African Journal of Biotechnology Vol. 12(13), pp. 1459-1466, 27 March, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2927
ISSN 1684–5315 ©2013 Academic Journals

*Full Length Research Paper*

# Genotoxic and cytotoxic effects of food flavor enhancer, monosodium glutamate (MSG) using *Allium cepa* assay

**Oyenike A. Adeyemo* and Ayomide E. Farinmade**

Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos State, Nigeria.

Accepted 6 February, 2013

**Genotoxic and cytotoxic effects of monosodium glutamate (MSG) used as flavor enhancer in foods was analyzed using the *Allium cepa* assay. Onion bulbs were grown at different concentrations (1, 3, 5 and 7 g/L) of MSG dissolved in distilled boiled water and also evaluated when dissolved in distilled water without boiling; a control group was also set up. The macroscopic (morphology and color of roots) and microscopic (mitotic index and chromosomal aberrations) parameters of *Allium* root tips were studied. MSG inhibited growth of *A. cepa* root tips in all concentrations and was significant on days 2 to 5 in distilled water without boiling and on days three to five when dissolved in boiled distilled water. MSG also reduced the number of roots growing from primodium in all test concentrations as compared to control and the least was observed in 5 g and 7 g/L. Color of root tips range from brownish to dark brown or black in higher MSG concentrations. Sticky chromosomal aberration at telophase was most commonly induced in all the MSG test concentrations. MSG decreased mitotic index of *A. cepa* cells at all the test concentrations but this result was not statistically different. There was no significant difference in total chromosomal aberrations in all experimental set up as compared to control.**

**Key words:** Monosodium glutamate, genotoxicity, cytotoxicity, *Allium cepa* assay.

## INTRODUCTION

Food additives have been used to keep the quality, texture, consistency, taste, color, alkalinity or acidity of foods. Humans are daily exposed to these chemical substances in their foods. Monosodium glutamate (MSG) is a food additive widely used as flavor enhancer of many foods like meats, poultry, seafood, snacks, soups and stews (Fuke and Shimizu, 1993). MSG is sodium salt of the amino acid glutamate and provides a flavoring function similar to naturally occurring free glutamate in foods (Yamaguchi and Ninomiya, 2000). Glutamate is a major component of most natural protein foods such as meat, fish, milk and some vegetables and plays an essential role in human metabolism (Filer and Stegink, 1994; Fernstorm and Garattini, 2000; Freeman, 2006). MSG is manufactured industrially by a fermentation process of molasses from sugar cane, sugar beets, starch and corn sugar. The breakdown and change of natural bound glutamate into various free forms of glutamate leads to the production of a white crystalline powder, when present in the free form, has a flavor enhancing effect in food (FSANZ, 2003). This distinctive taste is known as “Umami”, a word coined by the Japanese to describe the taste imparted by glutamate. Westerners often describe this flavor as savory, broth-like or meaty (Fuke and Shimizu, 1993). The optimal palatability concentration for MSG is between 0.2 and 0.8% with the largest palatable dose for humans being about 60 mg/kg body weight (Yang et al., 1997).

Since 1960s, MSG has received great opposition against its use as a flavor enhancer. There is a general belief that it has harmful health effects. The high

*Corresponding author. E-mail: adeyemona@gmail.com. Tel: 2348055073665.

**Abbreviation: MSG,** monosodium glutamate.

consumption of MSG, has led to the description of a variety of discomforts described by some non-oriental people after eating at a Chinese restaurant as “Chinese restaurant syndrome” (e.g. flushing, tightness of the chest or difficulty in breathing) after the consumption of Chinese foods (Morselli and Garattini, 1970). There are considerable reports about various adverse effects or intake of MSG as food additive (Stevenson, 2000; Hermanussen et al., 2006; Ortiz et al., 2006; Farombi and Onyema, 2006; Paulovic and Cekic, 2006). However, there are claims that there has been suppression of information on the toxicity/safety of MSG (Samuel, 1999). Also, there has not been a general acceptance that MSG could be toxic to the humans. With all the controversies surrounding the safety of MSG, it is still being consumed in large quantity in fast and packaged foods. It is imperative to carry out safety assessment of this additive. To our best knowledge, there are no published data on cytotoxicity and genotoxicity of MSG using the plant test systems.

