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# Mutagenic effects of environmental industrial chemical agents in inducing cytogenetical changes in wheat

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## Abstract

**Six environmental industrial chemical agents belonging to three separate groups: aziridines [Metepa (0.4 and 0.2%) and Thiotepa (0.2 and 0.01%)], nitroso compounds [MNG (0.1 and 0.05%)] and alkane sulphonic esters [MMS (0.1 and 0.05%) and EMS (0.5%)] and used in textiles, drug manufacture and as chemosterilants were compared for their mutagenicity and related cytogenetical effects on two wheat varieties - a tetraploid, durum wheat var. HD 4502 and a hexaploid bread wheat var. NP 880. Reduction in germination, survival, root and shoot length under the chemical treatments were observed in both the varieties, the tetraploid var. HD 4502 showing more sensitivity than the hexaploid var. NP 880. Among the chemicals Thiotepa followed by MNG showed drastic reduction in all the characters studied in $M_1$ generation. They also showed drastic effects in terms of cytological parameters eg., cell division and chromosomal aberrations. High mutagenic effectiveness of Thiotepa was also indicated by the absence of any dividing cells in the sensitive var. HD 4502 and very high frequency of abnormal cells, clumping and chromosome breakage in hexaploid var. NP 880. Higher frequency and wider spectrum of viable mutations was also observed in var. HD 4502. On the overall basis (varieties pooled over mutagens) highest number of abnormal cells and shattering of chromosomes were observed under Metepa followed by Thiotepa. The other two chemical agents, MNG and MMS also exhibited larger chromosomal abnormalities than EMS and controls. Thus the present studies clearly demonstrate that environmental chemicals are much more potent mutagenic agents which could also prove to be hazardous to human health due to their genotoxic and carcinogenic properties.**

**Key words:** Wheat, environmental mutagen, induced mutations, chromosomal aberrations, genotoxicity

## Introduction

A large number of industrial chemical agents abundantly present in the environment are not only leading pollutants but are also reported to be mutagenic and carcinogenic and hazardous to genetic material. Exposure to these environmental chemicals is suspected of causing several serious ailments in human body, including genetic disorders and neurodevelop- mental deficits. According to a recent US report [1] 1.2 billion pounds of chemicals were reported by industries to be released into air and water in 1998. These figures may be much higher because it has been estimated that only 5% of chemicals released into the environment are actually reported officially. The hazardous effects of these chemical agents have a great potential for disturbing the ecological balance and harmony. The toxicants that act on DNA cause damage to the genome, induce alterations in the nucleic acids and result in the modification or inactivation of a cell's genome are classified as 'genotoxic'. There is an universal call for testing of the effects of food additives, pesticides, drugs, and other industrial chemicals on human health. There has been an unprecedented growth and expansion in the chemical industry during the last few decades. Thousands of new industrial chemicals are introduced annually into commercial use and the exposure to these hazardous chemicals by the product users or due to accidents is common nowadays, which may be of great concern to human health. It is also recognized that many chemicals in our environment are potential causes of cancer and/or mutagenic risk [2]. Mutagens of environmental significance have gained special attention on account of severe environmental pollution and possible genetic hazards after their leakage tragedies reported from several huge chemical plants e.g., the Methylisocyanate (MIC) gas tragedy at the Union Carbide Factory, Bhopal [3]. It is in this context that as some of the industrial environmental mutagenic chemicals such as: aziridines - Metepa (used in the textile industry, hardening of photographic emulsion and clinical utility in the temporary palliation of certain cancers), Thiotepa (used as flame-retardent, crease- resistant, water-proof fabrics, in manufacturing dyes, adhesives, drugs and induces a very high frequency of sister-chromatid exchange); Nitroso-compounds - MNG (used in cancer therapy as antitumor and antileukemic); an ester - MMS (used in cancer chemotherapy, in sterilization in house fly and also has antifertility properties), have been included in the present study to compare their mutagenicity and related effects with a known potent mutagen EMS -

an ester and also untreated control. The chemostrilants, Metepa and Thiotepa have been studied for the first time to ascertain their possible mutagenecity and the effects on the recovery of mutations in a cereal crop wheat.

