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Biological control of phytophages by different strains of *Bacillus thuringiensis* in apple orchards

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ABSTRACT

The study assessed the use of effective microorganisms for the control of apple tree pests. Biological control of phytophagous pests is an environmentally safe alternative to chemical impact on ecosystems. The most successful bioagent used to control insect pests is Bacillus thuringiensis. The objects of research were apple trees of the Jonagold variety. The quantitative composition of the main insect pests of the apple tree by species was determined. The strains of microorganisms were isolated from dead insects of natural populations of leaf-eating phytophagous insects of the orders Coleoptera, Hemiptera, and Lepidoptera. The effectiveness of new bioagents was compared with chemical and biological insecticides in the course of the studies. In the course of studies of the isolated strains, they were found to be able to inhibit the growth of some phytopathogenic fungi. It was shown that the studied strains BT-43, BT-18, and BT-52 have a fungistatic effect, and strain BT-10 has a fungicidal effect against Botrytis cinerea and Alternaria mali Roberts. The effectiveness of the treatment of apple orchards affected by phytophages with bioinsecticides based on selected promising strains of *B. thuringiensis* was tested. The strain BT-10 proved to be particularly effective against pests, the effect of which is almost equal to or superior to that of deltamethrin. Due to the high efficiency and entomocidal effectiveness against insects of Lepidoptera, it is advisable to use this biological product in the future in the system of control of leaf-eating phytophages. The treatment of apple trees with biological products contributed to a significantly higher yield than the control, which was the highest when treated with the BT-10 strain. Investigating the cost-effectiveness of the used apple protection products based on BT strains, it was shown that treatment with a chemical insecticide reduced the cost by 18% compared to the control. Thus, the research has shown that the treatment of apple plantations with pest and disease control agents is an integral element of the technology of growing and storage of fruits.

Keywords: *B. thuringiensis*, biological products, entomocidity, biomethod, biocenosis of apple orchards, apple trees.

INTRODUCTION

In recent years, farming and agricultural production have been experiencing seriously disturbing trends, such as decreasing soil fertility, high crop losses due to pests, pathogens, ruderal and segetal phytobiota, environmental degradation of agricultural landscapes, and pollution caused by anthropogenic impact, including chemicals, which leads to a decline in the quality of agricultural products. The technology of applying effective microorganisms is environmentally friendly, helps to improve soil and plant health, and thus ensures the production of environmentally sustainable products.

Improving the quality of the environment is essential to stimulating economic development, social stability and sustainable development. Greenhouse gas emissions, toxic industrial waste, and pesticide contamination endanger the planet and human health. In such a context, the industrial sector is increasingly trying to use cleaner production technologies and environmentally friendly environmental engineering approaches based on microorganisms. The production of safe food through the use of microbial products, which, being biological pesticides, contribute to environmental protection, biodiversity conservation and sustainable development, which is in accordance with the EU policy and adopted strategies. Despite the potential harm, pesticides play a crucial role in modern agriculture, protecting crops from pests and diseases, thus guaranteeing higher yields and better product quality (Levishko et al., 2023). The absence of pesticides would have a profound impact on global food production, increasing food insecurity and raising consumer prices. Government incentives are pushing for updated and stronger regulations in major agricultural producing countries. However, the toxicity, high persistence, and prevalence of these substances in the environment cause a number of challenges.

Apple is the most common fruit in the structure of perennial fruit plantations in Ukraine. Its fruit production among pome crops averaged about 90% or 1179.6–1278.9 thousand tonnes per year in 2015–2023. According to the State Statistics Service of Ukraine, in 2023, the total area of apple trees in fruiting age was 75.6 thousand hectares. Overall, Ukraine ranks therteenth in global apple production and fourth in Europe and has all the signs of a sustainable market (Salo, 2020).

One of the most important factors for highly efficient apple production is ensuring the proper phytosanitary condition of plantations and compliance with agro-technological growing practices (Yakovenko et al., 2023; Demyanyuk and Synenko, 2024). For example, fruit yields are reduced by 35–45% and fruit commerciality by 45–60% due to the harmful effects of pests (phytophages) and untimely or incorrect selection of products for protective measures against harmful insect and mite species (Yanovskyi, 2021).

The peculiarity of perennial fruit plantations is that they form specific agrocenoses with a relatively stable complex of living organisms. With changes in the structure of crops, the introduction of intensive varieties and hybrids, the use of large quantities of chemical plant protection products and climate change, there is an inevitable change in the status of pests. Many pests have expanded their habitats, developed resistance to pesticides, and become dominant. As a result, the harmfulness of most insect pests and pathogens is usually increasing at all stages of development, which causes significant economic losses to producers, including through increased financial costs for plant protection products (Gumeniuk et al., 2022).

