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Effect of culture media, temperature, pH, carbon and nitrogen sources on growth of *Botryodiplodia theobromae* causing Java black rot of sweetpotato tubers

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Java black rot is a dry rot of sweet potato in storage caused by *Botryodiplodia theobromae* Pat. The fungus was found to grow best on papaya dextrose and czapek dox liquid culture media under static and shaking condition respectively, whereas on solid media, the best growth was noticed on papaya dextrose. The optimum temperature which favoured the growth of *B. theobromae* was between 25°-30°C while the optimum pH for growth was found in the range of 5.0-6.0. The fungus utilized a wide range of carbon sources including dextrose, fructose, maltose, sucrose etc. Growth was highest with maltose as carbon source followed by fructose and soluble starch. Organic N sources (beef extract, tryptone, yeast extract) were better suited for growth of *B theobromae* than the inorganic sources (ammonium chloride, ammonium sulphate, sodium nitrate, etc.). Among the various salts (Ca^{2+}, Fe^{2+}, Na^{2+}, Mg^{2+}, B^{2+}) tested on pycnidial production by *B. theobromae*, only boron (50-200 μg/ml) inhibited and all other salts stimulated the sporulation.

Key words : *Botryodiplodia theobromae*, sweet potato, effect of some growth conditions.

INTRODUCTION

Brotryodiplodia theobromae Pat. causes Java black rot in sweet potato during storage and transit (Jenkins, 1981, 1982; Ray and Misra, 1995; Ray and Punithalingam, 1996). The spoilage characteristics of the disease has been described (Ray and Punithalingam, 1996). The affected roots show externally dark patches within which develop several pycnidia and internally, the tissues turn black. Ultimately, the spolied roots become shriveled and brittle, and show externally mummified appearance. Studies have been made on the effect of various physiological factors, such as light, pH, temperature, sources of carbon and nitrogen, vitamin requirements (Honda and Aragaki, 1978; Rao and Singhal, 1978) on the growth of some strains of *B. theobromae* isolated from different crops other than sweet potato (Punithalingam, 1980). Since the physiological behaviour of the same organism may varies within races/strains isolated from different crops (Punithalingam, 1980). The present study was undertaken on the effect parameters like culture media, temperature, pH, C and N sources on growth of *B. theobromae* (Code No. IMI 361230) isolated from sweet potato under static and shake (shaking at 120 r.p.m in an orbital shaker incubator) conditions.

MATERIALS AND METHODS

Fungus

The isolate of *B. theobromae* (IMI 361230) used in this study was earlier isolated from the post harvest decayed sweet potato tubers (Ray and Misra, 1995). Spore suspensions of *B. theobromae* were prepared from 10 days old cultures grown at room temperature (25 ± 2 °C) on potato dextrose agar (PDA). Spores were harvested in sterile distilled water and diluted to concentration of 5.5 x 10^6 spores/ml. The same concentration was used in all the experiment.

Effect of culture media

Five different media were used for studying the growth

of *B.theobromae*. The pH of the media was adjusted to 6.0 by addition of dilute (0.1 N) HCL. For liquid culture, one ml of the spore suspension (5.5 x 10^6 spores/ml) of the fungus was inoculated into 250 ml Erlenmeyer flasks containing 100 ml of the media and the flasks were incubated for 8 days at 30°C either under static or shaking (shaking at 120 r. p. m. in an orbital shaker incubator) conditions at 30°C. After 4 and 8 days of incubation, the contents of growth in the flasks for *B. theobromae* were pooled and filtered. The mycelial mats (cell masses after filtration) corresponding to each harvest were dried in an oven at 80°C to constant mass.

To study the growth on solid medium, the culture media supplemented with agar-agar (20 g/l) and sterilized at 127°C for 20 minutes were plated on sterilized petriplates. Mycelial discs (1 cm. dia), cut off from 10 days old culture of *B.theobromae* grown on PDA, were put on the centre of culture media in petriplates. The plates were incubated at 30°C. The linear growth of colony was measured from 3rd day of incubation up to the 6 day. Six replicates were maintained for each treatment.

