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**Research Article**

# Isolation, Identification and Mass production of five *Trichoderma* spp. on Solid and Liquid Carrier Media for Commercialization

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## Abstract

*Trichoderma* spp. are fast growing fungi, widely used as bio-control agents for controlling soil borne diseases of plants, as well as growth promoters. The present investigation was carried out to isolate and identified fast growing *Trichoderma* spp. and mass production using low cost widely available solid and liquid carrier materials. Several fungal spp. were isolated and colony and microscopic characterization were observed in order to identify *Trichoderma* to generic levels following identification keys. Fast growing *Trichoderma* spp. were confirmed up to species level following ITS-PCR and sequencing. Identified five *Trichoderma* spp. were inoculated in solid (coir dust, saw dust and parboiled rice) and liquid (coconut water and rice washed water) carrier materials separately under sterile conditions. Viability and stability of each *Trichoderma* spp. was tested for 180 days at 40 days intervals by calculating the colony forming units per milliliter (CFU/ml). Twelve *Trichoderma* isolates were identified up to generic level and five *Trichoderma* isolates were identified as fast growing species based on their growth rates. Following molecular characterization, identities of these five *Trichoderma* spp. were confirmed as *T. viride*, *T. asperellum*, *T. virens*, *T. longibrachiatum* and *T. harzianum*. *Trichoderma* spp. incubated in solid carrier materials showed higher ($10^6$ CFU/ml) values than the liquid carrier materials ($10^4$ CFU/ml) after $180^{th}$ days and it was significantly different. The highest stabilization potential was recorded for parboiled rice ($5 \times 10^6 - 8 \times 10^6$ CFU/ml) from day $20^{th}$ to$180^{th}$ for all five *Trichoderma* spp. Locally available solid and liquid substrates can be used for the mass multiplication of selected *Trichoderma* spp. to maintain the viability and virulence for long term commercial availability.

**Keywords:** *Trichoderma;* mass production; liquid; solid; carrier media.

## Introduction

*Trichoderma* species are free living, filamentous fungi that multiply rapidly on many substrates such as soil, decaying wood materials and as root colonizers (Grondona *et al.*, 1997). The diverse metabolic capability and aggressively competitive nature help them to succeed in their habitats (Barari, 2016). When considering the morphological characterization such as conidia, they form mainly compact pustules which are typically in green shades, grey or brown (Sriram and Savitha, 2015). Use of colony morphological characters is the conventional method to identify *Trichoderma* species. Therefore, microscopic characteristics of *Trichoderma* isolates were studied in order to identify them at least up to generic level and to separate the different isolates from each other (Samuels *et*

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*al*., 2002). Information from microscopic studies are not specific to precisely identify *Trichoderma* species level because they have relatively few morphological characters and limited variation, that it may cause overlapping and misidentification of the isolates (Chennappa *et al*., 2017). Therefore, it is essential to use a molecular technique to compensate for the limitations of morphological characterization.

*Trichoderma* species have a high potential for reproduction and sporulation, as well as competitive ability and saprophytic survival (Howell, 2013). *Trichoderma* strains grow rapidly when inoculated in the soil, since they are naturally resistant to many toxic compounds, including herbicides, fungicides and pesticides (Seethapathy *et al*., 2017). The genus *Trichoderma* comprises of numerous strains that act as efficient Biological Control Agents (BCA). Several *Trichoderma* species are used as BCA against plant pathogenic fungi such as *Sclerotinia*, *Verticillium*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *Thielaviopsis paradoxa* under both greenhouse and field conditions (Herath *et al*. 2015). The most effective species of *Trichoderma* are *T. virens*, *T. viride*, *T. koningii*, *T. polysporum*, *T. hamatum* and *T. harzianum*. *Trichoderma* species possess several control mechanisms to act against phytopathogenic organisms. These bio-control mechanisms include competition with plant pathogens, mycoparasitism, antibiosis, production of lytic enzymes and secretion of secondary metabolites (Woo *et al.*, 2007). In addition to that, competition for nutrients or space, inducing resistance in host plants and inactivation of pathogen's enzymes are also utilized to control pathogens (Harman, 2000).

*Trichoderma* spp. control deleterious microorganisms which are cause a number of diseases and they produce growth stimulating factors such as plant hormones and growth factors (Singh *et al.*, 2014). They increase the nutrient uptake through enhanced root growth or by promoting the availability of necessary nutrients. While also helping to reduce the concentrations of inhibitory substances of plant growth in soil (Veeken, *et al*., 2005). As most of the *Trichoderma* spp. are strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic producers, they are important in designing effective and safe bio-control strategies (Kumar *et al*., 2012).

Mass scale production of *Trichoderma* spp. would have great potential for commercial use. Mass production of *Trichoderma* spp. has become a focus of research, in the search for alternatives to pesticides and chemical fertilizers for control of plant diseases and growth promoters of plants (Parkash and Saikia, 2015). Many studies have reported on the use of conventional synthetic media like glucose, cellulose, soluble starch, and molasses to produce *Trichoderma* spp. However, the cost of these raw materials for commercial production of BCAs is one of the major limitations behind the restricted use. In recent years, considerable success has been achieved by the use of fungal bio control agents. But so far there is a lack of practicable and effective formulation of bio-agents to exploit it commercially (Subash *et al.*, 2013).

