Biologization of grape growing technologies to obtain safe and high-quality products

Elena Ostroukhova^{1,*}, *Natalia Aleinikova*¹, *Irina Peskova*¹, *Evgenia Galkina*¹, *Elena Bolotianskaia*¹, and *Olga Zaitseva*¹

¹Federal State Budget Scientific Institution All-Russian National Research Institute of Viticulture and Winemaking Magarach of the RAS

Abstract. Viticulture is impossible without the use of chemical plant protection agents. Their use follows to ecological destabilization of ampelocenoses and constitutes real danger to human health. Last years there is a tendency to biologize the protection technologies that the relevance of studies of the influence of biopreparations on the quality of grapes and wines. The article presents the results studies of fungicide action of the preparations Biocomposite-correct and Biocomposite-protect. Their high anti-mycotic activity (97-100%) against *Trichothecium roseum* and *Botrytis cinerea* has been established. The high efficiency in the control of grey rot on grapes cultivar Merlot showed a two-fold application of the Biocomposite-protect – the damage to grapes decreased by 2 times in comparison with the control and amounted to 0.3%. Using of biofungicides did not affect the of mush/must fermentation. The test wines were distinguished by a higher (by 13-28%) concentration of phenolic substances. The use of Biocomposite-protect followed to an increase of anthocyanins concentration (by 1.5 times) and the total dry extract (by 12%) in wines; decrease – of titratable acids; Biocomposite-correct – decrease the total dry extract (by 10%). Using of biological preparations for grape protection did not influence the organoleptic quality of wines.

1 Introduction

An important agrotechnical approach to preserve grape yield is its protection from pests and diseases. A significant role in solving this problem belongs to pesticides, which use guarantees the stability of yielding power and quality of grape products. However, recent studies have shown the negative consequences of use of chemical fungicides and insecticides - their accumulation in such ecosystem objects as soil, water, living organisms; the loss of useful fauna and microflora; the emerging of populations of harmful organisms resistant to chemical preparations [1, 2]. As a result, there are massive epiphytotics of disease, requiring the increasing use of chemicals to control them. All these negative consequences of pesticide using have led to understanding the necessity to improve plant protection, the transition from individual techniques and methods to their integration in the system, the development of more sustainable means and methods [3]. One of the

* Corresponding author: bioxim2012@mail.ru

alternatives to chemical plant protection agents is the use of biological preparations [4, 5]. Microorganisms isolated from nature and introduced again into natural conditions as plant protection agents allow to avoid undesirable changes in biocenoses and to preserve useful species. The main advantage of microbiological plant protection agents based on naturally occurring viruses, bacteria, fungi is their specificity – the ability to infect certain objects without harming humans, animals, birds and useful insects [6, 7].

A wide range of biological preparations is now presented in Russian market. The developments of the company “Shchelkovo Agrokhim” are among them – Biocomposite-correct, SC and Biocomposite-protect, L. However, despite the growing popularity of biologized plant protection agents, there is a lack of studies concerning their use on grape plantations and the effect on the composition of grape berry and wine, which determines the urgency of this problem [8, 9].

The purpose of the present research was to study the antifungal efficiency of use of biological preparations Biocomposite-correct, SC and Biocomposite-protect, L against pathogenic micromycetes; their effect on the main quality characteristics of grapes and wines, the rate of fermentation.

2 Materials and methods

The research was carried out in 2018-2019 on the basis of laboratories of plant protection and still wines of the FSBSI Institute Magarach of the RAS. The studied biological preparations, representing a set of highly effective strains of bacteria and products of their metabolism, were kindly provided by JSC “Shchelkovo Agrokhim”: Biocomposite-correct, SC (the consortium of strains of several types of beneficial bacteria) and Biocomposite-protect, L (strain 11RW *Pseudomonas asplenii*).

A preliminary assessment of antimycotic efficiency of biological preparations was carried out in laboratory conditions in accordance with the methods for assessing the biological activity of biological control agents *in vitro* [10].

