

Review Article

Potential and Prospects of *Trichoderma* in Plant Protection

Ritesh Kumar ¹, Pratik Samanta,¹ Susanth Vijay Raj,¹ Pratush Bera,¹ and Mohammed Naimuddin ²

¹Department of Plant Pathology, M. S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Bhubaneswar, Odisha, India

²Department of Applied Biology, School of Applied Natural Sciences, Adama Science and Technology University, Adama, Ethiopia

Correspondence should be addressed to Mohammed Naimuddin; mnaimuddin@gmail.com

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In order to feed the growing population, agriculture is a vital component of any country; however, pests pose a constant threat to it. Chemical pesticides are employed to safeguard the crops from the enormous yield loss. These chemical pesticides are boundless in killing crop pests; however, they have detrimental effects on the health of both humans and the environment. Therefore, biological control techniques are being utilised after identifying an environmentally suitable substitute. Due to its well-known biological control mechanism, *Trichoderma* spp. have been utilised extensively in agricultural applications. The host plant's soil and rhizosphere serve as shelter place for *Trichoderma*. It has the ability to create a variety of secondary metabolites and secretion of important enzymes. Clarification of *Trichoderma*'s importance in the prevention and treatment of plant diseases is thus important in order to advance sustainable agriculture. The mechanisms, which include mycoparasitism, antibiosis and competition aid in the management of insect pests and plant pathogens in the soil, seeds, roots, stems, leaves, etc. It is crucial to create new formulations of biocontrol microorganisms with a greater level of stability and survivability in order to implement biocontrol technology in the field and enhance its commercialisation.

1. Introduction

By 2050, it is anticipated that there will be 9.1 billion people on the planet earth, consequently, an increase in agricultural food output of around 70% is required to sustain this growing global population [1]. The problems like global warming and environmental degradation led to plants experiencing a variety of biotic and abiotic stress, that are responsible for yield loss of the plants. Additionally, the plant pathogens are forced to undergo genetic alterations as a result of the indiscriminate use of fungicides, which ultimately lead to the selection of fungicide-resistant biotypes. Agronomists and business sectors have recently demonstrated a strong interest in the creation of eco-friendly and economical techniques for the management of plant diseases [2].

Recent strategy that uses naturally occurring plant growth-promoting fungi rather than pesticides in order to achieve the good soil and plant health is an excellent illustration. There have been numerous reports on the use of environmentally

benign biocontrol agents to control plant diseases, insect pests, bioremediation and enhance plant growth. In addition to the mycorrhizal fungi, endophytic fungi, endophytic bacteria, plant growth-promoting rhizobacteria, plant growth-promoting fungi, and other organisms have been reported for the enhancement of plant growth under various stress conditions [3].

One of the many biocontrol agents used to manage biotic and abiotic stress in plants are *Trichoderma* species, which predominate in the soil microflora [4]. Due to their significant antagonistic action against a variety of phytopathogens, they can biologically control a variety of plant pathogens that cause diseases, including a range of soil and air-borne plant diseases [3]. The ability to act as a biocontrol agent against other microbes, specially plant pathogenic fungi is a result of the production of antibiotics and mycoparasitism that affects the growth of other microbes [5]. In addition to producing antibiotics, enzymes, volatile and non-volatile chemicals, *Trichoderma* also induces systemic resistance in plants [5]. *Trichoderma harzianum* solubilises several soil nutrients that

TABLE 1: The classification taxa of *Trichoderma* [11].

| | |
|-------------|--------------------|
| Kingdom | Fungi |
| Division | Ascomycota |
| Subdivision | Pezizomycotina |
| Class | Sordariomycetes |
| Order | Hypocreales |
| Family | Hypocreaceae |
| Genus | <i>Trichoderma</i> |

are present in an inaccessible form and transforms them into available forms for the plants, which increases the efficiency of CO₂ and O₂ utilisation in plants [6]. Also, it is particularly helpful for the potential of *Trichoderma* to promote plant development and produce resistance through controlling the expression of genes in plants, in addition to having a direct impact on fungal plant diseases [7]. Maximizing the potential of *Trichoderma* as bioinoculants for agriculture, requires proper identification of the native strains that are most effective there, which is a prerequisite for the development of a bioformulation product for agricultural use.

The versatility and growth of *Trichoderma* is suitable on a variety of substrates like grains, millets, husk, etc. under in vitro condition. Naturally, presence of these organisms has been reported in a wide range of soil types, including agricultural soils, forest soils, salt marsh soils and desert soils and also in various climatic zones [8]. On rotting wood and in soil, due to their heterotrophic interactions, including parasitism, decomposition and even opportunistic endophytism, *Trichoderma* species are known to be widespread. It has been shown that *Trichoderma* grows on plants as epiphytes, endophytes and even in the rhizosphere.