Micropropagules of *Trichoderma* spp. in the form of conidia are preferred over chlamydospores and mycelial biomass because of the viability and stability in field application. Addition to that solid state fermentation is a cost effective system for sporulation of fungi (Cavalcante *et al.*, 2008). In the investigation of Babu and Pallavi (2013) describe the way of use of household waste, vegetable waste and pother waste are good resources for formulation and stabilization of different *Trichoderma* spp. For commercial production, it is essential for the product to possess certain qualities. There is need for optimizing the operating parameters to increase sporulation or conidia production by adapting to cheaper and alternative substrates to achieve economically better yield. The dose requirement should be minimum, must have large shelf life, easy to apply, free from contamination, and should have economic feasibility with positive monetary return.

Therefore, stabilization and mass production of *Trichoderma* species as commercially available bio control agents in mostly available carrier material is very frequent in temperate countries and some tropical countries. There are no any published research work in Sri Lanka for the formulation and stabilization of *Trichoderma* species in different carrier materials in order to introduce them commercially except the investigations on their activities in research levels. There are only few published articles and *in vitro* experiments available for the formulation and stabilization of different *Trichoderma* spp. as bio control agents and plant growth promoters. So that the present investigation is carried out in an attempt to develop a better organic carrier material for the five species of *Trichoderma* (*T. viride*, *T. asperellum*, *T. virens*, *T. longibrachiatum* and *T. harzianum*) which is required by the *Trichoderma* spp. to maintain the viability and the bio controlling ability for a long duration. In order to introduce them into the market as bio control agents and plant growth promoters the knowhow of their formulation and stabilization in different carrier materials are significant.

## Materials and Methods

***Isolation and Identification of Trichoderma spp.***

The soil samples with decomposing plant litter material were collected from Sinharaja, Hakgala and Kitulgala rain forests and surrounding areas in Sri Lanka. *Trichoderma* spp. were isolated into PDA plates using serial dilution and spread plate techniques. The plates were incubated at room temperature (28 °C) for 2 days. Suspected *Trichoderma* colonies were sub-cultured on new PDA plates to obtain

pure cultures for further observation. By preparing sticky tape slides and using graticular calibrated phase contrast microscope, spore shape, spore size and mycelial width were measured and recorded. Identification was carried out according to the morphological and microscopic characteristics following the identification keys in Compendium of soil fungi (Domsch *et al.*, 1980).

***Growth Rates of Trichoderma Isolates***

The growth rate of each *Trichoderma* isolate on PDA plates were studied (9cm diameter). Mycelial plugs (3 mm) from the margin of growing fungal colonies were cut and placed at the center of the agar plates. The diameter of each colony was measured at every 24 hour-intervals until the agar plates were fully colonized. The growth trial was repeated once and the average diameter of the colonies was taken from the three independent growth trials.

***Molecular Characterization of Trichoderma spp.***

Total DNA was isolated from 15 mg of each sample and 5µl of extracted DNA was subjected to the Polymerase chain reaction (PCR) using primers ITS1 and ITS4. Amplified DNA was subjected to DNA sequencing and the obtained DNA sequences were analyzed homologies NCBI BLAST (Basic Local Alignment Search Tool) data base compared with already existing DNA sequences in GenBank in order to identify the isolates into species level (Altzchul *et al.*, 1990).

***Stabilization and Mass Production of Trichoderma spp.***

Mass production of selected *Trichoderma* spp. were done on naturally available solid substrates carrier materials (coir dust, saw dust and paddy seeds) and liquid carrier materials (coconut water and rice washed water) separately.

***Solid carrier media***

Two kilograms of coir dust, saw dust and paddy seeds were washed thoroughly and then the paddy seeds were soaked for 24 hours in water and boiled, until husk was split. Coir dust and saw dust were sieved using a 3 mm mesh. To each carrier material 2.5 g of dry poultry manure and 2.5 g of sugar was added (Table 1). The moisture level of each medium was adjusted to 40% by adding distilled water. The prepared media were put in polypropylene autoclave bags (15 x 20 cm), and five replicates of each treatment was prepared. All media were prepared and autoclaved at 121 $^{o}$C (15 psi) for 25 minutes. Tetracycline (500 mg) was added into 250 g of each medium to inhibit bacterial growth. Afterwards, each *Trichoderma* spp. grown on PDA mat (2cm ×2 cm) was inoculated separately in to all carrier media under sterile conditions and incubated at room temperature (28$^{o}$C) for 180 days.

***Liquid Carrier Media***

Potato dextrose broth, coconut water and rice washed water were tested as liquid carrier material for mass production of *Trichoderma* spp. Coconut water and rice washed water were adjusted by adding sieved dry cow dung (2.5 g per 250 ml) (Table 2). Prepared liquid carrier media were filled into 250 ml of Erlenmeyer flask with screw cap individually. Five replicates of each treatment were prepared. All media were sterilized by autoclaving at 121$^{o}$C (15 psi) for 25 minutes and cooled to room temperature before inoculation. Tetracycline (500 mg) was added to each flask to inhibit bacteria growth. Five *Trichoderma* spp. grown on PDA (2cm ×2 cm) were inoculated into all liquid media under sterile conditions and incubated at room temperature (28 $^{o}$C) on a shaker at 60 rpm for 180 days.

