The use of a microbiological preparation based on Bacillus subtilis in organic viticulture

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Abstract. In organic farming, microbiological preparations are an alternative to chemical pesticides and mineral fertilizers. The article summarizes the experience of a three-year use of the biofungicide Extrasol in combination with colloidal sulfur in the vineyards of the Crimea. The use of the biofungicides makes it possible to control the development of powdery mildew of grape at the level of chemical plant protection products, including during the years of epiphytoties: on the cv. Bastardo magarachskiy grapes – 7.1–14.7 %, on the Italy grape cultivar – up to 5.2 %. The degree of influence of the biofungicide Extrasol on the phenolic and oxidase complex of grapes depends on the background level of development of Uncinula necator and the biological effectiveness of the treatment. In comparison with chemical means of protection, the smallest effect of processing cv. Bastardo magarachskiy grapes with a biological product in relation to the accumulation of phenolic compounds was observed at a level of powdery mildew development of 30-50 %; the maximum increase in the technological reserve of phenolic compounds was 59 %, anthocyanins – 12 %; the activity of polyphenol oxidase increased 1.1–3.3 times. The use of the biofungicide Extrasol on Italian grape cultivar led to an increase in the weight of the bunch by an average of 11 %, yield – by 25.6 %, titratable acids – by 9.6 % relative to chemicals; improved the aroma, taste and texture of the berry.

1 Introduction

In the world, there is a growing interest of the population in the consumption of environmentally friendly, natural products, and the desire to reduce the negative anthropogenic impact on the ecosystem of our planet. In this regard, there is a need to develop environmentally friendly technologies, including organic farming. Organic farming involves limiting or completely eliminating the use of chemical plant protection products and synthetic root fertilizers [1]. In the system of protection of vineyards, which provides for repeated treatment of plants with pesticides to obtain a healthy harvest, these issues are especially relevant. At the same time, the natural self-regulatory processes of ampelocenoses, as well as the management of grape metabolism, including the

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immobilization of its own protective mechanisms, by environmentally friendly methods (agrotechnical, physical, biological, etc.), combined into a system of precision farming [2], become the fundamental basis of organic viticulture.

In this context, a promising alternative to chemical pesticides and mineral fertilizers is the use of biofungicides containing living cells of microorganisms and their metabolic products. Competing with rhizospheric and epiphytic microflora, the live culture of preparations intensively reproduces and populates developing plant tissues, exerting a multifunctional effect on it: it stimulates growth processes, induces immunity, and also, due to the synthesis of antibiotics, lytic enzymes and other biocidal metabolites, suppresses a wide range of infections. [3, 4]. Biopesticides have many advantages in comparison with their chemical counterparts – less environmental impact due to high efficiency in small doses, less toxic, more selective, biodegradable, reduce the effect of resistance [4, 5]. The effectiveness of the biofungicide Extrasol, created on the basis of rhizospheric bacteria, has been proven on a number of agricultural crops – potatoes, corn, wheat, oats, etc. [6–8]. It has been established that the treatment of corn seeds with this preparation contributes to an increase in grain yield by 7.3–9.1 % relative to the control; the use of the drug on vegetative plants provides an increase in the leaf surface by 15.3–33.2% at a density of 45 thousand plants/ha and by 39.2–56.7 % at a density of 55 thousand [6]. The use of the Extrasol preparation on potatoes in a closed ground made it possible to obtain an additional income of up to 230 thousand rubles/ha, due to an increase in the productivity of microplants up to 64.4 g/bush and a higher yield of the standard mini-tuber fraction up to 2.6 pieces/bush [7].

At the same time, the information available in scientific publications on the effectiveness of control over grapevine pathogens, the effect on beneficial microbiota, the synthesis of primary and secondary metabolites, the productivity of the grape plant and the quality of the crop is limited and contradictory [5, 9, 10]. The latter is associated not only with the different microbiological composition of preparations, but also with the peculiarities of the interaction of microorganisms and plants in a particular ampelocenosis, due to varietal specificity, climatic, orthographic factors, and phytopathogenic situation [3, 4, 11]. In some cases, it is inevitable to combine the biocontrol of fungal infections with other natural fungicides allowed in organic viticulture [12].

