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Coconut Water - A Medium for Commercial Production of *Trichoderma viride*

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***Abstract* – The suitability of coconut water as a medium for the mass production of *Trichoderma viride* was studied to find out a cheap and quality substitute for the commonly used media, potato dextrose broth. Among the different concentrations of coconut water tested *viz*., 100, 75, 50 and 25 per cent 100, 75 and 50 per cent were statistically on par in supporting the growth of *T.viride.* But only 100 per cent coconut water is as good as potato dextrose broth in supporting growth of *T.viride*. The possibility of reducing the concentration of coconut water by supplementing with different concentrations of components of potato dextrose broth was studied. A combination of 50 per cent coconut water with 25 g potato and 2 g dextrose per litre (10% concentrations of components of PD broth) was equally good in supporting the growth of biocontrol agent as PD broth. The solid media with different concentrations of coconut water and agar was tested in comparison with the Potato Dextrose Agar. 50 per cent coconut water agar was sufficient to support the mycelial growth of *T. viride* to an extent that obtained in 100 per cent coconut water and PDA. The study reveals the possibility of utilizing coconut water, a waste product from coconut processing industries as a cheap nutrient media for commercial production of biocontrol agent *Trichoderma viride* .**

Keywords* – Biocontrol Agent, Mass Production, Coconut Water, Nutrient Media, *Trichoderma viride.

I. INTRODUCTION

Commercial success of a biocontrol agent depends not only on its bio efficacy or shelf life but also the ease with which it can be mass multiplied on a suitable substrate, which is easily available and relatively cheap. To make the biocontrol agent available for reasonable price is very important for its commercialisation and its adoption by the farmers. For this, the media used for mass production should be cheap and the production process should be cost effective. Search for less expensive and locally available materials for mass production of bio control agents has resulted in the use of several naturally occurring plant derived organic substrates.

Trichoderma is an important biocontrol agent used for the management of various fungal diseases of crop plants [4]. *Trichoderma sp* has been grown on a wide range of solid substrates. But such formulations are bulky and require extensive space for processing and storage. There is greater risk of contamination also. The liquid state fermentation is devoid of such problems and large quantities of biomass can be produced within few days [6]. Utilization of coconut water as a medium for mass multiplication of beneficial microbes has been reported by several workers [2] [3] and [5]. Coconut water is free from any microbial contamination and is highly nutritious, rich in amino acids, vitamins and minerals.

If it is possible to substitute common nutrient media such as potato dextrose broth with coconut water, it would be very cheap. Coconut water is a byproduct of coconut processing industry and is a waste material in most of processing units. In this context, the study was conducted with the objective to test the suitability of coconut water as a medium for mass production of *Trichoderma viride.*

II. MATERIALS AND METHODS

The study "Evaluation of coconut water as a medium for mass production of *Trichoderma viride*" was carried out during 2010 at the department of Plant Pathology, Regional Agricultural Research Station, Pattambi.

The pure culture of *Trchoderma viride* maintained at the Department of Plant Pathology , Regional Agricultural Research Station, Pattambi was utilised for the study. All the experiments were conducted following completely randomised design with four replications.

A. Evaluation of coconut water for growing Trichoderma viride

Different dilutions of coconut water *viz*., 25% (T_1) , 50% (T_2), 75% (T_3) and 100% (T_4) and Potato dextrose broth (Potato 250g + dextrose 20g / litre of water) (T_5) were prepared and 300 ml each were sterilized in bottles in autoclave at 15 lb pressure and 121^0 C or 20 minutes. These were inoculated with 10 mm sized culture discs of *T. viride* and incubated for 10 days. The bottles were kept in rotary shaker. Shaking was given at regular interval of half an hour daily for getting proper aeration and growth. After 10 days of growth, the biomass of the fungus along with the liquid broth was properly mixed using wet mixer grinder. Then mixed inoculum was mixed with the carrier, sterilized talc in a ratio of 300 ml : 1 kg. The formulated product made from different treatments were air dried to reduce the moisture to 12 per cent. The number of colony forming units per gram of the formulated product was determined by serial dilution technique using Trichoderma specific medium (TSM). Observations on number of colonies of Trichoderma were recorded using colony counter.

