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Efficiency of Zeolite as Alternative Product for Controlling Downy Mildew (*Plasmopara viticola*) in Table Grape

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ABSTRACT

Downy mildew is a disease caused by obligate parasite *Plasmopara viticola* on grapevine worldwide. Chemical fungicides are widely used for the control of downy mildew. However, the use of copper based fungicides has been limited by European Commission regulation since 2002, and pathogen- resistance to some of systemic fungicides have been reported. Therefore, the development of environmentally friendly and sustainable products to protect against downy mildew in table grape is necessary. In this study, we tested several formulations of zeolite in semi-field and field experiments to evaluate their efficaciouses to control downy mildew. The most effective zeolite products on grape yield and quality were investigated in two locations. This study could be help the development of a novel environmentally friendly product, not only yield loss and also sustainable control of downy mildew on grape. The potential of zeolite to control of downy mildew on grape have been demonstrated for the first time.

Keywords: *Plasmopara viticola,* fruit quality, table grape, yield, zeolite

ÖZ

Sofralık Üzümlerde Mildiyö Hastalığı (Plasmopara viticola) Kontrolünde Alternatif Ürün Olarak Zeolitin Etkinliği

Mildiyö obligat parazit olan *Plasmopara viticola* tarafından oluşturulan ve dünyada bağ alanlarında oldukça yaygın bir hastalıktır. Kimyasal fungisitler mildiyö ile mücadelede oldukça yaygın olarak kullanılır. Bununla birlikte, bakır esaslı fungisitlerin kullanımı Avrupa Komisyonu tarafından 2002 yılından beri sınırlandırılmış ve patojen-direnç gösteren bazı sistemik fungisitler belirtilmiştir. Bu nedenle, sofralık üzümde mildiyö ile mücadele yapmak amacıyla, çevre dostu ve süsdürelebilir ürünler geliştirmek çok önemlidir. Bu çalışmada, zeolit içerikli bazı formülasyonların hem yarı arazi hem de arazi koşullarında mildiyö ile mücadeledeki etkinliği araştırılmıştır. Mücadelede en fazla etkin olarak belirlenen formülasyon, iki farklı lokasyon üzerine meyve verim ve kalite üzerine etkileri araştırılmıştır. Bu çalışma yalnızca çevre dostu değil, aynı zamanda üzümde ürün kaybına sebep olmayan ve mildiyö ile mücadele edebilecek yeni bir ürünün elde edilmesine imkan sağlamıştır. Üzümde mildiyö ile mücadelede kullanılabilecek potensiyel zeolit ürünü ilk kez belirlenmiştir.

Anahtar Kelimeler: *Plasmopara viticola*, meyve kalitesi, sofralık üzüm, verim, zeolit

INTRODUCTION

Downy mildew in table grape (*Vitis vinifera* L.) is caused by *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni, and it mutiplicates and spreads rapidly on susceptible table grapes (Emmett et al., 1992). It is a highly destructive disease of grapevines, especially in a warm and wet climate, in all vineyards in the world (Ash, 2000; Caffi et al., 2010).

Although *Muscadinia* species and several wild *Vitis* species exhibit varying levels of resistance to *P. viticola, V. vinifera* cultivars are highly susceptible to the disease (Davidson, 2008; Zyprian et al., 2009). A number of species of *Vitis* are resistance to the pathogen and these have been used as sources of resistance in the breeding programs (Doazan, 1980; Alleweldt and Possingham, 1988). However there is no report on very important commercial resistant variety to downy mildew in table grape (Brown et al., 1999). The resistant hybrids are also unsuitable for the production of high quality grapes due to their unpleasant flavors and aromas (Toffolatti et al., 2012).

Fungicides are widely used to control of downy mildew (Chen et al., 2007). Bordeaux mixture (copper sulfate and lime) was firstly sprayed to the control downy mildew in the vineyards of France in 1885 (Ash, 2000). Burgendy mixture invented in 1887 (Masson, 1887) contains sodium carbonate (Na_2CO_3) instead of lime. Although two mixtures are similar, growers prefers the Bordeaux mixture due to costs and ease of use in the worldwide. There are lots of products for controlling downy mildew, Bordeaux mixture has been preferred by most of the growers since long years (Gessler et al., 2011). However, the misuse of chemical pesticides has led to environmental pollution, residues on product and the development of resistant populations of pathogens (Clerjeau and Simone, 1982; Wong and Wilcox, 2000; Chen et al., 2007). We need to change fungicides to avoid resistance development against pathogen (Gessler et al., 2011). Therefore, development of new alternatives that are both effective and consistent as synthetic fungicides are required to control of downy mildew (Perazzolli et al., 2008; Dagostin et al., 2011).

