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Research Article Cytological Effects of Bleaching Agent (Quneex) on Plant Cells and Plant DNA

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Abstract

Background and Objective: There have been a number of reported drawbacks and efficacy issues regarding the use of bleaching agents in the plant industry. This study was conducted to determine the cytological effects of the bleaching agent (Quneex) on the plant cells and plant DNA using the *Allium cepa* assay. **Materials and Methods:** It was subjected sixteen root meristems of *A. cepa* to different concentrations of the bleaching agent (0.1, 0.2, 0.3, 0.4 and 0.5%) with different periods of time (6, 12 and 24 h). Recovery was done for 6, 12 and 24 h after exposure. **Results:** The mitotic index significantly decreased with time and also decreased with increase in the concentration of the bleaching agent. Abnormal chromosomal changes reflecting mutagenesis including stickiness, laggards, bridges, C-metaphase, star-metaphase, binucleation, polyploidy, disturbance and multinucleation were observed in the different concentrations and periods of time. After recovery, a slow increase in the mitotic index was observed. All treatments with or without recovery for 12 and 24 h resulted in reduction in the amount of DNA. **Conclusion:** Bleaching agents similar to Quneex containing sodium hypochlorite have mutagenic properties that can be potentially hazardous to the environment and also to humans. Thus, there is a need to regulate the use and disposal of such chemicals into the environment particularly to the sewers, to prevent contamination of potable water, plant and biodiverse aquatic animals.

Key words: Quneex, Allium cepa assay, bleaching agent, cytological effects

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Quneex, is a bleaching liquid that is used to clean white clothes and remove stain. It also kills germs that cause bad odor. Its ingredients include water, caustic soda and chlorine. It has a specific gravity of 1.08-1.09 and a pH¹ of 13-14. Liquid caustic soda is around $\leq 2\%$ sodium carbonate, $\leq 4 \mu g g^{-1}$ of Arsenic (As₂O₃), $\leq 30 \mu g g^{-1}$ of heavy metal Lead (Pb) and $\leq 0.1 \mu g g^{-1}$ of Mercury (Hg) content, with 95.0% sodium hydroxide (NaOH), which is an inorganic compound (NaOH) with a highly caustic metallic base and alkali salt². NaOH is one of the strongest alkalis and is highly reactive. It dissolves in water with evolution of huge amount of heat, in which its vapors is highly toxic. NaOH together with chlorine forms sodium hypochlorite (NaOCI), which is the active ingredient of Quneex.

NaOCI is often used as a disinfecting agent to contain infection due to pathogenic bacteria in hospitals although its efficacy has been shown to be lesser than that of chlorhexidine³. In plant industry, NaOCI is often used as a disinfectant that will affect seed germination and growth of plants. It was shown that diluted bleach baths with NaOCI controlled the exacerbation of atopic dermatitis, with a reduction of *Staphylococcus aureus* density, however with some patients to have intolerance to the NaOCI baths⁴.

NaOCl at total active chlorine concentration of 0.002% has been shown to successfully sterilize the medium for plant growth, which is cost-effective⁵. Furthermore, NaOCl at concentration of 0.2% decreased microbial contamination in the propagation of sugarcane, but the survival and growth of shoots were affected adversely⁶. NaOCl in a concentration of 500 ppm was also found to be effective against wild yeast strains growing on plants⁷. On the contrary, there have been a number of reported drawbacks and efficacy issues regarding the use of these agents in the plant industry⁸. This study was aimed to determine the cytological effect of the bleaching agent (Quneex) on the growth of plant cells and plant DNA.

MATERIALS AND METHODS

This experimental research was conducted from October, 2017-March, 2018 in the Scientific sections of the Girls College, Department of Genetics, Faculty of Agriculture, Mansoura University, Egypt.

Sixteen actively growing young onion roots (*Allium cepa*) with primary root meristem (2-3 cm long) were collected and treated separately with the bleaching solution (Quneex) that contain 5.25% sodium hypochlorite, water and caustic soda. Different concentrations of the bleaching solution were used (0.1, 0.2, 0.3, 0.4 and 0.5%). The Quneex that was used has a pH (1% solution) of 13-14, specific gravity of 1.08-1.09 at 28°C, with no flash point. The stability was one year from production date with acceptable chemical degradation when stored properly. Treatment duration was 6, 12 and 24 h. Recovery experiments were carried out for each period and the concentration in distilled water for 6, 12 and 24 h after treatments. A control (untreated root tips) was simultaneously conducted for both treatments and recovery experiments.

