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Efficiency of biological preparations against scab and powdery mildew of apple trees

Abstract. Scab (caused by *Venturia inaequalis* Cooke (Wint.)) and powdery mildew (*Podosphaera leucotricha* (Ellis & Everh. Salmon)) are harmful diseases of the apple tree, the protection against which is based on repeated spraying with chemical fungicides. To reduce the pesticide burden on agrocenoses, the use of biological protection products is relevant. The purpose of this study was to investigate the effectiveness of biological preparations based on fungi and bacteria (mycoparasites and antagonists) against scab and powdery mildew of apple trees. For this, a set of methods was used, including phytopathological diagnostics and disease monitoring, phenological observations, yield recording, and statistical analysis. The biological preparations Ampelomycin BT (6 l/ha) based on the fungus of the genus *Ampelomyces* Ces ex Shlecht., Gliocladin BT (10 l/ha), which contains mycelium and spores of the fungus of the genus *Gliocladium*, and Fluorescin BT (8 l/ha), which is based on bacteria of the genus *Pseudomonas*, were investigated. Their use led to a decrease in the spread and development of apple scab, and the technical efficiency of the preparations ranged from 68% to 79%. The biological preparations also reduced the damage to plants by the powdery mildew pathogen with a technical efficiency of 59-76%. Treatment of apple plants with Gliocladin BT, Ampelomycin BT, and Fluorescin BT reduced the number of scab-affected fruits by 22.7-35.6%. The technical effectiveness of the preparations was 62-78%. The use of the biological preparations under study ensured the preservation of the yield within 3.7-4.4 t/ha. The results obtained indicate the prospects of using biological preparations against scab and powdery mildew of apple trees for the ecologisation of plant protection

Keywords: apple tree diseases; fungi; microorganisms-antagonists; technical efficiency; yield

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INTRODUCTION

The apple tree (*Malus domestica* Borkh) is the most common fruit crop in temperate regions. Its fruits are used for fresh consumption and in the food industry, as well as to produce medicines. At the same time, one of the obstacles to increasing the yields of various crops is biotic stress, specifically, plant pathogens. The apple tree is particularly susceptible to diseases such as scab and powdery mildew. These diseases are among the most harmful to apple trees and can cause considerable yield losses, which makes it important to investigate the ability of biological preparations to limit plant damage.

According to FAOSTAT (n.d.), apple fruit production ranks second in the world with a share of 9.6%. According to The State of Food Security and Nutrition in the World (FAO *et al.*, 2020), the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) recommend a minimum consumption of 400 g of fruit and vegetables per day. In Ukraine, only half of the population's demand for apples is met.

One of the obstacles to increasing crop yields is plant damage caused by pathogens. Modern research shows that plant diseases cause major losses to the global economy. According to FAO estimates (Climate change..., 2023), they amount to more than \$220 billion annually. Many scientists have devoted their research to the study of apple diseases. M.S. Mullett *et al.* (2023) point out that the fungus *Phytophthora × cambivora*, which is an invasive pathogen in Europe and North America, but also poses a serious threat to agricultural fruit tree ecosystems. Research by M. Papp-Rupar *et al.* (2023) indicates that European canker caused by *Neonectria ditissima* (Tul. and C. Tul.) is widespread on apple trees worldwide. However, the apple tree is particularly susceptible to diseases such as scab and powdery mildew. According to M. Chatzidimopoulos *et al.* (2022), scab (caused by the fungus *Venturia inaequalis* Cooke (Wint.)) and powdery mildew (caused by the fungus *Podosphaera leucotricha* (Ellis & Everh. Salmon)) are among the most harmful diseases of apple trees and can cause yield losses of more than 70%.

According to H. George (2023), the powdery mildew pathogen, infecting young shoots and leaves, inhibits plant growth, which ultimately

leads to weakening of trees and reduced productivity and fruit quality. D.A. Strickland *et al.* (2022) point out that fungicides have to be applied multiple times during the growing season to control scab and powdery mildew. Studies conducted in the Precarpathian province of the Carpathian mountainous zone of Ukraine have established that in conditions of significant pest infestation of apple stands, it is advisable to use a chemical protection system that includes 12 treatments (Hunchak *et al.*, 2022). At the same time, various alternative methods of controlling apple diseases are being developed to reduce the pesticide burden on agrocenoses. L.A. Shuttleworth (2021) emphasises that in this aspect, it is important to use biological agents that should be cost-effective, ensure environmental sustainability and food safety.