In recent years, researches on mycoparasite (Bakshi et al., 2001; Musetti et al., 2007; Perazzolli et al., 2008; Dagostin et al., 2011), plant extracts (Cohen et al., 2006; Godard et al., 2009; Pereira et al., 2010; Žežlina et al., 2010; Dagostin et al., 2011), synthetic substances (Riches and Holmes, 2005; Hamiduzzaman, 2005; Žežlina et al., 2010; Dagostin et al., 2011; Selim, 2013) and inorganic substances (Žežlina et al., 2010; Dagostin et al., 2011; Selim, 2013) are tested them as alternative biological control to downy mildew (*P. viticola*) in viticulture. The aim of this study is to provide new alternatives to the standard fungides for controlling downy mildew.

MATERIALS and METHODS

Semi-field Trails

Trakya Ilkeren table grape variety was used as host plant in this trial. Experiments were carried out in the vineyard of Bati Akdeniz Agricultural Research Institute (BATEM) in Antalya (Turkey) according to Dagostin et al. (2011). The trials were started when grapevine plants have 20-30 fully developed leaves on their shoots. The experiment was set according to completely randomized block design with three replicate. Ten plants were used for each replicate. The semi-field trials were carried out in 2013 and 2014. Firstly, five substances (a formulation of zeolite (zeolite 4A), two microorganisms (*Trichoderma harzianum* and with 4 A plus *T. asperellum*), a pigment (Blue ultramarine) and a plant extract (Peppermint) with different doses were tested in 2013. Trial was constructed as an untreated control and a standard treatment of copper hydroxide (Kocide® Opti, DuPont 46.1% copper hydroxide). Therefore, new 4 substances which have two formulations of zeolite [Zeolite 800 MSC (80% Zeolite) and Zeolite 850 WP 100% Zeolite)], a pigment (Blue ultramarine) with zeolite 850 WP, a microorganism (*T. asperellum*) formulated with zeolite 850 WP) were tested in 2014. Trials were performed as an untreated control and a standard treatment of copper hydroxide (Kocide), a commercial microorganisms (Remedier; *T. asperellum* icc 080 2.00% + *T. gamsii* icc 012 2.00%) and commercial standard zeolite (Wetstop). Plants were treated by hand sprayers until near run-off, with sufficient coverage of the lower and upper surfaces of the leaves. The protocol from Dagostin et al. (2011) was used in experiments. All the disease assessments were made using EPPO standard scale (EPPO, 2004).

Field Trials

Trials were conducted in two experimental vineyards (BATEM/Antalya location and Fruit Research Institute, Egirdir/Isparta location) according to EPPO guidelines. BATEM location trials were carried out in vineyard of Bati Akdeniz Agricultural Research Institute (Antalya-TURKEY). This location has subtropical climate. Trakya Ilkeren table grape variety grafted onto the rootstock 110R was used. Planting distance is 3×2.5 m. Egirdir location trials were carried out in vineyard of Fruit Research Station (Egirdir, Isparta-TURKEY). This location has temperate climate. Trakya Ilkeren table grape variety (grafted onto the rootstock *Chasselas X Berlandieri* 41 B Millardet et de Grasset (41 B) was used in Egirdir. Planting distance was 3x2 m. In both location guyot trellis training system were used. The experiment was set in a completely randomized block design with three replicate. Six plants were used for each replicate. Four substances used in semi-field trials were tested on field trials in both locations in 2014. Trial was designed as an untreated control and a standard treatment of copper hydroxide (Kocide), a commercial microorganisms (Remedier) and Wetstop. Plants were sprayed with garden-type spraying pump until near run-off, with sufficient coverage of the lower and upper surfaces of the leaves.

Field treatments were carried out according to modified Dagostin *et al.* (2011) protocol. The treatments were applied at 2-3 weeks intervals depending on weather conditions, plant growth and the risk of *P. viticola* infection. In BATEM location, first application was made buckshot berries stage in 6 May 2014. Second application was made bunch closure stage in 23 May 2014. In Egirdir location, first application was made buckshot berries stage in 27 June 2014. Second application was made bunch closure stage at 22 July 2014. Symptoms in leaf and fruits were assessed on a categorical scale based on the percentage of damaged surface according to EPPO standard scale (EPPO, 2004).

