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Evaluation of DNA Damage by Methyl Methane Sulfonate in *Allium cepa* Root Cells by Comet Assay

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Abstract:

The genotoxic effect of Methyl methanesulfonate (MMS), an alkylating compound or radiomimetic agent, was evaluated over a wide range of concentrations, 0, 50 and 100 µM (least toxic); 250 and 500 µM (moderately toxic); 1000, 2000 and 4000 µM (highly toxic) in *Allium cepa* root cells using a mild alkaline comet assay (Single Cell Gel Electrophoresis). Single Cell Gel Electrophoresis has recently been applied to plant systems for genotoxic study. In A*. *cepa* root growth assay, a significant increase in comet tail length, %Tail DNA, Tail Moment (TM) and Olive Tail Moment (OTM) was noticed in root cell nuclei, indicating MMS induced DNA damage. %Tail DNA increased significantly (*P*≤0.05) in the range of 3.7%–91.7% over 0–4000 µM MMS. OTM of 0.678 µm was recorded in *A. cepa* roots under control conditions and it increased significantly upon increasing the dose of treatment, and was 130.29 µm upon exposure to 4000 µm MMS. The increase in TM and OTM with increasing dose of MMS in the current investigation indicated Deoxyribonucleic acid (DNA) damage induced by MMS.

Keywords: Comet assay, DNA damage, Methyl Methane Sulfonate, Olive Tail Moment, Radiomimetic agent, Tail Moment

1. Introduction:

Methyl methanesulfonate, MMS (MW=110.1), a possible human carcinogen, was first listed in the Sixth Annual Report on Carcinogens (1991). As per Environmental Protection Agency (EPA, USA), MMS has been listed as a hazardous constituent of waste (Anonymous, 2011). It is widely used for research purposes as a solvent catalyst in polymerization, alkylation, and esterification reactions. Additionally, it has been also used as a reversible insect and mammalian pest chemosterilant and as a human male contraceptive (IARC, 1974). MMS has been reported to be clastogenic and mutagenic in various genetic systems (Brewen et al., 1975; Singh and Gupta, 1983; Lambert et al., 1984; Edwards et al., 1993; Vasudev et al., 1997; Kaya, 2003; Doak et al., 2007; Pottenger, 2009; Mahadimane and Vasudev, 2013).

MMS is well-known DNA damaging agent, induces mutagenesis, and is widely used in recombination experiments. MMS causes mispairing of bases and replicates blocks by modifying both guanine (to 7-methylguanine) and adenine (to 3-methlyladenine) (Beranek, 1990; Lundin *et al*., 2005).

In this era of chemical science, various radiomimetic agents are used to increase the agricultural yield (Vasu and Hasan, 2011). These radiomimetic agents are characterized with their bi-functional alkyl reactive groups which react with DNA, causing DNA damage at the genetic level. Thus, the present study was planned to evaluate genotoxicity in *Allium cepa* (onion) roots with the use of MMS as a radiomimetic agent. For its evaluation, a wide range of MMS, 0 (control), 50 and 100 µM (least toxic); 250 and 500 µM (moderately toxic); 1000, 2000 and 4000 µM (highly toxic) were selected. MMS induced DNA damage was evaluated in terms of Head DNA (%), Tail DNA (%), Tail Moment (µm) and Olive Tail Moment (µm) after 24h of exposure in *A. cepa* roots. *A. cepa* serves as a good indicator for evaluating genotoxic potential of MMS, due to its sensitivity and good correlation with mammalian test systems (Chauhan *et al*., 1999; Yildiz *et al*., 2009). Not only this, *A. cepa* is widely used as a bioassay species to study genotoxic changes due to its relatively large-sized cells and higher percentage of dividing cells having fewer numbers of large-sized chromosomes that stain well (Fiskesjö, 1985).

