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Studies on clastogenic activity caused by synthetic red food colour dye on the root tips of *Allium cepa* L.

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Abstract
The present study was carried out to determine the effect of synthetic red food color on chromosomal behaviour on root tips of *Allium cepa* L. The common synthetic red food colour at different concentrations, 500, 1000, 2000 and 3000ppm was prepared and used to study various behavioral changes of chromosomes. Initially the mitotic cell division was much affected and mitodepressive effect was found to be increased in all the studied concentration and simultaneously the frequency of abnormality was found to be higher when the compared with control. It is also observed many important abnormal stages like DNA vacuolation, Elongated nucleus, Clumping, Diagonal Metaphase, Disorientation, Laggards, Chromosomal bridges, Diagonal Anaphase, Binucleated condition in different stages of mitotic stages. Most predominantly the DNA vacuolation (22.97%) and elongated nucleus (12.97%) were found in the study and synthetic food colour considered to be highly toxic to chromosomes and cell division.

Keywords: chromosomal abnormalities, DNA vacuolation, laggards

1. Introduction
Food colours were become the integral part of the food industry. Selection and purchase the food on various parameters is increased day by day. The chemical mutagens was causing many abnormal behaviour of chromosomes which may leads to drastic in the normal movement of chromosomes Numerous natural and synthetic dyes are used as unavoidable ingredient on food items to increase the consumer's attraction. During nineteenth century food dyes are prepared by naturally available vegetable and fruit extracts were used normally. Due to high cost and lack of availability of natural coloring materials people change to prepare the synthetic food colours in modern food processing. Synthetic food dye occupies a large food market due to its less production cost and easy availability. Synthetic food dyes had been an integral constituent of foods since long and are still indispensable to the consumers of modern era. They are derived from various toxic substances like aniline, petroleum and heavy metals, which have harmful effects on human health, resulting in diseases like hyperactivity, allergic reactions and even cancer.
Red food powder is an azo dye and is permitted food color in India. Today virtually all dyes are commercially and freely available in markets [2]. Food colors are water soluble and they are in the form of liquid, power, gels and pastes which are used in various concentrations. Food colors are extensively used in almost every type of edible preparations like soft drinks, jams, jellies, ice creams, foodstuffs, sweets, candies, sauces and pickles [4].
The present study was carried out to evaluate effect of red food color on the root tips of *Allium cepa* L and its chromosomes. The onion root tip is a convenient system to determine both many parameters including chromosomal behaviours. The study envisages cytotoxic aspects of synthetic red food color using *Allium cepa* L. assay.

2. Materials and Methods
2.1 Collection of food color
The commonly available synthetic red food colorant was purchased from local market in Pollachi.

2.2 Selection of test material
Clean and healthy *A.cepa* bulbs were selected as study material to detect the changes in chromosomal behavior caused by synthetic red food color. The onion bulbs were collected from local vegetable market in Pollachi. It has been the most efficient test material to indicate the mutagenicity due to its kinetic characters [9, 3].

2.3 Methodology
The synthetic red food color was prepared by various concentrations *viz*., 500ppm, 1000ppm, 2000ppm and 3000ppm as test solution. Healthy onion bulbs were cultured on solution and allowed to grow in three replications. The onion root tips were well grown to 1-3cm length. The roots were excised and stored in 3:1 acetic acid-ethanol (carnoy's fixative). The root tips were selected randomly from triplicates and slides prepared for each concentration. The fixed root tips were soaked in Acetocarmine stain for few minutes to allow the excised roots for absorption. These root tips with stained and heated gently to facilitate the uptake of stain the heated slides were allowed to cool. Coverslip was sealed over the root tips and squashed gently by giving thumb impression gently on coverslip till the cells were spread sufficiently. Slides were observed under microscope and calculated the percentage of Mitotic index and

frequency of abnormalities. The percentage of Mitotic Index and frequency of abnormality was calculated by following formulae.

2.4 Mitotic index

$$\frac{\text{Total number of dividing cells}}{\text{Total number of cells observed}} \times 100$$

2.5 Frequency of abnormalities

$$\frac{\text{Number of abnormal cells dividing}}{\text{Number of cells dividing}} \times 100$$

Statistical analysis was calculated by mean and standard deviation of mitotic index and frequency of abnormality. Frequency of abnormalities in all stages were tabulated and analyzed.

3. Results and Discussion

Allium cepa L. exhibits species level genomic constitution (2n=16). Table 1 represents characteristic normal and disturbed phases of cell cycle during mitotic cell cycles. Spectrum of mitotic manifestation is found to be concentration dependent of synthetic red dye. The study on synthetic red dye on the root tips of *A. cepa*. Five different concentrations were prepared accordingly 500, 1000, 2000 and 3000 ppm. The mitotic index was observed to be higher when compared with control. The maximum of mitotic activity of 82.78 % was found in control without addition of synthetic red dye and much reduced mitotic activity of 18.93 % was found in maximum concentration of 3000 ppm concentration. These concentrations of red dye were much influenced towards the mitotic activity. The reduction in mitotic indices was also noted [5] that the inhibition of certain cell cycle due to absence or less secretions of specific enzyme such as DNA polymerase, which is essential for DNA replication, might have caused antimitotic effect.

