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Induction of Chromosomal Aberrations in *Allium cepa* Root Tips Cells Exposed to Azo Dye Scarlet RR.

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ABSTRACT

Various chemicals were reported to be mutagenic because they are detrimental and causes inheritable changes in the genetic material. Diverse tests have been developed and employed for biomonitoring the pollutants to evaluate their toxic and mutagenic effects that are being discharged in natural environment. This study is aimed at assessing the potential of azo dye Scarlet RR to induce chromosomal and nuclear aberrations in *Allium cepa* test systems. A continuous exposure of root tips to the dye of different concentrations (10µg/L, 100µg/L & 1000µg/L) and for different time exposure (24hr, 48hr, 72hr) was carried out. Cells in divisional phases were examined for mitotic index and chromosomal aberrations. Our results revealed that the dye scarlet RR produces depression in the mitotic index and various chromosomal aberrations in a dose and time dependant manner as compared to control. These results are useful in gathering the data that the dye is cytotoxic and mutagenic and may therefore lead to the damage in the genetics of the organisms.

Keywords: Azo dye, Scarlet RR, Mitotic index, Chromosomal aberrations.

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INTRODUCTION

Pollution of aquatic system is the major concern in today's world because of introduction of residual dyes from different colour using industries (e.g., textile industries, paper and pulp industries, dye and dye intermediates industries, pharmaceutical industries, tannery, and Kraft bleaching industries, etc.). Textile industries are the major contributor as they are generating huge volume of waste water with complex composition of effluents [1]. These untreated effluents add up potentially mutagenic chemicals in the water system thereby disturb ecological balance and also damage the genetic system of the organisms without being immediately expressed [2].

Extensive uses of synthetic dyes are reported in textile, paper, pharmaceutical, food and cosmetic industries [3]. Azo dyes account for 70% of synthetic dyes used [4] and are applied in textile, paper, leather, food stuff and cosmetic industries. Azo dyes are characterized by one or more azo bonds [5] with substitutes such as sulfonic (-SO3H), chloro (-Cl), methyl (-CH3), nitro (-NO2), amino (-NH2), hydroxyl (-OH) and carboxyl (-COOH) group [6]. These are highly soluble in water and are persistent once discharged in the natural environment [6]. They reduce the water transparency and poses toxic effect to aquatic flora and fauna [7]. The azo dyes and their biotransformation products are toxic mainly by causing DNA damage therefore release of such chemicals in aquatic system is hazardous for both ecosystem and human health [5].

Cytogentic analysis have been introduced to find the harmful effects of substances in aquatic system for biomonitoring the extent of pollution and to evaluate the effects of toxic and mutagenic substances [8, 9]. Plant genetic organization constitutes the major test system to evaluate the environmental pollution. They are sensitive and simple and are regarded as the best bio indicators of genotoxicity and mutagenicity caused by toxic substances that contaminate the natural resources. *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, easy to manipulate, sensitive, cheap and other properties, which help its analysis for deletion or damage to the DNA structure [10].

The present study was undertaken to find the clastogenic effect of dye scarlet RR on chromosomes of *Allium cepa* root tip cells as no report is available on the toxicity of scarlet RR. The objective of study was to determine the dose and time dependant clastogenic effect of scarlet RR on the root tip cells which may find their way in the aquatic system as during the processing 10- 15% of total dye remains left as spent dye bath [7].

MATERIALS AND METHODS

The dyes which were used in this study were Scarlet RR which is presumed to be potential genotoxicant on plant model. The dye was procured from Prabhat Dying Mills, Tajpur Road, Ludhiana and JCT Mill, Phagwara, Punjab where most of the azo dyes are used.

Experimental plant organism

Allium cepa is the experimental plant employed. Genotoxicity have been assessed by *Allium cepa* root system, 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 sharp pair of forceps 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. After that the root tips were exposed to three different concentrations 1000µg/L, 100µg/L and 10µg/L for three different time period 24, 48 and 72 hrs. Control test were carried out with distilled water. The experimental and control group were containing 5 onion bulbs for each. The temperature was maintained at about 25^0C. After the time exposure the roots tips were collected and slides were prepared immediately.

