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Copper-Citrate as a Possibility for Control of Some Phytopathogenic Bacteria

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Abstract

Copper citrate is a complex compound of copper, which is characterized by a high degree of dissociation in relation to other copper compounds that have applied so far and can be used in lower concentration. This compound had no toxic effects on fish, birds, mammals and bees in his introduction of the application and can be used as a environmentally acceptable agent in plant protection. The aim of this study was to evaluate the effectiveness of copper-citrate applied to six economically important plant pathogenic bacteria.

Agar diffusion disk method has been used in the determination of Growth Inhibition Pathogens and the Minimum Inhibitory Concentration. Dilution series of copper-citrate was prepared from the initial concentration of 1% to 0.01% from the end. In this study we tested efficiency of copper-citrate on the following pathogenic bacteria: *Erwinia amylovora*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas savastanoi* pv. *phaseolicola*, *Xanthomonas campestris* pv. *campestris*, *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas campestris* pv. *vesicatoria.*

Obtained results showed that the copper-citrate had bactericidal effect to all tested bacteria. Tested concentrations of 0.5-1% formed inhibition zone

around the diameter of 15 mm, a concentration of 0.07-0.4% zone diameter of about 10 mm. Concentrations that are not inhibited colony growth in tested isolates were ≤ 0.06%.
Based on these results, we can conclude that the copper-citrate can be used as an effective agent for control of plant pathogenic bacteria.

Key words: Copper, plant pathogenic bacteria, control

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Introduction

Plants are constantly exposed and threatened by a variety of pathogenic microorganisms present in their environments. Diseases caused by pathogens, including plant pathogenic bacteria, significantly contribute to the overall loss in crop yield worldwide (Strange and Scott, 2005; Savary *et al*., 2006). Bacterial pathogens and their control are a serious problem in agriculture practice. For many years, copper-containing compounds have been used in vegetable and fruit crops as a bactericides to limit the spread of plant pathogenic bacteria (Mappes *et al.*, 1984; Scheck and Pscheidt, 1998; Geider, 1999; Vidaver and Lambrecht, 2004). Their relatively high toxicity to plant pathogens, low cost, and low toxicity to mammals have made them economically important. Copper compounds are the most common bactericides for control of plant bacterial diseases, especially since antibiotics are not registered for use on most edible crops. The interference of copper ions with bacteria appears to be complex (Cooksey, 1993). An excess of intracellular copper ions seems to inhibit many enzymatic activities. Copper may accumulate in the outer membrane or in the cytoplasm of Gram-negative bacteria (Geider, 1999). A toxic effect of copper can be seen as a growth inhibition of bacterial cultures which can result in an immediate change in the cell metabolism.

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 (Fishel, 2011). Effectiveness of copper-containing often being measured by the absence of bacterial growth on a solid medium (Rózycki, 1992).

Copper-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 (Popović *et al.*, 2012). Therefore, Cu-citrate can be used in the application of lower concentrations compared to other copper products, and how, according to Fishel (2011) expressed no toxic effects to fish, birds, mammals and bees, it can be introduce in environmentally acceptable strategy in plant protection.

The purpose of our study has been *in vitro* screening of the efficacy of copper-citrate at different concentrations to six economically important plant pathogenic bacteria.

Material and Methods

Copper-citrate efficacy test were conducted *in vitro*. The Growth Inhibition Pathogens (IPP) and the Minimum Inhibitory Concentration (MIC) was evaluated using the Agar diffusion disk method (Klement et al., 1990).

Following plant pathogenic bacteria were tested: *Erwinia amylovora* - TEad1 isolate originating from quince, *Pseudomonas syringae* pv. *syringae* - IZB162 isolate originating from peach, *Pseudomonas savastanoi* pv.

phaseolicola - TP1 isolate originating from beans, *Xanthomonas campestris* pv. *campestris* - TKu1 isolate originating from cabbage, *Xanthomonas axonopodis* pv. *phaseoli* - TX11 isolate originating from beans and *Xanthomonas campestris* pv. *vesicatoria* - TXv6 isolate originating from pepper. The isolates were grown on Nutrient Agar (NA) for 48 h at a temperature of 28°C. Petri dishes (ø 90 mm) with NA medium were inoculated with one hundred microliters of bacterial suspensions (3 x 10^8 cells/mL) of each tested strain.