2. Materials and methods:

2.1 Materials

Equal sized *A. cepa* bulbs (25−30 mm in diameter; 2n=16) were procured from the local cultivators. Methyl methanesulfonate, MMS (MW=110.1; Sigma Co., St. Louis, USA; 100% purity) was used for treating *A. cepa* roots. All the other analytical grade biochemical reagents used in the study were purchased from Sisco Research Laboratory Pvt. Ltd., India; Sigma Co., St. Louis, USA; Merck Ltd., India; Loba-Chemie Pvt., Ltd., India.

2.2 Raising of Onion root tips

To expose the apices of the root primordia to MMS, onion bulbs of uniform size were scrapped and their dry scales were peeled off. The bulbs were set for rooting in distilled water in the dark for 4 days (Fig. 1). Finally, the onion bulbs were subjected to 0 (control), 50, 100, 250, 500, 1000, 2000 and 4000 μM MMS treatment, respectively, for 24 h in an environmentally controlled growth chamber under a 14 h photoperiod of 240 µmol m^{-2} s^{-1} PFD at 18/8 (±2)^{o}C and 75±2% relative humidity. In all, there were eight treatments, including control, with five independent replications.

2.3 Comet assay (Single Cell Gel Electrophoresis, SCGE)

Root meristem cells of *A. cepa* were exposed to different concentrations of MMS and used for cytogenetic analysis during comet assay (Tice *et al.*, 2000). Before beginning with the assay, microscopic slides (with one-fourth frosted ends) were prepared by dipping into 1% normal melting point agarose (NMPA; SD Fine Chemical Limited, India) at 50ºC. These slides served as the agarose base-coated slides. The treated root tips of *A. cepa* were placed in a watch glass kept over an ice base and gently sliced using a sharp razor blade to isolate the nuclei in Phosphate Buffer Saline (PBS, pH 7.4) and the suspension of nuclei (100 µl) mixed with 50 µl of low melting point agarose in PBS kept at 37°C was pipetted over the agarose base coated slides. Slides were covered with a cover slip and left in a metal tray kept on ice. Nuclei were left for 10 min and after removing the cover slip, the slides were immersed in Lysis solution for 1 h in dark. Thereafter, the slides were placed in a horizontal gel electrophoresis tank containing freshly prepared cold electrophoresis buffer (1 mM EDTA and 300 mM NaOH, pH≥13). The nuclei were incubated for 30 min to facilitate DNA unwinding prior to electrophoresis at (25 V, 300 mA) for 25 min (Fig. 2). After rinsing thrice with distilled water, electrophoresed slides were stained with ethidium bromide (20 µg mL^{-1}) for 5 min in dark, dipped in ice-cold water to remove the excess ethidium bromide, and covered with a cover slip..

A computerized image analysis system (CASP; Comet Assay Software) was employed. % Head DNA, % Tail DNA, Tail moment (TM=%Tail DNA x Tail Length) and Olive Tail Moment [OTM= % Tail DNA x (Tail Mean X−Head Mean X)] were used as the measure of DNA damage. A minimum of five roots were taken for each treatment and from each root three SCGE slides were prepared. From each slide 25 nuclei were randomly chosen and were analyzed using a fluorescence microscope with an excitation filter of BP 546/10 nm and a barrier filter of 590 nm.

3. Results and Discussion:

MMS induced genotoxicity was evaluated by comet assay. The nuclei were isolated from roots of onion bulbs treated with 0−4000 µM MMS and the comet assay was performed (Fig 3). It was observed that the comet length increased with increasing dose of MMS (Fig. 3). Results obtained with CASP are summarized in Table 1 and Fig. 2. Upon increasing the dose of treatment (MMS), a notable decrease in % head DNA was observed. It was found to be 96.3%, 68.3%, 51.7%, 40.2%, 34.2%, 32.4%, 21.6% and 8.3% in *A. cepa* roots treated with 0, 50, 100, 250, 500, 1000, 2000 and 4000 µM MMS, respectively. However, the % tail DNA increased significantly ($P \leq 0.05$) in the range of 3.7%−91.7% in response to 0−4000 µM MMS. With increase in % tail DNA, a significant decrease was observed in % head DNA with respect to increasing dose of MMS (Table 1). Increase in % tail DNA linearly relates to DNA break frequency over a wide range of concentrations. This indicates damaging potential of this alkylating agent (MMS). The occupational or environmental exposure to such genotoxic agents needs to be monitored.

