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Mass Multiplication of *Trichoderma viride*

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The discovery and use of synthetic chemicals has contributed greatly to the increase of food production industry by controlling pest and diseases. However, the use of synthetic chemicals has raised number of ecological problems apart from increasing the cost of cultivation. In recent years, scientists have directed their attention in exploring the potential of beneficial microbes for plant protection measures. Biocontrol agents exhibit antagonistic activity against plant pathogens and activate resistance mechanisms in the host despite improving plant growth. In agricultural soils the nutritional condition is depleted hence beneficial microbes decrease and increase the pathogenic microbes. To ensure the sustained availability of biocontrol agents mass production technique and formulation development protocols has to be standardized which also increases the shelf life of the formulation. Though these agents have a very good potential in the management of Pest and Diseases it can not be used as cell suspension under field conditions. The cell suspensions of these biocontrol agents should be immobilized in certain carriers and should be prepared as formulations. The organic carriers used for formulation development include peat, turf, talc, lignite, kaolinite, pyrophylite, zeolite, alginate, pressmud, sawdust, vermiculate, etc.

Characteristics of an ideal formulation

- It should have increased shelf life
- It should not be phytotoxic to the crop plants
- It should dissolve well in water and should release the bacteria

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- It should tolerate adverse environmental conditions
- It should be compatible with other agrochemicals
- It should protect the biocontrol agents from desiccation and death of cells

Trichoderma is the most widely used biocontrol agent. Among the various isolates of *Trichoderma*, *T.viride, T.harzianum, T.virens* and *T.hamatum* are used against the management of various diseases of crop plants. *Trichoderma viride* is an important *Trichoderma* species which is a potential biofungicide exploited to manage many of the soil and seed-borne plant pathogens. It is found on well decomposed organic matter, root surfaces of various plant and decaying bark. It possesses innate resistance to most agricultural chemicals which includes fungicides. The mechanism of biological control includes mycoparasitism, antibiosis, competition for nutrients and space, induced resistane, inactivation of pathogen's enzymes and making the crop tolerance to stress through enhanced root and plant development.

Molasses Yeast Medium

Yeast extract : 5g

Molasses: 30g;

Water: 1000 ml

Mass multiplication of *T. viride*

Nucleus culture

- The nucleus culture of *Trichoderma viride* (Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore) is maintained in potato dextrose agar slants.
- *T. viride* is multiplied on potato dextrose agar in 9 cm diameter petriplates and the culture is used for subsequent inoculation in 100 ml of molasses's-yeast medium in each of 250 ml conical flasks distributed and autoclaved at 15 lbs/sq. inch pressure (121°C) for 1 h.
- Eight mm mycelial disc of *Trichoderma viride* from the petridish is taken and transferred to each flask that has been incubated for 10 days.
- This mother culture is used for inoculating medium in fermenter.

Mass multiplication

- Prepare Molasses-yeast medium (50 lt.) in 50 lt. fermenter and sterilize at 121°C, 15 lb/sq. inch. and cool it to room temperature.

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- Add the inoculums of 2.5 lt. (mother culture) grown in conical flasks aseptically into the sterilized medium to the fermenter and incubate for 10 days.
- Draw samples at 10 days after incubation and assess the spore load in the broth.
- The spore load should be at least 10^8 spores / ml at the time of sampling.
- Presence of greenish fungal growth indicates *Trichoderma viride* and this broth culture contains fungal mat, spores and antibiotics.
- Mix 1 lt broth culture with 2 kg talc powder and carboxy methyl cellulose @ 5g/kg.
- Talc based *Trichoderma viride* formulation is ready for use after air drying and sieving (moisture content 12%).
- Pack the formulation in opaque polythene bags and store in a cool dry place before use.
- The product can be safely stored for four months without losing its viability.

Methods of application

Biocontrol agents can be applied to various plant parts like seed, root, foliage, inflorescens and also the soil. For this purpose several methods have been developed effectively and enhanced the yield considerably.

Seed treatment

Seed treatment is an alternative method for introducing bio-control agents into soil plant environment. The efficacy of seed treatment depends on the ability of the antagonist to multiply in the rhizosphere region. Seed treatment with *T.harzianum* reduced the disease caused by *R. solani* on cotton in field conditions (Elad *et al.*, 1982). Treatment of black gram, green gram, pigeon-pea, cowpea, groundnut, sunflower, sesamum and cotton with *T.viride* @ 4g/Kg reduced the incidence of root rot and wilt (Nakkeeran and Renukadevi, 1997).

Solid Matrix Priming

It is a process in which moistened seeds are mixed with an organic carrier and the moisture content of the mixture is brought to a level just below that required for seed sprouting. Solid matrix priming can further enhance the efficacy of antagonist in treated seeds. *Trichoderma* grow on seed surface during priming process and increase in numbers and other microbes may not easily dislodge it. Slurry of *Trichoderma* is first added to seeds followed by addition of lignite and

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water. *Trichoderma* required less moisture for growth. The primed seeds were incubated for 4 days at 80-100% RH. During this period *Trichoderma* colonises seed and sporulation was evident. In *Pythium ultimum* infested soil the stand was increased to 70-80% as against 10% in control. In tomato seed the population of *Trichoderma* increased by ten fold due to solid matrix priming (Harman *et al.*, 1989).

Soil

For effective management of soil borne disease, a high population of the antagonist in the soil is necessary. Adams (1990) defined effeciency of biocontrol agents as the ratio of number of propagules of mycoparasites required to obtain disease control to the typical inoculum density of a plant pathogen. Therefore attempts were made to develop suitable delivery system to soil. For controlling *R. solani*, *Trichoderma* population of 5×10^6 was required for each propagule of *R. solani*. Addition of wheat bran based inoculum to soil gave 80 per cent reduction of root rot over control in chickpea and bean (Elad *et al.*, 1986). Incidence of urdbean root rot was reduced by 91.3 per cent by adding *T.viride* + *T. harzianum* to soil (Kousalya and Jeyarajan, 1988). Delivering *T. harzianum* through soil, during sowing increased the per centage of survival of peanut (90%), while in control none of the plants survived (Muthamilan and Jeyarajan, 1996).

Cut Stump Application

Ricard, (1981) developed a special shears to apply *T. viride* simultaneously with prunning to protect fruit trees against silver leaf disease caused by *Chondrostereum purpureum*. The shears have a reservoir for suspension of *T. viride*. Each prunning cut was covered by spore suspension.

Hive insert

Thomson *et al.* (1992) developed an innovative method of application of bio-control agent right in the infection court at the exact time of susceptibility. *Botrytis cinerea* (fruit rot of straw berry) was effectively controlled by *T. virens* through honey bees which acted as vector. Peng *et al.* (1992) developed a novel and more efficient method of applying inoculum of *T. virens* on talc and corn meal (5×10^8 cfug/g), which was placed inside the beehive in a dispensor. Bees acquired the conidia on their legs and bodies as they crawled. Each bee had a spore load of 88 to 1800×10^3. Each flower got 1.6 to 27×10^3 conidia of antagonist. It suppressed *B. cinerea* on stamens (54%) and petals (47%) and controlled fruit rot.

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