

Isolation of *Trichoderma* from Soil Sample for Production of Fungicide

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Abstract -

This study is investigated that *Trichoderma viride* was show antifungal activity. *Trichoderma*, soil-borne filamentous fungi, are capable of parasitising several plant pathogenic fungi. Twelve isolates of *Trichoderma* spp. isolated from different locations of Sangli were characterized for their cultural, morphological and antagonistic activity against soil borne and foliar borne pathogens. The sequencing of these isolates different species. The isolates revealed differential reaction patterns against the test pathogens viz., *Trichoderma* were most effective in percentage inhibition of mycelial growth of test pathogens. Significant chitinase and β -1,3-glucanase activities of all *Trichoderma* isolates has been recorded in growth medium. *T. viride* was found with highest chitinase whereas *T. harzianum* was recorded with highest β -1,3-glucanase activities.

Keywords: *Trichoderma*, Spice, Biocontrol, Sangli

Introduction

In the modern context of sustainable agriculture, biofertilizers have emerged as a pivotal solution to the increasing demand for environmentally friendly and sustainable agricultural practices. Traditional chemical fertilizers, although effective in boosting crop yields, have led to numerous environmental and health issues, such as soil degradation, water pollution, and the disruption of beneficial soil microorganisms.

Biofertilizers, derived from natural materials and living organisms, offer a sustainable alternative that not only enhances soil fertility but also promotes plant growth and resilience against diseases. Among various biofertilizers, *Trichoderma* species have garnered significant attention due to their multifaceted benefits and eco-friendly nature. *Trichoderma* has a diverse Range of strains and species and impacts the functioning of The ecosystem, such as the decomposition rates.

Depending On the strains, the use of *Trichoderma spp.* In agriculture Provides numerous advantages (Sood *et al.* 2020; Vinale *et al.* 2008. From different Paddy fields in the Tra Vinh province of Vietnam. The high Potential cellulose degrading *Trichoderma* was identified and used to produce biofertilizers, which have the ability to Decompose agricultural wastes.

In the past decade, Vietnamese production of rice, bioactive compounds such as biofertilizers. Microbial fruits, and vegetables have grown substantially and steadily technology has been used effectively in the treatment of (Ngoc et al. 2021).

The rice sub-sector in particular is the agricultural and municipal waste (Hidalgo et al. 2022) .Foundation for comprehensive development and the main The treatment of agricultural by-products by microbial technology, especially cellulase enzyme and extracellular contributor to Vietnam's economy. Rice straw, a residual by-product of the rice harvest, has been estimated at around peroxidase from microorganisms, have yielded several 76 million tons for 4 million hectares of rice field.

Fungi are one of the groups of rice straw has been used as cooking fuel, animal feed, microorganisms capable of decomposing organic materials roofing, and compost (Nguyen *et al.* 2019).

Trichoderma spp. Colonize roots, however the plant .Protection benefits could also be obtained by Applying them on fruits, flowers and foliage.(Dhruva Prasad Gauchan and *et al.*2020). *Trichoderma asperellum*, one of the plant growth-Promoting fungi (PGPF), is a well known antagonist, And one of the most documented biological control . Agents of various pathogens(D Rai and N Singh2023) .

He genus *Trichoderma*, *anamorphic Hypocrea (Hypocrea-Ceae)* is present in all soils, where they are the most prevalent Culturable fungi, although exhibiting low species diversity. Many species in this genus can be characterized as opportu-Nistic avirulent plant symbionts. Strains Of *Trichoderma spp.* Are also widely used in the biocontrol of Soilborne plant pathogenic fungi. In addition, *T. atroviride* and *T. viride* are good bioremediators for some.Heavy metal ion (Qi-Rul li. and *et al.* 2012.)

Types of biofertilizer :

Biofertilizers are carrier-based microbial inoculants containing sufficient cells of efficient strains of specific microorganisms, that help in enhancing the soil fertility by fixing atmospheric nitrogen, solubilization/ mineralization of phosphorus or decomposing organic wastes, by augmenting plant growth promoting substances with their biological activities.