The root tips from all the treatments and control were fixed in ethanol and glacial acetic acid in a concentration ratio of 3:1. Cytological examination was done using the acetocarmine squash method as described by Arzani *et al.*9. The frequency of mitotic index (MI) and the frequencies of each type of mitotic aberration were calculated by dividing the total number of cells containing the aberration to the total number of cells 10. Study also estimated the amount of DNA ($\mu g g^{-1}$) in the apical meristems using calf thymus DNA as the standard Goswami and Chatterjee 11.

Data was analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS Inc., Armonk, New York, USA). Results were presented as numbers and percentages for categorical variables and as mean and standard deviation for continuous variables. Independent t-test was used to determine significant differences in means. A p-value of <0.05 was considered statistically significant.

RESULTS

There were significant differences in the mitotic index (%) between treatments without recovery versus treatment with recovery. The mitotic index (%) increased with treatments over time with recovery. The mitotic index was significantly greater between treatments with recovery versus without recovery (p<0.001). Concentration \times times of mitotic index also increased over time with recovery and was significantly greater with recovery at 24 h compared to without recovery (p<0.05). The percentage and the concentration of abnormal

Table 1: Mitotic index (%) and abnormal cells induced by Quneex in *Allium cepa* cells

	Mitotic inde	x (%)			Abnormal ce	ells (%)		
		With reco	very			With recove	ery	
	Without				Without			
	recovery	6 h	12 h	24 h	recovery	6 h	12 h	24 h
Replicates	5.13	6.18	3.31	7.92	188.57	266.77	43.89	301.16
Treatments	17.84	12.08	12.25	29.87	1735.22	3788.48	1349.28	1493.73
Concentration	29.41	23.07	16.54	24.21	4353.90	6582.90	2869.32	2537.12
Times	5.92	3.79	24.29	54.07	243.52	4072.97	492.95	1436.66
Concentration × times	14.43	8.25	7.69	27.87	724.17	2334.37	760.54	983.45

cells with treatments was also significantly higher at 6 h without recovery compared to treatment with recovery (p<0.01) (Table 1).

Treatment with different concentrations of Quneex over time with recover showed significant decrease in the mitotic index of *A. cepa* root meristem cells along with the significant increase of percentage of abnormal cells. (Table 2-5). The chromosomal abnormalities (%) was found to be directly related to each of treatments and recovery. Treatments at 6 h and recovery at 24 h gave lower abnormal cells (%) at 0.1% compared to other concentrations. (Table 5) The maximum abnormalities were seen in treatment with 0.4% for 24 h and recovery at 6 h (Table 3).

Table 2-5 also shows the effect of Quneex on the cells including stickiness, laggards, bridges, C-metaphase, star-metaphase, binucleation, polypoid, disturbance and multinucleation, the most common of which was stickiness. Stickiness was observed highest at 0.2% treatment 24 h without recovery (Table 2) and treatment 12 h with recovery (Table 5).

Anaphase bridges were observed at 0.5% at 24 h treatment without recovery (Table 2). Spindle formations, star-anaphase, binucleation, polypoid chromosomes and other chromosomal abnormalities were also observed at different concentrations and durations of treatment (Table 2-5). This study has shown that star metaphase was observed at 0.1% concentration at 6 h treatment and at 12 h recovery (Table 4) and polyploidy was observed at 6 h and 12 h recovery (Table 3 and 4).

The reduction in the amount of DNA in *A. cepa* cells was observed with increasing concentration of Quneex and with longer duration of exposure to the bleaching agent (Table 6, Fig. 1-3).

DISCUSSION

The interaction between concentration and time showed significance at no recovery for mitotic index and the

abnormal cells (%), which is similar to the results reported using chlorophenols (present in toilet cleaning agents) on the cells of the root meristem of onion seeds¹². The finding of significant decrease in the mitotic index of A. cepa root meristem cells along with the significant increase of (%) abnormal cells is similar to the cytological effect of flurochloridone, as explained by several authors particularly on the increase in the interphase duration due to the inhibition of DNA synthesis and increase in the G₂ period of plant cells^{13,14}. However, the mitotic index was found to steadily increase in the recovery sets from 6-24 h similar to findings from previous studies 14-16. The relationship between mitotic index and the amount of chromosomal abnormalities has been reported by previous studies where cell division is inhibited with a stronger or higher concentration of solutions such as dyes, preservatives and bleaching agents with induction of a wide range of mitotic abnormalities^{17,18}.

