Original scientific paper

BIOCONTROL POTENTIAL OF *TRICHODERMA ATROVIRIDE* AND *BACILLUS SUBTILIS* AGAINST *ERYSIPHE NECATOR* UNDER FIELD CONDITIONS

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ABSTRACT

Powdery mildew of grapevine, *Erysiphe necator*, is a very destructive grape disease, causing great economic production losses. That is why, regular fungicide applications are required. In recent years, due to the rise of the environmental awareness, alternative methods in controlling various plant diseases have slowly taken over the primacy from the classic chemical control. The aim of these experiments was to study the possibility for biological control of powdery mildew of grapevine with novel biofungicides Vintec (a.i. *Trichoderma atroviride* strain SC1) and Serenade Aso (a.i. *Bacillus subtilis* strain QST 713). Experiments were conducted during 2023 in the regions of Demir Kapija and Negotino, on two grape varieties, Riesling and Vranec. In the untreated variant in Negotino region, very high level of disease severity (44.8%) on bunches was observed, which demonstrated the destructive potential of this disease in our country. In Demir Kapija region, significantly lower level of disease severity was observed in the untreated variant (7.6%). In conditions of very high levels of disease severity, both of the tested biofungicides showed comparable and good fungicide effectiveness, above 90% (Serenade Aso – 94.64 % and Vintec – 97.03 %). In the region of Demir Kapija, where the level of disease severity was lower, both biofungicides reached absolute efficacy performance of 100%.

Key words: biological control, powdery mildew of grapevine, Serenade Aso, Vintec.

INTRODUCTION

The causal agent of grape powdery mildew, *Erysiphe necator* Schw. (syn. *Uncinula necator* (Schw.) Burr.), represents one of the most important parasitic fungi on this host. Powdery mildew of grapevine is economically very important disease, causing a significant loss in the quantity and quality of yield (Gadoury *et al.* 2001; Ahmed, 2018; Yparraguirre et al., 2020). It attacks all green parts of the plant, i.e. leaves, stems, inflorescences, young berries, and pedicles (Sawant et al., 2012). In our climate conditions it occurs every year, both on wine and table grape varieties, with a stronger or weaker intensity of attack, sometimes developing with epiphytotic proportions, generally causing more damage on the wine varieties.

Management of powdery mildew in grapes is largely dependent upon the use of chemical fungicides, and interestingly, grapes are considered to be one of the high pesticide-demanding crops (Pertot et al., 2017; Arestova and Ryabchun, 2021). Worldwide, an average of 35% of all pesticides produced are used in viticulture (Essling et al., 2021).

Though disease management still relies on various classes of chemical fungicides, their rational use based on pathogen life cycle, observed disease development risk (crop stage, weather) and the risks associated with fungicide (resistance, residue) is always promoted (Sawant et al., 2019). The persistent use of chemical fungicides develops pathogen resistance and environmental pollution (Chen et al., 2007; Zang et al., 2020). The economic costs and negative environmental impact associated with these applications has led to a recent search for alternative strategies, involving biological stimulation of host defence mechanisms (Ait Barka *et al.* 2002).

The development of resistance in pathogens and the environmental and health concerns associated with the residual toxicity of chemical fungicides have driven researchers and grape growers to explore alternative strategies (Yildirim and Dardeniz, 2010; Miles et al., 2012; Fernández-González et al., 2013; Çetinkaya and Fadime, 2016). With the increasing removal of active ingredients and chemical fungicides from the list of acceptable compounds, coupled with the rising demand for residue-free grapes, there is an urgent need to identify effective alternatives for disease management in organic viticulture systems (Carisse et al., 2009; Yildirim and Dardeniz, 2010; Lu et al., 2020).

In this context, the use of microbe-based approaches to control powdery mildew has emerged as a promising solution. These methods are reported to be environmentally friendly, residue-free, and safer for effectively managing the powdery mildew pathogen (Hayes, 2015; Kumar et al., 2021; Pathma et al., 2021; Sellitto et al., 2021). Recently, several microbial biological control agents have been evaluated and utilized for managing powdery mildew in grapes. Notable examples include: *Ampelomyces quisqualis*, *Trichoderma harzianum*, *T. asperellum*, *T. virens*, *Pythium oligandrum*, *Pseudozyma flocculosa*, *Bacillus subtilis*, *B. licheniformis*, *B. brevis*, *B. cereus*, *Pseudomonas fluorescens*, and *Streptomyces cacaoi* (Rao et al., 2015; Damalas and Koutroubas, 2018; Thakur et al., 2020; Salimi and Hamedi, 2021).