Genotoxicity studies are designed to determine chemicals that can perturb genetic material causing gene or chromosomal mutations. A large number of assay systems, especially *in vitro* systems, have been devised to detect the genotoxic effect of different substances. Genotoxicity test results are usually taken as indicators for mutagenic effects. However, the differential responses to chemical substances between animal and plant assays will be as a result of differences in their metabolism. To an extent, results of plant bioassays can reveal potential health hazards in humans. Among plant test systems, *Allium cepa* is one of the most commonly used species for the study of chromosomal aberrations. *A. cepa* test was introduced by Levan (1938) to study cytogenetic short term bioassay and has proven to be a useful tool in basic research to detect the chromosomal aberrations caused by chemicals and complex mixtures such as industrial waste water (Fiskesjo, 1985; Odeigah et al., 1997a). *A. cepa* test represents an alternative first-tier assay to experiments on animals for preliminary toxicity screening in accordance with the Council Directive 86/69 (EEC art .23) encouraging research on alternative techniques not requiring the use of animals. This study was conducted to evaluate the genotoxic and cytotoxic potential of monosodium glutamate using the *A. cepa* assay (Fiskesjo, 1985, 1993). The objectives of this study were: (i) to assess the effect of MSG on the growth of *A. cepa* root tips; (ii) to assess genetic effects of MSG on mitotic index and chromosome damage to root meristem cells of *A. cepa*.

## MATERIALS AND METHODS

Monosodium glutamate flavor sachets as the test substance were obtained from Iyana Ipaja local market in Lagos, Nigeria. The brand of MSG sachets was Vedan made in China. In this study, dry healthy *A. cepa* (125 bulbs) of 1.5 to 2.0 cm in diameter were purchased. The outer papery brown layer of each onion was peeled away and the dried basal root plate was cleaned. The MSG were dissolved in distilled water without boiling and also in boiled distilled water at concentrations of 1.0, 3.0, 5.0 and 7.0 g/L (concentration used in foods) at room temperature. MSG solution (150 ml) was dispensed into the culture bottles and the onion placed on the culture tubes; root growth was observed for five days. Controls were included for each experiment set up. In addition, pure white and purple color onion bulbs were used for each experiment to validate the results obtained in the first sets of experiments.

For macroscopic study, root tips of equal length were used in all five replicates per group. The root length was measured to the nearest millimeter using a graduated ruler every day for five days. The root form was studied to check for presence of twists (crocket hooks), presence of swellings (c-tumors), broken root tips, normal roots (straight) and color of root tips (white, pale and dark brown or black). Digital photographs of growing onions were taken.

For microscopic studies, mitotic stages and chromosomal aberrations in *Allium* root cells were determined using light microscope. The root tips were fixed in fixative (95% ethanol: acetic acid, 3:1 v/v) for 90 min, hydrolyzed in 1 N HCL, stained in 1% aceto-orcein stain and squashed in 45% glacial acid on microscope slides. The mitotic index (MI) was determined as the percentage of number undergoing mitosis relative to the total number of cells examined by scoring at least 900 to 1000 cells per group. Chromosomal aberrations were also scored from these cells. Photomicrographs of cells were taken.

### Statistical analysis

GraphPad Prism was used to construct graphs and 2-way analysis of variance (ANOVA) was used to compare means of root length. The significance difference of root length in test experiments and their control were determined by Tukey’s test (P=0.05%). Data on cells at mitosis were compared using analysis of variances (ANOVA) and the significance difference of number of chromosomal aberrations and MI in test experiments and their controls were determined by Tukey’s test (P=0.05%). The analyses were performed using GraphPad Prism Statistical Software version 6.

## RESULTS

### Macroscopic studies

Monosodium glutamate dissolved in water without boiling and in boiled water inhibited growth of *A. cepa* root tips in all concentrations and growth periods as compared with the control (Figures 1 and 2). However, root tips were more inhibited when *Allium* bulbs were grown in MSG concentrations dissolved in water without boiling. This inhibition was significant in all concentrations on days two to five when MSG with different concentrations were dissolved in water without boiling and on days three to five when MSG was dissolved in boiled water. MSG reduced the number of roots growing from primodium in all test concentrations as compared to control and the least was observed in 5 g and 7 g/L. The root shape of *A. cepa* showed some swellings in 5 and 7 g/L concentrations. Color of root tips range from brownish in 5 and 7 g/L dissolved in distilled water without boiling to dark brown or black in 7 g/L concentration dissolved in boiled distilled water. Plate 1 shows mild crochet hooks,