**Table 1**: Composition of different solid carrier materials – inoculum bags

| Solid media | Composition (250 g) |
|---|---|
| 1 - M.C.D | Coir dust (245 g)+ Dry poultry manure (2.5 g) + Sugar (2.5 g) |
| 2 - M.S.D | Saw dust (245 g)+ Dry poultry manure (2.5 g) + Sugar (2.5 g) |
| 3 - M.P.C | Parboiled rice (245 g) + Dry poultry manure (2.5 g) + Sugar (2.5 g)<br>Dry poultry manure (5 g) + Sugar(5 g) |

**Table 2**: Composition of different liquid carrier materials

| Liquid media | Composition ( 250 ml) |
|---|---|
| 1- P.D.B | Distilled water (250 ml) +Potato (50 g) + Glucose (5 g) |
| 2 -C.W.B | Coconut water (250 ml) +Dry cow dung (2.5 g) |
| 3- R.W.B | Rice washed water (250 ml)+ Dry cow dung (2.5 g) |

***Determination of the Viability of Trichoderma spp. in the Formulated Products***

Viability and stability of each *Trichoderma* spp. was tested for 180 days at 40 days intervals by calculating the number of colony forming units per one milliliter (CFU/ml) of carrier material. From each solid carrier material 1g was mixed thoroughly in 10 ml of sterile distilled water and was allowed to stand. From the supernatant, a serial dilution was prepared up to $10^{-5}$. From the $10^{-4}$ and $10^{-5}$ dilutions, 1 ml of suspension was taken without disturbing the surface of the medium and spread separately on plates using a spreader. For liquid carrier media, same procedure was followed using 1ml of carrier material and CFU/ml was calculated. Three replications were maintained for each formulation. *Trichoderma* inoculated petri dishes were incubated at room temperature (28ºC) for three days and CFU were calculated using following formula (Khan *et al.*, 2011).

$$\text{CFU/ml} = \frac{\text{Numbers of colonies per ml plated}}{\text{Dilution factor}}$$

## Results and Discussion

***Isolation and Identification of Fast Growth Trichoderma spp.***

A total of 24 fungal cultures were successfully isolated from different soil and decomposition litter samples. Based on the preliminary screening and colony morphology on PDA plates, 12 fungal isolates were identified as *Trichoderma* species. Besides macroscopic characteristics and growth rate, microscopic features of *Trichoderma* isolates are also important morphological keys in the identification of *Trichoderma* species (Gams and Bissett, 2002). The microscopic features that are frequently studied include the shapes and sizes of conidia, the branching patterns of conidiophores, the shapes and sizes of phialides (Samuels *et al*., 2002). Different terms have been used to describe the shapes of the conidia because no systematic rule has been established to define the shapes of the conidia. Also, the description of the shapes of conidia may be subjective. Therefore, the descriptions alone of the shapes of conidia are not a good criterion in identifying most of the isolates. However, the measurements of spore and phialide sizes are useful in identification of possible *Trichoderma* species. Using the identification key in the Compendium of soil fungi (Domsch *et al.*, 1980) and online identification key provided by Samuels *et al*, (2002). Isolates were identified up to generic level both macroscopic and microscopic characteristics. Conformity of the isolates was obtained which revealed that all 12 isolates belonged to *Trichoderma* species.

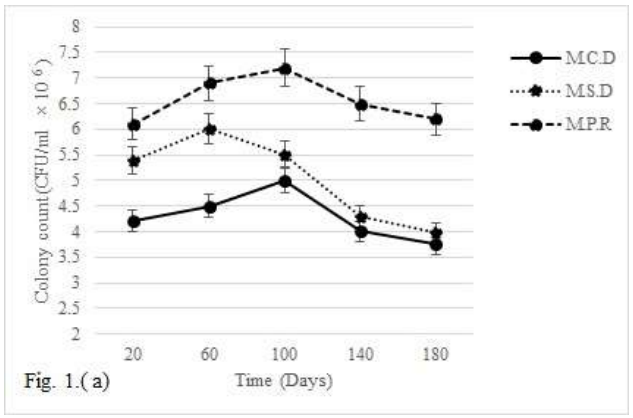
Almost all *Trichoderma* isolates grown in PDA plates showed confluent growth within 4-5 days because of their higher growth rates with slight variations among some of the isolates. Among the twelve *Trichoderma* isolates, five *Trichoderma* species were identified as fast growing species based on their growth rates. The resulting ITS sequences were edited using Bio Edit version 7.2.0 and analyzed for homologies in NCBI BLAST database based on previously published database sequences. Identities of these five Trichoderma spp. were confirmed as *T. viride*, *T. asperellum*, *T. virens*, *T. longibrachiatum* and *T. harzianum*. The DNA sequences were submitted to GenBank under the accession number AF218788.1, KT588246.1, KT852821.1 KP132794.1 and KP985643.1 respectively.