The assessment of efficiency of biological preparations Biocomposite-protect, L (1 Lha⁻¹) and Biocomposite-correct, SC (2 Lha⁻¹) in relation to grape rots in field conditions was carried out on wine grape cultivar Merlot (Uglovoye village, Bakhchisarai district, Republic of Crimea), cultivated in accordance with agrotechnical actions recommended for this zone of viticulture, of 2014 planting year, rootstock Berlandieri × Riparia Kober 5BB, planting scheme – 2.5 × 1.5 m, bush training – vertical cordon on a middle trunk, open-earth culture, on drip irrigation. The establishment of experiments and registration were carried out according to the methods and techniques generally accepted in viticulture and plant protection. Eight recording bushes in four replicates (2 bushes per replicate) were selected. [11, 12]. The experimental scheme included two testing variants and the control (system of grape protection and treatment adopted in the enterprise, without using of anti-rot preparations (CPA) (Table 1). The studied biological preparations were used twice: 1st treatment - 03/08/2019 – “softening of berries”; 2nd treatment – 17/08/2019 – “berries ripe for harvest”.

Wine production was carried out according to the scheme generally accepted for the production of red table wines. Mush/must fermentation of the control batch and the batch obtained using the Biocomposite-correct preparation was carried out using the yeast strain I-652; in case of using the preparation Biocomposite-protect batches – on the yeast strain I-25 from the CFC Collection of Microorganisms of Winemaking Magarach. The fermentation process was controlled by the temperature of fermenting must and changes in its consistency. Samples of the must were taken for analysis once every day, up to complete fermentation of sugars. Evaluation of intensity of alcoholic fermentation of the mush / must

in control and experimental batches of grapes was carried out on the basis of the dynamics of sugar utilization.

Table 1. Experimental scheme.

Growth stage (date of treatment)	Destructive objects	Biological preparation	Biological preparation	Management system
“Softening of berries” (03/08/2019)	Oidium	no	no	Falcon, EC (0.3 L ha ⁻¹) Tiovit Jet, WDG (3.5 kgha ⁻¹)
	Rots	Biocomposite-protect, L (1 Lha ⁻¹)	Biocomposite- correct, SC (2 Lha ⁻¹)	no
“Berries ripe for harvest” (17/08/2019)	Oidium	no	no	Universal, WP (0.3 kg)
	Rots	Biocomposite-protect, L (1 Lha ⁻¹)	Biocomposite- correct, SC (2 Lha ⁻¹)	no

Experimental and control samples of wines were prepared in 2-3 replicates: the sample size consisted of 9 batches. The organoleptic evaluation of wines was carried out in accordance with ISO 8589:2007.

The analysis of chemical composition of grapes / wines was provided using methods of analysis adopted in oenological practice [13]. All chemical tests were performed in triplicate.

The obtained experimental entries were processed by statistical methods of descriptive statistics and analysis of variance [12] using the statistical software of Excel electronic worksheets and SPSS Statistics 17.0 program.

Figures and text of the article show the arithmetic mean values of parameters, the standard deviation of which did not exceed 8%.

3 Results and discussion

The results of laboratory tests of the effectiveness of biofungicides against pathogenic micromycetes isolated from grape plants into the pure culture (*Alternaria* sp.; *Botrytis cinerea* Pers.; *Macrophoma flaccida* (Viala et Rav.) Cav.; *Trichothecium roseum* (Pers.) Link; *Fusarium* sp.) showed, that both of the studied biological preparations performed a consistently high anti-mycotic activity (97-100%) against *Trichothecium roseum*, starting from the fifth day of cultivation (Fig. 1). It was noted that the effectiveness of fungicide action of the Biocomposite-correct, SC preparation against other microorganisms was growing with an increase in the time of cultivation up to 10 days and ranged from 40% (*Alternaria* sp.) to 59% (*Macrophoma flaccida*). As far as Biocomposite-protect, L preparation is concerned, preservation of high anti-mycotic activity (100%) against *Botrytis cinerea* was observed, regardless the duration of cultivation. In relation to other microorganisms, a decrease in the fungicide effect by 20% (*Alternaria* sp.), 12% (*Macrophoma flaccida*) and 22% (*Fusarium* sp.) was noted. This fact requires further research.

