

Farm Level Evaluation of *Trichoderma* Enriched Organic Substrates for Improved Field Establishment and Yield Enhancement in Chillies (*Capsicum annuum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author SK designed and conducted the field study, performed the statistical analysis, wrote the first draft of the manuscript, managed the literature searches and obtained corrections and suggestions for modifications from co-authors and prepared the final manuscript. Author MB involved in design of study, provided the microbial formulations for the study, supported in selecting substrate combinations, performed microbial analysis, managed literature searches, correction of final manuscript and editing of manuscript after reviewing. Author JM managed the physico-chemical analysis of soil and correction of final manuscript. Author SI assisted in microbial analysis and preparation of microbial formulations and overall support. All authors read and approved the final manuscript.

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ABSTRACT

Trichoderma sp. is extensively used for controlling several soil and seed borne fungal plant pathogens and for enhancing crop growth and yield. But the field efficacy depends on several factors viz., proper formulation, an efficient delivery system, methods of application and environmental conditions. The performance and persistence in field to a greater extent is decided by the carriers or substrates used for mass multiplication. In a field trial undertaken on the varied performance of *Trichoderma* sp. involving different substrate combinations on the yield of chillies by ICAR-CPCRI for the period, April, 2014 to June 2016 two combinations, viz., Cowdung + Neem

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cake (4:1) and Coir pith compost + Neem cake + Poultry Manure + Cowdung (2:1:1:1) were found to be superior when enriched with *Trichoderma harzianum* (CPTD 28) as well as native isolate, *Trichoderma* sp. KKTD 6.

Keywords: Enrichment; organic substrates; *Trichoderma* sp.

ABBREVIATIONS

ICAR : Indian Council of Agricultural Research.

CPCRI : Central Plantation Crops Research Institute.

1. INTRODUCTION

Many of the beneficial microbes have been proved as effective biocontrol agents. Some microbial agents act as biofertilizer and/or plant growth promoters by fixing N, solubilizing P, chelating Fe, producing hormone-like substances, degrading 1-aminocyclopropane-1-carboxylate (ACC) deaminase, degrading organic matter and releasing nutrients in soil [1]. About 70% of fungal BCAs market comprises of *Trichoderma* sp. and it has been included in the gazette of India as potential bio-control agents on 26th March 1999. *Trichoderma* sp. is extensively used for controlling several soil and seed borne fungal plant pathogens [2] and it enhances the crop yield by playing multiple roles such as promoting healthy growth in early stages of plant with its application as biofungicides [3].

Most often farmers use microbial agents along with several locally available substrates and in some of them like poultry manure and goat manure, the establishment of the microbes was observed to be poor. Hence, a field trial was undertaken on the varied performance of *Trichoderma* sp. (maintained at ICAR-CPCRI and best native isolate) involving varied use of organic substrates under the NABARD supported Farm Innovation and Promotion Fund Project on "Community based Bio - resource management for sustaining production and livelihood security under coconut based farming systems" implemented by ICAR-CPCRI in Kanjikuzhy Block for the period, April, 2014 to June 2016. Even though some of these antagonists show excellent potential for wider use (particularly *Trichoderma*), they continue to be greatly underutilized, primarily due to strict regulations regarding their use and the technical difficulties associated with introducing and maintaining a specific strain of fungi in the soil [4]. Some technical problems to be overcome are: the identification of the factors affecting their survival rates in soils, the best strain for each

crop and field conditions, the best methods of field application, the best formulation for delivery, the most appropriate farm management practices to enhance biocontrol, and the education of farmers on the use of the technology.

The optimal conditions required to market *Trichoderma* as a biocontrol agent against soil-borne fungi and nematodes were reported earlier, which includes a proper formulation, an efficient delivery system, and alternative methods of application [5]. Several possible ways to improve its efficacy were reported elsewhere as: the increase of the applied quantity per unit of soil, which is commonly limited by the cost of the commercial product, the use of carriers or substrates which can guarantee a longer persistency (i.e. cellulose, or chitin rich substrates), to give sufficient time to *Trichoderma* to reduce inoculum before planting, to apply when the temperature of the soil is sufficiently high, to combine with other practices, for example as bioinoculum after solarisation or anaerobic soil disinfestation or biofumigation [1]. Decomposed coir pith was observed as one of the best substrates for the preparation of bio-manure using *T. viride* [6]. This potential soil fungus, degraded the cellulose and lignin in coir pith and reduced the C/N ratio in decomposed coir pith from 38.76 to 23.15. In general, mass multiplication of bioagents depends largely on the substrate used for mass multiplication and the method of culturing.