## Matrials and methods

Two hundred uniform size seeds of two wheat varieties: - one tetraploid durum var. HD 4502 and another a hexaploid var. NP 880, were presoked in distilled water for two hours. Subsequently, the treatments were given in aqueous solutions of different mutagens for 2 hrs at 20 ± 1°C. The four environmental mutagenic agents comprising of Metepa [tris (2-methyl-1-aziridinyl) phosphine oxide] 0.4 and 0.2%; Thiotepa [tris (1-aziridinyl) phosphine sulfide thiophosphoramide] 0.2% and 0.01%; MNG (N-methyl-N-nitro-N-nitrosoguanidine) 0.1% and 0.05% and MMS (methyl methane sulphonate) 0.1% and 0.5% were compared for their effectiveness with a single dose of 0.5% of the potent alkylating agent EMS (ethyl methane sulphonate), and also with the distilled water presoaked untreated control. The approximate comparable biological dose for different chemical agents was worked out previously on the basis of a pilot study of shoot growth at 20°C. The root and shoot development was recorded on the 10 day old seedling under laboratory conditions. The data on germination was recorded from field on the 10th day of sowing. The relative effects on chromosome structure and mitotic index were scored and estimated from a minimum of five temporary slides in each case. All comparisons were done with their respective controls. Different types of chromosomal abnormalities were separately scored and for the sake of brevity, the values were pooled up and indicated as percentage of abnormalities observed.

## Results and discussion

The immediate effects of different environmental agents could be ascertained from some of the $M_1$ parameters and it was observed that in general, there was a reduction in germination, shoot and root length in both the varieties. Maximum shoot reduction was observed in Thiotepa (0.01%) followd by Metepa (0.4%) and MMS (0.1%) in case of var. HD 4502 and Thiotepa, Metepa, MNG and MMS in NP 880 (Table 1). The effect of Thiotepa was found to be more drastic than Metepa especilally in lower concentrations. Seed germination and survival was reduced in almost all the treatments. It was reduced to 4.73% of control with Thiotepa 0.2% and to 7.43% with MNG 0.1% in var. HD 4502. These two treatments also showed the lowest survival rate, though var. NP 880 was found to be more tolerant.

**Table 1.** Effects of environmental chemical mutagens on $M_1$ parameteres in wheat

| Treatments | No. of germinating plants | Germination (%) | Survival No. | Survival (%) | Root length (cm) | Shoot length (cm) |
|---|---|---|---|---|---|---|
| | | | Tetraploid var. HD 4502 | | | |
| Control | 148 | 100.00 | 61 | 100.00 | 14.95 | 13.66 |
| Metepa 0.4% | 37 | 25.00 | 22 | 36.06 | 3.00 | 6.47 |
| Metepa 0.2% | 48 | 32.43 | 43 | 70.49 | 4.15 | 8.26 |
| Thiotepa 0.2% | 7 | 4.73 | 4 | 6.55 | 4.46 | 7.74 |
| Thiotepa 0.01% | 43 | 33.59 | 30 | 49.18 | 4.24 | 5.04 |
| MNG 0.1% | 11 | 7.43 | 10 | 16.39 | 6.72 | 10.98 |
| MNG 0.05% | 23 | 15.54 | 16 | 26.22 | 11.92 | 13.16 |
| MMS 0.1% | 41 | 27.70 | 26 | 42.62 | 3.28 | 6.34 |
| MMS 0.05% | 54 | 36.49 | 35 | 57.37 | 7.34 | 11.64 |
| EMS 0.5% | 47 | 31.76 | 25 | 40.98 | 6.52 | 8.95 |
| Mean | 34.56 | | 23.44 | | 5.73 | 8.73 |
| SE | 16.88 | | 4.80 | | 2.79 | 2.71 |
| | | | Hexaploid var. NP 880 | | | |
| Control | 128 | 100.00 | 122 | 100.00 | 15.50 | 17.48 |
| Metepa 0.4% | 70 | 54.69 | 52 | 42.62 | 4.79 | 6.64 |
| Metepa 0.2% | 107 | 83.59 | 67 | 54.91 | 9.18 | 8.00 |
| Thiotepa 0.2% | 45 | 35.16 | 29 | 23.71 | 2.70 | 4.89 |
| Thiotepa 0.01% | 57 | 44.53 | 45 | 36.88 | 1.93 | 3.00 |
| MNG 0.1% | 47 | 36.72 | 39 | 31.95 | 5.72 | 5.71 |
| MNG 0.05% | 81 | 63.28 | 58 | 47.54 | 8.16 | 7.41 |
| MMS 0.1% | 104 | 81.25 | 49 | 40.16 | 6.70 | 7.20 |
| MMS 0.05% | 94 | 73.44 | 49 | 40.16 | 6.72 | 8.33 |
| EMS 0.5% | 76 | 59.34 | 43 | 35.24 | 5.35 | 7.29 |
| Mean | 75.66 | | 48 | | 5.69 | 6.49 |
| SE | 3.50 | | 10.93 | | 2.35 | 1.87 |

