

# Beneficial and Harmful Aspects of Trichoderma : A Review

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## ABSTRACT

Microorganisms or their metabolites are being employed for the plant health and nutrient acquisition. Trichoderma species is one such fungal organism that is recognised for its biocontrol potential against various plant pathogens especially in rhizosphere. Their ability to protect in wide soil environments and mechanisms are widely studied. It is known to have various strategies and potential metabolites that influence plant and the pathogens, such as mycoparasitism and induced resistance. Not only beneficial but Trichoderma is also known to have some harmful effects. This review focuses on the both the beneficial and harmful aspects of Trichoderma for the environment and as well as some of its molecular aspects during its interactions.

**Keywords :** Trichoderma, Biocontrol, Myco-Parasitism, Biofertilizer, Rhizosphere

## I. INTRODUCTION

Trichoderma genera is known for its antagonistic properties especially against many soil borne plant pathogens. Persoon (1794) first proposed the name 'Trichoderma', he described that these fungi appear as mealy powder enclosed with a hairy covering<sup>[1]</sup>. They are classified as imperfect fungi since they lack sexual stage<sup>[26]</sup>. These species are filamentous fungi and belong to the Hypocreales of Ascomycota Phylum. Trichoderma species has high level of diversity, in total 104 species have been recorded internationally (www.isth.info.in). Members of Trichoderma are known to attack other fungal organisms by producing various antibiotics and exhibiting mycoparasitism<sup>[2, 3]</sup>. This species is known to occur in different types of soils such as temperate and tropical soils, agriculture, forest, prairie, salt marsh and desert soils across all the climatic zones. They are also found to be colonizing roots, litter, decaying wood, decaying bark and plant materials in all climatic zones. Trichoderma was shown to be three percent of the total fungal propagules from various forest soils and 1.5 percent of pasture soils grown with different crop species<sup>[6, 7, 8]</sup>. They were found to produce various extracellular enzymes and some of these are implicated in biological control of various plant diseases and for their ecological role in degradation of plant material<sup>[4, 5, 12, 13, 14]</sup>. Different classes of chitinolytic or glucanolytic enzymes are produced from Trichoderma that are known to be synergistic and antifungal in action

<sup>15, 16, 17, 18</sup>. These fungal species were shown to help in plant nutrient acquisition by improving plant growth and yield during single or co-inoculation, and as well as induce the plant disease suppression (pathogen control) in soil systems<sup>9, 10, 11, 19, 20</sup>. Trichoderma was shown to have direct effects on plants by inducing systemic and localized resistance in plants by protecting against various plant pathogens (fungi, oomycetes, bacteria and viruses)<sup>21, 22</sup>. During the evolution *Trichoderma* might have developed various tools that made it as good competitor<sup>26</sup>.

### A. Nutrient acquisition

Trichoderma is known to be a very successful biocontrol agent due to high propagative capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi, and most importantly efficiency in promoting plant growth and defense mechanisms<sup>28</sup>. These fungal species are also known to colonize roots by penetrating the epidermis and then into the cortex<sup>23, 56, 58</sup>. Such endophytic activity may be useful in stimulating plant growth and hinder the pathogen entry into the plant<sup>56</sup>. Trichoderma produces a series of metabolites that limit its growth after its entry into the plant roots, this process may be similar to other root endophytic fungi such as binucleate *Rhizoctonia* species and nonpathogenic *Fusarium*<sup>24, 25</sup>. These associations are



known to alter gene expression thereby altering the plant physiology, such alterations can be used for nitrogen acquisition, resistance to abiotic and biotic stress and photosynthetic efficiency<sup>59, 60</sup>. Of all the known species of *Trichoderma*, *T. koningiopsis*, *T. stilbohypoxyli* and *T. stromaticum* were classified (phylogenetic analysis) distinctly among the known endophytic species suggesting the endophytic character as a recently evolved trait<sup>58,62,63,64</sup>. One of the efficient way to control fungal phytopathogens is by limiting the nutrients, for e.g. competition for carbon is shown to be an effective mode in *Trichoderma* and as well as *Fusarium*<sup>35, 36, 37</sup>. Under iron limiting conditions, *Trichoderma* isolates also produced siderophores to mobilize iron from the environment and inhibited the growth of *Pythium* and *Botrytis cinerea*<sup>38, 39</sup>. Its survival in soil is improved by its ability to generate energy from sugars that are generally available, such as cellulose, glucan, chitin and others<sup>28</sup>.

