

Isolation, characterization & biomass production of *Trichoderma viride* using various agro products- A biocontrol agent

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ABSTRACT

*In the recent years, the environmental contamination caused by excessive use of chemical pesticides increased the interest in integrated pest management, where chemical pesticides are substituted by bio-pesticides to control plant pests and plant diseases. *Trichoderma* spp, is a potential fungal biocontrol agent against a range of plant pathogens. Present study deals with use of household waste, vegetable waste and other wastes and assessment for their suitability as substrates for mass multiplication of *Trichoderma viride* and develops effective production methodology which can be easily adopted. For the production of biomass of fungal grain media, organic media and non-synthetic media have been used. The maximum biomass production of *Trichoderma* spp. was observed in pulses (19.98 gm in 250 ml).*

Key Words: Pathogenic fungi, Grain Medium, Non Synthetic Medium and Organic Medium, Biomass production.

INTRODUCTION

The conventional chemical pesticides has not only enhanced the food production, but also adversely affected the environment and non-target organisms. Due to the side-effects of chemical pesticides, the sustainable crop production through eco-friendly pest management is essentially required in recent scenario. Among the several micro-organisms viz., bacteria, fungi, virus, protozoans and entomopathogenic nematodes, a few have been systematically studied for their effective beneficial characteristics. *Trichoderma viride* is very promising method against soil borne plant parasitic fungi. The fungal pathogens play a major role in the development of diseases on many important field and horticultural crops; resulting in severe plant yield losses. Intensified used of fungicides has resulted in accumulation of toxic compound potentially hazardous to human and environment an also in the buildup of resistance of the pathogens. In order to tackle these national and global problems, effective alternatives to chemical control are being employed. Biological control by an antagonism is a potential, no chemical and ecofriendly approach for managing plant diseases. [1]. *Trichoderma* is one of the common fungal biocontrol agent, is being used world wide for suitable management of various foliar and soil borne plant pathogens.[2] Biocontrol agents like *Trichoderma* spp. are acclaimed as effective, ecofriendly and cheap, nullifying the ill effects of chemicals. Therefore, of late, these biocontrol agents are identified to act against on array of important soil borne plant pathogens causing serious diseases of crops. Therefore considering the cost of chemical pesticides and hazardous involves, biological control of plant diseases appears to be an effective and ecofriendly approach being practice world over. Further biological control strategy is highly compatible with sustainable agriculture and has a major role to play as a component of integrated pest management (IPM) programme. Large scale production, along

with shelf life and establishment of bioagents in targeted niche, determine the success of biological control. Therefore cost effective large scale production, shelf life of formulation, establishment of bioagent in to targeted niche and consistency in disease control are the primary concern with augmentative biological control. Adaptation of technology in the biocontrol arsenal needs to be investigated. Development of acceptable easily prepared and cost effective formulations for delivery should be major goal.

Present study was carried out to evaluate grains such as rice, wheat, pulses and maize at different temperatures, and naturally available solid media such as carrot, ladyfinger, jack seeds, rice husk, and saw dust for the biomass production of *Trichoderma viride*. For mass multiplication of bio agent through liquid state fermentation technology an enormous quantity of spore biomass is needed. Various substrates like sugarcane baggase, fruit juice waste, vegetable waste, rotten wheat grains etc. are being used for mass multiplication of *Trichoderma viride* with various degree of success. [3] Moreover a huge amount of solid waste like sugarcane baggase, fruit juice wastes, vegetable waste and rotten wheat grains increasing pollution and disposal problems. Therefore looking towards need for large scale cost effective production of ecofriendly biopesticide, present investigation is carried out to evaluate locally available cheaper substrates for mass multiplication of *Trichoderma viride* for sustainable environment and sustainable agriculture.

MATERIALS AND METHODS

Isolation and Identification of *T. viride*

Fungal species *Trichoderma viride* was isolated from soil samples by using potato dextrose agar (PDA) medium. Samples were inoculated over plates by multiple tube dilution technique (MTDT) and the plates were incubated at 26°C for 4 days. The fungal colonies which were picked up and purified by streaking and incubated at 26°C for 7-8 days. Green conidia forming fungal bodies were selected and microscopic observation was identified to be *Trichoderma viride*. The culture was maintained on PDA slants [4].

Maintenance of culture

A loopful of inoculum from sub cultured plates of *Trichoderma viride* were transferred to Potato Dextrose Agar (PDA) slants and maintained as pure culture.

