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Genotoxicity of Synthetic Food Colors on Nitrogen-Fixing Bacteria in Agricultural Lands Irrigated with Wastewater of Corresponding Industries

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Abstract: Food colors are considered the most important component of foodstuff for enhancing the aesthetic appeal of the products. The rapid increase in population raised the demand for food materials, while wastewater from as-related processing industries is used for irrigation. This study was conducted to examine the genotoxicity of industrial wastewater on the plant growth-promoting rhizobacteria (PGPR). Three predominantly used synthetic food colors, including Azorubine E-122, Tartrazine E-102 and Allura Red AC E-129, were used during this project. Rhizobacteria were isolated from agricultural soils and treated with various concentrations of Azorubine E-122, Tartrazine E-102 and Allura red E-129 for a 24 and 48 h duration. DNA extraction and quantification were performed through a modified CTAB method, spectrophotometry and agarose gel electrophoresis. A comet assay was used to check DNA damage. According to the results, all the food colors had caused significant damage to DNA depending upon the concentration and exposure time. The extent of DNA damage caused by Azorubine E-122 was relatively greater compared with the other colors, so the fragmentation rate of 86% and 92% was obtained at 1.25% concentration for 24 and 48 h, respectively. The current results have revealed the devastation capacity of food colors by accelerating distortion risk to soil micro-flora, hence the fertility of the soil.

Keywords: synthetic food colors; genotoxicity; plant growth-promoting rhizobacteria (PGPR)

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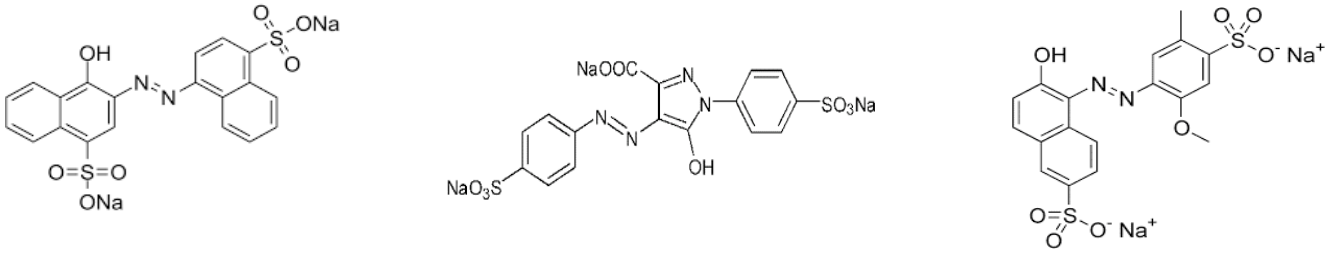
1. Introduction

Food colors are an important sensory property in the food production and beverage industries. The global synthetic dye market in 2022 was forecasted to be USD 15.46 billion. Population growth and increasing demand for food products have exerted pressure on food processing industries for the past several decades [1]. Synthetic food colors are widely used in all food products to sustain the sensory characteristics of the food products, which vanish in processing, and also in order to maintain the preferred appearance [2]. Natural food colors are less stable and also are not toxic in nature compared with synthetic food colors [3]. Most of the synthetic colorants are azo compounds in nature, which have been added to various edible items, imparting the latest color and also making them palatable and attractive [4]. The most selling and daily need-based products, such as colas

or carbonated drinks, would not be brown without color additives, and similarly, the margarine would not be yellow, and mint ice cream also would not be green. In candy manufacturing, synthetic food colors are more frequently used as compared to natural food colors. Synthetic food colors are considered to be the most important part of processed food. They are usually water-soluble chemical substances and are directly used in the items without further processing. The coloring ability of synthetic dyes is because of the azo group [5]. Azo dyes are commonly synthetic aromatic compounds, having a functional azo group (N=N) [6]. Most food dyes (azo dyes) are hydrophilic in nature [7]. In Pakistan, there are eighteen synthetic and five natural permitted food colorants [8]. The United States permitted only seven food colors, including Fast Red (which was forbidden in India), whereas the European Union permitted sixteen synthetic food colors. Iran and Australia legalized thirteen synthetic food colors [9]. Synthetic food colors have a potential effect on the health of living organisms. Most synthetic dyes used in conventional toxicity experiments showed a high rate of toxic effects. The majority of food-borne diseases resulted from the intake of prohibited textile colors [10,11]. They cause DNA damage, skin diseases, cancer of gastrointestinal tracts, colon cancer, and disorders of the liver, kidney and abdomen. Synthetic food colors have explicit genotoxicity and cytotoxicity impacts on humans, such as the impacts of sunset yellow and tartrazine on DNA, by increasing the level of DNA damage and the percentage of apoptosis. The adverse effects of synthetic food colors have also been studied on reactive oxygen species (ROS) [12]. The food color Allura red E-129 (azo dyes) and other vigorous synthetic food colors are the major reason for gut, oral, cardiovascular and neurodegenerative disorders [13]. Because these azo dyes reduce the aromatic amines by the intestinal micro-flora, that is why they cause different carcinogenic diseases in the human body. Epidemiologic research on synthetic food colors has been conducted to identify the neurobehavioral outcomes in both humans (children) and animals. In another finding, azo dye (synthetic food color) azorubine/carmoisine was used as an investigating component; in the results, it showed a high rate of toxicology in animal body weight, hematology, organ weights and biochemistry. It was also reported that the expression of BCL-x and PARP genes was intensively increased, whereas the expression of the p53 gene declined in the mouse liver tissues treated with carmoisine. Consequently, carmoisine was reported to cause renal failure, hepatotoxicity and a suspected reason for liver oncogenesis [14].

