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Trichoderma viride: A Biocontrol Agent and its Role in Disease Management in Sericulture

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Abstract

The fungus Trichoderma was described as early as 1794 by the mycologist Persoon. It includes a broad group soil fungus, remarkable for their rapid growth and antagonistic behavior over many of the plant pathogenic fungal species. *Trichoderma viride* holds potential as a biocontrol agent in sericulture, offering a sustainable and environmentally friendly solution for managing fungal diseases and promoting the health and productivity of mulberry plants, thus supporting the silk production process.

The fungus Trichoderma

The fungus Trichoderma was described as early as 1794 by the mycologist Persoon. It includes a broad group soil fungus, remarkable for their rapid growth and antagonistic behavior over many of the plant pathogenic fungal species. Presently this genus is considered mainly as saprophytic fungi, fast grower, green or white molds with hyaline phialides and single-celled, green or hyaline conidia. They are believed to be one of the predominant components of the mycocenoses in various soils of all climatic zones of the world (Danielson and Davey, 1973a), where they have significant importance in decomposition of woody and herbaceous materials and also considered as necrotrophic parasites against the primary wood decomposers (Rossman *et al*., 1999). They produce a wide range of extracellular enzymes (mainly hydrolases and oxidases) and can use most naturally occurring organic substrates for their growth and development.

Biology and nomenclature: The genus Trichoderma belongs to the class Deuteromycetes. It was classified as an imperfect fungus, where the sexual stage of the fungus is unknown (Gams and Bisset, 1998). Misra and Gupta (2009) differentiated the genus into nine species viz., *Trichoderma aureoviride, T. hamatum, T. harzianum, T. koningii, T. longibrachiatum, T. piluliferum, T. polysporum, T. pseudokoningii* and *T.viride* on the basis of conidiophore branching patterns and conidium morphology and other microscopic characters. Bissett (1991) proposed a sectional classification for Trichoderma where it was grouped into five sections *viz.*, Trichoderma, Longibrachiatum, Saturnisporum, Pachybasium and Hypocreanum.

Characteristics of the Genus Trichoderma: The genus Trichoderma is characterized by rapidly growing colonies bearing tufted or postulate, branched conidiophore containing lageniform phialides and hyaline or green conidia born in slimy heads (Bissett, 1984). The primary branches of conidiophore produce smaller secondary branches that also may produce tertiary branch and so on (Babu *et al*., 2007). Conidia are generally hyaline or more usually, green, smooth walled or roughened. Hyaline chlamydospores are usually present in the mycelium of older cultures (Yuan *et al*., 2021). Phialides are ampulliform to lageniform, usually constricted at the base, swollen near the middle, and abruptly enlarged near the apex into short sub-cylindrical neck. They are disposed in verticals terminally on branches of the conidiophore, or less frequently singly or in whorls directly beneath septa along the conidiophore and its branches (Bissett, 1991).

Growth medium and sporulation: Trichoderma species are saprophytic fungi that utilize wide range of compounds containing carbon and nitrogen sources. Monosaccharides and disaccharides mainly satisfied their carbon and energy requirements (Papavizas, 1985). Danielson and Davey (1973) suggested that ammonium is the mostly utilized source of nitrogen by Trichoderma spp. in buffered media to support their vegetative growth.

In 1981, Elad *et al*. found special Trichoderma selective media (TSM) which is recommended for the quantitative isolation of Trichoderma from soil. Selectivity was obtained by using chloramphenicol as a bacterial inhibitor and pentachloronitrobenzene (PCNB) and rose Bengal as fungal inhibitors. Trichoderma colonies grow rapidly and mature in 5 days on potato dextrose agar at 25°C and the colony is woolly and soon develops to a compact mass. Initially the colonies are white in color which subsequently turned to blue-green or yellow green as the conidia are scattered into patches with prominent visibility.

