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# A study on cytotoxicity and genotoxicity induced by egg yellow color (an artificial food color) using *Artemia salina* and *Allium cepa* models

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## Abstract

Food adulteration is a major problem throughout the world where food items are being processed indiscriminately using excessive amount of artificial food colors. Consequently, consumers are suffering from various physical complications. The present study was aimed to investigate the hazardous impacts of egg yellow food color through cytotoxicity assay *in Artemia salina and* genotoxicity assay in *Allium cepa models*. Two different doses of egg yellow food color were selected including high dose: 30 mg/L and low dose: 15 mg/L. Toxicity level of egg yellow food color was checked out through cytotoxicity assay using A. salina model. High dose showed severe toxicity where 87.67% of larvae died (survival rate 12.33%), low dose showed moderate toxicity where 50.66% larvae were alive but in the control group, 98.66% larvae survived. Root growth inhibition assay of *A. cepa* was conducted after 72 hours of root germination where high dose significantly inhibited root growth compared to the control. Mitotic indexing (MI) was performed and several mitotic abnormalities including disrupted prophase, irregular anaphase, anaphase bridge, polyploidy, nuclear destruction, star metaphase, sticky metaphase, and anaphase with bridge, nuclear blebbing, nuclear notch etc were found. MI decreased significantly when treated with high dose than low dose compared to the control group. The findings of this study suggest that excessive consumption of egg yellow food color should be controlled immediately by making laws with proper implementation and social awareness.

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## Introduction

Consumption of good quality foods is the prerequisites of maintaining sound health and to fulfill the daily nutritional requirements. Human beings need wide varieties of nutrition in accordance with their age, sex and capabilities (Garber Jr, Hyatt, and Starr Jr 2000). Besides, people also desire that their daily edible foods would be more delightful, attractive and healthy in nature (Pérez-Gálvez, Viera, and Roca 2020). To make food more appealing it is customary to add vibrant color to it. However, although all types of food color has its permissible limit, some food merchants indiscriminately add much more synthetic colors in the food items during production and making them alluring to consumers (Oplatowska-Stachowiak and Elliott 2017). Food colors do not add any nutritional values but usually used food for food management purpose (Ranjan *et al.* 2019).

Excessive usage of synthetic food colors in daily food items or food adulteration has become one of the great concerning issues throughout the world. Consequently, public health is suffering with numerous diseases. This hazard aggravated further more due to the use of artificial food colors which are not classified (unclassified and low grade colour) and their prolonged consumption (Babatunde and Bakare 2006).

Egg yellow color made up of four different types of chemical components including Sunset yellow, tartrazine, sodium chloride and sodium sulfate. In accordance with the European Food Safety Authority (EFSA), the acceptable daily intake (ADI) of Sunset Yellow is 4 mg/ml bw/day and the ADI level of tartrazine is 0-7.5 mg/ml bw/day (EFSA Journal 2009;7(11):1331.[52pp.].http://dx.doi.org/10.2903/jefsa.2009.1331). But in Bangladesh the locally available egg yellow food colors are not of good standard and grade. The bakery industries indiscriminately use these uncategorized egg yellow food color exceeding the recommended ADI level. They use generally 2-4 folds more egg yellow color compare to the recommended ADI level. As a result, people are being affected with severe health related problems.

Therefore, the present studies were carried out to explore the danger or risk of using local egg yellow food color using brine shrimp (*A. salina*) lethality assay and the *A. cepa* root tip genotoxicity assay.

## Materials and methods

*Food color selection*

Egg yellow food color was collected from a local bakery to carry out the current study because of its frequent use in the bakery products in Bangladesh.

*Dose selection*

Based on the information obtained from bakeries and food industries located in different parts of Bangladesh, two different doses were selected to carry out the present study including low dose: 15 mg/L (equivalent to 2 fold of ADI) and high dose: 30mg/L (equivalent to 4 fold of ADI).

*Place of the study*

All experimental works were carried out in the Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Bangladesh.

*Organism for cytotoxicity test*

Eggs of brine shrimp (*A. salina*) were used to test the cytotoxicity of egg yellow food color.

*Organism for genotoxicity test*

Roots of onion (*A. cepa*) were used for assessing the genotoxic effects of this particular food color. This species had been selected as a test material because of their fewer chromosome number (2n=16) and transparent physiological features (Kong and Ma 1999; Patra and Sharma 2002; Rank and Nielsen 1997; Serra de Lima Moraes and Jordão 2001).