Azo dye, such as Red 3 (azorubine/carmoisine), was reported to cause cancer in animals, and in addition to Red 40, Yellow 5 and Yellow 6 have been found to be contaminated with benzidine and other carcinogenic compounds. Whereas Blue 1, Red 40, Yellow 5 and Yellow 6 also resulted in causing hypersensitivity reactions [15]. While having adverse effects on living organisms, synthetic dyes are widely used in textile, food, and pharmaceutical industries and cause significant negative effects on the environment and human health when discharged without prior treatment. The untreated effluents released by the industries contain a diverse range of organic pollutants [16]. Industries that release their wastewater without degrading the free azo dyes in it can cause serious ecotoxicological threats as well as toxic effects on living organisms. Farmers in developing countries used untreated industrial wastewater as a primary source of irrigation, which had a negative impact on crop germination rates and soil quality and ended in prompting genotoxicity in nitrogen-fixing bacteria by causing a reduction in the soil fertility when it reached the soil horizons [17,18]. Several degradation techniques or methods of dye compounds have been introduced to overcome the toxicity concern of contaminants discharged from different industrial activities. Among them, photocatalytic technologies are revealed to be promising candidates because they play an important role in breaking down different hazardous compounds into innocuous products [19–21]. Synthetic food dyes are abundantly present in industrial effluents, and when they are discharged into the environment (water and soil), they reduce light penetration and inhibit the performance and growth of algae, aquatic plants and agricultural crops. Synthetic dyes ingested by living organisms can be metabolized in their bodies into toxic intermediates, causing adverse effects in their bodies [22].

Plant growth-promoting rhizobacteria (PGPR) are the rhizosphere bacteria that have the power to raise the availability of nitrogen concentration in the soil atmosphere [23]. Bacterial oxidoreductase enzymes play an important role in the degradation of synthetic food colors. Bacterial metabolism permits them to exploit complex xenobiotic compounds of the dyestuff as a substrate. During this procedure, they are degraded to minute complex metabolites [24]. Rhizobacteria are more in the rhizosphere of plants than in the adjacent soil, and this perfusion depends on the crop species [25]. The process of nitrogen fixation is among the most important biological processes. Biological nitrogen fixation assists in improving soil fertility and crop production [26]. Rhizobacteria are useful microorganisms and can apply as bioinoculants and also for studying the nitrogen fixation process. They are sensitive to acidic pH, high salts and temperature [27]. It has beneficial effects on crop growth and yields through the biosynthesis of biologically active substances, stimulation of rhizospheric microbes and by producing phytopathogenic inhibitors [28]. Rhizobacteria have the capacity to continuously grow and quickly fix large amounts of nitrogen and are able to alter atmospheric nitrogen into ammonia, which is consumed by plants. Moreover, nitrogen plays an explicit role in plant growth as it is a fundamental plant nutrient and is broadly used as a nitrogen fertilizer to improve the yield of agriculturally important crops. In addition to nitrogen fertilizer, other chemical fertilizers also play an important role in increasing the economic yield of crops [29]. In addition, the efficiency of PGPR has been found to decrease with increased nitrogen levels [30]. The finest grouping was verified with NH_4Cl (Ammonium chloride) at 0.1 g/L, whereas the action of copper in rhizobacteria was found to be toxic, even in small amounts [31]. The rhizobacteria may suffer due to extreme amounts of nitrates and the acidic environment created because of chemical fertilizers and toxic industrial effluents [32]. It is investigated that the result of herbicide 2, 4–D and its products, p-chlorophenoxy-acetic acid and p-chlorophenol were exploited by *Azotobacter croococcum* as a carbon source, which eventually stimulates the nitrogenase enzyme. The organophosphorus insecticides profenofos and chlorpyrifos had contrary effects on the aerobic nitrogen fixers and lessened their nitrogen-fixing ability. The intake and absorption of some toxic heavy metals by aquatic flora and soil can cause detrimental health issues in animals and, ultimately, in humans via the food chain [33]. In this study, three synthetic food colors, such as Azorubine E-122, Tartrazine E-102 and Allura red AC E-129, were selected to study the rate of genotoxicity on the genome of PGPR. The food color Azorubine E-122, commonly known as carmoisine or Cl food Red 3 [34], has a structural formula in which two subunits of naphthalene were present ($C_{20}H_{12}N_2Na_2O_7S_2$). The food color Tartrazine E-102 was a synthetic lemon-yellow azo dye, also known as "Cl food yellow 4" with a chemical formula ($C_{16}H_9N_4Na_3O_9S_2$) [35], and the synthetic food color Allura red AC E-129, known as "CL Food Red 17", was a synthetic red azo color, having the chemical formula $C_{18}H_{14}N_2Na_2O_8S_2$, as shown in Figure 1 [35]. Although it was commonly delivered as a sodium salt, it can normally be used as potassium and calcium salts.



