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# EVALUATION OF CYTOTOXIC AND GENOTOXIC EFFECT OF THE TEXTILE DYE DIRECT BROWN ON *ALLIUM CEPA* L

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## ABSTRACT

In the present study, the cytotoxic, genotoxic and mutagenic effects of the textile dye Direct Brown was evaluated using root tip cells of *Allium cepa.* Root length, mitotic indices and chromosomal aberrations were used as the test parameters. There was significant difference ($p<0.05$) between mean root lengths of *A. cepa*exposed to different concentrations of the dye and the control. Root growth inhibition was concentration dependent and $IC_{50}$ value was found as 200 ppm using regression plot. The mitotic index decreased significantly with dye treatments. Chromosomal aberrations such as sticky metaphase, disturbed metaphase, anaphasic bridge, disturbed anaphase, laggards and chromosome fragments were observed.

**KEYWORDS:** Chromosomal Aberrations, Cytotoxicity, Direct Brown, Genotoxicity, Textile Dyes

## INTRODUCTION

Water pollution is a major threat which deepens the ecological crisis of the day. Excessive and indiscriminate use of synthetic textile dye stuffs has become increasingly a subject of environmental concern. These dyes can enter the environment through the industrial effluents of dye manufacturing plants.Moreover labors are getting exposed to these dyes during manufacturing and from textile dyeing and printing operations. Assessment of genotoxicity of dyes is therefore of utmost importance. Azo dyes, compounds characterized by the presence of one or more azo groups (-N=N-) – constitute the most important class in the textile industry [3], because they are one of the most easily synthesized dyes, showing both excellent fixation properties and permanence in fibers [24]. Azo dyes are known to be recalcitrant and highly toxic and also become harmful to the environment by the formation of aromatic amines (anilines), which are carcinogenic and/or mutagenic [14]. They are also used as reagents and biological stains in laboratories, are used in food industries and have more recent uses in laser, liquid crystal displays, ink-jet printers and electro-optical devices [16,4].

Plants can be considered as biosensors of genetic toxicity of the environmental pollutants. To the best of our knowledge, the diazo textile dye Direct Brown is not yet studied for its cytogenotoxic effects. Therefore, in the present investigation, the toxicity potential of the textile dye Direct Brown is studied by assessing their cytotoxicity, genotoxicity and mutagenicity on *Allium cepa*. Root length was used as the macroscopic parameterto assess the $IC_{50}$ (inhibitory concentration) value and determine the cytotoxic effect of the dye on *A.cepa*.Microscopic parameters such as mitotic index and chromosomal aberrations were used to study the genotoxicand mutagenic effects induced by Direct Brown on *A.cepa*.

The Alliumtest is a sensitive test showing good correlation to other test systems. Thus positive results in the Alliumtest should be considered as a warning and also an indication that the tested chemical may be a risk to human health and to our ecosystem. Further,chromosome aberration studies are important for a better understanding of the action of chemicals on biological systems [5].

## MATERIALS AND METHODS

### Test Material

The textile diazo dye Direct Brown (DB) and healthy onion bulbs of almost same size and age were used for the study.

### Macroscopic Studies for Cytotoxicity Evaluation of Direct Brown on *A. cepaf*

Commercially available onion bulbs, *A. cepa* L. (2n = 16) were used as test system. A modified protocol of Fiskesjo[7] was used for *A.cepa* test setup. The roots already present at the base of the onion bulbs were carefully shaved off to expose the fresh meristematic tissues. For root growth evaluation, the bulbs were exposed directly to 50 ppm, 100 ppm, 200 ppm and 400 ppm treatment solutions of the test chemical (DB) prepared with distilled water.

For each treatment group, treatment solutions and control were changed every 24 hours till 72 hours. After 72 hours exposure, the roots of the bulbs were removed with a forceps and their lengths measured in centimeter with a meter scale. Data was subjected to statistical analysis and values are expressed as mean ± S.D. Root growth inhibition was measured as $IC_{50}$, which is defined as the inhibitory concentration required for root length reduction to 50% of the control. $IC_{50}$ value of Direct Brown on *A. cepa*was assessed using the regression plot constructed with the help of Microsoft Excel computer programme. The significant difference (at $P< 0.05$) in the mean root lengths, was determined using analysis of variance (ANOVA) coupled with Q test [9].

