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Research Note

Effect of various organic substrates on the mass multiplication of *Trichoderma viride*

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ABSTRACT: Four different organic substrates, *viz.*, decomposed coir pith, dried cow dung, rice bran and tea waste, were tested for the growth of *Trichoderma viride* (isolate 1). The population (CFU) of *T. viride* on various substrates was estimated at 20th, 30th, 40th and 60th day after inoculation by serial dilution plate technique. The population of *T. viride* was highest on 20th day in sterilized tea waste (236.30 x 10^6 cfu), followed by rice bran (152 x 10^6 cfu), decomposed coir pith (44.35x 10^6 cfu), talc (36 x 10^6 cfu) and dried cow dung (20 x 10^6 cfu). Sterilized substrate was favourable for the multiplication of *T. viride* except for dried cow dung. The population increased in all the substrates except talc on 30th day and declined from 40th day onwards.

KEY WORDS: Colony Forming Unit (CFU), organic substrates, talc, *Trichoderma viride* (isolate 1)

Trichoderma spp. are saprophytic and highly interactive fungi in root, soil and foliar environments enhancing the root growth and crop productivity. *Trichoderma* induces resistance to biotic and abiotic stresses and also helps in the uptake of nutrients (Harman *et al.*, 2004). *Trichoderma viride* is one of the most widely used biocontrol agents against several root pathogenic fungi throughout the world. The commercial exploitation of a biocontrol agent depends on the substrate on which it can be mass multiplied, cost effectiveness and easy access to the substrate with appropriate nutrient balance (Singh *et al.*, 2001). The commercial formulations available in the market use talc as a carrier. Talc being an inert material, does not support the multiplication of the antagonist. The present study was aimed to find out a suitable substrate for the mass multiplication of *T. viride*.

Screening and identification of effective *T. viride* strain

Twenty-one isolates of *Trichoderma* sp. were obtained by serial dilution plate technique from soils collected from different regions of Tamil Nadu.

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The isolates obtained were maintained on PDA slants. These isolates were tested against the pathogens of vanilla, *viz.*, *Fusarium oxysporum* f. sp. *vanillae*, *Phytophthora meadii* and *Colletotrichum vanillae* by dual culture technique. The isolate *T. viride* 1 (from Candura estate) was found antagonistic to the above pathogens. Assays for protease, ß-1, 3 glucanase and chitinase activity were carried out for all the twenty one isolates of *Trichoderma* sp. Among these, *T. viride* 1 was found to produce more of protease, ß-1, 3 glucanase and chitinase enzymes and found highly antagonistic to vanilla pathogens and hence was used for the study.

Preparation of the substrates for multiplication of *T. viride*

Various organic substrates, *viz.*, rice bran, tea waste, decomposed coirpith (DCP) and dried cow dung, were tested for the multiplication of *T. viride*. One hundred gram of each substrate was weighed in polypropylene bag and moisture content was adjusted to 60% (W/V) by gravimetric method (Dutta and Das, 1999). One set of treatments of the above substrates was sterilized and another set was kept without sterilization. All the treatments were replicated four times and arranged in factorial completely randomized design (FCRD). For sterilization the substrates were autoclaved at 15lb PSI for 1h on two consecutive days. The substrates were inoculated with four mycelial discs of 9 mm each, cut from the periphery of seven-day-old sporulating cultures of *T. viride*. The substrates were incubated at 28 ± 2 ° C under alternate 12h light and 12h darkness condition (Sangle and Bambawale, 2005).

Assessment of population of *T. viride* in various substrates

One gram sample from each substrate was drawn at regular intervals and the population of colony forming units (CFU) of *T. viride* was assessed at 20th, 30th, 40th and 60th day after inoculation by serial dilution technique in *Trichoderma* selective medium (Pramer and Schmidt, 1965; Elad and Chet, 1983). The data was statistically analysed (Rangaswamy, 1995). The data on population (CFU) was log transformed (Gomez and Gomez, 1984) before analysis of variance (ANOVA) was carried out. Duncan's Multiple Range Test (DMRT) was carried out to compare the treatment means. The package used for analysis

Table 1. Population of *Trichoderma viride* (cfu x 10^6) in various substrates after different inoculation days at 10^{-6} dilution

