



Cocoa sweating, a promising culture medium for mass production of fungal bioagents

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Abstract

Cocoa sweating collected on the first day of fermentation of beans was used for the preparation of culture media. When cocoa sweating as collected from the fermentary was used as liquid medium to grow *Trichoderma harzianum* and *Metarhizium anisopliae*, there was no growth. Therefore, growth and sporulation of both the fungi in different dilutions of cocoa sweating were compared with that in potato dextrose broth (PDB) and potato jaggery broth (PJB). Growth and sporulation of both the fungi were the highest in cocoa sweating (CS) diluted with tap water (TW) in the proportion CS: TW 1:4 (CS 1:4). Both the biocontrol agents did not exhibit any difference in growth and sporulation in CS 1:4 and in CS1:4 supplemented with 15 g dextrose and pH adjusted to 6.5. Growth and sporulation of both the fungi in CS1:4, PDB and PJB increased with number of days of incubation and was the highest on 20th day after inoculation. When 800 ml CS 1:4 was used to grow the biocontrol agents and to prepare talc formulations using 2 kg talc powder, the moisture content was 8 % after 72 h of drying. Shelf life of talc formulations of both the biocontrol agents could be maintained for 5 months with very high colony forming units(CFUs). The CFUs /g of talc formulations of both the fungi stored for 6 months were less than that in the 5th month.

Keywords: Cocoa sweating, colony forming units, *Metarhizium anisopliae*, mycological culture media, talc formulation, *Trichoderma harzianum*

Introduction

In India, cocoa (*Theobroma cacao L.*) is grown in the states of Kerala, Karnataka, Tamil Nadu and Andhra Pradesh, mainly in the interspaces of arecanut and coconut gardens. The area under cocoa cultivation was 16185 ha during 2001-2002. The expansion of area under cocoa cultivation is steadily improving year after year.

The total area under cocoa cultivation in India was estimated 46,318 ha with an annual production of 11,198 mt dry beans during 2009-10 whereas the area under cultivation during 2003-04 was 25,157 ha. With the increase in production, there is proportionate increase in cocoa wastes/byproducts. During fermentation of cocoa beans, the watery content of pulp called 'sweating' drains out from the fermentation boxes. Though this sweet and nutritious liquid has several uses, it is not commercially used for any purpose in India. Thus, it is

merely wasted from the fermentaries as a major waste of cocoa industry. Therefore, the present study was undertaken to develop a technology for mass multiplication of biocontrol agents such as *Trichoderma harzianum* and *Metarhizium anisopliae* using cocoa sweating as the medium.

Materials and Methods

Cocoa sweating collected on the first day of fermentation process from a cocoa fermentary near the place of study, was used for the preparation of culture medium. The pH of cocoa sweating was determined. Cocoa sweating as collected from the fermentary was dispensed into 250 ml conical flask @ 100 ml/flask and sterilized at 121°C for 20 min. Sterilized cocoa sweating medium was inoculated with 5 mm mycelial disc cut from the periphery of five day old culture of *T.harzianum* and *M.anisopliae*. The inoculated flasks were incubated

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at room temperature for 10 days. Three replications were maintained for each fungus.

As there was no growth of both the fungi in pure cocoa sweating, it was diluted with tap water in different proportions. The pH of each dilution of sweating was determined (Table 1).

Table 1. Dilution of cocoa sweating

Sl.No.	Cocoa sweating	Tap water	Proportion	pH
1	50 ml	50 ml	1:1	3.5-3.8
2	33.3 ml	66.7 ml	1:2	3.7-4.1
3	25 ml	75 ml	1:3	4.4-4.8
4	20 ml	80 ml	1:4	4.5-4.9
5	16.7 ml	83.3 ml	1:5	4.6-4.9
6	14.3 ml	85.7 ml	1:6	4.6-4.9
7	12.5 ml	87.5 ml	1:7	4.6-4.9
8	11.1 ml	88.9 ml	1:8	4.8-5.0

