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Title: Inoculation of Trichoderma

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Abstract

The novel technologies present in all areas of agriculture have improved agricultural production, but there are some modern practices affect the environment. The recent challenge faced by advanced farming techniques is to achieve higher yields in environment-friendly manner. Thus, there is an immediate need to find eco-friendly solutions such as vast application of biocontrol agents. The main antagonist applications in disease control in Agriculture is the fungus Trichoderma Viride, a low cost biocontrol agent that has abilities to establish itself in different pathosystems, has moderate effects on soil balance and does not harm beneficial organims that contribute towards pathogen's control. This biocontrol agent has not harmful effects on humans, wild life and other beneficial microorganisms. Trichoderma is a safe and effective biocontrol agent in both natural and controlled environments that does not acquisition in the food chain and to which it has not been described resistance.

Trichoderma is a biocontrol agent and can act as a 1) colonizing the soil or parts of the Plant and occupying a physical space with avoiding the multiplication of the pathogens; 2) producing cell walls that degrading enzymes against the pathogens; 3) producing antibiotics that can kill the pathogens and harmful organisms; 4) promoting the plant development and inducing the defensive mechanisms of the plant.

Key words: *Trichoderma Viride, Sorghum seeds, biological control.*

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1. INTRODUCTION

The conventional chemical pesticides have not only increased the food production, but also counter affected the environment and non-target organisms. Due to the side-effects of chemical pesticides, the defendable crop production through eco-friendly pest management is imperative required in recent scenario. Among the various micro-organisms viz., bacteria, fungi, virus, protozoans and entomopathogenic nematodes, a few have been detailed studied for their effective beneficial characteristics. *Trichoderma viride* is very encouraging method against soil borne plant parasitic fungi. The fungal pathogens play a vital role in the development of diseases on many important field and floriculture crops; resulting in serious plant yield losses. Increase used of fungicides has resulted in collection of toxic compound potentially hazardous to human and environment an also in the growth of resistance of the pathogens. In order to outfit these national and global problems, effective substitute to chemical control are being employed. Biological control by an antagonism is a potential, no chemical and ecofriendly approach for control plant diseases. *Trichoderma* is one of the common fungal biocontrol agent, is being used universal for suitable management of diverse foliar and soil borne plant pathogens. Biocontrol agents like *Trichoderma* spp. are praise as effective, ecofriendly and economical, nullifying the ill effects of chemicals. Therefore, of late, these biocontrol agents are recognize to act against on line-up of important soil borne plant pathogens causing serious diseases of crops. Therefore considering the cost of chemical pesticides and unsafe involves, biological control of plant diseases to be an effective and ecofriendly approach being practice globe over. Further biological control policy defendable 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 initiation of bioagents in targeted niche, determine the success of biological control. Therefore cost effective large scale production, shelf life of formulation, initiation 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

explored. Development of tolerable easily prepared and cost effective formulations for carriage should be major goal.[1]

Trichoderma is a fungal genus that was narrated in 1794, including anamorphic fungi isolated primarily from soil and deteriorated organic matter (Per soon 1794). Strains within this genus include a extensive spectrum of evolutionary solutions that range from very effective soil colonizers with high biodegradation potential, to non-strict plant symbionts that colonize the rhizo sphere. Species concepts within *Trichoderma* are very vast, which has resulted in the identification of many infra specific groups. Some groups of biotypes within this conglomerate are allowed to antagonize phyto pathogenic fungi by using substrate colonization, antibiosis and/or mycoparasitism as the main mechanisms. This antagonistic potential is the base for productive applications of different *Trichoderma* strains as a replacement to the chemical control against a wide set of fungal plant pathogens. As a outcome of the variety of activities displayed by the *Trichoderma* strain conglomerate, a large range of applications have been grow.[2]

