

USAGE OF MICROBIAL FUNGICIDAL PREPARATIONS IN APPLE ORCHARDS AND THEIR EFFECT ON FRUIT STORAGE UNDER REFRIGERATION CONDITIONS

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Abstract. The article presents the results of using microbial fungicidal preparations developed by the Engineering and Technological Institute “Biotekhnika” the National Academy of Agrarian Sciences of Ukraine in apple orchards and their effect on fruit storage under refrigeration conditions. The study employed microbial pesticides, including *Ampelomitsyn* BT (producer *Ampelomyces Ces ex Shlecht*), *Hliokladyn* BT (producer *Gliocladium rozeum*), *Fluorestsyn* BT (producer *Pseudomonas fluorescens st. 2*). The application of microbial preparations in apple orchards had a positive effect on the suppression of microorganisms causing the main apple diseases, namely powdery mildews and apple scab. The economic efficiency of this agricultural practice ranged from 53.0% to 86.0%. The study of the effect of treating apple trees with microbial fungicides on fruit infection during storage by fungal diseases, such as storage scab (*Venturia inaequalis*), bitter rot (*Gloeosporium fructigenum* Berk.), blue mold (*Penicillium expansum* Thom), and fruit rot (*Monilia fructigena* Pers. et Fr.), showed that the incidence of fruit infection by pathogenic microorganisms was 2.1% when *Fluorestsyn* BT and *Hliokladyn* BT were applied, and 4.2% when *Ampelomitsyn* BT was used, compared to 10.4% in the control (untreated) variant. Thus, the use of microbial fungicides for the protection of apple orchards against major diseases positively affects fruit storage under refrigeration conditions, reducing losses from microbiological spoilage by up to 40%. This ensures the preservation of fruit quality and provides an opportunity to increase profits from sales.

Keywords: apple fruits, microbiological spoilage, microbial preparations, storage, pathogenic microorganisms.

Introduction

From 2010 to 2020, global apple production increased by more than 20%, and the apple market is expected to grow annually by 4% between 2019 and 2027 [1]. Currently, fruit growers employ modern cultivation technologies, the strict adherence to which allows for harvesting crops with a share of high-quality fruits reaching 95-98%, meeting the requirements of European regulatory standards [2].

In EU countries, apples of various cultivars are grown, which complicates the development of a unified system for harvesting, storage technologies, and the rational use of transport for storage and distribution. Each apple cultivar requires development of a detailed storage technology that takes into account its specific characteristics and responses to particular storage conditions [3-5].

Fruits have the ability to ripen after being detached from the parent plant; therefore, winter cultivars are selected for long-term storage, as they acquire desirable taste, specific aroma, and appropriate texture during storage [6; 7].

The economic losses caused by food spoilage are extremely high. According to various estimates, up to one quarter of global food production is lost due to microbial activity. Losses of fruit and vegetable products are particularly significant, ranging from 15% to 70% [8; 9]. These losses begin at the agricultural enterprise and continue throughout the entire technological chain – from harvesting and storage to raw material supply, wholesale and retail trade, and final consumption.

One of the main causes of losses during the storage of fruits and vegetables is diseases that manifest in the postharvest period [8]. Losses caused by harmful agents vary from year to year and average between 6.2% and 23.2% of total plant production.

A large number of microorganisms are present on the surface of fruits and vegetables, colonizing plant materials during cultivation, harvesting, transportation, and storage [10]. The microbial communities on fresh apples are closely associated with fruit diseases, storage processes, and quality control. The study of microbial communities on apples from different production regions may reveal microbial specificity and contribute to disease prevention and quality management.

Preharvest and postharvest diseases, primarily caused by micromycetes, can lead to significant losses in both yield and quality [11; 12]. Therefore, a promising area of research is to determine the impact of microbial fungicides on the long-term storage of apples.

Materials and methods

The study was conducted during 2021–2023 in apple orchards of the *Renet Simirenko* cultivar. The experimental plots measured 15 m², with a randomised layout and three replicates. The tree spacing was 5.0 × 1.0 m, with 2,000 plants per hectare.

Spraying of apple trees with biopreparations against diseases was carried out using a Stihl motorized sprayer at the following growth stages according to the BBCH scale: the first treatment – 67, the second – 72, the third – 74, the fourth – 75, and the fifth – 85. The assessment of disease incidence and severity, as well as the determination of the technical efficiency of the biopreparations, were performed according to the standard methodology of Trybel [13].

