PRODUCTION TECHNOLOGY OF MYCOLOGICAL CULTURE MEDIUM USING COCOA BEAN SHELL

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Introduction

The expansion of area under cocoa cultivation is steadily increasing year after year with an increase in production. In addition to the highly flavoured cocoa, which is the main commercial product of cocoa cultivation, processing of cocoa both at primary and secondary levels leaves a large quantity of waste materials. The disposal of these is one of the major problems in major cocoa growing tracts especially in cocoa fermentaries. The important waste materials are pod husk, sweatings and bean shell.

Different synthetic and non-synthetic culture media are extensively used in all mycological laboratories for isolation and maintenance of microorganisms and for their cultural and morphological studies. If any one of the cocoa wastes/ by-products can be utilized as a raw material for preparation of non-synthetic mycological culture medium, it will be a cheap medium and a substitute for many costly culture media. Though CBS, a byproduct of chocolate factory is available in large quantity, it is not properly used for any purpose in our country. Therefore, investigations were undertaken to develop a mycological culture medium using cocoa bean shell.

Materials and Methods

a. Cocoa Bean shell (CBS) based culture medium

Cocoa Bean Shell (CBS), the brown coloured covering of the cotyledon, is removed from dry beans by a process called kibbling at the factory level. In the factory the cocoa beans are first cracked by passing through rollers. The lighter shell is blown away by an air current. Thus CBS @120 kg per ton of dry beans is obtained as a by product at factory level.

CBS collected from the chocolate factory was oven dried at 60ºC, for 2 days and powdered using a mixer grinder. To standardize the quantity of CBS required for the preparation of one litre medium, water extracts of 10, 15, 20, 30 and 40 g of CBS powder were tried keeping the quantity of agar constant at 20 g.

In the second set of experiment 20 g dextrose was also added to CBS powder extract prepared using various quantities of CBS powder in addition to 20 g agar for one litre medium. A third set of media was prepared using three different quantities of dextrose (20, 30, and 40 g) along with 20 g agar in 1000 ml distilled water to find out the effect of dextrose alone on growth and sporulation of the test fungi. The compositions of different media prepared are given below:

Composition of CBS based culture media and dextrose agar media

A. Water extracts of CBS powder +Agar + Distilled water (CBSA)	
CBS powder	10/15/20/30/40 g
Agar	20 g
Distilled water	1000 ml
pH	5.2-5.8
B. Water extracts of CBS powder + Dextrose + Agar + Distilled water (CBSDA)	
CBS powder	10/15/20/30/40 g
Dextrose	20 g
Agar	20 g
Distilled water	1000 ml
pH	5.5 to 6

C. Dextrose + Agar + Distilled water (DA)	
Dextrose	20/30/40 g
Agar	20 g
Distilled water	1000 ml
pH	5.3 to 5.6

Standardization of quantity of agar requirement in Cocoa Bean Shell Dextrose Agar (CBSDA) medium

In order to find out the minimum quantity of agar required to solidify 1000 ml CBSDA medium various quantities of agar such as 5, 8, 10, 15 and 20 g were used as per the details given below:

Extract of CBS powder	30 g (standardized)
Dextrose	20 g
Agar(Agar powder-Bacteriological from Hi Media Laboratories Pvt. Ltd. Mumbai, India)	5/8/10/15/20 g
Distilled water	1000 ml
pH	5.5-6.0

The CBSDA medium was prepared with 5 different quantities of agar as mentioned above. The media were kept at room temperature (25ºC) to find out the minimum quantity of agar required for solidification of CBSDA medium. Solidification of the medium was assessed by visual observation and tilting and shaking the petri plates 2 hrs after pouring the media. It was found that 15 g agar in CBSDA was sufficient for very good solidification of the medium.

Standardization of quantity of dextrose in CBS based medium

To find out the minimum quantity of dextrose to be used in CBS-based medium for better growth and sporulation of fungi, different quantities of dextrose such as 5, 8, 10, 15 and 20 g were tried, keeping the quantities of CBS powder and agar constant at 30 g and 15 g respectively for 1000 ml medium.

