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RECEIVED 18 February 2025

ACCEPTED 08 April 2025

PUBLISHED 07 May 2025

## CITATION

Villavicencio-Vásquez M, Espinoza-Lozano F,  
Espinoza-Lozano L and Coronel-León J  
(2025) Biological control agents: mechanisms  
of action, selection, formulation and  
challenges in agriculture.  
*Front. Agron.* 7:1578915.  
doi: 10.3389/fagro.2025.1578915

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# Biological control agents: mechanisms of action, selection, formulation and challenges in agriculture

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The increasing global population has intensified the demand for food production, both in quantity and quality. To meet this challenge and prevent crop diseases, chemical pesticides have been widely used. However, their negative effects on human health and the environment have driven the search for sustainable alternatives. In this context, microbial-based biopesticides, particularly *Trichoderma* and *Bacillus*, have emerged as key biocontrol agents in agroecosystems. This review provides a comprehensive overview of their agricultural significance, focusing on their mechanisms of action, efficacy, and biotechnological advancements aimed at optimizing their performance. *Trichoderma* spp. act primarily through mycoparasitism, nutrient competition, and antibiosis, while *Bacillus* spp. employ mechanisms such as lipopeptide production, lytic enzymes, siderophores, and the activation of induced systemic resistance (ISR). Unlike traditional reviews that primarily describe their biological functions, this work offers a structured framework for their application, addressing key factors such as selection criteria for effective biocontrol agents, formulation strategies, product stability, viability, and regulatory challenges in commercial agriculture. Additionally, recent studies on genetic enhancement, including CRISPR-based modifications, are examined to improve their adaptability, metabolic activity, and effectiveness in pathogen control across diverse agricultural systems. By integrating biological, technological, and practical perspectives, this review aims to bridge the gap between scientific advancements and real-world agricultural applications, contributing to the development of scalable and sustainable disease management strategies.

## KEYWORDS

beneficial microorganisms, biological control agents, biopesticides, crop protection, sustainability

# 1 Introduction

Agriculture has been a fundamental pillar in the social and economic transformation of countries, particularly in developing nations, where it plays a crucial role in ensuring food security and contributing significantly to the economy. However, the prolonged use of agrochemicals has led to serious consequences for the environment, soil health, and human well-being, making the need for more sustainable alternatives ever more pressing (Zhao et al., 2020). According to the World Health Organization (WHO, 2022), over 1,000 pesticides are used worldwide, many of which accumulate in the adipose tissue of organisms, posing risks to both health and the environment (Souza, 2016; Zhang et al., 2019). Although pesticides are necessary for crop protection and food security, their inappropriate or excessive use can lead to serious consequences (Beyuo et al., 2024). These include the negative impact on non-target organisms, such as pollinators and beneficial species that naturally control pests (Elhamalawy et al., 2024), as well as the promotion of pest resistance, which complicates pest control and increases production cost (Kumar Chaube and Pandey, 2022).

In the face of these challenges, it is fundamental to explore alternatives such as Integrated Pest Management (IPM), which integrates biological control agents (BCAs) such as bacteria, fungi, and viruses (Thomine et al., 2022). Recent advancements in pesticide application technologies, such as precision application systems, allow for more accurate dosing, reducing pesticide use and minimizing environmental impact (Anastasiou et al., 2023). Furthermore, the increasing pressure from consumers and international organizations for clean food production has spurred the search for innovative solutions that minimize the harmful effects of agrochemicals (Thilagam et al., 2023). An example is the global commitment to achieving sustainable agriculture by 2050, aligned with the Sustainable Development Goals (SDGs) (Blazhevskaja, 2019).

In this context, BCAs have emerged as a promising solution to reduce dependence on synthetic pesticides and minimize their environmental impact (Damalas and Koutroubas, 2018). Notably, the genera *Trichoderma* and *Bacillus* have gained considerable attention due to their ability to suppress pathogens and promote plant growth naturally (Tyagi et al., 2024). These microorganisms employ several mechanisms to protect plants, including nutrient competition, production of antibiotic metabolites, induction of systemic resistance (ISR) in plants, and competition for space in the soil (Poveda, 2021; Prasad et al., 2023).

This article provides a comprehensive review of the potential of *Trichoderma* spp. and *Bacillus* spp. as BCAs, analyzing their mechanisms of action, selection processes, and the challenges associated with their formulation and large-scale implementation. The effectiveness of these microorganisms is influenced by various factors, including environmental conditions, proper formulation, and the selection of specific strains with desirable characteristics (Naamala and Smith, 2020). By reviewing the latest scientific literature, this paper aims to provide a holistic view of how these microorganism function in modern agriculture, highlighting their successes and the challenges that must still be overcome to ensure

their widespread adoption in sustainable agricultural systems. Moreover, this review compiles a list of biological products currently available on the Ecuadorian market (Supplementary Materials), offering valuable insights into the practical application of BCAs in different agricultural contexts. This information can help guide future efforts in integrating these biocontrol solutions more broadly into farming practices, contributing to a shift toward more environmentally sustainable agriculture.

# 2 *Trichoderma* and *Bacillus* in agriculture

The use of microorganisms in agriculture has gained significant attention as environmentally friendly alternatives to chemical pesticides. These microorganisms include bacteria, fungi, viruses, and protozoa, that enhancing plant health and performance. Various studies have highlighted the effectiveness of bacteria such as *Rhizobium*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Klebsiella*, *Bacillus*, and *Pseudomonas* in agricultural applications (Alizadeh et al., 2020). Also, endophytic microorganisms, which live inside plants without causing harm, are also part of the plant growth-promoting (PGP) group. Among endophytic fungi, species like *Penicillium* sp., *Guignardia mangiferae*, *Hypocrea* sp., *Neurospora* sp., *Eupenicillium javanicum*, *Lasiodiplodia theobromae*, and *Trichoderma* sp. have proven effective in inhibiting *Fusarium oxysporum* f.sp. *cucumerinum*, the principal causal agent of stem rot in cucumber under greenhouse conditions (Abro et al., 2019). Among these agents, fungi such as *Trichoderma* spp. and bacteria such as *Bacillus* spp. enhancing soil health, promoting plant growth, and controlling plant pathogens (Alizadeh et al., 2020).

*Trichoderma* is a filamentous fungus present in the rhizosphere and roots (Ghorbanpour et al., 2018; Jaroszuk-Ścisiel et al., 2019). According to the MycoBank classification, the *Hypocrea/Trichoderma* includes more than 300 species characterized both molecularly and morphologically (Tamandegani et al., 2020). These fungi colonize plant roots and produce metabolites with antimicrobial properties, contributing to plant health (Tyśkiewicz et al., 2022). *Trichoderma* spp. can degrade toxic contaminants like herbicides and fungicides through enzymatic processes that break down cellulose and lignin (Escudero-Leyva et al., 2022). *Trichoderma* species are renowned for their antagonistic activities against various fungal pathogens, including *F. oxysporum*, *Botrytis cinerea*, *Rhizoctonia solani*, and *Alternaria alternata* (Es-Soufi et al., 2020). *T. asperellum* and *T. harzianum* are frequently noted for their ability to inhibit the growth of *Fusarium* spp. through the production of hydrolytic enzymes and growth inhibition mechanisms (Vargas-Hoyos and Gilchrist-Ramelli, 2015; Miguel-Ferrer et al., 2021). The wide array of enzymes, including exo- and endo- $\beta$ -glucanases and proteases, which degrade the structural components of the pathogen's cell walls, further facilitating colonization (Alizadeh et al., 2020). In comparison, *T. spirale* and *T. atroviride* excel in antibiosis and the production of extracellular metabolites, which suppress pathogens like *Corynespora cassicola* and *Spodoptera frugiperda*, respectively (Contreras-Cornejo et al., 2018; Baiyee et al., 2019).

Economically, applying *Trichoderma* has led to reduced pesticide usage, decreased crop losses, and potentially higher yields, offering a cost-effective solution for sustainable farming, especially in developing countries (Morales-Mora et al., 2020; Martínez-Salgado et al., 2021). The data presented in Table 1 illustrates *Trichoderma*'s significant role in controlling numerous economically important pathogens across various crops, including tomatoes, wheat, maize, and avocados.