The rhizobacterium *Bacillus subtilis* is being investigated and used as a potential biocontrol agent (Leconte *et al.*, 2022). Some strains of *B. subtilis* are registered for commercial use against plant pathogens. H. Desmyttere *et al.* (2019) indicate that natural substances (fungicin, surfactin, mycosubtilin) produced by *B. subtilis* showed *in vitro* antifungal effects against strains of the apple tree scab pathogen *V. inaequalis*. In the scientific literature, there are also reports of the use of fungi *Gliocladium spp.* and *Ampelomyces spp.* and bacteria of the genus *Pseudomonas* against plant diseases (Rahman *et al.*, 2021). In Ukraine, studies have been conducted on the use of certain biofungicides on apple trees (Hradchenko, 2022). However, biological preparations based on microbial agents – fungi *Gliocladium spp.* and *Ampelomyces spp.* and bacteria of the genus *Pseudomonas* – against diseases are understudied, which may be an obstacle to their development and introduction into production.

Given the above, the purpose of this study was to determine the ability of biological preparations to limit the damage to apple plants by *V. inaequalis* and *P. leucotricha* fungi, which cause scab and powdery mildew, respectively.

MATERIALS AND METHODS

The study was conducted during 2021–2022 in the apple tree stands of the Renet Simirenko variety of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine

(IH NAAS). This variety is susceptible to the most common diseases and is a model object for studying the effectiveness of protection measures. The tree layout is 5.0×1.0 m, the number of plants per hectare is 2,000 pcs. Soil of the experimental plot: dark grey podzolised light loamy, pH – 6.2, humus content – 2.8%.

The effectiveness of the following biological preparations was studied against scab and powdery mildew of apple trees: Ampelomycin BT (6 l/ha), based on the fungus of the genus *Ampelomyces* Ces ex Shlecht., containing mycelium and spores (pycnospora) with a titer of at least 4.0×10^9 CFU/cm³ and biologically active substances; Gliocladin BT (10 l/ha), containing mycelium and spores of the fungus from the genus *Gliocladium* with a titer of at least 1.5×10^9 CFU/cm³ and biologically active substances; Fluorescin BT (8 l/ha), contains bacteria of the genus *Pseudomonas* with a titer of at least 5.0×10^9 CFU/cm³, as well as biologically active substances (phenazine-carboxylic acids, siderophores, cytokinins).

The apple trees were sprayed with biological preparations in the morning in the absence of dew on the surface of plants using a STIHL SR 450 sprayer (Germany). The working fluid consumption was 600 l/ha. Each variant is repeated three times. The placement of accounting trees of different repetitions of the same variant is randomised. Meteorological information during the apple tree growing season was obtained from the it Lynx weather station (Ukraine). Apple plants were sprayed with biological preparations against diseases in the following stages of crop development according to the BBCH scale of stages of development and growth of plants: the first spraying – 67 (Flowers fading: majority of petals fallen), the second – 72 (Development of fruit; fruit size up to 20 mm), the third – 74 (Fruit diameter up to 40 mm; fruit erect), the fourth – 75 (Fruit about half final size), the fifth – 78 (Fruit about 80% final size). Records of disease spread, development, and technical effectiveness of biological preparations were carried out according to generally accepted methods (Trybel & Sigareva, 2001).

The spread of scab and powdery mildew on apple trees was determined according to the following formula:

$$P = \frac{n \times 100}{N}, \quad (1)$$

where P is the disease prevalence (disease incidence), %; N is the total number of plants in samples, pcs; n is the number of diseased plants in samples, pcs.