With this back ground, the present study was undertaken to evaluate the efficacy of *Trichoderma* multiplied on various locally available organic substrates to evolve an economic and eco-friendly strategy to enhance the establishment and yield of solanaceous vegetables in coconut based farming system in coastal areas.

2. MATERIALS AND METHODS

A field evaluation trial on the efficacy of different organic substrate combinations enriched with *Trichoderma harzianum* (CPTD 28 obtained from ICAR- CPCRI Kasaragod) and the best native isolate of *Trichoderma* sp. (KKTD 6) from

Kanjikuzhy Block was conducted with chilly (Hybrid Chilly var.INDAM Aruna) as the test crop as part of the NABARD supported Farm Innovation and Promotion Fund Project on "Community based Bio - resource management for sustaining production and livelihood security under coconut based farming systems" implemented by ICAR-CPCRI for the period, April, 2014 to June 2016. The soil in the experiment site was sandy with pH, 6.47, organic carbon (0.59%), available P (138.75ppm), available K (36.5ppm), exchangeable calcium (125.71ppm), and exchangeable magnesium (48.96ppm) [7].

2.1 Production of Talc Based Formulation of *Trichoderma* spp.

Seven day old pure culture of *T. harzianum* (CPTD 28) and *Trichoderma* sp. KKT6 grown on potato dextrose agar (PDA) were used for inoculating Potato dextrose broth (PDB). Sterilized PDB inoculated with sporulating discs of *Trichoderma* spp. were incubated at room temperature (28 ± 2 °C) for 10 days. The broth containing mycelial mat and conidia were homogenized and mixed with sterilized talc powder in the ratio 1:2. The talc based formulation shade dried to 8% moisture level was used for mass multiplication of *Trichoderma* spp. in various substrates.

2.2 Mass Multiplication of *Trichoderma* spp. on Various Substrates

The following combinations of locally available organic substrates were used for the study conducted:

1. Coir pith compost + Neem cake (4:1)
2. Coir pith compost + Neem cake + Poultry Manure + Cowdung (2:1:1:1)
3. Cowdung + Neem cake (4:1)
4. Coir pith compost + Cow dung + Neem cake (2:1:1)
5. Coir pith compost alone

Selected substrates were weighed and the moisture content was adjusted to 50 per cent. The substrates were thoroughly mixed to ensure the uniform availability of moisture. The above five substrates were enriched with *T. harzianum* (CPTD 28) and native isolate KKT6 @ 1 Kg talc formulation per 100Kg substrate. The inoculated substrates were incubated for 12 days for multiplication of *Trichoderma* sp. The viable propagule count of *Trichoderma* sp. in enriched

substrates was enumerated. The ten enriched combinations were applied to 10 beds (2 m X 3 m size) each having of 12 chilly plants in each. There were 120 plants altogether.

Treatment nos. 1-5 are the above five substrate combinations (in the same order) enriched with CPTD 28 and treatment nos. 6-10 are the same combinations (in the same order) with KKT6 and one control plot was maintained (treatment-11).

The manure combinations were applied at the time of planting and at monthly intervals @ 500 g/plant. A control bed with 12 plants was also maintained and application of cow dung slurry once in a month and mulching with fresh Glyricidia leaves and dry leaves were practiced uniformly in all the beds including control. For enumeration of *Trichoderma* population soil samples were taken to a depth of 20 cm 30 days after application. Soil samples were collected at a depth of 0-25m using augor and hammer and were processed for the analysis of pH, organic carbon and available nutrients as per the standard procedures available in Jackson (1973).

2.3 Enumeration of *Trichoderma* sp. in Soil and Substrate

The population of *Trichoderma* in the inoculated substrates and treated soil was determined by serial dilution and plating technique [8]. The diluted substrate/ soil suspensions were plated on *Trichoderma* selective medium (TSM) [9]. The plates were incubated at room temperature (28 ± 2 °C). The number of *Trichoderma* colonies developed after three days of incubation in individual plates were recorded and colony forming units (CFU) per gram of substrate/ soil was determined.

