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Cytogenetic Effect of Food Preservatives Sodium Metabisulphite on *Allium Cepa* L.

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Abstract.

This study looked at the cytogenetic effects of the food preservative sodium metabisulphite on *Allium cepa* L. The parameters utilized to rate the various dosages of the drugs examined for this purpose were chromosomal abnormalities and Mitotic Index. The Mitotic Index (MI) decreased as sodium metabisulphite concentrations increased. Among the cytological anomalies identified were anaphase bridges, micronuclei, vagrant chromosomes, chromatid gaps, shattered chromosomes, and ring chromosomes.With increasing concentrations of dietary preservatives, the percentage of chromosomal abnormalities at mitosis rose. According to the findings of this study, these compounds caused permanent cytogenetic effects at certain dose levels.

KeyWords:*Allium cepa* L., Food Preservatives, Mitotic Index, Sodium Metabisulphite.

1. Introduction

Large amount of chemical compounds have been added to the meals by human for different purposes, such as: flavor, texture, shelf-life enhancement and appearance of food [2]. It is of extreme importance that food additives have incorporated in food supply. Any chemical or solution of substances that are added to the food under scientific regulation where it is not the main component of food is considered as a food preservative. The addition of food preservatives may occur in food manufacturing, or in processing and treatment, or in packing and transportation, or even in storing food. Thus, to conserve meals' taste and colour additives are used. Additives have helped humans in making a healthy, safe and beautifully prepared food given that humans have not cultivated their own food properly [9]. In order to save food in good conditions and keep it from being damaged by microbes, preservatives might be deployed. The shelf-life of food can be extended by preservatives [9]. Even though preservatives can preserve the food for specific period of time, but rather, they have negative implications on human's health especially those preservatives that have the properties against bacteria. This has been proved in different test systems. Therefore; scientists have studied the different potential of substances pertaining to a mutagen since they the ability to generate genetic alterations in DNA which are invisible to detect in its early stage. In plant cells, for example, food preservatives with increased concentrations and different intervals, have shown to change the mitotic index values in such plant. Thus, the entire proportion of mitotic stages has changed. In addition, the percentage of aberrations has increased [13]. The goal of this study was to see how sodium metabisulphite, a food preservative, affect the root tip cells of *Allium cepa* L.

Material and Methods

A clean and sharp blade was used to remove the discs and the dry outermost scales of healthy onion bulbs, the bulbs were cut very carefully avoiding the damage to the root system. Vials of 25 cm^3 were filled with water to place the *Allium cepa* L. bulbs inside for 2-4 days. As a test material, sodium metabisulphite, a food preservative, was employed. The impact of the test material on onion root tips was investigated using 0.0gm, 0.25gm, 0.5gm, 1.0gm, and 2.0gm /L sodium metabisulphite, with onion bulbs placed immediately on top. The onion bulbs were first grown in tap water, then moved to a succession of sodium metabisulphite concentrations for 24 treatment hours in another setup. For each concentration, five bulbs were utilized. The root tips were cut, cleaned, and treated with 0.05 percent colchicines solution for 3 hours after having been treated with sodium metabisulphite. At the end of each treatment period, one-inch of root tips was excised and immediately fixed in a 1:3 Acetic-Ethanol solution for 24 hours. To examine the samples, from each bulb, five root tips were removed. The root tip was then macerated for 5 minutes in drops of 1N HCl for hydrolysis. After that, two drops of giemsa dye were applied to the root tip and left for 20 minutes. After that, the cells were gently crushed and uniformly distributed [12]. Five slides were yielded in each bulb and one root was used for each slide. The stained substance was gently covered with a cover-slip on the slide to make sure that no trapping air in the process. To remove any extra discoloration, a piece of filter paper was placed over the slide and firmly pushed down. Sealing good slides with colorless nail polish protected them. To guarantee the consistency of the results, each treatment repeated five times.

The slides were examined with the use of light microscope. Mitotic cells and aberrations were recorded in five random fields each plate. Some slides were photographed with the X40 objective lens or the X100 objective lens (oil immersion lens). To obtain the mitotic index, the number of dividing cells was divided by total number f cells per field then multiplied the product by 100 [8].

The overall statistical analysis was carried out through graph pad prism 5.0 software after a few samples of the data were hand examined. The treatments were set up in a completely random order (C. R. D). The significance of (F) values from ANOVA tables was then evaluated at $P<0.05$ and $P<0.01$, respectively. The Dundan Multiple Range test was used to assess the significant difference between the various treatments in the trials and their controls (DMRT).

2. Results

3.1 Effect of Sodium Metabisulphite on Mitotic Index of *Allium cepa* L. Root Tips Cells.

The effect of sodium metabisulphite on the mitotic index of *Allium cepa* L. root tip cells are shown in table (3.1). The findings of the experiment indicated that sodium metabisulphite have a negative impact on the frequency of various phases of mitosis and the mitotic index. In comparison to the control, the inhibition was dose-dependent and increased substantially ($p<0.01$)) with increasing sodium metabisulphite concentration. The proportion of mitotic index was 7.03 percent in the control group and 2.06% in the dosage no. 4 group.