Sometimes concentric rings also appear in the patches. They are more readily visible on potato dextrose agar compared to other media (Pons and Sutton, 1998). Most species of Trichoderma are photosensitive, sporulating on many natural and artificial substrates. Exposure of agar cultures to light of 85-90 lux intensity for 20-30 seconds is usually sufficient to induce some sporulation (Papavizas, 1985).

Ecology: Trichoderma species are ubiquitous in the nature, mainly in soils. They are among the most frequently detected mitosporic fungi (teleomorph Hypocrea, Hypocreales, Ascomycota, Dykaria) in cultivation-based surveys. They were isolated from innumerable, diverse natural and artificial substrata that demonstrate their high opportunistic potential and their ecological adaptability (Druzhinina, 2013). They have been used in various human activities, including commercial applications in production of enzymes and biological control of plant diseases (Samuels, 1996). Trichoderma species are spread to all over the world (Maina *et al*., 2015) and found in all types of soils including humus layer of the forest as well as in agricultural orchard soils (Roiger *et al*., 1991) and their natural habitats, especially which contains more amount of organic matter (Papavizas, 1985). They are also present in the root surfaces of various plants and on decaying bark, especially when damaged by other fungi; and on sclerotia or propagules of other fungi (Papavizas, 1985). Trichoderma species can produce chlamydospores on natural substrates. Caldwell (1958) first observed that chlamydospores survive better than conidia in soil. These structures may play an important role in the survival of the genus in the soil (Papavizas, 1983). Lewis and Papavizas (1985) demonstrated the potential of various Trichoderma species aggregates to form chlamydospores readily and in great numbers in natural soil or in fragments of organic matter after the introduction of the fungus to the soil as conidia.

Recently scientist discovered the physiological trait of Trichoderma spp. that they can occur as endophytes. T. theobromicola was isolated form a healthy cocoa tree (Theobroma cacoa, Malvaceae) in Amazonian Peru and T. paucisporum was obtained in Ecuador from cocoa pods partially infected with frosty pod rot, Moniliophthora roreri. Both the species were produced diffusible, volatile antibiotic compounds that inhibited the development of M. roreri in vitro and on-pod trials (Samuels *et al*., 2006).

Modes of action of Trichoderma: There are several mechanisms of action suggested for Trichoderma spp. viz., mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced resistance, solubilization and sequestration of inorganic nutrients and inactivate the pathogen enzymes (Samuels, 1996). The first three were the most important, by which these fungi have always been considered to function (Harman, 2006).

Mycoparasitism: Mycoparasitism is considered an important mechanism of biological control and it depends on the production of lytic enzymes including β-1, 3-gluconase and proteases (Harman, 2006). Several chitinolytic enzymes have been reported in *T. harzianum* including endochitinases, exochitinases and 1, 4-β-N-acetylglucosaminidases which are induced during growth in liquid medium containing chitin as carbon source (Harman *et al*., 2006). Mycoparasitism is a complex process including several steps. The initial interaction shows that the hypha of the mycoparasites grows directly towards its host (Chet *et al*., 1998). When the mycoparasite reaches the host, its hypha coils it or attaches to it by forming a hook like structure (Viterbo and Horwitz, 2010). Following these interactions hypha penetrates the host mycelium, apparently, by partially degrading its cell wall (Elad *et al*., 1983).
Antibiosis: Several studies have demonstrated the importance of antibiosis activity in biological control. Dennis and Webster (1971) found that various types of volatile and non-volatile antibiotics are produced by different Trichoderma strains. Excluding the enzymes Trichoderma spp. produces 43 substances which have antibiotic activity (Sivasithamparam and Ghisalbetri, 1991). Among those, alkyl pyrones, isonitriles, polyketides, peptaibols, dikeyopiperazines, sesquiterpenes and steroids are associated with biocontrol activity of some species and strains of Trichoderma (Howell, 2003).
Competition: Competition and rhizosphere competence for space or nutrients has long been considered one of the classical mechanisms of biocontrol by Trichoderma spp. (Elad *et al*., 1981). The competition for nutrients, mainly carbon, nitrogen, and iron are 16 one of the methods of the biological control of soil borne plant pathogens (Scher *et al*., 1986).

