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Effect of Linear Alkyl Benzene Sulphonate on Germination of Spores of the Aquatic Fern *Ceratopteris thalictroides*

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Validity of fern spore germination bioassays for the effects of environmental pollution was established by many workers (Nakazawa 1960, Francis and Petersen 1983). Wada et al (1987) used fern spore germination bioassay and recommended this model as bioindicator of Sulfur dioxide. Fern spore bioassay for mutagenicity testing of water pollutants was suggested by Klekowski and Davis (1977). Yasmeen and Devi (1986) studied the phytotoxicity of linear alkyl benzene sulphonate (LAS) on the spores of Diplazium esculentum and observed that LAS levels above 0.001% are toxic to fern spores.

Water pollution due to synthetic detergents has been increasing continuously during the last few years due to their extensive use in domestic life, agriculture and industry. These detergents are among the most common pollutants responsible for water pollution. Adverse effects of the synthetic detergents on aquatic flora were studied by Swisher 1970 and Chawla et al 1986. Ernst et al (1971) used orchid seedlings for assessing the impact of detergents during germination, and observed lower rate of seed germination and survival as dose and duration of the detergent increased. In view of the above, the phytotoxicity of LAS on germination of an aquatic fern Ceratopteris thalictroides spores was studied. However, in these studies, only germination pattern was taken as index and no observations were made on the developmental stages.

MATERIALS AND METHODS

Linear alkyl benzene sulphonate was obtained from Industrial Toxicology Research Centre, Lucknow, India (Chawla et al. 1986). One percent (W/V) solution was prepared after adjusting the pH 7.0. Further dilutions were prepared accordingly and pH maintained at neutral. Different concentrations of detergent were prepared in triplicate with Knops medium (Miller and Greany 1974) containing only macromolecules. Medium was solidified by adding 1-2% of agar-agar.

Three petri dishes containing only Knops medium were kept as control set. Mature fresh spores of Ceratopteris 2.0 mg were sprinkled with a paper spatula over the culture medium, containing different con-

centrations of the detergent. Standardization experiments showed that 2.0 mg of the spores when suspended in water and an aliquot was counted, showed the number to be around 150 and using this as 100% the percent germination was calculated. Care was taken to avoid over crowding during spore sowing. The culture petri dishes were maintained under standard asceptic physiological conditions at 25± 0.5°C temperature, 16 hours light (1600 Ft. C fluorescent Philips tubes), and eight hours dark cycle. Incubated petri dishes were kept under constant observation and the germinated spores in each petri dish were scored under a binocular and their percent values calculated. Spore germination was indicated by the production of first rhizoid or first prothallial cell division. Length and width of the rhizoids were measured by stage and occular micrometer and for this measurement gametophytes were randomly selected from the petri dishes.

Fluorescence microscopy was done to study the structural differences in gametophytes, caused by detergent treatment. Gametophytes bearing mature antheridia were treated with 1 mM chlorotetracycline (Miller et al. 1983) and examined under a Leitz Laborlux D fluorescence microscope fitted with I_2 exciting filter (BP 450-490), beam splitting mirror (RKP 510) and suppression filter (LP 515). Photographic recording was made with a Leitz Vario-orthomat camera on a Agfa Black and White film DIN (24), ASA (200).

RESULTS

LAS at 10 ppm shows similar percentage of germination as control at 15 and 20 days, but antheridial development was more and faster in the treated spores. Higher concentrations of LAS decreased germination and development and also delayed antheridial formation (Table 1). Beyond 75 ppm, lethality was 100%. Eventhough strict dose time response was not manifested LAS was found to be toxic to fern spore germination. After 20 days no antheridial initiation was observed in control (fig 1) or in other concentrations, whereas fifty percent gametophytes in 10 ppm developed antheridia (fig 2). The number, width and length of the rhizoids of control were always higher than those of 10 ppm (Table 2).

After 20 days, cracking of spore coat in 50 ppm (LAS) was observed but no cell development took place and the cytoplasm was shrunken into a rounded mass. Similarly spores in 75 ppm developed no rhizoid, but cell differentiation was noted and fatty contents were seen in the form of small droplets (fig 3).