A six-point scale was used to determine the intensity of scab lesions (Trybel & Sigareva, 2001): 0 points – healthy leaves; 0.1 points – up to 1% of the leaf surface is covered by individual small spots; 1 point – 1-10% of the leaf surface is covered by individual small or medium-sized spots; 2 points – 11-25% of the leaf surface is covered by individual large spots (up to 5 mm) or small spots; 3 points – spots are mostly larger than 5 mm, often merging and covering 26-50% of the leaf surface; 4 points – spots are mostly larger than 10 mm, over 50% of the leaf surface is covered, leaves turn yellow, deformed, dry out. Powdery mildew of apple trees was recorded on the following scale (in points): 0 – no signs of damage; 0.1 – separate small spots of fungal mycelium with damage to the leaf surface of 0.1-1.0%; 1 – separate small, sometimes merging spots (1.1-10% of the leaf surface affected); 2 – small, blurry spots, merging; 11-25% of the leaf surface affected; 3 – large, blurry spots, merging with 26-50% of the leaf surface affected; 4 – large spots, with intense sporulation of the fungus, the leaf is deformed, dries up (over 50% of the leaf surface is affected).

Disease progression (lesion intensity, disease index) was determined according to the formula (Trybel & Sigareva, 2001):

$$Rx = \frac{\sum(a \times b) \times 100}{N \times K}, \quad (2)$$

where Rx is the disease development, %; $\sum(a \times b) \times 100$ is the sum of the product of the number of diseased plants and the corresponding damage score; N is the total number of plants (healthy and diseased), pcs; K is the highest score of the accounting scale.

The technical efficacy of the preparations (Te , %) was determined according to the formula (Trybel & Sigareva, 2001):

$$Te = \frac{100 \times (Dc - De)}{Dc}, \quad (3)$$

where D_c is the indicator of disease development in the control; D_e is the indicator of disease development in the experimental variant.

Experimental studies of plants (both cultivated and wild), including the collection of plant material, were following institutional, national, or international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973).

RESULTS AND DISCUSSION

An analysis of the meteorological conditions of the 2021 growing season (April–August) showed that the average daily air temperature and precipitation were substantially different from the long-term average (Table 1). Thus, only in April and May was the air temperature slightly lower than long-term indicators (by 0.5–1.2°C), while in June and August the excess of the average daily temperature reached 4°C. In April–July 2021, there was a shortage of precipitation.

Although there were 6 to 15 rainy days with precipitation per month, the shortfall compared to the long-term average was 20.4–66.6 mm. In just one month, during April–August, there was between 16.5 and 57.6 mm of precipitation. The largest number of them was recorded in August. An analysis of weather conditions (April–August 2022) showed that the average daily air temperature and precipitation data differed substantially from the long-term average (Table 1). Specifically, in April and May, the air temperature was slightly lower than long-term indicators (by 0.5–1.0°C), and in June and August, the excess of the average daily temperature ranged within 0.2–3.5°C. During the growing season of apple plants in April–August, from 19 to 26.3 mm of precipitation was recorded monthly. There is a substantial deficit of precipitation (26.7–59.6%) compared to the average long-term figures. In general, the meteorological conditions of 2021–2022 ensured the accumulation and spread of the inoculum of apple scab and powdery mildew pathogens and the manifestation of diseases.

Table 1. Characteristics of meteorological conditions during the apple tree vegetation (Institute of Horticulture of NAAS, 2021–2022)

Main indicators	Months and years									
	April		May		June		July		August	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
air temperature, °C										
average long-term	9.2		15.3		18.6		20.8		19.5	
of the current year	8.0	8.2	14.7	14.8	21.8	22.1	25.0	21	21.2	22.6
deviations from long-term	-1.2	-1.0	-0.6	-0.5	+3.2	+3.5	+4.2	+0.2	+1.7	+3.1
precipitation, mm										
average long-term	45.7		55.0		83.1		69.5		54.8	
of the current year	24.0	19	34.6	26.3	16.5	23.5	33.5	24	57.6	21.2
deviations from long-term	-21.7	-26.7	-20.4	-28.7	-66.6	-59.6	-36.0	-45.5	+2.8	-33.6

Source: weather station of the Institute of Horticulture of the National Academy of Sciences of Ukraine

During the period under study, apple scab was observed on leaves and fruits (Fig. 1a). On the affected leaf blades, the symptoms of the disease were characterised by the appearance of chlorotic spots, which eventually became covered with a velvety greenish-olive coating. On

diseased fruits, crustal spots of assorted sizes were formed, covered with a velvety olive coating. Powdery mildew developed on young shoots and leaves (Fig. 1b). A white bloom was formed mainly on the underside of the leaves, which curled over time. Subsequently, the symptoms

of the disease on the leaves and young shoots changed: the plaque acquired a dirty grey hue

with black dots – cleistothecia. Intensively affected shoots had shortened internodes.