The immediate effect of aziridines on cell division (Table 2) was adequately revealed by the absence of any dividing cells in tetraploid var. HD 4502, except with Metepa (0.2%) which gave some dividing cells. Both the treatments of Thiotepa were so drastic that no dividing cell was observed, although, some stimulation was noticed with MNG, MMS and EMS treatments. On the other hand, slight reduction in percentage of dividing cells was recorded in MNG and EMS treatments and with lower dose of Thiotepa In hexaploid var. NP 880. The effect of aziridines was also evident from the high percentage of abnormal cells which were highest with Metepa 0.2% in tetraploid (durum) wheat var. HD 4502. This treatment gave 33.3% nuclear shattering and 27.5% cells with clumping of chromosomes. Among the esters, EMS (0.5%) was equally effective in inducing shattering and clumping of chromosomes. Interestingly the treatment, Metepa 0.2%, which gave the highest percentage of shattering and clumping also gave highest chromosome breaks, while only chromatid breaks were observed in MMS 0.1% and EMS 0.5%. Highest incidence of fragmentation was recorded in Metepa 0.2%, MNG 0.1% and EMS 0.5%, thereby indicating the compartive efficiency of these three chemicals at different concentrations in inducing similar changes. EMS (0.5%) on the other hand was less drastic in terms of chromosomal damage than environmental mutagens, but showed some drastic effect similar to Metepa in var. NP 880. Breakage at primary constriction was common in almost all the treatments with varying degree. Negatively stained portions which were designated as gaps were common with MMS 0.05% and MNG 0.05% and 0.1% treatments.

Taking all the parameters together, it was found that the two concentrations of Thiotepa were more effective, whereas, Metepa treatment (0.2%) manifested minimum effects. Although abnormal cells were highest with aziridine treatments in var. NP 880 in comparison to var. HD 4502, where no dividing cells were recovered in any concentrations. Like var. HD 4502, the high incidence of shattering was observed with MNG 0.1% and Metepa 0.2% in comparison to EMS which was least effective. Similarly, highest clumping was recorded with Thiotepa (0.2%), Metepa (0.4%) and the least was recorded with EMS 0.5%. All the treatments gave higher