a: Azorubine E-122 b: Tartrazine E-102 c: Allura red AC E-129

Figure 1. Chemical structures of Azorubine E-122, Tartrazine E-102 and Allura red AC E-129.

Subsequently, this study revealed how synthetic food colors, which were released as industrial effluent by food processing industries, cause DNA damage in rhizobacteria.

Consequently, the damage of PGPR (rhizobacteria) results in inhibiting the biological process of nitrogen fixation and also the performance of rhizobacteria in the production and regulation of compounds and stress hormonal status. As a result, via entering the food chain, these adverse chemicals cause various serious disorders in the human body.

2. Materials and Methods

2.1. Sample Collection and Preparation

Soil samples were collected by using sterilized equipment from contamination-free fields more than 60 km away from urban settlements and industrial setups, along with rhizosphere soil surface and subsurface about 10–15 cm in depth, and along with the roots of the plant in a sterilized polybag. For isolation of bacteria from roots, 3–5 g of fresh roots were washed and, for surface sterilization, dipped in 5% NaOCl for one minute. After sterilization with double-distilled water was performed three times, the root sample was ground by using a sterilized mortar and pestle. For the preparation of diluent/peptone salt solution, 0.5 g of peptone was taken in a large glass bottle along with 4.25 g NaCl and 500 mL distilled water, and then the solution was well-shaken and autoclaved. A measurement of 225 mL of peptone solution was added into the stomacher bag and mixed into the solution to homogenize [36].

Bacterial growth media was prepared by following the protocol designed in [37] with some modifications (2.5 g of tryptone, 1.25 g of yeast extract and 2.5 g of NaCl added into 250 mL of distilled water). A total of 10 mL of LB broth (Luria Bertani) was added to each screw-capped test tube. One mL of prepared samples from both soil and root was in each test tube. After this, the enrichment broth was incubated at 37 °C for 24 h. Synthetic food colorants, such as Azorubine E-122 and Tartrazine E-102, were purchased from Essence.pk Company (important colors), whereas Allura red AC was bought from a local commercial brand (Italiano).

2.2. Morphological Identification of Bacterial Isolates

Colony morphology, shape, size, color and growth pattern were noted after 24 h of growth on LB (Luria Bertani) medium, as previously described [38]. The size of cells was observed by light microscopy, and the gram staining was completed by following the protocol in [39]. Several steps of biochemical tests were also performed to characterize bacteria by following the procedure in [40], such as the KOH solubility test, performed as previously described [41].

2.3. Application of Dilution of Synthetic Food Colors

Five dilutions were made from each synthetic food color (Azorubine E-122, Tartrazine E-102 and Allura red AC E-129). Precisely 0.25, 0.50, 0.75, 1.0 and 1.25 g [1] of Azorubine E-122, Tartrazine E-102 and Allura red E-129 were taken in each jar along with 100 mL of dH_2O and stirred to homogeneously dissolve the color. After dilution preparation, two mL of each diluted synthetic food color was applied to bacterial cultures. The experiment was conducted in two setups for each synthetic color at 24 and 48 h. Both setups contained the same concentration of dilutions, 0.25%, 0.50%, 0.75%, 1.0% and 1.25%, along with the control. After the application of dilutions, the bacterial cultures were incubated at 37 °C for 24 and 48 h.

