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Full Length Research Paper

Endosulfan: A potential genotoxicant on *Allium cepa* root tip cells

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The present study reports the potential genotoxic effects of a widely used insecticide, endosulfan (Endoin 35% EC), on root tip cells of onion (*Allium cepa L.*). Treatment of onion root tips at a concentration of 0.156 µg/L of endosulfan showed highest fluctuation of mitotic depression at various durations (0.38 to -181%) of exposure. The mitotic index decreased with the increase in the concentrations of endosulfan mostly at 48 h treatment. The maximum number of dividing cells (n) was observed to be 224 calculated as maximum MI value of 7.0 in lowest concentration (0.0078 µg/L) at 72 h. The abnormalities of common occurrence were unequal cytokinesis and karyokinesis and formation of binucleolated cells in many cases. Gradual decondensation or little condensed chromosomal arms were the common observations in abnormal metaphases and anaphases. The frequencies of all the mitotic abnormalities showed a good correlation with the concentration of the insecticide.

Key words: *Allium cepa*, endosulfan, mitotic index, aberrant cells, genotoxicity.

INTRODUCTION

Large-scale utilization of different agrochemicals in developed as well as developing nations has created significant impact on agro-productions to bring green revolution but on the other hand they have become agro-pollutants in the environment. Excessive use of broad-spectrum or non selective pesticides, insecticides and herbicides damages the ecosystem irreversibly, contaminates soil surface and ground water as well as food chains affecting health of the inhabitants of aquatic and terrestrial environment (Tilak et al., 1980; Subbarao, 1999). Human exposure to agricultural chemicals at the time of use in the field has been associated with an increase in cancer incidence (Saftlas et al., 1987; Brown et al., 1990; Blair and Zahm, 1991; Cerhan et al., 1998; Settimi et al., 2003) even if in some cases only a moderate association is reported (Abdalla et al., 2003). The mutagenic and carcinogenic action of herbicides, insecticides and fungicides on experimental animals is well known and several studies have shown that chronic exposure to low levels of pesticides can cause mutations and/ or carcinogenicity (IARC, 1990, 1991; Karabay and Gunnehir, 2005; Bull et al., 2006). As per Pimentel et al. (1998), genotoxicity and mutagenicity of pesticides for non-target organisms and their influence on ecosystems are of worldwide concern. Phytotoxic and genotoxic effects of different pesticide have been determined in different organisms (Sinha, 1989; Aktac et al., 1994; Dane and Dalgic, 2005; Tartar et al., 2006). Several studies have shown that chronic exposure to low levels of pesticides can cause birth defects and that prenatal exposure is associated with carcinogenicity (Heeren et al., 2003; Alavanja et al., 2004; Vogel, 2005; Ferretti et al., 2007).

Endosulfan is an insecticide belonging to the organochlorine group, under the cyclodiene subgroup, introduced in the 1950's. It's International Union of Pure and Applied Chemistry (IUPAC) name is 6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide. It is a derivative of hexachlorocyclopentadiene and is chemically similar to aldrin, chlordane, and heptachlor. It is used in vegetables, fruits, paddy, cotton, cashew, tea, coffee, tobacco and timber crops. It emerged as a leading chemical used against a broad spectrum of insects and

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mites in agriculture and allied sectors and acts as contact and stomach poison and has a slight fumigant action. Technical endosulfan is a 7:3 mixture of stereoisomers, designated α and β. It is acutely neurotoxic to both insects and mammals, including humans. It is a gamma amino butyric acid (GABA) gated chloride channel antagonist, and a Ca^{2+}, Mg^{2+} ATPase inhibitor. It is widely used and continues to pollute the human environment not only in developing countries but in developed countries as well (Simonich and Hites, 1995). Symptoms of acute poisoning include hyperactivity, tremors, convulsions, lack of coordination, staggering, difficulty in breathing, nausea, vomiting, diarrhoea and in severe cases, unconsciousness (WHO, 2000).

The plant (*Allium cepa*) as a test system was introduced by Levan (1938) while investigating the affects of colchiciene. *A. cepa* assay is an efficient method for chemical screening and *in situ* monitoring of genotoxicity of environmental contaminants. It is a short-term test with many advantages like low cost, ease to handle, good chromosome conditions for the study of chromosome damage or disturbance of cell division including the evaluation of risks of aneuploidy (Fiskesjo, 1985). It has been widely used to study genotoxicity of many pesticides and other compounds (Ma et al., 1995; Thais et al., 2007). The assay is good and sensitive for monitoring clastogenic effects (Rank and Nielsen, 1993; Ma et al., 1995). In contrast, a chromosomal aberration study is time consuming and requires very accurate observation by skilled worker (Siboulet et al., 1984).

