Chapter 10
Non-target Effects of *Trichoderma* on Plants and Soil Microbial Communities

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Abstract Biocontrol agents are currently considered as promising alternative to chemical fungicides because of the latter's negative impacts on consumer health, plant health, and the environment. In the current biopesticide world, *Trichoderma* spp. has been globally accepted to prevent the invasion of pathogens, viz., *Fusarium oxysporum*, *Verticillium dahliae*, *Pythium aphanidermatum*, *Rhizoctonia solani*, etc. The antagonistic activity of *Trichoderma* spp. is attributed to several mechanisms, viz., mycoparasitism, antibiosis, induction of host systemic resistance, and production of hydrolytic enzymes. They not only have plant growth-promoting properties but also exert transient or long-term impact on the resident soil microbiome and may pose risk to beneficial non-target soil communities. Some compounds released by them in higher amount increase the sensitivity of the plant, and may pose negative impact on their growth. Additionally, *Trichoderma* spp. affects microbial community functions. The current chapter summarizes *Trichoderma*-pathogen-plant interaction, and the impact of *Trichoderma* spp. on plant growth, soil enzyme activities, and soil microbiome.

10.1 Introduction

Plant diseases caused by various phytopathogens often considerably deteriorate quantity and quality of agricultural products. Also, the production of toxins by these microbial pathogens during postharvest or field infestation can have severe effects on health of livestock as well as humans (Brimner and Boland 2003). For green revolution, the use of chemical or synthetic pesticides was seen as a substantial measure to control the invasion of plant pathogens. Although these methods for

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managing disease in plants are effective, they are not free from adverse impacts on the environment and human health (Naseby et al. 2000). Furthermore, these chemicals are non-biodegradable and expensive, and repeated use of chemical fungicides is leading toward the development of new resistant pathogenic population (Goldman et al. 1994; Naseby et al. 2000). This has prompted the exploration of some new methods and technologies for disease management to bring down or eradicate the use of chemical pesticides. One such technology is the integration of biocontrol agents that have minimal impact on environment, and potential to control diseases (Chet and Inbar 1994).

Bioinoculants play a major role in controlling plant diseases, with several properties of acting as phytostimulators, biofertilizers, etc. Though these biocontrol agents are considered to be safer as compared to chemical pesticides, their possible impact on environment is related to the application of high densities of viable cells in host plant rhizosphere (Trabelsi and Mhamdi 2013). Also, a large number of inoculants may pose threats to non-target resident soil microbiome (Brimner and Boland 2003), most likely by altering the abundance of other microorganisms or soil enzyme activities.

Trichoderma spp., known for their antagonistic efficacy, are avirulent plant symbionts and opportunistic in nature. Many species of *Trichoderma* have been broadly studied for their potential to reduce disease severity in plant disease by showing inhibition of plant pathogens (Ros et al. 2017). They promote plant growth (Harman 2006) and are tolerant to biotic and abiotic stresses (Hermosa et al. 2012). Nonetheless, direct interactions of antagonistic species of *Trichoderma* or other biocontrol agents are not only restricted to targeting particular phytopathogens but also affect other saprophytic microbes of resident soil community. While biology and potential benefits of *Trichoderma* spp. along with their other aspects of plant growth enhancement have been discussed in several reviews, their non-target impacts have been largely ignored. In recent years, growing interest toward the use of reliable and sustainable biological method has led to the risk assessment of biocontrol agents in terms of their effects on environment and non-target microbiome (Brimner and Boland 2003; Szczepaniak et al. 2015). The present chapter attempts to summarize the non-target effects of *Trichoderma* on plant health, soil enzyme activities, and fungal and bacterial communities.

10.2 *Trichoderma*-Plant-Pathogen Interactions

Trichoderma is one of the most popular commercial biological pesticides. Worldwide, it shares 60% of the total registered biocontrol-based pesticides (Verma et al. 2007). It is a soil-borne microbe and has the capability of penetration and colonization of roots of host plant (Howell 2006). It builds an opportunist/facultative symbiosis with plant, utilizes nutrients from the plant, boosts plant's immunity against phytopathogens, induces host systemic resistance, and enhances plant growth (Mukherjee et al. 2012) (Fig. 10.1). *Trichoderma* evokes synchronized

