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Research Article

EVALUATION OF GENOTOXIC EFFECTS OF BAKING POWDER AND MONOSODIUM GLUTAMATE USING *ALLIUM CEPA* ASSAY

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ABSTRACT

Objective: To investigate the genotoxic effects of two food additives, baking powder (BKP) and monosodium glutamate (MSG) using *Allium cepa* root tip cells as a test system.

Methods: Roots of *A. cepa* were treated with four concentrations (0.01%, 0.05%, 0.1% and 0.5%) of these additives for different time durations (½h, 1h, 2h and 3h).

Results: Exposure to different concentrations of these chemicals showed an inhibitory effect on cell division in root tips of *A. cepa* and caused a general decline in mitotic index values. Additionally, a wide range of abnormal mitotic stages, both clastogenic and non-clastogenic were detected in treated cells when compared to the control. The total percentage of aberrations generally increased in a dose and time dependent manner. Among these, frequently observed chromosomal abnormalities were stickiness, chromosome fragments, polyploidy, disturbed meta and anaphases, laggards, ring chromosomes, chromosome bridges, multipolar anaphases, vagrant chromosomes *etc*.

Conclusion: The results of the present study clearly prove the sensitivity of *Allium* test and hence substantiate its use as a cytogenetic assay to assess the genotoxic effects of chemicals that are consumed as food additives or preservatives.

Keywords: *Allium cepa*, Baking powder, Chromosomal aberrations, Food additives, Monosodium glutamate, Mitotic index.

INTRODUCTION

Food additives are a large group of substances that are added to foods directly or indirectly either during the storage or processing of foods [1]. Diverse types of food additives serve different purposes such as preservatives, nutritional additives, flavouring agents, colouring agents, texturing agents *etc*. Baking powder (BKP) and Monosodium glutamate (MSG) are two widespread and regularly used food additives in a range of foods. MSG, known as a flavour enhancer, is commonly used particularly in West African and traditional Asian cuisine [2] and BKP, a texturing agent, is indispensable in baked foods and so is extensively used worldwide.

MSG can be defined as the monosodium salt of the naturally occurring L-form of glutamic acid, produced by the hydrolysis of vegetable proteins. It elicits a unique fifth basic taste that is quite distinct from the tastes of sweet, bitter, salt and sour, referred to as 'umami' [3]. The optimum concentration used is from 0.2 to 0.5% in normally salted food. Some of its names used in trade are Ajinomoto (most common), Glutacyl, RL-50, Vetsin, Chinese seasoning, Accent, Zest, Glutavene *etc*. Generally, MSG is accepted as a safe food additive that needs no specified average daily intake or an upper limit intake [4]. Ingestion of even large quantity of MSG did not present any adverse effect when taken with food [5]. There are however, divergent reports on the safety of the use of MSG in humans since there are many other studies that reveal MSG-induced toxicity as well [6-9]. An experimental study by Burde *et al*. [10] demonstrated that both subcutaneous injection and oral administration of MSG to immature rats and mice could damage the nerve cells of the hypothalamus. Mohammed [11] investigated the possible effects of MSG on the histology and ultra-structure of testes of the adult rats and found that MSG could induce severe damage in long term treatment. So the question of whether MSG is a harmless food additive remains highly controversial even now.

BKP, used as a leavening agent, lightens the texture and increases the volume of baked foods. Most commercially available baking powders are made up of an alkaline component (sodium bicarbonate, also known as baking soda), one or more acid salts (either sodium aluminium sulphate or sodium aluminium phosphate) and an inert starch (corn starch mostly or potato starch). There are no significant reports on any toxicological evaluations of BKP recently. Baking powders have so far been found to be safe for consumption and no definitive correlation has scientifically been proved regarding the health problems arising from regular intake of BKP.