The production and use of endosulfan is banned worldwide but still it is used by the farmers in many parts of India including the state of Odisha. As endosulfan is a well known genotoxicant, effort is required to assess the genotoxic and cytotoxic effect of this pesticide on vegetables and crops. Hence, in the present study an attempt has been made to evaluate the effects of endosulfan on the mitotic cell division and chromosomes in the growing root tip cells of *A. cepa*.

MATERIALS AND METHODS

The endosulfan used in the present study was purchased from the local market of Bhubaneswar, India, under the trade name ENDOIN 35% (390 µg/µl) EC. It comprises technical grade endosulfan (39%) based on purity 90% dissolved in other ingredients *viz.*- stabilizer epichlorohydrin (2%), emulsifier (6%), xylene solvent (5%), aromax solvent (48%). Concentration of technical endosulfan in ENDOIN 35% EC in the original pack purchased was: 39% (w/v). The test procedure involved original form of Allium test (Fiskesjso, 1985) where the root growth was initiated in tap water. When the roots have reached the length of 1 to 2 cm, treatments were performed at various duration and concentrations. The test tubes for each bulbs were filled with test liquids every day to compensate evaporation. Five concentrations (0.78, 0.312, 0.156, 0.078 and 0.0078 µg/L) of endosulfan were prepared in distilled water and employed to the growing root tips of *A. cepa* in separate experimental tubes. The pre-soaked bulbs in distilled water for 30 to 60 min were grown in different test tubes for separate treatment schedules (24, 48 and 72 h). Harvested root tips were fixed in 1:3 aceto-alcohols following treatment and then stored in 70% alcohol after 24 h for future use. The bulbs grown in ordinary day to day use tap water was utilized as the control. For each concentration and treatment schedule of endosulfan, 5 to 10 numbers of bulbs were planted from various purchase stock and from each bulb 5 to 10 root tips were collected for each analysis. Conventional squash preparation was adopted following the acid hydrolysis of cellulosic cell wall in warmed 1 N hydrochloric acid. Staining was done in 2% aceto-caramine in 45% glacial acetic acid to visualize the dividing cell stages. A total of 3 to 7 slides were prepared per each concentration of treatment. The slides were observed under the binocular light microscope (Olympus CX 31) using the 100 X objective lens with oil immersion. Micro photography of some representative selected stages was taken by Sony Digicam. A range of 300 to 3000 cells were scored from slides and the cells were recorded as normal or aberrant in the different stages of the cell cycle. In the root tip cells, chromosomal and mitotic abnormalities were scored. Mitotic indices (MI), mitotic depression (MD) and anaphase to metaphase ratio were calculated following the procedure of Das (1986) and Kar (1992). In addition, the proportion of specific cell abnormalities such as abnormal prophase, metaphase and anaphase was calculated in terms of percentage of the type of abnormality out of the total number of cells counted.

Statistical analysis

The mean value with standard deviation (SD) for each root length was calculated from value obtained from individual bulbs and it was compared to the corresponding control value. The 't' test was conducted manually to ascertain if the difference was statistically significant or not for root growth.

RESULTS

The effects of endosulfan, observed in this study were based on microscopic (cytogenetic effect) and macroscopic (growth inhibition effect) evaluations which includes observation on mitotic abnormalities like stickiness, spindle disturbances (orientation of spindle, unequal cytokinesis and karyokinesis, etc.) and binucleate cells.

Table 1 describes the effect of endosulfan on mitotic indices and depression in various treatment schedules. Number of onion bulbs used for each treatment ranged from 3 to 12 where as the number of cells scored (N) for MI calculation ranged from 355 to 3200 with an average number of 1409. The maximum number of dividing cells (n) was observed to be 224 calculated as maximum MI value of 7.0 in case of lowest concentration (0.0078 µg/L) for 72 h. Similarly, the minimum number of dividing cells were observed in the same concentration for 48 h (n = 9, MI = 2.53). In control conditions the MI varied from 2.53 to 6.9 which were very similar to those in the lowest concentration of endosulfan (0.0078 µg/L) (Table 1). The trend of mitotic index showed a gradual increase with increasing concentration of endosulfan.