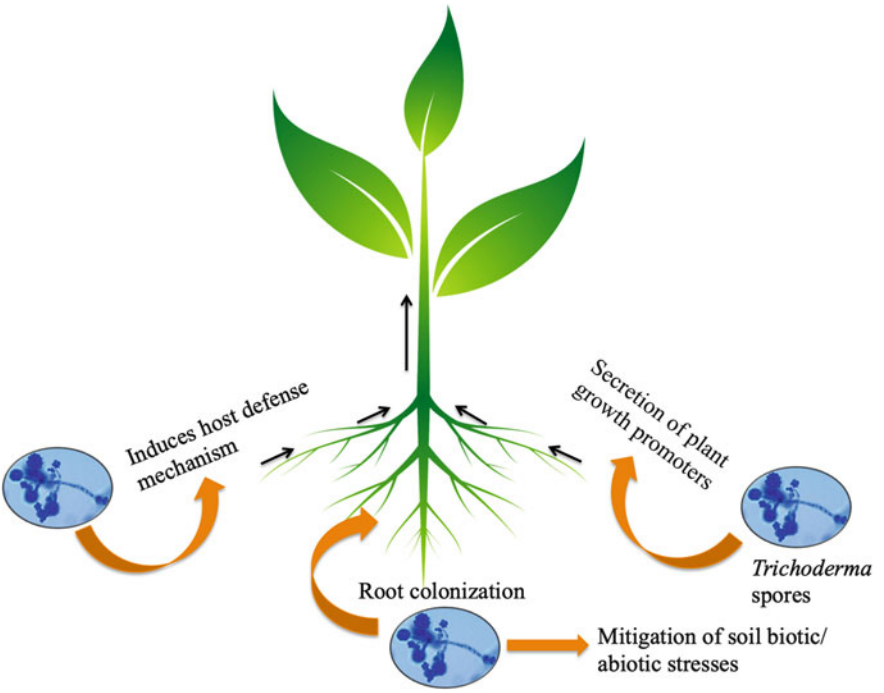

Fig. 10.1 Schematic representation of *Trichoderma*-plant interactions

metabolomic, proteomic and transcriptomic responses in plant system (Shoresh and Harman 2008; Lorito et al. 2010; Morán-Diez et al. 2012).

Among all its characteristics, mycoparasitism is one of the key factors for its antagonistic activity (Morán-Diez et al. 2012). Typically, mycoparasitic interactions between pathogen and *Trichoderma* involve sensing of the pathogen, attraction, attachment, hyphal coiling around pathogen hyphae, and production of lytic enzymes along with secondary metabolites for the lysis. The interactions between *Trichoderma* and plant pathogens are generally beneficial for plant disease management. However, *Trichoderma* exhibits some non-target impacts on soil environment also.

10.3 Non-target Effects on Plant

10.3.1 Effect on Plant Growth Parameters

It is a well-known fact that *Trichoderma* helps in plant growth promotion and enhances soil microbial population, but it also exhibits specific non-target effects. It was over two decades back when Cook et al. (1996) identified that toxicity and

competitive displacement of non-target soil microbes are two chief unintended impacts correlated with the application of biocontrol agents. When a native microbe gets replaced or expelled by biocontrol agent for space and nutrients, this is called competitive displacement. Pathogenicity and toxicity may occur when alkaloids or antibiotics produced by these biocontrol agents start acting against beneficial or nontarget microbes (Brimner and Boland 2003).

Naseby et al. (2000) assessed the potential of some strains of *Trichoderma* for disease control in pea plant against *Pythium ultimum* (IMI 308273). Among all the strains, *Trichoderma* strain To10 showed minor inhibitory effect on plant growth. The production of fungistatic volatile organic compounds, viz., pentenyl and pentylpyrones by *Trichoderma harzianum* (Lumsden et al. 1990), had a negative impact on plant growth at high doses (Naseby et al. 2000). Two native ecosystems of New Zealand were evaluated for non-target effects of bioinoculant *T. atroviride* on plant health of trees (McLean et al. 2014). The application of biocontrol agent significantly modified the levels of photosynthetic pigments in case of some native forest seedlings. Nevertheless, variation in the levels of carotenoids and total chlorophyll was unusual. Generally, in stressed conditions, plants tend to decrease chlorophyll and increase carotenoid content in leaves (Peñuelas and Filella 1998; Sampson et al. 2003). McLean et al. (2014) reported that the addition of biocontrol agent in the rhizospheric soil of *Coprosma robusta* and *Asplenium gracillimum* decreased content of both carotenoids and chlorophyll. However, carotenoid content was increased and decreased with *Plagianthus regius* and *Dacrycarpus dacrydioides*, respectively. Also, there was no significant difference in percent seedling emergence after inoculation of biocontrol agent for each representative species of tussock grassland (*Chionochloa rigida*, *C. rubra*, *Festuca novae-zelandiae*, and *Poa cita*), and podocarp forest (*Pittosporum eugenioides*, *P. regius*, *C. robusta*, *D. dacrydioides*, *Melicytus ramiflorus*, and *A. gracillimum*) except *C. australis* (McLean et al. 2014).

