# NUTRITIONAL STUDIES OF THREE SPECIES OF GLŒOSPORIUM

## I. Effect of Different Sources of Carbon and Some of Their Mixtures

BY R. N. TANDON AND R. K. AGARWALA
*(Department of Botany, University of Allahabad)*

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### INTRODUCTION

IT has long been established that carbon as well as a number of other elements are indispensable for the growth of fungi. Grewal (1954) has given a summarized account of the effect of different sources of carbon on the growth and sporulation of various fungi. A survey of literature clearly indicates that all the micro-organisms do not grow equally well on any single source of carbon and the best source for any organism has to be determined.

In nature the fungi usually find mixed sources of carbon. Horr (1936) investigated the growth of *Aspergillus niger* on a mixture of glucose and galactose. He found that galactose alone was unsatisfactory for the growth of *A. niger* but it could be utilized satisfactorily from the mixture. Steinberg (1939) also studied the effect of mixed carbon sources on the growth of *A. niger*. He found that a combination of various sources of carbon (*e.g.*, mannitol and lactose) supported more growth while others (*viz.*, glycerol and galactose) gave less growth than it could develop on any of them used singly. An attempt has, therefore, been made to find out the best source of carbon for three different species of Glœosporium and to determine the effect of mixtures containing different sources of carbon.

### MATERIAL AND METHOD

The three fungi *Glœosporium psidii* Delacr., *Glœosporium limetticolum* Claus. and *Glœosporium citricolum* Speg. were isolated from *Psidium gujava*, *Citrus aurantifolia* and *Citrus medica* respectively. The isolation and purification technique have already been described by the authors in their previous paper (1954). Five grams of glucose present in Asthana and Hawker's medium A* was replaced singly by different carbon compounds. Care was taken that the amount of carbon in each case was similar to that present in

* It contains $KNO_3$ 3·5 gm., $MgSO_4$ $7H_2O$ 0·75 gm., $KH_2PO_4$ 1·75 gm., Glucose 5 gm. and Distilled Water 1000 c.c.

TABLE I

*The Dry Weights in mgm. and Sporulation of Three Species of Glœosporium on Various Sources of Carbon*

| *Glœosporium psidii* | | | | | *Glœosporium limetticolum* | | | | *Glœosporium citricolum* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mannitol > 103·2 | Dulcitol > 84·8 | Glucose > 63·9 | Sorbitol > 58·1 | Galactose > 55·6 | Mannitol > 110·0 | Raffinose > 94·4 | Galactose 89·4 | Sorbitol > 88·0 | Mannitol > 103·9 | Lactose 94·6 | Raffinose 90·0 | Galactose 88·7 |
| R | R | ++++ | R | +++ | + | ++++ | ++++ | +++ | ++ | +++ | +++ | +++ |
| Raffinose 53·6 | S. Starch > 53·0 | Inulin > 49·6 | Maltose > 45·5 | Tartaric acid 44·3 | S. Starch > 78·0 | Sucrose > 71·4 | Malic acid 68·1 | Maltose 66·0 | S. Starch 87·1 | Sorbitol 84·2 | Dulcitol 84·0 | Malic acid 77·8 |
| ++ | +++ | R | ++ | ++++ | ++++ | ++++ | +++++ | +++++ | +++ | +++ | + | + |
| Sucrose > 43·6 | Rhamnose > 40·0 | Lactose 36·2 | Arbutin > 35·5 | Dextrin > 33·9 | Glucose > 64·4 | Tartaric acid > 59·0 | Dulcitol > 56·5 | Lactose > 50·7 | Dextrin 76·9 | Sucrose 76·7 | Tartaric acid 69·9 | Glucose 60·5 |
| ++ | R | + | + | ++ | ++++ | +++++ | ++++ | ++++ | ++ | ++ | + | ++++ |
| Malic acid > 27·9 | Arabinose > 25·7 | Glycerine > 18·9 | Xylose > 10·8 | No. carbon 0·0 | Rhamnose > 47·2 | Arbutin 35·9 | Dextrin 34·0 | Inulin > 33·0 | Rhamnose > 55·1 | Maltose 45·2 | Arbutin 41·8 | Inulin > 40·1 |
| + | ++ | R | R | – | +++ | + | + | R | ++ | +++ | ++ | + |
| | | | | | Glycerine 20·9 | Arabinose > 19·6 | Xylose > 4·2 | No carbon 0·0 | Arabinose > 29·3 | Glycerine > 18·5 | Xylose > 9·5 | No carbon 0·0 |
| | | | | | R | + | – | – | ++ | R | R | – |

