Journal of Food Legumes
39 (Special issue - NC Pulses 2026): 157-174, 2026
DOI: 10.53550/jfl.v39.ncpspl.333

Review Paper

Trichoderma spp. as a pillar of sustainable pulse production: ecological functions, mechanisms, and field applications

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ABSTRACT

***Trichoderma* spp. are free-living filamentous fungi widely distributed in agricultural, horticultural, and forestry ecosystems and are recognized for their ecological adaptability and multifunctional roles. Although extensively used as biological control agents against a wide range of phytopathogens, the broader potential of *Trichoderma* in sustainable agriculture, particularly in pulse-based production systems, remains relatively underexplored. Beyond disease suppression, *Trichoderma* enhances nutrient-use efficiency, induces plant defense mechanisms, supports organic matter decomposition and bioremediation, and reduces agrochemical pollution, thereby contributing to environmentally sustainable crop production systems. In Pulses based cropping systems, *Trichoderma* spp. facilitate nutrient mobilization through the secretion of organic acids such as citric and fumaric acids, which cause rhizosphere acidification and thereby improve solubilization of phosphorus, magnesium, and essential micronutrients. Soil application of *Trichoderma* significantly alters rhizosphere microbial dynamics, enhances nutrient availability in nutrient-deficient soils, and reduces dependence on synthetic fertilizers and chemical pesticides. Field and controlled-environment studies at the ICAR–Indian Institute of Pulses Research, Kanpur, have demonstrated significant improvements in plant growth, yield attributes, and overall crop health in chickpea and other pulse crops. Systematic research has focused on the exploration, molecular identification, and functional characterization of multi-trait *Trichoderma* isolates from diverse pulse-based agroecosystems. Selected isolates showed strong antagonistic activity against major soil-borne pathogens of pulses. GC–MS-based metabolomic analyses revealed a diverse array of volatile secondary metabolites associated with antifungal activity and plant growth promotion, thereby elucidating key biochemical mechanisms underlying pathogen suppression. Further studies revealed activation of reactive oxygen species (ROS) scavenging pathways, conferring enhanced tolerance to biotic and abiotic stresses, along with improved root system architecture and nutrient uptake efficiency. Promising multi-trait isolates have been registered, and talc-based formulations, DALHANDRRMA, Pulse Booster, Dalhan Bio-Consortia, and Pulse Bio Guard have been developed and evaluated under the All India Coordinated Research Project (AICRP) for on-farm validation and commercialization**

Key words: *Trichoderma*, Legumes, Biocontol, PGP, Phytopathogens, Environment friendly

INTRODUCTION

Pulse crops play a vital role in achieving global targets related to food and nutritional security. The complementation of cereal-based diets with legumes ensures a nutritionally balanced and protein-rich vegetarian diet. However, the productivity of legume crops is severely constrained by multiple biotic and abiotic stresses operating at different crop growth stages, leading to substantial economic losses worldwide (Singh and Pratap 2016). Among these, biotic stresses, particularly diseases and insect pests, are major yield-limiting factors, causing annual yield losses of approximately 30–40% (Dhaliwal *et al.* 2020). In Asia and Africa, leguminous crops may suffer devastating losses of up to 100% under favourable conditions for biotic stress development (Varshney *et al.* 2013, Singh and Singh 2014, Pandey *et al.* 2016, Singh *et al.* 2022, Mishra *et al.* 2025a & b). Chemical pesticides are commonly

employed to manage these stresses; however, their indiscriminate use has resulted in serious environmental contamination and health hazards. Agricultural chemicals are often toxic to humans, plants, domestic animals, and wildlife, rendering them ecologically unacceptable and frequently ineffective in the long term. Consequently, there is an urgent need for sustainable, cost-effective, and eco-friendly biological control strategies to mitigate these challenges.

Among biological control agents, *Trichoderma* species or *Trichoderma*-based formulations are among the most prominent and widely distributed soil fungi, extensively used for the management of a broad range of phytopathogens worldwide (Howell, 2002, Mishra *et al.* 2018, 2020a & b, 2023a & b. 2024 and 2025). The biocontrol potential of *Trichoderma* has been recognized since the early 20th century, following the pioneering work of Weindling (1934). In addition to disease suppression, *Trichoderma* spp. significantly improves soil and plant health by enhancing root architecture, including increased primary and lateral root length, thereby improving nutrient uptake efficiency (Naseby *et al.* 2000, Yedidia *et al.* 2001, Cai *et al.* 2013, Mishra *et al.* 2021).

Trichoderma spp. penetrates the first and second epidermal layers of root tissues and colonize intracellular spaces between the plasma membrane and cell wall, establishing a stable association with host plants. Root colonization by *Trichoderma* promotes seed germination, plant height, root and shoot growth, biomass accumulation, and overall plant vigour. Thus, *Trichoderma* species function not only as effective biocontrol agents but also as potent plant growth promoters (Mishra *et al.* 2020a & b, 2023 a & b, Figure 1).

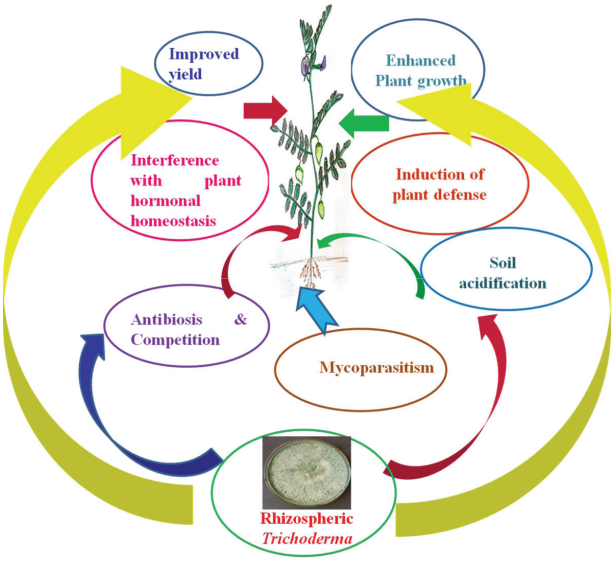

Fig. 1. *Trichoderma*-plant-soil-pathogen interactions

The antagonistic mechanisms employed by *Trichoderma* spp. have been extensively studied and include mycoparasitism, competition for nutrients and space, and the secretion of cell wall–degrading enzymes (Mishra *et al.* 2018, 2023; Praharaj *et al.* 2018; Table 1). Furthermore, *Trichoderma* is recognized as an important plant endophyte that interacts intimately with host plants, effectively colonizing roots and establishing long-term associations (Catska *et al.* 1975, Contreras-Cornejo *et al.* 2009, Mastouri *et al.* 2010, Vishnevetsky *et al.* 2010, Lopes *et al.* 2012).

Overall, *Trichoderma*-based biopreparations represent an effective, eco-friendly, and sustainable strategy for pulse crop protection and productivity enhancement. Research outcomes from ICAR–IIPR, Kanpur, provide robust scientific evidence for integrating *Trichoderma* into climate-resilient pulse production systems with broader applicability across diversified agricultural cropping systems.

SCENARIO OF *TRICHODERMA* RESEARCH IN PULSE

Global scenario

Trichoderma species have been known since 1865, but their taxonomy and identification came into light in 1969. Druzinia and Kubick 2006 have done a lot of work on the biodiversity of *Trichoderma* species (Rifai 1969). *Trichoderma* fungi are difficult to distinguish by morphological classification, so phylogenetic classification has rapidly gained popularity. *Trichoderma* is most commonly used as a biocontrol agent; however, it has adverse effects on immunocompromised mammals and on mushrooms, and these are the disadvantages of this fungus. Use of *Trichoderma* as a biocontrol agent has been exploited lately against phytopathogens (Baker and Cook 1974, Papavizas *et al.* 1982). Unlike many other fungi, *Trichoderma* spores can be used for application directly, which poses the advantage of protection from adverse environmental conditions. *Trichoderma* fungi are well-known antagonists against bacterial and viral pathogens (Table 2). Field performance of *Trichoderma* species against wilt disease complex of chickpea caused by *Fusarium oxysporum f. sp. ciceri* and *Rhizoctonia solani* was evaluated by Mujeebur *et al.* (2014). In *Pisum sativum,* application of *T. harzianum* N47 has been found