The human population is continuously been exposed to a plethora of diverse chemicals, and this long term exposure can cause several deleterious effects on specific biomolecules. Among them, DNA alterations are known to be the early signs of damage in the affected organisms [12]. Though a considerable volume of work has been carried out in the past investigating the possible effects of MSG and BKP on humans and experimental animals, only limited information is available on the effects of these food additives on sensitive plant systems. *A. cepa* assay is now considered as one of the most efficient and cost effective approaches to determine the toxic potential of chemical compounds in the environment because of its high sensitivity and good correlation with the results of mammalian test systems [13]. Study of the effect of several chemicals on plant mitosis may provide valuable information in relation to possible genotoxicity in mammals and especially in humans [14]. So the present study was designed to investigate the effects of BKP and MSG on the mitotic index and the frequencies of chromosomal abnormalities in the root tip cells of *A. cepa* L., with a view to detect their mutagenic potential.

MATERIALS AND METHODS

Samples of BKP, MSG used were well known commercial products purchased from a local super market. They were dissolved in distilled water and diluted to obtain different concentrations (0.01%, 0.05%, 0.1% and 0.5%) of test solutions. Onion bulbs weighing 15-30 g were purchased freshly from local markets. The bulbs were carefully unscaled and the old roots were removed. They were then placed on top of small jars containing distilled water and were allowed to germinate for 36 hours at room temperature (25±2˚C). When the emerged roots were 1-2 cms long, the bulbs were treated with the series of concentrations of the two test substances for ½, 1, 2 and 3 h. The control group was treated with distilled water.

After each treatment, a few healthy root tips excised from each bulb were fixed in ethanol/glacial acetic acid (2:1) fixative for 1 h. After hydrolysis in 1N HCl for 15 min at room temperature, mitotic squash preparations were made with improved techniques [15] using 2% acetocarmine. Two slides were made for each treatment and scoring

was done from five sites that were randomly selected from these slides to determine the mitotic index and the percentage of chromosomal aberrations. The mitotic index was calculated for each treatment as the number of cells in mitosis/total number of cells counted and expressed as percentage. The cells were also scored for cytological abnormalities and the percentage of chromosomal aberrations was measured as the ratio of number of aberrant cells to total number of cells observed. The relative frequency of the different aberrations at each dose was calculated. Preparations were scanned under Leica ICC 50 integrated camera attached to Leica DM 500 research microscope. The most frequent abnormalities are shown in photomicrographs (Fig. 1).

Statistical Analysis

The data of mitotic index (MI) and chromosomal aberrations (CA) are represented in percentage mean±SE of five scorings. For statistical analysis, one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMR) [16] were used. All statistical analyses were performed by using the computer software SPSS 20.0 for Windows. Results with $P < 0.05$ were considered to be statistically significant.

RESULTS

Exposure to different concentrations (0.01%, 0.05%, 0.1% and 0.5%) of the food additives significantly and dose dependently inhibited the mitotic index (Tables 1 and 2) in the root tip cells of A. *cepa*. There were significant differences in the mitotic index values as compared to the control ($P< 0.5$) at all treatments right from the lowest dose. The highest concentration of BKP (0.5%) at the 3 h treatment could bring about a considerable reduction in mitotic index (54.38%), whereas the same concentration of MSG caused a decrease of mitotic index to 37.12%.

The tables 1 and 2 also show the exponential relationship between the percentage of aberrations and the concentrations of the test compounds. Both the chemicals could significantly increase the percentages of chromosomal aberrations at all concentrations more or less in a dose dependent manner. The frequency of mitotic aberrations was significantly increased when exposure time was increased from ½ to 3 h. The highest number of mitotic aberrations was recorded in root tips subjected to 3 h treatment in 0.5% BKP (46.48%) whereas MSG could induce 27.38% aberrations at the same dose and duration of treatment.

Table 1: Mitotic index and chromosomal aberrations in *Allium cepa* root tip cells exposed to increasing concentrations of BKP for different periods.

