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Evaluation and assessment of shelf life of liquid substrates and talc formulation for mass production of native *Trichoderma spp*.

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Abstract
The efficient management of foliar as well as soil borne pathogens makes *Trichoderma* one of the most used biocontrol agents in the world. Development of formulations with enhanced shelf life and broad-spectrum activity could pave the way of rapid commercialization of these beneficial microorganisms. Two native isolates of *Trichoderma* species (Tr Isolate 2 and Tr Isolate 5) were collected from paddy and groundnut rhizospheres respectively. Their vigorous growth in different culture media and profound inhibitory effects on two potential soil borne pathogens namely *Rhizoctonia solani* and *Sclerotium rolfsii* as observed in dual culture experiment were the basis of selecting these two isolates among all the seven other isolates collected from different crop rhizospheres for mass production. Present study deals with evaluation of nine liquid substrates and one talc-based formulation. Colony forming units were counted following the serial dilution technique and a gradual reduction was observed over a period of six months. Among various liquid substrates put into test, Coconut water was observed to be the best for both of the isolates (Tr Isolate 2 and Tr Isolate 5) recording the maximum cfu count 170.00 x 10^7 cfu/ml and 99.00 x 10^7 cfu/ml of substrate in the first month respectively. So far as talc-based formulations are concerned, the maximum count 44.33 x 10^7 cfu/g was recorded in the first month in Tr Isolate 2. Similarly, in case of Tr Isolate 5 the highest cfu count of 48.00 x 10^7 cfu/g was recorded in first month after inoculation.

Keywords: *Trichoderma*, formulation, mass production, shelf life

Introduction
Plants diseases are one of the major limiting factors in crop cultivation causing large scale destruction every year. In disease management, the amplified use of chemicals has put a negative impact on environmental quality and resulted in upward trend of many living forms which are resistant to the chemicals (Kumar and Gupta, 2012) [1]. Bioaccumulation of pesticide residues in the food chain has become the reason behind poisoning and death of human as well as beneficial flora and fauna through impairment of vital body functions, malfunctions, carcinogenicity etc. To combat challenges like depleting biodiversity, environmental pollution and growing stress on organically produced food, the need of the hour is a major shift towards utilization of more and more biocontrol agents. *Trichoderma* is one such agent having a worldwide occurrence and is usually isolated from the soil and decaying plant organic matter. It not only kills the harmful fungi via mechanisms like mycoparasitism and antibiosis but also plays an important role in plant growth promotion. The term liquid state fermentation (LSF) is a process in which soluble materials in water are used for the microbial growth. This fermentation system has been adopted for the mass multiplication of fungal biocontrol agents. For mass multiplication, the selected medium should be inexpensive and readily available with appropriate nutrient balance. Potato dextrose broth, V-8 juice, Molasses-yeast medium and Wheat bran are generally used for the mass production of *Trichoderma spp*., through liquid fermentation technology (Prasad *et al*., 1999; Prasad *et al*., 2002) [2, 3]. Shelf life of the formulations decides the commercialization of biocontrol agents. They should retain their viability for increased periods under storage condition and the carrier material should be inert and should not influence the viable nature of the formulation. It is easier and more convenient on the part of farmers to handle and apply liquid biofertilizer formulations which could be considered as one potential strategy for improving the shelf life of biofertilizer. Gaur *et al.* (2005) [4] reported that *T.harzianum* in talc based powder formulation remained viable at temperature ranging between 0 to 40 ºC for 180 days. Sarode *et al.* (1998) [5] utilized five different substrates, i e FYM, peat soil, charcoal powder, talc powder and neem powder as carrier. Charcoal, FYM and talc were most suitable carriers for long storage of *Trichoderma*. Hence it is required that biocontrol formulations should possess several desirable

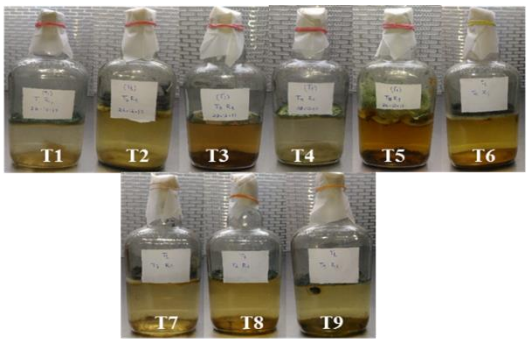
characteristics such as ease of preparation and application, stability, adequate shelf life, abundant viable propagules and low cost of production.