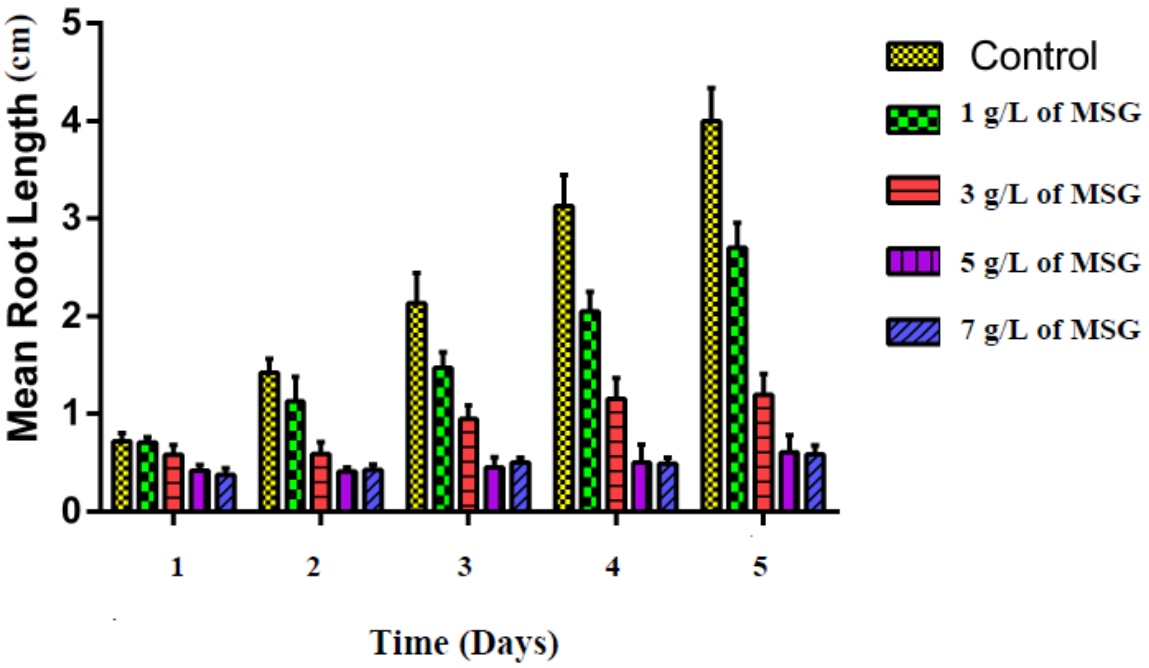


**Figure 1.** Mean root length of *A. cepa* grown in monosodium glutamate dissolved in distilled water without boiling.

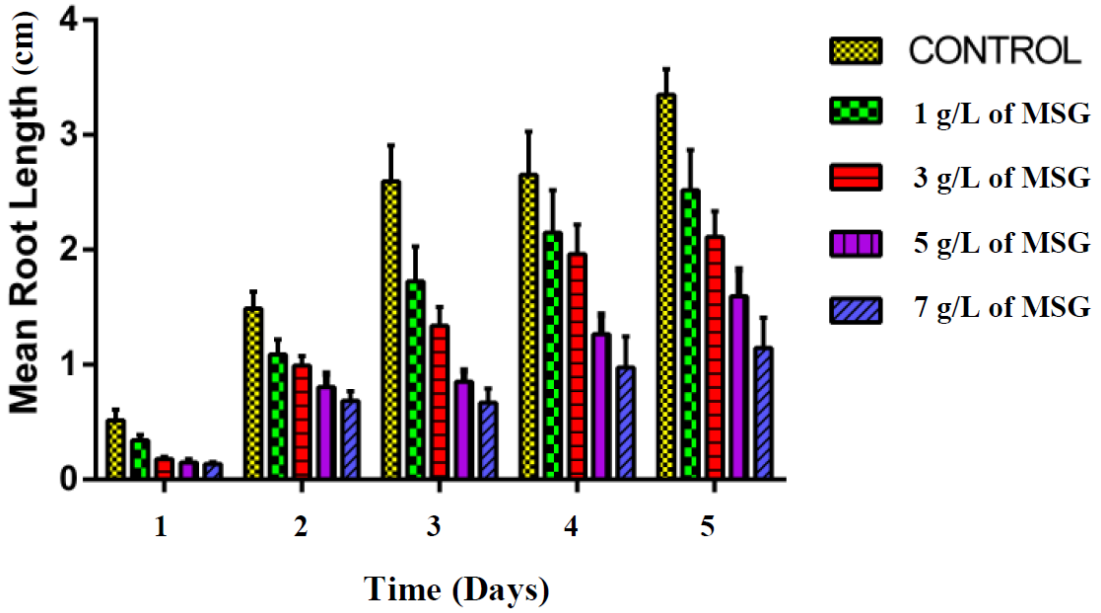


**Figure 2.** Mean root length of *A. cepa* grown in monosodium glutamate dissolved in distilled boiled water.

dark brown or black color of *A. cepa* roots.

MSG dissolved in distilled water without boiling and boiled water inhibited growth of roots in white and purple color onions in all test concentrations. When dissolved in boiled distilled water at concentration of 3.0 g/L, it inhibited root growth of purple and white onion bulb by 54 and 58%, respectively. Root growth inhibition was 100% at 5.0 and 7.0 g/L MSG when dissolved in boiled water. At concentrations of 5.0 and 7.0 g/L (dissolved in distilled water), MSG inhibited growth of onion roots by 52% (purple onion bulb); 88 (white onion bulb) and 89% (purple onion bulb); 80% (white onion bulb), respectively.

