GENOTOXIC EFFECTS OF TWO COMMONLY USED FOOD ADDITIVES OF BORIC ACID AND SUNSET YELLOW IN ROOT MERISTEMS OF *TRIGONELLA FOENUM-GRAECUM*

G. Kumar, *N. Srivastava

Plant Genetics Laboratory, Department of Botany, University of Allahabad, U.P., India

Received 15 October 2010; revised 23 September 2010; accepted 20 January 2011

ABSTRACT
Food additives are the substances that are intentionally added to modify visual appearance, taste, texture, processing or the storage life of food. There has been significant controversy associated with the risks and benefits of food additives. The effect of different concentrations of food additives viz. boric acid and sunset yellow on the chromosomes of *Trigonella foenum-graecum* L. was investigated. Four concentrations of the two food additives (0.25, 0.50, 0.75 and 1%) were used for 3 hours. All concentrations of boric acid and sunset yellow showed mitoinhibitory effect in root tips of *Trigonella foenum-graecum* and increase in chromosomal aberrations. Various types of metaphasic and anaphasic aberrations were scored and it was found that metaphasic aberrations were more prominent than the anaphasic aberrations. The most observed aberrations induced by boric acid were stickiness at metaphase, bridges at anaphase, stickiness at anaphase, and scattering at metaphase, while the most prevalent aberrations caused by sunset yellow were precocious movement, unorientation at anaphase, scattering at metaphase and unorientation at metaphase. The result of present study clearly establishes the genotoxic behavior of boric acid and sunset yellow.

Key words: *Trigonella foenum-graecum*; Food additives; Boric acid; Sunset yellow; Chromosomal aberrations

INTRODUCTION
There has been an increase in processed food since 19th century, which has led to increase in the use of food additives. Food additives such as food colors and preservatives are in use of varying levels of safety. Numerous food additives are in use which could affect adversely their consumers at varying degree (U.S. Food and Drug Administration, 1993). Food colors are water soluble and are extensively used in almost every type of edible preparations like soft drinks, foodstuffs, jams and jellies, sweets, candies, ice creams, sauces and pickles (Hallagan *et al,* 1995).

The possible harmful effect of coloring matters and all other food additives to foodstuffs are a subject of public concern. Accordingly there is an increase in application of legislative control so as to restrict the use of harmful coloring matters in food and to check certain permitted items which have not any harmful effect when subjected to rigorous examination (Tripathi *et al*., 2007).

Boric acid (H_3BO_3) is a boron compound which is soluble and circulates in plasma (Di Renzo *et al*., 2007).It is colorless and water soluble white powder which has been used as pesticide to kill mites, insects, fungi and algae and also the fleas, cockroaches, termites and wood decay fungi (Cox, 2004). Boric acid is widely used as food preservative (4gm/L) in food products like

*Corresponding author: E-mail: srivastava_nitisha@yahoo.com
Tel:

caviar (The ministry of Agriculture of Turkey, 1997) and also for medicinal and nonmedicinal purposes (Heindel *et al,* 1997). It is also used for preserving meats, caviar and dairy products (Arslan *et al*., 2008). Boron and its derivatives are used in a lot of aspects of our daily life (Beyer *et al*., 1983; Moore, 1997); due to this reason, any possibility of damage through boron to the living cells should be considered. There are many reports indicating its harmful effects on the organisms (Bringman and Kuhn, 1980; Martinez *et al*., 1986; Ghul, 1996) can cause health problems if the food is ingested by human. Boric acid and borates are toxic to cell (Yiu *et al*., 2008). For new born baby, the possible lethal doses are in the range of 3-6 g, whereas 15-20 g is assigned for adults (Litovitz *et al*., 1988). The common symptoms from several incidents of boric acid poisoning includes coughing, eye irritation, vomiting and oral irritation (Baker and Bogema, 1986). Moreover, boric acid is harmful to human health if consumed in higher amounts (See *et al*., 2010). However, due to unawareness of the risk of boric acid, it is continued to be used in many foodstuffs.

