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EVALUATION OF LIQUID MEDIA FOR MASS CULTURING OF TRICHODERMA

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Abstract: *The in vitro mass culturing of Trichoderma is done in sugarcane molasses or Potato Dextrose Broth. Lack of sugarcane industries at Kasaragode makes availability of molasses difficult, and use of Potato Dextrose Broth involves high cost. The district has plenty of Copra processing units where large amount of coconut water is getting waste daily. In vitro studies were conducted for evaluating coconut water and other liquid growth media such as rice gruel, potato extract etc. using dextrose, sucrose and jaggery as the carbon sources. Each treatment was replicated thrice. The results indicated that coconut water with 2% jaggery gave the highest mycelial dry weight of Trichoderma followed by coconut water with 2% sucrose. Sporulation of Trichoderma was also highest in coconut water with 2% jaggery.*

Key words: *mass multiplication, liquid media, Trichoderma.*

Introduction

The excessive use of chemical pesticides caused a negative impact on human health and environmental quality and hasalso resulted in the emergence of many fungicide-resistant pathogen strains. This impact graduallyled to reduction in use of chemical pesticidesand increased the need for biological methods of disease management.The fungal bioagent *Trichoderma* belonging to Hyphomycetesis one of the most common biocontrol agent being used worldwide for the management of various foliar and soil-borne plant pathogens (Dominguesa*et al.*, 2000). Among the Trichoderma species, *Trichodermaviride* isextensively used inthe disease management due to the better ability to promote plant growth and soil remediation activity compared to their counterparts, virus, bacteria, nematodes and protozoa (Mishra and Khan, 2015).

The *in vitro* mass culturing of *Trichoderma* is done in sugarcane molasses or Potato Dextrose Broth. Lack of sugarcane industries at Kasaragode makes availability of molasses difficult, and use of Potato Dextrose Broth involves high cost. Therefore, the present study is carried out to evaluate better substitutes for mass multiplication of Trichoderma which are locally available and cheaper. The district has plenty of Copra processing units where large amount of coconut water is getting waste daily. Utilization of coconut water, a waste product from coconut processing industry, as a medium for mass multiplication of beneficial microbes has been reported by many workers. So, the present study is conducted to investigate the suitability of cheaper carbohydrate liquid media such as rice gruel, potato extract and its combinations with dextrose, sucrose and jaggery for better performance of Trichoderma.

Materials and Methods

Four days old culture of *Trichoderma viride*was used for the study. The treatments included twenty one different liquid media, which consists of combinations of potato broth, coconut water andrice gruel using dextrose, sucroseand jaggery as carbohydratesources.

Each liquid media (50ml quantity) was taken in 100 ml conical flasks and sterilized. Each of this medium was inoculated with two 5mm size mycelial discs of thefour days old culture of Trichoderma. The flasks were incubated under room temperature (28±1ºC) for 10 days. Three replications were kept for each treatment. The culture was harvested by collecting mycelia on pre-weighed filter paper and

the mycelial biomass yield was assessed. The dry weight was determined after 24 hours of oven drying at 60°C. Number of conidia per ml of mycelial suspension wasdetermined by dilution method with the aid of a haemocytometer.The data was subjected to analysis of variance (ANOVA), and the Duncan's Multiple Range test at 5% level of probability was used to test the differences among mean values (Steel and Torrie, 1980).

Results and Discussion

The results of the present study revealed that the maximum dry weight of mycelial mat of 460 mg was produced onCW+ 2% jaggery followed by CW + 2% sucrose and CW + 2% dextrosewhereas,the highest spore count of $20.31x10^6$ was recorded in CW + 2% jaggery followed by CW + 2% Sucrose. The least dry weight of mycelial mat and spore count was obtained on PB (40 mg) (Table 1).