Since some of the most studied biocontrol agents are fungi belonging to the genus *Trichoderma* (Harman et al., 2004; Lorito et al., 2010; Woo et al., 2014), and also considerable work has been conducted on the interaction of plant pathogenic fungi with *Bacillus* sp. (Ben Maachia et al., 2010; Kuzmanovska et al., 2023; Sawant, 2023), in this study we have decided to focus on these two biocontrol agents, that is *T. atroviride* (Vintec) and *B. subtilis* (Serenade Aso), and the possibility of replacing the chemical with biological treatments**.**

MATERIAL AND METHODS

The research was conducted during 2023 in two vine growing regions: Demir Kapija and Negotino. In the region of Demir Kapija, the test was conducted on the Riesling grapevine variety and in the region of Negotino, the experiment was performed on the Vranec variety. Both of the varieties were grown on low-cordon trellises. The treatments were preventive. Each trial consisted of three different variants, (two variants tested with biofungicides Vintec and Serenade Aso and the untreated variant - control). The variants are shown in Table 1.

Table 1. Tested variants represented in the regions of Demir Kapija and Negotino in 2023

No.	Biofungicides	Active substance	Content of active substance	Producer	Rate of application
1.	**VINTEC**	*Trichoderma atroviride* strain SC1	1x10^{13} CFU/kg	BI-PA NV/SA Belgium	200 g/ha
2.	**SERENADE ASO**	*Bacillus subtilis* strain QST 713	13,96 g/L	Bayer Germany	4 L/ha
3.	**CONTROL**	Untreated			

A randomized block design with three replicates was used. In each variant, on an experimental block of 100 m^2, a total of 30 plants of grapevine were included. Application of pesticides was foliar, performed with the use of a hand-compression sprayer with a volume of 10 L. Total of 4 foliar applications were performed with the biofungicides Vintec and Serenade Aso, in an interval of 7-10 days. All of the treatments were performed in June, on the following dates: I treatment - 5.6.2023, II treatment - 14.6.2023, III treatment - 21.6.2023 and IV treatment - 28.6.2023. Efficacy of the tested biofungicides was conveyed 7 days after the fourth treatment (5.7.2023). From each variant, 200 leaves and 50 bunches were randomly evaluated, based on which the disease incidence and disease severity on leaves and bunches were calculated. The incidence of the disease (I=incidence) was determined according to the formula:

I% = Number of infected leaves/bunches/Total of leaves/bunches evaluated × 100%

The severity of the disease was classified on a scale of 0 to 5, where: 0 = no infection; 1 = 1-10% of leaf area infected with powdery mildew; 2 = 11-25%; 3 = 26-50%; 4 = 51-75% and 5 = 76-100% (Horsfall and Heuberger, 1942). The disease severity index was, after that, calculated according to the Townsend-Heuberger's formula (Townsend-Heuberger, 1943). The efficacy of the tested fungicides was calculated according to the Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION

During the vegetation in 2023, the disease incidence and severity of powdery mildew in grape vines in the untreated control trial blocks varied among regions and plant parts. In both of the tested regions, Demir Kapija and Negotino, no disease symptoms were observed on the leaves of the infected plants, only on the bunches.

There was also a significant difference of detected disease incidence and severity between the regions. In the region of Demir Kapija, a relatively mild infection with disease severity of 7.6%, and a low level of disease incidence of 20% were measured. In these conditions, both of the tested biofungicides, Vintec and Serenade Aso, used in their proposed rates, achieved maximal level of efficacy (100%) in the treated trial blocks (Table 2).

In the region of Negotino, a very high levels of disease severity (44.8%) and disease incidence (84%) were measured. In these severe disease conditions, both of the tested biofungicides, used in their proposed rates with proper application, showed comparable and very high fungicide efficacy, above 90%. For Serenade Aso, the fungicide efficacy measured was 94.64% and for Vintec was even superior, with 97.03% (Table 3). During the trials, no negative effects were detected during the usage of the biofungicides on the tested plants of grape vine in the proposed rates.