The induction of bridges could be attributed to breaks in the chromosomes and stickiness, c-anaphase multipolarity chromosomal aberrations are usually observed in anaphase-telophase cells¹⁹. Chromosomal lagging, on the other hand was found to provide a direct mechanistic link between extra centrosomes and chromosomal instability through promotion of multipolar anaphase, which results into many aneuploidy cells by abnormal cell division. This is also known to be present in some solid tumors²⁰. The formation of anaphase bridges is in direct link with the chromosomal instability²⁰. Binucleation can be due to the inhibition of the cell wall development as found in previous studies¹⁸. Star metaphase (bipolar configurations) of the chromosomes in which the centromeres clump with each other in the center of the cell and will take the shape of a star has something to do with inhibition of cytokinesis and the formation-deformation of the cell plate and may also be due to the effect of 3.45

6.45

3.22

Disturbance Multipolar 6.89 7.58 4.55 3.70 6.45 1.61 2.22 Polypoid 15.15 4.55 7.41 24.19 4.44 2.00 3.22 binucleation 16.13 99.9 17.24 10.61 Different abnormal cells (%) relative to the number of abnormal cells Table 2: Cytological abnormalities in root tip cells of Allium cepa induced by different concentrations of Quneex for different time exposure with no recovery Star-m 16.66 13.63 14.81 3.13 16.66 1.62 2.22 2.00 3.22 3.22 7.69 16.66 46.60 19.35 10.61 40.90 29.63 3.44 3.13 3.22 6.66 2.00 C-n bridges 6.66 3.22 15.38 1.52 4.55 10.34 6.25 3.22 1.62 2.22 4.00 Laggards 16.68 42.84 28.56 13.33 6.06 3.12 3.22 7.69 1.62 4.44 2.00 Stickiness 58.64 78.13 100.00 79.15 57.16 71.44 31.81 27.27 33.34 70.96 69.24 50.02 46.77 64.47 88.00 26.75 61.39 84.62 9.72 5.30 4.66 28.32 22.22 31.76 49.15 41.55 31.71 68.88 20.96 31.57 53.91 60.81 78.13 35.71 57.40 28.26 Abnormal cells % Š. 31 13 6 15 31 13 662227 29 32 13 624550 Mitotic index 7.00 6.17 4.82 3.88 6.36 2.72 4.055.531.11 9.07 5.47 5.03 4.54 3.84 3.84 2.87 3.43 3.17 Number of divided cells 223 132 150 233 99 85 115 74 64 597741 45 62 19 45 46 46 Number of cell exam 2568 1810 1690 1520 1210 1510 3185 2142 3110 1110 1120 1710 2510 1928 1665 1460 1573 1450 Concentration 12 24 **0.10 (%)** 12 24 **0.20 (%)** 0.30 (%) 0.40 (%) 12 24 **0.50 (%)** Time (h) Control

3.22 13.33

13.33

26.66

6.25

Multipolar Distur. 6.25 7.69 3.22 Polypoid 1.82 25.00 33.33 7.14 1.66 2.38 19.35 Binuc. Different abnormal cells (%) relative to the number of abnormal cells Table 3: Cytological abnormalities in root tip cells of Allium cepa induced by different concentrations of Quneex for different exposure with 6 h of recovery Star-m 21.42 60.6 2.70 7.69 2.38 6.45 - 6.66 28.57 4.55 7.69 2.94 7.14 12.90 1.82 20.0 7.69 ر-ا Brid. 5.41 7.69 3.2 25.00 22.70 7.69 2.70 Lag. 66.67 50.00 66.67 69.24 100.00 89.19 84.62 98.34 92.06 88.10 76.92 51.61 85.45 35.71 87.50 Stic. 2.65 3.31 2.56 15.05 50.00 25.28 43.33 50.00 84.09 24.52 92.31 82.92 51.2 56.52 96.43 73.80 82.09 16.30 Abnormal cells % 8 42 26 54 14 16 22 26 17 37 13 60 34 31 55 15 Mitotic index 7.29 6.38 6.19 5.281.734.91 4.35 2.83 2.17 2.76 7.64 4.55 3.7 3.77 4.24 2.75 2.92 7.44 Number of divided cells 113 121 117 82 46 56 93 32 87 60 34 44 536541 426793 Number of cell exam 1550 1896 1890 1761 1850 1772 1380 1200 2030 1920 850 900 2215 1220 1320 1530 2293 1250 Concentration 24 **0.50 (%)** 0.10(%) 24 **0.20 (%)** 0.30 (%) Time (h) Control (mL %)