2.4. Genomic DNA Extraction

The genomic DNA of rhizobacteria was extracted by the CTAB extraction method [42] with some modifications.

2.5. Quantification of Genomic DNA

Quantification of rhizobacterial DNA was measured after being treated with Azorubine E-122, Tartrazine E-102 and Allura red AC E-129 for 24 and 48 h by a Single Beam

Scanning UV-VIS Spectrophotometer-2373 [43]. Agarose gel electrophoresis was used to quantify the presence of genomic DNA [44].

2.6. Comet Assay

The comet assay was accomplished according to the described protocol [45], with some modifications. Precisely 1% of NMPA (Normal Melting Point Agarose) was prepared in distilled water. After this step, the solution was placed into a water bath to maintain the temperature at 65 °C. The slides were washed with 70% methanol to make them contamination free. For labeling, paper tape was used on one side of the slide. In the next step, the slides were dipped in NMPA and placed on a clean surface overnight to dry completely. A total of 50 µL of bacterial genomic DNA sample were dissolved into 50 µL of 1% LMPA (Low Melting Point Agarose prepared in PBS).

The samples were mixed homogeneously by pipetting them up and down, making sure to catch the speed at this stage because the agarose can be quickly solidified. After this step, we immediately added 50 µL of the sample on the slide and put a coverslip on it, and then placed the slides at 4 °C for 30 min. A lysis buffer cannot be applied if DNA extraction has been previously performed (which is known as an indirect method of the comet assay).

In the next step, single-gel electrophoresis was carried out to run the experimental slides. The tank was placed in the freezer before running the slides for almost 1 h, then chilled alkaline electrophoresis solution was added to the chilled tank (place the tank on ice to try to maintain the temperature because the most important thing in this procedure is temperature management). Then, the slides were placed on the tank, and the slides were run at 25 V, 300 mA for 30 min at 4 °C. The excess alkaline electrophoresis solution was removed with the help of blotting paper. The slides were washed with a neutralizing buffer for almost 10 min 3 times. The slides were placed in 70% ethanol for almost 5 min at room temperature.

The next step was the staining of the slides. To make sure the slides were completely dry, we placed a drop of Ethidium bromide (CAS No. 1239-45-8) on the gel attached to the slide and placed a cover slip on top of it.

Quantification and Visualization of DNA Damage

The slides were placed under a fluorescence microscope (Olympus BX50, Hamburg, Germany) at 10× magnification to take pictures of the stained slide. For image analysis using the Comet software (casplab, comet score, Comet Assay IV), the results of the comet assay were calculated by using Comet scoring software [46].

2.7. Statistical Analyses

A one-way ANOVA was carried out for the statistical examination of data with Microsoft Excel for analyzing the significant difference of DNA tails between three of the synthetic food colors.

3. Results

3.1. Quantification of DNA after Treatment with Azorubine E-122, Tartrazine E-102 and Allura Red AC E-129

Quantification of DNA samples after 24 and 48 h was performed by using a spectrophotometer. Readings were recorded at both A260 nm and A280 nm wavelengths to analyze the purity index of DNA in experimental samples. An absorbance of one unit at 260 nm corresponds to 50 µg genomic DNA per mL (A_{260} = 1 for 50 ug/mL; based on standard 1 cm path length), whereas the ratio of A_{260}/A_{280} nm indicates sample purity as most of the results were above ≥1.8, which is a standard ratio for purified DNA as shown in Table 1.

Table 1. Quantification of DNA samples of three synthetic food colors, Azorubine E-122, Tartrazine E-102 and Allura red AC E-129, showing purity.

Time	Dilutions	Azorubine E-122		Tartrazine E-102		Allura Red AC E-129	
		Concentration (µg/mL)	Ratio 260/280	Concentration (µg/mL)	Ratio 260/280	Concentration (µg/mL)	Ratio 260/280
24 h	Control	48	1.83	52	1.89	39	1.82
	0.25%	40	1.75	36	1.4	53	1.6
	0.50%	29	1.59	40	1.83	24	2.2
	0.75%	42	1.66	52	1.75	28	1.4
	1.00%	37	1.5	48	1.63	38	1.93
	1.25%	36	2.2	33	1.66	42	1.6
48 h	Control	34	1.81	37	1.79	60	1.8
	0.25%	40	1.6	42	1.5	25	1.5
	0.50%	30	1.5	27	1.75	53	1.63
	0.75%	41	1.22	50	1.73	44	1.57
	1.00%	50	1.96	34	2.2	34	2.2
	1.25%	44	1.99	29	1.8	49	1.75

3.2. Quantification of DNA through Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to quantify the presence of genomic DNA of rhizobacteria by using 1% Agarose gel, in which 90 voltage is applied for 25 min to quantify the genomic DNA against 1 KB DNA ladder as in Figure 2. The presence of bands near the wells indicates the presence of genomic DNA.