Table 1. History and background of *Trichoderma* research

1794	*Trichoderma* Introduced
1928	In India, it was first isolated
1932	First ever evidence of *T. lingorum* (Tode) Harz. (*H. virens*) as a mycoparasite having biocontrol potential against *Rhizoctonia solani* was established
1934	Discovery of gliotoxin as the first antimicrobial compound from Trichoderma species
1939	*Trichoderma viride* Pers. ex Fries, and notes on *Hypocrea*
1969	Species aggregates
1972	*T. harzianum* suppressing *Sclerotium rolfsii* in the field was reported
1979	Superior cellulase production by *T. reesei*, start of the genetic improvement
1983	Cloning of first cellulase of *T. reesei*
1986	Mycoparasitism of *Trichoderma* strains first applications in biological control of plant pathogenic fungi
1989	First commercial formulation of *Trichoderma*, Binab T, for biological control of plant diseases was registered
1991a, b, c	Reported five sections and 27 morphological species
1998	Studied molecular phylogeny based on ITS 1 and 2 (27 species)
2002	Reported multigene phylogeny (46 species)
2003	Reported details of Hypocrea/*Trichoderma* (Ascomycota, Hypocreales, Hypocreaceae): Species with green ascospores
2005	Reported oligonucleotide barcode for species identification and database for verified for type sequences (100 species)
2008	Release of *H. fecorina*/*T. reesei* genome
2012	Reported molecular phylogeny and species delimitation in the section *Longibrachiatum* of *Trichoderma.* Reflects genomes of *T. harzianum, T. asperellum, T. longibrachiatum, T. virens, T. atroviride, T. asperellum, and T. citrinoviride* sequenced using NGS
2015	Draft whole-genome sequence—*Trichoderma harzianum*
2017	Complete genome sequence, repeat-induced point mutation, and partitioning of CAZyme gene clusters— *Trichoderma reesei*
2018	*Trichoderma helicum*, *T. koningiopsis*, *T. pleuroticola* and others under process
2018	Draft whole-genome sequence: *Trichoderma harzianum* species complex
2019	Evolution and comparative genomics of *Trichoderma* species
2020	A new species of *Trichoderma* and gliotoxin role: A new observation in enhancing biocontrol potential of *T. virens* against *Phytophthora capsici* on chili pepper
2021	*T. botryosum* and *T. pseudopyramidale* discovered Evalation of newly identified endophytic fungus *Trichoderma phayaoense* for plant growth promotion and biological control of Gummy stem blight and wilt of mskemelon.Draft whole-genome sequence—*Trichoderma afroharzianum and T.asperellum*
2021-22	Draft whole-genome sequence: *Trichoderma afroharzianum* and *T. asperellum*
2022-23	Talc based formulation of DALHANDERMA (*T. asperellum*) and Pulse Booster (*T. afroharzianum*)
2023-24	Dalhan Bio-consortia

to increase kernel size and root lengths (Naseby *et al.* 2000). Reduction in disease incidence (26%) has been observed by the combined application of *Trichoderma* and FYM (Ebenezar and Yesuraja, 2000). In a study by K. M. Khalequzzaman found that *Trichoderma harzianum* isolate TH-18 inhibits the *S. roflsi* growth by 83.6%. In a study conducted by Naglaa M. El-Benawy *et al.* (2020), in common bean found that *T. atroviride* is capable of inhibiting the mycelial growth of *M. pahseolinna* and *R. soleni*.

Elham Khalili et *al* (2015) conducted a study and found that three *Trichoderma* species are effective in inhibiting the mycelial growth of *M. phaseolina,* causing charcoal rot disease in soybean. Soil application of *Trichoderma harzianum*, *hamatum,* and *viride* is effective in controlling the wilt and root rot disease of pulses. *Trichoderma* species produce various antibiotics like trichodermin, gliotoxin, viridin, CWDEs. These substances inhibit the pathogen mycelial growth (Chet and Baker 1981, Khan *et al.* 2004, 2011). These *Trichoderma* species are effective in controlling the pathogen like *Fusarium, Pythium*, and *Rhizoctonia* (Papavizas *et al.* 1984; Mohiddin *et al.* 2010). Strains of *T. virens, viride,* and *harzianum* are effective in controlling the root rot and wilt pathogens. Lifshitz (1986) observed that *Trichoderma koningii* and *T. polysporum* exhibited limited efficacy in the management of wilt disease. *T.harzianum, T. hamatum,* and *T.viride* are potent in combating the soil-borne pathogens such as *Fusarium, Pythium,* and *Rhizoctonia* (Papavizas *et al.* 1984, Mohiddin *et al.* 2010). Various experiments have been conducted to check the efficacy of *Trichoderma* isolates against *S. sclerotiourum* (Geraldine *et al.* 2013). In a study conducted by Zeng *et al.* (2012)

Table 2. Role of *Trichoderma* in the management of different legume crop disease

Plant name	Disease causing agent	*Trichoderma* species used	References
Chickpea	*Fusarium oxysporum, Rhictonia solani, Aspergillus niger, Chaetomium spp., Sclerotium rolfsii, Penicillum spp. Macropho phaseolina, S. sclerotiorum, Fusarium oxysporum f. sp. ciceri and Rhizoctonia solani, Fusarium oxysporum f. sp., cicero, Ascochyta rabiei*	*T. lignorum, T. virens, T. hamatum, T. harzianum* and *T. pseudokoningii T. asperellum, T. harzianum T. harzianum, T. asperellum, T. harzianum, T. afroharzianum, T. Longibrachiatum, T. aureoviride* T122, *T. harzianum* T66 and T334, and *T. viride* T124 and T228, *T. hamatum and T. koningii, T. asperellum (IIPRTh-31)*	Haware *et al.* 1999; Mujeebur Rahman Khan et al 2014, Shabir-U-Rehman et al 2013, Poveda 2021, Mishra et al 2020a andb
Lentil	*Fusarium oxysporum f.sp.lentis, Macrophomina phaseolina, F. oxysporum*	*T. koningii, T. aureoviride, T.longibrachiatum, T. hamatum*	El-Hassan *et al.* 2013, El-Hassan *et al.* 2013
Cowpea	*Erysiphe flexuosa*	*T.virens*	Omomowo *et al.*2018
Bean	*Rhizoctonia solani, Fusarium oxysporum F. sp. Phaseoli, Rhizoctonia solani, S. Sclerotium F. graminiarum and R. solani*	*T. asperellum T. atroviridae*	Asad SA *et al.* 2014, Jane A. Otadoh *et al.* 2011, Sara Mayo *et al.* 2015, Mischke, 1997 Askew, *et al.* 1994, Júnior *et al.* 2007, Calin M *et al.* 2019
Faba bean	*Fusarium solani, Black Root Rot (Fusarium solani*	*T. asperellum, T. harzianum, T.afroharzianum,T. Longibrachiatum, T. harzianum*	Belay Habtegebriel *et al.* 2016, Eshetu Belete *et al.* 2015
Soybean (*Glycine max (L.*)	*Myrothecium, Anthracnose and Rhizoctonia, M. phaseolina, Colletotrichum truncatum*	*T. lignorum, T. virens, T. hamatum, T. harzianum* and *T. pseudokoningii* (*Rifai*), *T. viride, T. Harzianum, T. koningiopsis*	Kuchlan *et al.* 2017, Silva *et al.* 2020
Cajanus cajan	*F. udum*	*T. harzianum, T. virideT.asperellum,T. longibrachiatum*	Hassan Mir *et al.* 2011, Mishra *et al.* 2023
Peanut	*F. solani*	*T. hazianum ITEM 3636 T. longibrachiatum ITEM 3635*	Rojo *et al.* 2007, Calvet *et al.* 1990
Green bean	*Sclerotium rolfsii, Pythium spp, R. solani, F. oxysporum, F. solani*	*T. hazianum*	El-Mohamedy and Alla 2013
Cowpea	*Erysiphe flexuosa*	*T. harzianum*	Omomowo *et al.* 2018
Mungbean	*M. phaseolina*	*T. harzianum*	Swehla *et al.* 2020
Common bean	*M. Phaseolina, R. solani*	*T. atroviride*	El-Benawy *et al.* 2020

reported that *T.harzianum* T-22 inhibits the disease severity index of *S.clerotina* by 38.5%. Urszula and Baeta (2018) have found that *Trichoderma* spp. are effective in controlling the disease incidence caused by *Sclerotinia sclerotium*. In 2004, Vinale conducted field efficacy trials of *T.harzianum* and *T.atroviride* which improved the growth of lettuce, tomato, and pepper plants. Around 300% increase as compared to the control was observed in *Trichoderma*-treated plants. There are many reports that showed the positive response of *Trichoderma* under field and pot trials (Harman *et al.* 2003). The bio-pesticides h 5% of the total crop protection market globally, which was valued at about $3 billion worldwide (Olson 2015). The majority of *Trichoderma*-based bio-pesticides globally are dominated by *T. harzianum and T. viride* (Gardener and Fravel 2002, Koul 2011) (Figure 2).

Indian scenario

Pulses, commonly known as poor man's meat, are a rich source of proteins, which significantly contribute to the nutritional security of the world. Pulses are grown in many countries like China, India USA, Brazil, Australia, Argentina, Myanmar, Mexico. In India, the pulse production is around 19.3 million tons. India is the largest producer and consumer of pulses in the world. In India, pulses are grown over three seasons- including Kharif (*Cajanus cajan, Vigna mungo, Vigna radiata, Vigna unguicultae Macrotyloma uniflorum, Vigna acontifoila*), Rabi (*Cicer arietinum, Pisum sativum, Phaseolus vulgaris)* and Summer (*Vigna radiata, Vigna mungo,* and *Vigna*

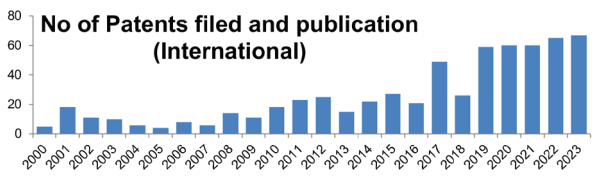

Fig. 2. International growth of *Trichoderma* research

unguiculate). Various biotic factors like, wilt, dry root rot, *Phytophthora* stem blight, collar rot, stem rot, *Aschochyta* blight, Botrytis grey mold, powdery mildew, rust etc., are responsible for the yield loss around 20-100% in different legume crops (Horst 1990). In today's world, the main problem is to meet the nutritional needs of a growing population. Plant diseases are an important factor that reduces crop productivity. It has been estimated that in the United States, soilborne plant pathogens are responsible for about 90% of the 2000 major diseases of the principal crops. Various approaches have been used for the control of soil-borne pathogens, but the use of biocontrol agents is the most effective, economical, and efficient approach (Mishra *et al.* 2021b). In India, available literature clearly showed that significant work has been done for the exploitation of biocontrol agents for the management of soil-borne pathogens. The biocontrol agent *Trichoderma* is not only able to control the phytopathogens but can also improve plant growth. This *Trichoderma* induces seed germination, seedling emergence, and promotes plant growth (Dubey *et al.* 2007, Joshi *et al.* 2010, Mukhopadhyay and Pan 2012, Elham K *et al.* 2016 Nirmalkar *et al.* 2017, Mishra *et al.* 2023). *Trichoderma* species secondary metabolites are capable of inhibiting pathogens' growth and are used for the geographic allocations (Keswani *et al.* 2014). A small group of scientists has tested the efficacy of *Trichoderma* against fungicides so that *Trichoderma* species can be applied in areas where heavy fungicide application has been done (Keswani *et al.* 2014).