**Table 2.** Effects of environmental chemical mutagens on cell division and chromosomal aberrations in wheat

| Treatment | Total cells observed | Cells in division | | Abnormal cells % | Shatte-ring% | Clumping % | Chro-mosome* breaks B″ | Chro-matid* breaks B′ | Gaps % | Frag-ments % | Centric breaks % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | % | | | | | | | | |
| | | | | | Tetraploid var. HD 4502 | | | | | | |
| Control | 762 | 143 | 18.76 | 2.44 | - | - | - | - | - | 3.2 | 0.80 |
| Metepa 0.4% | No proper cell in division | | | | | | | | | | |
| Metepa 0.2% | 983 | 132 | 13.42 | 75.82 | 33.33 | 27.54 | 75.36 | - | 17.39 | 89.86 | 31.88 |
| Thiotepa 0.2% | No proper cell in division | | | | | | | | | | |
| Thiotepa 0.01% | No proper cell in division | | | | | | | | | | |
| MNG 0.1% | 850 | 176 | 20.71 | 41.88 | 20.41 | 8.16 | 85.71 | - | 32.65 | 93.88 | 44.90 |
| MNG 0.05% | 719 | 202 | 28.10 | 57.02 | 24.61 | 26.15 | 63.07 | - | 30.76 | 73.84 | 23.07 |
| MMS 0.1% | 613 | 118 | 19.25 | 73.28 | 23.35 | 36.47 | 52.94 | 2.35 | 10.59 | 58.82 | 24.71 |
| MMS 0.05% | 507 | 152 | 29.98 | 47.71 | 4.10 | 54.79 | 63.01 | - | 32.88 | 63.01 | 32.88 |
| EMS 0.5% | 816 | 189 | 23.17 | 46.86 | 33.33 | 25.32 | 36.00 | 17.33 | 17.33 | 29.33 | 33.33 |
| Mean | 786 | 158.85 | | | | | | | | | |
| | | | | | Hexaploid var. NP 880 | | | | | | |
| Control | 1321 | 304 | 23.01 | - | - | - | - | - | - | - | - |
| Metepa 0.4% | 684 | 138 | 20.18 | 84.76 | 30.34 | 40.45 | 40.45 | - | 7.86 | 58.43 | 25.84 |
| Metepa 0.2% | 588 | 120 | 20.41 | 97.58 | 50.41 | 33.06 | 24.79 | - | 5.79 | 43.80 | 24.79 |
| Thiotepa 0.2% | 770 | 182 | 23.64 | 83.58 | 25.89 | 45.53 | 44.64 | - | 14.29 | 59.82 | 20.54 |
| Thiotepa 0.01% | 828 | 156 | 18.84 | 81.25 | 40.66 | 32.97 | 51.65 | - | - | 70.33 | 34.07 |
| MNG 0.1% | 879 | 146 | 16.61 | 49.28 | 52.94 | 25.00 | 41.18 | - | 19.12 | 50.00 | 20.59 |
| MNG 0.05% | 1133 | 170 | 15.01 | 67.88 | 43.01 | 20.43 | 49.46 | - | 3.24 | 43.80 | 15.33 |
| MMS 0.1% | 511 | 117 | 22.80 | 67.32 | 18.45 | 33.98 | 59.22 | - | 14.56 | 70.87 | 25.24 |
| MMS 0.05% | 732 | 163 | 22.27 | 36.81 | 26.41 | 5.66 | 41.51 | 3.77 | 11.32 | 56.60 | 39.62 |
| EMS 0.5% | 1778 | 203 | 27.22 | 16.28 | 16.28 | 4.65 | 32.56 | 34.88 | 2.33 | 41.86 | 37.21 |
| Mean | 922.4 | 16.99 | | | | | | | | | |

*Chromosome breaks (B″): When both the chromatids were broken from the same locus and the two broken parts were clearly separated.
*Chromatid breaks (B′): When one of the two chromatids was broken, while the other remained intact on the same locus.

percentage of chromosome breaks (B″) in comparison to EMS 0.5% (32.56%). No chemical was effective in inducing chromatid breaks (B′), except MMS 0.05% which gave 3.77% (var. NP 880) and EMS 0.5% which gave 34.88% (var. NP 880). Chromosome fragments were found in every treatment with varying degree, but centric breaks were highest with EMS and MMS treatments.

The highest percentage of viable mutations were observed in tetraploid var. HD 4502 with aziridine treatments, which mostly affected the seedling height, but such results were not available in hexaploid var. NP 880. Mutagens like MNG, MMS and EMS gave wide spectrum of mutation affecting height, maturity in var. HD 4502 and height, maturity, spike and leaf characters in var. NP 880. The pooled data for different morphological characters in var. HD 4502 and var. NP 880 suggest high incidence of mutation in tetraploid var. HD 4502 than the hexaploid var. NP 880.

The dosage effect of chemical mutagens is conditioned by many parameters, of which the most important are concentration, presoaking, duration of treatment, pH and temperature. The treatment time may be more critical for faster reacting chemicals than for slow reacting ones like EMS. Keeping in view the optimum reactivity, regular infusion of chemicals and increased effectiveness of different mutagens [4], the presoaking and treatment duration of two hours was kept constant for all treatment in the present study.

There was considerable reduction in root development and in some cases there was no shoot emergence with aziridine treatments (Thiotepa 0.2 and 0.01%). The influence on shoot growth has been related to many factors which include chromosomal abnormality with height reduction [5], reduction in auxin levels, inhibition of auxin synthesis, failure of assimilation mechanism and chromosomal damage-cum-mitotic inhibition [6].

In the present investigation a significant reduction in germination percentage was observed. The high proportion of seed lethality due to mutagen treatment has been associated with weakening of intra-chromosomal linkage or to accumulation of deleterious mutations in different genomes]. The reduction in germination could also be due to the alkylation of sulphahydral (-SH) group of important proteins causing death of the seeds [7]. The present data indicate reduction in survival (30-50%) in all the treatments in comparison to their respective controls. Although no change in survival percentage after treatment with EMS in bread wheat has been reported earlier [8], the reduction in survival in the present study could be due to inhibition of auxin synthesis or inability of cells to utilize the material available [6].

