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Growth and sporulation of *Alternaria radicina* under various carbon and nitrogen sources

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Carrot black rot pathogen *Alternaria radicina* grew best on a liquid nedium containing xylose and potassium nitrate as the carbon and nitrogen sources respectively. Starch, sucrose and dextrose also supported good mycelial growth. Ammonium salts as inorganic nitrogen sources were poorly utilized. Among organic nitrogen sources, casein hydrolysate produced good growth, followed by glycine. Abundant sporulation occurred on xylose. It was very good on nitrates, fair on casein hydrolysate and poor on other nitrogen sources. Maxmium vegetative growth was observed in medium containing a carbon-nitrogen ratio of 16 : 1.

Key words : *Alternaria radicina*, carrot, nutritional requirements

INTRODUCTION

Black rot of carrot (*Daucus carota* L) caused by the fungal pathogen *Alternaria radicina* is a major post-harvest storage disease of the crop inflicting considerable economic loss of vegetable vendors. The disease was first repoted in India by Patel *et al.* (1949) from Pune and then by Sharma and Sumbali (1993) from North India and by Guha Roy (1996) from West Bengal. In view of little information available regarding nutritional requirements of the pathogen for growth and sporulation, a study was undertaken on these aspects, the results of which are presented in this paper.

MATERIALS AND METHODS

The carrot black rot pathogen *Alternaria radicina* Meir. Drech. and Eddy maintained in the stock culture collection of the Department of Botany, Kalyani University was used in the present study. For nutritional studies, the fungal pathogen was grown on a liquid basal synthetic medium containing dextrose, 20.0 g ; KNO_3, 3.5 g ; KH_2PO_4, 1.75 g ; $MgSO_4\,7H_2O$, 0.75 g per liter of distilled water. To determine the effect of various carbon sources on growth and sporulation of the pathogen, dextrose was omitted from the basal medium and other test carbon compounds were added individually to the medium, keeping the total carbon content equivalent to that in 20 g dextrose. In a similar manner, while testing the suitability of different nitrogen sources, KNO_3 was replaced by other nitrogen compounds, keeping the total nitrogen content as in 3.5 g KNO_3. Twenty five milliliters of media adjusted to pH 6.0 were distributed in 100 ml flasks which were sterilized at 121°C for 15 minutes. Flasks containing either no carbon or no nitrogen sources were kept as controls. Inoculation of media, incubation and measurement of growth in terms of mycelial dry weight were done as described by Roy and Samajpati (1995). Sporulation was estimated by mounting a small portion of fungal mat in lactophenol and examining it microscopically after teasing.

RESULTS AND DISCUSSION

Among the different carbon compounds tested, representing pentose, hexose, di-and polysaccharides, xylose produced maximum mycelial dry weight of 213 mg (Table 1) measured after 21 days of incubation at 28°C in dark. Other carbon sources in order of decreasing preference were sucrose (183 mg), starch

(180 mg) and maltose (140 mg). Little growth was noticed in the control devoid of any carbon compound. Sporulation was highest in xylose followed by dextrose and sucrose. No sporulation occurred in the control.

Table 1. Mycelial growth and sporulation of *Alternaria radicina* on different carbon sources recorded at weekly intervals.

Carbon sources	Incubation period (days) 7 Gr (mg)[1]	Sp[2]	14 Gr (mg)	Sp.	21 Gr (mg)	Sp.
Dextrose	49	G	95	E	123	E
Sucrose	98	G	129	E	186	E
Maltose	83	G	93	G	140	E
Lactose	25	G	58	G	110	G
Galactose	43	G	78	G	89	G
Fructose	47	G	89	G	106	G
Xylose	106	E	167	E	210	E
Starch	95	P	103	F	180	G
Control (No carbon)	7	N	8	N	8	N

= Average mycelial growth recorded as dry weight in mg per 25 ml liquid medium.

= Sporulation (E=Excellent, G=Good, F=Fair, P=Poor, N=Nil).

Table 2. Effect of nitrogen sources on growth and sporulation on *Alternaria radicina*

Nitrogen sources	Incubation period (days) 7 Gr (mg)[1]	Sp[2]	14 Gr (mg)	Sp.	21 Gr (mg)	Sp.
Potassium nitrate	97	E	150	E	205	E
Sodium nitrate	27	P	102	E	131	E
Ammonium nitrate	12	N	19	N	24	N
Ammonium sulphate	12	N	25	N	28	N
Ammonium phosphate	7	N	14	N	16	N
Asparagine	7	N	12	N	69	N
Caseinhydro-lysate	45	F	174	G	195	G
Glycine	100	P	160	P	180	F
Glutamic acid	5	N	6	N	8	N
Control (No. nitrogen)	5	N	5	N	7	N

Legends : 1 and 2 as in Table 1.

Carbon compounds are equally important for fungi as in other living organisms. However all carbon sources are not equally acceptible to any fungus for its growth and sporulation. This might be due to their differences in structure and chemical configuration. Utilization of carbon compound xylose maximally by the carrot pathogen agrees well with reports of previous authors (Beckman *et al.*, 1953 ; Tandon, 1967) as they also observed xylose to be more satisfactory than glucose for the growth of certain fungi. Panwar (1972) also reported xylose as superior to dextrose for some species of *Curvularia*. Later on Jain and Gupta (1978) obtained maximum mycelial growth and sporulation of *Helminthosporium speciferum* on xylose. Good growth of the pathogen on starch indicated its ability to produce amylase necessary for splitting the macromolecules to assimibable form.

