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Beyond biocontrol agent: A review on the future of *Trichoderma*

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Abstract
Trichoderma is a well-known biocontrol agent that is gaining popularity in multiple fields. This fungus is found everywhere, but different species perform different functions. Despite having a wide range of uses in agriculture, its acceptance among farmers is limited, perhaps due to a lack of knowledge, awareness, and quality of the product. Therefore, it is essential to choose products from certified sources to ensure the quality and productivity of crops. With sustainable and eco-friendly agriculture being the main agenda of the world, *Trichoderma* alone or in combination with other bioagents will have a vast scope in agriculture.

Keywords: *Trichoderma*, biocontrol agent, eco-friendly and sustainable

Introduction
Trichoderma is a type of fungus that is commonly known as green spore fungi. It has a wide distribution and a high biotechnological value (Lorito, M. and Woo, S.L., 2014) [18]. This fungus was first named by Persoon in 1801, who referred to its conidial state (Manoharachary, C. and Nagaraju, D., 2020) [20]. However, *Trichoderma* gained popularity after Weindling discovered its pathogenicity properties on other fungi. For many years, the taxonomy of *Trichoderma* was complex, and it was thought to consist of only one species, *T. viride*, which is also known as green mold. But now it is clear that *Trichoderma* has many species, and its perfect stage mostly falls under Ascomycota, class Sordariomycetes, Order Hypocreales, and family Hypocreaceae.

Trichoderma possesses several properties that make it an excellent model for studying biocontrol. These properties include its ubiquity, ease of isolation and culture, rapid growth on various substrates, impact on a broad range of plant pathogens, and minimal pathogenicity on higher plants (Begum *et al*., 2010) [3].

Trichoderma is commonly used as a biocontrol agent, but recent research has shown that it can also be used as a growth promoter and yield enhancer in agriculture. Not only that, but it is also utilized in various industries such as Organic manure, Textile, Brewery, pharmaceuticals, food, toothpaste, and animal feed (Sood *et al*., 2020) [32]. This suggests that *Trichoderma* has a vast potential for future applications in multiple fields.

Some *Trichoderma* rhizosphere-competent strains have been shown to have direct effects on plants, increasing their growth potential, nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and stimulation of plant defenses against biotic and abiotic damage (Shoresh *et al*., 2010) [31]. Therefore, the selection of *Trichoderma* strains depends on the function for which it is to be used. The *Trichoderma* genus consists of many species having species-specific roles in various fields. Several *Trichoderma* species are currently used as biological control agents against phytopathogenic fungi, oomycetes, and nematodes (Monte, E and Hermosa, R. 2021) [22]. Some important familiar species and their description function is given below.

Trichoderma viride
This is a type of filamentous fungus that can colonize the root system of plants. It acts as an effective biocontrol agent against plant pathogens that can damage crops in agriculture and horticulture.

This fungus can produce several helpful enzymes such as chitinases, proteases, and secondary metabolites. These enzymes help inhibit the growth of pathogenic fungi and bacteria. The fungus is widely studied for its potential industrial and biotechnological applications. It is commonly used as a decomposer of lignin, cellulose, and protein. Market availability: Multiplex Nisarga

1. ***Trichoderma harzianum*:** It is also a filamentous fungus with basic properties similar *to T. viride* but this produces harzianic acid which has antifungal properties Market availability: Multiplex Safe root
2. ***Trichoderma hamatum*:** It is a fungus that feeds on dead organic matter and is commonly found in soil, humus, plant rhizosphere, and litter. (Lodi., *et al.*, 2023) [17].
3. ***Trichoderma virens:*** This soil-dwelling saprophyte is useful for controlling *Pythium ultimum* and *Rhizoctonia solani*, which cause damping-off disease in greenhouses.
4. ***Trichoderma asperellum:*** Available as Farm root Trichia

