ghareeb and George (1997) suggested that Temik, an insecticide, may be found to cause an identical effect on mitosis and meiosis, increasing similar anomalies. Armbruster et al. (1991) also suggested that the induction of meiotic and mitotic abnormalities seems to be a common effect of most pesticides. In these experiments, it was observed that the chromosomal abnormalities in pollen mother cells (PMCs) increased proportionally to the increased mitotic aberrations. Hence the increased heterozygosity in PMCs resulted in decreasing pollen fertility. Chary and Bhalla (1988) and Kumar et al. (2003) also reported an increase in pollen sterility with an increase in mutagenic treatment. Rana and Swaminathan (1964) and Ramanna (1974) reported that any deviation in karyokinesis or cytokinesis could produce non-viable microspores. Sinha and Godward (1969, 1972) described translocations to be responsible for decreased pollen fertility.

The mitotic index is a reliable predictor of the cell proliferation in the tissue. Compared to control, a decrease in the mitotic index in root tip meristems of *Trigonella foenum-graecum* was observed in treated populations. The mitodepressive effect of the pesticide may be due to the interference of pesticide in the normal process of mitosis by reducing the number of the dividing cells (Habib et al., 1988). The inhibition in mitotic index (MI) may be due to the interference of Temik pesticide in the normal sequence of cell division, which prevented or reduced the number of the cells to enter prophase stage (Ghareeb & George, 1997). Concerning with the reduction of MI, several pesticides belonging to different chemical groups were found to cause an inhibition of cell division, such as Gespax (Badr et al., 1985), Sencor (Haliem, 1990), and Cycluron (Ghareeb, 1991).

As a result of mutagenic treatment of seed with maleic hydrazide, the root tip cells of *Trigonella foenum-graecum* showed varying degrees of mitotic irregularities like stickiness, univalents, multivalents, fragments, precocious separations of chromosomes, bridges with or without fragment which generally increased with the increasing concentrations of MH. Several mutagens with mutagenic activity have been shown to induce such changes in the

chromosomes of plant cells (Amer & Ali, 1969, 1974). All the pesticidal treatments impaired spindle mechanisms to varying degrees leading to chromosome vagrancies as well as C-metaphase. Inhibition of ATPases by the pyrethroids might be the cause of spindle disorganization (Reddy & Bashamohideen, 1991). Precocious separation was also observed in tomato (Bose & Saha, 1970) and concluded that univalents separating precociously seemed to be as a result of desynapsis. According to Roy et al. (1971) precocious separation of bivalents in *Cucumis sativus* L. was attributed to the failure of chiasma formation in pairs.

Rao and Laxmi (1980) attributed univalent formation to the partial and complete lack of homologous chromosome pairing. According to Katiyar (1978) alteration in the chromosome associations, composed of uni-, tri-, tetra-, and multi-valent formations, were possibly the outcome of the non- or irregular pairing of chromosomes due to translocations.

The stickiness of chromosomes was the most common abnormality observed during the present investigation. Bivalents were found clumped into a single group due to stickiness. Similar results have also been reported by Abbasi and Anis (2002) in *Trigonella foenum-graecum.* Stickiness could be due to depolymerization of nucleic acid caused by mutagenic treatment. Stickiness in chromosomes is a result of partial dissociation and altered pattern of organization of nucleoprotein (Evans, 1962).

Presence of chromosome bridges may be due to stickiness or formation of dicentric chromosomes caused by breakage and reunion (Bempong, 1973). The stickiness of chromosomes makes their separation and free movement incomplete and thus they remain connected by bridges (Kabarity et al., 1974). This may also be due to the defective formation of the spindle apparatus (Badr, 1986; Abraham & Rajalakshmy, 1989).

Fragmentation might have arisen due to stickiness of chromosomes and consequent failure of separation of chromatids to poles and was also observed by many workers (Amer, 1965; Permjit & Grover, 1985 etc.). Fragments may also be acentric chromosomes that are formed as a result of inversion.

It may be concluded that MH causes toxic effect on root tip cells and other vegetative, as well as spore mother cells of *Trigonella foenum-graecum*. This toxicity induces different types of genic and chromosomal variations and enhances the rate of variations in morphological and yield characters for selection of better qualities and also increases the speed of evolution.

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References

Abbasi N & Anis M (2002). Clastogenic effect of chemical mutagens in *Trigonella foenum-graecum L. J Cytol Genet* 3 (NS): 109-114.

Abraham S & Rajalakshmy BN (1989). Production of mitotic abnormalities by magnesium sulphate in *Vicia faba* L. *Cytologia* 54: 559-563.

Acharya S, Srichamroen A, Basu S, Ooraikul B & Basu T (2006). Improvement in the nutraceutical properties of fenugreek (*Trigonella foenum-graecum L.*). *Songklanakarin J Sci Techno* 28-1:1–9.

Acharya SN, Thomas JE & Basu SK (2007). Improvement in the medicinal and nutritional properties of fenugreek (*Trigonella foenum-graecum L.*). In: Acharya SN, Thomas JE (Eds.) *Advances in Medicinal Plant Research*, Research Signpost, Trivandrum, Kerala, India (In press)

Agarwal R & Ansari MYK (2001). The effect of Aniline on root tip cells of *Vicia faba* L. *J Cytol Genet* 2 (NS): 129-134.

