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Agrobiotechnological protection of grapes from diseases using microbiological preparations

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Abstract. The article highlights the aspects of the use of microbiological preparations for the protection of vineyards from the most common disease - mildew. The results of the use of microbiological preparation "Mikosan B" for protection against mildew on private vineyards in the Transcarpathian region are shown. According to the intensity of pesticide use, grapes take one of the first places. During the growing season, 10-15 sprayings are carried out on the vineyards. With this existing technology of vineyard cultivation, there is a negative impact on both grape production and the overall production. In the world, there is a trend of gradually reducing the use of toxic pesticides and replacing them with environmentally friendly drugs. In European countries with the concept of ecologically sustainable agriculture, the gradual removal from the market of more than 60% of highly toxic pesticides is foreseen. Recently, there has been an increased interest in microbiological preparations, which activates an intensive search, study of biological properties and selection of promising microorganisms for biologization and greening of plant protection. Thus, the replacement of chemical meliorants, pesticides, agrochemicals with biological preparations created on the basis of microorganisms and their metabolites began to be implemented in the practice of plant protection.

Keywords: agrobiotechnological protection, grapes, Transcarpathia, microbiological preparations, mildew, Mikosan-B, quadris.

1. Introduction

According to ancient chronicles, Transcarpathia belongs to the oldest wine-growing regions, which were born due to the proximity of the northern borders of the Roman Empire, which ran along the Danube. In the northern provinces of the empire, Roman legionnaires had to plant grapes according to the order of the Roman Emperor Probst (276-282).

Given the favourable soil and climatic conditions of the lowland zone, as well as the slopes protected from the north by mountains, viticulture began to develop actively throughout the Middle Danube Lowland in the twelfth and thirteenth centuries.

In Ukraine, the largest area of grape plantations is in Odesa region and the Autonomous Republic of Crimea, with the rest concentrated in Mykolaiv, Zakarpattia and Zaporizhzhia regions.

The historical centre of Transcarpathian winemaking is Chorna Hora near Vynohradiv, where grapes have been grown since ancient times. It was Sevliush, which means "grape", that was first mentioned in the first mention of viticulture in Transcarpathia.

At the beginning of the twentieth century, the area of vineyards in Transcarpathia was about 3000 hectares, and over time, in the 20s and 30s, it increased to 4500 hectares During the Second World War, agriculture was severely damaged. At the time of the reunification of Transcarpathia with Soviet Ukraine, 3,500 hectares remained. There were 7.2-7.5 thousand bushes per hectare. In 1953 the area increased to 5400 hectares, in 1965 it was 14500 hectares. After the anti-alcohol campaign of the second half of the 1980s, the area decreased by more than 10 thousand hectares, and currently 4809 hectares remain (Viticulture in Ukraine 2009).

The aggravation of the global environmental crisis has led to changes in agrobiology. The chemicalisation of agriculture inherent in intensive technologies is giving way to adaptive agriculture, which is based on optimising and minimising chemical intervention.

Along with the selection of resistant crops, it is important to study the physiological characteristics of the impact of phytopathogenic microorganisms on plants when using various components of organic farming, including alternative soil revitalisation technologies using microbial biodestructors. In this direction, it is important to determine the effect of the natural consortium of microorganisms in the composition of the preparation Extrakon, fungi Mikosan-B intended for soil rehabilitation on the immunity of grape plants under artificial infection with separate and combined infection and against rot.

2.Object and subject of research

Find a selection of varieties, their acclimatisation and agronomy in Ukraine.

In Ukraine, mildew is widespread and very harmful, characterised by high damage in different viticulture zones. The disease develops almost annually in vineyards in the Transcarpathian region.

Among the diseases affecting grapes, fungal diseases (mildew (Plasmopara viticola Berl.Et de Toni), oidium (Oidium Tuckeri Berk.), grey rot (Botrytis cinerea Pers.), white rot (Coniothyrium diplodiella Sacc.), etc.) cause great damage [7]. Unfortunately, plant diseases are causing significant losses in viticulture today, mildew - downy mildew, the pathogen - is an obligate monophagous pathogen and parasitises only the vine. The source of primary infection of the disease is oospores that overwinter in fallen infected leaves and berries. Oospores are formed in late summer and autumn. Oospores germinate in spring, in conditions of high humidity. Two to three days of strong moisture promotes their active germination. The temperature is from 11°C to 38°C, the optimum temperature is 25°C. At 17-25°C, they germinate in 2-3 days, and at 11°C - in 8 days.

Mycelium develops in the intercellular spaces. Sterigmas are thin outgrowths of mycelial branches with the help of which conidiogenes are attached to the mycelium. They are ovoid, unicellular, with a small papilla at the apex. Conidiophores (formed on sterigmas) are repeatedly branched at right angles. Oospores are rounded with a four-layered shell. Diameter 25.0-35.0 μm. Zoosporangia - monopodial branching. Each forms 200 zoosporangia with 4-5 zoospores [9,13].

