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EVALUATION OF MITODEPRESSIVE EFFECT OF SUNSET YELLOW USING *Allium sativum* ASSAY

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Abstract: The influence of a permitted yellow food colour, Sunset yellow –FCF on root tips of *Allium sativum* was investigated with a view to ascertaining its mitodepressive effect. Root tips of *Allium sativum* were treated with five concentrations of Sunset yellow like 100 ppm,250 ppm,500 ppm,750 ppm and 1000 ppm and with distilled water as control. The time of action of the respective concentration of solutions were 2 hrs,4 hrs and 6 hrs. Exposure to different concentrations of Sunset yellow showed an inhibitory effect on cell division of *Allium sativum* root tips. It caused a general decline in mitotic index value. Sunset yellow diminishes the mitotic index in correlation with the increased concentration and duration of treatment. The results implicated Sunset yellow – FCF as a mitotic depressor.
Keywords: Sunset Yellow- FCF, *Allium sativum*, Mitotic Depressor, Mitotic Index.

Introduction

Ever since prehistoric times, man was fascinated to colour the objects of daily use. Earliest recorded use of food colour comes almost simultaneously from India, China and Egypt in 1500 BC. Since then the trend of consumption of food coloured with food dyes has been increasing day by day. Colours play an important and strong role in food identification and in assessment of their fitness for consumption. The colours make the food items more delightful. Before the mid 19th century the colour dyes of natural origin were used. Substances with colouring properties were extracted from natural sources, mainly from vegetables and animals. But due to high cost, lack of availability of natural colouring material and difficulty in incorporating those in modern western technology of processing food, might have resulted in the shift to using synthetic food colours. Today virtually all dyes commercially available are synthetic substance.

Worldwide the controlled use of food dyes is based on ADI (Acceptable Daily Intake),which is based on the results of International research and recommendation of Codex Committee on food additives (Bessonov *et.al.,*2011). However in spite of control demanded by different

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regulatory agencies, the manufacturers of different food industry are using synthetic food colours indiscriminately, ignoring their ADI to produce attractive food. In this era of increasing human population, there are very few people who actually bother to check the label on the food they eat.

Synthetic food colours are conceded to be one of the most difficult problem, which may cause some toxicological effects on human being especially on children, who are attracted more towards coloured food items. The danger of artificial dyes are often an issue in food safety, with many claiming them to be toxic and a factor to the rise of ADHD (Attention Deficient Hyperactivity Disorder) in recent years. Artificial colours have been linked to behavioural changes, especially in children, as well as allergic reactions and a host of other adverse effects. Synthetic food dyes are most dangerous food additives. The use of dyes in food stuff raises a series of doubts as to their cytotoxicity, because in literature there is limited work evaluating the cytotoxicity of these compounds (Feng *et.al.* ,2012). So the possible harmful effect of synthetic food colours are a subject of public concern and has a critical attitude to their use in food stuffs.

Sunset yellow FCF is a permitted food colour in India. It is extensively used in soft drinks, sugar confectioneries, marmalades, canned fruits and vegetables and a number of food preparations. It often exceed the permissible limit. Sardi *et.al.* (2010) mentioned that Sunset yellow can cause anaphylactic shocks, angioedema, vasculitis and thromboxan synthesis inhibition in people sensitive to its composition. It can also trigger serious allergic reactions in people and can enhance aggressive behaviour in children.

A number of studies have been done for mutagenicity and clastogenicity of this dye. (Haveland –Smith 1980, Price *et.al.* 1978, Ishidate *et.al.* 1984, Cameron *et.al.* 1987, Roychoudhary and Giri, 1989). It produced consistent negative results in mutagenic and DNA damage assay (Garner and Nutman,1977, Bonin and Baker ,1980). Sunset yellow was positive in clastogenic test *in vitro* using Chinese Hamster fibroblast (Ishidate *et.al.,*1984), micronucleus assay (Vaidya and Godbole, 1978). It was reported as non clastogenic *in vivo* in different laboratory animals (Wever *et.al.,*1989). Very little information is available regarding its effect at plant chromosomal level. Genotoxic effect of Sunset yellow in root meristem of *Trigonella foenum graecum* was done by Kumar and Srivastava (2011) and noticed many chromosomal aberrations.