Nitrogen fixing:

Nitrogen is most abundant and ubiquitous in the air, yet becomes a limiting nutrient due to difficulty of its fixation and uptake by the plants.

Phosphate solubilizing:

The phosphorus-solubilizing bacteria (PSB) can increase phosphorus availability to plants by dissolution of bound phosphates in soil by secreting organic acids characterized by lower pH in their vicinity.

Phosphate mobilizing:

The mycorrhizal fungi form obligates or facultative functional mutualistic symbioses with more than 80% of all land plants, in which the fungus is dependent on host for photosynthates and energy and in return provides a plethora of benefits to its host. The mycelium of the fungus extends from host plant root surfaces into soil, thereby increasing the surface area for more efficient nutrient access and acquisition for the plant, especially from insoluble phosphorus sources and others like calcium, copper, zinc, etc.

Mineral-Solubilizing Biofertilizers –

Potassium solubilizing: Certain rhizobacteria can solubilize insoluble potassium forms, which is another essential nutrient necessary for plant growth. Eg. *Bacillus edaphicus*, *B. mucilaginosus*, and *Paenibacillus glucanolyticus*.

Silicate and zinc solubilizing: Another important mineral is zinc, which is present at a low concentration in the Earth's crust, due to which it is externally applied as the costlier soluble zinc sulfate to overcome its deficiencies in plant. Certain microbes can solubilize insoluble cheaper zinc compounds like zinc oxide, zinc carbonate, and zinc sulfide in soil. Similarly, microorganisms can hydrolyze silicates and aluminum silicates by supplying protons (that causes hydrolysis) and organic acids. Eg. *Bacillus subtilis*, *Thiobacillus thiooxidans*, and *Saccharomyces sp.*

Compost Biofertilizers:

Compost is a decomposing, brittle, murky material forming a symbiotic food web within the soil, which contains about 2% (w/w) of nitrogen, phosphorus, and potassium, along with microorganisms, earthworms, and dung beetles.

Compost is produced from a wide variety of materials like straw, leaves, cattle shed bedding, fruit and vegetable wastes, biogas plant slurry, industrial wastes, city garbage, sewage sludge, factory waste, etc.

The compost is formed from these materials by different decomposing microorganisms like *Trichoderma viridae*, *Aspergillus niger*, *A. terreus*, *Bacillus spp* etc. that have plant cell wall-degrading cellulolytic or lignolytic and other activities, besides having proteolytic activity and antibiosis (by production of antibiotics) that suppresses other parasitic or pathogenic microorganisms

Benefits of Biofertilizer:

1. Biofertilizers are means of fixing the nutrient availability in the soil. Generally, Nitrogen deficiencies.
2. A bio-fertilizer is technically living, it can symbiotically associate with plant roots. Involved microorganisms could readily and safely convert complex organic material into simple compounds, so that they are easily taken up by the plants. Microorganism function is in long duration, causing improvement of the soil fertility. It maintains the natural habitat of the soil. It increases crop yield by 20-30%, replaces chemical nitrogen and phosphorus by 30%, and stimulates plant growth. It can also provide protection against drought and some soil-borne diseases.
3. It has also been shown that to produce a larger quantity of crops, biofertilizers with the ability of nitrogen fixation and phosphorus solubilizing would lead to the greatest possible effect.
4. They advance shoot and root growth of many crops versus control groups. This can be important when implementing new seed growth.
5. Biofertilizers also promote healthy soil, leading to greater farming sustainability

Mechanisms of Trichoderma as a Biofertilizer

The beneficial effects of Trichoderma as a biofertilizer can be attributed to several key mechanisms:

Soil Health and Nutrient Cycling: Trichoderma species play a crucial role in decomposing organic matter and breaking down complex soil nutrients into forms that are readily available to plants. They produce a range of extracellular enzymes, including cellulases, chitinases, and proteases, which contribute to the degradation of organic matter and the release of essential nutrients. This enhances soil fertility and promotes a healthy soil microbiome.