The traditional chemical pesticide has not only increases the food production, but also contra affected the environment and non-target organisms. Due to the many side-effects of chemical pesticides, the supportable crop production through eco-friendly pest management is essentially required in recent framework. Among the various micro-organisms viz., bacteria, fungi, virus, protozoans and entomopathogenic nematodes, a few have been systematically evaluated for their effective favourable characteristics. *Trichoderma viride* is very encouraging method against soil borne plant parasitic fungi. The fungal pathogens play a vital role in the development of diseases on many important pasture and horticultural crops; resulting in drastic plant yield losses. Increase in used of fungicides has resulted in cumulation of toxic compound possibly hazardous to human and environment an also in the build up of resistance of the pathogens. In order to outfit these national and global problems, effective options to chemical control are being employed. Biological control by an antagonism is a prospective, no chemical and ecofriendly approach for managing plant diseases. *Trichoderma* is one of the usual fungal biocontrol agents, is being applicable worldwide for suitable management of diverse foliar and soil borne plant pathogens. Biocontrol agents like *Trichoderma* spp. are praise as effective, ecofriendly and cheap, nullifying the ill effects of chemicals. Therefore, these biocontrol agents are recognize to act against on formation of important soil borne plant pathogens causing serious diseases of crops. Therefore acknowledge the cost of chemical pesticides and hazardous involves, biological control of plant diseases become visible to be an effective and ecofriendly approach being practice world over. Further biological control policy 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 throughout shelf life and establishment of bioagents in targeted niche, determine the success of biological control. Therefore cost effective extensive production, shelf life of formulation, establishment of bioagent in to targeted niche and stability in disease control are the primary concern with augmentative biological control. Adaptation of technology in the biocontrol arsenal required to be investigated. Development of acceptable easily prepared and cost effective formulations for conveyance should be major goal. Present study was carried out to assess 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 compounding of bio agent through liquid state fermentation technology an vast quantity of spore biomass is needed. Different substrates like sugarcane baggase, fruit juice waste, vegetable waste, rotten wheat grains etc. are being used for mass multiplication of *Trichoderma viride* with diverse degree of success. Moreover a huge quantity of solid waste like sugarcane baggase, fruit juice wastes, vegetable Waste and rotten wheat grains increasing pollution and destruction problems. Therefore looking towards required for large scale cost effective production of ecofriendly biopesticide, present inspection is carried out to evaluate locally available economical substrates for mass multiplication of *Trichoderma viride* for sustainable environment and sustainable agriculture.[3]

2. MATERIALS AND METHODS

2.1METERIALS

2.1.1 Trichoderma mother culture (1 tube)
2.1.2 Carboxy methyl cell (20 gm.)
2.1.3 Sorghum (1 kg)
2.1.4 Sterilized Distilled water (2 lit.)
2.1.5 Sucrose (20 gm)
2.1.6 Talcum powder (2 kg)
2.1.7 Spirit

2.2 APPERATOUS

2.2.1 Conical flask
2.2.2 Petri plate
2.2.3 Pipette
2.2.4 Test tube
2.2.5 Beaker
2.2.6 Spreader
2.2.7 Polypropylene bags
2.2.8 Plastic ring
2.2.9 Rubber bands
2.2.10 Silver foil
2.2.11 Cotton
2.2.12 Candle

2.3 EQUIPMENT

2.3.1 Autoclave
2.3.2 B.O.D. incubator
2.3.3 Microscope
2.3.4 Mixer
2.3.5 Weighing balance

3. PROCEDURE

Take Trichoderma mother culture in Petri plate. Add 1 ml of sterilized distilled water in a Petri plate and mixed with Trichoderma mother culture. Then prepare mixture mix

properly with 100 ml of sterilized distilled water in conical flask. Close conical flask with the help of cotton. Take 500gm sorghum seeds and wash it gently in water. Make 2% sucrose solution (20gm sucrose with 1000ml sterilized distilled water) and add sorghum seeds into it. Leave it for 6 hrs. At room temperature. Remove the water after 6 hrs. And take sorghum seeds into an polypropylene bags. Pack it with cotton and cover by silver foil. Put sorghum in autoclave at 15 psi. For half hrs. After half hr. Take out that bags and leave them at room temperature for cooling. After temperature of bags reaches at room temperature (25-30^{0}c) Take 1 ml pure Trichoderma suspension in laminar flow and inoculate it in sorghum solution bags and mix it well. Pack that bags with cotton and close it by silver foil. Place it in B.O.D. incubator at 25-27 ^{0} C for 15-20 days. Trichoderma eat that sorghum and sucrose which acts as supplements for Trichoderma. After 15-20 days Trichoderma grow and cover all sorghum seeds (generally Trichoderma is in greenish colour) Now whole bags reach with Trichoderma culture. Take that inoculated sorghum seeds and grind it in a grinder. After grinding take it out and mix with talcum powder in 1:9 ratios. Add carboxy methyl cellulose (C.M.C.) into that mixture in 5gm per kg proportion and mix it properly. Pack it in polypropylene bags.

