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Genotoxic Effect of Paraben and Oxybenzone on Root Tips of *Allium cepa*

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Abstract

Paraben is widely used as preservative in foodstuffs, cosmetics and pharmaceutical drugs. Oxybenzone is the most popular UV filter in skin protecting formulations or sunscreens. When washed into the water bodies both paraben and oxybenzone act as environmental contaminants. Though there are reports on cytotoxicity of Paraben and Oxybenzone on aquatic animals, studies on genotoxic and cytotoxic behavior of these on plant cells are few. This study aims to investigate cytotoxic and genotoxic impacts of Oxybenzone, Methyl paraben and Propyl paraben on root tip cells of *Allium cepa,* using it as an indicator organism, exposing the onion root tips to different concentrations of Oxybenzone (0.1, 0.2, 0.3, 0.4, 0.5, 0.6%), Methyl paraben and Propyl paraben (0.01, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30%) that are usually used as per FDA guidelines. The maximum root length (28.6±0.3mm) was observed in 0.01% concentration of MP after 24 h, then (28.9±0.3mm) in 0.01% concentration of MP after 48 h and then (18.5±0.7mm)in 0.1% concentration of OB after 72 h. respectively. The average decrease in root length was far more prominent in MP concentrations than in OB and PP. The mitotic index of the control was found to be 32.4±0.7, 36.9±0.6 and 40.5±0.7 after 24, 48 and 72 h respectively. Mitotic index reduced to 8.9±0.5, 8.2±0.3 and 7.5±0.6 in 0.6% OB and 6.5±0.3, 5.9±0.9 and 5.1±0.8 in 0.30% MP 9.2±0.3, 8.8 ±0.4 and 8.1±0.6 in 0.30 PP after 24, 48 and 72 h of exposure. A high negative correlation was observed between Mitotic index and concentrations of OB, MP and PP. Higher concentrations OB, MP and PP disturb stages of cell division causing chromatin bridge formation, stickiness, disturbed metaphase, multiple chromosomal breaks and cell disintegration. The present study also suggests that plants being an important component of the ecosystems need to be included in evaluating the overall toxicological impact of the parabens and oxybenzone in the environment.

Keywords: Allium cepa; Chromosomal aberrations; Cytotoxicity; Paraben; Mitotic index; Oxybenzone

Introduction

Parabens and oxybenzone are growing human and environmental contaminants (Petrie et al., 2015). Parabens are a class of preservatives widely used in cosmetics (makeup, commercial moisturizers, sunscreens, tanning solution, sprays, shaving gels, shampoos, toothpaste etc.), topical pharmaceutical products and packaged food. The aqueous medium of majority of these products (foods, cosmetics and pharmaceuticals) shortens their shelf life as it makes them susceptible to the growth of mold, fungi, bacteria and yeast and may lead to spoilage, discolorations, malodor or chemical breakdown. To enhance shelf life and prevent other deteriorations of these products parabens are used as additives (Garner et al., 2014). The permissible limits of parabens are 0.01-0.3% in cosmetics and 0.1% in food products as per U.S. FDA guidelines (USFDA, 2022).

Through regular use and wash offs these chemicals enter ecosystems. Parabens are a group of parahydroxybenzoates or esters of parahydroxybenzoic acid. The methyl, ethyl, propyl, butyl and heptyl are common forms of parabens. Whereas less common forms of parabens are isobutyl, benzyl and their sodium salts. Low concentration levels of parabens are always present in effluents of wastewater treatment plants despite several treatments. Though Parabens are biodegradable and ubiquitous in their nature, their accumulation and direct contact with plants may pose many hazards.

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Methylparaben and propylparaben predominate, reflecting the commonly used components of parabens in consumer products (Haman et al., 2015). Oxybenzone or benzophenone-3 (BP-3) is 1 of 16 approved (by U.S. FDA, 1978) active sunscreen ingredients that protect human beings from harmful effects of both UVA and UVB radiation either by absorbing, scattering or reflecting these radiations. Oxybenzone is a conjugated, aromatic hydrocarbon with its hydroxyl group being hydrogen bonded to its ketone group. This interaction enhances the light absorption properties of oxybenzone even at lower energies. It is one of the oldest active sunscreen ingredients playing a vital role in protecting consumers against skin cancer and premature skin aging (Mirsky et al., 2018). FDA has approved the use of oxybenzone as an active ingredient in sunscreens and other cosmetic products up to concentrations of 6% and 10% respectively.

Table 1 *Values for particular treatment Comparative root length (mean ± standard deviation; level of significance determined by ANOVA: *p _< 0.05) of Allium cepa exposed to various concentrations of oxybenzone*

Concentration (%) of Oxybenzone	after 24h Exposure		after 48 h Exposure		after 72 h Exposure	
	Root length (mm)	Percentile Root length	Root length (mm)	Percentile Root length	Root length (mm)	Percentile Root length
0 Control	80.1±0.9	100	98.7±1.1	100	107.2±2.0	100
0.1	70.0±1.3	87.3	78.4±1.6	79.4	83.5±1.1	77.8
0.2	63.3±0.8	79.0	65.4±1.7	66.2	69.9±1.5	65.2
0.3	75.4±2.3	94.1	80.0±1.1	81.0	81.7±0.6	76.2
0.4	68.5±2.1	85.5	73.3±0.8	74.2	74.8±1.1	69.7
0.5	71.6±1.1	89.3	77.4±1.0	78.3	78.3±0.2	73.0
0.6	65.3±1.8	81.5	70.1±0.7	71.0	69.5±0.3	64.8

