# BIOLOGICAL CONTROL METHODS AGAINST PLANT DISEASES: MODERN APPROACHES AND APPLICATION PROSPECTS

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**Abstract:** Plant diseases are among the major factors limiting agricultural productivity and crop quality worldwide. The excessive use of chemical pesticides has resulted in environmental pollution, the development of pathogen resistance, and negative impacts on human health, increasing the demand for environmentally friendly alternatives. The present study evaluated the effectiveness of biological control agents, particularly *Trichoderma harzianum*-based biopesticides, in reducing disease incidence and improving the growth and productivity of tomato plants under greenhouse conditions. Comparative analyses were conducted between control and treated variants by assessing disease incidence, disease severity, vegetative growth parameters, and yield characteristics. The application of *Trichoderma* significantly reduced disease incidence from 48.0% to 18.0% and disease severity from 35.0% to 12.0%. Moreover, treated plants exhibited improvements in plant height, root length, vegetative biomass, fruit number, and overall yield. Yield increased from 4.8 to 6.5 kg $m^{-2}$, while the proportion of marketable fruits increased from 74% to 90%. The positive effects of the biological preparation were mainly associated with mycoparasitism, competition for nutrients and ecological niches, production of antimicrobial metabolites, and activation of induced systemic resistance in plants. In addition to suppressing soil-borne pathogens such as *Fusarium* spp., *Rhizoctonia solani*, and *Pythium* spp., biological treatments improved soil microbiological activity and reduced dependence on chemical pesticides. The results obtained demonstrate that *Trichoderma*-based biopesticides represent an effective, environmentally safe, and sustainable alternative for disease management in tomato production and support their wider application within integrated plant protection systems.
**Keywords:** *Trichoderma harzianum*, tomato, biopesticide, induced systemic resistance, sustainable agriculture, integrated pest management.

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## Introduction

***Plant diseases and their characteristics.*** The widespread use of chemical fertilizers and pesticides has led to increased productivity in agriculture. However, the long-term use of these substances has had negative effects on the environment, the biological balance of the soil, and human health. Therefore, there has been increased interest in environmentally safe and sustainable control methods in modern agriculture.

Plant diseases are disruptions in the normal physiological function of plants as a result of pathogenic organisms or unfavorable environmental factors. These diseases weaken plant development, reduce yield, and sometimes lead to plant death.

Plant diseases are mainly divided into three groups:

- **Fungal diseases** are the most widespread group of diseases. It spreads through spores and is characterized by leaf spotting, root, stem and fruit rot.
- **Bacterial diseases** are caused by bacteria entering the plant through wounds or natural openings. The main symptoms are softening of tissues, watery spots and rot.
- **Viral diseases** occur as a result of viruses disrupting the functioning of plant cells. Mosaic symptoms are observed with color changes, impaired development, and fruit deformation.

The spread of diseases is influenced by climatic conditions, soil and water quality, as well as agrotechnical errors. Especially high humidity, improper irrigation, and violation of the crop rotation system accelerate the development of pathogens. Similar pathology is observed in plants due to the influence of several different factors (see Table 1).

Plant diseases cause reduced productivity and economic losses. At the same time, excessive use of chemicals to combat diseases leads to pollution of soil and water resources, a decrease in beneficial microorganisms, and risks to human health. For this reason, the application of biological control methods has become increasingly important in recent years (Cook & Baker 1983; Lahlali et al. 2022).

***Causes of the emergence and spread of diseases.*** The spread of plant diseases occurs as a result of the interaction between the pathogen, the host plant, and environmental factors, and this process is explained by the principle of the "Disease Triangle". The main factors influencing the occurrence and epidemiological dynamics of diseases are:

**Table 1.** Similar symptoms of infectious and non-infectious diseases

| Plant name | Non-infectious disease | Cause of the disease | Infectious disease with similar symptoms |
|---|---|---|---|
| Tomato (*Solanum lycopersicum* L.) | Blossom end-rot | Frequent changes in humidity, lack of calcium in the soil | Bacterial Blossom end-rot (*Bacterium lycopersici*) |
| Pepper (*Capsicum annuum* L.) | Wilting and chlorosis | Iron deficiency | Stolbur (viral disease) |
| | Blossom end-rot | Frequent changes in humidity, lack of calcium in the soil | Bacterial Blossom end-rot (*Bacterium lycopersici*) |
| | Wilting and Root Browning | Low temperature in the soil | Bacterial Blossom end-rot (*Clavibacter michiganensis* subsp. *michiganensis*) |

**a) Climatic factors:** Temperature, humidity, and precipitation play an important role in the development of plant diseases. High humidity and optimal temperature conditions accelerate the spread of fungal and bacterial diseases. Wind and air currents allow spores to be carried over long distances.

**b) Soil characteristics:** Soil structure, nutrient supply, pH, and drainage characteristics affect plant resistance to disease. Poor drainage and excessive moisture promote the development of root rot and other soil-borne diseases.

**c) Irrigation regime:** Excessive or improperly organized irrigation causes increased humidity in the area and creates favorable conditions for the development of pathogens. Water droplets that remain on the leaf surface for a long time, in particular, accelerate the spread of infection.