In India, only 15 types of bio-pesticides with more than 970 products are registered, and more than 63 Indian private companies, including Pest Control (Pvt) Ltd; Multiplex Biotech Ltd., International Panacea, Biotech International Ltd; etc. have their registered products as bio-pesticides. The microbial pesticides market in India is dominated by the sale of *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus thuringensis* and *Trichoderma* alone shared 52.5 % in the Indian bio-fungicides market in 2015 (Ken research Report 2015). Funding for the research and production of bio pesticides and their promotion is being led by the Department of Biotechnology (DBT) and Indian Council for Agricultural Research (ICAR), who supports 31 biocontrol production facilities respectively in India. For reducing complications in registration, some amendments are made in the Insecticide Act of 1968 for the speedy development of bio-pesticides. In India, the bio-control products are promoted by the government through subsidies, demonstrations, training, etc. and strict quality control protocols are followed to maintain their quality and efficacy. However, the major constraints for popularizing and marketing of bio-control agents (BCAs)/ bio-pesticides in India can be directly correlated with factors such as lack of awareness about the use of bio-pesticides and related products, high cost and time-consuming registration procedures, unreliable quality standards, and product availability. Moreover, the pesticide market is led by unorganized stakeholders and synthetic pesticides are more easily available than bio-pesticides. R&D for the improvement of a particular microbial bio-pesticide by using unique sources (nanosilica, chitosan) will be needed. Development of a consortium of two or more species or strains of *Trichoderma* in addition to liquid formulations is another area for increasing shelf-life and an efficient delivery system at farmer's field. *Surekha et al.* (2013) showed that seed treatment done by *T. viride* enhances the efficacy of *Trichoderma viride* to induce disease resistance and increase disease resistance against *Fusarium* wilt and *Alternaria* in legumes. Anupam *et al.* (2011) conducted a study and found that *Trichoderma* viride isolate Tr8 showed excellent pathogenic activity against *Rhizoctonia solani, Sclerotium rolfsii, Macrophomina phaseolina, Alternaria alternata, Fusarium solani* and *Colletotrichum capsici* of moong bean. Dry root rot, also known as charcoal rot disease, caused by *Macrophomina phaseolina* (Asexual stage *Rhizoctonia bataticola*), is a serious disease of pulses. This disease causes around 10-12% loss in the yield of pulses. For the control of this disease, seed and soil treatment by *Trichoderma* is the most effective method. In a study done by Kumari *et al.* (2012), application of *Trichoderma* with Bavistin and *Trichoderma harzianum* reduced the seedling mortality rate by 5 percent. Seed application of *T. harzianum* with phosphate-solubilizing bacteria can reduce the disease incidence by 26% (Deshmukh *et al.* 2016). Jegathambigai (2010) found that *Trichodrma* isolate inhibits the *S. rolfsi* growth under *in vitro* conditions. Similarly, Shiigan (2008) also tested the efficacy of *Trichoderma* against *S. roflsi* under *in vitro* conditions.

Phytophthora species belongs to Oomycetes, a group of fungi characterized by having a cellulosic cell wall. *Trichoderma* is exclusively used for the management of soil-borne disease. Earlier studies also showed that the integration of bio-agents and fungicides was found to be effective against several diseases caused by Pythiacious fungi,

such as stem and root rot (*Phytophthora vignae*) of cowpea (Fernando and Linderman 1994). In a study conducted by Birendra and Dubey (2020) found that co-inoculation of *Trichoderma hamatum* and *Trichoderma viride* has the potential to reduce *Phytopthora* blight incidence, and it also increased seed germination of plants. Bioagent-based integrated management of *Phytophthora* blight of pigeonpea

T.viride has been exclusively used for the control of *M. phaseolina* in green gram and blackgram (Raguchander *et al.* 1997 and 1998). In pigeonpea seed and soil application of *T.viride* has been found effective for the control of Fu and *M.phaseolina* (Nakkeeran *et al.* 1996). Nakkeeran and Doraisamy 2001 found that seed and soil application of *T.viride* is effective in controlling the *M.phaseolina* disease incidence in cowpea. Similar results were observed by Dinakaran and Marimuthu (1997) in blackgram.

Rudresh *et al.* (2005) reported significant control of wet root rot and Fusarium wilt of chickpea by soil application of *T. harzianum* (PDBCTH) and *T. virens* (PDBCTV12), respectively. Kumar et al. (2008) conducted a study and found that *T. virens* and *harzianum* are effective in controlling the *Rhizoctonia*. *T. harzianum, T. viride,* and *hamatum* are well known for their antagonistic and plant growth-promoting activities. *Trichoderma* species have been extensively used in agriculture for the management of various soil-borne diseases.

In a study conducted by Rudresh *et al.* found that soil application of *T. harizanum* (PDBCTH) and *T. virens* (PDBCTV12) is effective in controlling wet root rot and *Fusarium* wilt of chickpea. Kumar et al. (2008) reported that *Trichoderma virens* was more potent in reducing disease incidence caused bycompared to *T. harzianum*, indicating its superior antagonistic potential against the pathogen. There are various studies that have shown that the application of *T. harzianum, T. viride, and T. virens* is effective in controlling root rot and wilt disease. (Khan and Gupta 1998; Ganesan *et al.* 2007; Kumar *et al.* 2008). Mujeebur *et al.* (2014) conducted a study and found that the effects of *Trichoderma harzianum, T. hamatum, T. viride, T. polysporum, and T. koningii* on the wilt disease complex of chickpea caused by *Fusarium oxysporum f. sp. ciceri* and *Rhizoctonia solani* were investigated under field conditions, and observed that disease incidence of *Foc* and *Rhizoctonia* decreased by 56 and 67%.

Mishra *et al.* (2020a) have tested the efficacy of different isolates of *Trichoderma* spp. against the wilt disease of pulses. Further, he developed an efficient *Trichoderma*-based formulation, Dalhanderma for the management of wilt diseases of pulse crops (Mishra *et al.* 2020b). The efficacy of *Trichoderma* seed treatment is greatly affected by soil pH, moisture, temperature, and density of inoculum (Mathre *et al.* 1994). Bioagents are effective as they colonize plant roots, increase plant root biomass and health and constantly increase yield (Mukhopadhyay *et al.* 2012) (Figures 3, 4).

In ICAR-IIPR Kanpur, a *Trichoderma* library containing 160 *Trichoderma* isolates has been characterized from major pulse-growing states (Uttar Pradesh, Madhya Pradesh, Bihar, Jharkhand, Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, Gujarat, Rajasthan, Telangana, Delhi) of the country. All these 160 isolates belong to the 9 species. Out of these whole genome sequences of two *Trichoderma* isolates, IIPRTh-31 and IIPRTh-33, belonging to *T.asperellum* and *T. afroharzianm* species, have been submitted (JAJAWE010000000 and JAJAWF010000000)

IDENTIFICATION AND CHARACTERIZATION OF *TRICHODERMA* SPP. FROM THE RHIZOSPHERE ENVIRONMENT

Morphological characterization is commonly used as a preliminary method for identifying *Trichoderma* species; however, it lacks precision for accurate species-level identification. *Trichoderma*

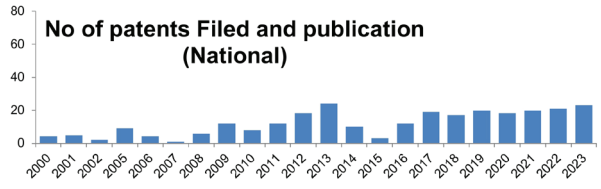

Fig. 3. Growth of *Trichoderma* research in India

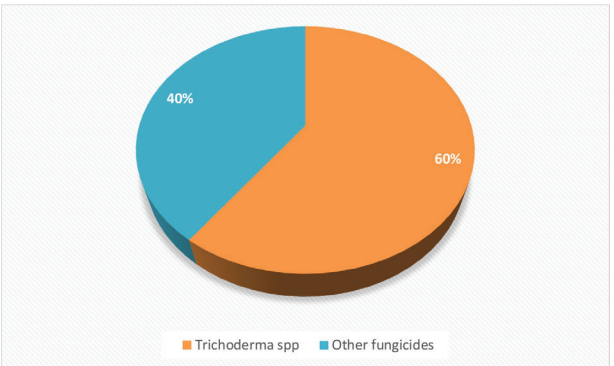

Fig. 4. Share of *Trichoderma* in bio-pesticide market

species are fast-growing with the optimum temperature range of 25-30ºC (Latifian *et al.* 2007). *Trichoderma* species use C and N sources for growth and sporulation. *Trichoderma* species produce a shower of green colored conidia, which is the characteristic of this genus. The conidiophore of *Trichoderma* is not well defined; however, it is generally branched and bears unicellular conidia produced from phialides at the top of its branches. Conidia ellipsoidal, oblong, globose to sub-globose in shape (Bissett *et al.* 2003; Jaklitsch *et al.* 2006). Conidia morphology is different among species, but mostly the colony colour varies from green to gray, white, or yellow (Jaklitsch *et al.* 2006).

