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Original Research

Analysis of Synthetic Food Colors and Food Preservatives Inducing Genotoxicity in Garlic (*Allium sativum* L.) Root Tip Cells

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Abstract

Certain synthetic food additives have been found to induce DNA damage in plant cells, primarily in the form of mutations and chromosomal abnormalities. This research aimed to assess the genotoxic potential of two commonly used food colors (Allura red and tartrazine) and two preservatives (boric acid and sodium benzoate) on the root cells of garlic (*Allium sativum* L.). Garlic roots were treated with various concentrations of food colors (0.1, 01, and 10 g/L) and food preservatives (1, 2, and 4 g/L) for 24 h. Root tip cells were observed under the microscope to check chromosomal abnormalities. The mitotic index observed a maximum of 11.32% at a 10 g/L concentration of Allura red and 19.88% for tartrazine at a 10 g/L concentration. The mitotic index for boric acid and sodium benzoate was maximum (9.80 and 10.95%, respectively) at a concentration of 4 g/L each. An abnormality index was recorded for Allura red (3.01%) and (2.98%) for tartrazine at a 10 g/L concentration. The abnormality index for boric acid was highest (5.88%) at a concentration of 4 g/L. The abnormality index for sodium benzoate was maximum at 5.17% at 4 g/L. Overall, genotoxicity increased with the increasing concentration of food additives.

Keywords: genotoxicity, chromosomal abnormalities, garlic roots, mitotic index, abnormality index

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Introduction

Food additives are substances that have no nutritional value but enhance the flavor, taste, and quality of food products. They include food colors, preservatives, and flavoring agents [1]. Depending on their sources, food additives may be natural or synthetic. Natural sources include ascorbic acid, carotenoids, and polyphenols, whereas additives, including tartrazine, monosodium glutamate (MSG), acetic acid, and benzoic acid, are synthesized artificially [2].

The most common additives are artificial flavors that are used to improve particular food flavors. Synthetic flavoring compounds can mimic natural flavors, such as benzaldehyde for cherry or almond flavor. Monosodium glutamate (MSG) is a flavor enhancer that brings out the flavors of other ingredients in meals. Preservatives are added to food products to inhibit the growth of microorganisms such as bacteria, yeast, and molds [3]. These are used as antioxidants as well as antimicrobials. Antimicrobial preservatives are used extensively, including sodium nitrate, sodium nitrite, calcium propionate, sulfur dioxide, potassium hydrogen sulfite, and sodium bisulfite. In contrast, food preservatives, such as alcohol, salt, vinegar, and sugar, are used traditionally [4]. Food additives directly reach terrestrial and aquatic ecosystems through 2 main pathways: the disposal of food waste in landfills and the applications of food waste-derived soil amendments. Significantly, about 90% of ingested food additives are excreted. Inefficient wastewater treatment indirectly allows these excreted additives to reach the environment by disposing of recycled water and sewage sludge on agricultural land. These food additives, toxic to soil and aquatic organisms, can enter the food web through plant uptake and animal transfer [5]. In the past 2 decades, concerns regarding food additives have increased due to studies that increasingly document endocrine disruption and other adverse health effects [6].

Color is an essential part of food that plays a substantial role in realizing a product in the market. Natural food colors are derivatives of natural sources such as paprika, saffron, and turmeric [7]. The primary purpose of using colors in foods is to provide an attractive look and convince buyers to purchase more food products [8]. Natural colors are more sensitive to light, pH, temperature, and heat [9]. Many natural colors, including caramel, comprise 80% of food colorants [10]. Carmine is a raw food color from an insect (*Dactylopius coccus*) [8]. Riboflavin is a standard natural food color used in sweets, drinks, and ice cream. It is most commonly used in cereals due to its mild odor and bitter taste [11]. Annatto is a standard food color derived from the waxy coating of achiote seed. It is the oldest natural carotenoid used in several products (beverages, snacks, cheese, butter, and margarine) to impart a yellow-orange color [10]. William Henry Perkin obtained the first synthetic lilac color from coal tar in the 18th century. Based on solubility, artificial colors are categorized into three groups, such as water-soluble, fat-soluble, and lake colors [12].

