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Research Article

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Effect of azo dye corafix on root tip chromosomes of *Allium cepa* L.

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ABSTRACT

The aim of this study was to evaluate the cytotoxic effect of the azo dye corafix on Allium cepa L. The dye was evaluated at the doses of 10, 100 and 1000µg/l at the exposure times of 24, 48 and 72. A total of 5000 cells was analyzed for each dose cellular aberration study. The mitotic index was calculated, and statistical analysis was performed using the Chi-squared test ($p < 0.05$). 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

Key words: Genotoxicity, Chromosomal aberrations, Mitotic index, *Allium cepa* Corafix

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INTRODUCTION

Pollution of aquatic system due to industrial discharge poses threat not only to the freshwater, marine water but ground water also. The discharge may be direct or indirect disbalances the ecological system and also genotoxic effects. Along with the other living systems, humans are also exposed to a large number of polluted material causing varieties of health hazards.

Dyes have several categories, which is based on the chemical structure of chromophoric group. These dyes are responsible for environmental pollution, as their disposal in aquatic bodies prevent the penetration of sunlight in them [1]. About 10, 0000 different dyes are being used in textile industries, these days [2]. Estimated production of these dyes at present world-wide is 800,000 tons/year to 50,000 tons/year loss in effluent during application and manufacture [1], and in India production is close to 800, 00 tons [3]. Dyes range from 60 to 70 % of all dyes used in food and textile industries are azodyes, are largest class of synthetic dyes with variety of colour and structure [4]. Azodyes are the largest class of dyes with the greatest variety of colors and is characterized by the presence of one or more azo group, and find extensive applications in textile, leather cosmetic food stuff and paper industries [5, 6]. These are most easily synthesized and have the properties of excellent fixation and permanence in fibers. During the processing 10- 15% of total dye remains left as spent dye bath [7, 8]. Because of inefficient industrial effluent treatment facilities available, these dyes find their route in the aquatic system which reduces the aesthetic value of the water bodies. They reduce the water transparency and poses toxic effect, genotoxicity to the aquatic flora and fauna [1,7]. The toxicant effects of the azo dyes have resulted in poor germination rates and biomass [9]. The azodyes have direct action on the cells by forming metabolites, react with DNA and therefore they damage the genetic system of living organisms.

Plant materials are important material for testing, sensitive and simple as compared to animal system and are regarded as the best bio indicators of cytotoxicity, genotoxicity and mutagenicity. *Allium cepa* is considered as an efficient system test for genotoxic evaluation, due to its kinetic proliferation properties and less no. of (2n = 16) large chromosomes and other properties, which help its analysis for deletion or damage to the DNA structure [10]. *Allium cepa* has been successfully used both for toxicity and genotoxicty assay and has been broadly utilized as a standard for Biomonitoring of ecological contamination

EXPERIMENTAL SECTION

Test dye

The dye which was used in this study was Corafix which are presumed to be potential genotoxicant on plant model. The dye was procured from Prabhat Dying Mills, Tajpur Road, Ludhiana, Punjab, India.

Experimental plant organism

Allium cepa is the experimental organism employed. Genotoxicity have been assessed by treating *Allium cepa* root system with the azo dye, which is known to give similar results to in vivo cytotoxicity test. Equal sized and healthy onion bulbs were chosen. Disease and dried bulbs were not used.

Test procedure

The outer dry scales and old roots were removed with the help of a pair of sharp forcep so as to expose root primodia. The bulbs were germinated in the coupling jars containing distilled water till new roots reached about 1 cm in length. The temperature was maintained at about $25^0C \pm 1$. After that the root tips were exposed to three different concentrations 1000µg/L, 100µg/L and 10µg/L for three different time periods 24, 48 and 72 hrs. The control test was carried out with distilled water. The experimental and control group was containing 5 onion bulbs for each. After the time exposure the root tips were collected and squash were prepared immediately.

