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RESEARCH ARTICLE

Mass Multiplication and Shelf life of Liquid Fermented final Product of *Trichoderma viride* in Different Formulations

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ABSTRACT
In present study, Trichoderma viride was produced in bulk quantity by using liquid fermentation technology wherein molasses soy flour broth was used as medium. Maximum biomass of Trichoderma viride was obtained after 72 hours of fermentation, when optimum fermentation conditions were temperature 27ºC; pH 7.0 dissolved oxygen 40%, stirring rate 250 rpm for first two days and 450 rpm on 3rd day. Initially, polyethylene glycol (10%) was used as antifoam agent but later to reduce production cost, silicone was used and its concentration was standardized as 0.1% by volume. Dissolved oxygen and pH decreased as the growth and conidiation of biocontrol agents started. After harvesting the mass of T. viride was formulated in de-oiled castor cake, gypsum, talc power, vermicompost and well decomposed farmyard manure. Shelf life of these formulated products was studied at 30ºC. Vermicompost, de-oiled castor cake and farmyard manure formulations retained shelf life for 220,190 and 180 days respectively as compared to gypsum and talc powder where the cfu/g declined by 80 days after storage. The results of present research indicate that vermicompost, de-oiled castor cake and farmyard manure formulations supported the growth of Trichoderma viride during storage, which is a major advantage for the marketing of these biocontrol agents.
KEY WORDS: *T. viride*, mass multiplication, shelf life, and formulations.

INTRODUCTION

Economically important agricultural, horticultural and ornamental crop plants are attacked by various soilborne and foliar pathogenic fungi, resulting in billions of dollars in cumulative crop losses. Currently, the most widely used control measure for suppressing these diseases are the use of fungicides. However, problems encountered, such as development of pathogen resistance to fungicides, and inability of seed-treated fungicides to protect the roots of mature plants. The chemical method developed to control too has its own limitations such as high capital investment, non-remunerative, poor availability, selectivity, temporary effect, efficacy affected by physico-chemicals and biological factors, development of fungicide resistance, pollution of food and feeds, health hazards, environmental pollution, etc. Soil borne diseases are very difficult and uneconomical to control with chemicals. Considering these limitations biological control is an important approach in this direction. *Trichoderma* species are important potential bioagents against several soil borne fungal pathogens. *Trichoderma*-based biocontrol agents possess better ability to promote plant growth and soil remediation activity compared to their counterparts (virus, bacteria, nematodes and protozoa [1,2]. Their capability to synthesize antagonistic compounds (proteins, enzymes and antibiotics) and micro-nutrients (vitamins, hormones and minerals) enhance their biocontrol activity. *Trichoderma* spp. have gained wide acceptance as effective biocontrol agents against several commercial phytopathogens. These antagonistic fungi are most common among fungal biocontrol agents because of their multiple characteristics, namely, antagonism and plant-growth stimulation [3].Thus, mass-scale production of *Trichoderma* spp. would have great potential for commercial use. There is abundant literature on the use of conventional synthetic media like glucose, cellulose, soluble starch and molasses to produce *Trichoderma* spp. [4]. However, the cost of these raw materials for

commercial production of biocontrol agents is one of the major limitations behind the restricted use. To overcome the cost limitation, many researchers have successfully used substrates like corn fiber dry mass, sewage sludge and compost. In several crops, bioagents were successfully used [5-8] biocontrol agents using low-cost technology is the logic requirement for successful exploitation of biocontrol agents. Similarly the production process must result in biomass with excellent shelf life even under adverse storage conditions. Therefore, efforts were made to produce *Trichoderma viride* in bulk quantity using low cost technology.

A prototype fermentation system developed by Papavizas *et al* [9] could be successfully used for fungal biocontrol agents. Generally, biocontrol agents are mass multiplied using solid fermentation technology. Some of the disadvantages of solid fermentation method are 1.High volume of the substrate-required 2.Contamination during fermentation and 3. Long time required for fermentation. On the contrary, liquid fermentation will help in the mass multiplication of microorganisms under axenic conditions within a short time. *T. harzianum,* a proven biocontrol agent against soil-borne plant pathogens, was mass multiplied using molasses as substrate [7]. Use of such inexpensive substrates such as molasses helps in reducing the cost of production of biomass. Objectives of this study were-1. Mass multiplication of promising isolate of *Trichoderma viride* using liquid fermentation technology and 2. Evaluate shelf life of final product in different formulations.

MATERIALS AND METHODS

Location of Study

The present study was carried out in Department of Plant Pathology, Directorate of Groundnut Research (formerly National Research Center of Groundnut), Junagadh, Gujarat, during 2009. Mass multiplication of *T. viride* was carried out by using a fermenter (Model BIOFLOW-2000) with a working volume of 10 liters.

