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# Genotoxic effects of mono-, di-, and trisodium phosphate on mitotic activity, DNA content, and nuclear volume in *Allium cepa* L.

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**Abstract —** The effects of the food additives monosodium phosphate (MSP), disodium phosphate (DSP) and trisodium phosphate (TSP) have been studied on root tips of *Allium cepa* L. Roots of *A. cepa* were treated with a series of concentrations, ranging from 300 to 500 ppm for 24, 48 and 72 h. The results indicated that these food preservatives reduced mitotic division in *A. cepa* when compared with the respective control. Mitotic index values were generally decreased with increasing concentrations and longer treatment times. Additionally, variations in the percentage of mitotic stages were observed. The total percentage of aberrations generally increased with increasing concentrations of these chemicals and longer period of treatment. Different abnormal mitotic figures were observed in all mitotic phases. Among these abnormalities were stickiness, anaphase bridges, C-mitosis and micronuclei. These food additives remarkably depressed the DNA content in the root meristems of *A. cepa.* The interphase nuclear volume (INV) also varied between the untreated (controls) and the treated plants.

**Key words:** chromosomal abnormalities; DNA content; genotoxicity; mitotic index; nuclear volume; sodium phosphates.

## INTRODUCTION

For centuries, man has treated food to prolong its edible life, and nowadays both traditional and modern preservatives are used widely to ensure the satisfactory maintenance of quality and safety of foods. However, when the food additives are given to organisms in excessive amounts, they may cause toxic reactions. It has been reported that certain food additives are genotoxic in different test systems (Rencüzoğullari *et al.* 2001a; Rencüzoğullari *et al.* 2001b; Gömürgen 2005; Türkoğlu 2007).

It is well documented that certain types of foods and beverages for human consumption may pose toxic, genotoxic or carcinogenic hazards (Aeschbacher 1990; Wakabayashi 1990). The sources of these hazards can be divided into four categories. First, certain food additives may have harmful effects (IARC 1983). Secondly, food staffs may be contaminated either by environmental pollutents or by microbial toxins (Williams 1986). Thirdly, the processing of food (e. g. cooking, broiling, smoking, pickling, etc.) may produce carcinogenic compounds (Sugimura *et al.* 1986). Fourthly, certain natural constituents foods are also known to possess mutagenic and / or carcinogenic potential (Ames 1986).

Monosodium phosphate (MSP - E339i), disodium phosphate (DSP - E339ii), and trisodium phosphate (TSP - E339iii) are used as antimicrobials, pH control agents (buffers), boiler water additives, cleaners, coagulants, dispersants, leavening agents, stabilizers, emulsifers, sequestrants, texturizers, nutrients, and dietary supplements (online).

Both sodium and phosphates are essential ions in human metabolism. Some studies offered by the database suggest that sodium taken in the diet as sodium phosphates may be correlated to displacement of calcium from the body (Boivin and Kahn 1998), but there is equally compelling data which suggest that perhaps this is not a significant threat (Whiting *et al.* 1997). On the other hand, there is nothing presented in the database or by the petitioner that indicates any positive nutritional or health effect from use of sodium phosphates in processing of food.

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Sodium phosphate in all of its chemical forms has been approved as a dietary supplement (Ash and Ash 1995). However, recent evidence suggests that there might be a relationship between high dietary levels of protein and phosphate and this relation caused increment of urinary calcium excretion. Additionally, a recent report indicated that oral sodium phosphate when taken for bowel preparation may cause electrolyte shifts in patients resulting in death of elderly or seriously ill patients (Boivin and Kahn 1998). Additionally, a study comparing bladder tumor promoting characteristics of sodium phosphates and sodium diphosphate with preformed nitrosamines showed that sodium phosphates, a urine alkalizer, demostrated tumor promoting activity in rats initiated by N-butyl-N-4 hydroxy butyl nitrosamine (BBN) (Shibata *et al.* 1993). The authors suggested further study to better understand how the sodium phosphates in the presence of carcinogens such as nitrosamines function to possibly act as tumor promoters.

Sodium phosphates have been used in food in the preparation of beverage powders, as leavening acids in chemical leavening systems, in cheese and dairy product beverages, puddings, coffee whiteners, whipped toppings, ice cream products, cream cheese and cheese spreads, and egg products (online).