The infection affects all reproductive and vegetative organs of grapes except the roots. These are the green organs of the plant: shoots, whiskers, leaves, berries, and inflorescences. The most striking signs of harmfulness are observed on grape brushes and leaves (Fig. 1.) There are several forms, appearing on young leaves in the form of large oily spots, rounded, visible only in the light. As the spots develop, they become pale green in colour. A whitish fluffy coating forms on the underside of the leaf in the area of spot formation (Fig. 2).

a) large oily spots, b) young flowers of pale green colour.

Figure 1. Signs of mildew on grapes.

Over time, the affected tissues become necrotic. The mosaic form is formed on leaves that have finished growing. In this case, the phytopathogen creates angular, small, vein-limited specks covered with a white coating on the back of the leaf blade. Grey rot, as a form of mildew, forms on flowers and berries that are no larger than a pea. Infected tissues are covered with abundant grey sporulation and die quickly. Brown rot, a form of mildew, appears on berries that have already reached normal size.

a) necrosis в) brown rot, c) milliard

Figure 2. Mildew disease.

When the infection process occurs in the stem area, they first wither, then turn brown and shrivel. The berries are mummified. The damage covers individual berries or the entire bunch. As the tissues age, the development of the phytopathogen is limited. Green shoots and antennae are rarely damaged at low disease intensity, and when growing, they are covered with a white coating of spores [9].

Effective protection of vineyards from mildew is ensured by a set of agronomic and chemical measures. Agronomic measures can create conditions unfavourable for the development of the disease. [15]. These include the placement of vineyards on southern, well-ventilated slopes, placement of rows in the direction of prevailing winds, timely tying, pruning, pinching, chasing, and systematic weed control to reduce the development of the disease.

Also, the development of diseases is significantly delayed by the application of phosphorus and potassium fertilisers, and the use of foliar feeding with microelements [10].

The main method of moth control is the treatment of plantations with fungicides. Fungicides are chemicals designed to prevent the development or destruction of pathogenic fungi and bacteria that cause numerous diseases [5,6]. This technology of vineyard treatment has a negative impact on product quality and the environment in general. In the global practice of chemical protection of crops from pests, there is a tendency to gradually reduce the use of toxic pesticides and replace them with environmentally friendly products. Ecological methods of developing the viticulture industry are being created. In the USA, France, Germany and other countries, they are based on balanced viticulture [10]. The principles of this method are to preserve soil fertility, protect the environment, reduce health hazards, and use natural compost and mulch. In European countries, the concept of environmentally sustainable agriculture envisages the gradual removal of more than 60% of highly lethal pesticides from the market.

Recently, there has been an increased interest in microbiological preparations, and this has activated an intensive search, study of biological properties and selection of microorganisms suitable for biologisation and ecologisation of plant protection [11,12]. Biological products based on microorganisms can be used in high doses with virtually no limit on the number of treatments against a range of pathogens on vegetable, fruit and berry crops and in horticultural plantations [4].

Therefore, it is promising in plant protection practice to replace chemical ameliorants, pesticides and agrochemicals with biological products based on microorganisms and their metabolites (biological insecticides, rodenticides and fungicides). The degree of visual damage caused by them ranges from 1-2 to 20-35 on average. There are a number of ways to regulate the activity of pathogens using biologically active substances that can induce defence responses and stimulate the plant immune system [1]. One of these areas is the use of biologically active substances based on fungal polysaccharides: β-glucans, chitin and its derivatives. As a result of studying various compositions of these biopolymers, it was found that they have a prolonged mechanism of action against crop diseases, providing restriction of the development of phytopathogenic fungal pathogens. According to research conducted by the Institute of Plant Protection and Ecological Agriculture of the Academy of Sciences of the Republic of Moldova, in experiments on the Pinot Black grape variety, biological preparations increased plant resistance to drought and disease [2]. In protecting against mildew and oidium, they were less effective than fungicides, but they were more effective against grey rot [3].

3. Target of research

Target of research is to find a simple alternative to the use of pesticides, to improve the environmental safety of protecting grapes from diseases by using biological products based on microorganisms in crop protection**.**

4. Literature analysis

I.L.Markov, O.V.Bashta, D.T.Gentosh, O.P.Dermenko, M.Y.Pikovsky and other countries have developed a number of studies, methods and a complex of approaches to protect grapes from growing diseases. Among them is mildew, zbudnik (Plasmopara viticola Berl.Et de Toni). Scientists have provided information on the distribution, diagnostic characteristics, biology of pathogens and the harmfulness of the main diseases of agricultural crops, grapes and tree species of shelterbelts. Systems for protecting measures used in intensive cultivation technologies are given.

