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Research article

Butylated hydroxytoluene and Butylated hydroxyanisole induced cyto-genotoxicity in root cells of *Allium cepa* L.

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ABSTRACT

This study aims to evaluate the effects of preservatives on somatic cells of *Allium cepa*. For the evaluation of cytotoxicity, root meristems of *A. cepa* were treated with 1000, 1500, 2000, 2500 ppm concentration for 5, 10, 15 days. The root tips showed concentration dependent growth retardation in all the used concentrations, as well as root texture, also has been changed mitotic index, total protein content decreased and frequency of chromosomal aberrations increased after 5 days treatment. Additionally, the RAPD-PCR method was applied to evaluate genotoxicity and found the highest concentration (2500 ppm) was comparatively more distant to the control group. Results concluded that BHT and BHA showed positive results and cytotoxic.

1. Introduction

Food preservatives are the substances that food manufacturers intentionally add to food in small quantities during production or processing to improve the taste quality and self-life of the food (Magnuson et al., 2013). These are substances not normally consumed as food nor usually used as typical food ingredients but used as additives in food or pharmaceuticals to achieve specified chemical effects in the food product (Sharma, 2015). They are used for various purposes and classified based on their functions, such as preservatives, antioxidants, coloring, non-nutritive sweeteners, ingredient improvers and many more (Güngörmüş and Kılıç, 2012). Earlier it has been reported that natural preservatives such as sugar and salt are as effective as synthetic preservatives (Doyle, 2007). Continuously increasing the world population, users concern towards to procure new food sources and preserve them for a longer period without affecting their quality (Kumar and Panneerselvam, 2007). The frequently used preservatives responded to be the best effect for a longer shelf-life of food sources. Unfortunately, the chemicals used to preserve foodstuff are reported as genotoxic (Luca et al., 1987), but simultaneously, antioxidant properties in different doses applied (Kahl, 1984; Ito and Hirose, 1989). Although few reports are available on the genotoxicity of chemicals used in preservatives, yet a list of chemicals are in queue to report their effects on test organisms as well as on human health.

Presently, the chemicals Butylated hydroxyanisole, BHA [(1,1-dimethylethyl)-4-methoxyphenol] and Butylated hydroxytoluene, BHT [2,6-di-tert-butyl-4-methylphenol] are used to report its genotoxicity on the test organism (*Allium cepa* L.). Both the chemicals were chosen because of its large scale use in the food industry especially in cereals, chewing gum, potato chips, vegetable oils, cosmetics, pharmaceuticals, rubber and petroleum products (Freitas and Fatibello-Filho, 2010). In the past, there were no reports on chromosomal aberration (in plants) and DNA damage (*B. subtilis*) for the chemicals (Kinae et al., 1981). Both the chemicals have antioxidant properties and it was used as low-cost antioxidant from a long time in food sources and cosmetics without any adverse effect (WHO, 1987). Recently, both chemical has been reported as genotoxic (Pandey et al., 2014) and BHT was reported as anticarcinogenic and well known to implement health risks in human (Shahidi and Ambigaipalan, 2015). The panel on food additives and nutrient sources (EFSA, 2012) suggested for the level of acceptable daily intake (ADI) is 1 mg/kg bw/day and 0.25 mg/kg bw/day for BHA and BHT respectively.

Allium test has been chosen to study the genotoxicity of BHA and BHT for the following reasons: 1) its genome has been considered as an important genetic material to test and monitoring the environmental pollution (Grant, 1978); 2) It has been considered as bioindicator (Leme and Marin-Morales, 2009; Rank et al., 2003) because; a) root grows in direct contact with exogenous substance of interest; b) used for the prediction of possible DNA damage in eukaryotes; 3) The cell division and DNA damage in the *Allium* and Mammalian cells closely correlated (82%) reported by Rank and Nielsen (1994) and Camparoto et al. (2002).