The Ministry of Agriculture of Turkey has suggested that MSP, DSP, and TSP may be used in food at 2500-5000 mg/kg (1998). No studies have been carried out, to our knowledge, on the cytological effects of these chemicals in plants, animals, and human, despite in the fact that it is commonly used. Therefore, it was considered desirable to test the mutagenic potential of the MSP, DSP, and TSP in mitotic cells of *Allium cepa*. This information will thus be helpful in understanding the mechanism of cytological damage.

Higher plants provide a useful genetic system for screening and monitoring environmental chemicals. Mutagenic activity of chemicals has been analyzed with different plant systems such as *Allium cepa* and *Vicia faba*. With these plant systems, chromosomal aberration assays, mutation assays and cytogenetic tests have been performed (Li and Meng 2003; Bolle *et al.* 2004; Marcano *et al.* 2004; Mastrangelo *et al.* 2006; Pandey and Santosh 2007; Türkoğlu 2007, 2008; Yildiz and Arikan 2008). Plant bioassays, which are sensitive and simple in comparison with animal bioassays, have been validated in international collaborative studies under the United Nations Environment Program (UNEP), World Health Organization (WHO) and US Environmental Protection Agency (US EPA), and proven to be efficient tests for genotoxic monitoring of environmental pollutants (Grant 1982, Ma 1982, 1999). Since the study of the effect of several chemicals on plant mitosis may provide valuable information in relation to possible genotoxicity in mammals and especially in human.

In the present study, it was reported the cytogenetic effects of three food additives on the root meristem cells of *A. cepa*, with an aim to provide (1) information which authenticates the genotoxicity potential of these compounds; and (2) possible explanation to validate the use of *Allium* test as alternative model to mammalian test systems for similar studies, by comparing the results of the present study with the outcome of earlier reports published on the mammalian tests.

## MATERIALS AND METHOD

*Chemicals* - In this study, the root tips cells of *Allium cepa* (2n=16) were used as the test system and the food additives monosodium phosphate, disodium phosphate and trisodium phosphate as the test substances. These substances are obtained from Tunçkaya Ltd. The chemical properties of these substances are shown as below:

*MSP (Cat no: N 11-30).* Chemical formula: $NaH_2PO_4 2H_2O$. Chemical name: Monosodium Dihydrogenmonophosphate. Molecule weight 155.96 g/mol. CAS No: 7558-80-7. *DSP (Cat no: N 52-82).* Chemical formula: $Na_2HPO_4$. Chemical name: Sodium Phosphate, Dibasic. Molecule weight 141.77 g/mol. CAS No: 7558-79-4. *TSP (Cat no: N 53-40).* Chemical formula: $Na_3PO_4$. Chemical name: Sodium Phosphate, Tribasic. Molecule weight 163.94 g/mol. CAS No: 7601-54-9.

*Plant material, experimental design, mitotic activity and aberrations* - Bulbs of *A. cepa* were placed in small jars with their basal ends dipping in distilled water and germinated at room temperature (25±2°C). When the newly emerged roots were of 1.00-2.00 cm in length, they were used in the test. Roots of *A cepa* were treated with a series of concentrations of three substances, ranging from 300 ppm, 400 ppm, and 500 ppm for 24, 48, and 72 h. These substances are diluted with distilled water. Control groups were treated with distilled water. Root tips were placed in a solution of ethanol (99%) and glacial acetic acid (3:1) for 24 h, washed with distilled water three times, and they dyed using aceto-orcein. The squash technique was applied for the study of the mitotic

index (MI) and chromosomal aberrations. Three replicates were performed for each treatment and scoring was made from the 3 roots of each replicate. Minimum 1000 mitotic cells were counted from each the slides. The MI was calculated for each treatment as a number of dividing cells /100 cells. The cytological abnormalities were scored in the mitotic cells and the results were shown in the tables and figures. The most abnormalities were presented with micrographs.

*Estimation of in situ nuclear DNA content* - For Feulgen cytophotometric estimation of 2C nuclear DNA contents the fixed root-tips were hydrolyzed in 1 N HCl for 12 minutes at 60 0C and stained Feulgen solution for one hour. The stained root-tips were washed in three changes of SO2 water for 10 minutes each and dried briefly on absorbent paper. Darkly stained root-tips were squashed in a drop of 45% glacial acetic acid. Cytophotometric measurement of 2C telophase nuclei were made, using a Reichert-Zetopan microspectrophotometer, at a wavelength of 550 nm. On average, 100 2C telophase nuclei were measured in each of three replicates in every dose of MSP, DSP and TSP (Van't Hof 1965).