Treatment duration (h)	Concentrations (%)	Mitotic index (mean±S. E.)	(%)Abnormalities (mean±S. E.)
	Control	24.74±0.23^{a}	0.00^{a}
½	0.01	22.64±0.77^{a}	10.5±0.80^{b}
	0.05	18.31±0.15^{b}	13.46±0.21^{c}
	0.1	13.21±0.38^{c}	12.84±0.23^{c}
	0.5	13.56±2.15^{c}	18.83±0.55^{d}
	Control	23.48±0.28^{a}	0.00^{a}
1	0.01	21.77±0.93^{ab}	16.46±1.08^{b}
	0.05	20.19±0.18^{b}	15.62±0.68^{b}
	0.1	17.39±0.18^{c}	15.94±0.71^{c}
	0.5	13.09±0.21^{d}	21.33±0.24^{c}
	Control	25.09±0.19^{a}	0.00^{a}
2	0.01	19.48±2.09^{b}	13.69±0.94^{b}
	0.05	15.3±0.36^{c}	23.79±0.47^{c}
	0.1	16.25±0.46^{c}	28.5±0.66^{d}
	0.5	13.88±0.30^{c}	34.81±1.27^{e}
	Control	24.92±0.12^{a}	0.00^{a}
	0.01	20.07±1.20^{b}	29.52±0.42^{b}
3	0.05	15.52±2.25^{c}	37.57±0.46^{c}
	0.1	14.7±0.18^{cd}	42.08±1.19^{d}
	0.5	11.37±0.39^{d}	46.48±0.63^{e}

Each value (mean ± S. E.) represents mean of five replicates.

Means in a column followed by the same superscript letters are not significantly different ($P < 0.05$, one-way ANOVA, DMR test)

Table 2: Mitotic index and chromosomal aberrations in *Allium cepa* root tip cells exposed to increasing concentrations of MSG for different periods.

Treatment duration (h)	Concentrations (%)	Mitotic index (mean±S. E.)	(%) Abnormalities (mean±S. E.)
	Control	24.74± 0.23^{a}	0.00^{a}
½	0.01	23.59 ± 1.27^{ab}	6.56 ±0.48^{b}
	0.05	22.51±0.12^{ab}	10.30 ±0.13^{c}
	0.1	18.13 ± 1.82^{c}	13.76 ±0.43^{d}
	0.5	20.32 ± 0.63^{bc}	15.38 ±0.80^{e}
	Control	23.48±0.28^{a}	0.00^{a}
1	0.01	23.84 ±0.26^{a}	8.60 ± 0.35^{b}
	0.05	21.31 ± 1.00^{b}	13.38 ±0.16^{c}
	0.1	19.54 ±0.37^{bc}	17.74± 1.12^{d}
	0.5	18.77 ±0.54^{c}	19.07 ± 1.89^{d}
	Control	25.09±0.19^{a}	0.00^{a}
2	0.01	20.71 ± 0.51^{b}	5.13 ± 1.15^{b}
	0.05	17.02 ± 1.38^{c}	14.89±0.51^{c}
	0.1	16.43 ±0.12^{c}	19.90 ± 2.10^{d}
	0.5	16.23 ±0.47^{c}	22.19 ±1.03^{d}
	Control	24.92±0.12^{a}	0.00^{a}
3	0.01	18.66 ± 0.24^{b}	9.14 ±0.92^{b}
	0.05	16.40 ± 0.87^{c}	15.68 ± 2.15^{c}
	0.1	17.16 ±0.60^{bc}	21.39 ± 0.94^{d}
	0.5	15.67±0.89^{c}	27.38± 1.90^{e}

Each value (mean ± S. E.) represents mean of five replicates.

