Short Communication

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EFFECT OF pH, TEMPERATURE AND THE FUNGICIDE BAVISTIN ON CELLULASE ACTIVITY OF *MACROPHOMINA PHASEOLINA*

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Effect of pH, temperature and the fungicide Bavistin on cellulase activity of *Macrophomina phaseolina* was studied *in vitro*. Production of cellulase (CMCase) increased with the age of culture. The optimum CMCase activity of 8 days old culture was found at pH 6 and temperature 35°C. Bavistin at 100 µg/ml concentration reduced CMCase activity by 47.2%.

Key Words: *Macrophomina phaseolina, cellulase, Bavistin, pH, temperature.*

Cellulose microfibrils form a frame work of plant cell wall. The basic unit of cellulose is a linear polymer of D-glucose. Cellulases break down cellulose. Secretion and activity of cellulases are influenced by physico-chemical factors. Informations on the effects of physico-chemical factors on enzymes are essential in plant disease control if the fungus is a pathogen. *Macrophomina phaseolina* (Tassi) Goid. is a root rot pathogen of soybean and many other crop plants. In previous studies assay of various enzymes (Dubey and Dwivedi, 1988) and reduction in survival of *M. phaseolina* by Bavistin (Dubey, 1991) were obtained. However, inhibition in secretion and activity of cellulases by fungicides have also been reported by many workers (Kannaiyan and Prasad, 1979; Mandles and Reese, 1965; Mehta *et al.*, 1990). This paper reports the effect of pH, temperature and the fungicide Bavistin on cellulase (CMCase) activity of this pathogen.

M. phaseolina was isolated from the diseased roots of soybean. Cellulase was produced on soybean seed extract (SSE) broth. SSE was prepared by boiling 100 g seed in 1 litre distilled water. After 15 min, pH of filtrate was maintained to 5.6. Aliquots of 50 ml was poured into 250 ml Erlenmeyer flask each containing 0.5% carboxymethyl cellulose (CMC) (Whatman Ltd, England) and blended in buffer at 50-60°C. Flasks were autoclaved at 15 lb/inch^2 for 30 min and inoculated with an agar block (5 mm diam) of *M. phaseolina* actively growing on potato dextrose agar (PDA). After 4,8 and 12 days incubation (25±1°C) culture was filtered through Whatman filter paper No. 1. Culture filtrates were centrifuged at 5,000 rpm for 15 min. This filtrate was taken to assay cellulase.

The cellulase acrivity was determined in terms of CMCase through measuring the reducing sugar by DNS reagent (Miller, 1959). To assay the effect of pH, citrate buffer of different pH levels viz., 4,6,8, and 10 were prepared. CMCase activity was measured after incubation for I h at 30°C. For optimization of temperature 25, 30, 35 and 40°C were used, whereas for Bavistin (methyl-2 benzimidazole carbamate) 50 WP 5, 50 and 100 µg/ml concentrations in the final volume of the reaction mixture were maintained. The CMCase activity was measured as described earlier. The results are expressed as per recommendations of International Union of Pure and Applied Chemistry (Ghose, 1987).

Table 1 reveals that CMCase production increased with age of culture. Maximum amount of enzyme was estimated in 12 days old culture where the rate of glucose liberation was 0.34 IU/ml. Maximum CMCase activity was recorded at pH 6 (30°C). CMCase activity gradually increased with temperature upto 35°C, but decreased at 40°C. Bavistin inhibited CMCase activity differently at different concentrations. It was least effective at 5 µg/ml level. At 100 µg/ml concentration CMCase activity was reduced by 47.2% (Table 2).

It is well known fact that enzyme activity is influenced by pH, temperature and certain chemicals (Mandels and Reese, 1965), but activity of the same differs from microbe to microbe. *In vitro* production of cellulase by *M. phaseolina* has earlier been

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Table 1. Effect of age on cellulase (CMCase) production by *Macrophomina phaseolina*.

Culture age (days)	CMCase activity (IU/ml)
4	0.126
8	0.21
12	0.30

Table 2. Effect of pH, temperature and Bavistin on CMCase activity of 8 days old culture of *Macrophomina phaseolina**.

Factors		CMCase (IU/ml)
pH	4	0.09
	6	0.192
	8	0.02
	10	0.01
Temperature (°C)	25	0.178
	30	0.197
	35	0.204
	40	0.168
Bavistin (µg/ml)	Control	0.21
	5	0.174
	50	0.12
	100	0.11

*. A constant temperature (35°C) is applicable only for pH and Bavistin.

studied by Dubey and Dwivedi (1988). Chauhan and Nagar (1979) have found maximum cellulase activity of *Fusarium moniliforme* var. *sublutinans* at pH range of 5 and 6. *M. phaseolina* is a high temperature loving fungal pathogen. Therefore,CMCase activity at 35°C conforms its great potentiality to secrete cellulase at this temperature.

Presence of cellulolytic activity in *M. phaseolina* explains its saprophytic ability or pathogenesis. On inhibition of these enzymes its saprophytic survival or disease development may be affected. Inhibitory effect of Bavistin on cellulase activity agres with Kannaiyan and Prasad (1979) who found 100% inhibition in cellulase activity of *Rhizoctonia solani* at 100 ppm of Bavistin. Complete inhibition of cellulase activity of *Fusarium oxysporum* by Brassicol (100 ppm) has also been reported (Mehta *et al.*, 1990). It may be attributed that inhibition in CMCase activity is due to combining of Bavistin with cellulase. In nature also probably the fungicides enter in the fungal cells and interrupt the metabolism, the intensity of which may help in breaking the saprophytic ability or intensifying the control.

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