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Mass production of *Trichoderma viride* a sustainable strategy for large-scale production

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Abstract
The growing dependence on synthetic agrochemicals has raised significant ecological concerns, driving the shift towards more sustainable and environmentally friendly agricultural practices. Among the various biocontrol agents, *Trichoderma viride* stands out due to its remarkable antagonistic activity against a broad spectrum of phytopathogens and its capacity to enhance plant growth. This study investigates the mass production of *T. viride* on maize grains as a substrate under controlled laboratory conditions. The initial stage involved the cultivation of *T. viride* on Potato Dextrose Agar (PDA) to establish a pure culture, which was then used to inoculate sterilized maize grains for large-scale production. The growth of *T. viride* on the maize substrate was monitored, revealing a consistent colonization pattern, with an average growth rate of 56.6% by the tenth day. The fungal biomass was subsequently formulated into a talc-based carrier, and its viability, environmental tolerance, and compatibility with commonly used agrochemicals were evaluated. The results demonstrate that *T. viride* can be efficiently mass-produced on maize, with the potential to serve as a sustainable, eco-friendly alternative to chemical pesticides in integrated pest and disease management strategies. This research underscores the viability of *T. viride* for large-scale application in organic and sustainable agriculture.

Keywords: *Trichoderma viride,* biocontrol agent, sustainable agriculture, eco-friendly alternative

Introduction
The creation of broad-spectrum insecticides was driven by the necessity of the agricultural community to manage pests and illnesses of farmed crops. Evaluation of pesticide effectiveness in management was the sole metric for progress. But now that population levels are steady and new agricultural technologies are proving successful, food production is no longer a big issue. The agrochemical sector, which formerly saw farmers as its primary clients, today fails to see the general population as a significant client. The public's view is that there are more drawbacks to pesticides than advantages. Biological disease control has seen a dramatic growth, thanks in large part to the growing awareness of environmental concerns over pesticide usage. The use of antagonistic microflora in disease management has been supported by the following factors: the rise of fungicide-resistant infections, contamination of groundwater and food supplies, and the emergence of oncogenic hazards, Over fifty commercial formulations including fungus and bacteria have been launched as a result of decades of laboratory trials, which have exploded the field of biocontrol (Baker and Cook, 1974; Whipps, 1997) [1, 2]. *Trichoderma* is an antagonist that has provided new opportunities for the commercialization of plant diseases and plays an important role in plant disease control (S Nakkeeran *et al*., 2011) [11].

Bartlett first introduced the term "integrated control" (Bartlett, 1956) and Stern and colleagues expanded on it (Stern *et al*., 1959) [5]. Later used to encompass coordinated use of all cultural, artificial, and biological activities, the word was originally in relation to integrating the use of biological and other controls in complementary ways (van den Bosch and Stern, 1962) [6]. The term "Integrated Pest Management" (IPM) has been used by several authors to describe a comprehensive approach to pest control that aims to integrate all relevant practices into a single systemic strategy for production. To save beneficial insects and forestall the return of pest populations that have developed resistance to pesticides, (Alastair, 2003) [4] proposed integrated pest management (IPM) as a means of rationalizing pesticide use. Alternative classifications that do not include conventional pesticides have emerged in recent years due to environmental and food safety concerns (C0 Ehi-Eromosele *et al*., 2013) [8].

Method uses in IPM
To control pests, IPM uses four types of methods:

1. **Cultur. al method:** It refers to the type of practices and techniques to prevent or manage pest like crop rotation, sanitation, irrigation management, pruning.
2. **Mechanical/physical method:** This method involves using the physical barriers like blue sticky traps, yellow sticky traps, hand-picking, fruit/fly traps.
3. **Biological method:** This method involves living organisms to control pest like predators, parasitoids and pathogen.
4. **Chemical method:** This method involves only when the pest is out of control like spraying, soil treatment, seed treatment.

Biopesticide
Pesticides known as biopesticides are made from substances found in nature, including plants, animals, microbes, and even minerals, that control pests through various mechanisms, including toxicity, inhibition of growth, and disruption of mating and other behavior's (EPA, 2020).

Bio-fertilizer

Applying a bio-fertilizer to seed, plant surface, or soil allows the microorganisms to colonize the plant's rhizosphere, or inner growth zone, and stimulate growth by increasing the plant's nutrition supply (J.P Sharma, 2002) [10].

