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Trichoderma Species as Potential Biological Control Agents in Sustainable Agriculture

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Abstract
Several species of fungus are present in the world, which cause various plant diseases. But some fungal species play significant roles as biocontrol agents like *Trichoderma* species, *Trichoderma* fungus is working in the form of best biocontrol agent as other fungal pathogens. Mostly fungus in plants produces diseases in that we use fungicides and chemicals which are harmful to our environment. But most fungus is like *Trichoderma* species which work the farm of bio-control agents. The farmers are easily growing *Trichoderma* species-which is No harmful to our environment and protection of crops and growth of production. *Trichoderma* species are fungal organisms that can help farmers grow better crops and have higher income while protecting the soil environment. They are living freely in soil in agricultural and natural environments worldwide. Commercial products have been developed for plant protection that contain special strains of living *Trichoderma*. They have been formulated in a way that farmers and growers can easily apply them in the field. It is a safe and effective biocontrol agent (BCA) that can control a variety of fungal, (and bacterial) diseases, such as wilt, damping off, stem rot, and downy mildew. But not only that, *Trichoderma* is a yield-booster that makes crops look healthier, grow bigger, and show better root development.

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Introduction

Trichoderma works best in the soil. It is a biological degrader and competitor of fungal plant pathogens, which has evolved mechanisms for attacking other fungi in the root zone of a plant. Beyond that it also improves nutrient uptake of plants. There is also evidence that *Trichoderma*-based products are effective for controlling bacterial (vascular) wilts; for instance, bacterial wilts of cucurbits (caused by Erwinia, eg in cucumber and pumpkin) or bacterial wilts of Solanaceous crops (caused by Ralstonia, e.g. tomato, eggplant, potato). However, bacterial wilts are best man-aged with products containing biocontrol agents such as Pseudomonas fluorescence or Bacillus subtilis, or by combining these with *Trichoderma* spp. Products containing *Trichoderma* species are best used as soil amendments, sometimes also as foliar sprays. The fungus grows rapidly and can out compete other microorganisms. The main indicators for measuring economic sustainability from a producer's point of view (livelihood) include revenue, costs, and income. *Trichoderma*, soil-born filamentous fungi are capable of parasitizing several plant pathogenic fungi. Morphological and antagonistic activity against soil borne and foliar borne pathogens. *Trichoderma* is a genus that belongs to the class sordariomycetes, a large fungal class that contains more than 1100 genera and 10,000 species. Multifarious actions of *Trichoderma* strains have been recognized long back as biological agents. However, the choice of active species is the most widely studied mycoparasitic fungus.

Review of Literature

Trichoderma Plant Interaction

Trichoderma works as it grows in the rhizosphere and is capable of penetrating and internally colony formation in the plant's root system. The presence of *Trichoderma* in the rhizosphere evokes a coordinated transcriptomic, proteomic and metabolomic response in the plant. Some proteins like an expansion, swelling in are helps to degrade or loosening the cell wall plant and some other proteins like hydrophobin help to adhere on root surface.

Genus Alternaria

Alternaria fungus has about one hundred species which can be found in various places all over the world. Many of them are important pathogens plants and cause important economical disease in a wide range of hosts. Some of them live saprophytic and are the main part of the fungi population in the soil and dead or dying plant tissue. Polyphagous nature and ability of them in producing toxic and carcinogenic materials indicates that Alternaria is potentially hazardous to human and animal health. This pathogen has a wide range (more than 380) of hosts in iron including citrus, pistachio, apple, pear, tobacco, tomato, and beans. *Alternaria alternata* has an important place among specimens of this genus because of a wide range of hosts including garden plants, fields, crops, vegetables, and ornamentals. This fungus is one of them important pathogens of citrus which cause brown spot by path type, leaf spot by rough lemon path type, and black rot of harvested fruits. Genus *Aspergillus*.

