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Efficacy of copper citrate in grapevine disease control

Goran Aleksić[1]*, Zoran Milićević[1], Slobodan Kuzmanović[1], Mira Starović[1], Miloš Stevanović[1], Goran Delibašić[2] and Svetlana Živković[1]

[1]Institute for Plant Protection and the Environment, Teodora Drajzera 9, 11000 Belgrade, Serbia
[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia
**Corresponding author: alegoran@gmail.com*

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SUMMARY

The control of *Plasmopara viticola* and *Botrytis cinerea*, two of the most dangerous pathogens on grapevine, requires frequent treatments with chemicals based on weather conditions. Numerous applications of fungicides have resulted in developing fungicide resistance. Active ingredients based on copper have been used very successfully for a long time to protect grapevines against these pathogens. Application of a copper citrate formulation with high degree dissociation at a very low concentration (1%) was evaluated in field trials. The efficacy of two concentrations of copper citrate, 0.5 and 1.0%, were tested against *P. viticola* on grapevine in three locations, and against *B. cinerea* in two locations during 2018. Our results demonstrated that the concentration of 1.0% copper citrate was highly effective against *P. viticola* (87.4%) and *B. cinerea* (63.7%), compared to standard treatment (90.6 and 53.1%), under a high level of infection.

Keywords: grapevine; downy mildew; grey mould; fungicides; copper citrate

INTRODUCTION

Downy mildew, which is caused by *Plasmopara viticola* (Berk. & Curt.) Berl. and de Toni, and grey mould, caused by *Botrytis cinerea* Pers, are very important and harmful diseases of grapevines.

Chemical control of fungal diseases of grapevines, and many other crops, must be adequate and durable. The development of pathogen strains that are resistant to new fungicides aggravates current grapevine protection efforts. Estimating the consequences of resistance is difficult. It is therefore necessary to avoid excessive use of any new fungicides and to monitor the development of resistance in the field (Leroux & Clerjeau, 1985). Finally, to reduce the impact of resistance in practice, cooperation between manufacturers, distributors, extension services, registration agencies and vine-growers is essential, especially when introducing new inorganic chemical compounds.

Copper has been used in viticulture for more than 150 years, in quantities of 80 kg ha^{-1} annually, which leeds to the accumulation of copper ions in vineyard soils (Rusjan et al., 2007). Although it has a damaging ecotoxicological profile (Flemming & Trevors, 1989), the use of copper is still acceptable due to its unique properties as a wide-spectrum fungicide and bactericide. The future of viticulture is dependent on the use of copper unless alternatives can be found (Dagostin et al., 2011).

EU regulations limit the use of copper fungicides in organic agriculture to 6 kg ha^{-1} annually (European Commission, 2002). Copper compounds are mostly applied as fungicides and bactericides to fruit, vegetable and field crops (Agrios, 2005; Aleksić et al., 2012; Rusjan, 2012). Copper compounds used to control plant diseases are Bordeaux mixture and fixed copper compounds.

Some copper formulations used to control phytopathogenic fungi in the field can cause phytotoxic effects (Jamar & Lateur, 2007; Kurnik et al. 2011; Aleksić et al. 2012). According to investigation results reported by Gavrilović et al. (2017, 2018a) copper citrate can be used as a defoliant on nursery apple and stone fruit trees.

Copper citrate is a copper compound with a higher degree of dissociation than other copper products, which causes no toxic effects on fish, birds, mammals and bees (Fishel, 2011) and can be used for plant protection at lower concentrations as an environmentally acceptable agent (Georgopoulos et al., 2001). Copper citrate has already shown a notable inhibition of mycelial growth and germination of *Venturia inaequalis* ascospores (Aleksić et al., 2013) and inhibition of mycelial growth of *Monilia laxa* (Popović et al., 2014). Gavrilović et al. (2018b) demonstrated that a low concentration of copper citrate was highly efficient in protecting grapevine against *P. viticola*.

The aim of this study was to investigate the effects of low concentrations of copper citrate on *P. viticola* and *B. cinerea* in grapevine.