Materials and Methods

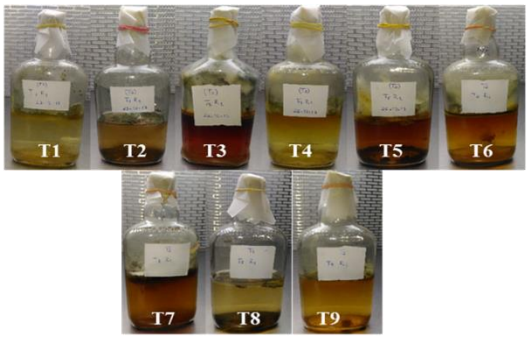
Serial dilution technique was undertaken to obtain seven native *Trichoderma* isolates from the soil collected from different crop rhizospheres. The isolates were identified through observation with respect to colony colour, structure of their phialides and spores. They were maintained by periodic sub-culturing on Potato Dextrose Agar (PDA). The two most virulent isolates namely *Trichoderma* isolate 2 (Tr. isolate 2) and *Trichoderma* isolate 5 (Tr. Isolate 5) were selected basing on their growth habit on different media and antagonistic nature against two soil borne pathogens i.e *Rhizoctonia* sp. and *Fusarium* sp. Following nine liquid substrates were freshly prepared as per procedures cited in literatures: -

1. Carrot- Barley broth (100g carrot extract + 25g Barley)/1 litre
2. Carrot glucose broth (100g Carrot extract+25g Glucose)/1litre
3. Coconut water (50%)
4. Potato dextrose broth (300g potato extract + 25 g dextrose)/1 litre
5. Molasses-brewer's yeast medium (molasses 30g, yeast 5g and water 1 litre)
6. Black gram soaked water
7. Coconut water (50%) + Potato dextrose broth (10%)
8. Jowar- Maize- Glucose broth (Jowar extract 100g, maize extract 100 g and glucose 25 g)/ 1 litre
9. Richard's medium + V-8 Juice

The viability of isolates of *Trichoderma* was also tested with talc by mixing it with pre-inoculated coconut water liquid substrate in the ratio of 1:2 (substrate: talc) which was then thoroughly mixed and air dried. All above liquid substrates were transferred to glass bottles, autoclaved at 121.6^0 C (15 p.s.i) for 20 minutes and plugged with non-absorbent cotton. Talcum formulation was transferred as such into sterilised bottles and plugged (Figure-2). Six replications per treatment were made for 6 months observation. Previously collected isolates were inoculated into PDA plates by hyphal tip method. The inoculated petriplates were incubated for 10-12 days at 26.5^0C. Discs of 10 mm diameter were cut from these petriplates using a cork borer. Bottles were then inoculated in aseptic condition @ 1 disc / bottle (Figure-1). Antibiotic was added to the sterilized potato dextrose Rose Bengal agar medium @ 250 mg/l and was poured into glass petridishes. After a month of inoculation, the entire content of the bottle with liquid substrate was emptied in to a sterile beaker, blended and serially diluted to get the final dilution of 10^{-7}. For talc, entire content was mixed thoroughly and serially diluted to get the final dilution of 10^{-7}. One milliliter from this solution was taken and spread over the petriplates containing medium. This entire procedure was repeated at monthly intervals upto 6^{th} month for cfu count. The inoculated petriplates were incubated at 27 + 1^0C for atleast three days in a BOD incubator. The colonies formed were marked with a permanent marker and observation was noted.