The growth and sporulation of *T.harzianum* and *M. anisopliae* in different dilutions of cocoa sweating (CS) was compared with potato dextrose broth (PDB, pH 5.5-5.8) and potato jaggery broth (PJB, pH 5.5 - 5.7). Cocoa sweating (8 dilutions), PDB and PJB were taken separately in 250 ml conical flask @ 100 ml/flask and autoclaved at 121°C for 20 min. Upon cooling, each flask was inoculated with 5 mm mycelial disc cut from the periphery of actively growing colony of each fungus. Two sets (3 flasks/set) of flasks for each treatment and each fungus were maintained. Thus three replications were maintained for each treatment and each fungus to determine the mycelial growth and degree of sporulation.

One set of inoculated flasks of each treatment was incubated at room temperature for 7 days. Mycelial dry weight was determined after the incubation period.

Spore production in different media

The second set of flasks was incubated at room temperature for 10 days after inoculating with the fungi. To determine the degree of sporulation the medium along with the mycelial mat was blended in a mixer grinder for about 2 min, filtered through muslin cloth and volume was made up to 1 litre. The total number of spores/ml was assessed with the help of a haemocytometer. Among the different dilutions of sweating, 1:4 dilution (20 ml CS: 80 ml tap water) was found to be the best.

Influence of pH and dextrose on growth and sporulation of *T. harzianum* and *M. anisopliae* in cocoa sweating (1:4)

This study was undertaken to further improve the growth of the fungi in CS 1:4. To study the influence of

pH and dextrose on growth and sporulation of *T. harzianum* and *M. anisopliae*, both fungi were grown in undiluted and diluted cocoa sweating (1:4) with and without adding dextrose and with and without adjusting the pH as per the details given in Table 1. Dry mycelial weight and sporulation were recorded. The growth of both the biocontrol agents could not be improved by adding dextrose or adjusting pH of CS 1:4. Hence, for further studies CS 1:4 without dextrose was used.

Influence of days of incubation on growth and sporulation of *T.harzianum* and *M.anisopliae* in diluted cocoa sweating (1:4)

Cocoa sweating (1:4), PDB and PJB were taken in 250 ml conical flask @ 100 ml/ flask, sterilized and inoculated with *T. harzianum* and *M. anisopliae* separately. The inoculated flasks were incubated at room temperature for 10, 15, 20 and 25 days. Three replications were maintained for each treatment (incubation period). Dry mycelial weight and sporulation were recorded.

Mass production of biocontrol agents using cocoa sweating

Since cocoa sweating was found to be a very good medium for mass multiplication of biocontrol agents, further studies were conducted on preparation of talc formulations of *T. harzianum* and *M. anisopliae* using cocoa sweating as the medium for mass multiplication. Cocoa sweating being a new substrate for mass production of biocontrol agents it was compared with the cheap media commonly used in southern parts of India viz., mature coconut water and rice water (from 500 g of rice boiled in 2 l of water).

Talc formulation of *T. harzianum* and *M. anisopliae* - Standardization of quantity of cocoa sweating (1:4)

Different quantities of diluted sweating (CS 1:4) such as 700 ml, 800 ml and 1000 ml were taken separately in 2 l capacity conical flask and sterilized at 121° C for 20 min. PDB was also prepared (1000 ml/ flask) and sterilized. Each flask was inoculated with 5 mm mycelial disc taken from five - day - old cultures of *T. harzianum* and *M. anisopliae* grown on PDA medium. The inoculated flasks were incubated at room temperature for 20 days. Three replications were maintained for each treatment.

Preparation of talc based formulation

Talc powder was filled in heat resistant poly propylene bags of 40×35 cm size @ 2 kg/ bag. The mouth of the bag was sealed and sterilized in an autoclave at 121°C for 20 min.