Benefits of using *Trichoderma*

- **Role in Plant Nutrition:** Nutrients are essential for plant productivity. The availability of nutrients to plants is influenced by microorganisms present in the soil and the rhizosphere, as noted by Mehetre and Mukherjee in 2015 [21]. *Trichoderma* was previously known only as a biocontrol agent, but it has now been found to play a significant role in plant growth and productivity. This is because *Trichoderma* dominates over minor pathogens in soil, resulting in stronger root growth, proliferation of secondary roots, better nutrient absorption, higher seedling fresh weight, and larger foliar area, all of which lead to higher crop production, as noted by Harman in 2006 [11] and Sharma *et al*. in 2011 [28]. Additionally, *Trichoderma* produces phytohormones like IAA, gibberellic acid (GA), and abscisic acid (ABA), as noted by Lei and Zhang in 2015 [15], which help regulate plant growth, as noted by Celar and Valic in 2005 [4].
- It is recommended to use *Trichoderma* in crop production of potassium-loving plants to produce high-quality yields. For example, in sugarcane, *T. harzianum* increases the uptake of potassium more than nitrogen and phosphorus, which results in improved quality and weight of the canes. In many crops, the application of *T. harzianum* has enhanced the uptake of nitrogen and phosphorus, resulting in increased seed production (Gupta and Baig., 2001). In paddy fields, *Trichoderma* increases the availability of phosphorus and zinc, which leads to increased yield. The application of *Trichoderma* also improves the nutrient use efficiency of nitrogen.
  In the alkaline soils of Punjab and Haryana, although iron is present in the soil, it is not always available to plants. In such cases, the application of *Trichoderma* can release fixed iron from the soil and make it available to the plant. This is because *Trichoderma* produces organic acids such as citric acid, gluconic acid, and oxalic acid, which are weak acids that can release fixed phosphorus from the soil to the plant. Moreover, these acids can lower the pH of the soil, which in turn makes micronutrients available to the plant .
- **Role in the growth and development of plant:** *Trichoderma*, when applied to plants, can enhance their morphology and physiological processes. It can increase root and shoot length, height, biomass, healthy leaves, and chlorophyll pigment content. Moreover, it can boost the number of fruits, tillers, and branches, leading to increased crop yield for commercial crops such as mustard, wheat, corn, tomato, sugarcane, and okra. *Trichoderma* is also beneficial for floriculture crops, as it can increase the number and weight of flowers. Studies have shown that *Trichoderma* application can have significant positive effects on plant growth and development (Sood *et al.*, 2020) [32].
- **Role in overcoming stress conditions:** *Trichoderma* is a type of fungus that has been found to improve the ability of field crops to tolerate salt. It also synthesizes various phytohormones, which are beneficial to plant growth (Sood *et al*., 2020) [32]. In addition, *Trichoderma* can help plants combat biotic and abiotic stresses by inducing systemic responses and promoting growth (Hidangmayum & Dwivedi, 2018) [13]. Research has also shown that *Trichoderma* enhances root growth and potassium availability, which improves the plant's ability to withstand abiotic stress during growth (Yildirim *et al*., 2020) [37].
- **Role as a biocontrol agent:** *Trichoderma* is effective in controlling soil-borne, airborne, and foliar pathogens, as well as post-harvest pathogens which cause severe diseases (Sharma *et al*., 2011) [28]. While chemical fungicides can be effective, they can also be expensive. *Trichoderma*, on the other hand, is an equally potent and more cost-effective bio-fungicide. It also enhances the plant's immune system, making it better equipped to defend against pathogens by hindering the development of pest resistance (Yedidia *et al*., 2000) [36].
  Trichoderma is resistant to most pesticides, making it a great alternative to fungicides like benomyl and captan for effective pathogen control. *Trichoderma* has also been proven to act as an entomopathogenic insecticide against various insects and mites, such as red mites, termites, sucking pests, flies, beetles, borers, and caterpillars, which are major crop pests. It can parasitize and kill these pests directly or alter their physiology by producing secondary metabolites, acting as an antifeedant. *Trichoderma* can also indirectly boost plant defensive mechanisms and encourage natural enemies (Poveda, J., 2021) [23]. Furthermore, some strains of *Trichoderma*, such as *T. harzianum* and *T. asperellum*, can act as bionematicides, adding to their usefulness in pest management (Al-Ani, L.K.T., 2018) [1].
- **Role as a Bio-remediator:** *Trichoderma* is a highly effective bioremediation agent for persistent pollutants such as DDT and endosulfan. It can also remediate the persistence of heavy metals like chromium and cadmium in water and soil (Sood *et al*., 2020) [32]. According to reports, *Trichoderma* is capable of degrading highly persistent chemical pesticides like carbendazim, chlorpyrifos, penthiopyrad, Dichlorvos, and DDVP (Alghuthaymi *et al*., 2022) [2]. Using biological control agents like phytopathogenic fungi, such as *Trichoderma*, can be an alternative management

strategy for producing safe food and reducing environmental pollution (Cubillos-Hinojosa *et al*., 2011) [6].