Amer SM (1965). Cytological effects of pesticides. I. Mitotic effects of N-methyl-1-napthyl carbonate "Sevin". *Cytologia* 30: 175-181.

Amer SM & Ali EM (1969). Cytological effects of pesticides. IV. Mitotic effects of some phenols. *Cytologia* 34: 1-4.

Amer SM & Ali EM (1974). Cytological effects of pesticides. V. Effects of some herbicides on *Vicia faba*. *Cytologia* 39: 633-643.

Armbruster BL, Molin WT & Bugg MW (1991). Effects of the herbicide Dithiopyr on cell division in wheat root tips. *Pesticide Bio-chem Physiol* 39: 110-120.

Badr A (1986). Effects of the S-Triazine herbicide tubutryn on mitosis chromosomes and nucleic acids in root tips of *Vicia faba*. *Cytologia* 51: 571-577.

Badr A, Hamoud MA & Haroun SA (1985). Effect of the herbicide "Gespax" on mitosis, mitotic chromosomes and nucleic acid in *Vicia faba* L. root meristems. *Proc Saudi Biol Soc* 8: 359-369.

Bempong AM (1973). Cytological effects of nogalmycin in *Tradescantia paludosa* microsporocytes. *Mutation Res* 21: 323-326.

Bose S & Saha A (1970). Cytogenetic effects of X-rays and chemicals on tomato studies in M_2 generation. *The nucleus* 13: 29-40.

Chary SN & Bhalla JK (1988). Mutagenic effectiveness and efficiency of gamma rays and EMS on pigeon pea (*Cajanus cajan* (L) Millsp). *J Cytol Genet* 23: 174-182.

Choudhury AK & Singh VV (2001). Inheritance pattern and growth habit in fenugreek (*Trigonella foenum-graecum* L.). *Indian J Genet* 61(4):369–371.

Darlington CD & La Cour LE (1976). The Handling of Chromosomes. 6th Edition. Allen and Unwin, London. p. 201.

Darlington CD & McLeish J (1951). Action of maleic hydrazide on the cell. *Nature* (London). 167: 407-408.

De Marco A, De Simone C, Ragglione M, Testa A & Trinca S (1992). Importance of the type of soil for the induction of micronuclei and the growth of primary roots of *Vicia faba* treated with the herbicides atrazine, glyphosphate and maleic hydrazide. *Mutat Res* 279: 9-13.

Evans HJ & Scott D (1964). Influence of DNA synthesis on the production of chromatid aberrations by X-rays and maleic hydrazide in *Vicia faba. Genetics* 49: 17-38.

Evans HJ (1962). Chromosomal aberrations induced by ionizing radiations. *Int Rev Cytol* 13: 221-321.

Fazli FRY & Hardman R (1978). The spice fenugreek (*Trigonella foenum–graecum*), its commercial variety of seed as a source of diosgenin. *Trop Sci* 10: 66-78.

Gaul H (1961). Mutation and plant breeding. In: *Proceedings of the Symposium on the Mut Plant Breed*, Nov–Dec, Cornell, USA

Ghareeb A (1991). Cytological effect of the herbicide Cycluron on root tips mitosis and nucleic acids in *Vicia faba. Egypt Sci,* Zagazig Univ. 6: 225-231.

Ghareeb A & George NM (1997). Cytotoxicity of insecticide Temik 15 G (Decarb) in mitotic and meiotic cells of *Vicia faba* plant. *Cytologia* 62: 259-263.

Gomez KA & Gomez AA (1984). Statistical Procedure for Agricultural Research. 2nd Edition, John Wiley and Sons, New York.

Griffiths DJ & Johnson TD (1962). The use of irradiation technique in oat breeding. *Radiat Bot* 2: 41-51.

Habib AA, El-Bayourni AA & Eweda MA (1988). Effcet of the fungicide Bupirimate on the mitotic activity in meristematic cells and germination rate of *Allium cepa* and *Vicia faba* seeds. *Ain Shams Sci Bull* 27: 261-283.

Haliem AS (1990). Cytological effects of the herbicide Sencor on mitosis of *Allium cepa. Egypt J Bot* 33: 93-104.

Isabelle D, Leon-Kloosterziel KM, Koomneel M (2000). Influence of the testa on seed dormancy, germination and longevity in *Arabidopsis. Plant Physiol* 122: 403-413.

Jain SC & Agarwal M (1993). Change in the morphological characters in *Trigonella foenum-graecum* L. due to chemical mutagen. *J Indian Bot Soc* 72: 277-280.

Kabarity A, El-Bayourni AA & Habib AA (1974). Effect of morphine sulphate on mitosis of *Allium cepa* L. root tips. *Biol Plant* 16: 275-282.

Katiyar RB (1978). Radiocytogenetical studies on *Capsicum*. Meiotic anomalies. *Cytologia* 43: 415-421.

