Coconut water amended coirpith - a conducive medium for mass multiplication of biocontrol agent *Trichoderma* spp.

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Abstract

Exploitation and recycling of naturally available agricultural waste is an important component of sustainable disease management. In the present work we have used the commonly available agricultural waste media such as coirpith and coconut water as growth and carrier medium for multiplication of fungal biocontrol agent *Trichoderma* spp. The raw coirpith was decomposed for two months using *Pleurotus platypus* and urea. In order to study the proliferation and population dynamics of *Trichoderma*, three strains of *Trichoderma* viz., *T.viride*, *T.viride* albino mutant and *T.harzianum* P26 were used. In partly decomposed and sterilized coconut coirpith, the population of *Trichoderma* increased dramatically from 10^4 colony forming units (cfu) to 10^7 cfu per gram of coirpith in 10 days. However, the population decreased in un-sterilized coconut coirpith and got stabilized at 10^4 cfu per gram of coirpith after 50 days of growth. When the coirpith was enriched with coconut water, the multiplication of *Trichoderma* was observed in both sterilized and un-sterilized medium. The increase in *Trichoderma* population was from 10^3 cfu per gram to 10^7 cfu per gram in five days in sterilized coirpith amended with coconut water. Experiments were also performed to know the effect of coir extract (aqueous extract) on multiplication of *Trichoderma*. There was no significant change in the population of *Trichoderma* in extract / liquid coir extract throughout the incubation period. Exponential multiplication in coirpith medium could be due to versatile nature of *Trichoderma* for carbon nutrient and also due to major and minor nutrients available in coir compost. From the results it is clear that the coconut water + coirpith medium could be exploited for mass multiplication of *Trichoderma* for management of soil borne diseases of plantation crops.

Key words : coconut water, coirpith, mutants, *Trichoderma*

Introduction

Major diseases of spice crops such as black pepper, cardamom and ginger are caused by soil-borne fungal and bacterial plant pathogens belonging to the genera *Pythium*, *Phytophthora*, *Ralstonia* and *Rhizoctonia* (Sarma *et al* 1994). The importance of biological and ecological methods of disease management in sustainable agriculture need not be overemphasized. Progress in biological disease control using introduced microorganisms in the past years prompted many researchers, administrators and farmers to incorporate such strategies in the integrated disease management. The success of biological control of soil-borne plant pathogens depends mainly on the ability of the introduced microorganism to competitively colonize the rhizosphere region of host plant which is influenced by the availability of nutrient from the substrate or carrier medium through that the BCA is applied. (Papavizas & Lewis 1981). Several techniques have been employed for the multiplication and delivery of biocontrol agents. For example, biocontrol agents have been applied in liquid (Marois *et al.* 1982), in organic matters (Kumar & Marimuthu 1997), as seed or seed piece treatment (Cotes *et al.* 1996) and in vermiculite or in clays such as pyrax (Fravel *et al.* 1983).

Several agricultural waste have been successfully used for growth of biocontrol organisms like *Trichoderma* sp, *Pseudomonas* etc. (Kausalya & Jeyarajan 1990, Anandaraj & Sarma 1997, Suseela Bhai *et al.* 1994, Kumar & Marimuthu 1997). The byproducts of coir industry such as coir dust or coirpith and mature coconut water which are being thrown out as waste have

been successfully used as a multiplication medium. The uses of composted coconut coirpith as a organic manure is well documented (Nagarajan *et al.* 1985).

In the present work the effect of mature coconut water amended decomposed coconut coirpith on growth and multiplication of *Trichoderma* sp. has been studied. Experiments were performed in sterile and nonsterile coirpith medium with three strains of *Trichoderma* for precise quantification of population.

Materials and methods

Preparation of decomposed coconut coirpith (DCCP)

The DCCP was prepared by inoculating the raw coconut coirpith with *Pleurotus platypus* (Theradimani and Marimuthu 1992). Two months old compost was used for the study.