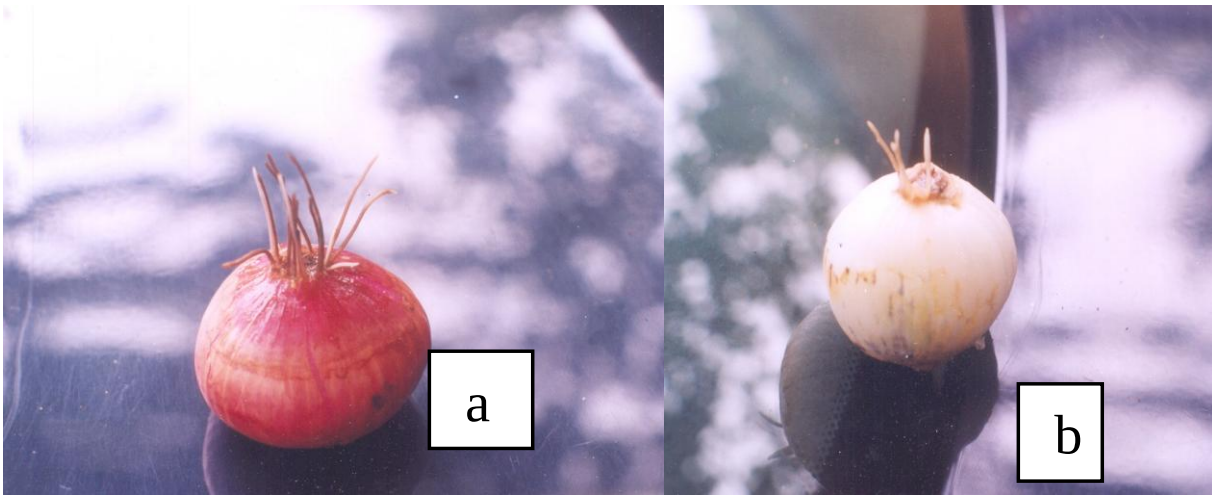


**Plate 1.** Root growths among experimental onions showing: (a) brown color root tips, mild crochet hook caused by 1.0 g/L and (b) reduced root growth caused by 3.0 g/L MSG dissolved in boiled water.

**Table 1.** Cytological effects and chromosomal aberrations observed in the *A. cepa* root cells grown in MSG (dissolved in distilled water without boiling).

| Concentration of MSG (g/L) | Number of dividing cells per mitotic stage | | | | Mitotic index (%) | Chromosomal aberration | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Prophase | Metaphase | Anaphase | Telophase | | Stickiness | C-mitosis | Bridge | Vagrant |
| Control | 6 | 17 | 19 | 19 | 6.1 | 0 | 0 | 0 | 0 |
| 1 | 7 | 9 | 8 | 17 | 4.1 | 11 | 0 | 5 | 6 |
| 3 | 5 | 9 | 9 | 16 | 4 | 12 | 0 | 6 | 7 |
| 5 | 5 | 14 | 7 | 9 | 3.7 | 9 | 1 | 4 | 11 |
| 7 | 7 | 2 | 15 | 9 | 3.5 | 7 | 2 | 4 | 8 |

**Table 2.** Cytological effects and chromosomal aberrations observed in the *A. cepa* root cells grown in MSG (dissolved in boiled distilled water).

| Concentration of MSG (g/L) | Number of dividing cells per mitotic stage | | | | Mitotic index (%) | Chromosomal Aberrations | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Prophase | Metaphase | Anaphase | Telophase | | Stickiness | C-mitosis | Bridges | Vagrant |
| Control | 12 | 23 | 8 | 11 | 5.4 | 0 | 0 | 0 | 0 |
| 1 | 3 | 15 | 10 | 17 | 4.6 | 8 | 1 | 7 | 8 |
| 3 | 5 | 16 | 8 | 4 | 4.5 | 9 | 0 | 5 | 7 |
| 5 | 2 | 13 | 4 | 18 | 3.9 | 13 | 1 | 5 | 5 |
| 7 | 5 | 8 | 10 | 12 | 3.8 | 10 | 0 | 8 | 7 |

## Microscopic studies

MSG decreased MI of *A. cepa* cells at all the test concentrations when dissolved in distilled water without boiling and in boiled water at day 5 as compared to the control (Tables 1 and 2). MI reduction was observed most at concentrations of 5.0 and 7 g/L in both experimental set-ups. However, at all concentrations, MI was not significantly different from the control. In this study, MSG induced chromosomal aberrations such as anaphase bridges, C- mitosis vagrant and sticky chromosomes. Sticky chromosomes at telophase stages occurred most at 1.0 and 3.0 g/L with MSG in distilled water without boiling and at 5.0 and 7.0 g/L concentrations in boiled water (Tables 1 and 2). C- mitosis was observed at 5.0 g/L concentration of MSG. Sticky chromosome aberrations were also pronounce in all purple and white onion bulbs experimental groups. There was no significant difference in total chromosomal aberrations in all experimental set up as compared to control. The