2.4 Statistical Analysis

Analysis of variance and comparison of means were calculated for yields under different treatments using SPSS. The mean yields were compared using the least significance difference (LSD) test. The significance of difference between the pairs of treatment means was evaluated by Tukey B Test at 95% confidence levels ($P \leq 0.05$). Multiple correlations were worked out to find the association among different variables, viz., yield, *Trichoderma* population and nutrient status in manure and soil.

3. RESULTS AND DISCUSSION

3.1 Field Evaluation of the Organic Manure Combinations Enriched with *T. harzianum* (CPTD 28) and Native Isolate of *Trichoderma* sp. (KKTD 6)

The *Trichoderma* population and nutrient status of the above manurial combinations enriched with *T.harzianum* (CPTD 28) and KKTD 6, the nutrient status of the soil after application of the enriched manures as well as the treatment-wise yields were recorded and provided in Table 1.

Table 1 revealed T3, Cowdung + Neem cake (4:1) enriched with *T.harzianum* (CPTD 28) as superior, followed by T2, the combination of Coir pith compost + Neem cake + Poultry Manure + Cowdung (2:1:1:1)enriched with *T.harzianum* (CPTD 28). Among the combinations enriched with KKTD 6 also, highest yields were obtained for the treatments having the above two organic mixes, but lesser than that with *T.harzianum* (CPTD 28). However, the trend in growth rate of the two strains were similar in the above two combinations.

One way ANOVA for comparison of yields (Table 2) under various treatments revealed significant variation among treatments at 1% level of significance.

Results of the Tukey post hoc test for multiple comparisons (Table 3) revealed significant difference among all treatments and with control, except treatment no.5.

The highest mean yield of chillies was observed in T3, with cowdung and neem cake as the substrate combination, but based on the Tukey post hoc test, T2 was found to be on par with T3, where both the organic substrate combinations were treated with *T. harzianum* CPTD28. The local isolate KKTD 6 also performed well with the same substrate combinations (T7 and T8), which were also on par. This can be further validated by analysing the yield levels from the native isolates wherein, highest yields were recorded from the same combinations, even though lower than those enriched with CPCRI culture.

Sustainability in yield was plotted for different treatment combinations in Fig 1.

Higher yields under T2 and T3 can be attributed to the optimum levels of microbial population in the enriched organic mix as well as soil coupled with higher nutrient levels in the treated plants. Yield was higher in T2 up to 6 harvests, with timely application of manures at monthly intervals. Thereafter the yield levels were higher in T3, when the interval of application was prolonged. The sustainability of yield under T3 and T8 can be attributed mainly to the higher

Table 1. Comparison of the effect of substrate combinations enriched with *T.harzianum* (CPTD 28) and KKTD 6 on field performance of chillies

T. No	<i>Trichoderma</i> Population (Manure) ($\times 10^5$ CFU /g)	<i>Trichoderma</i> Population (soil) ($\times 10^3$ CFU /g)	Nutrient status – manure (%)			Nutrient status – Soil (Kg/ha)			Total yield (g)
			N	P	K	N	P	K	
T1	27.6	17.2	1.17	0.5	0.81	48.49	18.50	241.00	4416.10
T2	20.6	22.4	1.16	1.5	0.91	63.04	44.75	280.00	8066.80
T3	27.2	35.2	1.44	0.88	0.42	37.18	49.75	192.50	8812.20
T4	19	12.2	1.05	1.15	0.83	51.73	26.00	212.00	4836.70
T5	12.6	14.4	0.87	1.68	1.76	53.34	94.00	157.50	3126.70
T6	17.6	15	0.8	0.83	1.15	35.56	34.00	239.50	4158.30
T7	13.2	15.8	0.91	1.16	0.81	53.34	40.75	167.50	5922.30
T8	18.4	16.4	1.26	0.73	0.34	51.73	46.25	81.00	6007.40
T9	15.8	13	1.09	0.98	0.81	38.79	57.00	171.00	4064.30
T10	11	11.6	1.13	1.98	1.42	75.97	33.50	124.50	4172.80
C		0.6				20.10	17.25	68.20	1679.10

Table 2. One way ANOVA for comparison of yields under various treatments

		Sum of squares	df	Mean square	F	Sig.
YIELD	Between Groups	3376399	10	337639.9	16.291	.000
	Within Groups	2507754	121	20725.241		
	Total	5884153	131			

Table 3. Multiple comparison of mean yields of chillies under different treatments