- **Role in Animal Nutrition:** According to Sood *et al*. (2020) [32], *Trichoderma* can be used in animal feed to increase body weight and milk yield.

Mode of action

Trichoderma is a type of fungus that uses various methods to control other plant parasitic pathogens. It produces a chitinase enzyme to attack the cell walls of the pathogens and penetrate them. Additionally, it produces antibiotics to control them chemically. One of the methods employed by *Trichoderma* is Mycoparasitism, wherein it physically parasitizes other fungi. The fungus protects itself by producing chitinase. The mechanism involved in this process is shown in the figure.1 and 2.

Trichoderma has a unique ability to derive energy from complex compounds such as chitin from fungi or cellulose from plants which other organisms find difficult to break down. It produces siderophores to acquire iron more competitively than other microbes in the soil, thereby inhibiting the growth of other microbes. *Trichoderma* can create a good microenvironment and enhance the immunity of plants. It can colonize the root system of a wide range of plants, and this process involves steps such as recognition, adhesion, and colonization.

Trichoderma uses hydrophobins for adhesion and swollen for colonizations, respectively (Ruocco, *et al*., 2015). The colonization of the *Trichoderma* is the basic characteristic of its multifarious beneficial functions as far as agriculture is concerned.

Mode of application

The efficacy of a product is greatly influenced by its commercial formulation and proper application methods. If the correct formulations are not applied consistently, the efficient strain may fail to display its full potential (Puyam, A., 2016) [24]. *Trichoderma* can be applied in various ways, such as seed treatment, seed biopriming, seedling dip, foliar, and soil applications when referring to application methods.

1. **Seed treatment:** Seed treatment of talc-based *Trichoderma* @ 4g/kg is generally recommended. This *Trichoderma* germinates on the seed surface and colonizes in the rhizosphere as and when the plant grows thereby enhancing the seed germination by 82.7% in maize and reducing the crop requirement of nitrogen to the extent of 30-50% (Harmon 2011: Shoresh *et al*., 2010) [31]. It is also effective in the management of seed/soil-borne diseases (Tewari, 1996) [33] because it terminates the infection if occurred at early stages when applied as seed treatment.
   Available products: Multiplex Nisarga, Multiplex safe root, IFCO Tricho powder.
2. **Seed biopriming:** Biopriming is the process of treating seeds with biocontrol agents and then incubating them under warm and moist conditions until just before the emergence of radicals. This method requires less quantity of biocontrol agents compared to seed coating, and it helps to tolerate adverse soil conditions and promote uniform germination. Nowadays, the use of plant-beneficial microorganisms as biofertilizers and bioprotectants is becoming increasingly popular for achieving high crop production with low ecological impact.
   Available products: Multiplex Nisarga, Multiplex Saferoot, Farmroot *Trichoderma*
3. **Seedling root dip:** The process involves treating cuttings or seedlings with a *Trichoderma* suspension. This method is typically used in transplanted paddy and vegetable cultivation. It has been found to enhance plant vigor and increase chlorophyll content, which in turn reduces the shock of transplantation. There are several *Trichoderma*-based products available for use, such as Multiplex Nisarga, Multiplex Saferoot, and Farmroot *Trichoderma*.
4. **Soil treatment:** For nurseries and greenhouses, *Trichoderma* can be used to treat soil. Directly applying *Trichoderma* to the soil can help inhibit the growth of other harmful pathogens (Kumar *et al*., 2014) [10]. Tea growers often face difficulties with raising their tea nurseries due to soil containing aluminum and iron complexes, which can hinder root establishment. However, after applying *Trichoderma* as a drench in tea nurseries, root development can be enhanced, and root biomass can increase. When transplanting vegetable seedlings, *Trichoderma* can be applied directly to the furrow. It can also be used as a surface soil application for turf. For greenhouse and nursery planting, *Trichoderma* can be mixed with potting media. When transplanting trees or shrubs, *Trichoderma* can be directly applied into the planting hole. Available products: Multiplex Annapurna, Multiplex Nisarga, Multiplex safe root
5. **As a foliar spray:** *Trichoderma* can also be mixed with water and sprayed as a foliar spray. Available products: Multiplex Nisarga
6. **Post-pruning application:** As a post-pruning application *Trichoderma* in tea plantation. Available products: Multiplex Nisarga

Combination with other bioagents

Integrated disease management involves the use of various biocontrol agents such as *Pseudomonas, Trichoderma*, and *Bacillus* sps together. When used in combination, these agents have additive and synergistic effects which are more effective than when used individually. However, the commercialization of these agents is currently hindered by the fact that the CIBRC does not allow their combination.

