NUTRITIONAL STUDIES ON COLLETOTRICHUM GLOEOSPORIOIDES PENZ.

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Colletotrichum gloeosporioides, the cause of the "leaf spot" disease of *Polyscias balfuriana* has a world wide distribution. Its attack on various plants has been described from time to time (BURGER 1924, MITTER & TANDON 1930, CHAUDHURI & SINGH 1930, FAWCETT 1936, RAMSEY 1943, CHOWDHURY 1947, KHAN 1959, MALLIK & HASAN 1959, PRASAD & SINGH 1960, TANDON & CHANDRA 1962 and TANDON & VERMA 1962).

So far *Colletotrichum gloeosporioides* has not been isolated from *Polyscias balfuriana*. Moreover considerable variations in the behaviour of various isolates have also been reported. In considering all these facts it was decided to carry out a detailed physiological study of this organism. The paper deals with the effect of the different pH, carbon, nitrogen and sulphur sources on the growth and sporulation of this organism.

MATERIAL AND METHODS

The fungus was isolated from diseased leaves of *Polyscias balfuriana*. A single spore isolate obtained from this culture was employed for the present study. The methods were quite similar to those of TANDON & CHATURVEDI (1963). The effect of pH on growth and sporulation was studied by adjusting the pH of the basal medium from 2.0 to 9.0 with the help of Beckman's pH meter by adding 0.1 N HCl or NaOH. In order to study the effect of various carbon, nitrogen or sulphur compounds, they were substituted singly to the basal medium by replacing the original corresponding compound i.e., glucose, KNO_3 or $MgSO_4 \cdot 7H_2O$ respectively. The amount of the substance was so adjusted so as to contain the same quantity of carbon, nitrogen or sulphur which was present in the basal medium. The amount of peptone (used in experiment dealing with nitrogen requirements) was similar to that of KNO_3 used in basal medium.

OBSERVATIONS

Effect of pH

The growth and sporulation at different pH are recorded in table I.

TABLE I

Growth and sporulation of C. gloeosporioides on different pH values

No.	pH of the medium	Dry wt. of mycelium mg	Sporulation
1	2.0	—	—
2	3.0	20.4	Poor
3	4.0	37.4	Good
4	4.5	43.9	,,
5	5.0	51.7	Excellent
6	5.5	64.2	,,
7	6.0	59.2	,,
8	6.5	52.7	,,
9	7.0	44.5	Good
10	7.5	39.7	Fair
11	8.0	36.7	,,
12	9.0	24.9	Poor

Critical difference at 5 % level = 2.5
Standard error = 0.88

Growth was observed from pH at 3.0. to 9.0. However, it failed to grow at pH 2.0. The best growth was at pH 5.5. Excellent sporulation was observed at pH 5.0. to 6.5. It was good at pH 4.0, 4.5 and 7.0. Fair sporulation was recorded at 7.5 and 8.0 while it was poor on pH 3.0 and 9.0.

Carbon sources

The growth and sporulation on various carbon sources (Table II) indicate that *C. gloeosporioides* could utilize carbon from several carbon compounds. Pentoses were not satisfactory sources of carbon except arabinose which supported good growth. Leaving galactose (poor carbon source) all the other hexoses induced good growth. Disaccharides, trisaccharide and polysaccharides (except inulin) were found to be satisfactory sources. Alcohols supported good growth. The pathogen gave poor response on organic acids.

In some cases there was a close correlation between growth and sporulation. Sources which were good for growth (glucose, fructose, maltose and starch) were also good or excellent sources for sporulation. Similarly poor sources (rhamnose and tartaric acid) induced poor sporulation only. In certain other cases, however, good carbon sources (mannose, sucrose and glycerol) produced poor sporulation and vice versa (inulin and xylose). On rest of

the compounds, however, such correlations were not possible. The present organism failed to develop any spores on malic acid.