Chemical insecticides and their degradation products, which are often even more toxic than the pesticides themselves, accumulate in treated plants and do not have time to be completely removed from them by the time of harvest (Tkach et al., 2021; Pathak et al., 2022). A microbiological control of phytophagous pests is an environmentally safe alternative to chemical impact on ecosystems (Furdychko and Demyanyuk, 2015; Levishko et al., 2024 a). The most successful bioagent used to control insect pests is the *Bacillus thuringiensis* bacterium (Lisovyi et al., 2018; Ma, 2023). The mechanism of action of products based on them is completely natural and is an example of the effective use of microorganisms against pests (Tomar et al., 2024). Taking into account the need of consumers for safe agricultural food products, as well as the scale of their production, the market of biopesticides has been growing rapidly in recent years and, products based on *B. thuringiensis*-based products are steadily occupying a leading position due to their high activity against the larval stages of various types of insects (Fayad, 2022). It is the most successful microbial insecticide, and its proteins have been studied for many years for their toxicity against insects belonging mainly to the *Lepidoptera, Diptera* and *Coleoptera* orders (Domínguez-Arrizabalaga et al., 2020; Valtierra-de-Luis et al., 2020).

The scientific, theoretical and practical approaches to the effective use of microbiological methods of fruit and berry crops protection from leaf-eating phytophages with the participation of natural resources – entomopathogenic bacteria of the *Bacillus thuringiensis* group. In 1902, this bacterium was first discovered in *Bombyx mori* by the plant breeding engineer Ishiwatari Shigetane, who named it *Bacillus sotto.* Subsequently, in Thuringia, Berliner isolated a gram (+) microorganism in the larvae of the moth *Ephestia kuehniella* and, ignoring nomenclature, Ishiwatari named it *B. thuringiensis* and this name has been preserved to this day (Beegle and Yamamoto, 1992). One of the components of the integrated plant protection system is the microbiological method, which is based on the use of entomopathogenic microorganisms – natural parasites of harmful insects. The biological method has not yet become widespread in our country. However, the global market for protective biological products is represented by 90–95% of spore-crystal complexes of *B. thuringiensis* (Pinos et al., 2021). The most common trade names of these commercial products are: DiPel, Javelin, Thuricide, Worm Attack, Caterpillar Killer and Bactospeine, but many small companies also sell similar products under other trade names (Sanchis and Bourguet, 2008; Moldovan et al., 2023).

The aim of the work was to study the effectiveness of *B. thuringiensis* strains against the main pests in the agrobiocenosis of apple trees, on the yield and quality of fruits.

MATERIALS AND METHODS

The research was carried out during 2015–2023 in the laboratory of microbial ecology at the Institute of Agroecology and Environmental Management of NAAS and in apple orchards of the educational and production department of the Uman National University of Horticulture. The objects of research were apple trees of the Jonagold variety, rootstock M26, planted in 2005 according to the 5 × 2 m scheme.

Trees of the Jonagold table apple variety, bred at the Geneva breeding station (USA) by crossing Jonathan and Golden Delicious varieties, were used in field and laboratory experiments. The variety is late-ripening, listed in the Register of Varieties of Ukraine. Fruits are round, large and weigh up to 220 g. The fruit is simultaneously coloured yellow and green with a light red blush.

The quantitative composition of the main insect pests of the apple tree by species was determined by various methods, depending on the specific characteristics of the phytophage biology.

The material for the research was liquid products containing new strains of entomopathogenic, toxin-forming bacteria *B. thuringiensis* as bioagents: BT-43, BT-18, BT-52, BT-10. Strains of microorganisms were isolated from dead insects of natural populations of leaf-eating phytophagous insects of the *Coleoptera*, *Hemiptera* and *Lepidoptera* orders. The effectiveness of new bioagents was compared with chemical and biological insecticide in the course of the study. The reference exotoxinogenic strain of *B. thuringiensis* var. *thuringiensis* 994 from the collection of beneficial soil microorganisms of the Institute of Agricultural Microbiology and Agro-Industrial Production of the NAAS of Ukraine was used in the study. Natural and insect populations of insects with varying degrees of sensitivity to bacterial contamination, in particular housefly larvae (*Musca domestica* L.) for *B. thuringiensis* strains, were used as model biotests as a specialised test for exotoxinogenicity (determination of thermostable β-exotoxin).