The frequency of abnormality was found to be higher in 3000 ppm concentration of 12.16% in synthetic red dye and lower most frequency of 0.81% was observed in control. The observation clearly exhibits that synthetic red dye was significant activity towards disturbing chromosomes. The above findings are also noted and justified by [2] it is due induced mutagens that causes interferes the normal cell cycle leading to many changes in the chromosomes.

The chromosomal abnormality such as Elongated Nucleus, DNA Vacuolation, Clumping, Diagonal metaphase, Disorientation, Laggards, Chromosomal bridges, Diagonal Anaphase and Binucleated condition was observed in the studied concentration. The abnormality of DNA Vacuolation and elongated nucleus was found to be maximum of 22.97 % and 12.97% at 3000 ppm concentration respectively. The aberrations are reported by many workers and referred as physiological aberrations are the outcome of disturbance of spindle formation, while clastogenic aberrations are the direct indication of DNA damage. Laggard (s) are outcomes of partial spindle inhibition where one or more chromosome (s) detach from the spindle fiber and lie separately in the cell either at pole (vagrant) or near equatorial plate (laggard). The formation of bridges may be attributed to unequal exchange resulting in the formation of dicentric chromosomes, which are pulled equally to both poles of anaphase, resulting in bridge formation. Some of the abnormality was less frequently found in the study. The abnormalities such as laggards (2.97%), chromosomal bridges (3.51%) and diagonal anaphase (1.89%) was found in few cells in 3000 ppm concentration. Earlier studies also revealed that most of the synthetic food dyes contained different heavy metals like arsenic, chromium, copper, lead, manganese, nickel, etc [8-11]. Which can pose a serious threat to human beings by inducing various genetic disorders. From the present findings it clearly shows that synthetic red dye is having a hazardous nature at chromosomal level against normal cell division and movements of chromosomes.

Table 1

Concentration % Stages	Mitotic index	Frequency of Abnormality (%)	Elongated Nucleus	DNA Vacuolation	Clumping	Diagonal Metaphase	Disorientation	Laggards	Chromosomal bridges	Diagonal Anaphase	Binucleated condition
Control	86.78±0.86	0.81±0.08	1.02±0.04	1.22±0.03	-	-	-	-	-	-	-
500 ppm	40.22±1.01	3.33±0.22	2.94±0.04	7.06±0.27	0.98±0.07	1.18±0.07	0.78 ± 0.04	-	-	-	-
1000 ppm	30.94±1.85	4.21±0.03	5.51±0.05	7.94±0.10	0.32±0.02	0.49±0.04	0.97 ± 0.03	0.81±0.7	0.49±0.04	0.65±0.14	0.49±0.11
2000 ppm	27.78±0.75	5.43±0.1	7.13±0.03	9.17±0.03	0.51±0.03	1.19±0.05	1.53 ± 0.08	1.19±0.14	1.53±0.07	0.85±0.11	0.85±0.08
3000ppm	18.93±0.66	12.16±0.04	12.97±0.22	22.97±0.11	2.16±0.05	2.97±0.24	3.24 ± 0.07	2.97±0.34	3.51±0.13	1.89±0.14	-

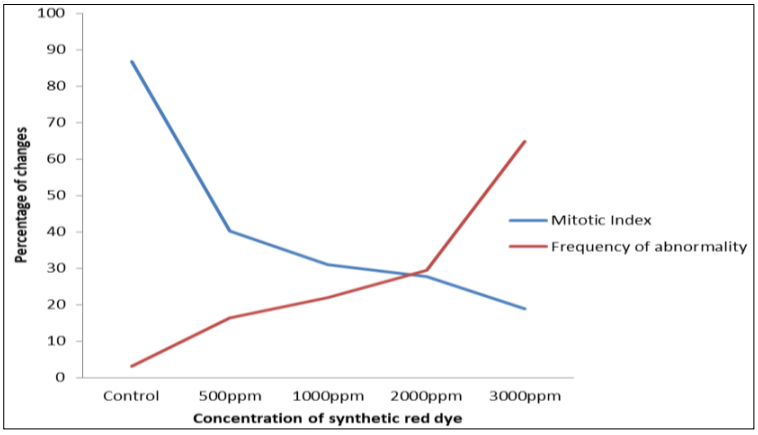


Fig 1: Showing the Mitotic Index and Frequency of abnormalities caused by the synthetic red dye on the root tips of *A.cepa*.

