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Growth of *Trichoderma* spp on Different Solid Substrates

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A B S T R A C T

Trichoderma is one of the common fungal biocontrol agents being used worldwide for efficient management of various foliar and soil borne plant pathogens. Unlike chemicals, the establishment of these biocontrol agents in the targeted niche requires support even after their application. Two *Trichoderma* species (Isolate 2 and Isolate 5) were isolated from paddy and groundnut rhizospheres respectively. These two isolates were the two most vigorous ones among all the seven *Trichoderma* isolates obtained from different crop rhizospheres on the basis of their growth in different media and their inhibitory effects on two potential soil borne pathogens namely *Rhizoctonia solani* and *Sclerotium rolfsii* as observed in dual culture experiment. Present study deals with evaluation of eighteen solid substrates. Colony forming units were counted following the serial dilution technique and a gradual decrease was observed over a period of six months. Among different solid substrates tested, vermicompost was observed to be the best solid substrate for isolate 2 recording the maximum cfu count 463.67 x 10^7cfu/g of substrate in the first month after inoculation and it retained spore viability even after six months of inoculation giving a fairly good cfu count of 97.33 x10^7cfu/g. For isolate 5, groundnut shell performed the best, recording the maximum cfu count of 467.67 x 10^7 cfu/g in the first month and 144.00 x 10^7 cfu/g was retained even in the sixth month after inoculation.

Introduction

Plant diseases are one of the major concerns in cultivation worldwide, consequential in loss of billions of dollars of farm produce. In disease management, the amplified use of chemicals have caused negative impact on environmental quality and resulted in upward trend of many living forms which are resistant to the chemicals (Kumar and Gupta, 2012). Under this changing agriculture scenario, the only technology that seems promising to manage the diseases without disturbing the equilibrium of harmful and useful composition of environment and ecosystem is the use of more and more biological control agents. As we approach the 21st century, there is an enlarging stress on organically produced food, conservation of biodiversity, unpolluted environment and sustainable agriculture. To compound these challenges, biocontrol agents and biopesticides have emerged as viable alternative in pest and disease management. The genus *Trichoderma* consists of anamorphic group isolated primarily from soil and decomposing organic matter with teleomorphs, when known, belonging to the division Ascomycota (Order Hypocreales). Fungal species belonging to this genus are worldwide in occurrence and easily isolated from soil, decaying wood and other plant organic matter. *Trichoderma* isolates are characterized by a rapid growth rate in culture and by the production of numerous spores (conidia) with varying shades of green.

Development of formulations with increased shelf life and broad spectrum of action with consistent performance under field conditions could pave the way for commercialization of the technology at a faster rate.

Mainly focus should be on formulation development, mass production, quality aspect, delivery system and its scope in commercialization in India for the management of plant diseases. Term solid state fermentation (SSF) is applied for the processes in which insoluble materials in water is used for the microbial growth. Solid-state fermentation (SSF) is an effective method for the mass production of fungal biopesticides since it provides micropropagules with higher conidia content. Various cheap cereal grains like sorghum, millets and ragi are used as substrates (Lewis, 1991; Jeyarajan, 2006).

The grains are moistened, sterilized and inoculated with *Trichoderma* and incubated for 10 to 15 days for production of *Trichoderma* which produces dark green spore coating on the grains. These grains can be powdered finely and used as seed treatment or the grains can be used as it is enriching with FYM for soil application.

Shelf life of the formulations decides the commercialization of biocontrol agents. Formulations should sustain the viable nature of the product for the increased period of storage. Bio control product should have the minimum shelf life of 8 to 12 months for industrialization.

