

A cheap nutritional liquid medium for enhancement of *Trichoderma harzianum* and *Pseudomonas fluorescens* population

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SUMMARY

Trichoderma harzianum and *Pseudomonas fluorescens* are widely used for the management of soil borne diseases. The chemically defined media used for the development of formulations are expensive. Cheap, abundant and readily available raw materials are required to produce low cost bioformulations. Hence, a study was conducted to find out a cheap liquid medium for the mass multiplication of bioagents for which different concentrations of coconut water along with nutritional supplements were used. The present study clearly indicated that 50 per cent coconut water or 25 per cent coconut water supplemented with sugar @ 15g / l, as cheap nutritional liquid medium for mass multiplication of *Trichoderma harzianum* and *Pseudomonas fluorescens*.

Key words :

Trichoderma harzianum,
Pseudomonas fluorescens,
Coconut water,
Nutritional supplements

Biocontrol of plant disease is an important component of integrated disease management. In view of hazardous impact of agrochemicals, biological control of plant diseases are gaining much importance in recent years. Biocontrol agents are widely utilized for the management of plant diseases in different crops. Among the various bioagents, *Trichoderma* spp. and *Pseudomonas fluorescens* are the widely used ones for the management of plant diseases. One of the important criteria for the bioformulation is that, the medium used for the mass multiplication should support fast multiplication of bioagents and must be cheap and easily available.

Earlier workers have tried various liquid media like molasses yeast medium, Czapek-Dox broth, V-8 broth, Potato dextrose broth for the mass multiplication of *Trichoderma* (Papavizas *et al.*, 1984; Harman *et al.*, 1991.) and King's B broth for the mass production of *P.fluorescens* (Vidhyasekaran and Muthamilan, 1995). Coconut water is an important by-product and a thrown away waste of the coconut processing industry. Matured coconut water consists of 5.4% total solids, 3% soluble sugars, 0.5 % minerals, 0.1 % protein, 0.1 % fat, 60 mg % acidity and pH 5.2 (Sathyavathy,1995).As the matured coconut water contains considerable amount of nutrients especially, sugars and minerals, it is

an ideal medium for the growth of microorganisms. The ability of undiluted coconut water for the growth of *Trichoderma* has been reported by Anandraj and Sharma (1997). The present study was carried out to reduce the concentration of coconut water without affecting the viable cell count of *T. harzianum* and *P. fluorescens*, for which, different dilutions of coconut water with nutritional supplements were used.

MATERIALS AND METHODS

The studies on cheap nutritional liquid medium for enhancement of *Trichoderma harzianum* and *Pseudomonas fluorescens* population were carried out at the Department of Plant Pathology, College of Horticulture, Trichur during 2008-09.

Different concentrations of matured coconut water viz; 100, 50, 25 per cent and 25 % supplemented with magnesium sulphate (2g/l), dextrose (15g/l) and sugar (15g/l) were prepared and sterilized at 1.1 kg cm² for 15 min. In addition, Tapioca powder @ 5g/l was also used in the case of *T. harzianum*.

These sterilized liquid media were inoculated with 7-d-old culture of *Trichoderma harzianum* and 48 h old *P. fluorescens* separately. Cell count and viability were estimated by serial dilution agar plating technique using selective media of the

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organisms (Johnson and Curl, 1972). Observations on number of colonies, growth, sporulation and fluorescence were recorded.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been presented under following heads:

Effect of different concentration of coconut water on the population of *Trichoderma harzianum*:

Data presented in Table 1 reveal that 100 per cent concentration of coconut water (CW) recorded highest sporulation and thick mycelium growth of *T. harzianum*.

