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STUDIES ON THE PHYSIOLOGY OF HIGHER FUNGI : II. EFFECT OF DIFFERENT CONCENTRATIONS OF CASEIN HYDROLYSATE ON SPORULATION AND CONIDIAL MORPHOLOGY OF *HELMINTHOSPORIUM ORYZAE* BREDA DE HANN

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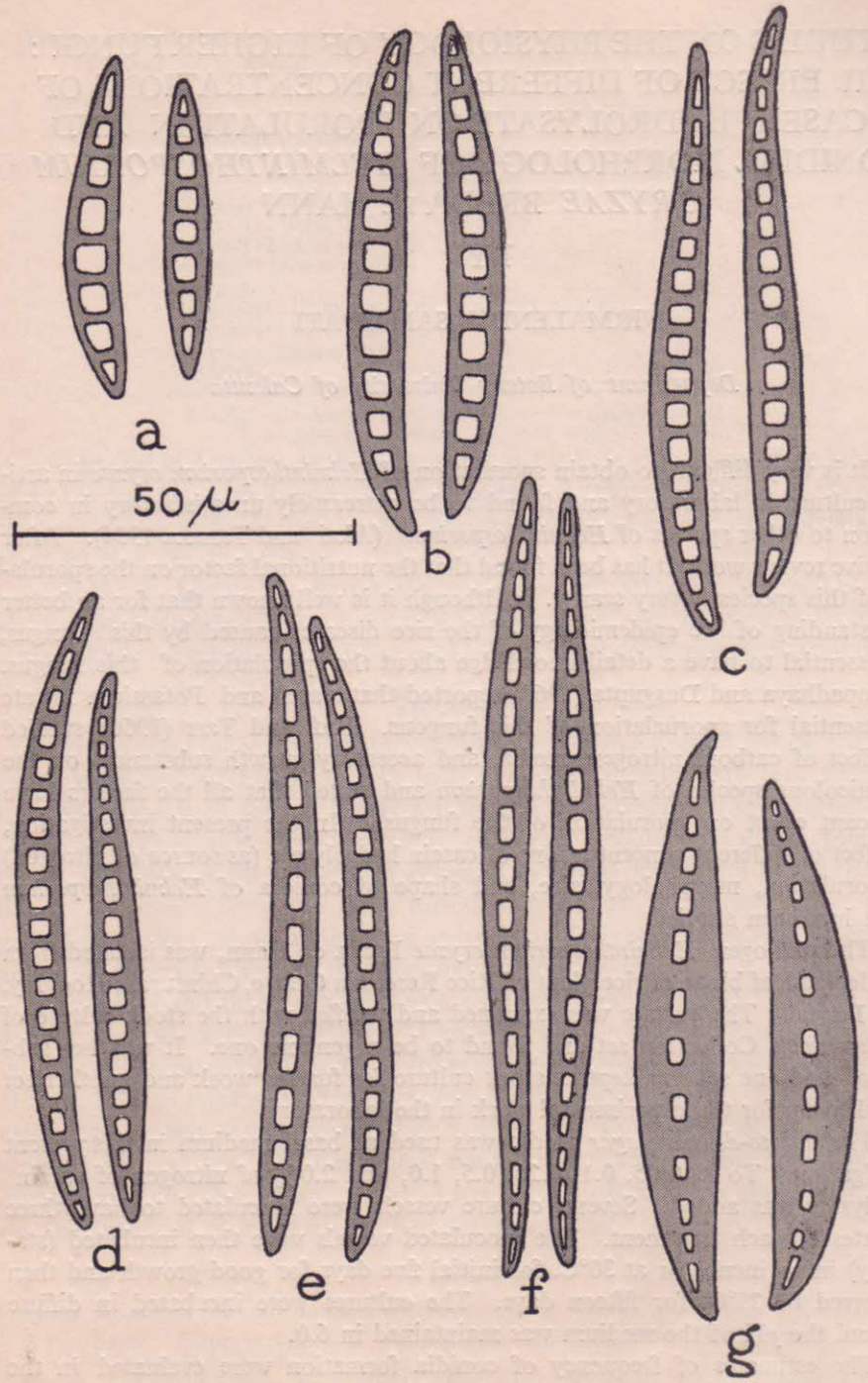
It is very difficult to obtain sporulation of *Helminthosporium oryzae* in artificial culture in laboratory and found to be extreamely unsatisfactory in comparision to other species of *Helminthosporium* (Akai and Tanaka 1954). After extensive review work, it has been found that the nutritional factor on the sporulation of this species is very scanty. Although it is well known that for an better understanding of the epidemiology of the rice diseases caused by this fungus, it is essential to have a detail knowledge about the sporulation of this fungus. Chattopadhaya and Dasgupta (1965) reported that starch and Potassium nitrate are essential for sporualation of this fungous. Kafi and Tarr (1966) studied the effect of carbon, nitrogen, media, and accessory growth substances on the graminicolous species of *Helminthosporium* and stated that all the factors have significant effect on sporulation of the fungus. In the present investigation, the effect of different concentrations of casein hydrolysate (as source of nitrogen) on sporulation, morphology, size, and shape of conidia of *Helminthosporium oryzae* has been studied.

