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Research Article

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Mycelium growth of *Trichoderma viride* (Biocontrol agent) on Different Agar Medium

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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 different kind of agar medium like PDA and MEA for the growth of mycelium of Trichoderma viride and develops effective production methodology which can be easily adopted. The best mycelium growth observed in PDA with 2.5 gm glucose and 1.5gm lactose respectively. Similarly, while using MEA medium the best growth recorded in MEA with 2.5g lactose.

Key Words: *Biopesticide, Mycelium growth, Trichoderma spp.*

INTRODUCTION

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, eco friendly 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 eco friendly 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 bio agents in targeted niche, determine the success of biological control. Therefore cost effective large scale production, shelf life of formulation, establishment of bio agent 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, the effects of different concentrations of glucose and lactose were examined on *Trichoderma viride* mycelium. Our purpose is to sight if there is any change in the development of *Trichoderma viride* mycelium in different media where different concentrations sugars and compare them with the control group.

MATERIAL 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.

Agar media

The potato dextrose agar (PDA), malt extract agar (MEA) (Gunay, 1995) were used as agar medium, these agar medium were used as control groups in the study. In these control groups, glucose and lactose were separately added as 0.50, 1.5 and 2.5 percentages. All prepared agar mediums were sterilized in the autoclave at 121oC for
15 min.

Mycelium transfers

The piece of tissue taken from the *Helvella crispa* were inoculated to PDA and MEA and vegetative prime mycelium was gained. From these primer mycelium agar discs in 8 mm (Ø) were taken. They were separately inoculated to potato dextrose agar (PDA) and malt extract agar (MEA) at the centre which is located in the 9mm Petri dishes. These are control groups of the study. In the same way also the 8 mm radius mycelium agars discs were separately inoculated to the agars where different concentration glucose and lactose added too. All prepared mediums with inoculated *H.crispa* mycelium were incubated at 28oC and dark. Prepared all different medium were given at Table 1. During the development the radial growth speed were taken as criteria.

Table 1. All agar medium in this study

Agar Medium Abbreviations	Carbohydrates		Concentrations
PDA (Control)	-	-	PDA-C
PDA	Glucose	0.5	PDA+0.5-Glu
PDA	Glucose	1.5	PDA+1.5-Glu
PDA	Glucose	2.5	PDA+2.5-Glu
PDA	Lactose	0.5	PDA+0.5-Lac
PDA	Lactose	1.5	PDA+1.5-Lac
PDA	Lactose	2.5	PDA+2.5-Lac
MEA (Control)	-	-	MEA-C
MEA	Glucose	0.5	MEA+0.5-Glu
MEA	Glucose	1.5	MEA+1.5-Glu
MEA	Glucose	2.5	MEA+2.5-Glu
MEA	Lactose	0.5	MEA+0.5-Lac
MEA	Lactose	1.5	MEA+1.5-Lac
MEA	Lactose	2.5	MEA+2.5-Lac

Potato dextrose agar (PDA)
Malt extract agar (MEA)

RESULTS AND DISCUSSION

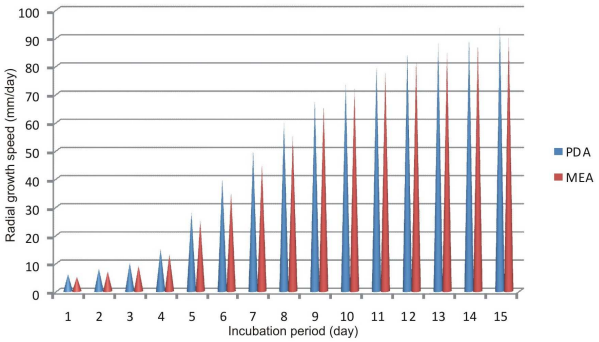
In the present study, the morphologic characteristics, radial growth ratios (RGR) and colonization periods (CP) of the control groups and the agar mediums which glucose and lactose were added at different concentrations to the potato dextrose agar and malt extract agar were researched. At the mycelium characterizations, mycelium growth ratio, variety of mycelium growths and pigmentation were also researched. All the results were given in Tables: 2. Control groups. When the control groups are examined that, during the incubation, 24 h later, the development of the mycelium started from the center, towards the edges proceeded in parallel. The colonization period was 15 d at the PDA-C and MEA-C agar. The mycelium characterizations of control groups were given at Table 2. During the incubation of mycelium development of *Trichoderma viride* was given Figure1.

Table: 2. The mycelium characterizations of control groups

Agar medium	CP (days)	RGR	Mycelial characterizations
PDA	15	Good	Concentric, dark green Pigmentation, compact hyphae
MEA	15	Good	Concentric, Yellowish-green Pigmentation, woolly hyphae

CP= Colonization period RGR= Radial growth radios

Figure 1. The mycelium development curve of control groups



As seen from Figure 1; first 5 d; mycelium developed more speed at the PDA-C agar according to MEA-C agar. At 6th d, mycelium development was equalized both the mediums, but at the last 9d mycelium developed more speed at the MEA-C agar according to PDA-C agar.