For the isolation of *Trichoderma,* various cheaper media like Czapek's Dox Agar, Corn meal Agar, Potato dextrose agar, carrot agar were used. There are some specific media also present; the main reason behind the use of selective media is that it favours the growth of *Trichoderma* over other fungi. So, specific media are being used for the isolation and characterization. The most commonly used specific medium for *Trichoderma* is *Trichoderma* specific medium (TSM), which is commonly used for the isolation and identification of *Trichoderma* species. Apart from TSM, other media used are Rose Bengal Agar and *Trichoderma harzianum.* Selective media used for the specific isolation of *Trichoderma*. The most common method used for the isolation of *Trichoderma* is the serial dilution method. The various methods used for the identification are morphological, cultural, and molecular. For morphological observations, cultures were grown on PDA and observed. For the more complex conidiophore development study *Trichoderma* cultures were used after 3 to 5 days of inoculation. For cultural characterization, *Trichoderma* isolates were grown on different media and incubated at different temperatures (15ºC, 20ºC, 25ºC, 30ºC, 35ºC). After 24 hours of incubation, colonies were examined for the conidiation pattern, odor, and pigmentation presence, colony diameter, mycelia colour, and reverse colony growth.

Identification keys provided by Rifai (1969) and Bissett (2003) were used for the identification of *Trichoderma* cultures and finally for the confirmation that molecular analysis is being done. ITS, RPB-2, and Tef Primers are used for the identification of *Trichoderma* species. Tef- genes are more variable among the *Trichoderma* groups, so these are the best choice to use for the identification of *Trichoderma* spp. (Table 3). After sequencing, the sequenced data were further analyzed by various online available tools like TrichOKEY and TrichoBLAST (Druzhinina *et al.* 2008, Kopchinskiy *et al.* 2005). Sequence data obtained from these genes is used for the identification of *Trichoderma* species.

MECHANISMS OF *TRICHODERMA* TO COMBAT PHYTOPATHOGENS

The mechanisms employed by *Trichoderma* against phytopathogens include: competition, mycoparasitism, antibiosis, induced systemic resistance, and plant growth hormone production.

Table 3. *Trichoderma* species specific primers (László Kredics et al 2018)

T. aggressivum	Th-F: CGGTGACATCTGAAAAGTCGTG Th-R:TGTCACCCGTTCGGATCATCCG
T. pleuroti and *T. pleuroticola*	FPforw1: CACATTCAATTGTGCCCGACGA FPrev1: ACCTGTTAGCACCAGCGC FPforw1: CACATTCAATTGTGCCCGACGA
T. pleuroti	PSrev1: GCGACACAGAGCACGTTGAATC
T. harzianum	HAR-1.6F: GTACCTCGCGAATGCATCTA HAR-1.6R: GGCTATGACCATGATTACGC
T. hamatum	HAM-450F: TTGACACGGTTCTATAATTACCAA HAM-450R: TGACTTAAGTAAGCCGGGTCAAG
T. harzianum	2F2: TGGCTCGTCGTAGTTCGGAGAAG 2R2: CCAGATCGGCCACCGAAGAAAC
T. harzianum	2F2: TGGCTCGTCGTAGTTCGGAGAAG 2R3: GCCACCCACCGCGGGATTCA
T. harzianum	2F2: TGGCTCGTCGTAGTTCGGAGAAG 2R2: CCAGATCGGCCACCGAAGAAAC
T. harzianum	ITS1 S: TACAACTCCCAAACCCAATGTGA ITS1 R: CCGTTGTTGAAAGTTTTGATTCATTT
T. virens	$TvCTT_{56}$f: CTTGATGACAAGCCAAAAGG $TvCTT_{56}$r: GAAGAGAGGACATAGGGTCTGG $TvCAT_{32}$f: GTGTAGCAGCCCAACAGTCC $TvCAT_{32}$r: CAGGTGTCGTGACAGATTCG $TvCTTT_{29}$f GGAAGATAGCACGATGAAGTCG $TvCTTT_{29}$r AACCGTGGAAGTAGGTGTCG $TvCTTTT_{27}$f: TCATCCACCCTGCTAACTCG $TvCTTTT_{27}$r: CGCTGCGTCATCCTAAACC $TvAAC_{21}$f: CACCATTCCATTATTACGCGACG $TvAAC_{21}$r: CTGCACTCCCTCCCAATGC $TvCAG_{13}$f: CCCAGGAAACCCTCAGAACG $TvCAG_{13}$r: TCTTTGCAGTTTCCAAGTCGG $TvGAAA_{34}$f: GGGGTGCTGAATAGCTAACG $TvGAAA_{34}$r: TGCCGTCTTGTCTTATTTTCG $TvTGTC_{18}$f: GTGGTGAGGACTTGCTTGG $TvTGTC_{18}$r: TCTGCCTGTCAGTTGTTTGC $TvGAT_{18}$f: GGGATCTGATTTGGCCTACC $TvGAT_{18}$r: ACTTCCCCCATCCAATAACG $TvCA_{39}$f: GCATCTGCACCTGATATATTCC $TvCA_{39}$r: CCTTGTACGATCTCCAGAACC $TvGTT_{23}$f: GCATCAAAGCGTGCTGTTGG $TvGTT_{23}$r: GCAAACACAAGCTGACAATGC $TvAG_{29}$f: TGTGCCCACTGAGATTTCG $TvAG_{29}$r: TCAGCATGAGATTACACATACCG
T. atroviride	Q01_4F: GCACACCAACTGCTGGAGCTT Q01_4R: CACGCTGACAATGACCGACAC
T. atroviride	X18_3F: AGGCACAGTCCCCTGTTTAGT X18_5R: TGACGATCCTGGTAAGGTTTG
T. atroviride	04_2F: TTACCCAGTGCGGAATCCAAA Z04_2R: TATACGGCGCCTTCCACATTG
T. atroviride	Q01_3F: AAGCAAGGGGGTTGGCAAGTA Q01_3R: GAGAAGGGGTTCCCTGCAGAA
T. atroviride	X18_1F: GACTAGGTGGTCACAGACGAAA X18_3R: GGAAACTCCATCACAAATCCA
T. harzianum	QTh_5F: GGGTTGTTCGGATGGAAG QTh_4R: GTTGGAGATGGGAGGAAGA

Trichoderma species employ these mechanisms alone or in combination to combat the phytopathogens' action (Figure 5). In the present time, 5% of the total biopesticide market is governed with a $3 billion market value. The majority of *Trichoderma* formulations are made up of *T. viride* and *T. harzianum*. *Trichoderma* employs various mechanisms to combat phytopathogen action.

Competition for food and space

Trichoderma species compete with other microorganisms responsible for causing plant disease for food and space. Due to competition, the weaker microorganisms die while the stronger one survived (Harman *et al.* 2004). *Trichoderma* species can produce siderophores, thus they can easily chelate the iron from the environment, while for pathogenic microbes, iron becomes unavailable. *Trichoderma* species also compete for the root exudates; these root exudates play an important role in propagule germination of microbes.

Root colonization and nutrient solubilization

Monocotyledon and dicotyledon plants treated with *Trichoderma* exhibit a high degree of resistance against pathogens (Harman *et al.* 2004). *Trichoderma* root colonization increases the plant's resistance against diseases. Plant colonization by *Trichoderma* alters its metabolic pathway, which alters the level of hormones, soluble sugars, phenolic compounds, and amino acids, the rate of photosynthesis, transpiration, and water content. In various studies, it has been observed that exchange of molecular signals between *Tricoderma* and plants also occurs, which has a high impact on plant disease management and crop yield (Rey *et al.* 2001). Plant colonization by *Trichoderma* increases the uptake of nutrients by different crop plants. It increases the absorption of phosphorus, Iron, magnesium, copper, zinc, and many other micronutrients. The process of solubilization is through the following process: acidification, chelation, redox production, and hydrolysis. *Trichoderma* increases the solubilization of phytase, iron oxide, copper oxide, and metallic zinc. However, magnesium oxide is not absorbed by *Trichoderma*. Gluconic acid and citric acid produced by *Trichoderma* play an important role in the solubilization of phosphates and micronutrients.

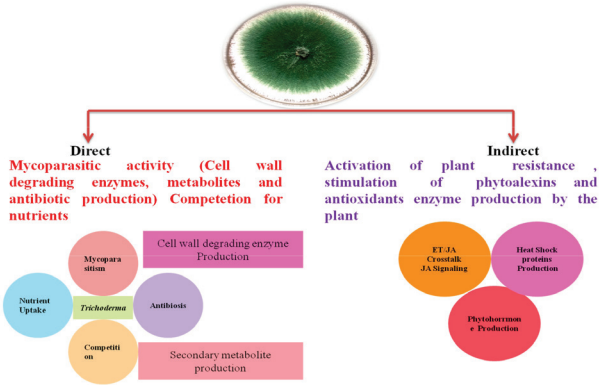

Fig. 5. *Trichoderma* mechanisms employed to control plant pathogens and disease control

Mycoparasitism

Weinding in 1932 first reported the mycoparasitism in *Trichoderma*. Wells et al. (1972) reported coiling of hyphae in *T.lignorum* against *R.soleni* and its death. The process of coiling is very complex; it involves chemotrophic attraction of *Trichoderma* towards the host and coiling around its hyphae and penetration at the contact point. After penetration, cells near the point of contact are lysed, and cells of the pathogen are digested enzymatically by the *Trichoderma*. *Trichoderma* produces a variety of enzymes (β, 1,3 glucanase, chitinase, lipase, and protease) and metabolites and PGP hormones (Table 4). *Trichoderma* species induce systemic resistance in the colonized plant, which triggers disease suppression (Figure 6).