The mitotic inhibition was calculated to be of negative value in many cases due to high MI values of negative control experiments. It was maximum of -181.1 in 0.0078

Table 1. Effect of endosulfan on mitotic indices (MI), mitotic depression (MD), root growth and % of abnormal cells in root tip cells of onion (*Allium cepa*).

S/N	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Treatment	C1	C2	C3	T1	T1	T1	T2	T2	T2
Number of bulbs	12	10	6	3	3	7	7	4	4
Conc. (µg/L)		0			0.0078			0.078	
Duration (h)	24	48	72	24	48	72	24	48	72
Average no. of cells scored (N)±SEM	592±8	787±12	1766±14	-	355±4	3197±2	1347±3	1400±12	830±12
No. of dividing cells (n)±SD	31 ±2	71±1	44±2	-	9±1	224±1	43±3	60±2	12±4
Mitotic Indices (M.I.) in %±SEM	5.23±1.2	6.9±0.9	2.49±0.1	-	2.53±0.3	7.0±1.5	3.19±1.0	4.28±2.2	1.44±1.3
Mitotic depression (M.D.)±SEM	-	-	-	-	63.3±0.2	-181.0±9.5	39.0±1.2	37.9±0.8	42.2±2.1
Mean root length± SD	5.0±3.8	11.8±7.4	9.4±6.4	--	5.7±4.1	25.4±16.3	8.3 ±4.9	18.9±8.3	13.6±9.6
t Values									
(p=0.005)	NA	NA	NA	NA	Ns*	Ns	Ns	Ns	Ns
(P=0.05)	NA	NA	NA	NA	Ns*	Ns	Ns	Ns	Ns
Abnormal cells (%**)**	0.18±0.2	0.25±0.4	0.39±0.1	-	0.28±0.7	1.24±1.1	0.89±0.1	1.07±0.9	0.24±0.6
% of each anomaly									
AP	0.00	0.00	0.05	-	0.00	0.00	0.00	0.00	0.00
AM	0.00	0.48	0.16		0.00	0.25	0.32	0.50	0.00
AA	0.6	0.38	0.00		0.00	0.59	0.57	0.42	0.24
AT	70.50	0.87	0.18		0.28	0.40	0.00	0.14	0.00
S/N	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Treatment	T3	T3	T3	T4	T4	T4	T5	T5	T5
Number of bulbs	6	3	6	4	6	4	5	5	5
Conc. (µg/L)		0.156			0.312			0. 78	
Duration (h)	24	48	72	24	48	72	24	48	72
Average no. of cells scored (N) ± SEM	1937±3	1574±6	1626±4	2071±9	1655±5	1554±6	1641±9	1189±11	434±16
No. of dividing cells (n) ± SD	101±1	70±2	90±2	115±1	95±2	65±2	107±3	43±7	14±6
Mitotic Indices (M.I.) in % ± SEM	5.21±2.1	4.44±0.3	5.53±0.8	5.55±2.4	5.74±0.2	4.18±1.3	6.52±1.7	3.61±0.4	3.22±0.5
Mitotic depression (M.D.) ± SEM	0.38±0.1	35.6±1.7	-122±7.9	-6.1±0.6	16.8±3.2	-67.9±0.1	-24.6±0.45	47.7±0.3	-29.3±0.7
Mean root length ± SD	4.7±2.3	14.1±5.9	7.6±5.4	6.1±3.7	11.2±6.9	17.4±8.5	6.3±4.3	6.5±2.3	11.0±8.09
t values									
(p=0.005)	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
(P=0.05)	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Abnormal cells (%**)**	0.92±0.8	1.90±0.1	1.59±1.2	1.86±0.2	0.90±0.1	0.96±0.2	2.43±0.34	1.00±0.98	1.38±1.8
% of each Anomaly									
AP	0.05	0.00	0.00	0.33	0.12	0.00	0.06	0.00	0.00
AM	0.20	0.44	0.30	0.09	0.00	0.32	0.67	0.51	0.00

Table 1. Contd.

AA	0.56	0.95	0.73	0.19	0.48	0.25	1.03	0.33	0.69
AT	0.10	0.31	0.55	1.15	0.30	0.38	0.54	0.16	0.69

Mitotic index (M.I) = n/N × 100; Mitotic depression (M.D) = {MI (Control) - MI (Treated)/ MI (Control)} × 100; NA: Not applicable; Ns: Not significant, * = significant; AP: Abnormal prophase; AM: Abnormal metaphase; AA: Abnormal anaphase; AT: Abnormal telophase; SD: Standard deviation; SEM: Standard error of mean.

