J. Mycopathol. Res.35(2) :131-136 (1997) Printed in India

Effect of physico-chemical factors on growth of *Drechslera oryzae*

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Effect of temperature, pH, culture media and fungicides has been studied on growth and sporulation of *Drechslera oryzae* which is a causal agent of brown spot disease of rice. The optimum temperature for growth and sporulation was 30° and 25°C, respectively. The best suited pH for radial growth and sporulation was pH 6. The corn meal agar medium supported the best growth and sporulation of the pathogen followed by potato dextrose agar and rice leaves agar. Out of eight fungicides screened *against D. oryzae,* Dithane M-45 showed the maximum inhibitory effect on growth of the pathogen followed by Thiram, Shield and Foltaf.

Key words : Culture media, *Drechslera oryzae,* fungicides, pH, temperature

INTRODUCTION

Development of plant diseases is largely dependent on physico-chemical factors such as temperature, pH and nutrients. The role of pH and nutrients affecting growth of the pathogen, has been reported by various workers (Mishra and Chatterjee 1963; Mistra and Mukherjee, 1965; Singh, 1980). Temperature is one of the factors which limits development of brown spot disease of rice (Mishra and Mukherjee, 1965). In this report the optimum temperature, pH and most suitable culture medium have been recorded for best growth and sporulation of *Drechslera oryzae* the incitant of Brown spot of rice. An attempt has also been made to test the efficacy of some of the potent fungicides which may be used either alone or in an integrated manner to control the disease.

MATERIALS AND METHODS

The effect of temperature on growth and sporulation of pathogen was observed at 15°, 20°, 25°, 30°, 35° and 40°C. A 5 mm diameter of agar block was cut from the margin of actively growing culture of the test pathogen with the help of a sterilized cork borer. The agar block was placed centrally in Petridish of 9 cm diameter containing previously sterilized and solidified 15 ml PDA medium in triplicate and the plates were incubated at the above said temperatures on BOD incubator. After 7 days of incubation the radial growth of the test pathogen was recorded by measuring the growth of the radii from the back side of the Petri dishes.

For the measurement of sporulation 5 mm diameter mycelial mat of the test pathogen was cut with the help of a steilized cork borer randomly at four different places from 10 days old culture and was macerated in 5 ml sterilized distilled water. An aliquot 0.1 ml suspension was placed on a clean glass slide and the number of conidia were counted per microscopic field under low power of the microscope.

Five, 4.0, 5.0, 6.0, 7.0 and 8.0 pH levels were adjusted in PDA medium separately with the help of dilute phosphoric acid and NaOH in different Erlenmeyer flasks. These flasks were autoclaved at 15 lb pressure for 20 min. The medium after cooling was poured in Petridishes in aseptic condition

and was allowed to solidify. The method of inoculatin of Petriplates has been described. After 7 days the radial growth and sporulation were recorded by the methods described earlier.

Potato dextrose agar, Czapek-Dox agar, and Corn meal agar media were prepared according to their standard formulae. For leaf extract medium, 200 g fresh leaves were collected from healthy plants of rice from experimental plots and were brought into laboratory. The leaves were washed thoroughly and were air dried for 4 h. Then the leaves were cut into small pieces and steamed in 500 ml sterilized distilled water for 15 min at 15 lb pressure. When cooled, the steamed leaf pieces and water were filtered through muslin cloth. This decoction was added to 17 g agar in another 500 ml sterilized disitilled water and the volume was made up to 1 litre. The pH of the medium was adjusted to 6.5 and the medium was autoclaved at 15 lb pressure for 20 min.

For rice straw agar medium the straws were cut into small pieces and soaked in sterilized distilled water for 12 h and were then boiled in the same water for 4 h. It was filtered through muslin cloth and was added with 17g agar and the volume was made unto 1 litre. The pH was adjusted to 6.5. This medium was autoclaved at 15 lb pressure for 20 min.