### Evaluation of Genotoxic and Mutagenic Effect of Direct Brown

Healthy bulbs of *A. cepa*were set for germination in sandy soil at $25^0$C to obtain roots. The root tips (1-1.5 cm length) from the germinated bulbs were treated with DB at different concentrations ranging from 50, 100, 200 and 400 ppm for 6 hours. The root tips treated in distilled water was used as control. After treatment, the root tips of control and experimental samples were thoroughly washed in distilled water and fixed in freshly prepared acetic acid: alcohol (1:3) solution. Chromosome preparations were made following haematoxylin squash technique [22]. Minimum of 5,000 cells from 5 root tips from 5 bulbs were analyzed to score the frequency of mitotic index (MI) and chromosomal aberrations (CA). The results are expressed as mean ± SD. The statistical significance between control and experimental data were analyzed using ANOVA Tukey HSD.

## RESULTS AND DISCUSSIONS

### Cytotoxicity Evaluation of Direct Brown on *A. cepa*

*A. cepa*offers a good experimental model for *in vivo* evaluation of cytotoxicity and genotoxicity of chemical compounds and complex mixtures. Root length was considered as the macroscopic parameter for testing cytotoxicity (root growth inhibition) of the textile dye Direct Brown on *A. cepa* and the results of the study are presented in Table 1 and Figure 1. It was observed that the root growth was inhibited in a dose dependent manner.

**Table 1: Effect of the Textile Dye Direct Brown on *A.cepa* Root Length**

| Concentration of the Dye (ppm) | Root Length (cm) after 72hrs |
|---|---|
| Control | 8.26 ± 0.29 |
| 50 | 6.52 ± 0.31* |
| 100 | 5.32 ± 0.28* |
| 200 | 3.84 ± 0.30* |
| 400 | 1.80 ± 0.19* |

Values are mean of five replicates and given as Mean± SD,
* significantly different from the control at P<0.05 by one-way ANOVA coupled with Q-test

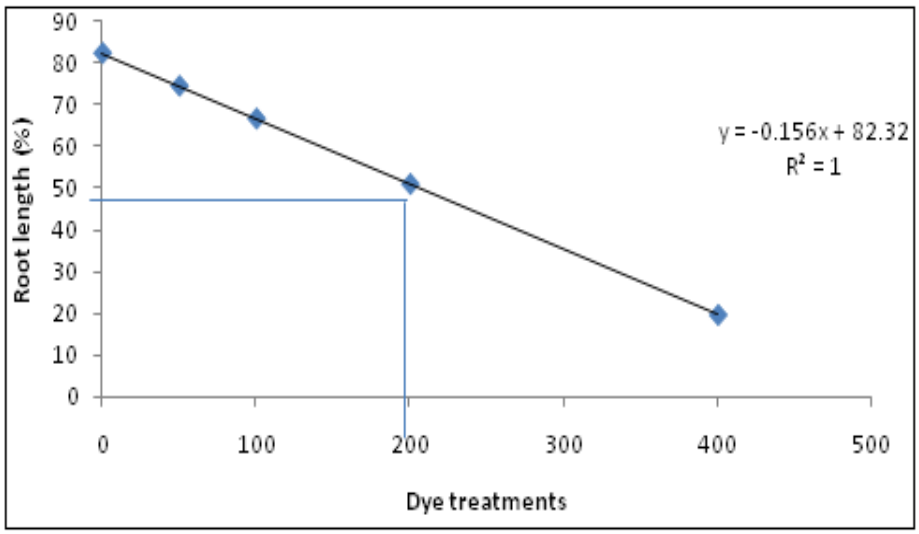



**Figure 1: Assessment of $IC_{50}$ Value of Direct Brown Using Root Growth Inhibition Studies on *A. cepa***

The regression equation was determined and the $IC_{50}$ value assessed from a plot of root length as percentage of control against the treatment concentrations by using Microsoft Excel computer programme. The estimated $IC_{50}$ value of the dye was 200 ppm. Statistical analysis using analysis of variance (ANOVA) showed that there was a significant difference (at $P< 0.05$) in the mean root lengths of *A. cepa* exposed to different concentrations of the dye. Further analysis using Q test revealed that the root length of the control group *A. cepa* (treated with distilled water) was significantly different (at $P < 0.05$) from the root length of *A. cepa* exposed to all the different dye treatments.