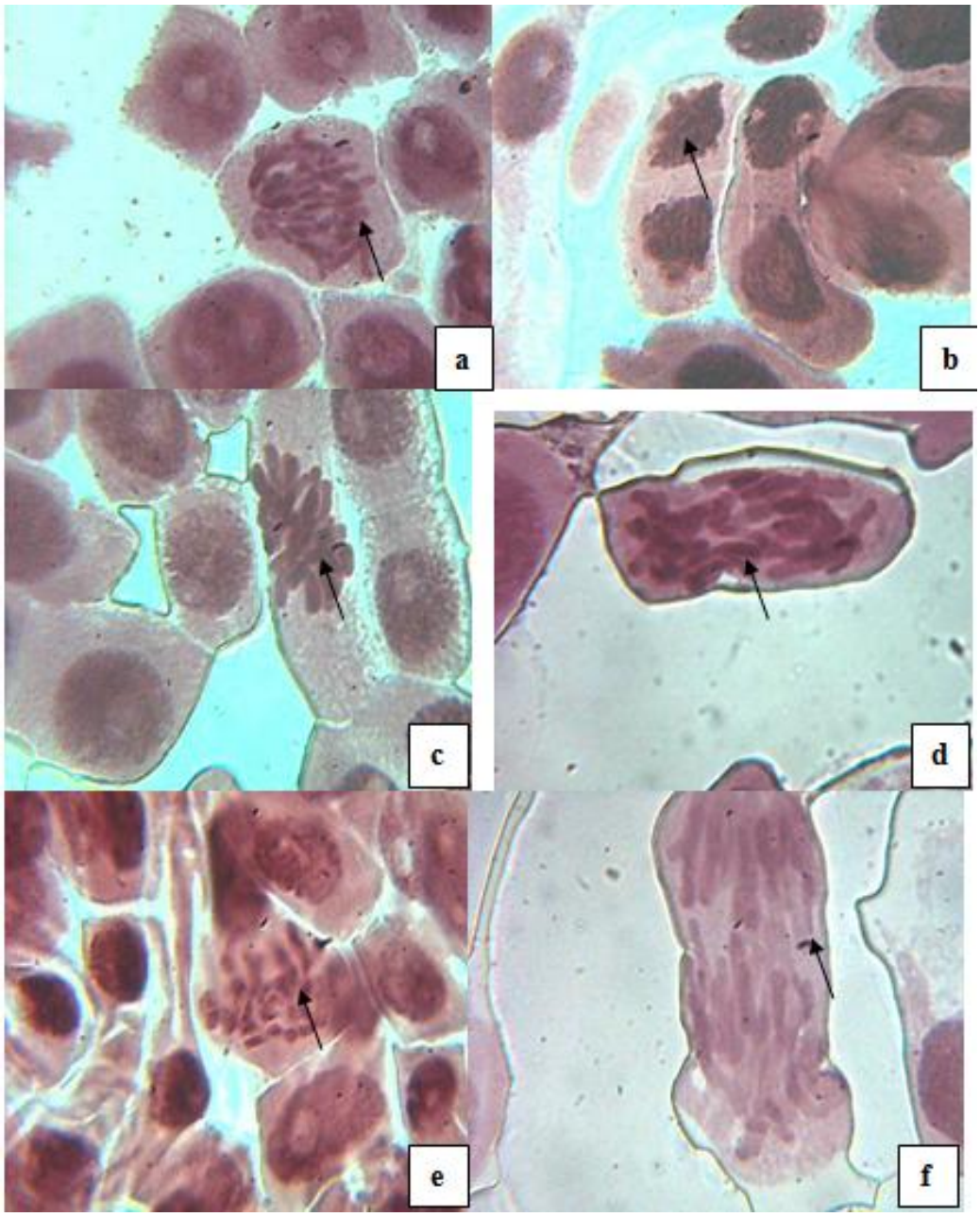


**Plate 2.** Chromosomal aberrations induced by monosodium glutamate in *Allium* root tips: (a) sticky chromosome and (b) vagrant chromosome at 3.0 g/L; (c) bridge anaphase at 5.0 g/L; (d) fragmented chromosomes at 7.0 g/L dissolved in boiled distilled water; (e) c-mitosis at 7.0 g/L; (f) bridge anaphase at 5.0 g/L dissolved in distilled water without boiling.

chromosomal aberrations observed in the study are shown in Plates 2 and 3.