Means in a column followed by the same superscript letters are not significantly different ($P < 0.05$, one-way ANOVA, DMR test)

A wide spectrum of both clastogenic and non-clastogenic aberrations (Fig. 1) were observed in the treated meristematic cells. The results regarding the type and frequency of chromosomal abnormalities induced by different concentrations of the test compounds are shown in Fig. 2. More or less similar types of aberrations were noticed in response to both the chemicals used. The most common clastogenic abnormalities noted were chromosome stickiness, pulverization, ring chromosomes, chromosome bridges, fragments, binucleate cells, polyploidy *etc*. The major non-clastogenic aberrations found were disturbed meta/anaphases, binucleate cells, laggards, vagrants, diagonal orientation *etc*. Stickiness, chromosome fragments, vagrants and disturbed metaphase and anaphase were the most frequently recorded aberrations in all treatments with both the test compounds (Fig. 2). In addition to that, a very high frequency of ring chromosomes, stickiness and laggards were noted in response to BKP, while MSG caused a high incidence of binucleate cells and fragmented chromosomes. Significant increase in the frequency of mitotic abnormalities was observed after 2 and 3 h of exposure to BKP treatments at all doses (Table 1).

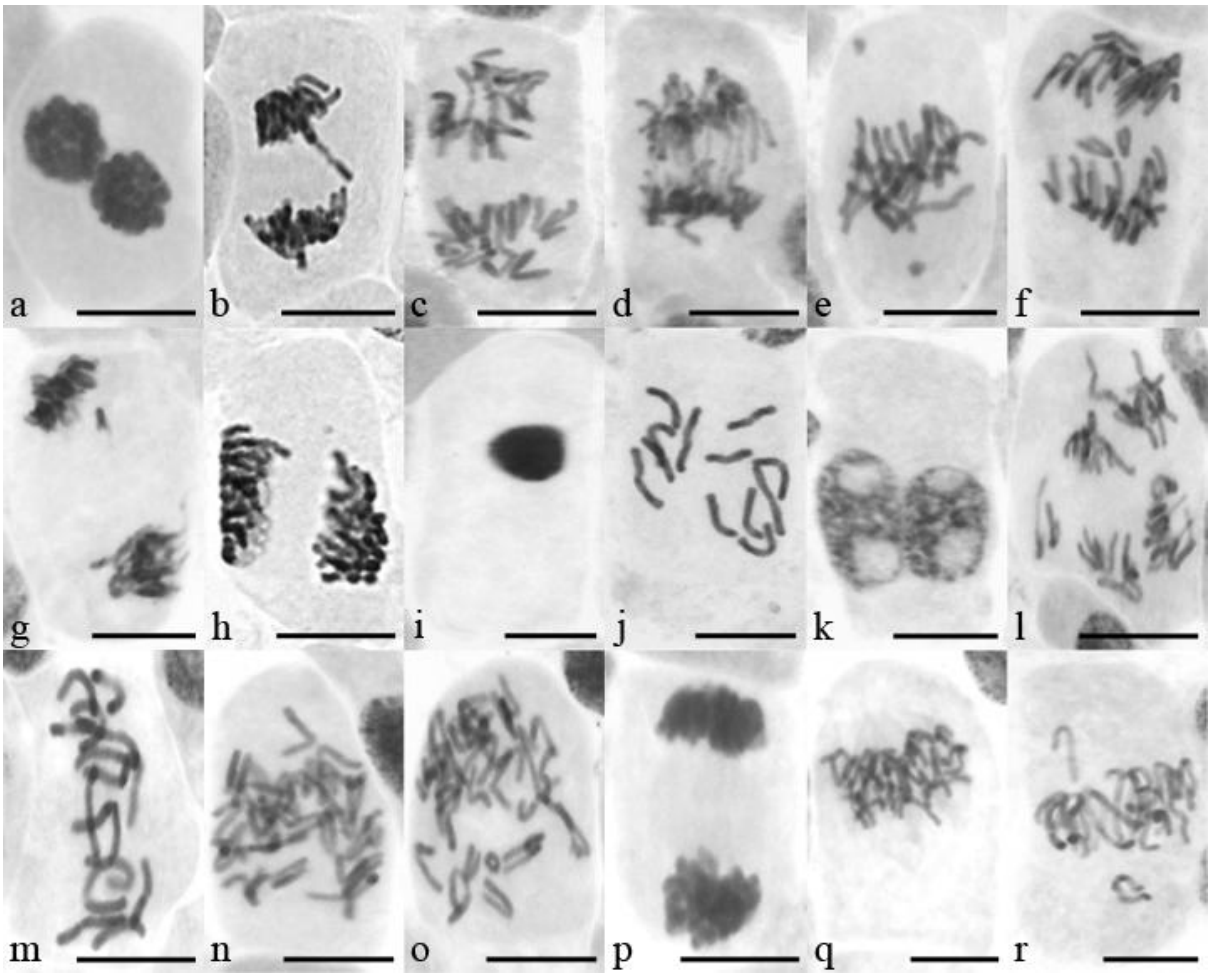
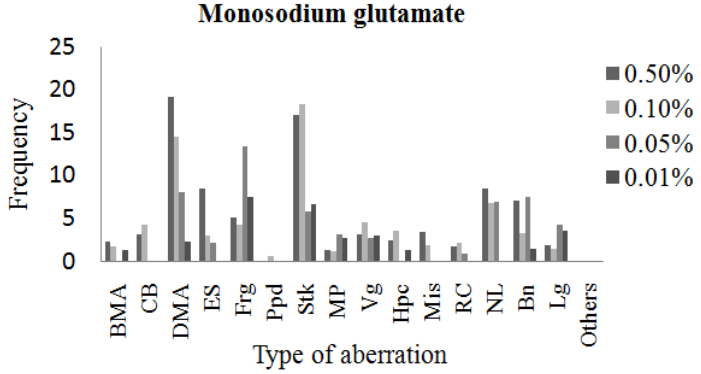


