



# Combined use of *Trichoderma* and beneficial bacteria (mainly *Bacillus* and *Pseudomonas*): Development of microbial synergistic bio-inoculants in sustainable agriculture

Jorge Poveda<sup>a,c,\*</sup>, Daniel Eugui<sup>b,c</sup>

<sup>a</sup> Department of Plant Production and Forest Resources, Higher Technical School of Agricultural Engineering of Palencia, University Institute for Research in Sustainable Forest Management (iuFOR), University of Valladolid, Avda. Madrid 57, 34004 Palencia, Spain

<sup>b</sup> Delso Fertilizantes Holding, Madrid, Spain

<sup>c</sup> Universidad Pública de Navarra (UPNA), Pamplona, Spain

## HIGHLIGHTS

- *Trichoderma*-bacteria co-inoculations have a synergistic effect on plant benefits.
- *Trichoderma*-bacteria biocontrollers have similar results than chemical pesticides.
- Compatibility and formulation are key steps in *Trichoderma*-bacteria co-inoculants.
- More studies are needed in *Trichoderma*-bacteria effects on abiotic stress in plants.

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## ABSTRACT

Agriculture nowadays is facing many challenges, with among the most important to be able to feed the increasing human population through more sustainable and environmentally friendly production. In this context, the use of microorganisms has been extensively studied, both with fungi such as *Trichoderma* spp. and with bacteria, such as *Bacillus* spp. or *Pseudomonas* spp. While inoculation with these microorganisms has a positive effect on crops, their combination offers even greater potential as plant growth promoters and as biocontrol agents, with diverse mechanisms that are thoroughly considered in this review. Synergies between *Trichoderma* and bacteria cause more benefits than the sum of their parts, and this makes them a promising alternative for managing crops and controlling diseases or pests in modern agriculture. However, more studies are needed to determine the specific mechanisms of this synergistic effect in certain lines of research, since there is extensive data about their use as plant growth promoters or biocontrol agents against diseases and certain pests, but little or no information is available about their use against diseases caused by viruses or the effect on plant tolerance to abiotic stresses.

## 1. Introduction

Actual growth calculations have projected that world population will increase from 7.4 billion in 2017 to 9.7–10 billion by 2050 (Fukase & Martin, 2020). One of the biggest problems we face is how to meet the increasing demand for food (Fukase & Martin, 2020; van Dijk et al., 2021). In a meta-analysis carried out on 57 global food security projection and quantitative scenario studies, it was determined that global food demand is expected to increase by 35 % to 56 % between 2010 and

2050 (van Dijk et al., 2021). The primary sector, the basis of food production, is currently located in rural areas, where the population is continuously decreasing. It is estimated that in 2050 about 70 % of the global population will live in cities or megacities (with 10 million or more inhabitants) (Knorr et al., 2018).

The agricultural advances of the 1930s have made it possible to feed an exponentially growing world population, achieving more calorie production *per capita* than was ever available before in history (Ramanakutty et al., 2018). However, this agricultural development has come

\* Corresponding author at: Department of Plant Production and Forest Resources, Higher Technical School of Agricultural Engineering of Palencia, University Institute for Research in Sustainable Forest Management (iuFOR), University of Valladolid, Avda. Madrid 57, 34004 Palencia, Spain.

E-mail address: [jorge.poveda@uva.es](mailto:jorge.poveda@uva.es) (J. Poveda).

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at a huge environmental cost, and agriculture is now a major cause of global environmental degradation (Kopittke et al., 2019). This is a consequence of the intensification of crops and the massive use of agricultural chemicals (fertilizers and pesticides), causing the loss of soil organic matter, soil erosion, release of greenhouse gases, pollution, acidification and salinization of soils and waters, and loss of genetic diversity; the damage is now irreparable in some places on the planet (Ramankutty et al., 2018; Kopittke et al., 2019). Therefore, the development of a sustainable agriculture system that can feed future generations requires the development of new biological strategies that respect the environment and health (Jhariya et al., 2019).

Sustainable agriculture must solve three fundamental problems in order to increase crop productivity and feed the world population: the supply of nutrients to plants, increasing tolerance to abiotic stresses, and reducing losses caused by pathogens and pests in crops and post-harvest management (Roberts & Mattoo, 2018). Plants need to acquire a large amount of primary macronutrients (nitrogen (N), phosphorus (P), and potassium (K)) and micronutrients from the soil to maintain their genetic production potential. A continuous external supply of nutrients to agricultural soils is necessary in the form of chemical fertilizers or through the use of organic and/or biological strategies. The inappropriate use of nutrients leads to a decrease in the efficiency of agriculture and carries additional costs related to the deterioration of the environment (Mironiuk & Izdorczyk, 2022). As far as abiotic stresses are concerned, agricultural productivity is vulnerable to different environmental and physical-chemical factors, such as drought, floods, extreme temperatures, radiation, salinity, nutrient deficiencies, extreme pH, and chemical contaminants. These stresses are major challenges for the production of crops, meaning that only 9 % of the world's agricultural land can be used for the establishment of crops, as the remaining 91 % are subject to different abiotic stresses. Although agricultural losses caused by abiotic stresses are currently estimated at 50 % of agricultural production, the current climate change scenario is continually increasing this percentage (Minhas et al., 2017). On the other hand, biotic stresses in plants are caused by pests (mainly insects and mites) and pathogens (viruses, bacteria, fungi, oomycetes, and nematodes) (Gimenez et al., 2018). It is estimated that pests reduce global agricultural productivity by between 18 and 25 % per year (Poveda, 2021), while pathogens are directly responsible for losses of between 10 and 15 % (Mohammad-Razdari et al., 2022).

Microorganisms play a fundamental role in the agrosystem. Endophytic, epiphytic, and rhizospheric microorganisms may form mutualistic relationships with plants, improving agricultural productivity through direct nutrient supply, production of plant-growth-promoting substances, increasing plant tolerance to abiotic stresses, direct biopesticide action against biotic stresses, and induction of plant defenses (Umesha et al., 2018; Yadav et al., 2020). Modifying plant and soil microbiota is essential to stimulate beneficial symbiotic relationships in agriculture. The exogenous application of microorganisms modifies, in a directed way, the populations of beneficial microorganisms present in the agrosystem (Kaul et al., 2021). According to European Union legislation (2019/1009), microorganisms used in agriculture are divided into two large groups: biostimulants and biopesticides (Poveda & González-Andrés, 2021). Microbial biostimulants, including biofertilizers from other world legislations, are microorganisms that are capable of directly providing nutrients to plants, improving access to them, or increasing tolerance to abiotic stresses (European Council, 2019). Microbial biopesticides include microorganisms that protect the crop from pests or diseases, either directly or indirectly (Poveda & González-Andrés, 2021; Poveda et al., 2022).

Microorganisms can be applied exogenously in the agrosystem, improving their viability and functions as much as possible, through the development of bio-inoculants. This line of research has been of great interest in the last decade, as suggested by the publication of the books *Microbial Inoculants in Sustainable Agricultural Productivity* (Vol. 1: *Research Perspectives*, and Vol. 2: *Functional Applications*) in 2016 (Singh

et al., 2016a, 2016b) and the Research Topic in the journal *Frontiers in Plant Science* "Biostimulants in Agriculture" in 2020 (Rouphael & Colla, 2020). The main bio-inoculants used in agriculture include biostimulants, such as plant-growth-promoting rhizobacteria (PGPR), and biological control agents (BCAs), such as *Bacillus thuringiensis* and *Trichoderma* spp. (Qiu et al., 2019). However, although the use of microbial bio-inoculants in agriculture can contribute to meeting current and future production demands, it is essential to develop formulations that allow microorganisms to survive in new environments and successfully colonize soil and/or plant tissues (Qiu et al., 2019).

Currently, the formulation of microbial bio-inoculants includes liquid formulations (cell suspensions in water with a surfactant), solid formulations (carriers such as peat, charcoal, bagasses, vermiculite, or lignite), polymeric formulations (with carriers such as alginate, chitosan, agar, pectin, bean gum, or carrageenan), and metabolite formulations (including only the microbial metabolites of interest) (Chaudhary et al., 2020; Chaudhary & Shukla, 2020). In addition to the "vehicle" formulation, microbial bio-inoculants must be encapsulated by a protective capsule or shell of synthetic and polysaccharide polymers using different techniques, such as spray drying, coacervation, or gel dissolving techniques (Chaudhary et al., 2020; Chaudhary & Shukla, 2020). The use of different microorganisms in the formulation of bio-inoculants is called co-inoculation, and it represents important improvements. The functions of some microorganisms can supplement the deficiencies of others; for example, avoiding dependence on an exogenous nitrogen supply. The use of various microorganisms can also have synergistic effects on plants (Chaudhary et al., 2020). The development of genomics techniques and knowledge in recent years has made it possible to determine and use the genetic potential of microorganisms in research, selecting the most suitable ones, and identifying the genetic and molecular mechanisms (Wang & Haney, 2020).

The objective of this review was to compile all existing studies to date where beneficial bacteria were co-inoculated with *Trichoderma*, in order to identify and discuss the positive effects, the mechanisms, and the possible problems. The development of combined *Trichoderma*-bacteria bio-inoculants may be a good strategy to develop within sustainable agriculture.

## 2. *Trichoderma* bio-inoculants in agriculture

*Trichoderma* is a filamentous fungi genus that includes 260 species, of which 35 have economic importance as BCAs in agriculture, or as producers of enzymes and antibiotics in industry (Sharma et al., 2019). Fungi within the *Trichoderma* genus are characterized by being present in the vast majority of ecosystems due to their rapid growth and tolerance to different abiotic stresses (Khan & Mohiddin, 2018). In its interaction with plants, *Trichoderma* can live as a rhizospheric, epiphytic, or endophytic microorganism, without ever colonizing the vascular bundles (Poveda et al., 2020a). Both in the laboratory and industry, *Trichoderma* is easily grown on different substrates, producing large amounts of green conidia (Khan & Mohiddin, 2018). In its use in agriculture, *Trichoderma* is capable of promoting plant growth, increasing plant tolerance to abiotic stresses, and acting as a direct and indirect BCA, and is one of the groups of microorganisms with the greatest agricultural and scientific potential in recent decades (Guzmán-Guzmán et al., 2019; Zin & Badaluddin, 2020).

In recent years, different species within the *Trichoderma* genus have become of great interest as plant-growth-promoting fungi (PGPF). Their capacity as PGPF is directly related to their production of siderophores, phosphate-solubilizing enzymes, plant-growth-promoting enzymes such as 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase), and phytohormones, mainly indole acetic acid (IAA) and cytokinins (CKs) (Viterbo et al., 2010; El Enshasy et al., 2020). *Trichoderma* is capable of promoting the growth and yield of crops, such as cereals (Mahato et al., 2018), oilseeds (Poveda et al., 2019), and vegetables (Fiorentino et al., 2018), and also improving the content in phyto-

substances of nutraceutical interest in derived plant products (Velasco et al., 2021).

