

INFLUENCE OF DIFFERENT PHYSICAL FACTORS ON GROWTH AND SPORULATION OF *BOTRYODIPLODIA THEOBROMAE* PAT. IN CULTURE

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Effect of different factors viz., pH, light and temperature on growth and sporulation of *B. theobromae* causing fruit rot of banana, were studied in liquid Czapek's medium. Among the different pH levels, pH 6.5 was found to be optimum for growth and sporulation of the test-fungus. Continuous darkness showed the most optimum effect of light treatment. 30°C proved to be the optimum temperature for growth and sporulation of the test-fungus among the treated temperatures. Sporulation was also proved to be maximum at the optimal conditions of pH, light and temperature.

INTRODUCTION

Activities of fungi viz., growth, spore germination and reproduction are known to be affected by different pH, light and temperatures. A fungus will grow maximally over a certain range of initial pH of the medium and will fail to grow above or below the extremes. Most of the phytopathogens grow best in media having initial pH reactions on various metabolic activities thereby affecting the shape of pH curve for growth. Like pH, light also played an important role in the fungal metabolism as reported by Inaba and Toshihiro (1972), Marsh *et al.*, (1959), and Page (1965). Literatures show that light has atleast some influence at certain stage of growth and development in fungi. The influence of temperature on growth and sporulation of fungi has attracted the attention of a number of investigators as reviewed by Daverall (1965); Hawker (1950) and Cochrane (1958). For each fungus there are minimum, optimum and maximum temperatures for its metabolic activities. The range of cardinal points of temperature over which fungi will grow varies considerably even among the different species of the same genus.

MATERIALS AND METHODS

The present investigation has been designed to study the effect of different physical factors like pH, light and temperature on growth and sporulation of *B. theobromae* isolated from rotted 'Champa' variety of banana fruits collected from Burdwan markets in W. Bengal.

Czapek's synthetic solution were selected to study the hydrogen ion concentration. Following Sorensen Scale of pH with 1/15 phosphate buffer, two stock solutions of Na_2HPO_4 , $2\text{H}_2\text{O}$ and K_2HPO_4 were prepared from these two stock solutions. In 100 ml of each pH solution the following ingredients of Czapek's medium were added (sucrose — 3gm, NaNO_3 —200mg, KC — 50mg, MgSO_4 , $7\text{H}_2\text{O}$ —50mg, FeSO_4 —1mg). Before harvesting the mycelium from the culture media, the final levels of pH of the media were noted.

To study the effect of light, the pH of the Czapek's medium was adjusted to optimum 6.5 by using N/15 phosphate buffer. One set of inoculated flasks were wrapped with black paper to make the condition dark and another set was kept in continuous light (100 wt.). An identical set were kept in alternate light and darkness (12 hrs. in each).

Growth studies were carried out at different temperature in Czapek's medium (pH—6.5). The selected temperatures were 22°C, 25°C, 28°C, 31°C, 33°C and 35°C.

In all the experiments the medium (25ml) was taken in each 250 ml conical flask, plugged and sterilized at 15 lb. pressure for 15 minutes. The media were inoculated with inocula (5mm) obtained from actively growing mycelium of the test-fungus on Czapek's agar medium. Three replicates were maintained for each treatment. The inoculated flasks were incubated for 4 days for recording mycelial growth. The mycelia were harvested, washed and dried to a constant weight at 60°C in an oven and weighed. Dry weight of mycelium were expressed in term of mg.

For sporulation, the flasks incubated for 21 days. After the incubation period the fungal mycelium were thoroughly mixed with distilled water (10ml) and the spores in suspension were counted with the help of a haemocytometer of 0.1 ml depth. The number of spores were calculated and expressed in million/ml spore.

RESULTS AND DISCUSSION

It was evident from Table 1 that the test-fungus showed optimum growth at pH 6.5 with much less growth towards more acidic alkaline levels. Cochrane (1958) reported that most of the plant pathogen grew best on media with an initial pH of 5.0 to 6.5 Shukla and Sarkar (1974) working on *Botryodiplodia theobromae* isolated from papaya fruits also reported pH 6.5 to be the optimum for growth and sporulation of the fungus. Shreemali (1973) however, reported that 6.0 to be the best : or growth of *B. theobromae* isolated from six different hosts. The probable existence of strain variations in the fungus may account for the slight differences in optimal pH. Sporulation of the test-fungus was also most luxuriant at pH 6.5. Considerable growth and sporulation of test-fungus in all the pH levels tested proved that the fungus possessed a wide range of pH tolerance. The final pH of the media in all the cases showed only insignificant changes from the initial pH.

The results have been recorded in the Tables 1, 2 and 3 respectively.

Table 1. Effect of different pH on growth and sporulation of *B. theobromae*

Initial pH	Final pH	Dry wt. of mycelium* (mg)	No. of spores (million/ml)
5.5	5.7	150.35	0.120
6.0	6.2	175.50	0.124
6.5	6.7	250.62	0.412
7.0	7.1	164.55	0.120
7.5	7.4	140.62	0.084

Table 2. Effect of continuous light, alternate light and darkness and continuous darkness on growth and sporulation of *B. theobromae*

Condition	Dry wt. of mycelium* (mg)	No. of spores (million/ml)
Continuous light	88.44	0.084
Alternate light and darkness	125.60	0.120
Continuous darkness	180.55	0.160

From the Table 2 it was evident that maximum growth took place in darkness much less in alternate light and darkness and minimum in continuous light. This was in full accordance with the earlier observations made in other fungi showing considerable inhibition of fungal growth by light (Page, 1952; Gessner, 1976 and Olutiola, 1976). Sporulation of test-fungus was also observed to be maximum in darkness, but continuous light showed considerable inhibition.

Table 3. Effect of temperature on growth and sporulation of *B. theobromae*

Temperature (°C)	Dry wt. of mycelium* (mg)	No. of spores (million/ml)
22	115.35	0.120
25	118.50	0.124
28	125.74	0.150
30	155.62	0.350
33	120.55	0.125
35	105.48	0.084

* Average of three replicates.

The results (Table 3) showed that at 22°C the growth of the test-fungus was very low which gradually increased with rise in temperature and reached maximum at 30°C and then gradually decreased at further rise in temperature. Nagaraja *et al.*, (1971) also reported 30°C to be the optimum for growth of *B. theobromae* isolated from coconut kernel. A number of workers studied the effects of different temperature on growth of other fungi and reported that an incubation temperature of 30°C was optima for their growth and development (Pal and Grewal, 1975; Tiwari, 1973 and Falloon, 1976). Sporulation of the test-fungus was also maximum at 30°C. The fungus also showed a wide range of temperature tolerance showing fair amount of growth and sporulation.

From the present study it is evident that the test-fungus, which happens to be a fruit rotting pathogen because of its wide range of pH and temperature tolerances survives better in environment and poses a serious threat to fruit preservation programme by causing effective fruit rot.

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