On the other hand, *Bacillus* spp. is recognized for promoting plant growth, enhancing soil fertility, and serving as a biocontrol agent. These Gram-positive, aerobic bacteria produce bioactive compounds that support plant health and reduce pathogen pressure (Etesami et al., 2023). *Bacillus subtilis* exhibits antifungal properties attributed to metabolites like surfactin, iturin, and fengycin (Méndez-Úbeda et al., 2018; Chen et al., 2021). They also act as biofertilizers or biostimulators by facilitating nutrient uptake and producing plant hormones (Dimopoulou et al., 2021). Integrating *Bacillus* strains into agricultural practices has led to reduced reliance on chemical pesticides and supported sustainable farming methods.

The selection of *Trichoderma* and *Bacillus* species for agricultural use requires careful consideration of factors such as their ability to produce effective metabolites, efficiency in nutrient acquisition, and stability under varying environmental conditions (Silva et al., 2019). Both genera offer distinct advantages for different agricultural systems, with *Trichoderma* providing broad-spectrum biocontrol against fungal pathogens and *Bacillus* contributing to disease management and plant growth promotion. Their integration into farming practices can lower production costs, enhance soil health, and improve overall crop productivity (Martínez, 2021). Table 2 highlights the effectiveness of different *Bacillus* spp. in managing plant diseases across various crops, underscoring their importance in sustainable agriculture.

### 3 Mechanism of action of *Trichoderma* spp.

As mentioned, BCAs offer significant benefits to the agricultural sector. However, their effective application depends on a thorough understanding of each microorganism's specific mechanism of action. This lack of clarity can raise concerns among farmers about whether the selected microorganism is truly appropriate for their needs. To address this issue, this section provides farmers and policymakers with a detailed description of the mechanisms of action of *Trichoderma* spp. The key biocontrol strategies that *Trichoderma* spp. develops in direct conflict with fungal pathogens, such as mycoparasitism, competition, and antibiosis (Figure 1).

#### 3.1 Mycoparasitism

*Trichoderma* spp. employs a dual strategy to degrade the pathogen's cell wall: enzymatic degradation and secondary metabolite production. It secretes extracellular enzymes such as

chitinases, glucanases, and proteases (Cortés Hernández et al., 2023), breaking down structural components of the pathogen's hyphae. In addition, bioactive compounds like peptaibols and gliotoxins further weaken the pathogen, enhancing penetration and colonization (Leiva et al., 2020; Pimentel et al., 2020). For example, *T. asperellum* produces chitinases and cellulases that significantly affect *F. oxysporum* in *Stevia rebaudiana* (Díaz-Gutiérrez et al., 2021). Similarly, *T. virens* has demonstrated effectiveness against *R. solani*, with electron microscopy revealing structural changes such as protuberances, coiling, and cell wall lysis (Inayati et al., 2020). Additionally, hyphal interactions between *T. virens* and *Sclerotium rolfsii* have been documented where *T. virens* acts targets sclerotia and conidia within the host fungal tissue (Mukherjee et al., 2022).

#### 3.2 Competition for nutrients and space

*Trichoderma* spp. exerts biocontrol through competition by effectively competing with pathogens for limited resources such as space, nutrients, water, and light (Khan et al., 2020). Its rapid growth allows it to efficiently consume nutrients, such as carbohydrates, nitrogen, and oxygen, reducing their availability for pathogens (Hariharan et al., 2022). This ability to quickly colonize decreases the amount of nutrients accessible to phytopathogenic fungi, contributing to their control (Cortés Hernández et al., 2023).

Additionally, *Trichoderma* spp. colonizes the plant rhizosphere, forming a protective layer around the roots and preventing pathogen establishment (Basińska-Barczak et al., 2020; Oszust et al., 2020). *T. harzianum* has a growth rate 2.0 to 4.2 times faster than *B. cinerea* (Risoli et al., 2022), *T. viride* competes with *Fusarium graminearum*, the causative agent of fusariosis in cereals, through mechanisms such as adhesion, entanglement, and interpenetration of its hyphae, damaging the structures of *F. graminearum* with enzymes like chitinases and glucanases (Dugassa et al., 2021).

The competition for nutrients between *Trichoderma* spp. and plants can be significant, particularly in soils with low levels of nitrogen, phosphorus, or potassium. Under these conditions, *Trichoderma* spp. may deplete essential resources before plants can absorb them, which is especially concerning in degraded soils or those with poor fertilization management (Pedraza et al., 2020). Additionally, excessive application of *Trichoderma* spp. can lead to high concentrations in the soil, intensifying competition with plant roots and potentially hindering early growth (Sood et al., 2020). Environmental stress factors such as drought or salinity further influence this competition, limiting nutrient availability and reducing the efficiency of beneficial symbioses, such as those with mycorrhizal fungi (Xiao et al., 2023). Moreover, in soils with high microbial activity, *Trichoderma* may compete with other beneficial microorganisms for space and resources, potentially disrupting the soil microbiota and indirectly affecting plant health.

To mitigate these challenges, several strategies have been proposed. First, co-applying *Trichoderma* spp. with plant growth-

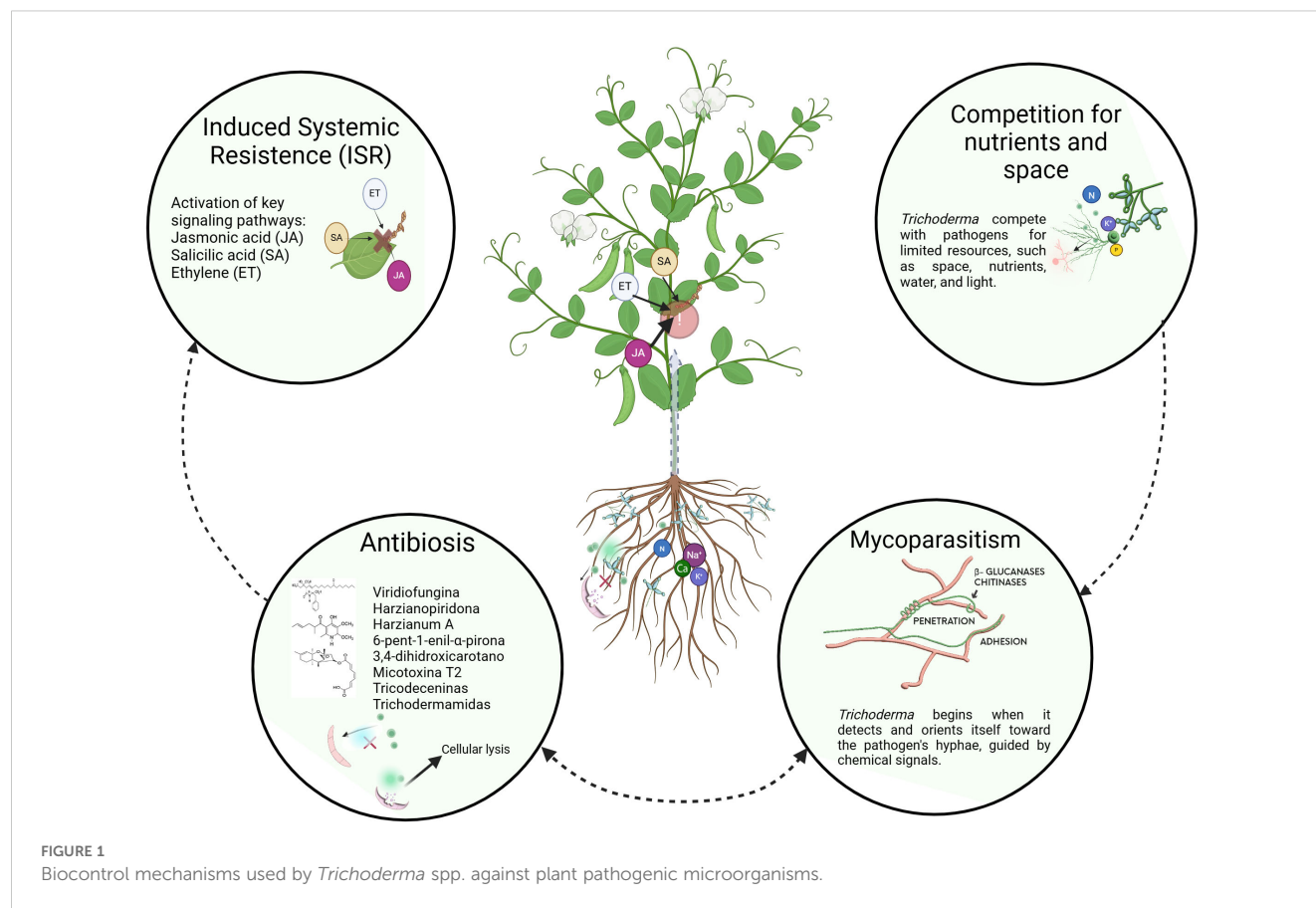
TABLE 1 Plant pathogenic fungi controlled by different strains of *Trichoderma*.