µg/L for 72 h and minimum of 0.38 in 0.156 µg/L for 24 h (Table 1). Treatment of onion root tips at a concentration of 0.156 µg/L of endosulfan showed highest fluctuation of MD at various durations (0.38 to -181%). The trend of MI and MD exhibited increasing MI with increasing concentration of endosulfan on onion root tip cells. In comparison to the control, the minimum and maximum MI values at 24 h treatment were recorded at 0.078 µg/L concentration (3.19) and at 0.78 µg/L concentration (6.52) respectively. At 48 h, there was a continuous increase in MI value from 0.0078 to 0.312 µg/L of endosulfan compared to the control (Table 1).

The mean root length varied from 4.7± 2.29 to 25.4±16.3 mm. Maximum root length (18.9 ± 8.31) was found in 48 h duration at any concentration. However, after 24 h treatment, maximum root length (8.3± 4.9) mm was observed at 0.078 µg/L and minimum root length (4.7± 2.29) mm was found at 0.256 µg/L (Table 1). Similarly, at 48 h treatment duration, maximum root length (18.9± 8.31) mm was observed at 0.078 µg/L concentration and minimum root length (5.7± 4.15) mm) at 0.0078 µg/L concentration of endosulfan. Finally, the 72 h treatment schedule showed maximum and minimum root length 25.4±16.3 and 7.6±5.44 mm at 0.0078 to 0.156 µg/L concentration of endosulfan. In negative control experiments, abnormal cells varied within a range of 0.18 to 0.39% where as in endosulfan treated onion root tips the value ranged between 0.24 and 2.43%.

Stastistcal analysis (t-test) was performed manually to correlate the effect of endosulfan on onion root tip growth (Table1). The t test for root growth observed was found to be insignificant in all cases in comparison to those at negative control experiments at 0.005 level of probability but was only significant in case of 0.078 µg/L at 48 h of exposure.

DISCUSSION

Concern for genotoxicity caused by environmental pollutants has led to the development of several biological tests for detecting and identifying genotoxicant in the air, water and soil. In this study, mitotic indices of onion root tip treated with endosulfan were reduced as compared to the negative control. Other chromosomal aberrations included rings, laggards, and bridges, disoriented and precocious chromosomes. Growth of roots did not occur uniformly. The common types of aberrant cells observed were chromosomal bridge and abnormal uncoiling of chromosomes during anaphase and metaphase. The insecticide treatment could have cause the failure of chromosomes to align at equatorial plate because of the dysfunction of spindle and energy deficiency causing delay in the division of centromeric region which might have also caused distorted chromosome (Jain and Sarbhoy, 1988). The most common type of aberration found was the irregular and transverse orientation of chromosomes in equatorial plate. In metaphase, chromosomes were arranged in metaphagic plate which was diagonally placed. In anaphase, chromosomal bridge and orientation problem in pole was observed. Stickiness is induced either by the effect of pesticides on chromosomal protein attributed to the improper folding of chromosome fibers which render the chromatid connected by means of sub-chromatid bridges (Badr and Ibrahim, 1987; Klasterska et al., 1976) or may be due to the action of pesticides on the polymerization process, resulting in the fragmentation of chromosomes and bridges at anaphase stage forms sticky chromosomes (El-ghamery et al., 2000). This stickiness presumably is due to intermingling of chromatin fibres, which leads to sub-chromatid connections between chromosomes (Klasterska et al., 1976).

In the present study, endosulfan affected cell plate formation was found to be under great disturbances and the orientation as well as functioning of the spindle. In several late anaphases, one group of chromosomes was found to take the extreme terminal position while the other was in the middle. Similar orientations of the two nuclei were also noticed in certain bi-nucleated cells. Some adjacent cells were also of unequal sizes, some diagonal and transverse metaphases and anaphases were also observed (Figures 1 to 3). These indicated abnormal orientation of mitotic apparatus (MA) or distorted mitotic spindles (DOS) causing dis-oriented mitosis (DOMi). Occurrence of disoriented