Figure 1. Symptoms of apple scab (a) and powdery mildew (b)

The use of biological preparations Ampelomycin, Gliocladin, and Fluorescin on apple trees led to a 15.0-21.9% reduction in the spread of apple scab, and the development of the disease was reduced by 9.5-11.1% compared to the control (Table 2). Therewith, the technical efficiency of the preparations ranged from 68%

to 79%. The tested biological preparations also controlled powdery mildew, reducing the prevalence of the disease by 5.6-7.0% and its development by 2.2-2.5%. The technical efficiency was within 59-76%. The most effective against the disease was the application of Ampelomycin at a rate of 6.0 l/ha.

Table 2. Efficiency of biological preparations against scab and powdery mildew of apple trees (Institute of Horticulture of NAAS, Renet Simirenko variety, average for 2021-2022)

Variant	Consumption rate, l/ha	Scab			Powdery mildew		
		DP	DD	TE	DP	DD	TE
Control (without processing)	–	37.7	14.0	–	11.6	3.3	–
Ampelomycin BT	6.0	15.8	2.9	79	4.6	0.8	76
Gliocladin BT	10.0	22.3	4.5	68	6.0	1.4	59
Fluorescin BT	8.0	20.1	3.9	72	6.6	1.3	61
LSD ₀₅			2.9			1.5	

Note: DP – disease prevalence, %; DD – disease development, %; TE – technical efficiency, %

Source: research results of the authors of this study

The study of the effect of biological preparations on scab damage to apple fruit helped establish their negative impact on the pathogen. The number of diseased fruits decreased by 22.7-35.6%, and the intensity of their damage was lower by 5.1-6.4% (Table 3). The technical efficacy of the preparations was as follows: Ampelomycin – 68%, Gliocladin – 62%, and Fluorescin – 78%.

The analysis of yields in variants involving biological preparations against apple diseases showed their positive impact on this indicator. The preserved fruit yield was within 3.7-4.4 t/ha compared to the variant where no preparations were used (Fig. 2). The highest yield of apple trees was observed in the case of spraying the plants with the biological preparation Ampelomycin at a rate of 6 l/ha.

Table 3. The effect of biological preparations on scab damage to apple fruit (Institute of Horticulture NAAS, Renet Simirenko variety, average for 2021-2022)

Variant	Consumption rate, l/ha	Fruits		
		DP	DD	TE
Control (without processing)	–	53.1	8.2	
Ampelomycin BT	6.0	30.2	2.6	68
Gliocladin BT	10.0	30.4	3.1	62
Fluorescin BT	8.0	17.5	1.8	78
	LSD ₀₅		1.9	

Note: DP – disease prevalence, %; DD – disease development, %; TE – technical efficiency, %