Synthetic colors are water soluble and are extensively used in almost every type of edible preparations (Hallagan *et al,* 1995). Possible toxicity must be the first consideration for choosing the food additive colors. By the early 1995, natural and synthetic color additives were used extensively to color foods, drugs and cosmetics (Hallagan *et al*., 1995). The possible harmful effects of synthetic colors are a subject of public concern and a critical attitude to their use in foodstuffs. The food synthetic colors are manufactured chemically and are most commonly used dyes in food, pharmaceutical and cosmetic industries. There are 3 categories of food colors: primary food colors , blended food colors and lake food colors. Lake pigments find wide usage in areas like foodstuffs, pet foods, drugs, pharmaceuticals and cosmetics e.g. sunset yellow, tartrazine, brilliant blue, etc (Saeed-ul-Hassan *et al,* 2006). Since the study of genotoxic effect of several chemicals on plant mitosis may provide valuable information in relation to possible genotoxicity in mammals especially in humans, therefore this study was based on the effect of food preservative, (boric acid) and food color, (sunset yellow) on genome of commonly used food ingredients (spice and vegetable), *Trigonella foenum-graecum*. *Trigonella* leaves and stem parts are used as vegetable in India like spinach. The seeds of *Trigonella* have great medicinal property which is used in curing stomach problems. *Trigonella* seeds are also very important component of spices. The chromosome number of *Trigonella* is low i.e. 2n=16, therefore it is easy for cytological studies; chromosomes do not show stickiness at normal mitotic condition. *Trigonella* also gives root growth easily on normal temperature so it is selected as a study material for the effect of food additives on mitosis.

MATERIALS AND METHODS

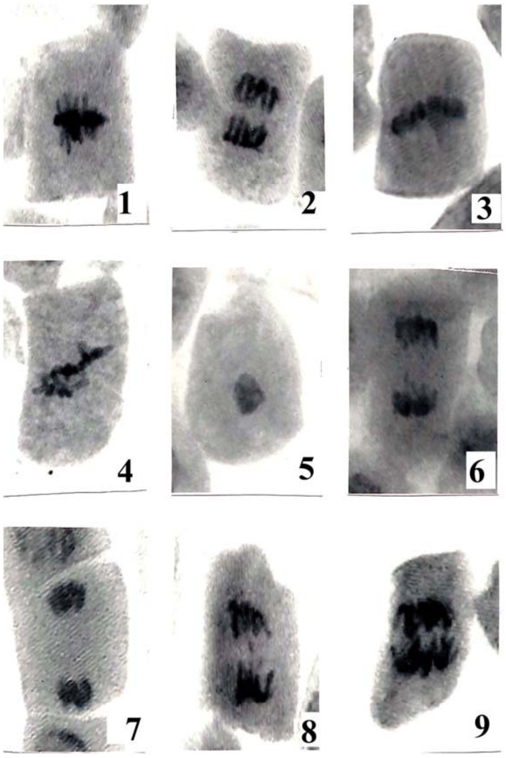
Seeds of *Trigonella foenum-graecum* were soaked in distilled water for 14 hr and allowed to germinate. Germinated seeds were treated with four concentrations (i.e. 0.25, 0.50, 0.75 and 1%) of boric acid and sunset yellow solutions for three hours. Boric acid and sunset yellow was purchased from Science Corporation, Allahabad-211003, U.P., INDIA. Some germinated seeds were kept in distilled water for control in each set. After 3 hr the treated seeds were washed with distilled water to remove boric acid and sunset yellow. The washed seeds were then fixed in carnoy's fixative (GAA: Abs. alcohol, 1:3) in their respective bottles. The root tips from distilled water were fixed for control set. The root tips were removed from fixative after 24 hr, stored in refrigerator and then used for cytological studies.

The root tips were hydrolyzed in 1N HCl at 60 ± 2ºC for 15-20 minutes. After hydrolysis, root tips were washed with distilled water for 4-5 times to remove HCl and then dried by filter paper. After drying, root tips were stained in 2% acetocarmine and the apical tips of these root tips were removed and used for slide preparation. Microscopic examination was done for mitotic index and chromosomal aberrations for each set of treatment and control. From each slide a minimum of 500 cells were scored and mitotic index and chromosomal aberration were scored. For statistical analysis, the one-way analysis of variance (ANOVA) was used.