It also yielded a high spore count of 7760×10^9 CFU / ml as compared to 50 and 25 per cent. Anandraj and Sharma (1997) also observed highest growth of *Trichoderma harzianum* and *Gliocladium virens* in undiluted coconut water. However, it was noted that, 50 % concentration was sufficient for attaining desired optimum population (1240 to 2230×10^9 CFU / ml.) of *Trichoderma*, even though, its sporulation was less than that of 100 per cent. It was found that, coconut water concentration could be still reduced to 25 % by addition

of nutritional supplements, which provides additional nutrients for growth and sporulation. The maximum population of 19000×10^9 CFU / ml. was recorded in the case of 25% CW + sugar (15g/l) followed by 25% CW + dextrose (15g / l) with 10200×10^9 CFU / ml. Even then, 25 % CW + $MgSO_4$ (2g/l) or Tapioca powder (5g/l) were also equally good on comparison with 50 % coconut water which can also be used as cheaper source. Earlier, Dagdag and Reyes (1991) reported that coconut water supplemented with sucrose gluman and PDA recorded spore count of 10×10^9 CFU / ml and 10×10^3 CFU / ml, respectively. Mathew (2006) also observed that addition of 10 % coconut water to Potato dextrose broth enhanced the conidial yield of *T. harzianum* from 248 to 504×10^9 CFU / ml.

Effect of different concentration of coconut water on the population of *Pseudomonas fluorescens*:

The enumeration of *Pseudomonas fluorescens* in different concentrations of coconut water indicated varied population and amount of pigmentation (Table 2).

In this case, undiluted coconut water recorded lowest population of *P. fluorescens*, however, it showed excellent fluorescent pigmentation. Both 50 % coconut water and

Table 1 : Effect of different concentrations of coconut water on the population of *Trichoderma harzianum* at 10 days after incubation

Sr. No.	Media	No. of colonies* ($\times 10^9$ cfu ml ⁻¹)	Growth	Sporulation
1.	100% Coconut water	7760	+ + + + +	+ + + + +
2.	50% Coconut water	1240	+ + + +	+ + + +
3.	50% Coconut water + antibiotic (Rosellin @ 40mg/l)	2230	+ + + +	+ + + +
4.	25% Coconut water	890	+ + +	+ + +
5.	25% Coconut water + tapioca powder (5g/l)	1140	+ + + +	+ + + +
6.	25% Coconut water + $MgSO_4$ (2g/l)	1090	+ + + +	+ + + +
7.	25% Coconut water + dextrose (15g/l)	10,200	+ + + +	+ + + + +
8.	25% Coconut water + sugar (15g/l)	19,000	+ + + +	+ + + + +
9.	Potato dextrose broth	364	+ + + +	+ + + +

+++++ Excellent, +++ + Very good, +++ Good * Mean of three replications

Table 2 : Effect of different concentrations of coconut water on the population of *Pseudomonas fluorescens* at 48 hours after incubation

Sr. No.	Media	No. of colonies* ($\times 10^{11}$ cfu ml ⁻¹)	Fluorescence
1.	100% coconut water	96	+ + + + +
2.	50% coconut water	1344	+ + + +
3.	25% coconut water	420	+ +
4.	25% coconut water + $Mg SO_4$ @ 2g / l	1140	+ +
5.	25% coconut water + sugar @ 15g / l	7530	+ +
6.	King's B broth	1440	+ + + +

+++++ Excellent, ++++ High, ++ Medium * Mean of three replications

King's B medium were equally effective after 48 h of incubation with a population of 1344×10^{11} CFU/ml. and 1440×10^{11} CFU / ml, respectively and also showed very good fluorescence. 50 per cent concentration could be still reduced to 25 % by addition of sugar (15g/l) which recorded the maximum count of 7530×10^{11} CFU / ml and addition of $MgSO_4$ (2g/l) yielded the population of 1140×10^{11} CFU / ml. Prabhakar *et al.* (2008) reported the use of coconut water for the growth of *Bacillus thuriengenesis*. Thus, the present study, suggested use of either 50 % coconut water alone or 25 % coconut water + sugar (15g /l) are the economically viable media for the mass multiplication of *Trichoderma harzianum* and *Pseudomonas fluorescens* at commercial level.

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