5. Research methods

The main research methods were: field - to determine the agrobiological indicators of the state of grape bushes, which was carried out according to the generally accepted methodology. The study of the development and spread of diseases was carried out on a 9-point scale on leaves and a 4-point scale on bunches in private plantations of Transcarpathia [10]; laboratory - to study the causative agent of grape disease in private plantations of Transcarpathia, as well as visual - for the general condition; measuring - for agrobiological records and determination of yield. The effectiveness of the preparations was determined by the presence of diseases before and after treatment of the bushes. The effectiveness of the biological product was determined by comparing it with the reference variant and the control, where no treatment was carried out.

6.Research results

In Transcarpathia, mildew is a causative agent of grape disease - a parasitic fungus Plasmopara viticola, which belongs to the kingdom Chromista, division Oomycota, order Peronosporales, is an obligate monophagous fungus and parasitises only on the vine. The disease is widespread in all areas where grapes are grown. Mildew is one of the most dangerous grape diseases. In the spring, during the initial period of bud growth, disease foci can already be seen on bushes infected in the previous year. [14]

During 2021-2022, the biological effectiveness of the biofungicide Mikosan B against mildew was observed in private vineyards on the Kishmish and Vostorg varieties**.**

The experiment included 4 treatments. In this variant, the development of the disease on leaves was 35.7%, on bunches - 38.8%, and in the control variant it was 17.6-95.4 and 95.4%, respectively (Table 1).

The effectiveness of "Mikosan V" compared to the standard, where four sprayings with fungicides approved for use in Ukraine (Quadris, Strobi, Topaz, Thiophene, Falcon and Flint) were carried out, was slightly lower. In the control variant, by the time of harvesting, there was a severe damage to the leaves of the grape bushes, and the harvest was of poor quality. In the variant with the use of "Mikosan V", the yield was 2.6 kg/bush, i.e. at the level of the reference variant, the difference was within the error of the experiment.

The experiment included 4 treatments. In this variant, the development of the disease on the leaves was at the level of 35.7.

Table 1. Effectiveness of "Mikosan V" in protecting grapes from mildew. (Kishmish variety, 2021)

Biological product	Date of effectiveness determination			
	26.05	13.07	21.08	31.08
Development of the disease on the leaves, %.				
Control (untreated)	17,6	35,4	68,8	95,4
Mikosan V	10,8	25,8	36,8	35,7
Reference	9, 3	18,0	24,0	24,8
HCP_{05}	3,9	2,3	6,2	5,3
Development of the disease on the bunches, %.				
Control (untreated)	3,5	17,7	72,1	97,1
Mikosan V	1,4	13,4	37,8	38,8
Reference	1,2	8,6	25,6	24,4
HCP_{05}	1,7	4,5	4,1	3,0

Continuation of Table 1

Effectiveness of the drug on leaves, %.				
Mikosan V	34,6	23,0	43,1	60,6
Reference	38,5	41,5	62,8	73,3
Effectiveness of the drug on bunches, %.				
Mikosan V	57,3	25,6	44,7	58,7
Reference	67,1	50,0	62,6	72,8

The experiment (Vostorg variety, 2022) included four treatments with "Mikosan V" at a rate of 10 l/ha (100 ml/10 l of water). Four sprayings with fungicides approved for use (quadris, strobi, topaz, thiophene) were carried out on a private standard. In this variant, "Mikosan V" restrained the development of the disease on the leaves within 0.3-17.3%, on the bunches - 2.7-14.3% (Table 2).

The preparation "Mikosan V" at four times spraying of grapes on a private plot against mildew before harvesting showed high efficiency on leaves - 77.4%, on bunches - 84.5% (Table 2).

Table 2. Effectiveness of "Mikosan V" in protecting grapes from mildew. (Vostorg variety, 2022)

Biological product	Date of effectiveness determination			
	07.05	21.05	04.06	18.06
Development of the disease on the leaves, %.				
Control (untreated)	2,7	6,2	17,8	64,5
Mikosan V	0,3	0,9	11,7	17,3
Reference	0,1	0,2	0,5	1,1
HCP_{05}	0,2	0,4	0,5	0,4
Development of the disease on the bunches, %.				
Control (untreated)	2,1	4,4	9,9	45,2
Mikosan V	0	2,7	7,1	14,3
Reference	0	0	0	0
HCP_{05}	0,2	0,4	0,3	0,4
Effectiveness of the drug on leaves, %.				
Mikosan V	92,7	83,4	43,2	77,4
Reference	95,9	96,5	96,9	98,3
Effectiveness of the drug on bunches, %.				
Mikosan V	100	37,2	30,4	84,5
Reference	100	100	100	99,8

8.Conclusions

Ecological methods of developing the grape industry and preserving soil fertility, protecting the environment and avoiding health hazards are called organic production, and the biological system of vineyard protection against diseases involves a combination of different biological products depending on the phytosanitary condition of plantations, as well as different weather conditions of the year and the spectrum of the product's action. A two-year study of the effectiveness of the microbiological preparation "Mikosan V" (10 l/ha) in protecting grapes from mildew showed high efficiency of the preparation on leaves - 77.4% to 84.5%, which allows to reduce the pesticide load and produce environmentally friendly products.

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