Table 2 Values for particular treatment Comparative root length (mean ± standard deviation; level of significance determined by ANOVA: *p _< 0.05) of *Allium cepa* exposed to various concentrations of Methylparaben

Concentration (%) of Methylparaben	after 24h Exposure		after 48 h Exposure		after 72 h Exposure	
	Root length (mm)	Percentile Root length	Root length (mm)	Percentile Root length	Root length (mm)	Percentile Root length
0 Control	95.0±0.7	100	112.4±1.1	100	132.1±1.2	100
0.01	85.1±1.9	89.5	90.0±1.7	80.0	94.1±0.4	71.2
0.05	74.0±1.1	77.8	78.3±1.6	69.6	80.0±1.3	60.5
0.10	81.4±0.4	85.6	82.3±0.4*	73.2	83.9±1.1	63.5
0.15	67.3±0.7	70.8	71.8±1.2	63.8	70.4±0.5	53.2
0.20	70.3±1.6	74.0	73.9±0.5	65.7	74.2±1.6	56.3
0.25	64.8±0.8	68.2	66.5±0.3*	59.1	66.9±0.4	50.0
0.30	76.7±1.8	80.7	78.3±1.1	69.6	77.9± 0.6	58.9

Table 3 *Values for particular treatment Comparative root length (mean ± standard deviation; level of significance determined by ANOVA: *p _< 0.05) of Allium cepa exposed to various concentrations of Propylparaben*

Concentration (%) of Propylparaben	after 24h Exposure		after 48 h Exposure		after 72 h Exposure	
	Root length (mm)	Percentile Root length	Root length (mm)	Percentile Root length	Root length (mm)	Percentile Root length
0 Control	95.0±0.7	100	112.4±1.0	100	132.1±1.2	100
0.01	73.7±1.8	77.5	81.6±1.7	72.5	85.2±0.5	64.4
0.05	77.1±1.4	81.1	78.3±0.7	69.6	80.0±1.9	60.5
0.10	81.6±0.8	85.8	82.4±1.6	73.3	84.7±1.6	64.7
0.15	67.5±1.1	71.0	71.5±0.6	63.6	70.9±0.3	53.6
0.20	70.6±0.7	74.3	73.1±0.8	65.1	73.9±0.8	55.9
0.25	64.1±1.0	67.4	66.1±0.9	58.8	66.3±0.6	50.1
0.30	76.5±0.7	80.5	78.1±0.9	69.9	78.6±0.5	59.1

Allium cepa assay is a standard test for rapid and sensitive screening of chemicals and pollutants indicating environmental hazards (Fiskesjö, 1993). It is an efficient test for *in situ* monitoring for genotoxicity of environmental pollutants and their chemical screening (Feretti et al., 2007). The root tip system of *Allium cepa* has particularly shown sensitivity to harmful effects of chemicals/pollutants as the root tip is often the foremost part of a plant that comes into contact with chemicals/pollutants found in water or soil. There are various studies that indicate toxic

effects of several chemicals with the *Allium cepa* test in less than 24 h or even in just 3 h (Yuzbasioglu et al., 2009). *Allium cepa* chromosomal aberration assay is employed to evaluate the genotoxicity of various chemical compounds and environmental pollutants. (Grant, 1982; Rank and Nielsen, 1997; Liman et al., 2014). *Allium cepa* test is a useful bio-indicator to detect genotoxicity also severity of pollutants (Shakyawal et al., 2020). Hindrance of root development and the appearance of dwarfed roots are indicators of growth retardation and cytotoxicity (Liman et al., 2015; Randhawa et al., 2019). Therefore, in this study we investigate the genotoxic and cytotoxic effects of exposures to varying concentrations of MP, PP and OB on *A. cepa* root cells.

Materials and methods

Test system and treatment

The *Allium cepa* root growth parameters and chromosomal aberration assays are highly sensitive and are capable of detecting any change in the environment (Tedesco and Laughinghouse IV, 2012). *Allium cepa* root growth assay is used to study macroscopic parameters such as root length, consistency of roots and presence of hooks (Fiskesjö, 1985). It is an established plant bioassay approved by the International Programme on Chemical Safety (WHO, 1985) and the United Nations Environment Programme (Grant, 1982) as a competent and standard test for chemical screening and In-situ monitoring for genotoxicity in the environment. Hence to assess the possible genotoxic effects of parabens and oxybenzone root tip cells were used as the test system.

Test model

Allium cepa as it is considered to be as a suitable plant indicator for the determination of potential genotoxic agents in the testing samples (Firbas & Amon, 2014).

Onion preparation

Small, healthy, uniform-sized onion bulbs (*Allium cepa*: 2n=16), weighing about 3–4 g were selected. After cleaning off the loose outer scales, the old roots were removed with the help of a forceps to expose the root primordia.