*2. 3. Estimation of interphase nuclear volume* - For the study of INV, it was obtained the mean of two diameters of each selected Feulgen-stained nucleus, observed at right angles to each other and measured under oil-immersion objectives. The INV was calculated using the Formula, $4/3\pi r3$, where r is the radius of the nuclei (Das and Mallick 1993).

*2. 4. Statistics* - Analysis of variance of the data was done with SPSS computer program. For statistical analysis, the one-way analysis of variance (ANOVA) and Duncan mean range test (DMR) was used.

## RESULTS AND DISCUSSION

The effects on MI and the frequency of mitotic phases are given in Tables 1, 2 and 3 for the treatments of MSP, DSP and TSP, respectively. These chemical substances significantly decreased the MI in the treatment groups at all concentrations and in all treatment period. Mitotic activity was reduced as the concentration increased and the period of treatment prolonged. Several other chemicals and food additives have been reported to inhibit mitosis (Türkoğlu and Koca 1999; Sudhakar *et al.* 2001; Rencüzoğullari *et al.* 2001b; Gömürgen 2005; Pandey and Santosh 2007; Türkoğlu 2007). The lowering of MI might have been achieved by the inhibition of DNA synthesis at S-phase (Sudhakar *et al.* 2001). Hidalgo et al (1989) reported that the inhibition of certain cell cycle specific proteins remains as a possible chemicals target site. Thus, inhibition of the enzyme DNA polymerase, which is necessary for the synthesis of DNA as well as other enzymes more directly involved with spindle production, assembly or orientation, could explain the reported antimitotic effect.

Table 1 — Cytogenetic analysis of *A. cepa* root tips exposed to different concentrations of monosodium phosphate for different periods.

| Time of treatment (h) | Concentrations (ppm) | Examined total cells | Total mitosis | % Prophase | % Metaphase | % Anaphase-Telophase | Mitotic index (Mean± S. E.*) | A. B. | S. | C-M. | (%) Total abnormalities* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | 1013 | 90 | 40.25 | 38.25 | 21.50 | 8.90 ± 0.05 a | – | – | – | 0.00 a |
| | 300 | 1015 | 61 | 45.00 | 17.55 | 37.45 | 6.08 ± 0.60 b | 2.94 | 3.77 | 3.43 | 10.14 b |
| 24 h | 400 | 1021 | 49 | 50.26 | 24.28 | 25.46 | 4.85 ± 0.29 b | 2.32 | 4.49 | 3.47 | 10.21 b |
| | 500 | 1032 | 31 | 61.31 | 28.17 | 10.52 | 3.03 ± 0.85 cd | 2.91 | 4.65 | 3.88 | 11.44 b |
| | Control | 1019 | 92 | 45.18 | 30.66 | 24.16 | 9.05 ± 0.25 a | – | – | – | 0.00 a |
| | 300 | 1007 | 57 | 55.20 | 22.30 | 22.50 | 5.73 ± 0.29 b | 2.00 | 4.00 | 4.50 | 10.50 b |
| 48 h | 400 | 1017 | 47 | 53.13 | 25.37 | 21.50 | 4.70 ± 0.43 bd | 2.50 | 4.37 | 6.88 | 13.75 c |
| | 500 | 1011 | 29 | 57.00 | 22.53 | 20.47 | 2.90 ± 0.53 c | 3.00 | 7.00 | 8.00 | 18.00 df |
| | Control | 1023 | 92 | 42.17 | 31.33 | 26.50 | 9.07 ± 0.16 a | – | – | – | 0.00 a |
| | 300 | 1016 | 56 | 53.62 | 20.10 | 26.28 | 5.56 ± 0.23 b | 4.74 | 7.50 | 6.47 | 19.11 d |
| 72 h | 400 | 1006 | 46 | 57.23 | 27.35 | 15.42 | 4.60 ± 0.50 bd | 6.33 | 7.20 | 2.93 | 16.46 e |
| | 500 | 1010 | 27 | 59.00 | 26.00 | 15.00 | 2.70 ± 0.61 c | 4.00 | 8.18 | 5.00 | 17.18 ef |

A. B., Anaphase bridges; S., Stickiness; C-M., C-Mitosis.
* Means with the same letters do not significantly differ at 0.05 level (DMR test).