Allura red is a synthetic azo dye known as disodium, and its common name is Red 40 [13]. Red 40 is used in snacks and alcoholic and non-alcoholic beverages. Its chemical formula is $C_{18}H_{14}N_2Na_2O_8S_2$, and its molecular weight is 496 g mol^{-1}. High concentrations of Allura red and other synthetic food colors might interfere with a plant's metabolic processes and growth and contaminate the water released into the soil, damaging the soil's quality [14]. Allura red is an allergic chemical that aggravates or causes asthma, rhinitis, or hives and has other potential negative consequences on health [15]. This food coloring may include carcinogenic residues that are thought to cause hyperkinesia [16]. Tartrazine is a synthetic azo dye, also known as trisodium salt. Its common name is lemon yellow. It imparts orange-yellow to food products such as noodles, corn chips, and tartar sauce. Among all the azo dyes, tartrazine seems to be the most allergenic [17].

Boric acid is a colorless, water-soluble powder used as a preservative in caviar, dairy products, and meat. It is also used in medicines and acts as a pesticide. It is extensively used in food products as a preservative; thus, boron damages the living cells [18]. According to the WHO expert committee [14], boric acid is unsafe for human health. Sodium benzoate is a food preservative used to prevent bacterial and fungal growth. It is used in jam, beer, salad creams, and soft drinks. It has been observed that sodium benzoate has cytogenetic effects on plants, inhibiting DNA replication, creating anaphase bridges, premature chromosome condensation, and chromatin erosion [19]. The increasing use of artificial food additives in processed foods has made them a health risk for people. A typical individual consumes between 3 and 5 pounds of food daily; thus, studying food preservatives is essential [20].

Synthetic food colors and preservatives induce genotoxicity and DNA damage in plants and humans [21]. Most studies explained the toxicity of synthetic food additives on *Allium cepa* L. root tips. Farheen et al. [22] conducted a study to evaluate the genotoxicity of artificial food colors sunset yellow, fast green, and tartrazine on the root tips of *Allium cepa* L. as a model plant. Their findings showed that synthetic colors reduced the mitotic index, and different mitotic aberrations were observed. Kumar et al. [23] determined many cytological defects like chromosomal stickiness, fragmentation, and bridge in the root apical meristem cells. The phase of abnormalities increased with the rise in the treatment concentration of respective dyes. An increase in food additive concentration significantly reduced the mitotic index. It also disturbed metaphase, creating laggards, stickiness, C-mitosis, fragmentation, precocious chromosomal movement, and unequal and late separation of chromosomes [1]. Nassar [24] experimented with studying the cytotoxic and genotoxic impacts of three food dyes (tartrazine, caramel dye class III and IV) on *Allium cepa* L. root tip

cells; results showed that these three food colors have cytotoxic effects (inhibition of root growth after 120 h of incubation) and genotoxic effects (reduction in mitotic index and increase in chromosomal abnormalities like chromosomal bridges, vagrant chromosomes, and stickiness). Sodium metabisulphite caused micronuclei, chromatid gaps, anaphase bridges, vagrants, and shattered and ring chromosomes in *Allium cepa* L. root tip cells [25].

Artificial food additives are increasingly used in the food industry, potentially harming health by causing genotoxicity. This study tested the mitotic abnormality index of *Allium sativum* L. root tip cells for two commonly used food colors and food preservatives. This will help us understand the genotoxic effects on crop plants.

Materials and Methods

Selection of Food Colors

Two food colors were selected: Allura red ($C_{18}H_{14}N_2Na_2O_8S_2$) and tartrazine ($C_{16}H_9N_2Na_2O_9S_2$). Based on product concentrations, three concentrations of each food color (0.01%, 0.1%, and 1%) were used, including ketchup, sauce, and beverages [26].

Selection of Food Preservatives

Two food preservatives (boric acid and sodium benzoate) were selected to study the mitotic aberrations. Three dilutions of each food preservative (1, 2, and 4 g/L) were prepared in distilled water. These three concentrations were selected based on concentrations used in food products [27].

Plant Root Growth

Before treatment, garlic bulbs were placed in a water-filled jar until their roots became 1-2 cm long [28]. For treatment, garlic bulbs were divided into three groups, i.e., group 1 (-ive control), group 2 (+ve control), and group 3 (experimental group). Group 1 garlic bulbs were treated with distilled water, while in the 2nd group, garlic bulbs were treated with 3 concentrations of $CuSO_4$ solution (2µM, 4µM, and 8 µM) [29]. The 3rd group of garlic bulbs was placed in food color (Allura red and tartrazine) and food preservative (boric acid and sodium benzoate) dilutions for 24 h [22].

