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Biocontrol potential of *Trichoderma spp.* and its mechanisms of interaction between plant and pathogen

Shweta Jaiswal [1]and Amit Kumar Tiwari[1]*

[1], Corresponding Author, Assistant Professor,Institute of Basic science,Vigyan Bhawan, Department of Botany, Bundelkhand university, Jhansi,amitstar82@gmail.com*

[1] Research Scholar (JRF), Institute of Basic science, Vigyan Bhawan, Department of Botany, Bundelkhand university, Jhansi,shwetajaiswal74@gmail.com

Abstract

Agriculture is a vital part of any country to feed the millions of people, but it faces a constant threat from pests. Recently, chemical pesticides have been used to protect crops from this enormous yield loss. Although chemical pesticides have shown effective results in killing crop pests, they have negative impacts on the environment and humans. In order to find an environmentally friendly alternative, biological control methods are therefore used. *Trichoderma spp.* can reduce the severity of plant diseases by inhibiting plant pathogens in soil through their potent antagonistic and mycoparasitic activity. *Trichoderma's* ability to control plant diseases was mainly based on the activation of single or multiple control mechanisms. It is known that *Trichoderma* based biological control mechanisms are mainly based on mycoparasitism, production of antibiotic and/or hydrolytic enzymes, competition for nutrients, and signaling pathways mediated by *Trichoderma spp*. be initiated and the most important are the heterotrimeric G protein, the MAP kinase and the cAMP pathway. G-protein and MAPK are mainly involved in the secretion of antifungal metabolites and the formation of infection structures. The cAMP pathway aids in the condition and enrollment of *Trichoderma* mycelium on pathogenic fungi and inhibits their proliferation.

Keywords: *Biocontrol, Mode of action, Plant pathogens, Signalling pathway, Trichoderma spp.,*

1. INTRODUCTION

Fungi of the genus *Trichoderma* are soil-borne sac fungi with green spores found throughout the world. Traditional methods of protecting crops from disease have largely relied on the use of chemical pesticides. The use of fungicides and fumigants can have drastic environmental and consumer impacts and are often used in agricultural production at higher rates than herbicides and insecticides. Chemical processes are not economical in the long term as they can pollute the atmosphere, damage the environment, leave harmful residues and with repeated use, lead to the development of resistant strains of the target organisms [1].

One of the biggest challenges facing agricultural production today is to meet the quality and quantity requirements for producers and consumers without harming the environment. Various pathogens attack the crops, including filamentous fungi, which are the most destructive, causing significant economic losses [2].

Fungi in the genus *Trichoderma* have been known since at least the 1920s for their ability to act as bio-control agents against plant pathogens [3]. *Trichoderma* has been researched worldwide for its biological effectiveness against pathogenic fungi and its ability to promote plant growth. *Trichoderma spp*. can reduce the severity of plant diseases by inhibiting plant pathogens through their highly potent antagonistic and mycoparasitic activity[4]. *Trichoderma spp.* has been studied as a biological control agent against soil-borne plant-pathogenic fungi [5]. Results from various studies showed that several *Trichoderma* strains had a significant reducing effect on plant diseases caused by pathogens such as *Rhizoctonia solani, Sclerotium rolfsii, Phythium aphanidermatium, Fusarium oxysporum, F. culmorum* and *Gaeumannomyces graminisvar var. tritici* under greenhouse and field conditions [6] [7] [8] [9].

2. PROPERTIES OF *TRICHODERMA* SPP.

The genus *Trichoderma* contributes a large number of its abilities among different phyla as multifunctional fungi found in a wide variety in ecosystems. Typically, they occur from forests or agricultural soils. *Trichoderma* is a genus of fungi in the Hypocreaceae family, usually belonging to the inhabitants of the rhizosphere. The difficulties encountered in identifying *Trichoderma* isolates at the species level become more significant because the morphological differences are rare and difficult to observe. In general, morphological features have been used to classify the species of *Trichoderma* [10] stated that species belonging to the genus *Trichoderma* have about 10,000 fastest growing species. Initially, *Trichoderma* strains appeared white and cottony, then developed into yellowish green to deep green compact clusters, particularly in the center of a growth spot or in concentric annular zones on the agar surface, as shown in Fig. 1.