Test procedure

Series of onion (*Allium cepa*) bulbs are grown in 100 ml glass flasks containing distilled water and allowed to germinate at room temperature (25±2°C) for pre-germination, away from direct sunlight. No initial treatment with distilled water was given to the bulbs; hence this method of treatment is more similar to natural conditions (Fiskesjö, 1979). After seven days when the roots length is about 3-4 cm, roots were exposed to the series of concentrations of Oxybenzone (OB) (0.1-0.6%), Methylparaben (MP) (0.01-0.3%) and Propylparaben (PP) (0.01-0.3%), in distilled water for 24 to 72 h at relatively same temperature, away from direct sunlight. The control and exposures were set up in duplicates.

Macroscopic parameters for toxicity

The root lengths of all the onion bulbs that were grown in 100 ml pots in distilled water (control) and aqueous solutions of OB, MP and PP at various concentrations were recorded (Fiskesjö, 1995). The mean values were calculated from ten measurements and relative growth values were expressed as percent of the control value. The other possible signs of toxicity, like change in color of root color, consistency and appearance, were also examined.

Chromosome slide preparation and fixation

The squash technique for chromosomal preparations of *Allium cepa* root tips, standardized by Al-Sabti and Kurelec (1985) and Al-Sabti (1989), was followed. After washing in distilled water for 15 min, the root tips of 4 mm length were placed into a Petri dish with 2 ml acetic acid and HCl solution. The root tips were then heated for 5 minutes at 60°C. Then, the sample was stained and squashed in 0.5% Aceto-carmine for 5-6 minutes at 60°C without hydrolysis and squashed in Aceto-carmine. The root tips were removed and placed on glass slides covered with a cover slip.

The root tips were then squashed by pressing slightly down and sealed with DPX. The slides were coded and the root meristematic cells were observed under light microscope. The slides were analyzed at 1000× magnification.

Microscopic parameter for toxicity

To measure and compare the toxic effects of parabens and oxybenzone, the Mitotic index (MI) and Chromosomal aberrations (CA) were analyzed.

Mitotic index (MI)

Aceto-carmine squash method was used to determine mitotic index and changes in chromosome morphology in *Allium cepa* root tips. The foremost part of a plant that comes into contact with chemicals/pollutants found in water or soil is root tip. *Allium cepa* root tips cells were used as the test system because they are highly sensitive and are capable of detecting any change in the environment (Tedesco and Laughinghouse IV, 2012). The effects were observed after every 24 h till 72 h. The root lengths of *Allium cepa* after exposure to various concentrations of MP, PP and OB were measured after every 24 h. Correlation between the root length and sample concentrations were calculated. Observations were made as to whether the samples suppressed root growth when compared with control or had no effect.

Table 4 MI (mean ± standard deviation) of the root meristematic cells of *Allium cepa* exposed to various concentrations of oxybenzone after 24, 48 h and 72h treatment.

Concentration (%) of Oxybenzone	MI %		
	24h	48h	72 h
CONTROL	32.4±0.7	36.9±0.6	40.5±0.7
0.1	19.2±0.8	18.9±0.6	18.5±0.7
0.2	14.8±0.4	14.3±0.6	13.7±0.5
0.3	13.3±1.1	13.1±0.3	12.5±0.2
0.4	12.9±0.6	11.8±0.5	11.6±0.5
0.5	10.1±0.6	9.9±0.4	9.3±0.3
0.6	8.9 ±0.5	8.2±0.3	7.5±0.6

Table 5 MI (mean ± standard deviation) of the root meristematic cells of *Allium cepa* exposed to various concentrations of methylparaben after 24, 48 h and 72h treatment.

Concentration (%) of Methylparaben	MI %		
	24h	48h	72 h
CONTROL	32.4±0.7	36.9±0.6	40.5±0.7
0.01	28.6±0.3	28.9±0.3	27.5±0.7
0.05	20.8±0.3	20.2±0.4	19.6±0.8
0.10	18.4±0.4	18.0±0.4	18.0±0.2
0.15	12.4±0.5	11.8±0.5	11.2±0.5
0.20	15.6±0.6	14.9±0.4	13.6±0.9
0.25	8.8±0.3	8.0±0.7	7.7±1.1
0.30	6.5±0.3	5.9±0.9	5.1±0.8

Table 6 MI (mean ± standard deviation) of the root meristematic cells of *Allium cepa* exposed to various concentrations of propylparaben after 24, 48 h and 72h treatment.

Concentration (%) of Propylparaben	MI %		
	24h	48h	72 h
CONTROL	32.4±0.7	36.9±0.6	40.5±0.7
0.01	29.5 ±1.1	29.9±0.8	28.7±0.7
0.05	19.3±0.8	18.9±0.5	18.1±0.8
0.10	12.4±0.6	12.0±0.5	11.5±0.3
0.15	11.6±0.6	11.8±0.5	11.2±0.5
0.20	11.3±0.4	11.9±0.4	11.6±0.4
0.25	11.2±0.5	10.5±0.3	9.9±0.3
0.30	9.2±0.3	8.8 ±0.4	8.1±0.6

Chromosomal aberrations (CA)

Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosome. The physiological and clastogenic aberrations like Chromosomal stickiness in metaphase and anaphase stages, breaks laggards, vagrants, fragments, chromosomal bridges, nuclear bud, nucleus alterations and morphological alterations of cell and nucleus were noted in the treated cells. In this study different kinds of chromosomal aberrations were observed with different concentrations of OB, MP and PP.

