

Short Communication

Evaluation of substrates for multiplication of bio-agent, *Trichoderma viride*

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An experiment was conducted using locally available substrates to mass multiply *Trichoderma viride* on different substrates. Experiment was conducted during June to August for 2 years (2011 and 2012). Result revealed that sporulation was observed after 5 days of incubation in cowdung, followed by all other substrates after 7 days of incubation. Maximum sporulation (51.50×10^8 cfu g⁻¹) was observed in cowdung on 15th day, which is followed by rice bran, talc powder, rice straw, banana leaf, arecanut leaf, coconut leaf, neem cake and vermicompost with 48.12×10^8 , 44.5×10^8 , 39.00×10^8 , 29.00×10^8 , 21.4×10^8 , 18.2×10^8 , 15.5×10^8 and 10.45×10^8 cfu g⁻¹, respectively. Nevertheless, up to 90 days of observation, significantly superior growth (39.1×10^8 cfu g⁻¹) was noticed on cowdung in comparison to other substrates.

Key words: *Trichoderma viride*, substrates, mass multiplication.

INTRODUCTION

Out of various strategies for management of diseases, chemicals have been so far found to be most dominating. Although this has resulted in increase in production of agricultural commodities but there is deleterious effects of chemical pesticides on crop ecosystems. Increase in public awareness about these problems, has stimulated research on biocontrol agents. Also, biological control of plant diseases has got momentum as it offers many advantages over the conventional methods of control (Mukhopadhyay, 1994). Among the biocontrol agents, *Trichoderma* spp. have shown exceptionally good promise in the management of a wide range of plant pathogens (Cook and Baker, 1983) and are acclaimed as

effective, eco-friendly and cheap, nullifying the ill effects of chemicals. However, it is also known that these biocontrol agents should be native for their good efficacy against their target pathogens. But the utilization of these bioagents has been hampered mainly because of non-availability of effective methods of mass production technology. Different workers have used different substrates such as composted coir pith, coffee (Rukmani and Mariappan, 1993), coffee wastes and poultry manures (Sawant et al., 1995), neem cake, coir pith, farmyard manure (FYM), and decomposed coffee pulp (Saju et al., 2002), well-decomposed FYM, dried cowdung, gobar gas slurry (GGS), sorghum grain floor,

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Table 1. Effect of different substrates on the population of *Trichoderma viride*.

S/N	Substrates	Mean population ($\times 10^8$ cfu g^{-1})						Per cent decrease over initial population
		Days of incubation						
		15	30	45	60	75	90	
1.	Areca nut leaf	21.40 ^f	19.2 ^f	15.6 ^f	13.4 ^f	11.2 ^f	9.4 ^f	56.07
2.	Banana leaf	29.00 ^e	28.4 ^e	26.2 ^e	24.1 ^e	21.2 ^e	17.7 ^e	38.96
3.	Coconut leaf	18.2 ^g	16.7 ^g	15.7 ^g	12.8 ^g	9.3 ^g	7.3 ^g	59.89
4.	Cowdung	51.50 ^a	49.3 ^a	47.7 ^a	44.6 ^a	41.8 ^a	39.1 ^a	24.08
5.	Neem cake	15.5 ^h	14.9 ^h	13.3 ^h	11.5 ^h	9.2 ^h	6.5 ^h	58.06
6.	Rice bran	48.12 ^b	47.9 ^b	45.8 ^b	41.4 ^b	38.2 ^b	35.2 ^b	26.85
7.	Rice straw	39.00 ^d	37.2 ^d	35.9 ^d	32.7 ^d	29.3 ^d	25.4 ^d	34.87
8.	Talc powder	44.5 ^c	42.3 ^c	40.7 ^c	38.2 ^c	36.8 ^c	34.7 ^c	22.02
9.	Vermicompost	10.45 ⁱ	9.4 ⁱ	8.6 ⁱ	6.8 ⁱ	5.7 ⁱ	4.9 ⁱ	53.11
	SEd \pm	0.07	0.08	0.08	0.08	0.08	0.08	
	C.D.(0.05)	0.15	0.17	0.17	0.17	0.17	0.17	

wheat bran, groundnut shell, molasses, saw dust, wheat straw, mushroom bed straw, neem cake, peat soil, fly ash and talc (Sangle and Bambawale 2005), cowdung, neem cake, coir pith, sorghum grain, saw dust, and rice bran (Rini and Sulochana, 2007), vegetable wastes, fruit wastes, crop wastes, FYM and poultry manure (Simon, 2011), vegetable waste, fruit juice waste, sugarcane bagasse, rotten wheat grains (Babu and Pallavi, 2013) for mass production of *Trichoderma* spp.

The present study was therefore carried out to identify a suitable, cheaper and locally available substrate for mass multiplication of *Trichoderma viride*.

MATERIALS AND METHODS

The trial was conducted in the laboratory of Central Plantation Crops Research Institute, Research Centre, Kahikuchi, Guwahati. Nine substrates viz., areca nut leaf, banana leaf, coconut leaf, cowdung, rice bran, rice straw, neem cake, vermicompost and talc powder were evaluated for mass multiplication of *T. viride* using the procedure of Kousalya et al. (1990). Rice straw, areca nut, coconut and banana leaves were chopped into small bits (2 to 3 cm) and then pre-soaked for overnight as per the procedure of Kousalya et al. (1990). The treatments were maintained following a completely randomized block design with 20 numbers of packets for each treatment for 3 months. After incubation, the samples were drawn and population of *T. viride* was enumerated in *Trichoderma* selective medium (Elad and Chet, 1983). Colony forming units (cfu $10^8/g$) were estimated by dilution plate technique (Pramer and Schmidt, 1956) after every 15 days interval and the data was analyzed statistically. The study was conducted during the period from June to August for 2 years (2011 and 2012).