RESULTS

Effect of boric acid and sunset yellow on mitotic index of root tip cells

On fenugreek, the somatic complement consists of 16 chromosomes (2n = 16). Mitotic index was recorded to be 14.04 ± 0.28% in control set with no chromosomal anomalies (Fig 1, 2). Mitotic index significantly decreased with increasing concentrations of boric acid and sunset yellow. Mitotic indices at different doses of boric acid and sunset yellow have been shown in Table-1. At lowest concentration (0.25%) of boric acid and sunset yellow, the mitotic indices were reduced to 9.38 ± 0.28 and 10.83 ± 0.30, respectively. However, further increase in concentration of these food additives resulted in a decline in mitotic index in a dose dependent manner. At 1 % concentration of boric acid and sunset yellow, the mitotic index was greatly reduced and found to be 2.74±0.14 and 5.58±0.20, respectively. Present study clearly showed that boric acid was found to be more mitoinhibitory in comparison to sunset yellow.



Figs: 1. Normal metaphase (2n = 16); 2. Normal anaphase; 3. Unorientation at metaphase; 4. Precocious movement at metaphase; 5. Intense stickiness at metaphase; 6. Laggards at anaphase; 7. Stickiness at anaphase; 8. Single bridge at anaphase; 9. Multiple sticky bridge at anaphase

Effect of boric acid and sunset yellow on chromosomal aberrations

The result of present investigation clearly suggests the chromotoxic behavior of boric acid and sunset yellow. Boric acid and sunset yellow both have shown a dose dependent increase in chromosomal aberrations with increasing doses of treatment. The treated root tips showed various types of metaphasic and anaphasic aberrations at each dose of treatment. One common observation was also studied in both cases that metaphasic aberrations were more pronounced than anaphasic aberrations. Increase in concentration of these two food additives significantly increased the mitotic inhibition and ensures the harmful effect of boric acid and sunset yellow on mitotic cycle. The highest abnormality percentage was observed at 1% of boric acid (15.50 ±0.28) followed by 1% of sunset yellow (12.60 ± 0.34). The most prevalent aberration caused by

Table.1: Effect of boric acid and sunset yellow on mitotic index of Trigonella foenum -graecum.

Treatments	Concentration (%)	Mitotic index
Control		14.04±0.28
Boric acid	0.25	9.38±0.28
	0.50	7.15±0.02
	0.75	5.43±0.09
	1.0	2.74±0.14
Sunset yellow	0.25	10.83±0.30
	0.50	9.71±0.57
	0.75	7.47±0.18
	1.0	5.58±0.20

Table 2: Effect of boric acid on root meristems of fenugreek

Doses of treatment (%)	Total no. of cells observed	Total no. of abnormal cells	Metaphasic abnormalities (%)				Anaphasic abnormalities (%)				Total abnormalities (%)
			Sc	St	Pr	Un	Br	Lg	St	Un	
Control	187.33±6.35	-	-	-	-	-	-	-	-	-	-
0.25	383.33±9.82	20±0.0	0.86	0.95	0.86	0.60	0.60	0.78	0.26	0.52	5.2±0.11
0.50	307.33±21.52	30±5.77	2.1	2.3	0.9	0.47	1.15	0.47	0.75	1.17	9.53±1.31
0.75	344.33±25.43	30.66±0.66	2.30	4.26	-	2.20	2.72	-	3.20	-	13.96±0.84
1.00	349.33±25.43	54.66±4.66	2.66	4.71	-	1.25	3.69	-	3.52	0.49	.28

Sc- Scattering, St- Stickiness, Pr- Precocious movement, Un- Un-orientation, Br- Bridges, Lg- Laggards.

Table 3: Effect of sunset yellow on root meristems of fenugreek

Doses of treatment (%)	No. of cells observed	No. of abnormal cells	Metaphasic abnormalities (%)				Anaphasic abnormalities (%)				Total abnormalities (%)
			Sc	St	Pr	Un	Br	Lg	St	Un	
Control	187.33±6.35	-	-	-	-	-	-	-	-	-	-
0.25	264.66±7.85	9.33±0.66	0.61	0.37	0.99	0.37	0.37	0.37	-	0.37	3.12±0.19
0.50	385.33±9.82	28.33±1.66	0.93	0.67	1.70	0.77	0.67	0.77	0.77	0.93	7.23±0.14
0.75	382.66±38.82	36.66±4.40	1.27	1.01	1.86	1.10	0.94	0.99	1.10	1.01	9.31±0.32
1.00	401.66±29.15	51.66±4.40	1.62	1.22	2.66	1.38	1.13	1.48	1.38	1.69	12.60±0.34