2. Systemic Classification of *Trichoderma*

The earliest description of *Trichoderma* was published in 1794 [9] and a relation to the sexual stage of a *Hypocrea*, the species was proposed in 1865 by Tulasne brothers [10]. The taxonomy of *Trichoderma* and *Hypocrea* has been well reviewed thorough examination of various life stages (Table 1). However, morphologically it was challenging to differentiate among the numerous species of *Trichoderma*. In fact, it has been suggested that just *Trichoderma viride* should be considered as a single species; an identification concept was not developed until 1969 [12, 13]. Harz first identified the species *Trichoderma harzianum* in 1871 after the study of its microscopic features, particularly the presence of phialides [14].

Internal transcribed spacer (ITS) region, restriction fragment length polymorphisms and random amplified polymorphic DNA sequence analysis are among the strategies to identify microbes using DNA markers. This has piqued the interest of *Trichoderma* workers to incorporate sequence data to the developing taxonomy of *Trichoderma* and *Hypocrea*. Several new *Trichoderma* that belongs to *Hypocrea* species were discovered, bringing an increase in number of phylogenetically recognised species [15]. *T. harzianum* was misidentified in some situations, mostly in early reports [16]. TrichoKEY, an oligonucleotide barcode, along with a

custom search tool and TrichoBLAST, developed by International Subcommittee on *Trichoderma* and *Hypocrea* have greatly helped in the identification of novel species [17, 18]. However, there are still a large number of *Trichoderma* strains for which sequences have not yet been identified [19]. Over 20 of the estimated 40 described taxa of *Trichoderma* have been found to have some agricultural applications [11].

3. Morphological Features of *Trichodermas* spp.

Trichoderma is a genus under fungi, which is mostly found in soil and root environments and are free-living, anamorphic, filamentous and mostly asexually reproducing [4, 8, 20]. In temperate and tropical soils, *Trichoderma* species form ascomycetes with green spores with hyaline and smooth septate vegetative hyphae (Figure 1(b)). The conidia are single celled and oval or globose in shape. Also, *Trichoderma* produce branched conidiophores on septate, hyaline and smooth-walled vegetative hyphae. The primary branches' lateral side branches may or may not be paired, and they occasionally may rebranch. In most cases, the branches develop at an angle of 90°. Phialides, also referred to as conidiogenous cells, can be cylindrical or almost subglobose in shape [20]. The mycelial colour is generally green, but can sometimes be colourless, grey or brownish as seen in Figure 1(a) [13, 21]. On Potato Dextrose Agar (PDA) and Cornmeal Dextrose Agar substrates, the colonies are white and transparent, respectively and when conidia are produced, sporadic blue-green or yellow-green patches can be seen. One or a few phialides normally emerge straight off from the tip of the typical conidiophore (Figure 1(b)). *Trichoderma* spores, which are propagative structures, can germinate and grow on the surfaces of plant roots when they are introduced to soil and even infect the outermost root cells [20]. For *Trichoderma*, characters including growth rate, colony colour and colony appearance are considered as taxonomically useful traits. Pale or yellowish colour of reverse of colonies, rapid growth at 25–30°C of majority of *Trichoderma* isolates and typically not growing at 35°C [22]. By growing at 35°C, *T. harzianum* can be differentiated from species like *T. aggressivum* and *T. atroviride* that share a similar morphology. While *T. harzianum* grows well and sporulates at 35°C, neither *T. aggressivum* nor *T. atroviride* can have colonies greater than 5 mm after 96 hr [22]. Additionally, some *Trichoderma* species, like *T. viride*, emit a distinctive sweet scent similar to 'coconut' odour [20]. Within 30 samples, morphological analysis identified two different conidiophore and phialide arrangement types. The first set of 24 isolates had conidiophores that were spread out at the top and wide near the base that were smooth or rounded. Phialides mostly appeared in densely populated areas, but they also had whorls of two to six on the terminal stems and an angle with conidiophores. The penicillate type second group of six isolates was distinguished by predominate conidiation, numerous divided ranches, collecting all finger to top and fertile to the apex [23]. Chlamydospores serve a role in survival and are vegetative cells with thickened walls and compacted cytoplasm [24]. These colourless, pale yellowish, or greenish, globose to subglobose structure are produced either within hyphae or at the hyphal tips [20].

