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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, $\leq 4 \mu\text{g g}^{-1}$ of Arsenic (As_2O_3), $\leq 30 \mu\text{g g}^{-1}$ of heavy metal Lead (Pb) and $\leq 0.1 \mu\text{g g}^{-1}$ 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 (NaOCl), which is the active ingredient of Quneex.

NaOCl 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, NaOCl is often used as a disinfectant that will affect seed germination and growth of plants. It was shown that diluted bleach baths with NaOCl controlled the exacerbation of atopic dermatitis, with a reduction of *Staphylococcus aureus* density, however with some patients to have intolerance to the NaOCl 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 cells¹⁰. Study also estimated the amount of DNA ($\mu\text{g g}^{-1}$) in the apical meristems using calf thymus DNA as the standard Goswami and Chatterjee¹¹.

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 index (%)				Abnormal cells (%)			
	Without recovery	With recovery			Without recovery	With recovery		
		6 h	12 h	24 h		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 studies¹⁴⁻¹⁶. 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 and 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

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

Concentration (mL %)	Time (h)	Abnormal cells			Different abnormal cells (%) relative to the number of abnormal cells										
		Number of cell exam	Number of divided cells	Mitotic index	No.	%	Stic.	Lag.	Brid.	C-m	Star-m	Binuc.	Polypoid	Distur.	Multipolar
Control															
6	1550		113	7.29	3	2.65	66.67	-	-	33.33	-	-	-	-	-
12	1896		121	6.38	4	3.31	50.00	25.00	-	-	25.00	-	-	-	-
24	1890		117	6.19	3	2.56	66.67	-	-	-	33.33	-	-	-	-
0.10 (%)															
6	1761		93	5.28	14	15.05	35.71	-	-	28.57	21.42	7.16	-	-	-
12	1850		32	1.73	16	50.00	87.50	-	-	-	-	-	6.25	6.25	6.25
24	1772		87	4.91	22	25.28	27.29	22.70	4.55	4.55	9.09	4.55	9.09	9.09	4.55
0.20 (%)															
6	1380		60	4.35	26	43.33	69.24	7.69	7.69	7.69	-	-	7.69	-	-
12	1200		34	2.83	17	50.00	100.00	-	-	-	-	-	-	-	-
24	2030		44	2.17	37	84.09	89.19	2.70	5.41	-	2.70	-	-	-	-
0.30 (%)															
6	1920		53	2.76	13	24.52	84.62	-	-	7.69	7.69	-	-	-	-
12	850		65	7.64	60	92.31	98.34	-	-	-	-	1.66	-	-	-
24	900		41	4.55	34	82.92	97.06	-	-	2.94	-	-	-	-	-
0.40 (%)															
6	2215		82	3.7	42	51.2	88.10	-	-	7.14	2.38	-	-	-	-
12	1220		46	3.77	26	56.52	76.92	-	-	11.54	-	-	-	-	-
24	1320		56	4.24	54	96.43	94.45	-	-	-	5.55	-	-	-	-
0.50 (%)															
6	1530		42	2.75	31	73.80	51.61	-	3.2	12.90	6.45	-	3.22	3.22	3.22
12	2293		67	2.92	55	82.09	85.45	-	-	1.82	-	1.82	-	-	-
24	1250		93	7.44	15	16.30	-	26.66	13.33	20.0	6.66	-	13.33	13.33	13.33

Table 4: Cytological abnormalities in root tip cells of *Allium cepa* induced by different concentrations of Queneex for different exposure with 12 h of recovery