Sc- Scattering, St- Stickiness, Pr- Precocious movement, Un- Un-orientation, Br- Bridges, Lg- Laggards

boric acid was stickiness at metaphase (4.71 %), bridges at anaphase (3.69 %), stickiness at anaphase (3.52 %), and scattering at metaphase (2.66 %). Precocious movement at metaphase (Fig. 4) and laggards at anaphase (Fig. 6) were found to be absent at higher doses of treatment of boric acid (0.75 and 1%). Table -2 shows percentages of different abnormalities caused by boric acid. However, the most prevalent aberrations caused by sunset yellow were precocious movement (2.66 %), unorientation at anaphase (1.69 %), scattering at metaphase and unorientation at metaphase (Fig. 3). Table 3 shows percentages of different abnormalities caused by sunset yellow. Thus, on comparing chromotoxic behavior of boric acid and sunset yellow, boric acid was found to be more chromotoxic than sunset yellow and showed more stickiness behavior of chromosomes.

DISCUSSION

In this era of increasing human population, there are very few people who actually bother to check the labels on the food they eat to see just what they are taking into their bodies. It is almost a certainty that few really know what it is, that is part of their foodstuffs, and yet may present treats and danger. Since absolute safety of any substance can never be proven, decisions about the safety of color additives or other food ingredients are made on the best scientific evidence available. A large number of methods have been investigated for food preservation and many food additives are being used for preservation of foods. These agents were reported to be mitotoxic and chromotoxic in several text systems (Nagao *et al*., 1977; Ishidate and Odasima, 1977; Matsuoka *et al*., 1979; Luca *et al*., 1987; Mukerjee *et al*., 1988). It is clear from present investigation that boric acid and sunset yellow significantly suppress mitotic behavior and both of them increase abnormality percentages with increasing doses of treatment. The similar cytotoxic behavior of these food additives was also reported by Donbak *et al* (2002). Similar result of cytotoxicity of food additive ajinomoto on root tips of *Allium cepa* has also been reported (Omanakumari *et al*., 2006). Njagi and Gopalan (1982) reported that several food additives inhibit DNA synthesis. The inhibition of DNA synthesis and decrease in mitotic index might be caused due to decrease in ATP (Adenosine triphosphate) level and the pressure from the functioning of energy producing centre (Epel, 1963; Jain and Andsorbhoy, 1988). Sadia and Vahidy (1994) reported that boron disturbs the normal cell cycle process by preventing biosynthesis of DNA and microtubules. Several studies on mice and rats reported that boric acid has toxicity to male reproductive system (Weir and Fisher, 1972). It was reported by several workers that boric acid suppressed the sperm release from the testes and when the animals were treated with high amount of boric acid; it inhibits DNA synthesis in sperm cells and hence reduces fertility and it impares fertility in male rodents (Cox, 2004).

It was reported by Amer and Ali (1983) that laggard and disturbed anaphase could be caused by effect of boron on microtubule formation. In the present investigation, boric acid produced a significant amount of chromosome stickiness with parallel increase in bridges. Gaulden, 1987, reported that sticky chromosomes may be resulted from defective functioning of one or two types of specific non-histone proteins, involved in the chromosome organization which is needed for chromatid separation and segregation. Chromosomal stickiness may also be due to disturbance in nucleic acid metabolism in cells (Chidembaram *et al.*, 2009). Stickiness (Fig. 5 and Fig. 7) of chromosomes reflects highly toxic effects and may lead to cell death (Turkoglu, 2008). Chromosomal bridges (Fig. 8 and Fig. 9) are formed by chromatin fibres that join sister chromatids at metaphase and hold the chromatids together until late anaphase or telophase; if these connections become too strong, chromatids might break at or near the points of connection at anaphase (Turkoglu, 2008). The similar results were reported in *Vicia faba* and *Allium cepa* mitosis after treatment with food additives (Njagi and Gopalan, 1982; Gomurgen, 2005; Torkoglu, 2007). Laggard chromosomes also increased with increasing dose of treatment of sunset yellow. Laggard chromosomes resulted due to failure of movement of chromosomes to their respective poles. Gomurgen (2005) and Turkoglu (2007) also reported the induction of laggard chromosomes following the treatment with food additives. Prevalent precocious movements of chromosomes observed in case of sunset yellow treatment may have resulted from univalent chromosomes at the end of prophase I or precocious chiasma terminalization at diakinesis or metaphase I. Univalents may originate from an absence of crossing over at pachytene or from synaptic mutants (Kumar and Rai, 2007). Unorientation at metaphase (Fig. 3) and scattering of chromosomes may be due to either the inhibition of spindle formation or the destruction of spindle fibres formed (Kumar and Rai, 2007).