**d) Agrotechnical errors:** Failure to follow crop rotation, monoculture, dense planting of plants, and use of contaminated tools and equipment increase the risk of disease spread. Pathogens can easily be transported from one area to another as a result of human activity.

**e) Modern monitoring methods:** PCR-based molecular diagnostics, Geographic Information Systems (GIS), GPS technologies, and remote sensing methods are widely used for early detection and prediction of the spread of plant diseases. These methods allow for the identification of disease foci and the planning of effective control measures (Agrios 2005).

***Economic and ecological impacts of plant diseases.*** Plant diseases cause reduced productivity in agriculture, deterioration of product quality, and serious economic losses. These losses are divided into two groups: direct and indirect. Direct losses include crop loss in the field, decay during storage, and loss of quality. Indirect losses are related to changes in market prices, shortages of raw materials in the processing industry, and limited export opportunities (FAO 2021).

Plant diseases negatively affect not only the quantity of the crop, but also its quality indicators. As a result of the weakening of photosynthesis, the amount of sugar, vitamins, and dry matter in the crop decreases. In addition, as a result of some fungal diseases, mycotoxins that are dangerous to human health accumulate in the product, which limits the use and sale of the product.

From an ecological perspective, the intensive use of chemicals to combat diseases leads to the pollution of soil and water resources, the decline of beneficial microorganisms, and the weakening of biodiversity. At the same time, the widespread spread of disease-susceptible varieties and the decline of local genetic resources increase the risk of losing important genetic material for future breeding efforts.

Global climate change creates conditions for the spread of new pathogens, which poses an additional threat to the sustainability of native plant species. Crop losses caused by plant diseases can negatively affect the economic situation of small and medium-sized farms, especially in rural areas, and can lead to social problems.

Thus, plant diseases are one of the problems of great importance not only from an agrobiological perspective, but also from an economic, ecological, and social perspective (FAO 2021).

***The role and problems of chemical control:*** Chemical methods have been used as the main means of combating plant diseases and pests for many years. Although these methods are highly effective in the short term, their widespread and continuous application poses serious risks to human health and the environment.

***Effects of pesticides on human health:*** Pesticides are potentially toxic substances and can have both acute and chronic effects on the human body. Acute effects are mainly characterized by poisoning, respiratory problems, and skin diseases. Long-term effects are associated with weakened immune systems, impaired reproductive health, and genetic changes.

***Pesticide residues in food products:*** The use of chemical products can result in pesticide residues on products. In some cases, these residues exceed the maximum limits set, posing a threat to human health. In particular, systemic drugs accumulate in plant tissues and cannot be completely removed by conventional washing processes.

***Environmental impacts:*** Most pesticides are released into the soil, water, and air before reaching the target organism. This leads to a decline in soil microorganisms, a weakening of biodiversity, and a disruption of ecosystem balance.

***Resistance problem:*** Prolonged use of the same chemical preparations leads to pathogens gaining resistance to those substances. As a result, there is a need to use higher doses and new drugs, which further increases the environmental burden.

***The importance of applying biological control.*** The health and environmental risks posed by chemical control have necessitated the development of alternative control methods. In this regard, biological control methods are of particular importance in terms of ensuring ecological safety, reducing pesticide burden, and contributing to the formation of sustainable agriculture (WHO 2023).

***The essence, definition and fundamental principles of biological control.*** Biological control or biocontrol is an environmentally safe method of combating plant diseases and pests based on the use of beneficial living organisms, their metabolites, and microbiological products. The main goal of this method is not only to destroy pathogens, but also to preserve and restore the natural balance in the agroecosystem. In modern agriculture, biological control is considered one of the key components of sustainable production and serves to reduce dependence on chemical pesticides.

The basis of biological control is competition between microorganisms for nutrients and habitat, antagonistic effects, and natural regulatory mechanisms. As a result of the activity of beneficial microorganisms, unfavorable conditions are created for the development of pathogens, and the risk of infection is reduced.

***The scientific nature and impact of biocontrol.*** The scientific basis of biological control is based on research conducted in the fields of microbiology, phytopathology, ecology, and molecular biology. Biocontrol agents settle in the rhizosphere and phyllosphere of the plant, creating a protective microbiological environment and limiting the development of pathogens in those areas.

Unlike chemical pesticides, biological agents increase the microbiological activity of the soil, accelerate the circulation of nutrients, and improve the overall physiological condition of the plant. In addition, some biocontrol microorganisms have a positive effect on increasing productivity by increasing the synthesis of hormone-like substances that stimulate plant growth.

***Fundamental principles.*** The effectiveness of biological control is based on the following main mechanisms:

- **Antagonistic impact** – beneficial microorganisms inhibit the development of pathogens by synthesizing antibiotics, enzymes, and other metabolites.
- **Competition for food and habitat** – beneficial microorganisms limit the development of pathogens by utilizing nutrients more quickly.
- **Induced systemic resistance (ISR)** – biological agents activate the plant's defense mechanisms, increasing its resistance to diseases.
- **Siderophore production** – some bacteria synthesize substances that bind iron ions, reducing the availability of elements essential for the development of pathogens.
- **Hyperparasitism** – some microorganisms grow on pathogens and directly destroy them. This feature is especially characteristic of Trichoderma species (Compant et al. 2010).

