

Abstract

Biocontrol agents have emerged as vital tools in the quest for sustainable agriculture, offering environmentally friendly alternatives to chemical pesticides for managing plant pathogens and pests. This comprehensive review explores the advancements in biocontrol agent research, focusing on the exploration of new agents, genetic engineering, and integration into Integrated Pest Management (IPM) systems. The discovery of novel biocontrol agents from diverse environments, including plant microbiomes and marine ecosystems, has expanded the arsenal available for agricultural use, while genetic engineering and synthetic biology have enhanced the efficacy of existing agents by improving their production of antimicrobial metabolites and stress tolerance. Additionally, the development of synthetic microbial consortia and innovative delivery systems, such as encapsulation and nanotechnology, has improved the stability and application efficiency of biocontrol agents in various agricultural settings. The review also highlights the importance of sustainable and eco-friendly approaches, such as the use of organic amendments and crop rotation, to enhance the effectiveness of biocontrol strategies. These methods not only improve soil health and biodiversity but also reduce the reliance on chemical inputs. However, the widespread adoption of biocontrol agents faces challenges, including environmental variability, host specificity, and the need for supportive regulatory frameworks. Harmonizing regulatory processes, promoting public awareness, and providing incentives for sustainable practices are essential for overcoming these barriers. Furthermore, international cooperation and knowledge exchange are crucial for advancing research and ensuring that biocontrol agents become integral components of modern agriculture. This review underscores the potential of biocontrol agents to contribute significantly to global food security and environmental sustainability, provided that ongoing research and innovation continue to address existing challenges and expand their practical applications in diverse agricultural contexts.

Keywords: *Biocontrol, Sustainability, Pathogens, Biotechnology, Microbiome, Genetic-engineering*

I. Introduction

A. Background on Plant Pathogens

Plant pathogens, including bacteria, fungi, viruses, nematodes, and oomycetes, cause significant agricultural losses worldwide. These organisms, through various mechanisms of infection and disease progression, lead to symptoms such as wilting, blights, rots, and galls on plants. According to the Food and Agriculture Organization (FAO), plant diseases account for 20-40% of global crop production losses annually, amounting to billions of dollars [1]. Bacterial pathogens like *Pseudomonas syringae* degrade plant cell walls, while fungal pathogens such as *Fusarium oxysporum* and *Phytophthora infestans* cause wilt and blight diseases. Viral pathogens, though small, have severe impacts, causing stunted growth and reduced yield. Nematodes, like *Meloidogyne spp.*, damage plant roots, reducing their nutrient absorption capabilities. The reliance on chemical pesticides for pathogen control, despite their effectiveness, raises concerns about human health, environmental impact, and the emergence of pesticide-resistant strains, necessitating alternative sustainable strategies [2].

B. Overview of Biocontrol Agents

Biocontrol agents, which include microorganisms such as bacteria, fungi, and viruses, as well as macroorganisms like insects and nematodes, are used to manage plant pathogens naturally. These agents work by utilizing one organism to suppress another, thus mitigating the impact of pathogens on crops. For example, *Trichoderma* fungi and *Bacillus subtilis* bacteria are effective biocontrol agents, combating fungal pathogens through mycoparasitism, nutrient competition, and the production of antifungal compounds. Bacteriophages target bacterial pathogens specifically, providing a natural disease control method, while predatory insects and parasitic nematodes reduce pest populations that spread plant diseases. Additionally, biocontrol agents can enhance plant growth by promoting nutrient uptake and inducing systemic resistance [3].

C. Importance of Biocontrol in Agriculture

Biocontrol is essential for sustainable agriculture, offering specific pathogen targeting without the broader ecological impact associated with chemical pesticides. This specificity minimizes the risk of collateral damage to non-target species and reduces the development of pathogen resistance. By promoting biodiversity and enhancing natural ecological balance, biocontrol agents contribute to soil health, reduce chemical inputs, and improve crop resilience to various stresses. Biocontrol is particularly important in organic farming, where synthetic chemical use is limited, enabling the production of healthy, pesticide-free crops. Moreover, the adoption of biocontrol practices aligns with consumer preferences for sustainably produced food, providing economic benefits to farmers [4][5].