**Genotoxic and Mutagenic Effect of Direct Brown**

Details of mitotic indices and frequency of chromosomal aberrations in *A. cepa* root tip cells after six hours of dye exposure are depicted in Table 2. Statistical analysis using ANOVA Tukey HSD comparison test revealed that all the dye treatments are significantly different from the control at $P<0.05$. Significant reductions were observed in the mitotic index of the dye treated samples, while chromosomal aberration frequencies increased. The control exhibited the highest mitotic index (MI) of 9.29%, while cells exposed to 400 ppm dye concentration had the lowest MI (3.61%).

Chakraborty *et al*. suggested that a decreased MI of meristematic cells of *A. cepa* may be considered as a reliable method to determine the presence of cytotoxic compounds [2]. ButHoshina[10], opined that increased or decreased MI may serve as an important parameter for determination of the cytotoxicity level of a test compound. The present investigation and earlier observations indicate that the dyes and textile effluents exert effects on chromosomes and cell division [8, 19, 20, 21, 11, 17, 24]

**Table 2: Mitotic Indices and Chromosomal Aberrations in *Allium cepa* Root Meristem Cells Treated with Direct Brown**

| Treatments | TAC | SD | AC | MN | Mitotic Index (Mean ± SD) | Chromosomal Aberration % (Mean ± SD) | Micronuclei (Mean ± SD) |
|---|---|---|---|---|---|---|---|
| Control | 5375 | 502 | 15 | 4 | 9.29 ± 0.75 | 3.02 ±1.50 | 0.76 ± 0.43 |
| 50 ppm | 5350 | 290 | 45 | 7 | 5.37 ± 1.69 * | 17.24 ± 8.66 * | 1.28 ± 0.82 |
| 100 ppm | 5275 | 311 | 56 | 8 | 5.86 ± 1.39 * | 17.37 ± 3.31 * | 1.55 ± 2.06 |
| 200 ppm | 5135 | 314 | 53 | 16 | 5.99 ± 2.57 * | 16.44 ± 3.20 * | 3.21 ± 2.63 |
| 400 ppm | 5300 | 195 | 51 | 5 | 3.61 ± 1.06 * | 26.38 ± 5.34 * | 1.01 ± 1.28 |

* - Significant at $P<0.05$ (Control *vs* treated)

TAC – Total analyzed cells; SD – Showing division; AC – Aberrant cells; MN – Micronuclei

Besides reduction in mitotic index, genotoxicity analysis showed that different kinds of chromosomal aberrations were generated by the textile dye Direct Brown. Table 3 and 4 gives the indication of types, number and frequency of aberrations generated by Direct Brown. The number of total aberrant mitotic cells caused by all the concentrations of textile dye was apparently different from that of the control (Figure 2). The highest level of chromosomal aberration was observed in 400 ppm dye treatment (26.38 ± 5.34). 50, 100 and 200 ppm treatments had almost similar frequency and effect of chromosomal aberrations, while 400 ppm had an increased effect of chromosomal aberrations, since the total number of dividing cells were drastically less when compared with other treatments. No significant increase was observed in the frequencies of cells with micronuclei.

Total number of cells with alterations was significantly higher in dye treated samples than that of control, which is indicative of genotoxic nature of the dye. The most common chromosomal abnormalities in all treatments were sticky metaphase, disturbed metaphase, anaphasic bridge, disturbed anaphase and laggards (Figure 3).

**Table 3: Frequency of Different Metaphasic Aberrations Observed in Dye Treated *Allium cepa* Root Cells**

| Dose (ppm) | AC | MA | | | | | |
|---|---|---|---|---|---|---|---|
| | | CM | | STM | | DM | |
| | | No | % | No | % | No | % |
| Control | 15 | 1 | 6.67 | 3 | 20.00 | 4 | 26.67 |
| 50 | 45 | 3 | 6.67 | 6 | 13.33 | 12 | 26.67 |
| 100 | 56 | 2 | 3.57 | 25 | 44.64 | 8 | 14.29 |
| 200 | 53 | 1 | 1.89 | 11 | 20.75 | 5 | 9.43 |
| 400 | 51 | 2 | 3.92 | 15 | 29.41 | 7 | 13.73 |

AC – Aberrant cells; MA – Metaphasic aberrations; CM – C-metaphase; STM – Sticky metaphase; DM – Disturbed metaphase