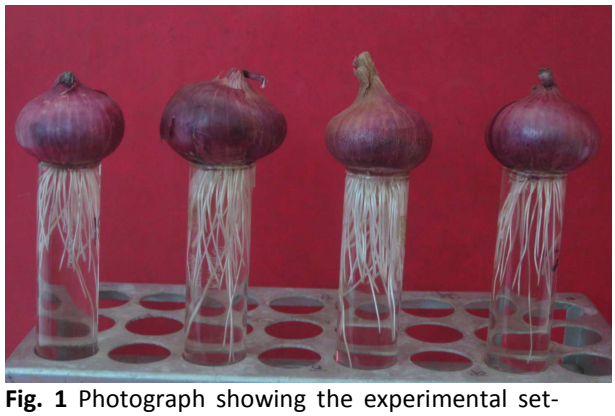

Fig. 1 Photograph showing the experimental set-up: bulbs with rooting in distilled water in dark after 4 days

Table 1: MMS-induced DNA damage in *A. cepa* roots as evaluated by Head DNA, Tail DNA, in Comet Assay (as assessed by CASP, Comet Assay Software).

MMS (µM)	Head DNA (%)	Tail DNA (%)
0	96.26±0.77a	3.74±0.22a
50	68.26±0.66b	31.74±0.57b
100	51.66±0.57c	48.33±0.43c
250	40.17±0.44d	59.83±0.67d
500	34.23±0.33e	65.77±0.74e
1000	32.37±0.57f	67.62±0.55f
2000	21.63±0.63g	78.37±0.63g
4000	8.33±0.33h	91.67±0.73h

Data were recorded after 24 h of exposure to MMS; represented as mean±S.E; means with common letters are not significantly different at $P \leq 0.05$, according to Tukey's test.

Comet analysis revealed by Tail Moment (TM) and Olive Tail Moment (OTM) indicated that DNA damage induced by MMS was significant ($P \leq 0.05$) as compared to control. Higher doses of MMS induced greater genotoxicity, which was evident from increasing values of TM and OTM. Exposure to 4000 µM MMS exhibited the highest value for TM and OTM followed by 2000, 1000, 500, 250, 100, 50 and 0 µM MMS (Fig. 4). The tail moment increased from 0.263 to 235.84 µm over 0−4000 µm MMS. OTM of 0.678 µm was recorded in *A. cepa* roots under control conditions and it increased significantly upon increasing the dose of treatment. OTM was found to be 8.99, 38.96, 52.05, 68.21, 75.38, 89.78 and 130.29 µm upon exposure to 0, 50, 100, 250, 500, 1000, 2000 and 4000 µm MMS, respectively.

The Comet assay, applied to *A. cepa* roots, proved to be a sensitive method for detection of DNA damage. It has been performed to study the kinetics of DNA damage as a function of increasing concentrations of MMS. The study revealed a linear relationship between DNA damage and increasing concentrations of MMS. The consequences of this radiomimetic agent on comet length, % head DNA, % tail DNA, TM and OTM in *A. cepa* root nuclei as performed in the present study, is important in providing useful information about the genotoxic effect of this widely used chemical substance.

