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EFFECT OF DIFFERENT SOURCES OF CARBON AND NITROGEN AND TISSUE MEDIA ON THE CULTURAL CHARACTERS AND PATHOGENICITY OF *HELMINTHOSPORIUM ORYZAE* BREDADE HAAN*

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Growth and virulence of fungal organisms are known to be influenced by the nutrient medium on which they are grown. Tanaka (1956), Misra and Mukherjee (1962) and Chattopadhyay and Das Gupta (1965) studied the effect of nutrition on growth and sporulation of *H. oryzae.* The present studies were undertaken to determine the effects of different sources of carbon and nitrogen and different tissue media on the cultural characteristics and pathogenicity of *H. oryzae.*

Material and Methods

H. *oryzae* was isolated from infected leaves and grains of paddy and it was purified by single spore isolations and maintained on potato-dextrose agar medium. Sabouraud's agar containing 9·416 g of glucose, 2'35 g of peptone, 15 g agar agar and 1000 ml of distilled water was used as the basal medium. Sucrose, maltose, lactose, fructose and starch were substituted for glucose as the different sources of carbon. Asparagine, urea, potassium nitrate, sodium nitrate, ammonium nitrate and ammonium sulphate were substituted in place of peptone to provide the different sources of nitrogon. The quantities of carbohydrate and nitrogenous compounds were adjusted so as to give 0'5 per cent carbon and 0'04 per cent nitrogen. The pH of the medium was adjusted to 6'0. The medium was dispensed at 15 ml each in test tubes and sterilized at 15 lb pressure for 20 minutes. The two types of tissue media tested were those containing grain and leaf extracts. They were prepared from 60 g of whole grain (powdered) and 200 g fresh leaves respectively with distilled water. The clear extract was decanted, filtered and made up to 1000 ml and 1·5 per cent agar agar was used in each case.

Pathogenicity of the organism cultured on different media was tested on four varieties of rice, viz., PTB 23, PTB 26, PTB 31 and Kochuvithu. Spore suspension was prepared from 20 days old cultures and 5 ml each of the inoculum was sprayed with an atomiser on four seedlings raised in pots. Control seedlings were sprayed with sterile distilled water. The inoculated seedlings were covered with alkathene bags for 36 hours to maintain humidity.

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The results were assessed in terms of cultural characters, growth and sporulation and pathogenicity (based on the number of spots developing on the inoculated plants) in respect of the organism raised on the different media.

Results

Cultural characters. The colony characters of the fungus grown on different media are given in Table 1.

The initial growth of the fungus was white with loose, raised mycelium. The colour gradually changed to greenish grey, dark olive green and finally black. The fungus growth was profuse on all media which contained different carbon sources while it was loose and thin on media with various nitrogen sources except peptone. Both the tissue media supported good growth of the fungus, but the growth was better on grain extract medium. The growth was slow, thin and non-spreading on media with ammonium salts as nitrogen sources.

Radial growth. Radial growth of the fungus on different media recorded on the sixth day is shown in Fig. 1. The growth was quick on host extract agar media covering the petri dishes fully on the sixth day. The rate of growth was slow on synthetic media and it was poor on media with urea, potassium nitrate, sodium nitrate and ammonium salts used as nitrogen sources.

Sporulation and size of spores. Observations made on sporulation and size of spores are represented in Table 2. Sporulation was observed on the fourth day on glucose-peptone agar; it was late on the other media and spores were observed on all cultures after fifteen days.

Sporulation was good on media containing host extract, glucose-peptone, sucrose-peptone and maltose-peptone. It was less on all other media with the different carbon sources. Average sporulation was observed on all media with the various nitrogen sources except on those containing ammonium salts on which the sporulation was sparse and poor.

As regards the spore size (measured from 20 days old culture) the conidia produced on host extract agar were bigger than those formed on all the synthetic media. The spores of the host extract media measured $55{\cdot}4\mu$ x 13.5μ and $62{\cdot}2\mu$ X $13{\cdot}4\mu$ for the grain and leaf extracts respectively. The average spore size varied from $50{\cdot}4\mu \times 12{\cdot}9\mu$ to $54{\cdot}3\mu \times 13{\cdot}2\mu$ on all synthetic media containing the different sources of carbon. The conidia found on media with different nitrogen sources, except that containing peptone, were small, the spores measuring from $47{\cdot}4\mu$ X $11{\cdot}9\mu$ to $50{\cdot}0\mu$ X $12{\cdot}6\mu$.