The above autoclaved media were poured into Petridishes in triplicate and inoculated as described earlier. After 7 days the radial growth and sporulation were measured. The per cent sporulation in each experiment was calculated by the formula given below :

$$\text{Percent sporulation} = \frac{\text{Average no. of conidia in treated plates}}{\text{Average no. of conidia in control plates}} \times 100$$

Eight fungicides viz., Agrosan GN, Blue Copper, Dithane M-45, Foltaf, Jkstein, Sheild, Sulphur 80 and Thiram were tested against radial growth of *D. oryzae* in laboratory by poisoned food technique (Flack, 1907). Twety five, 50, 100, 200, and 400 ppm concentrations of each fungicide were prepared in double strength on active ingredient basis in sterilized water under aseptic condition. Double strength PDA medium was also prepared in sterilized distilled water and autoclaved at 15 lb pressure for 20 min. Ten ml of each concentration was added to 10 ml of double strength PDA medium in a sterilized Pertidish and was mixed thoroughly and then was allowed to solidify. Each Petridish containing 20 ml of treated solid medium was inoculated with growing culture of *D. oryzae* as described earlier. Control was maintained in Petridishes containing 10 ml PDA + 10 ml sterilized distilled water. Experiment was performed in triplicate for each concentration and control. The plates were incubated at 25±1°C. Measurement of radial growth was done on 2nd, 4th and 7th day of inoculation. The per cent reduction in radial growth was calculated by the formula described previously.

RESULTS AND DISCUSSION

The optimum temperature for maximum growth and sporulation was 30° and 25°C whereas the minimum was 15° and 40°C respectively (Table 1A). Similar results were obtained by other workers also with regard to growth (Nishikado, 1929; Matsumra, 1927; Mukherjee, 1960) but not for sporulation. Mishra and Mukherjee (1965) noted that sporulation in *D. oryzae* is dependent on temperature (21°-26°C) and on optimum level of C : N ratio.

The optimum radial growth and sporulation occurred at pH 6 followed by pH 5. An inhibitory effect was seen at pH 4 and 8. Lilly and Barnett (1961) have mentioned that initial pH for optimal growth of majority of fungi is 5 to 6. The same pH range has been observed for best radial growth of the test pathogen by others also(Stevens, 1922; Nishikado, 1927; Matsumura, 1927, Mukherjee, 1960;

Table 1A. Effect of temperature on growth and sporulation of *Drechslera oryzae*

Temperature (°C)	Radial growth (mm)	Sporulation (%)
15	45	48
20	78	76
25	88	86
30	90	46
35	81	37
40	70	14

Mishra and Chatterjee, 1963). Maximum sporulation of *D. oryzae* was recorded at pH 6.0 followed by pH 5.0 (Table 1B). In nature, different races of *D. oryzae* are present which may differ in physiological process. Nishikado (1927) compared the American and Japanese strains of *D. oryzae* with respect to their morphology, physiology and pathogenicity and considered them as two distinct morphological forms. Tochinai and Sakamoto (1937) also recorded physiological specialization in

Table 1B. Effect of pH on radial growth and sporulation of *Drechslear oryzae*

pH	Radial growth (mm)	Sporulation (%)
4	56	35
5	80	86
6	89	91
7	79	66
8	42	21

D. oryzae on the basis of morphological and cultural characteristics and its pathogenicity. Hingorani and Prasad (1951) recorded small spored *Drechslera oryzae* along with normal spored *D. oryzae*. The former was referred to be a new variety of the latter.

The optimum radial growth of *D. oryzae* occurred on corn meal agar medium (90 mm) followed by patato dextrose agar and rice leaves agar media. However, the maximum sporulation was observed on patato dextrose agar medium (86%). Czapek-Dox agar medium was found to be the least suitable medium for both growth and sporulation (Table 1C). Stevens (1927) noted that linear growth and spore production were independent of each other and colonies with slow linear growth were high spore producing or vice versa. Singh (1978) reported that natural and semisynthetic media were more favourable for growth of *D. graminea* than synthetic ones.