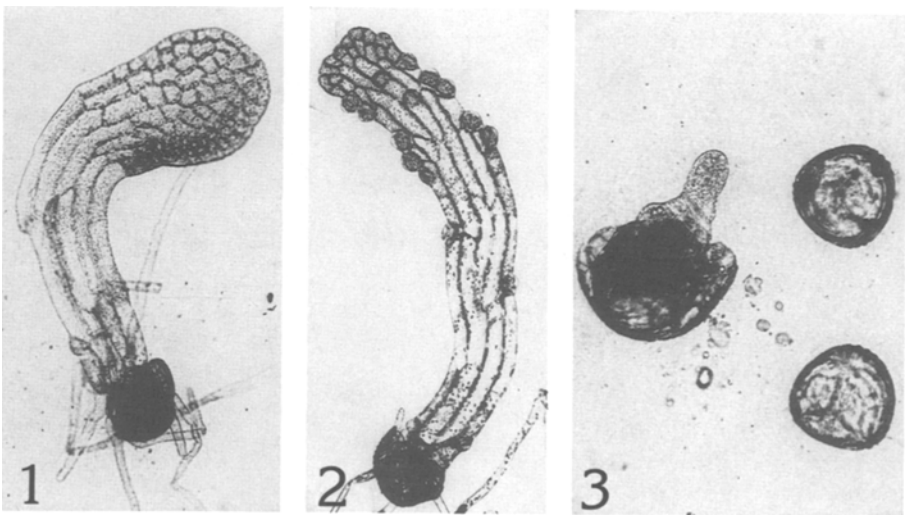
In control gametophytes, antheridial initiation started after 30 days, whereas 80% gametophytes in 10 ppm LAS developed antheridia. No change was observed in the structure of antheridia of treated and controlled gametophytes. However, in control, most of the antheridia were borne on marginal clefts of the thallus (figs 4, 5) while in treated ones, they were protruding from the margins (fig 2). Fluorescence microscopy studies also revealed no change in 10 ppm (LAS) and control gametophytes before and after the development of anthe-

Table 1. Percent germination and subsequent gametophyte development of spores of Ceratopteris.

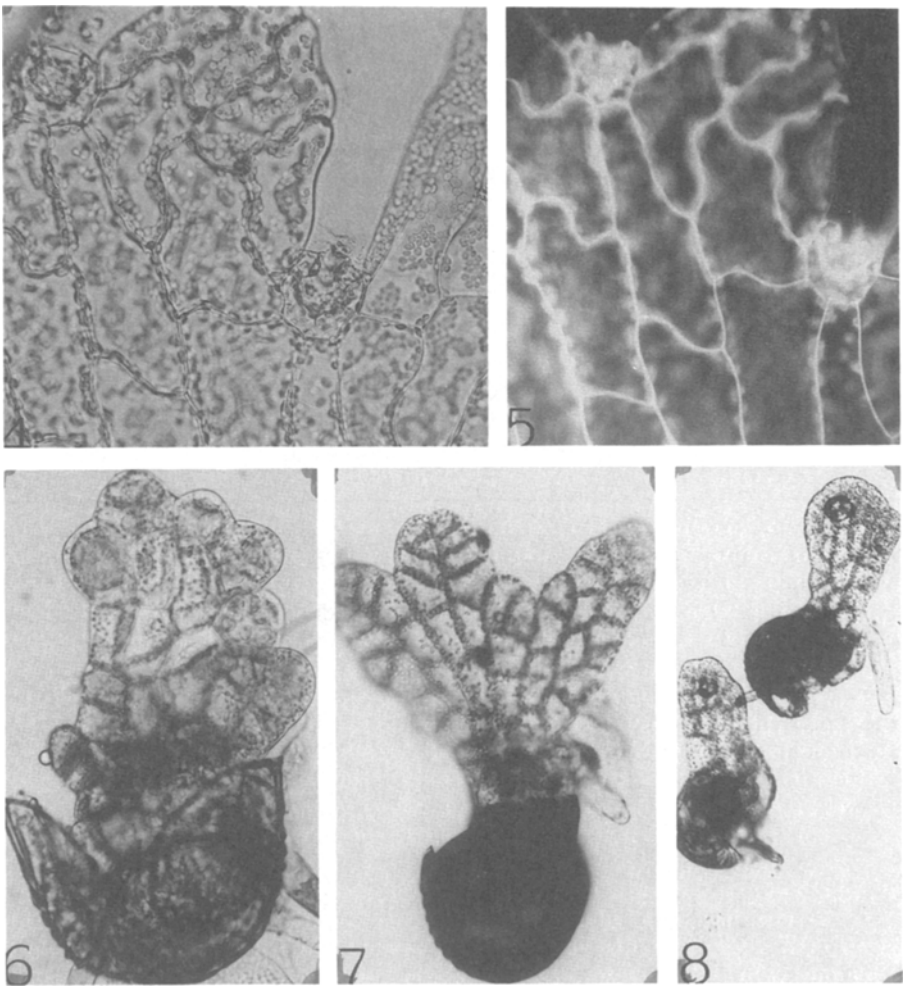
LAS ppm	After 15 days	After 20 days	After 30 days
Control	80 % germination 50% spores in 10-30 celled stage	95% germination	50% antheridia development
10	80% germination 50% spores in 10-30 celled stage	90% germination, 50% antheridia development	80% antheridia
20	40% germination 2-10 celled stage	60% germination	30% antheridia development
30	30% germination 1-5 celled stage	50% germination	10% antheridia development
75	10% germination	20% germination	5% antheridia development
100	5% germination	10% germination	20% germination

ridia. Red emission due to autofluorescence of chlorophyll pigments were also similar in intensity in both the cases. Antherozoids from 10 ppm (LAS), exposed gametophytes produced similar fluorescence characteristics and movement as that of control.