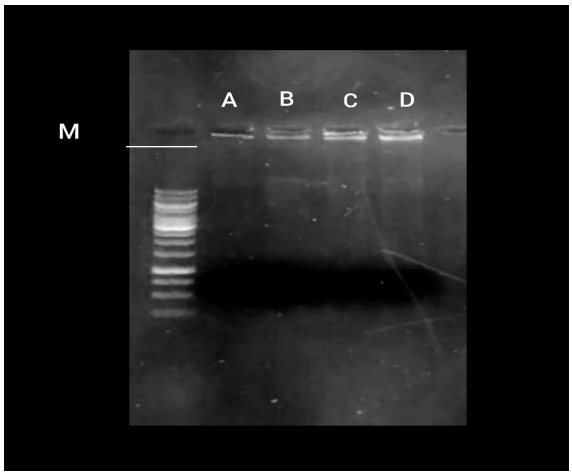


Figure 2. Quantification of genomic DNA of plant growth-promoting rhizobacteria (PGPR) on Agarose gel electrophoresis. M represents the DNA ladder of 1 kb, and the extracted genomic DNA samples of rhizobacteria are from well A to D.

3.3. Image Analysis by Using Comet Scoring Software

Comet slides (Azorubine E-122, Tartrazine E-102 and Allura red AC E-129) at both exposure time periods 24 and 48 h were visualized under a fluorescence microscope (Olympus BX50, Hamburg, Germany) at 10× magnification, and after visualization, images of comets were amplified by using the comet score software. In addition to measuring the amount of DNA damage, the ratio of fluorescence intensity was used inside the comet tail and the fluorescence intensity of the comet head. After putting the images in the software, other parameters, such as comet length, comet head radius, tail length and tail movement, were also measured. In Figure 3, the fragmented tail of comets showed adverse effects of synthetic food colors on the genome of rhizobacteria. The control of each dilution showed

negative results. The frequent rate of fragmentation in every image indicates the rate of genotoxicity which was induced by three of the studied synthetic food colors.