T. no.	Treatment details	Mean yield / plant(g)	SE
1	Coir pith compost + Neem cake (4:1) with <i>T. harzianum</i> (CPTD 28)	357.86 ^{bc}	19.56
2	Coir pith compost + Neem cake + Poultry Manure + Cowdung 660.57 ^a (2:1:1:1) with <i>T. harzianum</i> (CPTD 28)		43.59
3	Cowdung + Neem cake (4:1) with <i>T. harzianum</i> (CPTD 28)	706.85 ^a	80.88
4	Coir pith compost + Cow dung + Neem cake (2:1:1) with <i>T. harzianum</i> (CPTD 28)	389.41 ^{bc}	37.94
5	Coir pith compost alone with <i>T. harzianum</i> (CPTD 28)	249.24 ^{cd}	33.13
6	Coir pith compost + Neem cake (4:1) with native isolate KKTD 6	331.94 ^{bc}	27.79
7	Coir pith compost + Neem cake + Poultry Manure + Cowdung 479.11 ^b (2:1:1:1) with native isolate KKTD 6		39.24
8	Cowdung + Neem cake (4:1) with native isolate KKTD 6	485.28 ^b	26.11
9	Coir pith compost + Cow dung + Neem cake (2:1:1) with native isolate KKTD 6	333.69 ^{bc}	39.97
10	Coir pith compost alone with native isolate KKTD 6	339.85 ^{bc}	50.68
11	Control	137.36 ^d	21.87

Significance at .05 level

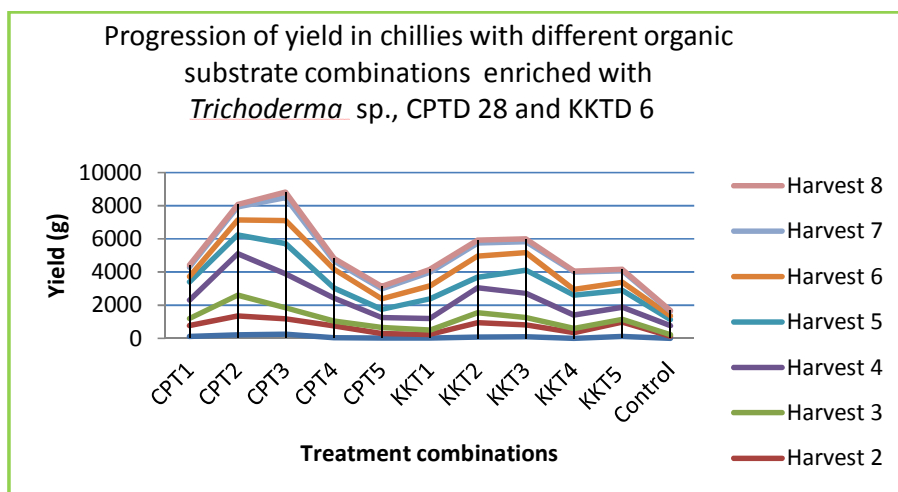


Fig. 1. Progression of yield in chillies with different organic substrate combinations enriched with *Trichoderma* sp., CPTD 28 and KKTD 6

survival and growth rates of *Trichoderma* sp. in soil when enriched with Cowdung + Neem cake in the proportion 4:1.

Correlation analysis also revealed that the population of *T. harzianum* in the manure as well as that in soil and Nitrogen content of the manure and soil were found to be significantly associated with the yield of chillies (Table 4).

It was observed that while enriching organics with *T.harzianum*, the population count in the enriched manure as well as soil were found to enhance the N content in the enriched manure

and K content in soil, thereby contributing to enhanced growth and sustained yield in chillies. Even though the K content in the manure was not influenced by the *Trichoderma* population, the soil K was found to be enhanced, may be due to the solubilizing effect. The potential of *Trichoderma* contained biofertilizer in crop cultivation to achieve attractive yield and reducing N-fertilizer application has been undoubtedly proved [10]. *Trichoderma*-enriched organic fertilization regime could maintain a stable tomato yield with reduced input of chemical fertilizers, which could be attributed to its novel effect on the improvements in soil

Table 4. Association of *T. harzianum* population and nutrient status in manure and soil with yield of chillies