C.D. at 5% level = 1·17. 2·33 8·75

5 grams of glucose (*viz.*, 2 grams of carbon). Even in media containing mixed sources of carbon the total amount from different sources was so adjusted as to include only 2 grams of carbon. The amount from each source was similar. The dry weights were determined by the usual method after the organism had grown on liquid media containing 50 c.c. of the nutrient solution in 150 c.c. pyrex flasks at 26° C. for 3 weeks. Double distilled water and purest available chemicals (Analar) were used. Fifteen different microscopic fields were examined before deciding about the degree of sporulation. The amount of sporulation has been indicated by the following sign (+).

\+ Poor
\++ Fair
\+++ V. Fair
\++++ Good
\+++++ V. Good
\++++++ Excellent

Every positive sign denotes the presence of four spores. R denotes rare sporulation (less than four).

Polysaccharides undergo some hydrolysis during autoclaving. They were, therefore, sterilized by fractional sterilization (*i.e.*, steaming them for ½ hour daily for 3 successive days).

## Experimental Results

The three species of Glœosporium were grown on 19 different sources of carbon. The statistical analysis indicated that the different sources could be arranged in the order recorded in Table I.

Mannitol, galactose, raffinose and sorbitol supported good growth of the three species of Glœosporium used in the present investigation. These four compounds were, therefore, mixed together in all possible comibinations and their effect on growth and sporulation of the three fungi was studied. The results are summarized in Tables II, III and IV.

It is evident from the above tables that the behaviour of different species of the same genus may not be essentially similar towards a mixture of various compounds of carbon. The growth of the organism on the mixture could be better or worse than on individual substances.

Table I shows that mannitol supported the best growth of all the three species of Glœosporium. It has been observed that any combination of

TABLE II

*Average Dry Weights in mgm. and Sporulation of* G. psidii *on Combinations of Carbon Compounds*

| Mixture of carbon compounds | Dry weight | Sporulation |
|---|---|---|
| Mannitol + raffinose | 107·9 | +++ |
| Mannitol + galactose | 104·0 | +++ |
| Mannitol | 103·5 | R |
| Mannitol + galactose+sorbitol+raffinose | 103·5 | +++++++ |
| Mannitol + sorbitol + raffinose | 97·1 | ++++ |
| Mannitol + sorbitol + galactose | 96·2 | +++ |
| Raffinose + galactose | 94·0 | ++++ |
| Sorbitol + raffinose + galactose | 92·0 | +++ |
| Mannitol + sorbitol | 90·1 | ++ |
| Sorbitol + raffinose | 86·4 | +++ |
| Sorbitol + galactose | 76·4 | +++ |
| Sorbitol | 58·0 | R |
| Galactose | 55·0 | +++ |
| Raffinose | 52·0 | ++ |

General mean = 86·86 ± 1·9.
C.D. at 5% level = 1·9.