The experiment was repeated thrice and the pattern confirmed. Since the number of spores in 2.0 mg was about 150 with possible variations, statistical evaluation was not attempted.



1. Control, no antheridia formation in gametophyte after 20 days. x 100. 2. Treated gametophyte with 10 ppm LAS, showing marginal antheridia x 100. 3. At 75 ppm no rhizoid development after 20 days. x 100.



4. Antheridia formation in marginal cleft of control gametophyte. x 500.
5. Fluorescence micrograph of the same (using chlorotetraycline as fluorochrome, blue excitation) x 500.
6. Antheridia formation in the gametophyte of 75 ppm after 30 days. x 100.
7. Branched gametophyte at 20 ppm. x 100.
8. Gametophyte with stunted rhizoids 30 ppm. x 100.

Table 2. Number, length and width of rhizoids of gametophytes treated with 10 ppm LAS.

	After 15 days		After 20 days		After 30 days	
	Control	Treated	Control	Treated	Control	Treated
Average number of rhizoids of ten gametophytes ± SD	5.5±1.08	5.4±1.07	7.7±2.6	6.8±1.4	16.1±2.02 ($p < 0.1$)	13.3±2.7
Average width x 10^{-3} µm±SD	2.7±0.9	1.98±0.6	2.3±0.86 ($p < 0.1$)	2.1±0.75	2.5±0.92	2.1±0.79
Average length µm±SD	0.13±0.03 ($p < 0.02$)	0.05±0.02	0.13±0.05 ($p < 0.1$)	0.06±0.03	0.14±0.04 ($p < 0.1$)	0.09±0.03

Each value is the arithmetic mean of ten individual cases, along with ±SD. The experiment was done in triplicates and results were confirmed. The data of one experiment only is given. P values between control and treated cases are given in parenthesis.

Antheridial formation took place in the gametophyte of 75 ppm and the gametophytes were neither cordate nor filamentous (Fig. 6). After 30 days in 100 ppm (LAS) branching of gametophytes is very common but no antheridial formation was observed (fig 7). Apical meristem formation was observed only in control (fig 1). Reduction in number and stunting of rhizoid were observed in 30 ppm LAS (fig 8). Size of the gametophytes were also smaller than that of the control.

DISCUSSION

Deleterious effects due to synthetic detergents to aquatic macrophytes are well documented. Muramoto and Oki (1984) reported significant decrease in the growth ratio of root length of Water Hyacinth, when treated with 30 ppm and higher concentrations of sodium dedecyl sulfate. In *Cladophora domereta*, alkyl benzene sulfonate caused cell death and chloroplast destruction (Hynes and Robert 1962). Swelling of chloroplast thylakoids and loss of chloroplast limiting membrane were seen after 4 hrs of LAS treated protocorms of *Phalaenopsis* (Healey *et al.* 1971). Chawla *et al* (1986) used fresh water alga *Scendesmus quadricauda* to evaluate phytotoxicity caused by LAS and recommended 0.05% as safe limit for the use, beyond which loss of protoplasm from the cells, collapsing of cell and chloroplast disruption were noted. At lower levels, LAS was found to enhance the algal biomass production. Lower doses as well as short term treatment of alkyl benzene sulphonate was proved to be beneficial for *Cladophora* and *Voucheria* also (Hicks and Neuhold, 1986).

Faster growth, earlier rhizoid development and higher frequency of antheridia formation than the control were noticed in Diplazium under the influence of LAS at .001% (Yasmeen and Devi 1986). Ernst et al (1971) observed growth stimulation and increase in fresh weight of Phalaenopsis seedling when treated with lower doses of surfactants. Similar stimulatory effects have been reported by Parr and Norman (1965). These detergents at lower concentrations serve as metabolic stimulators or helps in the uptake of water and (or) nutrients.

It can be concluded from the above studies that at 10 ppm, LAS positively promotes growth of gametophytes but at higher levels of LAS it not only reduces spore germination but subsequent development also. Unlike the earlier studies on fern spore bioassay, the present data shows possible effects on reproduction and likely effects on subsequent generations. Application of fluorescence microscopy could be useful in genotoxic and detergent toxicity. The use of spores of aquatic fern proves to be more desirable for such studies and the pathomorphological observations reported will help in identifying reliable indices of phytotoxicity.

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