Microscopic Analysis

Roots were excised from the experimental and control groups and placed in a farmer fixative (acetic acid: alcohol, 1:3 v/v) for one day (24 h). After 24 h, roots were separated from the farmer fixative and hydrolyzed in 1N HCl for 15-18 min at 60ºC [30]. Roots were washed with distilled water to neutralize the acid and stained with 2% aceto-orcin. The root tips were removed, macerated, and stained by placing a coverslip on the slide. The slides were observed under a compound microscope (Olympus CX 21), applying a 100X objective lens with oil immersion, with an attached image analyzer to evaluate distinct phases of mitosis for analyzing chromosomal aberrations [31]. Three slides were made for each factor [32].

Evaluation of Mitotic Index (MI)

The mitotic indices were evaluated by taking 3 to 5 root tips per experiment for each line, and root tip cells were unsystematically evaluated. The mitotic index was calculated by following the formula [33].

$$Mitotic\ index\ (\%) = \frac{Total\ number\ of\ dividing\ cells}{Total\ number\ of\ observed\ cells} \times 100$$

Evaluation of Abnormality Index (AI)

Using the following formula, the abnormality index (AI) is estimated:

$$Abnormality\ index\ (\%) = \frac{Total\ number\ of\ abnormal\ cells}{Total\ number\ of\ dividing\ cells} \times 100$$

Various chromosomal abnormalities, including chromosome bridges, abnormal metaphase, C-mitosis, micronuclei formation, and fragments, were observed, as described by [34].

Statistical Analysis

Data were obtained from three replicates for each concentration of food colors and food additives and normally distributed as means were determined. These means were used for making graphs. Results for various observed parameters were subjected to one-way analysis of variance (ANOVA) and post-hoc analyses based upon least significance difference (LSD) to assess the impact of different concentrations of food colors and food preservatives on the mitotic index and abnormality index using CoStat V. 6.45 (CoHort Software). The statistical significance was determined with $p<0.05$, which is considered significant [35].

Results and Discussion

Garlic (*Allium sativum* L.) was used to study the genotoxic effects of food colors and preservatives on root tip cells. Chromosomal abnormalities are indicators of a clastogenic action and can be used as a genotoxicity measurement. Mitotic and abnormality indices were calculated after 24 h of incubation to evaluate the genotoxicity of two synthetic food dyes and food preservatives with different concentrations. The mitotic index and chromosomal aberrations were observed by

applying food colors and food preservatives on the root tips of *Allium sativum* L. The top mitotic index was observed in 10 g/L of food colors and 4 g/L of food preservatives. The chromosomal abnormalities with the highest observation frequency were during metaphase, anaphase, and telophase. Severe abnormalities such as chromosomal bridges, stickiness, C-mitosis, unequal distribution, and irregular nuclei were observed concerning the increased concentration of food additives.

Mitotic Index (MI)

The mitotic index is the rate at which cells in a tissue divide mitotically. The data for MI was highly significant, as depicted by ANOVA and post-hoc analysis (Tables 1 and 2) (Fig. 1). In the case of food colors, MI values decreased with the increase in concentrations. MI values for Allura red were 17.24%, 15.68%, and 11.32% at 0.1, 1, and 10 g/L concentrations compared to the control (19.3%). Meanwhile, MI values were observed for tartrazine at 27.13%, 24.00%, and 19.88% at 0.1, 1, and 10 g/L concentrations compared to the control (27.45%) (Table 1) (Fig. 1A). In the case of food preservatives, MI values decreased with the increase in concentrations. MI values were 17.00%, 14.61%, and 9.8% at concentrations of boric acid (1, 2, and 4 g/L) compared to the control (17.48%). In the case of sodium benzoate, MI values were 17.32%, 14.00%, and 10.95% at 1, 2, and 4 g/L concentrations, while the control showed an MI value of 17.54% (Table 1) (Fig. 1B).