Pathogenicity. Results are presented in Table 3. It may be seen that the maximum number of leaf spots was produced by the organism grown on grain extract agar medium followed by that grown on leaf extract agar medium,

Table 3

Leaf spot counts on different varieties of paddy caused by *H. oryzae* cultured on different media

Media	Number of leaf spots on different varieties of paddy				Total
	Kochu-vithu	PTB 23	PTB 26	PTB 31	
Glucose-peptone	33	24	23	30	110
Sucrose-peptone	27	28	23	36	114
Maltose-peptone	16	32	16	27	91
Lactose-peptone	12	29	16	11	68
Fructose-peptone	12	25	25	20	82
Starch-peptone	23	30	35	28	116
Glucose-asparagine	10	12	8	22	52
Glucose-urea	8	11	10	9	38
Glucose-potassium nitrate	30	33	36	19	118
Glucose-sodium nitrate	23	20	32	26	101
Glucose-ammonium nitrate	16	18	17	33	84
Glucose-ammonium sulphate	11	9	7	17	44
Grain extract	54	139	292	247	732*
Leaf extract	38	47	101	140	326*
Total	313	457	641	665	2076

* Critical difference 36·63

and maltose-peptone agar media, Sporulation also was poor when ammonium salts were used as nitrogen sources.

The pathogen grown on grain and leaf extract agar media was significantly more virulent. No significant variation in virulence was observed when the fungus was cultured on media provided with different sources of carbon and nitrogen.

H oryzae. Good growth was observed when the fungus was cultured on media containing the different forms of carbon and starch was the best form favouring profuse and dense growth (Fig. 1), followed in the descending order by maltose, glucose, sucrose and fructose. Tanaka (1956) reported good growth on medium containing maltose, while Misra and Mukherjee (1962) obtained maximum growth on mannitol, sucrose and glucose. The abundant growth of the fungus on host extract agar media observed in the present studies was contrary to the observations of Misra and Ghatterjee (1963), who reported poor growth on host extract media. This disparity may be due to the variation in the isolates of the organism.

Peptone and asparagine were observed as the best sources of nitrogen for the growth of the organism and ammonium salts as very poor. Misra and Mukherjee (1962) reported good growth on peptone containing medium, while Subramanian (1967) on a comparative evaluation of the different species of *Drechslera,* recorded asparagine as the best source of nitrogen for growth.

Sporulation was best on host extract agar media as previously observed by Chattopadhyay and Das Gupta (1965). Of the carbon sources, glucose, sucrose and maltose were observed to be good for sporulation whereas Misra and Mukherjee (1962) recorded no sporulation on medium containing maltose and Chattopadhyay and Das Gupta (1965) reported glucose as a poor source of carbohydrate for conidial production. Among the nitrogen sources, peptone was the best for sporulation while ammonium salts were poor. However, the sporulation was not inhibited in any case as reported by Misra and Mukherjee (1962). Ammonium salts were earlier reported to be poor sources of nitrogen for conidial production (Chattopadhyay and Das Gupta 1965).

By culturing H. *oryzae* on rice grain and leaf extract agar media its virulence increased significantly more than on all the synthetic media and the increased virulence of the organism was significantly more after culturing on grain extract medium. This increase in pathogenicity could be attributed to the availability of some essential substance present in the host extract but absent in the synthetic media. White and Mc Intyre (1943) observed similar variation in the pathogenicity of *Ophiobolus graminis.*

Summary

The cultural characters of H. *oryzae* Breda de Haan were studied on 14 media ; 12 synthetic, with different sources of carbon and nitrogen and two host extract agar media. Profuse growth was observed on host extract media. The fungus growth was good on the various media containing different carbohydrates. Good mycelial growth was noted when peptone was used as nitrogen source ; while it was poor in media containing ammonium salts. Good sporulation occured on *host* extract, glucose-peptone, sucrose-peptone

Table 2

Sporulation and size of spores of conidia of *H. oryzae* cultured on different media

