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Evaluation of Cytogenotoxic Effect of Brilliant Blue FCF on *Allium cepa* Root Tip

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Abstract— *Effect of the synthetic food colorant brilliant blue FCF was tested on Allium cepa root tip meristematic cells. The concentrations of brilliant blue FCF used were: zero% (control), 50 mg/L, 100 mg/L, 300 mg/L, and 500 mg/L with two different time of exposure: 10, and 20 hours. The results revealed that increasing the dose concentration resulted in the inhibition of root growth up to 78.67% at 500 mg/L of brilliant blue FCF. The mitotic index decreased with increasing the concentration of the tested material and increasing exposure period from 8.95% in the control to 3.34% in 500 mg/L for 10 hours and from 9.44% in control to 2.95% in 500 mg/L for 20 hours. The mitotic abnormalities like laggard chromosomes, C-mitosis, multipolar anaphase, sticky metaphase, star-shaped anaphase, sticky anaphase, chromosome loss, chromosome fragments, and disturbed anaphase were increased with increasing brilliant blue FCF concentration and increasing time of exposure as compared with the control. The total abnormal cells were zero in the control group increased to 54 in 500 mg/L concentration after 10 hours, this value increased to 99 after 20 hours of treatment.*

Keywords— *food colorant, meristematic cells, mitotic index, abnormalities, treatment.*

I. INTRODUCTION

Food additives are used to preserve, blend, thicken, flavour and color food (Mpountoukas *et al.*, 2008). Brilliant Blue FCF is a synthetic colorant used in processed foods, medications, and cosmetics (FD&C Blue 1, 2019). It is water-soluble, blue-coloured (Lucova *et al*., 2013). Classified as a triarylmethane dye, the chemical formula is $C_{37}H_{34}N_2Na_2O_9S_3$ (Yousefi *et al*., 2017). The synthetic compounds present less color loss against external conditions and lower cost of manufacture as compared with the natural colorants, (Martins *et al*., 2016; Ou *et al*., 2018). The food containing synthetic colorants can have side effects when consumed in large amounts, such as toxicity, allergy, asthma, hay fever, rashes, vomiting, tight chest and possible carcinogenesis (Etteh, 2003; Amchova *et al*., 2015). Brilliant blue FCF was banned in most European countries due to its carcinogenic effect shown during tar-induced tumor study in rats (Gilani *et al*., 2017). Brilliant Blue acceptable daily intake (ADI) was established as 12.5 mg/kg bw/day by the European Union (EU) Scientific Committee for Food (SCF) in 1975 (SCF, 1975).

Then European Food Safety Authority (EFSA) revised the ADI of 10 mg/kg bw/day allocated by the Scientific Committee for Food (SCF, 1984) to be 6 mg/kg bw/day in 2010. Currently, in the EU, Brilliant Blue FCF is allowed to be use in various foodstuffs with a maximal level of 20–500 mg/kg, and in beverages less than 200 mg/L (EFSA, 2010).

Brilliant blue FCF is a synthetic dye used extensively in our various foodstuff in Kurdistan Region, Iraq because of the rare presence blue food colorants from natural sources and little is known about its adverse effects on our health, therefore. The objective of this study was to test the toxic effect of the synthetic brilliant blue FCF on the mitosis of *Allium cepa* root tip cells as the test system.

II. MATERIALS AND METHODS

2.1: Materials

Onion bulbs *(Allium cepa,* 2n = 16 chromosomes) free from fertilizers were purchased from a local store in Zakho/Duhok, Kurdistan regional government. Brilliant blue FCF material was obtained from ROHA/India (SRNO: 021, LOT NO: RP19050161), the concentrations of the test product were control (distilled water), 50 mg/L, 100 g/L, 300 mg/L and 500 mg/L of brilliant blue FCF for different times of exposure 10 and 20 hours. The different concentrations were diluted with distilled water.

2.2: Methods:

2.2.1: The root length calculation:

The method of Fiskesjo, (1985) was used for calculating root growth inhibition.

$$I\% = CG/TG*100 \quad (1)$$

Where I= inhibition
CG= Change in growth
TG= Total growth

2.2.2: Squash method for preparing onion root tip mitosis slides:

The bulbs of *Allium cepa L.* were seated on 25cm^3 vials filled with tap water until the root length reached about 1 cm in length then put in the test substance. The roots were harvested twice, once after 10 hours and another time after 20 hours.