Table 7 Different Chromosome aberrations in the root cells of *Allium cepa* exposed to various concentrations of Oxybenzone.

Concentration (%) of Oxybenzone	Time of exposure (h)	Chromosomal aberrations (CAs)					Cytological aberrations			% (CAs)
		Bridges	Breaks	Stickiness	Abnormal kinetics	TNA	Nuclear buds	MA	TNA	
Control	-	-	-	-	-	-	-	-	-	Nil
0.1	24	2	3	3	6	14	1	-	1	1.63
	48	2	5	3	8	18	2	1	3	2.07
	72	3	5	3	10	20	2	1	3	2.26
0.2	24	1	1	8	7	17	-	3	3	1.85
	48	2	1	9	14	26	-	6	6	3.15
	72	2	3	9	16	32	-	9	9	4.03
0.3	24	4	3	7	6	20	2	-	2	2.31
	48	6	5	11	18	40	1	6	7	4.63
	72	6	5	13	22	46	1	6	7	5.22
0.4	24	-	3	9	11	23	-	1	1	2.34
	48	2	3	10	16	31	1	3	4	3.44
	72	7	4	12	19	42	1	4	5	4.63
0.5	24	1	2	7	8	18	1	15	16	3.07
	48	9	6	8	13	36	2	18	20	5.51
	72	13	7	14	17	51	2	20	22	7.19
0.6	24	3	2	10	9	24	-	10	10	3.49
	48	8	4	12	11	35	2	16	18	5.22
	72	15	5	14	16	50	3	23	26	7.48

TNA: Total number of aberrant cells, **MA:** Morphological Alterations of cell. Out of 1000 cells examined for CA

Table 8 Different Chromosome aberrations in the root cells of Allium cepa exposed to various concentrations of Methylparaben.

Concentration (%) of Methylparaben	Exposure Time (h)	Chromosomal aberrations (CAs)					Cytological aberrations			% (CAs)
		Bridges	Breaks	Stickiness	Abnormal kinetics	TNA	Nuclear buds	MA	TNA	
Control	-	-	-	-	-	-	-	-	-	Nil
0.01	24	-	1	-	2	3	-	-	-	0.20
	48	-	3	2	8	13	-	-	-	1.28
	72	-	3	3	10	20	-	1	1	1.97
0.05	24	1	2	2	6	11	-	-	-	1.08
	48	1	5	3	9	18	-	1	1	1.97
	72	2	5	3	10	20	2	1	3	2.26
0.10	24	-	-	-	6	6	-	-	-	0.59
	48	1	-	4	7	12	1	-	1	1.28
	72	1	2	8	11	22	1	-	1	2.26
0.15	24	3	-	7	6	16	-	2	2	1.77
	48	3	2	11	14	30	1	3	4	3.34
	72	3	2	13	19	37	1	3	4	4.03
0.20	24	-	3	8	11	22	2	-	2	2.36
	48	2	3	9	15	29	1	4	5	3.34
	72	5	4	9	17	35	1	4	5	3.94
0.25	24	1	2	7	12	22	1	3	4	2.56
	48	6	6	11	15	38	1	4	5	4.23
	72	8	7	14	16	45	1	15	16	6.00
0.30	24	1	2	7	14	24	1	5	6	2.95
	48	7	6	8	16	37	2	8	10	4.63
	72	7	7	13	19	46	2	14	16	6.10

TNA: Total number of aberrant cells, **MA:** Morphological Alterations of cell. Out of 1000 cells examined for CA

Data analysis

Different stages of mitosis were counted and chromosomal aberrations were observed to calculate the mitotic index, phase indices and total abnormality percentage at different stages of cell division.

$$\text{Mitotic index (MI)} = TDC/TC \times 100 \quad (1)$$

$$\text{Phase index (PI)} = TC/TDC \times 100 \quad (2)$$

$$\text{Total percentage of abnormal cells} = Tabn/\ TDC \times 100 \quad (3)$$

Where TDC = total number of dividing cells; TC = total number of cells observed; Tabn = total number of abnormal cells (Kumari et al., 2009).

Toxic effects of OB, MP and PP were evaluated in terms of macroscopic and microscopic parameters.

Table 9 Different Chromosome aberrations in the root cells of Allium cepa exposed to various concentrations of Propylparaben.

Concentration (%) of Propyl paraben	Exposure Time (h)	Chromosomal aberrations (CAs)					Cytological aberrations			% (CAs)
		Bridges	Breaks	Stickiness	Abnormal kinetics	TNA	Nuclear buds	MA	TNA	
Control	-	-	-	-	-	-	-	-	-	Nil
0.01	24	-	-	-	5	5	-	-	-	0.49
	48	-	1	1	5	7	-	1	1	0.78
	72	-	3	3	6	12	-	1	1	1.28
0.05	24	1	2	2	5	10	-	-	-	0.98
	48	1	3	3	9	16	-	1	1	1.67
	72	2	5	3	10	20	2	1	3	2.26
0.10	24	1	1	7	8	17	-	2	2	1.87
	48	2	1	9	12	24	1	2	3	2.66
	72	2	2	9	13	26	1	2	3	2.85
0.15	24	1	2	3	9	15	-	2	2	1.67
	48	1	5	5	12	23	-	6	6	2.85
	72	5	5	8	17	35	1	8	9	4.33
0.20	24	2	3	8	11	24	-	2	3	2.66
	48	2	3	10	15	30	1	6	7	3.64
	72	5	4	9	17	35	1	9	10	4.43
0.25	24	4	3	7	7	21	1	3	4	2.46
	48	5	6	10	19	40	1	6	7	4.63
	72	6	5	14	21	46	1	10	11	5.61
0.30	24	3	2	10	9	24	1	5	6	2.95
	48	8	4	11	10	33	2	7	9	4.13
	72	15	5	14	16	50	2	12	14	6.30

