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Chromosomal aberrations in root meristems of *Allium cepa* L. induced by dyeing industrial effluent

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Abstract

The present study was investigated to study the response of onion plant genetic material to the presence of potential cytotoxic and genotoxic substances in the environment. So to evaluate the toxicity of dyeing industrial effluent collected from a dyeing industry in Surat, India, the Allium test procedure was used. 50 seeds of *Allium cepa* L. were taken in a petriplate and treated with 20%, 50%, 80% and 100% of dyeing industrial effluent. Tap water was taken as control. The data obtained showed that the mitotic index (MI) decreased with the increase in the effluent concentration along with diverse chromosomal aberrations. Lower concentration induced abnormality such as stickiness in chromosome, laggard chromosome and vagrant chromosome while higher concentration induced abnormality such as chromosomal bridge. At 100% concentration C-mitosis was observed.

Keywords: *Allium cepa*; dyeing industrial effluent; chromosome aberration; mitotic index

1. Introduction

Water pollution caused by industrial effluents has been one of the major issues of global environmental concern. Continuous disposal of wastewater into the water bodies has deteriorated quality of water surface because of the mixing of various chemicals of the effluent with water.

Due to non-availability of alternative sources of irrigation water, the use of effluents for irrigation is a worldwide practice in many developing countries including India due to non-availability of alternative sources of irrigation water. In such countries water treatment cost cannot be yet afford [1]. Such effluents have high level of several toxic metals. Many big industries have their own effluent treatment plants, but small scale industries do not follow the norms and guidelines prescribed for the industrial effluents. Most of the dyeing industries, either partially treat or do not treat their effluent and discharge them into the neighboring ecosystem [2]. This untreated or partially treated waste water from industries are continuously used in irrigating the agricultural fields in developing countries including India [3]. Even when treated, conventional waste treatment techniques do not completely decolorize and detoxify the dye effluents [4].

Various researchers have identified mutagenic effects of textile samples and waste water of the textile industry. Their findings show that dyes used for textile finishing are mainly responsible for the mutagenic effects observed [5, 6]. A large number of compounds have been identified for clastogenic, mutagenic and carcinogenic abnormalities also inducing genetic damage [7].

Plant roots are very useful in testing because the root tips are often the first to be exposed to chemicals in the soil and water [8]. No matter how many methods have been developed for mutagenicity assays; plant tests are still used routinely for genotoxicity testing all over the world [9].

The plants such as *Allium cepa*, *Zea mays*, *Vicia faba*, *Tradescantia*, *Nicotiana tabacum*, *Crepis capillaries* and *Hordeum vulgaris* have been the most common species used for cytogenotoxicity evaluation. This is because these species produce great number of seeds, the easy handling, has large chromosomes number and are highly sensitive to toxic compounds thereby increasing their application in cytogenetic studies [10, 11, 12, 13]

Important genotoxicity endpoints that are routinely used are chromosomal aberrations. To evaluate cytoxicity, mitotic index and nuclear abnormalities have been used and to verify mutagenicity of different chemicals micro nucleus are analysed [14]. The cytotoxicity levels of an agent can be determined by the increase or decrease in the MI [13].

Thus the aim of my study was to investigate the response of onion plant genetic material to the presence of potential cytotoxic and genotoxic substances in the dyeing industrial effluent.

2. Materials and Methods

2.1 Test organism

Seeds of onion were purchased from Agriculture Produce Market Committee, Surat –Gujarat.

2.2 Test agent

Dyeing industrial effluent was collected directly from the industrial wastewater discharge pipe of the factory. The effluent was collected in plastic container and stored in the refrigerator at 4°C for pending use. Before each test was carried out, the effluent was diluted with tap water to produce the series of dilutions investigated.

2.3 Procedure

Healthy seeds of *Allium cepa* were taken and germinated in petri plates. Seeds were treated with 20%, 50%, 80% and 100% of the dye effluent. Tap water was used as control for the study. 50 seeds were placed on cotton in each petri plates and 40 ml solution of each concentration was supplied once for seed germination.

The staining procedure followed was that of [15].

2.4 Scoring of slides

Photomicrographs were taken on Axioscope, Zeiss, Germany. The cells were recorded as normal or aberrant in the different stages of the cell cycle namely: prophase, metaphase, anaphase or telophase. All cells with aberrations were counted and the most representative ones for each abnormality were photographed.