The obtained results served as the basis for testing biological preparations in the conditions of field stationary experiment in 2019. The intensity of infection of grape bunches with gray rot (*Botrytis cinerea*) on the plot with chemical plant protection system, where fungicides against this disease were not used, did not exceed 0.6%. Two-fold

application of the biofungicide Biocomposite-protect, L (1 Lha⁻¹) contributed to a decrease in the disease progression by an average of 2 times (the affection was less than 0.3%). At the same time, the progression of disease on the plot treated with Biocomposite-correct, SC (2 Lha⁻¹) remained at the level of the variant without special treatment against the gray rot, and amounted to 0.6%.

Fig. 1. Fungicide activity of preparations Biocomposite-correct, SC and Biocomposite-protect, L for species of pathogenic micromycetes *in vitro*.

Evaluating the effect of biological preparations used on the indicators of grape yield, it should be mentioned that a good conditioned grape yield on both the experimental and the control variant was at the same high level and amounted to 2.6-2.7 kg per bush (Table 2).

Table 2. The effect of biological preparations Biocomposite-correct and Biocomposite-protect on the grape yield parameters, 2019.

Variant	Average bunch weight, g	Number of bunches, pcs per bush	Yield, kg per bush	Concentration of sugars, gL ⁻¹
CPA + Biocomposite-protect, L	118.7	21.9	2.6	242
CPA + Biocomposite-correct, SC	122.2	22.1	2.7	231
CPA	119.4	22.5	2.7	242
HCP ₀₅	5.8	1.6	0.3	1,0

According to the requirements of regulatory documents and information from scientific and technical literature, red table wines are recommended for production from grapes with concentration of sugars not less than 180 gL⁻¹, titratable acids – 5-8 gL⁻¹, pH of the must – 3.2-3.8. As can be seen from the data presented in Table 2, the studied grapes, obtained during the use of chemical protecting agents and ecologically sound systems of protective measures, in terms of carbohydrate-acid complex parameters, were meeting the requirements of regulatory documents and scientific recommendations for production of red table wines. At the same time, there was no significant difference in values of considered parameters of grapes in the control and experimental batches: the concentration of sugars was in between 231-242 gL⁻¹, titratable acids – 7.1-7.4 gL⁻¹, pH value – 3.30-3.35.

The concentration of phenolic substances in a berry is technologically important parameter of grapes used for production of red wines. Technological reserve of phenolic substances in grapes of the control batch consisted of $2621 \pm 131 \text{ mgL}^{-1}$. Using of microbiological preparations in the grape plant protection system has led to a decrease in technological reserve of phenolic substances in berries by 12% in the case of using the Biocomposite-correct, SC preparation, and by 16%, when using the Biocomposite-protect, L preparation, relative to the value of parameter in the control batches. At the same time, the use of biological preparations contributed to an increase in the concentration of anthocyanins: in case of using the preparation Biocomposite-correct, SC – by 10%; Biocomposite-protect, L – by 40%. Perhaps this is a consequence of intensification of the grape plant protective actions under the influence of biological preparations, to a large extent, due to the biosynthesis of anthocyanins stimulation. As a result, the content of anthocyanins in grapes of experimental batches consisted of $1076\text{-}1367 \text{ mgL}^{-1}$, and in the control – 978 mgL^{-1} . In general, the technological stock of phenolic substances and anthocyanins in grapes of both the control and experimental batches corresponded to the optimal for table dry wines production values: $\geq 2000 \text{ mgL}^{-1}$ and $\geq 500 \text{ mgL}^{-1}$, respectively [14].