	T. Population (Manure) V1	T. Population (Soil) V2	Manure N V3	Manure P V4	Manure K V5	Soil N V6	Soil P V7	Soil K V8	Yield Y
V1	1	0.814**	0.837**	0.065	0.053	0.198	0.023	0.676**	0.699**
V2		1	0.785**	0.214	0.022	0.172	0.28	0.496	0.894**
V3			1	0.452	0.23	0.552*	0.251	0.413	0.744**
V4				1	0.811**	0.848**	0.517	0.22	0.227
V5					1	0.606*	0.537	0.334	-0.152
V6						1	0.184	0.189	0.295
V7							1	-0.037	0.095
V8								1	0.45
Y									1

microflora and soil nutrient availability [11]. *Trichoderma*-enriched biofertilizer could save at least 50% N fertilizer uses for mustard and tomato and could reduce excessive uses of NPK for crop cultivation [10]. It was observed that the plants grown with Coir pith compost + Neem cake + Poultry Manure + Cowdung (2:1:1:1) mix get exhausted fast with decline in yield due to the initial higher yields compared to that of Cowdung + Neem cake (4:1) mix. Hence, frequent application, preferably at monthly intervals should be followed in case of Coir pith compost + Neem cake + Poultry Manure + Cowdung (2:1:1:1) mix for higher and steady yield, while the yields were sustainable in the Cowdung + Neem cake (4:1) mix treated plants when the treatment interval was prolonged. Experience from Farmers' field also revealed tolerance of chilly plants grown with *Trichoderma* enriched manures under short periods of water logging, ensuring sustained higher yields.

4. CONCLUSION

Trichoderma sp. plays an important role in sustainable crop production through its multiple roles as a bio agent against fungal plant pathogens and a growth promoter. *Trichoderma* contained biofertilizer in crop production has been undoubtedly proved as a better option in organic agriculture for reducing N-fertilizer application and to ensure attractive and sustainable yield. However, the efficacy in the field depends largely on the quality of the commercial formulation, the substrates used for multiplication and soil and environmental conditions. Hence, it is very much needed to improve the commercial production and marketing process along with optimization of the operating parameters by utilizing cheaper and locally available substrates. Two such substrate combinations viz., Cowdung + Neem cake (4:1) and Coir pith compost + Neem cake + Poultry Manure + Cowdung (2:1:1:1) were found to be ideal for enrichment with *T.harzianum* under field level evaluations. However further evaluations in multiple locations with different local isolates and further substrate combinations can give more insight on improving the efficiency of microbial agents and also their impact on growth and yield under varied stress conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pertot I, Alabouvette C, Esteve EH, Franka S. Focus group soil-borne diseases. Mini-paper – The use of microbial biocontrol agents against soil-borne diseases; 2015.
2. Kumari A, Kumar R, Maurya S, Pandey PK. Antagonistic Potential of Different Isolates of *Trichoderma* against *Rhizoctonia solani*. Eur Jour of Exp Biol. 2016;6(6):2-6.
3. Subhash Chandra and Singh. *Trichoderma* sp.: As potential bio-control agents (BCAs) against fungal plant pathogens. Indian J. L. Sci. 2016;5(2):105-111.
4. Cook RJ. Making greater use of introduced micro-organisms for biological control of plant pathogens. Annu Rev Phytopathol. 1993;31:53–80.
5. Spiegel Y, Chet I. Integrated Pest Management Reviews. 1998;3:169. Available: <https://doi.org/10.1023/A:1009625831128>
6. Ushamalani C. Development of biomanure for the management of turmeric rhizome rot. Ph.D Thesis, Tamil Nadu Agricultural University, Coimbatore. 2003;106.
7. Jackson ML. Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi. 1973;498.
8. Sriram S, Savitha MJ. Enumeration of colony forming units of *Trichoderma* in formulations – precautions to be taken to avoid errors during serial dilution. J. Biol. Control. 2011;25(1): 64-67.
9. Elad Y, Chet I. Improved selective media for isolation of *Trichoderma* spp. or *Fusarium* spp. *Phytoparasitica*. 1983;11:55-58.
10. Haque M, Ilias GNM, Molla AH. Impact of *Trichoderma*-enriched Biofertilizer on the Growth and Yield of Mustard (*Brassica rapa* L.) and Tomato (*Solanum*

- Lycopersicon* Mill.). The Agriculturists. 2012;10(2):109-119.
11. Cai F, Chen W, Wei Z, Pang G, Li R, Ran W, Shen Q. Colonization of *Trichoderma harzianum* strain SQR-T037 on tomato roots and its relationship to plant growth, nutrient availability and soil micro flora. Plant and Soil. 2015;388:337–350.
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