CYTOGENETIC EFFECTS OF TWO FOOD PRESERVATIVES, SODIUM METABISULPHITE AND SODIUM BENZOATE ON THE ROOT TIPS OF *ALLIUM CEPA* LINN.

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ABSTRACT

Allium cepa assay has been used extensively to determine the cytotoxicity and genotoxicity of compounds on plants and animals. The cytogenetic effects of two commonly used food preservatives, sodium benzoate and sodium metabisulphite were evaluated using the *A. cepa* assay. The parameters scored for the different concentrations of the compounds tested are: root length, chromosomal aberrations and Mitotic Index. The Mitotic Index (MI) decreased with increasing concentration of both sodium benzoate and sodium metabisulphite. Cytological aberrations observed were clumping, fragmentation, pulverization, lagging, binucleate cells and reduction in chromatin materials. Clumping and fragmentation were the most frequent aberrations. The percentage of chromosomal aberrations at mitosis increased with increase in concentration of the food preservatives. The effects of sodium metabisulphite at the different concentrations in this study were very detrimental as more aberrations were recorded even after the recovery experiment. The results of this experiment show that these additives had irreversible cytotoxic effects at some levels of dosage. It supports the call for the banning of these substances as food preservatives.

Key words: Chromosome Aberration, Food Preservatives, Mitotic Index; *Allium cepa* Assay.

INTRODUCTION:

Food additives are substances not normally consumed as food and not usually used as typical food ingredients but used as additives in foods or pharmaceuticals to achieve specified chemical effects in the final food product. Currently, there are over 3000 additives with different functions in use in the food industry and they are classified based on their functions. For example, they could be classified as preservatives, colourings, non-nutritive sweeteners, ingredient improvers and many more. As natural preservatives, they are as effective as synthetic preservatives (Etteh 2003; Doyle, 2007; Turkoglu, 2007; Daoliang and Chunjiang, 2009).

The need for food preservation will remain for all time if the world is to cater for the global population which is ever increasing at an alarming geometric progression (FAO/WHO, 1994). The need for food preservation will increase as new food sources are expected to cater for the ever-increasing global human population (Kumar and Panneerselvam, 2007). Traditional methods of preservation usually aim to shut out air, moisture, and microorganisms (Aworh, 2008). Synthetic/chemical preservation are generally seen as an almost perfect method of ensuring food availability. They are also commonly used because it has been reported that they have a longer shelf life and they assist with contamination by inhibiting the growth of moulds and bacteria.

Many scientific investigations have shown that some of these chemical preservatives used, especially those with antimicrobial functions have adverse effect on health in different test systems (Turkoglu, 2007). Numerous potentially mutagenic chemicals have been studied mainly because they can cause damaging and heritable changes in the genetic material, which are usually not immediately expressed. It has also been demonstrated that many of the chemical food preservatives are decomposed or converted into other by-products such as sulphites, disulphides or sulphides and many more have a variety of biological effects that could be antimicrobial, antioxidizing or chelating (Armando *and Pilar,* 2006).

Sodium benzoate occurs naturally in several fruits like the apples, cranberries, prunes and in spices like cinnamon and cloves. The presence of sodium benzoate in these foods does not make it function as a preservative. It is also present in beers, tomatoes and other sauces. It is usually produced chemically and added as a preservative in foods where it has major antimicrobial function, being most effective against yeast and mould. Sodium benzoate is a common preservative in soft drinks because it suppresses the growth of bacteria and fungi under the acidic

conditions found in carbonated beverages. It has excellent solubility in water and it is sparingly soluble in alcohol (Seager and Slabaugh, 2000). Sodium metabisulphite is also known as E223 in the food industry. Food items containing this preservative are some fruit juices, concentrated soft drinks, beer and wine. Sulphites are primarily used as antioxidants or antimicrobial agents to prevent or reduce the discolouration of light-coloured fruits and vegetables. Sodium metabisulphite is an excellent anti-melanosis additive for sea food (Omar, 1998). It can decrease the vitamin composition, especially of vitamin B_1 in food; as an additive, non-sensitives are safe (Luck *et al.*, 1997).

There are various reports on the abuse of some food additives but to prevent this and also to ensure that consumers have food products of suitable quality, national regulatory authorities of each country and the Codex Alimentarius Commission publish lists of permitted additives, the modes of their administration and recommended dosages in specified foods. In Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC) has adopted the Codex General Standard for Food Additives (GSFA) and uses the permitted list of additives attached to that standard (Etteh, 2003). The accepted daily intake (ADI) is an estimate of the amount of food additive expressed on a body weight basis that can be ingested daily over a life time without appreciable health risk. The ADI of benzoates is 0-5 mg/kg of body weight while that of sulphites is 0-0.7 mg/kg of body weight (FAO/WHO, 1994).