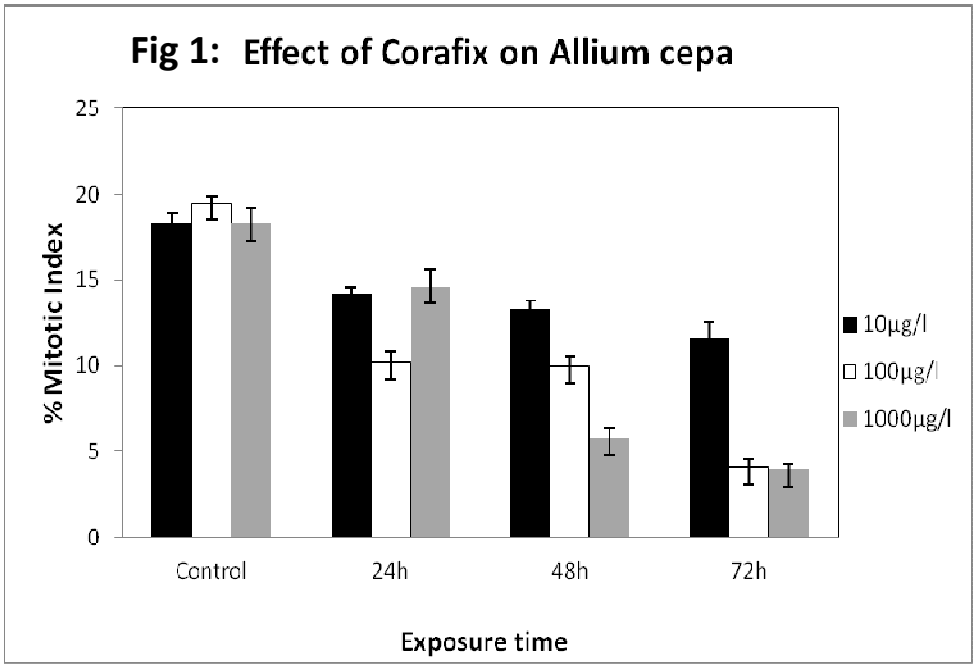
Squash preparation

For chromosomal analysis, the root tips were hydrolyzed in 1N HCl at 60 deg C for 10 minutes. Then they are again treated with 2% acetocarmine and heated again for 10 minutes in water bath. The root tip was then cut with a sharp blade and placed on a glass slide in a drop of acetocarmine and covered with coverslip. The root tips were squashed by tapping with a matchstick and sealed with nail polish. The cells were under the microscope for different types of chromosomal aberrations and photographs were taken.

An aggregate of 1000 cell bulbs of every experiment group was investigated totally 5000 cells per group. The mitotic index was calculated by the number of cells under division, divided by the total number of cells analyzed (MI = no. of dividing cells ×100/total no. of cells). The statistical analysis of the data was carried out by T - test at 5 % significance level.

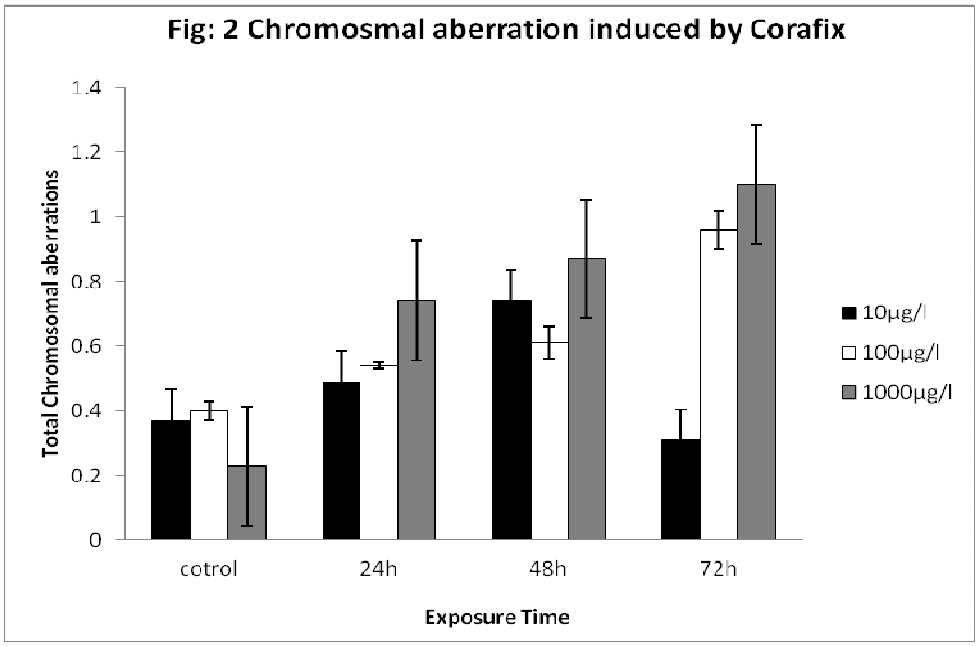
RESULTS AND DISCUSSION

To study the Mitotic indices, after the treatment 5000 cells, each was counted for each concentration and time period. Figure 1 represents the effect of the dye on the root tip meristems of *Allium cepa.* The general trend shows that with the increase in dose and time period the Mitotic indices reduce gradually for the dye taken into the study. In the experiment, it was also found that 100µg/l at 72h for all the dye shows less impact of toxicity on the root tip meristems. The 1000µg/l has the highest shock.



The dose dependent study states that the 1000µg/l was highly toxic and significantly inducing mitotic poisoning. The treatment dependent study shows that more the treatment time more the toxic effect of dye taken into study. The study of the time dependent that shows that 72h treatments were harmful taking Mitotic study into consideration.

The azodye taken in the study produces various types of chromosomal aberrations (vagrant chromosomes, metaphase with loss, multipolar anaphase, adherence, anaphase with bridge) the total chromosomal aberrations are shown in fig. 2.