Table 3.1: Effect of sodium metabisulphite on mitotic index of *Allium cepa* L. Root tips cells.

Treatments	no of examined cells	Prophase	Metaphase	Anaphase	Telophase	Total Mitosis	Mitotic index
0.0 ml/L	1422.0	37.0 ±1.73 a	23.0 ±3.51 a	18.0 ±1.15 a	22.0 ±3.60 a	100.0	7.03%
0.25 ml/L	1182.0	22.0 ±3.60 b	16.0 ±1.51 b	14.0 ±2.64 ab	21.0 ±1.15 a	73.0	6.01%
0.5 ml/L	1345.0	19.0 ±3.60 b	12.0 ±1.00 bc	11.0 ±1.15 bc	16.0 ±2.64 ab	58.0	4.33%
1.0 ml/L	1234.0	15.0 ±2.08 bc	9.0 ±1.52 cd	10.0 ±2.08 cd	10.0 ±1.15 bc	44.0	3.52%
2.0 ml /L	1116.0	10.0 ±1.00 d	3.0 ±0.57 d	4.0 ±1.52 d	6.0 ±1.73 c	23.0	2.06%
Significance		**	**	**	**		

3.2 Effects of Sodium Metabisulphite on Cytogenetic aberrations in *Allium cepa* L.

The effect of the different concentrations of sodium metabisulphite on the chromosome aberration in the root tip cells of *Allium cepa* L. are given in table (3.2) (figure 3.2). The sodium metabisulphite treatment caused a variety of cytogenetic consequences after 24 hours, which are represented by six types of abnormalities (bridges of anaphase, micronucleus, vagrant chromosomes, gaps in chromatid, chromosomal fragmentation and ring chromosomes).

The total of chromosomal abnormality were observed with increasing of sodium metabisulphite concentration and they were dose dependent greatly increased (Table 3.2). The total number of anomalies in control cells was 5, but in cells treated with 2.0 ml/L sodium metabisulphite, the number rose to 255.

Type of abnormality	Treatments					Significance
	0.0 ml/L	0.25 ml/L	0.5 ml/L	1.0 ml/L	2.0 ml/L	
Anaphase Bridges	2 ±0.57 d	12 ±2.00 c	18 ±3.70 c	23 ±6.02 b	45 ±3.60 a	**
vagrant chromosome	1 ±0.00 d	12 ±1.15 cd	22 ±3.70 bc	32 ±8.50 b	54 ±8.14 a	**
Ring chromosome	0 ±0.00 d	11 ±1.52 b	16 ±2.08 b	25 ±4.35 ab	36 ±5.13 a	**
Fragment chromosome	0 ±0.57 d	10 ±1.00 cd	19 ±2.60 c	29 ±5.00 b	49 ±5.00 a	**
Chromatid gaps	0 ±0.00 d	6 ±1.15 cd	15 ±2.57 bc	27 ±4.72 b	41 ±4.58 a	**
Micronucleus	1 ±0.00 d	8 ±5.30 c	13 ±3.05 bc	22 ±2.51 b	30 ±4.51 a	**
Total Abnormality	5	59	103	158	255	

The creation of a vagrant chromosome was the prominent anomaly, which accounted for 121 cases, following this, chromosomal fragment formation, which accounted for 107 cases, and the formation of a micronucleus, which accounted for 74 cases were observed. When compared to the control, there were significant statistical differences in the abbreviated values in all evaluated characteristics ($p<0.01$).

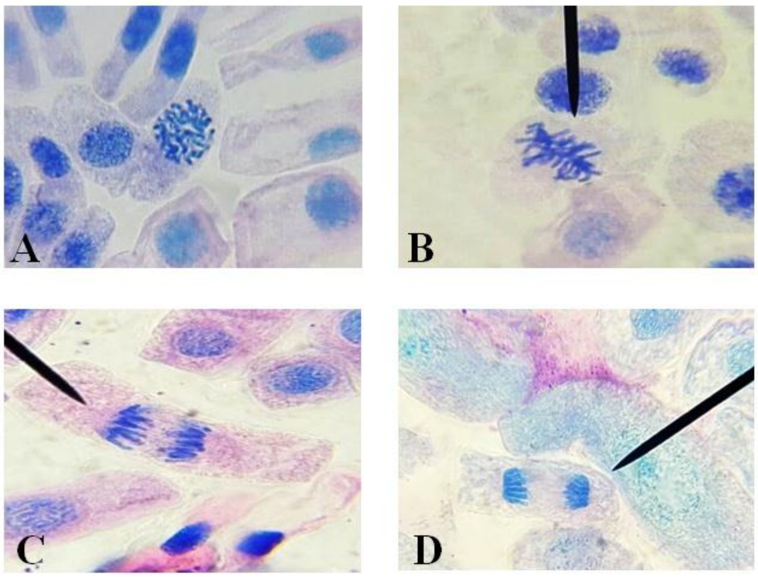

Figure 3.1: Normal cell division stage of *Allium cepa* L. (A) prophase, (B) metaphase, (C) anaphase, (D) telophase. (Magnification x1000).