D. Objectives of the Review

This review aims to provide a comprehensive analysis of biocontrol agents' role in suppressing plant pathogens, focusing on their mechanisms of action, application methods, and effectiveness across different agricultural contexts. It will synthesize current knowledge, highlight recent advances, and address challenges and limitations in using biocontrol agents. Additionally, the review will explore future research directions to enhance biocontrol strategies' efficacy and adoption, contributing to sustainable agriculture and addressing global food security and environmental sustainability challenges [6].

II. Types of Biocontrol Agents

A. Microbial Biocontrol Agents

Microbial biocontrol agents, including bacteria, fungi, and viruses, play a vital role in suppressing plant pathogens through mechanisms such as competition, antibiosis, parasitism, and induction of host resistance. These agents are integral to integrated pest management (IPM) programs due to their diversity and specificity.

1. Bacteria

Bacterial biocontrol agents, particularly species from the genera *Bacillus* and *Pseudomonas*, are widely studied for their effectiveness in agriculture. *Bacillus subtilis* and *Bacillus thuringiensis* produce antibiotics like iturins and surfactins, which inhibit pathogenic fungi and bacteria [7]. *Pseudomonas fluorescens* and *Pseudomonas putida* produce siderophores and antifungal compounds that restrict pathogen growth [8]. *Streptomyces spp.* are also effective, producing antibiotics like streptomycin and promoting plant growth through phytohormones.

Comment [h2]: This part is more suitable in section III Mechanism of biocontrol agents where it is already mentioned. So, delete from here

2. Fungi

Fungal biocontrol agents like *Trichoderma* species are known for their mycoparasitic activity, attacking the hyphae of pathogens like *Fusarium oxysporum* [9]. *Trichoderma virens* (formerly *Gliocladium virens*) produces gliotoxin, an antifungal compound effective against pathogens such as *Pythium* and *Rhizoctonia*. Additionally, *Beauveria bassiana*, an entomopathogenic fungus, controls insect pests and suppresses plant pathogens like *Botrytis cinerea* through enzyme production.

3. Viruses

Bacteriophages, viruses that infect bacterial cells, are explored as biocontrol agents, particularly against bacterial pathogens. Phages have been successfully used to manage diseases like fire blight caused by *Erwinia amylovora* and bacterial wilt caused by *Ralstonia solanacearum*. Their specificity is both an advantage and a limitation, though phage cocktails have broadened their applicability in agriculture [10].

B. Macro-organisms as Biocontrol Agents

Macro-organisms, including insects and nematodes, are significant in biological control, primarily through predation and parasitism.

1. Insects

Predatory insects like lady beetles and lacewings are used to control aphid populations, which are vectors for plant viruses such as Potato Virus Y (PVY) [11]. Parasitic wasps, such as *Trichogramma* and *Aphidius*, lay eggs in pests like aphids, reducing their populations and consequently the spread of plant pathogens.

2. Nematodes

Entomopathogenic nematodes, such as *Steinernema carpocapsae*, target insect pests that vector soil-borne pathogens. By infecting and killing these pests, nematodes help control the spread of diseases like root rot in crops [12].

C. Natural Plant Compounds and Extracts

Natural plant compounds, including alkaloids, terpenoids, and essential oils, are used as biocontrol agents. Neem oil, rich in azadirachtin, exhibits both insecticidal and antifungal properties, effective against pathogens like *Fusarium spp.* [13]. Essential oils, such as those from thyme and clove, disrupt fungal cell membranes, while phenolic-rich extracts from garlic and ginger inhibit pathogen growth by interfering with their metabolism. These plant-based agents are biodegradable, pose low toxicity risks, and are less likely to promote pathogen resistance, though their effectiveness can vary based on extraction and application methods.

III. Mechanisms of Action of Biocontrol Agents

Biocontrol agents, whether microorganisms or macro-organisms, suppress plant pathogens and promote plant health through mechanisms such as antibiosis, competition, parasitism, induced systemic resistance, and hyperparasitism. These mechanisms are crucial for optimizing biocontrol strategies in agriculture.

