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Effect of Synthetic Detergents on Germination of Fern Spores

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Plant based mutagenesis assay on onion root tips and Tradescantia stamens are routinely used in the predictive toxicological evaluation. Similarly algal bioassays (Walsh et al 1982, Claessen 1984, Edward 1972), pollen tube growth and seed germination are recommended as test systems (Pfahler et al. 1981). However, its applicability in the case of fern spores has not been brought to light (Devi 1980). Recent works by Klekowski and Davis (1977), Francis and Petersen 1983a, b, Petersen et al. (1980) permit investigations on fern spores as assay for environmental mutagenesis or as a model system for studying the effect of heavy metals. Synthetic detergents constitute one of the most important water pollutants by contaminating the lakes and rivers through domestic and industrial use. Considerable information is now available for the adverse effects of detergents on aquatic fauna including fish (Divo 1974, Misra et al. 1984, Lal et al. 1983), algae (Swisher 1970, Ernst et al. 1983, Markwell and Thornber 1983, Hicks and Neuhold 1966, Chawla et al. 1986) and higher aquatic plants (Degens et al. 1950, Klein et al. 1963). Marked inhibition of germination in orchids (Ernst et al. 1971) and brinjals (Kale 1966) and of seedlings growth in raddish (Kale et al. 1968) suggest that rapidly growing systems could be sensitive to detergent polluted water. The present study of the effect of linear alkyl benzene sulphonate on germination of the spores of a fern, Diplazium esculentum aims at the understanding of the effects of water pollution on pteridophytes and the development of spore germination assay for phytotoxicity evaluation.

MATERIALS AND METHODS

Linear alkyl benzene sulphonate was obtained from Industrial Toxicological Research Centre, Lucknow (Lal et al. 1983, Misra et al. 1984). One % (w/v) solution was prepared after adjusting the pH to 7.0. Subsequent dilutions as desired were made accordingly and pH maintained at neutrality. Different concentrations of detergent i.e., .01, .05, .1, .2 and .3 ppm were prepared in triplicate with the Knop's solution (Bold 1936). A petridish containing only Knop's solution was kept as control set. Fern spores (approximately 100 in number) were sprinkled with paper spatula over Knop's solution containing different concentrations of detergent under aseptic conditions and the petridishes were incubated at 25°C for 2-3 days and the germinating spores were observed daily under light microscope.

After a month of study the experiment was repeated with another set of concentrations in liquid as well as in solid Knop's medium. The different concentrations of the detergent were 0.001, 0.004, 0.008 ppm and a control set. The germinating spores were observed daily under the light microscope.

RESULTS AND DISCUSSION

The spores started germinating after three days of sowing in control. There was 80% germination. The sets containing different concentrations of detergent showed no sign of germination even after ten days except, in 0.01 ppm there was cracking in the spore coat, cytoplasm was disturbed and the fatty contents were coming out in the form of globules (Fig 3). In 0.05 ppm cracking of the spore coat started after twelve days. Here also the spore cytoplasm showed the same effect as that in 0.01 ppm (Fig 5). In other concentrations i.e., 0.1, 0.2 and 0.3 ppm the spores showed no change in the cytoplasm or spore coat.

In 0.1 ppm the spore coat started breaking after fifteen days in the similar manner while they showed no sign of germination although cytoplasm was shrunken. The percentage of cracking of spore coat was very low (10% in 0.01 ppm, 5-6% in 0.05 and 0.1 ppm). In 0.2 and 0.3 ppm there was no cracking of the spore coat even after one month. Nevertheless, the spore cytoplasm was disturbed and spore coat appeared to be fragile and weak. It was also observed with these concentrations that the spore coat did not break while the cytoplasm had shrunken to a globular mass including large oil globules (Fig 6). However, one hour after the spores were taken out of the solution and mounted in water for observation, they turned green and the spore coat cracked and the protoplasm was exposed (Fig 4).

In control set the spore germination was normal, starting with the emergence of a rhizoid (Fig 7). The average rhizoidal length was 88 microns in one day old sporeling, and 268 microns in two days old sporeling. Two rhizoids and two celled stages were observed after six days and three or four celled stages after seven days of germination. Multirhizoidal stages were also served with four celled stage. Though rhizoidal growth was very rapid in the liquid medium, the sporelings could not survive after 5 celled stage (Fig 2). Spores were sown in liquid as well as solid Knop's medium in another experiment which was performed with different concentrations of detergent i.e., 0.001, 0.004, 0.008 ppm and a control set. In liquid medium the spores started germinating after three days of sowing in control and 0.001 ppm while in 0.004 and 0.008 ppm there was no germination. There was no marked difference in the growth pattern of the two sporelings (Fig 1) in control and treated ones. In both the cases the germination started with the emergence of a rhizoid, while the percentage of germination was 60% in control and 50% in 0.001 ppm. The average length of the rhizoid in control and 0.001 ppm were about the same.

