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Cytotoxic Effect of Silk Dyeing Industry Effluents on the Mitotic Cells of *Allium cepa*

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ABSTRACT

The present study was carried out to evaluate the cytotoxic and genotoxic effects of the silk dyeing industry effluents on the mitotic cells of Allium cepa. A. cepa is selected for the purpose as the plants has large mono-centric chromosomes in reduced number and is accepted as suitable test material for the study of impact of the environmental toxic substances to be mutagenic. The plants were treated with the effluents at various dilutions and kept in the laboratory for 72 h with 11 sunshine hours and at room temperature at 27-30°C. There is a gradual increase in the mitotic index (MI) up to 30% dilution level and this is attributed to the fact that the chemicals present in the effluents may not interfere in the process of mitosis rather it enhances the synthesis of DNA and help in the mitotic activities. The genotoxic effect of the silk dyeing industry effluents induced micronuclei in the roots of Allium, indicating the efficiency of Allium MNC (Micro Nuclear) system in detecting clastogenic potential of the effluents. The present study reveals that if the effluents are released to the environment with dilution up to 30.0% then they will enhance the mitotic activities and thereby the growth of the plants. The higher concentrations of the effluents will harm the plans thereby reducing the mitotic activities.

Keywords: *Alium cepa*, Clastogenic, Cytotoxicity, Effluents, Genotoxicity, Mitoclastic, Mitotic index, Silk-dyeing industry, Waste-water

INTRODUCTION

All the developing countries in the world face a very acute problem ‘the pollution’, which create harm to all the life forms (both plants and animals). The pollutants are released from various sources which include the households, industries, distillery, tannery, agro industries, silk industries, etc. The effluents released from the sources are discharged to the local water bodies, thereby polluting the ecosystem. The developed countries however, have proper drainage system and dump the effluents to the large water bodies like large lakes, rivers and canals or drains [1].

It is well established that, pollution lowers the quality of life in various aspects. Ever since prehistoric times, man has fascination to color the objects of daily use by employing inorganic salts and natural colors obtained from flowers and fruits, pigments from natural products may be vegetative and animal origin. But today as science advanced, more than 1,00,000 synthetic dyes are known of which majority has been used in the textile industries to dye the fibers. Many of these synthetic dyes are found to be toxic in nature and cause havoc in life systems [2-4]. Except a few textile industries none of the industries have effluent treatment plants and release the effluents directly to the ecosystems. Dye wastewater contributes a numbers of contaminants including acids and caustic liquors, dissolved solids and color. The dyestuff molecules are normally biologically resistant and not degradable and thus many a times the conventional biological methods are practically impracticable. Besides the direct health effects, the subtle danger of pollutants lies in the fact that, they have many mutagenic or toxic effects and lead to several human afflictions like cancer, cardiovascular diseases and premature aging [1].

Most industrial effluents wastewater can be characterized as extremely complex mixtures containing numerous organic as well as inorganic compounds [5]. Studies show that, the untreated dye effluents contain substances that could endanger the aquatic lives [6,7]. The wastewater from various industries is also known to induce chromosomal abnormalities in plant cells [8-15]. Though the silk dying industries are in large numbers, their effluents have not been

studies for cytotoxicity and genotoxicity studies. The sewage effluents also show mitotic abnormalities and lower mitotic Index [16].

In the search for test systems which combined with the chemical analysis, can be used to provide data as a scientific basis for regulating the discharge of potentially hazardous substances into the environment or ecosystems and suitable for performances of toxicity evaluation, the *A. cepa* test seems to have advantages. Therefore, it is pertinent to mention here to have cytotoxic and genotoxic studies of the silk industry effluents, on the mitotic cells of *A. cepa*, as this plant is easily available and easily germinated in the laboratory for the purpose. It was proposed as a standard method in environmental monitoring toxicity screening of wastewater and river water [17-20]. This information will help in understanding the underlying mechanism of the abnormalities of cytotoxicity and other cell damages occur due to these effluents causing pollution.

It is highlighted that the species *A. cepa* is widely employed for the determination of cytotoxic, genotoxic and mutagenic effects of various substances and also environmental samples. The Allium test for genotoxicity was introduced by Levan [21] and has been used on wastewater in other studies. The mitotic index (MI) could be used as parameter for evaluating the cytotoxicity of various agents [22]. The test can be used without any considerations, purification and sterilizations of the wastewater. Furthermore, the test is easy to handle, it has low cost and it shows good correlation with mammalian systems [23].