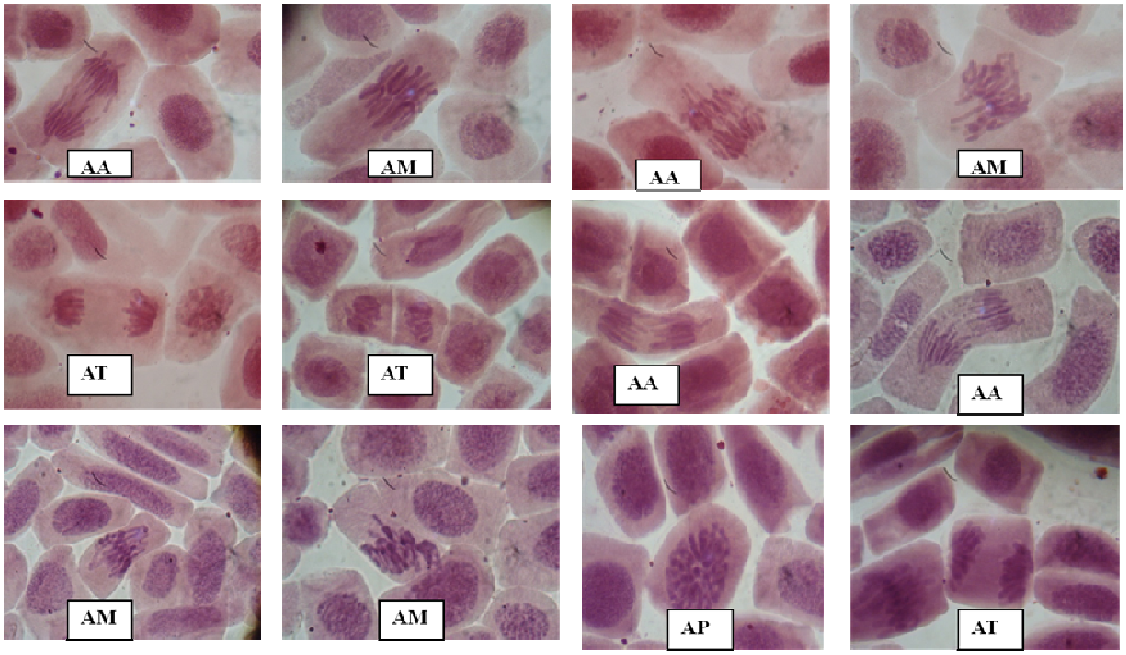

Figure 1. Chromosomal aberrations due to genotoxicity of endosulfan at 0.0078 µg/L-72 h. AP, Abnormal prophase; AA, abnormal anaphase; NP, normal prophase; NA, normal anaphase; DDOMi, diagonally disoriented mitosis; AMC, abnormal mitotic cell; AM, abnormal metaphase; AT, abnormal telophase; NM, normal metaphase; NT, normal telophase; DOMi, disoriented mitosis.