Role of Biopesticide and Biofertilizer in Agriculture

The use of chemical fertilizers and pesticides in Indian agriculture has skyrocketed in the last several years, reaching concerning levels in some regions that threaten human health, the environment, and groundwater. Therefore, it is critical to immediately implement eco-friendly strategies for enhancing soil fertility and controlling pests and diseases. One significant alternative to traditional fertilizers is biofertilizers. An eco-friendly alternative to traditional pesticides and fertilizers, biopesticides and biofertilizers help plants thrive in their natural environments. Their potential for reducing reliance on artificial fertilizers while yet providing plants with the nutrients they need is enormous. These bio-inputs, when applied to plants, boost their development and harvest. Biofertilizers are organic products that include concentrated forms of microorganisms that are either obtained from plant roots or the soil around a plant's roots. The use of chemical fertilizers and pesticides on crops has led to a cascade of negative consequences, including a breakdown in the sustainability of agricultural systems, skyrocketing cultivation costs, stagnant farmer income, and the terrifying prospect of food insecurity and contamination. Soil health has declined significantly due to the use of chemical pesticides, urea in particular, and other chemical fertilizers in an unbalanced and careless manner, as well as the lack of organic manures. Because they are selective, biopesticides do not leave behind any toxic byproducts. These are the actual creatures that may eliminate pests in farming. When used correctly, bio inputs aid in soil health restoration, which in turn offers a cost-effective method of managing agricultural productivity while simultaneously balancing the ecosystem (Jyoti S Kawalekar, 2013) [11].

***Trichoderma:* A Natural Defense Mechanism**

When it comes to agricultural systems, insects are the biggest problem since they eat crops and spoil them in storage. A new generation of effective and safe pesticides is urgently needed since the current method of controlling pest insects has relied on the widespread and unregulated use of chemical pesticides, which pose serious risks to human and environmental health. As there are many types of biocontrol agents which are used against pest in this, we learned about biocontrol agents which is *Trichoderma spp*. *Trichoderma* is a filamentous fungal genus that has been the subject of much research and study. Its capacity to parasitize pathogenic fungus, a process known as mycoparasitism, is one of its many modes of action that make it useful as a biocontrol agent in agriculture. There has been some indirect and direct discussion on the potential use of *Trichoderma* as a biocontrol agent for insect pests in recentyears. So far, research has shown that *Trichoderma* may act as a parasite and produce insecticidal secondary metabolites, antifeedant chemicals, and repellent metabolites, all of which help to manage insect pests. Also in a roundabout way, by luring in natural enemies or parasitic symbiotic microbes, or by activating systemic plant defence mechanisms. Thus, *Trichoderma* may be used as a plant pathogen and insect pesticide in agriculture, offering a potential future solution for sustainable farming (Jorge Poveda, 2021) [12].

Literature Review

Trichoderma viride, first described by Christiaan Hendrik Persoon in 1794, has historically been considered a single species. However, research by (Lieckfeldt 1999) [13] challenged this notion by revealing significant genetic diversity within strains identified as *T. viride*. Their study suggested that what was once considered a single species might encompass multiple distinct species (Leickfeldt et.al., 1999). However, *Trichoderma viride* is believed to have originated from a common ancestor with other *Trichoderma* species around 100-150 million years ago (Kullnig *et al*., 2000).

The filamentous fungus *Trichoderma viride* is an effective biocontrol agent for many plant diseases. It is known for its ability to produce secondary metabolites, induce plant resistance, and promote plant growth. The fungus is commonly applied in agriculture for its antifungal properties, particularly against soil-borne pathogens (Harman 2004) [15].

Among the many biocontrol agents used to combat plant diseases, *Trichoderma viride* is by far the most ubiquitous. This study aimed to determine how different formulations affected soil dilution and post-treatment persistence adjusting dilution. Among these compositions were a *Trichoderma viride* water-in-oil emulsion and a gel matrix derived from natural commodities. While all of the formulations outperformed the control, the bio gel and water-in-oil formulations stood out with their very high occurrence rates. Furthermore, the impact of metallic nanoparticles on the formulation's efficacy was investigated, and the results demonstrated that each of the formulations had potential. A study examined the effects of different formulations on the biocontrol efficacy of two soil-adapted microbes, *Fusarium oxysporum* and *Alternaria alternata* While all of the formulations exhibited a slowing effect on the growth of pathogenic fungal colonies, the bio gel formulation of *Trichoderma viride* treated with nanoparticles had the most beneficial effect on the breakdown of organic waste (S Karthick Raja Namasivayam 2024) [16].