The roots were washed with distilled water and cut about 1 cm and kept in Carnoy's fixative (3 parts absolute ethanol and one part glacial acetic acid). Then the roots were placed in IN HCl at 60ºC for 6 minutes then washed with distilled water and stained with Giemsa stain for about 30 minutes, then the root tips were cut and covered with a coverslip on the slide and the tissue squashed and spread evenly under the coverslip. The

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mitotic slides were examined under 100x light microscopy (Sharma and Sharma, 1999).

2.2.3: Mitotic Index Calculation:

Three slides of each treatment and about 1000 cells from each slide was counted and the mitotic indices were measured (Onyemaobi *et al.*, 2012).

M.I.= DC/TC *100 (2)

Where M.I.= Mitotic Index
DC=Dividing Cells
TC=Total No. of Cells

2.2.4: Mitotic Abnormalities Percentage:

The abnormalities percentage was calculated according to (Bhatta & Sakya, 2009).

A%= ADC/TDC*100 (3)

Where A%=Abnormalities percentage
ADC=Abnormally dividing cells
TDC=Total no. of dividing cells

2.2.5: Statistical Analysis:

All the collected data were saved in a new spreadsheet in Microsoft Excel program to be prepared for analysis. The, the data were projected to Statistical Package for Social Scientists (SPSS Version 14) to be analyzed. Residual plots confirmed by Shapiro-Wilk normality test that data were parametric; therefore, Analysis of Variance (ANOVA) analysis test was used for data. Tukey's pairwise test was used for post hoc comparison. Tables were prepared in Excel spreadsheet.

III. Results and Discussion

3.1: The Root Length Inhibition:

The root length of the different treatments after 72 hours showed a significant reduction in root growth as compared to the control group as shown in figure 1.

The inhibition percentage was calculated to be 13.3% at 50 mg/L treatment increased to 78.67% at 500 mg/L treatment as shown in table 1.

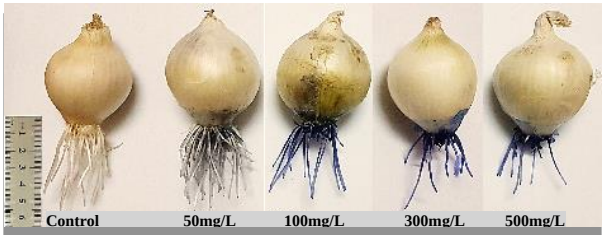

Fig. 1. The mean root length of *Allium cepa* L. before and after treatment with different concentrations of brilliant blue FCF.

TABLE 1. Mean root length and inhibition percentage caused by increasing doses of brilliant blue FCF to *Allium cepa* roots.

Concentration (mg/L)	Mean root length (cm)	Inhibition%
Control	6	0%
50	5.2	13.3% *
100	3.85	35.83% **
300	2.78	53.67% **
500	1.28	78.67% ***

Note: *= significant at p< 0.05, **= significant at p< 0.01, ***= significant at p< 0.001.

3.2: Mitotic Index:

The total cells counted were about 3000 cells for control and for each treatment then the mitotic indices calculated. The mitotic index of the control was about 8.95%, after 10 hours of treatment it decreased to 8.47% at 50 mg/L to 3.36% at 500 mg/L. The results of M.I. decrease were significant in 100,300 and 500 mg/L were significant at p<0.01 after 10 hours treatment as compared with the control. After 20 hours of treatment the mitotic indices of most treatments were significant at p<0.01 except for 500mg/L which was significantly different from the control at p<0.001.

TABLE 2. Mitotic indices of *Allium cepa* root tip cells after 10 and 20 hours of treatment with different concentrations of brilliant blue FCF.

Time of treatment (hours)	Concentrations (mg/L)	Total Examined cells	Prophase	Metaphase	Anaphase	telophase	Mitotic index%
10	C	3384	210	15	51	27	8.95%
	50	3330	165	33	39	45	8.47% n.s.
	100	3156	132	39	30	51	7.98% **
	300	3456	87	33	15	57	5.56% **
	500	3210	66	9	15	18	3.36 % **
20	C	3384	210	15	51	27	9.44%
	50	3159	129	27	36	27	6.93% **
	100	3173	87	21	27	49	5.8% **
	300	3198	87	21	12	27	4.59% **
	500	3660	57	24	9	18	2.95% ***

Note: C= control, n.s.= non-significant, **=significant at p<0.01, ***= significant at p<0.001.