Abnormality Index (AI) of Food Colors and Food Preservatives

The data for AI was highly significant, as depicted by ANOVA and post-hoc analysis (Tables 3 and 4) (Fig. 2). Regarding food colors, the AI value significantly increased with the increase in concentrations. AI values for Allura red were 0.51%, 1.17%, and 3.01% at 0.1, 1, and 10 g/L concentrations, respectively, compared to the control sample (0%) after a 24 h incubation period. Meanwhile, the AI values for tartrazine were 0.5%, 1.00%, and 2.98% at 0.1, 1, and 10 g/L, respectively, compared to the control sample (0%) **(**Table 3**)** (Fig. 2A). When boric acid was applied to the root tip of garlic, AI was observed at 0.4%, 2.88%, and 5.88% at 1, 2, and 4 g/L concentrations compared to the control sample (0%). Meanwhile, sodium benzoate was 0.8%, 2.4%, and 5.17% at 1, 2, and 4 g/L compared to control (0%) (Table 3) (Fig. 2B).

The two chromosomes that separate during anaphase or cytokinesis are joined by chromatin bridges, which are threads of chromatin. Chromosome bridges and breaks are indicators of a clastogenic action and can be used as a genotoxicity measurement. The chromatin bridges were concentration-dependent; the food color Allura red at 10 g/L and boric acid at 04 g/L showed many chromatin bridges, as shown in Fig. 3A. Chromosomal stickiness is ordinarily detected in the anaphase and metaphase phases, respectively. Root cells growing under high-concentration chromosomes initiated to accumulate at prophase or metaphase, forming sticky clumps

Table 1. MI at different concentrations of food colors and preservatives.

Food additives	Concentrations (g/L)	No. of observed Cells	No. of actively dividing cells	Mitotic Index (MI*)* (%)
Allura red	Control	572	110	19.23
	0.1	580	100	17.24
	01	510	80	15.68
	10	530	60	11.32
Tartrazine	Control	510	150	27.45
	0.1	538	146	27.13
	01	500	120	24.00
	10	503	100	19.88
Boric acid	Control	572	100	17.48
	01	500	85	17.00
	02	520	76	14.61
	04	510	50	9.80
Sodium benzoate	Control	570	100	17.54
	01	508	88	17.32
	02	500	70	14.00
	04	502	55	10.95

Table 2. ANOVA of Mitotic Index (MI).

Source	Df	Type III	SS	MS	F	P
Main effects						
Food Color	3	269.9453688	89.98179	391.30906	.0000	***
Concentrations	3	135.2583688	45.086123	196.06865	.0000	***
Error	9	2.06955625	0.2299507<-			
Total	15	407.2732938				
Model	6	405.2037375	67.533956	293.68886	.0000	***

R^2 = SSmodel/SStotal = 0.99491850735, Root MSerror = sqrt (MSerror) = 0.47953174498, Mean Y = 17.539375, Coefficient of Variation = (Root MSerror) / abs (Mean Y) * 100% = 2.7340298.

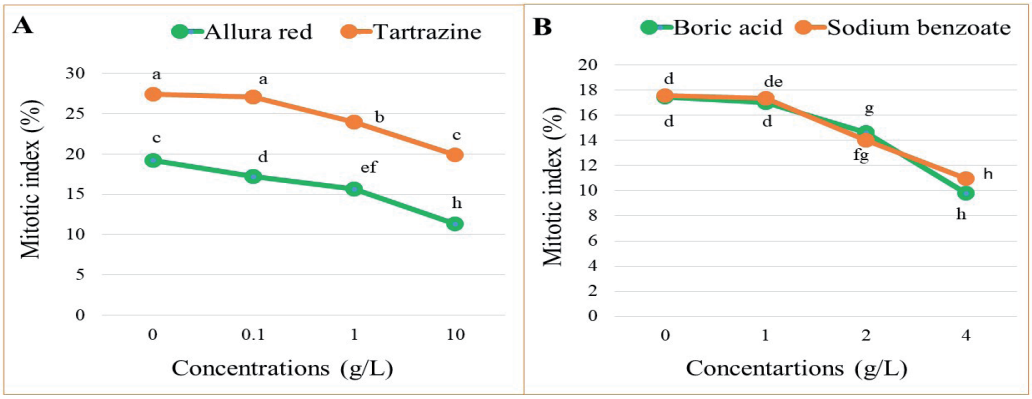

Fig. 1. A) Mitotic index (MI) in garlic root tips under the influence of different concentrations of food colors (Allura red and tartrazine). B) Food preservatives (boric acid and sodium benzoate). Symbols indicate statistical differences between the lighting conditions (one-way ANOVA; Tukey's post-hoc test, $p<0.05$).

