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Genotoxic effect of two commonly used textile dyes Reactive Blue 19 and Reactive Black 5 using *Allium cepa* L. as an indicator

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Abstract

In this study, the cytotoxic and genotoxic effects of Reactive Blue 19 and Reactive Black 5 were investigated using the Allium test and comet assay. These chemicals are fabric dyes used in textile industries in various parts of Turkey. Bulbs with roots of *Allium cepa* L. were treated with different concentrations (25, 50 and 100 ppm) of these textile dyes for 24 h. and 48 h. The root tips were processed for cytological studies by the aseto-orcein squash procedure. Distilled water and methyl methane sulfonate (MMS, 10 ppm) were used as an negative and positive control, respectively. Exposure of Reactive Blue 19 and Reactive Black 5 significantly decreased mitotic index values. Additionally, all treatments changed the frequency of mitotic phases when compared with the control groups. These dyes increased chromosome aberrations in test material. Among these abnormalities were anaphase bridges, c-mitosis, laggards, micronuclei and stickiness. A significant increase in DNA damage was also observed at all concentrations of both Reactive Blue 19 and Reactive Black 5 examined by comet assay.

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1. Introduction

Since ancient times in history, coloring agents have had a place in human life. While the coloring agents that are as old as human history were formerly obtained from natural products, they were later replaced by synthetic colorants and the industrial revolution, the development of organic chemistry, and the textile and pharmaceutical industries.

Today there has been a growing interest in synthetic products because natural coloring agents are costly, their acquisition is rather challenging, and their color scale is narrow. Rapid developments in the textile industry have brought with them an uncontrollable growth, due to which the places that have suffered at most have doubtlessly been the seas, lakes, rivers, and drinking-water basins contaminated by the wastes of textile factories. The living organisms that live in these resources have been affected by this contamination with a great deal, and the substances being the causes of such contamination reach even humans through the food chain. The fact that some of these substances spreading uncontrollably around the environment may be mutagenic or carcinogenic has been a matter of discussion for years. These substances left in nature by humans by abandoning them to their fate lead to several diseases, including cardiac diseases, early aging, cataract, hereditary and developmental birth defects. Besides such diseases, the hypothesis that they are the main cause of cancer is gaining strength by finding new supporters each passing day. The fact that the coloring agents used in the textile industry are emitted into the external environment through the factory wastes causes unhealthy conditions to occur. In particular, the wastewater into which the industrial water containing coloring agents are disposed of destroying the natural environment in terms of color, smell, and landscape, and thus, the natural flora and fauna existing within the environment are annihilated [1].

Al-Sabti [2], in a study he conducted by using a micronucleus test, reported that under the laboratory conditions, chlorotriazine Reactive Azo Red 120 textile dye, which is also found in the textile wastewater, posed a genotoxic effect by increasing the amount of micronucleus in the *Carassius auratus gibelio* erythrocytes. In another study conducted on *Clarias lazera,* the textile industrial wastewater was determined to increase the micronucleus formation [3].

Sumathi et al. [4], in a study they conducted by applying a comet test, reported that DNA damage occurred in the erythrocytes and liver cells of the fish belonging to *Cyprinus carpio* species exposed to the wastewater of textile dyes. It was also reported that the textile industrial wastewater given in different concentrations and at different periods had increased the amount of the micronucleus in the erythrocyte and

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branchial cells of *O. niloticus* and enhanced the other nucleus anomalies [5].

In their study, Moawad et al. [6] researched the effects of different dyes applied on clover, lettuce, wheat, and tomato plants grown widely on the Nile Delta on seed germination, root elongation, and genotoxicity. As a result of the research, the germination rate in the seeds at increased dye concentrations decreased. Separately, a high level of dye concentrations was determined to have inhibited the shoot/offshoot formation. The elongation of the rootlet was also suppressed at high concentrations. It was observed that among the plants that were used, wheat had shown much more resistance to the toxic effect of the dye when compared to other plants.

In toxicology, the changes that have occurred in the genetic mutations, the number, and the structure of the chromosomes have been of great interest, and a wide variety of *in vivo* and *in vitro* test systems have been developed to determine any of these mutations. The genotoxicity tests used frequently are the Allium test, Ames test, micronucleus test, sister chromatid exchange (SCE) test, and the single-cell gel electrophoresis test (comet assay).

