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Growth of three Isolates of Botryodiplodia Theobromae in Relation to Carbon, Nitrogen Sources and C/N Ratio

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Fungi differ in their ability in utilizing various carbon and nitrogen compounds for the growth and sporulation. Carbon occupies an unique position among the essential elements required by living organisms. The studies of Lilly and Barnett (1951) and Steinberg (1950) have clearly established that the fungi differ in their ability to utilize carbon and nitrogen sources. *Botryodiplodia theobromae* is an important pathogen causing considerable damage to different parts of the economic crop plants (Luke, 1982, Maholay and Sohi, 1986, Parakhia *et al.*, 1984. Shillingford, 1977, Vyas and Singh, 1977). To find out the nutritional requirements of three isolates in utilizing the different carbon, nitrogen sources and its optimum ratio, the fungal isolates causing fruit rot of Inidian almond (A), dieback in mango (B), drying of roots of coconut (C) were isolated and maintained on PDA. Richard's medium was used as basal medium. From the basal medium, sucrose and potassium nitrate were replaced by different carbon and nitrogen sources. Their quantities were determined on the basis of their molecular formula so as to supply an equivalent amount of carbon and nitrogen as was present in 50 g of sucrose and 10 g of potassium nitrate respectively per litre in the Richard's medium. The polysaccharices were added at the rate of 50 g per litre of medium. In order to determine best carbon nitrogen ratio. Richard's medium was supplemented with 2.5, 5.0, 7.5, 10.0, 12.5. 15.0 g of potassium nitrate per litre which supplied 0.3968, 0.693, 1.809, 1.385 and 1.732 and 2.079 of nitrogen respectively and 12.5, 25.0, 37.5, 50.0, 62.5 and 75 g sucrose per litre which supplied 5.25, 10.52, 15.77, 21.09, 26.29 and 31.57 carbon respectively. All the possible combinations of carbon and nitrogen were taken. The pH of the medium was adjusted to 6.0 before autocalving.

Fifty ml medium was dispensed in each of the 250 ml corning flasks and then autoclaved at 0.69 kg/cm^2 pressure for 20 minutes. Each flask was inoculated with a single disc of 4 mm diameter obtained from 7 days old culture grown on PDA. Flasks were incubated at 28 ± 2° C. The mycelial mate (15 days old) were filtered by using Whatsmen filter paper No. 42, washed with

TABLE 1

Effect of various sources of carbon on growth and sporulation of three isolates of *Botryodiplodia theobromae*.

Sr. No.	*Name of carbon source*	*Isolates*		
		Almond (A)	*Mango (B)*	*Coconut (C)*
1.	D. Glucose	260	438	318
2.	Galactose	443	618	566
3.	Sucrose	1485	1550	1471
4.	Maltose	479	628	509
5.	Lactose	408	458	233
6.	Fructose	414	446	397
7.	Starch	364	523	380
8.	Mannitol	666	586	426
9.	Control	90	65	71
	C. D. at 5%	95.4	82.4	102
	C. V. %	10.8	8	12.2

luke warm sterilized distilled water and dried at 62° C until it attained the constant weight. Colony character, pigmentation and sporulation were recorded.

It is evident from Table 1 that all the three isolates of the pathogen utilized a variety of compounds as a source of carbon. Sucrose proved to be the best source for the growth of all the isolates followed by maltose for isolate B and galactose for isolate C and mannitol for isolate A. Remaining carbon sources supported good growth of isolate A being the least on D glucose. The growth of isolate A and B was good on galactose and mannitol. Remaining carbon sources supported moderate growth of isolate B., the least being on lectose. Good growth of isolate C was observed on maltose and mannitol being the least on lectose. These results are in agreement with the results obtained by Shreemala, 1973.

No sporulation was observed in isolate A and B on any of the carbon sources tested but scanty sporulation was observed in case of isolate C on all the carbon sources. This supports the report of Shreemala 1973 while working on six different isolates of *B. theobromae* reported that some of the isolates of *B. theobromae* did not sporulate on medium having defferent carbon sources.

On perusal of Table 2, all the isolates of the fungus under study vary in utilizing the different nitrogen sources. Isolate A grew well where the ammonium molyblade was used

while the growth of isolate B and C was medium on calcium nitrate and magnesium nitrate respectively. Second best source was calcium nitrate for isolate A and C and ammonium dihydrogen orthophosphate for isolate B. The least growth of isolate A and C was observed on sodium nitrate and isolate B grew least on ammonium oxalate.

Results of carbon nitrogen ratio shows that the growth of all the isolate increased proportionately as concentrations of carbon in the medium increased up to 26.29 g/l. Higher quantity of carbon inhibited the growth of all the isolates i.e. (31.57 g/l carbon). Increase in nitrogen also improved growth in some cases while in some cases the growth was declined. Highest growth of isolate A and B was observed at C_5N_5 C/N ratio while isolate C grew at C_5 N_3 ratio. Sporulation was only observed in case of isolate C which was isolated from coconut in all the combinations of carbon and nitrogen.

TABLE 2

Effect of various sources of nitrogen on growth and sporulation of three isolates of *Botryodiplodia theobromae*.

Sr. No.	*Name of nitrogen source*	*Isolates*		
		Almond (A)	*Mango (B)*	*Coconut (C)*
1.	Potassium nitrate	387	539	466***
2.	Magnesium nitrate	484	579	614
3.	Ammonium chloride	351	355	372
4.	Ammonium sulphate	467	592	335
5.	Ammonium nitrate	469	472	382
6.	Ammonium dihydrogen Orthophosphate	402	615	469
7.	Ammonium oxalate	485	95	224
8.	Sodium nitrate	465	579	431**
9.	Calcium nitrate	627	618	598*
10.	Sodium nitrate	49	311	208
11.	Urea	485	581	346**
12.	Ammonium molyblade	792	493	575
13.	Control	000	000	000
	C.V. at 0.05%	116	126	93
	C. V. %	16.3	16.7	14.3

*Sporulation observed.

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TABLE 3

Effect of different C/N ratio on growth and sporulation of three of isolates of *Botryodiplodia theobromae*.

Carbon Level	Isolate	Nitrogen					
		N1	N2	N3	N4	N5	M6
C1	A	141	346	210	271	253	300
	B	233	296	260	341	196	386
	C	78	310	160	253	183	213
C2	A	263	256	260	311	355	326
	B	276	370	343	455	385	516
	C	150	360	413	310	356	373
C3	A	390	286	396	406	423	378
	B	436	396	460	601	583	616
	C	473	463	530	343	396	403
C4	A	430	503	533	435	406	360
	B	426	413	573	706	680	693
	C	453	423	500	410	410	413
C5	A	573	536	493	472	623	535
	B	633	760	726	686	985	915
	C	590	506	726	453	541	486
C6	A	480	446	735	578	618	620
	B	483	470	623	833	698	953
	C	600	533	615	503	600	580

		A	B	C
C. D at 0.05%	N	35	67	NS
	C	35	67	72
	NC	85	165	172

A = Indian Almond B = Mango C = Coconut

Looking to the nutritional requirements of the isolates and the capacity to use different carbon and nitrogen sources for the growth and sporulation, it seems that isolates A, B and C of *B. theobromae* do not differ in their ability to use carbon sources while all the isolates are differed in utilizing the nitrogen sources for their growth. They are also different as fas as the quantity of nitrogen source in C/N ratio is concern. They are definitely different as far as the ability of sporulation concern. Isolate A and B did not sporulate on medium having any sources or quantity of nitrogen or carbon.

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