The fungal biomass of *T. harzianum* and *M. anisopliae* along with the medium was mixed with sterilized talc powder. Three different quantities of the culture (cocoa sweating media with fungal biomass) viz., 700 ml, 800 ml and 1000 ml were mixed separately with each 2 kg talc powder. The PDB culture was mixed at the rate of 1000 ml culture (medium + fungal biomass) with 2 kg talc powder. While mixing the fungal biomass with talc powder, 10 g of carboxy methyl cellulose (CMC) was added for each 2 kg talc. After thoroughly mixing the culture with talc powder, the mixed product was air dried for 72 h at room temperature. After 72 h of drying, the clots in the formulation were broken and moisture percentage of the product prepared with different quantities of culture (in CS1:4 and PDB) was determined. Similarly the CFUs of each treatment were also found out.

Shelf life of talc formulations of *T. harzianum* and *M. anisopliae* grown in cocoa sweating (1:4)

T. harzianum and *M. anisopliae* were grown in diluted cocoa sweating (CS 1:4), PDB, coconut water and rice water and prepared talc formulations, as explained earlier. The commonly used media such as potato dextrose broth, coconut water and rice water were used for comparison. For preparation of talc formulations, 800 ml CS 1:4 and 1000 ml each of PDB, coconut water and rice water along with the fungal biomass were separately mixed with every 2 kg talc and 10 g CMC and allowed to dry. Three replications were maintained for each treatment.

The dried talc based bio-formulation of both *T. harzianum* and *M. anisopliae* were packed in clean polythene bags labeled and stored at room temperature for varying duration and the CFU at different intervals was determined to evaluate the shelf life. The CFUs were determined immediately after preparation and thereafter at 30, 60, 90, 120, 150 and 180 days intervals.

Results and Discussion

Cocoa beans taken out from the harvested ripe pods are surrounded by an aromatic pulp. The mucilaginous pulp is composed of spongy parenchyma cells containing cell sap rich in sugars (10 to 13%), pentosan (2 to 3%), citric acid (1 to 2%) and salts (8 to 10 %) (Lopez, 1986). Approximately 40 l of pulp could be obtained from 800 Kg of wet seeds (Figueira *et al.*, 1993). The sweating is removed through the process of fermentation. But no work has been done so far to utilize the sweating as a medium for growing pathogenic or beneficial fungi.

Growth and sporulation of *T.harzianum* and *M.anisopliae* in cocoa sweating

When undiluted cocoa sweating was used as a liquid medium to grow *T.harzianum* and *M. anisopliae*, there was no growth of any of these biocontrol agents. Growth and sporulation of *T.harzianum* and *M.anisopliae* in cocoa sweating diluted with tap water in different proportions as well as in PDB and PJB were assessed to select the best proportion of CS: tap water. The growth and sporulation of *T.harzianum* and *M.anisopliae* were the highest in CS 1:4 with a pH of 4.5 to 4.9 (Table 2).

Table 2. Standardization of cocoa sweating (CS) as a medium for growth and sporulation of *T. harzianum* and *M. anisopliae*

Sl.No.	Liquid media (100ml/flask)	<i>Trichoderma harzianum</i>		<i>Metarhizium anisopliae</i>	
		Dry mycelial weight (mg/100ml*)	Spore Count (10 ⁶ /ml) **	Dry mycelial weight (mg/100ml)*	Spore Count (10 ⁶ /ml) **
1	Undiluted cocoa sweating (CS)	0.000	0.000(0.000)	0.000	0.000(0.000)
2	CS 1:1	0.000	0.000(0.000)	0.000	0.000(0.000)
3	CS 1 : 2	0.947	11.787 (3.481)	0.827	6.32(2.241)
4	CS 1 : 3	0.987	12.7 (3.561)	0.830	8.993 (2.937)
5	CS 1 : 4	1.763	26.9 (5.172)	1.347	20.543 (4.583)
6	CS 1 : 5	1.430	19.533 (4.420)	1.277	13.657 (3.525)
7	CS 1 : 6	1.387	17.4 (4.420)	1.213	11.893 (3.421)
8	CS 1 : 7	1.253	14.8(3.757)	1.090	9.220 (3.012)
9	CS 1 : 8	1.167	11.307(3. 218)	0.890	8.623(2.8942)
10	Potato Jaggery broth	1.557	21.000 (4.538)	1.298	16.367 (4.114)
11	Potato Dextrose broth	1.553	17.597 (4.538)	1.283	15.507(3.979)
	Gen.Mean	1.09	13.91(3.710)	0.91	10.10(3.144)
	SE/Plot	0.01	1.04	0.03	0.57
	CV%	1.23	4.46	3.70	5.60
	CD (P =0.05)	0.02	1.77	0.06	0.96