For laboratory studies, the fungus was cultured on PDA medium. The medium was sterilized at 15 psi for 30 min in autoclave, poured to sterilized plates, cooled and inoculated with pure culture of the fungus under aseptic conditions. The plates were then incubated at room temperature (26±2°C) for ten days. After complete sporulation, conidia from the medium were harvested by washing them thoroughly with sterilized water containing Tween-20 (0.2%) for immediate use. Otherwise, spores were harvested with the help of a small sterile metal spatula. Harvested conidia were air dried under laminar air flow and stored in a small air tight screw cap vials (10 cm with 2.5 cm diameter) in refrigerator at 4°C before using for further studies. Colony forming units (cfu) were estimated by plating technique. Suspension of spores was made using distilled water with Tween-20 (0.2%) and filtered through a double layered muslin cloth. Spore count was made using a double rolled Neubauer's haemocytometer after necessary serial dilutions under phase contrast microscope. From the stock solution, further dilutions were made to obtain the required concentrations for further studies.

Grain Medium

Three Grains viz rice, wheat and pulses were used for estimating the biomass of *Trichoderma viride* at 26 °C. 20 g of each grain was washed well and boiled in distilled water for 1 hr. and then mesh properly and filter it, now makeup 1 liter with distilled water. Now these grain mediums were packed separately in individual 500 ml conical flask for *Trichoderma viride*. They were plugged with cotton wool and autoclaved at 15 psi for 1hr at 121°C. After cooling, 1 gm of the fungal culture was inoculated into each flask, separately. All these action were done under laminar air flow chamber. They were incubated in BOD incubator at 26°C for 3 weeks. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the culture and to break the mycelial mat.

After 14 days of incubation, the mycelial mat appeared in flasks. Now it was grow well for 21 days. The flasks were shaken in mechanical shaker for 10 min. The suspension was filtered through double layered muslin cloth and then taken biomass in each grain medium.

Non Synthetic Medium

Three Non Synthetic Medium viz saw dust, rice brawn and carrot were used for estimating the biomass of *Trichoderma viride* at 26 °C. 20 g of each grain was washed well and boiled in distilled water for 1 hr and then mesh properly and filter it, now makeup 1 liter with distilled water. Now this Non Synthetic Medium was packed separately in individual 500 ml conical flask for *Trichoderma viride*. They were plugged with cotton wool and autoclaved at 15 psi for 1hr at 121°C .After cooling, 1 gm of the fungal culture was inoculated into each flask, separately. All these procedures were done under laminar air flow chamber. They were incubated in BOD incubator at 26°C for 3 weeks. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the culture and to break the mycelial mat. After 14 days of incubation, the mycelial mat appears again in flasks. Now it was grow well for 21 days. The flasks were shaken in mechanical shaker for 10 min. The suspension was filtered through double layered muslin cloth and then taken biomass in each Non Synthetic Medium.

Organic Medium

Three Organic Medium viz yeast, potato dextrose and jaggery were used for estimating the biomass of *Trichoderma viride* at 26 °C. 20 g of each grain was washed well and boiled in distilled water for 1 hr. and then mesh properly and filter it, now makeup 1 liter with distilled water. Now this Organic Medium was packed separately in individual 500 ml conical flask for *Trichoderma viride*. They were plugged with cotton wool and autoclaved at 15 psi for 1hr at 121°C. After cooling, 1 gm of the fungal culture was inoculated into each flask, separately. All these procedures were done under laminar air flow chamber. They were incubated in BOD incubator at 26°C for 3 weeks. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the culture and to break the mycelial mat. After 14 days of incubation, the mycelial mat appears again in flasks. Now it was grow well for 21 days. The flasks were shaken in mechanical shaker for 10 min. The suspension was filtered through double layered muslin cloth and then taken biomass in each Organic Medium.

RESULTS AND DISCUSSION

In the present study, several naturally available substrates of both solid & liquid media were tested for mass multiplication of *Trichoderma viride*. The success of biological control depends not only the isolation, characterization & pathogenicity, but also on the successful mass production of the fungal agent in laboratory. Large scale availability of the pathogen is a primary requirement in the biocontrol programmed. For a successful integrated pest management programme, the agents like the pathogenic fungi should be amenable to easy & cheap mass multiplication. Current research efforts were directed at selecting native bio control fungi, characterizing them assessing their virulence and developing a formulation for them. The results obtained are discussed herein.

Grain Medium

Among the grain media, pulses medium produced significantly higher 19.98 gm per 250 ml of biomass production was recorded in *Trichoderma viride*. Abundance of minerals in the pulses medium may enhance the growth of fungi. Rice and wheat medium also supported the growth of both the tested fungi.