TABLE 2 — Cytogenetic analysis of *A. cepa* root tips exposed to different concentrations of disodium phosphate for different periods.

| Time of treatment (h) | Concentrations (ppm) | Examined total cells | Total mitosis | % Prophase | % Metaphase | % Anaphase-Telophase | Mitotic index (mean ± S. E. *) | A. B. | S. | C-M. | M. | (%) Total abnormalities* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | 1014 | 66 | 43.78 | 27.23 | 29.32 | 6.53 ± 0.28 ae | – | – | – | – | 0.00 a |
| | 300 | 1026 | 44 | 43.45 | 30.98 | 25.57 | 4.32 ± 1.73 b | 1.53 | 2.18 | 0.87 | – | 4.58 b |
| 24 h | 400 | 1013 | 25 | 43.06 | 37.15 | 19.79 | 2.52 ± 0.57 cd | 0.50 | 2.51 | 2.33 | – | 5.34 b |
| | 500 | 1020 | 23 | 54.50 | 28.75 | 16.75 | 2.26 ± 0.26 cf | 0.60 | 2.00 | 6.50 | – | 9.10 c |
| | Control | 1022 | 62 | 53.22 | 20.22 | 26.58 | 6.08 ± 0.51 ae | – | – | – | – | 0.00 a |
| | 300 | 1008 | 42 | 48.52 | 17.96 | 33.52 | 4.20 ± 1.24 bd | 1.21 | – | 1.28 | – | 2.49 d |
| 48 h | 400 | 1017 | 21 | 50.54 | 23.18 | 26.28 | 2.13 ± 0.60 cf | 1.19 | 2.60 | 6.17 | – | 9.96 c |
| | 500 | 1026 | 17 | 41.52 | 32.72 | 25.76 | 1.67 ± 0.28 c | – | 3.00 | 9.00 | – | 12.00 e |
| | Control | 1014 | 68 | 38.08 | 40.19 | 21.73 | 6.73 ± 0.30 a | – | – | 0.16 | – | 0.16 a |
| | 300 | 1020 | 50 | 52.13 | 17.98 | 29.89 | 4.91 ± 1.30 be | 0.20 | 0.38 | 4.32 | 4.20 | 8.90 c |
| 72 h | 400 | 1000 | 35 | 48.33 | 28.76 | 22.91 | 3.57 ± 1.08 bdf | 0.27 | 4.00 | 9.90 | 3.10 | 17.27 f |
| | 500 | 1030 | 19 | 40.27 | 39.00 | 20.73 | 1.86 ± 0.35 c | 1.38 | 4.75 | 12.00 | 3.60 | 21.73 g |

A. B., Anaphase bridges; S., Stickiness; C-M., C-Mitosis; M., Micronucleus.
* Means with the same letters do not significantly differ at 0.05 level (DMR test).

TABLE 3 — Cytogenetic analysis of *A. cepa* root tips exposed to different concentrations of trisodium phosphate for different periods.