*Cytotoxicity assay*

This experiment was conducted for evaluating the detrimental effects of egg yellow dye on survivability and hatching ability of brine shrimp eggs. 0.5 mg of

*A. salina* eggs was transferred to 1L 1% saline water and incubated at 28 °C for 48 hour to hatch. Oxygen supply was ensured with aerator. 20 hatched nauplli were then added into three separate test tubes containing saline water with six replications of each. Later, low dose and high dose of egg yellow food color were added into the separate test tubes except control groups and incubated at 28 °C for 72 hour. Later, different petri-dishes were used to keep the test samples for examining the mortality rate with the help of a clean lens where a white paper was used as a background platform for clear visualization of live and dead larvae. Larva mortality rate was calculated in the percentage form using the following formula (Sarah, Anny, and Mir 2017)

$$L_M\ (\%) = [(N-N_T) - (N-N_C)]/N \times 100\%$$

Here, N denoted the number of larvae used in the treated and control groups, $N_T$ is the number of survived larvae in each treated groups, and again, the survived larvae number of the control group was showed by $N_C$.

*Genotoxicity assay*

To carry out this experiment, 18 healthy young *A. cepa* bulbs were taken. Basal part of each bulb was scrapped and placed in individual test tube filled with water. 12 tubes containing high and low doses of egg yellow color (6 tubes each) and 6 tubes were used for control. The tubes with bulbs were kept in a dark place for 72 hours so that rootlets could emerge. After 72 hours of incubation in dark, roots length were measured in millimeter, excised and fixed in the mixer of ethanol and glacial acetic acid (3:1, v/v) and stored for 24 hours. Slides were prepared for studying mitosis in roots according to the protocol described by (Laughinghouse IV *et al.* 2012). Different stages of mitosis and mitotic abnormalities were studied under an inverted light microscope (Optika™, Italy) and pictures were captured using the photographic unit attached to it. Mitotic index (MI) was calculated by using a standard formula mentioned below:

(MI %) = (The full number of dividing cells ÷ full number of cells observed) × 100%.

Around 2000 cells of each group were observed for calculating mitotic index (MI) and mitotic abnormalities (MA) and compared the obtained results of treated groups with the control group.

*Statistical analysis*

Two doses of treatment were applied where each treatment consisted of six replications. The collected data were analyzed through exploitation of Microsoft Excel and IBM SPSS-20 software. Here, 5% significant level was considered in terms of DMRT analysis.

**Results**

Effects of egg yellow food color on brine shrimp (*A. salina*)

Brine shrimp lethality assay is suitable and widely used technique to detect general toxicity of chemicals, teratogenicity screening of drugs, ecotoxicology of biological organisms (Carballo *et al.* 2002; Kanwar 2007; Lee, Chen, and Chou 1999). Two different doses including low dose: 15 mg/L and high dose: 30 mg/L were used for *A. salina* lethality assay. In high dose *87.67% of larvae died and the survival rate of larvae was only 12.33% which indicates severe toxicity*. The survival rate was, 50.66% in low dose of egg yellow food color. However, 98.66% of the larvae were alive in the control group (Figure 1). The result indicates that locally used egg yellow food color causes high toxicity if used in high concentration.

**Table 1.** Evaluation of root growth of onion treated with egg yellow color at different time intervals.

| Treatment | Mean root growth (mm) inhibition rate at several intervals of time | | Total Treatment Mean ± SE |
|---|---|---|---|
| | 48 hour | 72 hour | |
| Control | 2.134±0.321 a | 3.947±0.593 a | 3.041±0.457 a |
| Low dose (15 mg/L) | 1.827±0.233 a | 3.43±0.558 a | 2.629±0.395 b |
| High dose (30 mg/L) | 1.672±0.276 a | 3.142±0.149 a | 2.407±0.213 b |

Values are indicated as mean ± SE. *Treatments are significantly different from control group at $P<0.05$.

*Root growth inhibition assay of A. cepa*

Current study revealed that root growth of onion was greatly affected by egg yellow food color. Root growth slowed down in dose dependent manner (Table: 1) compared to control.