Antibiosis

Trichoderma spp produce a variety of volatile compounds, which are toxic to the pathogenic microorganisms, and these toxic metabolites cause the death of pathogenic microbes. Antibiosis is a major mechanism of biological control in which the antibiotics, lytic enzymes, volatile substances or toxins produced by *Trichoderma* cause the death of the pathogen (Table 5).

Induction of disease resistance by Trichoderma spp.

Trichoderma species express various genes

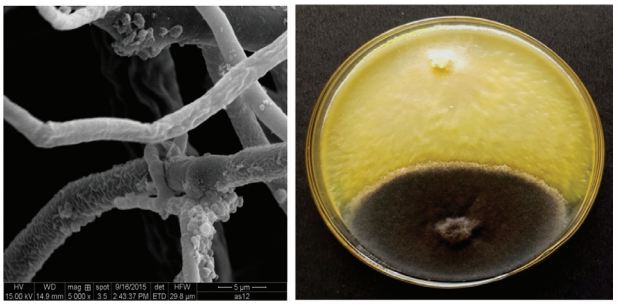

Fig. 6. A-Inhibition of *R.bataticola* by *Trichoderma* isolate B- SEM image of *Trichoderma*

Table 4. Primers used for the specific gene amplification

Chitinase	CHI-F 5'-ATG TTG GGC TTC CTC GGA-3'	
	CHI-R 5'-TTC GGG ATG GTT GTC ATA CTG-3'	
	MCH33f5′-GCTCTAGAATGCCTTCATTGACTGCTCTTGCG3′	
	MCH33r 5′-CGTCTAGATTACCTCAAAGCATTGACAACC-3′	
	CH33Pf5′-GGGTCTCGCATGCCTTCATTGACTGCTCTTGCG-3′ M13F5′-GCTAGTTATTGCTCAGCGG-3′	
	T7 promoter	5′-TAATACGACTCACTATAG-3′
	T7 terminator	5′-CGATCAATAACGAGTCGC-3
ACC deaminase	QAF 5′-CGGGAGGAAGCCGTATTACA-3′	
	QAR 5′-CGACCCTGTCACAGCACAAA-3′	
Xylanase	F1 5′-GCTGAATTCCAGACGATTCAGCCCGGCA-3′	
	R1 5′ ATGCGGCCGCTTAGCTGACGGTGATGGAA-3′	
Cellulose primers	ITS Forward	5′ TCC GTA GGT GAA CCT GCG G 3′
	ITS Reverse	5′ TCC TCC GCT TAT TGA TAT GC 3′
	cbh1 Forward	5′ CAT GTA TCG GAA GTT GGC CGT C 3′
	cbh1 Reverse	5′ GCT TTA CAG GCA CTG AGA GTA GTA AGG 3′
	cbh2 Forward	5′ CAT GAT TGT CGG CAT TCT CAC CAC 3′
	cbh2 Reverse	5′ CCT TAC AGG AAC GAT GGG TTT GC 3′
	bgl1 Forward	5′ GAA ATG CGT TAC CGA ACA GCA GC 3′
	bgl1 Reverse	5′ CTC GCT CCC ACG CTG ATG 3′
	egl4 Forward (qPCR)	5′ GTC ACA GGC ACT GGA GAC C 3′
	egl4 Reverse (qPCR)	5′ GAT CCG TCG CCT GGT AAA G 3′
	cbh1 Forward (qPCR)	5′ GCT ACG ACG GCA ATA CTT GG 3′
	cbh1 Reverse (qPCR)	5′ GTT CTT CGC GCA AGT CTC AT 3′
	bgl1 Forward (qPCR)	5′ CAG GCT ATC CGT CGT TCA A 3′
	bgl1 Reverse (qPCR)	5′ TGA CGT TGG TCT TGT GGT TC 3′
	sar1 Forward (qPCR)	5′ CTC TCC AAG GTT CCC TTC GT 3′
	sar1 Reverse (qPCR)	5′ CAG CTC GTC CTC GGA AAC 3′
	act Forward (qPCR)	5′ GCT GAG CGT GGT TAC ACC TT 3′
	act Reverse (qPCR)	5′ CTT GAT GTC ACG GAC GAT TTC 3′
Actin	Act-R 5 ATGTCAACACGAGCAATGG-3	
	Act-F 5 ATGGTATGGGTCAGAAGGA-3	

responsible for the induction of cell wall-degrading enzymes and growth hormones, which help the plants to fight pathogens. Biopriming of seeds with *Trichoderma* results in increased disease resistance and induction of systemic disease resistance. Alfano *et al.* 2007 have reported that treatment of tomato plants by *Trichodrma* results in increased resistance towards disease. Harman *et al.* 2004a and b found that *Trichoderma* species can induce localized or systemic resistance, which provides increased resistance towards disease. Defence mechanisms of *Trichoderma* have enabled it to be explored as an efficient biocontrol agent. *Trichoderma* species are very useful and popular species in the biofertilizer sector. Metabolites of *Trichoderma* are also used for the biocontrol of phytopathogens. Induction of local and systemic resistance by *Trichoderma* has been reported for both monocots and dicots (Harman *et al.* 2004). The induced resistance is mediated by the jasmonic acid and ethylene pathway and the induction of pathogenesis-related genes. The first report of induced resistance was reported by Bigirimana *et al.* (1977) against *Colletotrichum lindemuthianum* and *Botrytis cinera* causing foliage disease of beans. Yedida et al. (2001) studied induced resistance by *T.harziaznum* against cucumber seedling disease. Indirect evidence of induced systemic resistance by plants was described by Calderon *et al* (1993) through HR reaction. Later chang et al demonstrated the heat-stable mycelial extracts of *T.longibrachatum* to induce disease resistance against *Phytopthora parasitica*.In addition, there are many reports that have shown that *T.harzianum* T39 imparted resistance to leaves of bean plants against *B.cinera*. *Trichoderma* species treatment in plant increase its chlorophyll content, stress enzymes, and phytohorrmones which enable the plants to survive in extremely harsh conditions, providing them with strength (Rawat *et al.* 2011; Zhang *et al*. 2013, Hashem *et al.* 2014).

Trichoderma as a plant growth-promoting agent

Trichoderma use in agriculture is gaining momentum in the present time. Various researches are being carried out to understand the role of *Trichoderma* as a biofertilizer. *Trichoderma*-based biofertilizers are gaining importance because they are chemical-free and do not pose any threat to the environment or humans. *Trichoderma*-based biofertilizers make available the nutrients to the plants and improve soil properties and fertility. *Trichoderma*-enriched biofertilizers are more

Table 5. Secondary metabolites produced by *Trichoderma* species

Trichoderma species	Secondary metabolite	Function	*Trichoderma species*	Secondary metabolite	Function
T. hamatum	Mannitol	Antimutagenic	*T. harzianum*	Trichoharzin	Antifungal, Antimicrobial Plant growth Regulator
T. koningii	Methyl benzoate p-hydroxy benzyl alchohol		*T. longibrachiatum* *T. pseudokoningii*	Compactin	Antifungal
T. pseudokoningii	2-hydroxymalonic acid		*T. harzianum* IMI275950 *T. harzianum* IMI284726 *T. harzianum* ATCC20672 *T. koningii* IMI308475 *Trichoderma* spp *Trichoderma* spp. *T. viride* *T. viride* 0101	6-pentyl-a-pyrone	6-pentyl-a-pyrone
G. virens	Ferulic acid	Antiviral, bactericide, fungicide	*T. harzianum* IMI275950 *T. harzianum* IMI284726 *T. viride*	6-pent-1-enyl-a-pyrone	Antifungal
T. pseudokoningii	2,5-dimethoxybenzoquinone	Cytotoxic	*Trichoderma* spp.	Massoilactone	Antifungal Plant growth Regulator
G. roseum ACC 650	Dihydrocoenzyme Q10 Coenzyme Q10	Cardiotonic	*Trichoderma* spp.	d- decenolactone	Antifungal
T. pseudokoningii	Succinic acid Itaconic acid		*T. harzianum* IMI 311090	Koninginin E	Antifungal Plant growth Regulator
T. album	Pencolide		*T. harzianum* IMI 311090 *T. koningii* ATCC46314	Koninginin D	Antifungal
Trichoderma sp.	Carolic acid		*T. harzianum* IMI 311090 *T. koningii* ATCC 46314	Koninginin B	Antifungal Plant growth Regulator
T. viride ATCC74084	Viridiofungin A Viridiofungin B Viridiofungin C	Antifungal, Squalene synthase inhibitor	*T. harzianum* IMI 311090	Hydroxy koninginin B	Plant growth Regulator
T. pseudokoningii	Methyl-2,4,6- octatriene caarvizykate Trichodermene A		*T. harzianum* IMI 311090 *T. koningii* ATCC46314	Koninginin A	
G. zaleskii	2,4,6,8-nonatetrone- 2,8-bisethyleneketal, 2,3-dihydroxy-5,6-dimethyl Benzoquinone, 2-methoxy-3-hydroxy-5,6-dimethyl benzoquinone	Antibiotic, Phytotoxin	*T. koningii* ATCC46314	Koninginin C	
G. roseum	2,3-dimethoxy-5,6-dimethyl Quinhydrone, 2,3-dimethyl-5,6-dimethoxy- 2,3-dihydro benzoquinone	Antibiotic	*T. harzianum* IMI 311090	Seco-koninginin	
G. roseum CMI 93065 G. roseum	3,5-dihydroxy toluene, 1,2-dimethyl-3,4-dihydroxy Benzene, 2,3-dimethyl-4,6-dihydroxy benzoic acid	Antibiotic	*T. harzianum* IMI 311090 *T. koningii* ATCC 46314 *T. koningii*	Cyclonerodiol	
T. viride PRL 2233 *T. harzianum* IMI 311089	1-hydroxy-3-methyl anthraquionone	Bactericide Bactericide	*T. polysporum* *T. polysporum*	Cyclonerodiol oxide Epicyclonerodiol oxide	Antibiotic Antitumor
T. viride PRL 2233 *T. harzianum* IMI 311089	1,8-dihydroxy-3-methyl anthraquinone	Anticeptic, Viricide, Cytotoxic	*G. virens*	Gliocladic acid	Antibacterial and antibiotic
T. viride PRL 2233	1,6,8-trihydroxy-3-methyl anthraquinone		*G. virens*	Cadlene hydroxy acid	
T. viride	1,3,6,8-tetrahydroxy anthraquinone 1,3,6,8-tetrahydroxy-4-aacetyl anthraquinone	Antibacteria	*G. virens* *T. koningii* M3947	Heptelidic acid	Antifungal
Trichoderma spp.	Trichodermaol	Phosphodiesterase inhibitor	*T. koningii*	Tricho-acorenol, coccinol	K channel Agonist

