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Cu-CITRATE, A NEW SOURCE OF Cu ION AS A FUNGICIDE

Tatjana Popović[1], Zoran Milićević[1], Nenad Trkulja[1], Anja Milosavljević[1], Predrag Milovanović[2], Goran Aleksić[1], Žarko Ivanović[1]

[1] Institute for Plant Protection and Environment, Belgrade, Republic of Serbia
[2]Galenika-Fitofarmacija, Belgrade, Republic of Serbia
tanjaizbis@gmail.com

Pesticides containing copper have a historical significance in that the fungicidal properties of Bordeaux mixture, named after the Bordeaux region in France, were accidentally discovered. Today, there are approximately 15 various active ingredients registered for use over the world that contain some form of copper, depending on how their composition is defined. Cu-Citrate is a complex compound of copper, which is characterized by a higher degree of dissociation in relation to other copper compounds which are now applied as fungicides. Therefore, Cu-citrate can be used in the application of lower concentrations compared to other copper products, and how expressed no toxic effects to fish, birds, mammals and bees, it can be recommended to be used as an antifungal agent.
Brown rot blossom blight, caused by *Monilinia laxa* (Aderhold & Ruhland) Honey, is a devastating disease of sour cherry (*Prunus vulgaris* Mill.). The disease is endemic in Europe and United States. Depending on weather conditions, blossom blight can be controlled with one to three applications of protective or systemic fungicides during the bloom period in conventionally grown stone fruit orchards. Copper based fungicides are traditionally used to control brown rot blossom blight caused by *M. laxa*.
The aim of this study was to test *in vitro* efficacy of Cu-citrate comparing to other Cu-fungicides against *M. laxa* isolates.

In vitro evaluation of copper fungicides to check preferment of Cu-citrate in inhibitory of colony growth of the fungus *M. laxa* was done through *in vitro* method on potato dextrose agar (PDA) medium. The experiment was conducted in completely randomized design. Four fungicides, Cu-citrate, Cu-(II) hydroxide, Cu-oxychloride and Cu-sulfate were tested, each with different doses of i.e. (TABLE 1).
Four isolates of *M. laxa* were collected from symptomatic shoots affected with brown rot disease on several stone fruit from different locality in Serbia (TABLE 2). Isolates were collected from plum (*Prunus domestica* L.), sour cherry (*Prunus cerasus* L.), peach [*Prunus persica* (L.) Batsch] and apricot (*Prunus armeniaca* L.) orchards. All isolates were grown in 9-cm-diameter plastic Petri dishes on potato dextrose agar (PDA) medium at 22°C in the dark for mycelial production. Identification was conducted by combining culture characteristics, such as growth rate and colour, with morphological data such as the conidial dimensions.
After autoclaving of PDA medium, fungicides in different doses were added in separate 200 ml flasks and 10 ml of each concentration was poured in sterilized 50 mm Petri plates. The fungus, grown two weeks on PDA at 22°C were picked from purified culture in the form of a 3 mm agar disc and inoculated in the center of each Petri plate. Four replicate plates were inoculated for each fungicidal dose. The PDA medium without fungicide was kept as control. The dishes were incubated at 25°C. Mean colony diameter was measured after 7, 14 and 21 days.
First readings of colony growth (after 7 days) were taken for calculating EC_{50} (fungicide concentration which inhibits fungal development by 50%). Mean colony diameter (mm) of each isolate *M. laxa* was measured (minus the diameter of inoculation plug) by calculating the mean of two perpendicular colony diameters. The mean diameter of colonies was expressed as a percentage of colony diameters in control treatments and the relative growth (RG) was estimated. Based on relative growth EC_{50} value was calculated using MCSTAT ver.2.10.

TABLE 1. Tested copper fungicides on fungal cultures according to active ingredient, content of Cu ions and tested doses

Fungicides	Content of a.i. in formulations	Tested doses Cu^{++} (mg/L)
Cu-citrate	229 g/l	10; 25; 30; 40; 50; 70; 90; 100; 200; 400
Cu-(II) hydroxide	500 g/kg	32; 320; 640; 1000; 2000; 3000; 4000; 6000
Cu-oxychloride	350 g/kg	350; 700; 1500; 3600; 5000
Cu-(II) sulfate	100 g/l	250; 500; 1000; 2000; 5000

TABLE 2. Isolates of *Monilinia laxa* with information of host, locality and year of isolations

Isolates	Host	Locality	Year of isolations
KJ2	Apricot	Šabac	2010
SDV2	Cherry	Smederevo	2010
BRIC	Peach	Topola	2010
SD1	Plum	Grocka	2010

The fungicides showed variable response in inhibiting the colony growth of the pathogen according to their nature and specificity at different EC_{50} values. Obtained EC_{50} values indicate a very high toxicity of Cu-citrate compared to other copper fungicide. The mean EC_{50} for Cu-citrate was eight times lower than for Cu-sulfate, and 40 to 60 times lower compared to Cu-hydroxide and Cu-oxychloride, respectively (TABLE 3).
Although copper is non systemic however it kills fungal spores by combining with sulphhydral group of certain enzymes. Inactivation of fungal enzymes by copper ions gives good inhibition than other non-systemic fungicides. Due to possible negative effects on environment, investigations of alternative copper source are needed. Cu-citrate as a new form of Cu ions does not have a toxicities to fish, birds, mammals and bees.
Overall suppressive effect was displayed by all the tested Cu-fungicides and the colony growth decreased with increase of fungicidal doses. Considering the efficacy and cumulative performance, tested fungicides could be placed into three groups. First group, Cu-citrate constitutes highly effective fungicides. Second group includes Cu-sulfate with lower toxicity and appeared to be medium effective. Third group comprised of Cu-hydroxide and Cu-oxychloride was comparatively less effective. Use of cupric citrate as a growth promoter indicates the need to examine the possibility of introducing such compounds as a fungicides with the aim of protecting the environment.
Evaluation of EC_{50} values of copper fungicides also helped to standardize the best fungicides against fungi in the present study. This study could contribute in a future to devise fungicidal application schedule for commercial orchards.

TABLE 3. Calculated EC_{50} values for isolates *M. laxa* and mean values EC_{50} for all isolates to different copper fungicides

Fungicides	Isolates	EC_{50} (mg/l)	Mean EC_{50} (mg/l)
Cu-citrate	BRIČ	54.42	51.82
	KJ3	46.25	
	SDV2	52.57	
	ŠD1	54.06	
Cu-(II) hydroxide	BRIČ	2253.70	2082.62
	KJ3	2639.13	
	SDV2	1648.26	
	ŠD1	1789.40	
Cu-oxychloride	BRIČ	3535.09	3151.88
	KJ3	3816.31	
	SDV2	2315.45	
	ŠD1	2940.67	
Cu-(II) sulfate	BRIČ	417.04	401.62
	KJ3	417.02	
	SDV2	416.93	
	ŠD1	355.48	

EC_{50}: The effective concentration to inhibit mycelial growth by 50%