Yield and Quality Trials

Yield and quality trials were done using zeolite 850 WP (40000 ppm) and zeolite 800 MSC (40000 ppm). Two control (Wetstop 40000 ppm and untreated) parcels were included. Trials were carried out in two experimental vineyards (BATEM/Antalya and Fruit Research Station, Egirdir/Isparta location). The experiment was set in a completely randomized block design and treatments replicate with three times. Spray the plants on all leaves to both adaxial and abaxial leaf surfaces, until near run-off using garden-type spraying pump. Applications were repeated for two times. First application was made buckshot berries stage. Second application was made bunch closure stage.

Full blooming, veraison, harvest dates and pomological analysis were made according to OIV (Office International de la Vigne et du vin) and UPOV (The International Union for the Protection of New Varieties of Plant) guidelines (OIV, 1997; UPOV, 1996).

In soil analysis; pH in water and 0.01M Calcium chloride solution, cation exchange capacity (CEC), electrical conductivity (EC), total sodium (Na) and aluminium (Al) parameters were measured by EN standards in acridity laboratory (Eurofins, SOFIA).

Statistical Analysis

Statistical analyze were done on the effects of the different treatments of preparations on disease development with Abbott (1925) and multiple comparison, using disease incidence. Disease severity was also calculated. The disease severity were evaluated using the Townsend-Heuberger's formula (Townsend and Heuberger, 1943). Pomological analysis and soil analysis results were determined using SAS (Statistical Analysis System) statistical analysis program. Significant difference at p-level < 0.05 were determined using ANOVA and LSD test.

RESULTS and DISCUSSION

The purpose of this study was to development of a pool of new, efficient, environmentally friendly and sustainable products to protect against downy mildew on table grape. In this study, we evaluated the ingredients of plant extract, microorganisms, syntetic chemicals, inorganic materials and zeolite products.

Semi-field Trials

Firstly, 5 substances were used against to downy mildew in semi-field trials in 2013. All of tested substances were not effective. The results are summarized in Table 1. Efficcay of substance was calculated according to Abbott's formula, and the most effective substance was zeolite 4A (40000 ppm) with 43.67%. Likewise, disease severity was also determined, and the most effective substance against to downy mildew disease was zeolite 4A (40000 ppm) with 46.33%. All of the tested substances were not effective as much as Kocide for disease control. Therefore, different new four substances with different doses were evaluated and compared to non-sprayed control, as a commercial standard zeolite (Wetstop), a commercial *Trichoderma* (Remedier) and a chemical standard (Kocide) in semi-field trials in 2014 (Table 2).

In 2014, the highest effective substances were found as the zeolite 850 WP 60000 ppm + Blue Ultramarine 1000 ppm, zeolite 850 WP 60000 ppm + *T. asperellum* 1000 ppm, and zeolite 850 WP 30000 ppm + Blue Ultramarine 1000 ppm, 52.29%, 53.35% and 55.23%, respectively. Likewise, the highest disease severity were determined in the zeolite 850 WP 60000 ppm + Blue Ultramarine 1000 ppm, zeolite 850 WP 60000 ppm + *T. asperellum* 1000 ppm, and zeolite 850 WP 30000 ppm + Blue Ultramarine 1000 ppm; as 9.15%, 9.06 % and 8.65%, respectively.

Table 1. Disease incidence and disease severity in semi-field trials in 2013 year