*Determination of mitotic abnormalities of onion root tips treated with egg yellow color*

2000 cells were examined for control group in the current study where, there were 650 dividing cells including prophase-505, metaphase-75, anaphase-40 and telophase-30 and calculated mitotic index (MI) was 32.5%. No significant Mitotic Abnormalities (MA) was found. However, in low dose (15 mg/L), about 558 cells were in dividing stage out of 2000 cells while, prophase-443, metaphase-60, anaphase-35 and telophase-20 and obtained MI was 27.9% where, the amount of MA was 5.35%.

In high dose (30 mg/L) of egg yellow food color, 485 dividing cells (prophase-405, metaphase-35, anaphase-28 and telophase-17) were observed among 2000 cells and calculated MI was 24.25% where, the value of MA was 10.95%. Therefore, from the above data, it is statistically evident that, mitotic index (MI) was significantly decreased in high dose than the low dose of egg yellow food color where no remarkable changes were observed in control group (Table 2).

**Table 2.** Mitotic Index (MI) of *A. cepa* root tip cells treated with different concentrations of egg yellow food color.

| | Mitotic index | | | | | |
|---|---|---|---|---|---|---|
| Concentration mg/L | P (%) | M (%) | A (%) | T (%) | Total Mitotic Index(MI)% | Mean Mitotic Index ± SE |
| Control | 25.25 | 3.75 | 2 | 1.5 | 32.5 | 32.5±2.46764 |
| Low dose (15 mg/L) | 22.15 | 3 | 1.75 | 1 | 27.9 | 27.9±1.453009 |
| High dose (30 mg/L) | 20.25 | 3.75 | 2 | 1.5 | 24.25 | 24.25±1.154734 |

Values are indicated as mean ± SE. *Treatments are significantly different from control group at P<0.05. Here, MI = Mitotic index, P= Prophase, M =Metaphase, A= Anaphase, T= Telophase.

Concurrently, the value of MA was increased in case of the high dose compared to the low dose but control group had no abnormalities (Table 3). High dose caused several abnormalities in mitotic division of onion including disrupted prophase, irregular anaphase, anaphase bridge, polyploidy and nuclear destruction and low dose indicated disrupted interphase, star metaphase, sticky metaphase, and anaphase with bridge, nuclear blebbing, nuclear notch and laggard chromosome.

**Table 3.** Calculation of Mitotic Abnormalities (MA) of root tip cells of *A. cepa* treated with egg yellow food color.

| | Mitotic Abnormalities (MA) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration mg/L | Vg % | Bg % | Br % | Stk % | Lag % | Poly % | Mp % | Oth. % | Total % |
| Control | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Low dose (15 mg/L) | 0.9 | 0.65 | 0.55 | 0.70 | 0.45 | 0.3 | 0.35 | 1.45 | 5.35 |
| High dose (30 mg/L) | 1.10 | 1.15 | 1.5 | 1.35 | 1.25 | 1.45 | 1.4 | 1.75 | 10.95 |

Here, number of cells examined =2000 and the abbreviated forms are given below: MA= Mitotic abnormalities, Vg= Vagrant, Bg= Bridge, Br= Breaks, Stk= Stickiness, Lag= Laggard, Pol= Polyploidy, Mp= Multiple anaphase, Oth= Others.

**Discussion**

Food adulteration is one of the major problems throughout the world where synthetic colorants are being added to foods and consequently, causing numerous health-related problems. In the developing world, because of the illegal practices, boundless corruptions and lack of proper monitoring and control, manufacturers are deliberately using harmful colors in various food and consumable items (Perva-Uzunalić *et al.* 2004). In Bangladesh indiscriminate use of synthetic food colors in numerous food stuffs without maintaining quality control and safety measures (Nasreen and Ahmed 2014) has became a burning health issue.

