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EFFECT OF SYNTHETIC DETERGENTS ON INTERPHASE NUCELI IN *ALLIUM CEPA* L.

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ABSTRACT

Three commonly used detergents, Sansar, Sunlight and Key, were evaluated for their comparative efficacy in blocking the various stages of interphase: G_1 and G_2 arrest of interphase nuclei after detergents' treatment was recorded, which was found to be time- and concentration-dependent. However, no significant difference in the effects among three detergents was observed. The G_1- and G_2-arrest of cells is possibly due to nonsynthesis of proteins required for G_1–S transit and G_2–M transits, respectively. Further, at lower concentration of treatments, blockade in DNA synthesis at S phase was observed as indicated by reduction in G_2-cell populations.

Key words: Detergents, interphase, DNA, protein synthesis.

Pollution of aquatic environment by detergents through domestic and industrial discharge is attaining alarming proportions [1]. This pollution hazard is attributable to nonbiodegradation of ABS (alkyl benzene sulfonate), a common active ingredient of most Indian detergents, in sewers, lakes and rivers. Phytotoxicity of ABS [2, 3] and mitotic inhibitory property of synthetic detergents [4–6] has been established. However, the information available does not pinpoint the stage of cell cycle, affected by these detergents. The present study, therefore, aims to evaluate the relative efficacy of three commonly used detergents in blocking the cell cycle at different stages. The end point observed was the relative frequency of different sizes of interphase nuclei, which are indicative of cell cycle stages.

MATERIALS AND METHODS

Healthy bulbs of uniform size were chosen from a population of common onion, *Allium cepa* L., and grown on distilled water for 72 h. Three detergents, namely, Sansar (Karnataka Soap & Detergents Ltd.), Sunlight (Hindustan Lever Ltd.), and Key (Godrej Detergents Co.) were selected as test chemicals in view of their widespread household use in the country. Three concentrations (0.1%, 0.25%, 0.5% w/v) of each detergent were used in suspension of

distilled water. Germinated intact onion bulbs were dipped in different test chemicals so as to completely submerge the roots in the detergent solutions. A control was maintained in distilled water for each set. Observations were recorded after 6, 12 and 24 h of treatment.

After treatment 10 roots were cut in each set, washed, fixed overnight in 1:3 (acetic acid: ethanol) fixative, and standard acetocarmine squash preparations made.

About 300 interphase nuclei selected randomly were measured in each experimental set. The nuclear diameter was measured at x600 magnification using a calibrated ocular micrometer. The randomness of field of vision was achieved by following the method of Bhalla et al. [7]. Based on the interphase nuclear diameter, they were classified into four groups [8]: 15–19 μm corresponding to G_1 phase, 20–24 μm corresponding to S phase, 25–29 μm and 30–34 μm, both corresponding to G_2 phase (Table 1).

The experimental data were analysed statistically by 3-way ANOVA to see the effect of detergents, duration of treatment, and the detergent concentration and their interactions (Table 2).

RESULTS AND DISCUSSION

A significant increase was observed in the percentage of smaller (15–19 μm) nuclei, which is directly dependent on concentration and time. However, significant difference was not observed among different detergent treatments (Tables 1 and 2). It reflects a possible accumulation of interphase nuclei at G_1 phase having 2C-DNA value, because the relative DNA values have been shown to be positively correlated with nuclear volume of interphase nuclei in shoot and root meristems of different species, including *Allium cepa* L. [8, 9].

There was a significant reduction in the percentage of medium sized nuclei (20–24 μm) as compared to control. This decrease was also found to be concentration- and time-dependent, which implies that the nuclei are unable to reach S phase, possibly due to blockade at G_1–S transit point.

Again an accumulation of cells with large nuclei (25–34 μm) as compared to control was recorded. This indicates that the cells are unable to cross G_2 phase, possibly, due to blockade at G_2–M transit point. This blockade can be due to nonsynthesis of proteins like MPF (Maturation Promoting Factor), required for G_2–M transit. Accumulation of G_2-phase nuclei having 4C-DNA value as evidenced by the larger nuclear size itself along with reduction in the number of S-phase nuclei as compared to control also suggests that there is no blockade at S–G_2 transit point; the cells that are able to cross G_1 also complete S phase, then get arrested at G_2.