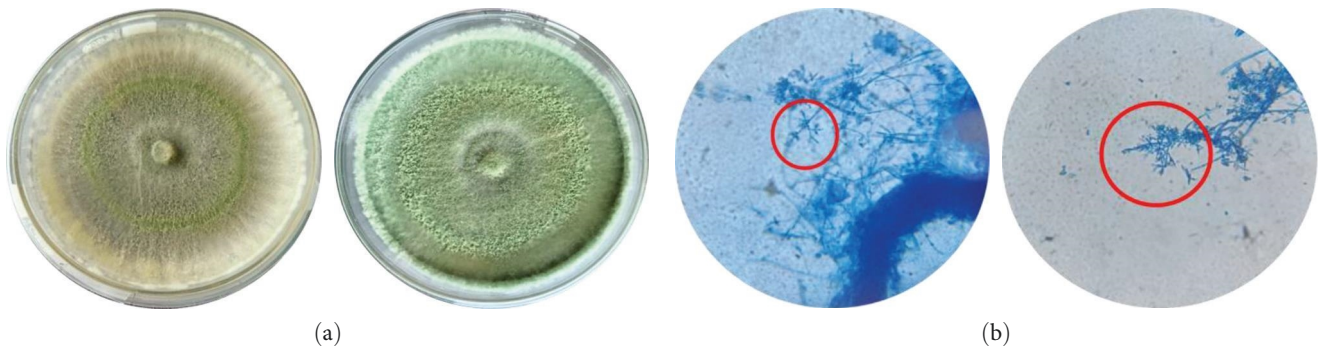


FIGURE 1: Mycelial (a) and microscopic (b) images of *Trichoderma* spp. (Structure in the red circle are denoting the Phialides of respective *Trichoderma*).

4. Identification of *Trichoderma* spp. Based on Morphology and Molecular Markers

Trichoderma isolated from soil samples, and incubated for 7 days at 28°C on PDA media shows green colony pigmentation as seen in Figure 1. Traditional methods have previously been used to identify *Trichoderma* spp. by its morphology like linear growth, colony colour, growth pattern, hyphal pigmentation and cultural structures include the arrangement of conidiophores, phialides and shape of conidia (Figure 1) [13, 23]. The rate of growth and microscopic characteristics of isolated *Trichoderma*, such as the sizes and shapes of conidia, the branching patterns of conidiophores and the sizes and shapes of phialides, are also crucial physical factors in identifying the various *Trichoderma* species [25]. Identification of *Trichoderma* species can also be benefited from observations of spore and phialide sizes, but most of the isolates cannot be identified using just the accounts of the conidial shapes as due to genetic, environmental and nutritional changes, the fungus has displayed varied morphologies on culturable media that makes the identification challenging. Molecular identification by using fungal ITS sequencing is a widespread method, which confers a reliable identification. Molecular identification utilising ITS region and their respective morphological characteristics of different *Trichoderma* species are summarised in the tabular format (Table 2). Additionally, the methods for identifying *Trichoderma* with DNA markers and the use of DNA sequence analysis became new ideas in the field of fungal systematics. Numerous researchers suggested using more variable phylogenetic markers, such as *tefl_int4*, *tefl_int5* and *rpb2*, that encode RNA polymerase subunit 2 and translation elongation factor 1-alpha (EF-1) [12, 13]. A significant amount of novel traits in terms of divergent base pairs are accumulated by DNA sequencing data and their incorporation into gene banks, contributing to cladistics analyses of most recent shared ancestry.

5. Effects of Temperature, Water and pH on Growth of *Trichoderma*

Trichoderma species can be grown on a wide variation in optimal temperatures [27]. Most *Trichoderma* strains are mesophilic [28], which means that they cannot protect germinating seeds from soil-borne plant diseases caused by

cold-tolerant plant pathogenic fungus. *T. aureoviride* and *T. viride* grow well at 5°C [29], while *T. harzianum* are adapted to warm climate [30]. The antagonistic potential of cold-tolerant *Trichoderma* strains was tested at different temperatures, and it was discovered that temperature had no effect on hyphal contacts [31]. When compared to *T. harzianum* strains, *T. aureoviride* and *T. viride* strains are more efficient in vitro antagonists as well as better cold tolerants. Extracellular β -1,4-N-acetyl-glucosaminidase (NAGase), trypsin and chymotrypsin-like proteases, β -glucosidase and other enzymes involved in the mycoparasitic process were produced at 10°C and found to be quite active at 5°C in the cold-tolerant strains [32]. The poor osmotolerance level of *Trichoderma* strains is one of the most significant drawbacks of using them as a biocontrol agent in field condition. Soil water conditions are limiting elements that affect fungal activity. As a result of natural drying between two rainfalls, dry conditions can develop even in soils that are usually moist. In dry soils, however, biocontrol chemicals may be required to combat plant pathogens. Water conditions have been shown to have a significant impact on *Trichoderma* activities, including spore germination and germ tube growth, mycelial growth [33, 34], saprophytic ability [35], interaction with other fungi and enzyme production [36]. Information on the effects of water conditions on the metabolic activities of *Trichoderma* strains is critical for biocontrol strategy development. At both 25 and 10°C, there is a linear relationship between water potential and colony development rate, with larger growth rates at higher temperature and water potential. Cellobiohydrolase and NAGase enzymes showed optimal secretion at higher water potential. Aside from those that are best for growth, β -glucosidase, β -xylosidase and chymotrypsin-like protease enzymes show the greatest activity at lower water potential values [36, 37]. A certain pH is required for the use of biocontrol *Trichoderma* strains in agricultural soils. Because of this, it is necessary to gather data regarding the effect of pH on mycelium growth and in vitro activities of extracellular enzymes that participate in nutrition competition and mycoparasitism in *Trichoderma* strains with respect to its biocontrol potentiality. *Trichoderma* strains can grow in a pH range of 2.0–6.0, with a preference towards pH 4.0. At this pH, there

