Effect of sulphite on spore germination and rhizoid development in the tropical fern 
*Lygodium japonicum* (Filicales: Lygodiaceae)

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Abstract: A 24 hr red light treatment followed by white light enhanced germination rate on spores of *Lygodium japonicum* Swartz. Sulphite adversely affected germination and caused abnormalities in rhizoidal growth. In sulphite-free media the apical growth left behind the swollen portion of the rhizoids. *L. japonicum* gametophytes are good bioindicators of SO₂ pollution.

Key words: *Lygodium japonicum*, pollution, sulphite, spore, germination, growth.

Sulphur dioxides is a major air pollutant in urban-industrial areas, leading in plants to growth retardation (Bell *et al.* 1979, Buckenham *et al.* 1982) and reduction of photosynthesis (Jones and Mansfield 1982) and carbohydrate contents (Heath 1980). Sulphur dioxide is reported to affect the germination of spores of some bryophytes, and of pollen in pine, lilly etc. (Keller and Beda 1984, Mitsugi *et al.* 1985, Nakada *et al.* 1970). Once inside the cells SO₂ dissolves in water to form H₂SO₃ which dissociates into SO₄⁻³, HSO⁻³ and H⁺ depending upon the pH of the medium (Puckett *et al.* 1973). It has been suggested that an aqueous solution of sulphite be applied as an alternative to SO₂ fumigation to study the effect on plants (Miller and Xerikos 1979, Garsed 1981). Since the life cycle of ferns is short and the developmental process of fern gametophyte is light-dependent (Furuya 1983) and easily observable under the microscope, Wada *et al.* (1986) proposed an excellent model system to investigate the toxicity of SO₂ to fern cells. Moreover, the cultures grown under liquid medium can easily be manipulated to observe the effects of exogenously added substances.

In the present study, the effect of sulphite on the germinating process of *Lygodium japonicum* Sw. spores and further development are analysed.

**MATERIAL AND METHODS**

Spores of *Lygodium japonicum* Swartz. a tropical fern cultivated in the University Botanical Garden were collected and imbibed in water for 24 hr before sowing aseptically in AF Dyer's nutrient media after sterilization with 0.3% sodium hypochlorite solution (Dyer 1979). The petri dishes (5 cm) were kept separately at 24±2°C under white light (8.7 Wm⁻²) and red light (4.8 Wm⁻²) to see the effect of several light treatment periods on spore germination (Table 1). For each treatment 100 spores were picked on glass slides from the aseptic cultures, mounted in Acetocarmine-Chloral hydrate stain and examined under 100 magnifications for germination counts (Edwards and Miller 1972). Spore coats often dissociated from germinating spores and
aborted or empty spore coats were not counted (Table 1).

Some modifications were carried out in the rest of the experiments. Approximately 0.5 mg of spores were imbibed for one day in darkness and then suspended in 6 ml of culture medium (potassium phosphate buffer, 1 mM, 10 mM and 100 mM; pH 6.0) to be cultured under continuous red light of 4.8 Wm\(^{-2}\) for 3 days. The definition of spore germination was the emergence of rhizoid or protonemal cell out of a spore coat. All spores under one microscopic field was scored for per-cent germination (three replicate samples per culture and three replicate cultures per treatment). Sample size for each observation varied from 20 to 60 spores per microscopic field. Approximately 300 spores were scored per treatment to calculate standard errors of the average per-cent germination. Sulphite was applied as a solution of Na\(_2\text{SO}_3\) (dissolved just before use in an autoclaved medium using sterilized glassware). Sulphite was not autoclaved to prevent destruction. Solutions of Na\(_2\text{SO}_4\) were also prepared in the above manner (Table 2).

To see the effect of sulphite treatment on spore germination and rhizoidal growth the spores were cultured under continuous red light (R) of 4.8 Wm\(^{-2}\) for four days after imbibition in dark (D) for 48 hrs (Table 4). Sulphite treatment of 3 mM (Na\(_2\text{SO}_3\)) in K-phosphate buffer was given for 24 hrs on consecutive days to each set during spore germination. Data were taken on the 7th day of spore inoculation and are presented as mean ± SE.

**RESULTS**

The treatment of 24 hr of red light followed by white light enhanced the rate of germination (Table 1). However, continuous exposure of red light could not bring any significant difference in the formation of antheridia, archegonia and sporophyte.