Materials and Methods

Seven isolates of *Trichoderma* were collected from different crop rhizospheres through serial dilution of soil. The isolates were identified through their colony colour, structure of their phialides and spores. They were maintained by sub culturing o n Potato dextrose agar.Two isolates namely *Trichoderma* Isolate 2 and *Trichoderma* Isolate 5 were selected from all the collected isolates basing on their growth habit on different media and antagonistic nature against two soil borne pathogens i.e *Rhizoctonia sp* and *Fusarium sp.*.Eighteen solid substrates were collected from different sources and evaluated against these two isolates. Three replications were maintained for each treatment. Bottles used for spawn production (Mushroom seed) were used for carrying out mass multiplication of *Trichoderma*. Grains were first washed properly and then half boiled in water depending on their size and thickness of seed coat. Excess water was drained out and the grains were shade dried to retain 60 % moisture approximately. After drying, grains were mixed properly with $CaCO_3$ (Calcium carbonate) powder @30g $CaCO_3$ per kg of grain. All other solid substrates except grains were filled in the bottles and sterile water was added in order to keep 60% moisture approximately. These bottles were then plugged with non-absorbent cotton.

The bottles already filled with substrates were steam sterilised in an autoclave at 121.6°C (15 p.s.i) for about 20 minutes. Previously collected isolates were inoculated into PDA plates by hyphal tip method. The inoculated petriplates were incubated for 10-12 days at 26.5^{0}C. 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 and 2).

Antibiotic was added to the sterilised 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 was mixed thoroughly using a sterile glass rod and was serially diluted to get the final dilution of 10^{-7}. One millilitre from this solution was taken and spread over the petriplates containing medium. This entire procedure was repeated at monthly intervals up to 6th month for cfu count.The inoculated petriplates were incubated at 27 $\pm$ 1^{0}C for atleast three days in a BOD incubator. The colonies formed were marked with a permanent marker and observation was noted.

Results and Discussion

All the eighteen substrates were found potent enough for mass multiplication of *Trichoderma* isolates. The two most virulent *Trichoderma* isolates were mass multiplied in the substrates and the cfu counts were taken at 10^{-7} dilution upto six months at monthly intervals. For Isolate 2, Vermicompost recorded the maximum cfu count 463.67 X10^7 cfu/g in second month of inoculation followed by 427.67 X10^7 cfu/g and 366.33 X10^7cfu/g in first month and third month respectively and minimum 97.33 X10^7 cfu/g was registered in the sixth month (Table-1, Figure 3a & 3b).

Table.1 Mean monthly cfu in solid substrates (Isolate 2)

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	Spent maize cob	118.00 (9.07)*	99.00 (9.00)	83.33 (8.92)	45.67 (8.66)	39.33 (8.59)	34.67 (8.53)
2	Maize grain	118.00 (9.07)	124.33 (9.09)	94.67 (8.98)	47.33 (8.67)	40.67 (8.61)	18.33 (8.26)
3	Wheat grain	255.67 (9.40)	167.67 (9.22)	98.33 (8.99)	64.33 (8.81)	42.33 (8.62)	32.00 (8.50)
4	Finger millet grain	133.67 (9.12)	103.33 (9.01)	79.33 (8.90)	66.00 (8.82)	38.33 (8.58)	16.67 (8.20)
5	Rice bran	272.00 (9.44)	137.00 (9.14)	121.67 (9.08)	104.33 (9.09)	88.33 (8.95)	70.67 (8.85)
6	Rice husk	333.70 (9.52)	251.70 (9.40)	210.33 (9.32)	174.33 (9.24)	119.33 (9.08)	90.33 (8.96)
7	Cotton Waste	12.67 (8.09)	83.00 (8.92)	32.00 (8.51)	22.00 (8.34)	16.00 (8.20)	9.33 (7.97)
8	Farm yard manure	252.33 (9.40)	139.33 (9.14)	98.33 (8.99)	66.33 (8.82)	47.00 (8.67)	30.00 (8.48)
9	Vermi-compost	427.70 (9.63)	463.67 (9.67)	366.33 (9.56)	181.00 (9.26)	137.00 (9.14)	97.33 (8.99)
10	Sorghum grain	130.00 (9.11)	88.70 (8.95)	60.00 (8.78)	46.33 (8.66)	34.00 (8.53)	25.33 (8.40)
11	Groundnut Shell	252.70 (9.40)	261.33 (9.42)	201.67 (9.30)	172.33 (9.24)	117.67 (9.07)	87.67 (8.94)
12	Bagasse	52.00 (8.72)	39.67 (8.60)	29.33 (8.47)	21.33 (8.33)	13.67 (8.13)	8.67 (7.94)
13	Coir pith	23.33 (8.37)	29.33 (8.47)	23.00 (8.36)	14.33 (8.16)	9.67 (7.98)	5.33 (7.73)
14	Charcoal	21.00 (8.32)	21.67 (8.34)	20.00 (8.30)	13.67 (8.13)	9.33 (7.97)	4.00 (7.59)
15	Saw dust	29.33 (8.47)	26.70 (8.43)	23.33 (8.37)	17.33 (8.24)	11.70 (8.06)	7.33 (7.86)
16	Spent mushroom substrate	115.67 (9.06)	90.33 (8.96)	78.67 (8.90)	55.33 (8.74)	35.67 (8.55)	25.00 (8.40)