The effect of treating apple trees of the *Renet Simirenko* cultivar with microbial fungicidal preparations on fruit storability was investigated. For this purpose, tree treatments were carried out using microbial fungicides developed on the basis of microbial cultures from the Collection of Industrially Valuable Microorganisms of the Engineering and Technological Institute “Biotekhnika” of the National Academy of Agrarian Sciences of Ukraine, which includes 134 strains [14]. The following preparations were used in the study: Fluorestsyn BT (producer *Pseudomonas fluorescens*), Ampelomitsyn BT (producer *Ampelomyces artemisia*), and Hliokladyn BT (producer *Gliocladium roseum*).

Fruits of the *Renet Simirenko* cultivar harvested at the stage of removable maturity were placed in cold storage (storage temperature 0-2 °C, relative air humidity 87-92%).

The effect of treating apple trees of the *Renet Simirenko* cultivar with microbial fungicides on fruit storability was evaluated according to the following scheme:

1. control – untreated;
2. *Fluorestsyn* BT – application rate 8 dm³·ha⁻¹; number of treatments – 4;
3. *Ampelomitsyn* BT – application rate 6 dm³·ha⁻¹; number of treatments – 4;
4. *Hliokladyn* BT – application rate 10 dm³·ha⁻¹; number of treatments – 4.

The commercial quality assessment of fruits was carried out by classifying fruits affected by fruit rot up to half of the fruit as technically defective, and fruits with more than half of the fruit affected by browning and decay as total loss.

Harmful agents were isolated from spoiled apple fruits using the method of inoculation onto selective nutrient media (meat-peptone agar (MPA), *Sabouraud* medium).

Results and discussion

The storage of plant products occurs under conditions of wide accessibility to microorganisms. All plants possess their inherent epiphytic microflora during their lifetime, while diseased plants also harbour specific pathogens. During harvesting, this microflora is supplemented by microorganisms from the environment, primarily from the soil. Therefore, each storage object contains a significant number of microorganisms capable, under certain conditions, of actively proliferating and affecting the quality of stored products.

In this regard, a series of studies was conducted to isolate harmful agents affecting apple fruits during storage under refrigerated conditions. Pathogenic microorganisms belonging to the following genera were identified: *Botrytis* (gray fruit rot), *Rhizopus* (soft rot), *Aspergillus* (black mold), and *Penicillium* (green mold).

Diseases manifested during storage are generally associated with the development of specific groups of microorganisms. Resistance to phytopathogenic microorganisms changes during storage. As the products ripen and age, their resistance to infection decreases.

Therefore, in order to ensure that fruits maintain resistance to phytopathogenic microorganisms over an extended period, even under elevated storage temperatures, and retain their ability to resist pathogen development, a preharvest treatment technology for apple fruits was developed.

This technology involves the application of working solutions of microbial fungicides *Fluorestsyn* BT, *Hliokladyn* BT, and *Ampelomitsyn* BT using fine-dispersed sprayers directly onto apple fruits on trees to suppress the development of major apple diseases (Tables 1, 2).

The data presented in the tables demonstrate that the preparation *Ampelomitsyn* BT exhibited high antagonistic activity against the main apple diseases. Its technical efficiency, averaged over the years of research, amounted to 75.0% for powdery mildew and 80.3% for apple scab. The preparation *Hliokladyn* BT showed slightly lower technical efficiency, amounting to 75.0% for powdery mildew and 67.6% for apple scab.

Table 1

**Effectiveness of microbial fungicides in suppressing
the development of apple scab**

Variant	2021			2022			2023		
	I	DS	TE	I	DS	TE	I	DS	TE
Control	37.7	13.5	–	37.7	14.5	–	24.7	6.1	–
<i>Ampelomitsyn</i> BT, 6 dm ³ ·ha ⁻¹	11.9	2.1	84	19.7	3.7	75	6	1.11	82
<i>Hliokladyn</i> BT, 10 dm ³ ·ha ⁻¹	17.3	3.4	75	27.3	5.6	61	6.67	2.03	67
<i>Fluorestsyn</i> BT, 8 dm ³ ·ha ⁻¹	16.9	3.4	75	23.3	4.4	70	10	2.8	54
LSD ₀₅		1.3			0.9			0.6	

Note. I – incidence (%); DS – disease severity (%); TE – technical efficiency (%); LSD₀₅ – least significant difference of tests at 5%