The physiological and biochemical properties of the strains were studied using differential diagnostic Hiss and Omelyansky media, taking into account the indicators of milk peptidation, acetylmethylcarbinol production, lecithinase, catalase, lipase, esculin degradation, starch hydrolysis, sugar fermentation, proteolysis on meat-peptone gelatin, and nitrate reduction to nitrite.

The entomocidal effect of insecticides against apple (*Hyponomeuta malinellus* Zell.), fruit moths (*Hyponomeuta padellus*) and apple leafminer (*Cydia pomonella*) was studied in field research at the educational and production department of the Uman National University of Horticulture. The registered area of the experimental plot was 250 m^2. Replication was carried out in triplicate. The plots were arranged in rows of 10 trees per row.

The Jonagold apple trees were treated in the evening in calm weather with a Gruntek BS-12-3BP sprayer in the most sensitive phase of phytophage development, immediately after flowering, as well as during the emergence of new generations of insects. Liquid preparative forms of the studied strains were used in production environments. The consumption rate of the working fluid in the orchard was 1000 dm^3/ha.

For comparison, the chemical systemic insecticide deltamethrin with contact and intestinal action was used. Trees treated with water served as a control. Field experiments were conducted according to the following scheme: control – without treatment; standard - chemical insecticide deltamethrin (0.3 L/ha); variants of trees treatment with liquid culture of *Bacillus thuringiensis* (BT) strains. The experiments were replicated three times. The number of phytophages was counted on shoots of the middle tier of apple trees from four sides (Trybel, 2001). The fungicidal effect of pathogens was determined by the method of perpendicular lines (Volkohon, 2010). The sugar content of apple fruits was detected according to DSTU 4954:2008 using an areometer. Total acidity was determined by titration.

The data were statistically processed using the methods of dispersion, regression and correlation analyses using Excel-2016 and StatSoft STATISTICA 8 software packages. Results are given as average value ± standard deviation (x ± SD).

RESULTS AND DISCUSSION

It is known that there are about 180 harmful insects and mites in industrial apple plantations in the Forest-Steppe of Ukraine, which cause significant damage [Borzykh 2021]. In the absence or untimely implementation of protective measures against the main pests in the orchard, the yield of standard products is reduced by 15–45%.

In our research, we conducted an initial analysis of the species composition of apple orchard pests (Fig. 1).

The dominant species in the years of research were sucking insects: apple codling moth, apple and fruit moths, other phytophages were not economically important. The most harmful phytophage in apple orchards in 2015–2023 was the apple codling moth, which developed in 2 generations in the conditions of the studied region of Ukraine. The weather conditions of the studied years were favourable for the development of the first and second generation of the pest. The most widespread and numerous in terms of species diversity was the order *Lepidoptera* – 50% of the total composition of insect phytophages, 30% of the species of the harmful entomocomplex of apple cenosis were attributed to the order *Hemiptera* and 20% of the total number to *Coleoptera* (Fig. 2).

Therefore, it is obvious that to increase the resistance of apple plantations to pests and diseases and to obtain a high yield, it is necessary to implement protective measures.The effectiveness of biological and chemical insecticides is part of

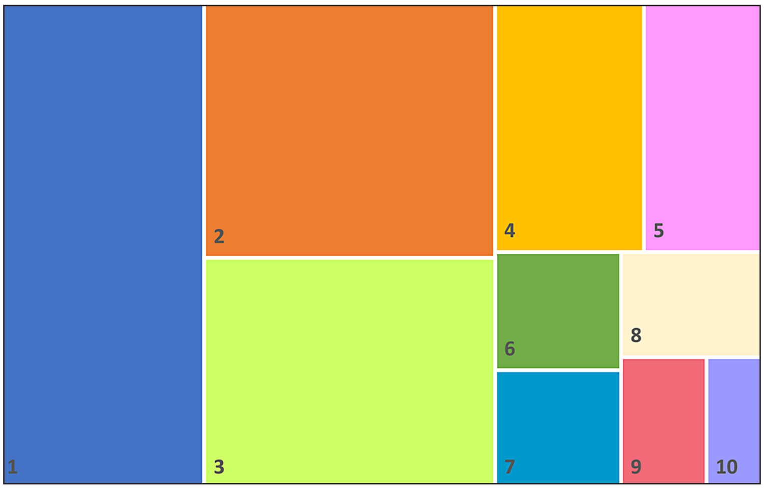

Figure 1. Species composition of phytophages of Jonagold apple orchards: 1 – *Cydia* (*Laspeyresia) pomonella* L.); 2 – *Hyponomeuta malinellus* Zell; 3 – *Hyponomeuta padellus*; 4 – *Aphis pomii* Deg.; 5 – *Dysaphis devecta*; 6 – *Spilonota ocellana* F. ; 7 – *Phyllobius oblongus* L.; 8 – *Anthonomus pomorum* L.; 9 – *Synanthedon myopaeformis* Bork; 10 – *Eriosoma lenigerum* Hausm