TABLE 2: Molecular identification and morphological characteristics of different *Trichoderma* species [26].

| S. No. | <i>Trichoderma</i> | Locus | Primer pair used | Amplicon | Morphological and cultural characteristics |
|--------|---------------------------|----------|---|------------------|--|
| 1 | <i>T. harzianum</i> | KC800922 | ITS1-AGAGTTTGGATCCTGGCTCAG ITS4-GGTTACCCTGTTACGACTT | 546 base pairs | The colour of mycelium varies from watery white to light green. The petri dish's reverse side displays areas of uncoloured rings. Conidiophores grow in loose tufts and are heavily branched. Phialides have a short, skittle-like form with a centre bulge and a base that is narrower |
| 2 | <i>T. asperellum</i> | KC800921 | ITS1-TCCGTAGGTGAAACCTGCGG ITS2-TCCTCCGCTTATTGATATGC | 1,200 base pairs | Mycelium is smooth and hairy with a cotton pattern that is often composed of 1-2 ringed concentrics in a yellowish green colour. Conidiophores have a compact morphology and are heavily branched. Phialides have a nine-pin appearance |
| 3 | <i>T. viride</i> | KC800920 | ITS1-TCCGTAGGTGAAACCTGCGG ITS4-TCCTCCGCTTATTGATATGC | 641 base pairs | The mycelium changes from green to a dark yellowish green colour after 2-3 days. Green conidia are spread out all throughout, with conidia production being lower in the centre than at the borders. Phialides are long, thin, horn-shaped, and bulging in the centre |
| 4 | <i>T. atroviride</i> | KC008065 | ITS1-TCCTCCGCTTATTGATATGC ITS2-GGAAGTAAAAGTCGTAAACAAGG | 627 base pairs | The mycelium mat looks watery white and appears translucent and smooth. Conidiophores are highly branched and arise in compact form. Phialides appear in ampulliform and oblong shaped, curved, and narrow at the base |
| 5 | <i>T. longibrachiatum</i> | JX978542 | ITS1-TCCTCCGCTTATTGATATGC ITS2-GGAAGTAAAAGTCGTAAACAAGG | 664 base pairs | Mycelium are mostly submerged translucent or watery white. Conidiophores are smooth, form irregular tufts and arise from substratum or from aerial hyphae. Phialides can appear individually or in verticils of two to three. They often have lageniform shapes and a narrow base |
| 6 | <i>T. koningii</i> | KC800924 | ITS1-TCCTAGGTGAAACCTGCGG ITS4-GGAAGTAAAAGTCGTAAACAAGG | 206 base pairs | The colour of mycelium is creamy white and shifts from white to terreverte. Colonies resemble glaucous membranes and are crusty and dense. Chlamydospores can develop terminally or intercalarily. At the base, phialides are thin. They seem like a nine-pin bowling ball when they emerge singly and laterally |
| 7 | <i>T. virens</i> | KC800923 | ITS1-TCCTCCGCTTATTGATATGC ITS4-GGAAGTAAAAGTCGTAAACAAGG | 635 base pairs | Mycelium does not have a distinctive smell and goes from being watery white to a lovely shade of green with granules that are dull blackish green. Conidiophores branch irregularly towards the tip, with each branch ending in a group of three to six tightly packed phialides. Phialides are lageniform to ampulliform in shape, with a bulging midsection and attenuated tip |

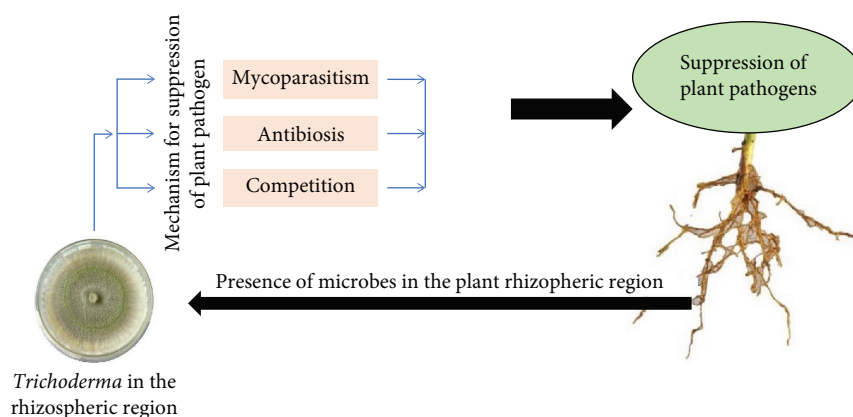


FIGURE 2: Mechanisms of *Trichoderma* spp. for suppression of growth and viability of plant pathogens.