The pathogen, *Helminthosporium oryzae* Breda de Hann, was isolated from the infected leaf blade of rice plant of Rice Research Cefitre, Chinsurah, Hooghly, West Bengal. The culture was examined and verified with the stock culture of Dr. Banerjee's Collection set and found to be a geniune one. It was then subcultured and one set was kept as stock culture for further work and another set was taken up for the experimental work in the laboratory.

The *potato-dextrose-agar* media was used as basal medium in this present investigation. To it, 0.05, 0.1, 0.25, 0.5, 1.0, and 2.0% of nitrogen of caesin hydrolysate was added. Several culture vessels were inoculated to have three replicates of each treatment. The inoculated vessels were then incubated (stationary) in an incubator at 30°C. for initial five days for good growth and then transferred to 25°C. for fifteen days. The cultures were incubated in diffuse light and the *p*H of the medium was maintained in 6.0.

The estimates of frequency of conidia formation were evaluated in the following procedure. The numbers of conidia were counted in each of five microscopic fields of 1 mm diameter using a Leitz objective (10X). The counts



TEXT-FIG. 1. Variation in from and size of conidia of *Helminthosporium oryzae* : a, control; b, 0·05%; c, 0·1%; d, 0·25%; e, 0·5%; f, 1·0%; g, 2·0%. of N/l.

were made from prepared slides from three zones (centre, margin, and middle portion) of the culture vessel. Each treatement were replicated three times.

The results obtained after the experimental period was given in Table 1 and TEXT-FIGURE 1. It has been found that the fungus differs widely in the behavior of conidial characterestic and sporulation against the different concentration of nitrogen.

Table 1. *Data showing the effect of different concentrations of caesin hydrolysate on length, width and septation of conidia and sporulation of* Helminthosporium oryzae.

Caesin hydro lysate (%N/1)	Conidia Length (μ)	Conidia Width (μ)	Septation	Sporulation (conidia/mm²)
0.0	49-98 (73.5)	12-17 (14.5)	5-9 (7)	3
0.05	41-128 (84.5)	9-16 (12.5)	4-13 (8.5)	8
0.1	40-141 (90.5)	8-16 (12)	4-15 (9.5)	14
0.25	37-178 (107.5)	10-18 (14)	4-17 (10.5)	20
0.5	35-210 (122.5)	10 18 (14)	4-17 (10.5)	28
1.0	31-234 (132.5)	10-18 (14)	4-17 (10.5)	39
2.0	38-140 (89)	9-20 (14·5)	3-14 (8.5)	17

Sporulation and conidial characteristic of *Helminthosporium oryzae* were significantly affected by the different concentrations of caesin hydrolysate as source of nitrogen. Shape, size, and septation of conidia were each affected to varying degrees. Length. septation, and sporulation increases directly with the increase in nitrogen concentration, but decreases to a considerable size at 2.0%/1. But in case of width the case is reverse. The width decreases directly with the increase of nitrogen upto 1.0%/1., but increases slightly again and maximum at 2.0%/1 of nitrogen (TEXT-FIGURE 1,a-f). The best sporulation was noticed at 1.0%/1 of nitrogen, followed by 0.5, 0.25, 2.0, 0.1 and 0.05%/1. In this present case, all the concentration of nitrogen studied, have stimulatory effect on sporulation. The length (31-234μ), septation (4-17) was maximum at 1.0%, but the width (9-20-μ) was at 2.0%. It has been found that the increase in thickness of wall of conidia also increases directly with the increase of available nitrogen sources and it is maximum at 2.0% of nitrogen, where the wall of conidia have almost completely replaced the cell lumen leaving a narrow central ramnant of the same.

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