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Trichoderma species a Bio control Agent for Sustainable Management of Soil Borne Diseases

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Abstract

The use of biological agents is becoming an increasingly important alternative to chemical control against insects, weeds and diseases in agriculture. The success of this method depends on the nature antagonistic characters and the mechanisms of action of microorganism. Fungi are the most important biological agents against plant pathogens. *Trichoderma* spp. are free-living, asexually reproducing and filamentous fungi, common in soil and root ecosystems. They produce or release a variety of compounds that induce localized or systemic resistance responses in plants. It is an exceptionally good model of bio control agent as it is easy to isolate and culture, multiply rapidly on many substrates, act as mycoparasite, strong opportunistic invaders, avirulent plant symbionts, competes for food and site with pathogens, prolific producers of spores, powerful antibiotics, antifungal compounds, secondary metabolites as well as enzymes. These properties make them ecologically very successful and are the reasons for their iniquitousness.

Introduction

Bio control or Biological control can be defined as the use of natural organisms or genetically modified, genes or gene products the effects of undesirable organisms to favor organisms useful to human, such as crop, trees, animals and beneficial microorganisms. This strategy of control is ecologically clean and compatible with different models of agriculture organic biological and pathogen management. Bio control agents are widely regarded by the general public as natural and therefore non-threatening products, although risk assessments

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must clearly be carried out on their effects on non-target organisms and plants. Moreover, knowledge concerning the behavior of such antagonists is essential for their effective use. One of the most interesting aspects of the science of biological control is the study of the mechanisms employed by bio control agents to effect disease control. Bacteria and fungi are the most important biological agents against plant pathogens. Particular bacterial strains in certain natural environments prevent infectious diseases of plant root. How these bacteria achieve this protection from pathogenic fungi has been analyzed in detail in bio control strains of fluorescent *Pseudomonads*. During root colonization these bacteria produce antifungal antibiotics, elicit induced systemic resistance in the host plant or interfere specifically with fungal pathogenicity factors.

Isolation from soil

Trichoderma is isolated from the soil by using *Trichoderma* selective medium developed by Elad and Chet (1983). Collect soil samples from the field, mix well and make it into fine particles. Soil samples should be collected in root zone at 5-15 cm depth and from rhizosphere wherever possible. Ten gram of soil sample is taken, and suspended in 100 ml of sterile distilled water and stirred well to get 1:10 dilution. Transfer one ml from this to 9 ml of sterile water in a test tube to get 1:100 dilution. Make serial dilutions by transferring one ml of suspension to subsequent tubes to get dilution of 1: 1 0,000. Transfer one ml of the desired soil suspension to sterile petriplates. Pour 15 ml of melted and cooled *Trichoderma* selective medium in the same petriplates. Rotate the plate gently and allow to solidify, incubating at room temperature for 5-7 days and observe for the development of fungal colonies. *Trichoderma* colonies will be white initially and turn to green. Count the number of colonies developing in individual plates. Transfer the individual colonies to potato dextrose agar slants.

Testing Method: Dual Culture Technique

It consists of growing the test organism and the pathogenic organism on the same plate. This can be done by the following procedure. Transfer 15-20 ml of melted and cooled PDA to sterilized petridishes. Allow it to solidify. Transfer 8 mm disc of test organism to one end of the petriplate. In the opposite end, 8 mm disc of the pathogenic culture is transferred in the same petriplate. Incubate the plate at room temperature. Observe the development of

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inhibition zone. Observe under microscope where both the test organism and the pathogen come in contact.

Mass production of *Trichoderma*

The success of a bio control agent depends much on the establishment of the product, the formulation and delivery system. Of the three kinds of propagules produced by *Trichoderma*, namely hyphae, chlamydospores and conidia, chlamydospores and conidia are used in making *Trichoderma* based products, as hyphae fail to withstand drying process. The production of propagules can be carried out in liquid or solid fermentation. In either case, the choice of the substrate is important and usually agricultural or industrial waste is considered to be economical. The type of formulation of the product depends on the type of application in the field. The propagules may be formulated along with the medium used for cultivation or they may be harvested and preserved with the addition of an inert base, such as talc. Addition of preservatives may be done for safe storage of the products. The products are generally stored at 40C for a maximum period of one year.