MATERIAL AND METHODS

The efficacy of two concentrations of copper citrate against *P. viticola* and *B. cinerea* on grapevine was tested under field conditions in locations: Ruma (45°00′15.32′′N, 19°82′27.17′′E) (cv. Italian Riesling, 16 years old), Smederevo (44°65′58.94′′N, 20°93′35.17′′E) (cv. Kladovka, 18 years old) and Miličinica (44°44′00.09′′N, 19°69′03.96′′E) (cv. Chardonnay, 10 years old) during 2018. (Tables 1 and 2). Weather conditions in the locations Ruma, Smederevo and Miličinica during June-August 2018 are presented in Table 3. The essay used a completely

Table 1. Dates of protective application against *P. viticola* on grapevine and its growth stages in Ruma and Smederevo locations in 2018

No.	Ruma		Smederevo	
	Date	Growth stages*	Date	Growth stages
1.	01.06.	68	31.05.	68
2.	06.07.	79	05.07.	79
3.	26.07.	83	20.07.	83
4.	11.08.	85	10.08.	85

* BBCH-scale

Table 2. Dates of protective application against *B. cinerea* on grapevine and its growth stages in Ruma and Smederevo locations (2018)

No.	Ruma		Smederevo		Miličinica	
	Date	Growth stages*	Date	Growth stages	Date	Growth stages
1.	25.05.	**65-6**8	21.05.	**65-68**	02.06.	68
2.	01.06.	68	31.05.	68	15.06.	**71**
3.	15.06.	**71**	15.06.	**71**	22.06.	**73-75**
4.	06.07.	79	23.06.	**73-75**	03.07.	77
5.	10.07.	**79**	05.07.	79	13.07.	**79**
6.	18.07.	**81**	20.07.	83	23.07.	**81-83**
7.	26.07.	83	31.07.	**83-85**	28.07.	**83-85**
8.	03.08.	**83-85**	10.08.	85	06.08.	85
9.	11.08.	85	-	-	-	-

* BBCH-scale

randomized block design in four repetitions, and 20 vines per plot treated with Cu hydroxide (0.4%) (Funguran OH) against *P. viticola,* and pyrimethanil (0.25%) (Pehar) against *B. cinerea* as standard treatments, and an untreated control. The results were evaluated three weeks after the last application.

Disease severity and efficacy were calculated as follows (Liu et al., 2010):

$$I = \sum(Gi \times Ui)/(Gm \times N)$$

where ***I*** is disease severity; ***Gi*** is the grade value assessed visually based on the percent lesion area (1 = 0% no symptoms; 2 = 1-5%; 3 = 5-25%; 4 = 25-50%; 5 = 50-100%); ***Ui*** is the leaf/bunch number of ***Gi***; ***Gm*** is the highest grade value of 5, and ***N*** is the total of ***Ui,*** and

$$EK = (IK - IT)/IK \times 100\%$$

where ***EK*** is efficacy compared to untreated control; ***IK*** is disease severity in untreated control, and ***IT*** is disease severity in treatment.

The results were analyzed using standard statistical methods. The significance of differences between treatments was measured using the analysis of variance with a 95% level of confidence, and Duncan's multiple range test for comparison between them. Statistical analysis was done using STATISTICA v.7 software (StatSoft. Inc.).

RESULTS AND DISCUSSION

Disease severity on grapevine leaves caused by *P. viticola* and the efficacy of fungicides applied in the locations Smederevo and Ruma are shown in Table 4 and Figure 1. Disease severity in the untreated control was 34.6% in Smederevo and 100% in Ruma. The results in both locations show that copper citrate was evidently very effective against downy mildew caused by *P. viticola* at 1% concentration in grapevine, demonstrating 71.6% efficiency even where the infection was intense (100% in the controlled field). Even higher effectiveness of copper citrate was observed in Smederevo and Miličinica locations: 75.7 and 87.4%, where pathogen infection was not as severe. These results indicate the reliability of copper citrate in protecting grapevine against *P. viticola*.

Table 3. Weather conditions in Smederevo, Ruma and Miličinica locations during June-August 2018 period

Location	Month	Means in 2018				
		Temperature (°C)			Rainfall	RH
		max	min	mean	(mm)	(%)
Smederevo	June	32.06	15.57	21.33	6.02	73.27
	July	27.87	17.02	22.48	4.56	75.73
	August	30.84	16.49	23.76	3.64	67.71
Ruma	June	26.68	15.51	21.12	4.71	75.30
	July	27.69	16.24	21.99	3.25	76.58
	August	30.94	16.81	24.19	3.63	70.10
Miličinica	June	25.95	16.42	21.21	7.80	77.20
	July	26.99	17.86	22.45	6.37	78.23
	August	29.45	17.69	23.82	1.76	74.03

Table 4. *P. viticola* – disease severity on grapevine leaves in locations Smederevo, Ruma and Miličinica in 2018

