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Research Article

Investigation of chromosomal abnormalities induced by red dye in root tips of *Allium cepa.*

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ABSTRACT

Food dye is a synthetic chemical that improves food color, taste, and flavor and is used as a preservative. It is considered that synthetic food dyes are toxic compounds that have several adverse effects on living organisms, including genotoxicity. The Allium cepa genotoxicity test was used to assess the genotoxicity of unprocessed red dye from a local grocery store. The Allium cepa test is a low-cost but reliable genotoxicity test in which Allium cepa roots are grown in varying concentrations of the test substance. A student t-test statistically evaluated the result of the mean root length. Our results were highly significant (p<0.01) and clearly showed that the toxicity of the red dye prompted* Allium cepa *root growth inhibition and that this effect was increased with a higher concentration of the dye solution. Moreover, the mitotic index (MI) decreased as concentration increased. However, the percent (%) of aberrations increased by increased concentration. At the cellular level, dividing cells could be found in different concentrations of effluents. Different kinds of chromosomal abnormalities were also observed, including vagrants, bridges, fragments, stickiness, and multiple anaphases, generating evidence that those dye effluents have genotoxic effects on eukaryotic cells. The results of this study's data demonstrated that plant bioassays might be a valuable test battery for identifying substances that may be genotoxic.

Key words: Red dye, *Allium cepa*, mitotic index, genotoxicity, genetic damage.

1. Introduction

Food Additives are natural and artificial substances which are deliberately added to foods to enhance the color, taste and appearance thus making the food products more attractive and palatable. These dyes have no nutritional value and are used to make food, process it, treat it, package it, ship it, or keep it for a long time. Most of the time, they are used to make cereals, soft drinks, foods, jams, jellies, sweets, candies, ice cream, sauces, and pickles (Hallagan *et al*., 1995). Different types of food additives or dyes are used in different purposes such as preservatives, flavoring agents, coloring agents, texturing agents etc.

A color additive is any dye, pigment or substance which when added or applied to a food, drug or cosmetic, or to

the human body, is capable (alone or through reactions with other substances) of imparting color. FDA is responsible for regulating all color additives to ensure that foods containing color additives are safe to eat contain only approved ingredients and are accurately labeled. The common and available food dyes are Blue #1 Brilliant Blue, Blue #2 Indigo Carmine, Citrus Red 2, Green 3, Orange B, Red 3, Red 40, Yellow 5, Yellow 6 (Center for Science in the Public Interest, Washington, DC 2000).

Bangladesh is an extremely over populated and developing country in the world. A large number of populations need more food for their survival. In contrast, to meet up the food requirement, a large number of food industries were made in here. Today, the use of different food colorants additives is spreading throughout the world along with industrial progress and they are often used to simulate the presence of healthful, colorful fruits and vegetables. Maximum food industries do not follow the food safety rule for children and human health. All industries produce a large number of foods with different color for inviting and appealing to people, especially to the children. They use various types of unclassified food dye that are not approved by FDA.

Synthetic coloring additives cause severe tension to the consumer as general health for their bad effect. The abuse of dyes having hazardous effects on human health which might lead to cancer (Tsuda *et.al*.,2001).A tiny percentage of people who drink the dye have hypersensitivity (allergy-like) responses, and youngsters may become hyperactive as a result (Das and Mukherjee, 2004).

Several biological experiments provide information about the harmful effect of food dye on human health. Several studies were conducted on the use of food dye showed some behavioral changes in children such as irritability, restlessness, and sleep disturbances.

2. Materials and Methods

Red dye

Red dye has been collected from Rajshahi district of Bangladesh. The liquid form of this dye was used in this experiment (Figure 1).

Biological Test Material

Onion bulbs from a population of commercial *Allium cepa* cultivars are the test material. This species possesses 16 big somatic chromosomes (2n = 16), each with distinct morphological characteristics that make them appropriate for quick mitotic research.

Genotoxicity, as used in genetics, refers to a feature of chemical agents that alters the genetic material in a cell, resulting in mutations that may cause cancer. Some food additives or coloring chemicals were genotoxicants that led to chromosomal abnormalities in mammalian cells, including human cells, according to IARC (1983), cited by Hassan (2010).The genotoxic substances induce damage to the genetic material in the cells through interactions with the DNA sequence and structure (Genotoxic effect could result in point mutation, changes in the DNA structure or damage on the chromosome.)

Plants have been introduced to detect the genotoxical and cytological effects of different types of chemical compounds due to their high sensitivity and simplicity. Due to its kinetic proliferation characteristics, low number (2n = 16) of large chromosomes, ease of manipulation, sensitivity, low cost, and other properties that aid in its analysis for deletion or damage to the DNA structure, *Allium cepa* is thought to be an effective system test for genotoxic evaluation (Fiskesjo, 1988).