It is easier and more sensitive to perform genotoxicity with a plant bioassay because it is an efficient and cost-effective test system. High plants may be exposed to environmental pollutants directly in laboratories [7] or in situ [8-9]. *Allium cepa* L. (*A. cepa*) is used frequently to evaluate the potential of genotoxicity in pesticides, food additives, or other chemicals [10-13]. A good correlation has been determined between the Allium test results and the mammalian test systems [14]. The popularity of the Allium assay comes from its ability to evaluate chromosomal aberrations in cytogenetic tests in an efficient way [15].

On the other hand, comet assay is frequently used in finding the strand breaks in DNA of single cells because the comet assay is simple to use, sensitive, quick, cost-effective, and easy. In addition, it is a user-friendly application and requires a small number of cells compared with the other testing systems [16-17]. With the genotoxicity studies conducted in laboratories in the short term, a new system has been developed, and this is called the "the Micronucleus Formation Assay (MN)" and is used in detecting cytogenetic changes quickly and analyzes the dividing root meristems in *A. cepa* [18]. In the cell division, laggard chromosomes or acentric fragments are excluded from the nucleus, and therefore micronuclei are formed. These micronuclei are easy to detect in the subsequent interface.

In this study, to determine the possible genotoxic effects of the coloring agents, Reactive Blue 19 and Reactive Black 5 used in the textile industry, 25, 50, and 100 ppm – doses of these substances were applied into *A. cepa* root tip cells for the period of 24 and 48 hours, and their effects on the mitotic index, chromosome, and DNA structure were investigated through the Allium and comet tests.

2. Material-Method

2.1.Chemicals

The testing materials were bought from the Sigma-Aldrich Company (Turkey). Reactive Blue 19: Cas no: 2580-78-1, Molecular formula: $C_{22}H_{16}N_2Na_2O_{11}S_3$, Formula Weight: 626.54. Reactive Black 5: Cas no: 17095-24-8, Molecular formula: $C_{26}H_{21}N_5Na_4O_{19}S_6$, Formula Weight: 991.82. *Allium cepa* (2n=16) onion bulbs, 25-30 mm in diameter, non-treated, were bought from a supermarket in the city of Sivas. All the chemicals used in the comet assay were purchased from Sigma-Aldrich.

2.2. Allium test

The onions (*A. cepa*, 2n= 16) were determined according to whether they received the chemicals. They were transferred to test tubes containing pure water for 24 h. The root growth inhibition was determinated via this method (% inhibition = changing in growth/total growth X 100). After one day, the EC_{50} values of Reactive Blue 19 and Reactive Black 5 were determined at room temperature with different concentrations. After a treatment period of 4 days, ten roots were obtained from each onion and took from measuring on them. EC_{50} concentration was determined as one of the concentrations decreasing the root growth by about 50 % compared with the control group. 25 ppm (1/2x EC_{50}), 50 ppm (EC_{50}), and 100 ppm (2x EC_{50}) concentrations of Reactive Blue 19 and Reactive Black 5 were determined and performed for the Allium test. Distilled water was used as the negative control. As the positive control group, Methyl methanesulfonate (MMS, 10 ppm) was used.

1/2x EC_{50}, EC_{50}, and 2x EC_{50} of concentrations were added into test tubes for 24 and 48 h when six onion bulbs germinate for 24 h. Tip of roots was collected from controls and treatment groups at the high level of maximum mitotic activity on sunny days and used Carnoy's fixative in ethanol: glacial acetic acid (3:1) for 24 h. 1 N HCL was used for hydrolyses at 60 ºC for 7 mins. After aceto-orcein staining for cytogenetic analysis and destaining with 45% acetic acid, total and damaged cells were detected on six different slides with a 40x light microscope. Five slides per treatment were randomly coded and scored blindly. MI and CAs were calculated by using the following equations:

MI= (total number of dividing cells/total cell numbers) x 100

CA= (total number of abnormal cells/100 ana-telophase cells) x 100

2.3. Comet assay

The root meristem cells of *A. cepa* were exposed to concentrations similar to those used for cytogenetic analysis. The method used for comet assay was carried out as described by Tice et al. [19]. In brief, the root tips of *A. cepa* exposed to Reactive Blue 19 and Reactive Black 5 (25, 50, and 100 ppm) were placed in a watch glass which is kept in an ice base and gently sliced using a sharp razor blade to isolate the nuclei in Tris buffer pH 7.5. The microscope slides are pre-treated by 40 ml of 0.3% normal melting point (NMP, Cas no: 9012-36-6) agarose prepared in phosphate-buffered saline (PBS) evenly spread were air-dried. The suspension of nuclei (15 ml) mixed with 150 ml of low melting point (LMP, Cas no: 39346-81-1) agarose in PBS kept at 37 °C was pipetted over the slides. Slides were covered and left in a metal tray kept on ice. Nuclei were left for 1 h, and slides were rinsed in TAE buffer (40 mM Tris-acetate buffer, 1 mM EDTA, pH 8) to remove the salt. All operations were conducted under dimmed with yellow light. The slides were placed in a horizontal gel electrophoresis tank containing freshly prepared cold electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH 4,13). The nuclei were incubated for 10 min to facilitate DNA unwinding before the electrophoresis at 0.72Vcm_1 (26 V, 300 mA) for 25 min at 4 °C. Electrophoresed slides were stained with 80 ml ethidium bromide (20 mgml_1) for 5 min, dipped in ice-cold water to remove the excess ethidium bromide, and covered with a coverslip. For each slide, 25 randomly chosen nuclei were analyzed using a fluorescence microscope with an excitation filter of BP 546/10 nm and a barrier filter of 590 nm. Three slides were evaluated for treatment, and each treatment was repeated at least twice. Each image was classified according to the intensity of the fluorescence in the comet tail and given a value of 0, 1, 2, 3, or 4 so that the total scores of the slide could be between 0 and 400 arbitrary units (AU microgel-1110) [20].

The Total Arbitrary Unit (AUT), which was used to express the extent of the DNA damage, may be calculated by using the equation below:

$$AU_T = N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3 + N_4 \times 4$$

where N_i is the number of nuclei scored in each treatment.

2.4. Statistical analysis

The data of mitotic index, chromosomal aberrations and comet scores, expressed as percentages, and the levels of significance in the different treatment groups were analyzed. Least significance difference (LSD) test were performed by using one-way analysis of variance (ANOVA) on SPSS 23.0 version (at $p<0.05$ levels). Windows-Microsoft Excel 2003 software was employed for data analysis and graphics.

3. Results

This study aims to determine the possible cytotoxic and genotoxic effects of the Reactive Blue 19 and Reactive Black 5; for this purpose, the Allium test and the comet assay were used.

Table 1. The effects of Reactive Blue 19 on mitotic index and mitotic phases in the root cells of *A. cepa*

Time	Doses	Total Cells Number	Mitotic index Mean±S.D.*	Prophase	Metaphase	Anaphase	Telophase
24 h	Control	5095	79.83±0,18 a	74.42	2,97	1,5	0,94
	MMS	5074	45.26±0,37 b	39.77	2,47	1,73	1,29
	25	5157	38.88±0,40 c	34.59	1,74	1,76	0,79
	50	5468	35.24±0,17 d	30.87	1,50	1,9	0,97
	100	5230	29.41±0,38 e	26.44	1,45	0,62	0,9
48 h	Control	5037	63.94±0,25 a	59,57	1,75	1,37	1,25
	MMS	5033	36.15±0,33 b	32,82	1,5	0,9	0,93
	25	5071	27.55±0,39 c	25,47	0,84	0,69	0,55
	50	5201	24.93±0,27 d	22,83	0,92	0,57	0,61
	100	5168	13.82±0,45 e	12,51	0,45	0,32	0,54

*: Means with same letter do not differ statistically at the level of 0.05

The effects of the Reactive Blue 19 on the MI and the mitotic phase in the root cells of the *A. cepa,* which received treatment for 24 hours and 48 hours, and the results show that the MI decreased in significant amounts in every Reactive Blue 19 concentration (Table 1).

The same is true for each time of exposure. For 24 hours, MI values of the Allium root tips, which were incubated in water (i.e., the negative control group), and which were also incubated in the 10 ppm Methyl Methane Sulfonate (i.e., the MMS, the positive control group), have been determined as being 79.83±0.18 and 45.26±0.37, respectively. When the results were evaluated, it became clear that, as expected, the MMS treatment led to a decrease in the MI values in the root meristems compared with the control group. The 24-hour application of 25 ppm gave the highest MI values: 38.88±0.40. The percentage of the MI at the 24-hour application, on the other hand, gave a lower result at 100 ppm: 29.41±0.38. At the 48-hours application, the important inhibition mitotic index was observed for all the dosages of the Reactive Blue 19, which was treated root meristem cells of *A. cepa*.