Mitotic index scored as percentage of dividing cells was significantly reduced with aziridine treatment (Metepa 0.2%). The aziridine treatments, Metepa 0.4%, Thiotepa 0.2% and 0.01% were so drastic that no proper cell at dividing stage could be recovered in var. HD 4502. Among other treatments, EMS 0.5% showed maximum effect on normal mitotic activity. It was interesting that some stimulatory effect was observed with the treatments of nitroso-compounds in var. HD 4502. Kalia *et al.* [9] reported similar action of LSD on mitotic rate in barley. The reduction of dividing cells could be due to blockage at G2 stage [10]. Khilman [11] indicated that potent DNA inhibitors are primarily responsible for reduction in mitotic activity or it could be due to inhibition of DNA synthesis or change in the oxidative phosphorylation activity. The drastic effect of aziridines in the present study could be due to its ability for immediate binding and reactivity at many sites, which could be considered analogous to "prophase poisoning" [12]. It is thus inferred that normal function of the cells is suppressed, either due to impairment of DNA synthesis or blockage at synthetic stage of interphase cells.

The relative efficiency of different treatments was determined by the detailed root tip cytological observations. In order to eliminate the product of diplontic selection in the course of subsequent mitotic cycles, roots growing at controlled temperature (20 ± 1°C) were fixed at M phase of first mitotic division. Few dividing cells recovered at proper metaphase with aziridine treatment, Metepa 0.2% gave about 75% abnormality with highest chromosome breaks and fragments whereas, NMG 0.1% gave predominantly chromosome breaks and fragments only in var. HD 4502. The high percentage of chromosome breaks indicate the possibility of mitotic cells affected at pre-synthetic stage. Chromatid breaks primarily restricted to ester (MMS) treatments, that too in lower concentrations suggest that cells were already in S or G2 stage. It is known that real chromatid breaks are relatively lower and were largely overestimated due to inclusion of achromatic gaps. Different mitotic observations and their possible genetic consequences associated has been discussed by Kalia and Singh [13]. The chemicals used in the present study are alkylating agents which are chemically reactive and combine with nucleophilic centres such as sulfahydral and ionised acid groups in biological systems. Esterification of phospahate groups in DNA has been reported to be responsible for cytological effects [14].

The chromosome fragments rendered by different treatments did not show any reunion of broken parts.

In this respect the effects of chemicals appear to differ qualitatively from those induced by radiations, where fragment reforming of chromosome fragments is observed and these observations support our earlier reports for LSD induced chromosomal changes in barley [9]. It may be assumed that chemicals besides producing extensive chromosomal aberrations, may simultaneously interfere with the normal functioning of repair enzyme and series of actions involved in the process of rejoining. In this context attention may be drawn to the achromatic gaps, which were mostly induced by esters, nitroso-compounds & aziridines.

Another interesting observation pertaining to chromosome breaks is the high percentage of breaks confined to primary constriction region. Such preferential attack at hetrochromatic region has been recorded by several workers who used varied mutagenic chemicals [9]. However, there is no firm basis to interpret localised chromosome damage at molecular level [15] although the use of strand break assays to detect mutagenic potential of chemical agents and radiation has also developed rapidly over the past many years [16, 17 & 18].

The frequency of viable macromutations recorded in $M_2$ generation was extremely low and the spectrum of mutations was very restricted (Table 3). There are many reports to indicate the wide spectrum of induced mutations with ionising radiations in comparison to chemical mutagens [8]. In plants like wheat which are either tetraploid or hexaploid, visible mutations can occur, if the phenotypic buffering induced by duplication does not exist.