The effect of many chemicals on plant mitosis may provide valuable information in relation to possible genotoxicity in mammals, especially in humans. The bioassay with the

plants have been considered quite sensitive and simple in the monitoring of the cytotoxic effects of the chemicals (Grant 1999, Iganci *et.al.* 2006). So the plants might be employed for screening of cytotoxic potential and may be a valid alternative to the laboratory animals. Therefore present study was planned to investigate the mitodepressive effect of Sunset yellow FCF, a permitted yellow food dye on root tips of *Allium sativum*.

Material and Methods

In the present study root tip cells of *Allium sativum* were used as test system and synthetic yellow food colour Sunset yellow - FCF as test substance. Among the most commonly used dyes by food industry are those contain an azo group (-N=N-). Several studies show that release of these azo dyes into the environment is alarming due to its toxic, mutagenic and carcinogenic properties. Sunset yellow is one of the most commonly used monoazo dye. Sunset yellow FCF (FD & C NO.6, colour index 1956 No.15985) is a disodium salt of 1-p-suphophenylazo-2-naphthol-6-sulphonic acid. The maximum ADI established by Joint FAO/ WHO Expert committee on Food Additives (1994) for Sunset yellow is 2.5 mg/kg (Bw). For the present study the dye was procured from Hickson and Dadajee Co.Ltd. Mumbai.

The cloves of *A.sativum* were grown in small jars containing sterile moist soil. After allowing roots to grow the cloves were treated with 100 ppm,250 ppm,500 ppm,750 ppm and 1000 ppm concentration of Sunset yellow for 2hrs,4 hrs and 6 hrs.Treated root tips were fixed in Carnoy's fluid (Ethyle alcohol: Glacial acetic acid 3:1). For control group, cloves of *A.sativum* were grown in sterile moist soil and roots of about 1 cm were directly fixed in Carnoy's Fluid. Root tips were then transferred in 70% alcohol for preservation. For slide preparation the root tips were submitted to acid hydrolysis in 1 N HCl for 3-5 min. and then stained with acetocarmine squash technique (Sharma and Sharma,1990). Microscopic examination was done for mitotic index for each set of treatment and control. Mitotic index was calculated by using following formula-

$$\text{Mitotic Index (MI)} = \frac{\text{Total number of dividing cells} * 100}{\text{Total number of cells counted}}$$

In this study a statistical analysis was done to estimate SD and SE of the results.

Results and Discussion

The effect of Sunset yellow – FCF on mitotic index of *Allium sativum* is presented in Table - 1. Mitotic index considered as a parameter that allows one to estimate the frequency of cellular division (Marcano *et.al.,*2004). Table-1 shows the number of cells in interphase,

number of cells in different phases of cell division (prophase, metaphase, anaphase and telophase) and the mitotic index (MI) values obtained from root meristmatic cells of *A. sativum*, when treated with synthetic food dye Sunset yellow under exposure time of 2h,4h and 6h.

Table -1 clearly indicates that in the present study Sunset yellow –FCF decreased the MI in root tip cells of *A.sativum.* Decrease in mitotic index is dose and duration dependent. Similar effects on mitotic index have been reported by many researchers following the treatment of *Allium cepa* roots with the leaf extracts of *Ricinus communis* (George and Geethamma, 1990), Sodium metabisulphite (Rencuzogullari *et al*., 2001a) and Potassuim metabisulphite (Kumar and Panneerselvam, 2007). While studying cytogenetic effect of Sodium nitrite on mitotic division of *Lycopersicum esculentum*, Padureanu (2010) also obtained same pattern. However very peculiar result was observed in this study with 100 ppm concentration of Sunset yellow. Short duration treatment of dye increased the % of mitotic index, whereas long duration treatment was mitodepressive in nature. Its 2 h. duration treatment enhanced MI upto 42.8%, which is more than the control value (42.3%), whereas by 6 h. treatment mitotic index decreased to 41.2%. Rest of four concentrations like – 250 ppm,500 ppm,750 ppm and 1000 ppm decreased the MI from control value. In higher concentration the MI is inversely proportional to concentration and duration of treatment. With the increase in concentration and duration of treatment the % of mitotic index gradually decreased (Table -1, Graph 1A). An aqueous solution of 250 ppm, 500 ppm, 750 ppm and 1000 ppm of dye when treated for 2 h. induced 41.5%, 39.2%, 35.7% and 30.4% MI respectively. During 6 h. treatment mitotic index decline to 40 % (250 ppm), 37.2% (500 ppm), 32 % (750 ppm) and 29.7% (1000 ppm) (Table 1, Graph 1A). The results clearly show that Sunset yellow – FCF is mitodepressive in nature and this effect was more pronounced in highest concentration of dye treatment (1000 ppm). Similar type of results were also obtained by many researchers in *Vicia faba* root tips by action of food preservatives (Nagwa *et.al.* (2011)), in *A.cepa* root tips by Donbak *et.al*. (2002) and Renjana *et.al.* (2013) with the treatment of boric acid and food additives respectively. The decrease in mitotic index could be due to blocking of G1 phase, suppressing DNA synthesis (Sheneiderman *et.al.,*1971) or due to blocking of G2 preventing the cell from entering mitosis (Van't,1968), or it may be achieved by the inhibition of DNA synthesis at S phase (Sudhakar *et.al.* 2001). Inhibition of DNA synthesis might be caused by the decreasing ATP level, which is essential for progress of mitosis. (Rencuzogullari *et.al.*, 2001 a).