TABLE III

*Average Dry Weights in mgm. and Sporulation of* G. limetticolum *on Combination of Carbon Compounds*

| Mixture of carbon compounds | Dry weight | Sporulation |
|---|---|---|
| Sorbitol + raffinose + galactose | 123·0 | +++ |
| Mannitol | 111·0 | + |
| Raffinose + galactose | 105·5 | +++ |
| Sorbitol + galactose | 104·6 | +++ |
| Sorbitol + raffinose | 98·4 | ++ |
| Raffinose | 94·0 | ++++ |
| Galactose | 89·8 | +++ |
| Sorbitol | 87·5 | +++ |
| Mannitol + galactose | 87·1 | ++ |
| Mannitol + raffinose | 85·7 | +++ |
| Mannitol + sorbitol+galactose+raffinose | 82·4 | +++ |
| Mannitol + sorbitol + galactose | 80·0 | ++ |
| Mannitol + sorbitol + raffinose | 75·4 | ++ |
| Mannitol + sorbitol | 62·4 | + |

General mean = 91·78 ± 4.833
C.D. at 5% level = 4·833.

TABLE IV

*Average Dry Weights in mgm. and Sporulation of* G. citricolum *on Various Combinations of Carbon Compounds*

| Mixture of carbon compounds | Dry weight | Sporulation |
|---|---|---|
| Mannitol .. .. .. .. | 104·5 | ++ |
| Mannitol + galactose .. .. .. | 99·3 | +++++ |
| Mannitol + galactose + sorbitol .. | 99·1 | ++++ |
| Raffinose .. .. .. .. | 89·4 | +++ |
| Galactose .. .. .. .. | 88·0 | +++ |
| Mannitol + sorbitol .. .. .. | 86·6 | ++++ |
| Mannitol + raffinose .. .. .. | 85·1 | ++++ |
| Sorbitol .. .. .. .. | 83·8 | +++ |
| Mannitol + raffinose + sorbitol+galactose | 77·6 | ++++ |
| Mannitol + sorbitol + raffinose .. | 75·2 | ++++ |
| Raffinose + galactose .. .. .. | 69·5 | +++++++ |
| Sorbitol + galactose .. .. .. | 65·0 | +++++++ |
| Sorbitol + raffinose + galactose .. | 57·0 | ++++ |
| Sorbitol + raffinose .. .. .. | 44·0 | ++++ |

General mean = 80·29 ± 5·45.

C.D. at 5% level = 5·45.

mannitol with galactose, raffinose or sorbitol significantly decreased the growth of *G. limetticolum* and *G. citricolum* but *G. psidii* could utilize it with significantly greater advantage when it was mixed with raffinose. There was no significant difference between mannitol and a combination of this substance with galactose or with a mixture of all the four substances. In all other combinations the growth was significantly less.

Individually galactose, raffinose and sorbitol were the poorest source of carbon for *G. psidii* but the growth was significantly better on any of their combinations. Similarly their mixtures were better for *G. limetticolum.* A combination of all the three substances gave best growth of this organism. The growth of *G. citricolum* on different combinations of raffinose, galactose and sorbitol was generally less than on their individual sources. Their presence even decreased the growth on mannitol.

There was a marked influence of the mixture of carbon compounds on sporulation. Excellent sporulation of *G. psidii* was observed on a mixture containing all the four substances. *G. citricolum* also behaved similarly as the sporulation was better on various mixtures. Excellent sporulation was observed on a mixture of raffinose and galactose as well as on sorbitol

and galactose. It was very good on a combination of mannitol and galactose. Other combinations gave good sporulation though none of the four compounds could support good sporulation when they were added singly to the medium.

In general the sporulation of *G. limetticolum* was poorer and it was less on the mixture of different compounds than on individual substances. The best sporulation was on raffinose. The effect of different combinations of carbon compounds was particularly marked on sporulation.

## DISCUSSION

All the three species of Glœosporium gave very good growth on mannitol but it supported poor sporulation. Hawker (1939) also found that mannitol was a poor source for the sporulation of *Melanospora destruens*. The higher alcohol (mannitol) if mixed with raffinose, galactose or sorbitol improved the sporulation of *Glœosporium psidii* and *Glœosporium citricolum*. The sporulation of *Glœosporium limetticolum*, however, was suppressed when mannitol was combined with either of the above three substances. This ability or inability to utilize certain combinations of carbon compound for reproduction can be best explained as an inherent quality of an organism.