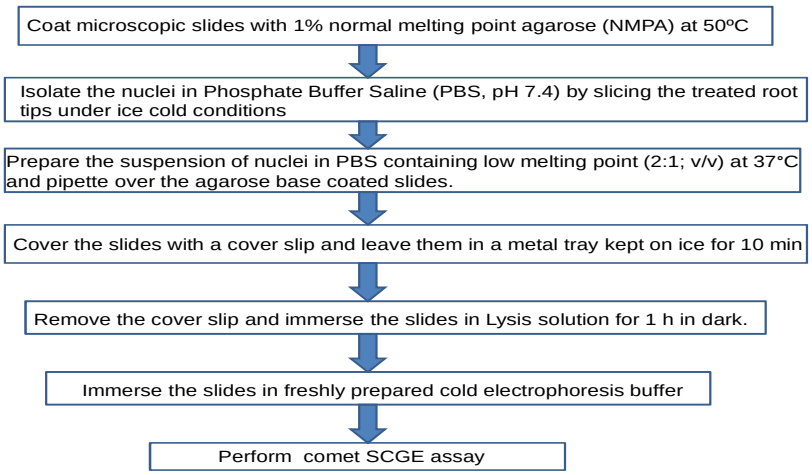

Fig. 2 Flow chart describing the sample preparation for comet assay

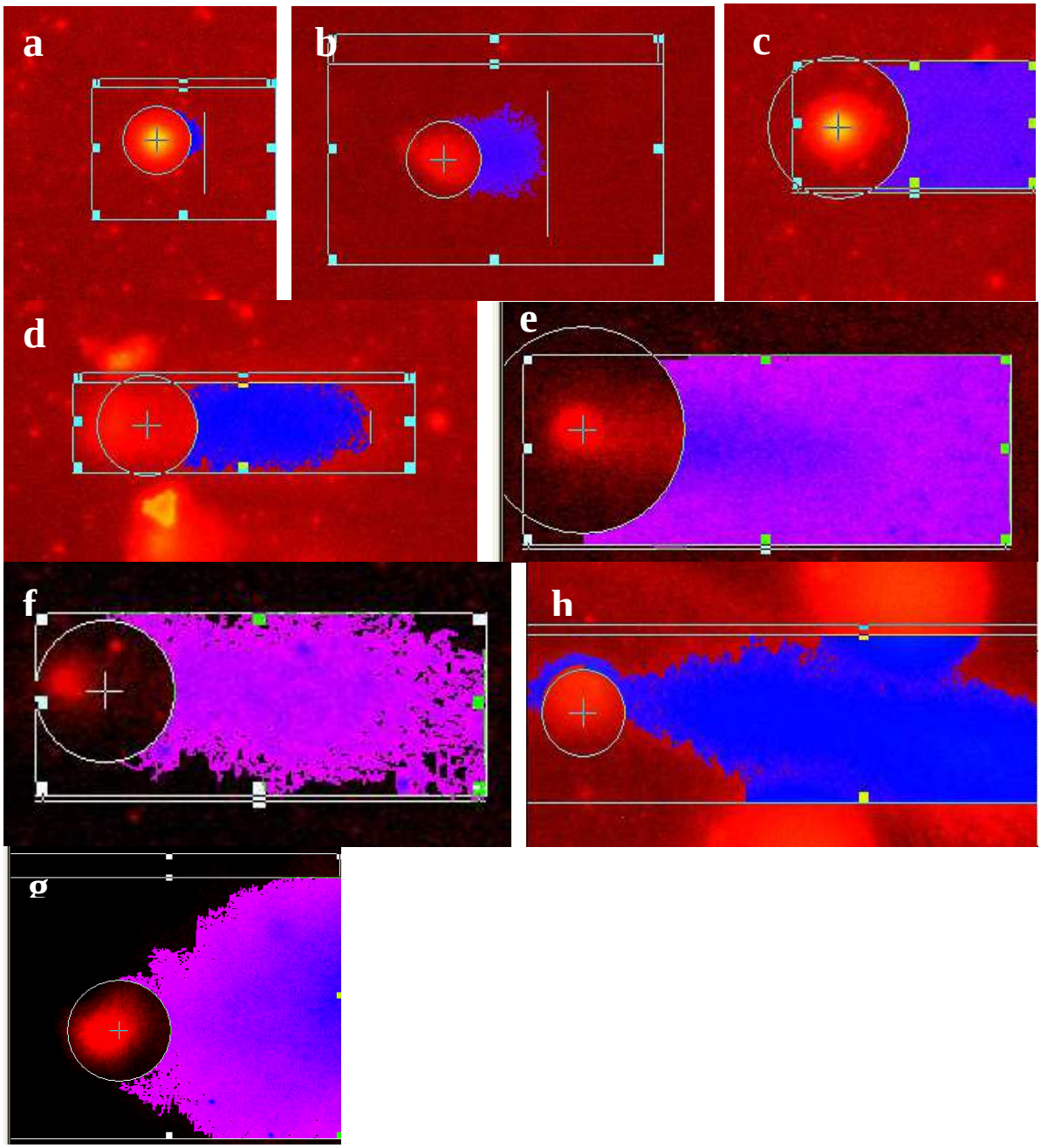

Fig.3 MMS induced DNA damage evaluated by Head DNA and Tail DNA in Comet Assay. Photographs showing comets in a) control, b) 50 µM, c) 100 µM, d) 250 µM, e) 500 µM, f) 1000 µM, g) 2000 µM, and h) 4000 µM in MMS treated root nuclei of *A. cepa*.