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DNA alterations are known to be the early signs of damage in the organisms (Mathew and Thoppil, 2012). For monitoring genotoxicity, it is important to use sensitive but nonspecific assays that indicate a wide range of DNA damage types such as RAPD. It is low cost and allows polymorphism to be detected in a simple and fast manner (Wu, 2000). Earlier RAPD technique has been used to show mutagenic effects of genotoxicants such as heavy metals, pesticides and UV radiation (Atienzar et al., 2002; Cenkci et al., 2009, 2010; Ozakca and Silah, 2013).

Previous studies solely focused to look at the result of BHT and BHA on mammalian cells (Chung, 1999; Panicker et al., 2014). Therefore, this study has been undertaken to investigate the mutagenic cytogenotoxicity of BHA and BHT in mitotic cells of *A. cepa* root tip.

2. Material and methods

Site of study was laboratory of Banaras Hindu University, Varanasi was selected for the experimental study. The dry *Allium cepa* bulbs L. (1.5–2.2 cm) in diameter were collected from the local market, were rinsed with tap water and outer dry scales and old root remnants were removed with care in order not to destroy the root primordial. The bulbs were germinated hydroponically in Hoagland medium (control) and concentration of BHT and BHA prepared in Hoagland medium (treatment) at room temperature (21 ± 2 °C) with 15 h light/9 h dark photoperiod for 48 h. After that roots were exposed to different concentration preservatives in the Hoagland medium (1000, 1500, 2000 and 2500 ppm) for 5, 10, and 15 days. The BHA and BHT (1, 1.5, 2.0, 2.5 g) was initially dissolved in 50% ethanol and then made a stock solution (10x) up to 100 ml (Jos et al., 2005). After that, the solution diluted in Hoagland medium of required (1000, 1500, 2000 and 2500 ppm) concentration. The best developed 10 roots of each onion in each group (control + treatment) were taken to calculate the mitotic index and measured mean root length.

2.1. Mitotic index (MI)

For fixation, root tips were fixed in fixative (95% ethanol: acetic acid, 3:1 v/v) and incubated overnight. On a subsequent day, root tips were washed with three changes of distilled water and preserved in 70% ethanol then stored at 4 °C for further use. Root tips were washed thoroughly in distilled water and treated with 1N HCl for 7–10 min at room temperature (RT). After repeated washing in distilled water, root tips were transferred on a clean glass slide containing a few drops of acetocarmine and slightly warmed. After placing the cover glass the slide was placed between filter paper folds and squashed gently (Sharma and Sharma 1980).

The slides were viewed under the light microscope with a magnification of 400X. Approximately more than five hundred cells were scored on every slide. The mitotic indices of the treated cells at every dose of preservatives were compared with the control group. Any dose of preservative was considered to be cytotoxic, if the mitotic index of treated cells at that concentration was half or less, compared to the mitotic index of control cells. Mitotic index and frequency of chromosomal abnormalities were calculated by formula 1 and 2 (Timothy et al., 2014; Türkoğlu, 2007)

$$\text{Mitotic index (MI)} = \frac{\text{Number of dividing cells (n)}}{\text{Total number of cells counted (N)}} \times 100 \quad (1)$$

$$\text{Frequency of chromosomal aberration (CA)} = \frac{\text{Number of aberrant cells}}{\text{total number of cells counted}} \times 100 \quad (2)$$

2.2. EC_{50} (effective concentration) value

EC_{50} value was taken into consideration for determining the 50% effective concentration of preservatives in the Hoagland medium (treatment) on root growth reduction of *A. cepa*.

2.3. Estimation of total protein content

Freshly weighed roots (500 mg) samples were homogenized in chilled mortar and pestle in 1 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% (w/v) PVP in liquid nitrogen. The homogenate was centrifuged at 9000X g for 15 min at 4 °C and the resultant crude supernatant was collected and stored at -20 °C for estimation of protein. Soluble protein content was determined according to the method of Bradford (1976) with bovine serum albumin as the standard.

2.4. Isolation of DNA

After treatment with preservatives for 5 days, DNA was isolated from *A. cepa* samples by Invitrogen Plant DNA Isolation Kit (HiMedia Chem. Ltd. India-MB502).