Disease Management

***Trichoderma viride*:** *T. viride* is a mold which produces spores asexually, by mitosis. It is the anamorph of Hypocrearufa, its teleomorph, which is the sexual reproductive stage of the fungus and produces a typical fungal fruiting body. The mycelium of *T. viride* can produce a variety of enzymes, including cellulases and chitinases which can degrade cellulose and chitin, respectively. The mould can grow directly on wood, which is mostly composed of cellulose, and on fungi, the cell walls of which are mainly composed of chitin. It parasitizes the mycelia and fruiting bodies of other fungi, including cultivated mushrooms, and it has been called the "green mould disease of mushrooms". The affected mushrooms are distorted and unattractive in appearance and the crop is reduced.

In the investigation on the "Efficacy of bio-agents and botanicals against leaf spot (*Cercospora arachidicola* Hori) of groundnut (*Arachis hypogaea* L.)", *Trichoderma viride* was found best treatment to manage for leaf spot (Cercospora arachidicola Hori) of groundnut. From cost benefit ratio *Trichoderma viride* treatment were statistically found as most economic method over control after chemical treatment. Yield obtained from plot as well as *Trichoderma viride* treated plot were comparable to that of chemical treated one. Since chemicals have many harmful effects on the environment as well as the human health, *Trichoderma viride* would be considered as better as it is eco-friendly also (Ramesh and Zacharia, 2017).

Application of biopesticides containing bioagents like *Trichoderma viride*/harzianum and Pseudomonas fluorescens as seed/seedling/planting material, nursery treatment and soil application has been recommended against Cercospora leaf spot in the AESA based IPM package of castor.

An experiment was carried out to evaluate the effect of different essential oils, bio-agent and their combinations in in vivo conditions for management of early blight of tomato caused by *Alternaria solani* and also to increase the growth parameters and yield. A total of eight treatments taken as seedling treatment namely Neem oil (5%), Castor oil (5%), Clove oil (5%), *Trichoderma viride* (5%), Neem oil + *T. viride* (2.5%+ 2.5%), Castor oil + *T. viride* (2.5%+ 2.5%) and lastly Clove oil + *T. viride* (2.5%+ 2.5%) including control were replicated three times. During evaluation, all the seven treatments were found to be significantly superior over control in managing early blight and also in increasing the growth parameters and yield (Madhuri *et al*., 2021). Among all the treatments Neem oil + *T. viride* (2.5%+ 2.5%) followed by Neem oil (5%), *Trichoderma viride* (5%) were significantly superior over other treatments in reducing early blight infection and also in increasing growth parameters of the crop (Madhuri *et al*., 2021). *Trichoderma viride* is most effective with the combination of essential oils than the single treatment to control early blight disease intensity and also in increasing the growth parameters respectively and also found to be economic.

A potential *Trichoderma viride* strain CZTV-1 was screened against 15 fungal plant pathogens of which nine pathogens *Diaporthe sp*., *Nectria haematococca*, Fusarium sp., *Aspergillus flavus, Lasidiplodia theobromae, Pseudofusicoccum adansoniae, Fusarium solani, Aspergillus niger* and *Fusarium brachygibbosum* were isolated from groundnut; three pathogens *viz., Alternaria alternata*, *Fusarium equiseti* and Fusarium oxysporum were isolated from cumin; and three pathogens *viz., Alternaria tenuissima*, *Chaetomium atrobrunnem* and *Alternaria porri* were isolated from castor. In dual culture, *T. viride* (CZTV-1) significantly reduced the mycelial growth of pathogenic fungi which was the least (29.0%) for Fusarium sp. (CZC-3) and which was the maximum (82.2%) for *Aspergillus niger* (CZGN-12) which was statistically on par with *Fusarium solani* CZGN-9 (80.7%) (Singh and Jadon, 2019).