For six decades, *Trichoderma spp.* are known to have the ability to attack other fungi. They are also known among researchers as potential biological control agents [11] [12] [13]. According to the findings of some researchers, *Trichoderma spp.* can kill plant pathogens and promote plant growth [11] [14a] [14b] [15].

The success of *Trichoderma spp*. in the soil ecosystem and its role as a natural decomposer is due to its ability to accelerate growth, its ability to uptake nutrients and its ability to modify the rhizosphere. It is also to tolerate unfavorable environments and has a strong destructive ability against plant-pathogenic microorganisms [16] [17].

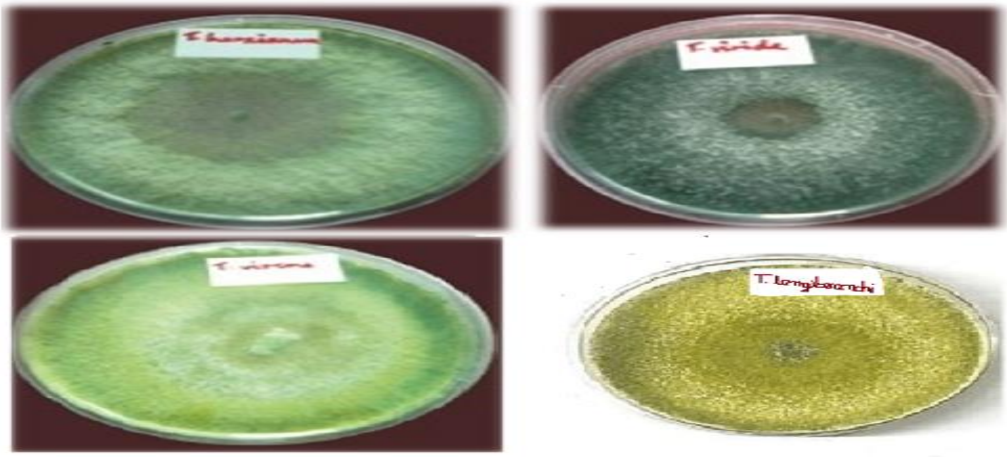


Figure 1. Colony of ***Trichoderma*** spp. isolates on PDA (7 days), up left: ***T. hazianum***; up right: ***T. viride***; down left: ***T. virens***; down right: ***T.longibranchiam.***

Trichoderma spp. are ubiquitous colonizers of cellulosic materials and are therefore often found wherever decaying plant material is available[18] [19] and in the rhizosphere of plants where they can induce systemic resistance to pathogens. *Trichoderma spp.* are very successful colonizers of their habitats, which is taken into account both by their efficient use of the available substrate and by their secretory capacity for antibiotic metabolites and enzymes. Techniques for genetic engineering of *Trichoderma* species of are used for industrial production of enzymes and as biocontrol agents. *T. reesei* is often referred to as an industrial workhorse due to its exceptionally high enzyme production and secretion rates of up to 100 g/l [2].

3. TECHNIQUES FOR GENETIC MANIPULATION OF *TRICHODERMA*

The physiology of the biomass must be conducive to long-term storage while maintaining a high level of viability under conditions of significant microbial competition after addition to the environment. However, the most important aspect of this hierarchy of biocontrol development is that a highly potent strain must be identified before further experimental conditions are established.

Trichoderma spp. are antagonistic to numerous plant pathogens [20] [21][22]. Strains of these genera have been isolated from native soils, including disease-suppressive soils, in many localities [21] [22][23].

Screening of these wild-type isolates has provided many strains with biological control ability[24][25]. The development of methods, particularly genetic engineering, to improve the effectiveness of biological control of these fungi is likely to encourage the use of biological control. Recent use of genetic engineering technology has produced a superior biocontrol strain of *Trichoderma* that has been registered as a biofungicide by the Environmental Protection Agency in the United States.

