

## RESEARCH COMMUNICATIONS

of the culture filtrate has a pronounced and distinct stimulating effect on the growth of the cultures. The culture containing culture filtrate had an absorbance of 0.96 on day 9, as against 0.63 absorbance of the control. This amounts to more than 50% increase in growth at this point of time. Higher proportions of filtrate produced no further stimulatory effect. Possibly, at 4% itself, whatever factor is stimulatory to growth had attained saturation point for the culture under the conditions of the cultivation. Ciferri<sup>3</sup> observed lysis of *Spirulina* whenever the inoculum size was very small under mixotrophic conditions and had termed it 'mixotrophic lysis'. No tangible explanation for this phenomenon is available. The present results demonstrate that *S. platensis* CFTRI excretes some unknown factor(s) that is stimulatory to its own growth. It is understandable, therefore, that if the inoculum size is small the growth-stimulatory factor coming from it is inadequate to support the growth of the culture. This would also mean that a certain threshold concentration of this factor would be essential.

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ACKNOWLEDGEMENTS. I thank the Department of Biotechnology, New Delhi, for financial assistance, and Dr. L. V. Venkataraman and Dr. R. Joseph of CFTRI, Mysore, for guidance.

17 March 1990

### Effect of fern extracts on growth and germination of fungi

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The effect of three fern extracts on growth and germination of *Alternaria brassicicola* and *Aspergillus niger* was studied. Extracts of all three ferns had an adverse effect on the growth and germination of both fungi. Rhizome extracts were more toxic compared to leaf extracts.

*ALTERNARIA BRASSICICOLA* causes dark spot disease in the genus *Brassica*<sup>1</sup>. *Aspergillus niger* is a saprophytic fungus that attacks seeds/fruits of a large number of plants in storage<sup>2</sup>. Spray or treatment with chemical fungicides is the method recommended for their control. But the use of fungicides has some inherent problems of toxicity. In view of this many workers have suggested the use of plant extracts to reduce the incidence of various plant diseases<sup>3,4</sup>.

Ferns are generally less prone to attack by fungal pathogens<sup>5</sup>. But nothing is known about the toxicity of fern extracts to fungi. Hence in the present study an attempt has been made to determine the effect of extracts of three ferns, viz. *Adiantum caudatum*, *Diplazium esculentum* and *Pteris vittata*, on growth, spore production and spore germination of *A. brassicicola* and *A. niger*.

The fern extracts were prepared by crushing 5 g green leaves and rhizome separately in 500 ml distilled water. The extract was steamed for 30 min, and strained through muslin cloth. Ten ml of extract was added to petri plates containing 20 ml of molten potato dextrose agar (PDA). The plates were then inoculated with the fungi. For control, plates with PDA alone were inoculated. The inoculated plates were incubated at 28°C for seven days. There were three replicates for each treatment and the experiment was repeated thrice.

Growth was determined by measuring the colony diameter after seven days of incubation (Table 1). For sporulation five agar discs, each of 5 mm diameter containing sporulated fungus, were selected at random from the fungus colony and transferred to 1 ml of distilled water. A suspension of 0.01 ml was placed on a clean slide and examined under the microscope. The number of spores per microscopic field was counted and average spore production was categorized as poor, fair, good or excellent (Table 1). For germination studies spores were taken in a test tube containing 5 ml distilled water and the suspension was mixed thoroughly. One drop was placed in a cavity slide containing 0.5 ml of fern extract. The cavity slides were incubated in a moist chamber at 30°C. Germination of the spores was observed after 15h and percentage of germination was calculated.

Table 1 shows that extracts of leaves and rhizomes of all three ferns had an adverse effect on germination and growth of both fungi. The highest toxicity was caused by

Table 1. Effect of fern extracts on germination, growth and sporulation in *Alternaria brassicicola* and *Aspergillus niger*.

Fern	Germination of spores (%) in extracts				Growth (mm)/sporulation in extracts			
	Leaf		Rhizome		Leaf		Rhizome	
	<i>A. brassicicola</i>	<i>A. niger</i>	<i>A. brassicicola</i>	<i>A. niger</i>	<i>A. brassicicola</i>	<i>A. niger</i>	<i>A. brassicicola</i>	<i>A. niger</i>
<i>A. caudatum</i>	60.3	58.6	5.1	7.2	36/(++)	32/(++)	27/(++)	23/(++)
<i>D. esculentum</i>	30.5	35.3	8.0	5.3	39/(++)	35/(++)	29/(++)	26/(++)
<i>P. vittata</i>	12.5	15.4	4.7	2.2	34/(+)	28/(+)	31/(+)	30/(+)
Control	97.2	95.3	97.2	95.3	41/(++++)	38/(++++)	41/(++++)	38/(++++)
LSD at 5%	1.73	1.38	3.84	2.80	2.38	2.87	2.26	2.58

+, Poor (1-10); ++, fair (11-30); +++, good (31-50); +++, excellent (above 50).