B. Evaluation of coconut water agar as a solid medium for growing Trichoderma viride.

Coconut water - agar media was prepared using different concentrations of coconut water *viz.*, 25% (T_1), 50% (T_2), 75 % (T_3) , 100 % (T_4) and agar 15 g / l. Potato dextrose agar media was the standard check (T5). Respective media were prepared, sterilized and plated. Using a cork borer 3 mm sized culture discs of *Trichoderma viride* were made. One such disk was placed at the centre of each plate and incubated. The diameter of mycelial growth was measured on third day.

C. Evaluation of coconut water along with supplements for mass production of Trichoderma viride

Treatments

T_1- Coconut water 50 %

T_2 - Coconut water 50 % + 50 % components of PD broth (125g potato + 10g dextrose)

T_3- Coconut water 50 % + 25 % components of PD broth (62.5g potato + 5g dextrose)

T_4 -Coconut water 50 % + 10 % components of PD broth (25g potato + 2g dextrose)

T_5- Potato Dextrose broth (Potato 250 g + Dextrose 20 g + 1000 ml of water)

To 50 per cent coconut water various concentrations of potato and dextrose were added. 300 ml media were autoclaved in bottles. It was inoculated with 3 mm sized discs of five days old culture of *Trichoderma viride* maintained on Potato Dextrose Agar plates. The inoculated bottles were incubated for 10 days in rotary shaker. It was formulated with talc as described earlier and allowed to dry to a moisture level to 12 per cent. The number of colony forming units per gram of the formulated product was determined by serial dilution technique using Trichoderma specific medium (TSM). Plates were incubated at room temperature. Observations on number of colonies of Trichoderma were recorded using colony counter on third day.

III. Results

A. Growth of Trichoderma viride in coconut water

The colony forming units (CFU) of *T. viride* in the talc based formulation from 100, 75 and 50 per cent coconut water were 20.25 X 10^6, 18.25 X 10^6 and 18.00 X 10^6 respectively which were statistically on par (Table1). However, the number of CFU in the formulated product from 50 per cent coconut water was significantly less than that of potato dextrose broth. Only the number of CFU in 100 per cent coconut water was statistically on par with that of potato dextrose broth. The CFU of the *T. viride* in the formulated product from the lowest concentration tested (25%) was significantly less than all other treatments.

Table 1: Effect of different concentrations of coconut water on number of colony forming units of *Trichoderma viride*

Treatments	Number of c.f.u x 10^6 / g of talc based formulation
T_1 - 25 % coconut water	13.50 ^{c}
T_2 - 50 % coconut water	18.00 ^{b}
T_3 - 75 % coconut water	18.25 ^{b}
T_4 - 100 % coconut water	20.25 ^{ab}
T_5 - Potato dextrose broth	22.00 ^{a}
CD (0.05%)	3.25

B. Evaluation of coconut water for growing Trichoderma viride in solid medium

The mycelial growth of *Trichoderma viride* showed statistically significant difference among different concentration of coconut water agar (Table 2). The mycelial growth of the fungus was lowest in 25 per cent concentration which was significantly less than that of PDA and all other treatments. The mycelial growth of the fungus in 50, 75 and 100 per cent coconut water agar were 8.6, 8.5 and 8.72 cm respectively which were statistically on par with that of PDA (9 cm). Hence 50 per cent coconut water agar is sufficient for growing *T.viride.*

Table 2: Effect of different concentrations of coconut water on mycelial growth of *Trichoderma viride*

Treatments	Diameter of mycelial growth (cm)
T1 - 25 % coconut water	3.33 ^{b}
T2- 50 % coconut water	8.60 ^{a}
T3 -75 % coconut water	8.55 ^{a}
T4 - 100 % coconut water	8.72 ^{a}
T5 - Potato dextrose broth	9 ^{a}
CD (0.05%)	0.53