In the present observations the ear mutants which normally form the most predominant class were conspicuously absent in all the treatments. Swaminathan *et al.*, [8] did not recover speltoid mutation in C 591 (6X) and H 389 (4X) wheat varieties in large EMS treated populations. It is rather difficult to pinpoint the exact reason for this non-occurrence of "Q" locus influenced mutations in this experiment. It may probably be due to the position of "Q" locus on the distal end of long arm of 5A. Singh [19] observed that mutants involving "Q" locus have a clear positive correlation with the total number of mutations, hence it can be used as an index for overall mutation rate. The genotypic differences seemed to have played an important role in manifestation of different types of mutations. This would imply that chemical mutagens have failed to react in this localised region of chromosomes. It may be due to the distribution of specific type of chromatin materials at this region. Further studies on genotoxicity with different genotype and additional chemical mutagens [20] would be required for establishing this type of behaviour where some chemicals fail to react and give expression in the recovery of particular type of mutants. The fruitfly *Drosophila malanogaster* has been extensively used in studies on antigenotoxicity of various environmental chemicals and mixtures [21]. A new range of tests called Somatic Mutation and Recombination Tests (SMART) has recently been developed and used for the purpose [22].

**Table 3.** Frequency and spectrum of viable mutations in $M_2$

| Mutagen & Treatment | Population | Height | | Maturity | | Ear shape | | | Leaf |
|---|---|---|---|---|---|---|---|---|---|
| | | Dwarf | Tall | Early | Late | Thick short | Thick long | Lax long | narrow erect |
| Tetraploid var. HD 4502 | | | | | | | | | |
| Control | 2855 | - | - | - | - | - | - | - | - |
| Metepa 0.4% | 1085 | 1 | - | - | - | - | - | - | - |
| Metepa 0.2% | 2030 | 5 | - | - | - | - | 1 | - | - |
| Thiotepa 0.2% | 1360 | 8 | - | - | - | - | - | - | - |
| Thiotepa 0.01% | 1540 | 9 | - | - | - | - | - | - | - |
| MNG 0.1% | 832 | 1 | - | - | - | - | - | 1 | - |
| MNG 0.05% | 805 | 1 | - | - | - | - | 2 | - | - |
| MMS 0.1% | 1248 | - | - | 3 | - | - | - | - | - |
| MMS 0.05% | 2703 | - | 2 | - | 2 | - | - | - | - |
| EMS 0.5% | 1696 | 1 | 2 | - | - | - | - | - | - |
| Grand total | 16154 | 26 | 4 | 3 | 2 | - | 3 | 1 | |
| Viable mutation | 0.24% | | | | | | | | |
| Hexaploid var. NP 880 | | | | | | | | | |
| Control | 2175 | - | - - | - | - | - | - | - | |
| Metepa 0.4% | 5075 | - | - | - | - | - | - | - | - |
| Metepa 0.2% | 7105 | - | - | - | - | - | - | - | - |
| Thiotepa 0.2% | 3255 | - | - | - | - | - | - | - | - |
| Thiotepa 0.01% | 4785 | 1 | - | 1 | - | - | - | 2 | - |
| MNG 0.1% | 1785 | - | - | - | - | - | - | - | - |
| MNG 0.05% | 2240 | - | - | - | - | - | - | - | - |
| MMS 0.1% | 2100 | 3 | - | - | - | - | - | - | 3 |
| MMS 0.05% | 2178 | - | - | 2 | - | 1 | 1 | - | - |
| EMS 0.5% | 1750 | 1 | - | - | - | 1 | - | 2 | - |
| Grand total | 32448 | 5 | - | 3 | - | 2 | 1 | 4 | 3 |
| Viable mutation | 0.05% | | | | | | | | |

The drastic effects on growth, germination, seedling height, survival, root development and mitotic activity have clearly indicated the quick reactivity of environmental mutagens. The lethal effects of chemo-sterility were adequately manifested in the early stages of plant growth but subsequently it was not reflected in recovery of viable mutations. It could be concluded that the treatments which were responsible for high toxicity, were not quite efficient in the ultimate recovery of viable mutations. Thus, the process of induction of chromosomal aberrations, which is directly linked with genetic alterations, may not entirely be correlated with the recovery of mutations in multicellular organisms. This assumption is in line with the views expressed by Gaul [23] that, in the process of diplontic selection the cells which are damaged or affected due treatment generally get eliminated. It, therefore, seems logical to assume that the cells with extreme

physiological or chromosomal alteration have less chance to pass through the mitotic sieve than non-damaged cells, hence the low recovery of visible viable mutations.

The experimental evidence gathered on the basis of $M_1$ parameters, chromosomal damage and mutation frequency in $M_2$ generation of the present study revealed that envronmental chemical agents, particularly chemosterilants - Metepa and Thioptepa are genotoxic and potent mutagenic agents.

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