***Methods of applying biological control.*** The effectiveness of biological control depends on the method of application of the biological agents used. The main application methods are as follows:

- **Inoculation method** – the introduction of a small amount of a biological agent into the soil or plant and subsequent formation of a protective effect through natural reproduction.
- **Augmentation method** – application of biological preparations multiplied in the laboratory to a large area.
- **Seed biopreparation** – the treatment of seeds with beneficial microorganisms before sowing and the creation of an initial protective microflora.
- **Soil treatment method** - application of biological preparations through an irrigation system or directly to the soil.
- **Foliar spraying** – the application of biological preparations to protect the aboveground organs of the plant from diseases.

The correct selection and combination of these methods increase the effectiveness of biological control and allows for the formation of a sustainable plant protection system (Glick 2012).

***Ecological and economic perspectives.*** Biological control plays an important role in the development of environmentally safe and sustainable agriculture. While intensive use of chemical pesticides leads to a decrease in soil biological activity and environmental pollution, biological methods help preserve soil fertility and biodiversity. In addition, reducing pesticide residues serves to protect human health and ensure food security. From an economic perspective, although the application of biological preparations requires additional costs at the initial stage, in the long term, economic efficiency is ensured by reducing pesticide consumption, minimizing crop losses, and preserving soil fertility. For this reason, biological control is considered one of the promising and sustainable directions of modern agriculture (Du et al. 2022).

***Taxonomic and functional characteristics of biological control agents.*** Biological Control Agents (BCAs) are beneficial microorganisms that limit the development of plant disease pathogens and increase the defenses of plants. These agents include bacteria, fungi, actinomycetes, and other microorganisms, and act through mechanisms such as antagonism, antibiosis, competition, and induction of systemic resistance.

***Molecular and biological potential of bacterial agents.*** Bacteria are one of the most widely used groups of microorganisms in biological control. They mainly inhabit the rhizosphere and phyllosphere, limiting the development of pathogens and stimulating plant growth.

**Characteristics of the genus *Bacillus*.** *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus pumilus* species are widely used in the preparation of biological preparations because they produce highly resistant spores (Fig. 1). *B. amyloliquefaciens* is one of the most common bacteria that colonize plants endophytically and plays an important role in the biocontrol of vascular plant pathogens (Choudhary & Johri 2009). These bacteria damage the cell wall and membrane of pathogens by synthesizing enzymes such as chitinase, β-glucanase, and protease, as well as antimicrobial substances such as iturin, surfactin, and fengycin. *Bacillus* species are particularly effective against pathogens belonging to the genera *Fusarium*, *Phytophthora*, *Alternaria* and *Rhizoctonia*, and are also involved in the synthesis of substances that stimulate plant growth.

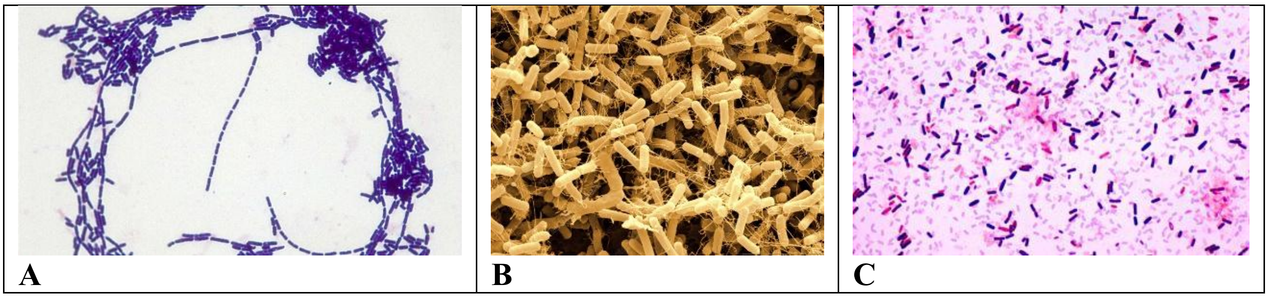


**Figure 1.** *Bacillus* genus bacteria: a) *B. subtilis* b) *B. amyloliquefaciens* c) *B. pumilus*.
**Source:** https://pmc.ncbi.nlm.nih.gov/articles/PMC10536829/

**Functional characteristics of the genus Pseudomonas:** Species such as *Pseudomonas fluorescens* form biofilms on the root surface, preventing the colonization of pathogens (Fig. 2). These bacteria bind iron ions through siderophores and synthesize various antibiotic substances and antimicrobial metabolites, limiting the development of pathogens. In addition, *Pseudomonas* species activate induced systemic resistance in plants, increasing their resistance to diseases and environmental stresses.