In a study conducted by Hashem et al. (2014), abiotic stress of salt reduced dry weight and length of shoot and root of *Ochradenus baccatus*. The inoculation of *T. hamatum* mitigated the detrimental effect of salt stress and increased dry weight of shoot and root. Seven strains of *Trichoderma* were tested for their ability to enhance plant growth. *T.* sp. "*atroviride* B" LU660 and *T. atroviride* IMI 206040 enhanced fresh weight of *Arabidopsis* by approx. 72% (Nieto-Jacobo et al. 2017). In contrast *T. asperellum* LU1370 exerted significant inhibition of growth of *Arabidopsis* with 74% reduction in biomass over non-inoculated soil, despite a positive effect observed in plate assay. Also, *T. novaeharzianum* LU1328, *T.* sp. "*atroviride* B" LU668, *Trichoderma* sp. LU668, and *T. trixiae* had no significant effect on growth of *Arabidopsis. Trichoderma* spp. is known to produce antibiotics of a diverse range. *T. longibrachiatum* SMF2 produced a peptaibol, trichokonin VI (TK VI), which displayed a dose-dependent effect on growth promotion of *Arabidopsis* seedlings (Shi et al. 2016). Supply of spores in soil at low concentrations ($\leq 5 \times 10^6$ cm^{-3}) enhanced the growth, whereas high dose ($\geq 1 \times 10^7$ cm^{-3}) of SMF2 inhibited the growth of *Arabidopsis* seedlings. It further reduced the number of meristematic cells, compressed cortex cells of the roots, and also inhibited the growth of primary roots.

A mutant Δ*Tpx1*, strain that did not produce TK VI, had no negative impact on seedling growth. The response of *Trichoderma* on plant growth is dependent on its ability of survival and development in the rhizospheric soil of host plant (Kleifeld and Chet 1992), and dose of inoculum.

10.3.2 Effect of Trichoderma *spp. on Soil Microbial Communities*

Rhizosphere of a plant is an unrevealed world with complex soil community structure and functional dynamics, which presents it as a real challenge in soil ecology (Trabelsi and Mhamdi 2013; Shrivastava et al. 2014). Various culture-independent and culture-dependent techniques are used in assessing the change in soil microbial communities. However, the analysis using culture-dependent methods is constricted to restricted samples giving a biased image, whereas culture-independent methods generally do not allow unambiguous identification of taxonomic groups. Some high-throughput sequencing techniques can be used that are more explanatory, but have limited affordability on economical basis (Trabelsi and Mhamdi 2013). Few popular techniques used to evaluate the changes in microbial communities have been listed in Table 10.1.

10.3.2.1 Effect on Bacterial Population

Trichoderma, as a biocontrol agent, has fundamental functions in stimulating the plant beneficial microbiome and inhibiting the invading pathogen through different mechanisms (Saravanakumar et al. 2017). The effect of *Trichoderma* as a bioinoculant cannot be generalized because negative, positive, as well as no effects have been reported in earlier studies. Gupta et al. (2014) assessed the non-target effects of *T. harzianum* MTCC 801 alone and in combination with strains of *Bacillus megaterium* and *Pseudomonas fluorescens* on major bacterial groups (actinomycetes and β-proteobacteria) in rhizosphere of *Cajanus cajan*. They found that *T. harzianum* alone, and in combination with *B. megaterium*, enhanced the population of actinomycetes significantly with time showing maximum abundance at maturity stage of the plant. Furthermore, they assessed the rhizospheric bacterial community profiles using ARISA and reported that the non-target effects of the inoculant (*T. harzianum* alone and *T. harzianum* + *B. megaterium*) were quite evident from a cluster of peaks observed in rhizospheric soil from harvest stage, which was absent in control at the same time point.