In 0.004 ppm the protoplasm was active and cracking started after two days of sowing. In about 30% of the cases, spores remained green in colour. Four to five days after the spore coat cracked, the rhizoidal portion was exposed. No further growth could be recorded. 0.008 ppm there was no visible change in spores except for the shrunken protoplasm. However, about 2% of spores turned green with cracked spore coat. The sporeling of control and 0.001 ppm grew normally upto 5 celled stage without any marked difference but could not survive after this stage.

In solid medium the germination started six days later than in the liquid medium, in all the concentrations of the detergent, but there was slight variation in the percentage of germination from control (Table 1). In 0.008 ppm the size of the protonema was bigger than the rest of the concentrations. In some sporelings bulbous rhizoids were also seen. There was no marked difference in the growth of the sporelings of the control and the treated ones. In the concentrations 0.001, 0.004, 0.008 ppm rhizoids appeared earlier as compared to the control, though the growth is faster in the control as compared to the treatments.

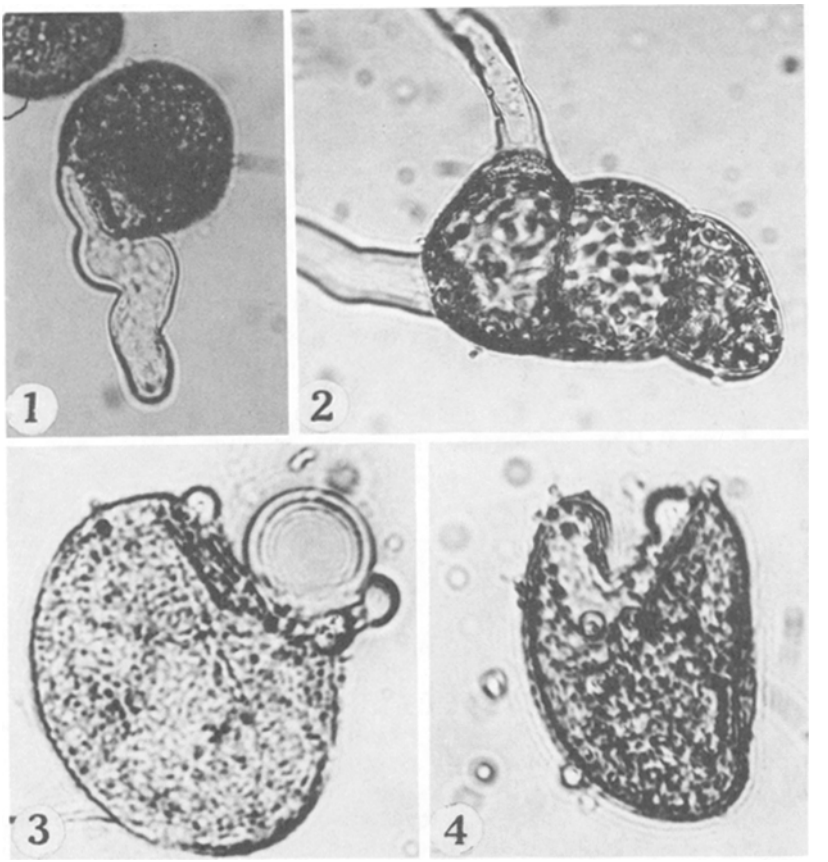
Table 1. Percentage of germination in Diplazium with different concentrations of synthetic detergent in solid medium.

Concentrations in ppm	% of germination
0.001	28±5
0.004	20±3
0.008	20±1
Control	35±3

Values are arithmetic mean ± of 5 determinations each.

The frequency of the antheridia was low in 0.008 ppm as compared to 0.001 and 0.004 ppm. It was also observed that the frequency of the antheridia was more in treated ones as compared to that of control. After antheridial development, the gametophyte could not survive.