Table 2 gives the effects of various Reactive Black 5 concentrations on cytogenetic parameters (MI and mitotic phases). There appeared important differences between the bulbs which received MI of the control group and the Reactive Black 5 treated bulbs in every exposure period. The test concentrations inhibited the MI in a concentration-dependent manner. The control group gave the highest values at 24 hours and 48 hours (84.72±2.01 and 78.01±1.68, respectively). The 100 ppm Reactive Black 5 gave the lowest values at 24 hours and 48 hours (34.69±1.29 and 20.36±1.20, respectively).

Table 2. The effects of Reactive Black 5 on mitotic index and mitotic phases in the root cells of *A. cepa*

Time	Doses	Total Cells Number	Mitotic index (Mean±S.D.)*	Prophase	Metaphase	Anaphase	Telophase
24 h	Control	5344	84,72±2,01 a	66,48	8,17	3,96	6,11
	MMS	5320	35,99±1,58 bd	32,23	1,75	0,57	1,44
	25	5257	42,31±1,01 c	38,57	1,23	1,92	0,59
	50	5229	36,81±0,77 b	34,24	0,48	0,82	1,27
	100	5312	34,69±1,29 d	32,12	0,63	1,28	0,66
48 h	Control	5307	78,01±1,68 a	64	7,41	3,29	3,31
	MMS	5238	54,61±2,01 b	48,47	2,21	1,18	0,75
	25	5248	40,63±2,27 c	37,31	1,25	1,38	0,69
	50	5351	33,19±1,98 d	30,19	0,58	1,41	1,01
	100	5355	20,36±1,20 e	17,4	1,05	1,23	0,68

*: Means with same letter do not differ statistically at the level of 0.05.

Table 3. Genotoxicity testing of Reactive Blue 19 at 24 h and 48 h exposure in *A. cepa*.

Time	Doses	Counted cell numbers	Anaphase bridge	Stickiness	C-mitosis	Laggard	Micronuclei	Total abnormallities (Mean±S.E.)*
24 h	Control	500	0,00	0,00	0,00	0,00	0,00	0,00±0,00 a
	MMS	500	3,51	1,54	1,83	0,10	1,03	8,01±1,46 b
	25	500	5,24	1,39	7,38	0,00	0,82	14,83±1,03 c
	50	500	4,90	1,86	7,65	0,00	1,79	16,20±1,22 d
	100	500	6,39	2,93	8,17	0,00	1,53	19,02±1,38 e
48 h	Control	500	0,00	0,00	0,00	0,00	0,00	0,00±0,00 a
	MMS	500	3,75	1,59	2,19	0,00	1,74	9,27±1,31 b
	25	500	5,47	2,26	10,14	0,00	2,10	19,97±2,21 c
	50	500	7,63	2,91	13,92	0,00	2,57	27,03±1,18 d
	100	500	7,00	1,43	18,13	0,00	3,18	29,74±1,25 e

*: Means with same letter do not differ statistically at the level of 0.05

Table 4. Genotoxicity testing of Reactive Black 5 at 24 h and 48 h exposure in *A. cepa*.

Time	Doses	Counted cell numbers	Anaphase bridge	Stickiness	C-mitosis	Laggard	Micronuclei	Total abnormalities (Mean±S.D.)*
24 h	Control	500	0,00	0,00	0,00	0,00	0,00	0,00±0,00 a
	MMS	500	5,23	3,48	4,39	1,5	1,29	15,89±0,71 b
	25	500	6,41	4,59	9,14	3,18	2,87	26,19±1,55 c
	50	500	5,00	5,13	18,2	2,47	2,46	33,26±1,29 d
	100	500	8,11	7,71	20,04	1,38	4,04	41,28±1,20 e
48 h	Control	500	0,00	0,00	0,00	0,00	0,00	0,00±0,00 a
	MMS	500	4,43	2,21	3,51	1,14	1,51	12.80±1,68 b
	25	500	2,96	12,02	10,54	2,18	3,23	30.93±2,72 c
	50	500	8,74	12,72	18,41	3,97	3,56	47,40±1,04 d
	100	500	14,93	14,11	16,19	3,06	4,48	52,77±2,23 e

*: Means with same letter do not differ statistically at the level of 0.05

The types and frequencies of the chromosome aberrations in *A. cepa* meristem cells exposed to the Reactive Blue 19 and the Reactive Black 5 for 24 hours and 48 hours are given in Tables 3 and 4.