Concentration				Abnorn	Abnormal cells	Different a	Different abnormal cells (%) relative to the number of abnormal cells	(%) relative t	to the numbe	er of abnorma	al cells			
(mL %)	Number of	Number of	Mitotic											
Time (h)	cell exam	divided cells	index	No.	%	Stic.	Lag.	Brid.	C-m	Star-m	Binuc.	Polypoid	Distur.	Multipolar
Control														
9	1570	124	7.89	9	4.84	89.99	16.66	,	16.6	1	1	,	1	,
12	1680	9/	4.52	9	7.89	89.99	16.66	,	16.6	1	1	,	1	1
24	1720	85	4.94	2	5.80	,	00:09	20.0	20.0	1	1	,	1	1
0.10(%)														
9	1136	83	7.31	13	15.66	30.79	,	ı	30.76	23.07	1	,	7.69	7.69
12	870	55	6.32	19	34.55	47.38	,	,	5.26	5.26	5.26	21.05	10.53	5.26
24	1930	51	2.64	22	43.13	100.00	,	,	,	ı	1	,	ı	,
0.20 (%)														
9	1540	45	2.92	19	42.22	27.28	60.6	60.6	18.18	,	60.6	,	-0.09	18.18
12	1120	43	3.83	24	55.8	100.00	,	,	,	,	,	,	,	,
24	1535	47	3.06	23	48.93	65.26	4.34	8.69	4.34	4.34	4.34	8.69	,	,
0.30 (%)														
9	1070	09	5.61	14	23.33	50.02	1	14.28	1	1	14.28	7.14	14.28	
12	1150	20	1.74	5	25.00	80.00	1	1	1	1	20.00	1	1	1
24	1550	23	1.48	12	52.17	75.00	16.67	1	1	1	8.33	1	1	1
0.40 (%)														
9	1890	94	4.97	33	35.11	51.52	90'9	90.9	15.15	90.9	1	12.12	3.03	1
12	1410	20	3.55	12	24.00	33.33	8.33	1	8.33	1	33.33	16.67	1	1
24	1700	87	5.12	51	58.62	100.00	1		1	1	1	1	,	1
0.50 (%)														
9	1550	74	4.77	36	48.65	83.33	2.78	2.78	2.78	1	2.78	5.55	,	1
12	1890	42	2.22	25	59.52	84.00	,	,	12.00	4.00	,	,	,	,
24	2100	66	4.71	21	21.21	61.92	1		14.28	9.52	4.76	4.76	4.76	1

(mL %)				Abnorn	Abnormal cells	Different a	ibnormal cells	(%) relative	Different abnormal cells (%) relative to the number of abnormal cells	er of abnorm	al cells			
	Number of	Number of	Mitotic											
Time (h)	cell exam	divided cells	index	Š.	%	Stic.	Lag.	Brid.	C-m	Star-m	Binuc.	Polypoid	Distur.	Multipolar
Control														
9	2620	158	6.03	1	,	1	,	,	,	1	,	,	,	1
12	2400	117	4.88	9	5.13	50.02	16.66	,	16.696	16.66	,	,	,	1
24	1825	106	5.81	1	1	1	1	1	1	1	1	1	1	1
0.10 (%)														
9	1323	146	11.03	17	11.64	35.32	5.88	5.88	17.64	5.88	17.64	,	5.88	5.88
12	930	49	5.27	22	44.89	40.91	4.54	,	60.6	4.55	4.55	22.72	60.6	4.55
24	1820	49	2.69	26	53.06	92.3	,	1	1	3.85	3.85	,	ı	1
0.20 (%)														
9	1810	06	4.97	25	27.77	40.00	,	8	32	16	4	,	ı	1
12	1030	35	3.39	18	51.42	100.00	1	1	1	1	1	1	1	1
24	1570	46	2.93	16	34.78	100.00	1	1	1	1	1	1	1	1
0.30 (%)														
9	096	22	2.29	6	40.91	55.55	22.22	1	11.11	1	11.11	1		1
12	1330	09	4.51	13	21.66	53.86	1	1	15.38	1	1	1	15.38	15.38
24	1510	40	2.65	14	35.00	71.43	,	,	28.57	1	,	,	ı	1
0.40 (%)														
9	2000	158	7.90	30	18.98	70.02	99.9	99:9	13.33	3.33	1	1		1
12	1670	38	2.27	23	60.53	100.00	1	1	1	1	1	1		1
24	1440	85	5.90	37	43.50	78.38	,	,	13.51	2.70	5.41	,	,	,
0.50 (%)														
9	1770	78	4.41	30	38.46	89.98	1	3.33	99.9	3.33	1	1		1
12	1770	62	3.64	35	56.45	80.05	1	2.85	1	1	1	5.70	5.70	5.70
24	1190	46	3.86	7	15.20	71 44	,	14 28	,	,	,		11.28	