Table 1C. Effect of various growth media on radial growth and sporulation of *Drechslera oryzae*

Growth media + agar	Radial growth (mm)	Sporulation (%)
Patato dextrose	89	82
Czapex-Dox	79	70
Corn meal	90	86
Rice straw	86	75
Rice leaf-extract	89	73

Table 1D. Effect of fungicides on per cent reduction/enhancement in radial growth of *Drechslera oryzae*

Name of the fungicides	Days of observation														
	2nd					4th					7th				
	25 ppm	50 ppm	100 ppm	200 ppm	400 ppm	25 ppm	50 ppm	100 ppm	200 ppm	400 ppm	25 ppm	50 ppm	100 ppm	200 ppm	400 ppm
Agrosan GN	-70	-60	-47	0	0	-16	-46	-21	0	0	-4	-9	0	0	0
Blue copper	43	55	60	72	91	25	49	55	68	82	16	45	53	64	73
Dithane M-45	60	100	100	100	100	75	100	100	100	100	77	100	100	100	100
Foltaf	83	84	85	85	100	88	89	89	90	100	90	91	91	93	100
Jkstein	50	60	70	70	85	43	44	61	69	72	28	44	44	52	70
Sheild	75	83	54	84	100	86	88	89	92	100	89	90	90	95	100
Sulphur 80	3	19	20	23	100	2	12	17	19	88	1	8	14	15	65
Thiram	71	92	100	100	100	56	90	100	100	100	53	90	100	100	100

- = Per cent enhancement of radial growth

However, Lilly and Barnett (1961) observed that natural and most semisynthetic media were of limited usefulness to study nutrition of fungi as their chemical composition is complex and not fully known. At the same time they cannot be reproduced exactly. Tewari and Premlathadath (1984) have reported that leaf extract medium of *Oryza sativa* encouraged the growth and sporulation of *D. oryzae*. Stevens (1922) and Mishra and Chatterjee (1963) have noted the best growth of the pathogen on patato dextroze agar and glucose peptone agar but poor growth on host extract agar.

Abundant production of conidia by *D. aryzae* was noted on potato dextrose agar by Hau and Rush (1980). Its sporulation was recorded maximum by Mishra and Mukherjee (1965) in media containing sucrose or glucose but it was completely inhibited in maltose medium containing high nitrogen (KNO_3). Singh (1978) found patato dextrose agar and Martin's medium as suitable ones for maximum sporulation of *Alternaria brassicae*. Tewari and Premiathadath (1984) reported that the best medium for good sporulation was rice leaves extract agar medium.

Dithane M-45 was found to be most effective in inhibiting the radial growth of the pathogen (Table ID). It was already reported as effective by Dharamvir *et al.* (1970) against seed borne infection by *Drechslera* spp. The inhibitory effect of Dithane M-45 and Thiram was reported by Sattar and Hussain (1982). The inhibitory effect of some fungicides including the former on the growth behaviour of *Aspergillus niger, A, flavus, Fusarium moniliforme* and *F. poae* was also reported by Agrawal (1978). Inhibitory effect of Dithane M-45, Blue copper, Bavistin, Mancojeb, MEMC, Thiram, Foltaf were reported against *Fusarium oxysporum* f. sp. *ciceri* by Bashar (1990).

An enhanced growth of the pathogen was recorded in case of the present study due to Agrosan GN. The possible cause of this result could be their degradation by the test pathogen. Although Agrosan GN is a recommended fungicide for seed dressing against brown spot disease of rice, In the present experiment the fungicide showed an unusual favourable effect on growth of the pathogen. More careful and thorough study is required to confirm the result obtained with regard to Agrosan GN.

ACKNOWLEDGEMENTS

The authors are thankful to the Head of the Department of Botany, Banaras Hindu University for laboratory facilities. Vinay Kumari is thankful to the CSIR, New Delhi, for financial support in the form of a Junior Research Fellowship,.

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(Accepted for publication 22 May 1997)