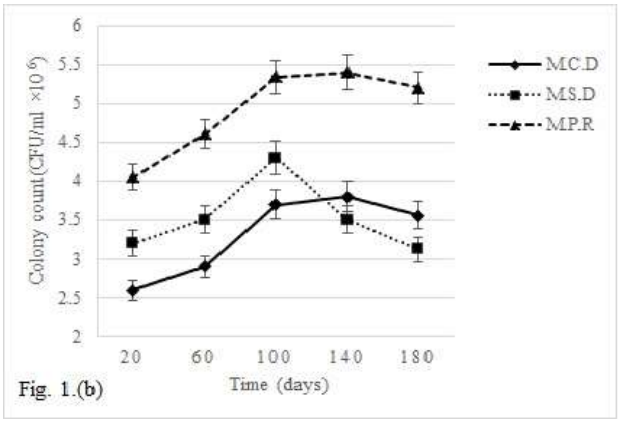
Molecular identification techniques were utilized to identify the isolates up to species level, since colony characteristics were not sufficient to do so. In this study, of 5.8S rRNA gene was amplified using primer pair ITS1 and ITS4. The two spacers, together with the 5.8S gene, are normally referred to as the ITS region. This is a very convenient tool of species identification because rRNA genes are universally conserved, while the ITS region is highly variable. The ITS region evolves rapidly and even within a genus it may vary among species (Kannangara *et al.*, 2016). Therefore, the sequences of these regions can be used for identification of closely related species. Restriction digestion of the amplified DNA using AluI enzyme was performed to differentiate the *Trichoderma* isolates depending on their genomic variability. At present, RFLP is recognized as a good technique to differentiate species, because of the cleavage of DNA molecules at specific sites (Sharma *et al.*, 2009). Therefore, the molecular characterization was also applied during the current study, in order to differentiate the isolated *Trichoderma* species.

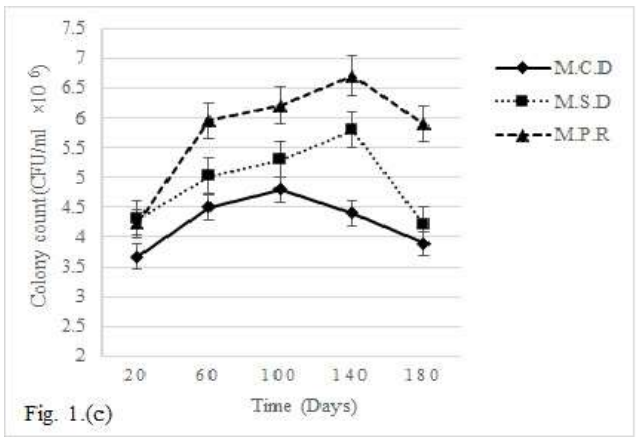
***Stabilization and mass production of Trichoderma spp.***

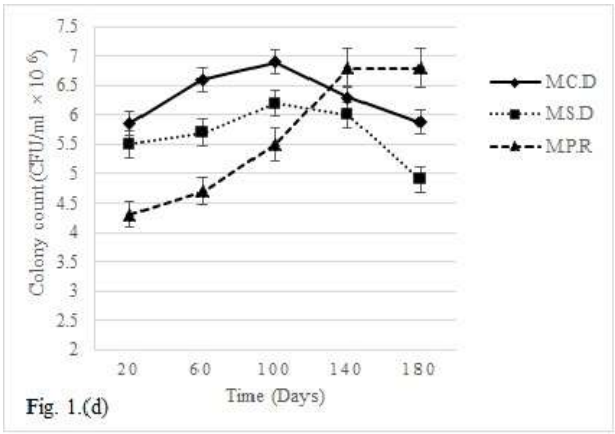
***Effects of Different Solid Carrier Media On Growth of Trichoderma spp.***

There was no significant difference among *T. viride, T. asperellum, T. harzianum, T. longibrachiatum* and *T. virens* with respect to the number of CFU/ml produced in the selected solid carrier materials (coir dust, saw dust and parboiled rice). All the five *Trichoderma* spp. grown in three different carrier materials showed more or less similar pattern of stabilization after 180$^{th}$ days (Figure 01). Following the CFU/ml values, the highest stabilization potential was recorded for parboiled rice after 180 days for all five *Trichoderma* spp. (a) *T. viride*; 6.2 x $10^6$ CFU/ml (b) *T. asperellum*; 5.2 x $10^6$ CFU/ml (c) *T. harzianum*; 5.4 x $10^6$ CFU/ml (d) *T. longibrachiatum*; 6.8 x $10^6$ CFU/ml and (e) *T. virens*; 6.8 x $10^6$ CFU/ml, respectively (Fig. 1).