Substance	Doses (ppm)	Disease incidence (%) (mean± SD)*	Disease severity (%) (mean± SD)*
Zeolite 4A	**4000**	25.67±11.54 defg	59.00±7.93 efg
Zeolite 4A	**20000**	35.00±2.64 bcd	53.00±3.60 gh
Zeolite 4A	**40000**	43.67±2.30 b	46.33±1.15 h
Zeolite 4A + *Trichoderma asperellum*	**1000**	36.33±6.11 bc	52.33±4.61 gh
Zeolite 4A+ *Trichoderma asperellum*	**750**	23.33±11.93 efg	63.00±7.93 cdef
Zeolite 4A+ *Trichoderma asperellum*	**500**	16.33±1.52 gh	68.67±2.88 bc
Zeolite 4A+ *Trichoderma asperellum*	**250**	17.00±5.29 gh	68.33±2.88 bc
Blue Ultramarine	**1000**	33.67±5.03 cd	54.66±4.04 g
Blue Ultramarine	**100**	16.33±2.88 gh	69.00±1.73 bc
Blue Ultramarine	**10**	7.67±3.78 hi	75.67±4.04 ab
Trichoderma asperellum	**2000**	19.33±6.65 fg	66.33±4.04 cde
Trichoderma asperellum	**4000**	30.00±6.55 cde	57.33±6.42 fg
Trichoderma asperellum	**6000**	18.33±2.02 fg	67.00±1.81 cd
Peppermint extract	**2.5**	27.33±5.50 cdef	59.66±2.51 defg
Peppermint extract	**12.5**	32.00±4.32 cde	55.67±1.73 fg
Peppermint extract	**25**	34.00±4.50 cd	54.00±6.02 g
Control (Water)	**-**	-	82.33±4.04 a
Control (Kocide)	**150 g/lt**	64.33±5.76 a	29.66±3.81 i

* Significant difference at $p < 0.05$; ANOVA and LSD test.

Field Trails

Field trials were carried out in two experimental vineyard locations (BATEM/Antalya and Egirdir/Isparta). In Egirdir location. However data could not be obtained, because downy mildew did not appear in fields. Therefore, data were obtained from BATEM location. Disease developments were weekly assessed for 6 times until harvest time. According to the substance efficiency and disease severity results, the highest effect was obtained from the zeolite 850 WP 60000 ppm + Blue Ultramarine 1000 ppm with 22.66% (Table 2).

On the other hand, disease severity were evaluated at harvest time (Figure 1). Under field conditions, only one of the experimental compounds, zeolite 850 WP (60000 ppm) was provided 90% control on fruit infection. Likewise, the standard copper hydroxide treatment significantly protected leaves and bunches at both sites in all years, with control rates of disease severity ranging between 77% on leaves and 90% on fruits. For each trial, disease level on plants treated with Kocide was significantly lower ($P < 0.05$) than the untreated control.

The misuse of chemical pesticides causes several problems, including environmental pollution, unwanted residues on food and the development of resistant populations of pathogens (Clerjeau and Simone, 1982; Wong and Wilcox, 2000; Chen et al., 2007). European Commission (2002) reported increasing of restricted use of copper in organic agriculture even in purpose of research. In recent years, the inquire dringing in minds has increased on development and to obtain new alternative control agents as new products. Because they are less effective and less consistent than synthetic fungicides in controlling grapevine downy mildew (Cohen et al., 2006; Perazzolli et al., 2008; Dagostin et al., 2011).

Yield and Quality Trials

Applications did not significantly affect phenological and pomological values. The data of phenological stages (full blooming, veraison and harvest dates) were given Table 3. Results of pomological analyses (total yield, titratable acid, brix, cluster weight, cluster length, cluster width, berry weight, berry length and berry width) were given in Table 4, 5 and 6.

Table 2. Disease incidence and disease severity on leaves in semi-field and field trials in 2014 year

Substance	Doses (ppm)	Disease Incidence (%) (mean± SD)*		Disease Severity (%) (mean± SD)*	
		Semi-field	Field	Semi-field	Field
Zeolite 850 WP	**60000**	42.65±18.44 abc	54.67±14.50 ab	11.04±3.40 bcd	24.33±6.02 de
Zeolite 850 WP	**30000**	30.83±13.06 bc	35.00±4.58 c	13.42±2.88 bc	35.33±2.51 c
Zeolite 850 WP + Blue Ultramarine	**60000 + 1000**	52.29±19.72 ab	58.33±6.65 a	9.15±3.60 cd	22.66±2.51 e
Zeolite 850 WP + Blue Ultramarine	**30000 + 1000**	55.23±21.62 ab	40.67±11.01 bc	8.65±4.16 cd	32.00±3.60 cd
Zeolite 850 WP + *Trichoderma asperellum*	**60000 + 6000**	53.35±8.93 ab	25.67±22.86 bc	9.06±2.03 cd	40.33±9.64 cd
Zeolite 850 WP + *Trichoderma asperellum*	**30000 + 6000**	35.95±11.07 abc	27.67±5.50 cd	12.43±2.45 bcd	39.67±2.51 bc
Zeolite 800 MSC	**60000**	38.39±16.52 abc	13.67±29.15 cd	11.94±3.36 bcd	46.67±12.58 bc
Zeolite 800 MSC	**30000**	30.70±25.16 bc	13.67±7.23 cd	13.37±4.65 bc	46.67±2.88 ab
Commercial *Trichoderma* (*Trichoderma asperellum* +*Tricoderma gamsii* Remedier, 1×10^8 cfu/g)	**2.5 kg/ha**	18.13±15.86 cd	30.00±11.78 c	16.08±2.91 ab	37.67±4.04 bc
Commercial Standart (Wetstop)	**60000**	39.68±7.67 abc	33.33±8.32 c	11.66±1.44 bcd	36.00±1.73 c
Commercial Standart (Wetstop)	**30000**	35.23±14.24 abc	27.67±8.50 c	12.50±2.50 bcd	39.33±6.02 bc
Control (Water)	**-**	-	-	19.34±0.65 a	54.33±4.04 a
Control (Kocide)	**150 g/lt**	56.68±4.42 a	57.6±7.76 a	8.37±0.70 d	23.00±5.00 de

