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Original scientific paper

BACILLUS SPP. – A POTENT BIOLOGICAL CONTROL AGENTS AGAINST DOWNY MILDEW OF GRAPEVINE

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ABSTRACT

Plasmopara viticola, the causal agent of downy mildew of grapevine, is one of the most devastating and economically most important grapevine pathogens worldwide. In the Republic of North Macedonia, control of downy mildew in grapevine is mainly achieved by application of synthetic fungicides. However, the long-term and continuous use of synthetic fungicides leads to appearance of resistant strains of the pathogen, residues and environmental pollution. Alternatives to chemical control such as the use of biological control agents or resistant cultivars, currently play a small role in controlling this disease. A promising alternative strategy that could replace or be combined with fungicides are biofungicides. The main goal of the experiment was to observe the possibility for biological control of grape downy mildew by the use of two novel biofungicides, Sonata (a.m. *Bacillus pumilus* QST 2808) and Serenade Aso (a.m. *Bacillus subtilis* QST 713). Experiment was conducted during the 2019 in two vineyard regions in the Republic of North Macedonia (Demir Kapija and Negotino), on three grape varieties (*Merlot*, *Riesling* and *Vranec*). Both tested biofungicides showed almost equal efficacy, with more than 94% reduction of disease severity index on leaves in Vranec and Riesling varieties and with more than 97% reduction of disease severity index on leaves in Merlot variety. On bunches, both biofungicides achieved 100% reduction of disease incidence and severity index in all three varieties. The results showed that *Bacillus pumilus* and *Bacillus subtilis* are potent biological agents and have a prospective use for control of downy mildew in grapevine.

Key words: *Bacillus* spp., control, downy mildew, grapevine.

INTRODUCTION

Plasmopara viticola, the causal agent of downy mildew of grapevine, is one of the most devastating and economically most important grapevine pathogens worldwide (Perazzolli et al., 2012). The introduction of this pathogen in the second half of the 19th century from North America to Europe resulted in severe losses and devastated wine industry in Europe (Gessler et al., 2011). Even today, this pathogen can be only controlled by repetitive applications throughout the season with synthetic fungicides in conventional viticulture, or by copper-based products in organic viticulture (Pezzotti et al., 2020). In addition, most commercially important cultivars of the European grapevine (*Vitis vinifera*) are highly susceptible to this pathogen (Zang et al., 2020). In the Republic of North Macedonia, control of downy mildew in grapevine is mainly achieved by

application of synthetic fungicides. However, the long-term and frequent use of synthetic fungicides leads to appearance of resistant strains of the pathogen, residues and environmental pollution (Pimentel et al., 1992; Chen et al., 2007; Zang et al., 2020). A promising alternative strategy that could replace or be combined with fungicides are biofungicides (Romanazzi et al., 2016). Recently, a huge number of microorganisms isolated from nature have been recognized as suitable biological control agents for developing biofungicides. Several biocontrol agents have been reported as promising against grapevine fungal pathogens, including *Plasmopara viticola* (Alfonzo et al., 2012). Moreover, some of them have been developed and launched as biofungicides in viticulture (Hamaoka et al., 2021). For example, some *Bacillus* sp. strains are already available on the market, including the Republic of North Macedonia, such as *Bacillus subtilis* QST-713 (product Serenade Aso®), which is mainly used for control of Botrytis bunch rot and *Bacillus pumilis* QST 2808 (product Sonata®), which is used for control of powdery mildew of grapevine. *Bacillus* species are known to involve various modes of action as biocontrol agents, including direct antibiosis, competition for niches and nutrients and induction of the host systemic resistance (Santoyo et al., 2012; Compant et al., 2013). They are also known to produce various bioactive compounds (cyclic lipopeptides), such as surfactin, iturin, and fengycin, which display strong suppressive effect on wide range of pathogens (Yan Li et al., 2019). In addition, recent study has shown that endophytic Bacillus can also inhibit grapevine downy mildew disease (Zhang et al., 2017).

The main goal of the experiment was to evaluate the biological efficacy of two novel biofungicides, Sonata (a.m. *Bacillus pumilus* QST 2808) and Serenade Aso (a.m. *Bacillus subtilis* QST 713) against *Plasmopara viticola* and to identify the possibility for biological control of grape downy mildew in the Republic of North Macedonia with promising biocontrol agents.