Blaya et al. (2013) showed changes in bacterial community of compost after inoculating it with *T. harzianum*. On the basis of DGGE profiles of 16S rRNA as marker, they found that treatment of *T. harzianum* enhanced bacterial community, with six phyla and a group of unclassified bacteria in comparison to three phyla and a

Table 10.1 Selected techniques employed to evaluate the changes in soil microbial communities

Techniques	Description	References
Next-generation sequencing (NGS)	Performs sequencing of entire genome or millions of small fragments of DNA in parallel	Zhu et al. (2018), Ros et al. (2017), Soliman et al. (2017)
Automated ribosomal spacer analysis (ARISA)	Uses a fluorescence-tagged oligonucleotide primer for PCR amplification and for subsequent electrophoresis in an automated system	Ondreičková et al. (2018), Wood et al. (2016), Gupta et al. (2014)
q-PCR (quantitative PCR)	Uses the linearity of DNA amplification to determine relative or absolute quantities of a known gene in a sample	Epelde et al. (2018), Wang et al. (2018), Kleyer et al. (2017), Zhang et al. (2017), Gupta et al. (2014)
Denaturing gradient gel electrophoresis (DGGE)	Separates DNA fragments of identical length but different sequence on the basis of their mobilities under increasingly denaturing conditions	Pacwa-Płociniczak et al. (2018), Wang et al. (2018), McLean et al. (2014)
Diversity and evenness indices, viz., Shannon and Simpson indices	Accounts for diversity, evenness, and richness of microbial communities	Epelde et al. (2018), Pascual et al. (2018), Louis et al. (2016), Blaya et al. (2013)
Community-level physiological profiles (CLPP)	Compares different communities on the basis of sole carbon source utilization patterns gathered using BIOLOG microplates	Amaresan et al. (2018), Epelde et al. (2018), Garcia et al. (2018), Li et al. (2013), Lladó and Baldrian (2017), Frac et al. (2012)
Terminal restriction fragment length polymorphism (T-RFLP)	Uses size of terminal restriction fragment for differentiation of microbial populations	Wu et al. (2015), Araújo et al. (2016), Cordier and Alabouvette (2009)

group of unclassified bacteria of control. In case of compost treated with *T. harzianum*, largest phylum Proteobacteria was 48.7% as compared to 41.2% in control. Cordier and Alabouvette (2009) also assessed the impact of *T. atroviride* on the native resident bacterial community using T-RFLP analysis of 16S rRNA genes. They reported that the inoculation of *T. atroviride*I-1237 significantly increased the bacterial populations only after 3 days of postinoculation. Pang et al. (2017) evaluated the response of resident bacterial community to *Trichoderma*-enriched organic fertilizer. They found that *Trichoderma* treated organic fertilizer showed higher number of operational taxonomic units (OTUs). Moreover, it had the highest Shannon diversity index for bacterial community.

In a study conducted by Naseby et al. (2000), it was found that all the tested strains of *Trichoderma* reduced the total soil bacterial population in the absence of *P. ultimum*; but *Trichoderma* strain TH1 reduced the population of soil bacteria in the presence of *P. ultimum.* However, the presence and absence of *P. ultimum* showed no significant difference in the population of fluorescent *Pseudomonas*, whereas the inoculation of *Trichoderma* strain To10 in the presence of *P. ultimum*

significantly increased the population of *Pseudomonas*. *P. ultimum* had pathogenic effect on plant by triggering nutrient leakage from the roots. The increased nutrient supplies to soil rhizosphere increased the population of soil bacteria including fluorescent *Pseudomonas*. Only strain TH1 was capable of lowering this effect of *P. ultimum* and hence reduced the bacterial population in its presence. It indicates that in the presence of *Trichoderma* strain, TH1 *P. ultimum* caused less damage to the roots of the host plant. However, the reduction of soil bacterial population in the absence of *P. ultimum* may be due to nutrient deficiency caused by inoculum of *Trichoderma* and rhizospheric soil fungal community that utilized a major portion of the soil niche (Naseby et al. 2000). In a study on PAH biodegradation, *Trichoderma viride* was inoculated together with bacterial consortium of 195 species (Szczepaniak et al. 2015). After the process of 12 months of biodegradation, only 73 bacterial species were identified from the consortium. *T. viride* proved to exert antagonistic effect on the bacterial consortium. However, the reason for this inhibitory effect was unexplained.