Media	Spore length in microns			Spore breadth in microns	Sporulation			
	Maximum	Minimum	Average					
Glucose-peptone	77·8	26·1	54·3	13·2	+	+	+	+
Sucrose-peptone	64·8	25·9	52·1	13·1	+	+	+	+
Maltose-peptone	71·3	32·3	51·8	12·9	+	+	+	+
Lactose-peptone	77·8	32·3	51·2	13·1	+	+	+	
Fructose-peptone	71·3	32·3	50·3	13·0	+	+	+	
Starch-peptone	71·8	38·9	52·9	13·0	+	+	+	
Glucose-asparagine	71·8	26·0	47·4	12·6	+	+		
Glucose-urea	64·8	26·0	48·5	12·4	+	+		
Glucose-potassium nitrate	64·8	22·9	47·4	11·9	+	+		
Glucose-sodium nitrate	71·3	20·8	49·0	12·2	+	+		
Glucose-ammonium nitrate	71·3	25·9	50·0	12·2	+			
Glucose-ammonium sulphate	64·6	19·4	48·1	11·9	+			
Grain extract	71·3	25·9	55·4	13·5	+	+	+	+
Leaf extract	77·8	32·4	62·2	13·4	+	+	+	+

++++ Good, +++ Satisfactory, ++ Average, + Poor.

the number of spots being 732 and 326 respectively. The pathogenicity of the organism varied when cultured on synthetic media containing different carbon and nitrogen sources but the variation in virulence was not significant. The data also revealed that there was no significant difference in the susceptibility of the varieties of paddy to the fungus. The interaction between the different media on which the fungus was grown and the varieties of paddy was also found to be statistically insignificant.

Discussion

The results presented show that variations in the sources of carbon and nitrogen in the culture media have influenced the growth and morphology of