3. EXPERIMENTAL SETUP

Fig.1: Trichoderma viride

Fig.2: Trichoderma viride

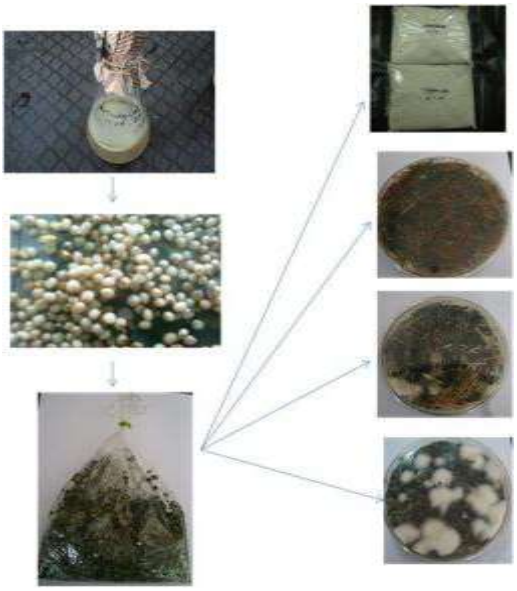

Fig.3: the process of cultivation of biocontrol agent, *Trichoderma*

Fig.4: the process of cultivation of biocontrol agent, *Trichoderma*

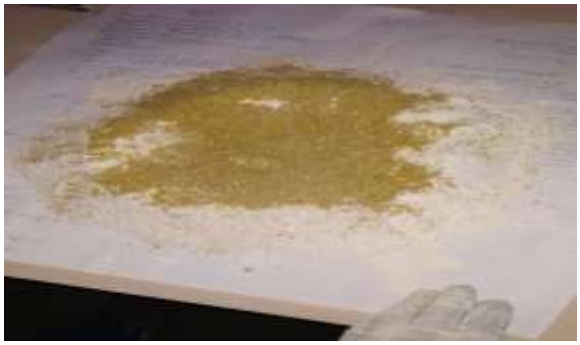

Fig.5: Trichoderma Bio-fertilizer Product

Fig.6: Trichoderma Bio-fertilizer Product

The fig.1 is Trichoderma viride in the test tube and Petri plate for the cultivation in the sorghum seed.

The fig.2 is process of cultivation of Trichoderma viride in the sorghum seeds, it also we can see the B.O.D. incubator equipment for the temperature maintenance.

The fig.3 is Trichoderma bio-fertilizer (product) which is contain grow Trichoderma in the talcum powder and carboxy methyl cellulose.

4. RESULT AND DISCUSSION

Cultivation of *Trichoderma viride:* The fungal mat formed in the mineral salts medium with whey medium was used to the inoculate sorghum seeds and spore biomass was prepared in boiled sorghum seeds. (Fig.2). The fungal mycelium slowly colonized the sorghum seeds and after 15 days the entire bag was fully of green colour growth with mycelium and spores on the seeds.

Large scale cultivation of *Trichoderma*: The inoculum developed on the sorghum seeds were used to immunize large scale culture of *Trichoderma*. In this study, agricultural wastes like paddy straw and sugarcane baggase were cast-off. As these two substrates are accessible in plenty in villages, farmers can use these waste for growth of bio control agent. Also we have used vermin compost which is prosperous in organic matter that supports the growth of *Trichoderma viride.* The substrates were immunizing with cultured sorghum seeds. The growth and asexual reproduction of *Trichoderma viride* was faster in sugarcane baggase followed by compost and then on paddy straw. There are two crucial methods of inoculum production of *Trichoderma*, solid state fermentation and liquid state fermentation. In solid fermentation, the fungus is grown on diverse cereal grains, agricultural wastes and by products. The solid state production is highly laboured rigorous and fit for cottage industry. These products are used mostly for straight soil application in nurseries /main fields to defeat the soil-borne inoculums. In liquid state fermentation, *Trichoderma* is grown in economical media like molasses and yeast medium in intense tanks on a commercial scale. Biomass from the liquid fermentation can be made into dissimilar formulations like, dusts, granules, pellets, powders. In the present study, various naturally available substrates of both solid & liquid media were tested for mass procreation of *Trichoderma viride.* The success of biological control depend not only the isolation, characterization & pathogen city, but also on the victorious mass production of the fungal agent in laboratory. Large scale accessible of the pathogen is a primary requirement in the biocontrol programmed. For a successful integrated pest board programme, the agents like the pathogenic fungi should be compliant to easy & cheap mass multiplication. Latest research efforts were directed at selecting native bio control fungi, characterizing them evaluates their virulence and developing a formulation for them.

5. DISCUSSION

Grain Medium: Among the grain media, pulses way produced remarkable higher 17.98g/200ml of biomass production was recorded in *Trichoderma viride*. Large quantity of minerals in the pulses medium may increase the growth of fungi. Rice and wheat medium also carry the extension of both the tested fungi.

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Non Synthetic Medium: between the Non Synthetic Medium, Beetroot produced higher 13.32g/20ml of biomass production was recorded in *Trichoderma viride* resp. Among the Organic Medium, Potato manufacture significantly higher 14.05g/200ml of biomass production was recorded in *Trichoderma viride* respectively.

Organic Medium: Among the Organic Medium, Potato produced significantly higher 14.05 g/200 ml of biomass production was recorded in *Trichoderma viride* respectively.