2.5 Calculation of Mitotic index and percentage of aberrant cells

Mitotic index and percentage of aberrant cells were calculated using following formula:

$$\text{Mitotic Index (\%)} = \frac{\text{No. of divided cells}}{\text{Total no. of cells studied}} \times 100$$

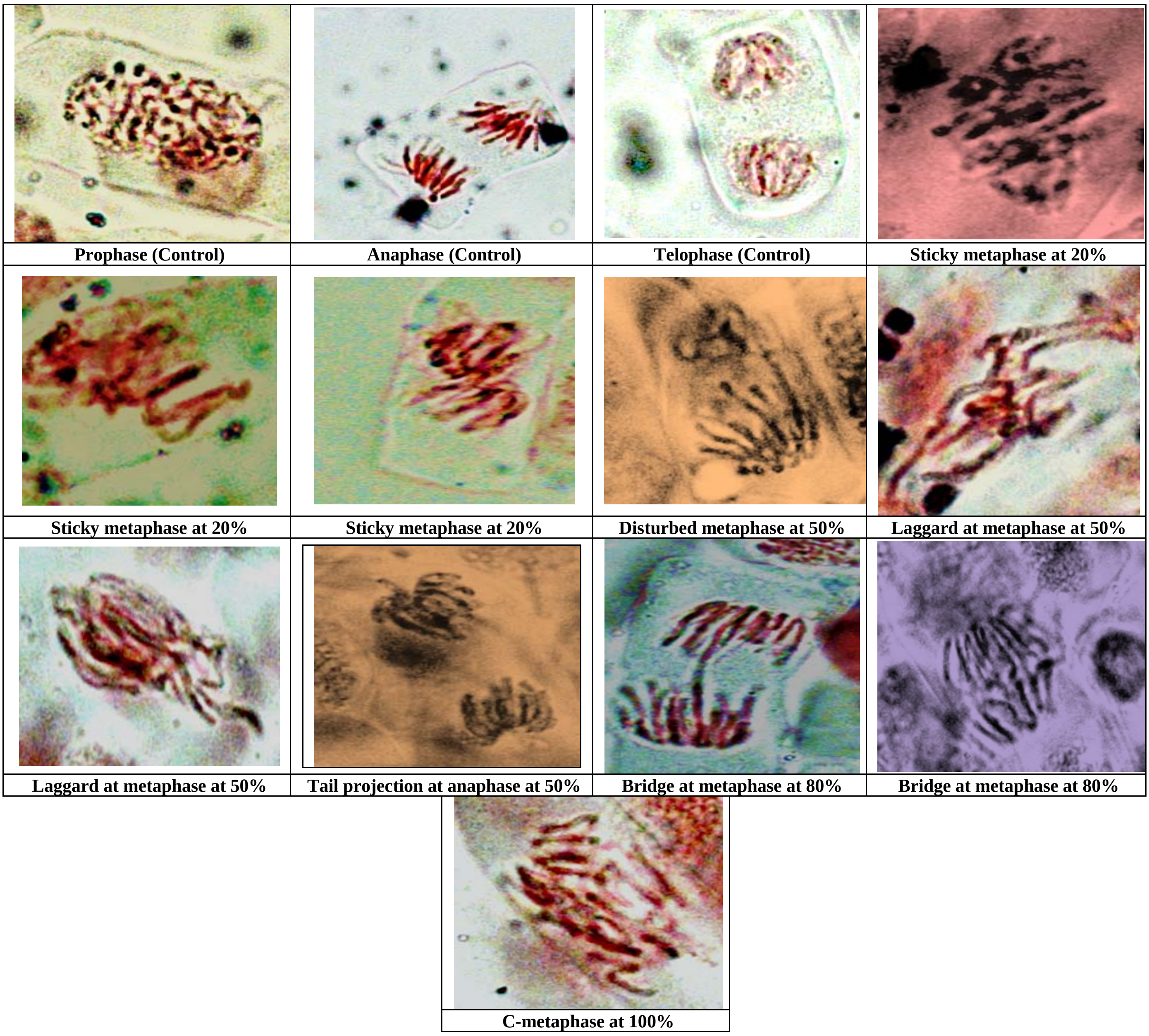
$$\text{Aberrant cells (\%)} = \frac{\text{No. of aberrant cells}}{\text{No. of dividing cells}} \times 100$$

3. Results & Discussion

Allium cepa has 2n=16 number of chromosomes. When the roots of *Allium cepa* were treated with different concentration of dyeing effluent, the cells exhibited various abnormalities showing its toxic effect on mitotic divisions. A number of cytological abnormalities viz., stickiness in chromosome, chromosome bridge, disturbed metaphase, tail like projection at anaphase, C-metaphase, etc. were observed in treated plants. The percentage of mitotic index decreased and percentage of aberrant cells increased with the increase in concentration while in control, percentage of aberrant cells was negligible (Table I).