* Significant difference at $p < 0.05$; ANOVA and LSD test.

Products based on plant extracts were used for downy mildew disease control in grapevine (Pereira et al., 2010; Dagostin et al., 2011). Peppermint, is a essential oil, including menthol, menthone and isomenthone (White et al., 1987) have suggested for medicinial and antimicrobial properties widely in the world (Shah and D'Mello, 2004; Sandasi et al., 2011). Essential oils and their components have activities against a variety of targets, particularly on the membrane and cytoplasm, and in some cases, they completely change the morphology of the cells (Nazzaro et al., 2013). In recent years, researches has increased to benefit from the microbial effect in agricultural areas (Kalaivani et al., 2012; Ghanbari and Ariafar, 2013). Downy mildew (*Sclerospora graminicola* [Sacc.] Schroet.) is also a serious agricultural problem for pearl millet (*Pennisetum glaucum* L.) grain production under field conditions. Extract of *Azadirachta indica*, *Argemone mexicana, Commiphora caudata, Mentha piperita, Emblica officinalis* and *Viscum album* were evaluated against pearl millet downy mildew. Among the plant extracts tested, *V. album* treatment was found to be more effective in resistance inducing against downy mildew disease (Chandrashekhara et al., 2010). In our study, *M. piperita* extract has been firstly tested for controlling downy mildew (*P. viticola*) in table grape. However, it was less effective to reduce the quantities of fungicides applied in table grape. On the other hand, three different tested doses caused blight on the leaves.

Soil Analysis

There was no significantly differences to EC. However, total Na, Al and CEC significantly altered by zeolite treatments compared to the control. On the other hand, it does not constitute environmental risk. Table 7 shows evaluated formulations against the soil concentration and environmental risk details of the field trials.

Table 3. Full blooming, veraison and harvest dates of grapes

	Full Blooming (day/month)		Veraison (day/month)		Harvest (day/month)	
	BATEM	EGIRDIR	BATEM	EGIRDIR	BATEM	EGIRDIR
Untreated	1/5	16/6	9/6	31/7	30/6	15/8
Wetstop40000 ppm	1/5	16/6	9/6	31/7	30/6	15/8
Zeolite 850 WP 40000 ppm	1/5	16/6	9/6	31/7	30/6	15/8
Zeolite 800 MSC 40000 ppm	1/5	16/6	9/6	31/7	30/6	15/8