Non Synthetic Medium

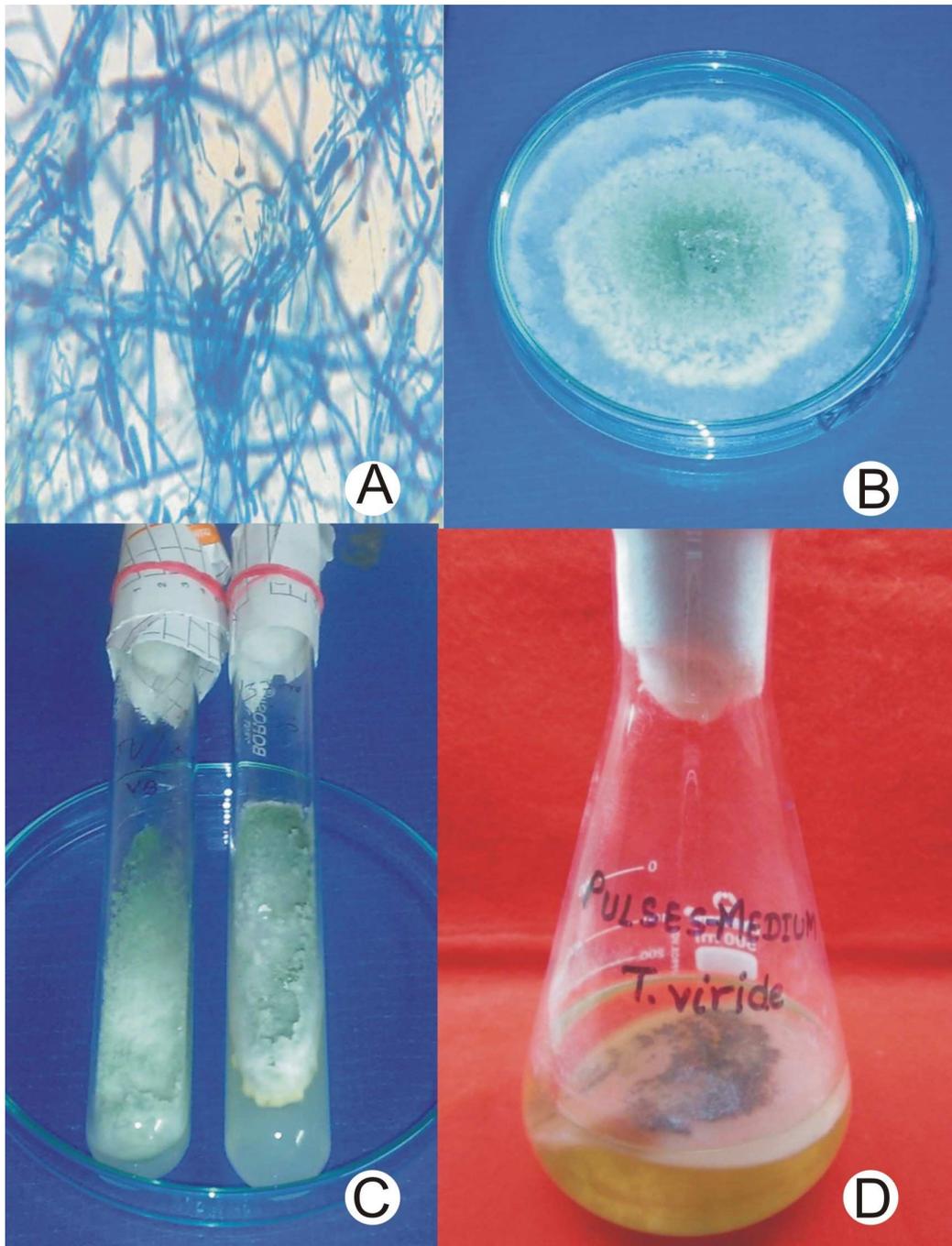
Among the Non Synthetic Medium, Beetroot produced significantly higher 14.32 gm per 250 ml of biomass production was recorded in *Trichoderma viride* respectively.

Organic Medium

Among the Organic Medium, Potato produced significantly higher 16.05 gm gm per 250 ml of biomass production was recorded in *Trichoderma viride* respectively.

In all three categories other media also supported biomass production in all tested fungi (Table-1, Figure-1 A-H, and Figure-2).

Figure-1 (A-H) Biomass production of *Trichoderma viride* fungi



A. Microscopic view of *Trichoderma viride*. B. Mother culture of *Trichoderma viride*.
C. Slants of *Trichoderma viride*. D. 3 weeks in Pulses media

Figure-2 Biomass production of *Trichoderma viride* fungi observed in various media.