(a) Isolate 2



(b) Isolate 5

T1: Coconut water (50%)
T2: Potato Dextrose broth
T3: Black gram soaked water
T4: Jowar-Maize-Glucose broth
T5: Richard's medium + V-8 juice
T6: Coconut water (50%) + Potato dextrose broth (10%)
T7: Molasses-Brewer's yeast medium
T8: Carrot- Barley broth
T9: Carrot Glucose broth

Fig 1: Bottles containing different liquid substrates inoculated with *Trichoderma* Isolate 2 and Isolate 5.

Fig 2: Bottles containing talc formulations of *Trichoderma* Isolate 2 and Isolate 5

Results and Discussion

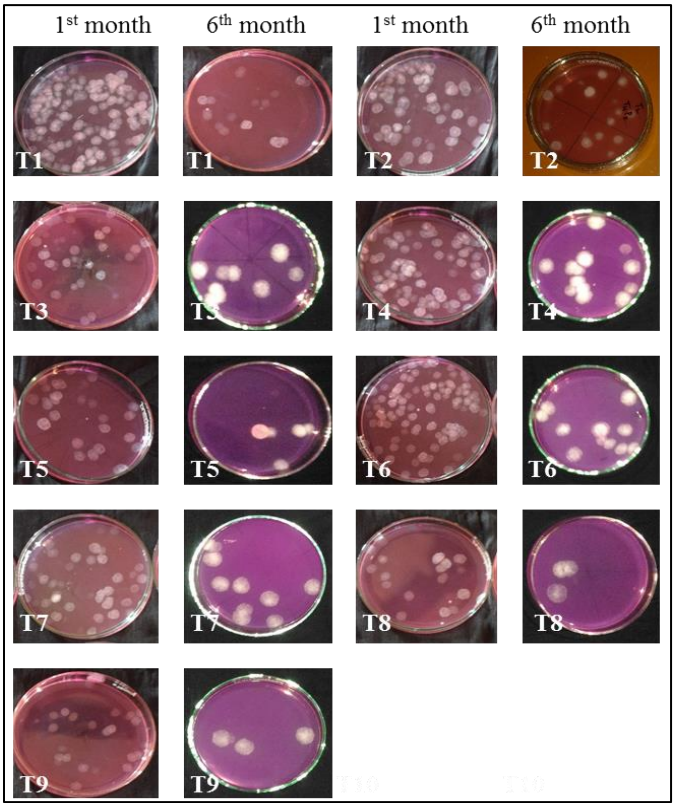
The results obtained for liquid substrates as per the materials and methods has been recorded in Table-1, Figure-3 and Table-2, Figure-4 for Tr isolate-2 and Tr isolate respectively. It was observed that coconut water recorded higher cfu count as compared to other liquid substrates and was considered to be the best performer for both the *Trichoderma* isolates. The maximum cfu count of 170.00 x 10^7 cfu/ml was recorded in

the first month in Tr Isolate 2 and the maximum cfu count of 99.00 x 10^7 cfu/ml was recorded in the first month in Tr Isolate 5. This finding supported the work of several workers. Dagdag and Reyes (1991) [6] reported that coconut water supplemented with sucrose and PDA recorded spore count of 10 x 10^9 cfu/ml and 10 x 10^3 cfu/ml respectively. Utilisation of coconut water as a medium for mass multiplication of beneficial microbes has been reported earlier by Anandraj and Sharma, 1997 [7].

Table 1: Mean monthly cfu in liquid substrates (Tr Isolate 2)