Crop	Pathogen	Strain- <i>Trichoderma</i>	Mechanism of action	References
Tomato ( <i>Solanum lycopersicum</i> )	<i>Fusarium</i> sp.	<i>T. asperellum</i>	Growth inhibition Hydrolytic enzymes	(Vargas-Hoyos and Gilchrist-Ramelli, 2015)
Wheat ( <i>Thriticum</i> spp.)	<i>F. culmorum</i> , <i>F. oxysporum</i> , <i>F. graminearum</i>	<i>Trichoderma</i> sp.	Mycoparasitism	(Jaroszuk-Ścisiel et al., 2019)
Chili pepper ( <i>Capsicum annum</i> L.)	<i>Colletotrichum truncatum</i>	<i>T. harzianum</i> <i>T. asperellum</i>	Systemic Resistance	(Yadav et al., 2021)
Tomato ( <i>S. lycopersicum</i> )	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	<i>T. asperellum</i>	Antagonism	(Mahmoud et al., 2015)
Lettuce ( <i>Lactuca sativa</i> L.)	<i>Corynespora cassiicola</i> , <i>Curvularia aeria</i>	<i>T. spirale</i>	Antibiosis (extracellular metabolites)	(Baiyee et al., 2019)
Maize ( <i>Zea mays</i> )	<i>Spodoptera frugiperda</i>	<i>T. atroviride</i>	Hydrolytic enzymes	(Contreras-Cornejo et al., 2018)
Jamaica ( <i>Hibiscus sabdariffa</i> L.)	<i>Phytophthora parasitica</i> , <i>F. oxysporum</i>	<i>Trichoderma</i> spp.	Mycoparasitism	(Alejandro et al., 2019)
Chili ( <i>C. annum</i> )	<i>Fusarium solani</i>	<i>T. harzianum</i>	Mycoparasitism	(Miguel-Ferrer et al., 2021)
Manzano chili ( <i>Capsicum pubescens</i> )	<i>Phytophthora capsici</i>	<i>T. harzianum</i>	Growth inhibition	(Valencia et al., 2021)
Onion ( <i>Allium cepa</i> )	<i>Fusarium</i> sp.	<i>T. harzianum</i>	Growth inhibition	(Martínez-Salgado et al., 2021)
Avocado ( <i>Persea americana</i> )	<i>Phytophthora cinnamomi</i>	<i>T. asperellum</i> , <i>T. hamatum</i> , <i>T. koningiopsis</i> , <i>T. harzianum</i>	Biocontrol	(Andrade-Hoyos et al., 2020)
Onion ( <i>A. cepa</i> )	<i>Alternaria porri</i>	<i>T. asperellum</i>	Defense response induction	(Camacho-Luna et al., 2021)
Strawberry ( <i>Fragaria</i> spp.)	<i>A. niger</i> , <i>Colletotrichum</i> sp., <i>R. stolonifera</i>	<i>T. harzianum</i>	Antagonism	(Morales-Mora et al., 2020)
Nopal ( <i>Opuntia ficus-indica</i> )	<i>Pythium</i> sp., <i>Fusarium</i> sp., <i>Colletotrichum</i> sp.	<i>T. harzianum</i> , <i>T. viride</i> , <i>T. atroviride</i> , <i>T. hamatum</i>	Antagonism	(Valencia De Ita et al., 2020)
Maize ( <i>Z. mays</i> )	<i>Sporisorium reilianum</i>	<i>Trichoderma</i> spp.	Bioestimulant	(Landero Valenzuela et al., 2019)
Wheat ( <i>Thriticum</i> spp.)	<i>F. pseudograminearum</i>	<i>T. afroharzianum</i> , <i>T. harzianum</i> , <i>T. gamsii</i>	Biocontrol	(Stummer et al., 2020)
Melon ( <i>Cucumis melo</i> )	<i>F. oxysporum</i> , <i>F. solani</i>	<i>T. asperellum</i>	Antagonism	(Espinoza Ahumada et al., 2019)
Tomato ( <i>S. lycopersicum</i> )	<i>F. oxysporum</i> , <i>B. cinerea</i>	<i>T. asperellum</i>	Biocontrol	(Herrera-Téllez et al., 2019)
Chili pepper ( <i>Capsicum annum</i> L.)	<i>C. truncatum</i>	<i>T. harzianum</i> , <i>T. asperellum</i>	Induction of Plant Resistance in Response	(Saxena et al., 2020)
Soft Fruit	<i>Colletotrichum</i> sp., <i>Botrytis</i> sp., <i>Verticillium</i> sp., <i>Phytophthora</i> sp.	<i>Trichoderma</i> spp.	Competition for Nutrients and Space	(Oszust et al., 2020)
Wheat ( <i>Thriticum</i> spp.)	<i>F. culmorum</i> , <i>F. oxysporum</i> , <i>F. graminearum</i>	<i>Trichoderma</i> sp.	Mycoparasitism	(Jaroszuk-Ścisiel et al., 2019)
Groundnut ( <i>Arachis hypogaea</i> )	<i>Sclerotium rolfsii</i>	<i>T. virens</i>	Hydrolytic enzymes	(Hirpara et al., 2017)

promoting bacteria (PGPB) helps balance nutrient competition while maintaining its biocontrol efficacy (Pedraza et al., 2020). Second, using controlled-release formulations, such as encapsulated systems, enables a gradual release of *Trichoderma* spp., preventing

excessive competition in the early stages of plant development and promoting a more stable soil establishment (Mukherjee et al., 2022). Additionally, optimizing fertilization practices ensures adequate levels of essential nutrients, reducing competition and allowing





*Trichoderma* spp. to function effectively as a biocontrol agent without compromising plant growth (Xiao et al., 2023). Finally, selecting *Trichoderma* strains adapted to specific soil conditions and crop nutritional needs enhances pathogen suppression while minimizing negative effects on plant nutrient uptake (Baker et al., 2020).

### 3.3 Antibiosis

*Trichoderma* spp. produce a wide range of secondary metabolites with antimicrobial properties that inhibit the growth of pathogens (Masi et al., 2018). These compounds include peptaibols, polyketides, and terpenes (Cortés Hernández et al., 2023). *T. virens* produces trichodermamides, while *T. koningii* synthesizes koniginins, both of which exhibit antimicrobial activity against pathogens such as *R. solani*, *F. oxysporum*, *Verticillium dahliae*, and *B. cinerea* (Manganiello et al., 2018). *T. atroviride* produces the volatile compound 6-pentyl-2H-pyran-2-one (6-PP), which not only promotes plant growth but also regulates sugar transport in the roots of *Arabidopsis* (Esparza-Reynoso et al., 2021) and *T. viride* synthesizes trichotoxins A and B, tricodectenins, trichorovins, and tricolcelins (Khan et al., 2020).

Among the bioactive compounds, *Trichoderma* also produces growth hormones such as auxins, cytokinins, and gibberellins, which stimulate plant development and overall growth (Estrada-

Rivera et al., 2019). It activates ISR, enhancing the plant's defense mechanisms against pathogens. *Trichoderma*'s ability to induce ISR in plants such as chili peppers and tomatoes against *Colletotrichum truncatum* and *F. oxysporum* (Yadav et al., 2021) strengthens the plant's defense system, providing long-term protection beyond direct antagonism. *T. asperellum* is particularly effective in controlling *Fusarium* spp. and inducing ISR, especially in tomatoes and onions (Camacho-Luna et al., 2021). Strains like *T. asperellum* are known to improve nutrient uptake, particularly phosphorus, by solubilizing ions that are typically inaccessible in many soils (Bononi et al., 2020). This nutrient solubilization, combined with ISR, enhances the plant's overall health, making it more resistant to both biotic and abiotic stresses. Another mechanism associated with the use of *Trichoderma* is its ability to produce antimicrobial compounds, lignify cell walls, and activate signaling pathways such as jasmonic acid (JA) and ethylene (ET), which are essential for the plant's response to biotic stress (Alizadeh et al., 2020).