**Table 4: Frequency of Different Anaphasic Aberrations Observed in Dye Treated *Allium cepa* Root Cells**

| Dose (ppm) | AC | AA | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | LG | | FG | | AB | | DA | |
| | | No | % | No | % | No | % | No | % |
| Control | 15 | 2 | 13.33 | 0 | 0 | 3 | 20.00 | 2 | 13.33 |
| 50 | 45 | 4 | 8.89 | 2 | 4.44 | 5 | 11.11 | 13 | 28.89 |
| 100 | 56 | 4 | 7.14 | 1 | 1.79 | 10 | 17.86 | 6 | 10.71 |
| 200 | 53 | 8 | 15.09 | 4 | 7.55 | 18 | 33.96 | 6 | 11.32 |
| 400 | 51 | 3 | 5.88 | 3 | 5.88 | 10 | 19.61 | 11 | 21.57 |

AC – Aberrant cells; AA – Anaphasic aberrations; LG – Laggards; FG – Fragments; AB – Anaphasic bridges; DA – Disturbed anaphase

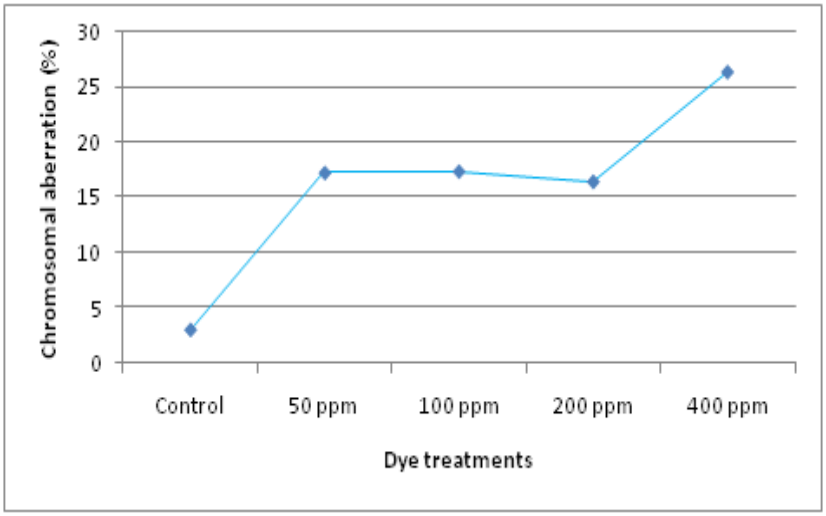
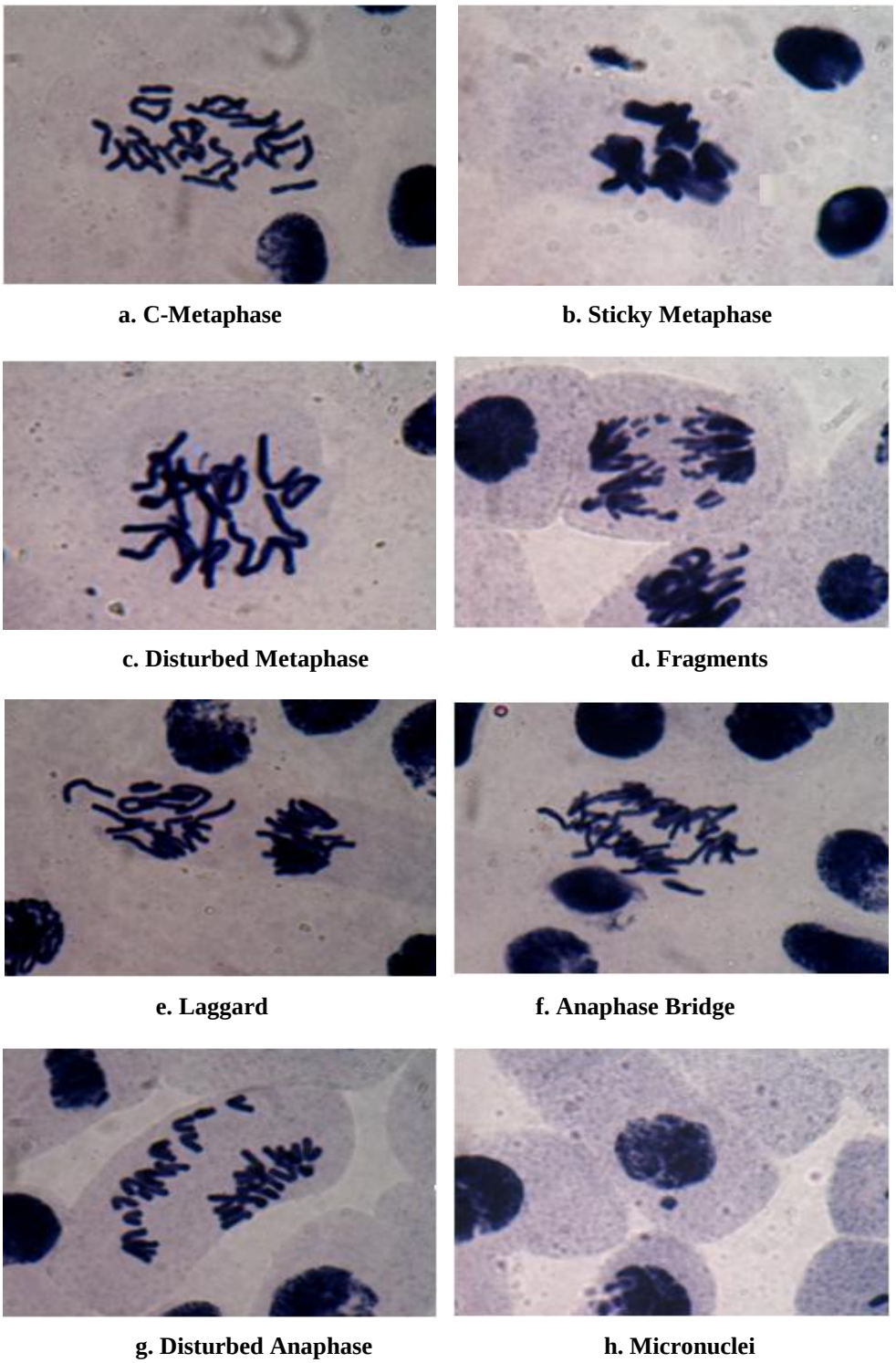