2.4.1. RAPD-PCR

RAPD-PCR was performed with 10 different RAPD primers to test amplification profiles for polymorphism, readability and reproducibility for treatment (5 days) as well as control by agarose gel electrophoresis (Atienzar et al., 2002). Sequences (5′-3′) of 6 primers successfully utilized were (OPA-01) CAGGCCCTTC; (OPA-02) TGCCGAGCTG; (OPA-03) AGTCAGCCAC; (OPA-05) AGGGGTCTTG; (OPA-07) GAAACGGGTG; (OPA-09) GGGTAACGCC, respectively. Amplification of genomic DNA was performed with GeNei Red Dye PCR Master Mix (Cat. No. 610667800041730) according to the manufacturer's instruction as 25 µl reaction mixture containing 11.2 Milli Q water, 12.5 µl 2X Red Dye PCR Master Mix, 0.3 µl of each primer and 1 µl template addition of DNA. PCR analysis was performed three times for control. Amplification was performed in Techne Thermal Cycler with 5 cycles for denaturation at 94 °C for 1 min, annealing at 27 °C for 30 s and extension 72 °C for 1 min, and then 45 cycles for denaturation at 94 °C for 45 s, annealing at 39 °C for 1 min and extension at 72 °C for 45 s, final extension at 72 °C for 15 min. Amplified products were kept in 4 °C. 9 µl each PCR product was electrophoresed on agarose gel (1.5%) in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 50 V for 2 h. The gel was stained by ethidium bromide and photographed.

2.4.2. Estimation of genomic template stability

Change that was observed in RAPD Profile was indicated as +1 arbitrary score. The stability is given as a percent and calculated according to the equation 100-(100 a/n), where ‘a’ is the number of polymorphic bands (appearing or disappearing in the sample) in the sample analyzed, n is the number of all DNA bands in the control sample.

2.5. Statistical analysis

Three replicated data of root lengths, MI and protein content analysis was expressed as Mean ± standard Error. EC_{50} was obtained from a plot of mean root lengths as a percentage of control and different concentrations of preservatives. Disparity between mean values was evaluated statistically using one-way analysis of variance (ANOVA). Duncan's multiple range tests were then used to determine which mean values were different at the 5% level of significance. All data were analyzed using SPSS 16.0. Graphical analysis was done using Microsoft Excel.

Table 1. Effect of food preservatives on root growth of *A. cepa*.

Chemicals	Applied Concentration	Mean root length (cm)±SD[a]		
		Day 5	Day 10	Day 15
	control	2.3 ± 0.27*b*	5.0 ± 0.44*d*	6.9 ± 0.78*d*
BHT	1000 ppm	1.9 ± 0.37*ab*	4.0 ± 0.46*cd*	4.1 ± 0.41*c*
	1500 ppm	1.7 ± 0.70*ab*	3.1 ± 0.07*bc*	3.4 ± 0.20*bc*
	2000 ppm	1.4 ± 0.21*ab*	2.0 ± 0.69*ab*	2.0 ± 0.62*ab*
	2500 ppm	0.8 ± 0.12*a*	1.6 ± 0.20*a*	1.6 ± 0.09*a*
	control	2.3 ± 0.27*b*	5.0 ± 0.44*c*	6.9 ± 0.78*b*
BHA	1000 ppm	1.7 ± 0.18*b*	2.6 ± 0.19*ab*	2.8 ± 0.19*a*
	1500 ppm	1.5 ± 0.37*ab*	2.9 ± 0.27*b*	2.9 ± 0.29*a*
	2000 ppm	1.4 ± 0.15*ab*	2.2 ± 0.66*ab*	2.3 ± 0.63*a*
	2500 ppm	0.7 ± 0.28*a*	1.4 ± 0.32*a*	1.4 ± 0.15*a*