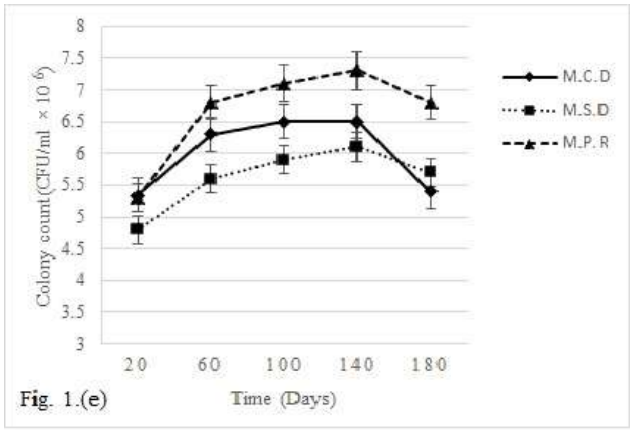




**Fig. 1:** Mean CFU/ml values of *Trichoderma* spp. on three different solid carrier materials with time; MCD- coir dust, MSD- saw dust, MPR- parboiled rice; (a) *T. viride*, (b) *T. asperellum*, (c) *T. harzianum*, (d) *T. longibrachiatum* and (e) *T. virens.*

The used carrier materials were rich in lignocellulosic organic compounds and contained nutrients essential for fungal growth such as carbon, nitrogen and mineral salts. In preparations of carrier materials, sugar was added as the carbon source and cow dung or poultry manure were also added as nitrogen source, since *Trichoderma* require different carbon and nitrogen sources for their growth and development (Kumar *et al*., 2014).

Results obtained for the CFU/ml values of *T. viride, T. asperellum, T. hazianum*, *T. longibrachiatum* and *T. virens* inoculated in parboiled rice at room temperature were higher after 180 days compared to the saw dust and coir dust. It may be due to the availability of higher carbon contents, higher nutrients, optimum C: N ratio and the neutral pH value in the parboiled rice that enhanced the production of fungal biomass of *Trichoderma* spp. According to Khan *et al*. (2011) water absorption capacity, texture and ability to form hydrogen bonds of the carrier materials also affect the production of high amount of fungal biomass. As per the results obtained, parboiled rice was considered as the best carrier material for the five *Trichoderma* spp. tested than coir and saw dust media.

In present study, survival and viability was determined up to 180 days, because the colony count gradually increased and started to reduce to constant level within that time. Maximum growth and higher survival stabilization of

*Trichoderma* can change after 180 days, therefore further study is required. Additionally, coir dust and saw dust based formulations were found suitable for growth and viability of *Trichoderma* spp. during storage. *Trichoderma* is a cellulotic fungus, therefore lignocellulosic substrates is a better source for sporulation, growth and development for long periods. Maximum growth and higher survival rate during storage in carrier material formulation is a major advantage for marketing these bio-control agents at commercial level.

Considering parboiled rice mass production, initial stabilization process slows down (low CFU/ml in initial stage) due to rigid rice husk. With time CFU/ml of *Trichoderma* increased due to stabilization, which resulted in an increment in mass. Instead of rice, other alternative substrates can be used for the mass production of *Trichoderma.* Parkash and Saikia (2015) used wheat straw, sorghum grains, lignite and stillage, molasses and brewer's yeast, and wheat bran medium for mass production of *Trichoderma* spp. In order to enhance growth rate and sporulation, various cellulosic residues were supplemented with chickpea flour as organic nitrogen supplement at two and four percent. According to the results, supplementation of all the substrates with chickpea flour enhanced growth and sporulation, and 4% chickpea flour supplementation gave maximum response in all the cases. In addition, paddy straw, sugarcane baggase and wheat straw, each supplemented with 4% chickpea flour, were found to be promising substrates for mass multiplication of *T. harzianum* (Parkash and Saikia, 2015).

***Effects of Different Liquid Carrier Media On the Growth of Trichoderma spp.***

All the five *Trichoderma* spp. grown in three different liquid carrier materials (PDB; potato dextrose broth, CWB; coconut water broth, and RWB; rice washed broth) showed more or less similar stabilization patterns over 180$^{th}$ days (Figure 02). There was no significant difference among the CFU/ml values of the three carrier materials for all *Trichoderma* spp. Among the CFU/ml values, PDB showed the highest stabilization potential (higher CFU/ml values) for all five *Trichoderma* spp. for multiplication from 20 to 180$^{th}$ days (Fig. 2).

In the laboratory, PDB used for multiplication of fungal colony showed higher CFU/ml compared to the other two liquid carrier materials. However, there was no significant difference in CFU/ml, among the three carrier materials for all *Trichoderma* spp. Coconut water, rice washed water and waste materials in household can be used as cost effective carrier materials instead of PDB.

Emerson and Mikunthan (2015) have used locally available substrates to improve the growth of *T. viride.* Results of this study also showed that locally available liquid substrates such as black gram soaked water, coconut water and rice mill effluent can be used for the mass multiplication of *T. viride* by small scale farmers. Similarly, in the present investigation, coconut water and rice wash water are identified as locally available liquid waste from domestic kitchens. There is a good potential for the use of these waste carrier materials for the mass production of *Trichoderma* spp. Panahian *et al*. (2012) have used formulated sugar beet molasses, broken corn seed, wheat bran, soybean meal, crushed potatoes, sugar beet pulp culture medium as carrier materials for *T. harzianum* and *T. virens*. Kumar *et al.* (2014) have used vegetable waste, fruit juice waste, sugarcane baggase and rotten wheat grains for mass multiplication of different *Trichoderma* spp. and have reported sugarcane baggase as the best carrier material that yielded the highest amount of mycelia, spores and higher CFU/g.