Moreover, the mitotic index and replication index are used as indicators of adequate cell proliferation (Gadano *et.al*., 2002), which can be measured by *Allium cepa* assay. Several changes of DNA structure can be easily analyzed by using this *Allium cepa L*. test system. Considering this, the present study was planned to investigate the mutagenic and genotoxic effects of food red dyes using *Allium cepa L*. root chromosomal aberration assay. For the adverse effect of food additive on human and animal, in this study we investigated the unclassified food dye red is either harmful to health or not. For this purpose, we conducted of biological experiment of genotoxicity for determining the chromosomal abnormalities on the root tips of onion (*Allium cepa*) that correlated to tumor or cancer development.

Test Procedure

Planting of Onion

The first step was to remove the brownish bottom plate and the outer scales that covered the bulbs. The rings of

the primordial root were not broken apart in any way. Following the procedure given by (Gadano *et al*., 2002) and (Fiskesjo, 1988), a number of cleaned small-sized bulbs of onion, also known as *Allium cepa*, were soaked in water and allowed to sprout. Following a period of 48 hours, those with the most developed root systems were chosen. The assay was done on different types of concentrated (100µg/ml, 50µg/ml, 25µg/ml) liquid form of Red dye. The onions were set up in each test solution. At the same time, some of the onions were grown in control medium (distilled water) for 48 hours.

Root length measurement

A ruler that had been calibrated was used on days 2, 3, 4, and 5 of the experiment to measure the root length of onion bulbs that had been exposed to each concentration. After calculating the total root length for each concentration and then dividing that number by four, we were able to get the mean root length for each treatment within each concentration. Additionally, the root length of the control was determined, and the result was graphed after being plotted. At a significance level of 5%, the results of the analysis of variance and the least significant difference (LSD) test were analyzed and assessed statistically. These tests were performed on the mean root length (Gadano *et al*., 2002).

Cytological study

After the second and fourth days of the experiment, the emerging root tips of the onion bulbs in the various concentrations of red dye solution were fixed in aceto-alcohol (1:3). The preparation of permanent slides of root meristems was done using the traditional Feulgen-squash technique (Sharma and Dphil, 1980). To soften the tissue, the root tips were submerged in 1 normal hydrochloric acid for five minutes. The tips were then macerated for 15 minutes and stained with 0.5% hematoxylin stain. The covered, crushed, and stained root tips were macerated, stained, and then examined under a microscope. Four duplicates of each treatment were used to record the mitotic activity, rate, and type of aberrations.

3. Result

Macroscopic effects

The result of microscopic parameter (root growth) used in testing the root growth of *A. cepa* exposed to different concentrations of red dye are present in Table 1 and Figure 1. It was observed that the root growth was decreased in the proportion of increasing treatment concentration except at concentration 25 µg/ml (Figure 2). Moreover statistical analysis using student t test showed that there was significant differences ($p<0.01$) in the mean root length of *A. cepa* between control and different treatment concentration (Table 2).

Figure 1: Red dye solution of different concentration

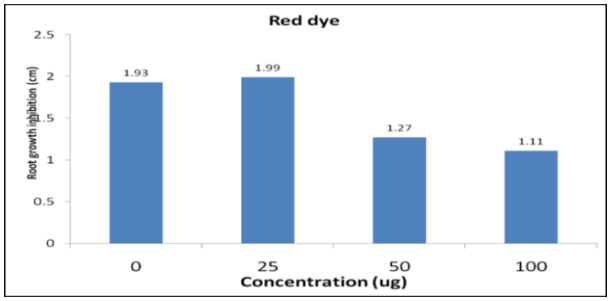

Figure 2: Growth inhibition of *A. cepa* roots exposed to different concentrations red dye

Table1: Root growth of *A.cepa* exposed to different concentrations of red dye solution

Concentration(µg/ml)	Mean Root growth (cm) ± SD
0 (Control)	1.93 ±0.30
25	1.99 ± 0.48
50	1.27 ± 0.29
100	1.11 ± 0.31

Microscopic effects

Table 3 presents a summary of the outcomes of the microscopic impacts. As the concentration of the treatments increased, the mitotic index dropped quickly, and it was favorably connected with root development. That is, both root development and the mitotic index reduced when the treatment concentration was increased. When compared to the control, the dye sample dramatically increased the amount of chromosomal abnormalities, according to the analysis of the chromosomes. In the chromosome of the *A. cepa* subjected to the control condition, there was no aberration seen (Figures 3).Different chromosomal aberrations were observed in different concentrations (Figures 4, 5 and 6).Some of them were vagrant, bridges fragment, stickiness, multiple anaphase etc.

