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CONTROL OF GREY MOLD DISEASE ON TOMATO WITH NOVEL BIOFUNGICIDES

Rade Rusevski[1], Biljana Kuzmanovska[1], Katerina Bandjo Oreshkovikj[2*]

[1]Faculty of Agricultural Sciences and Food - Skopje, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia
[2]Institute of Agriculture, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia
[*]corresponding author: kbandzo@yahoo.com

ABSTRACT

Grey mold disease on tomato (*Botrytis cinerea*) is widespread economically important disease all over the world with a very wide host range. This disease used to be controlled with regular fungicide applications, but in recent years, more ecological friendly methods are developed. One of them is development and use of new biofungicides, based on various beneficial organisms. The main goal of the experiments was to study the possibility for biological control of grey mold disease in tomato with the use of the innovative biofungicides Vintec (a.i. *Trichoderma atroviride* strain SC1) and Serenade Aso (a.i. *Bacillus subtilis* strain QST 713). Experiments were conducted during 2019 in the region of Bogdanci and the region of Dojran, on tomato hybrids grown in greenhouses. In the untreated variant in Dojran region, the disease severity index on leaves reached very high destructive level of 41.33%, while the disease incidence on the fruits was even higher (46.66%), and in Bogdanci region, a destructive level of 36% dead tomato plants caused by stem rotting was observed. In this disease conditions, both of the tested biofungicides in the region of Dojran showed similar results, with reduction of the disease incidence in fruits higher than 90% (92.86% for Vintec and 90.01% for Serenade Aso). In the region of Bogdanci, the biofungicide Serenade Aso provided significantly lower degree of incidence reduction of stem rotting (81.66%), compared with the biofungicide Vintec, which provided a full protection of 100%, difference which may be related to some beneficial organisms' preferences that should be investigated in the future.

Key words: grey mold disease in tomato, biofungicides, Vintec, Serenade Aso.

INTRODUCTION

Botrytis cinerea Pers., recognized as the causative agent of grey mold disease, is a phytopathogenic fungus that affects over 230 plant species. This fungus is particularly damaging in the global commercial greenhouse cultivation of vegetables, especially tomatoes and cucumbers, as highlighted by Borges et al. (2014) and Soliman et al. (2015). In the Republic of North Macedonia, the prevalence of grey mold in tomato greenhouses is particularly noticeable, leading to significant yield losses, often exceeding 70%, as reported by Kuzmanovska et al. (2012).

The primary strategy for managing grey mold disease has traditionally been the chemical application of fungicides. However, a major challenge in this approach is the tendency of *B. cinerea* to develop resistance to commonly used fungicides, a concern underscored by various researches, such as Zhao et al. (2010), Walker et al. (2013), and Hahn (2014). This resistance, coupled with difficulties in registering new fungicides and environmental considerations, has required exploring alternative control methods. One such method is biological control, as

indicated in studies by Barakat et al. (2014), Soliman et al. (2015), and Haidar et al. (2016). According to related reports, the main antagonistic microorganisms of tomato gray mold include bacteria, fungi, yeasts and actinomycetes (Raspor et al., 2010; Elad et al., 1994).

The *Trichoderma* species have been identified as promising fungal biocontrol agents against a variety of plant pathogenic fungi (Herrera-Parra et al., 2017). Among these, *Trichoderma harzianum* and *T. asperellum* have been the most researched and commercially utilized against an array of plant pathogens, including *B. cinerea* (Cheng et al., 2012; Bendahmane et al., 2012; Kuzmanovska et al., 2018). Nevertheless, given the effectiveness of the *Trichoderma* genus as biocontrol agents, there is an increasing focus on exploring the potential of other *Trichoderma* species, such as *T. atroviride*, *T. virens*, and *T. reesei*, as indicated by Poveda (2021).