TABLE II

Growth and sporulation of C. gloeosporioides on different carbon sources

No.	Source of carbon	Dry wt. of mycelium mg	Sporulation
1	Arabinose	61.5	Few spores
2	Xylose	40.8	Excellent
3	Rhamnose	35.3	Poor
4	Glucose	64.8	Excellent
5	Mannose	71.6	Poor
6	Fructose	65.8	Good
7	Galactose	40.8	Few spores
8	Sucrose	73.1	Poor
9	Maltose	56.4	Excellent
10	Lactose	52.0	Poor
11	Raffinose	62.9	Fair
12	Inulin	31.6	Good
13	Dextrin	53.3	Excellent
14	Starch	59.0	Good
15	Sorbitol	56.8	Few spores
16	Dulcitol	62.2	,,
17	Mannitol	86.2	,,
18	Glycerol	59.5	Poor
19	Tartaric acid	33.2	Poor
20	Malic acid	39.2	
21	No Carbon	0.0	—

Nitrogen sources

It is clear from table III that *C. gloeosporioides* was also capable of assimilating nitrogen from a large number of sources. The fungus could not grow on nitrites of potassium or sodium or media lacking any nitrogen. Aspartic acid was the best source and was followed by glutamic acid, peptone, Mg $(NO_3)_2$, KNO_3, leucine, arginine, glycine, $Ca(NO_3)_2$, alanine and acetamide. NH_4Cl, DL-Valine, $(NH_4)_2SO_4$, asparagine and $(NH_4)_2CO_3$ were moderate sources for the organism while significantly poor growth was recorded on $NaNO_3$, NH_4NO_3, histidine, phenylalamine, urea and thiourea.

Various nitrogen sources were capable of influencing the sporulation of *C. gloeosporioides* also. Occasionally good growth was associated with good or excellent sporulation (KNO_3, $Mg(NO_3)_2$, peptone) but instances are clearly brought out where good growth may be associated with fair or poor sporulation or with the development of few spores only. However, in certain other cases poor growth was associated with good or excellent sporulation ($NaNO_3$, NH_4NO_3,

histidine) while compounds like urea and phenylalanine were not only poor for growth but also supported poor sporulation.

TABLE III

Growth and sporulation of C. gloeosporioides on different nitrogen sources

No.	Source of nitrogen	Dry wt. of mycelium mg	Sporulation
1	KNO_3	65.8	Excellent
2	$NaNO_3$	40.8	
3	$Ca(NO_3)_2$	50.9	Fair
4	$Mg(NO_3)_2$	69.7	Excellent
5	$NaNO_2$	—	—
6	KNO_2	—	—
7	NH_4NO_3	37.6	Good
8	$(NH_4)_2CO_3$	41.5	—
9	$(NH_4)_2SO_4$	44.2	Fair
10	NH_4Cl	45.3	—
11	DL-Alanine	49.6	Poor
12	Glycine	58.3	—
13	DL-Leucine	64.8	Few Spores
14	DL-Phenylalanine	32.1	Poor
15	DL-Valine	45.0	,,
16	DL-Aspartic acid	82.8	—
17	L-Glutamic acid	78.3	Poor
18	L-Histidine	35.5	Good
19	L-Arginine	61.3	Few Spores
20	L-Asparagine	44.0	Fair
21	Acetamide	47.3	,,
22	Peptone	70.1	Excellent
23	Urea	23.4	Poor
24	Thiourea	21.0	Fair
25	NO Nitrogen	0.0	—

Critical difference at 5 % level = 2.9
Standard error = 1.05

Effect of Hydrogen-Ion concentration on the utilization of nitrite nitrogen

Nitrites serve more or less well as the sole source of nitrogen for a number of fungi. Failure to utilize nitrite, which is more common situation in fungi, is explained on the basis of its known toxicity in acid media. Since nitrite nitrogen was found to have a toxic effect at pH 5.5, its effect at various pH levels was fully investigated. The medium containing nitrites was adjusted at different pH levels, and the fungal mat was harvested after 15 days of growth. It was observed that there was no growth of the organism at different pH lower than 6.0 or at 11.0. The dry weight on KNO_2 and $NaNO_2$ at pH 8.0 was 47.2 and 35.6 mg respectively. Growth at pH 10.0 was much less than at pH 8.0 or 9.0 and was totally submerged and creamy. Thus the growth on the two nitrites was dependent on pH of the medium.

Sulphur sources

It is clear from the table IV that there was no growth in medium lacking sulphur or $ZnSO_4$. The growth was satisfactory on other compounds except thiourea which proved poor for the growth of this organism.