Squash preparation

For chromosomal analysis, the roots tips were hydrolysed in 1N HCl at 60 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 sharp blade and placed on glass slide in a drop of acetocarmine and covered with coverslip. The root tips were squashed by tapping with matchstick and sealed with nail polish. The cells were under microscope for different types of chromosomal aberrations and photographs were taken.

An aggregate of 1000 cell bulbs of every experiment group were investigated totally 5000 cells per group. Mitotic index was calculated by the number of cells under division, divided by total number of cells analysed (MI = no. of dividing cells ×100/total no, of cells). The statistical analysis of the data was carried out by t - test.

RESULTS

Mitotic study

To study the Mitotic indices after the treatment 5000 cells each were 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. On comparing it was found that 1000µg/L induces the highest effect on the root tip meristems. The dye at a dose 1000µg/L induces a significant ($p<0.001$) decrease in the value of mitotic index to that of its control. The dose dependent study states that the 1000µg/L for all the three different dyes was highly toxic and significantly inducing mitotic poisoning. In the study it was also found that the drop in the mitotic index from 10µg/L to 100µg/L is insignificant for all the time intervals taken in the study. However the drop from 100µg/L to 1000µg/L is highly significant for 24 hrs and 48 hrs exposure. The treatment dependent study shows that more the treatment time more the toxic effects of dye taken into study. The time dependent study shows that 72h treatments were more harmful taking mitotic study into consideration.

Chromosomal aberrations study

In the tests conducted with root meristems of *A. cepa,* the control group exhibited few abnormalities when compared with the treatments, as shown in Fig 2. Scarlet RR produces various types of chromosomal aberrations (vagrant chromosomes, metaphase with loss, multipolar anaphase, adherence, anaphase with bridge). It was observed that 1000µg/L induces the highest number of chromosomal aberrations for the entire time interval taken in the study Fig:2. Statistically significant ($p<0.001$) differences were found in the total chromosomal aberrations for 10µg/L, 100µg/L and 1000µg/L from that of their respective controls. The root tips treated with scarlet RR at 10µg/L shows maximum total aberrations at 48 hrs of time interval. Table 1 depicts the various types of chromosome aberrations wherein anaphase with bridge was found the most 29 out of 5000 cells at 1000µg/L for 72 hrs time interval. In the study it was also found that the second most chromosome aberrations were adherence in which it was highest at 1000µg/L for 72 hrs time interval.

DISCUSSION

The azo group dyes represents the class of the most commonly used synthetic dyes [11]. The observations which were seen in the present study is a clear indication of the clastogenic and mitoclastic property of the dyes and effluents used, which is apparent from the lowering of the mitotic index and increase in types and total chromosomal aberrations and can be used as a parameter genotoxic studies in environmental bio monitoring [12].

The effects of mutagens on eukaryotic nuclei can be studied through inhibition of cell growth or division and the induction of chromosomal aberrations [13]**.** In the present study the mitotic depression and chromosomal aberrations were studies on the *A. cepa* root tip cells.

A. cepa test system provides important information to evaluate action mechanisms of an agent about its effects on the genetic material [14, 15]**.**

Mitotic index is lower than that of the control for all the doses and time interval taken in the study. Azo dye Scarlet RR shows mitotic depressions in all concentrations. The result was also reported by [1, 2, 16]. Mitotic index is used as an indicator of cell proliferation; hence, the decrease in the mitotic index of *A. cepa* meristematic cells could be interpreted as cellular death reflecting a direct genotoxic effect of textile azo dye. Decrease in mitotic index can be an important indicator to pollution in affected environments, especially those contaminated toxic and cytotoxic compounds [17, 18]. Orange red dye has mito-depressive effect resulting in inhibition of cell access to mitosis [19].

In the present study it was found as the dose of the dye was increased from 10µg/L to 1000µg/L the mitotic index decreases. Mutagenic, cytotoxic and genotoxic effects of the azo dye CI Disperse Blue 291 was studied and the results clearly showed that the azo dye caused dose-dependent effects [2, 20]. Red 40 induces reduction in mitotic index in dose and time dependant manner [15]. The present study also depicts the same follow through as the dose and time is increased the value of mitotic index reduces significantly Fig 1.