The local people used to dye the silk fibers the collected from the local textile societies set up of by the state and central government. The dyes are also supplied by the societies in a major quantities and the people also prepare their own dyes from natural ingredients like *Punica granatum* (pomegranate), *Terminalia chebula* (Haritaki), lemon, Rosa (rose petals), *Tamarindus tropicana* (tamarind skins), *Clitoria ternatea* flowers (Aparagita), *Butea monosperma* flowers (Palas) and some commonly available chemicals like alum, eating soda, baking soda to fix the natural colors on the silk fibers. The ingredients are mixed in a fixed quantity to produce different colors, which they have inherited from their ancestors, are presented below:

1. Coffee color- Terminalia (Haritaki)-30.0%, Pomegranate-40.0%, Alum-20.0% and Ferrous Sulphate-10.0%.
2. Golden- Terminalia (Haritaki) -30.0%, Pomegranate-40.0%, Alum-20.0% and water-10.0%.
3. Light Yellow-Butea (Palas)-50.0%, Alum-05.0% and Water-45.0%.
4. Ghee (Cream)-Rose-80.0%, Eating Soda-05.0% and Water-15.0%.
5. Light Blue- Clitoria ternatea-90.0%, Water-10.0% and lemon ½.
6. Light Pink- Tamarind Peels-100.0%.
7. Light Grey- Paninai (A wild creeper)-100.0%.
8. Maroon- *Butea* (Palas flower)-20.0%, Myobalan-30.0%, Alum-10.0% and Water-40.0%.

MATERIALS AND METHODS

The silk industry effluents were collected from Nuapatna, a small town in the Cuttack district of Odisha, India. The wastewater collected from different places from the dying units located in and around Nuapatna. Standard methods [24] were employed during the collection, preservation and analysis of samples, were subjected to chemical analysis like, pH, alkalinity, hardness, suspended solids, dissolved solids, calcium content, magnesium content and turbidity following the standard methods employed by Jackson [25]. Suitable concentrations of the effluents were made with distilled water to 5.0%, 10.0%, 20.0%, 30.0% and 50.0%, 70.0%, 100.0%.

The onion bulbs were collected from the local market and screened for suitability and suitable bulbs were selected. Before set for the experiment the loose outer scales were carefully removed and the dry bottom plates were scrapped away without destroying the root primordial cells. Then the selected and dressed onion bulbs were kept in the diluted solutions in glass tubes and kept in the laboratory for 72 h. The onion bulbs germinated after 72 h were collected and the root tips were measured and 1.0 cm was cut from the root cap end and preserved in small injection vials as per Sharma [26].

The roots are treated with Carnoy's fluid for 15 min and then removed from the vials and kept in other vials and

treated with 1.0 N HCl and kept in a water bath for 5 min at 60°C [27]. After that the root tips were removed from the acid and stained with aceto-caramine for 15 min. After 15 min the root tips were kept on the slides and squashed potting a cover slip on it, following the methods of Koa [28]. The slides are then examined under a compound microscope and the cells were studied for mitosis. The cells were examined for all the stages Prophase, Metaphase, Anaphase and Telophase. For each slide 100 cells and 10 slides per dilution were examined and calculated the mitotic index (MI) by the following formula, for all the dilutions.

$$MI = \frac{P+M+A+T}{\text{Total Number of Cells}} \times 100$$

pH of the effluents were measured by employing a pH meter. Alkalinity was measured by titrimatic method using phenolphthalein and methyl orange as indicator following the standard methods [25] and calculated by the standard methods of calculation and presented in the table. The solid content (suspended, dissolved) were analyzed in the laboratory by filtering the fixed quantity of the effluents and by drying the effluents which was further measured. The BOD and COD were measured in the laboratory in the BOD incubator by keeping small quantity of effluents in the BOD bottles. Calcium and Magnesium were measured by employing standard titrimatic methods which were presented in the table. The mitotic index calculated for all the diluted samples subjected to statistical methods like standard deviation (SD), standard error (SE) and chi-square tests were conducted

RESULTS

The average values of physico-chemical parameters of the silk dyeing effluents were depicted in the Table 1. The pH of the effluents, were slightly alkaline which was 8.2. The effluent contains high quantity of total and dissolved solids due to the presence of high quantity of biological degradable substances, as the local dyers use the natural products for developing colors.

The effluent inhibited cell division in the treated root tips of *A. cepa* at higher concentrations (50%, 70% and 100%). A marked difference was noted in the gradual increase in the mitotic index, which is presented in Table 2. There was a gradual increase in the mitotic index percentage up to 30.0% dilution level (MI 28.0%) and there was decrease in the index at all higher concentrations 50.0%, 70.0% and 100.0%. At 70.0% dilution there was high decrease in the Mitotic index (4.0%) and at 100.0% of the effluent there was no cell division and no development of root system and the mitotic index is (00.0%) as shown in Figure 1. Examination of the squashed slides revealed that, there is great number of cells with two nuclei (bi-nucleated) and a wide range of chromosomal abnormalities in the higher concentrations of the effluents (50.0% , 70.0% and 100.0%) making to a percentage of 15.25%, 21.23% and 00.0% respectively, which is presented in Table 2 and shown in Figure 2.