Source: research by the authors of this study

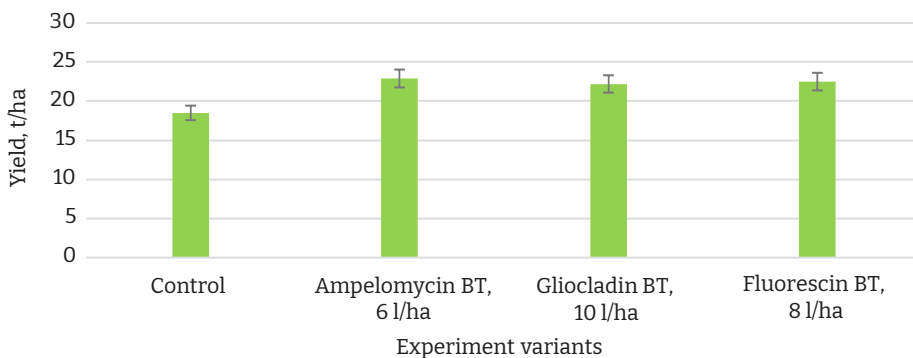


Figure 2. The impact of biological preparations on apple yields

Note: average for 2021-2022, Renet Simirenko variety

Source: developed by the authors

The presented findings indicate the ability of biological preparations Ampelomycin BT (based on the fungus of the genus *Ampelomyces* Ces ex Shlecht.), Gliocladin BT (containing mycelium and spores of the fungus of the genus *Gliocladium*) and Fluorescin BT (bacteria of the genus *Pseudomonas*) to reduce the spread and development of scab and powdery mildew of apple trees, which ultimately ensures the preservation of the crop yield. Notably, the scientific world has already conducted research on the control of powdery mildew fungi using representatives of the genus *Ampelomyces*. They were among the first mycoparasites to be used for this purpose, due to their ability to effectively inhibit the growth of pathogen mycelium and reduce the amount of infectious material (Prahl *et al.*, 2023).

The results of the study are consistent with the findings of Y. Kimura *et al.* (2023) on the effectiveness of *Ampelomyces* hyperparasites

against powdery mildew on different crops. Thus, in the conditions of protected soil *Ampelomyces* sp. strain Xs-q suppressed colonies of *Podosphaera xanthii*, the causative agent of melon powdery mildew. The strain *Ampelomyces quisqualis* CPA-9 (2.5×10^5 conidia/ml) was effective against powdery mildew of zucchini and reduced the development of the disease by 61.5%. A concentration of 7.3×10^7 conidia/ml reduced the incidence by 83% (Carbó *et al.*, 2020). T.D. Kushare *et al.* (2020) found that among the biological agents investigated for the control of grape powdery mildew, *Ampelomyces quisqualis* provided 52.67% disease control.

In the study by M. Hassine *et al.* (2022), a preparation based on *Gliocladium catenulatum* strain J1446 controlled grey rot of lettuce *in vitro* by 91%. The filtrates of cell-free cultures of *G. catenulatum* Gc1 and *G. virens* Gv1 limited the growth of mycelium of the anthracnose

pathogen – *Colletotrichum coccodes* and reduced the development of rot on tomato fruits. The use of *Pseudomonas fluorescens* bacteria on celery plants provided 61.2% control of powdery mildew (Ahmed *et al.*, 2021). Some studies have also confirmed the ability of the bacterium genus *Pseudomonas* to reduce the spread and development of scab and powdery mildew of apple trees. Thus, K. Paliwal *et al.* (2023) investigated the compatibility of a biocontrol agent (*Pseudomonas*) with a carbendazim-based fungicide against the root rot pathogen of soybean (*Rhizoctonia solani*). Scientists have found that *P. fluorescens* inhibited the growth of *R. solani* mycelium (43%) and could withstand 0.05–0.15% carbendazim concentration. This method reduced the development of the disease by 72% compared to the control.

L.A. Shuttleworth (2021), analysing alternative strategies for controlling apple diseases, notes the possibility of using bacterial strains as potential bioagents against scab, specifically the isolate *Pseudomonas syringae*, which suppressed the disease. There is also information in the literature about the ability of *Pseudomonas fluorescens* strains to inhibit the fungus *Alternaria brassicae* *in vitro* and *in vivo* (Gupta *et al.*, 2020). S.I. Hradchenko (2022) investigated biological preparations Kazumin 2L, RK, Phytocide-r, PhytoHelp, and MycoHelp in Ukraine, which were effective against apple scab at the level of 71–86% in years without epiphytic development of the disease. There are different opinions on the specifics of using biological preparations against *V. inaequalis*. Specifically, they can be integrated with forecasting models for the appropriate time of application (Berrie, 2019).

The studies of Z. Polat & H. Bayraktar (2021) and M. Chatzidimopoulos *et al.* (2022) indicated that the control of apple diseases is largely based on the frequent use of chemicals. Studies conducted in Greece have suggested that fivefold spraying of plants with fungicides ensured the intensity of scab development on apple fruits below 1.88% (Chatzidimopoulos *et al.*, 2020). D.A. Strickland *et al.* (2022) reported that in the US, powdery mildew management programmes that included flutriafol or myclobutanil in the previous season tended to reduce the incidence of apple disease in the following spring,

probably due to reduced inoculum overwintering. At the same time, scientists point out that pathogens are resistant to fungicides, which is a widespread problem (Polat & Bayraktar, 2021; Chatzidimopoulos *et al.*, 2022).