Commercialization and Adoption of *Trichoderma viride* in India

The fungus genus *Trichoderma* is among the most widely used biological control agents and plant growth promoters on the market. Literature reports more than 80 *Trichoderma* species. On the other hand, the most popular types of *Trichomonas* used as biocontrol agents include *asperellum*, *harzianum*, *viride*, and *virens*. Initiation of studies to investigate biocontrol agents' potential was motivated by the need to find a workable and affordable management solution Native biocontrol agents, including *Trichoderma harzianum*, *Aspergillus versicolor*, and *Bacillus* firmus, were isolated from various agricultural systems after testing a significant number of soil samples taken in the region's western regions. Both in and in investigations have demonstrated the antagonistic potential of these biocontrol drugs. The Indian government has approved the use of two *Trichoderma* species—*T. viride* and *T. harzianum*—as seed treatments and soil applications for the purpose of combating soil-borne plant diseases. Scientific articles have been published on the effectiveness of *Trichosporon asperellum* and *Trichosporon virens* in controlling diseases in India; however, these have not been registered with the Central Insecticide Board and Registration Committee (CIB | RC) Sustainable disease control is the main emphasis here, along with the commercialization acceptance, and usage of the numerous products from bacterial and fungal consortiums. (Ritu Mawar 2021) [17].

Research Methodology

Experimental Site of Study

The research was conducted in the Plant Pathology laboratory, Central Integrated Pest Management Centre, Gorakhpur a sub-office of Directorate of Plant Protection Quarantine, Ministry of Agriculture and farmer Welfare, Government of India.

Sterilization of Glassware

The experimental glassware was washed with dish detergent, immersed in chromic acid for 20 minutes, rinsed under running water, wiped to eliminate excess water, and set aside to dry and kept in hot air oven for 25-30 minutes at 120°C.

Media Preparation for Culturing

Potato Dextrose Agar (PDA)

The Formulation of PDA

1. Potato – 200gm
2. Dextrose – 20gm
3. Agar-Agar – 20gm
4. Distilled water – 1000ml

Steps Involved in PDA Preparation

1. Boil 200gm of peeled and sliced potatoes in 1000ml of distilled water for 30 min.
2. Strain the potato extract through muslin.
3. Discard the potato solid and keep the filtrate.
4. Add 20gm of dextrose and 20gm of agar to the potato infusion
5. Stir the solution until everything's dissolves.
6. Pour the mixture into flasks.
7. Autoclave at 121°C for 15-20 minutes to sterilize.
8. Allow the medium to cool to about 45-50°C.
9. Pour it into sterile petri dish, flask and test tube inside a laminar airflow cabinet
10. Allow the plates to solidify at room temperature.

Inoculation of *Trichoderma viride* in Flask, Petri Dish and Test Tube

The potato dextrose agar (PDA) was removed from autoclave before placing in the work area (laminar airflow cabinet) it was cleaned by 70% ethanol and then turn on the laminar airflow cabinet and let it run for 10-15 minutes. Flame sterilizes the inoculation loop using Bunsen burner until it turns red hot and allowed to cool down. After cooling, small proportion of *Trichoderma viride* taken from an actively growing culture using an inoculation loop and then placed the culture in fresh PDA which are kept in flasks, test tubes, and petri dishes. After that seal the flasks, test tubes with cotton and parafilm and petri dishes only with parafilm to prevent any contamination. Incubate at 25-28° C in a Biochemical Oxygen Demand (BOD) for 7-9 days. After 7-9 days observe the green pigments (*Trichoderma viride*) which indicates successful growth.

Mass production of *Trichoderma viride* on maize

- Maize – 4kg
- Mother culture of *Trichoderma viride* (provided by plant pathology lab CIPMC, Gorakhpur)
- Trays – 3
- Chart paper – 3
- Distilled Water as required
- Autoclavable polybags – 6
- Rubber bands
- Streptomycin
- Carboxyl methyl cellulose
- Gloves
- 70% ethanol to clean the trays
- Talcum powder

Inoculation of *Trichoderma viride* on Maize

In the beginning, the maize was soaked in water for at least 7-10 days before using it. After that 4kg of maize was filled into 6 autoclaved polybags in which each polybags contain 667g of maize, followed by the addition of streptomycin. Thereafter each autoclavable polybags are secured with Rubber bands.

Following the completion of these steps, the autoclavable polybags are placed in autoclave at 121°C for 20 minutes at 15 psi for sterilization. The laminar flow air chamber is cleaned with 70% of ethanol and is sterilized with UV for 45 minutes.

The autoclaved maize polybags undergo one hour of UV exposure to prevent contamination and then lets the polybags cool down. When the polybags cool down, mix them well so that there are no lumps, and then transfer them to a tray. After this, we will pour our culture *Trichoderma* culture which is mixed with distilled water (*Trichoderma viride* which are kept into flask, test tubes and petri dish) into the trays and mix it well. After mixing properly, while using the gloves and water as required so that it doesn't get dry and covered it with the chart paper. Then the tray will be placed at the temperature of 25-30°C for 12-15 days. Trays are kept away from direct sunlight.