Preparation of coconut water amended coirpith

Mature coconut water collected from Calicut was mixed with coirpith at a ratio of 4:1 (Moisture content after adding coconut water was 20%). The mixture (50g) was autoclaved for 1 hour at 121°C.

Multiplication and sporulation of Trichoderma *in coconut water, in shake and still cultures*

50ml of mature coconut water was inoculated with 500 µl of conidial suspension of *T.harzianum* or *T.virens* in 100ml flasks. The flasks were incubated at 24°C or 30°C in shaker (150 rpm) or in a BOD incubator. Population of *Trichoderma* was enumerated after 2 and 5 days of incubation using *Trichoderma* selective medium (Elad & Chet 1982).

Preparation of Trichoderma *inoculum*

Three strains of *Trichoderma* viz., *T.viride* NRLM, *T.viride* NRL, and *T.harzianum* P-26 were used to monitor the population proliferation in composted coconut coirpith. The descriptions of the strains are mentioned in (Table 1).

Trichoderma strains were grown on *Trichoderma* selective medium (Elad and Chet 1982) and discrete colonies of *Trichoderma* were used for preparation of conidial suspension in sterile distilled water. One ml of conidial suspension was inoculated in 50g of coirpith and mixed thoroughly. After inoculation the coirpith was incubated at 30°C for fifty days.

Enumeration of Trichoderma *from coirpith*

Population of *Trichoderma* in sterile and nonsterile coirpith was estimated immediately after inoculation and also at every 10 days interval up to 50 days. 5g of coirpith was suspended in 45 ml of sterile distilled water and was shaken well in a orbital shaker for 30 min. at 200rpm. Ten fold dilution was prepared to obtain 10^{-4} to 10^{-5} dilutions of coirpith suspension. One ml of aliquot from diluted suspension (10^{-4} or 10^{-5}) was plated on selective medium and the petri plates were incubated for 7 days at 30°C. Enumeration was also performed

Table 1. Characters of *Trichoderma* spp. used for enumeration studies.

Strain	Species	Phenotype	Host	Antagonistic to
NRLM	*T.viride*	White colonies	Mung bean Albino mutant obtained using uv irradiation.	*Macrophomina phaseolina*
NRL	*T.viride*	Dark green colonies	Mung bean	*M. phaseolina*
P-26	*T.harzianum*	Whitish-light green colonies	Black pepper	*Phytophthora capsici.*
P-12	*T.virens*	Whitish-dark green colonies	Black pepper	*Phytophthora capsici*

in unamended coirpith and also in unsterile coirpith.

Effect of coir extract on multiplication of Trichoderma

In order to study the effect of aqueous extract of coirpith on growth and survival of *Trichoderma*, 1ml of conidial suspension was inoculated in 20ml coir extract and the flasks were incubated in orbital shaker at 150rpm. The coir extract was prepared by homogenizing 200g of DCCP in 200ml distilled water in polytron homogenizer (Kinematica, Switzerland) at 25,000 rpm for 30min. The resultant extract was used for studying the survival of *Trichoderma.*

One ml of extract, immediately after inoculation and after every 10 days interval up to 40 days, was ten fold diluted to obtain 10^{-3} to 10^{-4} dilution and 1ml was plated on TSM. Number of colony forming units was calculated after 7 days.

Nutrient analysis of decomposed coconut coirpith

The nutrient composition of the coir compost was determined by total digestion of the material by diacid mixture & micronutrients by standard procedures (Jackson 1967, Hesse 1971). Organic carbon content was measured by wet oxidation method (Jackson 1967).