Due to industrial application of *Trichoderma spp.* the genetic method for this fungus is the most extensive of the genus, although also research with other species is not limited by technical obstacles and most technique can also be used for all species with slight modifications. Transformation of many species is possible, and different approaches such as protoplasting [26]. Agrobacterium-mediated transformation [27], or biolistic transformation [28], were developed. In *Trichoderma*, the single nucleus may undergo several mitotic divisions to give rise to multinucleate conidium. Segregation of unbalanced hetero- karyons within a wild population by single sporing to produce homokaryons may allow for isolation of new strains with novel genetic elements that could be more stable and have enhanced biocontrol characteristics.

Genetic manipulation of the genome of *Trichoderma* has been performed using chemical or physical mutagenesis. Used chemical mutagens, e.g. nitrosoguanidine, and ultraviolet (UV) irradiation to form mutant strains [29],used nitrosoguanidine or UV mutagenesis to produce several auxotrophic mutants of *Trichoderma*, which were used in somatic cell fusions and transformation. Mutation followed by selection has been used to produce strains resistant to benomyl and related fungicides[29] [30] [31]. Some mutant strains of *T. harzianum* and *T. viride* were benomyl resistant and more effective than the original strain-in suppressing damping-off (*Rhizoctonia solani*) of cotton and radish, damping-off and blight of bean (*Sclerotium rolfsii*), white rot (*S. cepivorum*) of onion and damping-off (*Pythium ultimum*) of peas. [30] showed that a few mutant strains selected for resistance to benomyl were also rhizosphere competent. Some of these first known rhizosphere competent strains increased plant vigor in laboratory trials.

4. INTRACTION OF *TRICHODERMA* WITH PATHOGEN

The main three mechanisms of biocontrol of *Trichoderma spp*. are for nutrients or space among others which may operate independently or together to suppress plant pathogens. *Trichoderma* prove antagonistic interaction with pathogen by following mode of action.

4.1 Mycoparasitism – [32] proposed the mycoparasitic activity (hyperparasitism) of *Trichoderma* as one of the main mechanisms involved in their antagonistic activities against phytopathogenic fungi. Mycoparasitism is a direct biological control mechanism that functions through parasitization, detection, growth, colonization, secretion of hydrolytic enzymes, penetration of hyphae, and lysis of the host Pathogen [33]. The effect of the mechanism of *Trichoderma* as mycoparasitism on biocontrol of wood rot fungi was reviewed by [34] who studied *T. harzianum* hyperparasitism by forming appressoria over the pathogenic hyphae of *F. solani* by gently wrapping themselves around them within 95 h of contact. They added that while *T. harzianum* was proliferated by conidiogens, the fungus was completely inhibited within 6 days. The know-how of microparasitizing other fungi has been widely used for biological control against pathogenic fungi. Some species of *Trichoderma* such as *T. asperellum*, *T. atroviride*, *T.virens*, *T.polysporum T.viride*, *T.hamatum*, *T-78 and T. harzianum* are widely used as biological control agents for pathogenic fungi [33].

Its strength to instantly kill pathogens and other plant-associated fungi with a wide host range in different ecologies [35]. These mycoparasitic abilities appear to be very complex, implying the detection of plant pathogens by chemotropism; lysis of the cell wall of the pathogen[36]. Pathogenic hyphal penetration through appresorial formation; Production of cell wall degrading enzymes (CWDEs) and peptaidols mediated by heterotrimeric G proteins and mitogen-activated protein (MAP) kinases [33] and parasitizing pathogens cell wall contents [34]. The breakdown of the cell wall of pathogens during mycoparasitism is mediated by a number of hydrolytic enzymes including -(1,6)-glucanases, chitinases and proteases. Adequate research has been done to identify and understand the enzymes induced by *Trichoderma* to recognize host pathogens [35]. The breakdown of a pathogen's cell wall is an important aspect of mycoparasitism and biological control of plant diseases.