Composition of media

Extract of CBS powder	30 g
Dextrose	5/8/10/15/20 g
Agar	15 g
Distilled water	1000 ml
pH	5.6-6.0

The quantity of CBS powder wherever mentioned in different combinations of media is the quantity used for preparing water extracts. To prepare CBS medium, CBS powder was boiled with distilled water for 30 minutes filtered through a double layered muslin cloth, and made upto 1000 ml with distilled water. Measured quantities of all the above mentioned media were dispensed into 250 ml conical flask plugged with non absorbent cotton, covered with aluminium foil and sterilized in an autoclave at 121°C for 15 minutes. The sterilized and cooled medium was poured into 90 mm diameter petri plates @15 ml medium per plate inside a clean air flow chamber. After solidification of the medium, it was inoculated separately with the test fungi and incubated at room temperature.

b. Performance of pathogenic fungi in CBS based media

Three cocoa pathogens *viz., Phytophthora palmivora, Colletotrichum gloeosporioides* and *Lasiodiploidia theobromae* were selected for the present study. Five mm mycelial disc cut from the periphery of five day old cultures of all these three pathogenic fungi were transferred to the centre of 90 mm petri plates containing each of the media mentioned above. PDA medium prepared using fresh potato and dehydrated PDA medium (Hi-media) were used for comparing the growth performance of the test fungi with that in CBS based media. Three replications were maintained for each fungus and each medium.

The inoculated plates were incubated at room temperature. The diameter of the fungal colony was recorded at 2, 3, 5 and 7 days intervals, expect in the case of *L. theobromae,* where the colony diameter was measured after 24 hrs of incubation. The colony diameter was measured in two directions at right angles to each other and the average of two such measurements was taken as the colony diameter in each plate.

Degree of sporulation was assessed on the 10^{th} day of inoculation. To assess the degree of sporulation, 5 mm diameter mycelial discs, three each from the periphery, middle and centre of the colony were cut with a sterile cork borer. The discs were transferred to a test tube containing 5 ml sterile distilled water and shaken well for 5 minutes on a mechanical shaker. From this 0.2 ml suspension was transferred to a slide and three such slides were prepared for each replication. The number of conidia per microscopic field (6.3 x 40X magnification) was recorded. The average count of conidia from 15 microscopic fields (5 from each slide) was considered as the degree of sporulation for each plate. The data were analyzed statistically.

Results

Performance of three pathogenic fungi in Cocoa Bean Shell (CBS) based media

Compositions of CBSA media (extract of cocoa bean shell powder + agar + distilled water) with and without adding dextrose are given in Table 1A. The growth and sporulation of *L. theobromae*, the causal organism of charcoal pod rot of cocoa on CBSA medium varied with the quantity of CBS. The growth and sporulation increased when dextrose was added to the CBSA medium. Among the treatments using different quantities of CBS without dextrose, the CBSA medium containing water extract of 30 g CBS powder supported better growth and sporulation of *L. theobromae.* When dextrose was added to the medium containing different quantities of CBS, the growth and sporulation increased and were the highest when 30 g CBS, 20 g dextrose, 20 g agar and 1000 ml distilled water were used for the preparation of medium (CBSDA). The performance of *L. theobromae* in the medium was on par with PDA prepared using fresh potato tubers. Thus, the growth and sporulation of *L. theobromae* in CBSDA medium containing 30 g CBS + 20 g dextrose + 20 g agar +1000 ml distilled water and freshly prepared PDA were significantly different from that on other different combinations of media. It was followed by dehydrated PDA in supporting growth of *L. theobromae*. But sporulation in this medium was less than that in the medium containing CBS 20 g + dextrose 20 g + agar 20 g + distilled water 1000 ml. Sporulation was the lowest in CBS 10 g + agar 20 g + distilled water 1000 ml and was on par with the medium containing only dextrose 20 g + agar 20 g + distilled water 1000 ml.

The growth and sporulation of *Colletotrichum gloeosporioides* also exhibited a similar trend as that of *L. theobromae*. When different combinations of CBS was used and compared with PDA the growth and sporulation of *C. gloeosporioides* were the highest in the medium CBS 30 g + dextrose 20 g + agar 20 g + distilled water 1000 ml (Table 1B). Thus the performance of *C. gloeosporioides* in this medium was significantly different from that in all other media including PDA. Among the different combinations of media tried, the lowest growth and sporulation were observed in the medium containing only 20 g dextrose + 20 g agar + 1000 ml distilled water.