Table 1. Effect of detergents on the size of interphase nuclei in root-tip cells of *Allium cepa*

Treatment duration (h)	Concentration (%, w/v)	Detergent	Interphase nuclei with different diameters (%)			
			15–19 μm	20–24 μm	25–29 μm	30–34 μm
6	0.1	Sansar	60.3 ± 0.7	33.8 ± 0.9	3.8 ± 0.2	2.1 ± 0.8
		Sunlight	62.1 ± 0.5	31.8 ± 0.8	4.2 ± 0.7	2.8 ± 0.4
		Key	61.5 ± 0.8	34.4 ± 0.3	2.5 ± 0.6	1.5 ± 0.9
	0.25	Sansar	64.3 ± 0.6	24.8 ± 0.4	6.8 ± 0.4	4.2 ± 0.3
		Sunlight	62.6 ± 0.5	27.2 ± 0.3	5.9 ± 0.7	4.3 ± 0.5
		Key	65.4 ± 0.7	25.3 ± 0.8	5.2 ± 0.9	4.1 ± 0.2
	0.50	Sansar	68.6 ± 0.4	18.5 ± 0.2	7.8 ± 0.5	5.1 ± 0.6
		Sunlight	65.8 ± 0.3	17.4 ± 0.7	10.2 ± 0.4	6.4 ± 0.4
		Key	67.2 ± 0.8	20.4 ± 0.3	8.1 ± 0.7	4.7 ± 0.5
		Control	54.4 ± 0.4	36.8 ± 0.4	5.0 ± 0.3	3.8 ± 0.2
12	0.1	Sansar	64.6 ± 0.3	28.2 ± 0.5	4.3 ± 0.4	3.0 ± 0.5
		Sunlight	65.2 ± 0.4	27.9 ± 0.4	5.9 ± 0.6	4.1 ± 0.4
		Key	62.7 ± 0.6	30.3 ± 0.5	4.1 ± 0.2	3.0 ± 0.5
	0.25	Sansar	70.5 ± 0.3	17.3 ± 0.5	7.2 ± 0.4	5.2 ± 0.3
		Sunlight	71.3 ± 0.4	20.5 ± 0.6	5.2 ± 0.3	3.1 ± 0.7
		Key	68.0 ± 0.2	17.6 ± 0.5	8.4 ± 0.4	6.0 ± 0.5
	0.50	Sansar	72.4 ± 0.1	15.3 ± 0.4	8.2 ± 0.3	4.2 ± 0.6
		Sunlight	75.4 ± 0.5	10.4 ± 0.6	8.8 ± 0.4	5.5 ± 0.3
		Key	74.2 ± 0.7	12.6 ± 0.2	9.3 ± 0.6	4.0 ± 0.7
		Control	52.4 ± 0.4	36.3 ± 0.5	5.2 ± 0.3	4.1 ± 0.2
24	0.1	Sansar	66.3 ± 0.7	22.4 ± 0.3	6.2 ± 0.4	5.1 ± 0.1
		Sunlight	69.5 ± 0.2	20.4 ± 0.5	6.0 ± 0.7	4.2 ± 0.3
		Key	64.3 ± 0.2	25.4 ± 0.4	5.5 ± 0.3	4.8 ± 0.2
	0.25	Sansar	72.5 ± 0.4	15.3 ± 0.3	8.2 ± 0.4	4.0 ± 0.5
		Sunlight	73.8 ± 0.1	17.4 ± 0.5	4.5 ± 0.6	4.3 ± 0.5
		Key	70.0 ± 0.3	15.6 ± 0.7	8.8 ± 0.5	5.7 ± 0.8
	0.50	Sansar	74.7 ± 0.2	10.8 ± 0.5	9.3 ± 0.4	5.2 ± 0.3
		Sunlight	78.0 ± 0.4	10.4 ± 0.3	8.6 ± 0.5	3.0 ± 0.6
		Key	76.6 ± 0.5	11.2 ± 0.6	8.3 ± 0.4	4.0 ± 0.4
		Control	58.3 ± 0.2	33.8 ± 0.4	5.7 ± 0.6	2.2 ± 0.2