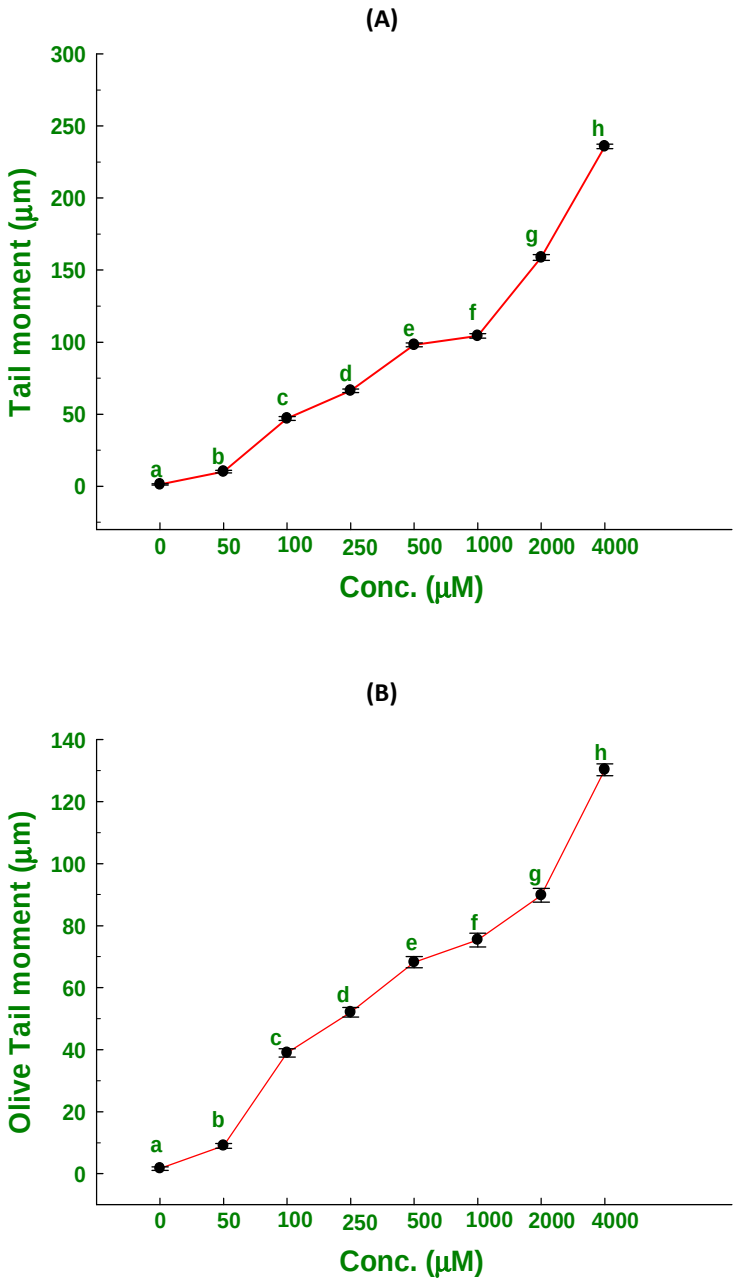

Fig.4 Influence of different treatments of MMS on DNA damage in root nuclei of *A. cepa* evaluated by (A) Tail Moment and (B) Olive tail moment in comet assay. Data were recorded after 24 h of exposure to MMS; represented as means of five replicates with standard errors shown by vertical bars; means with common letters are not significantly different at $P \leq 0.05$, according to Tukey's test.

Methylating agents methylate DNA mainly on oxygen or nitrogen residues present in either the DNA backbone or in the DNA bases. (Beranek, 1990). MMS is known for its efficiency to induce chromosomal aberrations in the comet assay (Yildiz et al., 2009). Vasu and Hasan (2011) opined that these radiomimetic agents generally target nucleus as the major site of injury. The used concentrations of MMS caused significant and dose-dependent reduction in % head DNA in *A. cepa* root nuclei. The % tail DNA increased linearly with stress intensity, the tail moments were also found to be dependent on the differences in DNA migration, which can be due to either the nature of DNA or the extent of DNA relaxation (Duez et al., 2003). Increase in TM and OTM with increasing dose of MMS in the current investigation indicated DNA damage induced by this radiomimetic agent. Cotelle and Férard (1999) reported a significant increase in DNA migration in *Vicia faba* root nuclei upon exposure to MMS. de Oliviera et al. (2012) reported a significant increase in the frequency of

DNA damage and induced micronuclei formation in V79 cells. Vajpayee *et al*. (2006) also reported a significant dose-dependent increase in DNA damage in *B. monnieri* upon exposure to MMS (0.05-100 µM) for 2 hr at (26 ± 2)° C. MMS was used as a positive control by Kaur *et al*. (2014) in their study pertaining to DNA damaging effect of Lead. Menke *et al*. (2001) and O'Connor (1981) opined