RESULTS AND DISCUSSION

The growth of *T. viride* on different substrates is enumerated in the Table 1. Sporulation was observed after 5 days of incubation in cowdung as compared to

7 days after incubation, in all other substrates. Maximum sporulation (51.50×10^8 cfu g^{-1}) was observed in cowdung on 15th day, which was followed by rice bran (48.12×10^8 cfu g^{-1}). The result is in consistent with the findings of Saju et.al. (2002). Other substrates in order of merit were; by talc powder, rice straw, banana leaf, areca nut leaf, coconut leaf, neem cake and vermicompost with 44.5×10^8 , 39.00×10^8 , 29.00×10^8 , 21.4×10^8 , 18.2×10^8 , 15.5×10^8 and 10.45×10^8 cfu g^{-1} , respectively. A significant difference was observed among all the substrates tried throughout the experiment period that is, starting from 15 to 90 days of incubation. The enhanced effect of cowdung might be due to the supplementation of NPK and other nutrients to the substrate. FYM was reported to be an effective substrate for growth and multiplication of both *Trichoderma harzianum* and *T. viride* (Kousalya and Jeyarajan, 1988). Sethuraman (1991) reported that the increase in *Trichoderma* population was due to application of organic amendments viz., rice bran and FYM in soil. Nevertheless, up to 90 days of observation, significantly superior growth (39.1×10^8 cfu g^{-1}) was noticed on cowdung followed by rice bran (35.2×10^8 cfu g^{-1}), talc powder (34.7×10^8 cfu g^{-1}) and rice straw (25.4×10^8 cfu g^{-1}). Vermicompost recorded continually lower population as compared to other substrates (Table 1). The study also indicated that there is decreasing trend of population of *T. viride*. The decrease in population of *T. viride* was observed after 15 days onwards. In all the substrates, invariably, there was a reduction in the population at 90 days after incubation from initial level, which ranged between 22.02% in talc powder to 59.89% in coconut leaf.

The result of study indicated that the locally available substrates viz., cowdung, rice bran, rice straw, banana leaf and areca nut leaf has got immense potential for growth and sporulation of the antagonistic fungi, *T. viride*,

which can be successfully used in agricultural field for management of soil borne diseases.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

- Babu KN, Pallavi PN (2013). Isolation, identification and mass multiplication of *Trichoderma*- an important bio-control agent. *Int. J. Pharm. Life Sci.* 4(1):2320-2323.
- Cook RJ, Baker KF (1983). *The nature and practice of biological control of plant pathogens*, APS Books, St. Paul, MN, USA, P. 539.
- Elad Y, Chet I (1983). Improved selective media for isolation of *Trichoderma* and *Fusarium* Spp. *Phytoparasitica.* 11:55-58. <http://dx.doi.org/10.1007/BF02980712>
- Kousalya G, Jeyarajan R (1988). Techniques for mass multiplication of *Trichoderma viride* Pers. Fr and *Trichoderma harzianum* Rifai. Abstracts of papers presented in National Seminar on Management of Crop Disease with Plant Products / Biological Agents. *Agric. Colleg. Res. Institut. Madurai*, pp. 32-33.
- Kousalya GK, Jeyarajan R (1990). Mass multiplication of *Trichoderma* spp. *J. Biol. Cont.* 4(1):70-71.
- Mukhopadhyay AN (1994). Biocontrol of soil borne fungal plant pathogens-current status, future prospects and potential limitations. *Indian Phytopathol.* 47:119-126.
- Pramer D, Schmidt EL (1956). *Exp. Soil Micro.* Burges Publishing Company, Minneapolis, USA, P.107.
- Rini CR, Sulochana KK (2007). Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. *J. Trop. Agric.* 45:21-28.
- Rukmani S, Mariappan V (1993). Influence of organic amendments with *Trichoderma viride* on the control of root rot of black gram. *Plant Dis. Res.* 5:244.
- Saju KA, Anandaraj M, Sarma YR (2002). On farm production of *Trichoderma harzianum* using organic matter. *Indian Phytopatho* 55:277-281.
- Sangle UR, Bambawale UM (2005). Evaluation of substrates for mass multiplication of *Trichoderma* spp. *Indian J. Plant Prot.* 33(2):298-300.
- Sawant IS, Sawant SD, Narayan KA (1995). Biological control of *Phytophthora* root rot of coorg mandarin by *Trichoderma* spp. grown on coffee waste. *Indian J. Agric. Sci.* 65:842-846.
- Sethuraman K (1991). Biological control of sesamum root rot caused by *Macrophomina phaseolina* (Tassi) Gold. *M.Sc (Agril) thesis.* TNAU, Coimbatore, India.
- Simon SA (2011). Agro-based Waste Products as a Substrate for Mass Production of *Trichoderma* spp. *J. Agric. Sci.* 3(4):169-171.