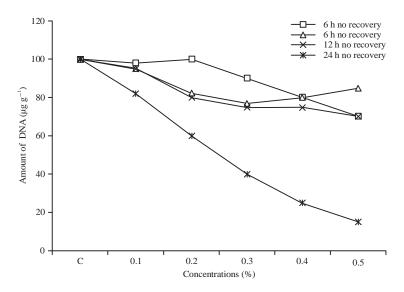


Fig. 1: Amount of DNA at different concentrations at 6 h and their subsequent recovery

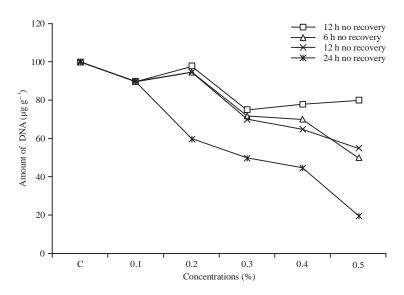


Fig. 2: Amount of DNA at different concentrations at 12 h and their subsequent recovery

Table 6: Amount of DNA at different concentrations of Quneex at different times and subsequent recovery

	Amount of DNA ($\mu g g^{-1}$)			
Parameters	Without recovery		(With recovery)	
Treatments	2439.79	2263.91	2408.61	2925.91
Concentrations	3532.15	4047.79	4476.19	9019.49
Times	9362.86	6662.20	7154.24	1497.69
Concentration×times	649.01	492.30	425.69	164.76
Errors	75.52	82.18	84.21	64.05

agents such as Quneex on the formation of mitotic microtubular organizing centers²¹. Polypoid chromosomes which arise from unreduced gametes by non-disjunction, are usually observed in both plants and animals whereby the

entire chromosome is multiplied, but mostly die²². The reduction in the amount of DNA in *A. cepa* cells was similar to the findings from previous studies on *A. cepa* using different solutions^{23,24}.

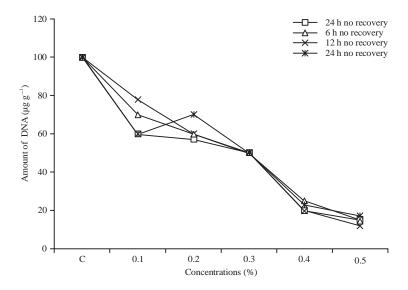


Fig. 3: Amount of DNA at different concentrations at 24 h and their subsequent recovery

SIGNIFICANCE STATEMENT

This study discover the advantages of using NaOCl as a disinfectant and sterilizing solution particularly in the plant and healthcare industry that can be beneficial for reducing potential infections and contaminations. However, this study will help the researcher to uncover the critical areas of the potentially hazardous and mutagenic effects of these agents that many researchers were not able to explore. Thus a new theory on the use of NaOCl may be arrived at particularly on its use in the plant industry and in the protection of the environment.

CONCLUSION

Bleaching agents similar to Quneex containing sodium hypochlorite have mutagenic properties that can be potentially hazardous to the environment and also to humans. There is a need to regulate the use and disposal of such chemicals into the environment particularly to the sewers, to prevent contamination of potable water, plant and biodiverse aquatic animals to some extent.

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