A mixture of *Trichoderma, Bacillus,* and *Pseudomonas* has been developed into microbial fertilizers that have a broad-spectrum antagonism against pathogens. This product is used to counteract the decline in soil physical and critical properties that result from excessive use of chemical fertilizers. Studies suggest that a combination of Rhizobacteria, *Pseudomonas fluorescens*, and *T. harzianum* is more effective in suppressing diseases, and it also improves the percentage of seed germination (Yadav *et al*., 2013) [35].

Another combination of *T. harzianum, P. fluorescence,* and *B. megaterium* has been found to manage mahali disease/fruit rot of areca, caused by *Phytophthora meadii*. This mixture also contributes to the development of new roots and leaves and enhances the yield of the crop.

Soil solarization combined with *T. harzianum*, Bavistin, and neem has been found to reduce wilt by up to 86%, according to Chakraborty *et al*. (2009) [5]. A mixed combination of

Trichoderma harzianum and *Pseudomonas fluorescence* formulation is more effective than individual formulations, both under greenhouse and field conditions. *Trichoderma*, along with *Azotobacter*, enhances root colonization, growth attributes, and the availability of macronutrients and micronutrients by 10-40%, as per Velmourougane *et al*. (2019) [34]. Lentil seeds treated with *Trichoderma harzianum* and *Rhizobium* inhibit foot and root disease pathogens, including *R. solani, S. rolfsi*, and *F. oxysporum*, while also increasing germination and yield attributes (Khatun *et al*., 2020) [9, 14].

Studies have shown that certain combinations of microorganisms can improve crop yield and health. For example, *Pseudomonas fluorescens* and *Trichoderma harzianum* have been found to reduce root-knot nematode infections in papaya (Rao, M.S., 2007) [25]. A combination of *Trichoderma harzianum* and Azospirillum brasiliense has been shown to enhance yield and reduce the need for nitrogen-phosphorus fertilizers in wheat and corn production (El-Katatny, M.H. and Idres, M.M., 2014) [8]. Additionally, co-inoculation of vesicular-arbuscular mycorrhizae (VAM) and *Trichoderma* has been found to promote growth and nutrient uptake in vegetable crops (Lucini, *et al*., 2019) [19]. Another study found that a combination of *Trichoderma harzianum* and *Pseudomonas fluorescens* resulted in the highest nitrogen uptake in vanilla (Sandeep *et al*., 2013) [30].

Demerits

- *Trichoderma* is not as effective when used to control diseases that have already developed.
- *Trichoderma* has a slower mode of action compared to chemicals, which results in a less noticeable effect.
- The effectiveness of *Trichoderma* depends on the surrounding environment.
- *Trichoderma* is not typically harmful to humans but can be harmful to mushroom cultivation causing green mold diseases. The responsible species include T. *aggressivum, T. pleurotum, T. pleuroticola* (Samuels *et al*., 2002) [29].
- *T. viride* and *T.harzianum* produce antibiotics like Trichodermin/ harzianum A which act as antibiotics for humans (Degenkolb *et al*., 2008) [7]. It also acts as an antibiotic Trichothecene.
- Gliotoxin produced by *T. virens* is known as immunosuppressive in humans
- *T. longibrachiatum* is a type of toxic mold that produces trilongines, a harmful compound that affects neurons, the heart, and anemocytes.

Precautions while using *Trichoderma*

When handling large quantities of *Trichoderma*, it is advised to wear a dust/mist filtering respirator and protective eyewear for safety figure.3.

Commercialization of *Trichoderma*

Trichoderma can be multiplied through two methods: solid-state fermentation or submerged fermentation. The fungus grows on either solid or liquid substrates, respectively, under specific conditions of temperature, pH, and aeration. The process typically starts by inoculating a starter culture into the chosen medium, allowing it to grow, and then transferring it to a fresh medium to continue the multiplication process.

Multiplication of *Trichoderma* species on liquid fermenter

Trichoderma species can be multiplied by cultivating them in a suitable growth medium under controlled conditions. The process involves inoculating the medium with a starter culture of *Trichoderma* and providing optimal conditions such as temperature, oxygen, pH, and nutrients for growth. The fungus is then allowed to proliferate for a certain incubation period. After this, the biomass can be harvested and formulated for use in various applications.