3.3: Mitotic Abnormalities:

Regarding the mitotic abnormalities, the control group contained no abnormal dividing cells. Figure 2 shows normal cell in interphase in which the cell is not actually dividing but preparing for mitosis, and different mitotic phases, prophase, metaphase, anaphase and telophase which were all normal.

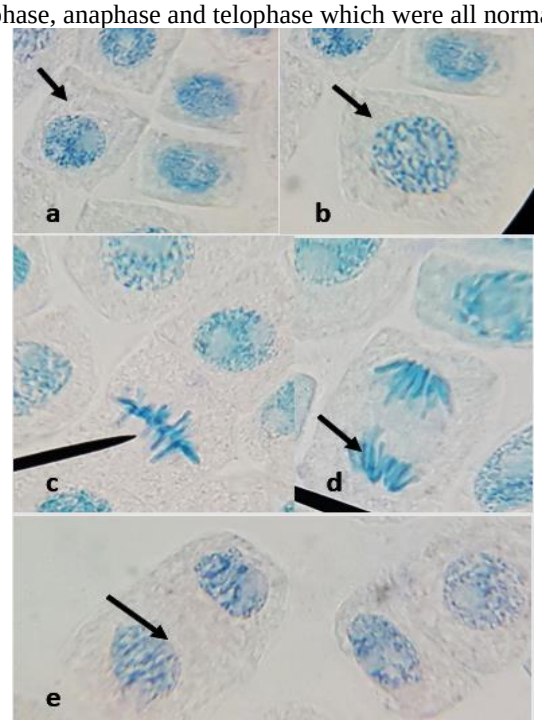

Fig. 2. Normal Mitosis in *Allium cepa* root tip cells of the control.
a- Interphase, b- Prophase, c- Metaphase, d- Anaphase, e- Telophase.

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Regarding the mitotic abnormalities observed after 10 hours were laggard chromosomes, C-mitosis, multipolar anaphase, sticky metaphase, star-shaped anaphase, sticky anaphase, and chromosome loss as shown in figure 3 and table 3, the least abnormalities percentage was observed at 50 mg/L and was significantly different from the control at $p<0.05$, but the highest abnormalities percentage was at 500mg/L which was significantly different from the control at $p<0.001$.

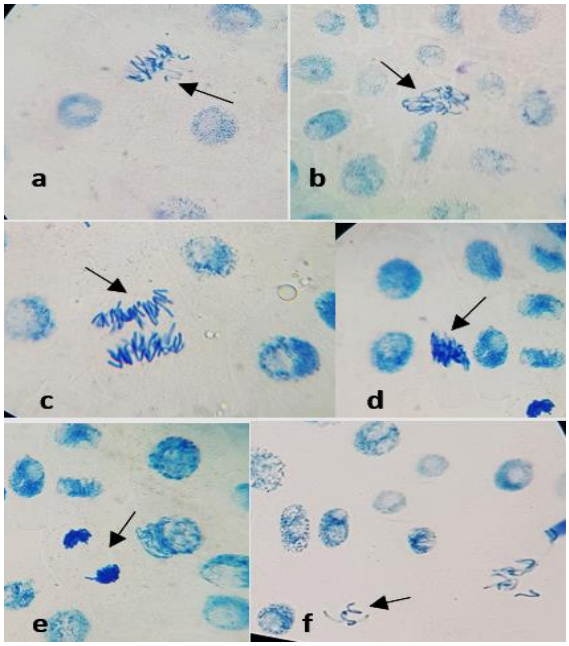

Fig. 3. Mitotic abnormalities in *Allium cepa* root tip cells after 10 hours treatment with brilliant blue FCF. a- Laggard chromosomes, b- C-mitosis, c- Multipolar anaphase, d- Sticky metaphase, e- Sticky anaphase, f- Chromosome loss.