17	Pigeonpea husk	66.00 (8.81)	58.33 (8.76)	46.33 (8.67)	30.67 (8.49)	23.00 (8.36)	18.33 (8.26)
18	Organic waste	21.67 (8.33)	47.67 (8.68)	40.33 (8.60)	24.00 (8.38)	18.67 (8.27)	13.67 (8.13)
	SE(m)+	0.03	0.02	0.02	0.02	0.03	0.04
	C.D(0.05)	0.07	0.06	0.06	0.07	0.09	0.11

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

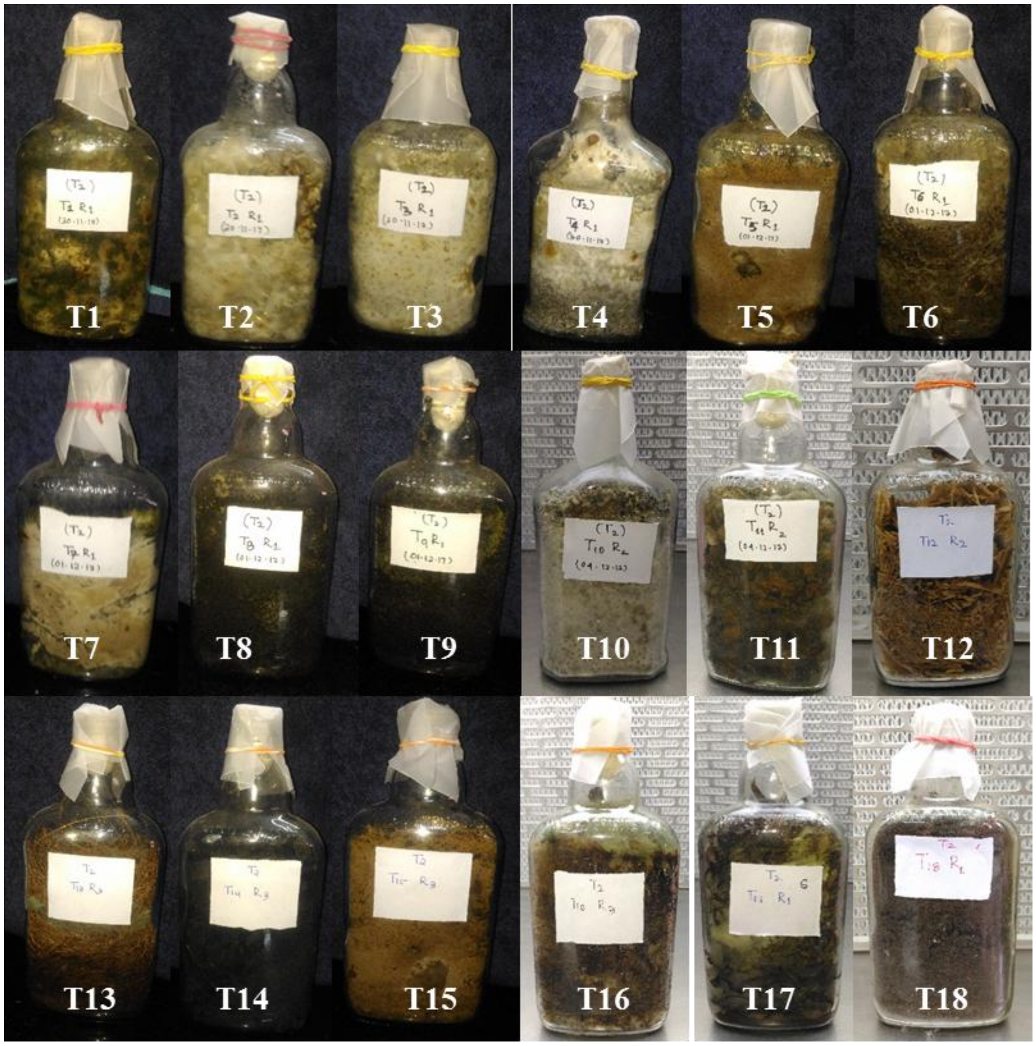
Table.2 Mean monthly cfu in solid substrates (Isolate 5)