Table 3. Abnormality index (AI) at different concentrations of food colors and preservatives.

Food additives	Concentration (g/L)	No. of observed cells	No. of abnormal dividing cells	Abnormality Index (%)
Allura red	Control	572	0	0
	0.1	580	3	0.51
	1	510	6	1.17
	10	530	16	3.01
Tartrazine	Control	510	0	0
	0.1	538	3	0.55
	1	500	5	1.00
	10	503	15	2.98
Boric acid	Control	572	0	0
	1	500	2	0.4
	2	520	15	2.88
	4	510	30	5.88
Sodium benzoate	Control	570	0	0
	1	508	4	0.8
	2	500	12	2.4
	4	502	26	5.17

Table 4. ANOVA of the Abnormality Index (AI).

Source	Df	Type III	SS	MS	F	P
Main effects						
Food Preservatives	3	4.78106875	1.5936896	3.0895395	.0825	NS
Concentrations	3	43.84151875	14.61384	28.330507	.0000	***
Error	9	4.64250625	0.515834<-			
Total	15	53.26509375				
Model	6	48.6225875	8.1037646	28.330507	.0000	***

R^2 = SSmodel/SStotal = 0.91284148918, Root MSerror = sqrt(MSerror) = 0.71821586433, Mean Y = 1.649375, Coefficient of Variation = (Root MSerror) / abs(Mean Y) * 100% = 43.544728%.

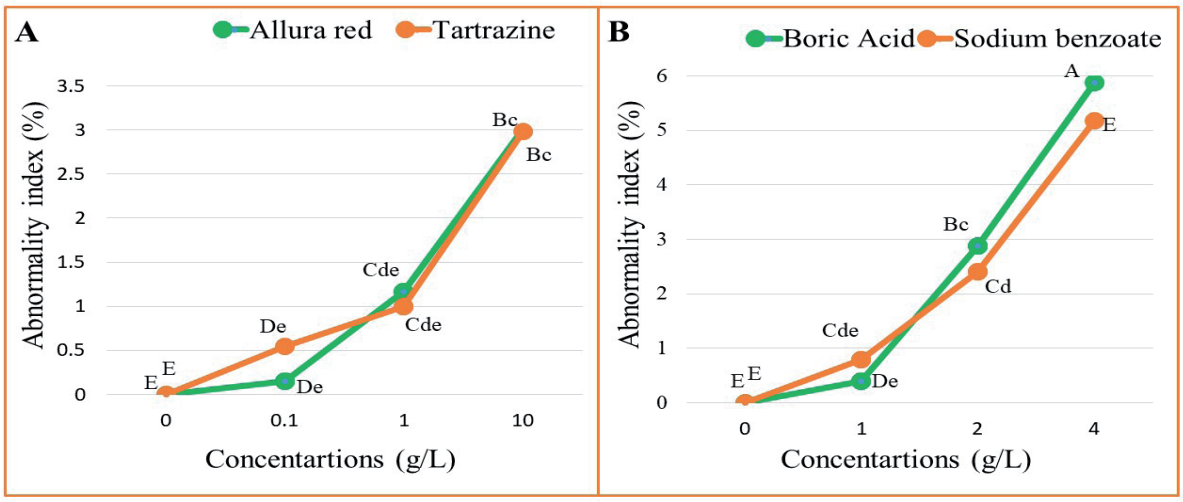

Fig. 2. A) Abnormality index (AI) in garlic root tips under the influence of different concentrations of food color (Allura red and tartrazine). B) Food preservatives (boric acid and sodium benzoate). Symbols indicate statistical differences between the lighting conditions (one-way ANOVA; Tukey's post-hoc test, $p<0.05$).

that did not adjust themselves on the equatorial plate (Fig. 3B). Anaphase disjunction was uneven or frequently unsuccessful, and chromosome fragmentation occurred from the prophase onward, resulting in c-mitosis. The reduction in mitotic activity could be due to the inhibition of DNA synthesis shown in Fig. 3C. The late anaphase was also detected due to the mutagenic event under the influence of food additives, as shown in Fig. 3D. The prediction of genotoxicity is concerned with chromosomal aberrations, morphological alteration of the nucleus, binucleated cells, and micronucleus formation. The results showed the presence of micronuclei and binucleated cells, as shown in the Fig. 3E and Fig. 4, which are considered the most influential mutagenic events.