that MMS, a monofunctional alkylating agent, preferentially induces N-alkyl lesions. Schubert and Rieger (1987) reported very efficient chromatid type chromosomal aberration and sister chromatid exchanges in *Vicia faba*. According to these researchers, 2.5–5 mM MMS yielded up to 48% of first post-treatment metaphases with structural aberrations in root meristems. Further, this DNA damage could be attributed to the higher accumulation of reactive oxygen species and alteration in the antioxidant scavenging enzymatic machinery upon exposure to MMS. Cunningham (1997) and Rucinska et al (2004) suggested that higher concentrations of stress causing agents enhance the production of free radicals and reactive oxygen species beyond the capacity of antioxidant systems, which in turn may induce DNA modification. Globally, researchers prefer comet assay for monitoring plant genotoxicity and in continuation, future studies shall focus on deciphering the role of ROS in mediating MMS-inhibited mitotic activity, and disruption of oxidative metabolism. In view of this MMS induced genotoxicity, it is necessary to look for some anticlastogenic agents. Ahmad *et al. (2014) reported Terminalia catappa* as an anticlastogenic agent against MMS-induced genotoxicity. This domain can be explored further..

4. Conclusion:

Methyl methanesulfonate (MMS), an alkylating compound or radiomimetic agent, was found to be genotoxic over at ≥100 µM in *A. cepa* root cells nuclei and caused greater DNA damage at higher concentrations.MMS caused significant decrease in % head DNA and a notable increase in % Tail DNA, thereby indicating MMS induced DNA damage. TM and OTM increased linearly with increasing stress intensity of this radiomimetic agent. The study can be extended further to evaluate the cytogenetic effect of this radiomimetic agent and some anti clastogenic agents can be looked forward.