The *Allium* assay was introduced by Levan in 1938; it is a short term biological assay and has been proposed as a standard method for toxicity testing. Some advantages of *Allium* assay include the fact that *A. cepa* is readily available all year round, it is relatively easy to handle, it provides good mitotic spreads for analysis, it is economical and shows a good correlation with a number of other test systems (El-shahaby *et al.,* 2003; Fiskesjo, 1985). *Allium cepa* roots contain oxidase enzyme which activates the conversion of promutagens into mutagens.

The 1974 World Health Organization Report Series on food additives reported that benzoate was toxic to mice, rats, rabbits, guinea pigs and dogs. It has also been reported that sodium benzoate and sodium sulphite in the roots of *Vicia faba* inhibit DNA synthesis and cause the formation of anaphase bridges, premature chromosome condensation leading to pycnotic nuclei and chromatin erosion in interphase nuclei (Njagi and Gopalan, 1982). The genotoxicity tests for benzyl alcohol, benzoic acid and sodium benzoate have been reported to have mostly negative effects but some assays were positive (Turkoglu, 2007). Ishidate and Odashima (1977) reported positive chromosomal aberration tests *in vitro* on Chinese hamster cells grown in culture with sodium benzoate.

Rencuzogullari *et al.* (2001a) in their work on the effect of this food preservative in human lymphocytes recorded induced chromosome aberrations and sister chromatid exchanges, decreased replication and mitotic indices that were dose-dependent. Sodium metabisulhpite was also found to have genotoxic effect on the bone marrow of rats (Kayraldiz and Topaktas, 2007). In plants, it was also recorded that sodium metabisulphite induced a significant reduction of frequency of dividing meristematic cells in *Calendula officinalis* (pot marigold) root tips. The most frequent aberration observed was the anaphase-telophase bridges. This incidence of aberrant cells increased proportional to increase of food additive concentration (Marc and Capraru, 2008).

MATERIALS AND METHODS

The root tips of *Allium cepa* (Linn.) were used as the test system. The dry outer scales were removed from healthy onion bulbs and the discs were trimmed at the base with a clean, sharp blade taking great care not to destroy the root primordia. The prepared bulbs of *Allium cepa* were seated on $25cm^3$ vials filled with tap water for 2-4 days. The food preservatives, sodium benzoate (E211) and sodium metabisulphite (E223) were used as the test substances.

The effect of the test substances on the onion root tips was conducted using 0.25M, 0.10M, 0.05M and 0.025M of the substances and the onion bulbs were placed directly on them. In another setup, there was the initial growth of the onion bulbs in tap water, the bulbs were transferred to a series of concentrations of the two test substances for 3, 6, 9 and 24 treatment hours. Five bulbs were used for each concentration and duration of treatment as well as for the control setup left in tap water. The lengths of the newly emerged roots were measured before transfer to the test solutions and also after the 6

hours and 24 hours treatment times. A concentrationresponse curve was drawn with the EC_{50} determined by simple interpolation (Taiwo *et al.,* 2006). After each treatment time, some root tips were removed and fixed immediately in 1:3 Acetic-Ethanol for 24 hours.

Five root tips were cut from each bulb. After fixation, the root tip was placed at the centre of a clean microscope-slide and cut into smaller bits using dissecting needles. The root tip was macerated in drops of 1N HCl for hydrolysis for 5 minutes. After maceration, the HCl was mopped up with filter paper; the root tip was then stained with two drops of Lactic Acetic Orcein and left for 20 minutes. Afterwards, the cells were squashed gently and evenly spread (Sharma and Sharma, 1999). Only one root was used per slide and five slides were made from each bulb. The stained material on the slide was carefully covered with a cover-slip ensuring that no air was trapped in the process. The slide was covered with a sheet of filter paper and pressed down firmly to remove any excess stain. Good slides were preserved by sealing them with colorless nail varnish. Each treatment was done five times to ensure that the results obtained were consistent.

A recovery test was also conducted in order to determine whether the effects induced by treatments with the test substances were permanent or whether the roots could recover from the treatment according to Williams and Omoh (1996). For this test, the onion bulbs with newly emerging roots of harvestable lengths were transferred to 0.25M and 0.025M solutions of both sodium benzoate and sodium metabisulphite for 3 hours. After exposure for 3 hours with the test solutions, the onion bulbs were transferred to tap water and the water changed after 3, 6, 9, and 24 hours. Five slides for each concentration were made for 6 hours and 24 hours recovery in water. Another test was set up whereby 30ml of 0.25M and 0.025M concentration of sodium benzoate and sodium metabisulphite (the highest and lowest concentrations) was prepared separately and then mixed in a 1:1 ratio. Five slides for each concentration were made for 3 hours, 6 hours, 9 hours and 24 hours.

The prepared slides were placed on a Zeiss light microscope for viewing. Five random fields were used per slide for recording of mitotic cells and aberrations. Photomicrographs of some slides were made under the X40 objective lens or the X100 objective (oil immersion lens). The mitotic indices were calculated by dividing the number of dividing cells per field by the total number of cells per field and multiplying the results by 100. The software package: Statistical Package for Social Scientists (SPSS Version 17) was used for other data analysis.

