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Exploitation of Trichoderma spp *as biocontrol agent for plant disease management*

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Traditional methods used to protect crops from diseases have been largely based on the use natural sources or by-products of the farms. After green revolution consumption of the pesticides has been increased. Applications of fungicides and fumigants can have drastic effects on the environment and consumer, and are often applied in greater quantities than herbicides and insecticides in agricultural production. Chemical methods are not economical in the long term applicable because they pollute the atmosphere, damage the environment, leave harmful residues, and can lead to the development of resistant strains among the target organisms with repeated use.

Today, there are strict regulations on chemical pesticide use, and there is pressure from green peace/ environmentalist to ban the most hazardous chemicals from the market. A reduction or elimination of synthetic pesticide applications in agriculture is highly desirable. One of the most promising means to achieve this goal is by the use of new tools based on Bio-control Agents (BCAs) for disease management. Biocontrol technologies have gained momentum in disease management of crop plants in twenty first era as these technologies not only minimize or replace the usage of harmful chemical pesticides but also found to be cheaper and efficient in certain disease control programmes. Successful use of biocontrol agents for the control of soil borne diseases caused by pathogens like, *Rhizoctonia, Sclerotium, Fusarium, Pythium,* and *Phytophthora* in several crops have been reported (Cook and Baker, 1983).

A number of BCAs have been registered to date and are available for commercial use, among these biocontrol agents *Trichoderma* spp are under intensive research because of their abundant natural occurrence, biocontrol potential against fungal and nematode diseases as well as host defense inducing ability and capable of promoting growth of certain crops (Haraman and Kubicek, 1998). The annual requirement of *Trichoderma* has been estimated as 5,000 tones to cover 50 per cent area in India (Jeyarajan, 2006). To ensure that the products of *Trichoderma* do not affect the environment, human beings and other living organisms adversely and to prevent the sale of poor quality products to the farmers, the Central Insecticide Board (CIB) of Government of India has made registration of microbial pesticides mandatory before commercial production/import/sale. Guidelines and data requirements for registration of microbial pesticides have been provided in the annexure of Insecticide Act. Quality control parameters set by CIB are adequate for knowing potentiality of a bioagent. It should be also taken the counts of live propagules in the formulation, bioefficacy as a quality parameter to ensure availability of better products to farmers. A number of *Trichoderma* spp such as *T. harzianum* and *T. viride* have been registered and are available for commercial uses.

Mass production of *Trichoderma* spp:

There are two major methods of inoculum production of *Trichoderma* spp are:

– Solid state fermentation

– Liquid state fermentation.

Solid fermentation is a very common method for mass production of *Trichoderma*. Various cheap cereal grains like, sorghum, millets, ragi are used as substrates. These grains are moistened, sterilized at 121^{0}C and inoculated with *Trichoderma* and incubated for 10-15 days. *Trichoderma* produces dark green spore coating on the grains. These grains can be powdered finely and used as seed treatment or it is for enriching FYM for soil application.

In liquid fermentation system, *Trichoderma* is grown in liquid media in stationary/shaker/ fermentor cultures and formulated and used for field application.

Maximum biomass of *Trichoderma* spp. can be realized in short-time by using appropriate substrate/ medium in a fermentor with aeration, agitation, temperature, pH and antifoam controls than in shake-flask cultures and will be more suitable for industrial production of *Trichoderma* spp.

Other substrates used for mass production of *Trichoderma* through solid and liquid fermentation are following:

Substrates for solid state fermentation	Substrates for liquid state fermentation
Sorghum grain, wheat grain	Molasses and brewer's yeast
Wheat bran, rice bran, rice straw and rice husk	Molasses soy medium
Groundnut shell medium	Jaggery-soy medium
FYM, press mud, biogas manure and poultry manure	Potato dextrose broth, V -8 juice
Coffee fruit skin, decomposed coconut coir pith	

Formulations of *Trichoderma* spp:

Formulation is the blending of active ingredients such as fungal spore with inert carriers such as diluents and surfactants in order to improve the physical characteristics. For example, grain or other types of organic matter upon which *Trichoderma* is grown are simply dried, ground and added to the area to be treated. The carrier material may be inert or a food base or a combination of both. The final formulation must have a long shelf-life, at room temperature, be easy to handle and must be stable over a range of -5 to 35^{0}C. *Trichoderma* spp can be formulated as granules, pellets, dusts and wettable powders and fluid drill gels. Granular or pellets preparations and *Trichoderma* enriched FYM have been used for soil application directly and have provided effective control of diseases both nurseries and field conditions. At National Agriculturally Important Micro-organism, Banglore, three carrier materials namely talc, kaoline and bentonite were tested for their effect on shelf life of *Trichoderma*. Kaoline and talc were identified as better carriers of *Trichoderma.*

Talc based formulation:

In India, talc based formulations of *Trichoderma* has become quite popular in India for management of several soil-borne diseases of various crops through seed treatment at 4-5g/kg seed. It was developed at Tamil Nadu Agricultural University, Coimbatore for seed treatment of pulse crops and rice (Jeyarajan *et al*., 1994). *Trichoderma* is grown in the liquid medium is mixed with talc powder in the ratio of 1:2 and dried to 8% moisture under shade. The talc formulations of *Trichoderma* have shelf life of 3-4 months. Several private industries produce large quantities of talc formulations in India for supply to the farmers. A list of commercial formulation of *Trichoderma* spp has been given in Table 1.

Standards for *Trichoderma* formulations:

– Colony Forming Units (CFUs) of *Trichoderma* spp. should be a minimum of 2 x 10^6 CFU per ml or gm on selective medium.

– Pathogenic contaminants such as *Salmonella*, *Shigella* or *Vibrio* should not be present. Other microbial contaminants not to exceed 1 x 10^4 count ml/g.

– Moisture content should not exceed 20% in the final product.

– Population per ml of the broth should be 2 x 10^8 cfu /g.

Shelf life of *Trichoderma* formulations:

Shelf life of the formulated product of a bio-control agent plays a significant role in successful managing of the diseases and longer storage periods. In general the antagonists multiplied in an organic food base have longer shelf life than the inert or inorganic food bases. Shelf life of *Trichoderma* in coffee husk was more than 18 months. Talc, peat, lignite and kaolin based formulation of *Trichoderma,* have a shelf life of 3-4 months. The viable propagules of *Trichoderma* in talc formulation were reduced by 50% after 120 days of storage.

Delivery of *Trichoderma* for plant disease management:

For successful diseases control, delivery and establishment of *Trichoderma* to the site of action is very

Table 1 : List of commercial formulation of *Trichoderma* spp and their target pathogen

Commercial product	Source	Target pathogen / disease
Root Shield	*Trichoderma harzianum* strain-KRL.AG2 (T-22)	*Pythium, Rhizoctonia, Fusarium*
BINAB T	*Trichoderma harziumum/Trichoderma polysporium*	Wood decay fungi
Promote	*Trichoderma harzianum* and *Trichoderma viride*	*Pythium , Rhizoctonia Fusarium* (Transplanted trees)
Trichodex	*Trichoderma harzianum*`	*Plasmopara, Colletotrichum, Monilin,* (Various plant)
F- stop	*Trichoderma harzianum*	*Rhizoctonia, Pythium,* (Ornamental and food crops)
Soil Gard (Glio Gard)	*Gliocladium* (*Trichoderma virens* GL-21	*Rhizoctonia solani, Pythium* (Ornamental and food crops)
Monitor SD	*Trichoderma* spp.	Soil borne plant pathogens
Monitor WP	*Trichoderma* spp.	Soil borne plant pathogens
Root Pro	*Trichoderma harzianum*	*Rhizoctonia solani, Pythium* spp. *Fusarium* spp and *Sclerotium rolfsii*
Supresivit	*Trichoderma harzianum*	Various fungi
Trieco	*Trichoderma viride*	*Rhizoctonia* spp., *Pythium* spp, *Fusarium* spp, graymold
Trichoderma 2000	*Trichoderma* spp.	*Rhizoctonia solani, Sclerotium rolfsii, Pythium* spp., *Fusarium* spp.
T-22, T-22 Planter box	*Trichoderma harzianum* Strain KRL AG2	*Pythium* spp, *Rhizoctonia solani, Fusarium spp.* and *Sclerotinia* spp.