## DISCUSSION

Root morphology, mitotic index and chromosomal aberration analysis of *A. cepa* root tip assay are used to detect potential cytotoxicity and genotoxicity of chemical substances (Kumar and Panneerselvam, 2007; Abu and Mba, 2011). Various chromosome disturbances effects on cell division in addition to breaks are important for a better understanding of the action of any substance. The *A. cepa* assay is well known and has been used severally and has repeatedly been suggested as a standard test material (Fiskesjo, 1985). Positive results of *Allium* test should be seen as a signal of warning and also an indication that the test chemical may constitute a

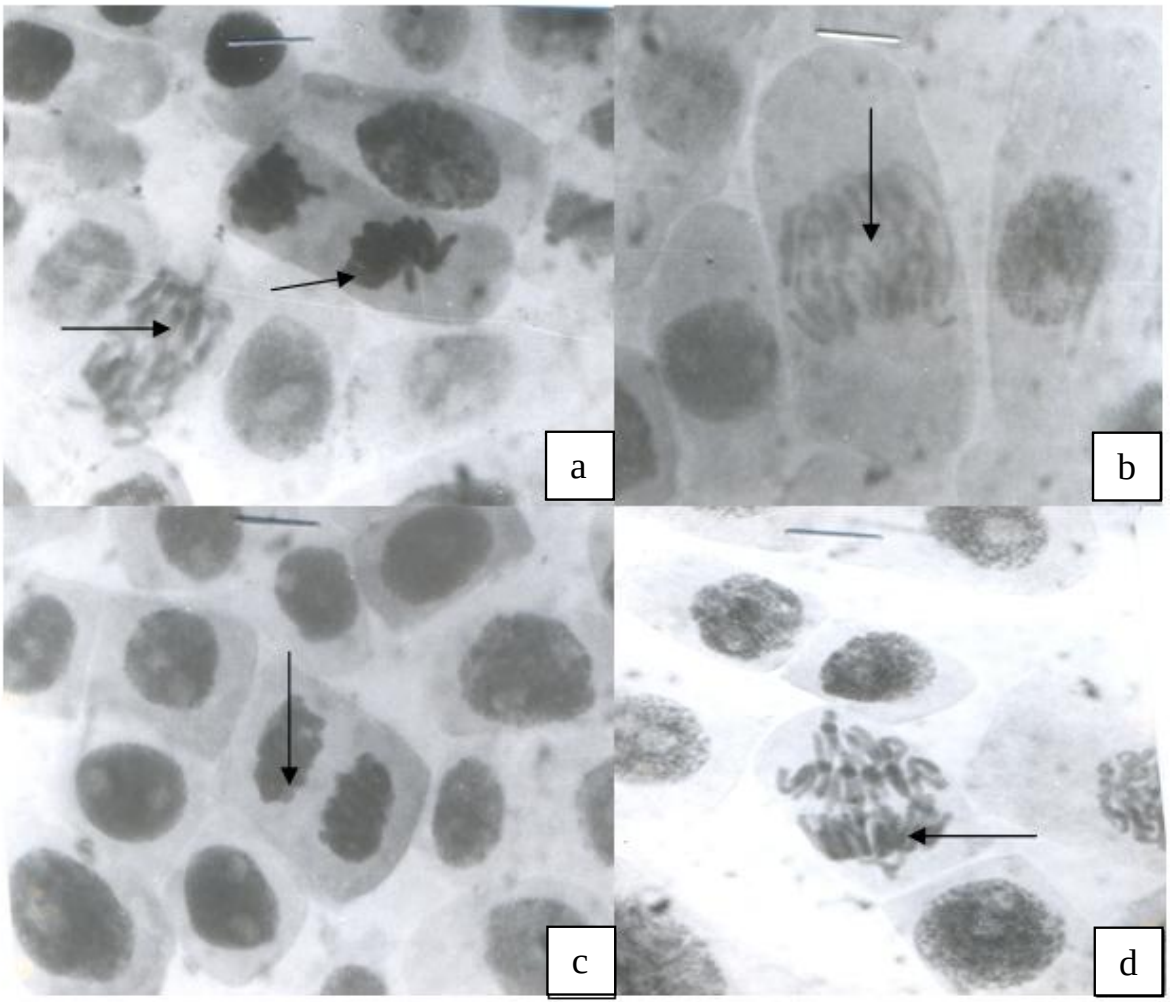


**Plate 3.** Chromosomal aberrations induced by monosodium glutamate in purple onions root tips: (a) Bridge anaphase and sticky telophase, (b) bridge anaphase in 3.0 g/L dissolved in water without boiling, (c) sticky telophase in 3.0 g/L dissolved in boiled distilled water and (d) anaphase with multiple bridges in 3.0 g/L dissolved in distilled water without boiling.

potential health risk (Fiskesjo, 1985). MSG is a flavor enhancer that is added to food and has been used extensively for nearly a century by many people. In many countries, MSG is consumed in large amount in restaurants and packed foods; the high intake of MSG may have effects on cell growth, chromosomes and may lead to cancer. Many food additives have been reported to be genotoxic (Kumar and Panneerselvam, 2007; Türkoğlu, 2009; Nagwa et al., 2011). MSG needs to be studied with respect to food safety to humans, in the present study, the genotoxic and cytotoxic potential of monosodium was evaluated using *A. cepa* assay. It will continue to be examined in the light of current scientific knowledge and methods of testing.