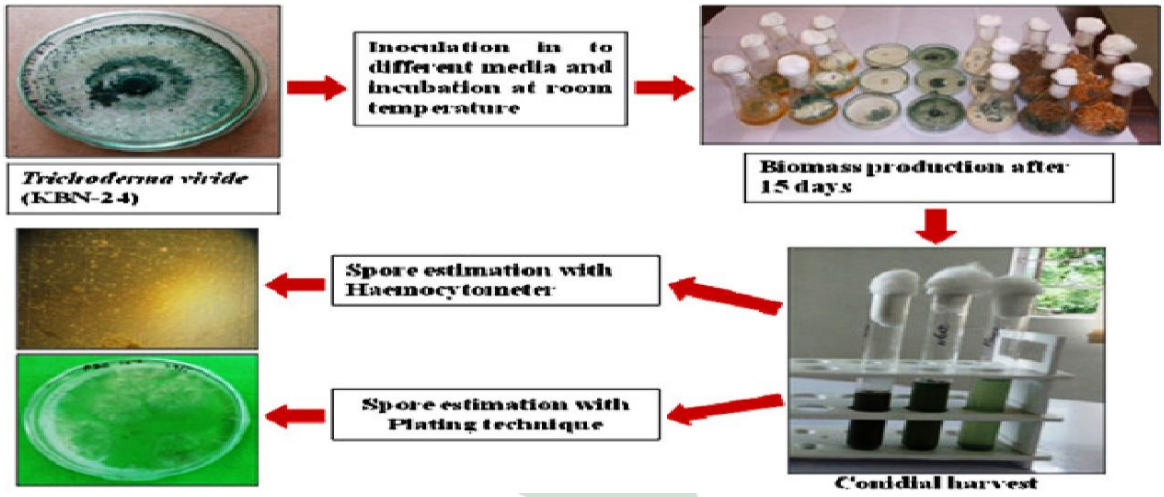

Fig.* 1. Biomass production of *Trichoderma

Commonly used growth media for production of *Trichoderma* in liquid state fermentation

Molasses yeast medium :- Molasses yeast medium (Molasses 30g + yeast 5g + water 1000ml) is prepared in conical flasks and sterilized at 1.1 kg/cm2 for 20 minutes. *T. viride*

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culture is inoculated by taking a fungal disc from 10 day old culture and incubated for 10 days. This serves as mother culture. Molasses yeast medium is prepared in a fermenter and sterilized. Then, the mother culture is added to the fermenter @ 1.5 litre/50 litres of medium and incubated at room temperature for 10 days. The fungal biomass and broth are mixed with talc powder at 1: 2 ratio. The mixture is air dried and mixed with carboxy methyl cellulose (CMC) @ 5g / kg of the product. It is packed in Polythene covers and used within 4 months.

Quality Control Specifications

1. Fresh product should contain not less than 28 X 106 cfu / g
2. After 120 days of storage at room temperature, the population should be 10 x 106 cfu / g.
3. Maximum storage period using talc as carrier is 120 days.
4. Size of the carrier (talc) should be 500 microns.
5. Product should be packed in whitePolythene bags.
6. Moisture content of the final product should not be more than 20%

Benefits and Uses of *Trichoderma*

1. Plant Disease Control: Several plant diseases caused by fungi can be potentially controlled *Trichoderma species. Trichoderma harzianum* strain (Pusa Th3) based bioformulations is very effective under different agro-climatic conditions in 32 crops including cereals, pulses, vegetables, spices etc. to control various fungal diseases

Table 1. Plant disease control by different *Trichoderma* spp

Name of the Crop	**Name of the Disease**	**Disease causing micro-organism**	***Trichoderma* spp**
Cabbage,Tomato, soybean chili, peanut,Potato	Damping off	*Pythium spp.*, *R. solani*	*T. hamatum, T.harzianum, T. virde, T. virens*
Rice, maize	Sheath blight,	*R. solani*	*T.harzianum, T. virde*
Tomato, chili, peanut, Potato, black pepper, Banana, Cotton	Wilt	*Fusarium spp*	*T. hamatum, T.harzianum, T. virde, T. virens*
Citrus, Tobacco, Pineapple, Rubber, black pepper, Litchi	Root rot	*Phytophthora spp.*	*T. virde T.harzianum*

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2. Plant Growth Promoter: *Trichoderma* strains solubilize phosphates and micronutrients. The application of *Trichoderma* strains with plants such as grasses increases the number of deep roots, thereby increasing the plant's ability to resist drought.