Babu and Pallavi (2013) recorded similar results when *Trichoderma* were tested for linear growth rates at different temperature levels (5-25 ºC). Optimum bio-mass was produced at temperatures between 20-30 ºC. *Trichoderma* spp. were found to be best grown at a temperature range of 25 to 30 ºC. Singh *et al*. (2014) showed that the temperature optimum of xylanase production was 28 ºC. In another study, Kumar *et al*. (2014) tested the effect of different temperatures on the mycelial growth of *Trichoderma* isolates. They showed that mycelial growth differed statistically with respect to the tested temperatures. Optimum temperature 25 ºC supported the highest mycelial growth of *T. harzianum* and *T. hamatum*, whereas the mycelial growth of *T. virens* was highest at 30 ºC. The present study reveals that, *Trichoderma* can sustain and survive the room temperature (28$^0$C) to obtain sporulation, higher stabilization and mass production.

Niranjana *et al.* (2009) demonstrated that pH can play a role in the regulation of extracellular enzyme, β-1, 6-glucanase production in *T. harzianum.* Biomass and conidia production by *T. harzianum* were examined in a defined medium at different pH values. Biomass production was observed within pH 3.5-9.5 with maximum growth between pH 5.5-7.5. Subash *et al.* (2013) had observed highest growth rates at pH 4.0 in a study where pH dependence of the linear mycelial growth and activities of extracellular enzymes of *Trichoderma* strains was tested. Kumar *et al*. (2014) reported that growth of *Trichoderma* spp. was maximum at pH 6 whereas, the growth was minimum at pH 4. Therefore, it is important to examine the effects of pH on mycelial growth and on the *in vitro* activities of extracellular enzymes contributing for competition and mycoparasitism of *Trichoderma* spp. with bio control potential. Considering the observations of previous studies, selected carrier materials were incubated at room temperature 28ºC and at 6-7 pH, for high bio mass production.

According to the results obtained for the five *Trichoderma* spp. on stabilization in solid and liquid carrier materials, contradictory results (CFU/ml) were obtained. *Trichoderma* spp. incubated in solid state carrier materials showed higher ($10^6$ CFU/ml) values than the liquid state carrier materials ($10^4$ CFU/ml) after 180 days and it was significantly different. This can be due to several characteristics of carrier materials ie; availability of higher carbon contents, optimum C: N ratio and the neutral pH value that enhance the production of higher fungal biomass of *Trichoderma* spp.

As described in the Panahian *et al.* (2012) propagules of *Trichoderma* can be produced in mass scale in both liquid and solid fermentation methods. As low cost materials to maximize the yield, crop residue, household waste, livestock waste and industrial waste can be used. As the cost of synthetic media for the mass multiplication at any bio-control agent is high, mass multiplication with the use of organic waste materials was found to be economically advantageous and indicates the fact that these media are cost effective and hence support commercialization of *Trichoderma* spp. According to Kumar *et al*. (2014) CFU of *Trichoderma* should be a minimum of 2 x $10^6$ CFU per ml or gram in selective medium of *Trichoderma* formulations as per the standards. Pathogenic contaminants should not be present and other microbial contaminants should not exceed a number of 1 x $10^4$ count ml/g. The viability of the *Trichoderma* formulations should be maintained for a considerable period of time.

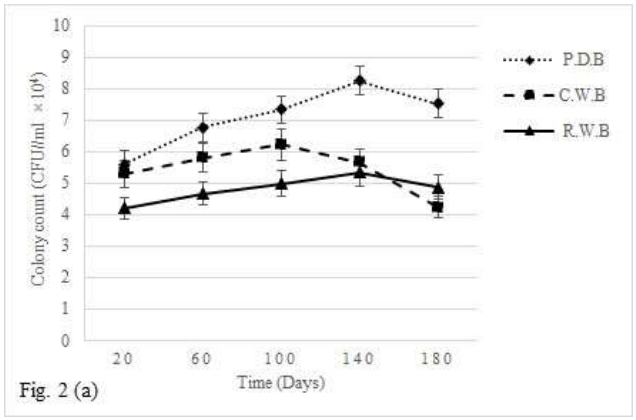
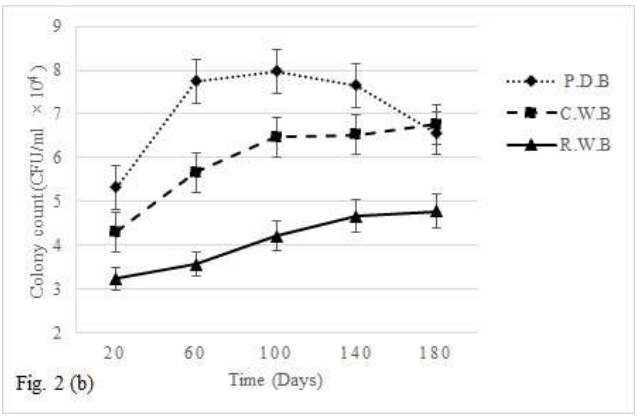


Fig. 2 (a)