Fig. 1: Different Chromosomal aberrations induced by BKP and MSG in root tip cells of *Allium cepa*.

a) Diagonal ball anaphase, b) Broken chromosome bridge at anaphase, c) Centromeric attraction at anaphase, d) Chromosome bridges at anaphase, e) Chromosome fragments at anaphase, f) Chomosome laggards at Anaphase, g) Diagonal sticky anaphase with a laggard, h) Equatorial separation at anaphase, i) Hyperchromasia, j) Hypoploid cell showing cytostasis at metaphase, k) Lesions in a binucleate cell at interphase, l) Multipolar anaphase, m) Disturbed pole to pole arrangement of chromosomes at metaphase, n) Polyploid cell at metaphase, o) Ring chromosomes in disturbed metaphase in a polyploid cell, p) Sticky anaphase, q) Unipolar anaphase, r) vagrant chromosomes at metaphase.

Bar represents 5µm.



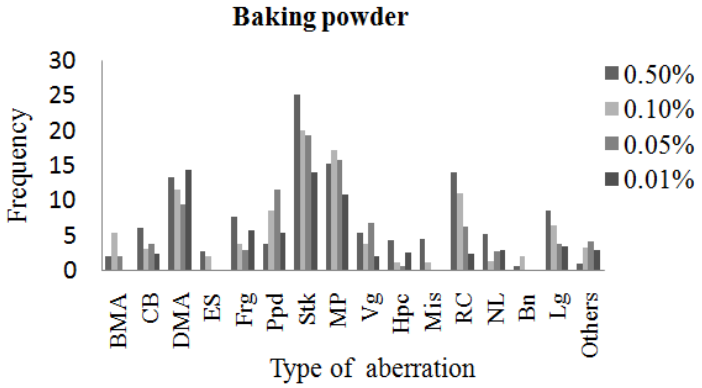


Fig. 2: Relative frequency of different chromosomal aberrations in root meristematic cells of *Allium cepa* following treatments with different concentrations of the food additives.