Effect of Bioagents on silkworm: Study on assessing the residual toxic effect of fungal and bacterial bio agents used against powdery mildew of mulberry on silkworms was conducted. The results of the toxicological studies on growth, development and cocoon parameters revealed that, *Trichoderma harzianum* had positive effect on both larval weight and its length with increase of 21.46 g and 5.18 cm respectively. Similarly, cocoon, pupal and shell weight of 14.99g, 11.86 g and 3.17 g respectively, shell ratio of 21.17 per cent and cocoon yield of 524.66 g/dfl was higher compared to other treatments. The filament length of 769.05 m, filament weight of 0.16 g and denier of 1.80 per cent fineness were found to be superior in *Trichoderma harzianum* treated mulberry leaves fed to silkworm. The present investigation clearly revealed that mulberry powdery mildew effective bio agents, *Trichoderma harzianum* and *Trichoderma viride* at 15 per cent concentration, *Bacillus subtilis* and *Pseudomonas fluorescens* at 10, 15 per cent concentration were found to be safe to silkworms when leaves fed to them three days after treatment with culture filtrate of bio agents (Manjunatha *et al*., 2018).

Berini *et al*. (2016) performed a biochemical characterization of a commercial mixture of chitinolytic enzymes derived from *Trichoderma viride* and analyzed it"s in vitro and in vivo effects on the PM of the silkworm *Bombyx mori* L. The peritrophic matrix (PM) is formed by a network of chitin fibrils associated with proteins, glycoproteins, and proteoglycans that lines the insect midgut. It is a physical barrier involved in digestion processes, and protects the midgut epithelium from food abrasion, pathogen infection and toxic materials. It was found that these enzymes have significant in vitro effects on the structure and permeability of the PM of this insect. A bioassay supported these results and showed that the oral administration of the mixture determines PM alterations, leading to adverse consequences on larval growth and development, negatively affecting pupal weight and even inducing mortality.

Antagonistic potentiality of four isolates of *Trichoderma* species viz., *Trichoderma viride* isolate A, *Trichoderma viride* isolate M, *Trichoderma harzianum* isolate C and *Trichoderma harzianum* isolate L against Beauveria bassiana causing white muscardine disease of silkworm, Bombyx mori L., was studied in vitro by studying the mycelial interaction between them as well as by the effect of culture filtrate of biocontrol agents on sporulation and spore germination of pathogen. *Trichoderma viride* isolate A and *Trichoderma viride* isolate M produced the maximum inhibition zone of 68.00 and 44.25 mm area respectively, followed by *Trichoderma harzianum* isolate L with inhibition zone of 39.60 mm and Trichoderma harzianum isolate C with 33.50 mm. In dual culture all the isolates of *Trichoderma* spp. over grew the pathogen and covered the entire medium surface. Its mycelia coiled around the hyphae of test pathogen and finally disintegrated it. The inhibition in mycelial growth by the cultural filtrate of *Trichoderma harzianum* isolate L was 55.35%. The cultural filtrate of *Trichoderma viride* isolate A has inhibited the spore production and germination by 63.65 and 83.70 % respectively. The mechanism of antagonism was found to be hyper parasitism and antibiosis. The studies conducted revealed that all the tested biocontrol agents possessed antagonistic properties against *Beauveria bassiana* in vitro and could be used in controlling the white muscardine disease of silkworm (Ahmad and Ahmad, 2020).

Conclusion

Trichoderma viride is generally considered safe for silkworms when used as a biocontrol agent in mulberry cultivation. Unlike chemical pesticides, Trichoderma viride is a naturally occurring fungus and does not contain toxic chemicals harmful to silkworms or other non-target organisms. However, it's important to note that while Trichoderma viride is generally safe for silkworms, proper application practices should still be followed to minimize any potential risks. This includes adhering to recommended application rates, avoiding direct contact with silkworms or their food sources, and conducting any necessary compatibility tests before widespread use. Additionally, local environmental conditions and specific mulberry cultivation practices may influence the interactions between Trichoderma viride and silkworms, so monitoring is recommended when implementing any new pest management strategy.

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