Table 3. Average mycelial dry weight and sporulation of *Alternaria radicina* in liquiid medium having different concentration of carbon and nitrogen after 15 days

Concentration of nitrogen (g/l) KNO_3		Concentration of carbon (g/l) as xylose. 8.0	4.0	2.0	1.0
0.50	A	185	105	75	64
	B	++++	+++	++	++
0.40	A	171	94	51	43
	B	++++	++	++	++
0.20	A	134	68	49	45
	B	+++	++	++	+
0.10	A	125	57	39	28
	B	++	++	++	+
0.05	A	110	41	25	20
	B	++	++	+	+

A : Average mycelial day weight in mg.

B : Sporulation (++++ = Excellent, +++ = Good, ++ = Fair + = Poor).

Good to excellent sporulation of the pathogen observed at later period of incubation may be attributed to exhaustion of nutrients following prolonged vegetative growth on small amounts (25 ml) of medium in flasks. Little growth and sporulation in the control indicated that carbon is required for both the processes.

Among inorganic nitrogen compounds maximum growth occurred on potassium nitrate (205 mg), followed by sodium nitrate (131 mg) while ammonium salts were inhibitory (Table 2). The pathogen grew efficiently also on organic nitrogen sources. Casein hydrolysate produced highest mycelial dry weight (195 mg) followed by glycine (180 mg) but asparagine supported poor growth. Negligible growth was observed on glutamic acid as also in the control. Sporulation was abundant on nitrates, fair on casein hydrolysate and poor or absent on other nitrogen sources.

Fungi differ in their ability to utilize different forms

of nitrogen. However, in general, nitrates are well utilized by most of them for growth (Garaway and Evans, 1984) as also by *A. radicina* of the present study. Tandon (1967) also reported that nitrates are utilized by most of Deuteromycetes and potassium nitrate is preferred. Poor growth on ammonium salts was possibly due to sharp drop of pH of the culture medium making it strongly acidic (Garaway and Evans, 1984). Such low pH is associated with minimal nitrogen utilization and poor growth (Morton and MacMillan, 1954). Casein hydrolysate supported better growth suggesting greater suitability of shorter polypeptides and similar other smaller breakdown products of casein for the pathogen. This indicated the secretion of peptidases by *A. radicina*. Sporulation was abundant on nitrates which was also observed by previous authors (Khandar *et al.*, 1985 ; Pande and Varma, 1992) in other related fungi. Casein hydrolysate also induced good sporulation and this was perhaps due to presence of traces of vitamins and other unknown growth-promoting compounds in it.

It is generally held that a proper balance among carbon and nitrogen sources or C : N ratio is quite important for growth in fungi. To test this for *A. radicina*, media were prepared with varying amounts of carbon (1 to 8 g/liter) and nitrogen (0.05-0.5 g/liter) in the form of xylose and potassium nitrate respectively. After incubation for 15 days in still culture at 30°C, mycelial dry weights were determined. From the data (Table 3) it appeared that a correlation existed between C : N ratio for optimum mycelial growth and sporulation. Maximum growth was noted when C : N ratio was 16 : 1 with 8.0 and 0.5 g of carbon and nitrogen respectively per liter medium. As the carbon content was lowered gradually, keeping the nitrogen level unaltered, mycelial dry weight declined. But comparable growth reduction was not observed when nitrogen content was lowered in steps, keeping carbon content fixed, specially at higher levels of carbon. The results thus indicated greater importance of carbon content for growth of carrot pathogen. Good sporulation also occurred at the same C : N ratio.

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REFERENCES

Beckman, K.S. ; Kuntz, J. F. and Ricker, A. J. (1953). The growth of oak wilt fungus with various carbon and nitrogen sources. *Phytopathol.* **43** : 441-447.

Garaway, M. C. and Evans, R. C. (1984). Fungal Nutrition and Physiology. John Wiley & Sons. New York. pp 96-123.

Guha Roy, H. S. (1996). Post-harvest diseases of some fruits and vagetables in some markets of Birbhum district in West Bengal. *J. Mycopathol. Res.* **34** : 135-140.

Jain, J. P. and Gupta, B. M. (1974). Effect of carbon, nitrogen and their ratio for the growth and sporulation of *Helminthosporium spiceferum*. *Ind. Phytopath.* **27** : 190-193.

Khandar, R.R., Bhatnagar, M. K. and Rawal, P.P. (1985). Cultural conditions affecting growth and sporulation of *Cercospora canescens*, incitant of mungbean leaf spot and germination of its spores. *Ind. J. Mycol. Plant Pathol.* **15** : 165-171.

Morton, A. and Macmillan, A. (1954). The assimilation of nitrogen from ammonium salts and nitrate by fungi. *J. Exptl. Bot.*, **5** : 232-252.

Pande, Alka and Verma, K.V.R.V. (1992). Influence of carbon and nitrogen sources on growth, sporulation and aggressiveness of three seed-borne fungi in pigeonpea. *Ind. Phytopath.* **45** : 213-216.

Panwar, K. S. (1972). Utilization of monosaccharides by four species of *Curvalaria*. *Ind. Phytopath.* **25** : 225-230.

Patel, M. K., Kamat, M. N. and Bhinde, V. P. (1949). Fungi of Bombay, Supplement 1. *Ind. Phytopath.* **2** : 142-155.

Roy, S. and Samajpati, N. (1995). Effect of carbon and nitrogen sources on growth of *Lentinus squarrasulus, L. polychrous, Agrocybe broadwayi* and *Astraeus hygrometricus* in liquid culture. *J. Mycopathol. Res.* **33** : 85-90.

Sharma, S. K. and Sumbali, Geeta (1993). Post-harvest fungal decay of some vegetables from North India. *Ind. J. Mycol. Plant Pathol.* **23** : 241-246.

Tandon, R. N. (1967). Certain aspects of fungal nutrition. Presidential Address, Section-Botany. 54th Indian Science Congress, Hyderabad.pp.1-14.

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