Location	DI			
	Cu citrate (%)		Cu hydroxide (%)	Control* (%)
	0.5	1	0.4	-
Smederevo	8.4 a**	5.4 a	11.1 b	34.6 c
Ruma	55.8 b	28.4 a	27.3 a	100.0 c
Miličinica	19.0 b	7.25 a	5.4 a	57.5 c

* untreated control
** different letters next to each number indicate significant difference between means ($p<0.05$)

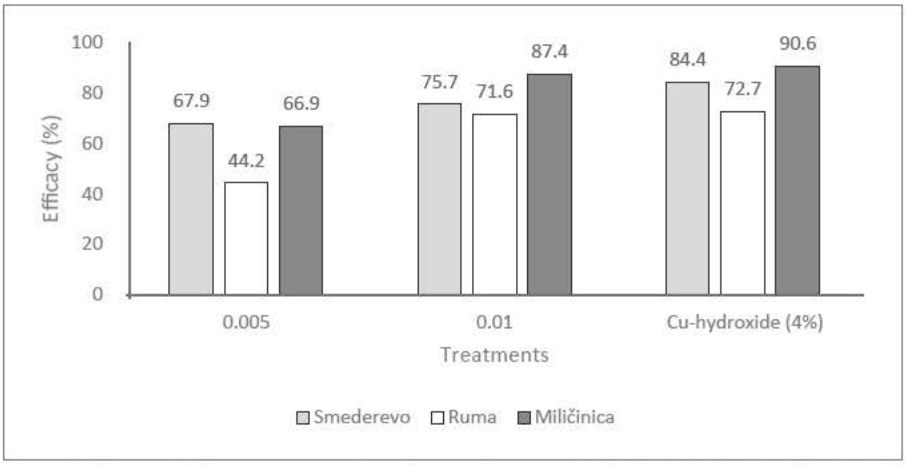

Figure 1. Efficacy of different concentrations of copper citrate against *P. viticola* in three locations in 2018

Figure 2. *P. viticola* - treatment with Cu citrate concentrations: 0.5% (a), 1% (b) and untreated control (c) (location Miličinica, 2018)

Table 5. *Botrytis cinerea* – disease severity on bunches in locations Smederevo and Ruma

Location	DI			
	Cu citrate (%)		Pyrimethanil (%)	Control (%)*
	0.5	1	0.2	
Smederevo	37.5 b**	26.3 a	34.0 a	72.5 c
Ruma	57.5 b	43.8 a	36.2 a	97.3 c

* untreated control
** different letters next to each number indicate significant difference between means ($p<0.05$)

There was no significant difference between the efficacy of copper citrate 1% concentration and the standard copper hydroxide, except in Ruma location. However, Cu citrate 0.5% concentration showed a significantly lower effectiveness than 1% concentration and the standard (Table 4, Figures 1 and 2). The concentration of 1% was therefore more effective.

Literature sources show that copper accumulates in soil over many years, causing a modification of soil characteristics. For this reason, other natural products are being used to control plant (such as grapevine) diseases, including propolis, heavy metals, mixtures of microorganisms, plant extracts and phosphates. A new copper-based foliar fertilizer is now used in which

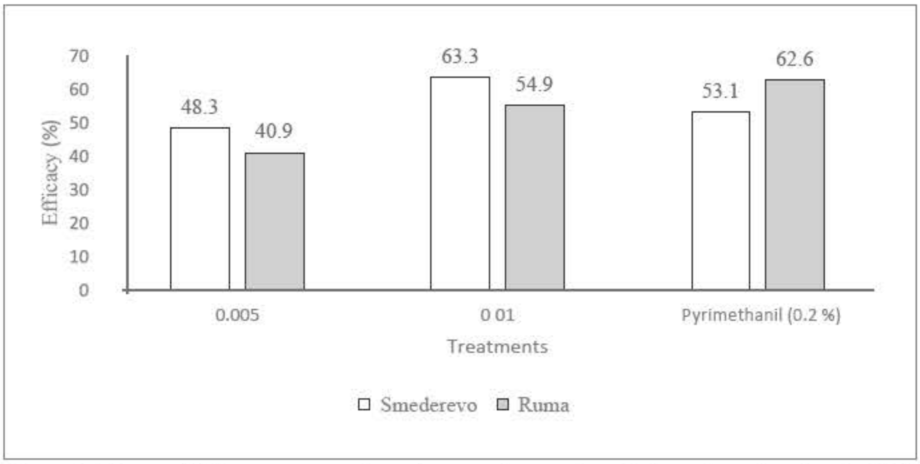

Figure 3. Efficacy of different concentrations of copper citrate against *B. cinerea* in two locations in 2018

Figure 4. *B. cinerea* – treatment with Cu citrate concentrations: 0.5% (a), 1% (b) and untreated control (c) (location Smederevo, 2018)

copper is chelated with gluconic acid and amino acids of plant origin, thereby significantly reducing the dose rate of copper. Bavaresco et al. (2019) noted a satisfactory reduction in downy mildew infection when copper concentration in grapevine was under 2 ppm.