A. Antibiosis

Antibiosis involves biocontrol agents producing secondary metabolites or antibiotics that are toxic to pathogens, inhibiting their growth or killing them. For example, *Bacillus subtilis* produces lipopeptides like iturins, which form pores in fungal cell membranes, causing cell death. *Pseudomonas fluorescens* produces phenazines that disrupt fungal metabolic processes, leading to oxidative stress and cell death. Similarly, *Streptomyces spp.* produce antibiotics like streptomycin that inhibit pathogen protein synthesis and cell wall formation [14][15].

B. Competition

Competition occurs when biocontrol agents outcompete pathogens for essential resources, such as nutrients and space. *Pseudomonas fluorescens* uses siderophores to sequester iron, depriving pathogens like *Fusarium spp.* of this critical resource. *Trichoderma harzianum* rapidly colonizes the rhizosphere, outcompeting pathogenic fungi for space and nutrients. Some biocontrol agents also produce volatile organic compounds (VOCs) that create unfavorable conditions for pathogens [16][17].

C. Parasitism

Parasitism involves biocontrol agents directly attacking and feeding on pathogens. *Trichoderma* species parasitize other fungi by secreting enzymes that degrade their cell walls, allowing *Trichoderma* to invade and digest the pathogen. *Lecanicillium lecanii* parasitizes insect pests that vector plant pathogens, reducing their populations and indirectly controlling the spread of diseases. Entomopathogenic nematodes, such as *Steinernema* species, release bacteria that kill insect hosts, reducing the spread of soil-borne pathogens [18].

D. Induced Systemic Resistance

Induced systemic resistance (ISR) occurs when biocontrol agents trigger a plant's immune responses, making it more resistant to future pathogen attacks. Plant growth-promoting rhizobacteria (PGPR), such as *Bacillus* and *Pseudomonas* species, produce elicitors that activate the plant's defense pathways, leading to the production of defensive compounds. *Trichoderma harzianum* can induce resistance in plants like cucumbers, enhancing their defense against pathogens like *Pseudomonas syringae* [19][20].

E. Hyperparasitism

Hyperparasitism, or secondary parasitism, involves one organism parasitizing another parasite. For example, *Ampelomyces quisqualis* targets powdery mildew fungi, growing inside their cells and killing them. *Coniothyrium minitans* parasitizes *Sclerotinia sclerotiorum*, degrading its sclerotia and reducing its ability to survive and cause disease. Hyperparasitic agents are valuable in reducing pathogen populations while minimizing resistance development [21].

IV. Application Methods of Biocontrol Agents

The effectiveness of biocontrol agents depends significantly on the method of application, which ensures that these agents reach and establish themselves in the environment to suppress pathogens effectively. Key application methods include seed treatment, soil amendment, foliar application, and post-harvest treatment, each with its own set of advantages and challenges.

A. Seed Treatment

Seed treatment involves applying biocontrol agents directly to seeds before planting to protect seedlings from soil-borne pathogens. *Trichoderma harzianum* is commonly used for this purpose, rapidly colonizing the root system to outcompete pathogens like *Pythium* and *Fusarium spp.*. Similarly, *Pseudomonas fluorescens* is used in seed treatments to produce siderophores and antibiotics that protect against soil-borne diseases, enhancing seedling vigor and yield [22][23].

B. Soil Amendment

Soil amendment involves applying biocontrol agents to the soil to manage soil-borne diseases like root rot and wilts. *Trichoderma spp.* and *Bacillus subtilis* are widely used in soil amendments. *Trichoderma* colonizes plant roots, outcompeting pathogens and degrading their cell walls, while *Bacillus subtilis* forms resilient endospores that produce antibiotics to suppress pathogens like *Phytophthora* and *Pythium spp.*. The effectiveness of soil amendments can be influenced by factors such as soil type and environmental conditions [24][25].

C. Foliar Application

Foliar application involves spraying biocontrol agents onto plant leaves and stems to combat foliar pathogens. *Bacillus subtilis* and *Pseudomonas fluorescens* are commonly used in foliar applications to produce antibiotics and lipopeptides that inhibit pathogens like *Botrytis cinerea* and *Xanthomonas campestris*. Fungal agents like *Trichoderma harzianum* are also effective in foliar applications, preventing diseases such as powdery mildew. The success of foliar applications depends on factors like application timing, coverage, and environmental conditions [26].