Results and discussion

Many substrates have been evaluated and found suitable for mass multiplication of *Trichoderma* (Prakash *et al.* 1999, Sawant & Sawant 1990). The most ideal substrate is one which support maximum growth and sporulation in short time; which is cheaply available and is environment friendly. Since coconut based agricultural waste is available plenty in Southern states viz., Kerala, Karnataka, Tamilnadu and Andhra Pradesh, studies were conducted to know the suitability of two products from coconut viz., coirpith and coconut water for mass multiplication of biocontrol agent *Trichoderma*. In mature coconut water both *T.harzianum* and *T.virens* multiplied exponentially. Between 2 and 5 days after inoculation, *T.harzianum* sporulated to the order of 10^7 to 10^9 cfu per ml of liquid medium. The increase in number of propagules (cfu) is 3.8 to 21.1 fold for P 26. Similarly the multiplication of *T.virens* P 12 is in the order of 10^7 to 10^9 under all incubation conditions. Increase in population is 3.8 to 417.1 fold for P 12. (Table 2).

Table 2. Multiplication of *Trichoderma* sp. in autoclaved coconut water

Incubation	*T.harzianum* P 26			*T.virens* P 12		
	2 DAI*	5 DAI	Ratio**	2DAI	5 DAI	Ratio
Still culture 24°C	97.3 x 10^6 (18.39)^{F}	2053 X 10^6 (21.45)^{A}	21.1	0.917 x 10^6 (13.66)^{K}	383 x 10^6 (19.73)^{DE}	417.7
Shake culture 24°C	54.4 x 10^6 (17.81)^{FG}	205 x 10^6 (19.147)^{E}	3.8	15.25x10^6 (16.13)^{HI}	467x10^6 (19.96)^{CD}	30.6
Still culture 30°C	100.0 x 10^6 (18.42)^{F}	787 x 10^6 (20.48)^{BC}	7.9	80.75x 10^6 (18.20)^{F}	303x10^6 (19.52)^{DE}	3.8
Shake culture30°C	8.83 x 10^6 (15.99)^{HI}	50x 10^6 (17.39)^{G}	5.7	15.67x10^6 (16.55)^{H}	145.7x10^6 (21.10)^{AB}	9.3
Shake culture 35°C	2.5 x 10^6 (14.60)^{J}	35.0 x 10^6 (17.34)^{G}	14.0	5.33 x 10^6 (15.46)^{I}	76.7X10^6 (18.14)^{F}	14.4

* Days after inoculation

**Increase in propagule = $\frac{\text{Population at 5 DAI}}{\text{Population at 2 DAI}}$

Figures in the paranthesis are natural log transformed

Data with same letter designations are not significantly different according to Duncan's Multiple Range Test at p=0.05

Accurate quantification of fungal population in complex organic matter is possible with the help of genetically marked strains. In the present work a mutant strain of *T. viride* that is distinct from wild type strain was used to study the population proliferation. This strain enabled us to differentiate it from locally available native strains. Use of genetically marked strains have been suggested for precise tracking of introduced microorganisms in soil and other agricultural environment.

When *Trichoderma* was inoculated in sterilized coirpith the population of NRLM has increased from 15.6 x 10^4 to 9.7 x 10^6 cfu per gram of coirpith in 10 days (Fig. 1a). Similarly the population of *T.viride* wild type and *T.harzianum* P 26 has increased from 10^4 to 10^6 cfu per gram (Fig. 1b & 1c). After 50 days of incubation the population was stabilized at 10^4 or 10^6 cfu which indicates the shelf life of *Trichoderma* in sterile coirpith. Availability of large quantity of macro and micronutrients from coirpith (Nagarajan *et al.* 1985) and absence of competing microorganisms might have contributed for the successful colonization of *Trichoderma* in sterile coirpith. When *Trichoderma* was inoculated in unsterile coirpith the population declined from 10^6 cfu to 10^4 cfu per gram of coirpith. Decline in the population of *Trichoderma* in unsterile coirpith could be due to the presence of large variety of fast growing bacteria and other fungi (Table 3). The difference in the population of *Trichoderma* in sterilized and unsterilized media clearly indicate the poor competitive nature of *Trichoderma*. This finding supports the view of Adams (1990) who observed poor competence of *Trichoderma* in rhizosphere of plants. However, the population was stabilized at (3.5 to 6.9 x 10^3) 10^3cfu after 50 days of inocubation.