4.2 Antibiosis - Antibiosis is an antagonistic interaction involving diffusible, low molecular weight secondary metabolites or antibiotics produced by a microorganism that are deleterious to the growth of host pathogens. *Trichoderma spp*. produces a variety of antibiotics such as gliovirin, gliotoxin, viridin, viridol, koninginins, pyrones and peptaiboles against fungal phytopathogens[36] [37] . Trichokonins VI, a species of peptaibol from *Trichoderma* pseudokoningii SMF2, demonstrated antibiotic activities by inducing extensive apoptotic programmed cell death in fungal pathogens [38]. [39] reported a series of antibiotics produced by *Trichoderma* species, which has been suggested as the mode of action of *Trichoderma* fungus against plant pathogens. Other metabolites consist of tricholine, rosinic acid, viridian, gliosopramine, heptelic acid, 6-pentyl-a-pyrone and massoilactone [40b]. All of these are natural products that are biosynthesized by many fungi and work in concert with cell wall degrading enzymes to inhibit or completely prevent the growth of pathogenic fungi and/or induce the development of induced plant resistance to pathogens [41]. Research has shown that these compounds exhibit potent promoting effects on plant growth and resistance of plants to abiotic and biotic stress [42]. Due to advances in analytical studies, many secondary metabolites of *Trichoderma spp*. were isolated and identified. Over 120 secondary metabolites of *Trichoderma* have been identified and reported by [44]. Trichoderma spp. In contrast to a number of pathogenic fungi, they have been reported to produce both volatile and non-volatile antibiotics [45]. Studies on the production of volatile and non-volatile antibiotics showed that *T. harzianum* and *T. viride* were highly effective in reducing radial growth of *S. rolfsii* [46].

4. 3 Bio-Control through Competition –

4.3.1 Fungistasis - Few antagonists are usually able to defeat the fungistatic effect of soil resulting from the presence of metabolites produced by other species, including plants, and under very extreme ruthless conditions to survive. The bioactive *Trichoderma* strains grow rapidly when inoculated into soil as they are inherently resistant to many toxic compounds including herbicides, fungicides and insecticides such as DDT and phenolic compounds [47], (TRicho-CRMB1 paper) Resistance against toxic compounds can be integrated with the presence of ABC transport systems in *Trichoderma* strains [48]. For this reason, taking *Trichoderma* strains is very efficient in combating multiple phytopathogens.

4.3.2 Competition for nutrients - *Trichoderma* competes with pathogens for nutrients and space and is thus the detrimental effect of one microorganism on another due to the use or removal of some resources from the environment. Competition for nutrients has been implicated as a biocontrol mechanism by *Trichoderma*. These fungi produce multiple siderophores that chelate iron and stop the growth of pathogenic microorganisms. Importantly, *Trichoderma* strains can compete for space and important exudates from seeds that stimulate germination of dispersal organs of plant-pathogenic fungi in soil [49]. In *Trichoderma spp*. diversity in siderophore production is due to modification of non-ribosomal peptide synthetase (NRPS) products rather than different NRPS-encoding genes [50]. In *Trichoderma spp.* explains the ability of tetramic acid to bind Fe3+ with good cohesion, the mechanism of iron solubilization, which significantly changes the nutrient content in the soil for other microbes and the host plant. Reduced nutrient concentrations result in reduced conidial germination and slowed germ tube growth of a pathogen, reduced number of infection sites and the extent of plant disease development [51].