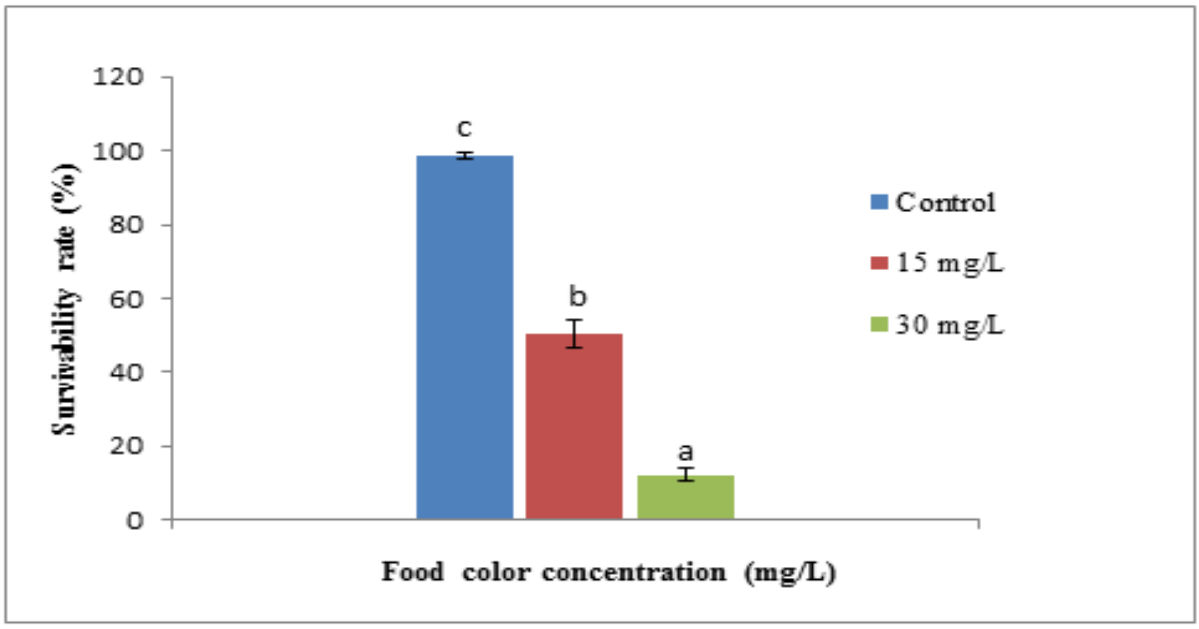


**Fig. 1.** Effects of egg yellow food color on *A. salina* to assess the toxicity. Each column indicates the mean ± SE. *Treatments are significantly different from control group at P<0.05.

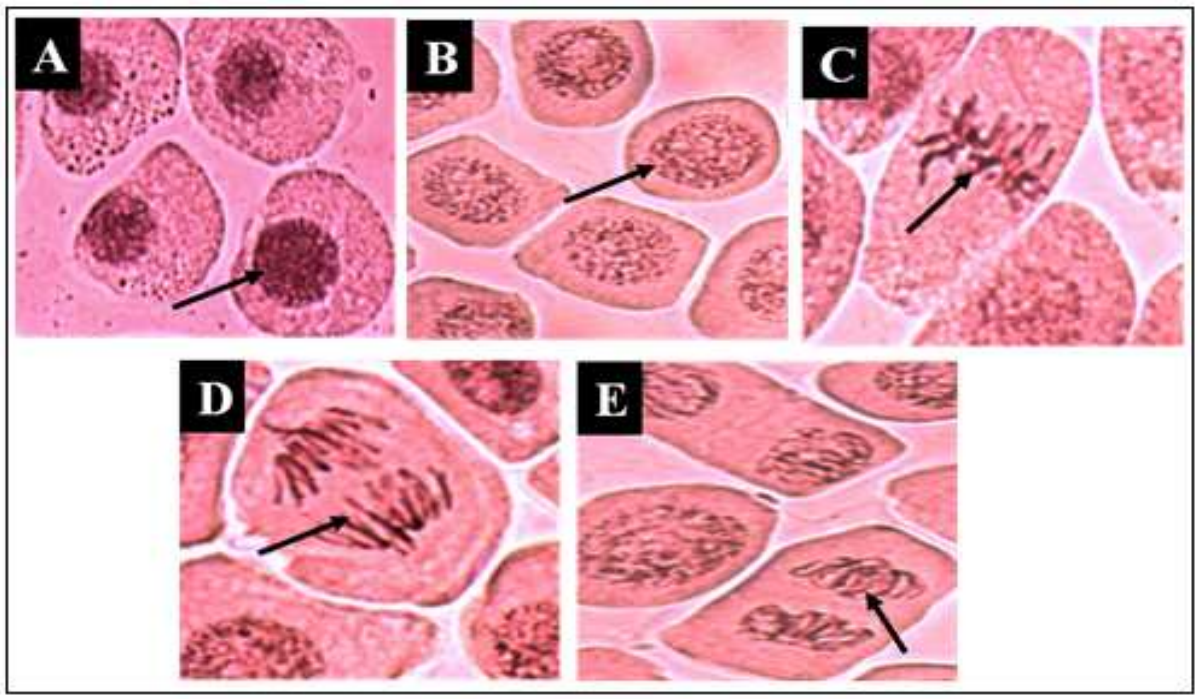


**Fig. 2.** Photographs of control group of *A. cepa* root tip cells showing normal divisional stages. Here, A: Interphase stage, B: Prophase stage, C: Metaphase stage, D: Anaphase stage, E: Telophase. Slides were stained with hematoxylin-eosin solution and visualized using inverted light microscope (Optika™ Vision Pro) at 400 X magnification.