In this study, inhibition of root growth at all test concentrations and changes in color of root tips by MSG was observed. This inhibition was significant at higher concentrations. This suggests an inhibitory or stimulatory effect on the cell cycle on *Allium* root tip cells and also an indication of likely toxicity by MSG. Kumar and Srivastava (2011) reported that boric acid and sunset yellow food additives had inhibitory effect on *Trigonella foenum-graecum* root tips. Root length is an important parameter, reflecting the toxicity of the additive, the elongation zone of the onions may serve as a sensitive external signal of ongoing internal cellular events. Plant roots interact with each of the physical factors in their environment and this interaction can lead to modification of the roots. In the *A. cepa* assay, inhibition of root growth has been shown to indicate retardation of growth and cytotoxicty (Odeigah et al., 1997b, Grant, 1999). Also, growth inhibition can be caused by reduction in mitotic activities and occurrence of various chromosomal aberrations.

In the results of our experiment, MSG induced decreased mitotic index. This might occur at pre-prophase where cells are prevented from entering

prophase or there be prophase-arrest where cells enter into mitosis but are arrested during prophase, resulting in a high frequency of prophase cells. In addition, this reduction in mitotic cell division, probably suggests that it may be a potential harmful food flavor enhancer. This might occur at pre-prophase where cells are prevented from entering prophase or there be prophase- arrest where cells enter into mitosis but are arrested during prophase, resulting in a high frequency of prophase cells. Furthermore, they may bind to tubulin and prevent the formation of the mitotic spindle. Nagwa et al. (2011) observed that food preservatives, sodium metabisulphite (SMB) and potassium metabisulphite (KMB) significantly decrease the mitotic index (MI), and increased the mitotic abnormalities in *Vicia faba* roots.

In the cytogenetic study, the high frequency of sticky chromosome aberrations was observed in all experimental set ups. The occurrence of sticky chromosomes may account for reduction of mitotic stages. Food additives have been found to cause chromosomal aberrations. Türkoğlu (2007) reported that citric acid induced chromosome stickiness, c-mitosis and anaphase bridges in *A. cepa*. Türkoğlu (2009) in another work reported that monosodium phosphate (MSP), disodium phosphate (DSP) and trisodium phosphate (TSP) have induced similar results in *A. cepa* cells. Other food additives such as ammonium acetate and zinc acetate were evaluated in the root meristem cells of *V. faba* and were found to induce micronuclei (Nishi and Anand, 2012).

Moreover, the inhibition of spindle formation may lead to several abnormalities such as stickness, unequal distribution and anaphase chromosomal bridges. Sticky chromosomes are indicative of a highly toxic, usually irreversible effect, probably leading to cell death. Bakare and Adeyemo (2004) have reported that sticky chromosomes can lead to cell death. It was also observed that there was gradual decrease in the number of chromosome aberrations at higher MSG concentration. This suggests increased toxicity and an inhibitory effect on metaphase and anaphase stages of cell division. Vagrant chromosomes show weak C-mitosis effect, indicating risk of aneuploidy, while anaphase bridges and fragments are the results of chromosome breaks and potential carcinogenic effects.

Flavonoids are compounds responsible for the colour of flowers, fruits and leaves. Some may contribute to the color by acting as co pigment. Flavonoids protect the plants from ultraviolet (UV) damaging effects. Resistance of pigments onion varieties to various microbial diseases has been ascribed to the presence of some water-soluble substances which are absent in white bulbs. The components from such colored bulbs which have been identified as catechol and protocatechnic acid, could arise from some flavanoid precursors of the inner wet scales of the bulbs. The role of flavonoids of onion bulbs in toxicity has not been reported. Non-significant difference in the effect of MSG among the two cultivars showed that flavonoids do not prevent the effect of toxic compounds.

In conclusion, MSG has potential cytotoxic and genotoxic effects in the root tip cells of *A. cepa*. It can lead to certain irreversible cytogenetic effects in plants and even in higher organisms. Further research should be conducted for the comparison of result from other test systems used to detect genotoxic potential of chemicals (cell line, micronucleus analysis of human lymphocytes, comet assays or single cell gel electrophoresis).

## REFERENCES


Abu NE, Mba KC (2011). Mutagenicity testing of pharmaceutical effluents on *Allium cepa* root tip meristems. J. Toxicol. Environ. Health Sci. 3(2):44-51.