6. OBSERVATION

All the treatments and control plant were contrast by observing the rate of infection and other biometric variables namely shoot length, number of leaves, number of flowers, growing period, and number of fruits were observed.

7. USES

Multiple uses of Trichoderma fertilizer by different ways:

7.1 Soil treatment:

Soil is the repertoire of both beneficial and pathogenic microbes. Delivering of *Trichoderma* spp. to soil will increase the population dynamics of augmented fungal antagonists and thereby would suppress the establishment of pathogenic microbes onto the infection court. There are several reports on the application of biocontrol agents to the soil either before or at the time of planting for control of a wide range of soil-borne fungal pathogens Soil application of *T. viride* besides alone and in combination with other treatments significantly reduced red rot caused by *C. falcatum* suggested that the soil application of *T. viride* was found to be best in controlling seedling blight, colour rot, stem rot and root rot disease of Jute. Soil application of organic preparation of *Trichoderma* was effective in managing seed borne pathogenic fungi *F. oxysporum, F. moniliforme, F. solani, B. theobromae, A. alternata* and *R. solani* and in seedling establishment of *Dalbergia sissoo* Roxb *Trichoderma* is capable of colonizing farm yard manure (FYM) and therefore application of colonized FYM to the soil is more appropriate and beneficial. This is the most effective method of application of *Trichoderma* particularly for the management of soil-borne diseases. We provide these fertilizers to soil. It increases the nitrogen percentage in soil. Nitrogen already present in soil but crops requires more nitrogen for better yield. Trichoderma increase the nitrogen percent in soil as well as decreases the quantity of harmful material.

7.2 Foliar spraying/Wound dressing:

The effectiveness of biocontrol agents for foliar diseases is greatly affected by fluctuation of microclimate. Philosopher is subjected to diurnal and nocturnal, cyclic and non-cyclic variation in temperature, relative humidity, dew, rain, wind and radiation. Hence water prospective of phylloclade microbes will be varying constantly. It will also vary between leaves or the periphery of the canopy and on sheltered leaves. Higher relative humidity could be observed in the shaded, dense region of the plant than that of peripheral leaves. The dew formation is significant in centre and periphery. The attentiveness of nutrients like amino acid, organic acids and sugars exuded through stomata, lenticels, hydathodes and wounds varies mostly. It influences the efficacy and survival of antagonist in phylloplane. suspension of *Trichoderma* has been victoriously applied to the aerial plant parts for the biocontrol of *Alternaria* leaf spot of *Vicia* emphasized on the functionality of *T. viride* in foliar sprays and talc-based formulations for decreasing disease incidence of sheath blight of in the form of powder and liquid bio formulation and found it successful in controlling disease in feasible due to enhance dosage and economy realized from the crop. Hence, dosage and usually of application has to be standardized based on the crop value, which could be as a reliable and practical approach .The liquid Trichoderma mix with water in the ratio of 2:8 (200gm Trichoderma in 800gm water) and spray it through the crops it protects the crop from pathogens like fungal bacterial and viral or nematodes can damage plants. Trichoderma increase the yield of overall plants by act as pesticides and antifungal, bacterial agent.

7.3 Seed treatment:

Seed coating with *Trichoderma* is one of the effortless and effective methods of delivering the antagonist for the management of seed/soil-borne diseases. Seed is coated with dry powder/dusts of *Trichoderma* just before sowing. For promotion purpose, dry powder of antagonist is used at 3 to 10 g per kg seed based on seed size. Propagates of biocontrol agents germinate on the seed surface and colonize roots of germinated seedlings and rhizosphere *T. viride* were found to be effective seed protestants against *Pythium* spp. and *R. solani* In another study Trichoderma provide extra nutrition and prevent from germs because of that seeds germinates excellently.

7.4 Root treatment:

Seedling roots can be treated with spore or cell suspension of antagonists also by drenching the *Trichoderma* in nursery beds or by intense roots in *Trichoderma* suspension before transplanting. Root dipping in antagonist's not only reduces disease severity but also enhances seedling growth in rice, tomato, brinjal, chilli and capsicum There are also reports on the decreases of sheath blight disease of rice by root dip of seedlings pervious to transplantation .

8. CONCLUSION

There were four treatments including control. The *Trichoderma viride* protected the seedlings from damping off disease. The growth promotion attributes like height, plant vigour, fruit formation were found good in the plants treated with *Trichoderma viride* grown in sugarcane baggase and talcum powder treated pots. A simple easy and cost effective method to mass culture the bio control agent by *Trichoderma viride* using sugarcane bagasse and talcum powder formulation.

The surplus 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 counter productively 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 substantial natural mortality agents and environmentally safe bio-pesticides. Bio pesticides are more successful than chemical pesticides in long term use, and also

cost effective. Pathogenic fungi have been reported as an excellent bio-pesticide.

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