Table 2: Result of Calculated value and tabulated value in *t*-test (Red dye)

Concentration (µg/ml)	Degrees of freedom	Calculated value	Tabulated value
			1%
25	16	7.303***	2.921
50	16	9.545 ***	2.921
100	16	11.876***	2.921

******* Highly significant

In the control experiment, 347 dividing cells (prophase-97, metaphase- 158, anaphase-107, and telophase-70) were found among 5000 cells. The calculated value of mitotic index was 6.94 in control. In this case, no chromosomal aberration was observed.

In case of 25µg/ml red dye the number of dividing cells (prophase-90, metaphase-65, anaphase-56, and telophase-54) was 256 and mitotic index value was5.22. In this experiment the percentage of aberration was 2.01.

In 100µg/ml red dye experiment the number of dividing cells (prophase-65, metaphase-52, anaphase-39, and telophase-35) were 175 among 5000 cells. 3.42 was the calculated value of mitotic index in this case. The percentage of chromosomal aberration was 3.92.

It was proved that the number of dividing cells is decreased with the proportion of increasing concentration of dye. It indicated that the dye was too toxic for the cells to inter cell division.

4. Discussion

The effects of dyes have more adverse effects on root growth. The root length was decreased with the increasing concentration of food dye. The examination of the chromosomes revealed that the dye solution led to a considerable increase in the number of chromosomal abnormalities in comparison to the control. The t-test was used to conduct statistical analysis, and the results indicated that there was a significant difference in the mean root lengths of *A. cepa* after it was subjected to various concentrations of treatments. MI (mitotic index) is the indicator to determine the levels of cytotoxicity of an agent (Fernandes *et al*., 2007). DNA repairing time can be reduced by the increased cell proliferation activity (Evseeva *et al*., 2005). With more cells entering and leaving mitosis than in the controls, this may point to a change in the cell cycle. If ITO (Inositol Trisphosphate Receptor) affects cytosolic signal transduction components to disrupt the homeostatic regulating system, as documented by Hasegawa *et al*., abnormal cell proliferation is amplified (2012) the spindle poisons have been occurred duo to the presence of C-mitosis.It is indicator of weak toxic effect that may be reversible indicating of risk of aneuploidy. C-metaphase which is a reversible effect may occur due to the presence of disturbed microtubules by ITO or an imbalance of the proteins responsible for the structure of nuclear chromatin and can result in multinuclear cells (Fernandes *et al*., 2007).

Table 3: Effects of different concentrations of red dye on dividing cell

Concen-tration (µg/ml)	Mitotic index	Number of cell Observation	Number of dividing cell	Chromosomal aberration					
				Vagrant	Bridges fragment	Stickiness	Multiple anaphase	Others	% Aberration
0	6.94	5000	347(P_{146}+M_{74} +A_{64}+T_{63}	0	0	0	0	0	0.00
25	5.22	5000	256(P_{90}+M_{65} +A_{56}+T_{54}	26	13	22	10	17	2.01
50	4.55	5000	223(P_{84}+M_{60}+ A_{49}+T_{45})	24	11	20	15	15	3.33
100	3.42	5000	175(P_{65}+M_{52}+ A_{39}+T_{35})	29	14	19	28	13	3.92

Here, P = Prophase, M = Metaphase, A = Anaphase, T = Telophase.

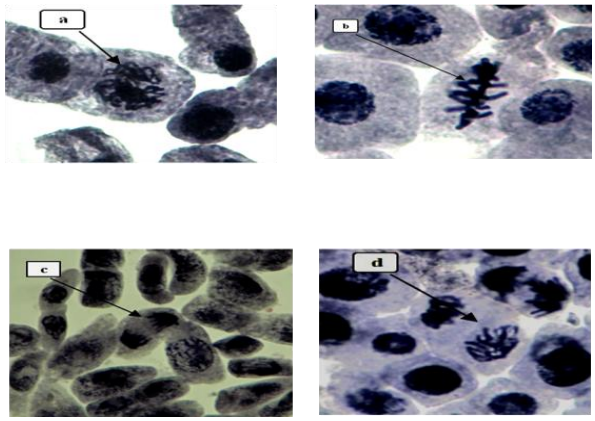

Figure 3: *A. cepa* meristematic cells in control medium (a) Normal prophase (b) Normal metaphase (c) Normal anaphase (d) Normal telophase