TNA: Total number of aberrant cells, **MA:** Morphological Alterations of cell. Out of 1000 cells examined for CA

Statistical analysis

Data were subjected to standard deviation, and were presented as mean ± standard deviation. Analysis of variance (ANOVA) test was used to determine the significance level of the difference between the samples at the 5% significance and the Linear Regression analysis was used in SSPS statistics version 16.0 and GraphPad Prism 8 software.

Results

Macroscopic analysis

The effects on *Allium cepa* root tip, after exposure to various concentrations of OB, MP and PP, were observed by analyzing macroscopic parameters like root lengths, root growth and root shape. The root length parameter showed significant differences after 24, 48 and 72 h exposure (Table-3.A-C). The maximum root length (28.6±0.3mm) was observed in 0.01% concentration of MP after 24 h, then (28.9±0.3 mm) in 0.01% concentration of MP after 48 h and then (18.5±0.7mm) in 0.1% concentration of OB after 72 h. The root length and concentration were negatively correlated for OB, MP and PP. In case of OB after exposure of 24 h ($r = -0.4805$, $n = 7$, $P < 0.05$) , 48 h ($r = -0.5811$, $n = 7$, $P < 0.05$) and 72 h ($r = -0.7110$, $n = 7$, $P < 0.05$).In case of MP after exposure of 24 h ($r = -0.6859$, $n = 8$, $P < 0.05$) , 48 h ($r = -0.7071$, $n = 8$, $P < 0.05$) and 72 h ($r = -0.6888$, $n = 8$, $P < 0.05$). In case of PP after exposure of 24 h ($r = -0.5799$, $n = 8$, $P < 0.05$), 48 h ($r = -0.6314$, $n = 8$, $P < 0.05$) and 72 h ($r = -0.6353$, $n = 8$, $P < 0.05$). The average decrease in root length was far more prominent in MP concentration than OB and PP and significant differences in root lengths were found in 0.10 and 0.25% concentrations of MP. It was observed that OB, MP and PP suppressed root growth and the presence of twists (crochet, hooks) in root was noticed in higher concentrations after 48 and 72 h.

Microscopic analysis

Mitotic Index (MI)

The mitotic index is a reliable parameter which allows estimating the frequency of cellular division (Marcano et al., 2004; Fernandes et al., 2007; Singh Z and Singh I, 2019). The reduction in MI was dose and duration dependent. The MI in the case of OB, MP and PP decreased subsequently with increasing concentrations and duration of treatment. This suggests a highly cytotoxic behavior of these chemicals. In our studies, we have found a high negative correlation between MI and concentrations of OB, MP and PP. In case of OB ($r = -0.9655$, $n = 6$, $P < 0.05$) after

24 h,(r =-0.9710, n = 6, P < 0.05) after 48 h and (r = -0.9674, n = 6, P < 0.05) after 72 h (Table 4). In case of MP (r =-0.9444, n = 7, P < 0.05) after 24 h,(r =-0.9410, n = 7, P < 0.05) after 48 h and (r =-0.9534, n = 7, P < 0.05) after 72 h (Table 5). In case of PP (r =-0.8271, n = 7, P < 0.05) after 24 h,(r =-0.8271, n = 7, P < 0.05) after 48 h and (r = -0.8322, n = 7, P < 0.05) after 72 h (Table 6)

The linear relationship between concentrations (0.1, 0.2, 0.3.0.4, 0.5and 0.6) MI of OB, (0.01, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30) MI of MP, (0.01, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30) MI of PP was obtained by regression analysis (Fig. 1)