Aspergillus flavus has a worldwide distribution and normally occurs as a saprophyte in soil and many kinds of decaying organic matter, however, it is also a recognized pathogen of humans and animals. It is a causative agent of otitis keratitis acute and chronic invasive sinusitis and pulmonary and systemic infections in immune compromised patients. A. flava is second only to A. fumigatus as the cause of human invasive aspergillosis (Hedayati *et al*., 2007). *Aspergillus* species are ubiquitous imperfect filamentous fungi. This *Aspergillus* also infects agricultural crops and contaminates stored grains while producing the most toxic and potent carcinogenic metabolites such as aflatoxins and other mycotoxins. *Trichoderma* spp. work as a strong contestant for food, space, antibiosis and number of devices which promotes the plant growth and provides immunity. *Trichoderma* species act as a bio-control agent in the nature of strong hostility against fungal pathogens. *Trichoderma* may suppress the growth of the pathogen population in the rhizosphere through competition and thus reduce disease development. It produces antibiotics and toxins such as trichodermin, which have a direct effect on other organisms. The antagonist (*Trichoderma*) hyphae or coil around it and secrete different lytic enzymes such as chitinases, glucanase and pectinase that are involved in the process of mycoparasitism. Examples of such interactions are T. acting against Fusarium, Fusarium, Fusarium solani, Phytophthora colocasiae and Sclerotium rolfsii. In addition, *Trichoderma* enhances yield along with quality of produce, boosting germination rate. Promote healthy growth in early stages of crop. Increase dry matter production substantially.

Provide natural long-term immunity to crops and soil. *Trichoderma* free sustenance fungi that are highly interchange in root, soil and foliar environment can parasitic other fungi (Harman *et al*., 2004). They are asexual filamentous fungi who share its anamorph with the genus hypocreac. They are also opportunistic virulent plants. They are known for prolific production of extra-cellular proteins and fungi toxic substances. They have been utilized largely as model microorganisms to analyze and improve the understanding of the role that these antagonistic fungi have been playing an instance with crop plants and phytopathogens (Marra *et al*., 2006).

Method of preservation of *Trichoderma* spp.

Trichoderma spp. A low nutrient medium is not a good choice for isolating since it does not act in lab conditions. A best medium for the obscure and growth of *Trichoderma* is potato dextrose agar medium. Spores of *Trichoderma* spp. are very small and very hard to isolate from soil. In growth of fungi there are various barriers because several other pathogens like other fungal viruses or bacteria grow in the media and pollute the media resulting in competition for food starting off extempore and proper growth does not arise and it can infect the preserved colonies. On the basis of fungal species, methods are chosen reasonably and user friendly in any laboratory. The number of mutagens present in environment safe keeping is very important. That is why we require the right method of protection (the cell goes into the go stage) of the respective fungus.

Trichoderma species used as biofertilizers

There are a number of *Trichoderma* fungi that are used as biofertilizers. *Trichoderma* has many advantages in forming, agriculture and home gardening. They are microscopic and are generally considered a virulent plant. *Trichoderma* spp. is the most common type of fungi in soils and they are present in nearly all soil types. This symbiotic connection is what biofertilizers try to utilize. In other words, they form a symbiotic or mutually beneficial relationship with plants. Physiologically and morphologically change in plant use to *Trichoderma* species Effect on seed germination

Seed treatment various plant species with *Trichoderma* activate the and No. of enzymes which personally alter the soil microflora complicated in seed germination. It also furthers the growth of germination and the seedling vigor procured earlier. Some examples are maize, beans, mustard, chilli, soyabean chickpea, okra, tomato, etc. (Mukhtar, 2008, Okoth *et al*., 2011. Rahman *et al*., 2012; Lalita *et al*., 2012; Kumar *et al*., 2014; Baby Chan and Simon, 2007), the high-speed growth of many seedlings. Avoid some diseases (Matsouri *et al*., 2010). Bezvidehlhun *et al*. (2012) reported that gliotoxin is a plant metabolite that works like a gibberellic acid (phytohormone that is included in seed germination) released by *Trichoderma* species.