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References:

1) Ahmad, M.S., Ahmad, S., Arshad, M., Rai, K.B. and Afzal, M. (2014): *Terminalia catappa*, an anticlastogenic agent against MMS induced genotoxicity in the human lymphocyte culture and in bone marrow cells of Albino mice. *Egypt. J. Med. Hum. Genet.*, 15(3): 227−233.
2) Anonymous (2011): Methyl Methanesulfonate. In Report on Carcinogens, 12th ed. U.S. Department of Health and Health Services. Public Health Services. National Toxicology Program. USA. 268. Available online at http:// ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/methylmethanesulfonate.pdf
3) Beranek, D.T. (1990): Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents. *Mutat. Res.*, 231(1): 11–30.
4) Brewen, J.G., Payne, H.S., Jones, K.P. and Preston, R.J. (1975): Studies on chemically induced dominant lethality. I. The cytogenetic basis of MMS-induced dominant lethality in post- meiotic male germ cells. *Mutat. Res.*, 33: 239–250.
5) Chauhan, L.K.S, Saxena, P.N. and Gupta, S.K. (1999): Cytogenetic effects of cypermethrin and fenvalerate on the root meristem cells of *Allium cepa. Environ. Exp. Bot*., 42: 181–189
6) Cotelle, S.and Férard, J.F. (1999): Comet Assay in Genetic Ecotoxicology: A Review. *Environ. Mol. Mutagen*., 34:246—255.
7) Cunningham, R.P. (1997): DNA repair: caretakers of the genome? *Curr. Biol.*, 7: 576–579.
8) Doak, S.H., Jenkins, G.J.S., Johnson, G.E. Quick,E. Parry, E.M. and Parry, J.M. (2007): Mechanistic influences for mutation induction curves after exposure to DNA-reactive carcinogens. *Cancer Res.*, 67(8):3904−3911.
9) Duez, P., Dehon, G., Kumps, A. and Dubois, J. (2003): Statistics of the Comet assay: a key to discriminate between genotoxic effects. *Mutagenesis*, 18: 159–166.
10) Edwards, A.J., Moon, E.Y., Anderson, D. and McGregor, D.B. (1993): The effect of simultaneous exposure to bromodeoxyuridine and methyl methanesulfonate on sister chromatid exchange frequency in human lymphocytes, *Mutat Res*., 289: 139−144.
11) Fiskesjö, G, (1985): The *Allium* test as a standard in environmental monitoring. *Hereditas*, 102: 99−112.