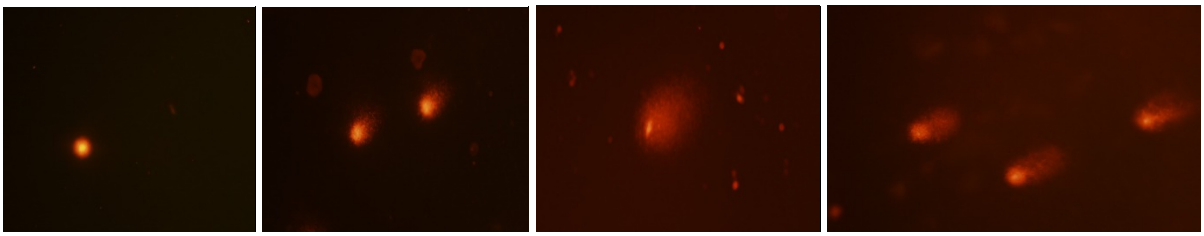
The dyes were reactive and showed an increase in a concentration-dependent manner in chromosome aberrations frequency. Sticky and the anaphase bridge were the frequently observed chromosome aberrations. C-mitosis and micronucleus were also among the chromosome abnormalities. According to the results of our study, the number of the cells with anomaly was increased at an important level after 24-

hour and 48-hour applications with all dyes that were tested compared with the control group. Table 4 gives a summary of the comet assay results.

Table 5. Detection of DNA damage in nuclei of *A. cepa* root meristems exposure to Reactive Blue 19 and Reactive Black 5 using the Comet assay

Time	Doses	DNA Damages (Arbitrary Units±S.D.)*	
		Reactive Blue 19	Reactive Black 5
24 h	Control	35.40 ±3.92 a	39.18 ±1.57 a
	MMS	64.03 ±3.64 b	62.66 ±2.80 b
	25	42.58 ±2.17 c	56.32 ±3.12 c
	50	49.66 ±1.60 d	74.15 ±1.36 d
	100	65.43 ±2.39 b	82.42 ±0.83 e
48 h	Control	35.40 ±3.92 a	39.18 ±1.57 a
	MMS	64.03 ±3.64 b	62.66 ±2.80 b
	25	47.63 ±1.65 c	69.74 ±0.27 c
	50	59.98 ±1.19 d	83.98 ±2.61 d
	100	63.84 ±0.77 b	96.13 ±1.84 e

*: Means with same letter do not differ statistically at the level of 0.05.



A B C D

Figure 1. Assessment of genotoxicity DNA damage estimated by comet assay in root nuclei of *A. cepa*.
A: control, B: 25 ppm, C: 50 ppm, D: 100 ppm

According to the results, the damages caused by the DNA are high at an important level in every concentration of the Reactive Blue 19 and the Reactive Black 5 when these values are compared with those of the control group ($p<0.05$). The length of the comet's tail increased with the reactive dye concentrations, which also showed an increase after the prolonged exposure. The damage caused by the DNA in the Reactive Black 5 was determined as being higher than that of the Reactive Blue 19.

4. Discussion

Investigating the effects of the chemical materials used and spread around the environment unconsciously on the test organisms can be evaluated as an indicator of possible toxic effects in humans and other living species.

The monitoring and screening of the genotoxic effects of the potentially toxic chemicals have a good indicator: High plants. The *A. cepa* is a high plant used commonly in evaluating the effects of the chemicals on genetic materials [21-22].

According to the findings of this study, the benefits of the *A. cepa* root meristem cells in textile dyes are obvious. The significant parameter in selecting the testing concentrations for the genotoxicity assays is the value of EC_{50}. In the tests conducted to reveal the inhibition of the Allium root growth, the important inhibition, dependent on the dosage, refers to the potential cytotoxicity of the chemical used for the

experiment. A reduction in the number of the dividing cells accompanies this inhibition of the root growth at all times [23], the cell elongation inhibition in the extension regions [24], and also the inhibition of the synthesis of the protein [25].