| Time of treatment (h) | Concentrations (ppm) | Examined total cells | Total mitosis | % Prophase | % Metaphase | % Anaphase-Telophase | Mitotic index (Mean ± S. E. *) | A. B. | S. | C-M. | M. | (%) Total abnormalities* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | 1021 | 118 | 36.19 | 27.72 | 36.09 | 11.61 ± 0.27 a | – | – | – | | 0.00 a |
| | 300 | 1013 | 75 | 45.11 | 28.14 | 26.75 | 7.45 ± 0.81 b | – | 3.12 | 4.99 | 2.66 | 10.77 b |
| 24 h | 400 | 1006 | 65 | 49.00 | 22.26 | 28.74 | 6.55 ± 1.13 bc | – | 1.06 | 6.13 | 4.24 | 11.43 b |
| | 500 | 1019 | 56 | 42.00 | 41.77 | 16.23 | 5.56 ± 0.33 cdef | – | 1.46 | – | 3.11 | 4.57 c |
| | Control | 1010 | 113 | 32.26 | 49.78 | 17.96 | 11.20 ± 0.58 a | – | – | – | – | 0.00 a |
| | 300 | 1012 | 65 | 43.98 | 33.56 | 22.46 | 6.47 ± 0.97 bcd | 1.42 | 1.78 | 4.24 | 1.38 | 8.84 d |
| 48 h | 400 | 1007 | 44 | 41.44 | 38.44 | 20.12 | 4.37 ± 0.43 ef | 1.98 | 2.77 | 7.05 | 5.00 | 16.80 e |
| | 500 | 1016 | 60 | 41.87 | 27.06 | 31.07 | 6.00 ± 0.42 bde | – | – | 5.92 | 9.16 | 15.08 f |
| | Control | 1014 | 72 | 36.00 | 41.28 | 22.72 | 7.18 ± 0.74 bd | – | – | – | – | 0.00 a |
| | 300 | 1011 | 49 | 48.27 | 21.31 | 30.42 | 4.92 ± 0.18 cef | 1.83 | 1.47 | 6.11 | 4.43 | 14.07 f |
| 72 h | 400 | 1006 | 39 | 51.40 | 25.00 | 23.60 | 3.97 ± 0.24 f | – | 8.87 | 5.73 | 17.24 | 31.84 g |
| | 500 | 1018 | 20 | 38.00 | 18.02 | 43.98 | 1.98 ± 0.93 g | 1.02 | 4.69 | 6.23 | 8.97 | 20.91 h |

A. B., Anaphase bridges; S., Stickiness; C-M., C-Mitosis; M., Micronucleus.
* Means with the same letters do not significantly differ at 0.05 level (DMR test).

From the results in Tables 1, 2 and 3, it was noted that the rate of each one of the mitotic stages was affected by the treatments in *A. cepa*. MSP increased the percentage of prophase and decreased the percentage of metaphase when compared with the control groups after the all treatment periods. The percentage of the anaphase-telophase stage decreased in the 48-h and 72-h treatment periods (Table 1). DSP increased the percentage of prophase in all treatment periods and metaphase in 24-h and 48-h treatment periods. In the 72-h treatment period, the percentage of metaphase decreased (Table 2). TSP increased the percentage of prophase in the all treatment periods. The percentage of metaphase increased in the 24-h. On the other hand, the percentage of metaphase was decreased in the 48-h and 72-h. The percentage of anaphase-telophase stages decreased 24-h, but increased these stages after 48-h and 72-h treatments (Table 3).

The treatment conducted with these chemicals showed an effect on the percentage of the different mitotic stages where the percentage of prophase increased with a corresponding decreased in the percentages of the other stages. This may attributed to the blocking of cell division by the food additives at the end of the prophase stage. In this case, these chemicals may be accepted as premet-

aphase inhibitors. Similar results have been reported after treatment of *A. cepa* root-tip cells with various other food additives (Rencüzoğullari *et al.* 2001b; Dönbak 2002; Gömürgen 2005; Türkoğlu 2007).

Mitodepressive action of the used chemicals on the cell division may be reason of the reduction of MI in *A. cepa*. Recorded inhibition of DNA synthesis and nuclear volume may be attributed to this mitodepressive action. This inhibition could be due to either the blocking of G1 suppressing DNA synthesis (Schneiderman 1971) or a blocking in G2 preventing the cell from entering mitosis (Sohbi and Haliem 1990; El-Ghamery and El-Yousser 1992; El-Ghamery *et al* 2000).

The types and frequency of chromosomal anomalies produced by the different treatments of MSP, DSP and TSP are shown in Tables 1, 2 and 3. The photographs of these abnormalities are illustrated in Figure 1. Their percentages increased as the concentration of the applied food additives and the duration of treatment increased when compared with the control groups.

MSP induced several chromosome abnormalities in the root tips of *A. cepa*. The percentage cells showing abnormalities are given in Table 1. The abnormalities noted in metaphase cells were anaphase bridges (Fig. 1a), stickiness (Fig. 1b) and C-mitosis (Fig. 1c). The frequency of sticky was greater than other aberrations.

Anaphase bridges, stickiness and C-mitosis were observed after the different treatments with DSP solution (Table 2). In addition to these types, a few cells with micronucleus (Fig. 1d) were observed at interphase stage for 72-h treatment period in *A. cepa*. The C-mitosis was the most prominent among these aberrations. The frequencies of abnormal cells had a positive relation with the length of exposure time and concentration of treatment solution.