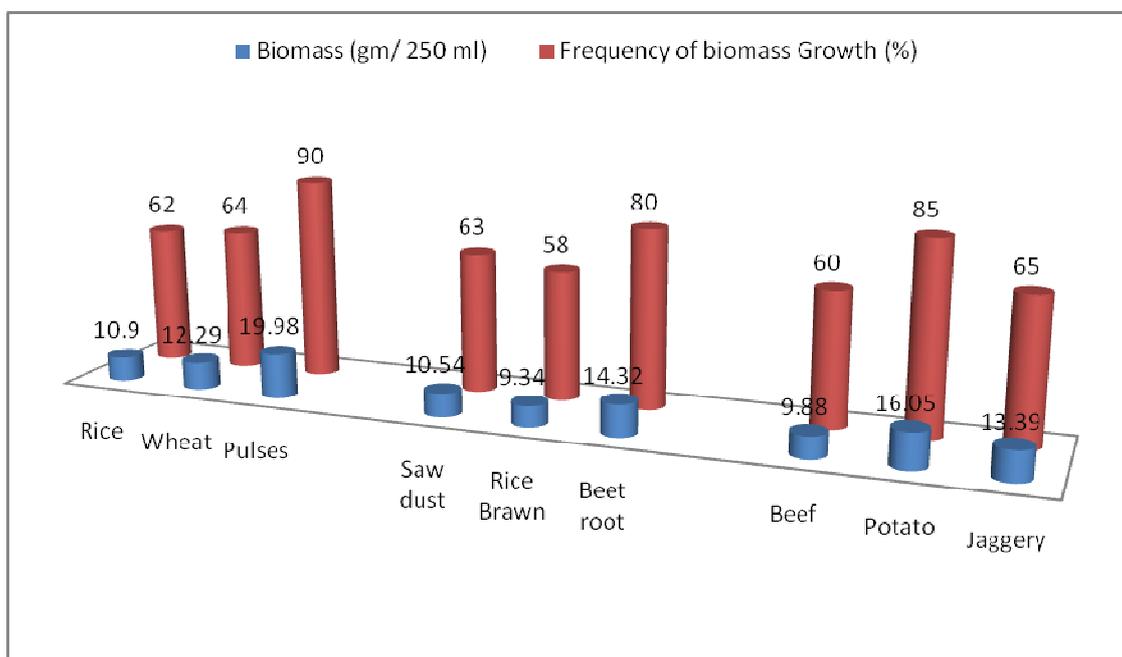


Table-1 Biomass production of *Trichoderma viride*

Media	Microorganism (Temperature 26 °C, Duration 3 weeks)	
Grain	<i>Trichoderma viride</i>	
Rice	Biomass (gm/ 250 ml)	Frequency of biomass Growth (%)
	10.90	62
Wheat	12.29	64
Pulses	19.98	90
Non Synthetic		
Saw dust	10.54	63
Rice Brawn	9.34	58
Beet root	14.32	80
Organic		
Beef	9.88	60
Potato	16.05	85
Jaggery	13.39	65

CONCLUSION

The excess use of chemical pesticides not only polluted the environment but also deteriorated the overall fertility of the soil. There can be benefits using pesticides at initial level but later use can counterproductively increase pest resistance and kill the natural enemies of pests and also adversely affect the fertility of soil. In turn, bio-pesticides not only increase the fertility of soil, but also are eco-friendly and do not affect the other beneficial microorganisms. The pathogenic fungi are considered natural mortality agents and environmentally safe bio-pesticides. Bio-pesticides are more effective than chemical pesticides in long term use, and also cost effective. Pathogenic fungi have been reported as an excellent bio-pesticide. From all above methods we were concluded that pulses showed a high growth rate for biomass production of entomopathogenic fungi (*Trichoderma viride*) as compare to beetroot and potato medium (Table-1, Figure-1 A-H, and Figure-2)..

Acknowledgement

We are grateful to Department of Microbiology and Pathology division, Vital Biotech Research Institute, Kota for providing laboratory facilities and also thankful to Mr. Jitendra Mehta of Vital Biotech for sincere efforts in writing

this research paper. We are also grateful to Mrs Mridula Khandelwal, Miss Sakshi Datta, Miss Ritu Naruka, Miss Komal Makhijani, Mr. Gajendra Sharma, Mr. Rajesh Kumar and Mr. Subhas Chandra for their continuous team work. We would also like to thank Mrs. Jaya Mehta and staff members of Vital Biotech Research Institute for encouragement and we would like to thank the reviewers of this paper for their excellent comments.

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