Plant Growth Promotion:

Trichoderma fungi produce growth-promoting substances, such as indole-3-acetic acid (IAA), gibberellins, and cytokinins, which stimulate plant growth and development. These hormones enhance root elongation, increase root biomass, and

improve overall plant vigor. Additionally, Trichoderma colonization of plant roots can lead to increased nutrient uptake, particularly of nitrogen, phosphorus, and micronutrients.

Induced Systemic Resistance (ISR):

Trichoderma species can activate plants' immune systems, inducing systemic resistance against a broad spectrum of pathogens. This ISR mechanism involves the production of elicitors and signaling molecules that prime the plant's defense responses. As a result, plants become more resilient to infections by fungi, bacteria, and viruses.

Biological control:

The term "biological control" has different meanings. We will use the definition proposed by Baker and Corn. "biological control is the reduction of inoculums or through one or more organism other than man". This definition is broad enough to encompass classic approaches to biocontrol that influence pest population as well as newly emerging biocontrol strategies.

One important difference between known biological agents and synthetic chemical is that biological are almost always protectants. While many modern fungicides are systemic and also may be used after infection has occurred.

Biocontrol organisms often continue to produce their active ingredients using their active biosynthetic pathway. Whereas, chemical and biological currently degrade synthetic chemical means some products address long-term root health, an area that is beyond the scope of most modern fungicides.

Effective use of biological products will require an understanding of plants microbe ecology and the mechanism of action of the biological control agents. Biological control agents have both direct action on the pathogen and indirect mechanism of activity. Indirect mechanism involves alteration in plant physiology. Some of these indirect mechanisms include; The production of plant growth of nutritional status of the plant because of a more efficient root system and increased nitrogen fixation because of enhanced *Rhizobium* nodulation. These mechanisms can lead to escape from diseases or improved tolerance to stress.

Direct mechanism of biological control involves antibiosis competition for nutrients or niches and parasitism.

Development of Product :

The integration of biological control into IMP systems will request the availability of product from use. Biocontrol products must meet the same development requirements as commercial pesticides. First the demand for the product and market size must justify investment in research, development, regulatory compliance, and marketing. To date most biological controls have a relatively narrow spectrum of efficacy. It is difficult to economically justify development for a single pathogen on single crop where ecological considerations limit performance. However development for a single pathogen cost of biological may prove to be much lower than the \$20- \$40 million now commonly associated with development of a synthetic chemical pesticides combination of biological control agent with spectrum of disease controls may efficacy environment tolerance (PH, temp, host genotype.) or combination of biological, chemical or cultural control may eventually be developed that will allow broad crop adaptation as well as multiple pathogen suppression and be cost effective from both a development and a farm benefit standpoint.

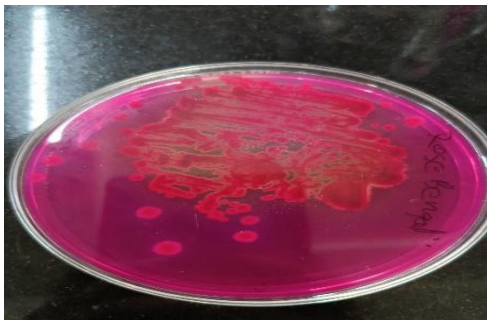
Efficacy of future biocontrol products must be high and consistent. biological control products or agent have not had the high degree of consistent reliable performance commonly demanded of pesticides. Performance standard can only be achieved when greater levels of funding and greater scientific understanding of mode of action and ecology are integrated into biological control products development utilize this occurs, biological will be limited to niche markets where no effective chemical are registered where growers desire no synthetic chemical inputs (e.g. organic farmers) or where high value crops are grown under controlled conditions high level of quantity quality control in fermentation or culture and formulation will be required so that efficacy is mentioned from manufacturing through field application.

Biological control products also must acceptable standard for environmental and toxicological safety. Biological agents used in control of plant diseases are primarily microbial agents, because they are used for pest control they are regulated as pesticides by the EPA and must satisfy environment and test protocols similar to synthetic pesticides. In addition, biological agents must satisfy USDA-APHIS (Animal and Plant Health Inspection Service) creative for interstate movement and release into the environment because they are considered genetically novel or exotic organisms .