Table 2

**Effectiveness of microbial fungicides in suppressing
the development of apple powdery mildew**

Variant	2021			2022			2023		
	I	DS	TE	I	DS	TE	I	DS	TE
Control	13.3	2.3	–	18.3	5.5	–	15.3	7.1	–
<i>Ampelomitsyn</i> BT, 6 dm ³ ·ha ⁻¹	6.0	0.6	73	8.0	1.3	77	8.0	1.8	75
<i>Hliokladyn</i> BT, 10 dm ³ ·ha ⁻¹	10.0	0.3	86	8.3	2.4	57	4.0	1.4	82
<i>Fluorestsyn</i> BT, 8 dm ³ ·ha ⁻¹	8.7	0.4	82	7.0	1.6	71	6.7	3.3	53
LSD ₀₅		0.5			0.2			0.6	

Note. I – incidence (%); DS – disease severity (%); TE – technical efficiency (%); LSD₀₅ – least significant difference of tests at 5%

The lowest efficiency was observed with the use of *Fluorestsyn* BT (8.0 Dm³·ha⁻¹), which was at the level of 68.6% for powdery mildew and 66.3% for apple scab.

Subsequently, the effect of applying microbial fungicidal preparations in apple orchards on fruit storage under refrigeration conditions was investigated, particularly the incidence of fungal diseases affecting apple fruits.

Monthly inspections of apple fruits were conducted in October, November, and December 2022. During these inspections, commercial quality indicators were assessed, including infection by fungal diseases such as storage scab (*Venturia inaequalis*), bitter rot (*Gloeosporium fructigenum* Berk.), blue mold (*Penicillium expansum* Thom), and fruit rot (*Monilia fructigena* Pers. et Fr.).

According to the results of commodity analysis conducted after three inspections following 85 days of storage, it was found that the incidence of fungal diseases in apple fruits was 2.5-5 times lower in the variants treated with microbial fungicides compared to the control (Table 3).

Table 3

**Effect of treatments of apple trees of the *Renet Simirenko* cultivar with microbial fungicidal
preparations on the incidence of fungal diseases in fruits during storage, %**

Control	Variants		
	<i>Fluorestsyn</i> BT	<i>Ampelomitsyn</i> BT	<i>Hliokladyn</i> BT
10.4	2.1	4.2	2.1

Significant losses of plant products during long-term storage may occur due to dehydration and microbiological spoilage.

The results obtained from the study of the effect of treating apple trees of the *Renet Simirenko* cultivar with microbial fungicidal preparations on fruit storability demonstrated the prospects of using microbial fungicides based on living microorganisms and their metabolites. These preparations proved effective both in suppressing the development of major apple diseases and in reducing the incidence of fungal diseases in fruits during refrigerated storage.

A comparison of the results with those reported by other authors indicates that pathogenic microbiota significantly affects the duration of fruit storage. Study [15] notes that the development of microbiological diseases, primarily fungal rots, is promoted by high relative air humidity in storage facilities, which must be maintained to preserve fruit weight. In addition, as a result of hydrolytic processes occurring in fruits under the action of enzymes during storage, a favourable environment for fungal growth and development is formed within apple tissues. The effectiveness of microbial fungicides in suppressing the main apple diseases was demonstrated to range from 53.0% to 86.0% for powdery mildew and from 54.0% to 84.0% for apple scab.

The authors of [16] also report that fivefold application of the biological preparations *Ampelomitsyn*, *Hliokladyn*, and *Fluorestsyn* within the apple disease protection scheme reduced the incidence and development of apple scab by 1.6-2.9 and 3.4-6.5 times, respectively, and powdery mildew by 2-2.7 and 2.6-4.4 times, compared to the control.

Conclusions

1. The effectiveness of microbial fungicides in suppressing the main apple diseases was as follows: *Ampelomitsyn* BT at an application rate of $6.0 \text{ dm}^3 \cdot \text{ha}^{-1}$ – 75.0% for powdery mildew and 80.3% for apple scab; *Hliokladyn* BT at $10.0 \text{ dm}^3 \cdot \text{ha}^{-1}$ – 75.0% for powdery mildew and 67.6% for apple scab; *Fluorestsyn* BT at $8.0 \text{ dm}^3 \cdot \text{ha}^{-1}$ – 68.6% for powdery mildew and 66.3% for apple scab.
2. The study of the effect of treating apple trees of the *Renet Simirenko* cultivar with microbial fungicidal preparations on the incidence of fungal diseases in fruits during storage showed that the level of infection was 2.5-5 times lower in the variants treated with microbial preparations compared to the control.