For the assessment of hazardous as well as toxic effects of synthetic colorants on biological organisms, the use of *A. salina test system is a simple yet convincing experimentation carried out worldwide (Carballo et al. 2002; Kanwar 2007; Lee, Chen, and Chou 1999; Waterston et al. 2002). In this study, cytotoxicity assay was carried out using A. salina to evaluate the perilous level of egg yellow food color where, high dose (30 mg/L), low dose (15 mg/L) and control group were used. According to our findings,* both

high and low dose represented severe toxicity (*87.67%* and 49.34% larval death respectively) compared to control (Figure 1).

This study is in concord with the result of (Himri *et al.* 2013) who showed that increasing concentration of tartrazine caused significantly higher mortality rate of larvae and mortality rate significantly decreased while treated with lower concentration of tartrazine during *A. salina* lethality assay. Another study also reported that the toxicity level of sunset yellow food color on brine shrimp larvae represented significantly increased mortality rate when treated with higher concentration but the lower concentration revealed significantly decreased mortality rate (Vakili-Saatloo *et al.* 2015). It has been also reported that the effect of sunset yellow dye on *A. salina* was dose dependent as this showed severe effects at higher concentration compared to the lower concentration (BUŞURICU *et al.* 2019).

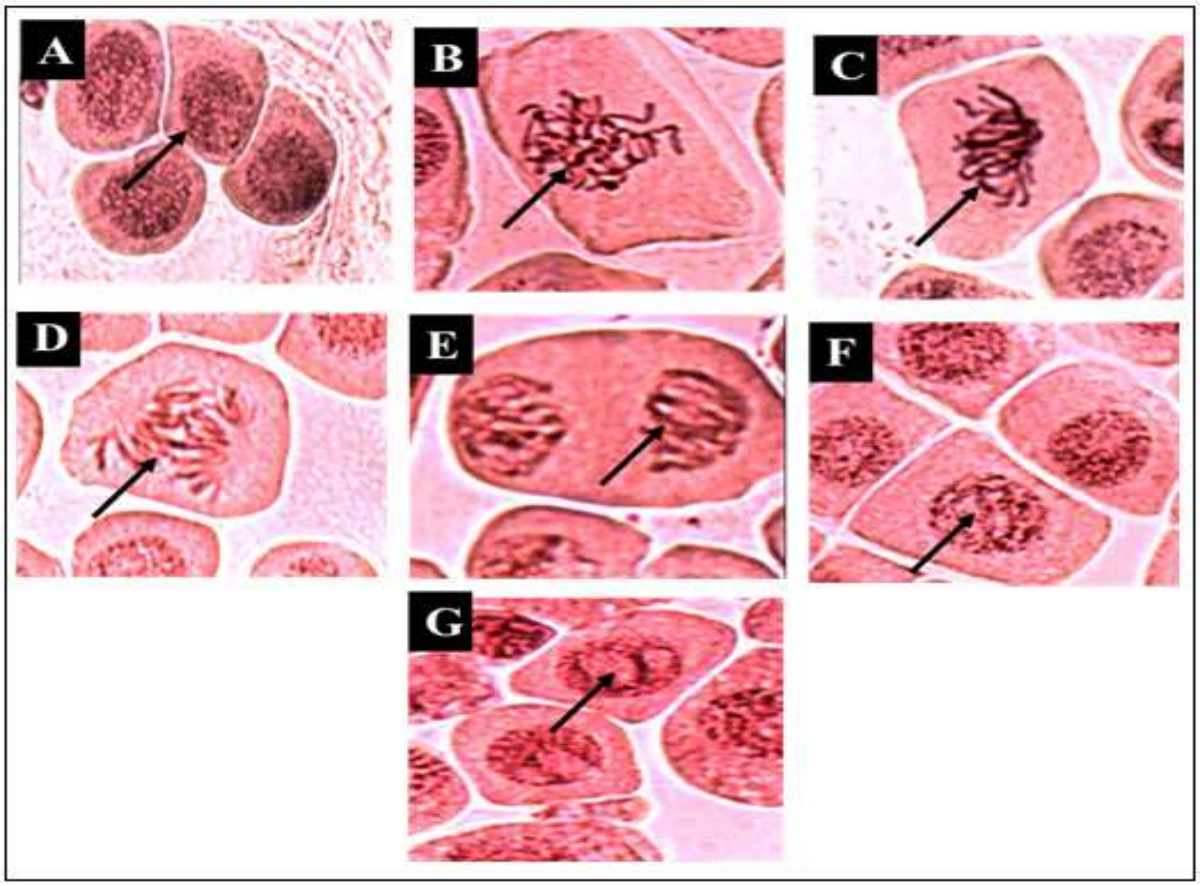


**Fig. 3.** Effects of low dose (15 mg/L) of egg yellow food color on root tip cells of *A. cepa*. Here, A: disrupted interphase, B: star metaphase, C: sticky metaphase, D: anaphase with bridge, E: laggard chromosome at telophase, F: nuclear blebbing, G: nuclear noch. Mitotic Slides were stained with hematoxylin-eosin solution and visualized using inverted light microscope (Optika™ Vision Pro) at 400 X magnification.