Population of *Trichoderma* increased exponentially when the fungus was inoculated in coconut water amended coirpith. The data on the population of *Trichoderma* in coconut water amended coirpith is presented (Fig. 2a to 2c). The colony forming units (cfu) of *Trichoderma* increased from 10^3 to 10^5 or 10^7 in 5 days of incubation in sterilized medium. It is interesting to note that even in unsterilized medium the population increased to 10^5 cfu per gram of coirpith. After 40 days the population of *Trichoderma* was stabilized at 10^3 cfu per gram of unsterilized coirpith and 10^6 cfu per gram of sterilized coirpith. Multiplication of *Trichoderma* spp. in coconut water amended coirpith could be due to nutritive liquid endosperm. In another study,

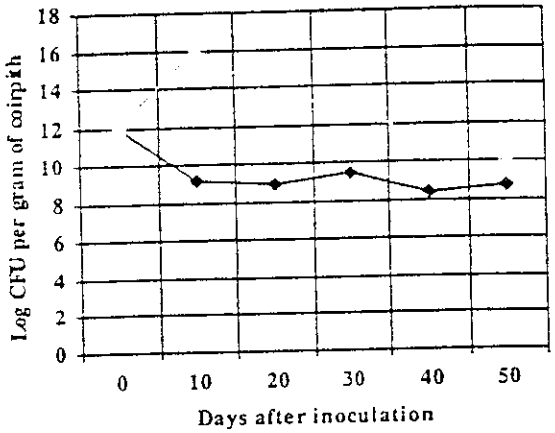


Fig 1a. Population dynamics of *T. viride* NRLM in decomposed coconut coir pith

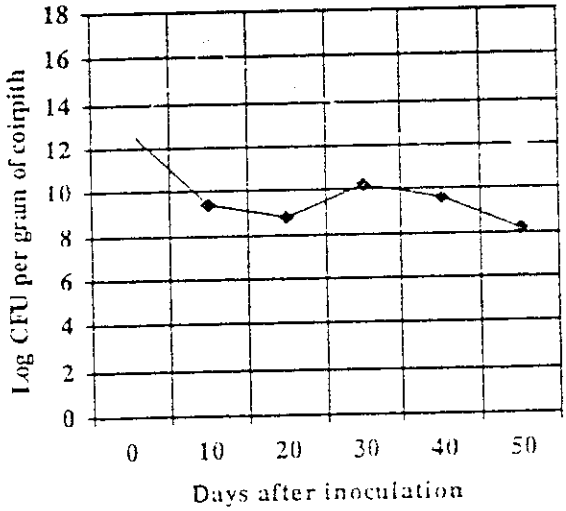


Fig 1b. Population dynamics of *T. viride* NRL in decomposed coconut coir pith

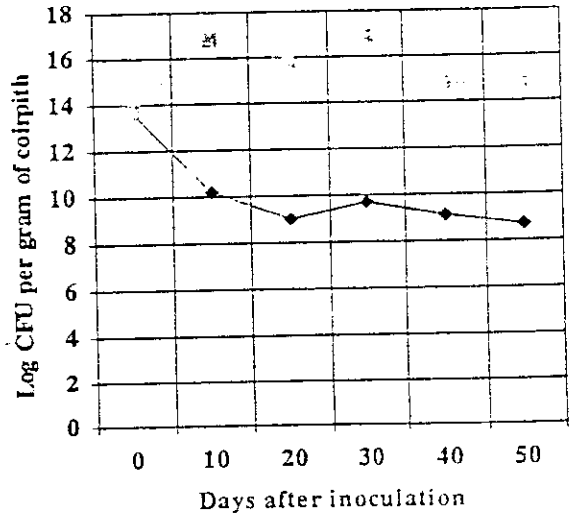


Fig 1c. Population dynamics of *T. harzianum* P 26 in decomposed coconut coir pith

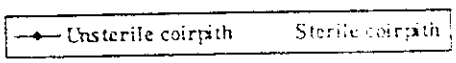


Table 3. Population of indigenous fungi isolated from coconut coirpith (10 DAI)