D. Post-Harvest Treatment

Post-harvest treatment with biocontrol agents is crucial for preventing decay in stored fruits and vegetables. Yeasts such as *Candida oleophila* and *Pichia guilliermondii* are used to control post-harvest pathogens like *Botrytis cinerea* and *Penicillium spp.* by competing for nutrients and producing antifungal compounds. These treatments can be combined with other methods like refrigeration or low-dose fungicides to enhance effectiveness and prolong the shelf life of produce while reducing chemical residues [27][28].

V. Case Studies of Successful Biocontrol Programs

Biocontrol programs have demonstrated significant success in managing plant diseases across various agricultural settings, reducing the reliance on chemical pesticides.

A. Biocontrol of Soil-Borne Pathogens

Soil-borne pathogens cause severe diseases like root rot and wilt, but biocontrol agents have proven effective in managing these threats. One notable success is the use of *Trichoderma harzianum* against *Fusarium oxysporum*, which causes Fusarium wilt in crops like tomatoes and bananas. In tomato plants, *T. harzianum* was applied to soil, where it colonized the rhizosphere, competing with *F. oxysporum* and significantly reducing disease incidence through mycoparasitism and antifungal metabolite production [29]. Similarly, *Bacillus subtilis* has been effective against *Rhizoctonia solani*, responsible for damping-

off and root rot in crops like lettuce and cotton. In lettuce fields, *B. subtilis* applied as a soil drench reduced disease incidence by producing lipopeptide antibiotics that disrupt pathogen cell membranes. *Pseudomonas fluorescens* strain CHA0 also successfully controls soil-borne pathogens like *Pythium* and *Fusarium spp.* in crops such as sugar beet by producing antifungal metabolites [30].

B. Biocontrol of Foliar Pathogens

Foliar pathogens affect the above-ground parts of plants, but biocontrol agents have shown considerable success in managing these diseases. For example, *Bacillus subtilis* strain QST 713, marketed as Serenade®, effectively controls powdery mildew in grapes and cucurbits. Applied as a foliar spray, it colonizes the leaf surface and produces lipopeptides that inhibit the growth of powdery mildew fungi, significantly reducing disease incidence in vineyards [31]. *Pseudomonas syringae* strain ESC-11, marketed as Bio-Save®, effectively controls bacterial speck and spot in tomatoes by competing with pathogenic bacteria on the leaf surface. *Trichoderma harzianum* has also been used to control botrytis blight in ornamental plants, reducing disease severity by colonizing the leaf surface and degrading pathogen cell walls.

C. Biocontrol in Greenhouse Environments

Greenhouses offer a controlled environment ideal for the application of biocontrol agents. One successful example is the use of *Trichoderma harzianum* in hydroponic systems to control *Pythium* and *Rhizoctonia* species in cucumbers. Applied as a root inoculant, *T. harzianum* colonizes the root system, reducing disease incidence and improving yield [32]. *Bacillus subtilis* strain FZB24, marketed as Taegro®, has also been effective in controlling bacterial and fungal diseases in greenhouse-grown tomatoes and peppers. Additionally, *Beauveria bassiana*, an entomopathogenic fungus, has been used to control whiteflies and aphids in greenhouses, reducing the spread of plant viruses by killing these insect pests [33].

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D. Biocontrol in Field Conditions

Field conditions present unique challenges, but biocontrol agents have been successfully implemented. *Pseudomonas fluorescens* strain Pf-5 has been used to control take-all disease in wheat, caused by *Gaeumannomyces graminis var. tritici*. Applied as a seed treatment, it colonizes wheat roots and produces antifungal metabolites that suppress the pathogen, leading to reduced disease incidence and increased yields [34]. *Trichoderma harzianum* has also been used in cotton fields to control Fusarium wilt, improving plant health and yield by outcompeting the pathogen and degrading its cell walls. Additionally, *Candida oleophila*, used as a post-harvest biocontrol agent, has effectively reduced post-harvest losses in fruits like apples and pears by controlling blue mold and gray mold on the fruit surface.

VI. Challenges and Limitations of Biocontrol Agents

Despite their potential in sustainable agriculture, biocontrol agents face several challenges and limitations, including environmental factors, host specificity, production and formulation difficulties, and regulatory and commercialization hurdles. Addressing these challenges is crucial for integrating biocontrol agents into mainstream agricultural practices.