Even though the highest growth and sporulation of *Phytophthora palmivora* were observed on freshly prepared PDA (Treatment 12), it was followed by CBSDA medium (Treatment 9) containing 30 g CBS, 20 g dextrose, 20 g agar and 1000 ml distilled water (Table 1B). Treatment-12 was on par with Treatment-9. As observed in the case of *L. theobromae* and *C. gloeosporioides*, the performance of *P. palmivora* was the poorest on dextrose medium (dextrose 20 g + agar 20 g + distilled water 1000 ml).

In general, all the three pathogenic fungi *viz., L. theobromae*, *C. gloeosporioides* and *P. palmivora* performed better on CBSDA medium containing water extract from 30 g CBS powder, 20 g dextrose and 20 g agar/L medium. Since cocoa plant parts contain lot of mucilage, a pilot study was conducted to find out the minimum quantity of agar required for good solidification of CBSDA medium. When different quantities of agar powder were used for the preparation of CBSDA medium, the solidification of the media varied. It was found that 15 g agar powder/L CBSDA medium was sufficient for very good solidification of the medium.

When different quantities of dextrose such as 5, 8, 10, 15 and 20 g were added to CBS based medium containing extract of 30 g CBS powder and 15 g agar/L of medium, the growth and sporulation of the pathogenic fungi *viz., L. theobromae, C. gloeosporioides* and *P. palmivora* varied with the quantity of dextrose. *L. theobromae* and *C. gloeosporioides* exhibited a similar trend in growth and sporulation. The growth and sporulation of both the fungi were the highest in CBSDA medium containing 20 g dextrose. It was on par with CBSDA medium containing 15 g dextrose. Both these treatments significantly differed from other treatments (Table 2 A and B). The lowest growth and sporulation of both these fungi were observed in CBSA medium (Treatment-1) where dextrose was not added. Though the highest growth of *P. palmivora* was observed in freshly prepared PDA medium it was on par with CBSDA containing 20 g/15 g dextrose. All these three treatments were significantly different from other treatments (Table 2B). The highest sporulation of *P. palmivora* was also observed in freshly prepared PDA medium which significantly differed from all other treatments. It was followed by dehydrated PDA and CBSDA with 20 g/ 15 g dextrose. These three treatments were on par. The lowest growth of all the three pathogenic fungi was in CBSA medium (without dextrose). Thus, in general CBSDA containing 15 g dextrose/L of medium was found to be ideal for growth and sporulation of fungi

Composition of Standardized CBSDA medium

CBS	30 g (for preparing extract)
Dextrose	15 g
Agar	15 g
Distilled water	10 ml
pH	5.5-6.0

Discussion

Though small quantities of by-products are utilized for some purpose in some of the cocoa growing countries, they are not put into any proper use in India. No attempt has been made so far in any of the cocoa growing countries to utilize major cocoa wastes to develop mycological culture media except a preliminary study on the growth of *Phytophthora palmivora* in the media prepared using fresh pod husk, bean and mucilage (Awuah and Frimpong, 2002). Cocoa bean shell was reported to have been used for making shell wine (Greenwood-Barton, 1965), food colour (Anon, 1966), livestock diet (Gohi, 1981) and to lighten heavy soil (Prasannakumariamma *et al.,* 2002). Based on a very preliminary study, Senyah *et al.* (1989) reported that bean shell can be used as a substrate for *Pleurotus ostereatus* cultivation. But nobody has further pursued this aspect of bean shell utilization. The quantities of CBS, agar and dextrose required for the preparation of solid media were standardized and the performances of three pathogenic fungi in cocoa bean shell based media were compared with that in PDA, a commonly used mycological culture medium. In general 20 g agar is used for the preparation of one liter culture medium.

Considering the presence of mucilage in the cocoa plant parts, a pilot study was conducted to find out the minimum agar requirements for solidification of cocoa bean shell (CBS) based medium. The study revealed that 15 g of agar was sufficient for solidification of one litre of CBS based medium. The cocoa bean shell dextrose agar (CBSDA) medium developed using 30 g CBS, 15 g dextrose, 15 g agar and 1000 ml distilled water was found to be better than or on par with freshly prepared PDA medium in growth and sporulation of fungi. PDA (Masago *et al.,* 1977, ChandraMohanan, 1982), V8 juice agar medium (Miller, 1955, Chowdappa *et al.,* 2003), carrot agar medium (Al-Headaithy and Tsao, 1979, Chowdappa and ChandraMohanan, 1993), corn meal agar medium (Eckert and Tsao, 1960, 1962) and several selective media (Shew and Benson, 1982) were successfully used for isolation and maintenance of *Phytophthora* sp. Shelar *et al.* (1998) tried various media for the growth of *L. theobromae* and found Richard's medium as suitable one for the growth and sporulation. PDA, OMA, Coon's medium (Singh and Shankar, 1971), Basal medium (Lal and Tandon, 1972), and Richard's solution (Rangaswami, 1972) were reported as better media for growth and sporulation of *C. gloeosporioides*. All these media reported earlier as good for the growth and sporulation of the three pathogenic fungi are much costlier than CBSDA. Hence, the CBS based media being economical and eco friendly have great potential in different fields of its applications.