Bakare AA, Adeyemo AR (2004). The potential mutagenic and cytotoxic effects of leachate from domestic wastes and Aba-Eku landfill, Nigeria on *Allium cepa.* Nature Environ. Pollut. Technol. 3(4):455-462.

Farombi EO, Onyema OO (2006). Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: Modulatory role of vitamin C, vitamin E and quercetin. Hum. Exp. Toxicol. 25(5):251-259.

Fernstrom JD, Garattini S (2000). International symposium on glutamate. J. Nutr. 130 (Suppl 4):891S–1079S.

Filer LJ, Stegink LD (1994). Report of the proceedings of the glutamate workshop. Crit. Rev. Food Sci. Nutr. 34(2):159-174.

Fiskesjo G (1985). The *Allium* test as a standard in environmental monitoring. Hereditas 1-2:99-112.

Fiskesjo G (1993). The *Allium* test in waste-water monitoring. Environ. Toxicol. Water Quality 8:291-298.

Food Standards Australia New Zealand (FSANZ) (2003). Monosodium Glutamate, a Safety Assessment. Technical Report Series No. 20. http://www.foodstandards.gov.au

Freeman M (2006). Reconsidering the effects of monosodium glutamate. A literature review. J. Am. Acad. Nurse Pract. 18(10): 482-486.

Fuke S, Shimizu T (1993). Sensory and preference aspects of umami. Trends Food Sci. Technol. 4:246-251.

Grant WF (1999). Higher plant assays for the detection of chromosomal aberrations and gene mutations-A brief historical; background on their use for screening and monitoring environmental chemicals. Mutat. Res. 426:107-112.

Hermanussen MG, Sunder AP, Voigt MM, Salazar V, Tresguerres JAF (2006). Obesity Voracity, and short stature: the impact of glutamate on the regulation of appetite. Eur J. Clin. Nutr. 6:25-31.

Kumar G, Srivastava N (2011). Genotoxic effects of two commonly used food additives of boric acid and sunset yellow in root meristems of *Trigonella foenum-graecum.* Iran. J. Environ. Health. Sci. Eng. 8(4):361-366.

Kumar LP, Panneerselvam N (2007). Cytogenetic studies of food preservative in *Allium cepa* root meristem cells. Med. Biol. 14(2):60-63.

Levan A (1938). The effect of colchicine on root mitosis in *Allium*. Hereditas 24:471-486.

Morselli PL, Garattini S (1970). Monosodium glutamate and the Chinese Restaurant Syndrome. Nature 227:611-612.

Nagwa RAH, Magda AME, Atef AAH, Elham AAAH (2011). Relative Mutagenecity of Some Food Preservatives on Plant Cells. Aust. J. Basic Appl. Sci. 5(12):2817-2826.

Nishi KA, Anand MJ (2012). Genotoxicity testing of food additives by employing Vicia MN assay. J. Phytol. 4(5):42-45.

Odeigah PGC, Makinwa J, Lawal B, Oyeniyi R (1997b). Genotoxicity screening of leachates from solid industrial waste evaluated *Allium* test. ATLA 25:311-321.

Odeigah PGC, Nurudeen O, Amund O (1997a). Genotoxicity of oil field waste water in Nigeria. Hereditas 126:161-167.

Ortiz GG, Bitzer-Quintero OK, Beaszarate C, Rodriguez-Reynoso S, Larios-Arceo F, Velazquez-Brizueh IE, Pacheco-Moises FO, Rosales-Corral SA (2006). Monosodium glutamate-induced damage in liver and kidney: A morphological and biochemical approach. Biomed. Pharmacother. 60(2):86-91.

Pavlvovic V, Cekic S (2006). The effect of monosodium glutamate on rat thymocyte proliferation and B protein expression. Arch. Med. Sci. 24:247-251.

Samuel A (1999). The toxicity/safety of processed free glutamic acid (MSG). A study in suppression of information. Account. Res. 6:259-310.

Stevenson DD (2000). Monosodium glutamate and asthma. J. Nutr. 13:1-67S-1-73S.

Türkoğlu Ş (2007). Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. Mutat. Res. 626(1-2):4-14.

Türkoğlu Ş (2009). Genotoxic effects of mono-, di-, and trisodium phosphate on mitotic activity, DNA content, and nuclear volume in *Allium cepa* L. Caryologia 62(3):171-179.

Yamaguchi S, Ninomiya K (2000). Umami and food palatability. J. Nutr. 13:921S-926S.

Yang WH, Drouin MA, Herbert M, Mao Y, Karsh J (1997). The monosodium glutamate symptom complex: assessment in a double-blind placebo-controlled, randomised study. J. Allergy Clin. Immunol. 99:757-762.