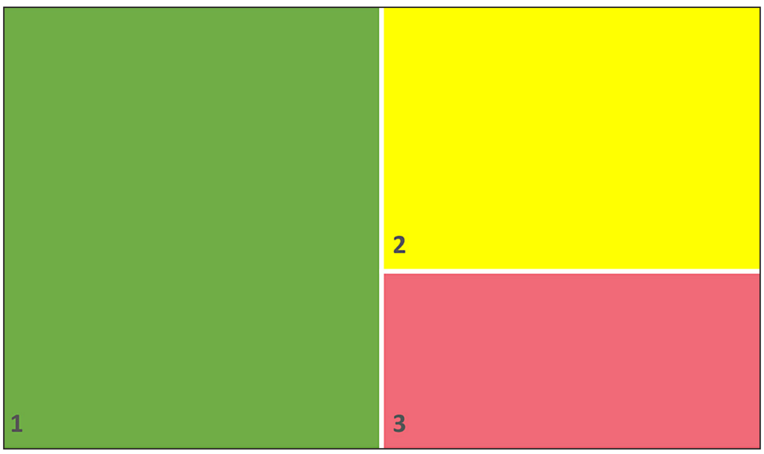

Figure 2. Frequency of phytophage species abundance in Jonagold apple orchards: 1 – *Lepidoptera*; 2 – *Hemiptera*; 3 – *Coleoptera*

an integrated method of protecting apple trees. Biological protection of apple trees helps to reduce the number of harmful insects and does not harm the environment.

An alternative to chemical protection is the use of microbial insecticides, the main advantages of which include selectivity of action, safety for animals, humans, and aquatic organisms, and low development, registration, and production costs. However, the disadvantages of existing microbial products are a narrow spectrum of insecticidal action directed against one and sometimes several groups of pests. This system is environmentally friendly, does not carry a pesticide burden on the orchard ecosystem and the environment, and allows to protect apple trees pests and diseases. Protective measures help to improve both the quantity and quality of the crop. In modern conditions, due to the intensification of horticulture, protection systems involve the maximum use of highly toxic fungicides and insecticides. Therefore, the peculiarity of the protection strategy should be the greening of the fruit crop protection system with the maximum use of biological agents, reduction of chemical treatments, and improvement of the pesticide range. The biological method of plant protection is not an alternative to the chemical method. Its use allows to reduce the number of pests to a level that does not exceed the economic level of harmfulness and partially eliminate the use of chemicals (Cacaj and Hasanaj, 2024).

Bacillus thuringiensis is a member of the *B. cereus* group, which also includes *B. cereus*, *B. anthracis* and *B. mycoides* (Helgason et al., 2000). The feature that distinguishes *B thuringiensis* from other members of the group is its entomopathogenic properties (Kupriyashkina et al., 2024). This species of bacteria produces insecticidal proteins (δ-endotoxins) during the sporulation phase in the form of parasporeal inclusions, which mainly consist of one or more proteins (Cry and Cyt toxins). These protein toxins are highly selective for phytophages, harmless to humans, vertebrates and plants, and completely biodegradable (Bel et al., 2020; Heckel, 2020). The entomopathogenic activity of *B. thuringiensis* is mainly due to Cry toxins with high selectivity for phytophages (Hung et al., 2016, Levishko et al., 2024 b).

Strains of microorganisms (BT-43, BT-18, BT-52, BT-10) were isolated from dead insects of natural populations – leaf-eating phytophagous insects from the *Coleoptera* and *Lepidoptera* orders. In the organism of sick and dead insects of agrobiocenoses, along with bacteria that form endotoxin crystals, spore bacteria were found that did not have crystal inclusions and were basically not pathogenic to phytophages, but were close to crystallogenic bacilli in their biochemical properties. Thus, it was necessary to isolate pure cultures from the same epizootic material in order to select strains with useful phytoprotective properties. Selection based on traits and properties of productivity, quantitative formation of endotoxin crystals made it possible to obtain effective, technological strains. As a result of the research, bacterial cultures with high entomopathogenic activity against *Hyponomeuta malinellus* Zell., *H. Padellus* and *Cydia pomonella* were selected: BT-43, BT-18, BT-52, and BT-10.