Sl.No	**Treatments**	**1st month** (cfu x10^7)/g	**2nd month** (cfu x10^7)/g	**3nd month** (cfu x10^7)/g	**4th month** (cfu x10^7)/g	**5th month** (cfu x10^7)/g	**6th month** (cfu x10^7)/g
1	Spent maize cob	210.67 (9.32)*	166.67 (9.22)	152.33 (9.18)	119.67 (9.08)	77.00 (8.89)	60.33 (8.78)
2	Maize grain	112.67 (9.05)	98.00 (8.99)	78.33 (8.89)	59.33 (8.77)	38.33 (8.58)	25.67 (8.40)
3	Wheat grain	161.00 (9.21)	118.00 (9.07)	104.00 (9.02)	73.00 (8.86)	38.67 (8.59)	25.00 (8.40)
4	Finger millet grain	205.00 (9.31)	153.00 (9.18)	128.67 (9.11)	101.00 (9.00)	66.00 (8.82)	41.33 (8.614)
5	Rice bran	375.33 (9.57)	284.00 (9.45)	215.67 (9.33)	190.00 (9.28)	141.67 (9.15)	98.67 (8.99)
6	Rice husk	353.33 (9.55)	286.00 (9.46)	237.33 (9.38)	210.00 (9.32)	177.00 (9.25)	107.00 (9.03)
7	Cotton Waste	121.00 (9.08)	98.00 (8.99)	67.33 (8.83)	51.67 (8.71)	26.67 (8.42)	19.00 (8.28)
8	Farm yard manure	201.33 (9.30)	160.33 (9.21)	132.33 (9.12)	98.67 (8.99)	73.00 (8.87)	49.33 (8.69)
9	Vermi-compost	602.00 (9.78)	355.00 (9.55)	274.00 (9.44)	205.33 (9.31)	169.33 (9.23)	120.00 (9.08)
10	Sorghum grain	227.00 (9.36)	187.67 (9.27)	155.67 (9.19)	117.00 (9.07)	92.67 (8.97)	62.67 (8.80)
11	Groundnut Shell	467.67 (9.67)	332.00 (9.52)	282.67 (9.45)	230.00 (9.36)	198.67 (9.30)	144.00 (9.158)
12	Bagasse	46.67 (8.67)	37.33 (8.57)	31.33 (8.50)	21.33 (8.33)	12.33 (8.09)	8.00 (7.89)
13	Coir pith	11.67 (8.07)	12.67 (8.10)	11.33 (8.05)	6.00 (7.76)	2.33 (7.36)	1.33 (7.10)
14	Charcoal	7.67 (7.87)	13.00 (8.11)	12.33 (8.09)	4.33 (7.62)	2.00 (7.30)	1.33 (7.10)
15	Saw dust	14.33 (8.14)	20.00 (8.30)	17.33 (8.24)	11.33 (8.05)	7.67 (7.88)	3.33 (7.50)
16	Spent mushroom substrate	166.00 (9.22)	155.33 (9.19)	142.00 (9.15)	109.00 (9.04)	83.00 (8.92)	55.67 (8.75)
17	Pigeonpea husk	47.67 (8.68)	34.33 (8.54)	28.00 (8.45)	19.67 (8.29)	12.33 (8.09)	8.00 (7.90)
18	Organic waste	52.67 (8.72)	43.67 (8.64)	31.67 (8.50)	21.67 (8.33)	15.00 (8.17)	8.67 (7.93)
	SE(m) +	0.03	0.02	0.01	0.03	0.03	0.05
	C.D (0.05)	0.09	0.06	0.04	0.10	0.08	0.15