The rapid development of industrialization has a significant impact on the emerging world. However, the worldwide community is greatly concerned about the high-cost treatment process of wastewater [36]. Consequently, releasing unprocessed and moderately treated emissions into the environment directly or indirectly eventually affects all organisms [37].

Current research work evaluated the genotoxic potential of food dyes and food preservatives. The *Allium* test is an effective and quick test system for genotoxicity detection [37]. Different parameters of *Allium sativum* L., such as mitotic index and chromosome abnormalities, were used to estimate the genotoxicity of different compounds [38]. The impact of food colors and preservatives was studied on the root tip cells of *Allium sativum* L., and a substantial decrease in mitotic index and enhancement of irregularity percentage was observed. The inhibition of mitotic activity was extensively used to evaluate the cytotoxicity of substances (Bellani [39]). Two food colors (Allura red and tartrazine) and two food preservatives (boric acid and sodium benzoate) were used in different concentrations to evaluate their genotoxic potential on the root tip cells of garlic. The findings of this work confirmed that synthetic food colors and food preservatives have genotoxic potential on the mitotic chromosomes of *Allium sativum* L. The influence of high concentrations of food colors (10 g/L) and food preservatives (4 g/L) showed

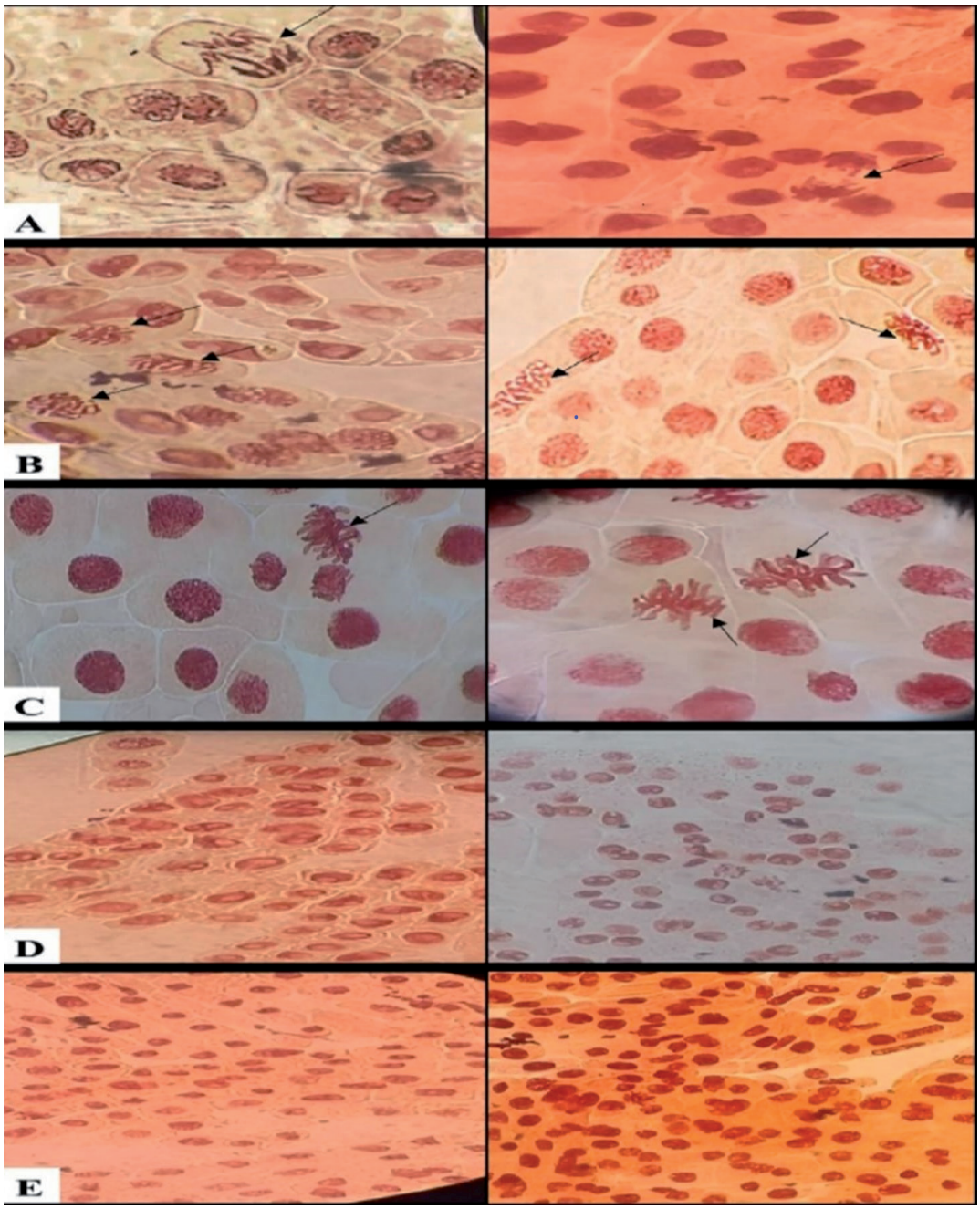