The investigation revealed that dyeing effluent was mito depressive and induced various types of chromosomal aberrations in the root tips of *Allium cepa*. Mitotic index was decreased with the increase in the concentration of dyeing effluent. This reduction in the mitotic index could be due to inhibition of DNA synthesis or a blocking in the G2 phase of the cell cycle, preventing the cell from entering mitosis [16]. Reduction in the mitotic index by the treatment of paint effluent on the root tip cells of *Allium cepa* was reported by [17].

Fig 1: Normal mitotic stages and chromosomal aberrations at different concentration of dyeing effluent



At lower concentration sticky metaphase normally appeared. Stickiness can be due to physical adhesion of the proteins of the chromosome [18]. Stickiness at metaphase was reported in root tips of *Ricinus communis* L. due to the influence of cycle industrial effluent [19].

At 50% laggard chromosome and vagrant chromosome were observed. Lagging chromosomes may be due to abnormal spindle formation [20], while vagrant chromosome may be due to disturbance of mitotic spindle [21].

At 80% Chromosome Bridge were observed. This chromosome bridge could be due to the breaks in chromosomes and/or chromatids indicating the clastogenic effect [22]. According to [25], influence of paint effluents on the root tips of *Allium cepa* caused bridges which may be the result of unequal chromatid exchange or chromosome break.

Another interesting feature noticed at 100 % was C-metaphase which is due to the inactivation of spindle apparatus connected with the delay in the division of centromere [22]. C-metaphase was also observed in the *Allium cepa* root system due to the effect of silk dyeing industry [16].

Table 1: Effect of dyeing industrial effluent on Root tip cells of *Allium cepa.*

z	Mitotic Index (%)	Aberrant cells (%)
Control	39.25	1.29
20	25.38	37.52
50	21.52	51.08
80	11.09	62.08
100	3.28	96.3

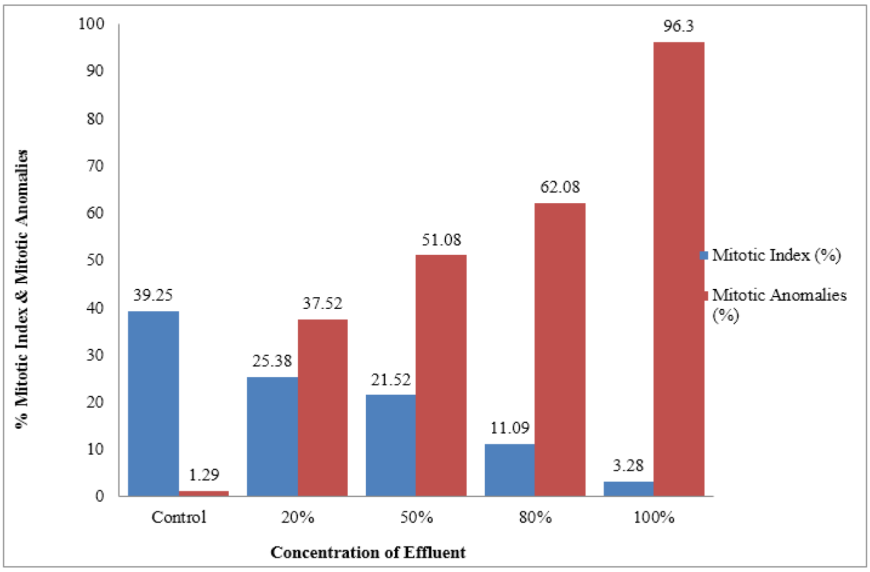


Fig 2: Graph indicating % of mitotic index and % of mitotic anomalies of dyeing effluent at various concentration

4. Conclusion

Present investigation detected the presence of potential cytotoxic and genotoxic substance in the dyeing effluent resulting in various forms of chromosomal aberrations. Results of this study have also shown that *A. cepa* chromosomal assay is a reliable tool for monitoring the genotoxic effects of industrial effluents and wastewaters; it would undoubtedly serve as a useful tool for monitoring the genotoxic effects of industrial effluents and wastewaters before they are discharged into the environment.

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