The number of total dividing cells decreased as the concentration and duration of treatment increased. The synthetic yellow food dye Sunset yellow –FCF also caused a change in the frequencies of different mitotic stages. It reacted differently for the four phases (prophase, metaphase, anaphase and telophase) of mitotic cell cycle. In all the four mitotic stages the cell proportion was below the control values (Table -1,Graph 1B). *Allium sativum* root tip cells showed a large number of cells in prophase and least number of cells in anaphase. Similar response of prophase and anaphase stages were observed by Onyemaobi *et.al.*(2012), while studying cytogenetic effects of food preservatives on *A.cepa* root tips. This might be due to cells are prevented from entering prophase or there is prophase arrest, where cells enter into mitosis, but are arrested during prophase resulting in a high frequency of prophase cells. Furthermore food dye may bind to tubulin and prevent the formation of mitotic spindle. Both metaphase and anaphase further showed a gradual decrease in their number with increase in concentration and duration of dye treatment. In all the concentration of dye treatment (100 ppm,250 ppm,500 ppm,750 ppm and 1000 ppm) the value of metaphase and anaphase cells were more in 2 h. treatment, which decreased in 6 h. treatment. The lowest number of cells with metaphase (20) and with anaphase (12) were found in 1000 ppm concentration of dye, when treated for 6h. In telophase the cell proportion was diminished over control in all the concentration of Sunset yellow treatment, especially at 1000 ppm dye concentration (Graph 1B).

Poul *et.al.* (2009) did not find any genotoxic effect in micronucleus gut assay in mice on oral exposure to Amaranth, Sunset yellow and Tartrazine. Immunological studies on Amaranth, Sunset yellow and Curcumine was done in albino rats by Hashem *et.al.(*2010). They concluded that all the three dyes has a depressing effect on the cellular, but not humoral immune response when used upto ten times the ADI. In an another study Sunset yellow –FCF alters functional responses of splenocytes at non toxic dose (Yadav *et.al.*, 2013). Effect of Sunset yellow on testis of rats was done by Mathur *et.al.* (2005) and reported that this dye can induce damaging effect on seminiferous tubules. Sasaki *et.al.* (2002) noted no DNA damage by Sunset yellow *in vivo* comet assay in mice. Saxena and Sharma (2014) studied serological changes induced by blend of Sunset yellow, metanil yellow and Tartrazine in Swiss albino rats and concluded that prolonged consumption of blend may cause adverse effect on human health.

Cytotoxicity of different food dyes were conducted by Oliveira *et.al.(*2013) in *A.cepa.* cell cycle and the results verified that a synthetic food dye red 40 can promote a significant

reduction in cell division and induce many aberrations. A study concluded by Sayed *et.al.(*2012) demonstrated that the mutagenic action of Sunset yellow dye in mice with significant chromosomal aberrations in liver and germinative cells, DNA fragmentation and increase of morphological abnormalities in spermatozoids of mice.

The result of the present study shows that Sunset yellow FCF significantly suppresses mitotic cycle. The similar genotoxic behaviour of Sunset yellow was reported by Kumar and Srivastava (2011) in root meristems of *Trigonelle foenum –graecum*. Cytotoxicity of Sunset yellow on *A.cepa* root meristem cells was done by Gomes (2013) and noticed same result. The inhibition of mitotic index of root tips of *Vicia faba* by treatment of Sunset yellow was confirmed by Bhattacharjee and Yadav (2005). All these studies corroborate the data obtained in the present study on the depression of mitotic index by Sunset yellow.