The selected strains are gram (+) spore-forming rods with rounded ends, motile. Facultative anaerobes. The minimum growth temperature is +10 °C, the maximum is +40 °C, the optimum temperature is +27–29 °C, and the optimum pH of the medium is 7.0–7.2. The size of the cells of the one-day culture on meat-peptone agar (MPA) was 3.00–4.80 × 1.22–1.45 µm. After 2 days of culture on MPA, BT formed matte, flat, soft creamy fine-grained colonies, d = 2–6 mm. A film was formed on the surface of the meat-peptone broth, the medium column under the film was transparent, and a sediment was present. On day 4, subterminal oval spores 1.38–1.70 × 1.10–1.60 µm in size and parasporeal crystals 0.50–1.40 × 1.00 µm were formed in the cells on MPA.

The processes of spore and toxin formation of selected strains on MPA and Luria Bertrani (LB) nutrient medium were studied in laboratory tests. The obtained data showed that strain BT-10 was the most active producer of entomotoxins. Evaluation of the exotoxinogenicity of new BT strains in the laboratory on the population of housefly (*Musca domestica* L.) larvae showed the predominant death of larvae (89–100%) when infected with BT strains in dilutions of culture fluid 1:4 (25 µl/g feed) and 1:8 – 12.5 µl/g feed (Table 1).

The new strains are not inferior to the reference strain in terms of productivity and level of exotoxinogenicity for housefly larvae. Thus, strains BT-43, BT-18, BT-52, BT-10 are promising bioagents that combine practically valuable properties, namely: high initial manufacturability

Table 1. Assessment of the level of exotoxinogenicity of BT strains on larvae *Musca domestica* L. (x ± SD, n = 5)

Strains	Spore titer 10^9 in 1 mL	% death of larvae during infection in the concentration of exotoxin, µl/g feed				LD_{50}, µl/g feed
		25	12.5	6.25	3.12	
BT-43	2.7±0.1	100±0.0	100±0.0	80.5±1.3	74.1±1.5	3.0
BT-18	2.9±0.3	100±0.0	100±0.0	81.6±1.2	72.3±1.1	3.0
BT-52	2.8±0.2	100±0.0	88.7±1.2	80.3±1.6	71.4±1.7	3.0
BT-10	3.2±0.1	100±0.0	100±0.0	82.0±1.3	77.2±1.1	3.0
B. thuringiensis 994 (reference strain)	3.3±0.1	100±0.0	89.1±1.3	82.2±1.4	78.2±1.6	3.2

– a stable titer of 3 × 10^9 spore/mL of culture fluid; exotoxicogenicity – according to LD_{50} for second instar larvae of *M. domestica* L. up to 3.0 µl/g of feed. At different doses of infection, the LD_{50} by the oral method was in the range of 2.4–2.8 µL/g feed for the studied strains. Using the contact method, the LD_{50} values in the experiment were 26–32% (Patyka et al., 2003). The bioinsecticide is necessary to be applied at very early stages of infection so that it gets on the leaves or fruit and is eaten by the caterpillar. The enzymes in the caterpillar's intestine break down the natural "capsule" surrounding the bacterium, and the toxin is released. The caterpillar stops eating and soon dies. Bt is recommended for the control of scale pests of apples and pears, including *Helicoverpa* (Kadoić et al., 2020; Lone et al., 2017). It is not suitable for use as an emergency treatment and its residual activity is short. However, if applied before the infestation becomes established, it will provide control that will not harm beneficial crops. The importance and safety of this bacterium lies in the fact that the conversion of the prototoxin to the active toxin occurs only under the action of specific proteinases. Infection of a healthy insect occurs through plant food contaminated with *Bacillus* spores. Once in the intestine, the spores germinate into vegetative cells, where they produce spores and crystalline endotoxin. The formation of a thermostable exotoxin increases the insecticidal effect of the product, as it has an ovicidal and teratogenic effect on insects.

Also, in the course of studies of the selected strains, they were found to be able to inhibit the growth of some phytopathogenic fungi, so we studied the selected promising BT strains against the following pathogens: *Botrytis cinerea* (grey rot of apple trees) and *Alternaria mali* Roberts (apple tree alternaria).

Studies of the antagonistic effect of highly active entomopathogenic BT strains against phytopathogenic microsporidia showed that the studied strains BT-43, BT-18 and BT-52 have a fungistatic effect, and strain BT-10 has a fungicidal effect against selected phytopathogenic micromycetes (Table 2).