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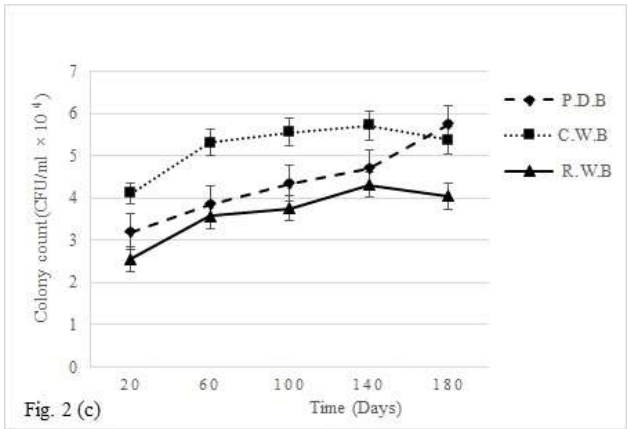

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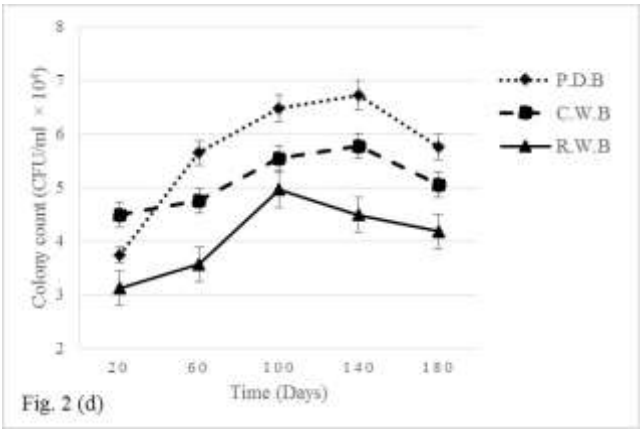

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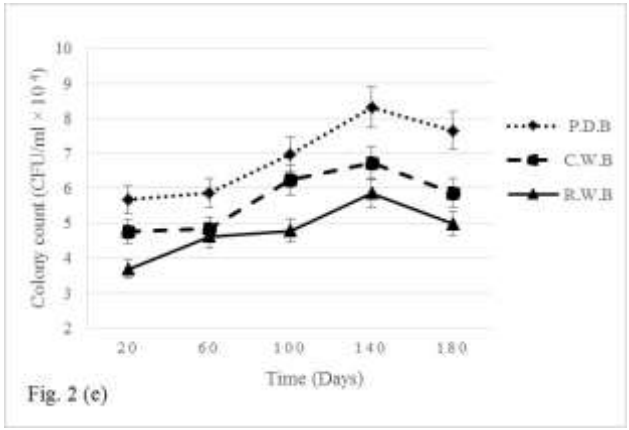

Fig. 2 (e)

**Fig. 2:** Mean CFU/ml values of *Trichoderma* spp. on three different liquid carrier materials with time; PDB- potato dextrose broth, CWB- coconut water broth, RWB- rice washed broth; (a) *T. viride*, (b) *T. asperellum*, (c) *T. harzianum*, (d) *T. longibrachiatum* and (e) *T. virens.*

## Conclusion

Selected *Trichoderma* spp. were successfully mass produced in naturally available solid carrier materials (coir dust, saw dust and parboiled rice) and liquid carrier materials (coconut water and rice washed water). Among the three solid substrate carrier materials, parboiled rice was identified as the most effective organic carrier material which maintained the viability and virulence of all *Trichoderma* spp. throughout the study period (180 days).

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## References

Babu KN and Pallavi PN (2013) Isolation, identification and mass multiplication of *Trichoderma*- an important bio-control agent. *Int J Pharm Life Sci* **4**(1): 2320-2323.

Barari H (2016) Bio-control of Tomato *fusarium* wilt by *Trichoderma* species under in *vitro* and in *vivo* conditions. *Cercetari Agronomice in Moldova* **1**(165): 91-98. DOI: 10.1515/cerce-2016-0008

Cavalcante RS, Lima HLS, Pinto GAS, Gava CAT and Rodrigues S (2008) Effect of moisture on *Trichoderma* conidia production on corn and wheat bran by solid state fermentation. *Food Biopr Technol* **1**: 100-104. DOI: 10.1007/s11947-007-0034-x

Chakraborty BN, Chakraborty U, Saha A, Dey PL and Sunar K (2010) Molecular characterization of *Trichoderma viride* and *Trichoderma harzianum* isolated from soils of north bengal based on rRNA markers and analysis of their PCR-RAPD profiles. *Global J of Biotechnol and Biochem* **5**(1): 55–61.

Chennappa G, Naik MK, Amaresh YS, Ashwathanarayan DS, Patil MG, Divya N and Shruthi P (2017) Morphological, molecular characterization of *Trichoderma*s species isolated from different rhizosphere soils and its anti-pathogenic properties. *Imperial J of Interdisciplinary Research (IJIR)* **3**(4): 2454-1362.

Domsch KH, Gams W and Anderson TH (1980) Compendium of soil fungi, Vol. 1, I.H.W. Verlag, Eching, Germany.

Emerson FL and Mikunthan G (2015) Small scale production of *Trichoderma viride* on locally available liquid waste and other substrates. *American-Eurasian J of Agri and Environ Sci* **15**(8): 1666-1671.

Gams W and Bissett J (2002) Morphology and identification of *Trichoderma*. In: Kubicek, C. P. and Harman, G. E. (Eds.). *Trichoderma* and *Gliocladium*: Basic biology, taxonomy and genetics. *Taylor and Francis Ltd* pp. 3-31.