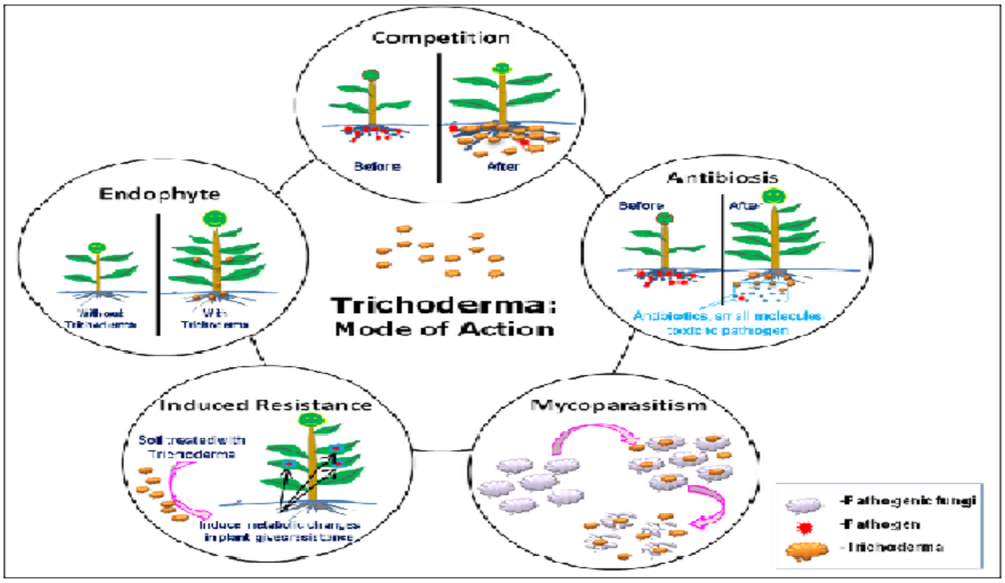


Figure 2. Model depicting mode of action of *Trichoderma spp.* against pathogen and plant growth improvement (Waghunde et al. 2016)

5. INDUCTION OF SYSTEMIC RESISTANCE AND PROTECTION AGAINST PHYTOPATHOGENS

ISR is an important mechanism by which rhizospheric Trichoderma plants are primed for improved defenses against pathogens and herbivorous insects [52]. The ISR activated by these fungi often involves the perception of Trichoderma triggers released into the rhizosphere, which are subsequently detected by plants or deposited directly in the apoplast of the root cell. Trichoderma triggers comprise a broad class of metabolites with different chemical natures (Table 1).

Effector	**Interaction**	**Role**	**Reference**
Enzymatic activity			
Ethylene-inducing xylanases(EIXs)	T. viride - tobacco and tomato	Potent effectors of the hypersensitive response and ET-dependent defense.	[53] [54]
Cellulases	T. logibrachiatum – melon	Their enzymatic activity promote the accumulation of JA and ET emission. Oxidative burst and defense dependent of SA also have been detected.	[55] [56]
ThPG1	T. harzianum T34 - tomato	This enzyme is required for the root colonization. Hydrolyzes pectin and produces oligogalacturonides that act as inducers of plant immunity.	[57] [58]
Alamethicin	T. viride - lima bean	Triggers the emission of plant VOCs mainly via the JA pathway although some SA-induced compounds are also released.	
Trichokonins	T. pseudokoningii – tobacco	Induce the accumulation of ROS and plant immunity against the tobacco mosaic virus through molecular components of the SA, JA and ET signaling pathways	[59]
18mer peptaibols	T. virens	Elicitation of cucumber systemic defences against P. syringae	[60]
TVHYDII1	T. virens	Involved in colonization of plant roots	

Table 1 Trichoderma elicitors of plant defense and physiological responses

In some cases the elicitors (effectors) were identified in the indicated Trichoderma strain and the pure fungal metabolite was tested on the indicated model plant.