Sl. No	Treatments	1st month (cfu x 10^7)/ml	2nd month (cfu x 10^7)/ml	3rd month (cfu x 10^7)/ml	4th month (cfu x 10^7)/ml	5th month (cfu x 10^7)/ml	6th month (cfu x 10^7)/ml
1	Coconut water	170.00 (9.23) *	128.33 (9.11)	105.67 (9.02)	77.00 (8.89)	47.00 (8.67)	29.00 (8.46)
2	Potato Dextrose broth	100.00 (9.00)	82.67 (8.92)	60.67 (8.78)	42.67 (8.63)	27.00 (8.43)	15.00 (8.17)
3	Black gram soaked water	53.33 (8.72)	32.00 (8.50)	22.33 (8.35)	18.67 (8.27)	10.67 (8.03)	6.33 (7.79)
4	Jowar-Maize -Glucose broth	101.33 (9.01)	83.33 (8.92)	62.67 (8.80)	37.33 (8.57)	23.67 (8.37)	13.67 (8.13)
5	Richard's medium + V-8 juice	55.33 (8.74)	33.00 (8.52)	23.00 (8.36)	16.67 (8.22)	10.67 (8.03)	5.67 (7.74)
6	Coconut water + PDB	107.67 (9.03)	98.67 (8.99)	71.67 (8.86)	44.33 (8.64)	28.00 (8.45)	16.33 (8.21)
7	Molasses-brewer's yeast medium	52.00 (8.71)	40.00 (8.60)	30.67 (8.49)	24.67 (8.39)	17.00 (8.23)	8.33 (7.91)
8	Carrot- Barley broth	25.33 (8.40)	18.00 (8.25)	12.67 (8.10)	10.00 (8.00)	6.00 (7.76)	2.33 (7.30)
9	Carrot glucose broth	27.67 (8.44)	19.67 (8.29)	13.33 (8.12)	14.00 (8.14)	8.33 (7.92)	3.33 (7.49)
	SE(m) +	0.03	0.02	0.03	0.04	0.04	0.08
	C.D (0.05)	0.10	0.07	0.08	0.12	0.13	0.25

*Figures in the parenthesis are Log_{10} transformed value



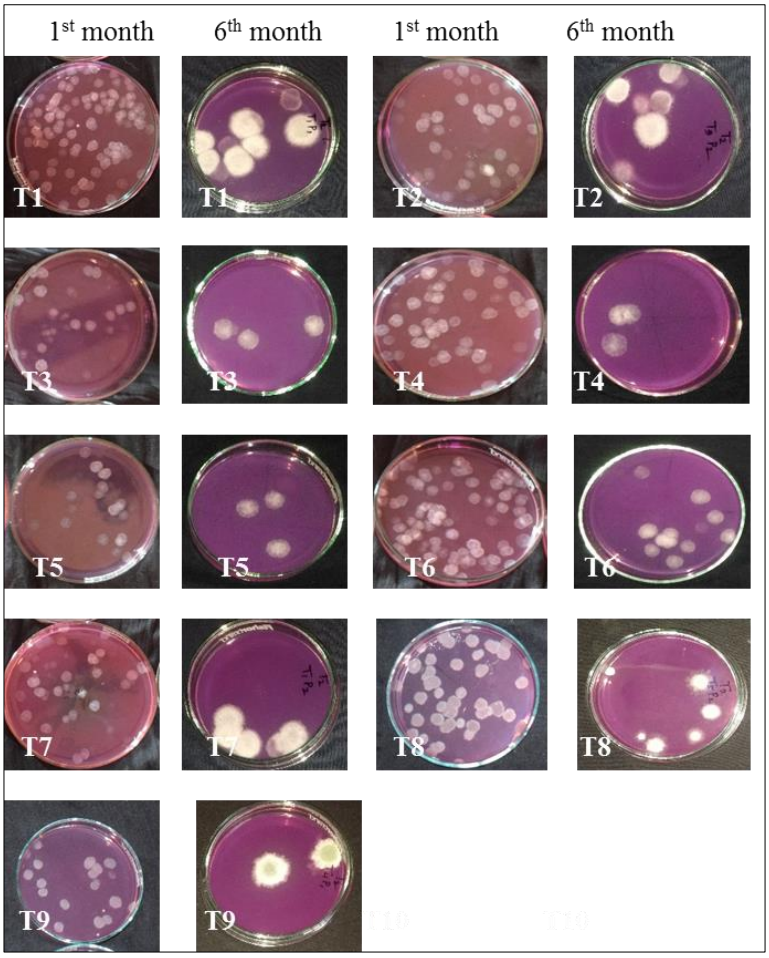
T1: Coconut water (50%)
T2: Potato Dextrose broth
T3: Black gram soaked water
T4: Jowar-Maize-Glucose broth
T5: Richard's medium + V-8 juice
T6: Coconut water (50%) + Potato dextrose broth (10%)
T7: Molasses-brewer's yeast medium
T8: Carrot- Barley broth
T9: Carrot glucose broth