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

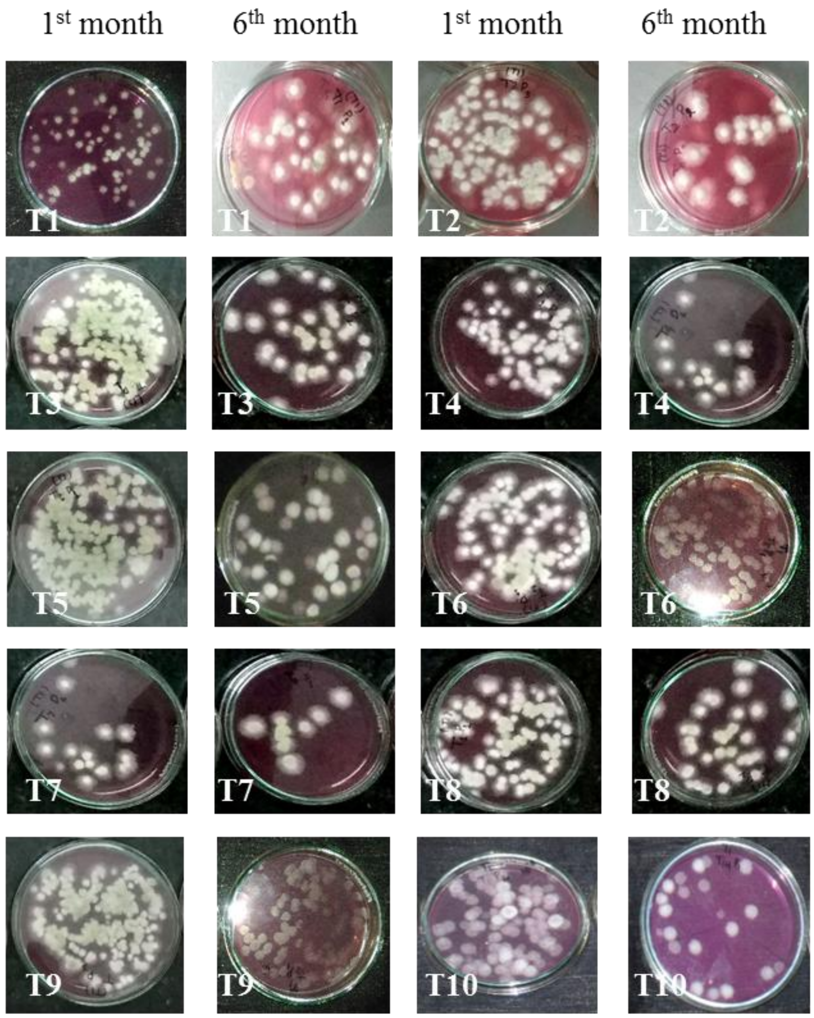
T1: Spent maize cob
T2: Maize grian
T3: Wheat grain
T4: Ragi grain
T5: Rice bran
T6: Paddy husk
T7: Cotton waste
T8: Farm yard Manure
T9: Vermicompost
T10: Sorghum grain
T11: Groundnut shell
T12: Sugarcane bagasse
T13: Coir pith
T14: Charcoal
T15: Saw dust
T16: Spent mushroom substrate
T17: Pigeon pea husk
T18: Organic waste

Figure 1- Bottles containing different solid substrates incolulated with *Trichoderma* Isolate 2