In the present work, it has been possible to observe different abnormalities after TSP treatments (Table 3). There had been observed anaphase bridges, sticky metaphases, C-mitosis and micronuclei. The most common type of abnormality observed with all the concentrations and periods of TSP treatments was micronucleus.

In this study, most common aberration was C-mitosis at all treated roots at MSP, DSP and TSP concentrations. C-mitosis was first described by Levan (1938) in root tips of *A. cepa* as an in activated of the spindle followed by the random scat-

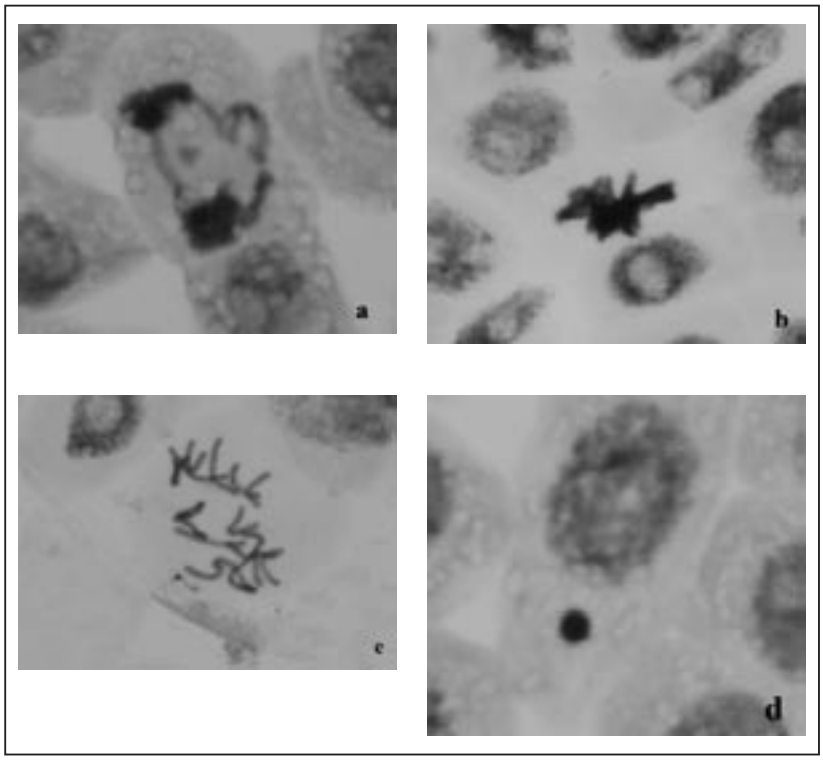


Fig. 1 — Different types of aberrations induced by the food additives in *Allium cepa* root tips. a. Anaphase bridge (MSP, DSP and TSP); b. Sticky metaphase (MSP, DSP and TSP); c. C-mitosis (treated with MSP, DSP and TSP); d. Micronucleus at interphase (DSP and TSP).

TABLE 4 — Effect of different concentrations of MSP, DSP and TSP on DNA contents and interphase nuclear volumes in *A. cepa* root tips after treatments for different durations.