Effect of temperature and pH

For this experiment, three different liquid media (PD broth. CD and CD + yeast extract (YE)] were taken. Each culture medium, taken in 250 ml Erlenmeyer flasks (in triplicates), were inoculated with the spore suspension of *B.theobromae* and incubated at different temperature (20°C – 40°C) for 8 days. The flasks were incubated in two batches : one batch under static condition and the other batch under shaking condition as described earlier. Likewise, the effect of growth medium pH was studied by incubating the growth flasks (in triplicate) containing 100 ml CD broth medium of different pH (3.0-9.0), adjusted by addition of dilute 0.1 N HCI or 0.1 N NaOH and incubated at 30° C.

Effect of C and N sources

To study the effect of carbon sources on growth of *B. theobromae*, different carbon compounds were incorporated into CD broth medium (in 250 ml Erlenmeyer flasks) at 3 percent concentration. Three flasks were kept for each treatment. Likewise, different nitrogen sources at 0.05% concentration were used to study the growth of *B. theobromae*. The other conditions (procedure for inoculation, incubation, harvesting cells etc.) were same as in the previous experiment.

Effect of salt concentrations on conidial production

Different salts were tested for their effect on pycnidial production of *B.theobromae*. Fifteen ml of salt solutions (50-200 μg/ml) taken on pertriplates, were inoculated with 0.5 cm diameter discs of 10 days old *B.theobromae* cultures grown on PDA and incubated at 30°C. Six plates were kept for each treatment. Mycelial discs grown on 1.5 ml distilled water kept on petriplates served as controls. The petriplates were observed under microscope at low power (10 X) and the number of conidia formed/plate were recorded at 24 th intervals from the 15th day of incubation up to the 19th day.

Analysis of variance (ANOVA) was performed by INDOSTAT software following completely randomized block design. Mean comparison with treatments was performed by least significant difference (LSD) at p = 0.05 level (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

In our earlier reports (Ray and Misra 1995; Ray and Punithalingam, 1996) it was observed that all infections in sweet potato due to *B.theobromae* occurred at harvest and during subsequent handling and transport. The affected roots initially showed no sign of rottage at least externally but as the infection progressed, dark patches developed in the skin within which developed numerous pycnidia and tissues truned black.

Table 1. Comparative growth of *B. theobromae* in different liquid culture media

Growth medium	Cell mass (mg)			
	Static culture		Shake culture	
	4 d	8 d	4 d	8 d
Papaya dextrose	368.2±2.3	801.3±7.2	163.3±8.6	505±8 6
Potato dextrose	268.4±8.0	793.0±4.9	275±43.3	410±10.9
Czapek dox	188.6±5.6	686.7±8.4	930±69.2	835±17.8
Rose Bengal	203.8±3.0	443.7±20.2	595±54.0	900±17.3
Asthana & Hawker	80.9±0.3	270.3±14.4	117.7±3.8	314.7±4.5

± Standard error
LSD between treatments at 5% level is 53.6

Table 2. Comparative growth of *B. theobromae* on different solid culture media

Growth medium	Linear growth on solid media [colony radius (mm)]				
	Days after inoculation				
	3	4	5	6	Mean
Papaya Dextrose	9.0 ± 0.6	23.7 ± 0.9	33.7 ± 0.9	44.0 ± 0.6	27.6 ± 6.7
Potato Dextrose	8.7 ± 0.3	24.0 ± 0.6	33.0 ± 1.7	43.0 ± 0.6	27.2 ± 6.6
Czapek Dox	8.3 ± 0.3	23.0 ± 0.6	32.7 ± 1.9	42.7 ± 0.9	26.7 ± 6.7
Rose Bengal	5.0 ± 0.6	18.3 ± 1.2	24.0 ± 1.2	33.0 ± 0.9	20.2 ± 5.4
Asthana & Hawker	5.0 ± 0.3	9.0 ± 0.6	14.0 ± 0.6	18.7 ± 0.3	11.6 ± 2.3

± Standard error

LSD between treatments at 5% level in 1.2

Table 3. Effect of temperature on growth cell mass of *B. theobromae* on different liquid culture media