**Mycoparasitic activity of antagonistic fungi.** Among biological agents of fungal origin, the most widely used genus is *Trichoderma*. Characteristics of the *Trichoderma* genus: *Trichoderma harzianum, T. viride,* and *T. asperellum* are fungi that are widely distributed in the soil and have high antagonistic activity. These species limit the access of pathogens to food sources and disrupt their hyphae through the mechanism of mycoparasitism. The chitinase, β-glucanase, and protease enzymes synthesized by *Trichoderma* destroy the cell wall of pathogens, resulting in their growth being halted. These fungi are particularly effective against soil-borne pathogens such as *Fusarium* spp., *Sclerotinia* spp., *Pythium* spp., and *Rhizoctonia solani*. In addition, *Trichoderma* species activate systemic resistance in plants, improve nutrient uptake, and have a positive effect on plant development.

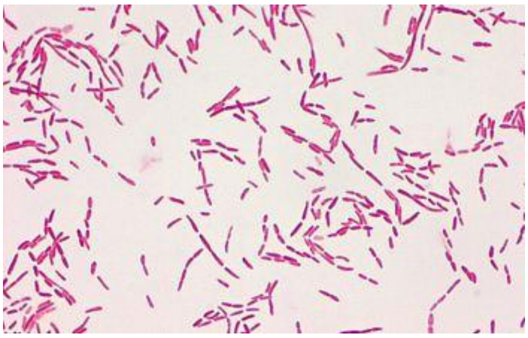

**Figure 2.** *Pseudomonas fluorescens.*

***Actinomycetes and antibiotic production:*** Among actinomycetes, the most important biological control agents are species belonging to the genus *Streptomyces*. These microorganisms limit the development of pathogens by synthesizing streptomycin, neomycin, and other antibiotic substances. *Streptomyces* species participate in the decomposition of organic matter, improve soil structure, and accelerate humus formation. In addition, they synthesize various enzymes and growth stimulants, which have a positive effect on plant development and increase their resistance to diseases.

***Bacteriophages:*** specific biological defenses. Bacteriophages are viruses that infect only bacteria and have high specificity against phytopathogenic bacteria. They target only certain bacterial pathogens without affecting beneficial microorganisms, making them an environmentally safe biological control tool. After bacteriophages enter a bacterial cell, they multiply inside it, eventually causing the cell to lyse. This mechanism allows the pathogen population to decrease in a short time. *Bacteriophages* are mainly used against bacterial pathogens such as *Erwinia amylovora, Xanthomonas* spp., *Pseudomonas syringae*, and *Ralstonia solanacearum*. The development of molecular biology and genomic technologies has expanded the application possibilities of bacteriophages in biological control. Their high specificity and environmental safety increase their importance in modern plant protection.

***Mechanisms of action of biological control agents***. Biological control agents limit the development of pathogens through various biological and biochemical mechanisms. In most cases, several mechanisms of action act simultaneously, weakening the development of diseases and increasing the defenses of plants. The main mechanisms of action include antibiosis, competition, parasitism, and induced systemic resistance.

***Antibiosis mechanism.:*** During antibiosis, antibiotics and other biologically active substances synthesized by beneficial microorganisms weaken or completely stop the development of pathogens. Iturin, surfactin, and fengycin synthesized by *Bacillus* species, as well as phenazines and other metabolites produced by Pseudomonas species, disrupt the cellular structure of pathogens, leading to their destruction.

***Competition mechanism:*** Beneficial microorganisms compete with pathogens for nutrients and living space. They rapidly colonize plant roots and leaf surfaces, making it difficult for pathogens to settle. Reduced availability of iron ions as a result of siderophore production also limits the development of pathogens.

***Parasitism and mycoparasitism:*** During parasitism, biological agents grow directly on the pathogenic organism and destroy it. This mechanism is particularly characteristic of *Trichoderma* species. In the process of mycoparasitism, enzymes such as chitinase, β-glucanase, and protease break down the cell wall of pathogenic fungi and stop their vital activity. This mechanism of action is highly effective against pathogens such as *Fusarium* spp., *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Pythium* spp., and *Botrytis cinerea*. The activity of bacteriophages against bacterial pathogens is also considered a special form of parasitism mechanism (Haas & Défago 2005).

***Induced systemic resistance:*** Some biological control agents activate the defense system of plants, increasing their resistance to diseases. This process is called induced systemic resistance (ISR). As a result of ISR, the synthesis of defense proteins and antioxidant enzymes is enhanced, the plant's response to infections accelerated, and the level of disease development is reduced. Thus, the multifaceted mechanisms of action of biological control agents ensure their high effectiveness in plant protection and play an important role in the development of sustainable agriculture.

***Induced systemic resistance***. Induced systemic resistance (ISR) is one of the most important mechanisms of biological control and is formed as a result of the interaction between beneficial microorganisms and plants. Signal molecules secreted by beneficial bacteria and fungi activate the plant's defense system, enhancing the synthesis of various protective proteins, phytoalexins, and antioxidant enzymes. As a result, the plant becomes more resistant to pathogen attacks, disease development is weakened, and crop losses are reduced. It has been determined that *Bacillus subtilis, Pseudomonas fluorescens,* and *Trichoderma harzianum* species play an important role in the formation of the ISR mechanism. Induced systemic resistance increases the resilience of plants not only to biotic stress factors, but also to abiotic stress factors such as drought, salinity, and temperature changes. This feature demonstrates the importance of biological control agents in terms of both plant protection and productivity enhancement (Köhl et al. 2019).