Because scientific knowledge is constantly evolving, federal officials often review earlier decisions to assure that the safety assessment of a food substance remains up-to-date. Any change made in previous clearances should be recognized as an assurance that the latest and best scientific knowledge is being applied to enhance the safety of the food**.** Owing to mitotoxic and genotoxic effects of these food additives, it is important to monitor and determine the level of these food additives in our food.

ACKNOWLEDGEMENTS

Authors are thankful to all the members of Plant Genetics Laboratory for their cooperation.

REFERENCES

Amer, S.M., and Ali, E.M., (1983). Cytological effects of pesticides XVII.Effects of the insecticide dichlorvas on root mitosis of *Vicia faba*. Cytologia, **51**: 21-25.

Arslan, M., Topatas, M., Rencuzogullari, E., (2008). The effect of boric acid on sister chromatid exchange and chromosome aberrations in cultured human lymphocytes. Cytotechnology, **56**: 91-96.

Baker, D.M., and Bogema, S.C., (1986). Ingestion of boric acid by infants. Am. J. Emergency Ned., **4**: 358-361.

Beyer, K.H., Bergfeld, W.F., Berndt, W.O., Boutwell, R.K., Carlton, W.W., Hoffmann, D.K., Schroeter, A.L., (1983). Final report on the safety assessment of sodium borate and boric acid. J Am Coll Toicol, **2**: 87-125.

Bringma, G., and Khin, R., (1980). Comparison of toxicity threshold of water pollutants to bacteria, algae and protozoa in cell multiplication inhibition test. Water Res, **14**:231 -241.

Chidembaram, A., Sundaramoorthy, P., Murugan, A., Sanker Ganesh, K., Baskaran, L., (2009). Chromium induced toxicity in Blackgram (*Vigna mungo* L.). Iran. J. Environ. Health. Sci. Eng., **6(1)**: 17-22.

Cox, C., (2004), Boric acid and borates. J. Pesticide Reform, **24**: 10-15.

Di Renzo, F., Cappelletti, G., Broccia M.L., Giavini, E., Menegola, E., (2007) Boric acid inhibits embryonic histone decatylases: A suggested mechanism to explain boric acid related tetragonicity. Toxicol. Applied pharmacol, **220**: 178-185.

Donbak, L., Rencuzoguliari, E., Topaktas, M., (2002). The cytogenetic effect of the food additive boric acid in *Allium cepa* L. Cytologia, **67**: 153-157.

Epel, D., (1963). The effect of carbon mono oxide inhibition of ATP level and the date mitosis in Sea Urchin egg. J. Cell Biol, **17**: 315-319.

Gaulden, M.E., (1987). Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis, **2**: 357-365.

Ghul, W., (1996). Ecological aspects of boron. SOFW-Journal, **118**(18/92): 1159-1168.

Gomurgen, A.N., (2005). Cytological effect of potassium metabisulphite and potassium nitrate food preservative on root tips of *Allium cepa* L. Cytologia, **70**: 119-128.

Hallagan, J.B., Allen, D.C., Borzelleca, J.F., (1995). The safety and regulatory status of food, drug cosmetics color additives exempt from certification. Food Chem. Toxicol., **33**: 515-528.

Heindel, J., Fail, P., Goerge, J., Grizzle, T., (1997). Reproductive toxicology of boric acid. Environ. Health Perspect., **105**: 275-276.

Ishidate, M.J.R., and Odasima, S., (1977). Chromosome test with 134 compounds of Chinese hamster cells in vitro-screening for chemical carcinogens. Mutat. Res., **48**: 337-353.