Table 2. Efficacy of the biofungicides Sonata and Serenade Aso in the control of *Erysiphe necator* on Riesling variety in the region of Demir Kapija

	VINTEC		**SERENADE ASO**		**CONTROL**	
	Leaves	Bunches	Leaves	Bunches	Leaves	Bunches
Disease incidence (%)	0	0	0	0	0	20
Disease severity (%)	0	0	0	0	0	7.6
Efficacy of fungicides (%)	/	100	/	100	/	/

Table 3. Efficacy of the biofungicides Sonata and Serenade Aso in the control of *Erysiphe necator* on Vranec variety in the region of Negotino

	VINTEC		**SERENADE ASO**		**CONTROL**	
	Leaves	Bunches	Leaves	Bunches	Leaves	Bunches
Disease incidence (%)	0	8	0	8	0	84
Disease severity (%)	0	1.33	0	2.4	0	44.8
Efficacy of fungicides (%)	/	97.03	/	94.64	/	/

Other authors also found biofungicides based on *Trichoderma* and *Bacillus* species to be very effective against powdery mildew. Sawant (2023) found that induction of the resistance mechanism of grapevines by soil application of specific *Trichoderma* and *Bacillus* strains was another excellent opportunity for reducing disease build-up and spread of powdery mildew and other pathogen diseases. He emphasized that *Bacillus* species had the unique ability to produce endospores, which rendered resistance capacity to adverse environmental conditions.

Due to their capabilities, Gram-positive bacteria, particularly *Bacillus* species, have gained significant attention as effective biological control agents and offer formulation advantages over Gram-negative bacteria (Emmert and Handelsman, 1999; Schisler et al., 2004). *Bacillus* spp. have been shown to control various plant diseases, primarily through the production of broad-spectrum antibiotics and their extended shelf life, which is attributed to their ability to form endospores (Emmert and Handelsman, 1999).

Bacillus biofungicidal effects were also observed by Ben Maachia et al. (2010). In this study, 29 bacterial strains have been tested, from which 6 isolates with high biocontrol activity against *U. necator* were selected. Dario et al. (2008) reported that commercial formulations of *Bacillus subtilis*, such as Serenade and Milastin K, demonstrated effective and reliable suppression of *U. necator* under both greenhouse and field conditions. Among these, Milastin K showed the highest effectiveness in disease control and yield improvement when used in rotation with fungicides (Dario et al., 2008; Sawant et al., 2011). Commercial formulations of *Bacillus pumilus* and *Bacillus subtilis* have also been tested in Republic of North Macedonia (Kuzmanovska et al., 2023), showing that biopesticides are potent biological agents and have a prospective use in Integrated Pest Management.

Although *Bacillus* species are undeniably important for plant protection, Mukhopadhyay and Kumar (2020) demonstrated that *Trichoderma* was highly effective at inhibiting the growth of phytopathogens and proved to be the most efficient bioagent against a variety of soil and foliar pathogens. Studies have also shown that applying biocontrol agents to the phyllosphere can induce systemic resistance in plants (Sawant et al., 2020), with field applications of *Trichoderma* strains boosting grapevine resistance to powdery mildew. Weekly preventive treatments with a commercial product containing *Trichoderma viride* strain CCBLA103, from the flowering stage to berry ripening, achieved over 80% disease control on *Vitis vinifera* cultivar Italia (Yparraguirre et al., 2020). In Egypt, Ahmed (2018) applied a mixture of *Trichoderma* species—*T. harzianum*, *T. hamatum*, and *T. viride*—to manage powdery mildew in a Thompson Seedless vineyard and observed a notable reduction in disease severity and incidence under conditions of moderate to low disease pressure.

Biocontrol agents do not always have to be observed separately. Kokare and Saha (2024) have suggested that *Trichoderma asperelloides* and *Ampelomyces quisqualis* in alternation with sulphur had the potential to be used as an alternative to chemical fungicides for the control of grape powdery mildew. Other authors have also observed that biocontrol agents used together with sulphur application indicated that the biocontrol agents could reduce the persistent fungal mycelia from the infected vines, (Sawant and Sawant, 2010; Ahmed 2018). Malviya et al. (2022) have observed that application of bioformulations individually or in combination with sulphur, significantly decreased powdery mildew disease on leaves and bunches and increased the quality parameters in grapes under this pathogenic stress.

These results demonstrate that even severe diseases like powdery mildew can be effectively managed with biofungicides, offering an environmentally friendly and health-safe alternative, marking a new era in plant protection. Overall, it can be concluded that biocontrol agents have bright future in replacing chemical fungicides, used alone, or in combination with other agents.

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