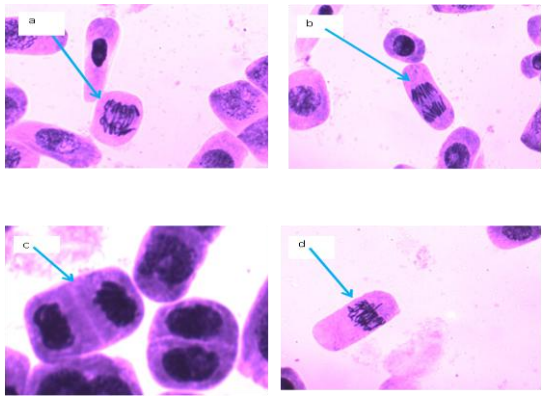

Figure 4: *A. cepa* meristematic abnormal cells in 100 (µg/ml) dye concentration (a) Anaphase bridge (b) Spindle abnormalities in anaphase (c) Stickytelophase (d) disturbed metaphase

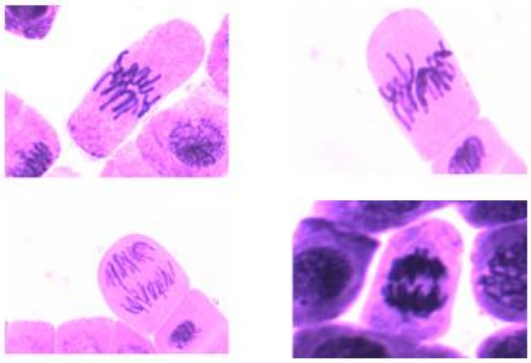

Figure 5: *A. cepa*meristematic abnormal cells in 50 (µg/ml) dye concentration. (a) C-metaphase (b) metaphase with numerical alteration (c) anaphasebridge (d) Laggard chromosome.

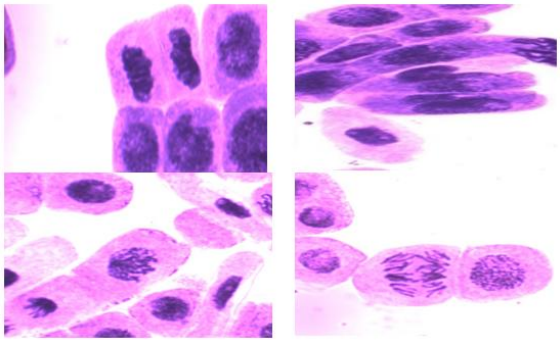

Figure 6:*A. cepa* meristematic abnormal cells in 25(µg/ml) dye concentration (a) Sticky telophase (b) Binucleate cells (c) Irregular prophase (d) Multiple anaphase

When the translocation of unequal chromatid exchanges, di-centric chromatids formed, the breakage and fusion of chromosomes and less active replication enzymes are found, the structural chromosomal mutations are occurred which are indicated by anaphase bridges (El-Ghamery *et al*.,2000).

In our experiment, presence of stickiness was high in all of the treatments especially at higher concentration that indicates an effect on proteins of chromosomes and reflects a toxic effect, usually an irreversible type condition that probably leading to cell death that are similar to the result of El-Ghamery *et al.*,(2000).

5. Conclusion

In this study, we showed that the red dye has adverse effect on root tips of *Allium cepa*. In genotoxicity, the root growth, Mitotic Index and percentage of chromosomal aberration were increased with the increasing concentration of food dye that lead to uncontrolled root growth by chromosomal mutation that are correlated to tumor and further development of cancer. Results demand further investigations on animal model including histopathological, molecular andblood biochemistry for determining more close correlation to tumor or cancer development.

6. Acknowledgement

We thank all the laboratory staffs of the Biochemistry, and Biotechnology laboratory for their assistance in sample collection and other research activities. We would like to offer special thanks to Mohammad Zakerin Abedin, head of the department of Microbiology and Mohammad Shahangir Biswas, head of the department of Biochemistry and Biotechnology, Vagrant chromosomes were observed in root tip cells after treatment with red dye even at low concentration. Binucleated cells were observed only in case of high concentration. Inhibition of cell plate formation and mitotic irregularities are responsible to form binuclear cells (Grant, 1978). When the data were compared, we found that the overall abnormalities as well as the percentage of abnormalities were high at larger concentrations and longer time periods. This suggests that higher doses of dye are genotoxic to live cells. In addition, the finding demonstrates that the lowest proportions of anomalies are found in concentrations that are low. The proportion of anomalies increased from high to low concentration in the sequence of 2.01, 3.33, and 3.92 as the concentration increased.
Khwaja Yunus Ali University, for their expert advice and encouragement.

7. Author's contributions

Jamiatul Husna Shathi and Rubait Hasan conceived, designed and conducted the study. Others collected and analyzed the data. Jamiatul Husna Shathi wrote the first drift of manuscript and all authors read and approved the final draft of manuscript.

8. Conflict of interest

The authors declare there is no with conflicts with due respect to the publication of this article.

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