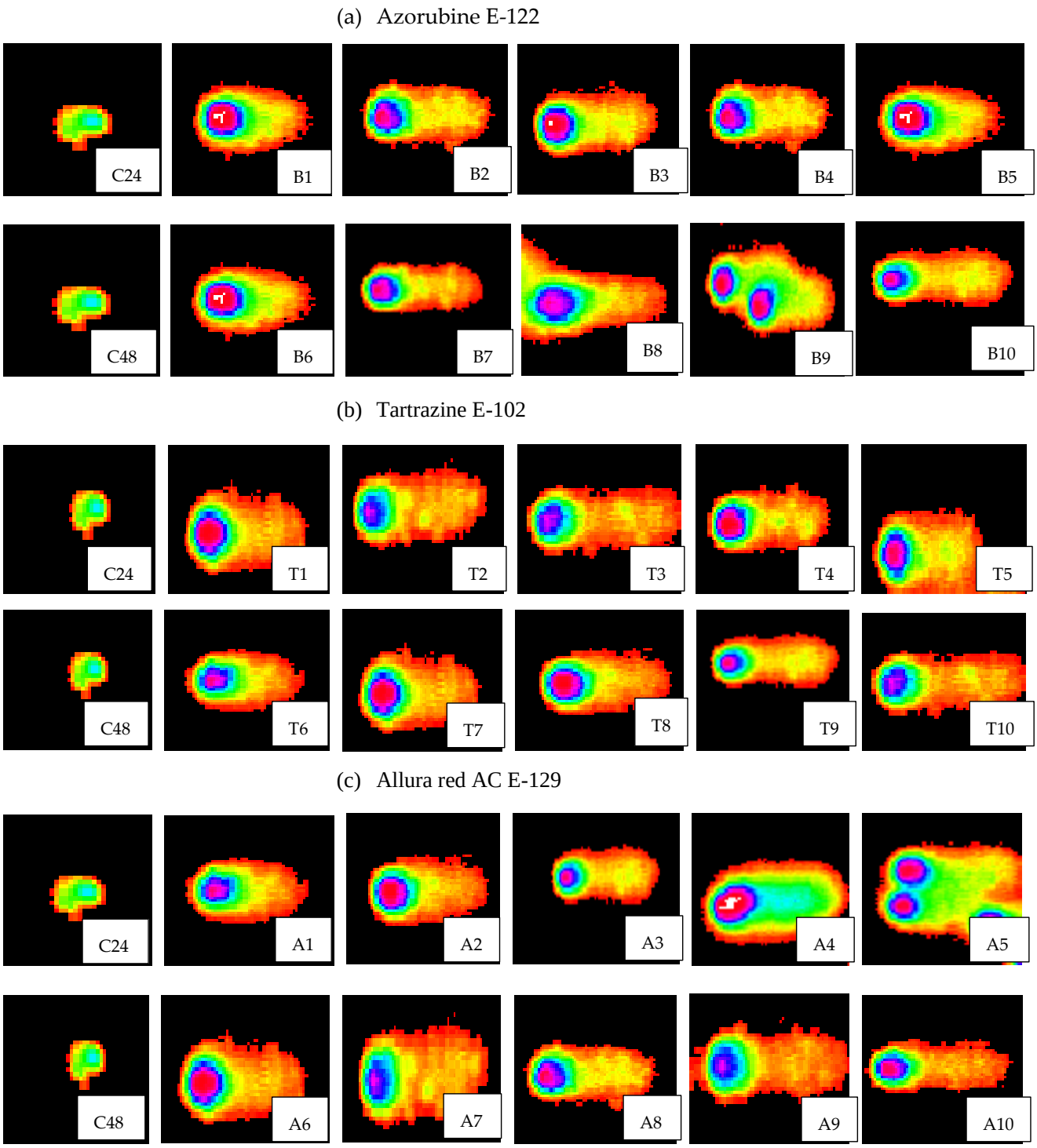


Figure 3. Interpretation of comet scores by using the comet scoring software. Images from B1 to B5 and B6 to B10, along with controls, show the comets of Azorubine E-122 at both 24 and 48 h exposure time, whereas the comets of Tartrazine E-102 are shown from T1 to T5 and T6 to T10 and Allura red AC E-129 are shown from A1 to A5 and A6 to A10 at both 24 and 48 h exposure times. Dilution concentrations were 0.25, 0.50, 0.75, 1.0 and 1.25.

3.4. Implementation of Comet Measurement to Evaluate the Rate of Genotoxicity on DNA Caused by Synthetic Food Colors

Single-cell gel electrophoresis or comet assay was used to measure the detection of DNA damage in the DNA cells of rhizobacteria. The total genotoxicity produced by Azorubine E-122, Tartrazine E-102 and Allura red AC E-129 was found to be dependent on concentration and exposure period. For measuring the significant units of a comet (comet length, head and tail length and movement), the comet score software was used. However, the result indicates that each of the three synthetic food colors causes significant genotoxicity. The most DNA damage was recorded in Azorubine E-122 rather than Tartrazine E-102 and Allura red AC E-129, as shown in Table 2.

Table 2. Comet measurement results determine the DNA damage, which indicates the size of the head and tail of comets at different concentrations of synthetic food color.

Time	Concentration of Dilution	Azorubine E-122					Tartrazine E-102					Allura Red AC E-129				
		Head Length	Tail Length	Comet Length	DNA Head	DNA Tail	Head Length	Tail Length	Comet Length	DNA Head	DNA Tail	Head Length	Tail Length	Comet Length	DNA Head	DNA Tail
24 h	Control	15	3	18	100	−0.1	8.0	1.0	9.0	100	−0.1	5	3	6.0	100	−0.1
	0.25%	10	4	12	100	3	7.0	2.0	9.0	83	18	10	3	12	99	1
	0.50%	8	39	46	18	83	14	14	28	61	40	9	7	14	69	32
	0.75%	19	35	54	30	71	31	35	66	50	52	20	24	44	61	41
	1.00%	10	25	35	31	70	10	25	35	31	70	23	55	78	73	28
	1.25%	4	15	18	15	86	5.0	11	16	36	65	11	33	44	28	73
48 h	Control	33	3.0	36	100	−0.1	20	1.0	21	100	−0.1	14	2	15	100	−0.1
	0.25%	5	15	19	88	13	15	4.0	19	88	13	13	33	46	81	20
	0.50%	16	17	33	36	65	14	15	29	54	47	7	15	21	25	76
	0.75%	20	23	43	31	70	19	19	38	21	80	10	19	29	45	56
	1.00%	3	19	21	11	90	10	12	22	24	77	10	34	43	22	79
	1.25%	6	52	57	8	93	4.0	9.0	13	22	79	8	45	52	9	91