Conclusion

The results of the current study clearly indicates that Sunset yellow –FCF, a popular and frequently used permitted synthetic food dye in India has a strong mitodepressive effect on *Allium sativum* root tip cells. There is need to extend investigations with varied doses, exposure time and test organisms to accurately evaluate the potential risk of this food dye. In view of above discussion, efforts are required to strength the food quality monitoring and surveillance to protect our society by continuous and unregulated use of Sunset yellow. In order to reduce the possibility of harmful effect of Sunset yellow the dye should be avoided as far as possible in food items.

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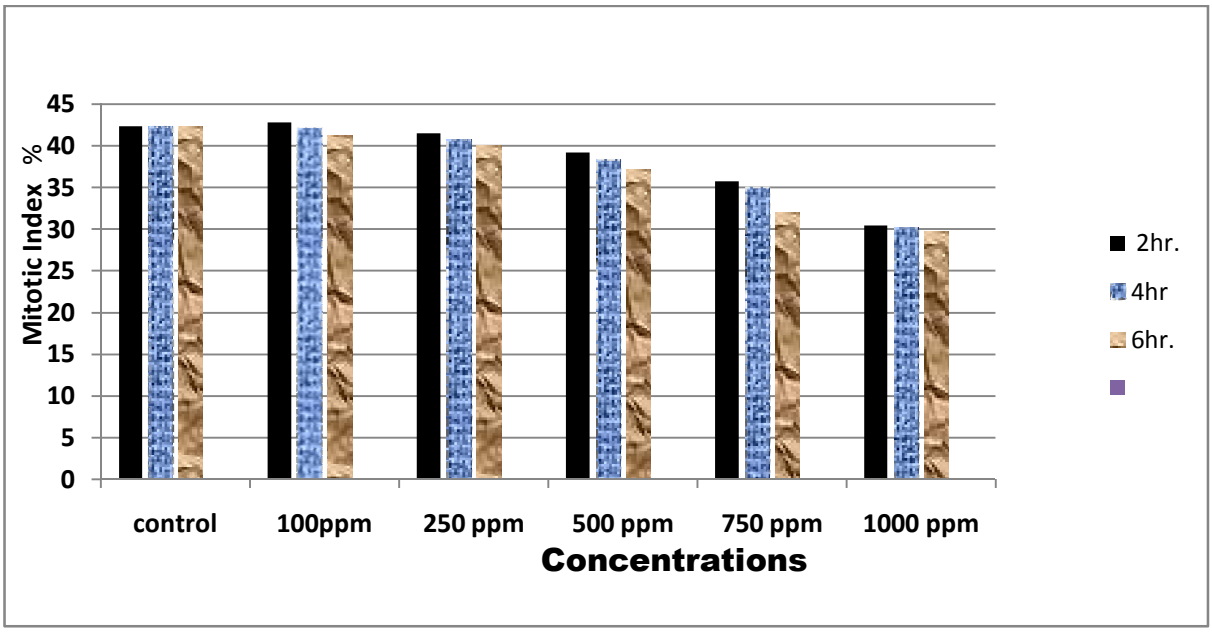
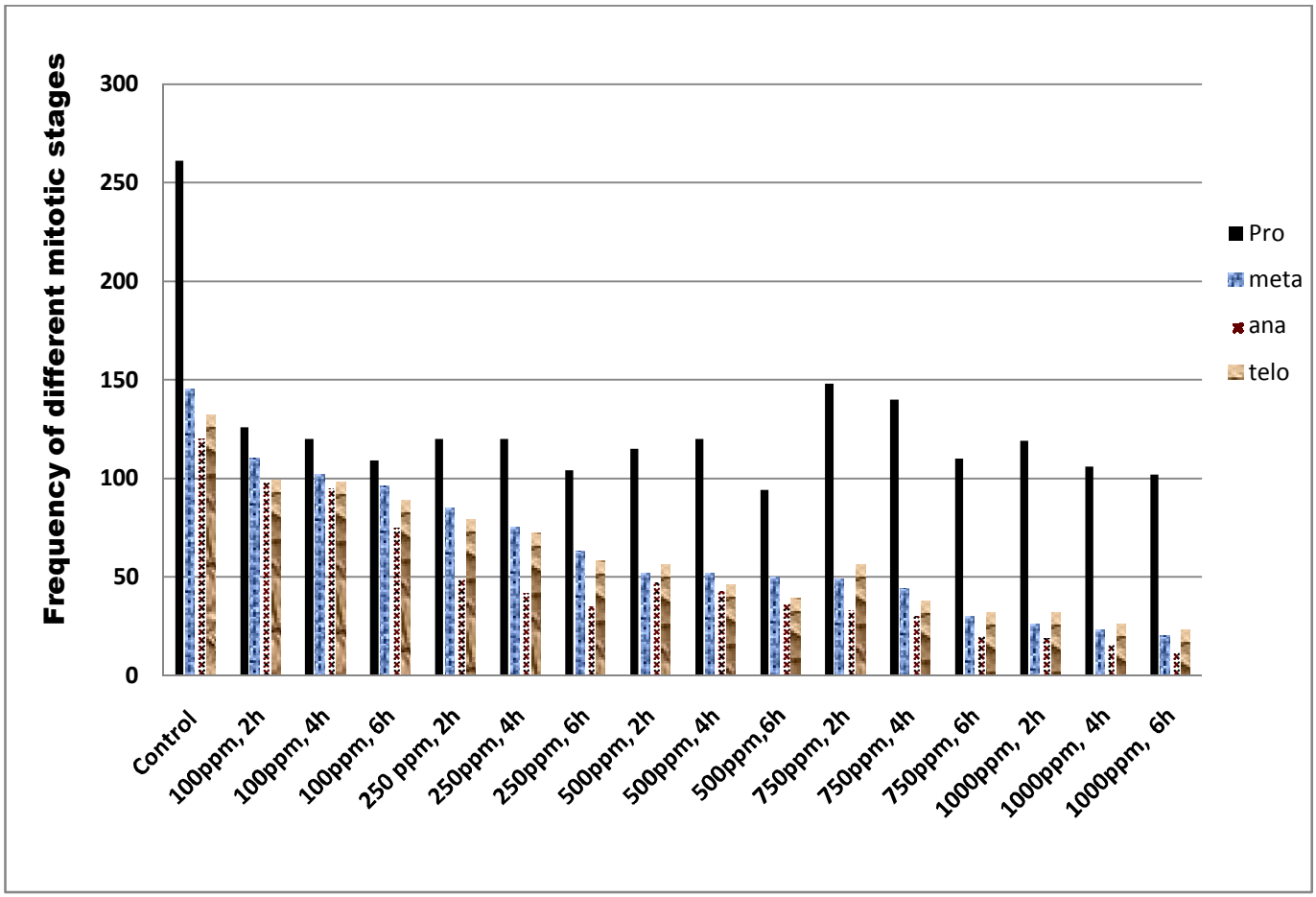