Fig 3: Petriplates showing colony forming units of different liquid substrates inoculated with Trichoderma Isolate 2 (T1-T9)

Table 2; Mean monthly cfu in liquid substrates (Tr Isolate 5)

Sl. No	Treatments	1st month (cfu x10^7)/ml	2nd month (cfu x10^7)/ml	3rd month (cfu x10^7)/ml	4th month (cfu x10^7)/ml	5th month (cfu x10^7)/ml	6th month (cfu x10^7)/ml
1	Coconut water	99.00 (8.99) *	82.33 (8.92)	59.33 (8.77)	35.00 (8.54)	24.33 (8.38)	16.33 (8.21)
2	Potato Dextrose broth	61.33 (8.79)	34.33 (8.53)	26.67 (8.43)	18.33 (8.26)	12.67 (8.10)	6.67 (7.82)
3	Black gram soaked water	29.67 (8.47)	20.67 (8.31)	13.67 (8.14)	9.33 (7.97)	6.33 (7.79)	2.33 (7.30)
4	Jowar-Maize -Glucose broth	39.00 (8.59)	25.67 (8.41)	17.00 (8.22)	13.67 (8.13)	7.67 (7.88)	2.67 (7.40)
5	Richard's medium + V-8 juice	24.67 (8.39)	18.67 (8.27)	15.33 (8.18)	12.00 (8.08)	5.33 (7.72)	2.33 (7.32)
6	Coconut water + PDB	76.00 (8.88)	60.33 (8.78)	39.33 (8.59)	29.33 (8.47)	20.33 (8.31)	13.00 (8.11)
7	Molasses-brewer's yeast medium	23.67 (8.37)	18.00 (8.25)	14.67 (8.16)	11.00 (8.04)	6.67 (7.82)	3.67 (7.56)
8	Carrot- Barley broth	26.00 (8.41)	18.00 (8.23)	16.00 (8.20)	14.00 (8.14)	8.33 (7.92)	4.33 (7.62)
9	Carrot glucose broth	20.33 (8.31)	14.67 (8.17)	13.67 (8.13)	10.00 (8.00)	5.33 (7.72)	1.67 (7.16)
	SE(m) +	0.02	0.05	0.04	0.02	0.03	0.11
	C.D (0.05)	0.07	0.14	0.11	0.07	0.10	0.32

*Figures in the parenthesis are Log_{10} transformed value



T1: Coconut water (50%)
T2: Potato Dextrose broth
T3: Black gram soaked water
T4: Jowar-Maize-Glucose broth
T5: Richard's medium + V-8 juice
T6: Coconut water (50%) + Potato dextrose broth (10%)
T7: Molasses-brewer's yeast medium
T8: Carrot- Barley broth
T9: Carrot glucose broth

Fig 4: Petriplates showing colony forming units of different liquid substrates inoculated with *Trichoderma* Isolate 5 (T1-T9)

The results obtained for talc formulations as per the materials and methods has been recorded in Table-3, Figure-5 for Tr isolate 2 and Tr isolate 5 respectively. The maximum count of 44.33 x 10^7 cfu/g was recorded in the first month in Tr Isolate 2. Similarly, in case of Tr Isolate 5 the highest cfu count 48.00 x 10^7 cfu/g was recorded in first month after inoculation. This finding is in accordance with the findings of Karunanithi *et al*., (2001) [8]. They reported the shelf life of talc-based formulation is upto 150 days at room temperature. Kumar *et al*., (2013) [9] reported the shelf life of talc-based formulation upto 120 days which is in agreement with the present findings.