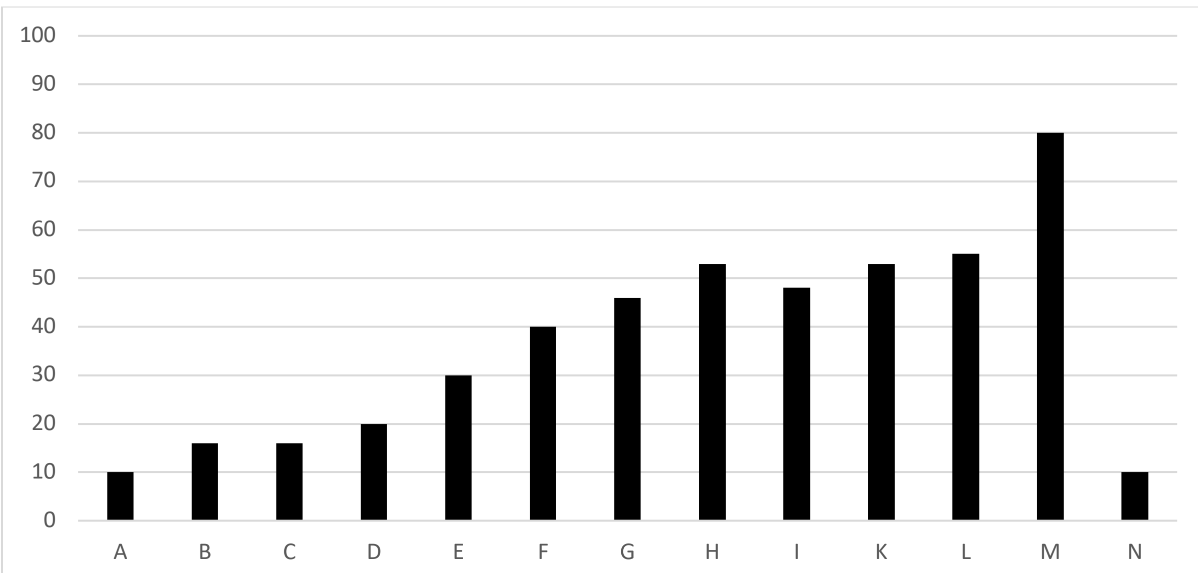

Figure 1. Disease severity on fruits at harvest time in field trials (%). A: Zeolite 850 WP (60000 ppm), B: Zeolite 850 WP (30000 ppm), C: Zeolite 850 WP (60000 ppm) + Blue Ultramarine (1000 ppm), D: Zeolite 850 WP (30000 ppm) + Blue Ultramarine (1000 ppm), E: Zeolite 850 WP (60000 ppm) + *T. asperellum* (6000 ppm), F: Zeolite 850 WP (30000 ppm) + *T. asperellum* (6000 ppm), G: Zeolite 800 MSC (60000 ppm), H: Zeolite 800 MSC (30000 ppm), I: Commercial Trichoderma (Remedier), K: Wetstop (60000 ppm), L: Wetstop (30000 ppm), M: Control (Water), N: Control (Kocide)

Table 4. Total yield, titratable acid and brix values of grapes

	Total Yield (kg/ha)		Titratable Acidity (%)		ºBrix	
	BATEM	EGIRDIR	BATEM	EGIRDIR	BATEM	EGIRDIR
Untreated	17346.6	32333.3	0.90 a	0.88 a	13.1 a	12.7 a
Wetstop 40000 ppm	16583.3	30078.3	0.80 ab	0.80 ab	11.8 b	11.7 b
Zeolite 850 WP 40000 ppm	17218.3	30773.3	0.82 ab	0.84 ab	12.3 ab	12.4 a
Zeolite 800 MSC 40000 ppm	16543.3	29513.3	0.80 ab	0.82 ab	12.1 ab	12.3 a
P>F	ns	ns	*	*	*	*

* Significant difference at p-level < 0.05, ns = not significant difference at p-level < 0.05; ANOVA and LSD test.

Table 5. Cluster weight, cluster length and cluster width of grapes

	Cluster Weight (g)		Cluster Length (cm)		Cluster Width (cm)	
	BATEM	**EGIRDIR**	**BATEM**	**EGIRDIR**	**BATEM**	**EGIRDIR**
Untreated	415.0	408.3	22.3	19.6	12.3	13.3
Wetstop 40000 ppm	325.0	438.3	21.0	20.0	11.0	13.6
Zeolite 850 WP 40000 ppm	393.3	406.6	21.3	19.6	12.3	14.3
Zeolite 800 MSC 40000 ppm	363.3	430.0	19.3	19.3	12.3	13.0
P>F	ns	ns	ns	ns	ns	ns

ns: not significant difference at p-level < 0.05; ANOVA and LSD test.

Table 6. Berry weight, berry length and berry width of grapes

	Berry Weight (g)		Berry Length (mm)		Berry Width (mm)	
	BATEM	**EGIRDIR**	**BATEM**	**EGIRDIR**	**BATEM**	**EGIRDIR**
Untreated	4.7	3.8	19.3	17.7	18.9	17.8
Wetstop 40000 ppm	4.7	3.8	19.0	17.2	19.3	17.6
Zeolite 850 WP 40000 ppm	4.8	3.9	19.2	17.9	19.2	17.9
Zeolite 800 MSC 40000 ppm	4.7	3.8	18.6	17.3	18.7	17.7
P>F	ns	ns	ns	ns	ns	ns

ns: Not significant difference at p-level < 0.05; ANOVA and LSD test.