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

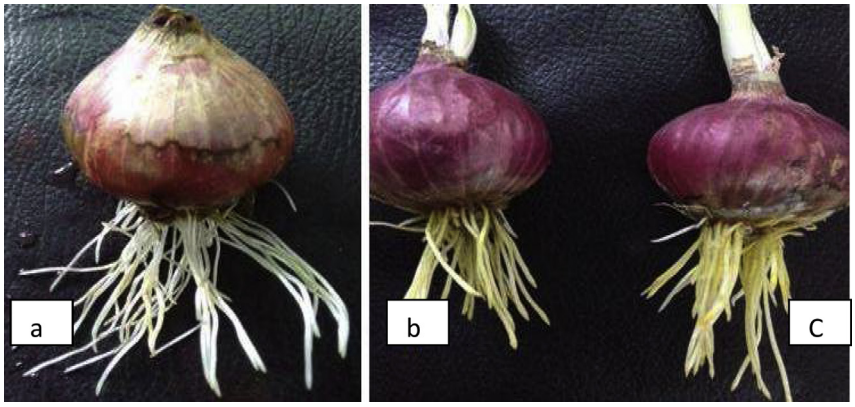

Figure 1. Root growths among experimental onions showing: (a) root growth in control (b and c) yellow colour roots after treatment with Butylated hydroxytoluene and Butylated hydroxyanisole.

3. Results

The dose-dependent reduction in mean root length has been observed in BHT was significant at ($p \leq 0.05$). Duncan's test was performed to observe the significant or insignificant variation among the data collected for control as well as the treatment group. The dissimilar alphabets showed some significant variation at $p \leq 0.05$. The reductions in mean root length at higher concentrations significantly differ from the control but it may not be significant within the same group. The doses between 2000-2500 ppm for 5 days showed a reduction in growth significantly differ with control but insignificant with each other whereas concentration between 1000-1500 ppm was found insignificant. The result of 10 days of analysis for root growth had shown significant reduction at all concentrations. The result of 15 days treatment, only the highest concentration (2500 ppm) was significantly differing with control. On the other hand, data for the BHA showed, all the root length was significantly different from control but insignificant with each other on days 5. The results of root length growth in different concentrations showed that all values are insignificant with each other but significantly differ with control for 10 and 15 days treatment for 10 and 15 days respectively. In concentration 1500 2000 and 2500 ppm the mean root length completely reduced or stopped at day 10 and 15 (Table 1).

Morphologically, the roots of onion bulbs were growing well hydroponically in control. The roots are healthy, longer, shiny, dense growth and growing very fast indicating high cell division which helps growth and development of the root. The root morphology showed obvious differences in its appearance as it became, less number of roots, not dense stiff, turned to yellow and change in texture in the treatment group (Figure 1).

Comparative analyses of EC_{50} value of treated roots with BHT and control were showed a sharp inhibition of root growth at effective concentration values ($EC_{50} = 2278.15$ ppm, $EC_{50} = 1809.26$ ppm and $EC_{50} = 1471.66$ ppm) for 5, 10 and 15 days respectively. Similarly, 50% reduction in root growth length for BHA was recorded at $EC_{50} = 2100.80$, 1624.44 and 1310.03 ppm for 5, 10 and 15 days respectively (Figure 2).

Total protein content (mg/g FW) in root samples showed a bit higher at 1000 ppm as compared to control after treatment with BHT and BHA, thereafter, a gradual reduction was observed in protein content of BHT treated root samples from 1500-2500 ppm, but a sudden decrease (approximately 75%) in protein content was observed in BHA treated root samples from 1500-2500 ppm (Figure 3).

Table 2 showed the exponential relationship between the percentage of aberrations and the concentrations of preservatives. Both the preservatives significantly increased 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 concentrations were increased from 1000 to 2500 ppm. The highest number of mitotic aberrations was recorded in root tips subjected to 2500 ppm treatment in BHT (18.36%) whereas BHA induced 22.34% aberrations at the same dose and duration of treatment. The mitotic index was significantly decreased when exposure of concentrations was increased from 1000 to 2500 ppm. The lowest number of mitotic index was recorded in root tips subjected to 2500 ppm concentration treatment for BHT (40.08 ± 0.44) whereas in the case of BHA 32.20 ± 1.25 MI was recorded at the same dose and duration of treatment.