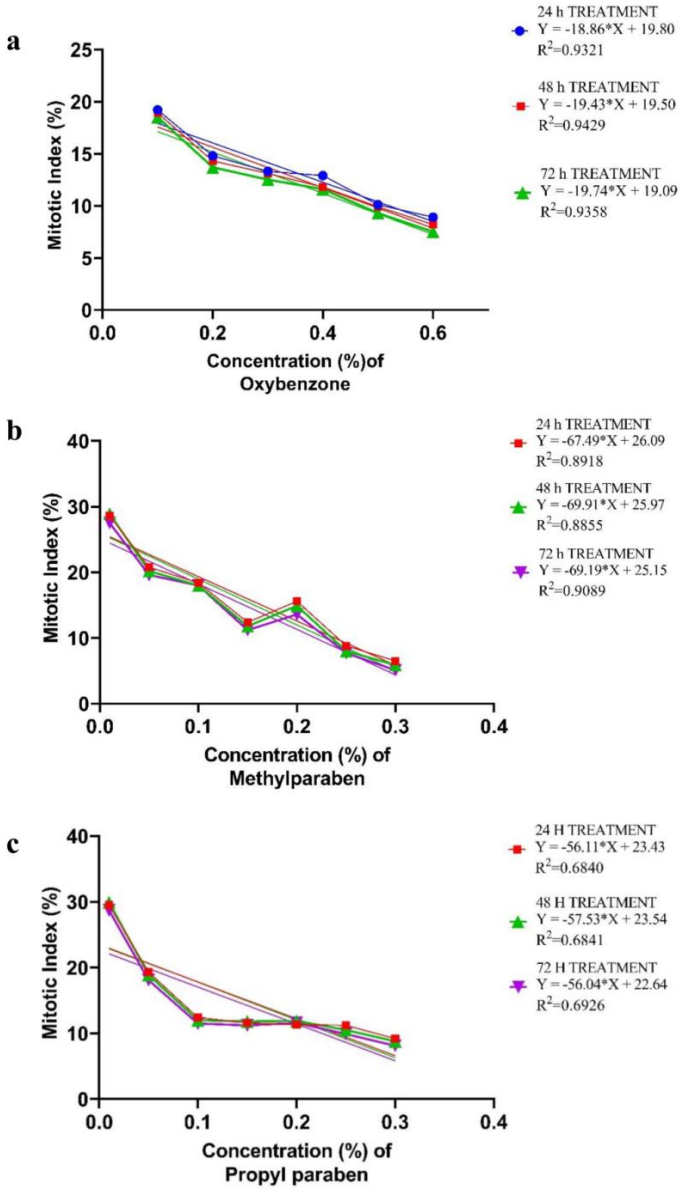

Fig. 1 Relationship between different concentrations of a) OB, b) MP and c) PP after 24h, 48h & 72h with MI in Allium cepa root chromosomal aberration assay.

Chromosomal Aberrations (CA)

The effects of various concentrations of OB, MP and PP on the root cells of *Allium cepa* after exposure to parabens and oxybenzone for 24, 48 and 72 h were exhibited by chromosomal damage. Resulting cytological and chromosomal aberrations (Bridges, breaks, stickiness and abnormal kinetics) observed in the root tip cells of *Allium cepa* treated with various concentrations of OB, MP and PP are shown in Tables 7-9. CAs increased with increasing concentrations. The occurrence of chromosomal aberration or abnormalities was more prominent in higher concentrations of OB, MP and PP. The lowest frequency of CA was observed

in 0.01% concentration of MP after 24 h, 0.01% concentration of PP after 48 h and 0.01% concentration of PP after 72 h. The highest frequency of CA was observed at 0.6% concentration of OB after 24 h, 0.6% concentration of OB after 48 h and 0.6% concentration of OB after 72 h.