As far as abiotic stresses are concerned, the use of *Trichoderma* as a biostimulant in agriculture represents the least studied function of this group of fungi, compared to its use as a BCA or PGPF. Despite this, the study of *Trichoderma*'s ability to increase plant tolerance to abiotic stresses has continued (Zaidi et al., 2014; Hidangmayum & Dwivedi, 2018). By colonizing the roots, *Trichoderma* induces local and systemic expression of abiotic stress tolerance-related genes (abscisic acid and ethylene-related genes), which causes better plant responses under stress situations (Poveda, 2020). These plant responses include better photosynthetic performance, higher pigment concentration, higher proline content, induction of lateral root development, oxidative inhibition reduction, heat-shock protein production, lipid peroxidation rate reduction and electrolyte leakage, increased antioxidant enzymes, and increase of plant tolerance to drought, salinity, extreme temperatures, or anthropogenic pollution conditions (Zaidi et al., 2014; Hidangmayum & Dwivedi, 2018).

The use of *Trichoderma* species as BCAs was initially described in the 1930s against the phytopathogenic fungus *Rhizoctonia solani*, with this being the main function for which this group of fungi is studied and used in agriculture to this day (Weindling, 1934; Weindling & Fawcett, 1936). *Trichoderma* is capable of reducing plant diseases caused by pathogens through different direct (mycoparasitism, antibiosis, competition for space and/or nutrients, and production of lytic enzymes) or indirect mechanisms of action (induction of plant defenses) (Al-Ani, 2018). The main mode of action against viruses and bacteria is the activation of plant defenses. Against fungi and oomycetes, *Trichoderma* uses all the mechanisms of action described, obtaining important benefits in its agricultural use (Al-Ani, 2018). In the case of nematodes, *Trichoderma* acts directly through parasitism, paralysis, antibiosis, production of lytic enzymes, and competition for space in the rhizosphere and root, and indirectly through the induction of plant defenses, which can be inherited by seeds (Poveda et al., 2020b). Furthermore, *Trichoderma* has recently been described as a powerful entomopathogenic agent with great potential in agricultural pest management. The direct mechanisms of action described in *Trichoderma* include parasitism and the production of insecticidal secondary metabolites, antifeedant compounds, and repellent metabolites; while indirectly, *Trichoderma* acts through the activation of systemic plant defensive responses, the attraction of natural enemies, or the parasitism of insect-symbiotic microorganisms (Poveda, 2021).

In order to use *Trichoderma* as an agricultural bio-inoculant, there are two fundamental aspects that must be developed: mass production and formulation. Mass production of *Trichoderma* inoculants requires the most efficient way to produce the largest possible number of conidia safely and profitably. For this, solid or liquid state fermentation can be carried out. Solid state fermentation is the most common method of *Trichoderma* mass production, based on the sterilization of wet grains (sorghum, corn, rye, millet, or rice) and their inoculation with the fungus. Liquid state fermentation is based on the growth of *Trichoderma* in liquid medium in a shaker, and it is a less used method due to the need for a greater number of steps to obtain spores and the ease of contamination (Srivastava et al., 2016; Waghunde et al., 2016). Once *Trichoderma* conidia are obtained, they are used to obtain the bio-inoculants through different formulations. The most widely used carrier in formulations with *Trichoderma* is talc, although there are many others, such as vermiculite, wheat bran, pesta granules, alginate prills, press mud, coffee husks, and oil or banana wastes. These formulations increase *Trichoderma* conidia viability to up to 18 months (Cumagun, 2014; Kumar et al., 2014). Furthermore, bio-inoculants can be based on consortia of microorganisms, which is an area with great potential in the case of a microorganism as versatile and adaptable as *Trichoderma* (Sharma et al., 2020).

### 3. Bacterial bio-inoculants in agriculture

There is a wide diversity of beneficial bacteria for plants that can be used to improve the productivity and health of crops. These bacteria can live in the rhizosphere, endophytic, or epiphytic, and include a wide variety of different genera, among which *Pseudomonas*, *Bacillus*, *Rhizobium*, *Agrobacterium*, *Burkholderia*, *Achromobacter*, *Micrococcus*, *Aerobacter*, *Flavobacterium*, and *Erwinia* stand out (Glick, 2015).

PGPRs act mainly through nutrient uptake (nitrogen fixation, phosphorus and potassium solubilization, or iron chelation) and production of phytohormones (IAA, CKs, gibberellins) (Verma et al., 2019). For the induction of tolerance in abiotic stress situations, bacteria produce different phytohormones, antioxidant compounds, enzymes, and exopolysaccharides (Verma et al., 2019). Furthermore, bacteria can also act as BCAs through competition for space and/or nutrients, production of secondary metabolites (antibiotics, antifungals, oomycetocides, nematocides, and/or insecticides), production of lytic enzymes, or through the induction of plant defenses (Verma et al., 2019).

The development and commercialization of agricultural bio-inoculants based on bacteria is in continuous growth, as shown by the increase in the number of patents worldwide, from less than 10 annual patents between 1980 and 2010 to more than 40 annual patents recently (Morales-García et al., 2019). Mass production of beneficial bacteria for agriculture is carried out in industrial liquid culture bioreactors with different culture media depending on the propagated bacterial species (Glick, 2020). For bacterial formulation in agricultural bio-inoculants, the main carrier used is peat, or for liquid formulations, the medium is rich in nutrients and cell protectors, with the advantages of easy application in irrigation, easy sterilization, and higher cell concentration (Santos et al., 2019). As with *Trichoderma* inoculants, co-inoculants based on bacteria in combination with other microorganisms are booming. This is because beneficial effects to crops are greater than in the case of mono-inoculants, derived from the synergistic effect of the isolated benefits of each mono-inoculant (Morales-García et al., 2019).

### 4. Combined use of *Trichoderma*-bacteria

The use of *Trichoderma* as an agricultural bio-inoculant presents an important indirect effect that can be beneficial or detrimental to crops: the modification of the rhizospheric microbiota. Root and rhizosphere colonization by *Trichoderma* significantly modifies the quantity and diversity of indigenous microbial populations, especially within bacteria. The application of *T. harzianum* in carrot crops significantly increases the populations of *Bacillus* sp. and *Pseudomonas* sp. (Patkowska et al., 2020). These effects on rhizospheric microbial populations occur over a short period of time, without quantifying differences 9 months after *Trichoderma* inoculation (Cordier & Alabouvette, 2009). However, in the absence of crops, no differences have been observed in the diversity of soil microorganisms after the application of *Trichoderma* (Ganuza et al., 2019). Therefore, there is a possible compatibility between *Trichoderma* and bacteria of agricultural interest in the rhizosphere, allowing the development of co-inoculants with better qualities.

Before the development of combined *Trichoderma*-bacteria bio-inoculants, the compatibility of both organisms must be studied in depth. A fundamental aspect in compatibility analysis is the formation of biofilms that encompass both microorganisms, where the bacteria grow intermingled within the fungal mycelia mat (Triveni et al., 2012). Several studies have shown the *in vitro* compatibility of *Trichoderma* and *Azotobacter*, achieving higher growth, aggregation, and biofilm formation together (Velmourougane et al., 2017a, 2017b).

In the case of bacteria with high antifungal capacity, the development of co-inoculants compatible with *Trichoderma* is complicated. As far as *Bacillus* is concerned, there are several species that can be used in the control of *Trichoderma* when it appears as a mycopathogen in the cultivation of edible fungi; a notable antagonistic capacity of the bacterium on *Trichoderma* has been reported (Chittihunsa et al., 2007; Kim

et al., 2008; Velázquez-Cedeño et al., 2008). Specifically, it has been observed that the production of iturin A by *B. subtilis* is involved in *T. harzianum* conidia lysis and in chlamydo spores formation (resistance structures) (Li et al., 2005). In the case of *Pseudomonas*, the effective antagonistic capacity of several species against *Trichoderma* has been described, inhibiting sporulation or micellar growth (Bin et al., 1991; Upadhyay et al., 1991). In addition, *Pseudomonas*–*Trichoderma* interaction has both positive and negative effects on their capacity as BCAs, since it increases the expression levels of some chitinases, but reduces those of others (Shirzad et al., 2012). However, co-cultures have been reported where *Pseudomonas* and *Trichoderma* were not antagonized, such as *P. fluorescens* and *T. harzianum* *in vitro* (Belkar and Gade, 2012), even with synergistic growth promotion in both microorganisms (Dandurand & Knudsen, 1993; Gangwar et al., 2013).

In the following sections, studies on the co-inoculation of *Trichoderma* with beneficial bacteria in crops are compiled, with the works grouped according to their role as PGPs, tolerance enhancers against abiotic stresses, BCAs, and other industrial uses. Fig. 1 summarizes through an infographic all the synergistic effects and mechanisms of action of *Trichoderma*–bacteria co-inoculation.

#### 4.1. Plant growth promotion

The co-inoculation of *Trichoderma* with different PGPRs has achieved important synergistic effects for promoting plant growth (Table 1), increasing nutrient uptake, and/or increasing yield in different crops. In

many studies it has been possible to determine the mechanisms of action of microorganisms for the effects observed in plants, but in many descriptive studies only the synergistic effect was reported. In greenhouse conditions, the cell suspension application of *T. atroviride* with *Bacillus* sp. and *Pseudomonas* sp. increased the biomass of banana plants by 37 %, above the individual increases reported for each microorganism (Chaves et al., 2009), as in black peppers with *T. harzianum* and *Pseudomonas fluorescens* (Thankamani et al., 2005). These results in *Trichoderma*–*Pseudomonas* interaction were linked to a higher nutrient uptake, mainly N and P (Sandheep et al., 2013). A promising *Trichoderma*–bacteria combination in greenhouse studies is *Trichoderma*–rhizobia. The application of *Trichoderma* together with *Rhizobium* or *Bradyrhizobium* species cell suspensions increases plant biomass, nutrient uptake, and crop yield in *Vigna mungo* (Badar & Qureshi, 2012a), peanuts (Neelipally et al., 2020), and sunflowers (Badar & Qureshi, 2012b), and is linked to a higher content of chlorophyll, carbohydrates, and foliar proteins. In addition, the synergistic effect can be increased by performing triple inoculations together with other beneficial bacteria, such as in faba beans with *T. harzianum*, *Rhizobium leguminosarum* biovar *viciae*, and *B. subtilis* (Firdu et al., 2021), or in apple seedlings with *T. viride*, *Azotobacter chroococcum*, and *Pseudomonas striata* (Raman, 2012), obtaining a synergistic effect not only on plant biomass but also on N and P uptake. On the other hand, the inoculation method can condition the survival of the microorganisms and, therefore, their effects on plants. The use of different carriers (oil cakes and maize granules) to formulate the *Trichoderma*–*Bacillus*/*Pseudomonas* co-

