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Research Article

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A Study of Azo Dye Reactive Red 120 Induced Genotoxicity on *Allium cepa* L.

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ABSTRACT

The effects of Reactive Red on the chromosome of Allium cepa are reported. A significant reduction in mitotic index was observed as the dose and time interval is increased as compared to control. The dye also induces different types of aberrations viz: anaphase with bridge, multipolar anaphase, metaphase with loss and stickiness. The dye at the highest concentration (1000 µg/L) induces highest number of chromosomal aberrations at different time exposure. Thus the results depict that under the dye at the evaluated doses and exposure times were mito-depressive and clastogenic. Cytotoxicity studies should be conducted for further evaluation of the effect of the dye in a natural system.

Keywords*:* Genotoxicity; Chromosomal aberrations; Mitotic index; *Allium cepa;* Reactive Red 120

INTRODUCTION

Contamination of natural environment due to industrialization has impacted to both flora and fauna. The wastewaters coming out of the industries pose threat to the aquatic resources [3, 18]. These wastewaters contain complex mixtures of chemicals from inorganic compounds to more complex organic molecules [26, 25] that enter water reservoirs and that might become hazardous [5].

Textile, paper, pharmaceutical, food and cosmetic industries uses azo dye at a large scale [24] and account for 70% of synthetic dyes used [7] and 5-10% of this amount is discharged into the environment [24]. Reactive dyes are very simple chemical structure and chemically constituted by azo compounds, anthraquinones and phtalocyanines [26]. Reactive Red-120 is frequently used dyes potentially threatening to the aquatic system because of its poor biodegradability [1]. These azo dye remains left as spent dye bath and reduces the water quality once discharged and poses toxic effect to the aquatic flora and fauna [22, 26] and also to human health [5].

Higher plants are the excellent genetic models to assess environmental pollution, which range from point mutations to chromosome aberrations (CA) in cells of different organs and tissues [17]. They are sensitive and simple as compared to animal system is regarded as the best bio indicators of contamination of the natural resources. Allium cepa is considered as an efficient system test for genotoxic evaluation [17, 9. 11, 4, 34, 2, 17] due to its size and no. of chromosomes (2n = 16) [19,20,8]. Furthermore, this test system has shown high sensitivity in detecting environmental chemicals [17]. The aim of the present study was to evaluate the genotoxic potential of reactive red 120 by employing root meristem cells of A. cepa.

MATERIALS AND METHODS

Materials

Reactive Red 120 (Mol. Formula- C44H24Cl2N14Na6O20S6, CAS Number: 61951-82-4) was procured from Prabhat Dying Mills, Tajpur Road, Ludhiana and JCT Mill, Phagwara, Punjab, India.

Allium cepa have been used as test organisms since they are genetically and physiologically homogenous and is also available throughout the year [8]. Equal sized and healthy onion bulbs were chosen. Diseased and dried bulbs were not used.

Methods

The treatment concentrations 1000µg/l, 100µg/l and 10µg/l- determined by using pilot tests. For the pilot-tests, 100, 10 and 1mg/L concentrations were tested, and the A. cepa roots development was analyzed. This is done to obtain a concentration at which the germination rate was above 60% and the roots were not so fragile for handling. The outer dry scales and old roots were removed with the help of sharp forcep so as to expose root primodia. The bulbs were germinated in the coupling jars containing ultrapure water at temperature 25+1 0C.

When the roots reached up to the length of 1 cm they were transferred to coupling jars containing different concentrations of Reactive Red 120 using one jar per concentrations. The roots were exposed to three different time periods (24, 48, 72 hrs) to deliberate upon the time effect of the dye. The control was prepared by exposing the seeds to ultrapure water only, after the required time exposure, the roots were collected and processed for squash preparation.

Squash preparation

Preparation of slides was done using the conventional staining technique for the chromosome aberration assay. For chromosomal analysis, the root tips were hydrolysed in 1N HCl at 60 0c for 10 minutes. Then roots were treated with 2% acetocarmine in water bath at 60 0C for 10 mins. After staining the root tips were squashed in one drop of 2% acetocarmine on a slide by tapping under cover slip and sealed with nail polish. The cells were observed under microscope for different types of chromosomal aberrations and photographs were taken.

In the analyses, the chromosome aberrations were considered: chromosome bridge, chromosome adherence, vagrant chromosome, chromosome bridge, multipolar chromosome (Carita and Marin-Morales 2008). Mitotic Index (MI) was another category analyzed. MI was also an indication of cytotoxicity [8, 17].