Jain, A.K., and Andsorbhoy, R.K., (1988). Cytogenetical studies on the effect of some chlorinated pesticide III. Concluding remarks. Cytologia, **53**: 427-436.

Kumar, G., and Rai, P.K., (2007). EMS induced karyomorphological variations in Maize (*Zea mays* L.) inbreds. Turk J Biol, **31**: 187-195.

Litovitz, T.L., Klein-Schwartz, W., Oderda, G.M. and Schmitz, B.F., (1988). Clinical manifestations of toxicity in a series of 784 boric acid ingestions. Am. J. Emerg. Med., **6**: 209-213.

Luca, D., Luca, V., Cotor, F., Raileanu, L., (1987). In vivo and in vitro cytogenetic damage induced by sodium nitrate. Mutat. Res., **189**: 333-339.

Martinez, F., Mateo, P., Bonilla, I., Fernandez-Valiente, E., (1986). Cellular changes due to boron toxicity in blue green algae *Anacystis nidulans*. Int J Exp Bot, **46**: 145-152.

Matsuoka, A., Hayashi, M., Ishidate, M.J.R., (1979). Chromosomal aberration test on 29 chemicals combined with S9 mix in vitro. Mutat. Res., **66**: 277-290.

Moore, J.A., (1997). An assessment of boric acid and borax using the IEHR evaluation process for assessing human developmental and reproductive toxicity of agents. Reprod Toxicol, **11**(1): 123-160.

Mukerjee, A., Giri, A.K., Talukder, G., Sharma, A., (1988). Sister chromatid exchange and micronuclei formation induced by sorbic acid and sorbic acid-nitrite in viv in mice. Toxicol. Let., **42**: 47-53.

Nagao, M., Honda, M., Seino, Y., Yahagi, T., Kawachi, T., (1977). Mutagenecity of protein pyrolates. Cancer Let, **2**: 335-339.

Njagi, M., and Gopalan, H.N., (1982). Cytogene effects of food preservatives-sodium benzoate and sodium sulphite on *Vicia faba* root meristems. Mutat. Res., **102**: 213-219.

Omanakumari, N., Shailja, P.N., Rejitha, P.G., (2006). Cytotoxic effects of the food additive Ajinomoto on root tip cells of *Allium cepa* L. J Cytol Genet, **7**(NS): 63-68.

Sadia, K.B., and Vahidy, A.A., (1994). Cytotoxic effect of herbicide ronstar on meristamic cells of Allium cepa L. Pak J Bot, **26**: 69-74.

Saeed-ul-Hassan, S., Bashir, S., Khokhar, I., Tehseen, M., (2006). Quality Assessment of Synthetic Food Dyes in Candies & Bubblegums. Pak. J. Med. Res., **45**(4).

See, A.S., Salleh, A.B., Bakar, F.A., Yosuf, N.A., Abdulamir, A.S., and Heng, L.Y., (2010). Risk and health effect of boric acid. American Journal of Applied Sciences **7(5)**: 620-627..

The ministry of Agriculture of Turkey (1997) Food Codex instructions Dunya Publications. : pp.197.

Tripathi, M., Khanna, S.K., Das, M., (2007). Surveillance on use of synthetic colors in eatables vis a vis Prevention of Food Adulteration Act of India. Food Control, **18**: 211-219.

Turkoglu, S., (2008). Evaluation of Genotoxic effects of sodium propionate, calcium propionate and potassium propionate on the root meristems cells of *Allium cepa*. Food and Chemical Toxicology, **46**: 2035-2041.

Turkoglu, S., (2007). Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. Mutat. Res. Genet. Toxicol. Environ. Muta., **626**: 4-14.

U.S. Food and Drug Administration. (1993). *Everything Added to Food in the United States*. Boca Raton, FL: C.K. Smoley (c/o CRC Press, Inc.).

Weir, R.J., and Fisher, R.S., (1972). Toxicologic studies on borax and boric acid. Applied Pharmacol, **23**: 351-364.

Yiu, P.H., See, J., Rajan, A. and Bong, C.F.J., (2008). Boric acid levels in fresh noodles and fish ball. Am. J. Agric. Biol. Sci., **3**: 476-481.