Coir pith	Population of fungi (cfu x 10^4)
DCCP uninoculated	2.502 (10.13)^c
DCCP + *T.viride* (Albino)	5.94 (10.98)^a
DCCP + *T.viride* (wild type)_	3.49 (10.44)^b
DCCP _+*T.harzianum*	4.01 (10.59)^b
Autoclaved DCCP + *T.viride*	0 (0)^d
Autoclaved DCCP + *T.viride*	0 (0)^d
Autoclaved DCCP + *T.harzianum*	0 (0)^d

* Days after inoculation

Figures in the paranthesis are natural log transformed
Data with same letter designations are not significantly different according to Duncan's Multiple Range Test at p=0.05

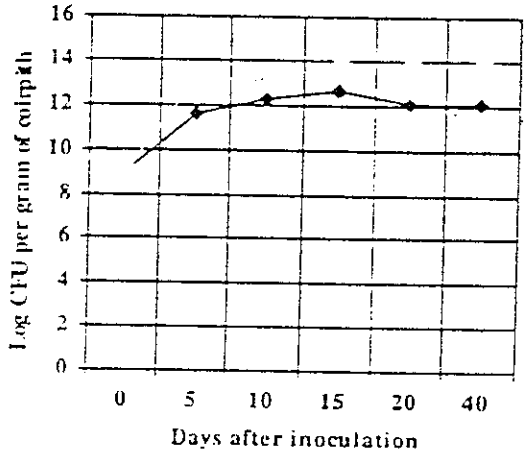


Fig. 2a Population dynamics of *T. viride NRLM* in coconut water amended coirpith

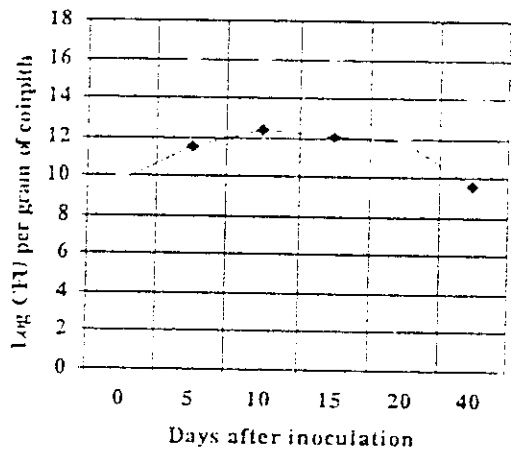


Fig. 2b Population dynamics of *T. viride NRL* in coconut water amended coirpith

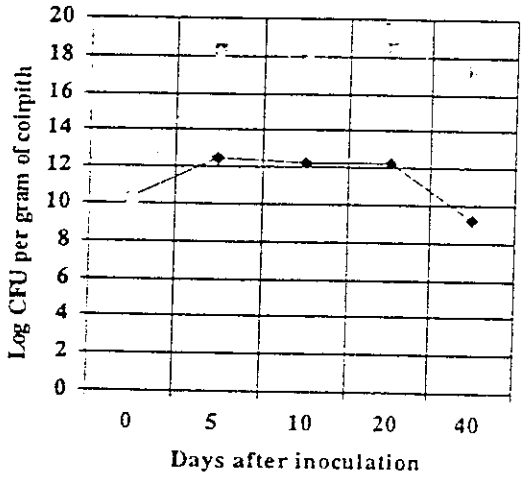
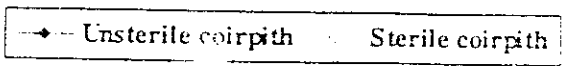


Fig. 2c Population dynamics of *T. harzianum P 26* in coconut water amended coirpith



population of 156 x 10^4 and 165 x 10^4 cfu per ml of coconut water was obtained for *T.harzianum* and *G.virens* respectively (Anandaraj & Sarma 1997). The nutrient rich coconut water might have contributed for exponential multiplication of *Trichoderma* in coconut water amended coirpith.

