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SOME FACTORS AFFECTING GROWTH, SPORULATION AND SPORE GERMINATION OF *HELMINTHOSPORIUM GRAMINEUM* AND DEVELOPMENT OF LEAE STRIPE OF BARLEY

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Optimum temperature for growth, sporulation and spore germination of *Helminthosporium gramineum,* the causal organism of leaf stripe of barley was 27°C. The fungus grew at a wide range of temperature (7°C - 37°C), but failed to sporulate and produce germ tubes at comparatively higher temperature. Maximum spread of lesion occurred at 27°C. Optimum relative humidities for growth and sporulation as well as spore germination were 75% and 100% respec'ively. Higher relative himidity favoured lesion development.

INTRODUCTION

Leaf stripe of barley, incited by *Helminthosporium graminenm* Rabh. (*Pyrenophora graminea* (Rabh.) Ito and Kuribay.), is one of the most serious and destructive diseases of the crop and occurs in nearly all the countries where barley is grown (Dickson, 1962). The casual fungus produces numerous conidia on lesions which serve as inocula for the secondary spread of the disease. Most of the barley varieties tested in India have been found to be moderately to highly susceptible to leaf stripe disease (Mohammad and Mahmood, 1973 ; Mondal, 1982) Since very little work has been done on the epidemiology of this disease in India it was considered worth while to study the effect of two environmental factors, viz., temperature and humidity, on the growth, sporulation and spore germination of *H. gramineum*, as well as lesion development on barley leaves.

MATERIALS AND METHODS

Leaf stripe affected barley leaves were collected from the field of the University Research Farm at Kalyani and *Helminthosporium gramineum* was isolated by plating surface sterilized infected leaf pieces on potato dextrose agar supplemented with 2% barley leaf extract (PDE), which also supported good sporulation (Singh and Sen Gupta, 1979). Single spore isolations were made from these cultures and kept at 4°C for future use.

Growth and intensity of sporulation were studied in Petri dishes (9 cm diameter) containing PDE which were inoculated with 6 mm agar discs containing mycelia of the fungus. For maintaining different humidities humidity chambers were prepared in sterilized Petri dishes (12 cm) by adding 13 ml solutions of different concentrations of sulphuric acid (McLean and Cook, 1965). The inoculated Petri dishes were placed aseptically in different humidity chambers. In all cases three replications were used for each treatment, irrespective of temperature and humidity and the growth was measured after 6 days of incubation. Usually 5 ml of distilled water was used in each Petri dish for dislodging spores from mycelia. The spore suspension, was strained through muslin and the concentration of spores was calculated by haemocytometer counts.

For studying sporulation on the infected leaves, leaf pieces (about 6 cm long) each containing 4-5 lesions, were washed in sterile distilled water to eliminate spores if any, and kept in moist chambers at desired temperature. For studying the effect of humidity, the leaf pieces were kept in different humidity chambers for 48 h at 25°C. The spores on the leaf lesions were separated with a hair brash in 5 ml distilled sterilized water and concentration of spores was calculated as described. Six replications (leaf pieces) were taken for each treatment.

Conidial germination was studied by placing a drop of spore suspension in sterile tap water (10^6 spores/ml) in each groove of double grooved cavity slides. The slides were placed in moist chambers and incubated at desired temperatures and humidities. After 20 h, percentage of spore germination was counted under a microscope.

To study the effect of temperature and humidity on lesion development, leaf pieces (4 cm long) each containing 1 lesion were taken and the length and breadth of each lesion were measured. The leaf pieces were then kept in moist chambers for 48 h. At the end of incubation period length and breadth of each lesion were noted again and the percentage spread of lesion was calculated.

RESULTS AND DISCUSSION

Temperature and humidity greatly affect the physiological processes of a fungus. Studies on the physiology of *H. gramineum* showed that the fungus grew at a wide range (7°C-37°C) of temperature (Table 1). Trend of sporulation was similar both in the culture and on infected leaf lesions at different temperatures. Although the optimum temperature for mycelial growth, sporulation and spore germination was similar the maximum temperature varied in these cases. Maximum spread of lesion occurred at a comparatively higher temperature (27°C-32°C). For sporulation and spore germination higher relative humidity (R.H. 75% and above) was

required (Table 2). It was noted that maximum growth occurred at 75% R.H., above which growth was reduced to some extent. Thus a high relative humidity appears to favour initial infection, establishment of the disease and spread of lesions. Spread of leaf stripe of barley due to irrigation or high humidity has also been demonstrated by Metz and Scharen (1979).

Table 1. *Effect of temperature on growth, sporulation and spore germination of* H. gramineum *and leaf stripe lesion expansion*

Temperature (°C)	Radial growth [a] (mm)	No. of spores (X10⁶/ml) In culture[b]	No. of spores (X10⁶/ml) On leaf lesion [c]	Germination (%)	Germ tube length (μm)	Spread of lesion(%) Length	Spread of lesion(%) Breadth
7	32.33	0.11	0.00	23.15	47.23	0 00	0.00
17	69.66	0.45	0.05	45.05	113.24	48.78	11.95
22	78.05	2.20	0.07	59.52	167 68	81.76	26.23
27	93.55	7.50	0.14	82.03	227.59	120.00	57.61
32	91.00	0.00	0.00	26.43	57.91	138.25	64.46
37	39.21	0.00	0.00	0.00	0.00	72.99	19.58

a & b After 6 days

c After 48 h

Table 2. *Effect of humidity on growth, sporulation and spore germination of* H. gramineum *and leaf stripe lesion expansion*

Relative humidity (%)	Radial growth[a] (mm)	No. of spores (X10⁶/ml) In culture[b]	No. of spores (X10⁶/ml) On leaf lesion[c]	Germination%	Germ Tube length(μm)	Spread of lesion(%) Length	Spread of lesion(%) Breadth
35	31.70	0.00	0.00	0.00	0.00	2.38	1.75
50	66.24	0.00	0.00	0.00	0.00	7.95	2.38
65	81.00	0.00	0.00	0.00	0.00	20.00	3.37
75	85.05	0.80	0.04	24.52	55.00	27.50	10.83
90	83.20	5.30	0.10	62.30	121.30	85.25	39.13
100	71.53	7.40	0.14	79.23	124.00	120 00	57.61

a & b After 6 days [c]After 48 h

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