There are three methods for the multiplication of *Trichoderma* species:

a) Small-scale production on a shaker (Figure 4.)
b) medium scale production on growth bags
c) Commercial scale production using a bioreactor or fermenter.

Submerged fermentation

Trichoderma can be grown in bioreactors or fermenters by inoculating it into a nutrient-rich liquid medium (Figure 5.). With proper control of temperature, pH, oxygenation, and agitation, it multiplies in the liquid.

Merits & Demerits of Liquid Fermentation

Merits

- Easy to scale up.
- Lower production cost
- less time per batch
- Commercially viable due to lower investment
- lower batch variation

Demerits

- Chance of contamination is more.
- lower yield when compared to Solid state fermentation.
- Low stability of CFU (Colony forming units)

II. Solid state fermentation of *Trichoderma*

- On small flask- Small-scale production (Figure 6)
- On Growth Bags - Medium Scale Production (Figure 7)
- Solid State Fermenter (SSF) – Commercial Scale Production

Solid-state fermentation (SSF) is a cultivation method where fungus is grown on a solid substrate. For *Trichoderma*, this method typically involves using solid materials such as grains, wheat bran, etc. In SSF, these solid substrates are moistened to a specific water content and are supplemented with additional nutrients. After that, they are inoculated with *Trichoderma* spores or mycelium. The conditions are optimized for growth, including factors such as temperature, humidity, aeration, and sometimes pH. *Trichoderma* grows and produces enzymes and secondary metabolites, which can be harvested after a designated fermentation period.

Merits

- The method is favoured due to its cost-effectiveness.
- high product yields
- feasible for different kinds of formulation
- potential for using waste materials as substrates.

Demerits

- Isolation and selection of high-yielding strains

- Identifying and using strains of *Trichoderma* that exhibit faster growth and
- higher yields can contribute to mass multiplication efforts.
- Maintaining sterile conditions, monitoring growth parameters, and regular checks for contamination are crucial during the mass multiplication process.

Fig 1: The production of secondary Metabolites of *Trichoderma* suppressing *Fusarium* growth.

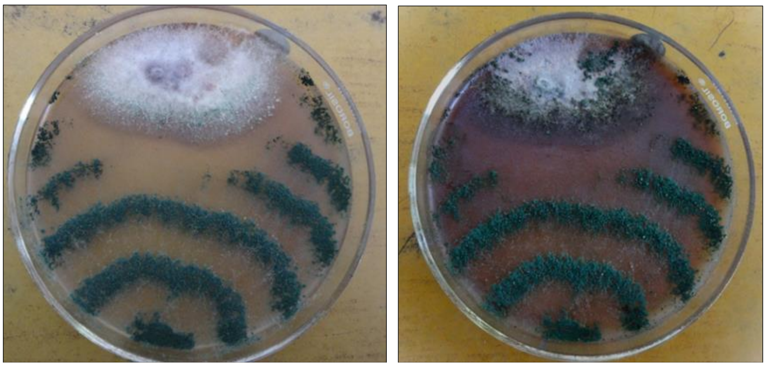

Fig 2: Antagonism effect of *Trichoderma* against the *Fusarium* fungus

Fig 3: Microbial culture work performed under the biological safety cabinet and with specialized personal protective equipment (PPE).

Fig 4: *Trichoderma* seed culture under continues Shaker

Fig 5: Commercial scale Mass Multiplication of *Trichoderma* in liquid state fermenter (Fermenter Capacity – 200 Lt to 5000 Lt)

Fig 6: Small scale Mass cultivation of *Trichoderma* in conical flask

Fig 7: Typical medium to large solid-state fermentation (SSF) cultivation of *Trichoderma* under total control system

Conclusion

Trichoderma is a type of fungus that is commonly found in soil. Unlike other fungi, *Trichoderma* doesn't harm beneficial microbes and the balance of the soil. Instead, it targets harmful microbes and acts as a safe, eco-friendly, and effective biocontrol agent for most crops. However, Indian farmers have been relying on synthetic pesticides for many years and are not familiar with the benefits of using *Trichoderma* due to a lack of knowledge and awareness. Therefore, effective promotion and education campaigns are necessary to encourage farmers to choose biocontrol agents over chemical fungicides. It's worth noting that many start-up companies fail due to a lack of marketing support.

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