### B. Plant pathogen control

In all the biocontrol applications against plant pathogens, 90% of them are members of *Trichoderma* genus. The most commonly used biocontrol agents are the strains belonging to *T. virens*, *T. viride* and *T. harzianum*<sup>27</sup>. Mixture of biocontrol agents with reduced fungicide application promotes disease suppression similar to a lone fungicide application<sup>26</sup>. *Trichoderma* is known to produce elicitors that activates certain plant defense genes and promote the growth of the plant and its nutrient availability, thereby improving the colonization and nutrient availability for the biocontrol agent; followed by enhancing the overall antagonistic potential against the plant pathogens<sup>80, 81, 22</sup>. Filtrates of *Trichoderma* strains were shown to control *Ceratocystis paradoxa* that causes pineapple disease of sugarcane<sup>40</sup>. Not only filtrates but also small diffusible compounds produced by *Trichoderma* inhibit the growth of microorganisms<sup>45</sup>. Strains of *T. virens* were shown to produce 'Gliovirin' that is involved in antibiosis<sup>47</sup>. It was shown that a mutant strain of *T. harzianum* strain 2413 exhibited an elevated level of extracellular enzymes and of  $\alpha$ -Pyrone that increased its resistance against *R. solani* than its wild type strain and as well as, in the assays of grape protection against *B. cinerea* under controlled environments<sup>48</sup>. Exogenous application of peptaibols from *T.virens* was shown to activate

defense responsive genes and reduce susceptibility to Tobacco mosaic virus in Tobacco plants<sup>49</sup>. The presence of volatile metabolic compounds that are inhibitory to pathogens such as harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-penthy- $\alpha$ -pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid were shown to be present in *T.viride*<sup>50,51</sup>. Pathways such as pyrone biosynthesis pathway, polyketide biosynthesis pathway, peptaibol biosynthesis pathway, flocculosin terpenoid/steroid biosynthesis pathway, gliotoxin and gliovirin biosynthesis pathways were shown to be playing a key role in the production of the *Trichoderma* metabolites<sup>52</sup>. But production of volatile compounds were not detected in *T. harzianum* isolates when tested with *Rhizoctonia solani*<sup>46</sup>.

*Trichoderma* employs mycoparasitism as one of its major strategy in directly attacking the pathogens. The process involves, chemotropic growth, recognition of host by *Trichoderma* hyphae, secretion of a wide array of extra-cellular enzymes, penetration of hyphae into the host and lysis of the host<sup>53</sup>. It grows towards its host by the sequential production of pathogenesis related proteins such as glucanase proteases and chitinases<sup>22</sup>. The low level secretion of exochitinases that degrade fungal cell wall releases oligomers and inhibit the growth of fungal pathogens<sup>54</sup>. Not only enzymes but also morphological changes, coiling and appressoria induces the penetration into the host cells. It coils around the pathogen and forms appressoria, thereby releasing its content that results in the production of peptides which help in both the entry of its hyphae into host cell and digestion of the host cell wall<sup>55</sup>.