| | | Monosodium phosphate | | Disodium phosphate | | Trisodium phosphate | |
|---|---|---|---|---|---|---|---|
| Time of treatment | Concentrations (ppm) | DNA content (pg ± S. E *) | Nuclear volume ($\mu m^3$± S. E *) | DNA content (pg ± S. E *) | Nuclear volume ($\mu m^3$± S. E *) | DNA content (pg ± S. E *) | Nuclear volume ($\mu m^3$± S. E *) |
| 24 h | Control | 98.37 ± 0.05 a | 568.38 ± 0.62 a | 89.75 ± 1.73 a | 539.78 ± 0.35 a | 82.13± 2.02 af | 498. 53 ± 0.66 ac |
| | 300 | 91.23 ± 0.58 ac | 522.57 ± 0.23 ab | 65.14 ± 0.16 bd | 501.35 ± 1.02 ab | 61.75 ± 0.75 bc | 403.18 ± 0.62 ab |
| | 400 | 93.58 ± 0.06 a | 400.15 ± 0.20 acf | 61.93 ± 0.57 bd | 493.67 ± 1.21 ab | 49.39 ± 2.14 be | 278.27 ± 0.96 bd |
| | 500 | 82.60 ± 0.04 ac | 412.97 ± 0.24 abc | 54.72 ± 0.34 bcd | 427.38 ± 1.31 ab | 31.16 ± 1.39 d | 235.33 ± 0.70 bd |
| 48 h | Control | 90.00 ± 0.13 ac | 577.00 ± 0.17 ac | 88.94 ± 0.26 a | 509.52 ± 1.11 ab | 98.26 ± 1.20 f | 574.27 ± 0.61 ce |
| | 300 | 87.06 ± 0.52 ac | 450.38 ± 0.45 bcd | 68.22 ± 0.13 b | 519.23 ± 1.80 ac | 69.77 ± 0.68 acg | 358.78 ± 0.59 ad |
| | 400 | 75.23 ± 0.14 bcd | 398.53 ± 0.18 bd | 59.21 ± 0.74 bd | 348.51 ± 0.22 be | 51.26 ± 1.64 bh | 303.48 ± 0.38 bd |
| | 500 | 62.45 ± 0.87 be | 403.03 ± 0.05 bd | 50.08 ± 0.51 de | 408.07 ± 1.66 ade | 35.26 ± 0.87 deh | 262.04 ± 0.44 bd |
| 72 h | Control | 85.85 ± 0.60 ad | 501.17 ± 0.25 ade | 90.15 ± 0.58 a | 527.00 ± 0.16 ad | 90.14 ± 1.29 f | 524.24 ± 0.42 ae |
| | 300 | 75.06 ± 0.35 bcd | 427.01 ± 0.69 bef | 60.04 ± 1.25 bd | 371.11 ± 0.56 bcd | 53.97 ± 0.76 bgk | 320.13 ± 0.85 bd |
| | 400 | 59.38 ± 0.29 be | 304.15 ± 0.24 be | 58.29 ± 0.28 bd | 392.34 ± 0.26 ade | 41.55 ± 1.74 dehk | 245.52 ± 0.63 bd |
| | 500 | 47.28 ± 0.65 e | 313.28 ± 0.15 be | 39.95 ± 1.31 ce | 357.28 ± 0.32 bce | 30.90 ± 0.52 d | 221.26 ± 0.99 d |

* Means with the same letters do not significantly differ at 0.05 level (DMR test).

tering of the condensed chromosomes. C-mitosis indicated that, the chemical inhibited spindle formation similar to the effect colchicine (BADR 1983), and induction of C-mitosis commonly associated with spindle poisons, indicating tubogenic effect (SHAHIN and EL-AMOODI 1991). Such C-mitotic cells were also reported to be induced by the treatments with various other food additives (RENCÜZOĞULLARI *et al.* 2001b; DÖNBAK 2002; GÖMÜRGEN 2005; PANDEY and SANTOSH, 2007; TÜRKOĞLU 2007, 2008). The action of the additives like colchicine may be explained by causing certain disturbances in the protein.

In this study, chromosome stickiness was the other major chromosomal aberrations and was recorded in a considerable percentage. Stickiness has been attributed to the improper folding of chromosomal fibers, which makes the chromatids connected by means of subchromatid bridges (BADR and IBRAHIM 1987). KLASTERSKA *et al.* (1976) and MC GILL *et al.* (1974) suggested that chromosome stickiness arises from improper folding of the chromosome fiber into single chromatids and the chromosomes become attached to each other by subchromatid bridges. Chromosome stickiness reflects highly toxic effects, usually of an irreversible type probably lading to death. According to AHMED and GRANT (1972), stickiness of chromosomes might have resulted from increased chromosome contraction and condensation or possibly from the depolymerization of DNA (DARLINGTON 1942) and partial dissolution of nucleoproteins (KAUFMAN 1958). Several chemicals have been reported to induce stickiness, and the results in this study are in agreement with those obtained after treating the different materials with different food additives (TÜRKOĞLU and KOCA 1999; RENCÜZOĞULLARI *et al.* 2001a; RENCÜZOĞULLARI *et al.* 2001b; GÖMÜRGEN 2005; PANDEY and SANTOSH 2007; TÜRKOĞLU 2007, 2008).