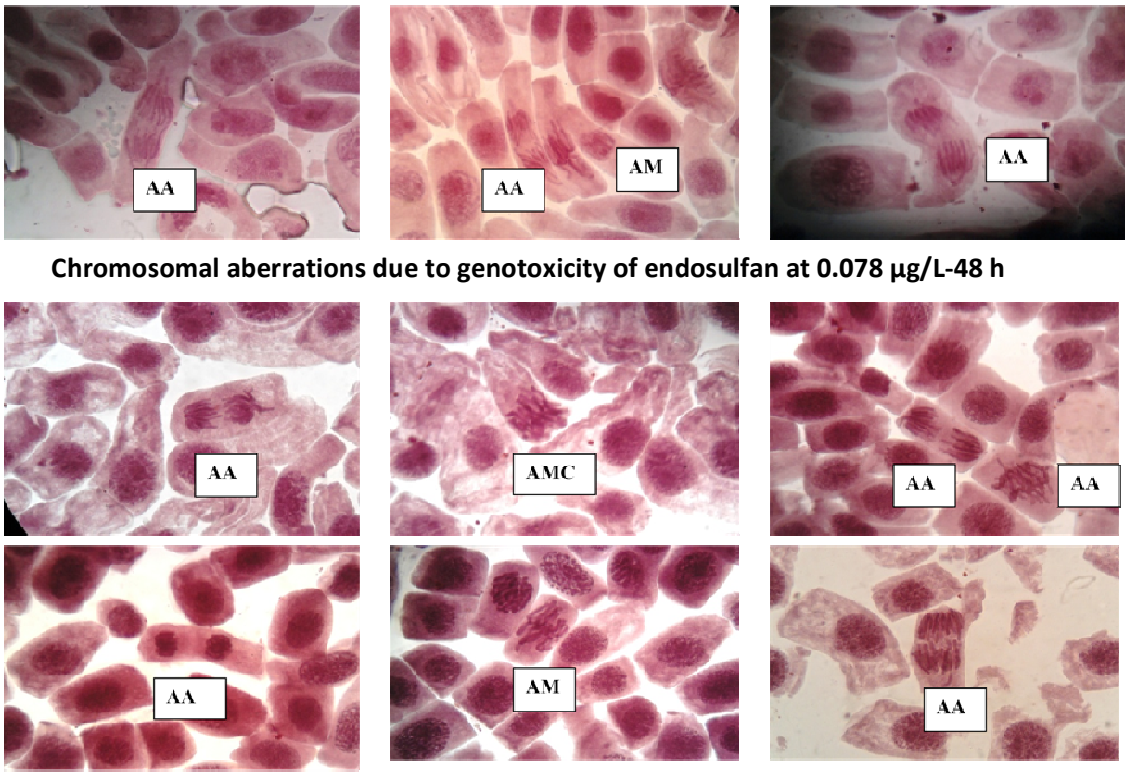

Figure 2. Chromosomal aberrations due to genotoxicity of endosulfan at 0.312 µg/L-48 h. AP, Abnormal prophase; AA, abnormal anaphase; NP, normal prophase; NA, normal anaphase; DDOMi, diagonally disoriented mitosis; AMC, abnormal mitotic cell; AM, abnormal metaphase; AT, abnormal telophase; NM, normal metaphase; NT, normal telophase; DOMi, disoriented mitosis.