***Biopesticides and phytobiological preparations.*** Biopesticides are preparations based on microorganisms, their metabolites, plant extracts, and other biologically active substances. These preparations are used to limit the development of pathogens, pests, and weeds. Unlike chemical pesticides, biopesticides do not accumulate in the environment, do not disrupt the microbiological balance of the soil, and ensure environmental safety.

***Biopesticides of microbiological origin***. Microbiological biopesticides are mainly made from bacteria, fungi, and viruses and are widely used in modern agriculture (Figure 3). Biopesticides are effective tools for controlling insects, pathogens, and weeds. However, biopesticides require more knowledge about application and the biology of target pests compared to broad-spectrum pesticides. Biopesticides are safer for human health and the environment, but they usually take longer to kill pests than traditional insecticides. Therefore, pest species may be observed for several days after initial biopesticide applications. Compared to traditional insecticides, biopesticides typically persist in the environment for a shorter period of time and therefore have fewer trade barriers. However, this may require more frequent application.

- Preparations based on *Bacillus subtilis* and *B. amyloliquefaciens* inhibit the development of phytopathogens by synthesizing antibiotic substances, lipopeptides, and hydrolytic enzymes. These drugs are especially effective against fungal diseases.
- *Trichoderma harzianum* based preparations inhibit the development of soil-borne pathogens such as *Fusarium*, *Rhizoctonia*, *Sclerotinia*, and *Pythium* through mechanisms of mycoparasitism and antagonism (Fig. 4).
- *Pseudomonas fluorescens* preparations limit the development of pathogens through siderophore production and synthesis of antibiotic metabolites, while also improving the development of the

plant root system and the absorption of nutrients.

- *Bacillus thuringiensis* (Bt)-based preparations are mainly used against insect pests. The crystal proteins synthesized by this bacterium affect the digestive system of insects, causing their destruction. The fact that these preparations are safe for humans, animals, and beneficial insects allows for their widespread use (Fravel 2005).

Thus, biopesticides of microbial origin, in addition to being an environmentally safe alternative to chemical preparations, play an important role in the development of sustainable agricultural systems.

***Phytobiological preparations and plant extracts.*** Plant-based biological preparations occupy an important place among natural defenses. Biologically active substances synthesized by various plants have a protective effect against pathogens and pests, and extracts obtained from these substances are used as biopreparations in agriculture.

Garlic extract has a strong antibacterial and antifungal effect due to the allicin it contains. Hot pepper extract limits the development of pests through capsaicin. Nettle extract not only improves plant nutrition but also increases their resistance to disease. Tobacco and chamomile extracts have insecticidal and antimicrobial properties.

In recent years, the use of preparations based on essential oils from plants such as mint, thyme, eucalyptus, rosemary, and tea tree has expanded. The terpenoids and phenolic compounds contained in these oils damage the cell membranes of pathogens, limiting their development. Therefore, plant extracts and essential oils are of great importance in the preparation of environmentally safe biological preparations.


**Figure 3.** Biopesticides.

***Advantages and application prospects of biopesticides.*** One of the main advantages of biopesticides is their easy integration into integrated crop protection systems. These preparations allow you to reduce the use of chemical pesticides, maintain the microbiological balance of the soil, and prevent the formation of pesticide residues in products. Additionally, they are considered safer for beneficial insects and pollinators.

The effectiveness of biopesticides depends on factors such as temperature, humidity, soil pH, and application method. Therefore, the correct implementation of agrotechnical measures during their use is of particular importance. Currently, the biopesticide industry is rapidly developing worldwide, and numerous bacterial, fungal, and plant-based preparations are being produced commercially. Advances in biotechnology and molecular biology have enabled the creation of new generation of biological preparations, and their role in agriculture is expected to increase in the future (Verma et al. 2024).

**Figure 4.** *Rhizoctonia*.
**Source:** https://vegpath.plantpath.wisc.edu/diseases/rhizoctonia/

***Application and advantages of biopesticides based on microorganisms.*** Biopesticides based on microorganisms are one of the most promising areas of modern plant protection. These preparations are made from living cells, spores, or their metabolites of bacteria, fungi, viruses, and other beneficial microorganisms. In addition to controlling plant diseases and pests, microbiological preparations also serve to preserve the biological activity of the soil and ensure the stability of agroecosystems. Their natural origin supports the activity of beneficial microorganisms, activates biological processes in the soil, and increases the resistance of plants to various biotic and abiotic stress factors. As a result of the development of biotechnology, it has become possible to prepare a new generation of highly effective microbiological preparations. These preparations have great promise in terms of ensuring environmental safety, reducing pesticide burden, and contributing to the development of sustainable agriculture.

***Application of bacterial biopesticides.*** Among bacterial biopesticides, the species *Bacillus subtilis, B. amyloliquefaciens,* and *B. pumilus* are of particular importance. These bacteria limit the development of phytopathogens by synthesizing enzymes, antibiotic substances, and lipopeptides, while simultaneously activating induced systemic resistance in plants. They are applied by seed treatment, soil bio enrichment, and foliar spray methods.