Substrates	Condition	20th day*	30th day*	40th day*	60th day*
Rice bran	Sterilized	152.47(2.19)$^{b}_{A}$	170.00(2.23)$^{b}_{A}$	156.44(2.20)$^{b}_{A}$	126.52(2.11)$^{b}_{B}$
	Unsterilized	7.00(0.90)$^{g}_{B}$	9.30(1.01)$^{g}_{A}$	5.00(0.77)$^{g}_{C}$	4.42(0.72)$^{g}_{C}$
Tea waste	Sterilized	236.30(2.38)$^{a}_{A}$	267.63(2.43)$^{a}_{A}$	253.35(2.41)$^{a}_{A}$	231.67(2.37)$^{a}_{A}$
	Unsterilized	21.67(1.35)$^{ef}_{C}$	33.33(1.54)$^{e}_{A}$	26.33(1.44)$^{de}_{B}$	18.93(1.30)$^{de}_{C}$
Coirpith	Sterilized	44.35(1.66)$^{c}_{C}$	71.00(1.86)$^{c}_{A}$	53.33(1.73)$^{c}_{B}$	32.00(1.52)$^{c}_{D}$
	Unsterilized	25.00(1.41)$^{e}_{B}$	41.33(1.63)$^{d}_{A}$	28.33(1.47)$^{d}_{B}$	18.93(1.30)$^{de}_{C}$
Dried cow dung	Sterilized	20.00(1.32)$^{f}_{B}$	32.67(1.53)$^{e}_{A}$	22.67(1.37)$^{ef}_{B}$	16.00(1.23)$^{ef}_{C}$
	Unsterilized	25.00(1.41)$^{e}_{B}$	34.33(1.55)$^{e}_{A}$	30.07(1.50)$^{d}_{A}$	20.00(1.32)$^{d}_{C}$
Talc	Sterilized	36.00(1.57)$^{d}_{A}$	27.33(1.45)$^{f}_{B}$	20.00(1.32)$^{f}_{C}$	16.00(1.23)$^{ef}_{D}$
	Unsterilized	33.00(1.53)$^{d}_{A}$	24.00(1.40)$^{f}_{B}$	20.75(1.34)$^{f}_{B}$	15.17(1.21)$^{f}_{C}$

* Mean of four replications; means followed by the same letters are not significantly different at both 5% and 1% level by DMRT; figures in parentheses are log-transformed values; small letters indicate the column wise comparison and a capital letters indicate the row-wise comparison

was IRRISTAT version 92-1 developed by the International Rice Research Institute Biometrics Unit, The Philippines.

Among the various substrates, the population of *T. viride* was highest in sterilized tea waste (236.30 X 10^6 cfu), followed by rice bran (152 X 10^6 cfu), decomposed coir pith (44.35 X 10^6 cfu), talc (36 X 10^6 cfu) and dried cow dung (20 X 10^6 cfu) on 20th day (Table 1 and Fig. 1).

Regarding unsterilized substrates, the populations recorded on dried cow dung and coirpith were on par with each other (25 X 10^6 cfu), followed by tea waste (21.67 X 10^6 cfu) and rice bran (7 X 10^6 cfu) on 20th day. Sterilized tea waste supported the growth of *T. viride* and gave the maximum population. This was in accordance with the findings of Singh *et al.* (2001), whereas Tiwari *et al.* (2004) used sterilized rice bran as a substrate for the mass multiplication of *T. viride* with 88.5% spore viability. Ushamalini (2003) observed decomposed coir pith as one of the best substrates for the preparation of bio-manure using *T. viride*. *T. viride*, a potential soil fungus, degraded the cellulose and lignin in coirpith and reduced the C/N ratio in decomposed coir pith from 38.76 to 23.15. The pectinolytic, cellulolytic and protease enzymes produced by *T. viride* enhanced the composting process.

Fig. 1. Growth of *T. viride* on rice bran

The glucanases and chitinases produced by *T. viride* help in the utilization of cellulose and chitin present in the organic waste (Papavizas, 1985). According to Dinakaran (1997), soil application of FYM in combination with *T. viride* was effective in reducing the root rot incidence of blackgram caused by *Macrophomina phaseolina* and increased the plant growth and pod yield. Antagonist-colonized compost not only provided better protection to crops, also improved crop growth as compared to the non-colonized cow dung (Zaidi and Singh, 2004). From this experiment, it is clear that sterilized substrates supported the maximum population compared to unsterilized substrates, the same trend was seen in all the substrates except cow dung. In case of cow dung, unsterilized condition supported the maximum population (25 x 10^6 cfu) compared to the sterilized cow dung (20 x 10^6 cfu) on 20th day. In all the substrates the population started increasing on 30th day after inoculation and then declined from 40th day onwards. It can be concluded that 30 days incubation period is optimum for the mass multiplication of *T. viride*.

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