Mosher *et al.* (1936), Corum (1942), Matsumato (1921) and Ledeboer (1934) reported that galactose was a good source for the growth of *Trichophyton interdigitale*, *Rhizopus suinus*, Rhizoctonia Species and *Ceratostomella ulmi* respectively. Horr (1936) and Edgecombe (1938), however, found that it decreased growth and reduced sporulation of *Aspergillus niger*, and Penicillium Species, etc., when it was used as the sole source of carbon. The three species of Glœosporium under investigation, however, developed significantly good growth on galactose. Lilly and Barnett (1953) also reported that galactose was a good source for *Colletotrichum phomoides* and *Colletotrichum lindemuthianum*. Horr (1936) as well as Hawker (1939) mentioned that *A. niger* and *Melanospora destruens* utilized galactose better when it was mixed with some additional source of carbon. In the present investigation also the growth of *G. psidii* improved when galactose was mixed with mannitol, raffinose or sorbitol but every combination of those substances failed to improve the growth than any of them alone while galactose and mannitol increased the growth of *G. citricolum*.

Raffinose is found in many plants. Lilly and Barnett (1953) have mentioned that *Fusarium medicaginis*, *Phoma betæ* and *Sphæropsis malorum* could grow well on this substance. Hawker (1936), however, found it to be an inferior source of carbon. According to her those results were obtained

because of the general unsuitability of galactose which is one of the products of hydrolysis of this sugar. In the present investigation galactose is found to be a good source of carbon and the hydrolysis of raffinose is thus not likely to have any adverse effect. Leonian (1925) found that raffinose was more favourable for the production of sporangia of *Phytophthora* sp. This substance could increase both the growth and sporulation of *G. psidii* and its combination with others improved the sporulation of *G. citricolum*. A combination of raffinose and mannitol decreased the growth of *G. limetticolum* though it was better for *G. citricolum*.

Wolf *et al.* (1950) found sorbitol to be useless for *Monosporium apiospermum* but Grewal (1954) reported it to be a good source of carbon for the sporulation of *Glœosporium papayæ* and *Colletotrichum papayæ*. All the three fungi grew well on sorbitol. It has already been mentioned that *G. psidii* grew better on a mixture of several carbon substances than on sorbitol alone which increased the growth of *G. limetticolum* in any combination of raffinose and galactose but *G. citricolum* gave improved growth in combination with mannitol alone and not with raffinose and galactose. This clearly indicates that addition of mannitol to any of the three substances improved growth of *G. psidii* and *G. citricolum* even though its addition suppressed the growth of *G. limetticolum*. According to Lilly and Barnett (1953) such organisms are able to produce small quantities of several enzymes more easily than a large quantity of anyone alone. Such fungi which like mixed carbon sources are probably better fitted for life under natural conditions where mixtures are frequent than larger quantities of any single form. Their capacity to sporulate better on mixtures of various sources of carbon may help them to survive in nature.

## Summary

1. Three species of Glœosporium were isolated from guava, lime and citron twigs. Their behaviour towards the mixture of various compounds of carbon were not similar.

2. *G. psidii* and *G. limetticolum* grew significantly better on any combination of galactose, raffinose and sorbitol while *G. citricolum* supported less growth than on their individual sources. Mannitol alone supported best growth of *G. citricolum* but it could produce excellent growth of *G. psidii* when it was mixed with raffinose.

3. Excellent sporulation of *G. psidii* was observed on a combination of all the four carbon substances, that of *G. citricolum* on a mixture of raffinose and galactose or sorbitol and galactose. *G. limetticolum* gave best sporulation on raffinose alone.

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* Not referred to in the original.