Thus, further, more detailed work is needed to understand the effectiveness of biological preparations on apple trees under different environmental conditions, which will help to develop more effective disease management strategies and reduce the pesticide burden on the agrocenosis.

CONCLUSIONS

The phytopathogenic fungi *V. inaequalis* and *P. leucotricha* infect apple trees and cause dangerous diseases such as scab and powdery mildew, which lead to a shortage of fruit yield, deterioration of its quality and a considerable decrease in plant productivity. The specific feature of pathogens is the production of many generations of sporulation and the development of various diseases throughout the entire period of plant vegetation. In this study, biofungicides from antagonist and hyperparasite microbial cultures were evaluated to limit the damage to apple plants by dangerous mycoses such as scab and powdery mildew. During the experiment, they were applied five times by spraying apple plants of the Renet Simirenko variety of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine (IH NAAS) in the following phases: BBCH 67, 72, 74, 75, and 78. Thus, microbiological preparations based on the pycnidial fungus of the genus *Ampelomyces* Ces ex Shlecht. (*Ampelomyces* BT), a micromycete from the genus *Gliocladium* (*Gliocladin* BT), and bacteria of the genus *Pseudomonas* (*Fluorescens* BT) controlled scab and powdery mildew of apple trees. *Ampelomyces* BT (at a rate of 6 l/ha) provided technical efficiency against scab 79% and powdery mildew – 76%, *Gliocladin* BT (10 l/ha) – 68% and 59%, respectively, *Fluorescens* BT (8 l/ha) – 72% and 61%. These biological preparations also reduced the development of scab on apple fruit, while the technical efficiency was within 62–78%. The use of biological plant protection products ensured the preservation of apple fruit yields from 3.7 to 4.4 t/ha. The highest rate was in the variant with the

biological product Ampelomycin BT. The results obtained can be used to develop a system for protecting apple trees from diseases.

Promising areas for future research include the study of the specific features of using biological preparations on apple varieties characterised by different degrees of susceptibility to scab and powdery mildew. The use of biological preparations based on biocontrol agents can

be an alternative to environmental protection against diseases and increase fruit production.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Ефективність біологічних препаратів проти парші та борошнистої роси яблуні

Анотація. Парша (збудник *Venturia inaequalis* Cooke (Wint.)) та борошниста роса (*Podosphaera leucotricha* (Ellis & Everh. Salmon)) є шкідливими хворобами яблуні захист від яких ґрунтується на багаторазовому обприскуванні хімічними фунгіцидами. Для зменшення пестицидного навантаження на агроценози актуальним є застосування біологічних засобів захисту. Мета роботи полягала у дослідженні ефективності біопрепаратів на основі грибів і бактерій (мікопаразитів та антагоністів) проти парші та борошнистої роси яблуні. Для цього використовували комплекс методів, які включали фітопатологічну діагностику та моніторинг хвороб, фенологічні спостереження, облік урожайності, статистичний аналіз. Вивчено біопрепарати Амтеломіцин БТ (6 л/га) на основі гриба роду *Ampelomyces* Ces ex Schlecht., Гліокладін БТ (10 л/га), який містить міцелій та спори гриба з роду *Gliocladium* та Флуоресцин БТ (8 л/га), основою якого є бактерії роду *Pseudomonas*. Їх застосування призводило до зменшення поширення та розвитку парші яблуні, а технічна ефективність препаратів становила від 68 до 79 %. Біопрепарати також знижували ураження рослин збудником борошнистої роси за технічної ефективності 59-76 %. Обробка рослин яблуні препаратами Гліокладін БТ, Амтеломіцин БТ та Флуоресцин БТ зменшувала кількість плодів уражених паршею на 22,7-35,6 %. Технічна ефективність препаратів при цьому становила 62-78 %. Застосування досліджуваних біопрепаратів забезпечувало збереження урожаю в межах 3,7-4,4 т/га. Отримані результати свідчать про перспективність використання біологічних препаратів проти парші та борошнистої роси яблуні для екологізації захисту рослин

Ключові слова: хвороби яблуні; гриби; мікроорганізми-антагоністи; технічна ефективність; урожайність