The results reveal that, three media *viz*., CW + 2% jaggery, CW + 2% sucrose and CW + 2% dextrose were superior in biomass production in terms of mycelial dry weight and spore count per ml.Several scientists observed similar results when used coconut water as a liquid medium forthe mass multiplication of Trichoderma. Emerson and Mikunthan (2015) observed high sporulation of *T. viride* in both coconut water and 1% palmyrahjaggery liquid media.Vidhya*et al.* (2015) recorded more colony forming units of *T. viride* in the talc based formulation from 100% coconut water.Anandraj and Sharma (1997) also reported highest growth of *Trichodermaharzianum* and *Gliocladiumvirens* in undiluted coconut water. Similar observations were also reported byMathew*et al.* (2010) who got better sporulation of Trichoderma with 25% coconut water + 1.5% sugar medium.

Most of the previously reported studies included mass multiplication of *Trichoderma* spp. on different concentrations of coconut water or undiluted coconut water, but in the present study, different carbohydrate sources were used along with coconut water, potato broth and rice gruel.

Out of the three liquid media, coconut water was found to be the best both in terms of dry weight and spore production. This is in accordance with the studies by Rachappa*et al.* (2005) who inferred that coconut water being rich in minerals, vitamins and essential nutrients might have stimulated the fungi to produce more spore yield. In the present experiment, jaggery was found to be the best source of carbohydrate which is in line with the studies by Talla*et al.*, (2015) who reported that jaggery was a better substitute to glucose for mass multiplication of Trichoderma.

The present study revealsthat coconut water, which is a waste product of Copra industries, can be effectively utilized for mass multiplication of Trichoderma by which we can assure more biomass production and sporulation of Trichoderma. Moreover, the commonly used carbohydrate source i.e., dextrose can be replaced by jaggery, which can support better sporulation, is cheaper and economical, and by which we can reduce the cost of mass production. Another observation made during the study was that sporulation and mycelial dry weight of Trichoderma in potato -jaggery broth was equivalent to or even better than that produced in potato dextrose broth. Thus, potato jaggery broth can also be used as a substitute for potato dextrose broth.

Conclusion

With the advancement of eco-friendly agriculture, *Trichoderma viride* is becoming a promising bio control agent used in management ofmany soil-borne plant pathogens. High cost of substrates or raw materials is a major constraintforlarge scale production of this bioagent. Therefore, the present study gives a promising recommendation that coconut water in combination with jaggery (2%) can be an ideal, economically feasible medium for mass multiplication of Trichoderma at commercial level.

Table 1: Mycelial dry weight of *T. viride* in different liquid media

Sl. No.	Treatments	Mean mycelial dry weight (mg) at 10 DAI*	Mean Spores count per ml (x 10^6)
1.	PB+ 1% Dextrose	153.31	10.60
2.	PB+ 2% Dextrose	243.35	12.78
3.	PB+ 1% Sucrose	150.00	9.91
4.	PB+ 2% Sucrose	273.33	12.65
5.	PB+ 1% Jaggery	220.00	10.78
6.	PB+ 2% Jaggery	253.27	12.86
7.	PB	36.68	3.26
8.	CW+ 1% Dextrose	393.36	15.52
9.	CW+ 2% Dextrose	396.67	18.13
10.	CW+ 1% Sucrose	303.40	16.14
11.	CW+ 2% Sucrose	413.26	18.82
12.	CW+ 1% Jaggery	376.69	16.64
13.	CW+ 2% Jaggery	460.00	20.31
14.	CW	336.57	14.32
15.	RG+ 1% Dextrose	190.00	9.26
16.	RG+ 2% Dextrose	196.659	9.87
17.	RG+ 1% Sucrose	213.41	9.90
18.	RG+ 2% Sucrose	230.00	10.46
19.	RG+ 1% Jaggery	240.00	9.93
20.	RG+ 2% Jaggery	266.72	8.38
21.	RG	220.00	8.38
	CD (0.05)	36.27	1.72
	SE (±)	12.18	0.58

DAI : Day after inoculationPB : Potato brothCW : Coconut waterRG : Rice gruel

* Figures followed by the same letter do not differ significantly according to one way ANOVA at P 0.05

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