Flavonoids, including anthocyanins, and oxidases play a significant role in the plant's own defense system: many researchers noted an increase in the biosynthesis of phenolic compounds and the activity of polyphenol oxidase, which catalyzes their oxidative transformations, in response to pathogen attacks [13–15]. It has been shown that bacterial endophytes induce systemic plant resistance due to the accumulation of phenolic compounds [16]. We observed an increase in the content of phenolic components in grapes when biological preparations were used in agrotechnological schemes [12, 17].

This publication presents the results of a study of the effect of complex treatment of grapes cultivated on the Crimean Peninsula with a bacterial preparation and a sulfur preparation on productivity indicators, the phenolic complex and oxidase activity of grapes.

2 Materials and methods

2.1 Grapes and growing conditions

The studies were carried out in 2019–2021 on grapes of the table direction (*V. vinifera*), the Italian grape cultivar growing in the mountain-valley coastal region of Crimea (the village Morskoye), and the cv. Bastardo magarachskiy grapes direction from the southern coast region (the village Livadia). The culture is uncovered. Planting scheme – 3.0 × 1.5 m. The shape of the bushes is a cordon on the middle stem. The reference system is trellis vertical.

The territory of vineyards in the mountain-valley coastal and southern coastal regions was characterized during the years of research by the sum of active temperatures above 10 °C – 4399–4480 °C and 3768–4510 °C; the amount of precipitation per year is 145–190 and 367–441 mm, respectively. The most common disease of grapes in the regions is powdery mildew (*Uncinula necator*), yield losses from which are 33-37 %, and in the years of epiphytoties – up to 100 % [18]. This predetermined the choice of drugs and grape processing schemes in the organic farming system.

2.2 Pesticides and treatment regimens

In the present studies, in the organic farming system (experimental variants), the biofungicide Extrasol (LLC Bisolbi-Inter, Russia) and the preparation of colloidal sulfur Thiovit Jet (LLC Syngenta, Russia) were used. Extrasol contains a live culture of *Bacillus subtilis* (Ch-13 strain) and metabolites obtained during its cultivation.

The processing of grapes was carried out starting from the stage of development "shoots 10–15 cm". On the experimental plots of the Italy grape cultivar, a four-time foliar treatment with biofungicides Extrasol (application rate 4.0 l/ ha) and Thiovit Jet (5.0 kg/ ha) was carried out. On the Bastardo magarachskiy grape cultivar in 2019, a six-fold treatment with the biofungicide Extrasol (4.0 l/ ha) and a three-fold treatment with Thiovit Jet (6.0 kg/ha) were used; in 2020 – eight treatments with Extrasol and six with Thiovit Jet at the same doses; in 2021 – five-fold treatment with Extrasol (4.0 l/ha) and Thiovit Jet (5.0 kg/ha).

In the traditional system of cultivation of vineyards (control variants), agro-measures were used that were adopted on the farm and included 8 treatments using chemical plant protection products (ChPS). The experiment with the cv. Bastardo magarachskiy grapes included a variant in which the grapes were not treated with anything – this grape cultivar was used to evaluate the background level of powdery mildew development.

2.3 Sample preparation and methods of analysis

The assessment of the biological effectiveness of treatment schemes in accordance with [19], their impact on the productivity of grapes and the quality of the crop was carried out at the time of the onset of technical maturity of the berries.

Yield accounting was carried out by weighing and counting bunches of grapes collected from 20 typical bushes of each variant in four repetitions. The average weight of a bunch was determined by dividing the weight of the crop of accounting bushes by the number of bunches collected from these bushes. The organoleptic evaluation of grape samples was carried out on a 10-point scale, including indicators: the appearance of the bunch and berries, the taste and aroma, and the texture of the skin and pulp of the berries.