**Figure 2: Effect of Dye Treatments on Chromosomal Aberrations**



**a. C-Metaphase** **b. Sticky Metaphase**

**c. Disturbed Metaphase** **d. Fragments**

**e. Laggard** **f. Anaphase Bridge**

**g. Disturbed Anaphase** **h. Micronuclei**

**Figure 3: Genotoxic and Mutagenic Damages Observed in Meristematic Cells of *Allium cepa* Treated with Direct Brown**

Determination of chromosomal aberrations can be considered as an important and efficient test for the investigation of genotoxicity potential of the textile dyes [1]. Sticky chromosomes indicate highly toxic, usually irreversible effect probably leading to cell death [5]. The presence of chromosome adherences (stickiness) may be a sign of genotoxic effect of the damage inducer, whose consequence of the action might lead to irreversible cell damage including cell death [13,23,12]. The present investigation also provides corroborative evidences to this. Highest frequency of sticky metaphase was observed in 100 ppm treatment of the dye (44.64% of the total aberrant cells). Highest frequency of anaphasic bridge was observed in 200 ppm dye treatments (34% of the total aberrant cells) along with highest frequency of

fragments (8%). Chromosome bridges and / or fragments result from chromosome- and chromatid- breaks. These aberrations may originate from either translocations or cohesive chromosome terminations [6].

The variation in the number of chromosomal aberration observed in this study was not dose dependent. This is not in accordance with Qian (2004), who reported that aberrant rate goes up with the concentrations [18]. Butthe results published by Odeigah*et al.*and Samuel *et al.*are supportive to the present investigation [15, 20]. According to Odeigah*et al.*, a possible explanation for this is that, with increasing concentration and consequently increasing toxicity, there is an inhibitory effect on cell division [15]. In the present study, even though the number of chromosomal aberrations were slightly less in the highest dose i.e., 400 ppm treatment when comparing with other treatments, it showed highest percentage of chromosomal aberrations and which is attributed to sharp decrease in mitotic index.

## CONCLUSIONS

Significant reductions were observed in the mitotic index of the dye treated samples, while chromosomal aberration frequencies increased. The test compound induced different types of chromosomal aberrations on *A. cepa*such as sticky metaphase, disturbed metaphase, anaphasic bridge, disturbed anaphase, laggards and fragments. Considering all the types of chromosomal aberrations observed through the present study, it is concluded that the textile dye Direct Brown is cytotoxic, genotoxic and mutagenic in higher organisms. The present investigation thus emphasizes the need for the development of non-mutagenic dyes and efficient methods for the treatment of industrial effluents contaminated with synthetic dye stuffsin order to avoid the harmful effects on biological systems.

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