*Trichoderma* biocontrol machinery consists of a wide array of lytic enzymes that play a significant role in its biocontrol property<sup>65, 66, 67, 68, 69, 70, 71, 53, 72, 73</sup>. Many of these cell wall degrading enzymes isolated from various *Trichoderma* strains were also characterized<sup>65</sup>. Various genes might play a role in the parasitism. *T. virens* or *T. atroviride* exhibit high degree of parasitism and a large genome, but whereas in comparison with *T.reesei* which exhibits low levels of mycoparasitism and less in genome<sup>115</sup>. Many factors and process affects this process. The role of glucanases and chitinases in mycoparasitism were studied well, the future studies on this aspect of *Trichoderma* will help us to understand this complex process<sup>52</sup>. Proteins that play an important role in root colonization by *Trichoderma* were also found to be playing role for niche competition with the



other root colonizers and to establish symbiotic relationship with host plants<sup>41, 42, 43, 44</sup>. Purified proteins from *Trichoderma* were tested in combinations and as well as alone, showed antifungal activity against various fungal and Oomycete pathogens (*Rhizoctonia*, *Fusarium*, *Alternaria*, *Ustilago*, *Colletotrichum*, *Pythium* and *Phytophthora*)<sup>66, 74, 75</sup>. At an industrial scale, these active components can be produced by modifying the growth conditions and media<sup>76, 77</sup>. Cell wall degrading enzymes were shown to be induced by the presence of mono or polysaccharides, colloidal chitin or fungal chitin<sup>78</sup>. Antifungal activity can be enhanced by a combined application of *Trichoderma* and its enzymes including various synthetic fungicides that particularly affects the cell membrane<sup>79, 67</sup>. It was shown, the purified mixtures of cell wall degrading enzymes with variable lytic activities increased antifungal activity against plant pathogens<sup>79, 67, 69, 70</sup>.

Electron microscopy studies showed that *Trichoderma* interaction with plant was limited to a few cell layers of plant epidermis and outer root cortex, which could be due to the deposition of callose by the surrounding plant tissue<sup>23</sup>. This interaction is more like a symbiotic rather than a parasitic one, as the fungus occupies the nutritional niche and the plant is protected from disease. The two-way interactions (plant-pathogen, plant-antagonist and pathogen-antagonist) are most studied in comparison with three way interaction of antagonistic-plant-pathogen especially *Trichoderma*<sup>109, 110, 111</sup>. Investigations focusing on the changes in gene expressions of all the partners in all possible combinations and furthermore, analysis of the compounds that play a role when the plants are exposed to various beneficial and harmful microbial partners would enable us in understanding such molecular cross-talk in three way interactions. But studies have been published in understanding such interactions using proteomics and gene reporter systems<sup>82, 83, 107</sup>. Creation of Green Fluorescent Protein (GFP) fusion mutants would also improve their understanding of interactions<sup>84</sup>.

*T. virens* or *T. atroviride* exhibits high degree of parasitism in comparison with *T. reesei*<sup>115</sup>. Sequencing of *T. reesei* showed small number of genes that encode for cellulases and hemicellulases<sup>83</sup> but whereas, highly parasitic *Trichoderma* species encode wide array of genes for enzymes such as chitinolytic and glucanolytic

and biosynthesis of small molecules such as non-ribosomal peptides, poliketides, terpenoids, and pyrones<sup>116</sup>. The amount and diversity of biosynthetic pathways utilized by parasitic *Trichoderma* species explain the size and complexity of their genomes. Expression studies of *Trichoderma* genes have proved to be inconclusive, one of the reasons perhaps may be due to the sole connection of such genes to particular microbes or to eukaryotes<sup>94</sup>. But strategies have been suggested to activate the silent genes by growing them in environments where competition exists thereby, their natural biosynthetic pathways are activated<sup>117</sup>. A detailed metabolomic-genomic study might help us in elucidating the roles of its numerous gene products towards its control of pathogens<sup>116</sup>.