Graph 1A: Percentage of Mitotic Index in *A. sativum* Root Tips Treated with Sunset Yellow FCF



Graph 1B: Frequency of Different stages of Mitotic Cycle in *A.sativum* When Treated with Sunset Yellow FCF

Table 1: Frequency of Mitotic stages and Mitotic Index Observed in Root Tips of *Allium sativum* Treated With Different Concentrations of Sunset Yellow FCF at Different Duration

Concentration Of Dye	Duration of Treatment	Total Cells Examined	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total Dividing Cells	MI	SD +/-	SE
Control		1556	898	261	145	120	132	658	42.3	0.9	0.3
100 ppm	2 hrs	1011	578	126	110	98	99	433	42.8	2	0.64
	4hrs	986	571	120	102	95	98	415	42.1	1.9	0.62
	6 hrs	896	527	109	96	75	89	369	41.2	1.9	0.62
250 ppm	2 hrs	803	470	120	85	49	79	333	41.5	1.5	0.49
	4hrs	756	447	120	75	42	72	309	40.8	2.7	0.86
	6 hrs	650	390	104	63	35	58	260	40	2.2	0.69
500 ppm	2 hrs	689	419	115	52	47	56	270	39.2	2.3	0.75
	4hrs	680	419	120	52	43	46	261	38.4	1.3	0.42
	6 hrs	600	480	94	50	37	39	220	37.2	2.9	0.93
750 ppm	2 hrs	800	514	148	49	33	56	286	35.7	3.3	1
	4hrs	720	468	140	44	30	38	252	35	3.7	1.1
	6 hrs	600	408	110	30	20	32	192	32	3.6	1.1
1000 ppm	2 hrs	645	449	119	26	19	32	196	30.4	3.7	1.1
	4hrs	562	392	106	23	16	26	170	30.2	3.9	1.2
	6 hrs	529	372	102	20	12	23	157	29.7	2.8	0.9