***Application of fungal biopesticides.*** Among mushroom-based biopreparations, *Trichoderma harzianum, T. viride,* and *T. asperellum* species are widely used. These fungi inhibit the development of pathogens through mycoparasitism, enzymatic activity, and competition for nutrients. They are especially effective against soil-borne diseases such as *Fusarium*, *Rhizoctonia*, *Pythium* and *Sclerotinia*.

***Viral biopesticides.*** The main representatives of virus-based biopreparations are baculoviruses. They are highly specific in that they only infect certain insect pests and do not pose a threat to beneficial organisms. This feature makes them an environmentally safe means of control.

***Effects of microorganism-based biopesticides.*** Biopesticides act through mechanisms of antibiosis, competition, parasitism, mycoparasitism, and induced systemic resistance. As a result of these processes, the population of pathogens decreases and the resistance of plants to diseases increases.

***Environmental and economic advantages of biopesticides.*** Biological preparations do not create toxic residues in the environment, protect soil microflora, and help maintain biodiversity. Their application reduces pesticide residues in products, reduces dependence on chemicals, and helps reduce production costs.

***Industrial production and prospects of biopesticides.*** As a result of the development of biotechnology, the industrial production of bacterial, fungal, and viral biopesticides has expanded. Although the effectiveness of these preparations depends on environmental factors, their role in sustainable agriculture is increasing and is expected to be more widely used in the future (Verma et al. 2024).

***Modern directions and prospects of biological control of plant diseases***. The intensive use of chemical pesticides against plant diseases has led to problems such as environmental pollution, the decline of beneficial organisms, and the emergence of resistance in pathogens. For this reason, biological control has gained widespread importance as an environmentally safe and sustainable alternative in modern agriculture. As a result of the development of biotechnology and microbiology, highly effective biopreparations have been developed based on microorganisms belonging to the genera *Bacillus*, *Pseudomonas*, and *Trichoderma*. These microorganisms not only limit the development of phytopathogens but also increase the absorption of nutrients and productivity of plants. The combined use of several beneficial microorganisms further enhances their effectiveness.

The study of the plant microbiome has opened new perspectives in biological control. The selection and application of beneficial microorganisms allow for the creation of more effective and sustainable biopreparations. At the same time, molecular biology and genomic technologies are enabling the identification of new strains with high activity.

Modern production technologies and microencapsulation methods have increased the shelf life of biopreparations and their resistance to environmental factors. In addition, the application of biological agents in integrated plant protection systems, together with agrotechnical measures and resistant varieties, provides high efficiency.

In recent years, the development of nanobiotechnology has created new opportunities to increase the duration of action of biological preparations and more effectively deliver active ingredients to plant tissues. The growing interest in ecologically clean production in the world and in Azerbaijan has led to the expansion of the application of biological control agents.

Thus, biological control will be one of the main directions of sustainable agriculture in the future, playing an important role in increasing productivity, managing diseases, and protecting agroecosystems (He et al. 2021).

## Materials and Methods

***Research area and conditions.*** The research was conducted on tomato plants in greenhouse conditions. Since the greenhouse environment allows for control of factors such as temperature, humidity, lighting, and irrigation, it has created favorable conditions for evaluating the effectiveness of biological control agents. Vegetable plants were selected as the object of the study, and they were cultivated according to standard agrotechnical rules. During the growing season, plants were watered, fed, and ventilated regularly, and special attention was paid to maintaining constant temperature and humidity levels within the greenhouse.

**Figure 5.** Grey leaf spot on tomato.

The randomization principle was applied when placing experimental variants, minimizing the impact of microclimate differences within the greenhouse on the results. The physical and biological properties of the substrate where the plants are grown were considered, and the stability of soil conditions was ensured. During the observations, plant height, leaf number, vegetative development indicators, prevalence of disease symptoms, and general phytosanitary condition were recorded during the observations. Changes observed in the leaf, stem, and root system were evaluated separately. During the research, sanitary and phytosanitary regulations were followed, the tools used were disinfected, and diseased plant residues were removed from the field in a timely manner. However, in some cases, several diseases are observed in tomato plants (Figs. 5, 6). Research conducted in greenhouse conditions allows for an accurate assessment of the effects of biological control agents and a scientific analysis of the results obtained.

**Figure 6.** Tomato internal blossom end-rot (IBER).

**Applied biological agents.** During the study, various biological agents were used to combat plant diseases (Table 2). Biological agents are distinguished by their environmental safety, their lack of toxic residues in crops, and their reduced risk of pathogen resistance. During the study, a comparative analysis was conducted between the control and study groups using the *Trichoderma* biopesticide. Microorganisms belonging to the genera *Bacillus*, *Pseudomonas*, and *Trichoderma* were used as the main biological agents in the study. *Bacillus* species have been shown to inhibit the growth of phytopathogens by synthesizing antibiotic substances, enzymes, and lipopeptides, while simultaneously stimulating plant nutrient uptake and growth (Lugtenberg & Kamilova 2009).