6. MOLECULAR IMPENITENCE OF GENUS *TRICHODERMA*

Understanding the properties of the genus *Trichoderma* at the molecular level would aid in the development of *Trichoderma* strains with excellent biological control properties. Several hundred genus and their products may be involved, giving the genus *Trichoderma* its biological control and plant growth enhancing trait. Hydrolytic enzymes such as glucanase, chitinase and protease are the main players that confer the mycoparasitic properties on *Trichoderma*[32]. It has been reported that 1,4-N-acetylglucosaminidases, endo- and exochitinases from *Trichoderma spp.* which help the fungus improve its biocontrol potential. β -glucanase produced by *Trichoderma spp.* breaks down β-Glucan the structural component of the attacking pathogen. 1,3-glucosidase purified from *T. harzianum* by [53] , which inhibited germ tube stretching and spore germination of Botrytis cinerea. Proteinase from *Trichoderma spp*. is also involved in mycoparasitism. PRA1, a trypsin protease purified from *T. harzianum* CECT 2413 strain, showed nematicidal effects by destroying the hatching eggs of Meloidogyne incognita [54]. Chitinase and protease activity produced by Trichoderma spp. helped control the *Sclerotium rolfsii* pathogen in peanuts [55].

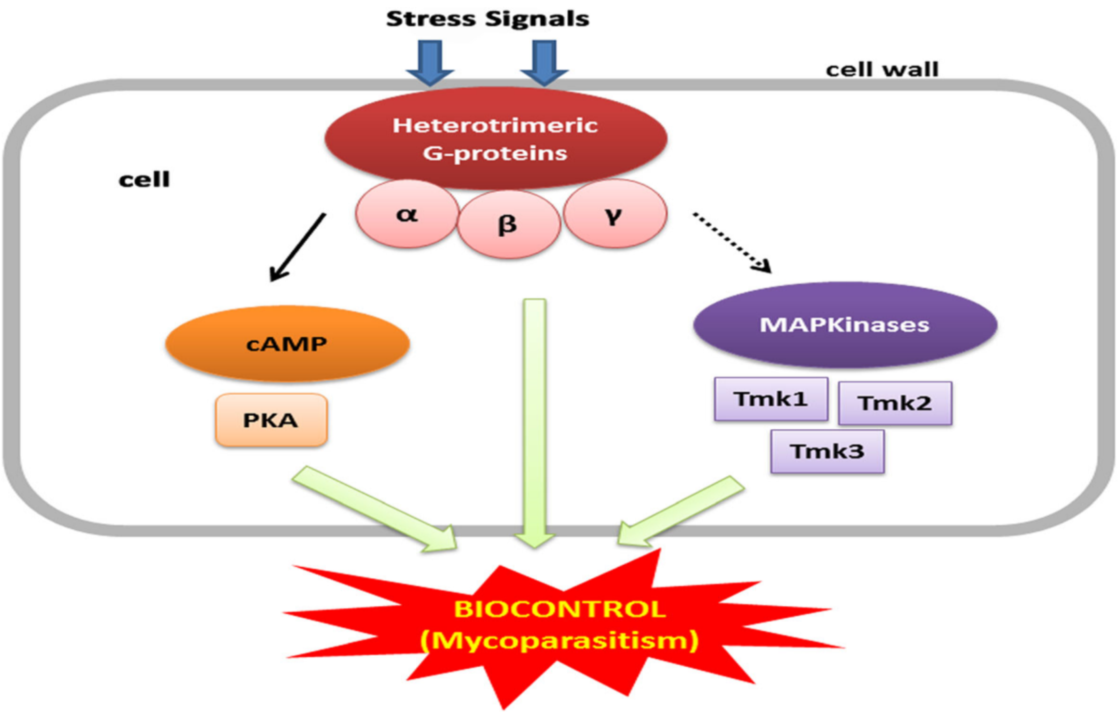
7. SIGNALING PATHWAYS BESMEARED IN THE BIOCONTROL MECHANISM

[56] showed that G protein signaling is fundamentally heterotrimeric and consists of 3 parts: a G protein-coupled receptor (GPCR), a heterotrimeric G protein (a, ß, γ subunits) and an effector. Heterotrimeric G proteins are signal transducers that couple cell surface receptors to cytoplasmic effector proteins and relay the signal to the MAPK and cAMP signaling pathways. In fungi, G proteins help in sexual and pathogenic development, secondary metabolism, and pathogen virulence. The Tga1 subunit Tga1 of T. atrovirid subgroup I was analyzed by overexpression and silencing of the tga1 gene, showing that it is involved in both coiling and state [57]. [58] characterized the Tga1 mutant and showed that the G protein subunit affects processes involved in *Trichoderma* biocontrol, such as the formation of chitinase and the production of antifungal metabolites important in *Trichoderma* biocontrol . Homologues of Tga1 from *T. atroviride*, i.e. H. TgaA and TgaB showed no such effects in *T. virens* and also showed differential effects in controlling pathogenic fungi such as *R. solani* and *S. sclerotiorum* [59] [60] showed that the Tga3 protein of *T. atroviride* is involved in the control of vegetative growth and conidiation and therefore tga3 knockout mutants produced significantly lower levels of intracellular cAMP compared to the parental strain. According to experiments by do[61], intracellular cAMP levels increased several times in T. reesei because it carries an activated allele of subgroup III G protein encoding Tga3 homologue gna3. TBRG-1, which carries the RasGTPase domain, helps in the conidation of *Trichoderma,* but its absence has beneficial effects on the antibiosis mechanism [62].