The mitotic index is a parameter allowing the prediction of the cellular division frequencies. These frequencies are beneficial in identifying the existence of cytotoxic chemicals [26]. The inhibition of DNA synthesis may reduce mitotic activity [27] or cause the S-phase synthesis of proteins [28]. According to the cell division ratio in the tips of the roots, the Reactive Blue 19 and Reactive Black 5 caused a reduction at an important level in the mitotic index when these values are compared with the control group and the MMS (Table 1 and 2). The decreased MI values were determined to be lower than the MMS after all the exposure values of the Reactive Blue 19 and the Reactive Black 5 (the only exception was determined at the 24-hour value). The MI inhibition (the important reduction on the prophase percentage at every concentration and treatment times used in the Reactive Blue 19, the Reactive Black 5, and the MMS) showed that the normal sequence of the cell division was interfered with by the treatment. Decreased cell division indices make us consider an inhibitory effect in the interphase level. The decrease in the DNA is observed together with this reduction and can happen because of the inhibition of DNA synthesis or the blocking in the cell cycle, G2 phase; this prevents the cell from entry to the mitosis [29].

Furthermore, the mitosis ratio has been determined as being related closely to the resulting level of ATP [30]. The mitotic indices in the treatments being lower and the decrease in the other stages make us consider that the treatments interfered with the respiratory pathway, leading to the lower levels of the ATP. In the current study, it was demonstrated that the Reactive Blue 19 and the Reactive Black 5 had a cytotoxic effect, and the reason for this was the decreasing level of the MI. In a substantially high number of studies conducted on *the A. cepa test in the literature,* the MI had similar influences [31-32].

Besides, there is the need for different test methods (e.g., flow cytometry) to determine how these chemical coloring agents impact the mitotic index and at what stage they affect the cell cycle.

Table 3 and Table 4 demonstrate the *A. cepa* chromosome aberration test, which was conducted to examine the genotoxic potential of the Reactive Blue 19, and the Reactive Black 5. The *A. cepa* root meristematic cells test was used in this testing. The following five forms of aberrations were determined in the root-tip cells: anaphase bridge, sticky, c-mitosis, laggards, and micronucleus (the root cells received treatment with Reactive Blue 19, and the Reactive Black 5).

It has been demonstrated and proven that the *A. cepa* chromosomal aberration assay is a cost-effective, sensitive and effective test. It is used in testing the potential mutagens in the mitotic and meiotic cells [33]. The chromosomal aberrations are classified under two sub-groups as the clastogenic aberrations (chromosomal break and chromatin bridge); and the physiological aberrations (c-mitosis, sticky, laggard). The anaphase bridges show structural chromosomal mutation and can occur during the translocation of chromatid exchange which is unequal because of the following reasons: presence of disentric chromosomes, replication enzymes that are not much active, breakage and fusion of chromosomes and chromatids [34]. After the treatments with Reactive Blue 19, and the Reactive Black 5, the property of sticky of the chromosomes was determined. The sticky is an effect which is toxic at a higher level and its effects are irreversible and usually causes cell deaths. The sticky chromosomes are indicative of the fact that the textile dye affects the chromatin organization. These effects occur due to the balance which is broken between the quantity of the histones or the other proteins, which are responsible for proper structure control of the nuclear chromatin [35]. These results are in accordance with the results of many other studies conducted so far and which investigated the effects of various chemicals on different material types [36-37]. The most widespread chromosome aberration has been determined as being the c-mitosis. It was observed with the common c-mitosis cells that the textile dyes break the mitotic spindle and arrest the cell division in the early prophase and results in random scattering of the chromosomes which are condensed. The behavior of these dyes remind us of the colchicine. It is known that colchicine binds to the intermeric interface between α-β tubulin dimmers and inhibits microtubule polymerization that causes c-mitosis by blocking cells in prometaphase. According to the results of our study, it has been confirmed that the Reactive Blue 19, and the Reactive Black 5 cause the inhibition of the spindle formation which is similar to the effects of the colchicine. The clastogenic and aneugenic activities are estimated by the existence of the micronuclei, which can be formed from the acentric fragments, which is a consequence of a clastogenic action or the loss of an entire chromosome because of an aneugenic activity [38]. The spontaneous origination of the micronucleus is possible. On the other hand, the induction of them is used for determining the genetic damages which stem

from exposure to a mutagenic agent. Because of the abovementioned facts, and in the light of the induction of the micronuclei it can be concluded that the Reactive Blue 19, and the Reactive Black 5 may either be spindle inhibitors or clastogens. The dyes might enter the nucleus of the cell and then bind to the purine, pyrimidine bases, or to the proteins (i.e. spindle). These interactions might denature the spindles. It is also possible that they might lead to a delay in the chromosome-spindle complex formation, and this might lead to the formation of the MN.