In the present study four main types of chromosomal aberrations were encountered besides micronuclei in anaphase-telophase aberrations: disturbed anaphase-telophase, chromosome laggards, stickiness, and anaphase bridges. Other aberrations like, disturbed nucleus, binuclear cell and vacuolated nucleus were also encountered during the study of the root meristem of *A. cepa*.

Table 1: Physico-chemical composition of the effluents (pH 8.2)

Chemicals	Composition (mg/L)
Total solids	3260
dissolved solids total	2526
Total suspended solids	730
B.O.D	420
C.O.D	346
chlorides	825
Alkalinity	360
Total hardness	525
Sodium	1080
Potassium	19
Calcium	256
Magnesium	48

Table 2: Mitotic index percentage and chromosomal aberration

Concentration (Percentage)	Mitotic Index Percentage	Chromosomal Aberration Percentage
Control	11 ± 1.42	0.0
5.0	19 ± 1.35	6.25 ± 1.83
10.0	21 ± 0.88	6.55 ± 1.68
20.0	23 ± 0.98	6.26 ± 1.24
30.0	28 ± 1.32	5.75 ± 1.35
50.0	08 ± 0.97	15.25 ± 0.97
70.0	04 ±. 095	21.23 ± 1.12

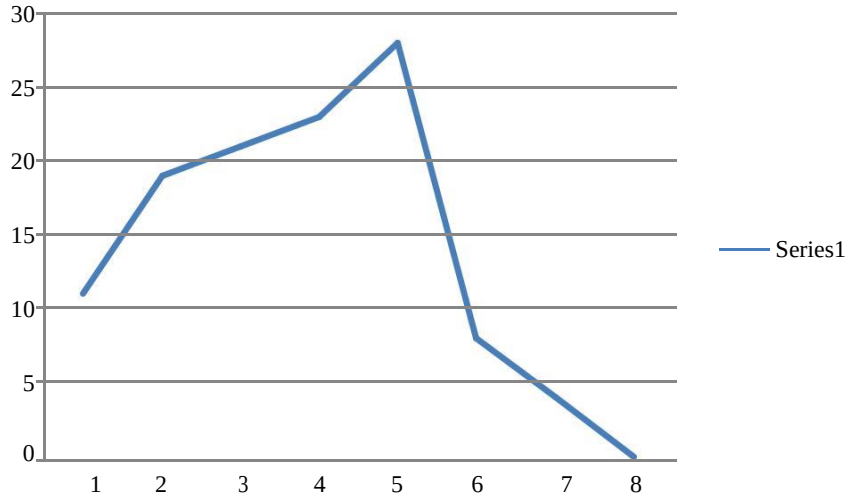

Figure 1: Showing the mitotic percentage at various concentrations of effluents: 1-Control, 2-5.0%, 3-10.0%, 4-20.0%, 5-30.0%, 6-50.0%, 7-70.0%, 8-100.0%

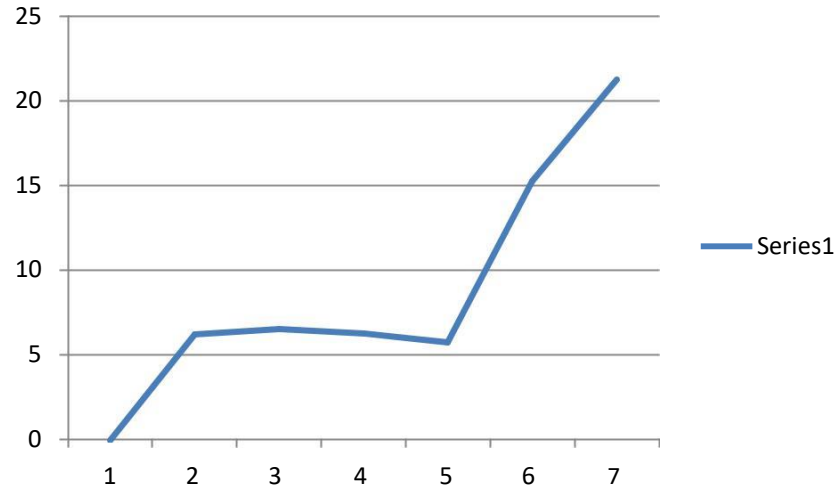

Figure 2: Showing the chromosomal abnormalities on *Allium cepa*- 1-Control, 2-5.0%, 3-10.0%, 4- 20.0%, 5- 30.0%, 6-50.0%, 7-70.0%

DISCUSSION

Plants are used for determination of environmental pollutants such as pesticides because they are indirect recipients of the pollutants and the chemicals of agro toxic. *Allium cepa* test has often been used for the determination of the Cytotoxic and or genotoxic effects of various substances [27,28]. Allium is selected for the purpose as the plants has

large monocentric chromosomes in reduced number and is accepted as suitable test material for the study of impact of the environmental toxic substances to be mutagenic [1,20,29,30].