The root tips treated with Corafix shows the aberrations increasing considerably in the dose dependent manner, i.e., increasing from 10µg/l and highest at 1000µg/l. The most number of aberrations was found at 1000µg/l at 72h. It is interesting to note that the 72h of 10µg/l treated root tips shows the least number of aberrations, which may depict the reduced harmful effect of the dye.

Table 1 depicts the data of various types of aberrations encountered. The most number of aberrations was Anaphase Bridge, which was found in the root tip cells exposed to effluents. The number of the total chromosomal aberrations was also higher in the treated effluents were highly genotoxic thereby they show disorganisation of the chromosomal elements in the root tip cells of *Allium cepa*.

Table :1 Number of chromosome aberrations obtained for the *Allium cepa* tests, for Corafix 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	8	16	16	11	12	10	11	5	14	15
MA	2	6	7	8	10	8	8	4	11	12
ML	6	4	5	10	5	5	10	4	9	7
AD	3	4	2	6	2	4	9	3	10	18
TB	0	0	0	0	0	0	0	0	0	0
BC	0	0	0	0	0	0	0	0	0	0
VC	0	0	1	9	2	4	6	1	5	4
TCA	19	30	31	17	31	30	44	17	49	56
TMO	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000
Mean of TCA	0.37±0.01	0.49±0.03	0.54±0.01	0.94±0.02	0.74±0.09	0.61±0.05	0.87±0.02	0.31±.0.01	0.96±0.63	1.10±0.04

AB-anaphase with bridge; MA- multipolar anaphase; ML-metaphase with loss; AD- adherence; TB- telophase with bridge; BC- binucleated cell; VC- vagrant chromosomes; TCA- total cells with alterations; TMO- total number of cells observed.

The azo group dyes contain azo 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 [11].The observation which was seen in the present study is a clear indication of the clastogenic and mitoclastic property of the dye, which is apparent from the lowering of the mitotic index and increasing chromosomal aberrations.

In this study, the toxic effects of azodye were evaluated by analysed onion root meristem cells. The highest concentration of dye was mostly found to reduce cell division. The potential cytotoxic and genotoxic parameters such as mitotic index and number of chromosomal abnormalities, including Bridges in anaphase, sticky chromosomes, multipolar anaphase. When dye concentrations were tested on *Allium cepa* cells to evaluate their action on the kinetic cell cycle, a decrease in mitotic indices was observed. At higher concentration high mitotic depression effects were seen [12, 13, 14]. The toxic effect was dose and time dependent. At concentration 1000µg/l and at 72h exposure time maximum mitotic depression was found.

The mitotic record diminishes in onion root meristem was discovered to be a dependable means for quick determination of the vicinity of cytotoxic contamination level in the natural environments and for assessment of water contamination levels. This parameter is sensitive additionally to be utilized for observing the contamination levels if somewhat contaminated water.

The dyes used in textile industries are of health concern for various organisms including humans. The present study is comparable to the similar other studies done earlier by various groups of workers in different non- human organisms [15, 16 and 17].

To measure the genotoxicity potential of dyes chromosomal aberrations provide an important tool [18]. The use of *Allium cepa* for genotoxicology studies has several advantages, it is sensitive, it is easy to manipulate, rapid response bioassays, it is cheap and good correlation with models that use mammalian cells for this type of study [19, 20].

Adhesion in the proteins of the chromosome may be the reason for stickiness of chromosomes which were found in onion roots [21]. Sticky chromosomes showed an irreversible highly toxic effect, probably leading to cell death [22]. 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 are responsible [23].

Unequal distribution of chromosomes results in formation of vagrant chromosomes, with paired chromatids which resulted from nondisjunction of chromatids in anaphase. A vagrant chromosome has mostly moved ahead of from its chromosomal group toward the poles, which leads to the unequal separation of number of chromosomes in the daughter cells [24].

Lagging chromosomes are formed when the chromosomes are not getting attached to the spindle fibre and to move to either of the two poles [25]. 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.

Introduction of genotoxicity research in the environment protection policies is of great importance, since it enables us to understand the impact and consequences of genetic substances present in water. Literature distributed on genotoxicity of industrial waste and effluents has as of late been checked [26]. From that audit it is seen that genotoxic impacts in wastewater from diverse commercial enterprises must be expected. Especially effluents from the dye related industries, pulp and paper mills, the refineries and related petroleum wastes, chemical manufacturing and the metal industries have been studied carefully. Most of them have shown to be from moderately to highly and extremely mutagenic when ranked in terms of mutagenic potency. The same result was observed with *Allium cepa* in the present study.

CONCLUSION

The azodye which may find their way in the aquatic system which reduces the aesthetic value of the water bodies. They reduce the water transparency and poses toxic effect, genotoxicity to the aquatic flora and fauna thereby disturbs the ecological balance. The study clearly depicts that azodyes (Corafix) is both cytotoxic and genotoxic. Further study is required to understand the mechanism of damage done by azo dye Corafix.

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