Tem-perature (°C)	Culture Media	Cell mass (mg)			
		Still culture		Shake culture	
		4 d	8 d	4 d	8 d
20	PD	226±14	719±34	260±12	742±28
	CD	136±7	549±62	169±14	579±38
	CD+YE	114±17	516±47	137±37	543±34
25	PD	235±26	734±20	266±59	788±13
	CD	155±14	562±25	177±7	604±34
	CD+YE	126±18	545±35	125±18	561±32
30	PD	245±27	758±24	286±61	801±10
	CD	169±10	586±23	177±27	614±58
	CD+YE	135±9	558±32	168±17	577±25
35	PD	234±12	669±24	272±17	698±36
	CD	164±3	640±25	201±12	673±24
	CD+YE	132±6	534±21	176±5	575±24
40	PD	172±6	190±3	186±7	248±4
	CD	145±3	165±6	163±4	286±4
	CD+YE	131±3	154±6	142±4	182±7

± Standard error

LSD between treatments at 5% level is 22.2

The data in Table 1 show the trend on growth (cell mass) of *B.theobromae* on five different liquid culture media i.e. Papaya dextrose, Potato dextrose broth (PD), Czapek dox, Rose Bengal and Asthana and Hawker under static as well as shaking conditions. Production of cell mass was greater on Papaya dextrose followed by PD under static conditions and on Czapek dox followed by rose bengal under shaking conditions. Minimum growth was observed on Asthana and Hawker medium either on static or shake culture. Similar result (Table 2) was obtained in solid culture media (static).

Table 4. Effect of pH of *B. theobromae* on CD medium after 8 days under shaking conditions

pH	Cell mass (mg)
3.0	575 ± 66.0
4.0	500 ± 31.0
5.0	595 ± 66.0
6.0	640 ± 63.4
7.0	540 ± 12.6
8.0	355 ± 16.0
9.0	200 ± 12.6

± Standard error

LSD between treatments at 5% level is 18.6

Table 5. Effect of carbon sources on growth (cell mass) of *B. theobromae* on CD medium under static and shaking conditions

Carbon source	Cell mass (mg)			
	Static Culture		Shake Culture	
	4 d	8 d	4 d	8 d
Sucrose	268 ± 18	567 ± 28	350 ± 21	690 ± 18
Maltose	567 ± 22	980 ± 35	720 ± 17	1110 ± 37
Lactose	255 ± 10	670 ± 26	308 ± 16	880 ± 33
Dextrose	270 ± 12	540 ± 15	312 ± 15	660 ± 12
Fructose	538 ± 14	888 ± 33	689 ± 20	1200 ± 52
Xylose	280 ± 15	680 ± 17	300 ± 18	760 ± 42
Soluble starch	260 ± 23	830 ± 40	312 ± 12	955 ± 18
Cassava starch	190 ± 11	760 ± 25	220 ± 14	580 ± 18

± Standard error

LSD between treatments at 5% level is 39.9

Suitability of different culture media for different fungi has been reported by Scott. (1976). However, PD has been reported to be most suitable media for a large number of fungi (Aneja, 1993 ; Patil *et al.*, 1988). Further, there was 1.2 to 4.9 fold increase in cell mass on CD media when the fungus was grown under shaking condition than on static condition. Interestingly, organic matter rich media like papaya dextrose and potato dextrose supported development of more cell mass under static condition than under shaking condition whereas the reverse trend was noticed in case of mineral media like CD, rose bengal etc. The reason could not be properly explained.

There are several under reports on the effects of temperature on the growth of *B.theobromae* under

Table 6. Effect of nitrogen sources on growth (cell mass) of *B. theobromae* on CD medium under static and shaking conditions

Nitrogen source	Cell mass (mg)			
	Static Culture		Shake Culture	
	4 d	8 d	4 d	8 d
Potassium nitrate	360 ± 17	720 ± 22	380 ± 9	890 ± 20
Ammonium nitrate	300 ± 12	620 ± 20	320 ± 18	820 ± 17
Sodium nitrate	190 ± 8	490 ± 10	220 ± 15	630 ± 22
Sodium nitrite	210 ± 7	280 ± 8	270 ± 12	290 ± 8
Peptone	218 ± 3	600 ± 22	280 ± 19	560 ± 16
Beef extract	280 ± 14	930 ± 33	320 ± 17	1180 ± 21
Tryptone	320 ± 16	1010 ± 35	340 ± 9	1360 ± 18
Yeast extract	660 ± 17	1140 ± 36	690 ± 10	1420 ± 17
Ammonium chloride	170 ± 15	490 ± 12	240 ± 12	630 ± 14
Ammonium molybdate	280 ± 13	388 ± 8	300 ± 17	573 ± 15
Ammonium sulphate	220 ± 17	730 ± 12	280 ± 16	800 ± 16