Effect on Plant Morphology

Trichoderma spp. is established in soil and they interchange with plant roots in the rhizosphere. *Trichoderma* spp. are expert to enlarge the, plant height (Chowdappa *et al*., 2013), recent root weight, root system architecture (Bjorkman *et al*., 1998; Harman *et al*., 2004), leaf number and tiller number (Doni *et al*., 2014), other so many growth element by secreting the number of metabolic mass such as phytohormones, a number of enzymes and proteins and secondary metabolite. All the metabolic effects alter the plant morphology.

Effect on Seed Treatment

Seed treatment with tail based and wheat bran based (WBB) formulations of 4g/kg of seed have been used (Jogani and John, 2014). The treatment of *Trichoderma* germination germinates on the seed surface. And when they are sown in the soil the germinating propagules colonize the seedlings root and rhizosphere (Tiwari, 1996). Seed coating with *Trichoderma* is one of less unmanageable and logical conveyance systems of the management of seed/soil borne disease. Seed is coated with dry powder/dusts of *Trichoderma* just in preparation for showing for commercial justification, dry powder of antagonism is used at 3 to 10 g per kg seed based on seed size (Mukhopadhyoy *et al*., 1992). Pythium spp. and R. solani (Mukherjee and Mukhopadhyay, 1995) and oilseed borne fungi like *Alternaria alternata*, Curvularia lunata, *Aspergillus* flavus, Fusarium oxysporum, which effect oil seed crops like soyabean, sesame and sunflower (Jat and Agalave, 2013).

Seedling Root Dip

It is largely practiced in the case of transplanted rice and vegetable crops. The cutting or seedling can be treated with the spore suspension prepared by mixing 10 g of *Trichoderma* powder with 100 g of well rotten gum per liter of water and dip for 10 minutes before planting root dipping in spore suspension before transplantation reduces sheath blight disease of rice.

Materials and Methods

Sample Collection

We collected many soil samples from various vegetable fields. We also took some samples from Godavari farm house Badi and botanical garden university college of science Udaipur and many other soil samples collected from RCA college Udaipur. We collected soil samples in poly begs. In store 4°C temperature. Until for the processing. Many fields are collected from tomato plant infected leaves in sterilization processing in the plant pathology laboratory.

Serial Dilution

First, we took five test tubes. 9 ml double add the all test tube. Then labeled 101, 102, 10 10. 10, 1 gm soil sample add in the first test tube add mixing well from test tube 10 add 1 ml in label test tube10 this some process labeled the all test tube. All the test tubes shake properly. Inoculation use for 10,10,10 tubes.

Formation of PDA

Firstly, we peeled the epidermis plants, weighing 200 gm potatoes. We make small pieces of potato. Boil the double distilled water up to 400 ml approx 30 minutes. As a filter the potato is extracted using Muslin cloth. Add 18 gm agar and 16 gm dextrose at appropriate amounts. We shake a lot. And set the PH using. IN, NAOH and IN, HCL. Normally set the 5.6 PH and up to 150 ml pour in a flask. For 15 minutes using autoclave on 121 and 15 psi pressure contribute steam sterilization.

Chemical amount (for 1 lit.)

Agar-18 gm
Dextrose-16 gm
Double Distilled Water-1000 ml
IN NaOH-10 ml
IN HCI-10 ml
Chlortetracycline-40 mg

Sterilization

Sterilization any process that removes kills decontaminates and other biological agents such as virus, bacterial and fungal spores. Their bacteria in viruses affected the production of fungal pathogens. So firstly, all the virus and bacteria should be removed from the PDA media and all the glass vials are autoclaved (instruments of stem sterilization) and remove all the contaminations at 15 psi pressure and 121°C temperature for 15 minutes.