Bacteria of the genus *Pseudomonas* formed beneficial microflora in the root zone, competing with pathogens for nutrients, and weakened the development of pathogens through the synthesis of siderophores and other metabolites. In addition, these bacteria have contributed to the activation of systemic resistance mechanisms in plants. Fungi belonging to the genus *Trichoderma* has been used against soil-borne diseases. These microorganisms limited the growth of pathogenic fungi by breaking down their hyphae and improved the development of the root system and the absorption of nutrients. The effectiveness of biological agents has depended on their proper selection, timing of application, and environmental conditions. Therefore, the preparations were applied under optimal temperature and humidity conditions, and agrotechnical measures were fully observed. Thus, applied biological agents have made a significant contribution to reducing the spread of diseases, improving plant development, and producing environmentally safe products

(Villavicencio-Vásquez 2025).

**Research method.** Studies were conducted in greenhouse conditions during the growing season to assess the effectiveness of biological control agents against plant diseases. The experiments were organized under the same agrotechnical conditions, the difference between the variants was only related to the biological agents applied. In the control variant, biological preparations were not used, while in the experimental variant, biological preparations prepared based on beneficial microorganisms were applied in accordance with the manufacturer's recommendations in the norms and periods. When applying powder or granule preparations, they are first dissolved in a small amount of water, then water is added to the required volume to prepare a homogeneous solution. Usually, *Trichoderma* preparations are prepared at a rate of 2–5 g of the preparation per one liter of water or according to the manufacturer's recommended dosage. The prepared solution should be used immediately without prolonged storage to preserve the viability and activity of microorganisms.

**Table 2.** Areas of application of biological control agents.

| Biological control agent | Active agent | Target disease or pathogen | Mechanism of action | Effect observed on tomato plants |
|---|---|---|---|---|
| **Bacterial** | *Bacillus subtilis* | *Fusarium oxysporum*, *Alternaria solani*, bacterial spot | Antibiosis, competition, induction of ISR | Disease spread is reduced; root development and vegetative growth are improved |
| **Bacterial** | *Bacillus amyloliquefaciens* | Root rot; leaf spots | Antibiotic and lipopeptide synthesis | Plant height and yield increase, disease pressure decreases |
| **Rhizosphere bacteria** | *Pseudomonas fluorescens* | Wilting diseases; root pathogens | Siderophore synthesis, nutrient competition, ISR | The root system is strengthened; nutrient absorption improves |
| **Fungal-based** | *Trichoderma harzianum* | *Fusarium* spp.; *Rhizoctonia solani*; *Pythium* spp. | Mycoparasitism, enzyme synthesis, competition | Reduction of root rot and acceleration of vegetative development are observed |
| **Fungal-based** | *Trichoderma viride* | Soil-borne fungal diseases | Cell wall breakdown and hyperparasitism | Plant viability and crop quality increase |
| **Plant extract** | Garlic extract (*Allium sativum*) | Fungal and bacterial diseases | Antimicrobial effects of allicin | The development of leaf spots and rots is weakened |
| **Plant extract** | Nettle extract (*Urtica dioica*) | For preventive protection purposes | Stimulation of plant immunity | Increases stress resistance and vegetative development of plants |
| **Essential oils** | Thyme and tea tree oils | Fungal diseases | Cell membrane damage | Pathogen development is limited |
| **Viral-based** | Baculovirus | Insect pests | Reproduction in the cells of the pest | The number of pests decreases; crop losses are minimized |

In tomato plants, *Trichoderma*-based biopesticides have been used as seed treatment, soil or substrate treatment, root system treatment, application during the vegetation period, and, if necessary, leaf surface spraying. During the observations, plant growth and development indicators, including plant height, leaf number, phytosanitary condition, and disease symptom development intensity, were assessed. The prevalence of diseases was determined as a percentage based on the ratio of infected plants to the total number of plants. Productivity and product quality indicators were also analyzed to assess the effect of biological preparations. The results obtained showed that biological agents reduce the development of diseases and increase the healthy development and productivity of plants. These results confirmed that biological control methods are a promising approach for environmentally safe and sustainable agriculture (Tariq et al. 2020).

**Results and Discussions**

During the conducted studies, the effect of biological control agents in preventing plant diseases and increasing productivity was evaluated. During the experiment, observations were made on the control and experimental variants, and the rate of disease development, vegetative development of plants, and yield indicators were compared. As a result of the application of *Trichoderma harzianum*-based biopesticide, the development of soil-borne pathogens, especially *Fusarium* spp., *Rhizoctonia solani* and *Pythium* spp., was significantly reduced in tomato plants. At the same time, positive changes were observed in root system development, vegetative growth and yield indicators. The effect of the preparation is mainly associated with mycoparasitism, competition for nutrients and activation of induced systemic resistance in plants.

Based on the results of the observations, it was determined that the intensity of disease spread in the variants where biological preparations were applied was lower than in the control variant. In particular, the use of preparations based on *Trichoderma* spp. and *Bacillus* spp. has weakened the development of pathogenic microorganisms in the soil and on the surface of plants, and the rate of reproduction of pathogens has decreased. The competitive ability, synthesis of antibiotic substances, and hyperparasitism properties of beneficial microorganisms have played an important role in limiting the development of diseases (Harman et al. 2004).