3. Biochemical Elicitors of Disease Resistance: *Trichoderma* strains are known to induce resistance in plants. Three classes of compounds that are produced by *Trichoderma* and induce resistance in plants are now known. These compounds induce ethylene production, hypersensitive responses and other defence related reactions in plant cultivates.

4. Transgenic Plants: Introduction of endochitinase gene from *Trichoderma* into plants such as tobacco and potato plants have increased their resistance to fungal growth. Selected transgenic lines are highly tolerant to foliar pathogens such as *Alternaria alternata*, *A. solani*, and *Botrytis cirerea* as well as to the soil-borne pathogen, *Rhizoctonia* spp.

5. Bioremediation: *Trichoderma* strains play an important role in the bioremediation of soil that are contaminated with pesticides and herbicides. They have the ability to degrade a wide range of insecticides: organochlorines, organophosphates and carbonates.

6. Industrial use: *T. reesei* and *T. harzianum* is used to produce cellulase and hemicellulase *T. longibratum* is used to produce xylanase. *T. harzianum* is used to produce chitinase that are utilized in cell wall degradation of cell which having chitin in their cell wall composition so utilized by fabric industries

Methods of application of *Trichoderma* for biological control in agriculture

Seed treatment: Mix 10g of Trichoderma formulation per litre of cow dung slurry for treatment of 1kg of seed before sowing, particularly for cereals, pulses and oilseeds.

Nursery treatment: Drench nursery beds with @ 5 Trichoderma formulations per litre of water before sowing.

Cutting and seedling root dip: Mix 10 g of *Trichoderma* formulation per litre of water and dip the cuttings and seedlings for 10 minutes before planting.

Soil treatment: Mix 1kg of *Trichoderma* formulation in 100 kg of farmyard manure and cover it for 7 days with polythene. Turn the mixture in every 3-4 days interval and then broadcast in the field.

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Trichoderma formulations: Important commercial formulations are available in the name of Sanjibani, Guard, Niprot and Bioderma. These formulations contain 3x106 cfu per 1 g of carrier material.

Biocontrol mechanisms

Antagonist microorganisms, such as *Trichoderma*, reduce growth, survival or infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion.

Competition: It is the phenomenon in which the pathogen and the introduced bio control agent (antagonist) compete for the availability of space and nutrients. During this process, the antagonist may suppress the growth of the pathogen population in the rhizosphere and thus reduce disease development. For example, *Trichoderma harzianum* reduces collar rot in elephant foot yam by 80-85%.

Antibiosis: *Trichoderma* strains are known to produce antibiotics and toxins, which are volatile or nonvolatile in nature, and have a direct effect on other organisms. Examples of such chemicals are trichothecin and a sesquiterpine, *Trichodermin* that has antimicrobial effect on bacteria and fungi.

Mycoparasitism: It is the phenomenon in which the antagonist fungi parasitize other fungi. The mechanism covers different stages of interactions.

First stage: Chemical stimulus of pathogenic fungi attracts the antagonist fungi and induces a chemotropic response of the antagonist.

Second stage: Recognition between the pathogen and the antagonist is due to the lectins.

Third stage: It is followed by the interactions between hyphae of the pathogen and the antagonist. The antagonist *(Trichoderma*) hyphae either grow along the host hyphae or coil around it and secrete different lytic enzymes such as chitinase, glucanase and pectinase that are involved in the process of mycoparasitism. Examples of such interactions are *T. harzianum* acting against *Fusarium oxyporum*, *F. roseum*, *F. solani*, *Phytophthara colocaciae* and *Sclerotium rolfsii*.

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Conclusion:

Trichoderma is free-living fungi that are common in soil and root ecosystems. They are highly interactive in root, soil and foliar environments. They produce or release a variety of compounds that induce localized or systemic resistance responses in plants. *Trichoderma* strains have long been recognized as biological agents, for the control of plant disease and for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, and uptake and use of nutrients.

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