Together, the multiple mechanisms of action of *Trichoderma* from the production of antimicrobial compounds to the stimulation of induced defenses and the optimization of plant nutrition not only make it a highly effective biocontrol agent but also reduce reliance on synthetic agrochemicals. This promotes more sustainable agriculture by minimizing environmental impact and enhancing crop resilience against diseases and adverse conditions.

TABLE 2 Plant pathogenic fungi controlled by different strains of *Bacillus*.

Crop	Pathogen	Strain - <i>Bacillus</i>	Mechanism of action	References
Potato ( <i>Solanum tuberosum</i> )	<i>Fusarium</i> sp.	<i>B. subtilis</i>	Antagonism	(Méndez-Úbeda et al., 2018)
Passion fruit ( <i>Passiflora edulis</i> )	<i>F. solani</i>	<i>B. subtilis</i>	Biocontrol	(Chen et al., 2021)
Maize ( <i>Z. mays</i> )	<i>Cephalosporium maydis</i>	<i>B. subtilis</i>	Siderophore production	(Ghazy and El-Nahrawy, 2021)
Rice ( <i>Oryza sativa</i> )	<i>Rhizoctonia solani</i>	<i>B. subtilis</i>	Antagonism Biocontrol	(Ghazy and El-Nahrawy, 2021)
Potato ( <i>S. tuberosum</i> )	<i>Phytophthora infestans</i> , <i>F. oxysporum</i>	<i>B. subtilis</i>	Growth inhibition	(Lastochkina et al., 2020)
Soybean ( <i>Glycine max</i> )	<i>Phytophthora sojae</i>	<i>B. subtilis</i> , <i>B. amyloliquefaciens</i>	Biocontrol	(Liu et al., 2019)
Tomato ( <i>S. lycopersicum</i> )	<i>B. cinerea</i> , <i>Cladosporium fulvum</i>	<i>B. subtilis</i>	Biocontrol	(Wang et al., 2018)
Pistachio ( <i>Pistacia vera</i> )	<i>Aspergillus parasiticus</i>	<i>B. subtilis</i> , <i>B. amyloliquefaciens</i>	Growth inhibition	(Siahmoshteh et al., 2017)
Maize ( <i>Z. mays</i> )	<i>Rhizomucor variabilis</i>	<i>B. amyloliquefaciens</i>	Lipopeptide Production	(Kulimushi et al., 2017)
Cucurbits	<i>Sphaerotheca fuliginea</i>	<i>B. amyloliquefaciens</i>	Systemic resistance	(Li et al., 2015)
Tobacco ( <i>Nicotiana tabacum</i> )	<i>R. solani</i> , <i>Phytophthora nicotianae</i>	<i>B. subtilis</i>	Induces Systemic Resistance	(Kim et al., 2015)
Citrus fruit	<i>Penicillium digitatum</i>	<i>B. megaterium</i>	Antagonism	(Mohammadi et al., 2017)
Wheat ( <i>Thriticum</i> spp.)	<i>Stagonospora nodorum</i>	<i>B. subtilis</i>	Antimicrobial metabolites	(Maksimov et al., 2020)
Turnip ( <i>Brassica campestris</i> )	<i>S. sclerotium</i> , <i>Plutella xylostella</i>	<i>B. thuringiensis</i>	Systemic Resistance	(Wang et al., 2020)
Tomato ( <i>S. lycopersicum</i> ) Soybean ( <i>Glycine max</i> )	<i>Phytophthora sojae</i> <i>Ralstonia solanacearum</i>	<i>B. velezensis</i>	Antimicrobial metabolites (fengycin, iturin, bacillomycin)	(Yu et al., 2023)
Rice ( <i>Oryza sativa</i> )	<i>Xanthomonas oryzae</i>	<i>B. atrophaeus</i> , <i>B. cabrialesii</i>	Antagonistic Activity	(Rajer et al., 2022)
Rice ( <i>Oryza sativa</i> )	<i>Aphelenchoides besseyi</i>	<i>B. thuringiensis</i>	Antagonistic Activity	(Liang et al., 2022)
Wheat ( <i>Thriticum</i> spp.) Maize ( <i>Z. mays</i> )	<i>F. graminearum</i> , <i>F. verticillioides</i>	<i>B. subtilis</i>	Antagonistic Activity	(Yu et al., 2021a)
Wheat ( <i>Thriticum</i> spp.)	<i>F. graminearum</i>	<i>B. atrophaeus</i>	Biocontrol	(Zubair et al., 2021)
Tomato ( <i>S. lycopersicum</i> )	<i>S. sclerotium</i>	<i>B. amyloliquefaciens</i>	Induce Systemic Resistance	(Farzand et al., 2019a)
Rapeseed ( <i>Brassica napus</i> )	<i>S. sclerotium</i>	<i>B. amyloliquefaciens</i>	Antifungal Activity	(Farzand et al., 2019b)

## 4 Mechanisms of action of *Bacillus* spp.

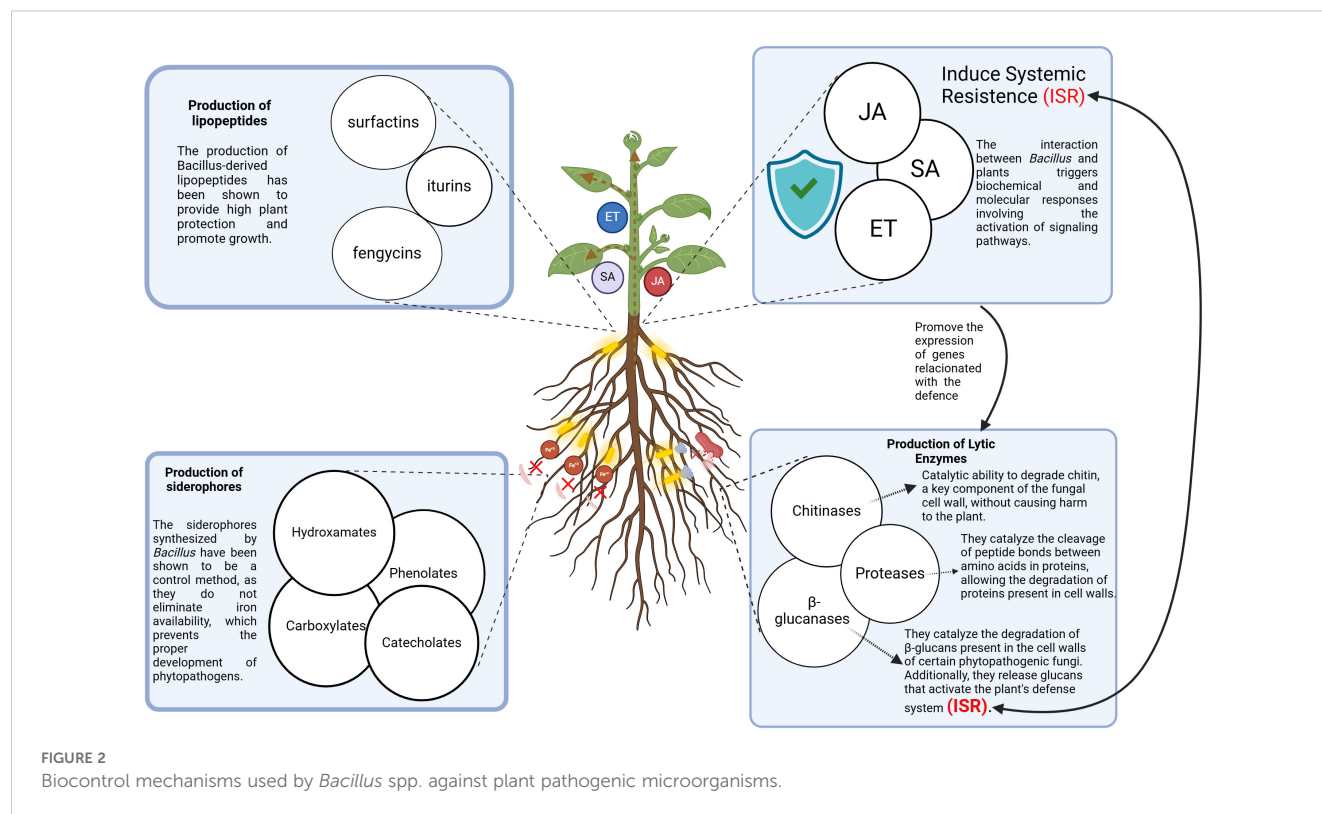
The main mechanisms of action of the *Bacillus* genus are production of lipopeptides, lytic enzymes, siderophores and induced systemic resistance (Figure 2).