While, after 20 hours of treatment the abnormalities increased and these were laggard chromosomes, C-mitosis, multipolar anaphase, sticky metaphase, star-shaped anaphase, sticky anaphase, and chromosome loss, chromosome fragments and disturbed anaphase shown in figure 4 and table 3. The least percentage of abnormalities results after 20 hours were at 50mg/L treatment and was significantly different from the control at $p<0.05$, but the highest abnormalities percentage was observed in 500mg/L and this value was significantly different from the control at $p<0.001$.

The largest number of abnormalities were observed at 500 mg/L treatment with brilliant blue FCF for 20 hours. The abnormality percent in the control group was 0% to 6.93% in 50 mg/L treatment and to 91.6% in 500 mg/L treatment.

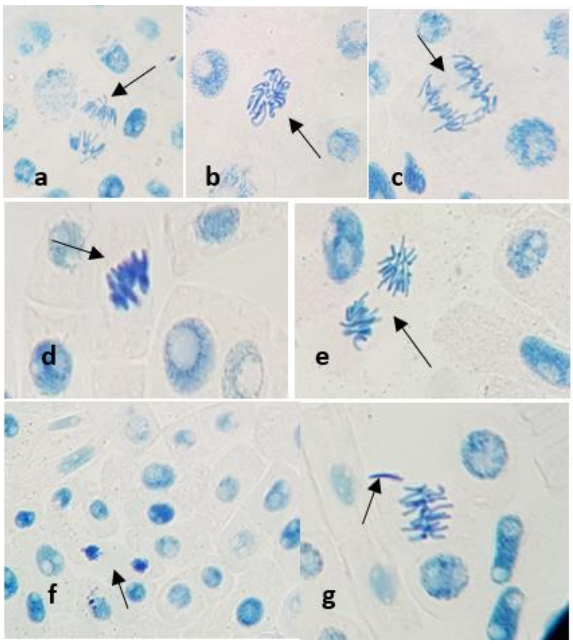

Fig. 4. Mitotic abnormalities in *Allium cepa* root tip cells after 20 hours treatment with brilliant blue FCF. a- Laggard chromosomes, b- C-mitosis, c- Multipolar anaphase, d- Sticky metaphase, e- Star-shaped anaphase, f- Sticky anaphase, g- Chromosome loss.

These observations are in agreement with the results of the Rencuzogullari *et al.* 2001 a; b and Samuel *et al*., 2010 which show that the number of aberrations increased with increasing the duration and concentration of the tested substance.

As well, similar effects of this substance on chromosomes were also described by other researchers such as Maneesha *et al*., 2007; Koç & Pandir, 2018. Other researchers reported similar results when they used other food additive such as sodium benzoate (Rekha and Dharman, 2011; Onyemaobi *et al.*, 2012); baking powder (Renjana *et al*., 2013).

TABLE 3. Mitotic abnormalities after 10 and 20 hours treatment with BB FCF.

Concentrations of BB FCF (mg/L)	Treatment duration (hours)	Total Examined cells	Total dividing cells	Laggard chromosomes	C-mitosis	Multipolar anaphase	Sticky metaphase	Star-shaped anaphase	Sticky anaphase	Chromosome loss	Total abnormalities	Abnormalities percentage
C	10	3384	303	0	0	0	0	0	0	0	0	0%
50		3330	282	3	3	0	3	0	0	0	9	3.19%*
100		3156	252	3	6	6	0	0	3	0	18	5.16%*
300		3456	174	3	9	9	6	0	0	0	27	15.5%**
500		3210	108	6	19	13	7	0	6	3	54	50%***
C	20	3384	303	0	0	0	0	0	0	0	0	0%
50		3159	219	3	0	12	6	0	0	0	21	6.93%*
100		3173	220	6	6	12	3	0	6	0	33	15%**
300		3198	147	3	6	8	15	6	3	1	42	28.57%**
500		3660	108	18	30	8	21	12	4	6	99	91.6%***

Note: C = control, BB = brilliant blue, *=significant at $p<0.05$, **= significant at $p<0.01$, ***= significant at $p<0.001$.

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IV. Conclusion

The food colorant brilliant blue FCF has a significant ability to inhibit root growth and decrease mitotic indices of root tips cells in *Allium cepa*. The results as well revealed that this substance has caused various mitotic abnormalities like multipolar anaphase, sticky metaphase, star-shaped anaphase which indicates its mutagenic activity.

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