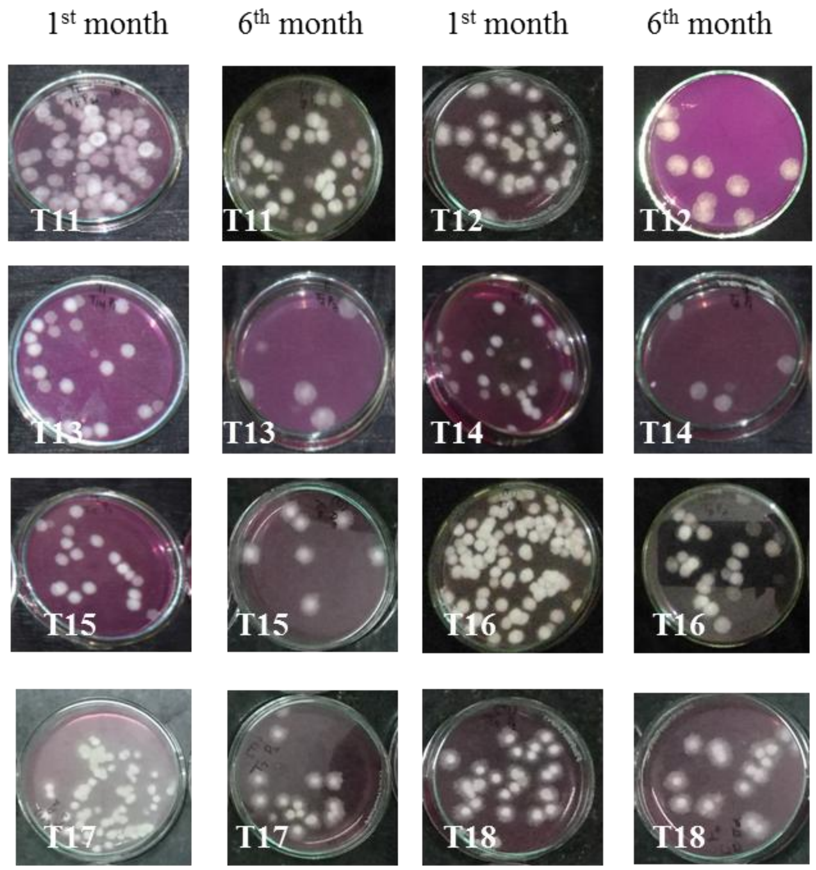
T1: Spent maize cob
T2: Maize grian
T3: Wheat grain
T4: Ragi grain
T5: Rice bran
T6: Paddy husk
T7: Cotton waste
T8: Farm yard Manure
T9: vermicompost
T10: Sorghum grain
T11: Groundnut shell
T12: Sugarcane bagasse
T13: Coir pith
T14: Charcoal
T15: Saw dust
T16: Spent mushroom substrate
T17: Pigeon pea husk
T18: Organic waste

Figure 2- Bottles containing different solid substrates incolulated with *Trichoderma* Isolate 5