is a regulation of the synthesis of extracellular enzyme β -1,6-glucanase in *T. harzianum* [38], also it has been seen that the optimal pH range for biomass generation in *Trichoderma* is between 4.6 and 6.8 [39].

6. Microbial Compatibility between *Trichoderma*, Different Bacteria and Plant Pathogenic Fungi

It has been discovered that numerous soil bacteria, such as fluorescent Pseudomonads, may have an impact on *Trichoderma* development in agricultural soils (primarily suppressive soil) [40]. There are numerous accounts on the enzymological underpinnings of the mycoparasitic activities of *Trichoderma* strains, but very few studies on bacteria that adversely affect *Trichoderma*'s biocontrol abilities. *Trichoderma*'s competitive success might be attributed to its sensitivity to the soil bacteria's inhibitory impact. Under inductive and noninductive conditions, the activities of NAGase, trypsin-like and chymotrypsin-like proteases were examined in the instance of five strains that demonstrated exceptional degrading abilities toward *Bacillus subtilis* [40]. All strains generated modest amounts of NAGase and proteases, which may be increased through induction using *B. subtilis* cells. NAGase and proteases were three to six times more abundant in inductive medium. The disintegration of bacterial cells appears to be significantly influenced by proteases, NAGases and muramidases. Testing *Trichoderma* strains' capacity to destroy bacteria might be helpful in addition to determining how well they can fight plant pathogenic fungus [41]. This characteristic may help the strains become the prevalent microorganisms in the environments where they are used. *Trichoderma*'s ability to parasitise other plant pathogenic fungi prompted the first research on biocontrol with *Trichoderma* [5]. However, it required another 40 years to prove that using *Trichoderma* in field might stop the spread of fungus-related plant diseases [42]. Numerous articles have since highlighted mycoparasitism as a crucial biological control mechanism in fungal diseases. Antagonistic tactics which include mycoparasitism, competition for nutrients and resources and antibiosis (Figure 2) are used by *Trichoderma* spp. to combat plant diseases. *Trichoderma*'s effectiveness

TABLE 3: List of genes involved in mycoparasitism.

| S. No. | Gene | Protein | Reference |
|--------|--------|-----------------------------|-----------|
| 1 | prb1 | Basic proteinase | [46] |
| 2 | ech42 | Endochitinase | [47] |
| 3 | chit33 | Endochitinase 33 | [48] |
| 4 | cre1 | Carbon catabolite repressor | [49] |
| 5 | egl1 | β -1,4-Endoglucanase | [50] |

against plant pathogenic fungus is due to its essential antagonistic property of mycoparasitism [43]. Cell wall degrading enzymes (CWDEs) and antimicrobial secondary metabolites make up the intricate process of mycoparasitism [44]. Endochitinase, β -1, 3 glucanase and chitobiosidase are the chitinolytic enzymes produced by *T. harzianum*, and are efficient against plant pathogenic fungi. Chitobiosidase and endochitinases can suppress the plant pathogenic fungal spore germination and hyphal lysis, while endochitinase and β -1-3 glucanase have been demonstrated for antifungal activity via lysis of spore cell walls, thallus and hyphal tips [45]. Various genes have been identified for mycoparasitism in biocontrol activity (Table 3).

Competition for resources and space, or ability to prevent spore germination, killing of cells (antibiosis), or altering the rhizosphere, such as by making the soil acidic, help *Trichoderma* spp. in preventing the growth of phytopathogenic fungi. As most plant pathogenic fungi require iron uptake for viability [51], competition for this (iron) limited nutrients results in the biological control of fungal phytopathogens. When iron is under scarce amount, the fungi release siderophores (low-molecular-weight ferric iron-specific chelators), to mobilise environmental iron [52]. Highly effective siderophores produced by *Trichoderma* chelate the iron and prevent the growth of fungi [53]. Interactions with low-molecular-weight diffusible substances or antibiotics produced by *Trichoderma* strains that prevent the growth of other plant pathogenic microbes result in antibiosis. Also, the production of 6-penthyll—pyrone, alamethicins, gliovirin, glisoprenins, harzianic acid, heptelidic acid, massoiltactone, peptaibols, tricholin and viridin are some of the volatile

and non-volatile metabolites secreted by most of the *Trichoderma* [54].