Amongst the bacterial fungicides, *Bacillus subtilis* is widely used in biological control (Stein, 2005). Physiological and biochemical characteristics of *B. subtilis*, a type of gram-positive bacterium, have been studied for decades (Sonenshein et al., 2002; Dubnau and Losick, 2010). Hundreds of *B. subtilis* strains have been purified and most of them can produce antibiotics. *B. subtilis* can also affect other microbial communities to a certain extent (Yavuztürk et al., 2018). Therefore, it has been increasingly used in the research on biological control. *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 (Li et al., 2019).

In light of the significant potential that biological control, particularly through the *Trichoderma* and *Bacillus* species holds for the future management of plant diseases, the primary goal of this study was to assess the biological control capabilities of *T. atroviride* (Vintec) and *B. subtilis* (Serenade Aso) against *B. cinerea*, a major economic threat in tomato cultivation.

MATERIALS AND METHODS

The research was conducted during the vegetative season in 2019 on tomato plants grown in greenhouses, in two regions: Dojran and Bogdanci. In the region of Dojran, the trial was conducted on the tomato hybrid HTP, grown on alluvial soil, while in the region of Bogdanci, the test was performed on the tomato hybrid Mamston, on hydroponics. The treatments were preventive, from the phase of first primary apical side visible, until the phase of full maturity (BBCH 21-89). Each trial consisted of three different variants, i.e. two variants with the tested biofungicides, compared with the untreated control (Table 1). A randomized block design with three replicates was used. In both regions, each variant consisted of 25 tomato plants, on an experimental block of 10 m^2. The 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 with Vintec and Serenade Aso were performed in an interval of 7 days, from the second half of April, till the second half of May: I treatment - 18.4.2019, II treatment - 25.4.2019, III treatment - 2.5.2019 and IV treatment - 9.5.2019. Efficacy of the tested fungicides was performed 7 days after the fourth treatment (16.5.2019).

In the region of Bogdanci, disease incidence was expressed as the percentage of infected stems (proportion of infected stems in relation to the total number of examined plants), because in this region B. cinerea caused stem rotting symptoms on the tested tomato plants. In the region of Dojran, *B. cinerea* caused leaf and fruit infections. That is why, on the infected fruits disease incidence was measured as the percentage of infected fruits (proportion of fruits that are infected in relation to the total number of fruits examined in 25 plants). The leaves infection was assessed by disease severity.

Table 1. Rating scale of leaf area infection

Notes	Decayed Leaf Area
0	0
1	1-5
2	6-15
3	16-50
4	51-95
5	100

* Based on Mouria et al. (2015)

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).

Table 2. Variants represented in the tested regions of Dojran and Bogdanci in 2019

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			

RESULTS AND DISSCUSION

During the tomato growing season in 2019, destructive levels of grey mold disease were detected in the untreated control trial blocks in both of the tested regions. In the region of Dojran, the disease severity index on leaves reached very high destructive level of 41.33%, while the disease incidence on the fruits was even higher (46.66%). In the region of Bogdanci, the incidence of the disease in the control (untreated) variant reached a destructive level of 36% dead tomato plants, caused by stem rotting.

In these conditions, where the disease was widely spread and the severity and the incidence measured on various plant parts reached very high levels, the tested biofungicides Vintec and Serenade Aso, used in their proposed rates, showed very high efficacy in the control of grey mold disease in tomato (*Botrytis cinerea*). Also, no negative effects were detected during the usage of the biofungicides on the tomato plants in the proposed rates.

In the Dojran region, both of the tested biofungicides showed comparable levels of efficacy. The biofungicides Vintec and Serenade Aso reduced the disease severity index on the leaves compared with the control by 88.72% and 87.10%, respectively. The reduction of the disease incidence on the fruits compared with the control was even superior (92.86% for Vintec and 90.01% for Serenade Aso) (Table 3). In the Bogdanci region, where grey mold manifested as stem rotting, the biofungicide Vintec showed noticeably better results, then the biofungicide Serenade Aso. While Vintec performed complete reduction and achieved full protection (100%) on the tomato plants, Serenade Aso reduced the disease incidence compared with the control by 81.66% (Table 4). Although both of the tested biofungicides, consisting different active ingredients (*Trichoderma atroviride* in Vintec and *Bacillus subtilis* in Serenade Aso) demonstrated evident results, it is interesting to notice that maybe there is a difference in the preferences amongst various beneficial organisms and many manifestations of the disease, in

our case stem rotting, performing evidently different levels of protection. This may present basis for future explorations.