important. The most common methods of application of *Trichoderma* are:

Seed treatment: Easy and effective methods of delivering the antagonist for the management of seed/soil-borne diseases. It is used at 6 - 10 g/ kg seed based on seed size.

Seed biopriming: Seed biopriming is treating of seeds with *Trichoderma* and incubating under warm and moist conditions until just prior to radical emergence. This technique has potential advantages over simple coating of seeds as it results in rapid and uniform seedling emergence. Seed biopriming was successfully used in tomato, brinjal, soybean and chickpea in Tarai Region.

Root treatment: Seedling roots can be treated with spore or cell suspension of antagonists either by drenching the bioagent in nursery beds or by dipping roots in bioagent suspension before transplanting. This method is generally used for the vegetable crops, rice where transplanting is practiced. Mix 10g of *Trichoderma* powder along with 100g of well rotten farm yard manure (FYM) per litter of water and dip the seedlings root at least 30 minutes before planting.

Soil application: Such applications are ideally suited for green house and nursery. *Trichoderma* is capable of colonizing 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. It can be applied at 1kg of *Trichoderma* formulation mix in 100 kg of farmyard manure and cover it for 7 days with polythene.

Foliar application: Liquid formulation of *Trichodema* performed better that powered formulation in case of foliar application. It can be applied at 5-10 ml/ L depending upon its concentration and application against particular disease.

Importance:

Growers are interested in reducing dependence on chemical inputs, so biological controls (defined in the narrow sense) can be expected to play an important role in Integrated Disease Management (IDM) systems. In this system, good cultural practices, including appropriate site selection, crop rotations, tillage, fertility and water management, provide the foundation for successful disease management by providing a fertile growing environment for the crop. However, such measures are not always sufficient to be productive or economically sustainable. In such cases, the next step would be to deploy biorational controls of insect pests and diseases. These include biocontrol agents, introduced as inoculants or amendments, as well as active ingredients directly derived from natural origins and having a low impact on the environment and non-target organisms. With the growing interest in reducing chemical inputs, companies involved in the manufacturing and marketing of BCAs should experience continued growth. However, stringent quality control measures must be adopted so that farmers get quality products. New, more effective and stable formulations also will need to be developed.

Future research:

The future research should focus on the following aspects for better utilization of *Trichoderma* as a biocontrol agent for crop disease management.

– Development of liquid/oil formulations suitable for foliar applications.

– Formulations with prolonged shelf life, field persistence and suitable for dry weather conditions.

– Scaling up of Solid state production systems with Industry collaboration.

– Large scale demonstration of biocontrol technologies in farmer's fields.

– Quality control laboratories and Fast Track Registration.

– Identification of strains suitable for various soil and environmental conditions (high temperature/ low moisture/saline conditions).

– Integration of more than two bio-agents for increasing the efficacy**.**

Conclusions:

Trichoderma spp. plays a major role as biocontrol agents, owing to their capabilities of ameliorating crop-yields by multiple roles, such as biopesticide and plant growth promotion. Information on the classification of the genus, *Trichoderma*, mechanisms of antagonism and role in plant growth promotion has been well documented. However, fast paced current research in this field should be carefully updated for the fool-proof commercialization of the fungi. In order to enhance marketability of *Trichoderma* as BCAs, feasible commercial production processes are of utmost importance. Pursuit for cheaper and alternative substrates and optimal operating parameters to increase conidia production is on, and several encouraging results are being reported by researchers worldwide. Thus, it is expected that in near future, exploitation of these interesting BCAs would be maximized. It would be frontline step in organic farming for managing of seed and soil borne pathogens.

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