T. harzianum MTCC 5179 was inoculated in the rhizosphere of black pepper (*Piper nigrum* L.) for evaluating its impact on the population, and functional dynamics of soil microbiome (Umadevi et al. 2018). The α-diversity of the metagenome was 455,862 and 489,569 species for control and treatment (*T. harzianum* inoculated), respectively. Upon analyzing the relative abundance, ten most abundant bacterial species in treatment were found to be *Candidatus koribacter versatilis*, *Acidobacteriaceae bacterium* KBS 96, *Ktedonobacter racemifer*, *Pedosphaera parvula*, *Candidatus solibacterusitatus*, *Sphingomonas* sp., *Chthonomonas calidirosea*, *Pyrinomonas methylalipathogens*, *Gemmatimonadetes* bacterium, and uncultured bacteria (*C. koribacterversatilis* and *Acidobacteriaceae bacterium*), whereas in control sample uncultured bacteria were more. The abundance of these bacteria indicated that the bioinoculant imparts rhizospheric competency to bacteria to colonize the roots of the host plant. Functional-level dynamics suggested that the rhizosphere in treatment sample (with inoculation of *T. harzianum*) had more abundant reads for disease, virulence and defense, metabolism and ion acquisition, and chemotaxis and motility as compared to control sample. Gasoni et al. (2008) studied the effect of *T. harzianum* on functional diversity of soil microbial community after inoculating in rhizospheric soil of tobacco plant. They observed that control soil showed high bioactivity with higher Yokoyama CLPP diversity index (DI, 4.41) as compared to treated soil (DI, 3.68). However, *T. harzianum* showed significant changes in the metabolic profiles. Also, the application of *T. harzianum* resulted in less number of metabolized compounds, whereas absorbance was much higher for a specific set of compounds in control. This indicated that the inoculation of *T. harzianum* contributed to growth stimulation of a specific soil bacterial population that altered the microbial community of the host rhizosphere (Gasoni et al. 2008).

10.3.2.2 Effect on Fungal Community

Significant increase in the fungal population of rhizospheric soil was found upon inoculation of *Trichoderma* TH1, T4, and T12 in the absence of *P. ultimum* (Naseby et al. 2000). This increased fungal population utilizing higher amount of nutrients explained well the observed reduction of bacterial soil population in the absence of pathogen. *Trichoderma koningii* Oudemans reduced the germination of resting spores of arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* (McAllister et al. 1994; Brimner and Boland 2003). Also, the volatiles produced by *T. koningii* reduced germination of *G. mosseae*. However, these volatiles had no effect on fungal mycelial growth of *G. mosseae* depicting that these compounds affected only the spores of resting stage. Inoculation of *Trichoderma* on mycorrhizal maize plants decreased population of *Azospirillum* in soil (Vázquez et al. 2000). However, this was observed only in natural AMF-mycorrhizal plants as compared to non-mycorrhizal control. Umadevi et al. (2018) analyzed the relative abundance of fungal species in the rhizospheric soil of black pepper (*Piper nigrum* L.). Upon treatment with *T. harzianum* MTCC 5179, most abundant fungi were observed to be *Fusarium oxysporum*, *Rhizophagus irregularis*, *Oidiodendron maius*, *Talaromyces stipitatus*, *Pseudogymnoascus pannorum*, *Pestalotiopsis fici*, *T. harzianum*, and *Mortierella verticillata*. *P. fici*, *T. stipitatus*, and *F. oxysporum* were abundant in treatment, whereas *R. irregularis* and *P. pannorum* (a human pathogen) and *O. maius* were higher in control sample. It depicted that the application of *T. harzianum* reduced effect of human pathogen in the amended soil, as compared to control (Umadevi et al. 2018).

Cordier and Alabouvette (2009) assessed the impact of *T. atroviride* I-1237 on native fungal soil community. They found that the inoculation of *T. atroviride* I-1237 resulted in a significant increase in the density of fungal community after 3 days of inoculation. However, T-RFLP analysis revealed that structure of fungal community evolved in a similar manner in both control and inoculated soil. Li et al. (2013) evaluated the impact of *T. longibrachiatum* T2 on functional diversity of rhizospheric soil microbiome with AWCD variations. They found that within 48 h of inoculation, the capacity of microbial community to utilize carbon source was highest in rhizospheric soil of treatment followed by non-rhizospheric soil of treatment and control. Moreover, the functional diversity index (Shannon index) was also highest in case of rhizospheric soil of treatment that depicted an enhancement in the richness of the soil microbiome. Pang et al. (2017) found higher number of OTUs in fungal community with *Trichoderma*-treated organic fertilizer. In addition, they found high diversity index for fungal community in *Trichoderma*-treated samples, as compared to chemical fertilizer and organic fertilizer.