*Mean of three replications on 7th day after inoculation

** Mean of three replications on 10th day after inoculation
(Figures in parenthesis are square root transformed values)

Growth and sporulation of both the fungi in CS 1:4 were higher than that in PDB and PJB. Dry mycelial weight and sporulation of *T.harzianum* and *M.anisopliae* in PDB and PJB did not vary. But sporulation of both the fungi in CS 1:4 was significantly higher than PDB and PJB. Both the fungi did not grow at all in undiluted CS (pH 3.5 to 3.8) and cocoa sweating diluted with tap water in 1:1 proportion (pH 3.7 to 4.1). This study indicates that large quantity of cheap and non synthetic medium can be prepared by diluting cocoa sweating four times with water.

Influence of pH and dextrose on growth and sporulation of *T.harzianum* and *M.anisopliae* grown in cocoa sweating

The data on growth and sporulation of *T.harzianum* and *M.anisopliae* grown in undiluted cocoa sweating and diluted CS (1:4) with and without addition of dextrose and pH adjusted to 6.5 are presented in Table 3. Among the eight treatments, cocoa sweating 1:4 supplemented with 15 g dextrose (pH 4.5 to 5.0) exhibited the highest growth and sporulation of both *T.harzianum* and *M.anisopliae*. But this medium was statistically on par with cocoa sweating 1:4 without the addition of dextrose (pH 4.5 to 4.9). Cocoa sweating (1:4) without adjusting pH and CS 1:4 after adjusting pH were also statistically on par for growth and

sporulation of both the fungi. There was only negligible growth of both the fungi in undiluted cocoa sweating with 15 g dextrose and undiluted cocoa sweating with pH adjusted to 6.5.

Growth and sporulation of *T.harzianum* and *M.anisopliae* in CS 1:4 at different intervals of incubation

The growth and sporulation of both the fungi increased with number of days of incubation and reached a peak on 20th day of incubation and thereafter decreased. Thus dry mycelial weight and sporulation of *T.harzianum* and *M.anisopliae* were the highest in all the three media (CS1:4, PDB, PJB) on 20th day after inoculation. Growth and sporulation of *T.harzianum* and *M.anisopliae* in CS 1:4 were higher than that in PDB and PJB. Thus the study further confirmed that CS 1:4 is better than the conventional culture media in growing bio control agents like *T.harzianum* and *M.anisopliae* and 20 day incubation period is sufficient to get better growth and sporulation (Fig. 1 and 2).

Talc formulation of *T. harzianum* and *M. anisopliae*

When different quantities of diluted cocoa sweating (CS1:4) were used to grow *T.harzianum* and *M.anisopliae* and prepared talc formulations, the

Table 3. Standardization of cocoa sweating (CS) as a medium for growth and sporulation of *T. harzianum* and *M. anisopliae*