3.5. Evaluation of DNA Damage through Statistical Analyses

Statistical analyses were used to evaluate significant differences in DNA tails among the three studied synthetic food colors at both exposure time periods (Azorubine E-122, Tartrazine E-102 and Allura red AC E-129). A one-way ANOVA was applied to find the rate of significance between the three studied synthetic food colors. In addition, the results showed that there is no significant difference between the DNA damage, which was caused by the application of synthetic colors (as the *p*-value of F statistics in both setups, 24 and 48 h, is higher than 0.05), as shown in Table 3. This means all three colors play a significant role in damaging the DNA of plant growth-promoting rhizobacteria (PGPR). However, the summary of the statistical analyses, in which the sum and average were calculated between two setups or groups (24 and 48 h), showed that most of the DNA damage resulted from Azorubine E-122 as compared with Tartrazine E-102 and Allura red AC E-129, as shown in Table 4.

Table 3. Statistical analyses of genotoxicity variance (one-way ANOVA) between two groups of treatment.

Groups of Treatment	Source of Variation	SS	df	MS	F	*p*-Value
24 h	Between groups	103	2	778	0.753	0.488
	Within groups	20,446	15	1034		
	Total	20,550	17			
48 h	Between groups	103	2	51.7	0.0379	0.963
	Within groups	20,446	15	1363		
	Total	20,550	17			

Table 4. Comparative statistical analyses of DNA damage caused by E-122, E-102 and E-129 at treatments 24 and 48 h.

	Groups	Count	Sum	Average	Variance
24 h	E-122	6	308	51.4	1617
	E-102	6	242	40.4	738.4
	E-129	6	172	28.6	747.5
48 h	E-122	6	327	54.6	1525
	E-102	6	294	48.9	1252
	E-129	6	319	53.2	1311

4. Discussion

This study was designed to investigate the factor of genotoxicity on plant growth-promoting rhizobacteria (PGPR) caused by synthetic food dyes (azo dyes), which were released as industrial effluents. The extent of employing rhizobacteria in research experiments as microbial inoculants is because of their growth-promoting ability and also their nitrogen-fixating capabilities, such as synthesizing indole compounds and producing hydrolytic enzymes [47]. It was found that all three colors show a significant rate of DNA damage on the genome of rhizobacteria, but among them, an exceptional rate of genotoxicity was shown in Azorubine E-122 as compared with Tartrazine E-102 and Allura red AC E-129 because the recorded ratio of DNA tail in Azorubine E-122 was 86% and 93% at both 24 and 48 h. Tartrazine E-102 and Allura red AC E-129 also showed significant DNA damage, such as 65% and 79% and 73% and 91% at both 24 and 48 h setups. The rate of DNA damage in both Tartrazine and Allura red AC was reported less as compared with Azorubine. Thereby, it was recorded that all of the dilutions had caused genotoxicity, but higher concentrations of dilution caused some major injuries or toxicity. A similar pattern was previously observed in which genotoxicity impact was studied on meristematic cells root tips of *Allium cepa*, induced by food coloring dyes. The highest frequency of genotoxicity was reported at various stages of the cell cycle [48]. In another research work, *Escherichia coli*, *Staphylococcus albus* and *Saccharomyces cerevisiae* were used to study the toxicity rate of synthetic food colors, in which the rate of toxicity or damage was directly proportional to the rate of concentration applied to the test organism [49]. The purpose of the current study is to determine the genotoxicity of synthetic food colors on PGPR, which contributes a prominent role in soil fertility and increasing plant growth and plays an important role in nitrogen fixation [50]. However, in a previous research work, an azo dye (used as a commercial dye) known as CI Disperse Blue 291 (DB291) was used to investigate the rate of genotoxicity of DB291. Primary DNA damage in blood, kidney, and liver cells, BAX, BCL2, SMAD4 and TNFα gene expression in leukocytes were recorded. Animals that are treated with 50 mg/kg bodyweight had shown an amplified frequency of micronucleated polychromatic erythrocytes (MNPCEs), whereas, on the other hand, neither genetic alteration, primary DNA damage nor aberrations in gene expression were reported [51]. In addition, according to the present results, the selected synthetic dyes (E-122, E-102 and E-124) also showed a significant rate of damage in the genome of rhizobacteria, similar to the primary damage that had been recorded by CI disperse blue. Industrial effluents are commonly discharged in nearby wetlands, crop fields and water bodies which, as a result, cause low fertility, dermal disease, low yields, boost insect attack on crops and increase the salt content and salinity of the soil [52]. Increased soil salinity resulted in adversely affecting the plant nutrient balance, protein synthesis, energy metabolism and photosynthesis and disturbing the whole biological process of nitrogen fixation overall [53]. Industrial effluents contain several carcinogenic heavy metals, such as Mercury (Hg), Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni) and Thallium (TI) [54]. Different food additives, which were used as food colors, include heavy metals, such as Lead (Pb), Mercury (Hg) and arsenic [55]. Synthetic dyes are azo chemicals in nature, and the oxidative end products have carcinogenic and mutagenic effects, which