The interpretation of the genotoxicity analysis was difficult due to the influence of the mutation and DNA damages on RAPD profiles. The electrophoretic bands of RAPD-PCR product from the control and treated

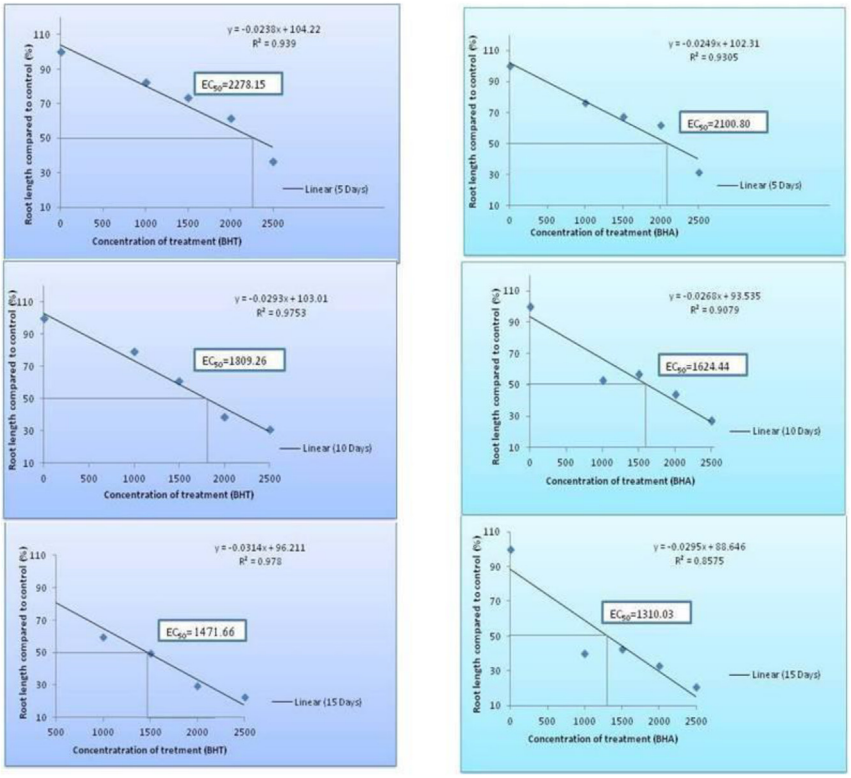

Figure 2. EC_{50} of a) BHT and b) BHA (5 days, 10 days and 15 days) at different concentration in *Allium* roots.

Table 2. Mitotic index and chromosomal aberrations in *Allium cepa* root tip cells exposed to increasing concentrations of Butylated hydroxytoluene and Butylated hydroxyanisole after 5 days treatment.

Treatment group	Conc.(ppm)	Total cells	Total mitosis	MI (mean ± S.E)	% of chromosomal abnormalities (mean ± S.E)
BHT	control	512.67 ± 1.45	314.00 ± 7.02	61.26 ± 1.50*d*	0.32
	1000	506.33 ± 2.03	223.33 ± 2.85	44.11 ± 0.59*c*	9.86
	1500	513.33 ± 2.91	213.67 ± 0.88	41.62 ± 0.16*b*	10.92
	2000	519.33 ± 1.20	208.67 ± 3.28	40.18 ± 0.55*a*	15.49
	2500	507.67 ± 1.76	203.33 ± 1.67	40.08 ± 0.44*a*	18.36
BHA	control	518.00 ± 3.46	267.77 ± 7.62	51.66 ± 1.13*e*	0.25
	1000	536.00 ± 1.73	226.33 ± 2.85	42.23 ± 0.65*d*	12.52
	1500	525.33 ± 2.91	200.67 ± 3.71	38.18 ± 0.62*c*	16.80
	2000	533.33 ± 2.40	184.67 ± 4.91	35.16 ± 1.06*b*	17.51
	2500	507.67 ± 1.76	171.67 ± 6.01	32.20 ± 1.25*a*	22.34

mi-mitotic index.
[a]Means with the same letters do not significantly differ at 0.05 level (Duncon's test).