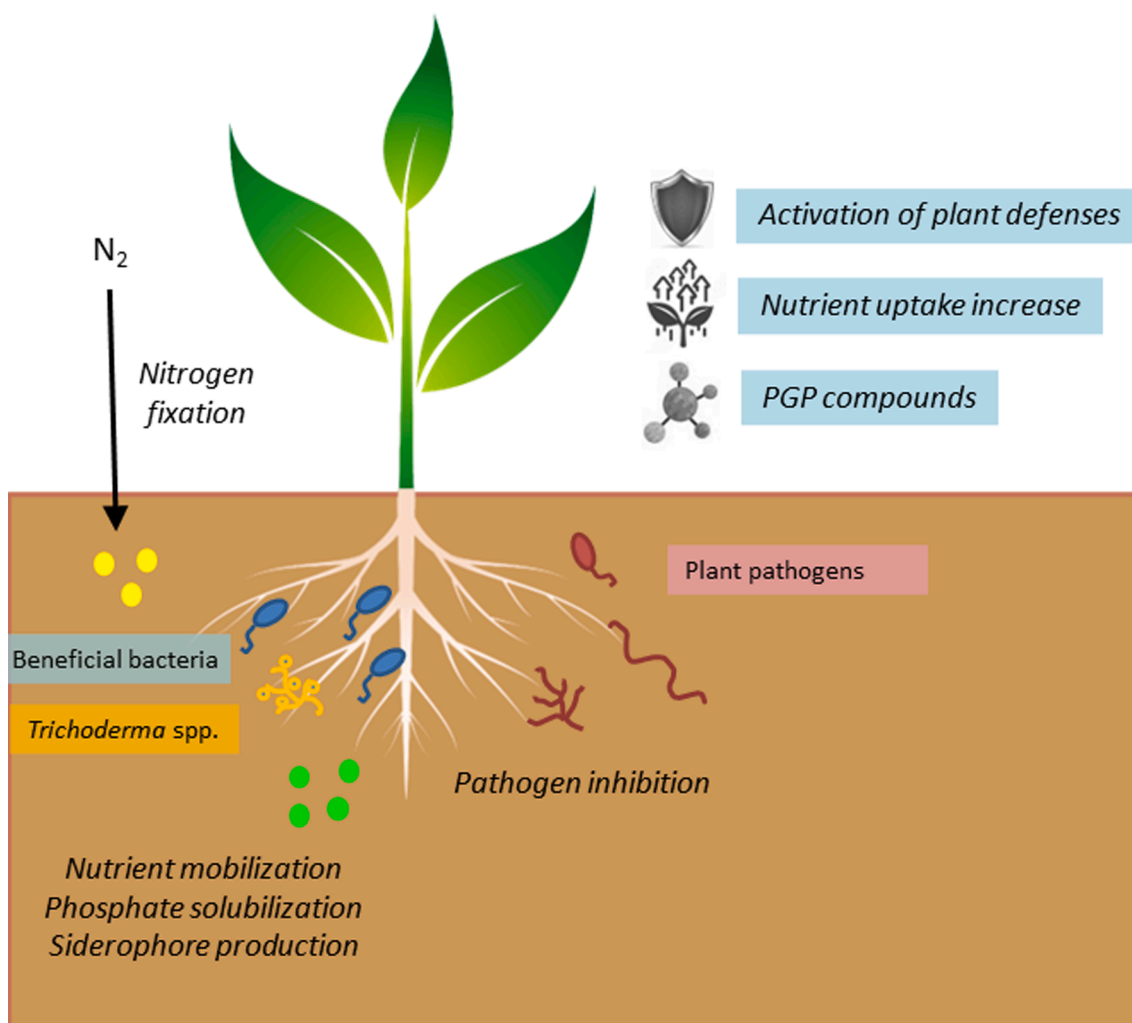


Fig. 1. Summary infographic with all the synergistic effects on plants and the mechanisms of action of *Trichoderma*–bacteria co-inoculation.

**Table 1**  
Effect of promoting plant growth and increasing yield in crops by co-inoculations with *Trichoderma* and beneficial bacteria.

<i>Trichoderma</i> SPECIES	BACTERIA SPECIES	EXPERIMENT	APPLICATION	CROP	SYNERGISTIC EFFECTS	MECHANISMS OF ACTION	REFERENCE
<i>T. asperellum</i>	<i>Pseudomonas fluorescens</i>	In greenhouse In field	Cell suspensions	Chickpea Bean	Plant growth promotion	Unidentified	Yadav et al., 2013
	<i>Rhizobium</i> sp. <i>Bacillus amyloliquefaciens</i>	<i>In vitro</i>	Cell suspensions	Wheat	Plant growth promotion	Production of plant growth promoting compounds (both)	Karuppiyah et al., 2019a
	<i>B. amyloliquefaciens</i>	<i>In vitro</i>	Cell suspensions	Maize	Plant growth promotion	Production of plant growth promoting compounds (both)	Karuppiyah et al., 2019b
	<i>Bacillus cereus</i>	In greenhouse	Cell suspensions	Oil Palm	Plant growth promotion	Siderophores production ( <i>T. asperellum</i> ), P supply ( <i>T. asperellum</i> ), IAA production ( <i>T. asperellum</i> and <i>B. cereus</i> )	Muhammad-Syafiq et al., 2021
<i>T. atroviride</i>	<i>Bacillus</i> sp. <i>Pseudomonas</i> sp.	In greenhouse	Cell suspensions	Banana	Plant growth promotion	Unidentified	Chaves et al., 2009
<i>T. hamatum</i>	<i>Rhizobium</i> sp.	In greenhouse	Cell suspensions	<i>Vigna mungo</i>	Plant growth promotion Increased nutrient uptake Increased crop yield	Unidentified	Badar & Qureshi, 2012 <sup>a</sup>
	<i>Rhizobium</i> sp.	In greenhouse	Cell suspensions	Sunflower	Plant growth promotion Increased nutrient uptake	Unidentified	Badar & Qureshi, 2012b
<i>T. harzianum</i>	<i>Clostridium butyricum</i>	<i>In vitro</i>	Cell suspensions	–	–	N fixation ( <i>C. butyricum</i> ), Cellulase activity ( <i>T. harzianum</i> )	Veal & Lynch, 1984
	<i>Azospirillum brasilense</i>	In greenhouse In field	Formulation with peat as carrier	Bean Wheat	Plant growth promotion Increased nutrient uptake Increased crop yield	P supply ( <i>T. harzianum</i> ), N fixation ( <i>A. brasilense</i> )	Ögüt et al., 2005
<i>P. fluorescens</i>	<i>Bacillus megaterium</i> sub sp. <i>phospaticum</i> <i>Rhizobium</i> sp.	In greenhouse In field	Formulation with talc as carrier and carboxyl methylcellulose as adhesive	Chickpea	Plant growth promotion Increased nutrient uptake Increased crop yield	N supply ( <i>Rhizobium</i> sp.), P supply ( <i>B. megaterium</i> ), Production of growth promoting substances ( <i>T. harzianum</i> )	Rudresh et al., 2005
	<i>Rhizobium leguminosarum</i>	In greenhouse In field	Cell suspensions Arabic gum encapsulated cells	Black pepper <i>Vicia faba</i>	Plant growth promotion Increased crop yield	Unidentified Production of growth promoting substances ( <i>R. leguminosarum</i> ), Increased formation of root nodules ( <i>R. leguminosarum</i> )	Thankamani et al., 2005 Saber et al., 2009
<i>A. brasilense</i>		In greenhouse	Calcium alginate-encapsulated cells	Tomato	Plant growth promotion	N supply ( <i>A. brasilense</i> ), Chitinase, $\beta$ -1,3-glucanase, carboxymethyl cellulase xylanase and polygalacturonase activity ( <i>T. harzianum</i> )	El-Katatny, 2010
<i>P. fluorescens</i>		In greenhouse	Cell suspensions	Vanilla	Plant growth promotion Increased nutrient uptake	Unidentified	Sandheep et al., 2013
<i>A. brasilense</i>		In greenhouse	Calcium alginate-encapsulated cells	Wheat Maize	Plant growth promotion Increased nutrient uptake Increased crop yield	N supply ( <i>A. brasilense</i> ), P supply (both)	El-Katatny & Idres, 2014
<i>Bacillus subtilis</i>		In greenhouse In field	Cell suspensions	Brinjal Beans Bitter gourd Bottle gourd Cabbage Chilli	Plant growth promotion Increased crop yield	Unidentified	Kumar et al., 2015a

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Table 1 (continued)

Trichoderma SPECIES	BACTERIA SPECIES	EXPERIMENT	APPLICATION	CROP	SYNERGISTIC EFFECTS	MECHANISMS OF ACTION	REFERENCE
				Carrot Cauliflower Pumpkin Ridged gourd Potato Papaya Tomato			
	<i>P. fluorescens</i>	In greenhouse	Formulation with oil cakes of neem and jatropa as carrier	Tomato	Plant growth promotion Increased crop yield	Unidentified	Tomer et al., 2015
	<i>Brevibacterium halotolerans</i>	In greenhouse In field	Cell suspensions	Mint	Plant growth promotion Increased nutrient uptake Increased oil yield	Unidentified	Singh et al., 2019
	<i>B. subtilis</i>	In field	Cell suspensions	Faba bean	Plant growth promotion Increased nutrient uptake	Unidentified	Firdou et al., 2020
	<i>Bradyrhizobium</i> sp.	In greenhouse	Cell suspensions	Peanut	Plant growth promotion	Unidentified	Neelipally et al., 2020
	<i>B. subtilis</i> <i>Rhizobium leguminosarum</i> biovar <i>viciae</i>	In greenhouse	Cell suspensions	Faba bean	Plant growth promotion Increased nutrient uptake	Unidentified	Firdou et al., 2021
	<i>B. halotolerans</i> <i>B. subtilis</i> <i>Achromobacter xylosoxidans</i> <i>B. cepacia</i>	In greenhouse	Cell suspensions	<i>Ocimum sanctum</i>	Plant growth promotion Increase abiotic stress tolerance	ACC deaminase (bacteria) Nutrient uptake Photosynthesis rate increase Starch and proline accumulation	Singh et al., 2020
<i>T. virens</i>	<i>B. megaterium</i> sub sp. <i>phosphaticum</i> <i>Rhizobium</i> sp.	In greenhouse In field	Formulation with talc as carrier and carboxyl methylcellulose as adhesive	Chickpea	Plant growth promotion Increased nutrient uptake Increased crop yield	N supply ( <i>Rhizobium</i> sp.), P supply ( <i>B. megaterium</i> ), Production of growth promoting substances ( <i>T. harzianum</i> )	Rudresh et al., 2005
<i>T. viride</i>	<i>P. fluorescens</i>	In field	Formulation with talc as carrier	Rice	Plant growth promotion Increased crop yield	Unidentified	Mathivanan et al., 2005
	<i>B. megaterium</i> sub sp. <i>phosphaticum</i> <i>Rhizobium</i> sp.	In greenhouse In field	Formulation with talc as carrier and carboxyl methylcellulose as adhesive	Chickpea	Plant growth promotion Increased nutrient uptake Increased crop yield	N supply ( <i>Rhizobium</i> sp.), P supply ( <i>B. megaterium</i> ), Production of growth promoting substances ( <i>T. harzianum</i> )	Rudresh et al., 2005
	<i>R. leguminosarum</i>	In field	Arabic gum encapsulated cells	<i>Vicia faba</i>	Plant growth promotion Increased crop yield	Production of growth promoting substances ( <i>R. leguminosarum</i> ), Increased formation of root nodules ( <i>R. leguminosarum</i> )	Saber et al., 2009
	<i>Azotobacter chroococcum</i> <i>Pseudomonas striata</i>	In greenhouse	Cell suspensions	Apple	Plant growth promotion Increased nutrient uptake	Unidentified	Raman, 2012
	<i>A. chroococcum</i>	In greenhouse	Cell suspensions	Chickpea	Plant growth promotion	P supply (both)	Velmourougane et al., 2017c
	<i>Rhizobium</i> sp.	In field	Cell suspensions	Bean	Plant growth promotion Increased crop yield	Unidentified	Negi et al., 2021
	<i>P. fluorescens</i>	In greenhouse	Cell suspensions	<i>Chrysanthemum indicum</i>	Plant growth promotion Increased nutrient uptake	Increased mycorrhizal fungi-root colonization: increased water absorption and P supply	Saini et al., 2019
<i>Trichoderma</i> sp.	<i>Bacillus</i> sp.	In greenhouse	Cell suspensions	Bean	Plant growth promotion Increased nutrient uptake	Increased formation of root nodules	Yobo et al., 2009
	<i>Azotobacter</i>	In greenhouse	Cell suspensions	<i>Amaranthus spinosus</i>	Plant growth promotion	IAA production	Kasa et al., 2015

(continued on next page)

Table 1 (continued)

Trichoderma SPECIES	BACTERIA SPECIES	EXPERIMENT	APPLICATION	CROP	SYNERGISTIC EFFECTS	MECHANISMS OF ACTION	REFERENCE
	<i>P. fluorescens</i>	In fields	Cell suspensions	<i>Stevia rebaudiana</i> Pepper	Increased crop yield	Unidentified	Duc et al., 2017
	<i>B. subtilis</i>	In greenhouse	Formulation with maize granules as carrier	Soybean	Plant growth promotion	Unidentified	Miftakhurrohmat, 2021

inoculants significantly increased their synergistic effect under controlled greenhouse conditions in tomatoes (Tomer et al., 2015) and soybeans (Miftakhurrohmat et al., 2021).