Table 1. Growth and sporulation of pathogenic fungi in different combinations of Cocoa Bean Shell Agar (CBSA) medium with and without dextrose

A. *Lasiodiploidia theobromae*

Sl. No.	Treatments	Colony diameter in mm Days after inoculation			Mean rate of growth/ day* (mm)	Degree of Sporulation***
		1	2	Mean		
1	CBS10g, Agar 20g, Distilled Water (DW) 1 L	25.30	57.30	41.300	26.975	3.600 (1.882)
2	CBS 15g, Agar 20g, DW 1 L	25.40	62.30	43.850	28.275	7.553 (2.737)
3	CBS 20g, Agar 20g, DW 1 L	26.30	68.60	47.450	30.300	10.433 (3.224)
4	CBS 30g, Agar 20g, DW 1 L	28.30	72.00	50.150	32.150	12.600 (3.546)
5	CBS 40g, Agar 20g, DW 1 L	25.30	65.00	45.150	28.900	10.133 (3.177)
6	CBS 10g, Dextrose 20 g, Agar 20 g, DW 1 L	36.00	78.00	57.000	37.500	12.867 (3.520)
7	CBS 15 g, Dextrose 20 g, Agar 20 g, DW 1 L	37.50	78.80	58.150	38.450	13.933 (3.722)
8	CBS 20 g, Dextrose 20 g, Agar 20 g, DW 1 L	38.00	83.00	60.500	39.750	16.100 (4.008)
9	CBS 30 g, Dextrose 20 g, Agar 20 g, DW 1 L	47.00	90.00**	68.500	46.000	18.800 (4.330)
10	CBS 40 g, Dextrose 20 g, Agar 20 g, DW 1 L	37.80	81.50	59.650	39.275	14.500 (3.806)
11	PDA (Hi-media)	42.16	87.80	64.980	43.030	14.933 (3.858)
12	PDA-freshly prepared: Potato 250 g, Dextrose 20 g, Agar 2 g, DW 1 L	44.50	90.00	67.250	44.750	17.233 (4.148)
13	Dextrose 20 g, Agar 20 g, DW 1 L	14.00	39.30	26.650	16.825	3.767 (1.885)
14	Dextrose 30 g, Agar 20 g, DW 1 L	21.30	60.00	40.650	25.650	4.433 (2.075)
15	Dextrose 40 g, Agar 20 g, DW 1 L	23.50	61.00	42.250	27.000	6.867 (2.605)
	Gen Mean			51.565	26.975	3.235
	S.E./Plot			0.689	28.275	0.275
	CV %			1.336		8.486

CD (P = 0.05)
colony diameter sporulation=0.198 medium=0.290 days=0.795 media x days=1.125
* mean of four observations, ** Size of petri plate used for culturing: 90 mm
***No. of spores/sporangia per microscopic field (mean of three microscopic fields)
Figures in parenthesis are square root transformed values