Table 7. The results of soil test value for grape

Substance and concentration	**pH ($CaCl_2$)**	**pH (Extraction)**	**EC (dS/m)**	**Na (%)**	**CEC (me/100 g)**	**Al (%)**
Untreated	6.90	7.01 ab	1.60	4.00 ab	18.52 ab	2.91 a
Wetstop (60000 ppm)	6.83	7.29 a	1.76	3.82 ab	20.32 a	2.05 bc
Zeolite 850 WP (600000 ppm)	6.48	6.62 b	1.62	3.83 ab	17.89 b	2.63 ab
Zeolite 800 MSC (600000 ppm)	6.78	6.82 ab	1.78	4.55 a	18.33 ab	2.29 bc
P>F	ns	*	ns	*	*	*

* Significant difference at p-level < 0.05, ns = not significant difference at p-level < 0.05; ANOVA and LSD test.

Use of microorganisms for biocontrol of plant disease is an effective alternatives to the use of chemical pesticides. For this purpose, many microorganism have been selected against to plant disease. However, only a few commercial microorganism have been succsessfully used as biofungicides (Arnone et al., 2008). The use of bio-fungicide for control downy mildew is much less in the vineyard. BCA1 (*Bacillus subtilis)*, BCA2 (*Fusarium* ssp.), BCA3 (*Thricoderma* spp.), Clonotri (*T. harzianum* + *Clonostachis rosea*)*,* Serenade (*B. subtilis*), Sonata AS (*Bacillus pumilis*) and Trichodex (*T. harzianum* T39) were used against to *P. viticola*. Clonotri and Trichodex provided more than 60% control of leaf infection. Trichodex provided control highers than 60% on bunches infection (Dagostin et al., 2011). In this study, *T. asperellum,* zeolite 4A plus *T. asperellum,* zeolite 850 WP plus *T.asperellum* with different doses and commercial standart *T.asperellum* +*T. gamsii* (Remedier, 1x108 cfu/g) as a control were used to control of *P.viticola*. Desease severity were changed from 33.67 to 68.67 % in semi-field conditions. According to studies, the effectiveness of *Thricoderma* spp. to the downy mildew have changed. The biocontrol activity of tested agents can be influenced by temperatures, relative humidity, surface wetness, and variety (Guetsky et al., 2001; Dagostin et al., 2011).

Some studies were carried out on the inorganic materials to control of downy mildew in the vineyard. For the first time, one of the inorganic substances, blue ultramarine has been used in our study to control of downy mildew in the vineyard. Ultramarine blue was originally made by grinding lapis lazuli into a powder form. Natural ultramarine blue was extremely expensive pigment until a synthetic ultramarine invented in 1826 by Jean Baptiste Guiment (Plesters, 1993). In recent years, the use of synthetic ultramarine blue has increased in agricultural areas (Shanco et al., 2008). However, ultramarine blue has been firstly tested for controlling downy mildew (*P. viticola*) in table grape, and the treatments provided positive results

Our study aims to development of a pool of new, efficient, environmentally friendly and sustainable novel zeolite products for table grape. These will bring a range of beneficial effects to control of downy mildew, reduction in pesticide use, increase of crop yield and tolerance to abiotic stress. For this purpose, in this study, synthetic zeolite 4A, zeolite 800 MSC and zeolite 850 WP were used. Zeolite 4A is an important adsorbent with a wide range of use besides the highest adsorption capacity. It has been proved to be an innocuous substance in terms of environmental impact and toxicity. Zeolite 4A is widely used in the agricultural field. Zeolite 850 WP and zeolite 800 MSC are new formulation made from FITO (Galenika-Fitofarmacija a.d., RS) that is ECO-ZEO project'parner.

Zeolite is one of the inorganic materials widely used in many areas such as agriculture, environmental pollution, industry, medicine (Polat et al., 2004; Szerement et al., 2014). Natural zeolite was invented from a copper mine in 1756 by a Swedish mineralogist Fredrich Cronstet (Polat et al., 2004). It is a mineral naturally formed in the reaction of volcanic ash with surface water or groundwater (Kumpiene, 2010). Nowadays, there are over 40 known types of zeolite natural. Clinoptilolite and chabazite are most commonly consumed. However, zeolite has been synthetically produced due to its intensive use and natural zeolite is more expensive. Some of the common synthetics are zeolite A, X, Y and ZSM-5 (Polat et al., 2004; Ramesh and Reddy, 2011; Szerement et al., 2014). Conducted so far, only one study has suggested using zeolites to control of downy mildew in grapevines. Dagostin et al. (2011) were used 3 commercial zeolite (two of the three has Clinoptilolite active ingeredients, one of active ingeredient is kaolin) for alternatives to copper for controlling grapevine downy mildew in organic viticulture. However, these products were less effective to reduce the quantities of copper applied in organic vineyards. In our study, Zeolite 850 WP was more effective to control of downy mildew in table grape.