Talc based formulation

Once the *Trichoderma* colonies are fully developed, the trays containing maize are subjected to drying for two days at a standard temperature. Following the drying process, the material is finely ground and subsequently mixed with talcum powder in a 2:1 ratio using a sieve to ensure uniformity and eliminate any lumps. The resulting mixture is thoroughly homogenized, and Carboxy Methyl Cellulose (5 g) is added as a supplementary nutrient. The final product is then packed into 200 g sealed packets. Under proper storage conditions, the formulation remains viable for a period of 3 to 4 months.

Observation and Results

(Table 1) Growth percentage of *Trichoderma viride* on maize

Day	R1	R2	R3	Avg %
2nd	15%	10%	15%	8%
4th	25%	20%	30%	15%
6th	50%	55%	50%	31%
8th	80%	75%	80%	47%
10th	98%	90%	95%	56.6%

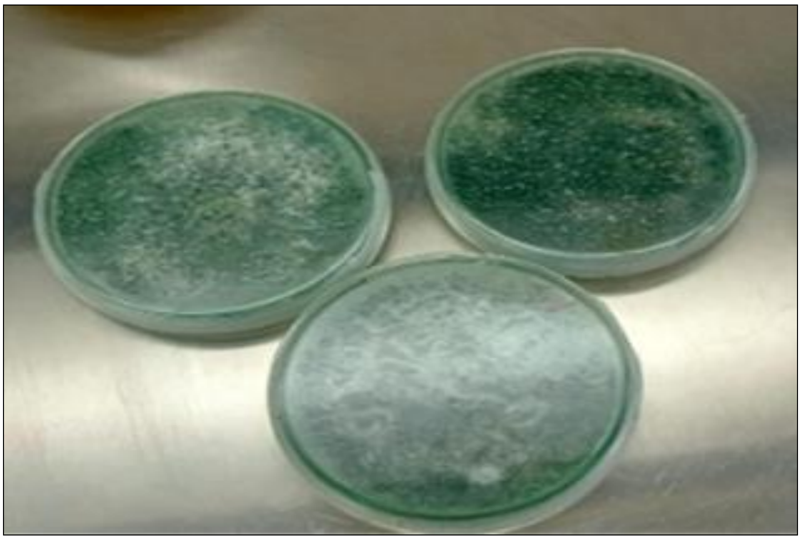

Fig 1: Growth of *Trichoderma viride* on PDA as shown above

Fig 2

Fig 3

Fig 4

Growth of *Trichoderma viride* on Maize as shown above images

Result

Mass production of *Trichoderma viride* was studied on maize. The data was collected on the 2^{nd}, 3^{rd}, 4^{th}, 6^{th}, 8^{th}, &10^{th} day respectively, then growth percentage were recorded.

Growth of *Trichoderma viride* on Maize

In above context, the table present the growth progression of *Trichoderma viride* on maize over a 10-day period across three experimental set (R1, R2, R3), with an average percentage growth.

- Initial growth is observed as early as the 2^{nd} day, with the average growth of 8%, (Table 1) indicating the early phase of *Trichoderma viride*
- The growth shows a slight increase compared to 2^{nd} day, with average growth of 15%, (Table 1) slow but steady due to the effect of temperature.
- On 6^{th} day we observed the growth with the average of 31%, (Table 1) because we maintained the standard temperature which enhanced the growth.
- The culture exhibits the rapid growth, with average of 47% (Table 1) on the 8^{th} day.
- The highest growth is observed at the 10^{th} day with values nearing 90-98% (Table 1) with the average of 56.6%.

Conclusion

The mass production of *Trichoderma viride* plays a crucial role in making biocontrol solutions more accessible for agricultural applications, offering an eco-friendly alternative to chemical pesticides. The data from this study demonstrate a gradual increase in the growth of *T. viride* over time, characterized by an initial lag phase followed by a period of rapid proliferation between the 6th and 10th days. Peak colonization was observed on the 10th day, indicating this as the maturity stage of *T. viride* development on maize.

When analyzed in terms of average percentage growth, the results further suggest that environmental conditions significantly influence the growth rate and overall development of *T. viride.* This underscores the importance of optimizing environmental parameters to enhance its mass production.This study confirms the efficacy of *Trichoderma viride* in colonizing maize, highlighting its potential as a reliable biocontrol agent. Moreover, it contributes to the broader fields of agriculture, biotechnology, and plant pathology by improving the practical application and field performance of *T. viride*. Overall, the findings add valuable insights to the existing body of knowledge on the mass production of *T. viride* and emphasize its potential for positive environmental impact.

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