Grondona I, Hermosa RM, Tejada MD, Gomis PF, Mateos PD, Bridge E and Garcia-acha I (1997) Physiological and biochemical characterization of *Trichoderma harzianum* a biological control agent against soil borne fungal plant pathogens. *App Envir Microbiol* **63**(8): 3189

Harman GE (2000) Changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant disease* **84**(4): 377-393. DOI: 10.1094/PDIS.2000.84.4.377

Herath HHMAU, Wijesundera RLC, Chandrasekharan NV, Wijesundera WSS and Kathriarachchi HS (2015) Isolation and characterization of *Trichoderma erinaceum* for antagonistic activity against plant pathogenic fungi. *Current Research in Environmental and Applied Mycology* **5**(2): 120-127. DOI: 10.5943/cream/5/2/5

Howell CR (2013) Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. *Plant diseases* **87**(1): 5-9.

Kannangara S, Dharmarathna RMGCS and Jayarathna D L (2016) Isolation, identification and characterization of *Trichoderma* species as a potential bio-control agent against *Ceratocystis paradoxa*. *The J Agri* **12**(1): 51-62.

Khan S, Bagwan NB, Iqbal MA and Tamboli RR (2011) Mass multiplication and shelf life of liquid fermented final product of *Trichoderma viride* in different formulations. *Advances in Bioresearch* **2**(1): 178-182.

Kumar K, Amaresan N, Bhagat S, Madhuri K and Srivastava RC (2012) Isolation and characterization of *Trichoderma* spp. for antagonistic activity against root rot and foliar pathogens. *Indian J Microbiol* **52**(2): 137–144. DOI: 10.1007/s12088-011-0205-3

Kumar KC, Babu A, Bordoloi M and Ali A (2014) Evaluation of culture media for biomass production of *Trichoderma viride* (KBN 24) and their production economics. *American J Agri and Forestry* **2**(6): 317-320.

Kumar S, Thakur M and Rani A (2014) *Trichoderma*: Mass production, formulation, quality control, delivery and its scope in commercialization in India for the management of plant diseases. *African J Agri Research* **9**(53): 3838-3852.

Niranjana SR, Lalitha S and Hariprasad P (2009) Mass multiplication and formulations of bio-control agents for use against *fusarium* wilt of pigeon pea through seed treatment. *Int J of Pest Manage* **55**: 317-324. DOI: 10.1080/09670870902919147

Panahian GH, Rahnama K and Jafari M (2012) Mass production of *Trichoderma* spp. and application. *Int Research J Applied and Basic Sci* **3**(2): 292-298.

Parkash V and Saikia AJ (2015) Production and multiplication of native compost fungal activator by using different substrates and its influence on growth and development of *Capsicum chinensis* Jacq. ‘‘Bhut Jolokia. *Int Biotechnol Research* Article ID 481363. DOI: 10.1155/2015/481363

Samuels GJ, Chaverri P, Farr DF and Mccray EB (2002) *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. *Mycologia* **94**: 146-170. DOI: 10.1080/15572536.2003.11833257

Seethapathy P, Kurusamy R and Kuppusamy P (2017) Soil borne diseases of major pulses and their biological management. *An Int J Agri* **2**(1): 1-11

Sharma K, Mishra AK and Misra A (2009) Morphological, biochemical and molecular characterization of *Trichoderma harzianum* isolates for their efficacy as bio-control agents. *J of Phytopath* **157**: 51-56. DOI: 10.1111/j.1439-0434.2008.01451.x

Sharma KK and Singh US (2014) Cultural and morphological characterization of rhizospheric isolates of fungal antagonist *Trichoderma. Journal of Applied and Natural Sci* **6**(2): 451-456. DOI: 10.31018/jans.v6i2.481

Singh A, Shahid M, Srivastava M, Pandey M, Sharma A and Kumar V (2014) Optimal physical parameters for growth of *Trichoderma* species at varying pH, temperature and agitation. *Virology and Mycology* **3**(1): 1-7.

Sriram S and Savitha MJ (2015) Morphological and molecular identification of *Trichoderma* isolates with bio-control potential against Phytophthora blight in red pepper. *Pest Manag in Horti Ecosys* **21**(2): 194-202.

Subash N, Viji J, Sasikumar C and Meenakshisundaram M (2013) Isolation, media optimization and formulation of *Trichoderma harizanum* in agricultural soil. *J of Microbiol and Biotechnol* **3**(1): 61-64.

Veeken AHM, Blok WJ, Curci F, Coenen GCM, Temorshuizen AJ and Hamelers HVM (2005) Improving quality of composted bio-waste to enhance disease suppressiveness of compost amended, peat based potting mixes. *Soil Biology and Biochem* **37**: 2131-2140. DOI: 10.1016/j.soilbio.2005.03.018

Woo SL, Ruocco M, Vinale F, Lombardi N, Pascale A, Lanzuise S, Manganiello G and Lorito M (2014) *Trichoderma*-based products and their wide spread use in agriculture. *The Open Mycology J* **8**: 71-126. DOI: 10.2174/1874437001408010071