To analyze the chemical composition of grape berries, a combined sample (total weight of about 1000 g) was taken from 5 bushes of each variant.

To determine the content of sugars, titratable acids, and the technological reserve of phenolic compounds (TR PhC), the berries were crushed on a Moulinex-LM600E (France). The pulp was centrifuged for 15 min at 5000 rpm (to determine the TA of the EF, the pulp was preliminarily thermostated for 30 min at a temperature of 70 ˚C). The resulting must was filtered through membrane filters with a pore size of 0.45 μm. The content of sugars was determined by the areometric method, titratable acids – by the titrimetric method in terms of tartaric acid, phenolic compounds – with the Folin-Ciocalteu reagent (in terms of gallic acid) [20]. Sample preparation and determination of the content of anthocyanins in berries was carried out according to the method of Glories Y. [21]. The activity of polyphenol oxidase (PPO) was evaluated in freshly squeezed must by the rate of formation of a blue-violet color of oxidized diethyl paraphenylenediamine sulfate.

2.4 Statistical data analysis

Chemical analysis of grapes was carried out in triplicate. The data were mathematically processed using the SPSS Statistics statistical software package. The text and tables present the arithmetic mean values of the determined indicators and standard deviations (SD).

3 Results and discussion

The conducted studies showed that in 2019 there was practically no development of powdery mildew on cv. Italy grapes both in the control and experimental plots: there were single damage to the clusters. In 2020, the same situation was observed on grape plants treated with chemical protection agents. Treatment of grapes with Extrasol and Thiovit Jet preparations restrained the development of powdery mildew at the level of 5.2 % (spread 20 %), downy mildew - up to 3.0 %, black rot – 0.5 %. There were single damages of clusters by thrips and cluster leafworm.

The data presented in Table 1 make it possible to analyze the effect of treatment of the Italian grape cultivar with the biofungicide Extrasol in combination with colloidal sulfur on the productivity of grapes and the quality of the crop.

It was revealed that experimental processing in the system of organic viticulture contributed to an increase in the mass of a bunch on average over two years of observation by 4.8 %, yield – by 11.6 % relative to the option involving the processing of grapes with chemical agents. The output of standard products in the experimental and control plots was estimated at the same level and averaged 85–86 %. The results of two-year studies did not reveal a single trend in the change in the concentration of sugars in grapes under the influence of Extrasol and Thiovit Jet: in 2019, an increase (in comparison with HSPP) was noted by an average of 3 %; in 2020 – a decrease of 15 %. This indicates the need to continue research in this direction. Along with this, the use of biofungicides led to an increase in the concentration of titratable acids by 7.8 – 11.3 %. The tasting assessment of grapes was carried out on a 10-point scale in terms of: appearance (beauty) of bunches and berries; taste and aroma of berries; skin properties and pulp texture. Tasters noted an improvement in the taste, aroma and texture of the skin and pulp in the grapes of experimental batches, the assessment of which averaged 9.2 points.

On cv. Bastardo magarachskiy grapes background level of development of powdery mildew (control – without treatment) was at the average level and amounted to 32.7 % (Table 2). On the contrary, in 2020–2021. the development of the disease was characterized by an epiphytotic level and reached 93.2–94.3 %. At the same time, the biological effectiveness of the combination of biofungicides Extrasol and Thiovit Jet in 2019 (four-fold treatment with each drug) and in 2020 (six-fold treatment) was 78.3 % and 84.2 %, exceeding in 2020 the biological effectiveness of the use of chemical protective agents in by an average of 2.5 %. The biological efficiency of the experimental treatment in 2021 decreased to 50.8 %, which is 20.2 % less compared to the use of chemical plant protection products. It can be assumed that in the system of organic farming, five-fold treatment with Extrasol and Thiovit Jet preparations of cv. Bastardo magarachskiy grapes during epiphytoty years is insufficient to control the development of powdery mildew.