Sl.No.	Liquid media (100 ml/flask)	<i>Trichoderma harzianum</i>		<i>Metarhizium anisopliae</i>	
		Dry mycelialweight (mg/100ml*)	Spore Count (10 ⁶ /ml) **	Dry mycelial weight (mg/100ml)*	Spore Count (10 ⁶ /ml)**
1	Undiluted Cocoa Sweating (CS) (pH = 3.5 to 3.8)	0.000	0.000 (0.000)	0.000	0.000 (0.000)
2	Undiluted Cocoa Sweating + 15g dextrose(pH = 3.5 to 3.8)	0.933	12.64 (3.551)	0.828	9.650 (3.075)
3	Undiluted CS (pH adjusted to 6.5)	0.973	15.607 (3.920)	0.857	11.390 (3.367)
4	Undiluted CS+Dextrose15g (pH adjusted to 6.5)	1.077	19.533 (4.342)	0.943	13.090 (3.322)
5	Cocoa Sweating20 ml+Tap water 80 ml (1:4) (pH = 4.5 to 4.9)	1.763	27.000 (5.213)	1.347	20.127 (4.392)
6	Cocoa sweating20 ml + Tap water 80 ml (1:4) + Dextrose15 g. (pH = 4.5 to 5.0)	1.767	27.573 (5.242)	1.347	21.367 (4.567)
7	Cocoa sweating 20 ml + 80 ml Tap Water (1:4) (pH adjusted to 6.5)	1.750	27.600 (5.242)	1.346	21.000 (4.553)
8	20 ml CS + 80 ml Tap Water (1:4) + 15 g of Dextrose (pH adjusted to 6.5)	1.753	27.633 (5.255)	1.352	21.500 (4.583)
	Gen.Mean	1.25	19.70 (4.462)	1.00	14.77 (3.913)
	SE/Plot	0.01	0.86	0.02	0.87
	CV%	1.08	4.38	1.54	5.90
	CD (P =0.05)	0.02	1.51	0.03	1.53

*Mean of three replications on 7th day after inoculation

** Mean of three replications on 10th day after inoculation
(Figures in parenthesis are square root transformed values)

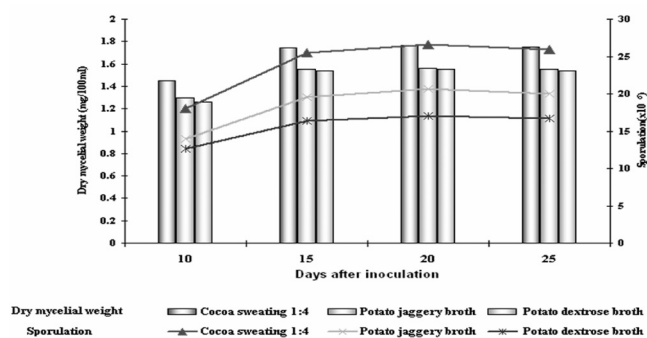


Fig. 1. Influence of incubation period after inoculation on growth and sporulation of *T. harzianum*.

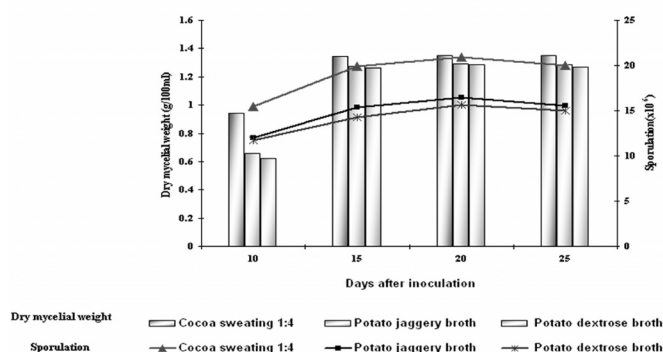


Fig. 2. Influence of incubation period after inoculation on growth and sporulation of *M. anisopliae*

the previous formulation was 9 % which was above the standard fixed for formulations. The CFUs of *T.harzianum* in the talc formulation prepared using 800 ml CS1:4 were $6 \times 10^6/g$ and that of *M.anisopliae* were $4 \times 10^8/g$ which were much above the minimum standard requirement. In this talc formulation the moisture content was 8 % (As per the standards it should not exceed 8 %). Thus 800 ml of CS 1:4 was found to be ideal for growing both bio control agents and preparing talc formulation using medium with fungal biomass +2 kg talc powder+10 g CMC.