According to the chromosomal analysis results obtained from this study, the reactive dyes used are believed to cause serious chromosomal abnormalities in A. cepa root meristems., which leads to decreases in the mitotic index.

The comet assay was performed to analyze the genotoxicity of the Reactive Blue 19 and the Reactive Black 5 in a single cell in *A. cepa* root tip. Table 6 gives a brief summary of the results which are obtained from the comet assay. According to the comet assay results, the DNA damage was higher at an important level in various concentrations of the Reactive Blue 19 and the Reactive Black 5 when they are compared with the negative control group. According to the chromosomal aberrations and mitotic index results, there is a good correlation with the comet assay. It is possible to explain the DNA damage with the increase in the activities of the free radicals and the reactive oxygen species in the treatments with reactive textile dyes. This situation leads to DNA strand break and DNA replication, repair, recombination, and transcription, which are irreversible [39].

In the comet analysis, when the increases in the injury rate observed in the DNA are evaluated, it can be stated that the textile dyes used in the study cause fractures in the DNA, due to which they may be considered as substances with clastogenic effect. Any organism can be affected by toxic chemicals in different ways. The damage rates may differ according to the amount and the type of the toxic material and its length of time to affect the organism. Tripathy and Patel [40] researched the effect of the coloring agent, reactive turquoise blue, on *A. cepa* root tip cells, and they stated at the end of the study that this substance had shown a genotoxic characteristic.

Studies with different biological tests have revealed that synthetic colorants may have genotoxic properties. In a study with *Vibrio fischeri*, a bioluminescent bacterium, the toxic property of Reactive Black 5 was revealed [41] (Gottlieb et al., 2003). In a study conducted by Şenel et al. [42], synthetic colorants Reactive Black 5, Reactive Blue 19, Reactive Red 74, Reactive Red 141, Reactive Yellow 84 were investigated. As a result of the research, it was determined that Reactive Yellow 84, Reactive Black 5 had a mutagenic effect at a concentration of 400 µg/mL in the presence of S9 fraction and Reactive Black 5 at a concentration of 400 µg/mL in the absence of S9 fraction. Salas-Veizaga et al. [43] investigated the genotoxic effects of Reactive Black 5 and found that this substance did not cause any change in the mitotic index in *Vicia faba*; in other words, it did not cause a cytogenetic effect; on the contrary, it increased the formation of micronuclei and thus had a genotoxic effect. Leme et al., in a study they conducted in 2015, investigated the effects of textile dyes Reactive Blue 19 and Reactive Red 120 on aquatic organisms and humans using different test methods [44]. At the end of the study, it was determined that the damage caused by both colorants in DNA was not at a level that would threaten human health. In another study conducted by Leme et al. [45] with Reactive Green 19 disperse red 1 and Reactive Blue 2 dyes on human dermal cells, it was determined that eactive green 19, one of the textile dyes used, showed a genotoxic effect, while the other two dyes did not. Researchers stated that textile dyes might show different organ-specific genotoxic effects, and the genotoxic effect may change due to the cell culture media used.

5. Conclusion

Considering all the data obtained as the result of the study, it is seen that both Reactive Blue 19 and Reactive Black 5 textile coloring agents reduce the mitotic index compared to the control, increase the rate of damage in the chromosomes and lead to fractures in the DNA. The significant decreases in the mitotic index suggest that the dyes used have a cytotoxic effect. The chromosomal anomalies indicate that the dyes are genotoxic, which is also supported by the comet test. Separately, it is accepted that chromosomal anomalies or DNA injuries are also strongly associated with mutagenic and carcinogenic events.

In light of the results, it can be concluded that the Allium test may give more comprehensive data if it is performed in interaction with the comet assay. In our study, it has been determined that the Reactive Blue 19 and the Reactive Black 5 have a genotoxic effect, and this effect occurs due to chromosomal aberrations and DNA damage. Furthermore, it has also been determined that the textile dyes induce the inhibition of *A. cepa* root, the growth, and the mitodepressive effect on the division of the cells (the cytotoxic effect). On the other hand, detailed cytogenetic studies should deal with the clastogenicity and genotoxicity of the textile dyes and conduct more comprehensive genotoxicity

assessments in animal models. Such studies may reveal significant results for the welfare of human beings. In order to protect the future generation of humankind, eco-friendly dyes should be used and encouraged.

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Conflicts of interest

The authors state that did not have conflict of interests

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