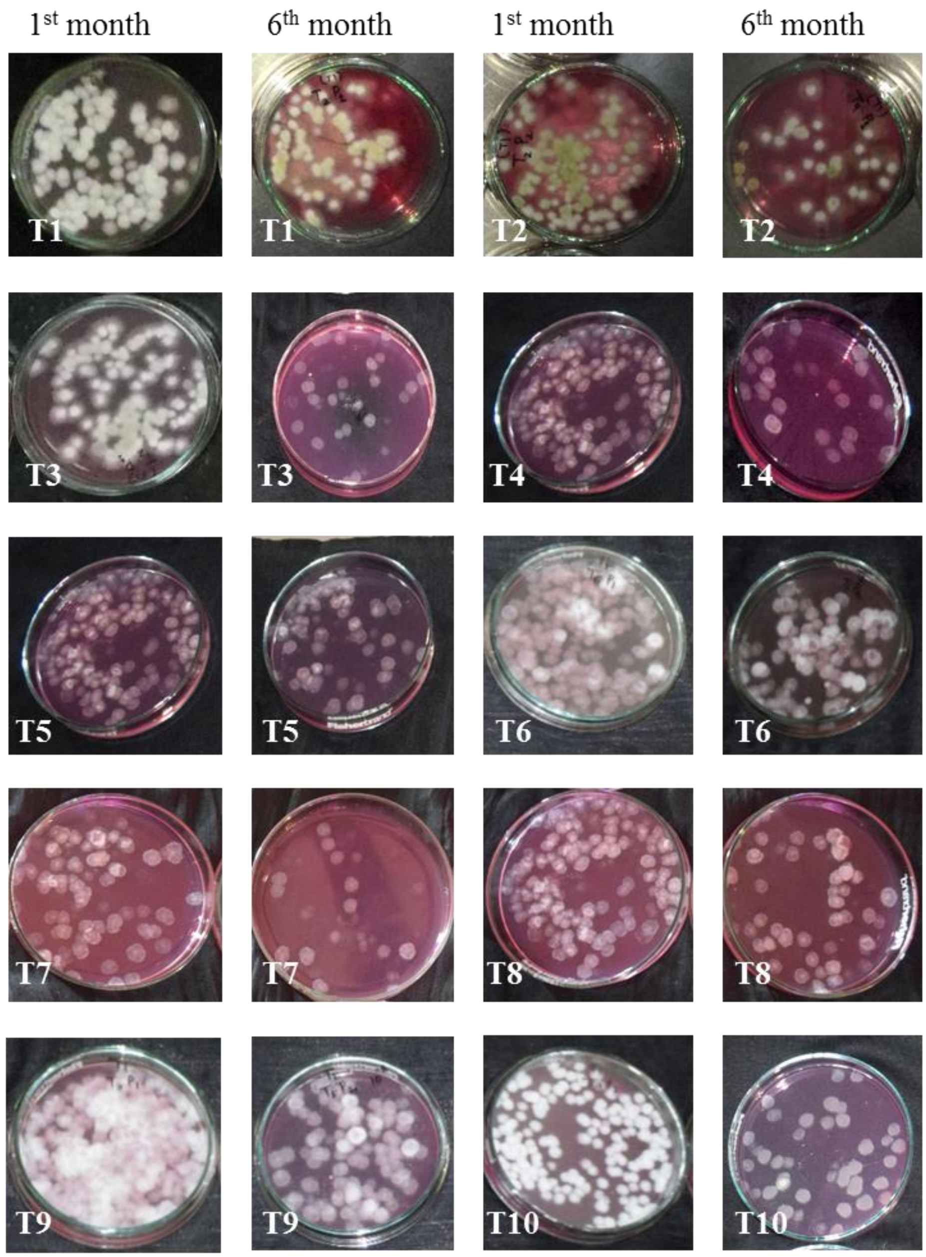
T1: Spent maize cob
T2: Maize grian
T3: Wheat grain
T4: Ragi grain
T5: Rice bran
T6: Paddy husk
T7: Cotton waste
T8: Farm yard Manure
T9: vermicompost
T10: Sorghum grain

Figure 3a – Petriplates showing colony forming units(cfu) of different solid substrates inoculated with *Trichoderma* Isolate 2 (T1-T10)