RESULTS

The bulbs placed directly on different concentrations of sodium metabisulphite and sodium benzoate showed no root germination even after five days exposure. In the setup with the initial germination of the onion bulbs in tap water for 24 hours before their transfer to the different concentrations used, it was observed that the roots emerged faster; the germinated roots were usually robust, upright and with a characteristic white colour. The roots of bulbs exposed in 0.25M of sodium metabisulphite are however sticky, staying attached to each other for support and a loss of the characteristic white colour that tapers to the root tips. This effect was also observed in other treatments with higher concentrations and duration of exposure of sodium metabisulphite and sodium benzoate. It was observed that there were decreases in the root length as the concentrations increased (Table 1).

The Effective Concentration 50 (EC_{50}) computed by interpolation was found to be 0.085M for sodium metabisulphite and 0.075M for sodium benzoate. Further analysis using Spearman's correlation was done and it revealed that sodium metabisulphite had a negative correlation with a correlation coefficient of ($r = -1.00$) that was significantly different at $p < 0.05$ from its control. Sodium benzoate on the other hand, also had a negative correlation ($r = -0.700$) which was not significantly different ($p < 0.05$) from the result of its control.

The food preservatives caused a change in the frequencies of different mitotic stages. The mitotic cells

Table 1: Effect of Different Concentrations of sodium Metabisulphite and Sodium Benzoate on Root Length of *Allium cepa*.

Concentration (M)	Mean root length before treatment (cm)		Mean root length after 24 hours treatment (cm)		Growth in % of control	
	Sodium metabisulphite	Sodium benzoate	Sodium metabisulphite	Sodium benzoate	Sodium metabisulphite	Sodium benzoate
Control (0)	1.55 ± 0.092	1.75 ± 0.229	1.84 ± 0.110	2.25 ± 0.147	100	100
0.025	1.54 ± 0.094	2.11 ± 0.180	1.15 ± 0.047	1.97 ± 0.185	62.50	87.55
0.050	1.78 ± 0.186	1.53 ± 0.169	1.12 ± 0.121	1.29 ± 0.136	60.86	57.33
0.100	1.63 ± 0.143	1.77 ± 0.190	0.90 ± 0.159	1.61 ± 0.207	48.91	71.55
0.250	1.70 ± 0.174	1.85 ± 0.192	0.74 ± 0.108	1.54 ± 0.187	40.21	68.44

Table 2. Mitotic Index (MI) of Sodium Metabisulphite(SMB) and Sodium Benzoate (SB) at Different Concentrations and Durations.

Concentration (M)	Time							
	3h		6h		9h		24h	
	SMB	SB	SMB	SB	SMB	SB	SMB	SB
0.000	13.228	12.339	13.228	12.339	13.228	12.339	13.228	12.339
0.025	3.112***	8.353	1.961***	8.034	1.259***	3.416*	0.882***	2.131**
0.050	2.061***	8.433	1.329***	4.215*	0.899***	2.078**	0.181***	1.305**
0.100	2.163***	2.311**	1.326***	1.850**	0.419***	1.451**	0***	0.796**
0.250	1.272***	1.363**	0.426***	0.749**	0***	0.170***	0***	0***

The Mitotic Index (MI) decreases in the two test substances, sodium metabisulphite and sodium benzoate as the duration of treatment and concentrations increases. When MI = 0, this means that at such treatment periods and concentrations, the test preservative have a toxic effect on the root cells of *A. Cepa*

Table 3 Frequenccies and the Different Classifications of Abnormal/Aberrant Cells Observed upon Treatment of *A. cepa* Root Tips with sodium metabisulphite and sodium benzoate at Different Concentrations and Durations.

Duration of treatment (Hours)	Concentrations (M)	Total no of cells examined		Clumping		Bridge		Fragmentation		Pulverization		Binucleate Cells		Lagging		Erosion		Reduction		Disintegration	
		SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB
3	0.000 (Control)	1013	1013	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.025	964	862	10	7	0	0	4	2	2	0	5	6	3	1	0	0	0	0	0	0
	0.050	825	747	13	8	2	1	9	15	11	3	6	2	1	3	0	0	0	0	0	0
	0.100	601	649	9	9	1	0	11	6	5	2	8	4	0	0	10	0	2	0	5	0
	0.250	629	587	14	16	2	0	6	0	12	10	4	0	3	0	15	4	2	0	8	7
6	0.025	867	473	11	8	1	2	9	6	5	1	7	5	3	2	3	3	0	0	1	0
	0.050	752	688	20	12	5	2	7	4	12	9	9	10	2	0	1	0	0	0	0	0
	0.100	528	649	12	10	0	2	14	3	13	8	5	9	5	0	8	5	10	6	5	2
	0.250	469	534	17	9	0	4	5	14	18	11	6	0	1	0	24	13	2	0	7	3
9	0.025	556	644	17	11	2	3	6	8	12	4	9	3	1	0	6	6	2	1	6	4
	0.050	556	722	19	12	1	0	9	6	15	8	7	3	1	0	11	6	6	2	4	2
	0.100	477	689	14	16	0	2	9	3	20	17	7	2	2	1	16	9	18	8	10	11
	0.250	448	588	4	8	0	3	7	5	15	13	10	7	0	0	30	12	8	7	2	0
24	0.025	567	704	14	14	4	2	13	11	7	8	11	2	0	0	7	6	3	1	6	0
	0.050	553	613	23	16	0	2	8	3	32	18	12	2	0	1	26	11	9	4	11	9
	0.100	353	628	16	8	0	2	7	4	23	15	12	6	0	0	31	14	12	6	10	19
	0.250	479	509	8	11	1	2	5	3	22	15	6	3	0	0	38	20	7	3	4	8