The effect of the studied strains on apple pathogens is demonstrated in different ways (fungicidal, fungistatic, neutral) and depends on the strains and toxins they produce. In studies of biological control agents for pests based on *B. thuringiensis* in an apple orchard, a decrease in plant disease severity was visually observed, which prompted a laboratory study of the effect of entomopathogenic strains on fungal pathogens of apple trees. It was found that all strains that synthesise exotoxin have a fungistatic effect on the studied fungi, especially strain BT-43. It is known that fungal diseases pose a particular threat to apples during their cultivation, as well as after

Table 2. Antifungal effect of *Bacillus thuringiensis* strains (x ± SD, n =5)

Strains	Spore-crystal complex titer, 10^9/mL culture fluid	Exotoxin content, mg/mL	Inhibition of culture growth, %	
			Botrytis cinerea	*Alternaria mali* Roberts
BT-43	2.7±0.1	8.9±0.1	20.3±1.5	17.9±1.4
BT-18	2.9±0.3	4.6±0.4	19.6±2.7	12.8±0.6
BT-52	2.8±0.2	6.5±0.7	12.1±0.8	10.6±1.1
BT-10	3.2±0.1	10.2±0.2	37.2±4.2	27.3±2.2
B. thuringiensis 994 (reference strain)	3.3±0.1	11.4±0.6	37.4±0.6	22.7±1.4

harvesting during storage. The difference in the antagonistic effect of the strains can be explained by the fact that *B. thuringiensis* strains with fungistatic or fungicidal properties, in addition to the specific entomocidal δ-endotoxin, synthesise a water-soluble exotoxin with a broad spectrum of action on biological objects (Sabra and Youssef, 2022). Treatment of apple trees with a culture of the entomopathogenic strain *B. thuringiensis* BT-10, an active antagonist of the studied phytopathogenic microbes, allowed to reduce the damage to trees by phytopathogens by 7.4%.

Also, the fungistatic effect can be explained by the influence of δ-endotoxin, the mechanism of action of which is to inhibit phosphorylation and respiration, on microorganisms from various taxonomic groups, including bacteria and micromycetes (Djenane et al., 2017; Sklyar et al., 2020). The antagonistic effect can also manifest itself not only antibiotically, but also as parasitism, using the fungal mycelium as a substrate for nutrition (He et al., 2020). The isolated strain BT-10, along with its insecticidal activity, had a significant antifungal effect. According to the researchers, the mechanism of Bt's antifungal effect is related to several factors. Bacteria produce and release hydrolytic enzymes such as protease and chitinase into the environment, so it can be assumed that they destroy the cell walls of phytopathogenic microbes. Lysis turns the contents of fungal hyphae into a source of food and energy for bacteria (Martínez-Zavala et al., 2020; Ajuna et al., 2023).

These strains can be effectively used as a basis for biological products with a complex entomofungicidal effect to control the number of harmful insects and fungi phytopathogens. Microbial products with multifunctional action can compete with chemical pesticides not only in terms of environmental safety but also in terms of economic performance, given the protective effect against phytophages (Gomis-Cebolla and Berry, 2023).

At the next stage of our work, we tested the effectiveness of treatment of the apple orchard affected by phytophages with bioinsecticides based on the selected promising strains of *B. thuringiensis*. The most common and harmful phytophages of apple trees are apple moth (*Hyponomeuta malinellus* Zell.), fruit moth (*H. padellus*) and codling moth (*Cydia pomonella*), so the effect on these phytophages was determined in this experiment. The biological treatment was carried out 2 times against each generation of pests with an interval of 5–10 days. Total number of treatments during the growing season: 2–4 times (Table 3).

The results obtained indicate that the selected bacterial strains are capable of causing the death of caterpillars of the studied pests (72.8–100% of insects died on the 10th day of the experiment). The *B. thuringiensis* BT-10 strain was particularly effective against pests, with an effect almost equal to or superior to that of deltamethrin. The BT-18 strain is also effective against apple caterpillars (92.3 to 99.6% of insects die), and the BT-52 strain (91.1% of insects die) and BT-10 strain (94.2% of insects die) against fruit moths. The selected strains of bacteria are particularly active against caterpillars of the apple leaf miner (codling moth) the insect mortality was 93.8–100%. Thus, the study of the obtained BT strains indicates high efficiency and entomocidality against insects of *Lepidoptera*, which shows that this biological product can be used in the future in the control system of leaf-eating phytophages.