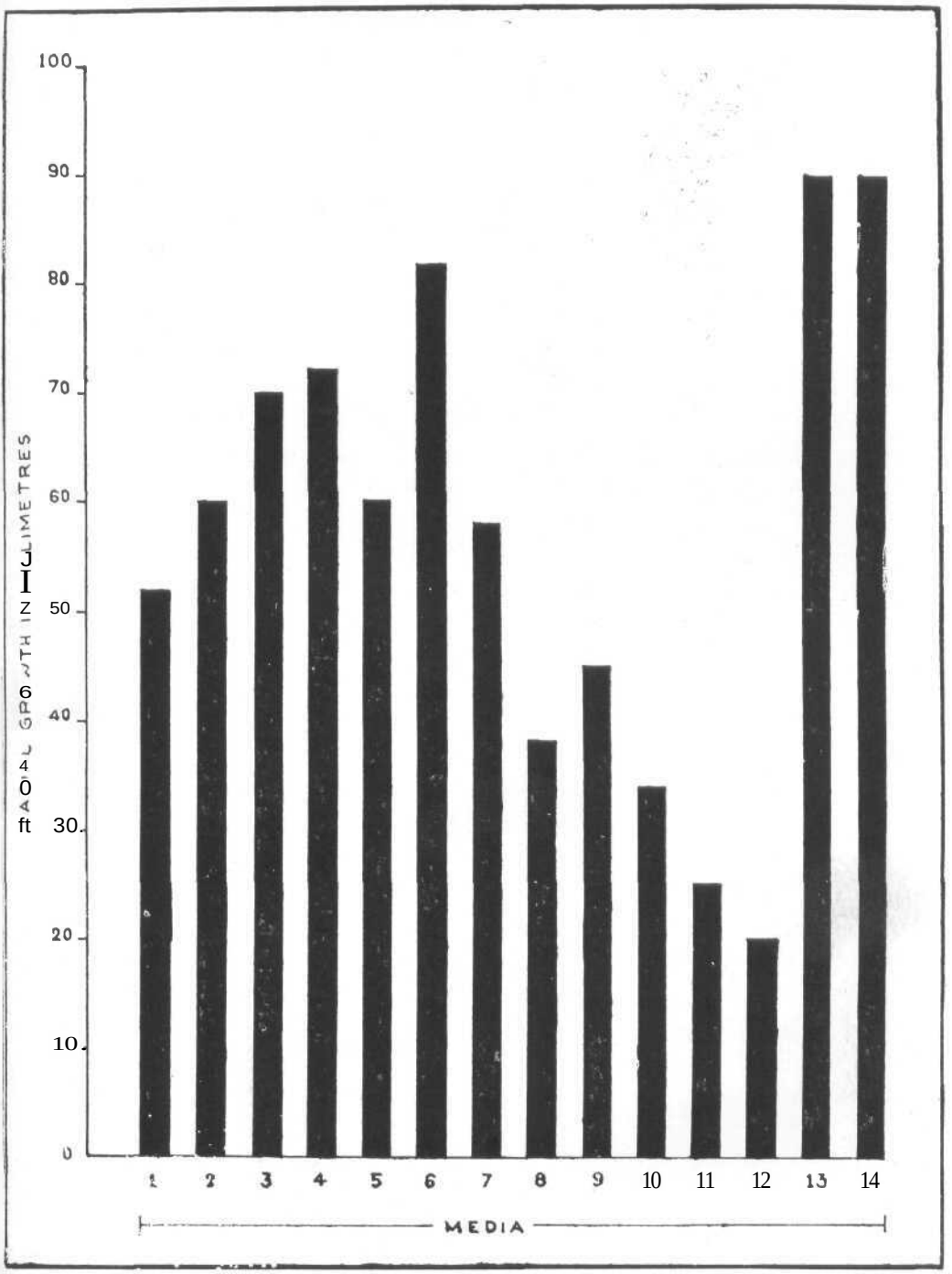

Fig. I. Radial growth of H. *oryzae* on different media.

1. Glucose-peptone
2. Sucrose-peptone
3. Maltose-peptone
4. Lactose-peptone
5. Fructose-peptone
6. Starch-peptone
7. Glucose-asparagine
8. Glucose-urea
9. Glucose-potassium nitrate
10. Glucose-sodium nitrate
11. Glucose-ammonium nitrate
12. Glucose-ammonium sulphate
13. Grain extract
14. Leaf extract

Table I

Colony characters of *H. oryzae* grown on different media

Glucose-peptone agar	Dark olive green, profuse and compact, aerial mycelium later turning black. Light coloured, irregular margins. Concentric zonations.
Sucrose-peptone agar	Moderate to dark olive green mycelium with inconspicuous zonations. Uneven, dark coloured and fringed border.
Maltose -peptone agar	Black green abundant mycelium. Uniformly thick growth with light coloured periphery.
Lactose-peptone agar	Black green, abundant mycelium, uniformly thick growth with light coloured periphery.
Fructose-peptone agar	Dark olive green mycelium. Uneven border, fringed in appearence.
Starch-peptone agar	Dark olive green, thick, compact and profuse mycelial growth with a few concentric zonations. Regular periphery.
Glucose-asparagine agar	Greenish grey, thin, aerial mycelium with 1 or 2 concentric zonations in the centre. Irregular and fringed border.
Glucose-urea agar	Light to dark green, thin aerial mycelium with irregular light coloured and fringed border.
Glucose-potassium nitrate agar	Olive green, thin, aerial mycelium with one or two concentric zonations close to the centre. Irregular and fringed periphery.
Glucose-sodium nitrate agar	Olive green, thin, aerial mycelium with irregular, fringed periphery.
Glucose-ammonium nitrate agar	Greenish, slow growing, thin aerial macelium, light coloured, wavy border.
Glucose-ammonium sulphate agar	Greenish, slow growing, thin aerial mycelium, Light coloured, wavy and fringed border.
Grain extract agar	Dark green, fluffy, abundant and compact aerial mycelium, later turning to black. Four to five concentric zonations. Regular periphery.
Leaf extract agar	Olive green to grey, fluffy, raised, abundant mycelium with concentric zonations. Light coloured, regular and fringed periphery.

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References

Chattopadhyay, S. B. and Das Gupta, C. 1965. Factors affecting conidial production of *H. oryzae. Indian Phytopath. 18:* 160-167

Misra, A. P. and Chatterjee, A. K. 1963. Comparative study of two isolates of H. *oryzae* Breda de Haan. *Indian Phytopath. 16:* 275-281

Misra, A. P. and Mukherjee, A. K. 1962. Effect of carbon and nitrogen nutrition on growth and sporulation of *H. oryzae. Indian Phytopath. 15:* 211-215

Subramanian, C. V. 1967. Nitrogen utilization by some plant pathogenic fungi. *Nat. Inst. Sci. India, Bull. No. 35:* 11-21

Tanaka, H. 1956. On the influence of carbon sources upon growth of *Cochliobolus miyabeanus. Forsch. Pflkr. Kyoto, 5:* 165-170

White, N. H. and Mc Intyre, G. A. 1943. The pathogenicity of single isolates of *Ophiobolus graminis* under field conditions. J. *Coun. Sci. Ind. Res. 16:* 98-94

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