One of the main advantages of zeolite is its additive property to fertilizers. Therefore, zeolites are used to promote better plant growth by improving the value of fertilizers (Polat et al., 2004; Szerement et al., 2014). Zeolite + urea combined fertilization has positive influence on the yield of spring barley (Vidican et al., 2013). Zeolite 4A fertilization significantly increased Mn content in olive leaf (Pasković et al., 2012). In accordance with literature, any article was found on the effects of zeolite to control of downy mildew in viticulture as soil application. In this study, applications did not occur significant change in phenological and pomological values. It has high cation exchange capacity (CEC), high water holding capacity, high adsorption capacity (Mumpton, 1999) and also improves the efficiency of water use by increasing the soil water holding capacity and its availability to plants (Bernardi et al., 2010; Susana, 2015). CEC significantly altered by zeolite 850 WP treatment compared to the control. However, it did not change by zeolite 800 MSC application.

On the other hand, it is important to know whether use of zeolite creates any environmental risks? For this reason, total EC, pH, Na and Al were measured, which was followed by zeolite treatments compared to the control and succesfull results were recorded by zeolite application. It had no any environmental risk. Electrical conductivity (ECe mmhos/cm) of the saturated extract from a root zone soil sample was maximum 12, whereas it has been informed as normaly <4 in grape (Fipps 2017). Breck (1974) reported that synthetic zeolites are manufactured at greater than 50°C temperatures and with alkali hydroxides (NaOH) as catalysts from Al_2O_3- containing substances, for instance; aluminium hydroxides, or aluminates, (HERA, 2005). HERA (2005) pointed out that environmental risk assessment provides a sound basis for the conclusion that the use of zeolites in detergent products does not pose a risk to the environment. Jeffrey et al. (1997) reported that the toxicity of aluminium are greatly influenced by many factors, including pH of water and organic matter content. Especially, its toxicity increases with decreasing pH (pH<5) (Jaishankar et al., 2014). It can cause nonproductivity in acid soils, but soils at pH 5.5 to 8.0 precipitate the ion and eliminate toxicity (Fipps, 2017). In fact, aluminium has no biological role and it is a toxic nonessential metal to microorganisms (Olaniran et al., 2013). Damages occures due to mainly enzymes such as hexokinase, phosphodiesterase, alkalic phosphatase and phosphoxidase that are inhibited by aluminium since it has a greater affinity to DNA and RNA (Barabasz et al., 2002). Sodium is not an essential element for plants but can be used in small quantities, similar to micronutrients, to aid in metabolism and synthesis of chlorophyll. Sodium toxicity appears as necrosis or scorching of the leaf tips and margins, similar to micronutrient toxicities. Therefore the plant may not acquire sufficient levels of a required beneficial element and can leads to its deficiency in the tissue. On the other hand, sodium concentrations are very important and inhibits of uptake of other nutrients that leads to negative effect such as K deficiency and inhibition of Ca uptake by plants (McCauley and Jacobsen, 2011; Fipps, 2017).

Under field conditions, the efficacy of natural products is often limited by their sensitivity to environmental factors and their inherent physico-chemical characteristics (Lange et al., 1993; Dagostin et al., 2011). Therefore, for the realistic assessment of their value as potential crop protection, the evaluation of compounds under a broad range of field conditions and in repeated field trials according to EPPO guidelines are necessary (Dagostin et al., 2011).

As results, overall analysis of the results indicates that zeolite 850 WP can be suggested as a new fungicide since it could reduce damage of downy mildew in table grape. Moreover, the results reported here may help researchers and farmers for choosing of effective products for disease control besides use of copper in table grape vineyards. However, there was a problem on the bunches obtained from plots where zeolite was applied. In this case, the marketing of the product decreased market value. Therefore, in future studies applications should be done avoiding of spoils on the bunches. Also, new formulations can be improved to diminish spoils.

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