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EFFECT OF DIFFERENT STIMULI ON THE SPORE GERMINATION OF *HELMINTHOSPORIUM ORYZAE* BREDA DE HAAN.

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Abstract

Chloroform (1 ppm), benzaldehyde (1 ppm), acetone (10 ppm), ethyl acetate (1 ppm) and extract of washed leaves of rice (100 ppm) stimulated spore germination of *Helminthosporium oryzae*. However, washings of rice leaves failed to stimulate spore germination at all its concentrations.

Introduction

Chemical stimuli play an important role in breaking the dormancy of fungal spores. By stimulating spore germination they help the development of certain plant diseases. Although information is available on the effect of chemical stimuli on spore germination in some fungi (Brown, 1946; Barton, 1957; Kerr & Flentje, 1957) no work, as far as the authors are aware, has been done on stimulation of spore germination in *Helminthosporium oryzae*. Ethyl acetate, malate and citrate, benzaldehyde, salicyladehyde, butyric acid, acetone, chlorform and various oils such as oils of Tansy and Cassia have been found effective for certain kinds of spores (Stakman & Harrar, 1957). The germination of spores of *Ascochyta rabiei* is very meagre, slow and uncertain in water but very good in N/50 to N/25 malic acid or in acidified carbon food (Sattar, 1933; Hafiz, 1952). Ling (1940) showed that certain chemicals affect both, the rapidity and percentage of germination of *Urocystis occulta*. Emerson (1948) reported that concentrations of furfural up to 1 ppm, activated the dormant ascospores of *Neurospora crassa*, but with higher dilutions the germination rate invariably dropped sharply. Gallic acid was found to promote germination of *Botryotinia ricini* (Orellana & Thomas, 1965).

Materials and Methods

Culture of *Helminthosporium oryzae* was obtained from the infected rice leaves and then multiplied on basal medium for further studies. In order to estimate the germination capacity of the spores of *H. oryzae*, four concentrations viz. 1, 10, 100 and 1000 ppm of four stimuli viz., chloroform, benzaldehyde, acetone and ethyl acetate were used. Moreover, extract of washed leaves and washing of leaves in the concentration of 10, 100, 1000 and 10,000 ppm; and 1:4, 1:8, 1:16 and 1:32 respectively were used for germination tests.

In order to have different concentrations of the extract of washed leaves, 10 gms of fresh rice leaves were cut into pieces and these were washed well with 95 per cent alcohol. The pieces were then washed well with distilled water and then dried at room temperature. The extract of the leaves was prepared and four different concentrations i.e. 10, 100, 1000 and 10,000 ppm were obtained in distilled water. For leaf washings, 10 gms of fresh leaves were put in a beaker containing 40 ml. of distilled water and the contents were stirred well. Taking this solution as 1:4, it was further diluted to have 1:8, 1:16 and 1:32 concentrations. "Hanging drop method" was used to test the germination of spores (Duggar, 1909). All the germination tests were carried out at room temperature.

TABLE 1. Effect of different stimuli on the germination of *H. oryzae* after 8 hours.

Concentration in ppm.	Chloroform		Benzaldehyde		Acetone		Ethyl acetate		Extract of washed leaves	
	Germination %age	Type of Germination	Germination %age	Type of Germination	Germination %age	Type of Germination	Germination %age	Type of Germination	Germination %age	Type of Germination
1000	6.7	Germ tubes small and mono-polar.	66.7	Germ tubes long, mono & bipolar.	78.9	Germ tubes short and mono-plar.	79.3	Germ tubes very short, usually mono-polar.	57.6	Germ tubes short, mono and bipolar.
100	21.4	Germ tubes small & mono-polar.	80.0	Germ tubes long, mono & bipolar.	80.6	Germ tubes short & mono-polar.	91.1	Germ tubes short, mono & bipolar.	86.2	Germ tubes long & bipolar.
10	64.7	Germ tubes long, mono & bipolar.	85.0	Germ tube long, mono & bipolar.	94.9	Germ tubes long, bifurcated mono-polar, some spores germinate laterally.	93.6	Germ tubes short, mono & bipolar. Some also germinate laterally.	80.0	Germ tubes long, mono & bipolar.
1	88.6	Germ tubes long, mono & bipolar.	92.6	Germ tubes long bifurcated mono & bipolar some spores with lateral germination.	89.8	Germ tubes long bifurcated & bipolar.	95.1	Germ tubes long bifurcated bipolar, a few germinate laterally.		Not seen
Check (distilled water)	79.4	Germ tubes mono & bipolar.	79.2	Germ tubes long, mono & bipolar.	79.4	Germ tubes long, mono & bipolar.	79.2	Germ tubes long, mono & bipolar.	80.0	Germ tubes long, mono & bipolar.

Results and Discussion

The results of these experiments are shown in Tables 1 and 2. Experiments on the effect of different concentrations of stimuli showed that as the concentration of chloroform increased, the percentage of spore germination decreased. Benzaldehyde stimulated spore germination at 1 and 10 ppm. Maximum germination of 89.6 per cent was obtained at 1 ppm after 8 hours. The germ tubes were very long, bifurcated and mostly bipolar. In some spores lateral germination was also observed (Table 1). Noble (1924) and Stakman, Cassell & Moore (1934) reported that benzaldehyde (1.5 ppm) was the most effective chemical in stimulating the germination of chlamydospores of *Urocyists tritici* and *U. occulta.*

Acetone in the concentrations of 1 ppm enhanced the germination and maximum germination of 94.9 per cent was obtained at 10 ppm. Long and bifurcated germ tubes were observed at these concentrations. The germination was mostly mono or bipolar but in some spores lateral germ tubes were also observed. Ethyl acetate accelerated the germination of spores at three concentrations namely 1, 10 and 100 ppm but the germ tubes remained small as compared to acetone.

TABLE 2. Effect of washings of leaves on the germination of *Helminthosporium oryzae* after 8 hours.

Concentration	Washing of leaves	
	Germination percentage	Type of germination
1:4	54.2	Germ tubes short, mono and bipolar.
1:8	64.5	Germ tubes short, mono and bipolar.
1:16	80.0	Germ tubes long, mono and bipolar.
1:32	79.0	Germ tubes long, mono and bipolar.
Check	80.0	Germ tubes long, mono and bipolar.

Extract of washed leaves inhibited the germination at concentrations higher than 100 ppm. Maximum germination of 86.2 per cent was obtained at 100 ppm. Whereas, minimum of 57.6 percent was obtained at 1000 ppm. Noble (1924) and Mathre & Ravenscroft (1966) also noted similar phenomenon in *Urocystis tritici* and *Thielaviopsis basicola.*

Spore germination at 1:16 and 1:32 concentration of washing of leaves was almost the same as in check but inhibition occurred at 1:4 and 1:8 concentrations (Table 2). It revealed that washing of leaves did not stimulate the germination of the spores. Noble (1924) believed that pre-soaking increased the permeability of the spores and allowed a more rapid intake of the stimulatory volatile substances, which increased the permeability of the protoplasmic membrane by changing their physical condition.

Whereas, Cheo & Leach (1950) stated that the stimulation in the germination of spores was probably due to alteration in the permeability of spore wall by the stimulatory substances. In some cases at least it has been shown that the effect is not on the permeability of the spore wall. According to Stakman & Harrar (1957) many of the substances are known to reduce the surface tension of water and it is possible that they may act on the content of the spores in such away as to permit greater hydration.

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