Trichoderma sp. SC2051	Dimeric xanthone		*G. virens* IFO9166 *T. virens* ATCC74180	3,4-dihydroxycarotane	Mycotoxin
T. longibrachiatum. ATCC2449	Sorbicillin Bisvertinolone Trichodimerol Trichodermolide Sorbiquinol	Inhibitor tumor necrosis factor	*T. viride* *G. flavofuscum* IMI 100714 *G. virens ACC 213* *G. virens GL-21* *T. koningii* *T. viride* *G. flavofuscum IMI* 2-epiviridin *100714*	epiviridin	
G. vermoesenii IMI40231	Nectriapyrone		*G. deliquesc. CMI101523* *G. fimbriatum CMI101525* *T. viride* NRRL 1828 *G. virens* GL-21 *G. virens* ACC 213	Viridiol	
G. vermoesenii IMI40231	Vermopyrone	Antifungal Plant growth regulator	*G. virens*	Virone	Antibiotic
T. harzianum IMI298371	Harzianopyridone	Antifungal	*T. pseudokoningii*	Epifridelenol	Antibiotic
T. harzianum IMI 311092, *T. harzianum* IMI 298371 *T. harzianum*	Harzianolide	Antifungal	*T. hamatum HLX 1379*	Isonitrinic acid F	Antibiotic Immunosuppressive
T. harzianum IMI 311092	Dehydro harzianolide	Antifungal	*T. hamatum* HLX 1360	Dermadin	Antibiotic
T. harzianum SY-307	Harzianic acid	Hypercholesterimic	*T. koningii* TK-1 *T. viride* UC 4875 *T. polysporum*	Dermadin methyl ester	Antibiotic
T. hamatum HLX 1379	Epoxy diol	Antibiotic	*T. hamatum* IMRL 3200	Isonitrin C, trichoviridin	Antibiotic Inhibit melanin Synthesis
T. hamatum HLX 1379	Spirolactone	Antibiotic	*T. koningii* IMRL 3201 *T. hamatum* HLX 1379	Tetrahydroxy isocyanide	Antibiotic
T. hamatum HLX 1379	Diol isocyanide	Antibiotic	*T. harzianum*	MR304A	Antibiotic Induction Of oospores
T. hamatum HLX 1379	Epidiol isocyanide	Antibiotic	*T. harzianum*	Isonitrin D	Antibiotic Induction ofoospores
T. hamatum IMI 3208 *T. hamatum* *T. harzianum* *IMI3198*	Isonitrin A	Antibiotic	*T. koningii*	Homothallin I	Antibiotic
T. koningii TK-163	Isonitrin B,	Antibiotic	*T. harzianum*	Homothallin II	Antibiotic and antifungal
Trichoderma Leo AK 5139	deoxytrichoviridin		*T. koningii*	Amine from Homothallin II	Antibiotic
Trichoderma sp. *T. viride* IFO 8951			*T. koningii*	Formamide from Homothallin II	Antibiotic and antiviral Acetolactate synthase inhibitor, Immunomodulator
T. hamatum HLX 1379	Hydroxy sporolactone	Antibiotic	*T. koningii*	N,N-dimethylamine from homothallin II	Antibiotic
T. album	3-methoxy-5hydroxy-5-allylcyclopentenone	PAF inhibitor	*G. fimbriatum* *G. virens* IMI 101525	Gliotoxin	Antibiotic and Antiviral
G. virens GL-21 *G. lingorum* *G. hamatum* *G. virens* IMI 101525	Gliotoxin E	PAF inhibitor	*G. virens* GL-21 *G. virens* IMI 101525	bisdethiobis(methylthio)glio toxin	
G. virens IMI 101525	Didehydrogliotoxin		*G. virens* IMI 101525	Bisdethiobis (methylthio) didehydrogliotoxin	
G. virens IMI 101525	Bis-N-norgliovictin		*G. deliqescens* *G. virens* IMI 101525	Phenol	
G. virens IMI 101525 *G. deliqescens*	Cyclo-(glycyl-O-3-methylbut-2-enyl-L-tyrosyl	Antibiotic	*G. virens* IMI 101525	3-methylbut-2-enyl ether	

G. deliqescens G. virens IMI 101525	3-hydroxymethylbut-2-enyl ether	Antitumor activity,	*G. virens*	Gliovirin	activity, Immunosuppressive Activity
G. deliqescens		Immunosuppressive activity	*T. koningii*	Cyclo-(L-pro-L-Leu)	Antitumor activity, Immunosuppressive Activity
Gliocladium sp. SCF-1168	Verticillin A		*T. polysporum*	Trichopolyn II	
Gliocladium sp. SCF-1168	Homoverticillin A	Antibiotic	*T. polysporum*	Trichopolyn I	
Gliocladium sp. SCF-1168	Hydroxyhomoverticillin A	Antibiotic	*G. deliqescens*	3-hydroxy-3,4-dimethylpentanoic acid	Melanin biosynthesis Inhibitor
G. deliqescens					
T. harzianum	Uracil		*T. polysporum*	Valinotricin	HIV inhibitor
T. harzianum	Melanoxadin		*Trichoderma* sp. *ATF*	Melanoxazal	HIV inhibitor
T. harzianum	Ceramide	Melanin biosynthesis inhibitor			

effective in increasing micronutrient composition and soil structure and fertility compared to chemical fertilizers.

Trichoderma species are well-known plant growth-promoting agents. Some strains of *Trichoderma* establish long-lasting and effective colonization with plant roots. They produce a variety of compounds that promote plant growth. *Trichoderma* root colonization enhances root development, crop productivity, and resistance towards abiotic and biotic stresses and mineral uptake. *Trichoderma* secretes several lytic enzymes that degrade the pathogen cell wall and increase plant growth. *Trichoderma* chitinolytic system contains five to seven distinct enzymes depending upon the strain (Haran *et al.* 1995). The *Trichoderma* chitinolytic system consists of two ² 1,4 N-acetylglucosaminidases and four endochitinases. The enzymes from *Trichoderma* degrade fungal cell walls. Colonization of *T. gamsii* in the cortical region has been found to have plant growth-promoting and biocontrol abilities. Through induced and acquired systemic resistance mechanisms, it supports plant growth (Shoresh *et al*. 2010). *T. gamsii* has been found to have bioactive compounds. In pulses *T.gamsii* a cold and Ph-tolerant strain, has been found to have growth-promoting traits (Rinu *et al.* 2003). The emphasis of *Trichoderma* research is on the plant growth of crops (Tomato, beans, eggplant, lettuce, pea, radish, pepper, bean, etc.) (Baker 1988, Chang *et al.* 1986, Kleifeld and Chet 1992, Lynch *et al.* 1991, Ousley *et al.* 1993, 1994a, Paulitz *et al.* 1986). For example, Lynch *et al*. (1991). *Tricoderma harzianum* and *T. gamsii* have been found to increase the plant growth in bean plants and also combat the plant disease (Minchin *et al*. 2000).

FORMULATION DEVELOPMENT AND MASS PRODUCTION

The most important point for *Trichoderma* formulation development is its shelf life. Shelf life maintenance of *Trichoderma* is the main issue related to the mass production of *Trichoderma*. For commercialization, the main hurdle is the lack of cost-effective substrates for mass production. Many carrier materials like grain bran, wheat straw, wheat bran, mushroom spent, wheat bran saw dust, sorghum grain, wheat bran saw dust, brewers yeast, lignite, pod pericarp, and stillage for mass multiplication of *Trichoderma* spp have been used to check the spore viability of *Trichoderma*. Different kinds of *Trichoderma* propagules like hyphae, chlamydospores, conidia, etc. are used for the formulation development. Conidia and chlamydospores are highly preferred for the formulation development as they can withstand the adverse environmental conditions (Howell 2003). Liquid formulations are being developed for the multiplication of fungal propagules in soluble materials (Culture broth, vegetable juice etc.). Various adjuvants like CMC, Tween-80, Mannitol, glycerol, etc., are added in the formulations for maintaining the viable spore count and shelf life.