Table 1. Indicators of productivity and crop quality of cv. Italy grapes

Variants	Productivity, t/ha	The average weight of the bunch, g	Yield of standard products, %	Concentration, gL^{-1}		Tasting assessment, score
				sugars	titratable acids	
2019						
ChPS	15.2±0.6	504±20.6	84.5±3.4	176.0±7.0	6.4±0.3	7.9±0.3
Extrasol + Thiovit Jet	19.1±0.8	535±21.4	85.3±3.4	182.0±7.3	6.9±0.3	9.1±0.4
2020						
ChPS	18.6±0.7	550±22.0	86.9±3.5	211.0±8.4	4.4±0.2	8.7±0.3
Extrasol + Thiovit Jet	18.5±0.7	570±22.8	85.5±3.4	180.0±7.2	4.9±0.2	9.2±0.4

Table 2. Development of powdery mildew and biological effectiveness of different treatment regimens.

Experience variant	Harvest year					
	2019		2020		2021	
	Development of the disease in bunches, %	Biological efficiency, %	Development of the disease in bunches, %	Biological efficiency, %	Development of the disease in bunches, %	Biological efficiency, %
Control (no processing)	32.7±2.1	-	93.2±5.7	-	94.3±6.4	-
ChPS	4.5±1.2	86.2	17.1±1.1	81.7	27.3±2.2	71.0
Extrasol+Thiovit Jet	7.1±1.7	78.3	14.7±1.2	84.2	46.4±1.6	50.8

The content of sugars in grapes of cv. Bastardo magarachskiy 2019-2021 yield on the control and experimental plots averaged 185±28 gL^{-1}, titratable acids – 7.3±1.1 gL^{-1}. Significant differences between grapes obtained in organic and conservative farming systems under experimental conditions in terms of the content of sugars and titratable acids were not revealed.

Table 3 presents the parameters of the phenol and oxidase complexes in grapes obtained in different farming systems. Analysis of the data allows us to state that the effect of the biofungicides Extrasol and Thiovit Jet in the system of organic farming on the accumulation of phenolic substances in grapes and the activity of polyphenol oxidase largely depended on the background level of development of powdery mildew and the biological effectiveness of the treatment.

At a high (93-94 %) level of development of powdery mildew , the absence of any protective treatment of plants led to an increase in the technological reserve of phenolic substances in grapes by 55–77 %, anthocyanins – by 12–47 % compared with grapes obtained using chemical protective funds. On the contrary, with a background level of disease development of 33 %, there was a decrease in indicators by 18 % and 10 %, respectively. Treatment of grapes with Extrasol and Thiovit Jet in 2019–2020 allowed to control the development of powdery mildew at the level of 7.1 % and 14.7 %, chemical means of protection – 4.5 % and 17.1 %. At the same time, the technological stock of phenolic components in grapes from the experimental plots was 4 % and 59 %, anthocyanins – 3–12 % higher than in the control batches of grapes. On the contrary, in 2021, when the development of powdery mildew after treatment of the vineyard with a biofungicide in combination with colloidal sulfur remained at the level of 46 %, there was a decrease in the content of the technological reserve of phenolic substances and anthocyanins in grapes by 7–10 % compared to the batches treated with chemical protection agents.

Figure 1 shows the change in the content of the components of the phenol complex in Bastardo magarachskiy grape cultivars obtained in the organic farming system (using biofungicides and without any treatment), relative to that in conservative farming, depending on the degree of development of powdery mildew . The dependence graph shows the area of the least efficiency of the organic farming system in terms of the accumulation of components (especially clearly expressed in the case of the technological reserve of phenolic compounds), corresponding to the development of powdery mildew at the level of 30-50 % (Figure 1).

Table 3. Indicators of the phenolic complex and oxidase activity of grape must.