Inoculation

Inoculation is the next step which is done under an instrument called a laminar air flow chamber. LAFC provides aseptic conditions to transfer an inoculum to appropriate media. In this step different concentrations of serial dilution test-tubes were taken and sample water spread throughout the media. The inoculation method is as follows. Firstly, we clean the laminar air flow chamber and operator's hand surface using cotton and absolute alcohol. After that give at least 20 to 30 min. UV treatment to all instruments which are used in laminar except- biological material because UV causes mutation. It is necessary to Turn off the UV light and, on the blower, and fluorescent light after than Pouring the PDA media in Petri plate and allow solidify and then spread water sample on the PDA media and sealed with paraffin and this Petri-plates Put into incubator for 3-4 days. Measure the growth using zone scale.

Sub-culture

Colonies obtained through inoculation; a number of species were obtained in a Petri plate. All the species of fungus are required to preserve its culture.

All the procedure is the same as the inoculation method but the inoculum was taken from a previously inoculated Petri plate using a cork-borer or needle. The petri plate is sealed with paraffin and placed into an incubator for 70 to 90 hours.

Antagonistic Activity

The antagonistic activity was conducted using Dual culture technique. The colonies of *Trichoderma* spp. and pathogen species were obtained. After 7 days, with the help of cork borer 6 millimetres mycelial plugs were taken from the edge of each colony (which species to be tested) transferred to PDA plates in the Laminar air flow chamber. The antagonistic activity applied in more than one Petri plate of each species. The controls consisted of pure culture of *Aspergillus* spp. and *Alternaria alternata*. The per cent inhibition of mycelia growth over control was calculated by following equation:

Percent inhibition (%) Growth in control-growth in treatment ×100 ÷ Growth in control

Results and Discussion

The in-vitro evaluation of *Trichoderma* spp. against two distinct plant pathogens, *Alternaria alternata* and *Aspergillus* spp., yielded significant data regarding its efficacy as a biocontrol agent. The study was conducted using the Dual

Culture Technique on Potato Dextrose Agar (PDA) medium. Antagonistic Effect on *Alternaria alternata*: The results indicated a high degree of antagonism. *Trichoderma* spp. demonstrated rapid mycelial growth, effectively colonizing the agar surface before the pathogen could establish itself. A clear zone of inhibition was initially observed, followed by the overgrowth of *Trichoderma* over the Alternaria colony. This suggests that *Trichoderma* utilizes mechanisms such as mycoparasitism and nutrient competition to suppress this specific pathogen.

Antagonistic Effect on *Aspergillus* spp.: In the case of *Aspergillus* spp., inhibition was also observed, but the percentage of growth inhibition was comparatively lower than that of Alternaria. While *Trichoderma* managed to restrict the spread of the *Aspergillus* colony, the latter showed more resilience, likely due to its rapid sporulation and robust metabolic defenses.

Observations: The antagonistic interaction was characterized by the physical contact of hyphae and the visible reduction of the pathogen's radial growth. The fast-growing nature of *Trichoderma* makes it a superior competitor for space and essential resources.

Table 1: *Trichoderma* spp. against Fungal Pathogens

Target Pathogen	Mean Radial Growth (Control)	Mean Radial Growth (Dual Culture)	Degree of Inhibition	Primary Mechanism Observed
Alternaria alternata	High (Full Plate)	Very Low	Significant/High	Mycoparasitism & Overgrowth
Aspergillus spp.	High (Full Plate)	Moderate	Moderate	Competition &

Conclusion

In our ecosystem plants act as an energy converter and spread this energy throughout the ecosystem thus we need a healthy plant and its life. Chemical pesticides are dangerous for our ecosystem as well as human-beings. That's why we chose the bio control agents to prevent the plant pathogens. This experiment explains that *Trichoderma* spp. act as a biocontrol agent and restricts the growth of *Aspergillus* spp. and *Alternaria alternata* pathogen on potato dextrose agar medium in the dual culture method.

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