TABLE IV

Growth and sporulation of C. gloeosporioides on different sulphur sources

No.	Source of sulphur	Dry wt. of mycelium mg	Sporulation
1	$MgSO_4.7H_2O$	64.1	Excellent
2	K_2SO_4	57.6	,,
3	$(NH_4)_2SO_4$	37.8	Fair
4	$ZnSO_4$	0.0	—
5	$NaHSO_4$	38.6	Excellent
6	$NaHSO_3$	44.6	Poor
7	$Na_2S_2O_8$	45.8	,,
8	$K_2S_2O_8$	34.9	,,
9	Thiourea	16.0	Fair
10	Cystine	45.6	Excellent
11	No Sulphur	0.0	—

Critical difference at 5 % level = 3.5
Standard error = 1.203

Excellent sporulation was observed on majority of sources except $NaHSO_3$, $Na_2S_2O_8$ and $K_2S_2O_8$ where it was poor. $(NH_4)_2\ SO_4$ and thiourea, however, induced fair sporulation.

DISCUSSION

It is evident from the above investigation that *C. gloeosporioides* could grow and sporulate at a wide range of pH. Growth was optimum at pH 5.5. The organism could therefore easily grow on host tissue as the pH of the leaves was also found to be nearly between 5.0 and 6.0. The pH of the medium affects the rate of growth of fungi, at least in part, by modifying the rate of certain enzymatic reactions. It appears quite probable that at pH 2.0, the enzyme system of the organism might have been completely inhibited so as to allow the pathogen to develop any mycelial growth.

The organism could utilize carbon from a variety of carbohydrates. Amongst monosaccharides xylose, rhamnose and galactose were unsuitabele for growth. This is possible because of the availability of lesser pathways for the utilization of these than for rest of the monosaccharides. The path way of the utilization of xylose is not known. It seems likely that it enters the phosphogluconate oxidation pathway as pentose phosphate. 'CH_3' grouping in rham-

nose may be responsible for the difference in its behaviour from the other pentose arabinose (TANDON & BILGRAMI 1959 as well as TANDON & CHANDRA 1962). Taking into consideration that galactose is a poor source, moderate growth of the organism on lactose was quite reasonable as galactose is one of the constituents of lactose hydrolysis. Inulin is not hydrolyzed by enzymes which act on starch or on sucrose. Poor growth on inulin seems to be due to the lack of synthesis of necessary enzymes by the organism which are essential for its hydrolysis. Poor growth on the organic acids can not be due to the rise of culture pH as the pathogen grew satisfactorily on alkaline pH. The low permeability of the cellwall appears to be the only other possibility for the poor growth of this organism on these organic acids.

The pathogen failed to grow on nitrites. Absence of growth could be explained by the fact that nitrites are in the form of undissociated nitrous acid at acid pH values and thus are toxic for the growth of the organism (COCHRANE & CONN 1950, NORD & MULL 1945 as well as TANDON & CHANDRA 1962). The toxicity of nitrites in acid medium is further confirmed in the present investigation as appreciable growth of the organism was recorded at alkaline pH values. All the amino acids except phenylalanine and histidine were good sources of nitrogen. Several other workers (LEONIAN & LILLY 1938, STEINBERG 1942, FOSTER 1949, LILLY & BARNETT 1951, TANDON & CHANDRA 1962) have also reported that amino acids are not of equal value in fungus nutrition. The difference of growth on various amino acids may be related to the different degree of availability of carbon skeleton of the amino acids for the synthesis of carbohydrates, proteins and fats (GOTTLIEB & CIFERRI 1956). The present results also indicate that the degree of utilization of amino acids varies with their chemical structure.

Results on sulphur requirements clearly suggest that *C. gloeosporioides* could assimilate sulphur from SO_4 as well as other sulphur compounds. Absence of growth on $ZnSO_4$ appears to be due to the toxic effect of Zn.

Results of the present isolate, compared with those of others (SATTAR & MALIK 1939, HAWKINS 1915, TANDON & CHANDRA 1962 and TANDON & VERMA 1962 as well as THIND & DUGGAL 1957) clearly suggest that there are physiological differences between them.