However, lower concentration (0.1%) of all the three detergents with 6 h treatment does not follow the same pattern. There was a significant drop in the percentage of G_2-sized nuclei, which indicates that S phase progress is also affected. Such situation can be explained due to low viscosity of the solution at 0.1% concentration, the detergents are able to penetrate the cell wall barrier more effectively than at higher concentrations, thereby affecting DNA synthesis also. The possibility of such pre- prophase inhibition of DNA synthesis by surfactants was also suggested by Nethery [10]. Further, similar decrease in protein and DNA synthesis by LAS (linear alkylbenzene sulfonate), a major active component of

synthetic detergents, was reported in the freshwater alga *Scenedesmus quadricanda* [11]. Nasar and Singh [8] also reported G_2 arrest by Sevin, a pesticide, in *Allium cepa* L. They suggested that the relative DNA values are positively correlated with the volume of interphase nuclei in the root meristem of *Allium cepa*. Nonsynthesis of the required proteins for crossing the G_2–M barrier was proposed by them as possible cause of G_2 arrest of cells after Sevin treatment.

Table 2. Three-way ANOVA showing level of significance

Source	Level of significance for different nuclear size groups			
	15–19 μm	20–24 μm	25–29 μm	30–34 μm
Treatment duration (A)	***	***	**	NS
Detergent concentration (B)	***	***	***	***
Detergent type (C)	NS	NS	NS	NS
A x B	***	***	NS	***
A x C	NS	NS	*	NS
B x C	NS	***	**	NS

*$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$.
NS—Nonsignificant.

It can be concluded that detergent treatment causes inhibition of protein synthesis during interphase as well pre-prophase DNA synthesis at lower concentrations, which accounts for overall mitotic inhibition by them due to arrest of interphase cells either at G_1 or G_2 phase.

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REFERENCES

1. V. Prakash and S. D. Vokil. 1983. Detergents and pollution. J. Sci. Indus. Res., **42**: 41–43.

2. R. Ernst, C. J. Gonzales and J. Arditti. 1983. Biological effects of surfactants. 6. Effect of anionic, nonionic and amphoteric surfactants of green alga (*Chlamydomonas*). Environ. Pollut. Ser. A, **31**: 159–176.

3. C. Rinallo, A. Bennici and E. Cenni. 1988. Effects of two surfactants on *Triticum durum* Desf. plantlets. Environ. Exptl. Bot., **28**(4): 367–374.

4. M. Kumar and S. P. Sinha. 1984. Changes in mitotic index in roots of *Allium cepa* exposed to detergents. Biol. Bull. Ind., **6**(3): 296–300.

5. M. Kumar and H. Kumar. 1987. Inhibition of germination and mitosis in *Vigna radiata* by some laundry detergents. J. Curr. Biosci., **4**(4): 135–138.

6. H. Kumar and M. Kumar. 1990. Effect of some detergents on germination and mitotic index in *Vigna radiata*. Ann. Agric. Res., **11** (3–4): 316–318.

7. P. R. Bhalla, T. S. Kocchar and P. S. Sabharwal. 1973. Induction of mitotic abnormalities in onion root-tips by tobacco smoke condensate (TSC). Cytologia, **38**: 707–712.

8. S. K. T. Nasar and S. Singh. 1980. G_2-arrest in onion by Sevin. Experientia, **36**: 948–949.

9. T. Mohandas and W. F. Grant. 1972. Cytogenetic effects of 2, 4-D and asnitrate in relation to nuclear volume and DNA content in some higher plants. Can. J. Genet. Cytol., **14**: 773–783.

10. A. A. Nethery. 1967. Mitotic inhibition by surfactants. Cytologia, **32**: 321–323.

11. G. Chawla, P. N. Vishwanathan and S. Devi. 1987. Biochemical studies on the toxicity of linear alkylbenzene sulphonate to *Scenedesmus quadricanda* in culture. Environ. Expt. Bot., **26**: 39–51.