### 4.1 Production of lipopeptides

Lipopeptides synthesized by species of the genus *Bacillus*, such as *B. subtilis*, *B. amyloliquefaciens*, and *B. velezensis*, are classified

into three main families: surfactins, iturins, and fengycins (Rabbee et al., 2019). These *Bacillus*-derived lipopeptides play crucial roles in both plant protection and growth promotion (Wang et al., 2024). They exhibit potent antifungal properties, effectively controlling a wide range of plant pathogens, including *Monilinia fructicola*, *Fusarium* spp., *B. cinerea*, *Alternaria* spp., and *Colletotrichum gloeosporioides*. They also induce systemic resistance in plants and promote biofilm formation, thereby enhancing rhizosphere antibacterial activity (Huang et al., 2019; Malviya et al., 2020).

The effectiveness of these lipopeptides can be attributed to their unique physicochemical properties. They disrupt lipid bilayers, reduce surface tension, and cause alterations in pathogen cell membranes. This disruption leads to the formation of pores,



osmotic imbalance, and, ultimately the death of phytopathogenic microorganisms (Saiyam et al., 2024). Furthermore, *Bacillus*-derived lipopeptides stimulate plant defense mechanisms by activating systemic acquired resistance (SAR) and ISR, thereby boosting plant immunity (Valenzuela Ruiz et al., 2024).

Among the various lipopeptides, iturins, particularly Iturin A, are known for their ability to disrupt fungal plasma membranes. This action effectively controls plant pathogens and even extends the shelf-life of fruit juices, offering a natural alternative to chemical preservatives (Shi et al., 2018). Surfactins, which are biosurfactants produced by *Bacillus* spp., help form protective biofilms that inhibit microbial growth, making them valuable for reducing fruit and vegetable decay (Huang et al., 2019). Fengycins, including types A, B, and C, also demonstrate broad-spectrum antimicrobial activity, inhibiting the growth of both pathogenic bacteria and fungi (Mnif and Ghribi, 2015).

Through the production of antimicrobial compounds, *Bacillus* spp. exhibit antagonistic mechanisms against various plant pathogens. For instance, *B. amyloliquefaciens* has shown strong antifungal properties against *Rhizomucor variabilis* in maize (Kulimushi et al., 2017). Similarly, *B. subtilis* produces antimicrobial mycosubtilin, which effectively controls *F. graminearum* in wheat and maize, while *B. velezensis* produces fengycin, iturin, and bacillomycin, which control *Phytophthora sojae* and *Ralstonia solanacearum* in soybean and tomato (Yu et al., 2021a).

In the case of *Sclerotinia sclerotiorum*, *B. amyloliquefaciens* suppresses its growth through the production of fengycin, inducing systemic resistance in crops such as rapeseed and

tobacco (Farzand et al., 2019b). This highlights the importance of selecting the appropriate *Bacillus* spp. for specific pathogen control, as different strains produce distinct bioactive compounds with varying efficacy. The diversity of lipopeptide producing *Bacillus* spp. thus offers a valuable tool for integrated pest management and sustainable agriculture.

## 4.2 Production of lytic enzymes

*Bacillus* spp. produce various lytic enzymes, such as chitinases, β-glucanases, and proteases, which contribute to the biological control of phytopathogens. Their mechanism of action involves disrupting the structural integrity of pathogens cell walls by hydrolyzing specific components, ultimately leading to their disintegration (Hakim et al., 2021). These enzymes achieve this by breaking the glycosidic linkages that bind the structural polymers of the cell wall (Santoyo et al., 2021; Ajuna et al., 2023).

Among these enzymes, chitinases have gained significant attention due to their ability to degrade chitin, a component of the fungal cell wall, without harming the host plant (Kumar et al., 2018). Similarly, β-glucanases catalyze the breakdown of β-glucans, polysaccharides present in the cell walls of certain phytopathogenic fungi such as *F. oxysporum* (Won et al., 2018). Notably, these enzymes not only degrade fungal cells but also enhance *Bacillus* spp. competitiveness in the soil. Besides, the released glucan fragments can trigger plant defense responses, such as ISR and the production of phytoalexins. For instance, *B. amyloliquefaciens* FS6 produces a β-1,3-1,4-glucanase with antimicrobial activity against *Alternaria*

*panax* and *B. cinerea* (Wang et al., 2021). Likewise, *B. velezensis* CE 100 synthesizes chitinase and  $\beta$ -1,3-glucanase, which degraded fungal cell walls and effectively inhibit the mycelial growth of *M. phaseolina* and *F. oxysporum* f. sp. *fragariae*, causal agents of charcoal rot and Fusarium wilt in strawberries  $\beta$ -1,3-glucanase (Hong et al., 2022). In addition to chitinases and  $\beta$ -glucanases, *Bacillus* spp. produces proteases, which catalyze the breakdown of peptide bonds in proteins, facilitating the degradation of fungal cell wall proteins (Rosazza et al., 2023). Beyond their antimicrobial function, these enzymes contribute to plant nutrition and may activate additional defense responses (Gray et al., 2019). An example of this is *B. subtilis* B315, which effectively reduced bacterial wilt in chili plants by 60.89% (Prihatiningsih et al., 2021).

### 4.3 Production of siderophores

*Bacillus* spp. is well known for its ability to produce siderophores, a variety of compounds in their competition for nutrients (Prasad et al., 2023). These molecules sequester ferric iron ( $\text{Fe}^{3+}$ ), giving *Bacillus* a competitive advantage over phytopathogens that lack siderophore production (Martínez-Canto et al., 2021). Notably, various *Bacillus* spp., including *B. subtilis*, *B. pumilus*, *B. cereus*, *B. licheniformis*, *B. amyloliquefaciens*, *B. velezensis*, *B. thuringiensis*, *B. halodenitrificans*, *B. mojavensis*, and *B. atropheus*, are known to produce siderophores, making them effective biocontrol agents against multiple phytopathogens (Andrić et al., 2020).

Several studies have demonstrated that siderophore-producing *Bacillus* strains can reduce the incidence of diseases caused by *Fusarium* spp. and *R. solani* in various plant species (Shafi et al., 2017; Dimopoulou et al., 2021). For instance, *B. amyloliquefaciens* synthesizes the catecholate siderophore bacilibactin under iron-limiting conditions, which effectively inhibits the growth of *Pseudomonas syringae* pv. tomato both *in vitro* and in plants (Nikolić et al., 2019). Similarly, *B. subtilis* MF497446 produces siderophores that not only restrict pathogen growth but also enhance resistance to *Cephalosporium maydis* in maize (Ghazy and El-Nahrawy, 2021).

### 4.4 Induced systemic resistance

Refers to a plant defense mechanism activated after interaction with specific microorganisms, such as *Bacillus* spp. This interaction triggers biochemical and molecular responses, involving the activation of jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) signaling pathways (Shafi et al., 2017). Among the key ISR elicitors produced by *Bacillus*, cyclic lipopeptides (CLPs), N-alkylated benzylamine derivatives (NABD), and pyoverdines stand out. These compounds enhance plant immunity by activating defense mechanisms, thereby increasing resistance against various pathogens without exerting direct antimicrobial effects.