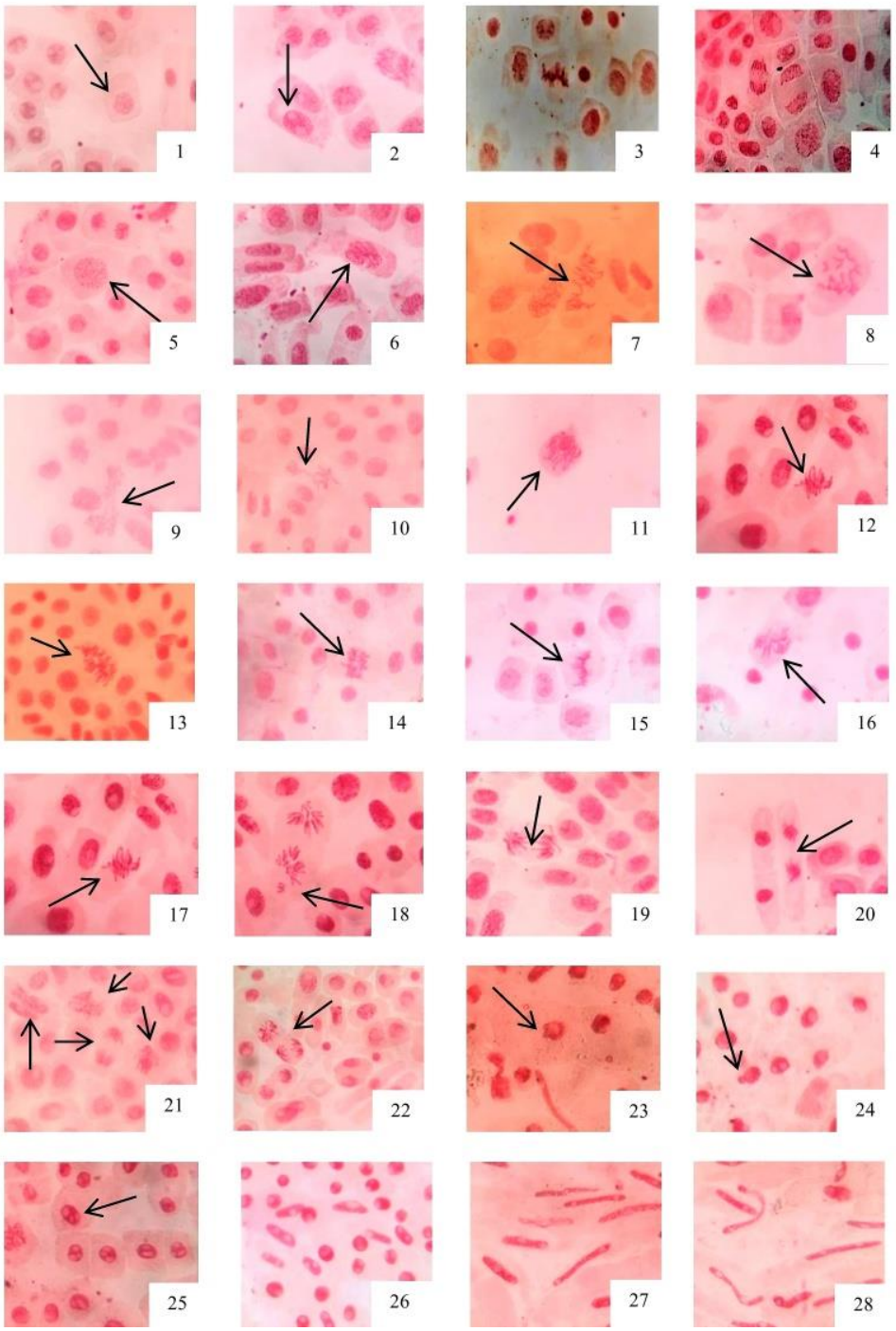

Fig. 2 Observed chromosomal aberration and cytological effects in Allium root cells. Images showing the normal mitotic Prophase, Metaphase, Anaphase, Telophase and Cytokinensis (1-4), micronucleus in prophase (5), stickiness (6), chromosomal break and abnormal kinetics (7-8), Chromosomal bridge (9-10), metaphase with chromosomal stickiness or adherence (11-16) polyploid metaphase (17), anaphase with chromosomal breakage (18), anaphase with chromosomal bridge (19), Telophase with laggard chromosome (20), stickiness, chromosomal loss and vagrant chromosomes (21-22), prophase with nuclear bud (23-24), nucleus alterations like binucleated cells at early prophase stage (25-26), Morphological Alterations (elongation) of cell and nucleus (27-28). Magnification for all images ×1000.

The percentage of CA showed positive correlation with concentrations of OB, MP and PP after 24, 48 and 72 h of treatment. In case of OB ($r = 0.9761$, $n = 6$, $P < 0.05$) after 24 h, ($r = 0.7994$, $n = 6$, $P < 0.05$) after 48 h and ($r = 0.9478$, $n = 6$, $P < 0.05$) after 72 h (Table 4). In case of MP ($r = 0.9546$, $n = 7$, $P < 0.05$) after 24 h, ($r = 0.9386$, $n = 7$, $P < 0.05$) after 48 h and ($r = 0.9562$, $n = 7$, $P < 0.05$) after 72 h (Table 5). In case of PP ($r = 0.9490$, $n = 7$, $P < 0.05$) after 24 h, ($r = 0.9566$, $n = 7$, $P < 0.05$) after 48 h and ($r = 0.9902$, $n = 7$, $P < 0.05$) after 72 h (Table 6).

The major chromosomal aberrations were noted and are shown in Fig. 2 chromosomal stickiness (5-6, 11-17), breaks laggards (7-8, 20), vagrants (21-22), chromosomal bridges (9-10,19) nuclear bud (23-24), nucleus alterations (25-26) and Morphological Alterations of cell and nucleus (27-28).

Discussion

Plants are important materials for genotoxic test and have been used for identification of environmental pollutants (Rank and Nielsen, 1997; Grover and Kaur, 1999). The *Allium cepa* assay is an efficient test for the chemical screening and *in situ* monitoring for genotoxicity of environmental pollutants (Feretti et al., 2007). The mitotic index, MI, is a reliable parameter which allows estimating the frequency of cellular division (Marcano et al., 2004; Fernandes et al., 2007). *Allium cepa* root assay for food preservatives sodium benzoate , boric acid , citric acid, potassium citrate and sodium citrate reduced mitotic division. Mitotic index decreased with increasing concentrations and longer treatment (Türkoğlu, 2007).

In the present study the Parabens and oxybenzone exhibited genotoxicity by decreasing the mitotic index in a dose and duration dependent manner. Similar effects on MI were reported by many earlier studies in the *Allium cepa* test (Özkara, 2015; Marcano et al., 2004; Pandey, 2008; Fındikli and Türkoğlu, 2010; Ozen et al., 2011; Andrioli et al., 2012).The decrease in MI reveals probable mitodepressive effect of Parabens and oxybenzone, i.e. it could hinder with the normal progression of mitosis, thus preventing a number of cells from entering the prophase and blocking the mitotic cycle during interphase inhibiting DNA/protein synthesis (El-Ghamery, 2000). Inhibition of mitotic activities is used for screening of cytotoxic agents (Linnainmaa et al., 1978; Sharma and Vig, 2012).

Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosome. Genotoxicity can be observed by chromosome aberrations in anaphase-telophase *Allium cepa* root tip cells (Grant, 1982; Rank and Nielsen 1993). Cytogenetic effects of the potassium metabisulphite in *Allium cepa* root meristem cells induced CAs such as breaks, gaps, multiple breaks and chromatid breaks (Kumar and Panneerselvam, 2007). CAs increase with increasing concentration of the tested chemical and for a longer period of treatment (Ragunathan and Panneerselvam, 2007).Different concentrations of glycidol induce CAs such as breaks, gaps, exchange, multiple breaks and chromosome fragments. Increasing concentrations of glycidol increased the number of CAs (Panneerselvam et al., 2012).Similar effects of CAs were noted in this study where different kinds of chromosomal aberrations were observed with different concentrations of OB, MP and PP. The physiological and clastogenic CAs like chromosomal stickiness in metaphase and anaphase stages, breaks laggards, C-mitosis, vagrants, fragments, chromosomal bridges, nuclear bud, nucleus alterations and morphological alterations of cell and nucleus were noticeable in the treated cells. Darlington and McLeish (1951) reported that damage or depolymerization of chromosomal DNA may lead to stickiness of the chromosomes. Stickiness has also been attributed to involvement of inter chromosomal chromatin fibers. Stickiness is probably an irreversible effect and a common indicator of detrimental effects of toxic elements on the chromosomes.

This study is an initial approach which revealed that parabens and oxybenzone are affecting plant growth if it is present in water or soil. Nevertheless, various studies found the harmful impacts of parabens and oxybenzone on animals. Previously, Tavares et al. (2009) reported that

in developed countries about 15% of human couples are affected by infertility, of which 50% are attributed to low sperm motility or/and sperm count, results from exposure to parabens. Schlumpf et al. (2008) observed that 78.8% of women were using products containing Oxybenzone. Out of these 76.5% of breast milk samples tested positive for oxybenzone. Urine samples of men and women using sunscreen with UV filters contained with mean concentrations of 44ng/mL and 81ng/mL (Jonjua, 2008).

Paraben and oxybenzone are not only harmful via direct contact; they can also have negative effects on the aquatic environment and the organisms found within these areas. Traces of oxybenzone and paraben were detected in marine environments (Downs et al., 2016; Jonkers et al., 2010) and in freshwater environments through direct and indirect sources (Giokas et al., 2007; Balmer et al., 2005; Carmona et al., 2014; Jonkers et al., 2009; Kasprzyk-Horden et al., 2009).''Sunscreens compounds'' oxybenzone cause coral bleaching (Danovaro et al., 2008)and is genotoxicant to corals (Downs et al.,2016) and show signs of bioaccumulation in predatory fishes (Gago-Ferreo et al., 2015).

Around 605 to 3450 ng/g (on a lipid weight (lw) basis) concentration of paraben (methylparaben) was surveyed in 3 fish species from marine water of Manila Bay (Kim et al., 2011). Studies revealed that bifurcations of different commercial sunscreens inhibit the growth of various phytoplanktons such as *Chaetoceros gracilis* in seawater (Tovar-Sanchez et al., 2013) and *Desmodesmus subspicatus* in freshwater environment (Sieratowicz et al., 2011). Herrero et al., (2012) studied the effect of di(2-ethylhexyl)phthalate, Triclosan and propylparaben on *Allium cepa* roots. They observed that Triclosan and propylparaben inhibit *A. cepa* root growth in a dose-dependent manner; Triclosan and di(2-ethylhexyl)phthalate caused alterations in the mitotic index of root-tip cells, whereas, propylparaben did not show any evidence of genotoxicity in assays for chromosome aberrations and micronuclei. But the present study demonstrated the genotoxic effects of propylparaben. Similar toxic effects were observed by Calma and Medina (2020) who demonstrated that exposure to naproxen and propylparaben disrupts the life cycle of *Aedes aegypti* L; by reducing its eclosion, larval survival, pupation and emergence. Mills et al., (2004) also determined that exposure to sodium metabisulfite and propyl-paraben strongly limited the mycelial growth and spore germination of various potato pathogens (*Alternaria alternata, Botrytis cinerea, Fusarium solani var. coeruleum, Phytophthora erythroseptica, P. infestans, Verticillium albo-atrum, and V. dahlia*)

Taking into account that *Allium cepa* test system evaluates the environmental risk present due to its high sensitivity and good correlation with tests using organisms (Fiskesjo, 1985; Fatima and Ahmad, 2006), as this study suggests that Paraben and oxybenzone can cause harmful effects in the organisms exposed to the water containing these chemicals.

Conclusion

Allium cepa test is an important bio-indicator of cytotoxicity genotoxicity and serves as an alert for the chemicals and pollutants that indicate environmental hazards. It can be concluded that parabens and oxybenzone induce cytotoxicity by decreasing the mitotic index in a dose and duration-dependent manner and induce damage in different manners (i) causing abnormal morphology in onion root tips at the cellular and nuclear level; (ii) arrest or prolongation of prophase and anaphase; and (iii) abnormal morphology and damage of chromosomes of metaphase. The cell division was arrested, at the metaphase stage, showing chromosomal bridges, stickiness and chromosomal breaks. This study suggests that plants, as an important component of the environmental and ecological systems, need to be included when evaluating the effects of parabens and oxybenzone in environment.

Abbreviations

A. cepa- *Allium cepa,* CA-Chromosomal aberrations, MA- Morphological Alterations of cell, MI-Mitotic index, MP-Methylparaben, OB-Oxybenzone, PP-Propylparaben, Tabn-total number of abnormal cells, TC-total number cells observed, TDC-total number of dividing cells, TNA-total number of aberrant cells.

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