BMA – Ball meta and anaphases, CB – Chromosome bridge, DMA – Disturbed meta and anaphases, ES – Equatorial separation, Frg – Fragments, Ppd – Polyploidy, Stk – Stickiness, MP – Multipolar anaphases, Vg – Vagrant chromosomes, Hpc – Hyperchromasia, Mis –Misorientation, RC – Ring chromosomes, NL – Nuclear lesions, Bn – Binucleate cells, Lg –Lagging chromosomes.

DISCUSSION

The results of the present study demonstrated clear evidence of the genotoxic potential of the tested food additives. The wide range of observed abnormalities proved that even short term exposure to relatively small doses of BKP and MSG significantly affects the mitotic index and chromosome structure and disturbs mitotic spindle formation. The cytotoxic effects of BKP and MSG are seen to be dependent more on their concentration than the time period, with even the low doses causing a significant rate of decrease of mitotic index and increase in percentage of mitotic abnormalities.

The lowering of mitotic index in the treated root tips could be due to inhibition of DNA synthesis [17], arrest of one or more mitotic phases [18] or blocking of G2 phase in the cell cycle [19] preventing the cell from entering mitosis. The reduction in mitotic activity with increasing concentrations clearly demonstrates the ability of the food additives to inhibit DNA synthesis. Significant genotoxicity observed at the highest concentrations (0.1% and 0.5%) can be another probable reason for the lower mitotic index scores noted at these doses.

The decrease in mitotic activity was accompanied by several clastogenic and non-clastogenic aberrations (Figs 1a – 1r). The two food additives are found to have more or less similar effects on the chromosomes. The most noticeable cytological aberrations detected in treatments were stickiness, fragments, bridges, multipolarity, disturbed meta and anaphase, polyploidy, vagrants *etc.*

BKP treatments showed a high incidence of spindle abnormalities like disturbed meta and anaphases, multipolar anaphases, polyploidy, binucleate cells, laggards, vagrants, diagonal orientation, unequal separation, unipolar movement, stellate anaphase *etc.*, demonstrating its acute damaging effects on the mitotic spindle. Apart from these, many clastogenic abnormalities like stickiness, ring chromosomes, fragmentation, bridges, ball meta and anaphases *etc.* were observed indicating its direct destructive effects on the chromosomes also. Most of the commercial baking powders contain aluminium and cytotoxicity of aluminium on meristematic cells of *A. cepa* and *Zea mays* has previously been reported [20]. Aluminium has been found to affect the control mechanisms of the microtubule cytoskeleton organization and also the normal chromosome movements through the spindle [21], leading to the occurrence of laggards, polyploidy and other physiological aberrations.

The aberrations induced by MSG are more or less comparable to those caused by BKP showing considerable cytotoxic effect. But the frequency of chromosomal aberrations induced was lesser. A high frequency of binucleate cells and chromosome fragments could be noted in response to MSG whereas BKP was found to be more cytotoxic which is evident from the remarkably high proportion of polyploid cells, fragments, stickiness and ring chromosomes it could induce.

Stickiness of chromosomes (Figs 1p, 1g) is one of the major abnormalities noted in the present study. We could observe different degrees of stickiness in all the treatments. Stickiness might have been caused by the physical adhesion of chromosomal proteins [22] or due to the disturbances in the nucleic acid metabolism of the cell or the dissolution of protein covering of DNA in chromosomes [23]. It is suggested that stickiness reflects a highly toxic and usually irreversible condition that probably leads to cell death [19, 24, 25]. Disturbed meta and anaphases (Figs 1m, 1o) are another most frequently observed abnormality noted in the treated cells. It may be caused by the loss of activity of microtubules in spindle fibres leading to complete inhibition of spindle formation [26]. Mitotic bridges (Figs 1b, 1d) are another commonly observed abnormality probably formed by the breakage and fusion of chromosomes [27-29]. Double and multiple bridges were also common. Chromosome bridges and fragments are signs of extreme lethal clastogenic effects resulting from chromosome and chromatid breaks [30]. Fragments (Fig. 1e) may arise due to stretching of chromosomes at metaphase followed by breakage at these fragile sites [31]. The induction of ring chromosomes (Fig. 1o) suggests the possibility of two breaks that occur in the same chromosome or may result from telomeric losses. According to Hall and Garcia [32], presence of ring chromosomes is a condition which is highly lethal to the cell.