12) Gulfishan, M., Khan, A. H., Jafri, I. F. and Bhat, T. A. (2012): Assessment of mutagenicity induced by MMS and DES in *Capsicum annuum* L. *Saudi J. Biol. Sci.*, 19 (2): 251−255.
13) I.A.R.C. (1974): Methyl methanesulfonate. In Some Anti-thyroid and Related Substances, Nitrofurans and Industrial Chemicals. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 7.: International Agency for Research on Cancer, Lyon, France. 253-260.
14) Kaur, G., Singh, H.P., Batish, D.R. and Kohli, R.K. (2014): Pb-inhibited mitotic activity in onion roots involves DNA damage and disruption of oxidative metabolism. *Ecotoxicology*, In Press, DOI 10.1007/s10646-014-1272-0
15) Kaya, B. (2003): Anti-genotoxic effect of ascorbic acid on mutagenic dose of Three alkylating agents. *Turk J Biol.*, 27: 241−246.
16) Lambert, B., Sten, M., Hellgrren, D. and Francesconi, D. (1984): Different SCE inducing effects of HN2 and MMS in early late G1 in human lymphocytes. *Mutat Res*., 139: 71−77.
17) Lundin, C., North, M., Erixon, K., Walters, K., Jenssen, D., Goldman, A.S.H. and Helleday, T. (2005): Methyl methanesulfonate (MMS) produces heat-labile DNA damage but no detectable *in vivo* DNA double-strand breaks. *Nucleic Acids Res.*, 3 (12): 3799-3811.
18) Mahadimane, P.V. and Vasudev, V. (2013): Effect of methyl methanesulfonate on Ehrlich Ascites carcinoma cells: dose effect relationships. *Int. J. Life Sci. Pharma Res.*, 3(2): 22−31.
19) Menke, M., Chen, I.P., Angelis, K.A. and Schubert, I. (2001): DNA damage and repair in *Arabidopsis thaliana* as measured by the comet assay after treatment with different classes of genotoxins. *Mutat. Res.*, 493: 87–93.
20) De Oliviera, P.F., Leandro, L.F., Montanheiro, G., Bastos, J.K., da Silva Filho, A.A., Tavares, D.C. (2012): Baccharin prevents genotoxic effects induced by methyl methanesulfonate and hydrogen peroxide in V79 cells. *J. Food Sci*., 77 (8):T138−42.
21) O'Connor, P.J. (2001): Studies on mechanism of action–interaction of chemical carcinogens with macromolecules. *J. Cancer Res. Clin. Oncol.*, 99: 167–186.
22) Pottenger, L.H., Schisler, M.R., Zhang, Fagen., Bartels, M.J., Fontaine, D.D., McFadden, LG. and Gollapudi, B.B. (2009): Dose–response and operational thresholds/NOAELs for in vitro mutagenic effects from DNA-reactive mutagens, MMS and MNU. *Mutat. Res.,* 678: 138–147.
23) Rucinska, R., Sobkowiak, R. and Gwozdz, E.A. (2004): Genotoxicity of lead in lupin root cells as evaluated by the comet assay. *Cell. Mol. Biol. Lett.*, 9: 519–528.
24) Schubert, I. and Rieger, R. (1987): Effects of hydroxyurea post-treatment in G2 on the frequency and distribution of mutagen-induced chromatid aberrations in a reconstructed karyotype of *Vicia faba. Biol. Zbl*., 106: 59–66.
25) Singh, B., and Gupta, R.S. (1983): Mutagenic responses of thirteen anticancer drugs on mutation induction at multiple genetic loci and on sister chromatid exchanges in Chinese Hamster Ovary cells. *Cancer Res*., 43: 577–584.
26) Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C., Sasaki, Y.F. (2000): Single cell gel/ Comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. *Environ. Mol. Mutagens.*, 35: 206−221.
27) Vajpayee, P., Dhawan, A., Shanker, R (2006): Evaluation of the alkaline Comet assay conducted with the wetlands plant *Bacopa monnieri* L. as a model for ecogenotoxicity assessment. *Environ. Mol. Mutagen*., 47(7):483-489.
28) Vasu, D. and Hasan, Z. U. (2011): Cytological abnormalities in *Trigonella* induced by EMS, MMS and MES. *Adv. Biores.*, 2 (2): 12–15.
29) Vasudev, V., Mahamood, R. Harish, S.K. and Guruprasad, K.P. (1997): Comparative analysis of error-free DNA repair (Adaptive Response) induced by EMS and MMS in *Poecilocerus pictus* and Mouse. In: Environmental Pollution and Genetic Risk. (Eds.) M. Hemaprasad and P.P. Reddy, M/s. Murthy Graphics, Hyderabad, 59−68.
30) Yildiz, M., Ciğerci İ. H., Konuk, M., Fidan, A. F. and Terzu, H. (2009): Determination of genotoxic effects of copper sulphate and cobalt chloride in *Allium cepa* root cells by chromosome aberration and comet assays. *Chemosphere*, 75(7): 934−938.