B. *Colletotrichum gloeosporioides and Phytophthora palmivora*

Sl. No.	Treatments	*Colletotrichum gloeosporioides* Colony diameter in mm Days after inoculation					Mean rate of / growth day (mm)*	Degree of sporula-tion**	*Phytophthora palmivora* Colony diameter in mm Days after inoculation					Mean rate of growth/ day (mm)*	Degree of sporula-tion**
		2	3	5	7	Mean			2	3	5	7	Mean		
1	CBS 10 g, Agar 20 g, DW 1 L	16.50	21.80	30.50	31.80	25.150	6.540	5.600 (2.354)	18.50	21.66	24.00	28.33	23.123	6.329	5.933 (2.380)
2	CBS15 g, Agar 20 g, DW 1 L	18.50	21.83	32.60	35.00	26.983	7.012	9.300 (3.044)	20.33	22.83	28.00	30.60	25.440	6.937	8.333 (2.839)
3	CBS 20 g, Agar 20 g, DW 1 L	20.80	25.00	41.00	42.00	32.200	8.233	10.333 (3.208)	23.00	26.33	33.00	34.60	29.233	7.955	11.600 (3.390)
4	CBS 30 g, Agar 20 g, DW 1 L	23.00	25.60	42.00	43.00	33.400	8.644	11.233 (3.350)	25.00	31.30	39.60	60.50	39.100	9.874	15.000 (3.867)
5	CBS 40 g, Agar 20 g, DW 1 L	16.50	21.83	38.00	39.00	28.833	7.175	10.333 (3.208)	21.50	23.16	28.00	30.00	25.665	7.089	10.000 (3.150)
6	CBS 10 g, Dextrose 20 g, Agar 20 g, DW 1 L	19.80	25.00	42.10	57.60	36.125	8.720	13.500 (3.668)	20.60	25.60	28.80	32.50	26.875	7.309	16.600 (4.069)
7	CBS 15 g, Dextrose 20 g, Agar 20g, DW 1 L	21.80	29.30	53.30	58.50	40.725	9.921	13.800 (3.706)	22.60	27.00	33.50	40.00	30.775	8.179	19.200 (4.533)
8	CBS 20 g, Dextrose 20 g, Agar 20 g, DW 1 L	24.50	29.30	53.30	61.50	42.150	10.366	13.867 (3.717)	26.16	34.00	40.66	49.10	37.480	9.890	20.600 (4.797)
9	CBS 30 g, Dextrose 20 g, Agar 20 g, DW 1 L	24.80	30.00	54.10	63.00	42.975	10.555	18.000 (4.184)	28.10	48.30	59.16	81.60	54.290	13.410	23.433 (4.889)
10	CBS 40 g, Dextrose 20 g, Agar 20 g, DW 1 L	19.50	25.00	43.00	57.16	36.165	8.712	12.667 (3.555)	25.16	30.50	39.30	42.30	34.315	9.162	18.667 (4.315)
11	PDA (Hi-Media)	19.10	27.00	45.16	59.83	37.773	9.032	14.133 (3.755)	26.60	34.16	49.50	70.60	45.215	11.162	23.067 (4.315)
12	PDA-freshly prepared: Potato 250 g, Dextrose 20 g, Agar 20 g, DW 1 L	24.50	28.10	48.16	60.50	40.315	9.973	16.667 (4.077)	28.50	47.60	59.60	82.80	54.625	13.466	25.733 (5.068)
13	Dextrose 20 g, Agar 20 g, DW 1 L	12.30	14.00	29.00	30.00	21.325	5.226	2.800 (1.664)	13.50	19.00	20.60	22.60	18.925	5.108	3.733 (1.896)
14	Dextrose 30 g, Agar 20 g, DW 1 L	13.50	15.50	30.60	31.60	22.800	5.638	4.633 (2.133)	15.16	19.66	22.30	24.83	20.488	5.535	5.667 (2.541)
15	Dextrose 40 g, Agar 20 g, DW 1 L	15.00	16.60	34.60	36.00	25.550	6.274	4.633 (2.316)	16.33	23.30	26.60	28.33	23.640	6.325	7.200 (2.667)
	Gen Mean					38.806		3.142					32.62		3.142
	S.E/Plot					0.957		0.269					0.775		3.636
	CV%					2.467		8.557					2.169		7.836

CD (P = 0.05)
colony diameter sporulation = 0.039, medium = 0.630
days = 0.398, media x days = 1.543

CD (P = 0.05)
colony diameter sporulation = 0.205, medium = 0-559
days = 0.323, media x days = 1.250

* mean of four observations

**No. of spores/sporangia per microscopic field (mean of three microscopic fields)

Table 2. Growth and sporulation of pathogenic fungi in CBSDA media containing different quantities of dextrose