Root growth inhibition assay is a very suitable experiment used in the present study to determine the toxicity level of any chemical. As the toxicity level greatly depends upon the concentration of applied dose. In the present study, root growth became inhibited significantly when applied with higher dose (30 mg/L) than the lower dose (15 mg/L) compared to the control group (Table 1). Earlier studies also stated that some free radicals may present in sunset yellow color those play inhibitory mechanisms on root growth by suppressing cell division while, completed an experiment on onion root tip cells (Bakare *et al.* 2013). Another studies of (Babatunde and Bakare 2006) reported that nitrate compounds might present in the carmosine that transforms into nitrosamine, a carcinogenic, mutagenic and

cytotoxic chemical that is capable of hinderence of root growth of *A.cepa*. Therefore, above findings supported that the use of egg yellow color is responsible for root growth reduction. The effects of high dose (30 mg/L) and low dose (15 mg/L) of egg yellow food color on mitotic cell division were observed by calculating the mitotic index (MI). In this study, mitotic abnormalities (MA) immensely influenced the MI as higher concentration of egg yellow food color showed greater MA than the lower concentration and hence, the value of MI was dose dependent (Table 2). Our study demonstrated that increasing concentrations of egg yellow food color enhance MA consequently, decrease the MI and induce several abnormalities of chromosomes. Various studies were also conducted before on *A. cepa* to observe the effects of different synthetic food colorants causing mitotic abnormalities. (Tripathy and Rao 2015) revealed that increasing concentration of allura red food color was responsible for decreasing MI in onion root tip cells. Reduction of MI was also studied by (John *et al.* 2020) who reported that brilliant blue, tartrazine and sunset yellow colorants revealed a negative correlation with MI, when these color concentrations were increasing the MI was decreasing and vice versa.