***Trichoderma viridie* as a biocontrol, antifungal agent.**

A) Taxonomic classification

Kingdom	- Fungi
Phylum	- Ascomycota
Class	- Euascomycetes
Order	- Hypocrisy
Family	- Hypocreaceae
Genus	- <i>Trichoderma</i>
Species	- <i>Viride</i>



Photograph of *Trichoderma*

B) Morphology –

Trichoderma is a filamentous fungus that is widely distributed in the soil, plant material, decaying vegetation and wood. *Hypocrea* spp. Are the teleomorphs of some *Trichoderma species*. On the basis of morphological feature *Trichoderma* show different species. The conidia and phialides help in differentiation of these species from each other species –

Trichoderma harzianum, *Trichoderma Viridea*, *Trichoderma Konnigi*, *Trichoderma longibranchiatum*, *I.pseudokoningi* etc.

Trichoderma represent the most studied fungus that shows antagonistic activities towards soil born plant pathogen. The first brilliant series of papers on parasitism of *Trichoderma viride* apperard in 1932 use of *Trichoderma* in biocontrol gave good results against *Fusarium* act on tomato. *Trichoderma viride* was demonstrated to protect the tomato plants against *Fusarium oxysprum* species lycopersici and cucumber. For 23 to 25 days. Similarly,*T.harzianum* was developed as a potential antagonistic against *F.oxysporum f.sp.T.harzianum* .*T.koningi* were known to improve germination and plant growth. There are indications of stimulation of enzyme production by *Trichoderma species*.

An endo- alfa-D(1->3)- glucan capable hydrolysing various alpha (1->3)- glucan has been isolated and purified *Trichoderma viride* and it's specificity has been tested only those with alpha (1->3) glucosidic linkage were attacked and enzymatic hydrolysis occurred with retention of configuration of the anomeric carbon atom involved in cleavage. Despite the fact that *Trichoderma* spp. are widely however, only the increasing interest in the biological control and with the development of biotechnology in agriculture. Do we began to see commercial products that utilize *Trichoderma* as a active biocontrol agent.

Methodology

1)Sample collection: Soil sample was collected from Islampur Tal-Walwa Dist -Sangli, farming soil and near pound in 15 cm in depth.

2) Enrichment of sample:

soil sample was enriched by Rose Bengal Broth and Sabouraud dextrose Broth In 1week incubation period at a 37°C room temperature.

3)Isolation method:

Isolation of microorganisms from soil.it was along enriched sample and spread on Rose Bengal Agar plates and Sabouraud dextrose Agar plates and incubate at room temperature for 4to 6 days.

4)Characterization of isolated microorganisms:

The isolated fungi were identified according to their micro-morphology as well as color pattern and conidial structure. Glass slide preparation was done by using lactophenol cotton blue as a stain. Microscopic examination of the prepared slide was done by using low power objective le

RESULTS AND DISCUSSION

Result:

Trichoderma viride was successfully cultivated on Sabouraud agar and Rose Bengal Agar. The initial inoculation showed visible growth after 4 to 5 days of incubation at 37°C. Colonies appeared green and yellow color, typical of *T. viride*.

Morphological and cultural characteristics of *Trichoderma viride* spp:

Sr.no	Size	Shape	Color	Margin	Elevation	Aerial myeceli
1.	3mm	Filamentous	Green	Irregular	Raised	Present
2.	4mm	Filamentous	Yellow	Irregular	Raised	Present

DISCUSSION

Some fungus have fungicidal properties which inhibit the growth of the other fungi, *Trichoderma viridie* is one of them. Many fungi cause various disease to the fruits and vegetables and damage to them.

Summary And Conclusion

Trichoderma viride was isolated from soil and it was characterized. *Trichoderma viride* showing lower mycelial growth and long filamentous comparison for *Aspergillus niger*.

Trichoderma viride used as Biofertilizer of various infection of fruits and vegetables. *Trichoderma viride* was effective insecticides is made in the modern era to control various fungal infections of plants.

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