A. Environmental Factors

The efficacy of biocontrol agents is highly influenced by environmental conditions such as temperature, humidity, soil pH, and moisture content. For instance, *Trichoderma* species perform optimally at temperatures between 20°C and 30°C, while *Pseudomonas fluorescens* shows reduced efficacy at high temperatures due to decreased antifungal metabolite production [35]. Similarly, high humidity levels favor some fungal pathogens, which can undermine biocontrol agents like *Bacillus subtilis* and *Trichoderma spp.*, especially under excessive soil moisture. Soil pH and microbial community composition also affect the survival and activity of these agents, making it challenging to predict their success across different agricultural settings [36].

B. Host Specificity

While host specificity can prevent non-target effects, it limits the applicability of biocontrol agents. For example, bacteriophages are highly specific, necessitating the development of phage cocktails to target different strains of a pathogen, increasing the complexity and cost of biocontrol strategies. Similarly, *Trichoderma* species are often effective against specific pathogens like *Fusarium*, but may not work against others, requiring the use of multiple agents in combination or rotation [37]. Additionally, there is a risk of unintended effects on beneficial microorganisms, which can disrupt the microbial community in the soil.

C. Production and Formulation Challenges

The production and formulation of biocontrol agents present significant challenges, particularly in maintaining viability and activity during mass production and storage. Scaling up production can lead to variability in product quality, and biocontrol agents are often sensitive to environmental conditions like temperature and moisture, requiring careful formulation to ensure stability and efficacy. The formulation must also be tailored to the delivery method, whether for seed treatments, soil applications, or foliar sprays, and must consider potential interactions with other formulation components [38][39]. The cost of production and formulation can be high, making biocontrol agents less competitive with chemical pesticides.

D. Regulatory and Commercialization Issues

Regulatory and commercialization challenges are major barriers to the widespread adoption of biocontrol agents. The registration process for biocontrol agents is often complex, time-consuming, and expensive, particularly in regions with stringent requirements like the European Union and the United States. This can delay the introduction of new biocontrol products and limit innovation, especially for small and medium-sized enterprises (SMEs) [40]. Additionally, farmers may be skeptical about the effectiveness of biocontrol agents compared to chemical pesticides, and the market for biocontrol products remains relatively small, discouraging investment in research and development [41]. Addressing these challenges requires harmonized regulatory frameworks, public-private partnerships, and education efforts to increase the acceptance and use of biocontrol agents.

VII. Advances in Biocontrol Agent Research

Recent advancements in biocontrol agent research focus on enhancing their efficacy and expanding their application in sustainable agriculture. These include genetic engineering of biocontrol agents, synergistic

use with chemical pesticides, integration into Integrated Pest Management (IPM) systems, and innovations in delivery systems.

A. Genetic Engineering of Biocontrol Agents

Genetic engineering has significantly improved biocontrol agents by enhancing their pathogen suppression abilities, adaptability, and host range. For instance, *Pseudomonas fluorescens* has been genetically modified to increase the production of antifungal metabolites like phenazines, improving its effectiveness against soil-borne pathogens such as *Fusarium oxysporum* and *Pythium spp.* [42]. Similarly, *Bacillus thuringiensis* (Bt) has been engineered to express multiple *cry* genes, broadening its insecticidal spectrum [43]. However, the use of genetically modified organisms (GMOs) faces regulatory and public acceptance challenges due to concerns about environmental impacts and potential gene transfer [44].

B. Synergistic Use with Chemical Pesticides

Combining biocontrol agents with chemical pesticides offers a synergistic approach to pest and disease management. For example, *Bacillus subtilis* used alongside reduced doses of chemical fungicides has shown improved control of diseases like Fusarium wilt in tomatoes and cucumbers, reducing the overall chemical load on the environment [45]. This strategy enhances pest control while mitigating the risk of resistance development and minimizing environmental impacts [46].