### C. Advantages

*Trichoderma* based agricultural formulations are being sold across the globe including India for sustainable agriculture<sup>31, 112</sup>. Mostly *T. harizianum* is being used as an active agent in commercially available biofertilizers and biopesticides<sup>32, 33, 34</sup>. These formulations were applied in vegetables such as onion, carrot, parsley, red beet, dill and radish comprehensively and to a smaller extent in other crops<sup>85, 86, 87, 88</sup>. Studies were also carried out in order to better formulate and improve the production processes<sup>89, 90, 91</sup>. Other than the plant protection, they help in plant nutrient acquisition by enhanced solubilization of nutrients, nutrient uptake including nitrogen, increased root hairs and deeper rooting<sup>22</sup>. When applied as a biocontrol agent there will be no development of pathogen resistance, no phytotoxic effects, suppresses plant parasitic nematodes, inhibits seed-borne diseases, causes no environmental pollution, induces host resistance and helps in plant nutrient acquisition<sup>22, 119</sup>. It minimizes the crop losses due to plant diseases and increases plant yield and quality<sup>22</sup>. *Trichoderma* species are also known to produce hydrolytic enzymes such as cellulases, chitinases and xylanases that play a role in food processing and pulp bleaching industries. *Trichoderma* can also be used to produce alternative sources of energy such as the second generation biofuels. These fuels are generated from agricultural wastes by using cellulases or hemicellulases produced by *T. reesei*<sup>83</sup>. This aspect of cellulase production should be studied so as to produce an economically viable and highly efficient process for the



production of these fuels. The enzymes are also being employed as a fabric detergent, animal feed production, alternative to conventional bleaching, in effluent treatment. *Trichoderma* is also being used for the production of enzymes and other metabolites<sup>92, 93, 94</sup>.

#### D. Disadvantages

Not only benefits but there are also negative or harmful aspects of *Trichoderma*. Some species are a threat to mushroom production. Mushroom yield has been reduced by 50% by *Trichoderma* infection<sup>108</sup>. It is known to seriously threaten the cultivation of champion, button, oyster and shiitake mushrooms. Particularly *T. viride* and *T. koningii* were reported to infest mushroom cultures<sup>100</sup>. Four types of biotypes of *Trichoderma* were reported (Th1, Th2, Th3, Th4) to be the causative organisms for losses in mushroom cultivation<sup>100</sup>. *Trichoderma* caused serious loss to button mushroom production in UK, Netherlands, North America, France, Spain, Hungary, Poland, Croatia, Mexico, and Australia<sup>95, 96, 97, 98, 99, 113</sup>. It was found that the members of *T. aggressivum* were responsible for the crop (mushroom) losses in Europe, Canada, USA and Mexico<sup>100, 101</sup>.

*Trichoderma* members were reported to affect human health<sup>26, 114</sup>. *Hypocrea orientalis* that is genetically close to *T. citrinoviride* Bissett, *T. harzianum*, and *T. longibrachiatum* were known to cause problems with individuals having reduced resistance, leukemia, HIV positive and in organ transplants<sup>103</sup>. It has also been reported that *Trichoderma*'s volatile metabolites may cause respiratory tract complications<sup>106</sup>. As a result of growing number of immunocompromised patients the numbers of opportunistic fungi are also increasing and it is already in the list of potential pathogens in immunocompromised hosts<sup>119, 61</sup>. Infections caused by *Trichoderma* members are generally diagnosed late and are hard to treat, as they are less sensitive to the commonly administered antifungal drugs and multiple drug treatments were shown to be effective<sup>104, 105</sup>. Not only humans but also to other chordates, *Trichoderma* members are known to be pathogenic<sup>29</sup>.

## II. CONCLUSION

Plant diseases are the key contributors in crop losses. Consequences of the applications of chemical based fertilizers and pesticides on crops are well known. Growers or farmers started to employ integrated disease or pest management so as to improve the sustainability in agriculture. *Trichoderma* has been proven to be a good candidate for improving plant health worldwide by controlling various seed and soil borne diseases. But still difficulties exist in understanding plant-antagonist-pathogen interaction systems to maximize their effects, use of high throughput systems and advanced molecular studies might reveal better about the molecular basis of interactions of *Trichoderma*. Such a studies will identify many new compounds and unravel the role of such compounds, before their application as biocontrol agents. In this way, the use of strains that are potentially harmful to plants or toxic to people are avoided. These studies needs to be multi-disciplinary with cooperation of mycologists, geneticists, biochemists, plant physiologists, toxicologists, and numerous researchers from other fields.

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