In this study, chromosomal aberrations assays were carried out to assess the potential of azo dye scarlet RR to induct chromosome aberrations. The most common aberrations observed in all treatments were anaphase with bridge, multipolar metaphase, metaphase with loss, adherence, vagrant chromosomes Table 1. We may infer that this dye presents a genotoxic action. Azo dye Disperse Blue 373 is clastogenic and caused nucleolar abnormalities which were assessed by FISH and chromosome banding technique [2, 21].

Chromosomal aberrations like bridges and chromosome laggards, in addition to alterations in telophase cells were also reported when the *A. cepa* root tip cells were exposed to industrial effluents contaminated with azo dyes [2, 20]. The present study revealed the fact that as the concentration of the dye and time is increased the chromosomal aberrations were also increased Fig 2. The industrial effluent induces mutagenic effect at a concentration of 10 and 100% [2].

Chromosome bridges are the result of cohesive chromosome terminations or structural rearrangements [22], or even from chromosome adherences [23, 24]. The present study depicts the effect of bridge formation due to the dye and is highest value was found in the root tips treated with 1000µg/L at 72 hrs of time interval. Cohesive chromosome terminations or structural rearrangements might results in bridge formation [22], or even from chromosome adherences [23, 24].

In all the experiments multipolar anaphase cells were also observed and the highest value was found in the root tip treated with 1000µg/L at 72 hrs of time interval. Non-significant values of multipolar cells were observed when *A. cepa* cells were treated with azo dye treatment and concentrations during anaphase and telophase [20].

Metaphases with chromosomal adherences were also found during the treatment of *A. cepa* cells with scarlet RR. It was found that the value is highest when the cells were treated with 1000µg/L at 72 hrs of time interval (Table1). The presence of chromosome adherences may be a sign of genotoxic effect of the inducer, which may lead to irreversible cell damage – including its death [25, 26] an effect also observed herein. This reinforces the aneugenic action of scarlet RR in *A. cepa* root tip cells. Damage to mitotic spindle then prevent the chromosome to migrate towards the pole and ultimately due to further condensation and coming closer to each other results in adherence of the chromosome [27].

Vagrant chromosomes were also observed in the diving cells of *A. cepa*. The variation was observed in this type of chromosomal aberrations wherein the cells treated with 1000µg/L at 24 hrs of exposure shows maximum value (Table 1). Vagrant chromosomes were caused because of unequal distribution of chromosomes which resulted from non-disjunction of chromatids in anaphase. In vagrant chromosome, a chromosome moves ahead of from its group toward poles and leads to the unequal separation [28].

Data related to mitotic index and all the different chromosomal aberrations it was observed that as the dose of the dye is increased it causes greater damage to the root tip chromosome that implies positive dose-response ratio of the *A. cepa* test organisms [21]. Chromosome aberrations produced by Acid violet 7 in root tip cells showed a significant increase in a dose-dependent manner [7]. This data confirms the cytotoxic and genotoxic effect of the dye scarlet RR. Since the highest concentration induced significant frequencies of