Table 4: Mitotic cells, % of Mitotic Stages and Mitotic Index Observed upon 3 hours Treatment of *A. cepa* Root Tips with 0.025M and 0.250M Concentrations of sodium metabisulphite and sodium benzoate at Different Durations of Recovery in Tap Water.

Recovery time (Hours)	Concentr-ation (M)	Total no of cells examined		Total Mitosis		Prophase		Metaphase		Anaphase		Telophase		% Prophase		% Metaphase		%Anaphase		% Telophase		Mitotic index (mean ± S. E.)	
		SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB
	0.000 (control)	1013	1013	134	125	60	62	18	24	25	15	31	24	44.78	49.60	13.43	19.20	18.66	12.00	23.13	19.20	13.207 ± 0.681	12.339 ±0.475
3	0.025	612	635	13	41	6	21	1	4	3	5	3	11	46.15	51.22	7.70	9.76	23.07	12.20	23.07	26.82	2.124± 0.305	6.456± 0.538
	0.250	738	701	7	5	4	3	0	0	1	0	2	2	57.14	60	0	0	14.29	0	28.57	40	0.948± 0.151	0.713± 0.145
6	0.025	499	572	16	69	7	25	2	8	3	23	4	13	43.75	36.23	12.50	11.60	18.75	33.33	25	18.84	3.206± 0.418	12.063 ±0.837
	0.250	655	868	13	10	6	5	2	1	3	1	2	3	46.15	50	15.38	10	23.08	10	15.38	30	1.985± 0.224	1.152± 0.173
24	0.025	635	743	26	96	12	33	2	17	6	22	6	24	46.15	34.38	7.69	17.70	23.08	22.92	23.08	25	4.094± 0.367	12.920 ±0.901
	0.250	582	702	20	15	9	7	3	1	4	1	4	6	45	46.67	15	6.67	20	6.67	20	40	3.436± 0.461	2.137± 0.255

Table 5: Frequencies and the Different Classifications of Abnormal/Aberrant Cells Observed Upon 3 hours Treatment of *A. cepa* Root Tips with 0.025M and 0.250M Concentrations of sodium metabisulphite and sodium benzoate at Different Durations of Recovery in Tap Water.

Recovery time (Hours)	Concentration (M)	Total no of cells examined		Clumping		Bridge		Fragmenta-tion		Pulverization		Binucleate cells		Lagging		Erosion		Reduction		Disintegration	
		SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB	SMB	SB
	0.000 (control)	1013	1013	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0.025	612	635	7	5	0	0	3	3	2	1	0	0	0	4	6	10	8	0	9	2
	0.250	738	701	12	8	0	0	6	3	0	0	0	2	0	0	14	7	6	0	10	5
6	0.025	499	572	10	3	0	0	0	0	0	0	3	1	0	0	4	3	0	0	0	0
	0.250	655	868	8	4	0	0	0	6	0	0	3	0	0	0	7	3	0	0	8	4
24	0.025	635	743	4	1	0	0	0	0	0	0	1	2	0	5	2	0	0	0	3	0
	0.250	582	702	5	2	0	0	0	0	0	0	2	0	0	3	9	4	0	0	0	0

decreased as the concentrations and duration of treatment increased, with a large number of the cells in prophase and the least cells at anaphase. There was decrease in the Mitotic Index as the concentration and duration of treatment increased for both test preservatives (Table 2). This reduction was more intense in sodium metabisulphite than in sodium benzoate. The Two-way ANOVA results of the Mitotic Index of sodium metabisulphite showed that the means of the Mitotic Index at all concentrations and treatment times were significantly different ($p < 0.001$) from the means of the control. Analysis on the Two-way ANOVA of the Mitotic Index of sodium benzoate showed that there was no significant difference between the means of the control experiment and the means of 0.025M, 0.050M at 3 hours treatment time and 0.025M at 6 hours treatment time. The means were, however, significantly different ($p < 0.05$) at concentrations of 0.050M, 0.025M and at 6 hours and 9 hours respectively.