Initial inoculum for inoculation of fermenter:

T. viride was grown on potato dextrose agar medium for seven days. Initial inoculum was prepared by scraping seven days old and fully sporulated cultures grown on PDA plate surface with sterile scalpel in sterile deionized water containing 0.1% Tween 20. One ml of this inoculum was added to 100ml of medium in 250ml Erlmenmeyer flask. The flasks were incubated at 28 ºC for 72h. The entire contents of the flasks were churned using a blender and one liter of initial inoculum was added to 9 litres of fermenter medium.

Mass multiplication of *T. viride*

Five broths viz. potato dextrose broth, glucose nitrate broth, maltose peptone broth, sabouraud dextrose broth and molasses–yeast extract broth were tested for mass production of *T. viride* in fermenter. Different parameters such as temperature, dissolved oxygen, pH, stirring rate, fermentation duration, and foam control which influence the growth of the biocontrol agent during fermentation were study for maximum biomass production of *T. viride*. After a series of trials, suitable broth and optimum conditions for fermentation were standardized.

Formulation development and shelf life of final product

The fermenter biomass was formulated in de-oiled castor cake, gypsum, talc power, vermicompost and well-decomposed farmyard manure. Upon harvest, the biomass was mixed with fine powder of these five carriers in 1:10 proportion and kept for three days under shade for drying. The final formulated products contain 10% moisture were packed in polythene bags @ 500 g/ pack and then stored at 30ºC for 10 months. Viability of these formulated products was tested for 10 months. Samples from these packets were drawn at 15 days intervals and tested for viability of the formulated product. Populations of these biocontrol agents were tested weekly taking 100mg of the product and diluted to 10^6. One hundred mg biomass was taken in 1ml sterile water and mixed thoroughly by vortex mixture and allowed to stand. Then from the supernatant, serial dilution was prepared up to 10^6. From the 10^6 dilution, 100 µl of suspension was taken and spread uniformly on Petri dishes containing potato dextrose agar medium using a spreader without disturbing surface of the medium. Five replications were maintained for each formulation. The petriplates were incubated at 28ºC for two days and numbers of colonies were counted.

RESULT AND DISCUSSION

Mass multiplication of *T. viride*

Potato dextrose broth, glucose nitrate broth, maltose peptone broth, sabouraud dextrose broth and molasses–yeast extract broth were tested for mass production of *T. viride* in fermenter. Among these, molasses yeast extract broth was found to be suitable for the maximum growth of *T. viride*. The molasses-yeast extract medium contained 30g molasses and 10g yeast extract per liter of medium. To reduce the production cost, yeast extract was successfully replaced by soy flour. The optimum fermentation conditions for obtaining maximum biomass of *T. viride* in molasses-soyflour broth were temperature 27ºC, pH 7.0, dissolved oxygen 40%, stirring rate 250 rpm for first 48h and 450 rpm for rest of the period. Silicon (0.1%) was used as an antifoam agent. Highest biomass of *T. viride* was obtained in molasses yeast extract broth after 72 hours of fermentation (Fig 1).

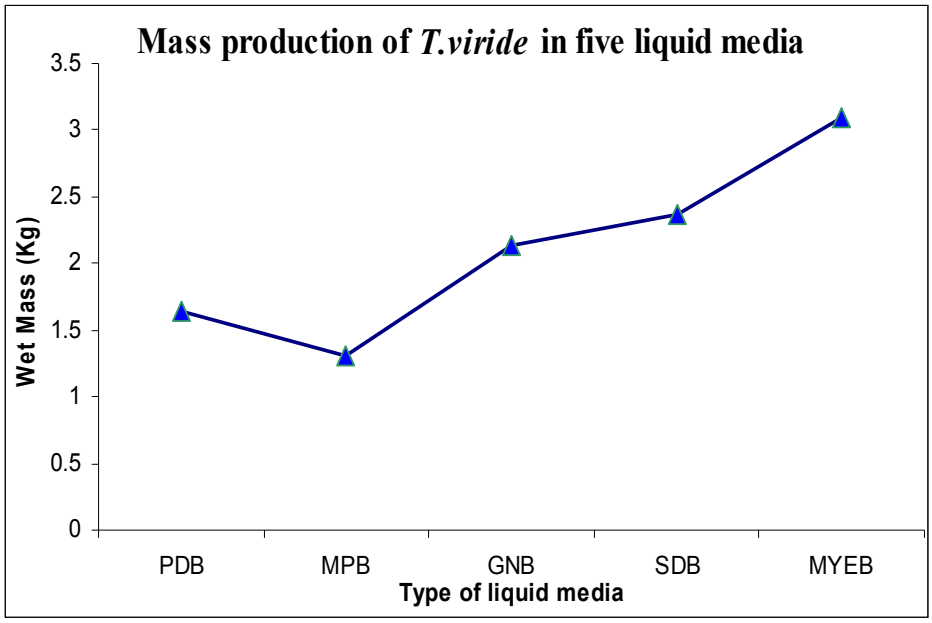

Figure 1: Mass production of *T.viride* in five liquid media (PDB-Potato dextrose broth, MPB-Maltose peptone broth, GNB-Glucose nitrate broth, SDB-Sabouraud dextrose broth, MYEB-Molasses yeast extract broth)