Metal ions of AF Dyer nutrient media promotes oxidation of sulphite; hence, for the growth of fern gametophytes a replacement with potassium-phosphate buffer was made in the present work (Fuzzi 1978, Beilke and Gravenhorst 1978, Heg and Hobbs 1978). Beyond 10 mM concentration of K-phosphate buffer the per-cent spore germination decreased irrespective of the presence or absence of sulphite or sulphate in the medium. At 1 mM concentration of buffer, spore germination was

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**TABLE 1**

*Effect of red light treatment of various duration followed by white light on spore germination of L. japonicum*

<table>
<thead>
<tr>
<th>Days</th>
<th>Germination</th>
<th>Days</th>
<th>Germination</th>
<th>Days</th>
<th>Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>93</td>
<td>5</td>
<td>92</td>
<td>5</td>
<td>90</td>
</tr>
</tbody>
</table>

**TABLE 2**

*Effect of K-phosphate buffer, sulphite, sulphate on fern spore germination of L. japonicum (values are average ± SE)*

<table>
<thead>
<tr>
<th>Concentration of K-phosphate buffer (M)</th>
<th>Control</th>
<th>Na(_2\text{SO}_3) conc. (M)</th>
<th>Na(_2\text{SO}_4) conc. (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>93.66 ± 1.52</td>
<td>19.66 ± 2.08</td>
<td>89.66 ± 4.04</td>
</tr>
<tr>
<td>10 mM</td>
<td>67.33 ± 2.08</td>
<td>57.33 ± 3.05</td>
<td>55.33 ± 3.05</td>
</tr>
<tr>
<td>100 mM</td>
<td>27.00 ± 2.00</td>
<td>12.66 ± 1.53</td>
<td>25.33 ± 2.08</td>
</tr>
</tbody>
</table>

(R) of 4.8 Wm\(^{-2}\) for four days after imbibition
Concentration Incubation Control 

Na₂SO₄: J Na₂SO₄

spore germination varied significantly with sulphite and sulphate (Table 93.66, p<0.001). 

control was demonstrated on application of germination was significantly inhibited by 0.01 increase sulphate between sulphate treatment and buffer concentrations (F = 204.50; p<0.001). Similarly, spore germination was significantly inhibited by sulphate (F = 289.42; p<0.001) and buffer concentrations (F = 327.40; p<0.001). Interaction between sulphate treatment and buffer concentration was significant (F = 23.52; p<0.001).

Sulphite and sulphate may change the pH of the K-phosphate buffer. Hence the buffering capacity of the medium was tested (Table 3). When using the 10 mM and 100 mM concentrations of K-phosphate buffer the dissolved sulphite showed very little variation in pH (with respect to the control). Nevertheless, after dissolving sulphite at 1 mM (buffer concentration) pH increased initially; a gradual decrease was observed by the 5th day of the experiment. The variation in the pH of buffer at all concentrations due to sulphite and sulphate did not differ from the control (Student's t-test, p>0.05).

TABLE 3

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Incubation time (days)</th>
<th>1 mM</th>
<th>10 mM</th>
<th>100 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>6.01</td>
<td>6.00</td>
<td>6.01</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.11</td>
<td>6.01</td>
<td>6.02</td>
</tr>
<tr>
<td>10 mM</td>
<td>0</td>
<td>6.97</td>
<td>6.59</td>
<td>6.21</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.38</td>
<td>6.23</td>
<td>6.20</td>
</tr>
<tr>
<td>Na₂SO₃</td>
<td>0</td>
<td>6.71</td>
<td>6.04</td>
<td>6.01</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.31</td>
<td>6.03</td>
<td>6.02</td>
</tr>
<tr>
<td>1 mM</td>
<td>0</td>
<td>6.27</td>
<td>6.06</td>
<td>5.99</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.39</td>
<td>6.08</td>
<td>6.02</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>0</td>
<td>5.87</td>
<td>6.12</td>
<td>5.99</td>
</tr>
<tr>
<td>1 mM</td>
<td>5</td>
<td>6.17</td>
<td>6.07</td>
<td>6.13</td>
</tr>
</tbody>
</table>

TABLE 4

Effect of sulphite treatment on spore germination and rhizoidal growth (D = Dark, R = Red light)