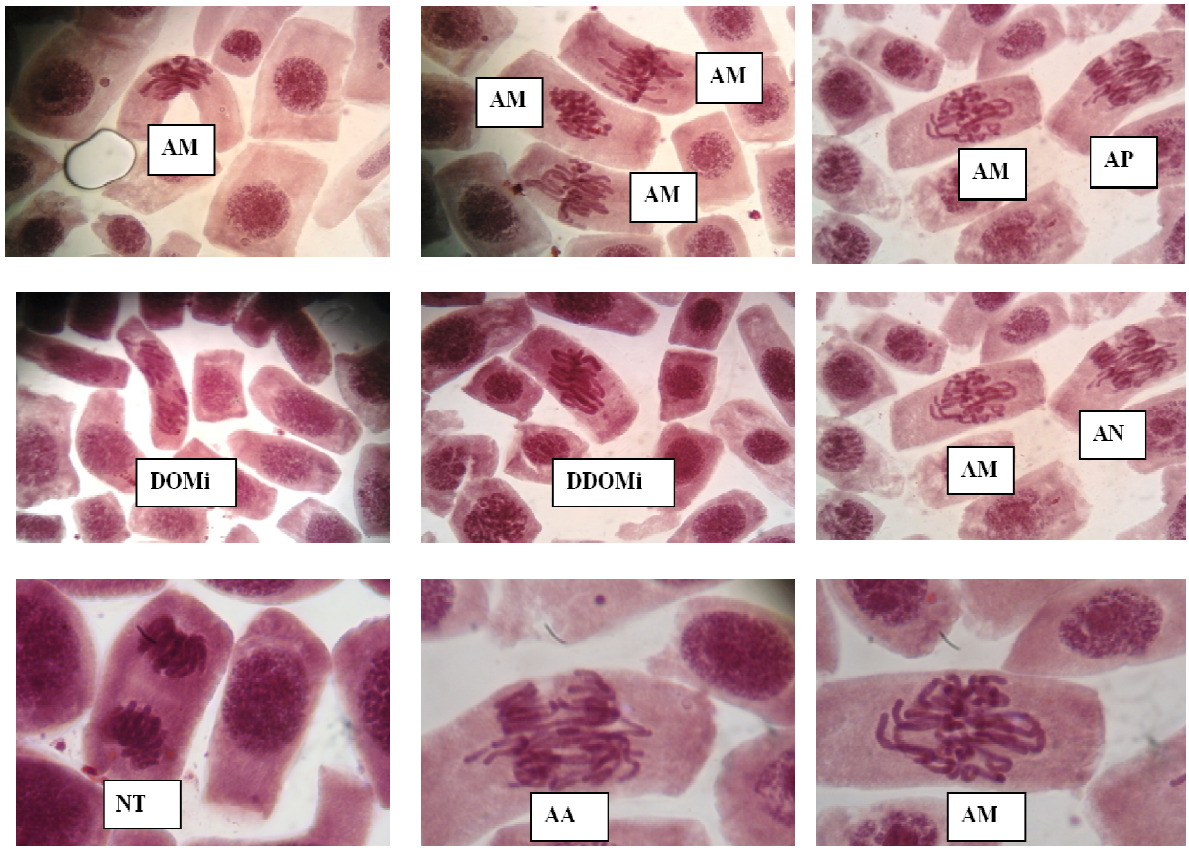

Figure 3. Chromosomal aberrations due to genotoxicity of endosulfan at 0.078 µg/L-48 h. AP, Abnormal prophase; AA, abnormal anaphase; NP, normal prophase; NA, normal anaphase; DDOMi, diagonally disoriented mitosis; AMC, abnormal mitotic cell; AM, abnormal metaphase; AT, abnormal telophase; NM, normal metaphase; NT, normal telophase; DOMi, disoriented mitosis.

chromosomes might have been brought about by action of endosulfan on the microtubules (MT) of the spindle fibres. The effects were assumed to be the genotoxic assault of endosulfan on chromosome condensation mechanism affecting the proteins like condensins and (or) cohesins during cell division resulting in unusual long arms. To describe the chromosomal condensation mechanism during cell cycle, Cooper and Hausman (2007) reported that, mitosis involves dramatic changes in multiple cellular components, leading to a major reorganization of the entire structure of the cell. The condensation of interphase chromatin to form the compact chromosomes of the mitotic cells is a key event in mitosis, critical in enabling the chromosomes to move along the mitotic spindle without becoming being broken or entangled with one another. The chromatin in interphase nuclei condenses nearly thousand fold during the formation of metaphase chromosome. Cooper and Hausman (2007) established that chromatin condensation is driven by protein complexes called condensins which are members of a class of 'structural maintenance of chromatin proteins', (SMC) that play key role in organization of eukaryotic chromosomes. Another family of SMC proteins called cohesins contribute to chromosome segregation during mitosis. In this study, observation of abnormally decondensed or elongated or stretched chromosomes in numbers of prophases, metaphases and anaphases are considered to be due to disturbance of chromosomal condensation mechanism due to endosulfan by disturbing the SMC proteins like condensins or cohesins.

Conclusion

Results of the study indicated the potential genotoxic effect of endosulfan on the root tip cells of onion. The insecticide showed highly significant effect on mitotic process. The frequencies of all the mitotic anomalies showed a good correlation with the concentration of the insecticide. In a long run, the use of this pesticide may have a negative impact on eukaryotic genome including plants and animals. So effort is required by the researchers to assess the genotoxicity of this pesticide

with more recent techniques.

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REFERENCES

Abdalla MH, Gutierrez-Mohamed ML, Farah IO (2003). Association of pesticide exposure and risk of breast cancer mortality in Mississippi. Biomed. Sci. Inst. 39:397-401.

Aktac T, Ekinci F, Sidal U, Sidal FE (1994). The effect of endosulfan on root tip cells of lentil (*Lens esculanta*). Turk. J. Biol. 18:27-37.

Alavanja MC, Hoppin JA, Camel F (2004). Health effects of chronic pesticides exposure: cancer and neurotoxicity. Ann. Rev. Pub. Health. 25:155-197.

Badr A, Ibrahim AG (1987). Effect of Herbicide Glean on Mitosis, Chromosomes and Nucleic Acids in *A. cepa* and *V. faba* root Meristems, Cytol. 52: 293-302.

Blair A, Zahm SH (1991). Cancer among farmers. Occup. Med. 6:335-354.

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, Burmeister LF, Van Lier SF, Dick F (1990). Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res. 50:6585-6591.

Bull S, Fletcher K, Boobis A, Batterrshill J (2006). Evidence for genotoxicity of pesticides in pesticide applicators. Mutagen 21(2):93-103.

Cerhan JR, Cantor KP, Williamson K, Lynch CF, Torner JC, Burmeister LF (1998). Cancer mortality among Iowa farmers: recent results, time trends, and lifestyle factors. Cancer Causes Cont. 9:311-319.

Cooper GM, Hausman RE (2007). In: The Cell, A Molecular Approach, Fourth Edition. Chapter 16: The cell cycle. pp. 672-673.

Dane F, Dalgic O (2005). The effects of fungicide Benomyl (Benlate) on growth and mitosis in onion (*Allium cepa* L.) root apical meristem. Acta Biologica Hungarica 56(1–2):119–128.