7. Plant Disease Suppression by *Trichoderma*

Mycoparasitism is the act of attacking another pathogenic fungus directly, in order to use it as a food source. The four phases of process are detecting prey, growing positively toward it, chemical and physical attacks, which usually destroy the hyphae of the prey and eventually consuming nutrients [13]. Combining mycoparasitism, competitive exclusion and antibiosis has an impact on *Trichoderma*-mediated disease suppression [55]. The physical attack during the contact phase results in morphological changes, including hyphal coiling, intense branching or the formation of appressorium-like structures, whereas the chemical attack occurs before contact, when enzymes that breakdown cell walls are produced and released in mass quantities. When the fungi used for biocontrol is administered to roots or seeds, *Trichoderma* spp. quickly colonise. Infectious pathogens are avoided by the rhizosphere (root zone) and the spermosphere (seed zone). In direct antibiosis, *Trichoderma*'s secondary metabolites or released enzymes prevent the germination or growth of pathogens [56]. The metabolites may also influence mycoparasitism and competition. Because mycoparasitism is difficult to study *in vivo*, there are fewer direct study of mycoparasitism's effects on plant diseases. The first detailed study of the mycoparasitic behaviour of *T. virens* (then known as *T. lignorum*) on *Rhizoctonia solani* showed that there is a coiling of hyphae, growth in wavy or straight lines, protoplast coagulation and loss of vacuolated structures [5]. Gliotoxin was ultimately discovered to be the cause of this *Trichoderma* strain's antagonistic activity and pot tests revealed that this strain effectively suppressed the *R. solani* strain [5].

8. Mycoparasitism and Cell Wall-Degrading Enzymes

CWDEs released by phytopathogenic fungi have been shown to play an important role when the cell wall of a plant cell is invaded by a pathogenic fungus. When the primary structural polysaccharide components of plant cell walls, such as cellulose, hemicellulose and pectin are broken down by fungal CWDEs (Figure 3), pathogenic fungus have the opportunity to utilise small polysaccharide molecules as a source of nutrients. Additionally, CWDEs from fungi have been identified as important sources of Microbe-Associated Molecular Pattern that trigger primary immune responses in plant cells, or fungal cell wall [57]. Also, induced systemic resistance is a type of active resistance that also relies on these types of structural and biochemical changes in plants. Various *Trichoderma* strains may be suggestive for the emergence of plant-induced systemic resistance [57]. In case of attack by the plant pathogens, sequential events, including host recognition, attack and subsequent penetration as well as killing of the host occur during mycoparasitism. *Trichoderma* spp. have the ability to recognise other fungus, migrate there and directly parasitise them. Plant pathogenic fungi such as *Botrytis cinerea* and some *Fusarium* spp. can be parasitised

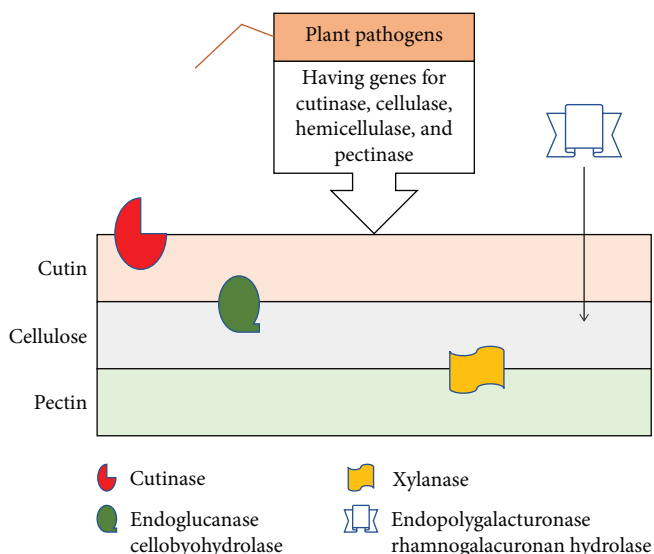


FIGURE 3: Interaction between a plant pathogen and host cell wall.

by *Trichoderma* spp. As lysis of the plant pathogenic fungal cell wall is one of the key steps during mycoparasitism and thereby controlling of plant pathogen, different strains of *Trichoderma harzianum* have been found to be effective in controlling the plant pathogens [58]. One of the main mechanisms put forth to explain the hostile activity of *Trichoderma* species against a variety of soil-borne plant pathogenic fungi, such as *Sclerotium rolfsii*, *Rhizoctonia solani* and *Pythium aphanidermatum*, is their direct mycoparasitic activity. [59]. *Trichoderma*'s chitinases are mainly involved in (i) mycoparasitism (ii) exogenous chitin degradation and (iii) recycling of the fungal cell wall [60]. The chitinolytic enzyme system of *Trichoderma* can be divided into subgroups A, B and C and is a member of GH family 18. Subgroup B is believed to be an endochitinase and several of its members contain a CBM1 domain at their C terminus, while subgroups A and C are predicted to be exochitinases that cleave close to the ends of the chitin chain and release N-acetylglucosamine dimers. CBM 18 and CBM 50 domains can be found at the N-terminus of the GH 18 module of subgroup C chitinases [60].