Fig. 3. Comparative chromosomal aberrations under 10 g/L food preservatives and 4 g/L color concentrations. A) Chromatin bridges formation in food preservatives and food colors. B) Chromosomal stickiness in food preservatives and food colors. C) C-mitosis. D) and E) irregular nuclei and binucleated cells.

genotoxicity; the cells went under stress conditions, resulting in different types of chromosomal changes, including chromosomal bridges, stickiness, c-mitosis, unequal distribution, and morphological changes in the nucleus. Kaur et al. [40] also indicated abnormalities, such as chromatin breaks, sticky chromosomes, chromosomal abnormalities, and morphological changes to the nucleus that are used to predict genotoxicity.

These chromosomal aberrations are caused by changes in chromosome structure and location. Most of these cellular aberrations are fatal, but several aberrations cause genetic or hereditary effects [41]. The stress-inducing agents, such as heavy metals, induce oxidative stress and cause genotoxicity [42]. The predominant chromosomal abnormalities observed in the current study were chromosomal bridge formation and stickiness, while unequal distribution and morphological changes in the nucleus were also observed in a few cells. Kaya has also stated similar observations [43]. Stickiness was found in chromosomes at the metaphase stage due to condensed chromosomes. Similar reports were given by Rosculete et al. [44]. Chromosomal stickiness may result from the fragmentation of chromosomes during the stress at anaphase movement [45]. Stickiness was