C. Integration into Integrated Pest Management (IPM) Systems

The integration of biocontrol agents into IPM systems has advanced significantly, providing a sustainable alternative to chemical pesticides. For instance, *Pseudomonas fluorescens* is successfully used in IPM programs for rice and wheat, where it suppresses soil-borne pathogens and is complemented by cultural practices like crop rotation and resistant varieties [47]. Similarly, *Trichoderma* species are integrated into horticultural IPM programs, reducing soil-borne diseases and improving overall farm sustainability [48].

D. Innovations in Delivery Systems

Innovations in delivery systems are critical for improving the effectiveness of biocontrol agents. Encapsulation technologies protect biocontrol agents from environmental stressors, enhancing their stability and controlled release in the field [49]. Biochar and nanotechnology are also being explored as carriers for biocontrol agents, improving their colonization and persistence in the soil. Additionally, biopriming seeds with biocontrol agents and priming agents like salicylic acid enhances seed resistance to pathogens during early plant development [50]. These advancements are crucial for ensuring that biocontrol agents are effectively integrated into modern agricultural practices.

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VIII. Future Perspectives and Research Directions

As agriculture shifts towards more sustainable practices, biocontrol agents are becoming increasingly vital in pest and disease management. To fully harness their potential, ongoing research and innovation are essential. Future directions include exploring new biocontrol agents, enhancing efficacy through biotechnology, adopting sustainable approaches, and developing supportive policy frameworks.

A. Exploration of New Biocontrol Agents

The discovery of new biocontrol agents is crucial for expanding pest and pathogen management tools. The plant microbiome, especially the rhizosphere, phyllosphere, and endosphere, offers a rich source of potential agents. High-throughput sequencing and metagenomics have identified novel *Pseudomonas* and *Bacillus* strains with unique antibiotic profiles [51]. Marine environments are also promising, with marine-derived *Streptomyces* showing antifungal activity against *Fusarium* and *Rhizoctonia* [52]. Additionally, bacteriophages and entomopathogenic fungi like *Beauveria* and *Metarhizium* are being explored for their potential in targeted biocontrol [53].

B. Enhancing Efficacy through Biotechnology

Biotechnology offers ways to enhance biocontrol agents' performance. Genetic engineering has been used to increase the production of antifungal metabolites in *Pseudomonas* and lipopeptides in *Bacillus*, resulting in more effective pathogen suppression [54]. Synthetic microbial consortia combine multiple agents with complementary actions, providing broader disease control. Molecular breeding and CRISPR-based genome editing are also improving traits like stress tolerance and pathogen specificity in biocontrol agents [55].

C. Sustainable and Eco-friendly Approaches

Sustainable biocontrol strategies focus on reducing environmental impacts while enhancing agricultural resilience. The use of organic amendments like compost and biochar improves soil health and supports biocontrol agent activity [56]. Combining biocontrol agents with cover crops and crop rotation enhances ecosystem health and reduces chemical inputs [57]. Developing biocontrol agents with enhanced stress tolerance is critical as climate change introduces more variable conditions [58].

D. Policy and Regulatory Frameworks

Supportive policy and regulatory frameworks are essential for biocontrol adoption. Current regulations can be complex and costly, especially for SMEs. There is a need for harmonized, streamlined regulatory processes that ensure safety while promoting innovation [59]. Policies that support research, encourage the integration of biocontrol agents into IPM systems, and provide incentives for sustainable practices are crucial for widespread adoption [60]. International cooperation and knowledge sharing will also play a key role in advancing biocontrol technologies globally.

IX. Conclusion

Biocontrol agents represent a promising avenue for sustainable agriculture, offering effective alternatives to chemical pesticides while minimizing environmental impact. Advances in biotechnology, such as genetic engineering and the development of synthetic microbial consortia, have significantly enhanced the efficacy and versatility of biocontrol agents. The exploration of new microbial and macro-organism biocontrol agents, alongside innovations in delivery systems, further expands their potential. However, challenges remain, including the need for resilient strains, improved formulations, and supportive regulatory frameworks. Integrating biocontrol agents into Integrated Pest Management (IPM) systems and adopting sustainable practices such as organic amendments and crop rotation are crucial for maximizing their benefits. Continued research, innovation, and international cooperation are essential to overcoming

existing limitations and ensuring the widespread adoption of biocontrol agents, ultimately contributing to global food security and environmental sustainability.

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Comment [h5]: References are not as per the journal guideline.....