Dilution series of copper-citrate was prepared from the initial concentration of 1% to 0.01%. Sterile filter paper discs (ø 5 mm) were placed on the surface of the Petri plates and supplemented with 20 mL of each tested concentrations of copper-citrate. There were four replicates for each bacterium and tested concentration. The plates were incubated at 28°C for three days, then examined and records recorded. The inhibitory effect of the treatment against each test bacterium was determined by measuring the diameter of zones of inhibition (in millimeters) and MIC was rated.

The results were subjected to analysis of variance (ANOVA) and means were compared by Duncan´s Multiple Range Test (P = 0,05) using the software COSTAT.

Results

Obtained results showed that the copper-citrate showed bactericidal effect because IPP was occurred for all tested strains. At 0.5-1% copper-citrate concentration inhibition zone were approx. 15 mm in diameter (Fig 1, 2). At lower concentration, 0.07-0.4% diameter were approx. 10 mm.

MIC was determined at a concentration of 0.07%. Concentrations that were not inhibited colony growth of tested strains were ≤ 0.06% (Fig 1, 2).

Figure 1. *Growth Inhibition of Phytopathogenic Bacteria by Different Concentrations of Copper-citrate*

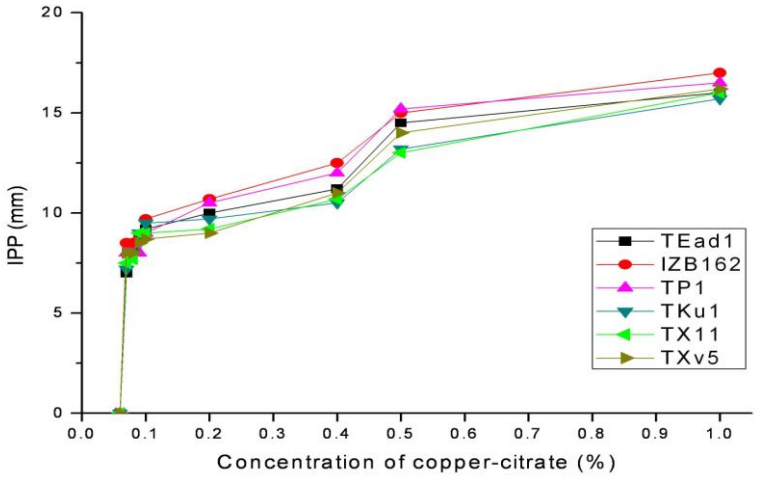
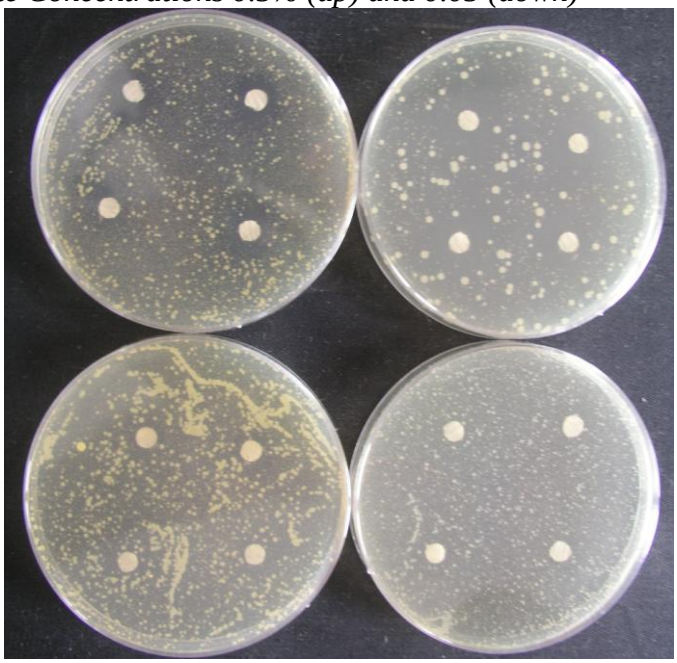