C. Evaluation of coconut water along with supplements for mass production of Trichoderma viride.

In order to increase the population of *T.viride* in the 50 per cent coconut water to the extent that supported by potato dextrose broth, it was supplemented with different concentrations of components of potato dextrose broth. When 50 per cent coconut water was supplemented with 10 per cent components of PD broth (T_5) the colony forming units obtained from the formulated product (27.13 X 10^6) was statistically on par with that obtained from PD broth (31.90 x 10^6). With further increase in concentrations of components of PD broth, there was a slight decline in CFU of *T.viride* (Table 3). It is inferred that 50 per cent coconut water supplemented with 25 g potato and 2 g dextrose per litre is an equivalent substitute media for mass production of *T.viride.*

Table 3: Effect of different treatments on number of colony forming units of *Trichoderma viride*

Treatments	Number of c.f.u x 10^6 / g of talc based formulation
T_1-Coconut water 50 %	24.75 ^{bc}
T_2-Coconut water 50 % + 50 % components of PD broth	16.75 ^{d}
T_3-Coconut water 50 % + 25 % components of PD broth	19.90 ^{cd}
T_4-Coconut water 50 % + 10 % components of PD broth	27.13 ^{ab}
T_5 - Potato dextrose broth	31.90 ^{a}
CD (0.05%)	6.07

IV. Discussion

The present study revealed the suitability of coconut water as a medium for mass production of *Trichoderma viride*. Anandraj and Sharma reported the suitability of 100% coconut water for growing *T. harzianum* [2]. The possibility of reducing the concentration of coconut water to 50% by supplementing it with sugar (0.15 g/l) for

growing *T. harzianum* was reported by Mathew *et al.*[5]. In many of the commercial production laboratories, potato dextrose broth is being used as medium for growing Trichoderma. In the state like Kerala, where coconut is a major crop and coconut processing industries are also common, the potential of utilizing coconut water for the mass production of *Trichoderma viride* is very high. The suitability of coconut water for growing plant growth promoting rhizobacteria such as *Azospirillum*, *Pseudomonas fluorescens*, and *Azotobacter* was found out by Akhilandeswari and Vetriselvi [1]. The suitability of coconut water supplemented with one per cent peptone for growing *Pseudomonas fluorescens* was reported by Senthilkumaran *et al*. [7].

While changing the conventional routinely used medium it is highly essential to ensure the quality of the alternate medium in supporting the growth of the biocontrol agent, in addition to the cost factor. In this context the results of this study is of high relevance. The results of this study revealed that 50% coconut water supplemented with 25g potato and 2g dextrose per litre is a quality medium for growing *Trichoderma viride* and is an equivalent substitute for potato dextrose broth. In this study the quality of the medium was tested by the number of CFU in the final formulated product which is the direct quality parameter in the case of commercial production of biocontrol agents. The results also showed that the undiluted coconut water is also an equally good medium for mass production of *T. viride*. So depending on the availability of coconut water, one can use either undiluted coconut water or 50% coconut water with supplements for mass production of *Trichoderma viride*

The results of this study suggests the possibility of utilization of coconut water, a waste product of coconut processing industry for the commercial scale production of biocontrol agent *Trichoderma viride*. If 50 per cent coconut water with supplements or undiluted coconut water is used, the cost of materials for production media can be reduced substantially to the tune of 90 -100 per cent compared to potato dextrose broth. 50 per cent coconut water agar can also serve as a cheap solid media for growing *Trichoderma viride*.

V. Conclusion

For the large scale production of *Trichoderma viride* undiluted coconut water or 50% coconut water supplemented with 25 g potato and 2 g dextrose can be used as a cheap and quality medium in place of conventional nutrient media such as potato dextrose broth. By the utilization of coconut water which is a waste product of coconut processing industries, an effective waste disposal is also possible. The results of this study will be useful for the biocontrol laboratories engaged in the production of *Trichoderma viride* for reducing their cost of production without affecting quality.

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