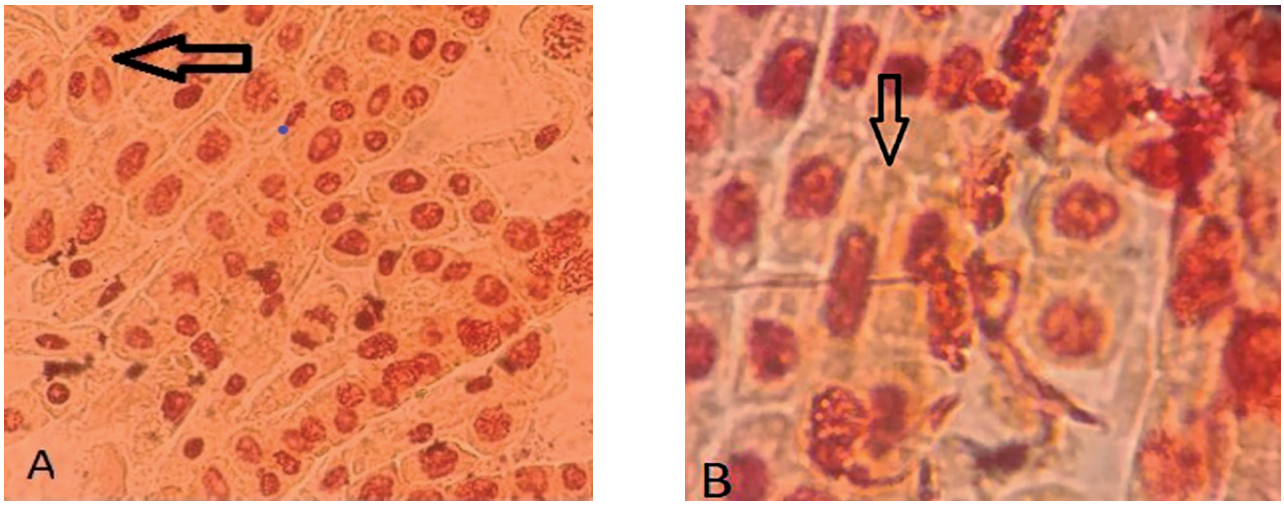

Fig.4. A) Binucleated cells in the +ve control group. B) Irregular-shaped nuclei

ordinarily detected in the anaphase and metaphase stages, respectively. Adhesion chromosomal abnormalities are those most triggered by the effect of food preservatives [46].

Chromosomal bridges are indicators of clastogenic action. It can be used for genotoxicity measurement. In our results, chromosomal bridges and stickiness were the common abnormalities found in root tips at anaphase and metaphase stages, respectively, in food color and preservatives at maximum doses. The metaphase stickiness resembles the results shown by Firbas and Amon [47]. Chromosomal bridges are strings of chromatin connecting the two segregating masses of chromosomes in anaphase or daughter nuclei in cytokinesis [48]. The microscopic study of the root tips of *Allium sativum* L. treated with food preservatives showed that these preservatives induced mitotic aberrations. Boric acid causes more adverse effects at a higher concentration than sodium benzoate. It has been reported that boric acid had inhibitory effects on the root tips of faba beans. [18]. Similar results were obtained when the root tips of *Allium cepa* L. were treated with paraben [49]. The mitotic index is universally regarded as a reliable indicator of cytotoxicity in all living organisms [50]. It is seen that changes in the mitotic index can reflect variations in the growth and development of organisms that have been exposed to chemicals [40]. The mitotic index is crucial in environmental monitoring and assessing cytotoxic chemicals with hazardous potential. In the current study, the mitotic index declined with the amplified concentration of food additives; this outcome correlates with earlier work done by Bellani et al. [39].

The decrease in mitotic index and inhibition of the DNA synthesized might be due to a reduction in ATP level [51]. It is reported that boron disturbs the normal cell cycle process by inhibiting the synthesis of DNA and microtubules [52]. It is evident from the current study's findings that increasing the concentration of food preservatives decreases the mitotic index and increases the abnormality percentage. Sheetal et al. [53] obtained similar results, which showed that greater dilution concentrations instigated some injuries, and the maximum rate of genotoxicity was testified to at different cell cycle phases. The results of the current investigation agree with Jhon et al.'s earlier reports [54]. The use of azo dyes caused abnormalities in chromosomes and induced mitotic aberrations. The decrease in mitotic index may be caused by a blockage in the cell cycle's synthesis phase, which hinders cell division [55]. Allura red and tartrazine exerted cytogenetic and mitodepressive effects on root tip cells of *Allium sativum* L. in a concentration-dependent manner. Nuclear structures can be signs or analytical markers of differentiation stages and pathological states [56].

The current study revealed irregular nucleus shapes under the influence of food colors and food preservatives; the same results were reported by Singh and Chadha [57]**.** Multinucleated cells were detected in the root meristematic cells, and the number of multinucleated cells was growing in different concentrations of food preservatives compared to food colors, which strongly indicates abnormal cell division [58]**.** The current study's findings suggested caution in using foods with these food colors and food preservatives and finding alternatives for the coloring and preservation of food products. However, further studies are required to validate the potential health risks of food color and preservatives.

Conclusion

Food industries release synthetic food colors and preservatives in wastewater, which reaches into the agriculture sector and adversely affects different crops. Farmers should know about the adverse impact of food additives on crops. Food colors like Allura red and tartrazine are extensively used in the cosmetics, food,

and medicine industries and have the potential for genotoxicity. The decrease in mitotic index and increase in chromosomal aberrations in root tip cells of *Allium sativum* L. showed genotoxic impacts. This study concluded that the frequent use of synthetic food color should be restricted due to its genotoxic effects on the crops.

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Conflict of Interest

The authors have no relevant financial or non-financial conflicts of interest to disclose.

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