To create sustainable yields with high quality indicators, it is necessary to create a favourable nutritional regime for plants. Yield is an integral indicator of the economic importance of individual agronomic components and the level of excellence

Table 3. Entomocidal activity of new strains of *B. thuringiensis* against common orchard pests (x ± SD, n =5)

Treatment	Mortality within 10 days after treatment, %		
	Hyponomeuta malinellus Zell.	*Hyponomeuta padellus*	*Cydia pomonella*
Control	5.3±0.4	4.5±0.1	2.4±0.2
BT-43	94.5±0.2	72.8±0.5	93.8±0.4
BT-18	92.3±0.3	90.4±1.1	100
BT-52	97.6±0.4	91.1±0.2	100
BT-10	99.6±0.2	94.2±1.3	100
B. thuringiensis 994 (reference strain)	99.2±0.4	91.2±0.7	100
Deltamethrin (0.3 L/ha)	99.7±0.3	90.4±1.2	100

of the cultivation technology in general. Research results show that foliar treatment with complex-acting products helps to increase the productivity of apple trees (Table 4).

Accordingly, the products more actively stimulate the processes of chlorophyll synthesis in apple leaves under conditions of increased water supply, which contributes to an increase in yield. When treating trees with a liquid culture of *B. thuringiensis*, spores and endotoxin crystals affect the physiological processes that occur in the cells of the leaves, which can be manifested in changes in the quality of apple products and yield. The treatment of apple trees with biological products contributed to a significantly higher yield compared to the control, and the highest yield was obtained with the BT-10 strain. The BT-43 strain, which had the lowest effectiveness against all phytophages studied, contributed to the lowest yield saving. The high technical efficiency of deltamethrin is manifested by the high yield of apple trees, which on average over the years of research is inferior only to the indicators obtained with the use of a biological product based on the BT-10 strain and the reference strain.

Organic acids are an important factor in the balanced taste of apples. A lot of them disrupts the sugar-acid balance. They are also one of the most important substances in the chemical composition of apples. High acidity hinders the use of fresh fruit and limits its suitability for processing. Organic acids, just like sugars, determine the taste and technological quality of fruits and are involved in plant physiological processes (Yang et al., 2021). The sugars found in fruits are easily absorbed by the human body, which determines their value. The amount of sugars has a significant impact on the quality of the product. Their accumulation in fruits depends on the growing conditions, the growing season, and the impact of foliar treatment with biological products. The effect of *B. thuringiensis* strains and chemical insecticide on the physiological characteristics of apple trees caused changes in yield and product quality: the content of sugars, organic acids and ascorbic acid, in particular. The treatment of apple trees with BT-18 strain resulted in a slight decrease in sugar content compared to the control. The concentrations of sugars in the fruits of trees treated with BT-43, BT-52, BT-10 were 1.02–1.06 times higher than in the control. The sugar content in fruits treated with the chemical insecticide deltamethrin was significantly lower than in the control – by 1.58% (Table 5).

Table 4. Yield of the Jonagold variety (average for the years 2015–2023)

Treatment	Specific surface density, g/dm^2	Fruit yield per tree, kg	Yield, t/ha
Control (water)	2.1	11.4	13.4
BT-43	2.3	12.3	15.7
BT-18	2.4	16.4	19.1
BT-52	2.5	16.9	19.5
BT-10	2.2	15.8	20.6
B. thuringiensis 994 (reference strain)	2.3	16.1	20.3
Deltamethrin (0.3 L/ha)	2.4	15.6	20.2
SSD_{05}	0.14	2.25	2.8

Table 5. Effect of insecticides on apple fruit quality indicators (average value) (x ± SD, n =5)

Treatment	Sugar content, %	Acidity, %	Sugar-acid index	Shelf life of fruits (days)
Control (water)	13.4±0.2	0.51±0.03	26.3	152±2
BT-43	13.6±0.1	0.45±0.02	30.2	196±3
BT-18	13.2±0.2	0.42±0.01	31.4	181±3
BT-52	13.7±0.2	0.55±0.03	24.9	178±4
BT-10	14.2±0.2	0.51±0.02	27.8	202±2
B. thuringiensis 994 (reference strain)	13.4±0.2	0.44±0.02	30.5	200±2
Deltamethrin (0.3 L/ha)	12.2±0.2	0.36±0.01	33.8	195±1

According to the results of the study, it was found that the content of organic acids in the fruits of the treated trees was in the range of 0.36–0.55%. Treatment of apple trees with BT-18 and BT-43 strains reduced acidity by 0.06 and 0.09%, respectively. The effect of strain BT-52 caused an increase in the acidity of apple fruit by 0.04%. This effect should be evaluated positively, because organic acids, in particular malic acid, have preservative properties, which helps to increase the shelf life of fruits.