Micronuclei were observed after treatments with DSP, TSP but not with MSP (Tables 1-3). Micronuclei are the results of acentric fragments or lagging chromosomes that fail to incorporate in to either of the daughter nuclei during telophase of the mitotic cells (ALBERTINI *et al.* 2000; KRISHNA and HAYASHI 2000). This means that DSP and TSP are clastogens that induce chromosome breaks and/or aneugens that induce lagging chromosomes. Similarly, micronuclei were recorded by many investigators following treatment with different food additives (LUCA *et al.* 1987; MUNZER *et al.* 1990; MENG and ZHANG 1992; GÖMÜRGEN 2005; TÜRKOĞLU 2007, 2008). Micronucleus formation implies loss of genetic materials.

In addition to the above-mentioned type of abnormalities, anaphase bridges were observed in the present investigation. The bridges noticed in the cells are probably formed by breakage and fusion of chromosomes and chromatids (HALIEM 1990). Such chromosome bridges were also reported to be induced by other chemicals. It was reported that the food preservatives sodium benzoate and sodium sulphite caused anaphase bridges in *Vicia faba* (NJAGI and GOPALAN 1982). PANDEY and SANTOSH (2007) and TÜRKOĞLU (2007, 2008)

also reported induction of anaphase bridges following treatments with several food additives in *V. faba* and *A. cepa*. According to GÖMÜRGEN (2005), chromosome bridges may be due to the chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to unequal translocation or inversion of chromosome segments.

The effects of the different treatments of MSP, DSP and TSP on the amounts of DNA and interphase nuclear volume (INV) are shown in Table 4. No studies have been carried out, to our knowledge, on the DNA content and INV of these food additives despite the fact that they are commonly used.

The amounts of DNA and INV were reduced in *A. cepa* under investigation on a result of root treatments with MSP, DSP and TSP when compared with control groups. The degree of the reduction was greater when the concentration was increased and the period of treatment was prolonged. The reduction in the DNA content was highly significant at root treatments with the DSP and TSP. Additionally, it was recorded that the MSP, DSP and TSP reduced the INV. The treatments conducted with these food additives showed no generally significant effect on the INV. Similar results were found after treatment with the several chemicals (BADR and IBRAHIM 1987; EL-GHAMERY and EL-YOUSSER 1992; TÜRKOĞLU and KOCA 1999; EL-GHAMERY *et al.* 2000; MOHANTY *et al.* 2004). The observations suggest that the amount of DNA decreases with decreases in nuclear volume. The effect of these food additives on DNA and nuclear volume, on the other hand, needs further investigations which may involve a study on the DNA polymeras.

The reduction in mitotic activity was accompanied with a depressive action on the amounts of DNA and INV. Increased concentration and prolonged period of treatments resulted in increased reductions in the amounts of DNA and INV. A remarkable decline in the DNA content and INV in the plant exposed to these food additives at the time of germination might be attributed to the intercalation of chemicals into the DNA double helix and the inhibition of DNA synthesis. Because cytophotometric estimation of DNA content involves Schiff's reaction of free aldehydes of DNA with fuchsine sulfurous acid to yield the typical magenta color (SHARMA and SHARMA 1980), the incorporation of these chemicals might cause rigidity of the DNA helix, thus preventing the Feulgen reaction.

The Allium assay is a good and sensitive test system for monitoring of clastogenic effects. Several other chemicals which have been demonstrated to induce chromosomal aberrations and micronucleus formation in allium root meristem cells are also found to produce similar effects in mammals (GIRI *et al.* 2002; HASEGAWA *et al.* 1984; MENG and ZHANG 1992; MURANLI and KAYMAK 2004; RENCÜZOĞULLARI *et al.* 2001a). This, together with the results of the present study, indicate the sensitivity of Allium test and correlates well the mammalian test systems and hence validates the use of Allium test as an alternative to mammalian test system for monitoring the genotoxicity potential of chemicals like pesticides or food additives. FISKESJÖ suggested that (1985), positive results in the Allium test should be considered as a warning and also an indication that the tested chemical may be a risk to human health and to our environment.

In conclusion, from the present test it appears MSP, DSP and TSP, which are used frequently in the food industry have clearly chromotoxic effects. For this reason it is necessary to be careful whether using these chemicals in food as additives. In addition, further cytogenetic studies dealing with clastogenicity and genotoxicity of these food additives may reveal further interesting results.

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