All the experiments were conducted in triplicate. About 5,000 cells were counted per tested concentration and in the three treatments (24, 48 and 72 hours). 500 cells per slide were counted, comprising a total of 10 slides. The same number of cells was analyzed in control tests. After obtaining the results, a statistical analysis was done using 't' test, accepting the 0.05 probability in order to indicate a significant effect [23].

RESULTS AND DISCUSSION

Study of Mitotic indices (MI)

A total of 5000 cells were observed for each concentration taken in the study (500 cells of each 10 slides).

Figure 1 depicts the MI becuase of the dye on the root tip meristems of Allium cepa. The figures give the understanding that the highest effect is pronounced at a concentration of 1000µg/L at 72hrs of treatment of the dye (7.27+0.48). The least effect was found at 10µg/L at 24 hrs time interval.

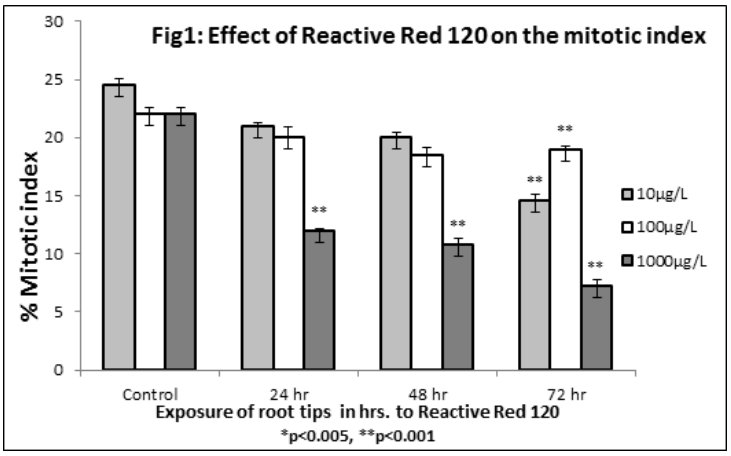

Figure 1: Effect of Reactive Red 120 on the mitotic index

Study of chromosomal aberrations

The test conducted with the root tips of *Allium cepa*. The control exhibited few abnormalitites when it is compared with that of the treatment groups as shown in Table 1, Figure 2. In all the concentrations the aberrations like anaphase with bridge, multipolar anaphase, metaphase with loss, stickiness and others. The

aberrations at 10, 100 and 1000µg/ L showed significant difference ($p<0.001$) at 24 hrs time treatment study Figure 2. Statistical analysis revealed the mutagenic effect of the treatment group compared to that of the control Figure 2, Table:1.

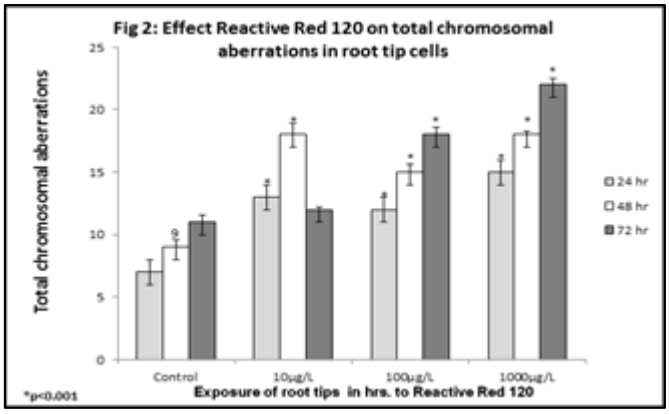

Figure 2: Effect Reactive Red 120 on total chromosomal aberrations in root tip cells

Table 1: Number and frequency of chromosome aberrations obtained for the Allium cepa tests, for Reactive Red in different concentrations.