Different chromosomal abnormalities were observed on the treatment slides but none was observed in the control. Some chromosome aberrations observed were chromosome clumping, chromosome bridge and chromosome fragmentation. An extreme type of fragmentation was also observed and cells in such state are said to show pulverization, abnormal condensation, erosion of the chromatin materials and total disintegration or reduction of the chromosome structure (Plates 1-4). Sodium metabisulphite was observed to have induced more aberrations. It was also observed that, at higher concentrations and exposure times, the frequencies of clumped, pulverized and eroded cells increased. The cytogenetic and toxicity effects of the test substances depended on their concentration and duration of exposure to the root tips.

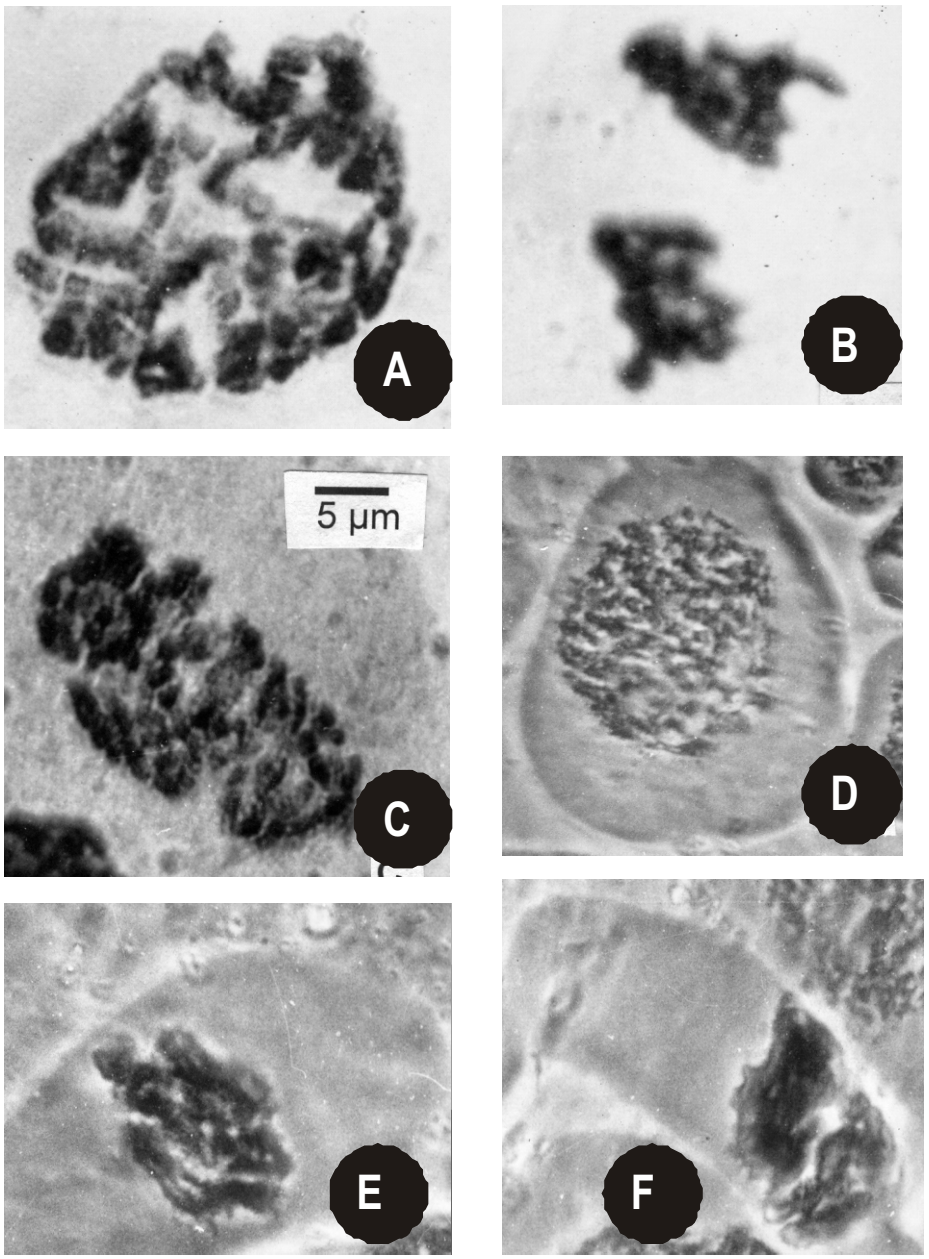

Plate 1: Aberrations Induced by Sodium Metabisulphite and Sodium Benzoate

A. Fragmentation at Prophase
B. Clumping at Anaphase
C. Abnormal chromatin fragmentation at early Prophase
D. Enlarged nucleus at Prophase;
E. Clumping at Metaphase