produce modifications of DNA [52]. Scientific work on zebrafish embryos was used as an indicator to study the effect of food colorant carmoisine, which showed a significant decrease in height and eye diameter of the embryos, a rise in free oxygen radicals in apoptotic cells and lipid accumulation and caused serious malformations [53]. Although in collaboration with the above finding, the present results showed that most of the DNA damage was caused at higher concentrations of dilution, such as at 1.0% and 1.25%, and at greater exposure time periods. The numeric and structural variations in chromosomes have become a key factor in appraising the genotoxicity of pollutants, heavy metals, water samples and food chemicals [56].

Despite the fact that the present study also positively relates to a research finding in which different bacterial isolates obtained from the soil are aggregated by means of industrial wastewater (secondary source of aggregation), the results showed adsorption of Cd and Ni at different concentrations of single- and bi-metal systems and indicated that these heavy metals not only affect the soil flora but also affect the agricultural system [57]. Similar to the presence of benzidine, 3,3′-dimethoxybenzidineand 3, or 3′-dimethylbenzidine were reported in the industrial wastewater from where the soil samples were collected. These chemicals are classified as carcinogens of category [58]. The presence of these carcinogenic chemicals in soil inhibits the activity of PGPR and causes highly toxic effects on the biological process of nitrogen fixation. Notable results of the comet assay proved that synthetic food colors, which were discharged as industrial effluents, play an explicit role in destroying the soil flora, which in turn leads to distortion of the food chain. Several food additives, such as sodium benzoate and sodium sulfite, were found in chromosomal aberrations, colon cancer, etc. This may be due to the clastogenic activity of chemicals on DNA [59]. Moreover, some studies hold the opposite opinion that azorubine/carmoisine is not a standard genotoxicity assay [60], and in contrast to the present research work, it was found that not only Azorubine E-122 but also Tartrazine E-102 and Allura red AC E-129 cause significant DNA damage at all dilutions other than the control. However, in another research finding, it was reported that synthetic food colors cause health problems, such as cancers, reduced hemoglobin concentrations, mutations and allergic reactions. Several confectioners also contain unidentified colors, such as textile dyes [2]. In addition, different industrial wastewater contains synthetic dyes or food colors in which almost 70% of azo dyes contain one or more azo bonds (R_1–N–N–R_2) in their chemical structure [61]. Some synthetic food colors are reported to contain carcinogenic chemicals, such as β-naphthylamine (which is also known as 2-naphthylamine), carrying the amino group at position two [62,63]. The chemical structure of Azorubine, such as disodium 4-hydroxy-2-[(E)-(4-sulphonato-1-naphthy)diazenyl]naphthalene-1-sulfonate, contain β-naphthylamine in their chemical structure. A report was issued by the Department of Health and Senior Services in New Jersey [64] in which it was stated that 2-naphthylamine is a carcinogenic chemical that is basically used in the manufacturing of different azo dyes (synthetic food colors). According to the report, 2-naphthylamine is no longer produced for commercial use and is only available for research purposes. The presence of β-naphthylamine or 2-naphthylamine in the chemical composition of Azorubine or carmoisine makes it carcinogenic, which is considered to be the most hazardous environmental pollutant [65]. In support of this present work, three of the studied synthetic food colors showed significant DNA damage, whereas Azorubine E-122 showed slightly greater DNA damage as compared with Tartrazine E-102 and Allura red AC E-129.

5. Conclusions

Synthetic food colors, such as Azorubine E-122, Tartrazine E-102 and Allura red E-129, induce significant damage to DNA in rhizobacteria. The Azorubine E-122 has shown explicit genotoxicity in the plant growth-promoting rhizobacteria (PGPR). Moreover, this compound (Azorubine E-122) causes higher fragmentation in DNA tails, where the fragmentation rate is 86% at 1.25% concentration for 24 h exposure time period, and at 48 h, the fragmentation rate is 93% at 1.25% concentration of synthetic food color. Hence, these

synthetic food colors are genotoxic chemical compounds. They are not only harmful to humankind but also to the environment in which they are released. The usage of synthetic coloring dyes should be prohibited, and natural food colors should be promoted at both industrial and commercial levels.

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