Glucose added agar media: The mycelium characterizations at glucose added potato dextrose agar (PDA) medium and malt extract agar (MEA) were given in Table 3 respectively. During the incubation of mycelium development of *Trichoderma viride* at different agar media with glucose were given Figure 2

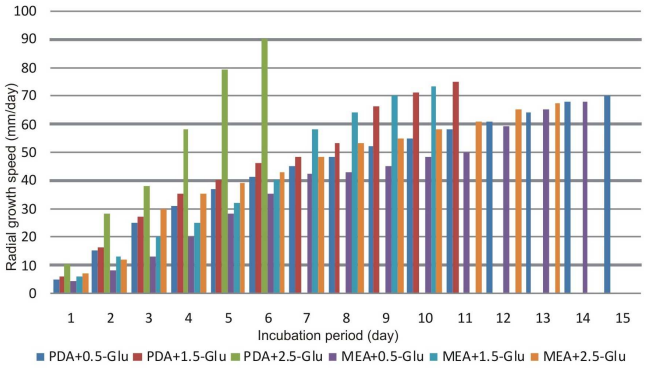
Table 3. The mycelium characterizations at different agar medium with glucose

Agar medium	CP (days)	RGR	Mycelial characterizations
PDA+0.5-Glu	15	Good	Yellowish- white pigmentation
PDA+1.5-Glu	11	Good	Dense, Light yellowish pigmentation
PDA+2.5-Glu	6	Very good	Very dense, Mycelium has grown in Concentric rings, dark green pigmentation
MEA+0.5-Glu	14	Medium	Yellowish and white pigmentation,
MEA+1.5-Glu	10	Good	dark Yellow-green pigmentation.
MEA+2.5-Glu	13	Medium	whitesh-green pigmentation

CP= Colonization period RGR= Radial growth radios

As seen from Table 3; at the all mediums with added glucose; mycelium developed more speed especially at the PDA+2.5-Glu according to all MEA mediums

Figure 2. The mycelium development curve of *Trichoderma viride* at PDA and MEA with glucose



Lactose added agar media: The mycelium characterizations at lactose added potato dextrose agar (PDA) medium and malt extract agar (MEA) were given in Table 4 respectively. During the incubation of mycelium development of *Trichoderma viride* at different agar media with glucose were given Figure 3.

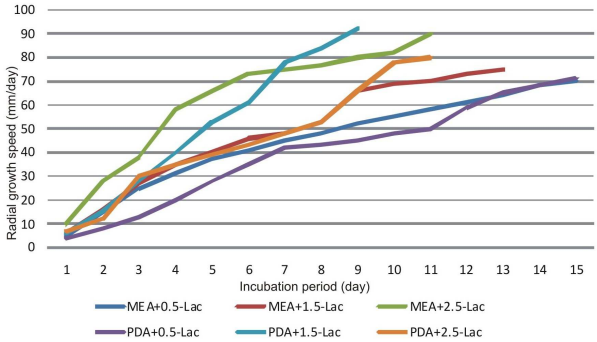
Table 4. The mycelium characterizations at different agar medium with lactose

Agar medium	CP (days)	RGR	Mycelial characterizations
MEA+0.5-Lac	15	Good	Linear, No aerial hyphae Yellowish-green pigmentation
MEA+1.5-Lac	13	Good	Dense, Light yellowish-white pigmentation
MEA+2.5-Lac	11	Very good	Very dense, Mycelium has grown in fully plate, green-white pigmentation,
PDA+0.5-Lac	15	Poor	Yellowish pigmentation
PDA+1.5-Lac	9	Very good	Dense, dark green pigmentation
PDA+2.5-Lac	11	Medium	Dense, green-yellow pigmentation

CP= Colonization period RGR= Radial growth radios

As seen from Table 4; at the all mediums with added lactose; mycelium developed more speed especially at the PDA+1.5-Lac according to all MEA mediums.

Figure 3. The mycelium development curve of *Trichoderma viride* at PDA and MEA with lactose



Some researchers done on Ascomycetes species, especially, have studied the mycelium development of *Morchella* conica at PDA colonization formation. Guler et al. (1995, 1996) have defined this development period as 5 days, Karaboz and Oner (1988) have stated the same period as 7 days. Kaul (1981) have investigated growth characters and rate of growth of *Morchella* spp on PDA. Iqbal et al. (1988) found that best growth of *Agaricus bitorquis* was on MEA and PDA. Takaaki and Hiroko (2004) found a method for culturing an edible fungus providing a liquid culture medium containing sucrose as a carbon source inoculating the medium with an *Agaricus* mycelium.

In this study glucose and lactose were used as carbon sources. These carbohydrates were added different concentration at PDA and MEA medium. As a result; when 2.5% and 1.5% glucose and lactose have been added, at the both PDA and MEA agar medium the mycelial growth was more rapid compared to control groups. There were no differences between the other groups to forming colonization Sati and Bisht (2006) in their study; have investigated four isolates of *Tetracheatum elegans, Tetracladium marchalianum, Pestalotiopsis submersus* and *Flagellospora penicillioides* for their carbon requirement, using glucose, fructose, sucrose, xylose, starch, cellulose, dextrin and lactose. They reported that glucose and sucrose were found to be the suitable sources of carbon for all four fungal isolates. In this study the *Trichoderma viride* mycelium developments that in different agar and different concentrations of monosaccharide (glucose) and disaccharide (lactose) were added to be investigated. The development mycelium was seen well almost all agar medium.

CONCLUSION

Trichoderma is an ecofriendly biopesticide and it do not affect the other beneficial micro organisms. In this study we used this microorganism to check its response on different agar medium like PDA, MEA with glucose and lactose.

Best mycelium growth of *Trichoderma* spp. observed in PDA+2.5-Glu (very dense, mycelium has grown in concentric rings, dark green pigmentation) on the 6th day incubation and PDA+1.5-Lac (dense, dark green pigmentation) on the 9yh day incubation. (Table 3, figure2 and table 4, figure3) And best growth of mycelium also observed in MEA+2.5-Lac (Very dense, mycelium has grown in fully plate, green white pigmentation) on the 11th day incubation period and MEA+1.5-Glu (dark yellow green pigmentation) on the 10th day incubation period. (Table 3, figure2 and table 4, figure3). PDA proved to be better choice than MEA because good response of mycelium occurred in PDA on less incubation period.

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