Several studies highlight the effectiveness of *Bacillus* in inducing ISR. For instance, *B. thuringiensis* enhances systemic resistance in

soybean plants against *Phytophthora sojae*, strengthening defenses not only in the roots but throughout the entire plant (Basu et al., 2021). Similarly, *B. velezensis* PEA1 exhibits both antifungal and antiviral properties, effectively suppressing *F. oxysporum* and cucumber mosaic virus (CMV) in *Datura stramonium* leaves (Abdelkhalek et al., 2020). Furthermore, *B. subtilis* SL18r reduces foliar damage in tomato plants caused by *Botrytis cinerea*, a benefit attributed to its ability to activate the expression of a long non-coding RNA associated with defense pathways (Zhou et al., 2021).

The versatility of *Trichoderma* spp. and *Bacillus* spp. in their mechanisms of action underscores their potential as effective biocontrol agents within sustainable agriculture. Both genera exhibit a multifaceted approach to pathogen suppression, employing strategies such as direct antagonism through the production of antimicrobial compounds, competition for nutrients and space, and the activation of plant defense mechanisms. Notably, *Bacillus* spp. has shown superior adaptability in iron-limited environments through siderophore production, whereas *Trichoderma* spp. excels in mycoparasitism through enzymatic degradation of pathogen cell walls. Comparatively, *Bacillus* spp. tends to provide more immediate and widespread antimicrobial effects due to its production of various bioactive metabolites, including surfactins, iturins, fengycins, and siderophores. These compounds exhibit broad-spectrum activity against bacteria, fungi, and viruses, enhancing their utility across diverse agricultural systems. However, *Trichoderma* spp. is particularly effective in directly parasitizing phytopathogenic fungi through chitinase and glucanase activity, mechanisms that are often strain-specific but highly efficient when appropriately matched to the target pathogen. In addition, while both microorganisms induce plant systemic resistance, the pathways activated differ slightly; *Trichoderma* spp. is more frequently associated with jasmonic acid and ethylene signaling, whereas *Bacillus* spp. often involves salicylic acid pathways as well.

The successful implementation of *Trichoderma* spp. and *Bacillus* spp. in agricultural systems requires careful consideration of their distinct mechanisms of action and environmental compatibility. Various factors, including soil composition, crop type, pathogen species, and climatic conditions, influence the efficacy of *Trichoderma* spp. and *Bacillus* spp. Moreover, the interaction between these biocontrol agents and native soil microbiota can significantly impact their performance. Farmers and policymakers must be aware that the indiscriminate or inappropriate application of these agents may yield inconsistent results or even counterproductive outcomes. Therefore, promoting an integrated approach that considers the specific attributes of each microorganism, supported by empirical evidence and tailored to local agricultural conditions, is important for optimizing their biocontrol potential. Ensuring the successful adoption of *Trichoderma* spp. and *Bacillus* spp. as biocontrol agents demands comprehensive research, clear guidelines, and educational efforts to inform practitioners about the conditions under which these microorganisms are most effective. Without such a nuanced understanding, their potential benefits may remain underutilized, compromising the progress toward more sustainable agricultural practices.



## 5 Laboratory scale, industrial scale-up, patents, and regulatory

The development of microbial fungicides follows a structured process to ensure their efficacy and safety in pest or disease control. The first step is identifying the target pathogen, which requires a deep understanding of its biology, life cycle, and environmental conditions that favor its proliferation (Basu et al., 2021). Once identified, a thorough review of previous studies is conducted to select microorganisms with proven effectiveness against the pathogen.

After selecting potential BCAs, they are isolated and cultured in the laboratory, where their efficacy is evaluated under controlled conditions. This evaluation considers various mechanisms of action to determine their potential (Zandi and Basu, 2016). For instance, it is important to assess the specificity of the BCA to ensure it does not affect non-target organisms, especially beneficial ones. Laboratory and field tests further validate their ability to reduce pest populations or disease severity (Jangir et al., 2021). Furthermore, compatibility with the agricultural environment, including climate, crop types, and agronomic practices, must be considered to enhance field performance (Teixidó et al., 2022).

Once a suitable BCA is identified, production begins through fermentation methods, primarily submerged fermentation (SMF) or solid-state fermentation (SSF) (Teixidó et al., 2022). SMF is preferred for bacteria and yeasts due to its high yield, ease of nutrient control, and cost-effectiveness. In contrast, SSF is more suitable for filamentous fungi, which struggles to sporulate in liquid media. To optimize fermentation, key parameters such as temperature, pH, agitation, aeration, inoculum concentration, and duration must be carefully adjusted (Vehapi et al., 2023). However, SSF presents challenges in nutrient addition due to its low water activity, making process control more complex (Fahim et al., 2013). The culture medium composition plays a critical role in microbial growth and viability. It typically includes inorganic (ammonium and nitrate salts) or organic nitrogen sources (amino acids, proteins, or urea), carbon sources such as simple sugars (sucrose, fructose), and complex carbohydrates (starch). Besides that, agro-industrial by-products like molasses, whey, yeast extracts, and peptones are commonly used to enhance growth (Clerici et al., 2021).

Following fermentation, the next step is formulating the BCA into a usable product. Various formulations have been developed for *Trichoderma* spp., including granules, pellets, wettable powders, capsules, water-dispersible granules, and emulsifiable liquids (Locatelli et al., 2018). However, some of these formulations pose challenges in application, affecting dosing precision and potentially increasing health risks for applicators (Ubando et al., 2020). In this context, Jangir et al., 2021, developed water-dispersible granules of *B. subtilis* and *T. harzianum* using response surface methodology (RSM) to control *F. oxysporum*, providing a more user and environmentally friendly option. Similarly, *B. megatherium* has been formulated in alginate microcapsules, pellets, or effervescent systems to enhance bacterial release (Wiwattanapatapee et al., 2013). In addition, microbial preparations can be combined with organic amendments to improve soil quality in semi-arid regions (Fendrihan et al., 2016).

Regarding formulation types, liquid formulations are often preferred due to their cost-effectiveness and ease of handling (Palmieri et al., 2022). They typically consist of an active ingredient, either microbial cells or their secondary metabolites combined with a carrier that ensures even distribution and influences release and stability (Berninger et al., 2018). Water is the most used carrier, often supplemented with additives to maintain stability during application (Bashan et al., 2014). Alternatively, oil-based formulations can be developed, provided they are non-toxic to microorganisms, plants, humans, or animals (Palmieri et al., 2022).

On the other hand, solid formulations offer advantages in storage and transport due to their stability and cost-effectiveness. Several dehydration methods are used to preserve microbial viability. Freeze-drying is particularly suitable for heat- and moisture-sensitive materials (Prakash et al., 2013). Spray drying rapidly converts liquid suspensions into powder by atomizing droplets in a hot air chamber (150°C–250°C), offering a fast and scalable process (Teixidó et al., 2022). Also, fluidized bed drying, is employed for granulating and coating, where particles are suspended in hot air to ensure uniform drying, often with cryoprotectants to enhance stability (Chaudhary et al., 2020; Teixidó et al., 2022). A combination of spray drying and fluidized bed drying can further improve uniformity and prevent clumping (Torres et al., 2014).

Scaling up production begins in the laboratory, where Erlenmeyer flasks are used to optimize culture media composition. The process then transitions to small bioreactors (2.0–5.0 liters) for further refinement of growth conditions. At an industrial scale, production is conducted in pilot plant bioreactors with capacities ranging from 100 to 300 liters (Fahim et al., 2013). Once fermentation is complete, the next phase involves recovering the microbial cells, spores, or supernatant containing secreted metabolites, depending on the BCA type. For instance, in *B. amyloliquefaciens*, it is recommended to recover both cells and metabolites, as both contribute to disease control (Gotor-Vila et al., 2017). Ensuring the viability and stability of the BCA throughout production, storage, and application for its effectiveness. Colony-forming unit (CFU) tests are commonly used to determine viable microorganism counts per milliliter or gram of product (Meyer et al., 2023). A sufficient concentration of viable cells is crucial to achieving the desired biological effects in the field. Furthermore, fungal spores must maintain a high germination rate, as prolonged storage or adverse conditions can reduce their viability and efficacy (Palmieri et al., 2022). Beyond cell viability, metabolic activity must also be assessed to confirm that the microorganisms remain functional. Measuring ATP (adenosine triphosphate) production serves as a reliable indicator of cellular energy and viability (Carrodeguas-González et al., 2022). Ensuring metabolic activity guarantees that BCAs will perform effectively upon application.