At ICAR-IIPR Kanpur, two talc-based formulations, Dalhanderma and Pulse booster, have been developed (Figure 6). For formulation development, *Trichoderma* spores are used. Technologies, when transferred from the lab to the field they take the form of a product. We all know that *Trichoderma* is very effective for the management of diseases. Thus, the culture of *Trichoderma* should be mixed with certain carriers for the ease of application, storage, commercialization, and field use.

Types of Trichoderma formulation

Different types of *Trichoderma* formulations are being developed using different carrier materials Figure 8. The efficacy of all the developed formulations is different (Vipul et al. 2023), (Table 6).

Talc-based formulation

In Tamil Nadu Agriculture University, Coimbatore, talc based *T.viride* formulation was developed (Jeyarajan *et al.* 1994). *Trichoderma* is mixed with talcum powder in ratio of 1:2. Talc based *Trichoderma* formulation is effective for the management of soil-borne disease. Several private industries produce a large quantity of talc-based *Trichodrma* formulation. According to an estimate, 5000 tonnes of *Trichoderma* formulation is required for meeting the 50% of agricultural land (Jeyarajan 2006). Pradhan et al. (2022) formulated a talc-based preparation of *T. viride* and assessed its effectiveness against Fusarium wilt disease in chickpeas. The results indicated that the talc-based formulation was effective for both seed and soil applications, significantly reducing the occurrence of wilt in chickpeas [Pradhan *et al*. 2022]. Similarly, Mishra

Table 6. Different carrier materials used for the mass production of *Trichoderma*

Substrate	Formulation	Efficacy
Grains (200 g + sugar (1%) + *Trichoderma harzianum*	Cereals (wheat, moong, maize)	12.96%
Vermicompost + cereals + pulses	Vermicompost fortified with *Trichoderma*	Reduction of 10.01% incidence of wilt in chili
Grinded grain + sugar solution (1%) + *Trichoderma harzianum*	Wheat seeds-based formulation	38×10^7 cfu/g
Decomposed cow dung + *Trichoderma* formulation	Cow dung enriched formulation	37.5×10^7 cfu/g
Talcum powder + CMC + *Trichoderma* culture	Talc-based formulation	37×10^7 cfu/g
Partial crushed grain + sugar (1%) solution + distilled water	Sorghum grain	6.1×10^4 cfu/g
Beech, fir, and chestnut + conidial suspensions of *T. atroviride* + distilled water + soy flour	Wood pellets-based formulation	Growth increase by ten-fold
Vermiculite (100 g) + wheat bran (33 g) + fermented *Trichoderma* biomass (20 g) + 0.05N HCl (175 mL)	Vermiculite–wheat bran-based formulation	Growth increase by 5-fold
Oat (20 g) + bentonite (50 mL) + vermiculite + *T. harzianum* + water (60 mL)	Vermiculite bentonite-based formulation	Maintained cfu after 8 weeks
Coffee fruit skin decomposed with cow dung + poultry manure + *T. harzianum* suspension	Coffee husk-based formulation	9×10^{11} to 3×10^{12} cfu/g substrate
Glycerol (1%), PVP (1%), Tween 20 (1%) as an emulsifying agent, $ZnSO_4$ (0.5%) to increase the shelf-life, coconut oil, and distilled water	Oil-based formulation	More than 180 days
Wheat flour (100 g) + fermenter biomass + sterile water (52 mL)	Pesta granules-based formulation	Viable for a long time
Banana waste (chopped 5–6 cm length) + rock phosphate	Banana waste-based	Six months
Trichoderma suspension + sodium alginate (0.6%) solution + $CaCl_2$ (1.5%)	*Trichoderma* sodium alginate encapsulation	Viable for more than six years at room temperature
Wheat flour (80 gm) + kalolin (20 gm) + fermenter biomass (52 mL)	Wheat flour- kaolin	Few months
Liquid formulation (NIPHM medium) *Trichoderma* filtrate (250 mL) + water (750 mL) + glycerol (3%)	T2- liquid formulation (NIPHM medium)	
Fifty grams of soil + rice straw (5 g) + *Trichoderma* biomass (500 mg)	*Trichoderma*-based compost activator	5.3×10^{10} cfu/g
B. subtilis and *T. harzianum* + 5% graphite 80 mesh + 1% silica NPs	Graphite and silica-based formulation	35–54% efficacy (in vitro)
Chitosan-PEG + *T. harzianum* spores + glacial acetic acid (0.1%)	Chitosan-PEG based formulation	More than six months
Sterilized rice powder + dextrose + talc powder + *Trichoderma viride*	Rice powder-based formulation	cfu/g 10×10^9 up to six months at room temperature
T. harzianum filtrate (500 g) + Paraffin oil (500 mL) + CMC (0.2%) + chitosan (0.1%)	Dextrin-based formulation	Efficacy 26.10%; 4.33×10^7 cfu/g) for six months
T. asperellum + paraffin oil	Oil-based liquid formulation	28.67×10^8 cfu/mL for 30 days
Molasses yeast extract (MYE) medium+ glycerol (3%) (V/V) +*T. harzianum* + Talc powder	Glycerol based formulation	Extended the shelf-life for 7 to 12 months
Starch (10%) + copper sulphate (20 ppm) + *T. harzianum*	Paste formulation of *T. harzianum*	11.6×10^{10} cfu/g for 120 days

et al. (2020) developed *Trichoderma asperellum* (IIPRTh-31) based bioformulation (Dalhanderma), which resulted in a notably low incidence of wilt disease, recorded at 25.33%.

Vermiculite wheat bran-based formulation

Trichoderma is also easily grown on molasses-yeast medium for 10 days. 100 g vermiculite and 33 g wheat bran are sterilized in an oven and mixed with 20 g of fermenter biomass and dried in shade (Lewis 1991,Vipul *et al.* 2023).

Pesta granules-based formulation

Trichoderma-based bioreactor biomass is mixed with 100g of wheat flour and mixed to form a dough. Then, 1mm sheets were prepared and air-dried. After air drying, the prepared sheets were rounded and meshed, and granules were collected (Connick *et al.* 1991, Martinez *et al*. 2023, Vipul *et al*. 2023, Wong *et al*. 2019).

Alginate prill-based formulation

25 g of sodium alginate is mixed with 750 ml of water, and 50 g of talcum powder is dissolved in 250 ml of water, autoclaved, and then mixed together. The prepared mixture is then added dropwise into the calcium chloride solution, and spherical beads are formed. Prepared beads were air dried and stored at 5°C (Fravel *et al.* 1985, Sharma et al 2023, Bhai 2020, Sudha *et al*. 2024).

Press mud-based formulation

Press mud is a byproduct of a sugar factory, and it is also used as substrate for the mass multiplication of *Trichoderma*. For the press mud-based *Trichoderma* formulation, 9 days old culture of *Trichoderma* is mixed with 120 kg of press mud. After incubation for 25 days, this culture is mixed with 8 tons of press mud and incubated for 8 days under shade before the application (Sabalpara 2014, Ram *et al*. 2024).

Coffee husk-based formulation

This *Trichoderma* formulation was developed by Sawant and Sawant (1996) in Karnataka. The coffee husk used in this formulation is a byproduct of the coffee industry. This kind of *Trichoderma* formulation is very effective for the management of the Phytophthora pathogen (da Silva Delabona *et al.* 2012, Oancea *et al.* 2017, Chilosi *et al.* 2020).

Oil-based formulations

In this type of *Trichoderma* formulation, *Trichoderma* spores harvested from solid and liquid state fermentaion mixed in diesel, mineral oil, and vegetable oil. Emulsifying agents were also added into the mixture to form a homogenous mixture (Batta 2007, Mane *et al.* 2021, Mbarga *et al.* 2014).

Vermiculite based

Vermiculite is classified as a phyllosilicate mineral within the mica group. It possesses an exceptional capacity for water retention, which is attributed to its interlamellar layers that undergo hydration and dehydration during material processing (Valkov and Simha 2012). The incorporation of *Trichoderma* into vermiculite is straightforward; this is achieved by introducing a concentrated spore suspension in water to the vermiculite and mixing until the desired concentration of colony-forming units (CFU) per gram is attained. Lewis *et al.* (1991) formulated a mixture by combining Terralite® grade 4, a commercially available form of vermiculite,

Fig. 7. Development of talc-based formulation (Dalhanderma and Pulse booster)

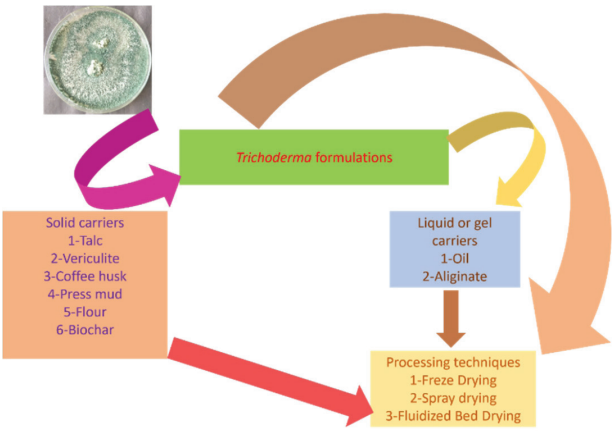

Fig. 8. Common *Trichoderma* carriers and processing techniques

with wheat bran and conidia from various Trichoderma isolates. Their research outlined a method for activating conidial growth, which involves incrementally increasing moisture levels and implementing acidification steps. The study evaluated the impact of both activated and non-activated formulations, alongside storage duration (ranging from 1 to 24 weeks) and temperature conditions (5 or 25 °C), on the overall survival and saprophytic growth of *Rhizoctonia solani*. Results indicated that applying the formulation to soil at a rate of 5% (w/w) significantly diminished the survival of *R. solani*, with enhanced effects observed when the activated formulation was stored at 5°C. In summary, vermiculite-based formulations are cost-effective, inert, and improve soil moisture levels, rendering them commercially viable for formulations of *Trichoderma* spp., whether as primary carriers or as adjuncts.