High frequency of polyploid cells (Figs 1n, 1o) was observed especially in treatments with BKP. Polyploidy has been attributed to the inhibition of complete disturbance of spindle mechanism [33]. Another important chromosomal aberration noted was presence of binucleate cells (Fig. 1k). The occurrence of binucleated cells was the result of inhibition of cytokinesis or cell plate formation [34, 35]. Lagging chromosomes (Figs 1f, 1g) also have been a regular feature of all the treatments of the present study. Occurrence of lagging chromosomes might be due to the hindrance of pro-metaphase movement of chromosomes, accompanied by adhesion of centromere to the nuclear membrane [36]. Ball anaphases (Fig. 1a) and ball metaphases observed in almost all the treatments with the food additives might be caused by the localized activity of the spindle apparatus at the centre. A considerable proportion of nuclear lesions (Fig. 1k) also could be recorded in most of the treatments which may be due to the disintegration of portion of nuclear material by the action of plant extracts.

Hypoploid cells (Fig. 1j) noted in the treatments might be due to the occurrence of multipolar mitosis or lagging chromosomes producing two hypoploid daughter cells [37]. Multipolar movement of chromosomes (Fig. 1l) observed is another major abnormality that results from severe disturbances in the spindle mechanism [33].

Abnormal pole to pole orientation of chromosomes (Fig. 1m) at metaphase leading to equatorial separation of chromosomes at anaphase (Fig. 1h) is an acute aberrant condition that arises as a result of irregular pathways of spindle assembly and abnormal spindle activity [38, 39]. Diagonal orientation (Figs 1a, 1g) was a

frequent abnormality that may be due to a slight tilt in the spindle apparatus. Scattering of chromosomes could be attributed to the interference of the food additives with the polymerization of the microtubular subunits [40]. Hyperchromasia (Fig. 1i) is one of the most distinguishable states of aberration which is an extremely condensed and deeply staining state of nucleus caused by the influence of toxic chemicals or during incompatible conditions. Gernand *et al*. [41] has suggested that hyperchromasia could be caused by gradual heterochromatinization in response to stress.

It is suggested that compounds shown to be reactive with DNA in one species, have the potential to produce similar effects in other species also [42]. The Significant reduction in mitotic index reported in the present study indicates that the food additives used are having clear antimitotic effects and so should be regarded as having strong cytotoxic potential. The chemicals could disturb nucleic acid metabolism and possibly inhibit the enzymes concerned with spindle production, assembly and orientation which is apparent from the vast array of abnormalities induced. According to Fiskesjo [43], a positive result in *Allium* test should be taken to indicate a potential biological hazard and thus the occurrence of the wide variety of abnormalities is an indication of the high mutagenic potentials of the food additive compounds tested.

CONCLUSION

The results of the current study points out that MSG and BKP that are frequently being used in the food industry possess high genotoxic risks. Both the chemicals could produce a very clear mito-depressive effect in onion root tips. The occurrence of cytological abnormalities like stickiness, breaks, bridges, multipolar anaphases and laggards in high frequencies clearly shows that BKP and MSG are potent clastogens and are having severe damaging effects on the mitotic spindle too.

The outcome of the present investigation suggests that attention should be paid to estimate the toxic potential of the regularly used food additives and other chemicals in consumable items and that *A. cepa* assay can be recommended as a practical and reliable cytogenetic assay for such toxicity assessments.

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