After production, proper storage conditions are critical to preserving product shelf life. Most BCAs require refrigeration (4° C–10°C) to maintain microbial viability, though some formulations remain stable at room temperature depending on the organism and formulation used (Alamprese et al., 2017). Humidity control is

equally important, as excess moisture can degrade powder or granule formulations, leading to contaminant growth or premature activation.

Finally, the commercialization of a BCA involves regulatory and patenting processes. Patent applications typically grant 20 years of protection to inventions that meet criteria for novelty, non-obviousness, and industrial applicability. A complete description of the BCA, including its production method and biological mechanisms, is required. Additionally, regulatory compliance varies by country; for instance, BCAs must be approved by entities such as the U.S. Environmental Protection Agency (EPA) in the United States or the European Union (EU) regulatory bodies before commercialization (Dourado et al., 2016). Proper labeling is also mandated, providing essential details on composition, application methods, safety precautions, and usage instructions (Palmieri et al., 2022). By following this structured approach from pathogen identification to formulation, production, quality control, storage, and regulatory approval, BCAs can be successfully developed and commercialized as effective alternatives for sustainable disease and pest management. Indeed, the market includes approximately 2,920 bacterial-based biopesticides, 1,658 fungal-based biopesticides, and 234 viral-based biopesticides. Of these, 227 are bacterial and 169 are fungal fungicides (Palmieri et al., 2022). Notably, 77 *Trichoderma*-based fungicides are available globally, with 7 approved by the European Commission for use in EU member states (Thambugala et al., 2020). These products demonstrate the rigorous regulatory approval processes that often involve high costs and lengthy approval periods. For instance, in countries like the U.S. and EU member states, BCAs must comply with strict manufacturing regulations and are subject to regular audits of production facilities. In contrast, many developing countries may have less stringent manufacturing controls, which raise concerns about product quality and effectiveness. These regulatory processes often include additional testing for safety, efficacy, and product stability. However, in countries with more flexible regulatory frameworks, such as some in Asia and Latin America, the registration process for BCAs may be quicker and less expensive, but this can come at the cost of reduced guarantees of product quality. Regulatory differences across countries and regions further complicate the international marketing and use of BCAs, so these challenges must be addressed carefully (Vekemans and Marchand, 2020).

Undoubtedly, this information offers valuable insights into how the efficacy and safety of these products are ensured within agricultural practices. For farmers, such knowledge is essential, empowering them to make well-informed decisions about selecting microbial fungicides based on crop compatibility, environmental impact, and field performance. Moreover, understanding optimal storage and application conditions allows them to maximize yield and enhance productivity. Beyond practical applications, this information highlights the scientific rigor underpinning the development of biological products, reinforcing that food produced through these methods is both safe and sustainable. It also fosters greater trust in biological alternatives to conventional agrochemicals, promoting a healthier and more environmentally responsible agricultural system.

## 6 Challenges and future perspectives in the use of BCAs in agriculture

The positive effects of BCAs in the field can vary significantly due to environmental conditions, competition with other microorganisms, and the genetic variability of pathogens. For example, high UV radiation, extreme temperatures, or high humidity can hinder BCA performance after application, limiting their effectiveness in certain regions or for specific crops (Umer et al., 2021). For this, the BCAs should be collected and screened to achieve relevant biocontrol results (Yu et al., 2021b). In addition to environmental limitations, scaling up BCA production presents another challenge. This is primarily due to the costs associated with microorganism propagation under optimal conditions and the need for suitable formulation technologies to ensure their efficacy. Formulations, such as controlled release or encapsulated systems, are necessary for maintaining prolonged stability in the field (Locatelli et al., 2018).

Beyond production challenges, another limitation lies in the interaction of BCAs with other organisms within the agroecosystem, such as pollinators, beneficial insects, and soil microorganisms. Understanding these interactions is vital to minimizing negative impacts and maximizing the benefits of BCAs (Palmieri et al., 2022). However, there is a lack of detailed information on these interactions. The available literature generally focuses on the ecological importance of relationships between plants, microorganisms, and insect communities, with some suggestions that negative interactions may arise when certain microorganisms or insects become parasites affecting plants (Barolia et al., 2023). On the other hand, beneficial microorganisms in the rhizosphere can improve plant health and indirectly support pollinators by enhancing nectar quality. For instance, *Lactobacillus kunkeei*, found in flowers, inhibits pathogens affecting bees, highlighting a positive interaction. In contrast, soil pathogens can harm plant health and reduce nectar availability for pollinators. This microbial cycle between soil, plants, and pollinators underscores the interconnectedness of ecosystem health (Liu et al., 2019).

Looking to the future of microorganisms such as BCAs offer a promising sustainable alternative to chemical pesticides. These agents address environmental pollution and biodiversity loss, as they do not negatively affect non-target organisms. Unlike chemical pesticides, which can lead to pathogen resistance over time, BCAs have multiple modes of action, reducing the likelihood of resistance development. For example, *Trichoderma* reduces pathogen resistance by producing secondary metabolites like peptaibols and gliotoxin, which exhibit antifungal activity. Besides, it triggers plant defense mechanisms through elicitors, enhancing systemic resistance and promoting mutualistic associations that improve nutrient uptake and plant health (Orozco-Mosqueda et al., 2023).

Utilizing specific *Trichoderma* isolates, such as *T. afroharzianum* TRI07, can effectively inhibit pathogens like *A. alternata*, enhance tomato plant defense mechanisms, and promote the production of defense-related enzymes and phenolic compounds (Philip et al., 2024). Similarly, *B. subtilis*, *B. mojavensis*, and *B. velezensis* reduce pathogen resistance of *F. oxysporum* by producing hydrolytic enzymes and

secondary metabolites that inhibit pathogen growth. These microorganisms also promote root development and create an unfavorable rhizosphere environment for pathogens, ultimately improving overall plant health and resilience (Auriza Rumandani, 2025).

Considering growing concerns about food safety, there is increasing demand for safer pest control methods that do not leave harmful residues on crops. One of the primary factors influencing consumer acceptance of BCAs is their level of awareness about these agents. Research has shown that individuals who are more informed about BCAs tend to have a more positive perception of their use. *Trichoderma* has gained acceptance due to its specificity, safety for beneficial organisms, and absence of toxic residues or environmental risks (Sharma and Gothwal, 2017). This indicates a positive trend toward acceptance among producers and consumers, as sustainable agricultural practices and biological control methods continue to be prioritized within IPM. However, despite these benefits, adoption remains limited in developed countries due to a general lack of awareness (Kumar Chaube and Pandey, 2022).

Biotechnological advances improving the efficacy, stability, and ease of use of BCAs include the development of specialized formulations using protective substances like sugars and polyols, which enhance stability during storage. Techniques such as freeze-drying and fluidized bed-spray-drying are employed to maintain viability (Chaudhary et al., 2020). The optimized packaging conditions, including vacuum packaging and modified atmospheres, help extend shelf life (Kumar et al., 2023). These innovations facilitate the mass production and application of BCAs, making them more viable alternatives to synthetic fungicides (Teixidó et al., 2022).