± Standard error

LSD between treatments at 5% level is 14.8

Table 7. Effect of Ca, Fe, Na, Mg and B on conidial production of *B. theobromae*

Salt	Conc. (ppm)	Spore count/Microscopic field (40X) i.e. 0.16 (mm)^2				
		Days after inoculation				
		15	16	17	18	19
Control		1.2	2.0	2.8	4.6	3.0
Ca	50	0.8	2.4	4.2	9.4	13.2
	100	1.0	2.2	6.0	12.2	14.6
	200	0.8	1.6	3.6	10.6	12.6
Fe	50	2.4	4.0	6.8	10.8	14.2
	100	2.0	3.4	5.8	8.2	10.0
	200	1.6	3.2	6.0	7.6	9.6
Na	50	1.8	4.4	8.0	14.2	16.4
	100	3.0	5.6	9.2	13.8	19.4
	200	3.4	8.0	10.8	16.8	19.6
Mg	50	2.0	5.6	9.2	13.8	19.4
	100	3.4	8.0	10.8	16.8	19.6
	200	3.6	7.4	11.0	15.4	17.6
B	50	0.4	1.0	1.6	2.2	2.6
	100	0.2	0.8	1.6	2.2	2.6
	200	0	0.4	1.0	1.4	1.6

LSD between treatments at 5% level in 0.97

static conditions (Srivastav and Tandon, 1968; Uduebo, 1974; Ray and Punithalingam, 1996). However, there is virtually no report on the effect of temperature on growth of *B. theobromae* under static and shaking condition simultaneously. The data in Table 3 show the effect of temperature on growth of *B. theobromae* in the range of 20-40°C on PD broth, CD and CD+YE liquid media under static and shaking condition. Among the three culture media, maximum growth was obtained on PD broth followed by CD and CD + YE, respectively. Further, the fungus could grow more at 30°C followed by 25°C whereas least growth was noticed at temperature of 40°C. The results agree with the findings of Somner (1982) that the post harvest pathogens grow best between 25°C and 30°C, depending on the species involved. Woolfe (1992) also reported the optimum growth temperature of *B. theobromae* was about 28°C. It appears the since sweet potato is harvested mostly in rabi (Sept-Mar) season when the prevalent atmospheric temperature in India is between 25°C-30°C, the situation in congenial for the growth of *B. theobromae* and initiating rot. The optimum pH which favoured the growth of *B. theobromae* under shaking condition was in the range of 5.0 to 6.0 (Table 4). The results are similar to those obtained under static conditions (Ray and Punithalingum, 1996).

The fungus utilized a wide range of carbon sources including sucrose, dextrose, fructose, maltose etc. for its growth (Table 5). Growth was highest with maltose followed by fructose and soluble starch under static as well as shaking conditions.

The organic nitrogen sources were found to be suited for growth of *B. theobromae* than the inorganic nitrogen sources (Table 6). The results are in contrast with the findingd of Soni *et al.* (1992) with *Fusarium oxysporum* that inorganic nitrogen sources supported fungal growth whereas organic N inhibited it. Shreemali (1973) also reported that inorganic nitrogen stimulated growth and sporulation of six different isolates of *B. theobromae* on liquid culture.

Various salts i.e. Ca, Fe, Na, Mg and B at different concentrations (50, 100, and 200 μg/ml) were tested for their effect on pycnidia formation by *B. theobromae* and the results are shown (Table 7). Among the salts tested all except boron enhanced pycnidial formation compared with control (water). This indicated that application of born to soil was detrimental for fungal sporulation whereas other salts were more or less stimulatory for pycnidial formation and consequently fungal growth.

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