Concentration (mL %)	Time (h)	Abnormal cells			Different abnormal cells (%) relative to the number of abnormal cells									
		Number of cell exam	Number of divided cells	Mitotic index	No.	%	Stic.	Lag.	Brid.	C-m	Star-m	Binuc.	Polypoid	Distur.
Control														
6	1570		124	7.89	6	4.84	66.68	16.66	-	16.6	-	-	-	-
12	1680		76	4.52	6	7.89	66.68	16.66	-	16.6	-	-	-	-
24	1720		85	4.94	5	5.80	-	60.00	20.0	20.0	-	-	-	-
0.10 (%)														
6	1136		83	7.31	13	15.66	30.79	-	-	30.76	23.07	-	7.69	7.69
12	870		55	6.32	19	34.55	47.38	-	-	5.26	5.26	21.05	10.53	5.26
24	1930		51	2.64	22	43.13	100.00	-	-	-	-	-	-	-
0.20 (%)														
6	1540		45	2.92	19	42.22	27.28	9.09	9.09	18.18	-	-	-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	8.69	-	-
0.30 (%)														
6	1070		60	5.61	14	23.33	50.02	-	14.28	-	-	7.14	14.28	-
12	1150		20	1.74	5	25.00	80.00	-	-	-	-	-	-	-
24	1550		23	1.48	12	52.17	75.00	16.67	-	-	-	-	-	-
0.40 (%)														
6	1890		94	4.97	33	35.11	51.52	6.06	6.06	15.15	6.06	12.12	3.03	-
12	1410		50	3.55	12	24.00	33.33	8.33	-	8.33	-	16.67	-	-
24	1700		87	5.12	51	58.62	100.00	-	-	-	-	-	-	-
0.50 (%)														
6	1550		74	4.77	36	48.65	83.33	2.78	2.78	2.78	-	5.55	-	-
12	1890		42	2.22	25	59.52	84.00	-	-	12.00	4.00	-	-	-
24	2100		99	4.71	21	21.21	61.92	-	-	14.28	9.52	4.76	4.76	-

Table 5: Cytological abnormalities in root tip cells of *Allium cepa* induced by different concentrations of Queneex for different exposure with 24 h of recovery

Concentration (mL %)	Time (h)	Abnormal cells			Different abnormal cells (%) relative to the number of abnormal cells										
		Number of cell exam	Number of divided cells	Mitotic index	No.	%	Stc.	Lag.	Brid.	C-m	Star-m	Binuc.	Polypoid	Distur.	Multipolar
Control															
6	2620	158	6.03	-	-	-	-	-	-	-	-	-	-	-	-
12	2400	117	4.88	6	5.13	50.02	16.66	-	16.696	16.66	-	-	-	-	-
24	1825	106	5.81	-	-	-	-	-	-	-	-	-	-	-	-
0.10 (%)															
6	1323	146	11.03	17	11.64	35.32	5.88	5.88	17.64	5.88	17.64	-	5.88	5.88	5.88
12	930	49	5.27	22	44.89	40.91	4.54	-	9.09	4.55	4.55	22.72	9.09	4.55	4.55
24	1820	49	2.69	26	53.06	92.3	-	-	-	3.85	3.85	-	-	-	-
0.20 (%)															
6	1810	90	4.97	25	27.77	40.00	-	8	32	16	4	-	-	-	-
12	1030	35	3.39	18	51.42	100.00	-	-	-	-	-	-	-	-	-
24	1570	46	2.93	16	34.78	100.00	-	-	-	-	-	-	-	-	-
0.30 (%)															
6	960	22	2.29	9	40.91	55.55	22.22	-	11.11	-	11.11	-	-	-	-
12	1330	60	4.51	13	21.66	53.86	-	-	15.38	-	-	-	15.38	15.38	-
24	1510	40	2.65	14	35.00	71.43	-	-	28.57	-	-	-	-	-	-
0.40 (%)															
6	2000	158	7.90	30	18.98	70.02	6.66	6.66	13.33	3.33	-	-	-	-	-
12	1670	38	2.27	23	60.53	100.00	-	-	-	-	-	-	-	-	-
24	1440	85	5.90	37	43.50	78.38	-	-	13.51	2.70	5.41	-	-	-	-
0.50 (%)															
6	1770	78	4.41	30	38.46	86.68	-	3.33	6.66	3.33	-	-	-	-	-
12	1770	62	3.64	35	56.45	80.05	-	2.85	-	-	-	5.70	5.70	5.70	-
24	1190	46	3.86	7	15.20	71.44	-	14.28	-	-	-	-	14.28	14.28	-