Moreover, other studies analyze advances in transformation tools for genetic improvement of *Trichoderma*, enhancing biomass, primary and secondary metabolite production, and enzyme activity, thereby improving efficacy and adaptability in agricultural applications, particularly against challenges posed by climate change and pathogens (Villao-Uzho et al., 2024). One of the most common techniques for genetic transformation is protoplast-mediated transformation using *Agrobacterium tumefaciens* (Wang et al., 2019). Early methods for transforming *Trichoderma* involved techniques such as electroporation and biolistics (Schuster et al., 2016). The CRISPR (regularly interspaced clustered short palindromic repeat)-Cas9 (CRISPR-related nuclease 9) system, has many innovative applications in *Trichoderma* (Wang et al., 2022). CRISPR allows the reduction of undesirable traits and the introduction of new, desirable traits that can target a wide range of pathogens and pests in various cropping systems (Tyagi et al., 2024). Analyses dedicated to the function of the genes of the beneficial *Trichoderma* spp. are generally attributed to the construction of recombinant DNA using conventional cloning techniques, which are based on digestion and ligation procedures (Fitz et al., 2018). The genomic organization, together with the regulation and enzymatic hydrolytic expression in the *T. harzianum* strain IOC3844, are closely related, which helps to increase plant degradation (Ferreira Filho et al., 2020). The layer-by-layer (LbL) encapsulation method using biobased lignin derivatives significantly enhances the stability and efficacy of *Trichoderma*

spores, protecting them from extreme temperatures and UV exposure, thus improving their performance as biocontrol agents in sustainable agriculture (Lövschall et al., 2024). Biopolymer-based emulsions, particularly those using agar and cellulose nanocrystals, enhance the stability and viability of *T. atroviride* conidia, allowing for prolonged storage and effective encapsulation, thus improving their efficacy (Martínez et al., 2023). Chemical mutagenesis using Ethyl Methyl Sulphonate (EMS) and gamma irradiation have enhanced the biocontrol capabilities of *T. viride*, resulting in stable mutants with improved antagonistic activities against soil-borne pathogens, thus increasing efficacy and ease of use in agricultural applications (Shewarega Foyate, 2024).

The genetic improvement of *Bacillus* spp. has enabled the identification of key genes that contribute to plant growth promotion and biopesticide production. Advances in genetic engineering have facilitated the development of strategies to optimize the production of enzymes, chemicals, and antimicrobial compounds, making *Bacillus* more competitive in the biotechnology industry (Mitra et al., 2021; Muras et al., 2021). One of the most extensively studied cases is *B. thuringiensis*, widely used in biopesticide development. Recombinant DNA techniques have enhanced its insecticidal potency, expanded its spectrum of action, improved its persistence in crops, and increased its fermentation yield (Ortiz et al., 2024). A notable example is the genetic modification of crops to express *cry* genes, which have been introduced into potatoes, tomatoes, tobacco, rice, corn, and broccoli to provide resistance against agricultural pests (Abbas, 2018). However, this practice has sparked controversy regarding its safety and potential impacts on human health.

Advancements in genetic engineering have also benefited other *Bacillus* species. For instance, in *B. amyloliquefaciens*, the manipulation of regulatory genes such as *codY*, *comA*, *degU*, and *spo0A* has proven to be an effective strategy for enhancing the production of antimicrobial lipopeptides (bacillomycin D, fengycin, and surfactin), resulting in strains with high potential for industrial applications (Sun et al., 2021). The application of advanced technologies, such as CRISPR, has revolutionized the genetic modification of *Bacillus* spp. It has been used in *B. subtilis* to enhance the production of the terpenoid artemisinic acid (Song et al., 2021) and in *B. licheniformis* to improve its production capacity (Zhou et al., 2019). Furthermore, genetic engineering allows not only gene modification but also the manipulation of their products, as seen in the case of *B. thuringiensis* Cry toxins, whose optimization has increased their insecticidal efficiency.

Biological products are increasingly integrated into existing agricultural systems as part of IMP strategies. This integration is particularly effective when combined with other sustainable agricultural practices, such as crop rotation, the use of resistant crops, or the application of organic fertilizers. These practices not only enhance the efficiency of biological control but also reduce reliance on chemical products (Baker et al., 2020). Moreover, when BCAs are integrated with other products like bactericides and organic fertilizers, their effectiveness is strengthened, contributing to crop sustainability and boosting agricultural productivity. By carefully considering interactions with the existing biota, the ecological functions of the system are optimized, leading to more

efficient and environmentally friendly pest and disease control (Vasquez-Lopez et al., 2021). In the case of *Trichoderma*, studies have shown that it is not only safe for the soil ecosystem but also helps maintain its balance and sustainability (Li et al., 2024). From an ecological perspective, *Trichoderma* is not considered an aggressive soil colonizer that disrupts existing microbiota. Instead, its mode of action is based on competition, which does not harm other beneficial microorganisms (Zin and Badaluddin, 2020). Given that *Trichoderma* is naturally found in soils, it serves to strengthen, rather than alter, the biological mechanisms in the soil. Importantly, it does not leave toxic residues or affect soil pH, making it an ecologically sustainable alternative (Zafra et al., 2015).

Based on the aforementioned, despite the ecological benefits of BCAs, their commercialization can be more costly compared to conventional chemical pesticides, which limit their adoption, particularly in resource-limited countries (Droby et al., 2016). These high production costs are attributed to the controlled and specific conditions required for multiplying microorganisms like *Trichoderma* and *Bacillus*. Such processes involve specialized facilities, expensive growth media, and highly skilled personnel (Palmieri et al., 2022). For this reason, there is a need to assess the impact of these organisms in a holistic manner, given the increasing demand for sustainable alternatives that has driven significant growth in the biofungicide market in recent years. This approach is supported by ongoing research demonstrating the effectiveness of microbial biocontrol agents in diverse crops. For instance, a study evaluated the effect of *Bacillus* and *Trichoderma* species in managing bacterial wilt in tomatoes (*Lycopersicon esculentum*). The *Bacillus* CB64 and *Trichoderma* T1 isolates achieved significant reductions in disease incidence and severity in the field, as well as a decrease of over 90% in the soil pathogen population (Kariuki et al., 2020). Additionally, research on bananas has shown that strains of *B. licheniformis* CSR-D4 significantly reduce the incidence of wilt caused by *F. oxysporum* f. sp. *cubense* in banana plants. The bioactive metabolites produced by this strain inhibited pathogen invasion, demonstrating its potential as an effective biocontrol agent (Izquierdo-García et al., 2024). Therefore, improving the connection and relationship between these findings highlights the importance of adopting integrated biocontrol strategies to enhance agricultural sustainability.

## 7 Conclusion

The agri-food industry bears a significant responsibility to meet global food demand with products that are not only nutritious and of high quality but also safe. As highlighted throughout this review, BCAs such as *Trichoderma* and *Bacillus* offer a viable and sustainable alternative to conventional chemical pesticides. These beneficial microorganisms have demonstrated their ability to enhance crop productivity, promote soil health, and improve resilience against various diseases, thereby contributing to eco-friendly agricultural systems. Understanding the mechanisms of action, selection criteria, formulation processes, and challenges

associated with BCAs is essential for optimizing their efficacy in diverse agricultural environments. This knowledge empowers farmers to select appropriate formulations that align with specific environmental and soil conditions, enhancing their productivity while reducing dependency on synthetic agrochemicals. Additionally, integrating BCAs with other sustainable practices can further bolster agricultural resilience and contribute to more environmentally responsible food production. Moreover, from a consumer perspective, BCAs present a safer alternative by reducing the presence of toxic residues in food products. Raising awareness and providing education about these biocontrol agents are essential steps in fostering their acceptance and encouraging wider adoption. Informed consumers are more likely to support sustainable agricultural solutions, which can drive market growth and innovation in this field. On a broader scale, challenges such as regulatory disparities between countries, economic barriers to production, and commercialization hurdles remain significant. Addressing these obstacles through collaborative efforts between researchers, industry, and policymakers is crucial to achieving the large-scale adoption of BCAs. Thus, this review has underscored the importance of developing strategies that harmonize regulatory frameworks and enhance economic feasibility across different agricultural contexts. While the positive impacts of BCAs on productivity and sustainability are evident, further research is needed to optimize formulations, improve application techniques, and assess long-term efficacy under various agroecological conditions. Continued efforts in these areas will be essential to unlocking the full potential of biological control agents and ensuring their successful integration into mainstream agricultural practices.

## Author contributions

MV: Data curation, Formal Analysis, Investigation, Writing – original draft. FE: Data curation, Formal Analysis, Writing – original draft. LE: Supervision, Validation, Writing – review & editing. JC: Conceptualization, Data curation, Resources, Supervision, Validation, Writing – review & editing.

## Funding

The author(s) declare that financial support was received for the research and/or publication of this article. The authors acknowledge the Biotechnology Research Center of Ecuador (CIBE) for supporting founding, Project CIBE-5-2023.

## Conflict of interest

The authors declare that the review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2025.1578915/full#supplementary-material>

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