Table 3. Efficacy of the biofungicides Vintec and Serenade Aso in controlling of *Botrytis cinerea* on tomato in Dojran region in 2019

No.	Variant	Disease severity on leaves (%)	Disease incidence on fruits (%)	Efficacy of fungicide (%)	
				Leaves	Fruits
1.	**VINTEC**	4.66 [a]	3.33 [a]	88.72 [a]	92.86 [a]
2.	**SERENADE ASO**	5.33 [a]	4.66 [a]	87.10 [a]	90.01 [a]
3.	**CONTROL**	41.33 [b]	46.66 [b]	/	/

Table 4. Efficacy of the biofungicides Vintec and Serenade Aso in controlling of *Botrytis cinerea* on tomato in Bogdanci region in 2019

No.	Variant	Disease incidence on stems (%)	Efficacy of fungicide (%)
1.	**VINTEC**	0% [a]	100% [a]
2.	**SERENADE ASO**	6.6% [b]	81.66% [b]
3.	**CONTROL**	36% [c]	-

Due to the prospective of the microbial organisms as effective biocontrol agents, their fungicidal potential has been studied (Alamoudi, 2024). Kuzmanovska et al. (2018) have considered the fungicide activity of two *Trichoderma* species: *T. harzianum* and *T. asperellum* against *B. cinerea* isolates. In her study, both tested *Trichoderma* species has shown similar values for inhibition of mycelial growth and inhibition of conidial germination, meaning that using either *T. asperellum* or *T. harzianum*, adequate level of biological control of grey mold disease in tomato can be achieved. Both antagonists can be considered as promising biological control agents for control of grey mold disease in tomato, proving the biofungicidal potential of the *Trichoderma* genus.

Trichoderma is also capable of activating systemic plant defenses against the attack of pests and/or pathogens (Poveda et al., 2020). The comparative analysis of the genomes of three species of *Trichoderma* widely used as biocontrol agents in agriculture (*T. virens*, *T. reesei* and *T. atroviride*) has shown that mycoparasitism represents the ancestral way of life of the fungal genus (Kubicek et al., 2011).

Wang et al. (2018) have studied the effect of *B. subtilis* strain isolated from the rhizosphere of healthy tomato plants in controlling of *B. cinerea* and *Cladosporium fulvum* on tomatoes. The results showed that this bacterial strain was the most effective one, amongst all of the other isolated rhizosphere bacterial strains, demonstrating efficacy of 74.70% and 72.07%, respectively and showing better results than the treatments with the fungicides pyrimethanil (preventing grey mold) and polyoxin (preventing leaf mold).

Kuzmanovska et al. (2023) have also studied the effect of the biofungicide Serenade Aso, together with another *Bacillus* biofungicide, Sonata (a.i. *B. pumilus* QST 2808), on *Plasmopara viticola* in grapevines, tested on 3 grape varieties. Both of the tested biofungicides gave similar results, causing 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.

CONCLUSION

Vintec (a.i. *Trichoderma atroviride* SC1) and Serenade Aso (a.i. *Bacillus subtilis* strain QST 713) are effective novel biofungicides, which can be used for control of the grey mold disease in tomato - *Botrytis cinerea.* Used in the proposed dosages, they successfully control and prevent the development of the grey mold disease in tomato on various plant parts. The high efficacy of the biofungicides was achieved with well-timed and quality application, emphasizing its preventive mode of action. No negative effects were detected during the usage of the biofungicides on the tomato plants. Difference in the protection provided by Vintec and Serenade Aso against stem rotting can be basis for future explorations.

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