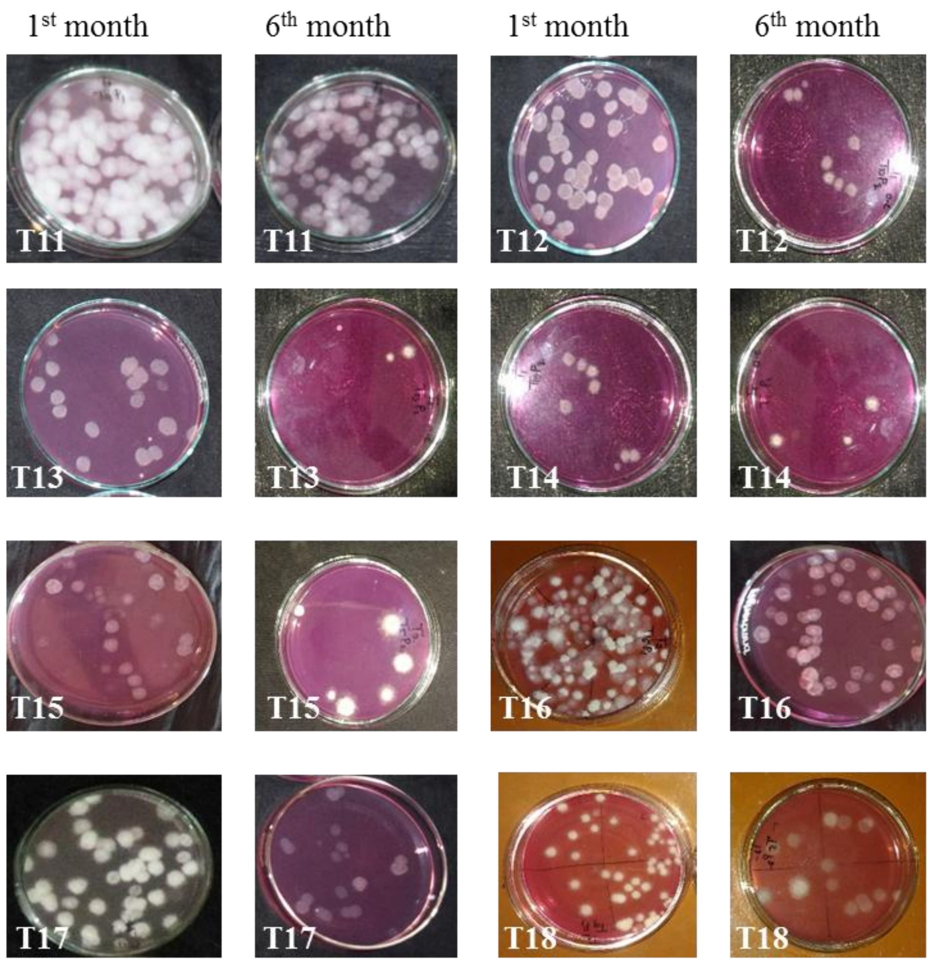
T11: Groundnut shell
T12: Sugarcane bagasse
T13: Coir pith
T14: Charcoal
T15: Saw dust
T16: Spent mushroom substrate
T17: Pigeon pea husk
T18: Organic waste

Figure 3b – Petriplates showing colony forming units of different solid substrates inoculated with *Trichoderma* Isolate 2 (T11-T18)



T1: Spent maize cob
T2: Maize grian
T3: Wheat grain
T4: Ragi grain
T5: Rice bran
T6: Paddy husk
T7: Cotton waste
T8: Farm yard Manure
T9: vermicompost
T10: Sorghum grain

Figure 4a – Petriplates showing colony forming units of different solid substrates inoculated with *Trichoderma* Isolate 5 (T1-T10)



T11: Groundnut shell
T12: Sugarcane bagasse
T13: Coir pith
T14: Charcoal
T15: Saw dust
T16: Spent mushroom substrate
T17: Pigeon pea husk
T18: Organic waste

Figure 4b – Petriplates showing colony forming units of different solid substrates inoculated with *Trichoderma* Isolate 2 (T11-T18)

This experimental finding is found in compliance with the finding of Khan *et al.*, (2011) where they used deoiled castor cake, gypsum, talc powder, vermicompost and well decomposed farmyard manure for preparation of *T. viride* formulation.

For Isolate 5, groundnut shell recorded to be the best performer with maximum cfu of 467.67 X10^7 cfu/g in the first month followed by 332.00 X10^7 cfu/g and 282.67 X10^7 cfu/g in the second and third month respectively and the least cfu count 144.00 X10^7 cfu/g was registered in the sixth month (Table-2, Figure-

4a & 4b), which is in agreement with findings of Kousalya *et al*.,(1988) who reported several substrates along with procedure for mass multiplication of antagonistic fungi and mentioned that, tapioca rind, well decomposed farm yard manure (FYM), well decomposed press mud, gobar gas slurry, mushroom spent bed, paddy husk, wheat bran and groundnut shell were found to be superior substrates for mass multiplication of *T. harzianum*. Therefore the effective substrates may be exploited for commercial production of *Trichoderma* sp.

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