Kaul BL & Choudhary DK (1975). Thiol modification of the biological damage of some non-alkylating mutagens. *Sci Cult* 41: 82-85.

Khan S (1997). Concepts in mutagenesis. In: Siddiqui BA & Khan S (Eds.) Plant Breeding Advances and in vitro Culture. 1st Edition. CBS Publishers and Distributors, N. Delhi, India. p. 98.

Kihlman BA (1975). Root tips of *Vicia faba* for the study of the induction of chromosomal aberrations. *Mutat Res* 31: 401—412.

Kumar G, Kesarwani S & Sharma V (2003). Clastogenic effect of individual and combined treatment of gamma rays and EMS in L*ens culinaris. J Cytol Genet* 4 (NS): 149-154.

Laxmi V, Gupta MN. & Dutta SK (1983). Investigation on an induced green seed coat mutant of *Trigonella foenum-graecum* L. *Cytologia* 40: 373-378.

Mir Z, Mir PS, Acharya SN, Zaman MS, Taylor WG, Mears GJ & Goonewardene LA (1998). Comparison of alfalfa and fenugreek silages supplemented with barley grain on performance of growing steers. *Can J Anim Sci* 78:343– 349.

Moyer JR, Acharya SN, Mir Z, & Doram RC (2003). Weed management in irrigated fenugreek grown for forage in rotation with other annual crops. *Can J Plant Sci* 83: 181–188.

Permjit K & Grover IS (1985). Cytological effect of some organophosphorus pesticides. II. Mitotic effects. *Cytologia* 50: 199-211.

Petropoulos GA (1973). Agronomic, genetic and chemical studies of *Trigonella foenum-graecum L.* Ph.D. Dissertation, Bath University, England.

Petropoulos GA (Ed.) (2002). Fenugreek—The genus *Trigonella*, 1st Edition. Taylor and Francis, London and New York, 1–127.

Ramanna MS (1974). The origin of unreduced microspores due to aberrant cytokinesis in meiocytes of potato and its genetic significance. *Euphytica* 23: 20-30.

Rana SR, Swaminathan MS (1964). Cytological aspects of pollen sterility. Recent Advances in Palynology. pp. 276-304. National Botanical garden, Lucknow.

Rao NB & Laxmi N (1980). Gamma rays induced meiotic abnormalities in *Capsicum annum L. Caryologia* 33: 509-518.

Reddy PMPGH & Bashamohideen H (1991). Inhibition of Mg^{+2} and Na^{+}-K^{+} ATPases in selected tissues of fish, *Cyprinus carpio* under fenvalerate toxicity. *Biochem Int* 23: 715-721.

Roy RP, Sinha BMS. & Thakur GK (1971). Irradiation studies in *Cucumis sativus* Linn. *J Cytol Genet* 6: 128-135.

Seregin V & kozhevnikova AD (2006). Physiological role of nickel and its toxic effects on higher plants. *Russian J Plant Physiol* 53: 257-277.

Siddiqui S & Hasan Z (2003). Morphological changes induced by chemical mutagens in *Trigonella foenum-graecum L. Ind J App Pure Bio* 18: 159-163.

Siddiqui S, Meghvansi MK, Khan SS & Aali, NS (2008). Mutagenic effect of herbicide (Maleic hydrazide) on seed germination and radicle length on *Trigonella foenum-graecum L. Ind J App Pure Bio* 23: 103-106.

Siddiqui S, Meghvansi MK & Hasan Z (2007). Cytogenetic changes induced by sodium azide (NaN_3) on *Trigonella foenum-graecum L.* seeds. *South Afric J Bot* (In Press).

Sideris EG, Nawar MM & Nilan RA (1971). Effect of gamma irradiation on gibberallic acid solution and gibberallic like substances in barley seedling. *Radiat Bot* 11: 209-214.

Sigurbjornsson B & Micke A (1974). Philosophy and accomplishments of mutation breeding. In: *Polyploidy and induced mutations in plant breeding. Proceedings of the International Atomic Energy Agency* (IAEA), Vienna, 1972, Bari, Italy, pp. 303–343.

Sigurbjornsson B (1983). Induced mutations. In: Wood DR (Ed.) *Crop Breeding*. American Society of Agronomy and Crop Science Society of America, Madison, WI, pp. 153–176.

Singh D & Singh A (1974). A green tilling mutant of *Trigonella foenum-graecum* L. (Methi). *Crop Improv* 1: 98–100.

Sinha SSN. & Godward MBE (1972). Radiation studies in *Lens culinaris*. Meiosis: abnormalities induced due to gamma radiation and its consequences. *Cytologia* 37: 685-695.

Sinha, SSN. & Godward MBE (1969). Radiation studies in *Lens culinaris*. Mitosis type of aberration and their elimination in root and shoot apical meristem. *J Cytol Genet* 3: 80-91.

Srivastava DP (1979). Effect of physical and chemical mutagens on *Solanum melongena* L. Ph.D. Thesis. Banaras Hindu University, Banaras.

Swietlinska JZ & Zuk J (1978). Cytotoxic effects of maleic hydrazide. *Mutat Res* 55: 15-30.