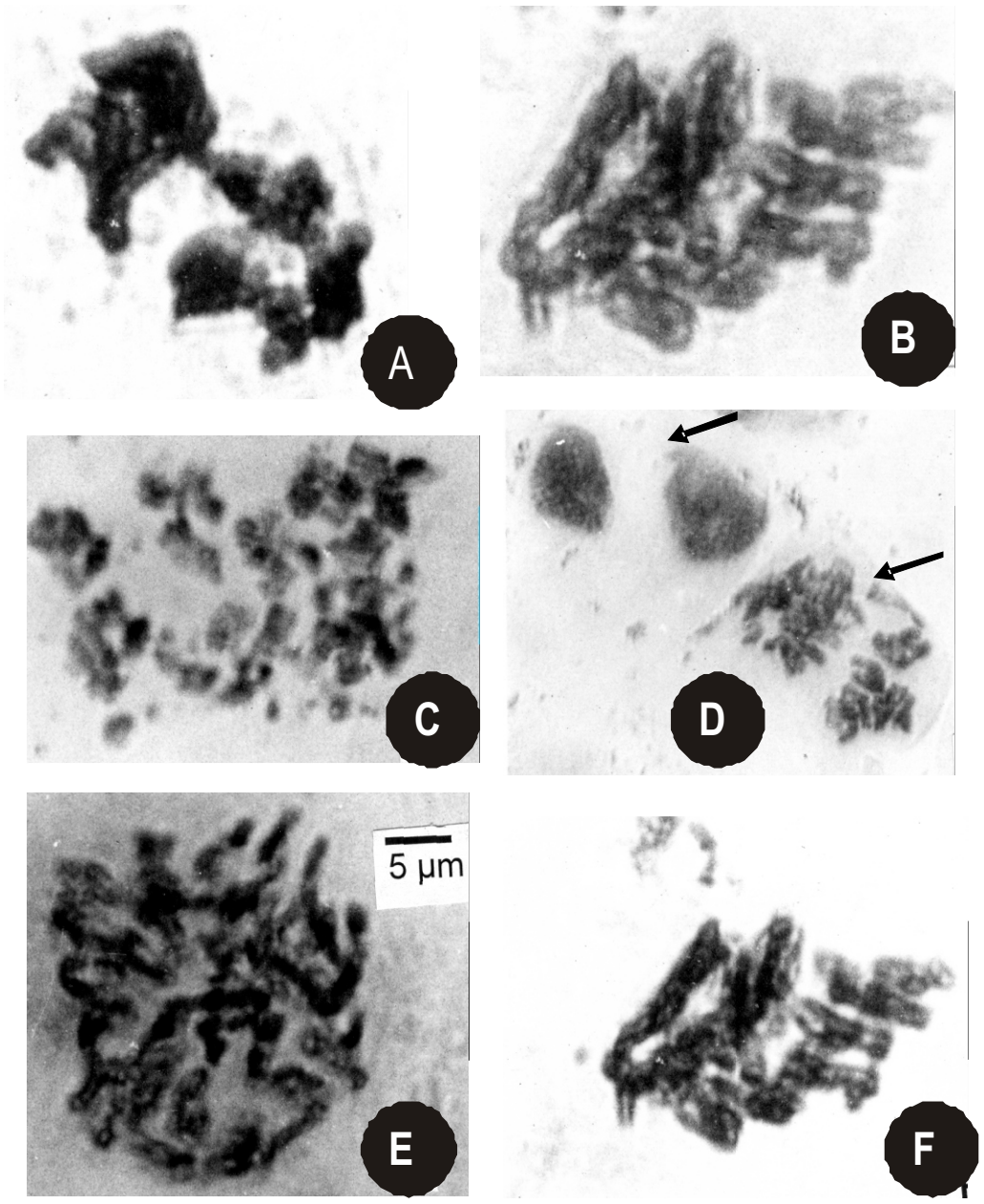

Plate 2: Aberrations Induced by Sodium Metabisulphite and Sodium Benzoate

A. Clumping at Metaphase
B. Clumping at Prometaphase
C. Intense clumping and fragmentation of chromosomes
D. Clumping at Anaphase (upper arrow) and fragmentation (lower arrow)
E. Fragmentation at Prometaphase
F. Wavy outlines of chromosomes at Prometaphase