When LAS was incorporated into the culture medium, even as a low level as 0.001 ppm caused a slight decrease in germination and rhizoidal length was slightly decreased, although sporeling features were similar. At 0.004 and 0.008 ppm there was no germination and cracking of spore coat was slower than in control. At concentrations above 0.1% the effect was even more marked. Thus LAS at levels .001 ppm distinctly inhibit germination indicating direct phytotoxicity. If this had been due to the reducing surface tension of medium by the detergent the lysis of spore coat would have been instantaneous. The detergent affecting the quality of the medium could also be a likely factor, even though pH was same. In solid medium incorporation of detergent was not as lethal as in liquid medium. LAS at 0.001, 0.004, 0.008 ppm caused 12.5, 37.5 and 37.5% less viability as compared to controls. Growth pattern and morphology characterisation were not much different in control and treated sporelings.



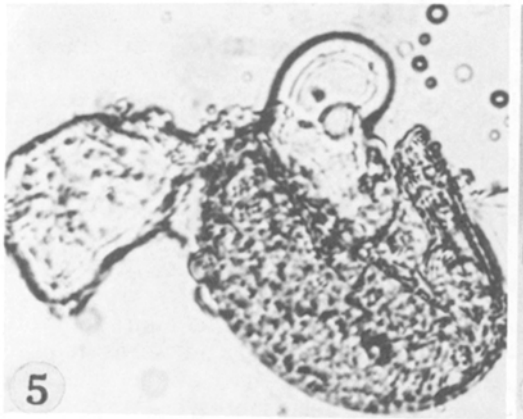
Spores treated with various concentrations of detergent.

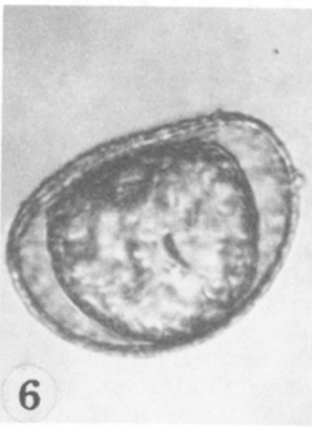
Figure 1. .001 ppm emerging rhizoid x 600

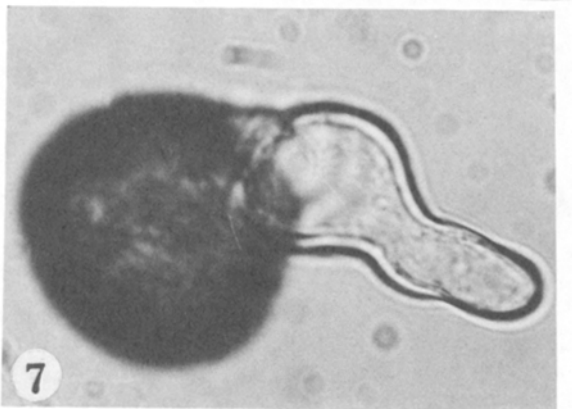
Figure 2. .001 ppm 5 celled stage x 600

Figure 3. .01 ppm fatty contents coming out from the cracked spore x 1000

Figure 4. .3 ppm cracked spore after mounting in water x 1000







Spores treated with various concentrations of detergent and control

Figure 5. .05 ppm fatty contents and cytoplasm coming out from the cracked spores x 1000

Figure 6. .2 ppm globular mass of cytoplasm x 1000

Figure 7. Control x 800

The adverse effect of LAS on spore germination is generally in analogy with that of heavy metals. It was observed that all equal weight combinations of Cu, Cd and Zn exhibited synergestic toxic responses in both Osmunda cinnamomea and Onoclea sensibilis, while at lower concentrations of HM combinations Onoclea sensibilis exhibited antagonistic toxic responses (Petersen et al. 1980, Francis and Petersen, 1983a,b).

Thus it is evident that the fern spore germination assay could be developed as a simple sensitive and reliable screening system for phytotoxicity. The test compounds can be incorporated into the liquid or solid media and viability growth pattern and morphology can be studied. It may also be possible to combine this with the mutagenicity test system of Klekowski and Davis (1977) so that the full potential of fern spores in toxicity testing can be realised. From the present results it is evident that synthetic detergents inhibit spore germination also as with seed germination (Kale 1966).

Since detergents pollute lakes and rivers they could cause phytotoxicity and ecological changes, among which aquatic ferns may be specifically affected. From the present data it may be concluded that LAS levels above 0.001% are toxic to fern spores.

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