samples have compared and such comparison depicted the result of appearing and disappearing of DNA bands due to mutation or DNA damage (Table 3). For the present study, the 10-mer oligonucleotide primers were used for screening the change in the genome in the form of presence and absence of bands. However, only 6 primers (OPA-01, OPA-02, OPA-03, OPA-05, OPA-07, and OPA-09) were suitable to amplify reproducible and informative (Figure 4). Electrophoretic bands showed obvious differences among control and treated roots samples with apparent changes. The total number of bands change was increased with increasing concentrations of BHT and BHA. Also revealed that genomic template stability (GTS %) values, a qualitative measure reflecting changes in RAPD profiles were calculated for each 6 primers (Table 4). Primer 5 and 9 showed only 50% GST value and less than 50% (20% and 40%) GTS values at 1500, 2000, and 2500 ppm respectively (Table 4). Similarly, GTS was recorded high (>60%) for all the primers used at all concentrations except primer 5 and 9 showed <50% at all concentrations except 1000 ppm (50%) indicate the unsuitability of the primer in respect to stable genomic character. Overall total RAPD assay showed the genomic template stability between the control group and BHT treatment groups (1000–2500 ppm) was 83.33%, 77.08%, 72.92%, and 72.92% respectively and for BHA it was 79.16%, 77.08%, 68.75%, and 68.75% respectively.

4. Discussions

Food preservatives have been used to keep the consistency, quality, texture, taste, color, alkalinity or acidity of the food. Humans are daily exposed to these chemical substances in their food items and neglected their effects for a longer period. The good taste, color, longer period consistency may be the reason to neglect its effects on Humans but,

Table 3. Changes of total bands in control, and of polymorphic bands and varied bands in *A.cepa* cells exposed to Butylated hydroxyltoluene (BHT) and Butylated hydroxyanisole (BHA).

primers	C	Treatments with BHT (ppm)																Treatments with BHA (ppm)													
		1000				1500				2000				2500				1000				1500				2000				2500			
		a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
OPA-01	11	2	1	0	0	2	1	1	0	1	2	1	0	0	1	0	0	2	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0
OPA-02	5	0	1	1	0	0	0	2	0	1	1	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	0
OPA-03	8	2	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	2	0
OPA-05	6	1	0	1	0	2	1	0	1	2	0	1	0	1	3	0	2	3	1	1	0	2	3	1	0	3	1	1	1	0	2	2	1
OPA-07	13	0	1	0	0	1	0	1	1	1	1	2	2	0	2	3	2	3	0	0	0	2	0	2	0	3	0	0	0	2	3	1	1
OPA-09	5	0	0	1	0	2	0	0	1	3	1	1	0	1	2	1	0	1	0	1	0	2	1	0	1	2	3	1	1	3	2	2	2
Total bands	48																																
a+b		8				11				13				13				10				11				15				15			
a+b+c+d		11				18				22				22				14				15				19				26			

C: indicates control group, a: indicates appearance of new bands, b: disappearance of normal bands, c: decrease in band intensities, and d: increase in band intensities. a+b denotes polymorphic bands, a+b+c+d, varied band.