Like other isolates, *C. gloeosporioides* in the present investigation (Isolate 1) could grow on a wide range of pH, but there are few notable differences where it is clearly marked out with others. SATTAR & MALIK (1939) reported that best growth of his organism (Isolate 2) was recorded at pH 7.5 while that of the one under investigation was at 5.5. TANDON & CHANDRA (1962) observed maximum growth of their isolate from *Punica granatum* (Isolate 3) at pH 6.0. Same pH was also reported by TANDON & VERMA (1962) for the maximum growth of their isolate from *Prunus persica*

(Isolate 4). The isolate 3 could grow at pH 2.0 but the present one failed to do so at such a low pH.

Growth response of various isolates (1,3 an 4) of *C. gloeosporioides* was similar on carbon sources like glucose, sucrose, fructose, maltose, sorbitol, mannitol (good sources) and organic acids (poor sources). On some compounds, however, their choice for carbon sources differed. Arabinose, mannose and lactose which were suitable sources of carbon for the present organism (Isolate 1) were quite unsuitable for the isolate 3. TANDON & VERMA (1962) observed poor growth of their isolate (Isolate 4) on raffinose and in this respect the present isolate differed with their isolate (Isolate 4) as raffinose was found to be a good source. The isolates 3 and 4 and one under present investigation also differed with each other for sporulation on various carbon sources.

The present isolate was similar to the isolates 3 and 4 in producing good growth on certain sources like KNO_3, $Ca(NO_3)_2$, DL-valine and glutamic acid. All the isolates, including one under present study grew poorly on urea. It, however, differed with isolate 3 which developed good growth on $NaNO_3$, NH_4NO_3, DL-phenylalanine & L-histidine. Similarly glycine and DL-alanine were good for the present organism while they were poor for isolate 3 studied by TANDON & CHANDRA (1962). Nitrites were toxic for the present pathogen at lower pH values. Similar results were obtained by THIND & DUGGAL (1957) as well as TANDON & CHANDRA (1962). TANDON & VERMA (1962), however, reported growth of their isolate even at acidic pH and in this respect the present isolate differed from the one studied by them. It also differed on $(NH_4)SO_4$ and peptone which were found as poor sources for the growth of their isolate (Isolate 4). The urea supported poor growth of the present organism but it was good for the isolates 3 (TANDON & CHANDRA 1962). Differences in sporulation on various nitrogen sources were also evident between the isolate under study and those investigated by TANDON & VERMA (1962) and TANDON & CHANDRA (1962) even though there was similarity on certain substances.

The differences in sulphur nutrition of the present isolate with other isolates can also be easily marked out. The present isolate and isolate 4 grew on $K_2S_2O_8$ but that of TANDON & CHANDRA 1962, (Isolate 3) did not grow at all on this course. $Na_2S_2O_8$ and $NaHSO_4$ were good sulphur sources for growth, but isolate 3 (TANDON & CHANDRA 1962) grew poorly on the former and was incapable of growing on the latter. All the three isolates (Isolates 1, 3 and 4), however, showed similar response on $(NH_4)_2SO_4$ and $MgSO_4$, $7H_2O$ which were good sources for growth.

From the above investigation it is clearly established that the different isolates differed with each other in some respects or the other as regards their carbon, nitrogen and sulphur requirements are concerned and this appears to be related with physiological differences in various forms.

Summary

Colletotrichum gloeosporioides isolated from the diseased leaves of *Polyscias balfuriana* could grow and sporulate on a wide range of pH (viz. from 3.0 to 9.0). Maximum growth was recorded at pH 5.5. Mannitol was the best carbon source for growth. Good growth as well as good or excellent sporulation was also recorded on glucose, fructose, maltose and starch. Organic acids (malic and tartaric) supported poor growth.

Present organism could utilize a number of nitrogen sources. Nitrates in general were comparatively better sources than ammonium compounds. Aspartic acid was found to be the best nitrogen source for growth. Nitrites were toxic at lower pH values though they supported growth at alkaline medium. Best growth of the organism was obtained on $MgSO_4$, $7H_2O$. The urea supported poor growth. $ZnSO_4$ inhibited the growth completely. The present organism was incapable of growing in media lacking carbon, nitrogen or sulphur.

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