Mitogen-activated protein kinases in Trichoderma Biocontro - MAPK cascades are evolutionarily conserved in all eukaryotes: they are typically organized in a three-kinase architecture consisting of a MAPK, a MAPK activator (MEK, MKK or MAPK kinase) and a MEK activator (MEK kinase = MEKK or MAPK kinase kinase). In yeast, there are five MAPK genes that transmit signals for mating, filament growth, cell integrity, response to osmotic stress, and ascospore formation [63]. In *Trichoderma*, the best observed MAPKs are yeast and fungal extracellular related kinases (YERK1) and also MAPKs like Fusarium oxysporum Fmk1, Magnaporthe grisea Pmk1, Ustilago maydis Ubc3/Kpp2 or B. cinerea Bmp1[64]. The MAPKs in different species of Trichoderma are more or less similar as described by [65]. According to their findings, there is almost 98% similarity in MAPKs of *T. atroviride* (Tmk1) and *T. virens* (TmkA/Tvk1).

MAPK signaling is important in Trichoderma to induce full systemic resistance in plants. TmkA MAPK loss-of-function mutants of T. virens can colonize the roots of cucumber plants against Pseudomonas syringaepv. lacrymans, but cannot induce systemic resistance to the pathogen [66]. MAPK cascades transmit signals through sequential phosphorylation of kinases. Inactivation of the MAP kinase gene tvk1 in the Trichoderma mutant line showed increased mycoparasitism and reduced growth of the fungus R. solani [67].

cAMP Signalling During *Trichoderma* Biocontrol - cAMP signaling in fungi aids in many cellular processes such as sexual development, virulence, control of differentiation, monitoring of nutritional status, and stress. But the most important function of the cAMP signaling pathway is the regulation of transcription and the cell cycle. The process of sporulation is the main mechanism for the survival and spread of Trichoderma in the environment. These processes are induced by environmental factors. cAMP plays a pivotal role in signaling during sporulation, as described in T. viride and T. atroviride by[68]. [69] observed that in T. reesei cAMP regulates cellulase expression depending on the carbon source. The cel7a and cel6a genes were expressed at higher levels in sporophase, which increased cAMP levels and regulated the secretion of celluloytic enzymes.



representation of signalling pathway involve in mycoparasitism in *Trichoderma*

CONCLUSION

Trichoderma can be an prerequisite component in IPM strategies. A combination of genetic, molecular, and biochemical approaches has led to important new insights into the signaling and transduction processes of secondary metabolites of *Trichoderma* in plants and other organisms. The identification of the plant receptors for *Trichoderma* trigger proteins and effectors that can reprogram a host's genetic machinery can serve as a basis for understanding how these symbionts trigger the host's defense response. The popularity of Trichoderma-based formulations needs to be increased among farmers for an environmentally friendly disease treatment.

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