Table 3: Mean monthly cfu in talc formulation (Tr Isolate 2 and Tr Isolate 5)

SL. NO	Treatments	1st month (cfu x10^7)/g	2nd month (cfu x10^7)/g	3rd month (cfu x10^7)/g	4th month (cfu x10^7)/g	5th month (cfu x10^7)/g	6th month (cfu x10^7)/g
1	Isolate 2	44.3	39.0	43.7	19.3	14.3	11.3
1	Isolate 5	48	36.7	27.3	19.3	14.3	11.3

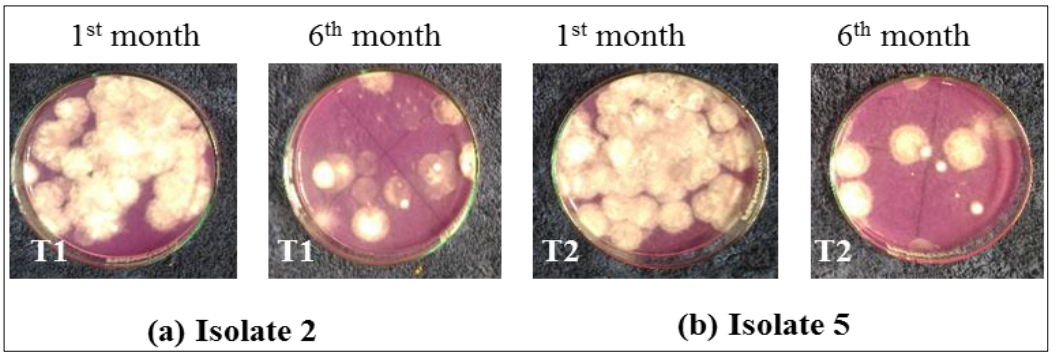


Fig 5: Petriplates showing colony forming units of different Talc formulations of *Trichoderma* Isolate 2 and 5, T1- Tr isolate 2, T2- Tr isolate 5

Conclusion

From the above experiment it is concluded that from all the nine substrates taken for mass multiplication of the *Trichoderma* isolates, coconut water proved to be the best. It was also deduced from the above results that in most cases Tr isolate 2 had a higher rate of mass multiplication than Tr isolate 5. Concluding about the results in talc formulations it was deduced that the viability of the biocontrol agent in the formulation starts decreasing from the 4th month and lasts maximum upto 6th month.

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Reference

1. Kumar S, Gupta OM. Expanding dimensions of Plant Pathology, JNKVV Research Journal. 2012; 6(3):286-93.
2. Prasad RD, Rangeshwaran R, Kumar S. Biological control of root and collar rot of sunflower. Journal of Mycology and Plant Pathology. 1999; 29(2):184-188.
3. Prasad RD, Rangeshwaran R, Sunanda CR. Jaggery- an easily available alternative to molasses for mass production of *Trichoderma harziaum.* Plant Disease, Research. 2002; 17:363-365.
4. Gaur RB, Sharma RN, Sharma RR. Shelf life of talc based formulation of *Trichoderma* and soil application for biological control of dry root rot of chickpea. Journal of Mycology and Plant Pathology. 2005; 35(2):380–4.
5. Sarode SV, Gupta VR, Asalmol MN. Suitability of carrier and shelf life of *Trichoderma harzianum*. Indian Journal of Plant Protection. 1998; 26(2):188–9.
6. Dagdag AP, Reyes RG. Growth response of *Trichoderma harzianum* Rafai in selected indigenous culture media. CLSU Scientific Journal. 1991; 12(1):1-5.
7. Anandraj M, Sharma YR. Mature coconut water for mass culture of bio control agent. Journal of Plantation crops. 1997; 25:112-114.
8. Karunanithi K, Muthusamy M, Seetharaman K. Gypsum - a suitable material for the mass multiplication of *Trichoderma viride*. Tropical Agricultural Research and Extension. 2001; 4(2):115-116.
9. Kumar S, Kumar R, Om H. Shelf-life of T*richoderma viride* in talc and charcoal based formulations. Indian Journal of Agricultural Sciences. 2013; 83(5):566-569.