MATERIALS AND METHODS

Experiment was conducted during the 2019 in two vineyard regions in the Republic of North Macedonia (Demir Kapija and Negotino). In the region of Demir Kapija, the trials were performed on *Merlot* and *Riesling* grapevine varieties, while in the region of Negotino only *Vranec* grapevine variety was included in the trials. All three grape varieties were grown according to the Guyot trellis system. In each grape variety, two different biofungicides were tested, compared with the untreated control. The active ingredients and rates of application are listed in Table 1. A randomized block design with three replicates was used. Each plot was consisted of 2 rows, with 5 plants of grapevine in a row. Tested biofungicides were applied by use of hand-compression sprayer with a volume of 15 L. Total of 3 foliar applications were performed with biofungicides Sonata and Serenade Aso during the growing season, with an interval of 7-10 days (14.6.2019, 21.6.2019 and 28.6.2019, respectively). Evaluation of grape downy mildew infections was carried out in all vines of each treatment on 300 leaves and 100 bunches randomly selected per plot, 7 days after the third treatment (5.7.2019). Disease incidence was expressed as the percentage of infected leaves and bunches (proportion of plant units that are infected (leaves and bunches) in relation to the total number of the units examined). The disease severity was assigned to 11 levels, according to the percentage of surface covered by downy mildew symptoms, from 0 (healthy leaf) to 10 (over 90% of leaf surface showing symptoms), as described by Romanazzi et al. (2016). Regarding the bunches, the disease severity was assigned to 8 levels, according to the number of infected berries and the percentage of the grape bunches showing symptoms, such as: 0=healthy bunch, 1=1 to 5 infected berries, 2=6 to 10 infected berries, 3=11 to 20 infected berries, 4=25% of bunch showing symptoms, 5=26 to 50% of bunch showing symptoms, 6=51 to 75% of bunch

showing symptoms and 7≥75% of bunch showing symptoms. The disease severity index was than calculated according to Townsend-Heuberger's formula (Townsend-Heuberger, 1943). Efficacy of the tested fungicides in the terms of reduction of disease incidence and disease severity index was calculated according to the Abbott's formula (Abbott, 1925).

The obtained data was subjected to ANOVA analysis and LSD test was used to compare the mean values for each trait from the analyzed grapevine varieties with the obtained mean values in the corresponding control varieties.

Table 1. Tested biofungicides in the regions of Demir Kapija and Negotino in 2019

No.	Biofungicides	Active substance	Content of active substance	Producer	Rate of application
1.	SONATA®	*Bacillus pumilus* strain QST 2808	14.3 g/L	Bayer Germany	5 L/ha
2.	SERENADE ASO®	*Bacillus subtilis* strain QST 713	13.96 g/L	Bayer Germany	4 L/ha
3.	CONTROL		Untreated		

RESULTS AND DISCUSSION

The results obtained from the trials are presented in Table 2. The destructive capacity of grape downy mildew was confirmed in the control (untreated) variants of all three tested grape varieties (*Merlot*, *Riesling* and *Vranec*). Accordingly, the disease incidence in the control variants of the three grape varieties ranged between 72% - 88% on leaves and 52% - 72% on bunches. The highest disease incidence on leaves was observed in Merlot variety (88%), while the highest disease incidence on bunches was detected in Vranec variety (72%). The disease severity index in the untreated (control) variants ranged between 27% - 38.33% on leaves and 8.6% - 24.6% on bunches, respectively. However, the downy mildew destructive potential was most clearly demonstrated in the control variant of Vranec variety, in which the highest disease severity index on leaves and bunches was observed (38.33% and 24.6%, respectively).

The tested biofungicides Sonata and Serenade Aso performed remarkably high efficacy in controlling the downy mildew disease of grapevine – *Plasmopara viticola*. During the assessment, both tested biofungicides displayed significant reduction of disease incidence on leaves and complete reduction of disease incidence on bunches, compared with the control (untreated) in all three grapevine varieties. Treatments with biofungicide Sonata had significantly reduced disease incidence compared with the control by 95.45% in Merlot variety and by 88.88 and 89.47% in Riesling and Vranec varieties, respectively. Similar trend was observed in treatments performed with the biofungicide Serenade Aso, where the disease incidence on leaves compared with the control was also significantly reduced by 96.59, 90.27 and 85.52% in Merlot, Riesling and Vranec varieties, respectively. In the same assessment, biofungicides Sonata and Serenade Aso achieved significant reduction of disease severity index on leaves and full protection of bunches, compared with the control, in all three grapevine varieties. Performed treatments with biofungicide Sonata had significantly reduced the severity index on leaves compared with the control by 98.11, 94.44 and 96.53%, whereas biofungicide Serenado Aso gave very similar results, with reduction of disease severity index compared with the control of 96.59, 95.70 and 95.22% in Merlot, Riesling and Vranec varieties, respectively. Regarding the disease severity index on bunches, both biofungicides displayed complete reduction and performed full protection on bunches (100%). The full protection on bunches was achieved under moderate to heavy disease pressure of the downy

mildew during the growing season, which resulted in high disease incidence and severity index on leaves and bunches in the control (untreated) of all three tested grapevine varieties. On the basis of the obtained results in the field trials, it was found that *Bacillus pumilus* strain QST 2808 (Sonata) and *Bacillus subtilis* strain QST 713 (Serenade Aso) are powerful and reliable biocontrol agents which can provide excellent protection against grape downy mildew, caused by *Plasmopara viticola*.

The potential of *Bacillus subtilis* and *Bacillus pumilus* as powerful biocontrol agents against grape downy mildew (*Plasmopara viticola*), has been also confirmed in several similar studies. In a study conducted by Zhang et al. (2017), the effect of *Bacillus subtilis* GLB191 and *Bacillus pumilus* GLB197, against *P. viticola*, was tested in the field trials during two successive years (2013-2014). The obtained results revealed significant decrease in disease severity after eight spray treatments, suggesting both strains as potential biocontrol agents of grape downy mildew.