In terms of yield, several studies have also been carried out where the synergistic effects of *Trichoderma*-bacteria co-inoculation were reported, but without identifying the mechanisms of action involved. The application of *T. harzianum*-*Bacillus subtilis* cell suspensions in the field has achieved synergistic increases in plant growth, yield, and quality of a wide variety of crops, including vegetables, legumes, and fruit trees (Kumar et al., 2015a; Firdu et al., 2020). In the case of *T. harzianum*-*Brevibacterium halotolerans* co-inoculation in mint plants, the increase in plant biomass and oil yield was a direct consequence of an increase in the rhizospheric populations of both microorganisms, derived from co-inoculation (Singh et al., 2019). As in the greenhouse, the inoculation of cell suspensions with triple combinations led to synergistic effects in plants in the field, increasing plant growth of chickpeas and beans (*T. asperellum* + *P. fluorescens* + *Rhizobium* sp.) (Yadav et al., 2013), and the yield in peppers (*Trichoderma* sp. + *P. fluorescens* + arbuscular mycorrhizal fungus) (Duc et al., 2017). Furthermore, the use of carriers in the field application of co-inoculants increases the effects observed, increasing the number of productive tillers, grains per panicle, and grain weight of rice with *T. viride*-*P. fluorescens* formulation, with talc as the carrier (Mathivanan et al., 2005).

Almost four decades ago it was described how, in the absence of a plant, the *in vitro* interaction between *T. harzianum* and *Clostridium butyricum* significantly and synergistically increased their activity as plant growth promoters, specifically in terms of bacterial N fixation and fungal cellulase activity (Veal & Lynch, 1984). To date, numerous studies have been carried out where *Trichoderma*-bacteria co-inoculation has shown important benefits for plants due to a microbial increase in nutrient supply: fixation of atmospheric N, solubilization of P and K, and siderophore production. The synergistic effect can be reported in one of the microorganisms or in both. In the co-inoculation of chickpea plants with *T. viride* and *Azotobacter chroococcum* cell suspensions, a synergistic increase in plant growth was reported as a consequence of a synergistic increase in P solubilization (Velmourougane et al., 2017c). However, co-inoculation with *T. asperellum* and *Bacillus cereus* versus only *Trichoderma* showed a synergistic increase in P solubilization and siderophore production in the oil palm rhizosphere (Muhammad-Syafiq et al., 2021). The use of different carriers in the formulations of the combined bio-inoculants has led to important results in the greenhouse and in field crops. The *T. harzianum* and *Azospirillum brasilense* formulation with peat as carrier synergistically promoted yield in beans and wheat through a synergistic increase in bacterial N fixation and fungal P solubilization (Ögüt et al., 2005). However, the triple combination of *Trichoderma* with *Bacillus megaterium* sub sp. *phosphaticum* and *Rhizobium* sp. with talc as the carrier and carboxyl methylcellulose as the adhesive did not increase the supply of nutrients to chickpea plants compared to just *Trichoderma* (Rudresh et al., 2005). The encapsulation of microbial cells with calcium alginate synergistically enhanced the nutrient supply of both microorganisms, as in cereal co-inoculation with *T. harzianum* and *A. brasilense*, increasing their bacterial capacity for N supply and microbial P contribution (El-Katatny & Idres, 2014).

Along with nutrient supply, one of the main mechanisms of action of

microorganisms as PGPs is the production of PGP compounds, such as the hormone IAA. In the *in vitro* co-inoculation of wheat and maize seedlings with cell suspensions of *T. asperellum* and *Bacillus amyloliquefaciens*, the production of these PGP compounds by both microorganisms led to a synergistic increase in root and shoot length, as well as fresh plant mass (Karuppiyah et al., 2019a, 2019b). Co-inoculation with *Trichoderma* and *Bacillus/Azotobacter* cell suspensions also led to a synergistic increase in the PGP capacity of both microorganisms in different crops, due to a synergistic increase in IAA production (Kasa et al., 2015; Muhammad-Syafiq et al., 2021). With the use of different formulations, it has been reported that only the production of PGP compounds increased synergistically in one of the microbial components of the co-inoculation. In a formulation with talc as the carrier and carboxyl methylcellulose as the adhesive, only *Trichoderma* produced PGP compounds synergistically (Rudresh et al., 2005) while, in the cell encapsulation with arabic gum, *Rhizobium leguminosarum* produced these substances, in addition to a synergistic increase in bacterial root nodule formation (Saber et al., 2009).

Other mechanisms of action implicated in promoting plant growth synergistically by *Trichoderma*-bacteria co-inoculation may be direct or indirect. Directly, the application of *T. harzianum* and *A. brasilense* in tomato plants resulted in a synergistic increase in plant growth as a consequence of a synergistic increase in the activity of *Trichoderma* enzymes, such as chitinase,  $\beta$ -1, 3-glucanase, carboxymethyl cellulase xylanase, and polygalacturonase (El-Katatny, 2010). Indirectly, it has been reported how *Trichoderma*-bacteria co-inoculation may not have a synergistic effect on the activity of both microorganisms, but it significantly increased that of other beneficial microorganisms. This occurred in *Chrysanthemum indicum* plant roots inoculated with *T. viride*, *P. fluorescens*, and arbuscular mycorrhizal fungi; an increase in root colonization by mycorrhizal fungi was observed, which meant a greater acquisition of water and P by the plant (Saini et al., 2019).

#### 4.2. Abiotic stress tolerance

Until now, few studies have been carried out analyzing the effects of *Trichoderma*-bacteria co-inoculation on the induction of plant tolerance under abiotic stress situations. Singh et al. (2020) studied the co-inoculation of *T. harzianum* with four different ACC-deaminase producing bacterial strains for cold stress alleviation in the plant *Ocimum sanctum*. The *Trichoderma*-*Achromobacter xylosoxidans* combination was the most effective, producing an increase in fresh weight and proline and starch content, and decreasing malondialdehyde (MDA) and ACC levels in cold conditions (Singh et al., 2020). Plants can also benefit from reducing heavy metal stress from the use of *Trichoderma*-bacteria consortia. The combined application of five *Trichoderma* strains and ten *Bacillus* strains that were heavy-metal resistant, through biochar impregnation and following application to sunflower plants, reduced the uptake of Cd, Cr, Cu, Ni, Pb, and Zn, while increasing Fe and Mg uptake, compared to biochar alone (Younas et al., 2022). More research is needed to further determine how combinations of different microorganisms may enhance plant stress tolerance to abiotic conditions.

#### 4.3. Other uses in industry

The combined use of *Trichoderma* with different bacteria has led to the development of interesting tools for various industrial processes. Through the use of a dual-chamber fungal microbial fuel cell, the ability of *T. harzianum* to degrade the soil and water contaminating drugs acetaminophen (APAP) and 4-aminophenol (PAP) was analyzed. While producing electrical energy, *T. harzianum* was able to degrade both contaminants in 7 h and produce a power density of 0.13 mW m<sup>-2</sup>. By adding *P. fluorescens* bacteria to the dual-chamber fungal microbial fuel cell, both microorganisms formed a biofilm. The *Trichoderma*–*Pseudomonas* system completely degraded APAP and PAP in 1.3 h, due to the bioremediation capacity of *P. fluorescens* on APAP. Furthermore, co-inoculation gave a power density ten times higher, of 1.7 mW m<sup>-2</sup> (Shabani et al., 2021).

*Trichoderma* is widely used in industry as a source of cellulase enzymes, which allow lignocellulose to be converted into products of industrial interest, with *T. reesei* being the most widely used species (Fang et al., 2021). In co-inoculation with different bacteria, an increase in cellulase activity has been reported for both microorganisms, increasing the production of reducing sugars (Weimer & Weston, 1985; Gow & Wood, 1988; Bothwell et al., 1993; Walker et al., 1992, 1993; Zhang et al., 2009), alcohols (Yu et al., 1985), gas (Morgavi et al., 2004), or malt (Hattingh et al., 2014).

In addition, at the molecular level, it has been possible to express bacterial and *Trichoderma* lytic enzymes in the yeast *Saccharomyces cerevisiae*, achieving synergistic activities with the use of a single microorganism. This is the case in the co-expression of the *Bacillus pumilus*  $\beta$ -xylosidase gene with the *T. reesei*  $\beta$ -xylanase 2 gene (La Grange et al., 2000), or the *Bacillus endoxylanase* and *Trichoderma endoglucanase* genes (Lee et al., 2007).