*P. vittata*, followed by *D. esculentum* and *A. caudatum*. Rhizome extracts were more toxic compared to leaf extracts. It is probably due to antimicrobial principles, as the rhizomes of various ferns have been reported to contain toxic compounds such as cyanogenic glycoside<sup>6,7</sup>. It is likely that this or related compounds might be adversely affecting the growth and germination of fungal spores.

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8 March 1990

## Reduced volatile aldehyde production in wheat by seed invigoration treatments

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**Highly significant negative correlations were noted between germinability of wheat (*Triticum aestivum* L. cv. Sonalika) seeds of different vigour status and volatile aldehyde production. Mid-term hydration-dehydration seed invigoration treatments, which effectively maintained vigour and viability of wheat seeds, showed a substantial reduction in post-ageing volatile aldehyde production.**

AN association between volatile aldehyde production and seed vigour has been demonstrated in several seeds<sup>1-6</sup>. These volatile aldehydes are clearly products of lipid peroxidation and can be produced by autoxidation or through the mediation of lipoxygenase found in a wide variety of germinating seeds<sup>1,5,7-9</sup>.

Lipid peroxidation is significantly reduced by hydration-dehydration of stored seeds<sup>10-13</sup>. If the higher percentage of germination of hydrated-dehydrated seed, compared to untreated seed, is attributable, at least in part, to reduced lipid peroxidation, it may be presumed that the production of volatile aldehydes is lowered by such seed invigoration treatments. To test this hypothesis, the effect of hydration-dehydration of seeds on the production of volatile aldehydes by germinating wheat has been studied.

The experiments were carried out with seeds of wheat (*Triticum aestivum* L.) cultivar Sonalika. Hydration-dehydration treatments, viz. soaking-drying, dipping-drying and moisture equilibration-drying, were given to 5-month-old medium-vigour wheat seeds (seed m.c. 9% on wet wt basis) following the method described by Basu and coworkers<sup>14-16</sup>. After treatment, one half portion each of treated and untreated seeds were stored in sealed glass vials in a refrigerator at  $-10^{\circ}\text{C}$  to arrest the ageing process (before ageing set); the other half portions of seeds of the different treatments were subjected to accelerated ageing at 95% RH and  $40^{\circ}\text{C}$  for seven days (after ageing set). Before and after accelerated ageing, germination tests were carried out following the inclined glass plate method developed by Punjabi and Basu<sup>17</sup>.

For the assay of volatile aldehydes in the gaseous emanations of germinating invigorated seeds before and after ageing, sterilized (by 0.05% mercuric chloride solution for 20 min) wheat seeds were placed in two rows (50 seeds in a row) on the upper portion of a moist blotter. The seeds were covered by a thin layer of moist absorbent cotton and placed on the inner surface of a wide-mouthed 1.3-litre capacity air-tight glass bottle containing 40 ml of water for continuous supply of moisture to the germinating wheat seeds. A 100 ml beaker containing 10 ml of 0.2% (w/v) 3-methyl-2-benzothiazolinone hydrazone (MBTH) solution was placed inside the larger bottle containing the germinated wheat seeds for absorption of volatile aldehydes. One control was taken in which there were no wheat seeds and only MBTH solution was placed. The wide-mouthed bottles were fitted with air-tight screw caps to prevent gas leakage. The bottles were kept in a temperature-controlled room at  $25 \pm 1^{\circ}\text{C}$ . After 48 h, germination percentage and seedling growth of wheat were recorded and the volatile aldehyde trapped by the aldehyde-absorbing reagent MBTH was determined following the method of Wilson and McDonald<sup>4</sup> with some minor modifications. A 3 ml aliquot of the aldehyde trapping solution was collected from each bottle and taken into a test tube containing 2.5 ml of 0.23% (w/v) ferric chloride solution and incubated for 10 min. Then 2.5 ml of absolute acetone was added to each tube and the tubes closed with tightly fitting corks. After 30 min, absorbance of the reaction mixture was read at 635 nm. Correlation coefficients (*r* values) between germinability and volatile aldehyde production of wheat were worked out following standard statistical methodology<sup>18</sup>.

The different hydration-dehydration treatments showed only minor improvements in germination percentage and seedling growth before ageing. But after accelerated ageing at 95% RH and  $40^{\circ}\text{C}$  for seven days, hydration-dehydration treatments resulted in greater vigour and viability of stored wheat seed (Figure 1). Soaking-drying gave greater germinability than dipping-drying and