A distinctive feature of the experimental grape batches is a high monophenol monooxygenase activity of the must – $0.163\text{-}0.234 \text{ c.u.}$, which is 1.4-2 times higher than in the control. Indirectly it indicates the intensification of defense mechanisms in the grape plant when using biological preparations [15].

The study of the effect of using biological preparations to protect grapes from pathogens on the course of the fermentation process has made it possible to establish the following. Analysis of dynamics of sugar utilization with yeast cultures I-652 and I-25 showed the same 2 days of duration of the period before the beginning of grape mush fermentation in the control and experimental batches (Fig. 2). During this period, $88.8 \pm 3.18 \text{ gL}^{-1}$ of mush were utilized in the control batches, and $73.3 \pm 3.18 \text{ gL}^{-1}$ in the experimental ones.

Fig. 2. Dynamics of concentration of sugars in the process of mush / must fermentation.

In the control and experimental batches (using the Biocompositec-correct, SC preparation), the greatest difference in the intensity of sugar fermentation using yeast I-652 was observed on the 3rd and 4th day of the process. The highest intensity of fermentation process in the experimental variant fell on the 3rd day, during which 90.5 gL⁻¹ of sugars were utilized; in the control variant – the largest amount of sugars was fermented on the 4th day – 109 g/dm³. To the end of 5th day, almost all sugars were fermented dry in both the control and experimental batches of must. In the control and experimental batches (using the Biocomposite-protect, L preparation), the difference in the amount of fermented sugars at each monitoring point ranged from 5 to 31 gL⁻¹. Moreover, on the 6th day, a smaller amount of utilized sugars was recorded in the experiment. After 7 days of fermentation, the amount of utilized sugars in the must of both the control and experimental grape batches was 237 gL⁻¹.

Thus, using of Biocomposite-correct, SC or Biocomposite-protect, L preparations in the grape protection system did not affect the mush / must fermentation process, carried out on I-652 and I-25 yeast cultures.

The results of analysis of the chemical composition of table dry wines from grapes of the control and experimental batches indicate that, regardless of the system of grape protection from diseases, the wines met the requirements of GOST 32030 in terms of the volume fraction of ethyl alcohol, concentration of sugars, titratable and volatile acids and total dry extract (Table 3). At the same time, we registered a decrease in the concentration of titratable acids in wines produced from the experimental grape batches and processed with the Biocomposite-protect, L preparation, by 21% relative to the control. Moreover, for the same experimental wines, an increase in the content of the total dry extract by 12% compared to the control variants was recorded. On the contrary, in experimental wine batches obtained from grapes processed with Biocomposite-correct, SC, a decrease in the concentration of the total dry extract by 10% was observed in comparison with the control. In wines of grape batches processed with the biological preparation Biocomposite-correct, SC, the pH value was 0.12 units higher, and with the biofungicide Biocomposite-protect, L - 0.11 units lower than the value of this parameter in the control.

Table 3. Parameters of chemical composition of table dry wines from grapes, obtained against the background of using various systems of protection.

Scheme of grape processing	Vol. fraction of ethyl alcohol, % vol.	Concentration						pH
		gL ⁻¹				mgL ⁻¹		
		sugars	titratable acids	volatile acids	total dry extract	phenolic substances	anthocyanins	
CPA	14.3	2.2	5.4	0.51	24.2	1005	114	3.39
CPA + Biocomposite-correct, SC	12.9	2.0	5.1	0.48	21.8	1140	118	3.51
CPA	14.4	2.5	5.8	0.49	22.5	1170	130	3.52
CPA + Biocomposite-protect, L	14.7	1.7	4.6	0.47	26.3	1499	190	3.41
GOST 32030	8.5-15.0	≤ 4.0	≥ 3.5	≤ 1.20	≥ 18.0	not rated		