#### 5. BCAs based on *Trichoderma*-bacteria co-inoculations: direct and indirect mechanisms

*Trichoderma* use in combination with different bacterial BCAs has gained increasing attention in recent years, and important synergistic effects have been reported in the control of agricultural pests and diseases (Table 2). Although many of the studies carried out to date describe the mechanisms of action involved in the synergistic action (both direct and indirect), in some interesting descriptive studies these mechanisms are unknown. In the case of soil pathogens, co-inoculant application is always carried out at the root level, but for foliar pathogens, the application can be carried out either by foliar spray or by root application, with different mechanisms of action being involved in each (Chien & Huang, 2020). Synergistic disease reduction data from the use of *Trichoderma*-bacteria co-inoculations as BCAs have reported reductions of up to 97 % in bacterial pathogens (Yendyo et al., 2017), between 70 and 90 % of fungal and oomycete pathogens (Rini & Sulochana, 2007; Yigit & Dikilitas, 2007; Zaim et al., 2018; Somani & Arora, 2010; Srivastava et al., 2010; Ali & Nadarajah, 2013; Izquierdo-García et al., 2020; Firdu et al., 2020, 2021), and between 60 and 90 % of plant parasitic nematodes (Chaves et al., 2009; Moradi et al., 2015). Compared to the use of chemical pesticides, the combined use of *Trichoderma*-bacteria achieves similar disease reduction results (Maketon et al., 2008). For example, against the oomycete *Phytophthora capsici* in chili, the *T. hamatum*–*Pseudomonas aeruginosa* combination was as effective as the use of the systemic fungicide Mefenoxam, noting that co-inoculation is an effective and sustainable alternative for chili pepper seed treatment (Chemeltorit et al., 2017). Against *Rhizoctonia solani* in rice, the *T. harzianum*–*P. fluorescens* combination was just as effective as the use of the broad-spectrum fungicide Carbendazim, although the BCAs also achieved an increase in grain yield (Singh et al., 2010). Even combining pesticides with BCAs, the synergistic effect of *Trichoderma*-bacteria co-inoculation remains higher. In rice, against the fungus *Magnaporthe oryzae*, *T. harzianum*–*P. fluorescens* combination caused

69.5 % disease reduction, displaying a synergy factor of 1.29; however, the *T. harzianum*–Carbendazim combination only obtained a synergy factor of 0.45 (Jambhulkar et al., 2018). In addition, *Trichoderma*-bacteria co-inoculations used as BCAs allows their combination with many other integrated control strategies. In tomato crops in the field, disease incidence reductions greater than 90 % have been reached with combinations of *T. harzianum*, *P. fluorescens*, arbuscular mycorrhizal fungi, and the contact fungicide Mancozeb (Kabdwal et al., 2019).

##### 5.1. Mycoparasitism

Mycoparasitism is the most well-known mechanism of action for BCAs within the *Trichoderma* genus (Mukherjee et al., 2022). The presence of bacteria can synergistically increase the activity of *Trichoderma* as a mycoparasite. *In vitro*, *T. viride*–*P. fluorescens* co-inoculation synergistically reduces the micellar growth of the oomycete *Phytophthora infestans*, due to an increase in mycoparasitism by *Trichoderma* (Zegeye et al., 2011). Furthermore, fungus–bacteria co-inoculation can lead to a synergistic increase in the mechanisms of action of each microorganism. This has been reported in peppers infected by *Phytophthora capsici*, where *T. harzianum* increased its mycoparasitic capacity by co-inoculating with the *Streptomyces rochei* bacteria, which increased the production of the non-volatile antifungal secondary metabolite 1-propenone, 1-(4-chlorophenyl) (Ezziymani et al., 2007).

##### 5.2. Antibiosis: volatile and non-volatile secondary metabolites

Both *Trichoderma* and different bacteria used as BCAs produce a wide diversity of volatile and non-volatile secondary metabolites which are highly efficient for phytopathogen management (Al-Ani, 2019). Co-cultured fermentation with *Trichoderma* and different bacterial BCAs synergistically increases the production of non-volatile secondary metabolites with biocidal activity against different agricultural pathogens (Siddiqui & Shaikat, 2004; Wu et al., 2018). The fermented culture filtrate of the *T. atroviride*–*B. subtilis* co-culture reduced the growth of the fungus *Fusarium graminearum in vitro* by 54 %, due to the synergistic production of the metabolites konigin A and mevastatin (Li et al., 2020). Other *Trichoderma*–*Bacillus* combinations have shown the synergistic production of effective oomycetocidal metabolites (pyrrolo [1,2-a] pyrazine-1,4-dione and hexahydro-3-(phenylmethyl)) (Jimtha et al., 2016) and antifungals (butyl acetate) (Emanuel et al., 2020). In addition to non-volatile metabolites, the *Trichoderma*-bacteria interaction may lead to a synergistic increase in the production of volatile antifungal metabolites involved in the reduction of disease caused by fungi, such as *F. oxysporum*, analyzed by transcriptomics (Ma et al., 2022) and metabolomics (Al-Waily & Hassan, 2019). In this sense, *T. viride*–*P. fluorescens* co-inoculation on seeds with carboxyl methylcellulose as the adhesive synergistically reduced seedling mortality caused by *Sclerotium rolfsii*, due to the hydrogen cyanide production by *P. fluorescens* and volatile antifungal metabolites by *T. viride* (Manjula et al., 2004). *Trichoderma* was also found to be effective in reducing *R. solani* and *F. oxysporum* infections in tomatoes when combined with *Pseudomonas* bacteria due to the secretion of volatile and non-volatile metabolites (Rini & Sulochana, 2008), and a more recent study pointed at these mechanisms combined with competition using *Trichoderma*–*Bacillus* consortia for reducing *F. oxysporum* disease in garlic (Poromarto et al., 2022). A combination of *Trichoderma* with bacteria consortia reduced potato common scab disease caused by *Streptomyces* sp. both *in vitro* and *in vivo*, inhibiting pathogen growth by up to 80 % and reducing tuber lesion size by up to 60 % (Porto et al., 2022a). The mechanism detected *in vitro* was a combination of both volatile and non-volatile antibiotic metabolites, although no specific metabolite was identified. These BCA combinations were further investigated in controlling potato common scab disease in the field, decreasing disease severity and yield losses compared to untreated control (Porto et al., 2022b). Other mechanisms of action can occur in combination with the



**Table 2**  
Biocontrol effects in crops by co-inoculations with *Trichoderma* and beneficial bacteria.

<i>Trichoderma</i> SPECIES	BACTERIA SPECIES	EXPERIMENT	APPLICATION	CROP	PATHOGEN/PEST	SYNERGISTIC EFFECTS	MECHANISMS OF ACTION	REFERENCE
<i>T. asperellum</i>	<i>Pseudomonas fluorescens</i>	In greenhouse	Cell suspensions	Pea	Fungus: <i>Erysiphe pisi</i>	Reduced disease incidence	Plant systemic resistance induction	Patel et al., 2016
	<i>Bacillus amyloliquefaciens</i>	<i>In vitro</i>	Cell-free filtering	–	Fungus: <i>Botrytis cinerea</i>	Inhibition pathogen growth	Non-volatile antifungal secondary metabolites production (both)	Wu t al., 2018
	<i>B. amyloliquefaciens</i>	<i>In vitro</i>	Cell suspensions	Wheat	Fungus: <i>Fusarium graminearum</i>	Reduced disease incidence	Non-volatile antifungal secondary metabolites production (both) Production of lytic enzymes (both)	Karuppiyah et al., 2019a
	<i>B. amyloliquefaciens</i>	<i>In vitro</i>	Cell suspensions	Maize	Fungus: <i>F. graminearum</i>	Reduced disease incidence	Plant local resistance induction	Karuppiyah et al., 2019b
	<i>B. amyloliquefaciens</i>	In growth chamber	Cell suspensions	Tomato	Bacteria: <i>Xanthomonas perforans</i>	Reduced disease incidence	Unidentified	Chien & Huang, 2020
<i>T. atroviride</i>	<i>Bacillus</i> sp.	In greenhouse	Cell suspensions	Banana	Nematode: <i>Radopholus similis</i>	Population reduction and root penetration capacity in nematodes	Unidentified	Chaves et al., 2009
	<i>Pseudomonas chlororaphis</i>	In greenhouse	Cell suspensions (bacteria) Mycelial-agar plug ( <i>T. atroviride</i> )	Avocado	Fungus: <i>Rosellinia necatrix</i>	Reduced disease incidence	Unidentified	Ruano-Rosa et al., 2014
	<i>P. pseudoalcaligenes</i>	<i>In vitro</i>	–	–	Fungus: <i>F. graminearum</i>	Inhibition pathogen growth	Non-volatile antifungal secondary metabolites production (both)	Li et al., 2020
	<i>Bacillus subtilis</i>	<i>In vitro</i>	–	–	Fungus: <i>F. graminearum</i>	Inhibition pathogen growth	Production of lytic enzymes (both)	Diby et al., 2005
<i>T. aureoviride</i>	<i>P. fluorescens</i>	<i>In vitro</i>	Cell-free filtering	Black pepper	Oomycete: <i>Phytophthora capsici</i>	Reduced disease incidence	Production of lytic enzymes (both)	Diby et al., 2005
<i>T. hamatum</i>	<i>Pseudomonas aeruginosa</i>	In field	Cell suspensions	Chilli	Oomycete: <i>P. capsici</i>	Reduced disease incidence	Unidentified	Chemeltorit et al., 2017
<i>T. harzianum</i>	<i>Enterobacter cloacae</i>	In greenhouse	Cell suspensions	Lettuce	Oomycete: <i>Pythium ultimum</i>	Reduced disease incidence	Competence for space (both)	Lynch et al., 1991
	<i>E. cloacae</i>	<i>In vitro</i>	Cell suspensions	–	Fungi: <i>Fusarium solani</i> , <i>Botrytis cinerea</i> and <i>Uncinula necator</i>	Inhibition pathogen growth	Production of lytic enzymes (both)	Lorito et al., 1993
	<i>P. fluorescens</i>	In greenhouse	Cell suspensions	Tomato	Nematode: <i>Meloidogyne javanica</i>	Reduced nematode population densities	Nematicidal secondary metabolites production ( <i>P. fluorescens</i> )	Siddiqui & Shaukat, 2004
	<i>P. fluorescens</i>	<i>In vitro</i>	Cell-free filtering	Black pepper	Oomycete: <i>P. capsici</i>	Reduced disease incidence	Production of lytic enzymes (both)	Diby et al., 2005
	<i>Paenibacillus lentimorbus</i>	In greenhouse	Alginate pellets ( <i>T. harzianum</i> ), Cell suspension ( <i>P. lentimorbus</i> )	Tomato	Fungi: <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> <i>Rhizoctonia solani</i> <i>Pyrenochaeta lycopersici</i>	Reduced disease incidence	Production of lytic enzymes ( <i>T. harzianum</i> )	Montealegre et al., 2005
	<i>P. fluorescens</i>	In nursery	Cell suspensions	Papaya	Nematode: <i>M. incognita</i>	Reduced nematode egg-mass	Space competence: root colonization (both)	Rao, 2007
	<i>P. fluorescens</i>	In greenhouse	Formulation with cowdung-neem cake ( <i>T. harzianum</i> ) and talc ( <i>P. fluorescens</i> ) as carrier	Chilli	Fungus: <i>R. solani</i>	Reduced disease incidence	Unidentified	Rini & Sulochana, 2007
	<i>P. fluorescens</i>	In greenhouse	Cell suspensions	Tomato	Fungus: <i>F. oxysporum</i> f. sp. <i>lycopersici</i>	Reduced disease incidence	Unidentified	Yigit & Dikilitas, 2007
	<i>Streptomyces rochei</i>	In field	Formulation with vermiculite as carrier	Pepper	Oomycete: <i>P. capsici</i>	Reduced disease incidence	Parasitism ( <i>T. harzianum</i> ), Non-volatile antifungal secondary metabolites production ( <i>S. rochei</i> )	Ezziyyani et al., 2007
	<i>B. subtilis</i>	In greenhouse	Cell suspensions	Tobbaco	Bacteria: <i>R. solanacearum</i> Oomycete: <i>Pythium aphanidermatum</i> Fungus: <i>Cercospora nicotiana</i>	Reduced disease incidence	Unidentified	Maketon et al., 2008
	<i>B. subtilis</i>	In field	Cell suspensions	Chickpea			Unidentified	Zaim et al., 2018

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Table 2 (continued)