Shelf life of *T. viride*

The results of this study indicate that vermicompost, de-oiled castor cake, and farmyard manure based formulations not only retained better viability but also promoted growth of *T. viride* during storage. A fermenter biomass in vermicompost formulation had longer growth and survival rate as compare to other formulations .Growth of *T. viride* continued in vermicompost formulation up to 195 and retained good viability for 255 days. There after, a reduction in colony forming unit (cfu) was recorded (Figure 2). Similarly de-oiled castor cake, and farmyard manure based formulations were also found suitable for growth and viability of *T. viride* during storage. De-oiled castor cake, and farmyard manure based formulations retained better viability. Talc and gypsum based formulations had poor growth and shorter shelf life. Maximum growth and higher survival rate during storage in vermicompost formulation is a major advantage for the marketing of these biocontrol agents on commercial level. The present study revealed that use of vermicompost formulation increases the shelf life of *T. viride*. Application of vermicompost formulation of *Trichoderma* in the field would help the farmers in controlling the soil borne and seed borne diseases and promising better yield.

Gangadharan *et al*, [10] found that tapioca rind, tapioca refuse and well decomposed farmyard manure formed good substrates for the mass production of *T. viride* and *T. harzianum*. Vermiculite-based fermenter biomass of formulation with an initial population of $205x10^6$ cfu/g stored in milky white bags showed an exponential phase up to 30 days ($309x10^6$ cfu/g). Further temperature of 20-30°C was optimum for the storage of the formulation at which even after 75 days, the product contained $206\text{-}271x10^6$ cfu/g [11]. Sankar and Jeyarajan [12] used similar techniques to develop seed dressing formulations of *T. viride*, *T. harzianum*, and *Gliocladium virens* for management of *Macrophomina phaseolina,* in sesamum. Spores of *Trichoderma* remained viable up to nine weeks of storage at room temperature on seeds when the seeds were coated and reduce the stem rot incidence

[13]. Illuyemi *et al.* [14] used palm kernel cake as substrate for mass production of *T. harzianum* among other fungi and reported good growth with changes in amino acids, fatty acids, and cellulose and hemicellulose fractions of the bioagent. Several isolates of *Trichoderma* spp. developed large amounts of biomass containing conidia and chlamydospores on substrates having inexpensive ingredients (15Lewis and Papavizas, 1984). Lo *et al*. (16) reported that application of a peat-based formulation of *T. harzianum* resulted in 100-fold increase in population compared to untreated plots, whereas in alginate formulations, *Trichoderma* numbers did not increase (Knudsen et al., 17991). *T. harzianum* propagules had increased 600-fold at harvest in wheat bran formulation (Ruppel *et al*., 183). Further research is required for mass multiplication of *T. viride* using different inexpensive culture media to reduce production cost and enhance shelf life.

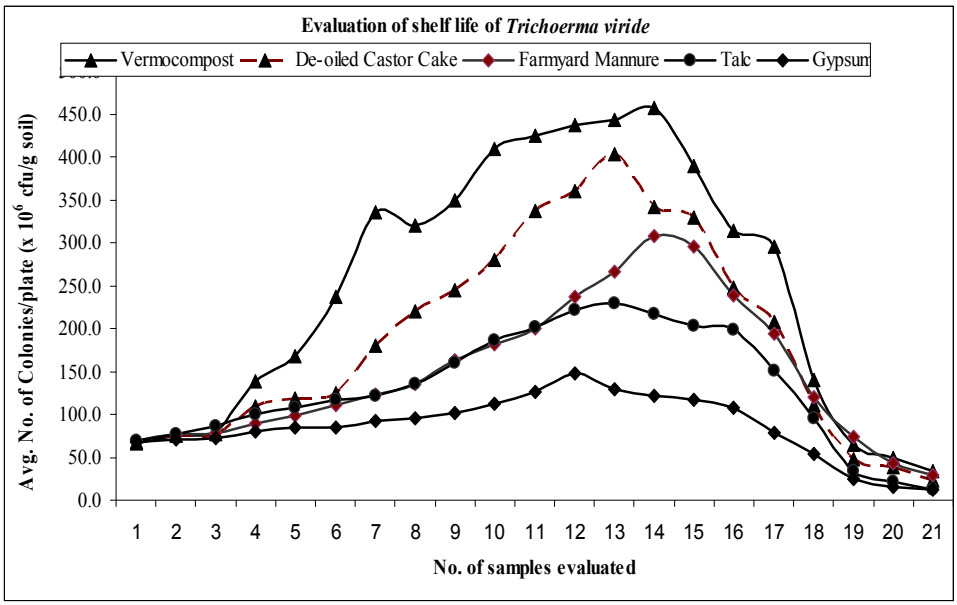

Figure 2: Evaluation of shelf life of *Trichoderma viride* in five different formulations at 15 days intervals.

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