Despite the fact that a decrease in the technological stock of phenolic substances was registered in the experimental grape batches in comparison with the control batches, an increase in the concentration of phenolic substances was observed in the experimental wines, which in the variant of processing with the Biocomposite-correct, SC preparation

amounted to 13%, with Biocomposite-protect, L – 28%. At the same time, in the samples of wine from grapes treated with Biocomposite-protect, L, the content of anthocyanins was 1.5 times higher than the value of the parameter in the control wines; in the variant with Biocomposite-correct, SC it was at the same level. Since grapes used in the research, regardless of the protection system, were technically ripe, the distinguished features of phenolic (including anthocyanin) complex of wines, obtained from grapes of experimental batches, and especially pronounced when processing grapes using the Biocomposite-protect, L preparation, should be considered as a positive fact.

Assessing the effect of using biological preparations for grape plant protection on organoleptic characteristics of the finished product, we should state the following. The control and experimental wine samples were characterized by dark ruby color, varietal aroma of berry direction with spicy hints, full-bodied, soft and / or tannin-velvety, harmonious flavor. The aroma of the control wines obtained using the I-25 yeast culture showed fruit tones and a pronounced cherry tone, which slightly smoothed varietal spicy-poppy hints. The average tasting evaluation of the control wines produced using the yeast culture I-25 was 7.74 points, and using the yeast culture I-652 – 7.83 points. The experimental wines made of grapes processed using the Biocomposite-correct, SC preparation and I-652 yeast culture were rated, on average, by 7.86 points; wines obtained from grapes processed with the use of Biocomposite-protect, L and the yeast strain I-25 – by 7.8 points. The difference in the average values of wine tasting assessment is 0.06 points, which does not exceed 10% and is considered insignificant as far as organoleptic analysis is concerned. Thus, using of microbiological preparations Biocomposite-correct, SC and Biocomposite-protect, L of JSC “Shchelkovo Agrokhim” against the background of using chemical plant protection agents did not significantly affect the sensory characteristics of wines.

4 Conclusion

In laboratory conditions, high antimycotic activity (97-100%) of preparations Biocomposite-correct, SC and Biocomposite-protect, L against *Trichothecium roseum*; Biocomposite-protect, L – against *Botrytis cinerea* (100%) was observed. It was shown that these preparations effectively control the development of such pathogenic micromycetes as *Alternaria* sp., *Macrophoma flaccida* Cavara; *Fusarium* sp. Field tests of biological preparations showed high efficiency of two-fold application of the biofungicide Biocomposite-protect, L in the control of gray rot progression – the intensity of grape affection decreased by half in comparison with the control; the disease progression on the plot treated with Biocomposite-correct, SC, remained at the level of the variant without specialized processing for protection against gray rot, and amounted to 0.6%. It was found that when biological preparations are included in the general system of grape protection from harmful organisms (experimental systems) in comparison with the standard protection system, there is a decrease in technological stock of phenolic substances in berries by 12-16%, but there is also an accumulation of anthocyanins during their ripening, increasing their potential amount by 10-40%. The use of biofungicides did not affect the process of mush / must fermentation; promoted to better extraction and preservation of phenolic substances during the processing of grape, leading to an increase in the concentration of phenolic substances in wines by 13-28%. Using of the Biocomposite-protect, L preparation has led to an increase in the content of anthocyanins in wines (by 1.5 times), total dry extract (by 12%) and a decrease in the concentration of titratable acids; using of the Biocomposite-correct, SC preparation did not affect the concentration of anthocyanins, titratable acids in wines and contributed to a decrease in the content of total dry extract (by 10%). The organoleptic quality of wines was not significantly affected by the use of

biological preparations to protect the grape plant. In general, the obtained results indicate the prospects of using biological preparations Biocomposite-correct, SC and Biocomposite-protect, L of JSC "Shchelkovo Agrokhim" for biologization of grape growing technology and obtaining safe and high-quality products.

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