Trichoderma SPECIES	BACTERIA SPECIES	EXPERIMENT	APPLICATION	CROP	PATHOGEN/PEST	SYNERGISTIC EFFECTS	MECHANISMS OF ACTION	REFERENCE
	<i>Rhizobium leguminosarum</i> bv. <i>vicia</i>	In field	Formulation with Arabic gum as carrier	<i>Vicia faba</i>	Fungus: <i>F. oxysporum</i> f. sp. <i>ciceris</i> Fungus: <i>Botrytis fabae</i>	Reduced disease incidence Reduced disease incidence	Plant systemic resistance induction	Saber et al., 2009
	<i>P. fluorescens</i>	In field	Formulation with talc as carrier	Rice	Fungus: <i>R. solani</i>	Reduced disease incidence	Unidentified	Singh et al., 2010
	<i>P. fluorescens</i>	In greenhouse In field	Formulation with talc as carrier and carboxyl methylcellulose as adhesive	Tomato	Fungus: <i>F. oxysporum</i> f. sp. <i>lycopersici</i>	Reduced disease incidence	Unidentified	Srivastava et al., 2010
	<i>B. subtilis</i>	In greenhouse	Cell suspensions	Bean	Fungus: <i>F. solani</i> f. sp. <i>phaseoli</i>	Reduced disease incidence	Increased abundance of beneficial fungi in the rhizosphere	Abeyasinghe, 2012
	<i>Pseudomonas</i> sp.	In growth chamber	Cell suspensions	Cucumber	Fungus: <i>F. oxysporum</i> f.sp. <i>radicis cucumerinum</i>	Reduced disease incidence	Plant systemic resistance induction	Alizadeh et al., 2013
	<i>P. fluorescens</i>	In greenhouse	Cell suspensions	Vanilla	Fungi: <i>F. oxysporum</i> , <i>R. solani</i> and <i>Sclerotium rolfsii</i>	Reduced disease incidence	Unidentified	Sandheep & Jisha, 2013
	<i>B. subtilis</i>	In greenhouse	Cell suspensions	Tomato	Fungus: <i>Alternaria alternata</i> Oomycete: <i>Phytophthora infestans</i>	Reduced disease incidence	Plant systemic resistance induction	Kumar et al., 2015a
	<i>B. subtilis</i> <i>Pseudomonas putida</i>	In greenhouse	Cell suspensions	Tomato	Oomycete: <i>P. infestans</i>	Reduced disease incidence	Plant systemic resistance induction	Kumar et al., 2015b
	<i>P. aeruginosa</i>	In greenhouse	Cell suspensions	Okra	Fungi: <i>R. solani</i> , <i>F. oxysporum</i> , <i>F. solani</i> and <i>Macrophomina phaseolina</i> Nematode: <i>M. incognita</i>	Reduced disease incidence	Plant systemic resistance induction	Shafique et al., 2015
	<i>Serratia proteamaculans</i>	In greenhouse	Cell suspensions	Tomato	Fungus: <i>R. solani</i>	Reduced disease incidence	Plant systemic resistance induction	Youssef et al., 2016
	<i>P. fluorescens</i>	In field	Formulation with talc as carrier and carboxyl methylcellulose as adhesive	Rice	Fungus: <i>Magnaporthe oryzae</i>	Reduced disease incidence	Unidentified	Jambhulkar et al., 2018
	<i>P. fluorescens</i>	In greenhouse	Cell suspensions	Maize	Fungus: <i>R. solani</i>	Reduced disease incidence	Plant systemic resistance induction	Madhavi et al., 2018
	<i>Mesorhizobium ciceri</i>	In greenhouse	Cell suspensions	Chickpea	Nematode: <i>M. incognita</i>	Reduced disease incidence	Unidentified	Rizvi et al., 2018
	<i>P. fluorescens</i>	In greenhouse	Formulation with manure as carrier	Pumpkin	Fungus: <i>F. oxysporum</i>	Reduced disease incidence	Antifungal secondary metabolites production (both)	Al-Waily & Hassan, 2019
	<i>P. fluorescens</i>	In field	Formulation with talc as carrier	Tomato	Unidentified	Reduced disease incidence	Unidentified	Kabdwal et al., 2019
	<i>B. subtilis</i>	In field	Formulation with diatomaceous earth as carrier	Potato	Bacteria: <i>Streptomyces</i> sp.	Reduced disease incidence	Increased abundance of beneficial bacteria in the rhizosphere	Wang et al., 2019
	<i>B. subtilis</i>	In field	Cell suspensions	Faba bean	Fungus: <i>B. fabae</i>	Reduced disease incidence	Unidentified	Firdu et al., 2020
	<i>B. subtilis</i>	In greenhouse	Cell suspensions	Faba bean	Fungus: <i>B. fabae</i>	Reduced disease incidence	Unidentified	Firdu et al., 2021
	<i>Bacillus velezensis</i>	In vitro	Cell suspensions	Rapeseed	Fungus: <i>Verticillium longisporum</i>	Reduced disease incidence	Plant systemic resistance induction	Hafiz et al., 2022
<i>T. longibrachiatum</i>	<i>B. amyloliquefaciens</i>	In greenhouse	Cell-free filtering	Tomato	Fungus: <i>F. oxysporum</i> f. sp. <i>lycopersici</i>	Reduced disease incidence	Antifungal secondary metabolites production ( <i>B. amyloliquefaciens</i> )	Ma et al., 2022
<i>T. pseudokoningii</i>	<i>Bradyrhizobium japonicum</i>	In greenhouse In field	Cell suspensions	Soybean	Nematode: <i>M. incognita</i>	Reduced disease incidence and nematode reproduction	Unidentified	Oyekanmi et al., 2007
<i>T. virens</i>	<i>Pseudomonas syringae</i>	In vitro	Cell-free filtering	–	Fungi: <i>R. solani</i> , <i>Alternaria alternata</i> , <i>A. solani</i> ,	Inhibition pathogen growth	Production of lytic enzymes (both)	Woo et al., 2002

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Table 2 (continued)

Trichoderma SPECIES	BACTERIA SPECIES	EXPERIMENT	APPLICATION	CROP	PATHOGEN/PEST	SYNERGISTIC EFFECTS	MECHANISMS OF ACTION	REFERENCE
T. viride	<i>P. fluorescens</i>	In vitro	Cell-free filtering	Black pepper	<i>Penicillium digitatum</i> and <i>F. graminearum</i> Oomycete: <i>P. ultimum</i> Oomicete: <i>P. capsici</i>	Reduced disease incidence	Production of lytic enzymes (both)	Diby et al., 2005
	<i>P. fluorescens</i>	In greenhouse	Cell suspensions	Tomato	Nematode: <i>M. incognita</i>	Reduced disease incidence and nematode reproduction	Unidentified	Moradi et al., 2015
	<i>Bacillus velezensis</i>	In greenhouse	Cell suspension ( <i>T. virens</i> ), Cell-free filtering ( <i>B. velezensis</i> )	Gooseberry	Fungus: <i>F. oxysporum</i> f. sp. <i>physali</i>	Reduced disease incidence	Unidentified	Izquierdo-García et al., 2020
	<i>B. velezensis</i>	In greenhouse	Cell suspensions	Tomato	Bacteria: <i>R. solanacearum</i>	Reduced disease incidence	Plant local resistance induction	Zhou et al., 2021
	<i>P. fluorescens</i>	In greenhouse	Cell application on seeds with carboxyl methylcellulose as adhesive	Groundnut	Fungus: <i>Sclerotium rolfsii</i>	Reduced disease incidence	Hydrogen cyanide production ( <i>P. fluorescens</i> ), Volatile antifungal secondary metabolites production ( <i>T. viride</i> )	Manjula et al., 2004
	<i>R. leguminosarum</i> bv. <i>vicia</i>	In field	Formulation with Arabic gum as carrier	<i>Vicia faba</i>	Fungus: <i>B. fabae</i>	Reduced disease incidence	Plant systemic resistance induction	Saber et al., 2009
	<i>P. fluorescens</i>	In field	Formulation with talc as carrier	Vanilla	Fungi: <i>Fusarium oxysporum</i> f. sp. <i>vanillae</i> and <i>Colletotrichum vanilla</i> Oomycete: <i>Phytophthora meadii</i>	Reduced disease incidence	Production of lytic enzymes ( <i>T. viride</i> )	Radjacomare et al., 2010
	<i>Bacillus cereus</i> <i>B. subtilis</i>	In field	Cell suspensions	Potato	Fungus: <i>R. solani</i>	Reduced disease incidence	Unidentified	Somani & Arora, 2010
	<i>P. fluorescens</i> <i>B. subtilis</i>	In greenhouse In field	Formulation with talc as carrier and carboxyl methylcellulose as adhesive	Nut	Fungus: <i>Lasiodiplodia theobromae</i>	Reduced disease incidence	Non-volatile antifungal secondary metabolites production (all of them), Siderophores production (all of them), Hydrogen cyanide production ( <i>T. viride</i> and <i>P. fluorescens</i> )	Latha et al., 2011
	<i>P. fluorescens</i>	In greenhouse	Formulation with talc as carrier	Chilli	Oomycete: <i>Pythium aphanidermatum</i>	Reduced disease incidence	Plant systemic resistance induction	Muthukumar et al., 2011
<i>P. fluorescens</i>	In vitro	Cell suspensions ( <i>P. fluorescens</i> ), Mycelial-agar plug ( <i>T. viride</i> )	–	Oomycete: <i>P. infestans</i>	Pathogen growth inhibition	Mycoparasitism ( <i>T. viride</i> )	Zegeye et al., 2011	
<i>B. subtilis</i>	In growth chamber	Cell suspensions	Cotton	Fungus: <i>M. phaseolina</i>	Reduced disease incidence	Plant local resistance induction	Triveni et al., 2015	
<i>P. fluorescens</i>	In greenhouse	Cell suspensions	Tomato	Nematode: <i>M. incognita</i>	Reduced disease incidence and nematode reproduction	Unidentified	Saeedzadeh, 2016	
<i>Azotobacter chroococcum</i>	In field	Cell suspensions	Chickpea	–	–	–	Plant systemic resistance induction	Velmourougane et al., 2017c
<i>P. fluorescens</i>	In greenhouse	Cell-free filtering	Peanut	Fungus: <i>F. oxysporum</i>	Reduced disease incidence	Plant local resistance induction	Rajeswari, 2019	
<i>P. fluorescens</i>	In greenhouse	Cell suspensions	Cowpea	Fungus: <i>F. oxysporum</i>	Reduced disease incidence	Unidentified	Ramasamy & Sundaram, 2020	
Trichoderma sp.	<i>Bacillus</i> sp.	In vitro	Cell-free filtering	Ginger	Oomycete: <i>Pythium myriotylum</i>	Reduced disease incidence	Non-volatile antifungal secondary metabolites production ( <i>Bacillus</i> sp.)	Jimtha et al., 2016
	<i>B. subtilis</i>	In vitro	Cell-free filtering	–	Fungus: <i>Colletotrichum gloeosporioides</i>	Pathogen growth inhibition	Non-volatile antifungal secondary metabolites production (both)	Emanuel et al., 2020
	<i>Bacillus</i> sp.	In vitro In greenhouse	Cell suspensions	Garlic	Fungus: <i>Fusarium oxysporum</i> f. sp. <i>cepae</i>	Reduced disease incidence Increased disease tolerance	Unidentified	Poromarto et al., 2022