Experiments were conducted to study the effect of aqueous extract of coirpith on population of *Trichoderma*. There was no multiplication of *Trichoderma* in coir extract. The initial population of 10^5 cfu per ml of extract has declined to 10^3 cfu per ml of extract after 40 days of incubation (Fig. 3). Very high concentration of phenols in the aqueous extract could be one of the possible reasons for failure of *Trichoderma* to grow in the extract.

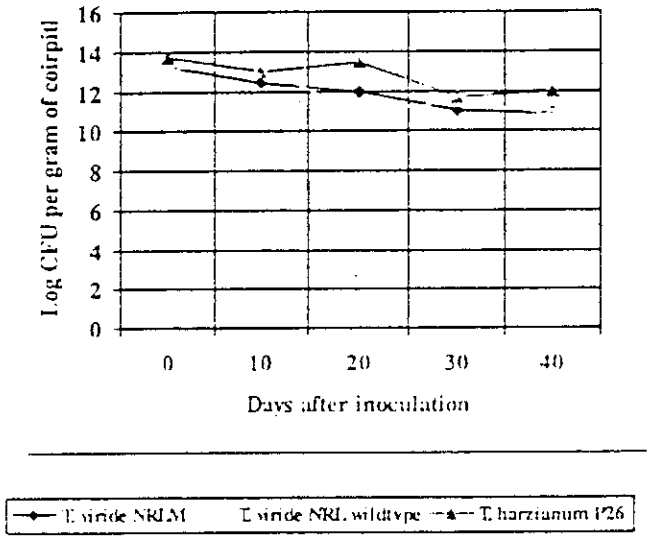


Fig. 3. Population dynamics of *Trichoderma* spp. in aqueous extract of coir pith.

In sterilized coirpith multiplication of *Trichoderma* indicate its versatility for carbon nutrition. The data on nutrient content of DCCP (Table 2) reveals its value as a organic manure and also as a mass multiplication medium for *Trichoderma*. On incubation the C/N ratio of the raw coir compost (8.96) has been brought down to the lowest of 7.72. The rate of reduction was high in sterilized decomposed coirpith as compared to unsterilized which could be due to exponential multiplication of *Trichoderma* in sterile coirpith. P 26 strain was highly efficient in both unsterilized and sterilized medium in utilizing the carbon source. The *Trichoderma* inoculated coir composts recorded increased 'P' availability after 50 days of incubation with highest availability in P 26 inoculated treatment (250 ppm). The 'P' solublisation capacity of P 26 might be the reason. Slight reduction in the contents of K,Ca, Mg and Zn in *Trichoderma* inoculated coir compost as compared to uninoculated control corroborate with the high

Table 4. Nutrient content of DCCP inoculated with *Trichoderma* spp.

Treatment	C/N	P (ppm)	K %	Ca %	Mg %	Fe	Mn ppm	Zn	Cu
DCCP*	8.96	180	0.48	0.11	0.13	320	10.5	10	3.2
DCCP +*T.viride* (albino)	8.11	230	0.42	0.06	0.10	288	11.0	8	5.0
DCCP +*T.viride* (Wild type)	9.76	180	0.44	0.08	0.12	499	11.4	9	4.2
DCCP-P 26 + *T.harzianum* (P-26)	7.79	240	0.45	0.09	0.12	479	12.1	7	4.5
Autoclaved DCCP + *T.viride*	7.76	190	0.44	0.085	0.12	470	11.2	9	4.5
Autoclaved DCCP+ *T.viride*	10.7	200	0.48	0.11	0.11	439	10.7	6	4.4
Autoclaved DCCP + *T.viride*	7.72	250	0.35	0.07	0.10	619	11.3	8	4.0

* Decomposed coconut coirpith.

rate of proliferation of *Trichoderma* population in those treatments (Table 4). Fe & Cu content in the coir compost due to higher proliferation of *Trichoderma*, there by release. From the results it is clear that the coconut water amended coirpith can be exploited for mass multiplication of *Trichoderma* that can be used for disease management in plantation and spice crops in India.

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