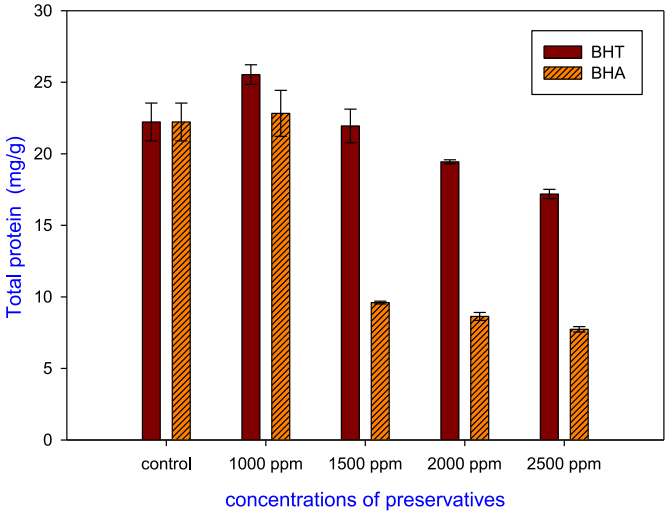

Figure 3. Total protein content in *Allium cepa* exposed to increasing concentrations of Butylated hydroxytoluene and Butylated hydroxyanisole.

nowadays, awareness for different kinds of diseases or abnormalities, it is necessary to focus on the investigation of their possible roles in human mutagenesis/carcinogenesis. The result of this study showed that BHA and BHT elicit inhibition of root growth and decrease the protein content in *A. cepa*. The inhibitions of *A. cepa* root growth by these chemicals were elicited at EC_{50} values as plotted in the graph (Figure 2) which is an indication of cytotoxicity (Sehgal et al., 2006). The same result of cytotoxicity induced by preservatives were also reported that include, the cytotoxicity in rat hepatocytes by linolenic acid (Sugihara et al., 1997) and (Gömürgen, 2005) also reported toxicity of potassium nitrate and potassium metabisulphite in the root tips of *A. cepa.*

Expansion of cells in the elongation zone of the root tip is the core cause of root growth where cellular differentiation occurs (Cordoba-Pedrosa et al., 2004). Biologically it would be explained that cellular expansion includes water uptake, nitrogen mobilization, increased sugar synthesis, and plasma and tonoplast membrane elasticity (Gonzalez-Reyes et al., 1995). Any alterations in these biological processes disrupted lipid biosynthesis and toxins have been coupled to reduced cell wall expansibility, loss of vacuolar homeostatic regulation, cellular toxicity, cell necrosis, and root growth inhibition (Gonzalez-Reyes et al., 1995). It has been also reported that shortening and decaying of roots are evidence of cytotoxicity (Sehgal et al., 2006).

mitotic index inhibition in the treated root tips could be due to inhibition of DNA synthesis or blocking of G2 phase in the cell cycle (El-Ghamery et al., 2000; Sudhakar et al., 2001), arrest of one or more mitotic phases preventing the cell from entering mitosis (Kabarity and Mallalah, 1980). The reduction in mitotic activity and increased value of percentage of aberrations with increasing concentrations demonstrates the capability of the BHT and BHA to inhibit DNA synthesis (Kannangara and Pathiratne, 2015; Boumaza et al., 2016) Considerable genotoxicity observed after increasing the concentration can be another possible cause for the lower mitotic index scores.

After treatment with preservatives, the total protein content in *A. cepa* system was found to be in stress condition. However, when the concentration of preservatives was 1500 ppm there was induction of total protein content. The reason could be justified that under stress condition some of the genes responsible for overcoming stress become highly active

Table 4. Genomic template stability (GTS %) in the root of onion bulbs exposed to BHT and BHA for 5 days.

	control	Treatment with BHT (ppm)				Treatment with BHA (ppm)			
		1000	1500	2000	2500	1000	1500	2000	2500
OPA-01	100	72.72	72.72	72.72	90.90	81.81	90.90	90.90	90.90
OPA-02	100	80	100	60	80	100	100	60	60
OPA-03	100	75	75	100	75	100	100	100	100
OPA-05	100	83.33	50	66.66	50	33.33	16.16	33.33	66.66
OPA-07	100	92.30	92.30	84.61	84.61	76.92	84.61	76.92	61.53
OPA-09	100	100	60	20	40	80	40	00.00	00.00
total	100	83.33	77.08	72.92	72.92	79.16	77.08	68.75	68.75

Genomic template stability for each 10-mers oligo was estimated by the formula GTS (%) = (1-a/n) X 100 where 'a' is the average number of changes in DNA profiles and n the number of bands selected in control DNA profiles.