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Author contributions

Conceptualization, N.P., A.Z. and A.B.; methodology, N.P., A.B., A.R. and O.B.; validation, A.B., A.A., A.R. and O.B.; formal analysis, O.B.; investigation, N.P., O.N. and A.B.; data curation, O.B.; writing–original draft preparation, N.P., O.N., O.B.; writing–review and editing, A.B., A.Z. and O.B.; project administration, O.B.; funding acquisition. All authors have read and agreed to the published version of the manuscript.

References

- [1] Shahbandeh M. Apple production worldwide 2010-2020. Hamburg: Statista, 2022.
- [2] Luzhansky V.V. The world market of apples and their processing products. *Economy and society*, 2024, 59. DOI: 10.32782/2524-0072/2024-59-145
- [3] Au W. et al. The Monash Apple Retrieving System: A review on system intelligence and apple harvesting performance. *Computers and Electronics in Agriculture*, vol. 213, 2023. 108164. DOI: 10.1016/j.compag.2023.108164
- [4] Zhang K. et al. An automated apple harvesting robot – from system design to field evaluation. *Journal of Field Robotics*, vol. 41, 2024, pp. 2384-2400. DOI: 10.1002/rob.22268
- [5] Kistechok A., Wrona D., Krupa T. Effect of storage conditions on the storability and nutritional value of new polish apples grown in Central Poland. *Agriculture*, vol. 14(1), 2024. DOI: 10.3390/agriculture14010059

- [6] Kassebi S., Korzenszky P. The effect of post-harvest storage on the weight of Golden Delicious apples. *Science, Technology and Innovation*, vol. 12(1), 2021, pp. 7-11. DOI: 10.5604/01.3001.0015.5265
- [7] Merganov A., Abdullaev Z., Halimboev A. (2024) The storage technology of local winter apples in controlled atmosphere storage. *European Journal of Technical and Natural Sciences*, No. 3-4, 2024, pp. 3-6. DOI: 10.29013/EJTNS-24-3.4-3-6
- [8] Argenta L.C. et al. Characterization and quantification of postharvest losses of apple fruit stored under commercial conditions. *HortScience*, vol. 56(5), 2021, pp. 608-616. DOI: 10.21273/HORTSCI15771-21
- [9] Etera O.F., Forsido S.F., Kebede M.T. Postharvest loss, causes and handling practices of fruits and vegetables in Ethiopia: scoping review. *Journal of Horticultural Research*, vol. 30(1), 2022, pp. 1-10. DOI: 10.2478/johr-2022-0002
- [10] Schweitze M. et al. Urban air quality affects the apple microbiome assembly. *Environmental Research*, vol. 262(1), 2024. 119858 DOI: 10.1016/j.envres.2024.119858
- [11] Li Z. et al. Characterization of fungal species isolated from cankered apple barks demonstrates the *Alternaria alternata* causing apple canker disease. *Journal of Fungi*, vol. 10(8), 2024, 536. DOI: 10.3390/jof10080536
- [12] Mishra R. et al. Effective microbial control, enhancing antioxidant activity and pesticide removal in fresh cut apples with plasma activated water. *Postharvest Biology and Technology*, vol. 228, 2025, 113660. DOI: 10.1016/j.postharvbio.2025.113660
- [13] Trybel S.O., Sigareva D.D., Sekun M.P., Ivashchenko O.O. et al. Methods of testing and applying pesticides. Kyiv: Svit, 2001. DOI: 10.36495/metodiki-Trybel.2001
- [14] Loban L., Pilyak N., Yaroshevsky V. Gene resource of industrially important microbial culture collection for agriculture biologization. Proceedings of International conference “Plant Protection – Achievements and Perspectives”, October 2-3, 2023, Chisinau, Moldova. *Information Bulletin EPRS/IOBC* vol. 58, 2023, pp. 168-173. DOI: 10.53040/ppap2023.26
- [15] Kondratenko P.V., Shevchuk L.M., Levchuk L.M. Methodology for assessing the quality of fruit and berry products. Kyiv: SPD “Zhytelev S.I.”, 2008.
- [16] Barabash L.O., Gradchenko S.I., Pikovsky M.Y. Economic efficiency of using biological means of protecting apple trees from diseases. *Bulletin of Agricultural Science*, 102(6), 2024, pp. 39-45. DOI: 10.31073/agrovisnyk202406-05