9. *Trichoderma* as an Insect Pest Biocontrol Agent

Numerous microorganisms that operate as entomopathogens and interact with plants in the soil can be employed as efficient biocontrol agents against various agricultural insect pests [61]. *Beauveria*, *Cordyceps*, *Hirsutella*, *Isaria*, *Lecanicillium*, *Metarhizium*, *Ophiocordyceps*, *Paecilomyces*, *Pochonia*, *Torubiella* and *Trichoderma* are some of the most investigated and utilised genus in agriculture [62]. *Trichoderma* is found to be effective against different type of insect pests (Table 4). The phytohormones salicylic acid (SA), jasmonic acid (JA) and ethylene are the signal transduction pathways that are activated by an arthropod attack on plants [70, 71]. The effects that these insects have on

TABLE 4: Biocontrol capacity of *Trichoderma* against different pests.

| S. No. | <i>Trichoderma</i> | Effective against | Reference |
|--------|--|------------------------------------|-----------|
| 1 | <i>T. asperellum</i> | <i>Tetranychus urticae</i> | [63] |
| 2 | <i>T. viride</i> , <i>T. harzianum</i> | <i>Odontotermes formosanus</i> | [64] |
| 3 | <i>Trichoderma</i> sp. | <i>Amrasca bigutulla bigutulla</i> | [65] |
| 4 | <i>Trichoderma</i> sp. | <i>Aphis gossypii</i> | [65] |
| 5 | <i>Trichoderma</i> sp. | <i>Myzus persicae</i> | [66] |
| 6 | <i>Trichoderma</i> sp. | <i>Locusta migratoria</i> | [67] |
| 7 | <i>T. viride</i> | <i>Corcyra cephalonica</i> | [68] |
| 8 | <i>T. viride</i> | <i>Helicoverpa armigera</i> | [69] |

TABLE 5: Various methods to prepare biocontrol formulation of *Trichoderma* [78].

| S. No. | Formulation type | Methodology |
|--------|------------------------------|--|
| 1 | Talc based | Sterilized talc powder is mixed with mycelia grown in liquid medium at 1 : 2 ratios. When the medium reaches 8% moisture, it needs to be dried under shade. The <i>Trichoderma</i> talc formulation can be stored for 3–4 months |
| 2 | Vermiculite-wheat bran based | In an oven, 33 g of wheat bran and 100 g of vermiculite are sterilised for 3 days at 70°C to multiply the mycelia. Then 20 g ferment or biomass is mixed with 0.05N medium and the entire concentrated biomass is mixed with HCl and dried in shade |
| 3 | Pesta granules based | The Fermentor biomass is mixed with 100 g of wheat flour. Then a layer of 1 mm thick dough is prepared and air-dried until it breaks crisply. After air drying, the dough sheet is ground and passed through an 18 mesh to collect the granules |
| 4 | Banana waste based | Different banana wastes are chopped into small pieces, such as the sheath, pseudo stem, and core, and layered in a pit. Different ingredients are added in five layers within the pit. One ton of banana waste, 5 kg of urea, 125 kg of rock phosphate, and 1 L of broth culture of <i>Trichoderma</i> are mixed thoroughly and that much of requirements for single layer. Five different layers are prepared in the same way and mixed thoroughly. Within 45 days, banana waste decomposes and is easily used in the field |
| 5 | Press mud based | One week old <i>T. viride</i> culture broth evenly mix with 120 kg press mud. To keep moisture in the mixture, water is sprinkled on. It is covered in gunny bags, which also help air circulation and trap the moisture. As the same procedure, eight tons of press mud are added to it, mixed it well, and incubated for 8 days under shade. In this way, 8,000 times more inoculums of biopesticides were added, then recommended, resulting in rapid and visible establishment of the biopesticides |

plants vary depending on how they attack them, with piercing/sucking species having different effects than chewing organisms [72]. Piercing and sucking type of insects like aphids trigger SA-related responses, while chewing type of insects like caterpillars activate the JA signalling pathway [73]. Despite the fact that their effects on plant pathogens have been extensively studied in the lab and in the field, investigations on their impact on insect pests are scarce and primarily recent. Research in the lab revealed that *Trichoderma* spp. negatively affect both chewing type of insects [74] as well as piercing and sucking type of insects [75]. The field study on *Trichoderma* spp. on the population of pests associated with maize [76] demonstrated that the abundance of piercing and sucking type of insects reduced under field, while the population of natural enemies increased, supporting laboratory findings. Entomopathogenic fungi typically infect insects directly through the cuticle, a process that necessitates adhesins and lytic enzymes (chitinases, proteases and lipases). In the end, the fungus forms and disperses new conidia from its dead host after defeating the insect's immune system and colonising its body. Entomopathogenic

fungi are required to create a wide range of insecticidal secondary metabolites throughout the procedure in order to complete their life cycle.