<table>
<thead>
<tr>
<th>Duration of sulphite treatment (hr)</th>
<th>Light condition</th>
<th>Germination (%)</th>
<th>Rhizoidal abnormalities (%)</th>
<th>Rhizoid length (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-24</td>
<td>D</td>
<td>76.33 ± 2.51</td>
<td>5.71 ± 1.04</td>
<td>36.33 ± 3.05</td>
</tr>
<tr>
<td>24-48</td>
<td>D</td>
<td>70.66 ± 2.08</td>
<td>7.10 ± 1.61</td>
<td>33.66 ± 1.53</td>
</tr>
<tr>
<td>48-72</td>
<td>R</td>
<td>65.00 ± 3.60</td>
<td>10.32 ± 1.40</td>
<td>35.66 ± 2.08</td>
</tr>
<tr>
<td>72-96</td>
<td>R</td>
<td>51.33 ± 3.05</td>
<td>13.08 ± 0.52</td>
<td>24.66 ± 1.69</td>
</tr>
<tr>
<td>96-120</td>
<td>R</td>
<td>74.66 ± 3.57</td>
<td>68.87 ± 4.57</td>
<td>20.00 ± 2.64</td>
</tr>
<tr>
<td>120-144</td>
<td>R</td>
<td>81.33 ± 3.05</td>
<td>71.60 ± 2.73</td>
<td>16.33 ± 1.53</td>
</tr>
</tbody>
</table>

93.66% but a slight inhibition with respect to control was demonstrated on application of sulphite and sulphate (Table 2). Inhibition in spore germination varied significantly with increase in sulphite (ANOVA, F = 289.42; p<0.001) and buffer concentrations (F = 204.50; p<0.001). Their interaction was also significant (F = 38.35; p<0.001).
A sulphite treatment (Na₂SO₃) of 3 mM was given to fern spores for 24 hr on consecutive days from day one to day six (Table 4). Observations were made on the 7th day to ascertain sulphite sensitivity on the processes of spore germination, induced rhizoidal abnormalities and rhizoidal length. Germination declined sharply on the third day of sulphite application i.e. the first day of red light treatment. Rhizoidal abnormalities were high on the third and fourth day of red light irradiation when the rhizoid showed active apical growth. Swollen rhizoids appeared on the seventh day. The per-cent spore germination (ANOVA F = 3.30; p< 0.005), rhizoidal abnormalities (F = 175.83; p<0.001) and rhizoid length (F = 12.39; p<0.001) varied significantly with sulphite treatment.

After the 7th day, spores were washed with sterile distilled water and re-transferred to a buffer medium and AF Dyer's nutrient media separately for growth. Normal protonemal growth was observed in both conditions i.e. after removal of sulphite from the medium.

**DISCUSSION**

Light has no effect on dormant dry spores, but regarding imbibition the photo-induced germination is enhanced under red light for two days. Phytochrome is one of the photoreceptors controlling the light induced spore germination (Sugai et al. 1977) Initial increase in the pH followed by a gradual decrease after dissolving sulphite to 1 mM concentration of K-phosphate buffer varies the phytotoxicity of sulphite because of a change in the pH of the medium (Hocking and Hocking 1977, Garsed 1981). A slight shift of pH (Table 3) to a higher value results in rapid increase in the ratio of sulphite (Puckett et al. 1973) and then to less toxic sulphate (Neiboer et al. 1976) causing acute to chronic injury symptoms (Environmental protection Agency 1976).

Little information is available on the precise mechanism of the toxicity of SO₂ on fern spore germination. The sulphite-sensitivity of these spores during germination was detected by a decrease in the germination rates and by abnormal rhizoids. The sulphite sensitive timing on the germination rate was the first day of red light treatment (Table 4). A delay of two days in germination was found in the spores kept in sulphite plus red light irradiated conditions. Sulphite affected early stages of germination much before the rupture of spore wall. Red light absorption by the phytochromes must have triggered a series of biochemical and physiological changes inside the spore for differentiation into protonema and rhizoids (Wada et al. 1987).

Apical growth of the rhizoids was retarded as soon as sulphite was applied and the growth in horizontal direction increased resulting a swollen shape of the apical tip. On the Other hand when sulphite was removed from the medium, the rhizoid resumed normal apical growth resulting in the swollen shape being left behind. This is possibly due to reversible changes in synthesis and/or arrangement of components such as microfibrils on the cell wall (Wada et al. 1987). Rhizoid growth is a very sensitive indicator of contamination of the medium (Dyer 1979)

The spore germination of *L. japonicum* is very sensitive to sulphur dioxide, therefore it can prove to be a good bioindicator of SO₂ pollution in the urban–industrial air sheds of India.

**REFERENCES**


Buckenham, A.H., M.A.T. Parry & C. Whittingham. 1982. Effects of aerial pollutants on the growth and yield of