		24h			48h			72h		
Analysis	**Control**	**10µg/l**	**100µg/l**	**1000µg/l**	**10µg/l**	**100µg/l**	**1000µg/l**	**10µg/l**	**100µg/l**	**1000µg/l**
AB	3	4	10	12	7	3	4	2	1	4
MA	1	2	5	7	2	2	2	1	2	3
ML	1	3	3	6	5	1	1	1	5	4
ST	1	2	2	3	2	3	3	3	0	2
OT	0	1	1	6	4	0	0	1	0	1
TCA	6	12	21	34	18	10	10	8	8	15
TMO	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000
Frequency of TMO	0.88±0.01	0.23±0.01	0.35±0.07	0.70±0.04	0.33±0.02	0.16±0.001	0.19±0.003	0.15±0.002	0.17±0.001	0.27±0.007
AB,anaphase with bridge; MA, multipolar anaphase; ML,metaphase with loss; ST, stickiness; TCA, total cells accounted; TMO, total number of cells observed; OT- Others										

DISCUSSION

The ace group dyes contain also bond (N=N) which connect the naphthalene ring to a second benzene ring. These rings can also contain one, two, or three sulfonic groups. Globally, it represents the class of the most commonly used synthetic dyes[23].

Aazodye (Reactive Red) shows mitotic depressions in all concentrations. The result was also reported by [8, 29, 25]. The mitotic index (MI) as a parameter of cytotoxicity in studies of environmental biomonitoring, has been used to check the cytotoxicity level of test compound [8]. Significant difference was not observed between the control group and the treatment of 10µg/L at 24 hrs and 48 hrs of exposure. The toxic effect was dose and time dependent. At concentration 1000µg/l and at 72h exposure time maximum mitotic depression was highest.

Decrease in MI can be an important indicator to monitor pollution levels in affected environments, especially those contaminated with potentially toxic and cytotoxic compounds [14]. Decrease in mitotic index in cells of A cape due to effluents are considered as the most reliable method to determine the presence of cytotoxic compounds in the environmental system [30]. The use of A. cepa for genotoxicology studies has several advantages, it is sensitive, it's easy to manipulate, rapid response bioassays, it is cheap and good correlation with models that use mammalian cells for this type of study [12, 6].

Mitotic index is utilized as a marker of cell proliferation biomarkers which measures the extent of the cells in the mitotic phase of the cell cycle. Hence, the decrease in the mitotic index of A. cepa meristematic cells could be interpreted as cellular death. The mitodepressive effect was often used in tracing cytotoxicity. The cells of A. cepa root tips after treatment with azodye (reactive red) showed decrease in mitotic index with increasing concentration. The mitotic index shows significantly different results between all treated groups and control group. The result may be due to abnormal conditions of the cells induced by the treatments [21].

In the present study chromosomal structural aberrations are considered as one of the important measures for determining the toxic effects of the effluents. Jadhav et al., 2011 reported that chromosomal aberrations provide an important measure for the genotoxicity potential of dyes.

The various types of the aberration observed were, anaphase with bridge, multiplier anaphase, metaphase with the loss and the stickiness of the chromosomal complements. Stickiness in the proteins of the chromosome may be the reason for stickiness of chromosomes which were found in onion roots [26]. Stickiness may also be interpreted as a result of depolymerisation of DNA, dissolution of nucleoproteins, exchange and breakage of the basic folded fibre units of chromatids and the stripping of the protein covering of DNA in chromosomes as reported by Mercykutty and Stephen (1980). According to Fiskesjo (1985), sticky chromosomes showed an irreversible highly toxic effect, probably leading to cell death.

Stickiness of chromosomes is responsible for the formation of chromosomal bridges which made their separation and free movements completely and thus they remained connected by bridges breakage and fusion of chromosomes and chromatids. For failure of free anaphase separation and inversion of chromosome segments stickiness of chromosomes is responsible [17]. Presence of chromosomal bridges at anaphase might be the result from chromosome stickiness which is caused by clastogeniic. Chromosome bridges may result from breaks in Chromosome bridge. The cell is called aberrant, if at least one chromosome gets damaged.

The present study depicts that the chromosomal aberrations in A. cepa is an efficient mechanism to investigate the genotoxic effect of the effluents. Based on the study it was observed that the effluents at different concentration induced mitotic depressions and chromosomal aberrations. The dose at a concentration of 1000µg/L produced highest chromosomal anomalies than that of the control values (Fig1 & 2 and Table 1). Time dependant study shown that for the entire dose taken in the study 72 hrs treatment produces maximum effect ($p<0.001$) detectable by the conducted tests.

CONCLUSION

The azo dyes show cytotoxic and genotoxic effect. They induce mitotic inhibition and various chromosomal aberrations. They are capable of causing ecological disruption in the receiving environment. It is obvious from this study that azo dyes have disastrous effects on living organisms, as it is the main receiving point of the effluents. These compounds can also contaminate water bodies, thereby making it unfit for drinking, irrigation.

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