(continued on next page)

Table 2 (continued)

<i>Trichoderma</i> SPECIES	BACTERIA SPECIES	EXPERIMENT	APPLICATION	CROP	PATHOGEN/PEST	SYNERGISTIC EFFECTS	MECHANISMS OF ACTION	REFERENCE
<i>Trichoderma</i> spp.	<i>P. fluorescens</i>	<i>In vitro</i>	Cell suspensions	-	Oomycetes: <i>Phytophthora colocasiae</i> and <i>Pythium</i> sp. Fungi: <i>R. solani</i> and <i>F. oxysporum</i>	Reduced pathogen population in soil	Unidentified	Jena, 2012
	<i>B. subtilis</i>	In greenhouse	Cell suspensions	Rice	Fungus: <i>R. solani</i>	Reduced disease incidence	Unidentified	Ali & Nadarajah, 2013
	<i>P. fluorescens</i> <i>B. subtilis</i>	In field	Cell suspensions	Tomato	Bacteria: <i>Ralstonia</i> spp.	Reduced disease incidence	Unidentified	Yendyo et al., 2017

production of these secondary metabolites, such as the production of siderophores that, in addition to providing a greater contribution of nutritional metals to plants, reduce their bioavailability for pathogens (Gu et al., 2020). This combined action has been reported in nuts co-inoculated with *T. viride*-*P. fluorescens*-*B. subtilis*, with talc as the carrier and carboxyl methylcellulose as the adhesive, synergistically reducing the disease caused by the fungus *Lasiodiplodia theobromae* due to the production of secondary antifungal metabolites and siderophores by the three microorganisms used as BCAs (Latha et al., 2011). The production of biocidal secondary metabolites can also be combined with the production of lytic enzymes (Karuppiah et al., 2019a). The combination of secondary metabolites and lytic enzymes was proposed as the main biocontrol mechanism of a consortium formed by *Trichoderma* and *Streptomyces* sp. in reducing soft rot disease incidence caused by *Erwinia* sp. in leek plants (Bustamam et al., 2022).

### 5.3. Hydrolytic and cell-wall degrading enzymes

*Trichoderma*, like different bacteria used as BCAs, has a great capacity to produce extracellular hydrolytic enzymes that will degrade the cell wall of different plant pathogens in a targeted manner. Among these enzymes, chitinases,  $\beta$ -1,3-glucanases, and proteases stand out (Kumari & Srividhya, 2020). The first study to describe the synergistic production of lytic enzymes by the *Trichoderma*-bacteria combination was in 1993, reducing *in vitro* the growth of the pathogenic fungi *Fusarium solani*, *Botrytis cinerea*, and *Uncinula necator* (Lorito et al., 1993). *Trichoderma*-bacteria co-inoculation has been described as a synergistic promoter in the production of lytic enzymes by *Trichoderma*; mainly chitinases,  $\beta$ -1,3-glucanases, and proteases (Montealegre et al., 2005; Radjacommare et al., 2010). However, in the *Trichoderma*-*P. fluorescens* co-inoculation in black peppers against *Phytophthora capsici*, the synergistic production of  $\beta$ -1,3-glucanases,  $\beta$ -1,4-glucanases, and lipase lytic enzymes by both BCAs was identified (Diby et al., 2005).

### 5.4. Competition for space and nutrients

The last direct mechanism of action for BCAs is competition for space and nutrients, widely described for microorganisms such as *Trichoderma* capable of establishing themselves very quickly in new niches, extensively colonizing the rhizosphere and superficial root tissues (Patel et al., 2019). It is possible to describe how the *Trichoderma*-bacteria co-inoculation in roots results in a synergistic increase in the endophytic and epiphytic colonization of plant tissues, which compete for fundamental space in the reduction of soil diseases. This is the case for the *T. harzianum*-*Enterobacter cloacae* combination in lettuce roots against the oomycete *Pythium ultimum* (Lynch et al., 1991), or *T. harzianum*-*P. fluorescens* in papaya roots against the nematode *M. incognita* (Rao, 2007). Another example of plant disease reduction through competition for space and nutrients was observed in a study conducted with combinations of *Trichoderma*, *Bacillus*, *Pseudomonas*, and *Streptomyces* microorganisms isolated from banana plants' rhizosphere, reducing *Fusarium* wilt incidence *in vivo* (Prigigallo et al., 2022). Among the combinations, the consortium formed by *T. virens*, *Pseudomonas chlororaphis*, and *B. velezensis* was found to be the most effective, combining competition for space and nutrients and antibiotic compounds.

### 5.5. Activation of host plant defenses

The main indirect mechanism of action by BCAs against agricultural pests and pathogens is the local and systemic activation of plant defenses. This is due to the microorganism-plant molecular dialogue, which causes plant recognition of microorganism-associated molecular patterns (MAMPs) and the activation of plant defenses against future possible biotic stress (Poveda et al., 2020b). At a local level, *Trichoderma*-*Bacillus* co-inoculation leads to the formation of biofilms that

colonize the root surface and induce an increase in SA-related defenses in these plant tissues (Karuppiyah et al., 2019b), and increases the activity of different defensive enzymes, such as polyphenol oxidase (PPO), peroxidase (POD), and superoxide dismutase (SOD) (Triveni et al., 2015; Zhou et al., 2021). Systemically, the activation of plant defenses by *Trichoderma*–bacteria co-inoculation includes both SA-related responses and JA-related defenses. For example, root inoculation with *T. asperellum*–*P. fluorescens* in peas synergistically reduced *Erysiphe pisi* conidial development on leaves as a consequence of systemic activation of JA-related defenses (Patel et al., 2016). The most widely studied plant synergistic defensive responses are the activities of different enzymes. Through this mechanism of action, *Trichoderma*–bacteria co-inoculation has shown reductions in disease incidence of over 60 %, including enzymes such as  $\beta$ -1,3-glucanase, PPO, SOD, phenylalanine ammonia lyase (PAL), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and catalase (CAT), along with other proteins, such as pathogenesis-related protein 1 (PR-1) (Alizadeh et al., 2013; Kumar et al., 2015b; Youssef et al., 2016; Madhavi et al., 2018). Together with the increase in enzymatic activity, plants respond systemically to biotic stresses through the synthesis of biocidal secondary metabolites. *Trichoderma*–bacteria co-inoculation achieves synergistic effects in reducing disease in different crops by close to 80 % mainly due to the addition to plant defenses of the systemic accumulation of phenolic compounds (Saber et al., 2009; Muthukumar et al., 2011; Kumar et al., 2015a; Shafique et al., 2015; Velmourougane et al., 2017c). For example, the combined use of *Trichoderma*–bacteria was successfully used to protect eggplants from the fungal pathogen *Verticillium dahliae* by activating plant defensive enzymes (Bilginturan & Karaca, 2021). It was also used in rapeseed against *Verticillium longisporum*, activating the JA and ET

pathways, which are first steps in activating the induced systemic resistance (ISR) response (Hafiz et al., 2022).

### 5.6. Rhizospheric microbiota modification

Another important indirect mechanism of action increasingly studied in different BCAs is the ability to modify the rhizospheric microbiota, increasing the diversity and population of other antagonistic microorganisms of plant pathogens (Hu et al., 2021). The root application of *T. harzianum*–*B. subtilis* has shown both an increase in the diversity and abundance of beneficial bacteria and beneficial fungi and great antagonistic capacity against pathogens such as *Fusarium solani* f. sp. *phaseoli* in beans or *Streptomyces* sp. in potatoes (Abeyasinghe, 2012; Wang et al., 2019). Despite the good synergistic results reported for *Trichoderma*–bacteria combinations, many other studies did not find evidence of a synergistic effect. Some examples are combinations with species within *Bacillus* (Yobo et al., 2011; Kamel and El-Khateeb, 2012), *Pseudomonas* (Mathivanan et al., 2005; Afzal et al., 2013; Duc et al., 2017; Kumar et al., 2017; Shafique et al., 2017; Madhavi & Devi, 2018), *Rhizobium* (Negi et al., 2021), *Mesorhizobium* (Dubey et al., 2015), or the *Burkholderia* genus (Meyer et al., 2001). These co-inoculations may even have a negative effect on the activity of both components. For example, the *T. asperelloides*–*Bacillus paralicheniformis* combination is less effective against *F. oxysporum* in tomato plants than the inoculation of each microorganism in isolation (Ramírez-Cariño et al., 2020). This may be due to significant inhibition of *Trichoderma* growth by bacteria, as has been described in combinations with *P. fluorescens* (Pan & Jash, 2011) and mycophagous bacteria of the genus *Collimonas* (Höppener-Ogawa et al., 2009). Therefore, the *Trichoderma*–bacteria combination appears

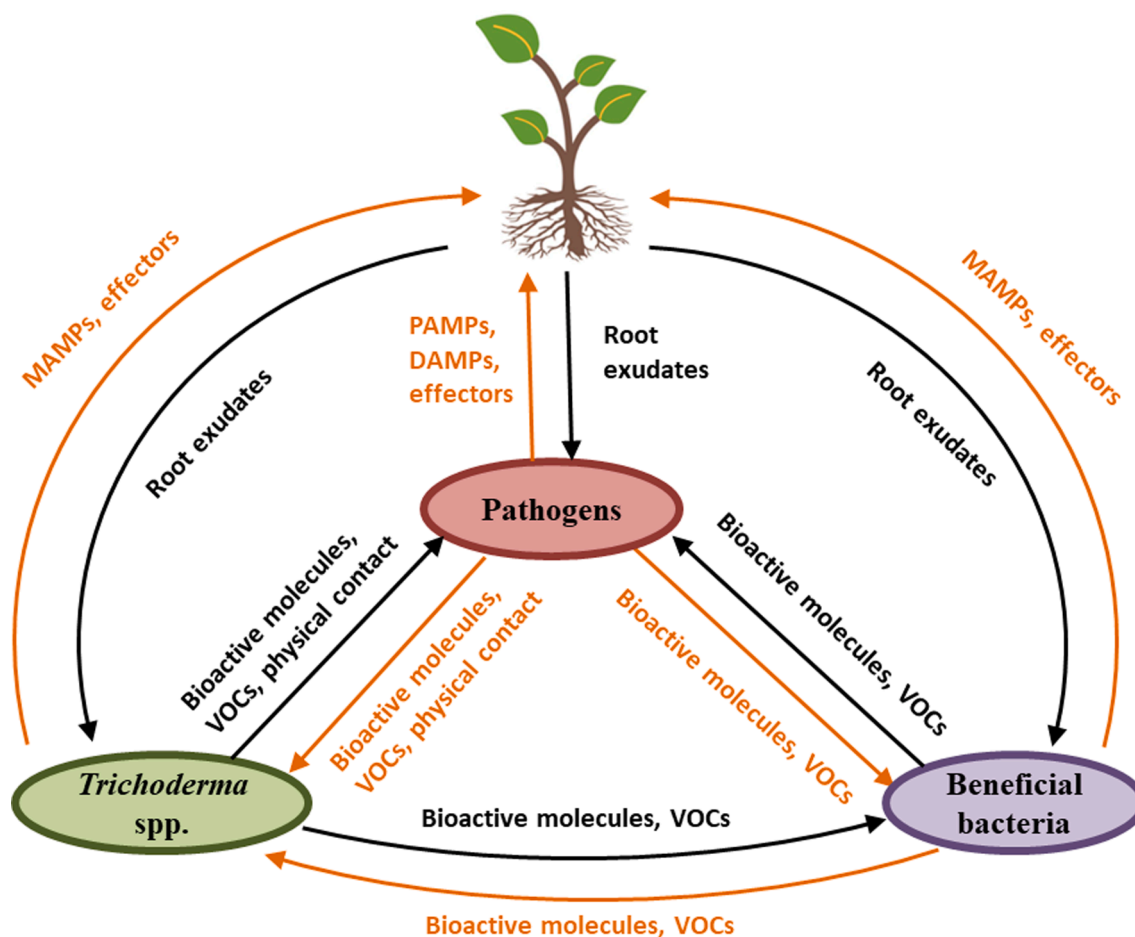


Fig. 2. Summary infographic with the recognition systems in the multitrophic relationship between plant, *Trichoderma*, beneficial bacteria and pathogen.