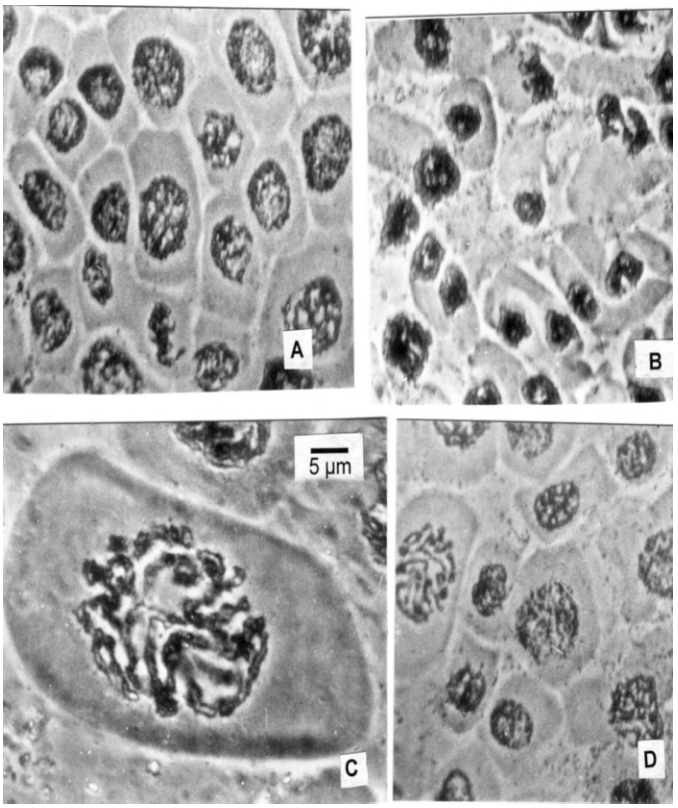

Plate 3: Aberrations induced by Sodium metabisulphite and Sodium benzoate
A. Nuclear outline normal B. Nuclear outline disrupted;
C. Wavy chromosome outlines; D. Normal Prophase

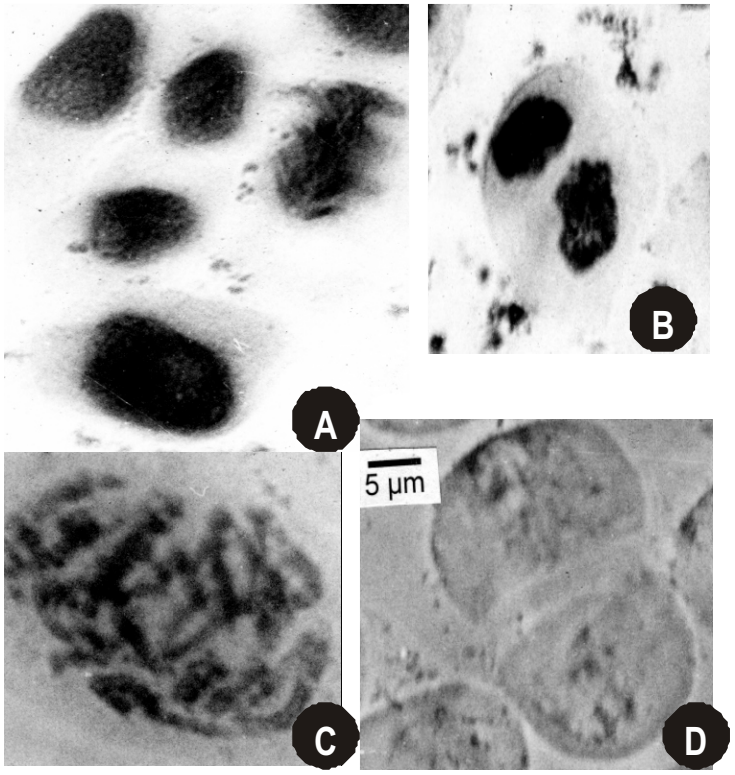

Plate 4: Aberrations induced by Sodium metabisulphite and Sodium benzoate
A. Clumping at Metaphase B. Binucleate cells C. Clumping and fragmentation at Prophase
D. Cells apparently devoid of chromatin material

The mitotic cells observed increased with the duration of recovery, with a large number of the cells in Prophase. After the 3 hours initial treatment in 0.025M of sodium benzoate, and 3 hours recovery in tap water, all the mitotic stages were observed but at 0.250M of the same 3 hours recovery time, metaphase and anaphase stages were not observed. But after the 24 hours recovery in tap water, the different mitotic stages were observed for both 0.025M and 0.250M (Table 5). Mitotic Index frequencies of both sodium metabisulphite and sodium benzoate increased as duration of recovery increased. Aberrations were observed on the recovery tests of both sodium metabisulphite and sodium benzoate but there was a decrease in their frequencies as the recovery times increased from 6 hours to 24 hours.

Results for the number of mitotic cells, percentage of mitotic cells, Mitotic Index and frequencies of aberrant cells observed on combinations of the test substances, sodium metabisulphite and sodium benzoate, are shown in Table 4. It was observed that the mitotic Index decreased as the duration of treatment increased. At the 3-hour treatment time, clumping, fragmentation and pulverization were the only aberrations observed while at the 24-hour treatment time, more aberrant cells were observed.

DISCUSSION AND CONCLUSIONS:

Plant systems are sensitive biomonitors of the cytotoxic and genotoxic effects of different chemicals both *in situ* and in the laboratory (Grant, 1999). Positive results monitored in higher plant systems like the *Allium* assay indicate the presence of cytotoxic and/or genotoxic attributes of some compounds. These also indicate the potential for direct or indirect risks for other living organisms (Fiskesjo, 1993).