Furuya et al. (2011) isolated the strain KS1 from grape berry skin, which was later identified as a new strain of *B. subtilis* according to morphological, biochemical, and genetic analyses. The biocontrol activity of KS1 against grapevine fungal diseases in vineyards, including against downy mildew was evaluated during 3 consecutive years (2007 to 2009). The results showed that grape downy mildew was significantly reduced on berry skins and leaves by using this treatment.

The study conducted by Li et al. (2019), have shown that *B. subtilis* GLB191 is an efficient biological control agent against grape downy mildew. The study clearly demonstrated that the GLB191 supernatant is highly active against downy mildew, due to the presence of cyclic lipopeptides (surfactin and fengycin). Furthermore, the study proved that the biocontrol activity of GLB191 results from both direct effect against the pathogen and stimulation of the plant defenses (induction of defense gene expression and callose production).

In addition, some other *Bacillus* species were also found to be effective in control of grape downy mildew (*Plasmopara viticola*). Thus, strain KOF112, which was isolated from the Japanese indigenous grapevine, was identified as a strain of *Bacillus velezensis*. This strain, in studies conducted by Hamaoka et al. (2021), showed biocontrol activity against several grapevine diseases, such as *B. cinerea, C. gloeosporioides* and *Plasmopara viticola*. In the case of *P. viticola*, KOF112 inhibited the release of zoospores from zoosporangia, but not zoospore germination. Moreover, the study showed that KOF112 has drastically upregulated the expression of genes encoding class IV chitinase and ß-1,3-glucanase in grape leaves, which suggests that KOF112 also works as a biotic elicitor in grapevine.

Table. 2 Efficacy of the biofungicides Sonata and Serenade Aso in the control of *Plasmopara viticola*

Efficacy of the biofungicides Sonata and Serenade Aso in the control of *Plasmopara viticola* during the 2019 growing season in Merlot variety

	SONATA		**SERENADE ASO**		**CONTROL**	
	Leaves	Bunches	Leaves	Bunches	Leaves	Bunches
Disease incidence (%)	4 [a]	0 [a]	5 [a]	0 [a]	88 [b]	52 [b]
Disease severity index (%)	0.66 [a]	0 [a]	0.83 [a]	0 [a]	35 [b]	8.6 [b]
Efficacy of biofungicides in the reduction of disease incidence (%)	95.45	100	96.59	100	/	/
Efficacy of biofungicides in the reduction of disease severity index (%)	98.11	100	97.62	100	/	/

Efficacy of the biofungicides Sonata and Serenade Aso in the control of *Plasmopara viticola* during the 2019 growing season in Riesling variety

	SONATA		**SERENADE ASO**		**CONTROL**	
	Leaves	Bunches	Leaves	Bunches	Leaves	Bunches
Disease incidence (%)	8 [a]	0 [a]	7 [a]	0 [a]	72 [b]	68 [b]
Disease severity index (%)	1.5 [a]	0 [a]	1.16 [a]	0 [a]	27 [b]	13.3 [b]
Efficacy of biofungicides in the reduction of disease incidence (%)	88.88	100	90.27	100	/	/
Efficacy of biofungicides in the reduction of disease severity (%)	94.44	100	95.70	100	/	/

Efficacy of the biofungicides Sonata and Serenade Aso in the control of *Plasmopara viticola* during the 2019 growing season in Vranec variety

	SONATA		**SERENADE ASO**		**CONTROL**	
	Leaves	Bunches	Leaves	Bunches	Leaves	Bunches
Disease incidence (%)	8 [a]	0 [a]	11 [a]	0 [a]	76 [b]	72 [b]
Disease severity index (%)	1.33 [a]	0 [a]	1.83 [a]	0 [a]	38.33 [b]	24.6 [b]
Efficacy of biofungicides in the reduction of disease incidence (%)	89.47	100	85.52	100	/	/
Efficacy of biofungicides in the reduction of disease severity index (%)	96.53	100	95.22	100	/	/

Values marked with different letters are significantly different on a level $p<0.05$

CONCLUSIONS

The results showed that *Bacillus pumilus* strain QST 2808 (Sonata) and *Bacillus subtilis* strain QST 713 (Serenade Aso) are potent biological agents and have a prospective use for control of downy mildew in grapevine. The high efficacy of the biofungicides Sonata and Serenade Aso was achieved by proper application (timely and quality spraying), by which their preventive and curative action against the downy mildew of grapevine was emphasized. Proper application of the biofungicides Sonata and Serenade Aso can guarantee reliable protection of grapevine from the cause of downy mildew – *Plasmopara viticola*. The treatments should be preventive and should start before the appearance of the first symptoms or when the weather conditions are favorable for development of *Plasmopara viticola*, with an interval of 7 to 10 days. During the trials, negative effects of biofungicides Sonata and Serenade Aso on grapevine were not observed.

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