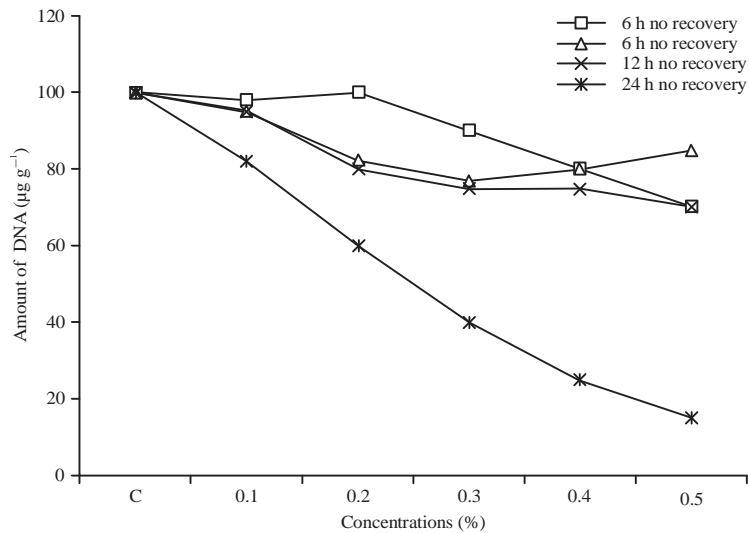


Fig. 1: Amount of DNA at different concentrations at 6 h and their subsequent recovery

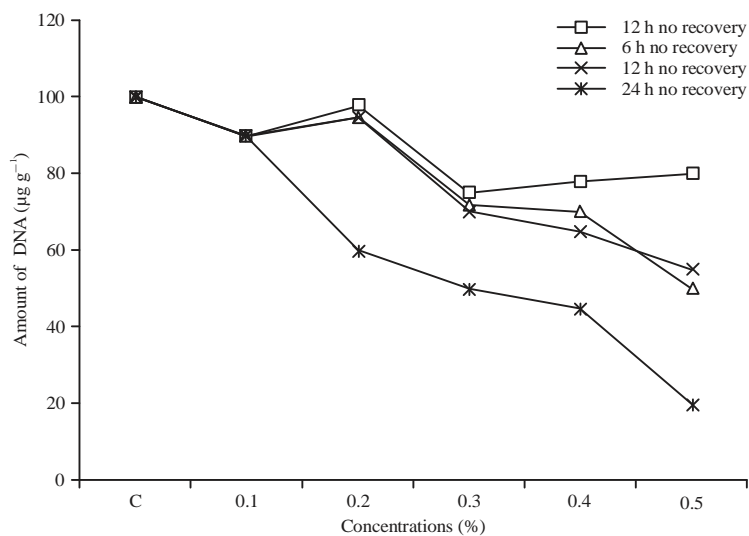


Fig. 2: Amount of DNA at different concentrations at 12 h and their subsequent recovery

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

Parameters	Amount of DNA ($\mu\text{g g}^{-1}$)			
	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 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. Polyploid 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}.

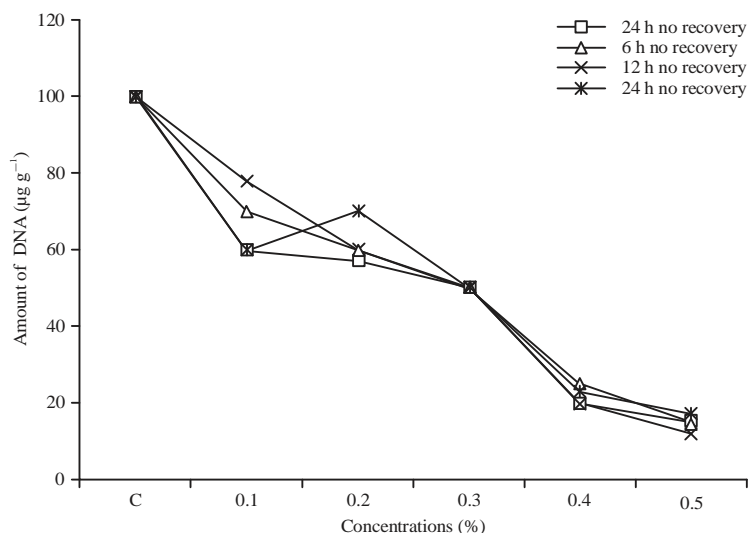


Fig. 3: Amount of DNA at different concentrations at 24 h and their subsequent recovery

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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