**Table 3.** Application of *Trichoderma*-based biopesticide on tomato plants.

| Indicator | Control variant | *Trichoderma* applied variant | Change (%) |
|---|---|---|---|
| Disease prevalence (%) | 48,0 | 18,0 | −62,5 |
| Disease development rate (%) | 35,0 | 12,0 | −65,7 |
| Plant height (cm) | 62,4 | 74,8 | +19,9 |
| Number of leaves (pcs/plant) | 21 | 28 | +33,3 |
| Root length (cm) | 14,5 | 19,2 | +32,4 |
| Vegetative mass (g/plant) | 285 | 358 | +25,6 |
| Number of fruits (pcs/plant) | 18 | 24 | +33,3 |
| Average fruit weight (g) | 82,0 | 91,5 | +11,6 |
| Yield (kg/m²) | 4,8 | 6,5 | +35,4 |
| Share of marketable product (%) | 74 | 90 | +21,6 |

During the study, positive changes were also observed in the morphological indicators of the plants. In plants treated with biological preparations, the development of the root system was better, the size of the leaf surface increased, and vegetative development was more intensive. Better development of the root system improved the absorption of nutrients and water, which had a positive effect on the overall physiological condition of the plants. Observations have shown that resistance to stress factors has also increased in plants treated with biological agents. Even in the face of temperature changes, humidity fluctuations, and other unfavorable environmental conditions, plant development has become more stable. This feature is explained by the fact that biological agents activate induced resistance mechanisms in plants. This feature is explained by the fact that biological agents activate induced resistance mechanisms in plants. Fruit formation has become more intensive, and product quality indicators have increased. Crop losses due to diseases have decreased, and the amount of marketable produce has increased. The appearance, color, and storage capacity of the fruits have improved, which has contributed to an increase in the market value of the product. Experiments have shown that the application of biological preparations also has a positive effect on the microbiological activity of the soil. The increase in the number of beneficial microorganisms has accelerated the decomposition of organic matter in the soil and improved the absorption of nutrients by plants. As a result, soil fertility has been preserved, and the biological balance of the agroecosystem has been maintained. The application of biological control methods has also allowed for a reduction in the use of chemical pesticides. This has reduced the risk of environmental pollution and prevented the accumulation of pesticide residues in soil and crops. Biological preparations are considered more favorable in terms of safety for human health and beneficial organisms. At the same time, minimizing the negative impact on soil microflora is of great importance for the formation of sustainable agricultural systems. During observations, it was determined that the effectiveness of biological control agents can vary depending on environmental conditions, temperature, humidity, soil properties, and application technology. However, when used under optimal conditions, biological drugs demonstrate high efficacy and provide significant results in disease management. The results obtained show that the application of biological control methods has a positive effect on reducing the spread of plant diseases, increasing productivity and product quality, protecting soil fertility, and ensuring ecological safety. Therefore, the widespread use of biological preparations in agriculture is considered one of the promising directions in terms of the development of sustainable production systems (Yao et al. 2023).

The results of the conducted studies have shown that the application of biological control agents is highly effective in controlling plant diseases, and the results obtained are consistent with the data presented in international scientific literature. Preparations based on *Trichoderma* spp. and *Bacillus* spp. limited the development of phytopathogens, while simultaneously increasing the vegetative development and productivity of plants (Harman et al. 2004).

The high efficiency of biological preparations is due to their ability to compete with pathogens, synthesize antimicrobial substances, and activate induced systemic resistance in plants. The development of the root system, increase in vegetative mass, and increased resistance to stress factors observed during the study were consistent with the results of other authors (Vinale et al. 2008).

However, the effectiveness of biological agents may vary depending on temperature, humidity, soil properties, and application technology. However, when applied under optimal conditions, they act as an environmentally safe and sustainable alternative to chemical pesticides. The results obtained indicate the feasibility of widespread use of biological preparations in integrated plant protection systems and the importance of directing future research towards the development of new microbiological preparations (Saldaña-Mendoza et al. 2023).

## Conclusion

The results of the present study confirmed that biological control agents can effectively reduce the incidence and severity of plant diseases while simultaneously improving plant growth and productivity. The application of *Trichoderma harzianum*-based biopesticides significantly suppressed the development of important soil-borne pathogens and contributed to improved vegetative and reproductive performance in tomato plants.

Compared with the control treatment, plants receiving biological applications showed lower disease prevalence, enhanced root development, increased vegetative biomass, and higher yield and fruit quality. These beneficial effects are primarily associated with the mechanisms of mycoparasitism, competition for nutrients, production of antimicrobial compounds, and induction of systemic resistance in plants. The study further demonstrated that biological preparations positively influence soil microbial activity and contribute to maintaining agroecosystem stability while reducing the need for chemical pesticides and minimizing environmental contamination.

Overall, *Trichoderma*-based biopesticides can be considered a promising component of sustainable agriculture and integrated plant protection systems. Future studies should focus on the development of improved microbial formulations, optimization of application technologies, and evaluation of their effectiveness under different environmental conditions and cropping systems.

**Conflict of Interest**

Authors declare that there is no conflict of interest.

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