The cytotoxicity level can be determined by the decreased rate of the mitotic index (MI), which indicates that the silk dyeing industry effluents contain some of the mitotic inhibitors like tannins and alkaloids which inhibit the process of mitosis by not allowing the mitotic cells to continue the division. The alkaloids and tannins inhibit the formation of the microtubules required for the spindle apparatus for the separation of the chromosomes at the metaphase, for which the Allium cells show a greater number of the metaphase stage. Significant reduction of the mitotic index (MI) may be due to the mitodepressive action of the substances used in the silk dyeing industry interfere in the normal mitotic activities in the cell cycle resulting in the reduced number of the dividing cells [30]. In addition it may be due to inhibition of DNA synthesis or blocking of the G-1, suppressing DNA synthesis or effects of test compounds at G-2 phase of the cell cycle [4,31,32]. My findings are agreed with the above findings.

There is a gradual increase in the mitotic index (MI) at the 5.0%, 10.0%, 20% and 30.0% dilution level and this is attributed to the fact that the chemicals present in the effluents may not interfere in the process of mitosis rather it enhances the synthesis of DNA and help in the mitotic activities. The BOD and COD values are high due to presence of bacteria in the effluents as the effluents contain a large amount of biological material which is suitable for bacterial colonization.

The genotoxic activities of the silk dyeing industry effluents induced micronuclei in the roots of Allium, indicating the efficiency of Allium MNC (Micro Nuclear) system in detecting clastogenic potential of the effluents. The induction of MNC in root meristem of *Allium cepa* is the manifestation of the chromosome breakage and disturbance of the mitotic process due to the malformation of the microtubules required for the formation of the spindle apparatus [1]. Micronuclei are considered as an indication of a true mutation effect [33], thus, in the present study the occurrence of the micronuclei in the root meristem are induced by the silk dyeing industry effluents.

In the present study four main types of chromosomal aberrations were encountered besides micronuclei in anaphase-telophase aberrations: disturbed anaphase-telophase, chromosome laggards, stickiness and anaphase bridges. Other aberrations like, disturbed nucleus, binuclear cell and vacuolated nucleus were also encountered during the study of the root meristem of *Allium cepa.* The present findings agree with the findings of Akinsemolu et al. [14] and Garcia et al. [15].

The total percentage of chromosomal aberrations, are increased with the increase in the percentage of concentration of the silk dyeing industry effluents which is presented in the Table 2 and Figure 2. Chromosomal laggards were the most common aberrations encountered during the present study, which attributes to the result in the malformation of the spindle fiber caused by different chemicals present in the effluents [14,34,35,].

Stickiness is considered to be a chromatid type of aberration [36]. Darlington and Mc-Leish [37] proposed that stickiness might be due to degradation of depolymerization of chromosomal DNA. Many researchers have reported that its occurrence during the study could be caused of sub-chromatid linkage between chromosomes [14,38-41].

The bridges involving one or more chromosomes were the most important and frequent type in addition to sticky chromosomes. These bridges cause structural chromosome mutations. Anaphase bridges could happen during the translocation of unequal chromosome exchange, due to the breakage and fusion of chromosomes and chromatids [42,43]. Bridges may also occur due to chromosome breaks, stickiness and breakage and reunion of the broken ends [44]. Other aberrations, such as, binuclear cell and disturbed nucleus, were also observed during the period of study. Binuclear cells are accepted as the inhibition of formation of cell plate during the telophase stage and inhibition of cytokinesis. Increased number of metaphase stages may occur due to disturbed microtubules [23,45] or might be due to disturbances in the spindle fiber formation [35].

CONCLUSION

In the present study four main types of chromosomal aberrations were encountered besides micronuclei in anaphase-telophase aberrations: disturbed anaphase-telophase, chromosome laggards, stickiness and anaphase bridges. Other aberrations like, disturbed nucleus, binuclear cell and vacuolated nucleus were also encountered during the study of the root meristem of *A. cepa.* The total percentage of chromosomal aberrations, are increased with the increase in the percentage of concentration of the silk dyeing industry effluents. Chromosomal laggards were the most common

aberrations encountered during the present study, which attributes to the result in the malformation of the spindle fiber caused by different chemicals present in the effluents. The present study reveals that if the effluents are released to the environment with dilution up to 30.0% then they will enhance the mitotic activities and thereby the growth of the plants. The higher concentrations of the effluents will harm the plans thereby reducing the mitotic activities.

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