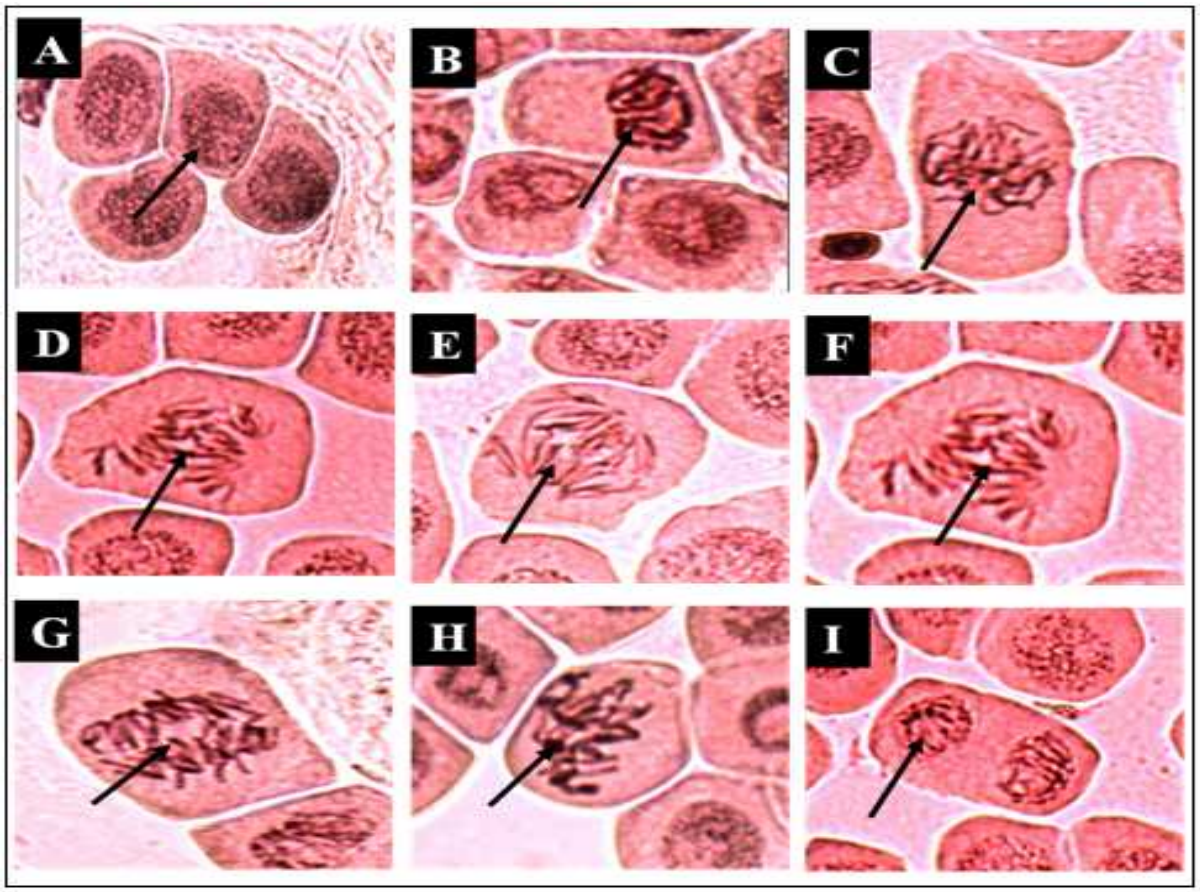


**Fig. 4.** Effects of high dose (30 mg/L) of egg yellow food color on root tip cells of *A. cepa*. Here, A: Disrupted interphase, B: Disrupted prophase, C: Irregular metaphase, D: Irregular anaphase, E: Multipolar anaphase, F: Anaphase-telophasebridge, G: Star telophase, H: Polyploidy, I: Nuclear destruction. Slides were stained with hematoxylin-eosin solution and visualized using inverted light microscope (Optika™ Vision Pro) at 400 X magnification.

They also reported that excessive use of these colors with higher concentration leads to different mitotic abnormalities including disrupted prophase, irregular metaphase, irregular anaphase, multipolar anaphase, anaphase-telophase bridge, star telophase and polyploidy etc. In the separate study, (Dwivedi and Kumar 2015) found that increasing concentration of azorubine food color on onion root tip cells

demonstrated several abnormalities in the mitotic cell division including chromosome breaks, chromosome bridges, polyploidy, lagging, stickiness, abnormal spiralisation, multipolarity and abnormal kinetics. Our findings are also in agreement with the above mentioned studies where we obtained several mitotic abnormalities due to the effect of egg yellow food color on *A.cepa* root tip cells including disrupted prophase, irregular anaphase, anaphase bridge, polyploidy, nuclear destruction, fragmented nucleus and scattered chromosome for high dose and low dose showed disrupted interphase, star metaphase, sticky metaphase, and anaphase with bridge, nuclear blebbing, nuclear notch and laggard chromosome but no notable abnormalities visualized in case of control group (Table 3).

**Conclusion**

In this study, it was observed that egg yellow food color poses severe negative impacts on development of brine shrimp larvae and mitotic cell division of onion. So, it may cause adverse impacts on human health also. Therefore, it is primetime for the competent supervisory authorities to ensure the proper use of quality egg yellow food color within ADI level and create social awareness. Besides, current law for food adulteration in Bangladesh should be reformed with more severe punishment options the food dyes may cause life threatening if used in excessive amount.

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