A. *Lasiodiplodia theobromae*

Sl. No.	Treatments	Colony diameter in mm			Mean rate of growth/ day (mm)***	Degree of sporulation****
		Days after inoculation				
		1	2	Mean		
1	CBS, Agar, DW 1 L*	39.50	76.30	57.900	38.825	13.133 (3.615)
2	CBS, Dextrose 5 g, Agar, DW 1 L	42.50	78.16	60.330	40.790	13.533 (3.675)
3	CBS, Dextrose 8g, Agar, DW 1 L	43.50	79.30	61.400	41.575	13.800 (3.709)
4	CBS, Dextrose 10 g, Agar, DW 1 L	45.13	81.80	63.465	43.015	14.767 (3.838)
5	CBS, Dextrose 15 g, Agar, DW 1 L	47.50	90.00**	68.750	46.250	20.500 (4.527)
6	CBS, Dextrose 20 g, Agar, DW 1 L	48.00	90.00	69.000	46.500	20.600 (4.536)
7	PDA-freshly prepared: Potato 250 g, Dextrose 20 g, Agar 20 g, DW 1 L	44.30	90.00	64.750	44.650	17.233 (4.148)
8	PDA (Hi-Media)	42.00	87.50	67.135	42.875	15.003 (3.868)
	Gen Mean			64.093		3.990
	S.E./Plot			0.429		0.268
	CV %			0.670		3.647

CD (P = 0.05)
colony diameter sporulation = 0.105
medium = 0.252
days = 0.505
media x days = 0.714

* Quantity of CBS and Agar (standardized) constant at 30 g and 15 g/L respectively in all the treatments in Table 3 A, B, C and D
** Size of petri plate used for culturing 90 mm
***mean of four observations
****No.of spores/sporangia per microscopic field (mean of three microscopic fields)

B. *Colletotrichum gloeosporioides and Phytophthora palmivora*

Sl. No	Treatments	*Colletotrichum gloeosporioides* Colony diameter in mm Days after inoculation					Mean rate of growth/ day (mm)**	Degree of sporula-ation***	*Phytophthora palmivora* Colony diameter in mm Days after inoculation					Mean rate of growth/ day (mm)**	Degree of sporula-tion***
		2	3	5	7	Mean			2	3	5	7	Mean		
1	CBS, Agar, DW 1 L*	15.50	18.50	20.80	26.00	20.200	5.448	11.233 (3.350)	18.83	35.80	48.80	70.50	43.483	10.295	15.133 (3.889)
2	CBS ,Dextrose 5 g, Agar, DW 1 L	16.50	20.83	26.80	38.00	25.533	6.495	11.600 (3.402)	21.60	39.00	50.50	72.00	45.775	11.046	18.867 (4.342)
3	CBS ,Dextrose 8 g, Agar, DW 1 L	19.60	26.50	31.60	39.00	29.175	7.631	12.600 (3.494)	23.80	42.16	52.80	74.00	48.190	11.771	19.333 (4.394)
4	CBS, Dextrose 10 g, Agar, DW 1 L	19.83	26.50	39.30	45.50	32.783	8.277	13.500 (3.666)	25.00	43.30	54.80	75.50	49.650	12.170	20.467 (4.522)
5	CBS, Dextrose 15 g, Agar, DW 1 L	24.50	30.00	55.30	65.50	43.825	10.667	19.400 (4.403)	27.83	48.30	59.50	81.30	54.233	13.382	23.067 (4.797)
6	CBS, Dextrose 20 g, Agar, DW 1 L	24.50	30.50	55.80	65.60	44.100	10.737	19.600 (4.426)	27.80	48.50	59.10	81.60	54.250	13.386	23.267 (4.819)
7	PDA- freshly prepared: Potato 250 g, Dextrose 20 g, Agar 20 g, DW 1 L	24.50	29.16	50.50	60.50	41.165	10.178	16.667 (4.077)	27.80	47.60	59.60	82.80	54.450	13.379	26.033 (5.098)
8	PDA(Hi-media)	19.10	27.00	43.16	49.80	34.765	8.574	15.033 (3.876)	26.60	34.16	49.50	70.60	45.215	11.168	23.467 (4.843)
	Gen Mean					33.94		3.837					49.41		4.588
	S.E./Plot					0.836		0.269					0.871		0.152
	CV %					1.855		7.006					1.535		3.317

CD (P=0.05)

colony diameter sporulation = 0.195, medium = 0.553,
days = 0.479, media x days =1.356

CD (P=0.05)

colony diameter sporulation = 0.110, medium = 0.633
days = 0.5000, media x days = 1.415

* Quantity of CBS and Agar (standardized) constant at 30 g and 15 g/L respectively in all the treatments in Table 3 A, B, C and D

**mean of four observations

***No. of spores/sporangia per microscopic field (mean of three microscopic fields)

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