Shelf life of talc formulation of *T.harzianum* and *M.anisopliae* grown in cocoa sweating and other conventional media

The colony forming units/g of talc formulation prepared using cocoa sweating 1:4, potato dextrose broth, coconut water and rice water were determined immediately after the preparation(after 72 h of drying)and at monthly intervals up to 6th month. The CFU of both the bio control agents slightly increased 30 days after packing and thereafter gradually decreased with the period of storage. The shelf life of talc formulations of both the fungi grown in different media could be maintained even six months after packing. However, it

Table 4. Talc Formulation of *T. harzianum* and *M. anisopliae* -Standardization of quantity of CS1:4

Sl.No.	Bioformulation	Moisture %	<i>T. harzianum</i>				<i>M. anisopliae</i>			
			Cfu/g in different dilution				Cfu/g in different dilution			
			10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	Mean	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	Mean
1	1:4 CS 700 ml, Talc powder 2 kg, CMC 10 g	7.8	5.500	5.250	5.000	5.250	3.500	3.250	2.750	3.167
2	1:4 CS 800 ml, Talc powder 2 kg, CMC 10 g	8	6.000	5.750	5.500	5.750	5.250	5.000	4.000	4.750
3	1:4 CS 1000 ml, Talcpowder 2 kg, CMC 10 g	9	6.250	6.000	5.750	6.000	5.500	5.250	4.500	5.083
4	Potato dextrose broth 1000 ml, Talc powder 2 kg, CMC 10 g	8	5.750	5.500	5.250	5.500	5.000	4.500	3.750	4.417
	Mean		5.875	5.625	5.375		4.812	4.500	3.750	
	Gen: Mean					5.625				4.354
	S.E/Plot					0.925				1.310
	C.V (%)					17.149				30.079

CMC- Carboxy methyl cellulose

CD (P=0.05)=1.084

CD (P=0.05)=0.733

moisture content of the 72 h dried talc formulations varied with the quantity of medium (Table 4). When 800 ml CS1:4 was used to grow the biocontrol agents and to prepare formulation using 2 kg talc powder and 10 g carboxy methyl cellulose (CMC), the moisture content was 8% after 72 h of drying. Though the CFU/g of talc formulation prepared using fungal mass grown in 1000 ml CS1:4+2 kg talc+10 g CMC were more than that prepared using 800 ml CS1:4, the moisture content in

may be better to use within six months as the CFU of *T. harzianum* were $5 \times 10^6/g$ in the 5th month of storage, whereas it was only $3.75 \times 10^6/g$ in the 6th month in the talc formulation using CS1:4 as the culture medium. The CFU/g of talc formulation of *M. anisopliae* prepared using CS1:4 were $3.25 \times 10^8/g$ in the 5th month whereas it was only $2.75 \times 10^8/g$ in the 6th month (Table 5). Thus cocoa sweating wasted from fermentaries in the cocoa growing regions of the country is found to be a very

Table 5. Shelf life of talc formulations of *T. harzianum* and *M. anisopliae* grown in diluted cocoa sweating (CS1:4) and other commonly used media

