

MATURE COCONUT WATER FOR MASS CULTURE OF BIOCONTROL AGENTS*

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Major diseases of spice crops like black pepper, cardamom and ginger are caused by soilborn plant pathogens belonging to the genera *Phytophthora* and *Pythium* (Sarma *et al.*, 1994). These organisms being weather dependent, occur during wet monsoon period and cause severe crop losses. One of the disease management strategies is biological control using antagonistic fungi such as *Trichoderma* and *Gliocladium*. Successful disease suppression is reported in black pepper, cardamom and ginger (Anandaraj and Sarma, 1994; Sarma, 1994; Suseelabhai *et al.* 1993; 1994). For large scale application of biocontrol agents in the field, a suitable medium is essential for mass multiplication. Various organic sources such as agricultural wastes and byproducts are reported to support growth of biocontrol agents (Kousalya Gangadharan and Jeyarajan, 1990). However,

agricultural wastes like coffee husk and farmyard manure are commonly used (Suseelabhai *et al.* 1994; Indu Sawant and Sawant, 1990). Inexpensive agricultural wastes or byproducts would be of great relevance for large scale multiplication of biocontrol agents. Coconut liquid endosperm, though nutritive in tender stages, becomes depleted of nutrients as the nut matures. From mature nut the liquid endosperm is seldom used. Mature coconut when split open for making copra, the coconut water is discarded as an agricultural waste (Jeyalakshmy *et al.* 1988; Nathanael, 1966). The suitability of mature coconut water for multiplying biocontrol agents was studied and the results are discussed.

Mature coconut water (CW) was collected when the nuts were readied for making copra. CW was used either as such

Table 1. Growth of biological control agents in coconut water with and without supplements

Composition of medium (for 100 ml)	Mycelial dry weight (mg)	
	<i>T. hamatum</i>	<i>G. virens</i>
Coconut water (CW)	713.20	837.13
CW 50ml+Distilled water (DW) 50ml	392.40	517.20
CW 25ml+DW 75ml	188.80	226.90
CW 10ml+DW 90ml	88.90	161.93
CW 50ml+DW 50ml+Micronutrients*	308.10	380.80
CW 50ml+DW 50ml+1g Glucose	376.36	492.23
CW 50ml+DW50ml+1g Glucose+1g Yeast extract	412.96	513.83
Rosebengal broth**	490.10	587.33
CD at 5%	17.59	115.54

*Micronutrients for 100ml Medium

Fe⁺⁺0.02mg

Zn⁺⁺0.02mg

Mn⁺⁺0.01mg

Thiamine Hcl 10µ g

**Constituents for 100ml

Dextrose 1.0g

Peptone 0.5g

KH₂PO₄ 0.1g

MgSO₄ 0.05g

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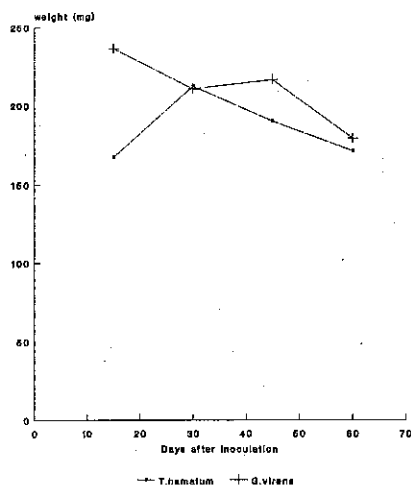


Fig. 1. Growth of biocontrol agents in coconut water

or diluted and supplemented with nutrients as shown in Table 1. This was autoclaved at 15psi for 20 minutes, cooled to room temperature and inoculated with 5mm culture discs of *Trichoderma hamatum* and *Gliocladium virens* cultured on potato dextrose agar. Growth was measured after 15 days by taking dry weight of the mycelium, and spore count was taken by blending the contents of a flask in a homogenizer and using a haemocytometer.

Growth of mycelia was highest in the undiluted coconut water and when it was diluted up to 50% the growth was on par with fungal culture medium for *G. virens* (Table 1). Further dilutions resulted in gradual reduction in the weight of mycelium.

The spore load was 156×10^4 and 165×10^4 for *T. hamatum* and *G. virens*, respectively. The time required for maximum growth was estimated by growing *T. hamatum* and *G. virens* in coconut water for periods ranging from 15-60 days. In case of *G. virens* maximum growth was obtained in 15 days (Fig. 1). Another experiment on the quantity of medium and growth of biocontrol agents has shown that growth is linear and surface area affects the growth of *G. virens*. The growth of *T. hamatum* was both on the surface and in to the medium whereas, in *G. virens* it was restricted to the surface. The surface area available in flasks and Roux bottle were 23.7, 38.46, 63.58, 86.5 sq.cm for flasks of 100 ml, 250 ml, 500 ml and 1000 ml,

Table 2. Growth of biocontrol agents as influenced by quantity of the medium and surface area.

Quantity of medium and type of container	Mycelial dry weight (mg)	
	<i>T. hamatum</i>	<i>G. virens</i>
50ml in 100ml flask	170.9 ^{d*}	150.8 ^b
100ml in 250ml flask	337.5 ^c	223.0 ^b
150ml in 250ml flask	359.0 ^c	239.3 ^b
200ml in 500ml flask	331.5 ^c	258.6 ^b
300ml in 500ml flask	500.6 ^b	280.6 ^b
300ml in Roux bottle	554.0 ^b	460.0 ^a
500ml in 1000ml flask	919.9 ^a	430.0 ^a
CD at 5%	115.71	108.5

*Means with the same superscript do not differ significantly in Duncan's Multiple Range Test

respectively. The surface area of Roux bottle was 200 sq.cm. When 300 ml medium was taken in 500 ml conical flask the dry weight of *G. virens* was 280.6 mg whereas, the same quantity in a Roux bottle yielded 460.0 mg mycelial dry weight which was on par with the weight obtained in 500 ml of the medium in 1000 ml conical flask (Table 2). Since,

G. virens grows only on the surface of the medium the availability of more surface area in Roux bottle contributed to the increased growth.

This study has shown that coconut water which is an agricultural waste could be utilised for large scale multiplication of *Trichoderma* and *Glilotadium*.

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India Institute of Spices Research
Kozhikode - 673 012, India.

M. ANANDARAJ
Y. R. SARMA