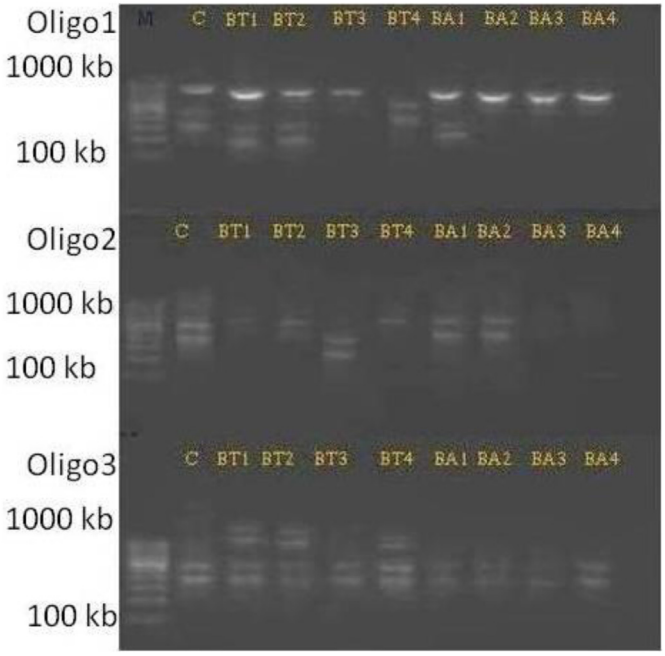

Figure 4. Oligo1, Oligo2 and Oligo3 RAPD Primer used in control group C, Butylated hydroxytoluene (BT) and Butylated hydroxyanisole (BA) exposed groups Where 1: (1000 ppm), 2: (1500 ppm), 3: (2000 ppm) and 4: (2500 ppm) represent the respective concentrations.

and lead to an increased in total protein content (Mahajan and Tuteja, 2005). Further severe decrease in total protein content might be due to inhibition of translocation reactions or polyptidyl transference reaction or to block the mRNA synthesis by binding to RNA or reported for α-amanitin (Perentesis et al., 1992) in ribosomes (Figure 3).

RAPD assay is the most widely used tools for assessment of the genetic variation (Singh et al., 1999). The treatment with BHT and BHA reflected the change in RAPD profile as variation in band intensity, disappearance or appearance of DNA bands. The present study reported that numbers of new appearing bands were greater at the highest concentration. The appearance of new bands, in DNA profile due to some mutation in oligonucleotide priming site and could become accessible to oligonucleotide primers after some structural change or some changes in DNA sequence (Table 3). The results indicated that genomic template stability (GTS) in onion bulbs was significantly affected by BHA and BHT. Genomic template stability is related to the level of DNA damage, the efficiency of DNA repair and replication. Therefore, a high level of DNA damage does not necessarily decrease the genomic template stability (in comparison to a low level of DNA alterations), because DNA repair and replication are inhibited by the high frequency of DNA damage (Atienzar et al., 2002). Based on the similar RAPD data, it has been indicated that *Thermopsis turcica* extract has DNA damaging effect on the root meristematic cells of onion bulbs at dose- and time-dependent manner (Ciğerci et al., 2016).

The carcinogenicity of BHA might be due to tert-Butylhydroquinone (TBHQ) formation resulting in oxidative damage to DNA (Gharavi et al., 2007) and BHT is toxic when given after initiating carcinogen for mouse lung (Witschi et al., 1997).

Overall, the present study suggests that BHT and BHA are capable to induce cytotoxicity and genotoxicity in root tip cells of *A. cepa*. It may be suggested that higher concentrations should not be used in packed foods and even low concentration would be harmful because the continuous use of these preservatives gradually accumulate in the body system may cause carcinogenic or mutagenic effect on humans. Therefore, it is essential to test the genotoxic effects of food preservatives on the other systems also.

Declarations

Author contribution statement

Himadri Pandey: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sanjay Kumar: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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