Growth retardation was observed in onion root tips exposed to high concentrations and longer duration of treatment for the two preservatives. Growth inhibition was estimated as EC_{50} which is the effective concentration of a chemical producing 50% of the total effect. The results obtained from the EC_{50} (0.075M for sodium benzoate and 0.085M for sodium metabisulphite) indicated that sodium metabisulphite was more toxic than sodium benzoate when tested with *A. cepa* using the root length. Neves *et al.* (2010) reported that, using soybean, the decrease in root length may be owing to the enhanced lignin production that solidifies the cell wall and restricts root growth. Using cinmethylin, a herbicide, it was reported that inhibited mitotic entry might also be the cause of growth inhibition in various plants (El-Deek and Hess, 1986).

The food preservatives used in this study caused a change in the frequencies of the different mitotic stages. Sodium metabisulphite and sodium benzoate increased the percentage of prophase at the different concentrations and duration of treatment. This is in agreement with the results obtained from the works of Rencuzogullari *et al.* (2001a) and Turkoglu (2007). However, at 3 hours exposure of *A. cepa* in 0.250M of sodium metabisulphite, telophase had a greater percentage; this is a deviation from recorded works and the distribution pattern of the daily mitotic pattern in *A. cepa* (Stephens, 1984).

The inhibition of mitotic activities is often used for tracing cytotoxic substances (Yildiz and Arikan, 2008). The two food preservatives used in this study caused a reduction in the Mitotic Index of *Allium cepa*. The concentration-dependent inhibition of the Mitotic Index illustrates the cytotoxic potentials of Sodium metabisulphite and Sodium benzoate in *A. cepa*. 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). Reduction in the mitotic activity could be due to inhibition of DNA synthesis which might be caused by the decreasing ATP level, which is essential for progress of mitosis and the pressure from the functioning of the energy production center (Rencuzogullari *et al.*, 2001a). A decrease in Mitotic Index could also arise as a result of a blockage at the G2-phase of the cell cycle, preventing the cell from entering mitosis.

In this study, nine types of chromosome aberrations were recorded: clumping, chromosome bridges, fragmentation, pulverization, binucleate cells, lagging, erosion of chromatin material, reduction in chromosome size and disintegration of chromosome materials. The numbers of aberrant cells observed in the roots treated with different concentrations and durations of treatment of the test food preservatives was different from those of the untreated control as no aberration was observed in the control. The percentage of aberrations

increased with increasing concentration and duration of treatment. There is no single overall theory which can explain all the aberrations since they are probably induced through different mechanisms. There is certainly no doubt that the depression of energy systems, interference with DNA synthesis at the S-phase, protein synthesis and binding/low uptake of Ca^{2+}, Mg^{2+} and Fe^{2+} which affects the integrity of the chromosome may have a role to play in fragmentation, pulverization and clumping of chromosomes. Bridges arise from joined ends of broken sister chromatids while lagging results from failure of chromosome movement or acentric fragments. Rencuzogullari *et al.*, (2001a) showed that sodium metabisulphite caused laggard chromosomes in *A. cepa*. Turkoglu (2007) also reported that potassium sulphite, potassium nitrate, boric acid, citric acid, potassium citrate and sodium citrate used as food preservatives caused laggards in *A. cepa*. The observation that the number of cells with aberrations increase with an increase in the duration of treatment and concentration of the test substances used, is in agreement with the results of the experiments by Rencuzogullari (2001a; b) and Samuel *et al.* (2010).

Observations from the recovery experiment showed that at the concentrations of sodium metabisulphite and sodium benzoate used, the longer the period spent in water after 3 hours pretreatment with the different test substances, the greater the recovery of the cells from the effects induced in them. More dividing cells and fewer cells with aberrations were observed at the end of the 24 hours recovery test period than there were at the 3 hours duration of treatment. According to Luck *et al.* (1997), a combination of sorbic acid and benzoic acid inhibited a number of bacteria strains better than either sorbic acid or benzoic acid alone. Harrington and Hills (1966) also reported that combining sorbic acid and diethyl pyrocarbonate produced an increased antimicrobial action. The latter named preservative ensured that the germs present are killed rapidly while the sorbic acid provides protection against re-infection. It is assumed that because of the advantages mentioned above, a combination of sodium metabisulphite and sodium benzoate is used as an antimicrobial and antioxidant preservatives especially in fruit juices (Luck *et al.*, 1997).

Research with other test systems has given useful information on the toxicity of sodium metabisulphite (Rencuzogullari *et al.*, 2001a) but more work is required on sodium benzoate to see if the results obtained from the *Allium* assay correlates with that of other test systems. Further work is needed to determine the effects of food preservatives when combined with other food preservatives. When this is done a recommended standard could also be obtained when food preservatives are combined in other to reduce any detrimental effects on consumers. The result of investigation support the banning of these two chemicals as food preservatives.

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