Biochar based

Biochar is a lightweight and highly porous material generated through the pyrolysis of biomass. Its composition primarily consists of ash and carbon, although the specific final makeup is influenced by the type of biomass utilized. Additionally, the characteristics of biochar are affected by the feedstock and the production methods employed (Lee *et al*. 2018). Common feedstocks for biochar production include corn, rice, fruit peels, agricultural waste wood, sludge, and microalgae (Zhao *et al*. 2019). Numerous studies have demonstrated the beneficial effects of biochar in biocontrol formulations, such as enhancing soil conditions for nutrient availability, thereby promoting plant growth (Biederman and Stanley Harpole 2013). Furthermore, biochar has been shown to increase the population of viable Trichoderma colony-forming units (CFUs) and to diminish the occurrence of diseases caused by phytopathogenic fungi (Muter *et al*. 2017, Akanmu *et al*. 2020). Graber *et al*. (2014) reviewed the existing literature on biochar's effects and proposed potential mechanisms by which biochar may reduce the incidence of pathogenic fungi in plants. It is likely that a combination of factors, including soil pH alkalization, enhanced water retention and nutrient content, improved soil structure, and a more diverse soil microbiome, all play a role in disease suppression. Additionally, some research has indicated a synergistic effect between biochar and Trichoderma species, which further promotes plant growth (de Araujo *et al*. 2019, Sani *et al*. 2020).

Nano-based Trichoderma formulation

Among the primary nanoparticles (NPs) utilized in agriculture, notable examples include silver NPs (AgNPs), zinc oxide NPs (ZnONPs), and copper oxide NPs (CuONPs), which can be produced through physical, chemical, and biological means. Physical and chemical techniques, such as ultraviolet radiation, aerosol technologies, lithography, laser ablation, ultrasonic fields, and photochemical reduction methods, are costly and involve the utilization and release of harmful substances during the process, leading to environmental contamination (Narayanan and Sakthivel 2010, Guilger-Casagrande and Lima 2019). Conversely, the biosynthesis of certain metal NPs offers a straightforward, cost-effective, large-scale, and environmentally friendly option (Vahabi *et al.* 2011, Mishra *et al.* 2014, Saravanakumar *et al.* 2017, Guilger-Casagrande and Lima 2019). Additionally, biosynthesized NPs are reported to exhibit reduced toxicity compared to those obtained through chemical methods due to their stabilization with organic compounds and the absence of toxic byproducts during synthesis (Fraceto *et al.* 2018). Furthermore, these novel eco-friendly synthesis approaches also enhance the biocompatibility of NPs (Guilger-Casagrande and Lima 2019). Trichoderma species are widely present in soil and are recognized as effective biocontrol agents and promoters of plant growth (Contreras-Cornejo *et al*. 2009, Hermosa *et al.* 2012). Moreover, these species have begun to be utilized in nanotechnology, particularly in the production of metal NPs. Trichoderma-mediated mycosynthesis of NPs can be facilitated by enzymes like reductases, which serve as bioreductive agents in the biomanufacturing of NPs (Elegbede *et al.* 2020). The properties and attributes of the various metallic NPs obtained indicate that *Trichoderma* represents a manageable source for the biological synthesis of NPs (Maliszewska *et al.* 2009, Kareem *et al.* 2020). Furthermore, Trichoderma spp. synthesizes nanoparticles that exhibit antimicrobial properties against various microorganisms, particularly phytopathogens like Fusarium, Aspergillus, Pseudomonas, and Xanthomonas. These nanoparticles are enhanced by enzymes, proteins, and secondary metabolites, contributing to their biological control of plant pathogens. It is crucial to optimize the parameters of mycosynthesis and consider the unique characteristics of fungal strains to achieve monodispersity, stability, and biocompatibility of the nanoparticles. Factors such as fungal growth conditions, reagent concentration,

reaction time, initial pH, and temperature play a significant role in controlling the nucleation, size, and shape of metallic nanoparticles. Trichoderma synthesizes a variety of nanoparticles, including gold (AuNPs), ZnONPs, copper (CuNPs) and CuONPs, selenium (SeNPs), and AgNPs, each with distinct characteristics and antimicrobial activities.

SHELF LIFE, QUALITY, AND STORAGE

One of the critical concerns in the commercialization of bio-agents is the decrease in viability of the propagules over time. The shelf life of the bio-control product is influenced by the storage temperature and carriers utilized in the formulation of bio-control agents. The shelf life of bio-control agents is a crucial factor in successful marketing. *Trichoderma* spp. are cultivated on bio-degradable substrates to prolong shelf-life and are also beneficial for field application. Bio-control agents are a biomass product, maintaining their viability at the end of the course (Adekunle *et al*. 2004). Talc-based Trichoderma virens conidia maintain 82% viability at 5°C in a refrigerator after 6 months, while at room temperature, viability was observed for 3 months (Chaube *et al*. 2003). The viable propagule of *Trichoderma* in talc formulation was reduced by 50% after 120 days of storage (Sankar *et al*. 1996). Increasing the shelf life of talc formulations of *Trichoderma* using various ingredients (chitin and glycerol) in the production medium fermentation extended the shelf life of talc formulation of Trichoderma up to 1 year (Sriram 2010, Sriram 2011). *Trichoderma* on coffee husk has a shelf life of more than 18 months. Talc, peat, lignite, and kaolin-based formulations of *Trichoderma* have a shelf life of 3–4 months. In the storage of polypropylene bags using various colors, *Trichoderma viride* showed the maximum shelf life in milky white bags of 100-micron thickness. The Trichoderma fungus in the storage temperatures is less than 4°C. In a study carried out by Patil and Raja in 2022, six different substrates (talcum powder, lignite, charcoal, sawdust, compost, and fly ash) were utilized to assess the shelf life of the most virulent isolates of *Trichoderma harzianum* (AkTr 2GM5) and *Trichoderma hamatum* (AkTm1GM5) mutants for up to 180 days. The highest cfu/g values of 154.50 x 10^6 and 45.50 x 10^6 were achieved at 30 and 180 days after storage in the *Trichoderma harzianum* talc-based formulation, respectively, while the lowest (16.25 x 10^6) was observed in the fly ash formulation. Additionally, *Trichoderma hamatum* talc-based formulations displayed maximum cfu/g values of 156.75 x 10^6 and 41.50 x 10^6 at 30 and 180 days after storage, respectively. Sankar and Jeyarajan (1996) and Bheemaraya et al. (2011) also conducted relevant studies. Sankar and Jeyarajan (1996) noted a 50% reduction in viable propagules of *Trichoderma* in talc formulation after 120 days of storage. Bheemaraya *et al*. (2011) observed a gradual decrease in cfu of Trichoderma from 30 to 180 days. The talc carrier in all packaging materials supported maximum growth and viable propagules required for the recommended concentration (x10^8 cfu/g) until the end of the storage period, with a gradual decline starting from the beginning of the shelf life. The population of the bioagent in the produce plays a crucial role for farmers in determining the quantity and quality of product needed for application in the field. Therefore, the experiment suggests that the bioagent can be stored in vermicompost and talc powder for up to 180 days without any loss of viability. Prasad *et al.* (2002) also stated that formulation in a talc-based carrier retained optimal amounts of viable propagules (>106 cfu/g) even after 180 days of storage at room temperature. The results of the current study are consistent with those of Das et al. (2006), who observed a gradual decline in multiplication and sporulation of *T. harzianum* in talc-based formulation from 30 days onwards.

CONCLUSION

Trichoderma is well known for its biocontrol potential against different plant pathogens worldwide. *Trichoderma* not only helps in biocontrol but also helps in plant growth promotion and high-yield production. The chief mechanisms involved are antibiotics, mycoparasitism, competition for nutrients, and systemic resistance development in plants. Besides reducing diseases and improving plant growth, *Trichoderma* species are also capable of decomposing wastes. In agriculture, the innovations lead to increased agricultural production. However, conventional agricultural practices lead to the destruction of agricultural products. The main challenge present in the modern agriculture system is to tackle the yield loss in an environmentally friendly manner. The interaction between *Trichoderma* and bacteria yields advantages that exceed their individual contributions, positioning them as a viable option for crop management and the control of diseases and pests in contemporary agriculture. Nevertheless, further research is essential to elucidate the specific mechanisms underlying this synergistic effect in particular research areas. While there is a wealth of information regarding their

roles as plant growth enhancers or biocontrol agents against various diseases and pests, there is a notable lack of data concerning their efficacy against viral diseases or their impact on plant resilience to abiotic stresses.

ACKNOWLEDGEMENTS

Authors are highly thankful to Indian Council of Agricultural Research (ICAR) for financial support and encouragement

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