The harmony of taste is determined not by the absolute content of sugars or acids, but by their ratio – the sugar-acid index (SAI). In fruits with high taste qualities and an optimally balanced content of sugars and acids, the SAI is 16–30 (Kondratenko, 2001). With the use of apple tree protection products in the studied variants, the SAI in fruits was in the range of 24.9–33.8. It was found that under the treatment of apple trees with deltamethrin, the concentration of ascorbic acid in fruits decreased by 1.4 times compared to the control.

Pectin substances are a valuable component of the chemical composition that plays an important role in the post-harvest period and during storage. Fruit shelf life significantly depends on the rate of conversion of protopectin to soluble pectin. In the fruits of winter apple varieties, the process of conversion of protopectin into soluble pectin is much slower than in summer and autumn varieties, which determines the ability of winter varieties to be stored longer (Zolotukhina, 2008). Harvested pome fruits are immediately cooled to a storage temperature of 0–4 °C. The optimum storage humidity for apples is 90%. In unripe fruit, pectin substances are mainly in the form of protopectin, which gives the fruit its firmness. The average soluble pectin content over the years of research ranged from 0.12 to 0.61%. The shelf life of fruits within the studied variants was within 152–202 days.

The shelf life of fruits is determined by the storage period during which they retain their good marketability, high quality taste and little weight loss. Fruit keeping quality depends on the variety, ripening speed and growing conditions (Lepaja et al., 2024). Fruit keeping quality is largely influenced by temperature and humidity during the growing season. Uniformly warm weather prolongs the onset of ripening and increases the shelf life of fruits, while very high temperatures, on the contrary, accelerate ripening and reduce the shelf life of fruits (Grabowski, 2021). The most favourable conditions for the formation of keeping quality fruit are uniform temperature and humidity (Dobrzański and Rybczyński 2002; Gidado et al., 2024). Long-term keeping quality of apples requires temperature stability. Fruit ripening takes place during storage. In this process, the flesh of apples becomes looser, and their taste, aroma, and colour improve. Due to the conversion of starch to sugar and the reduction of organic acids, the fruit becomes more sweet.

The treatment of apple plantations with pest control agents is an integral element of the technology of growing and storing fruit crops (Leng et al., 2022). However, during spraying, foreign biological and chemical agents are introduced into the biocenosis, which affect the natural background and can cause changes in the physiological processes of the plant.

It was also important to investigate the economic efficiency of the apple tree protection products based on BT strains. One of the most important indicators in determining production efficiency is the cost of production. This indicator makes it possible to establish the level of cost-effectiveness, which is the main characteristic of the efficiency of the technology used. For example, the highest cost per tonne of apples was in the control variant (water treatment) and when applying the BT-52 strain. Treatment with a chemical insecticide reduced the cost by 18% compared to the control. When treating apple trees with *B. thuringiensis* products, the cost of production was most reduced in the treatment with strain BT-18 – by 32% compared to the control, mainly due to an increase in yield. The level of profitability characterises the return on investment and is therefore the main indicator of the economic efficiency of production. We have determined that the highest level of profitability (339.7%) was obtained when treating the apple orchard with biological products based on BT-10 and BT-43 strains, which is 158 and 144% more than control. The use of a chemical insecticide showed a lower level of cost-effectiveness than the use of biological products. In general, the use of bioinsecticides based on BT strains showed the possibility of increasing the profitability of apple production by 47–120%. Thus, it was found that among the studied biological products based on new strains of entomopathogenic bacilli, it is economically most profitable to use the BT-10 strain, which has the lowest production cost, increases profits by almost two times, and the level of cost-effectiveness increases by 120–144% compared to the control.

CONCLUSIONS

The study conclude that the usage of biological products based on new local strains of entomopathogenic bacteria has shown changes in yields and product quality: the content of sugars, organic acids and ascorbic acid, in particular. This effect should be viewed positively, as organic acids, in particular malic acid, have preservative properties, which helps to increase the shelf life of products. The results of the research show that foliar treatment with complex-action products helps to increase the productivity of apple trees. The treatment of apple orchards with biological products contributed to a significantly higher yield compared to the control, and it was the highest in the treatment with the BT-10 strain. The work demonstrates that treatment with products based on strains of entomopathogenic bacteria *B. thuringiensis* is an effective, environmentally and economically feasible agricultural measure to protect apple orchards from insect phytophages (such as *Hyponomeuta malinellus* Zell., *H. padellus* and *Cydia pomonella*), which resulted in the high yields and product quality. The study of the received BT strains indicates high efficiency and entomocidality against insects of *Lepidoptera*, indicating that this biological product can be used in the future in the complex of control of leaf-eating phytophages.

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