10. *Trichoderma* as a Biopesticide

The search for eco-friendly biopesticides is critical, as well as being able to resist several types of plant pathogens. A highly effective biocontrol agent, high levels of productive and viable propagules and bioprotectant-friendly transport mechanisms give the biocontrol agent a competitive edge over other microflora [77]. Due to the importance of *Trichoderma*, it is more commonly used than other biopesticides in agriculture for the management of plant pathogens. It is risk-free to control plant diseases through biological means when resident antagonists are improved. To make adequate quantities of active and viable *Trichoderma* inocula, both solid and liquid forms are used. Hyphae, chlamydospores and conidia are the three types of propagules that can be used in formulations [56]. A comprehensive integrated pest management programme promotes disease management in a similar

TABLE 6: Different methods for the application of *Trichoderma*.

| S. No. | Methods | Application |
|--------|----------------|---|
| 1 | Seed treatment | Delivered in the infection court, i.e., surface of seed coat as protectant at the time of planting |
| 2 | Seed priming | Seeds are hydrated to allow the germination but not sprouting. Two types of priming are available. Solid matrix priming (SMP) to enhance biocontrol of <i>Trichoderma</i> by regulating water levels in the seed. Osmopriming makes use of aerated aqueous solutions of salts or polyethylene glycols to generate osmotic potential in the primary solution |
| 3 | Liquid coating | Application of <i>Trichoderma</i> to the seed with an aqueous, adhesive or binder |
| 4 | Double coating | <i>Trichoderma</i> is applied directly to the seed coat followed by a particulate to form the second layer |

manner to fungicides. The *Trichoderma* produces a variety of antimicrobial products that act as biocontrol agents and affect other microorganisms [69]. There are various methods developed for the mass production of *Trichoderma* (Table 5).

11. Compatibility of *Trichoderma* with Other Biological Systems and their Application

A single strain of *Trichoderma* might not be able to control all plant diseases and pests, therefore may require a combination of strains or another biological control agents as it is extremely disease specific [79]. The compatibility of the *T. harzianum* and *Glomus* trapped in alginate beads with no inhibitory effects on each other [80], control of sheath blight of rice caused due to *Rhizoctonia solani* by the combined use of *Pseudomonas fluorescens* and *T. viride* in talc formulation [81], reflected a synergistic type of combining ability of *Trichoderma* with other biocontrol agents.

Trichoderma's ability to biologically regulate plant pathogens depends not only on its ability to act as an antagonist, but also on how it is applied to the soil, roots and seeds. Additionally, for effective defence and control, all antagonists depend on where they are on the infection court [81]. *Trichoderma* is typically only effective as a preventative measure but can be integrated with other disease management options, particularly when the disease has already established, therefore timing of delivery and application is also essential. The following are illustrations of efficient *Trichoderma* transportation and application methods (Table 6).

12. Conclusion

A group of filamentous fungi known as *Trichoderma* has several species that has received extensive research investigations and are employed as biocontrol agents in agriculture. The sexual stage of *Trichoderma* species is the *Hypocrea*, and it forms ascomycetes with green spores, hyaline and smooth septate vegetative hyphae. The identification of various *Trichoderma* species has been substantially aided by TrichoBLAST and TrichOKEY, an oligonucleotide barcode. Additionally, successful identification of various *Trichoderma* species can be achieved by molecular methods that make use of the ITS region and the associated morphological traits. *Trichoderma* has been used as a biological control agent against a variety of plant pathogens and insect pests in recent years by employing a variety of antagonistic strategies to combat plant diseases, including mycoparasitism,

competition for nutrients and resources and antibiosis. The use of *Trichoderma* species should be promoted as a viable alternative to pesticides in the era of a green economy, focused on safeguarding both human health and the environment in light of the information provided in the present review. The existing methods for detecting and assessing these antagonists, which integrate multiple mechanisms of action and are quick, affordable as well as effective, must be improved upon and used widely. The current compilation of works also emphasises the commercialisation of these biocontrol-based biostimulation and bioremediation preparations for use in farming practises in a more natural way. However, more research is needed in this area and against more diverse groups of plant pathogens as well as insect pests in order to make *Trichoderma* a viable alternative for the future of agricultural plant health.

Data Availability

All the data are provided in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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