Scheme of processing	TR PhC, mgL^{-1}			Concentration of anthocyanins, mgL^{-1}			Polyphenol oxidase activity of the must, c.u. (×102)		
	2019	2020	2021	2019	2020	2021	2019	2020	2021[a]
ChPS + control	1822±182	2631±210	2345±197	845±80	1199±96	1055±103	16.3±1.6	25.0±2.3	6.7±0,4
Extrasol+	2228±134	1695±119	1321±124	938±49	814±48	938±98	6.6±0.5	15.0±1.7	6.2±0,3
Thiovit Jet	2328±93	2708±135	1233±147	1057±53	835±50	847±102	22.0±1.5	17.0±1.4	3.9±0,1

[a] the analysis was performed using only healthy bunches

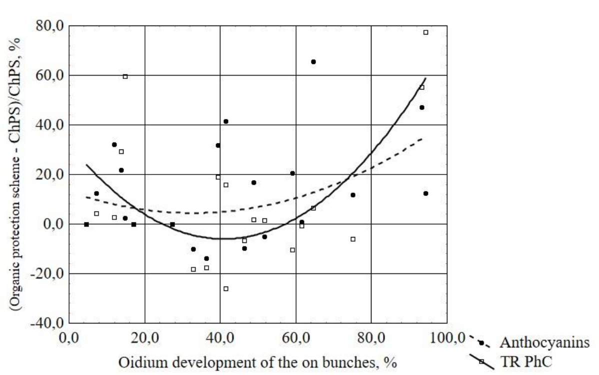

Fig. 1. Efficiency of an organic farming system in relation to the change in the content of phenolic components depending on the degree of development of powdery mildew (compared to ChPS treatment)

Polyphenol oxidase activity of grapes obtained in 2019 and 2020 in the system of conservative farming with the use of chemical means of protection, averaged 0.066 and 0.150 c.u. The absence of plant treatment in organic farming led to an increase in oxidase activity by 2.5 and 1.7 times, and the use of Extrasol and Thiovit Jet – by 3.3 and 1.1 times, respectively. In 2021, we evaluated the polyphenol oxidase activity of grapes not for the entire harvest, but only for healthy bunches selected for winemaking and further research. With this methodological solution, in all batches of grapes, a low level of enzymatic activity was recorded – 0.039–0.067 c.u.: this is consistent with the position that induction of polyphenol oxidase activity occurs at the site of plant damage by fungal infections [10].

The presented results can be summarized as follows: the use of microbiological preparations in combination with colloidal sulfur in organic farming makes it possible to control the development of powdery mildew on grapes even during epiphytoty years, among other things, by stimulating the phenolic and oxidase components of the grape defense system.

4 Conclusions

Based on a three-year study on the use of the biofungicides Extrasol based on a live culture of *Bacillus subtilis* in combination with colloidal sulfur in organic farming on table and wine grapes, the following conclusions were made.

The use of the biofungicides Extrasol, with a properly selected scheme, controls the development of powdery mildew on grapes at the level of a conservative farming system, which provides for the use of chemical plant protection products, including in the years of epiphytoties and with a reduction in the total number of treatments.

Treatment of grapes with Extrasol stimulates the plant's own defense system in terms of the formation of phenolic and oxidase complexes. The degree of influence of bacterial preparations on the parameters of the phenolic complex of grapes depends on the background level of development of powdery mildew and the biological effectiveness of the treatment: the least effect compared to the treatment with chemical protective agents in relation to the accumulation of phenolic substances, incl. anthocyanins, in cv. Bastardo magarachskiy grapes was observed at a level of development of the disease of 30–50%.

The use of the biofungicides Extrasol on the Italia grape cultivar was accompanied by an increase in bunch weight and yield at the output of standard products at the level of processing grapes with chemical protection agents, a slight increase in the content of titratable acids and an improvement in organoleptic characteristics.

Further research involves studying the effect of processing grapes with an extended range of microbiological preparations in the organic farming system on the dynamics of the quality of table grapes during storage and the quality of wines.

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