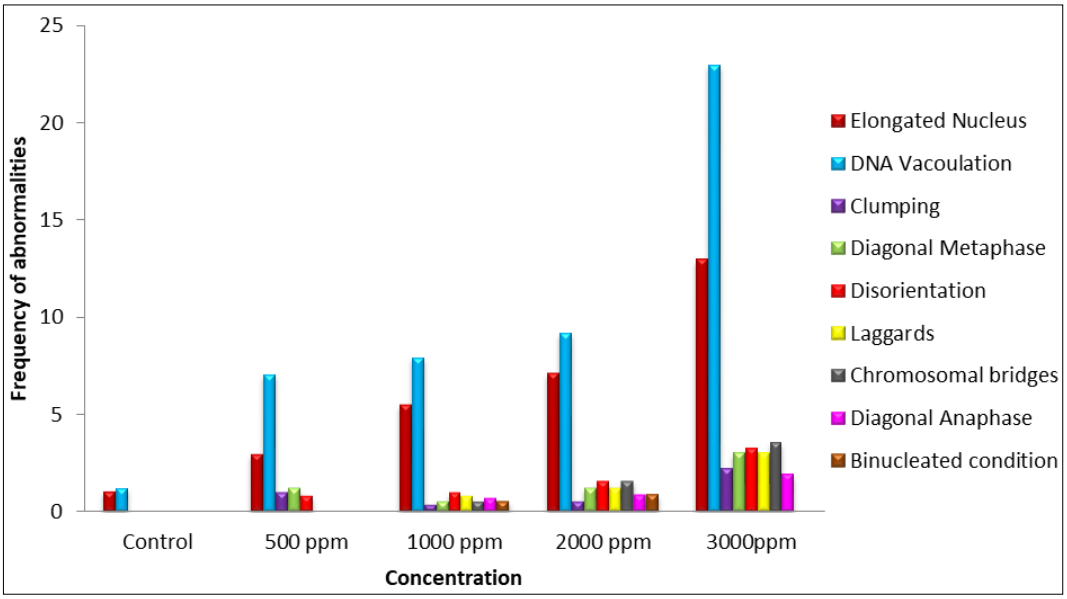
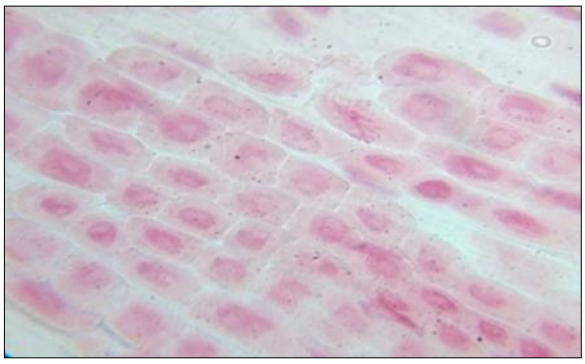


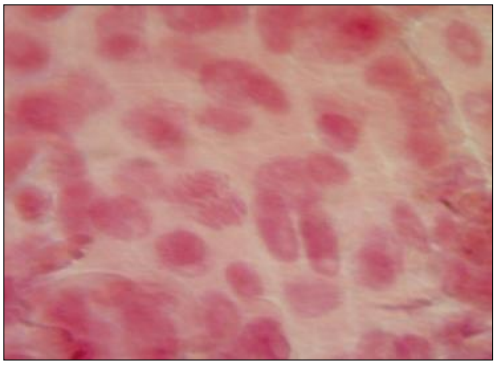
Fig 2: Showing the different chromosomal abnormalities occurred at various concentration of synthetic red dye on the root tips of *A.cepa*

a) Culturing of A.cepa bulbs

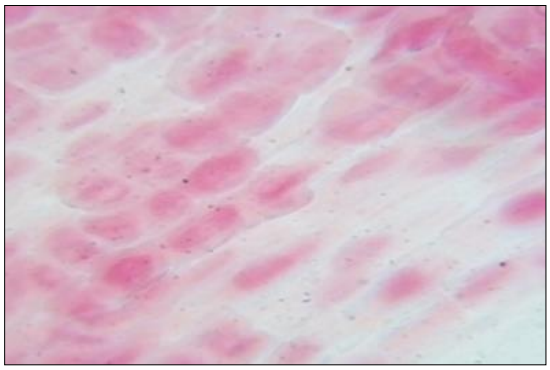
b) Well grown roots before excision



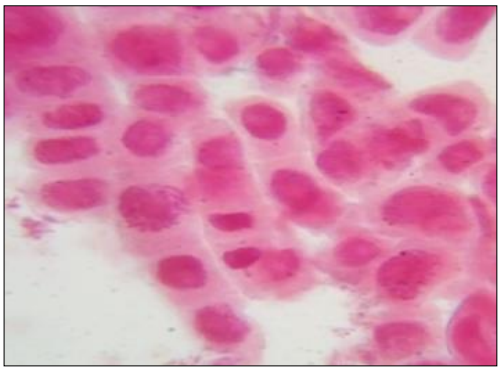
c) DNA Vacuolation



d) Clumping of chromosomes



e) Elongated nucleus



f) Binucleated condition

Fig 3

4. Conclusion

The study clearly showed that red dye which is commonly used for many food stuffs have considerable effect on chromosomal behavior. These abnormalities may lead to change in the chromosomal arrangements. The abnormalities like DNA vacuolation, laggards, stickiness may lead to loss of chromosomes and these changes will be transferred to allied species through pollination behaviours. Ultimately the loss chromosomes will occurs leading to the loss of even many conserved regions of the chromosomes. The similar effects may also be expected in higher animals which leads to unexpected changes both morphologically and physiologically.

5. References

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