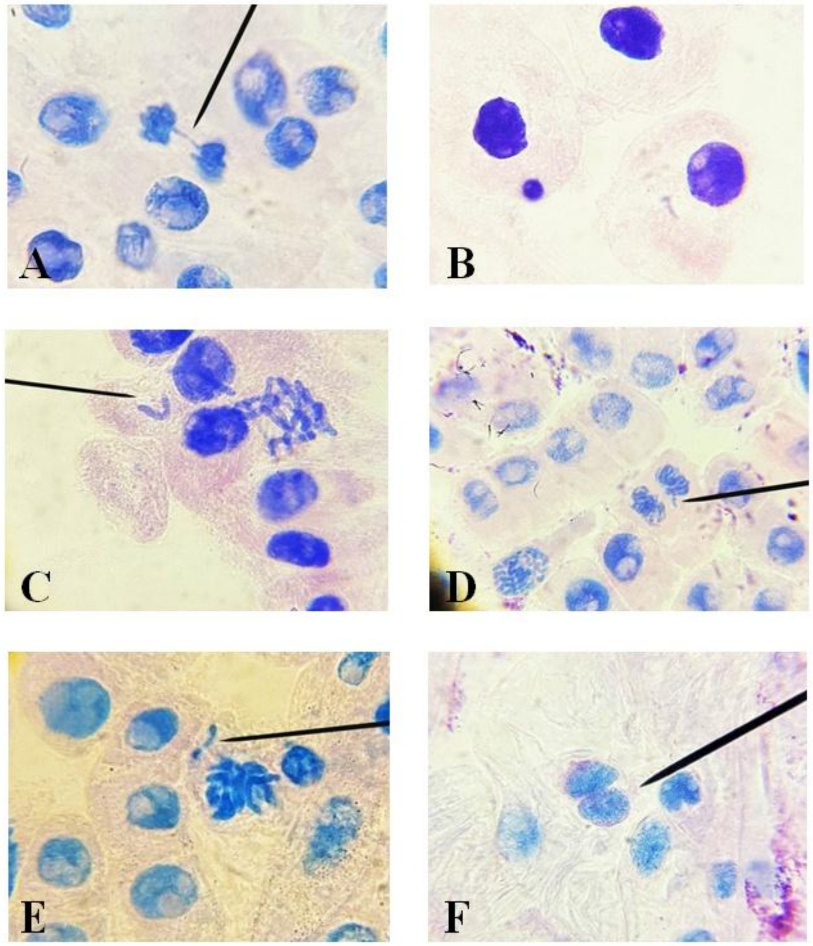

Figure 4.4: Abnormal cell division stage of *Allium cepa* L. and Cytogenetic aberrations:(A) anaphase bridges, (B) micronucleus, (C) vagrant chromosomes, (D) chromatid gaps, (E) fragmented chromosomes, (F)ring chromosomes. (Magnification x1000).

3. Discussion

The dietary preservatives employed in this investigation resulted in a shift in the frequency of certain mitotic phases. At various doses and treatment times, sodium metabisulphite enhanced the proportion of prophase. This is in accord with the findings of [10] and [14]. The cytotoxic potentials of Sodium metabisulphite in *Allium cepa* are demonstrated by the concentration-dependent suppression of the Mitotic Index. Many researches have found similar effects on Mitotic Index when *Allium cepa* roots were treated with *Ricinus communis* leaf extracts.

[3], Sodium metabisulphite [10] and Potassuim metabisulphite [1]. Similar result were obtained by [6].in *viciafaba*. The suppression of DNA synthesis which is caused by blocking of G1 stage may reduce in the activity of mitosis process [7].

In this study, nine types of chromosome aberrations were recorded: anaphase bridges, micronucleus, vagrant chromosomes, chromatid gaps, fragmented chromosomes and ring chromosomes.

Various concentrations and time frames of treatment of food preservatives have been treated a number of aberrant cells have shown to have different outcomes from those of control samples where lacking aberrations. [10] meanwhile, it is found that sodium metabisulphite caused *Allium cepa*to have lagging chromosomes. [14] also found that laggards in *Allium cepa* were recorded by food preservatives such as potassium sulphite, potassium nitrate, boric acid, citric acid, potassium citrate, and sodium citrate produced. Timing and concentration of the test chemicals that used increased and influenced an increase in abnormalities in the number of cells, this finding is consistent with the findings of [10] and [11] studies. These findings support the theory which state that the more sulphate concentrations, the more harmful effect it cause to cell division[4, 5].

Conclusion

Under the light of the results of this study, the following points can be concluded:

- Sodium metabisulphite can produce cytogenetic abnormalities in *Allium cepa* L. root tip cells.
- Sodium metabisulphite induce the frequency of micronuclei.
- The cytogenetic impact of sodium metabisulphite was shown by statistically significant abnormalities.
- Sodium metabisulphite may be regarded a possible mutagenic agent based on current results in *Allium cepa* L. root tip cells studies.

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