Sl.No.	Treatments	<i>T. harzianum</i>								<i>M. anisopliae</i>									
		CFU/gm in different months of storage ((x10 ⁶ /gm)									CFU/ gm in different months of storage(x10 ⁸ /gm)								
		0*	1	2	3	4	5	6	Mean	0*	1	2	3	4	5	6	Mean		
1	Diluted cocoa sweating (1:4) 800 ml	6.000	7.250	6.750	6.500	5.750	5.000	3.750	5.857	4.000	4.750	4.500	4.250	3.750	3.250	2.750	3.893		
2	Potato dextrose broth 1000ml*	5.750	6.750	6.500	6.000	5.250	4.250	3.250	5.333	3.756	4.250	4.000	3.750	3.500	3.000	2.250	3.393		
3	Coconut Water 1000 ml *	5.500	6.000	5.750	5.000	4.500	4.000	3.000	4.821	3.500	4.000	3.750	3.500	3.250	2.750	2.000	3.250		
4	Rice Water 1000ml *	5.000	5.750	5.250	4.750	4.250	3.750	3.000	4.536	3.000	3.500	3.250	3.000	2.750	2.250	1.750	2.786		
	Mean	5.562	6.438	6.000	5.438	4.938	4.250	3.250		3.562	3.938	3.875	3.625	3.312	2.812	2.188			
	Gen.Mean								5.125								3.330		
	Se/Plot								1.195								1.042		
	CV (%)								23.322								31.296		
	CD (P=0.05)																		
			CD for medium = 0.635									CD for medium=0.554							
			CD for months = 0.840									CD for months = 0.733							

Sl. No. 1-4: Each medium mixed with 2 kg talc and 10 g carboxy methyl cellulose and dried. Moisture content = 8 %

* On the day of packing after drying for 78 hours

promising medium for commercial production of the biocontrol agents. Large scale production of biocontrol agents can be taken up as a part of cocoa fermentation units and thereby the income from cocoa industry can be further increased.

Though it is reported that cocoa sweating can be used commercially for the preparation of cocoa jelly (Figueira *et al.*, 1993; Prasannakumariam *et al.*, 2002), binder in pharmaceutical pills (Moorthy and Subba Rao, 1976, 1978, 1980), alcoholic spirit and nata (Sansiah *et al.*, 1991) and acetic acid (Briton-Jones, 1934; Wood and Lass, 1985), it is not practically utilized to the expected level in any of the cocoa growing countries.

Several locally available cheap substrates/ agricultural wastes are used for multiplication of biocontrol agents. Coconut water wasted from copra making industry was found to be very effective medium for mass multiplication of *M. anisopliae*, because this substrate is easily available and can be used without any supplement or modification (Dangar *et al.*, 1991). Anandaraj and Sarma (1997) evaluated coconut water wasted from copra industry for the mass production of *T. hamatum* and *Gliocladium virens* and observed the highest mycelial growth of both the fungal biocontrol agents in coconut water without any supplements. Awuah and Frimpong (2002) prepared cocoa based culture media *viz.*, green cocoa pod husk agar, ripe cocoa pod husk agar, green cocoa bean agar, ripe cocoa bean agar, green cocoa mucilage agar and ripe cocoa mucilage agar and assessed for their clarity and for potential to support mycelial growth and sporulation of *Phytophthora palmivora*. Radial mycelial growth rate of 0.5 mm/day

was obtained on green cocoa mucilage agar. Such media can not be recommended for mass production of biocontrol agents as it may lead to loss of cocoa pods. Ahmed *et al.* (2005) conducted some preliminary studies on mass multiplication of *Trichoderma* sp. using areca bunch waste, areca husk, cocoa leaf and cocoa pod husk. The best growth and sporulation were recorded on areca bunch waste followed by areca leaf, leaf sheath and cocoa leaves. Though some initial attempts have been made to find out the suitability of cocoa waste for multiplication of fungi, these studies were not further continued. In India, large quantity of sweating flowing out of the fermentaries is not only wasted but also causes lot of environmental pollution problems and multiplication of harmful insects and microorganisms. The present study is the first attempt to utilize cocoa sweating wasted from cocoa industry for mass production of biocontrol agents *viz.*, *T. harzianum* and *M. anisopliae*. Thus the investigations revealed that cocoa sweating can be used as a cheap, non-synthetic mycological culture medium as well as for commercial production of biocontrol agents in the cocoa growing areas of the country. Commercial production of biocontrol agents will be economical if locally available and less expensive materials are used for its mass multiplication.

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