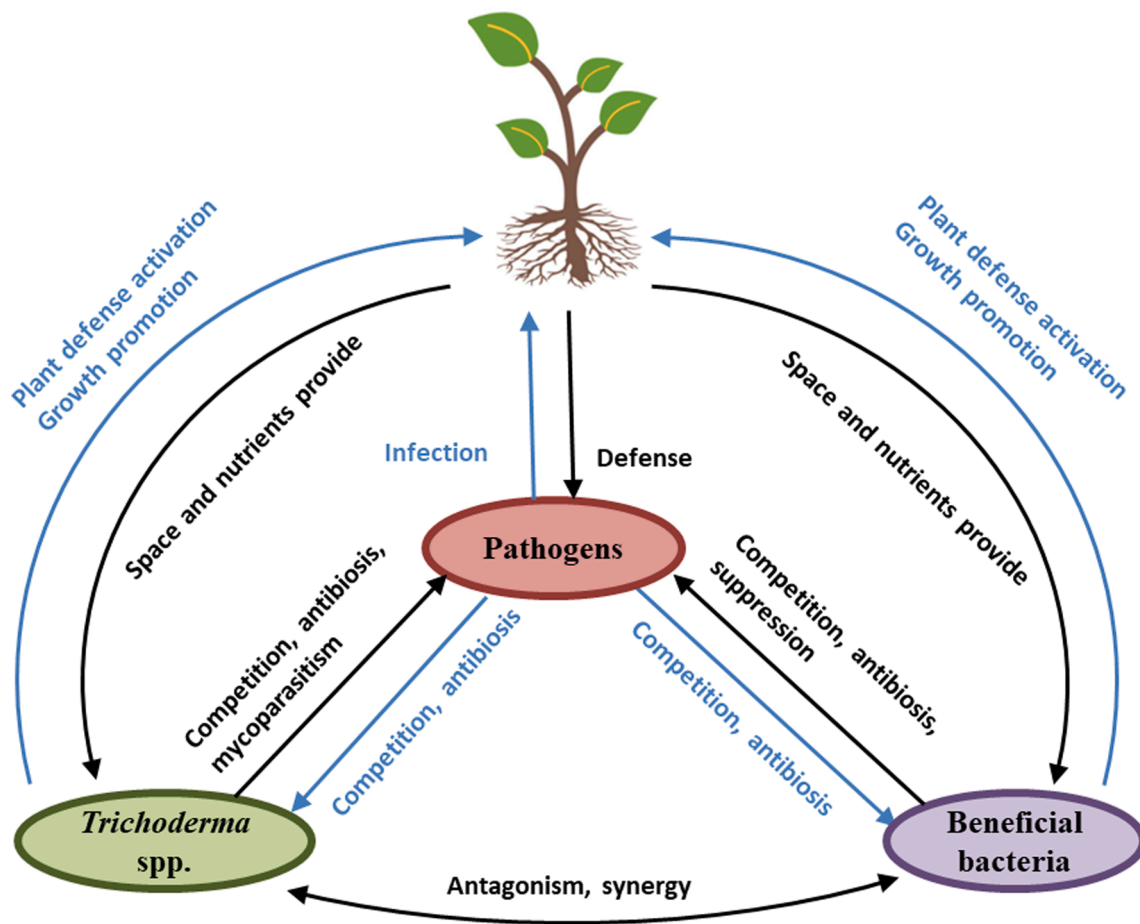


Fig. 3. Summary infographic with the interaction systems in the multitrophic relationship between plant, *Trichoderma*, beneficial bacteria and pathogen.

to be quite specific and requires in-depth research to achieve beneficial synergistic effects in agriculture.

Although there are several studies with very positive results of biocontrol efficacy in *Trichoderma*–bacteria co-inoculations, the molecular mechanisms involved have not been identified. In future works, it would be interesting to deepen our understanding of how microorganisms modulate the expression of genes related to biocontrol, such as hydrolytic enzyme producing genes, antimicrobial peptide genes, and siderophore producing genes.

### 6. Interactions between the host plant, *Trichoderma*, beneficial bacteria and pathogens

When using a BCA, it is of key importance to know how the microorganisms interact in the environment with the plant, the pathogen, and with the microbiota as a whole. Many works have focused on bitrophic interactions of BCA–plant, plant–pathogen, or BCA–plant, but there is much more to investigate.

A well-known example is the bitrophic interaction of the *Trichoderma* fungi with plants (Poveda et al., 2020a). In order to colonize the plant and establish the symbiotic relationship, the fungus must fulfill a sequence of steps: recognition and adherence to plant roots, penetration of the plant, and survival to the plant defense metabolites (Hermosa et al., 2012). The fungus recognizes the plant root through its root exudates, which act as signal for starting colonization, and adheres itself to the roots through the action of hydrophobins, which also protect the fungus from plant toxic metabolites once it has penetrated the roots (Viterbo & Chet, 2006). To establish itself in this environment, *Trichoderma* requires the actions of several molecules, such as swollenin, proteins with cellulase activity, and ISR elicitors (Brotman et al., 2008;

Saravanakumar et al., 2016). Once *Trichoderma* has established itself within plant roots, symbiotic interactions may start with one or more of these eventual outcomes: plant growth promotion, yield increase, nutrient and water uptake increase, or biotic and abiotic stress alleviation (Swain & Mukherjee, 2020).

Interactions between beneficial bacteria and plants follow a similar process to a certain degree, since bacteria also detect plants through organic acids and sugars exudated from roots and then attach to the root surface. A signaling exchange occurs in the rhizosphere and the bacteria switch to a colony-based lifestyle and start to attach and form a biofilm. The plant-associated microbiota overcome the host defenses by secreting effector proteins to avoid the generation of reactive oxygen species, or the induction of SA and JA signaling pathways (Trivedi et al., 2020).

Plant pathogens are also engaged in a continuous co-evolutionary race with its plant hosts. In this co-evolution, plants have developed two different strategies to detect pathogens through specific detectors, such as pattern recognition receptors (PRR), located on the external face of the cells. PRRs are capable of recognizing pathogen-associated molecular patterns (PAMPs), which are components of the pathogens such as fungal chitin, and also danger-associated molecular patterns (DAMPs), which are molecules released upon pathogen attack, such as cell wall fragments. Once these PRRs have detected these patterns, pattern-triggered immunity (PTI) is activated. To overcome this defense barrier, pathogens have developed molecular tools called effectors, able to inactivate this response in the host, with a vast range of these molecules across fungal and bacterial communities. The second defense strategy plants have developed is based on intracellular receptors able to recognize certain pathogen effectors, and trigger effector-triggered immunity (ETI). In this context, pathogens and host plants are involved in a

continuous and dynamic cycle, with plants recognizing pathogens through receptors and pathogens manipulating the defense response through effector molecules (Yuan et al., 2021).

Another biotrophic interaction that has been studied in this context is that of the BCA and the pathogen. *Trichoderma* fungi are the most well-studied BCA, yet it is not completely understood how it recognizes the pathogen. The current model suggest that these fungi recognize pathogens by their diffused bioactive molecules, but Li et al. (2018) suggested that two strains of *T. virens* and *T. viride* sense *F. oxysporum* specifically through volatile organic compounds (Li et al., 2018). After recognition interaction may occur, and the outcome of this interaction is usually the suppression of the pathogen by *Trichoderma* through mycoparasitism, antibiosis, or direct competition for space and nutrients (Swain & Mukherjee, 2020).

Separate biotrophic interactions cannot be extrapolated to field conditions however, since all agents involved (host plant, pathogen, *Trichoderma*, and bacteria) form a complex network of interactions that makes it difficult to predict the outcome (Rodriguez et al., 2019; Alfiky and Weisskopf, 2021). Many factors may influence the result of inoculating microorganism consortia, such as the interaction among species, the host plant, or the targeted pathogen, as well as environmental conditions such as the physiochemical and microbiological state of the soil or weather (Ben M'henni et al., 2022). Multitrophic crosstalk interactions should be further investigated to better understand how organisms influence each other.

In Figs. 2 and 3 we have tried to summarize by means of infographics the processes of recognition and interaction, respectively, that occur in this multitrophic relationship between plants, *Trichoderma*, beneficial bacteria and pathogens.

## 7. Conclusions and future perspectives

Inoculation of *Trichoderma*–bacteria consortia in plants may result in a growth promotion greater than the sum of its components; synergistic effects may occur when studied and developed properly, and for that to happen several factors should be considered. One of the first questions to be answered is the compatibility of the candidate microorganisms that will potentially form the bio-inoculant, which should be studied for each case individually. Some bacteria with antifungal capacity, such as some *Bacillus* and *Pseudomonas* species, could have an antagonistic effect on the fungus. The formation of biofilms containing both microorganisms is a key aspect for success. Another important step to develop is the formulation of the bio-inoculant. Many different techniques and formulations have been developed in recent years, but all must ensure the viability of both microorganisms' cells and capacities over an acceptable period of time, as well as a high cell concentration, absence of contamination, and ease of application in agriculture.

Research conducted on the combination of *Trichoderma* fungi with beneficial bacteria has focused mainly on the control of agricultural diseases, with less focus on control of pests and almost none on control of viruses. Furthermore, little research has studied the effect of the co-inoculation of such microorganisms in terms of the tolerance to abiotic stresses in plants. Therefore, more research is needed for these specific aspects.

Although plant growth promotion and biocontrol mechanisms are known in the case of *Trichoderma* fungi and beneficial bacteria, the mechanisms that trigger the synergistic effect of both are often unknown. In future studies, these mechanisms should be determined in order to develop efficient BCA formulations.

In the present context of agriculture, where the use of chemical pesticides needs to be limited while maintaining levels of pests and diseases below the economic loss threshold, the efficacy of novel alternatives should be similar to that of current options. Many studies considered in this review compared results obtained with the co-inoculation of *Trichoderma*–bacteria with current chemical pesticides, obtaining similar disease reduction values. The combination of such

microorganisms with chemical pesticides may have a further synergistic effect and enhance its effectiveness.

Extensive studies in the literature demonstrate how the combination of *Trichoderma*–bacteria has potential in agriculture management, both as a biofertilizer and BCA. However, more studies are needed regarding the effect of this strategy on abiotic stress tolerance in plants, and as BCAs in virus control.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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