Das RK (1986). *In vivo* cytogenetic assays for evaluating genotoxicity of pharmaceuticals. In Perspective in Cytology and Genetics (eds. Manna and U. Sinha) 5:13-19.

El-ghamery AA, El-nahas AI, Mansour MM (2000). The Action of Atrazine Herbicide as an Indicator of Cell division on Chromosomes and Nucleic Acids Content in Root Meristems of *A. cepa* and *V. faba*. Cytologia 65:277–287.

Ferretti D, Zerbini I, Zani C, Ceretti E, Moretti M, Monarca S (2007). Food Add. Contam.24(6):561-572.

Fiskesjo G (1985). The Allium test as a standard in environmental monitoring. Hereditas 102:99-112

Heeren GA, Tyler J, Mandeya A (2003). Agricultural chemical exposures and birth defects in Cape Province, South Africa. A case-control study. Environmental Health: Global Access Sci. Sour. 2:11-19.

International Agency for Research on Cancer, IARC (1990). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans 1(69):1969-1997.

International Agency for Research on Cancer, IARC (1991). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Occupational Exposures in Insecticide Application and Some Pesticides. 53:33-586.

Jain AK, Sarbhoy RK (1988). Cytogenetical studies on the effect of some chlorinated pesticides. III. Concluding Remarks, Cytologia 53:427-436.

Karabay NU, Gunnehir OM (2005). Cytogenetic and genotoxic effects of the insecticides, imidacloprid and methamidophos. Genet. Mol. Res. 4(4):653-662.

Kar NR (1992). Effects of textile mill effluent on root tips of onion (Allium cepa). A thesis submitted to Sambalpur University, Odisha, India for the partial fulfilment of the award of the degree on Master of Philosophy in Life Sci. pp. 06-07.

Klasterska I, Natrajan AT, Ramel C (1976). An Interpretation of the Origin of Sub-Chromatid berrations and Chromosome Stickiness as a Category of Chromatid Aberration, Hereditas 83:153–162.

Levan A (1938). The effect of colchiciene on root mitosis in *Allium*. Hereditas 24:471-486.

Ma TH, Xu Z, Xu C, Mc Connell H, Rabago EV, Arreola AG, Zhang H (1995). The improved *Allium/Vicia* root tip micronucleous assay for clastogenicity of environmental pollutants. Mutat. Res. 334:185-195.

Pimentel D, Greiner A, Bashore T (1998). Economic and Environmental Costs of Pesticides Use. In: Environmental Toxicology: current Developments. Gordon and Breach Science Publisher: London UK pp. 121-187

.Rank J, Nielsen MH (1993). A modified *Allium* test as a tool in the screening of genotoxicity of complex mixtures. Hereditas 118:49-53.

Saftlas AF, Blair A, Cantor KP, Hanrahan L, Anderson HA (1987). Cancer and other causes of death among Wisconsin farmers. Am. J. Ind. Med. 11:119-129.

Settimi L, Masina A, Andrion A. Axelson O (2003). Prostate cancer and exposure to pesticides in agricultural settings. Int. J. Canc. 104:458-461.

Siboulet R, Grinfeld S, Deparis P, Jaylet A (1984). Micronuclei in red blood cells of newt *Pleurodeles waltl* Michah: induction with X-rays and chemicals. Mutat. Res. 125:275-281.

Simonich SL, Hites RA (1995). Global distribution of persistent organochlorine compounds. Science 269:1851-1854.

Sinha SP (1989). Genotoxicity of pesticide. Perspect. Cytol. Genet. 6:749-753.

Subbarao NS (1999). Soil Microbiology, 4th edition, Science Publisher, Inc. pp. 303-324.

Tartar G, Kaymak F, Gokalp FM (2006). Genotoxic effect of avenoxan on *Allium cepa* L. and *Allium sativum* L. Caryologia 59:241-247.

Thais C, Dania Elisa C, Maria A (2007). Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin herbicide. Pest Biochem. Physiol. 88(3):252-259.

Tilak KS, Rao DMR, Devi AP, Murty AS (1980). Toxicity of carbaryl and 1 - naphthal to the freshwater fish *Labeo rohita.* Ind. J. Exp. Biol. 18(1):75.

Vogel JM (2005). Perils of paradigm: complexity, policy design and the endocrine disruptor screening program. Environmental Health: Global Access Sci. Sour. 4:2-13.

WHO (2000). International Programme on Chemical Safety, World Health Organization, Endosulfan (Poison Information Monograph 576), July 2000.