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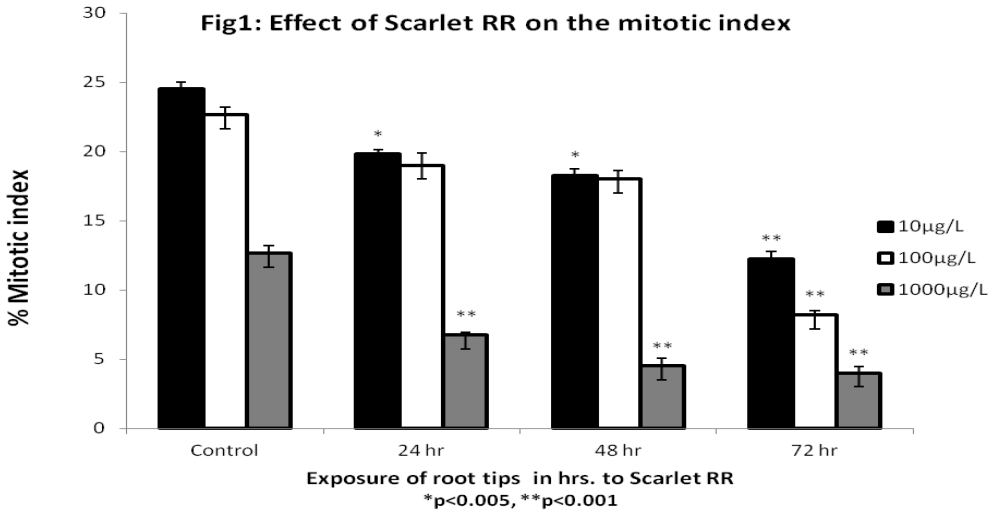
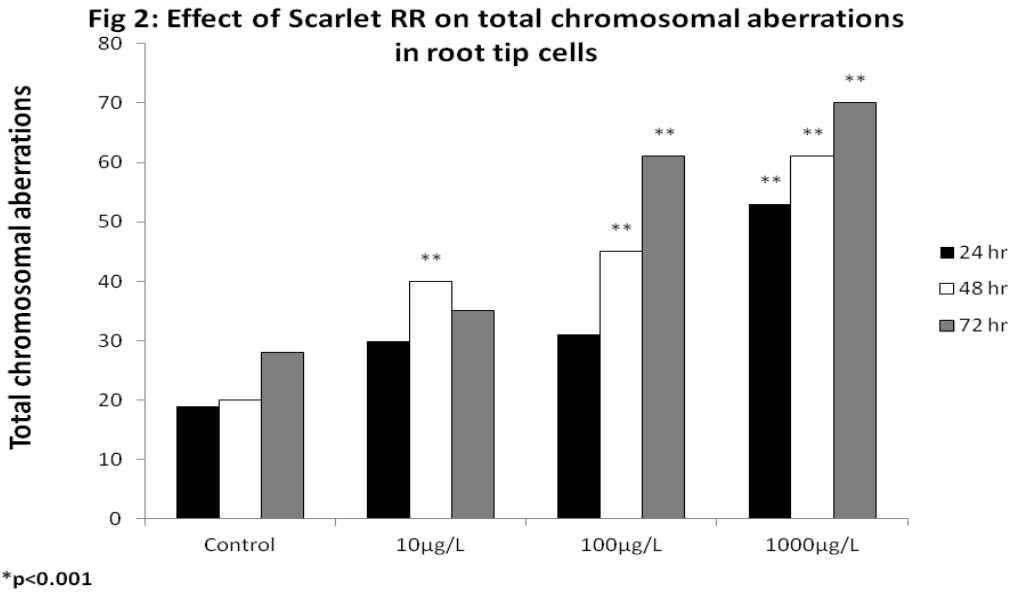
reduction in mitotic index and cell abnormalities, it was regarded as the highest toxic potential of the test organisms.

Table 1: Number and frequency of chromosome aberrations obtained for the *Allium cepa* tests

	24 hrs				48 hrs				72 hrs			
Parameters	**Control**	**10µg/L**	**100µg/L**	**1000µg/L**	**Control**	**10µg/L**	**100µg/L**	**1000µg/L**	**Control**	**10µg/L**	**100µg/L**	**1000µg/L**
Anaphase with bridge	8	16	16	20	10	21	24	28	12	23	26	29
Multipolar anaphase	2	6	7	8	4	10	8	8	4	4	11	12
Metaphase with Loss	6	4	5	10	7	5	5	10	6	4	9	7
Adherence	3	4	2	6	3	2	4	9	4	3	10	18
Vagrant chromosome	0	0	1	9	1	2	4	6	2	1	5	4
Total chromosomal aberrations (TCA)	19	30	31	53	25	40	45	61	28	35	61	70
Total metaphase observed	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000
Frequency of TCA	0.38	0.6	0.62	1.06	0.5	0.8	0.9	1.22	0.56	0.7	1.22	1.4





CONCLUSION

Our findings provide the understanding about the potential risk of azo dye scarlet RR that can induce various mitotic and chromosomal level aberrations. This may later alter cellular configuration and hence the dye may be considered as cytotoxic, genotoxic and mutagenic to the *A. cepa* test-organism. These results are of interest, because if the fixation of the damage in subsequent generation of cells with errors may affect the organism as a whole. It may lead to cell death and the organisms as a whole. From the results it is concluded that the associations of different cytogenetic methods may clarify the effect of environmental pollutants. Further studies are necessary, with different doses, time period and test organisms, to accurately evaluate the potential risks of cytotoxic agents that are generally discharged directly or indirectly causing severe threat to the aquatic bodies thereby disbalances the ecological system and also creates change in the genetics of living organism.

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