Table 5 shows data indicating the severity of *B. cinerea* infection on grapevine, and Figure 3 reveals the efficacy of fungicides in the locations Smederevo and Ruma. We inferred from the results for both locations that the tested 1% concentration of copper citrate was effective in reducing a severe infection with *B. cinerea* by 63.7% in grapevine. There was no significant difference in the efficacy of copper citrate and a standard fungicide (pyrimethanil), except in Ruma and at low doses (Table 5, Figure 3). Both the tested copper citrate and standard fungicide showed significant differences compared to untreated control grapevine (Figure 4).

Table 3 shows weather conditions measured at three locations: Smederevo, Ruma and Miličinica over the period June-August 2018. It is clear that the conditions were very favorable for grapevine leaf infection with *P. viticola*, the causal agent of downy mildew, and *B. cinerea*, the causal agent of grey mould. This is confirmed by data in Table 4, which shows 100% infection with *P. viticola* in Ruma, and Table 5, which shows 97.3% infection with *B. cinerea*. Similar results were obtained by Ouda (2014) in an investigation in which the concentration of 15 mg L^{-1} nanoparticles of copper was shown to cause the efficacy of over 50%.

Regarding *B. cinerea* infection, Table 5 shows that relative humidity (RH) was high (over 65%) in all locations throughout the period of observation, which contributed to the high level of infection.

Chemical control has been used for many years as standard practice against *B. cinerea*, which had quickly adapted to new chemicals in the past, first to dicarboxamide (Leroux & Gredt, 1982), before developing cross-resistance to pyrimethanil, cyprodinil and mepanipyrim fungicides (Hilber et al. 1999), which led to tolerant or more resistant strains and made it a classical 'high-risk pathogen' in terms of resistance management (Brent & Hollomon, 2007). It has also developed resistance to new derivatives, such as phenylpyrrole (Rosslenbroich & Stuebler, 2000). All these problems require constant development of new fungicides. The discovery of new active ingredients while still using the known, older compounds can ensure the implementation of an effective anti-resistance management strategy for disease control (Rosslenboich & Stuebler, 2000). A new fungicide, for example fenhexamid, with a different mechanism of action, does not show cross-resistance with any other fungicide.

Our results, which showed high efficacy of copper citrate at its concentration of 1% in grapevine protection against *P. viticola* (87.4%) and *B. cinerea* (63.7%), in relation to standard fungicide (90.6% and 53.1%), are important as they provide evidence of a reduced percent infection when copper citrate was applied even under high disease pressure. Copper citrate was tested as a plant protection compound in this study for the first time. The results indicate a possibility for introducing copper citrate into an anti-resistant strategy. The investigation should be repeated not only in grapevine but also in other crops which are vulnerable to fungicide resistance.

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Efikasnost bakar-citrata u zaštiti vinove loze od bolesti

REZIME

Zaštita vinove loze od prouzrokovača plamenjače vinove loze – *Plasmopara viticola* i sive truleži – *Botrytis cinerea*, je vrlo kompleksna i zahteva primenu većeg broja hemijskih tretmana, u skladu sa vremenskim uslovima. Učestala primena fungicida uslovljavala je pojavu rezistetnih izolata patogena na fungicide. Različite forme bakarnih jedinjenja primenjuju se u zaštiti vinove loze dugi niz godina prilično uspešno. Primena bakar-citrata – formulacije sa visokim stepenom disocijacije u niskoj koncentraciji (1,0%) ispitivana je u poljskim uslovima. Efikasnost dve koncentracije bakar-citrata – 0.5 i 1.0% je testirana u suzbijanju *P. viticola* i *B. cinerea* na vinovoj lozi na tri (dva) lokaliteta, tokom 2018 godine. Naši rezultati pokazuju da je ispitivana koncentracija od 1,0% bakar-citrata ispoljila zadovoljavajući efekat na *P. viticola* (87,4%) i *B. cinerea* (63,7%) u odnosu na primenjene standarde (90,6% i 53,1%) u uslovima visokih zaraza.

Ključne reči: vinova loza; plamenjača; siva trulež grožđa; fungicidi; bakar-citrat