Trichoderma-fortified compost was applied to pepper seedlings infected by *Phytophthora nicotianae* (Ros et al. 2017). Two successful techniques, viz., quantitative PCR and NGS, were used to evaluate the effect of *Trichoderma*-fortified compost on rhizospheric fungal and bacterial populations. The bacterial population showed no effect upon application of *Trichoderma* strain, whereas it was altered in

response to the pathogen. However, fungal population was independent of pathogen and inoculation of *T. harzianum*, but substrate impacted the same. In another study, it was observed that the addition of *T. atroviride* promoted arbuscular mycorrhizal (AM) spore density in the rhizospheric soil of host plant *Dacrycarpus dacrydioides* in comparison to control sample (McLean et al. 2014). DGGE analysis suggested that the biocontrol agent had no effect on the genetic diversity of AM soil population. This indicated that *T. atroviride* possibly contributed to proliferation of existing AM population, but not in enhancing diversity.

10.3.3 Effect on Soil Enzyme Activities

In addition to quantitative and qualitative alteration exhibited in population of soil microbial communities, another factor for evaluating the changes in the soil system is soil enzymes (Vázquez et al. 2000). Soil enzyme activities have been used as indicators to evaluate the impact of bioinoculants and can be used for gaining a better understanding of natural agitations caused to the ecological system (Naseby and Lynch 1998). The soil inoculated with *P. ultimum* showed high activity of soil enzymes as compared to control that indicated a significant increase in leakage of carbon and other nutrients from the roots due to the damage caused by pathogen (Naseby et al. 2000). *Trichoderma* strains TH1, T4, T12, and N47 substantially decreased NAGase, β-glucosidase, and chitobiosidase activities that are related to disease control (Naseby et al. 2000). Reduced activity of alkaline phosphatase was found with *Trichoderma* strains T4, T12, and TH1 that was relative to *P. ultimum* control. Activity of acid phosphatase and cellobiosidase could not be affected by any of the strains. The varying levels of reduction in soil enzyme activities signified the lowering of pathogenic effect. The application of *Trichoderma* in the rhizosphere of plants affected the soil enzyme activity. Inoculation of *Trichoderma* in AMF-colonized plants showed reduction (89%) in phosphatase activity, whereas evident increase (188%) was observed on inoculation in *G. deserticola*-colonized plants (Vázquez et al. 2000). *Trichoderma* also increased (121%) the phosphatase activity in the rhizospheric soil of *G. mosseae*-colonized plants. Significant increase in chitinase activity was found on inoculating *Trichoderma* in soil of natural AMF-colonized plants and non-mycorrhizal plant (121% and 151%). However, it significantly reduced (47%) the activity of trehalase enzyme.

10.4 Conclusion and Future Prospects

Typically, inoculation of *Trichoderma* spp. can be an environment friendly approach for disease management as they impart less lethal effects on plant, soil, and human health as compared to synthetic pesticides. However, they are not entirely free of risks to non-target species. Volatiles, toxins, and antibiotics produced by *Trichoderma* spp.

might affect not only pathogenic species but also beneficial microbes. As per some reports, the intensity of such effects depends on the time of inoculation of biocontrol agents and concentration of the toxins secreted. There is competition for nutrients that might also be responsible for alteration in the population of soil microbiome. Soil enzyme activities are considered indicators for abiotic or biotic stresses, where the presence of pathogen increases their levels. Normally, addition of biocontrol agents have reported to decrease the biotic stress of pathogen by lowering enzyme activities. But, it has some non-target impacts due to which *Trichoderma* spp. has also been observed to increase enzyme activities. It is challenging to monitor the effect of a bioinoculant on non-target soil microbial communities in rhizosphere and to understand the functioning of a biological system. Still, there is a need for future research to assess the ecological effects correlated with the application of biocontrol agents in the soil, mechanisms responsible for their non-target impacts, and development of methods for determination of these impacts.

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