Figure 2. *Growth Inhibition of X. c. pv. campestris and E. amylovora by Copper-citrate Concentrations 0.5% (up) and 0.05 (down)*



Discussion

In the present work, we have evaluated the efficacy of copper-citrate, a new source of copper ion, against economically important *E. amylovora, P. s.* pv. *syringae, P. s.* pv. *phaseolicola, X. c.* pv. *campestris, X. a.* pv. *phaseoli* and *X. c.* pv. *vesicatoria.* Our results showed that this compound exerted *in vitro* antibacterial activity and inhibits growth of these plant-pathogenic bacteria at low concentrations (MIC of $\leq 0.06\%$).

Many studies were conducted with copper against plant pathogens *in vitro* and *in planta* (Marco and Stall, 1983; Geider, 1999, Thirumalesh et al., 2011) and increasing resistance to the currently available compounds (Bender *et al*., 1990; Cooksey, 1990, 1996; Cooksey et al., 1990; Andersen *et al.*, 1991; Voloudakis *et al*., 2005). Products containing copper has reported to significantly reduce foliar leaf, fruit spotting of tomato (Gleason et al., 1993) or could me more active when mixed with mancozeb (Hausbeck et al., 2000).

The mechanism of action of copper ions against plant pathogenic bacteria is thought inhibit many enzymatic activities. Copper may accumulate in the outer membrane or in the cytoplasm of Gram-negative bacteria. *Pseudomonas* subspecies can express the 61 kDa CopA protein, which binds several copper ions per molecule. The CopC protein can accumulate in Cu-induced cells (Geider, 1999). Another copper-binding activity is suggested for CopB, which is bound to the outer membrane (Cha and Cooksey, 1991). The *cop*-mediated accumulation of copper is connected to resistance. The corresponding genes

are specifically induced by copper ions. The transfer of plasmids is a mechanism for the spread of copper resistance in nature (Bender and Cooksey, 1986, 1987).

Due to the increasing resistance of plant pathogens to the currently available copper compounds and the emerging need to eliminate toxic chemicals from agricultural use, these new copper form could serve as potential candidates to be developed as relatively economical broad-spectrum antibacterial agents in agriculture.

In summary, we present a strategy for eliminating some plant pathogenic bacteria by using economically available compound copper-citrate, in range 1% to 0.01%. Data are preliminary and extensive studies of the effects of copper-citrate on plants are planned for future work. Therefore, this complex might be considered as a potential compound for use in plant protection.

References

Andersen, G.L., O. Menkissoglou & S.E.Lindow (1991). ´Occurrence and properties of copper-tolerant strains of *Pseudomonas syringae* isolated from fruit trees in California.´ *Phytopath*. 81: 648–656.

Bender, C.L., D.K. Malvick, K.E. Conway, S. George & P. Pratt (1990). ´Characterization of pXV10A, a copper resistance plasmid in *Xanthomonas campestris* pv. vesicatoria.´ *Appl. Environ. Microbiol*. 56: 170-175.

Cha, J.S. & D.A. Cooksey (1991). ´Copper resistance in *Pseudomonas syringae* mediated by periplasmic and outer membrane proteins.´ *Proc. Natl. Acad. Sci.* (USA) 88: 8915-8919.

Cooksey, D.A. (1990). ´Genetics of bactericide resistance in plant pathogenic bacteria.´ *Annu. Rev. Phytopathol*. 28: 201-219.

Cooksey, D.A.(1996). ´Molecular genetics and evolution of copper resistance in bacterial plant pathogens.´ In: T. M. Brown (ed.), *Molecular genetics and evolution of pesticide resistance,* 79–88. Washington: American Chemical Society.

Cooksey, D.A., H.R. Azad, J.-S. Cha & C.-K. Lim (1990). ´Copper resistance gene homologs in pathogenic and saprophytic bacterial species from tomato.´ *Appl. Environ. Microbiol*. 56: 431-435.

Fishel, F.M. (2011). ´Pesticide Toxicity Profile: Copper-based Pesticides.´ Pesticide Information Office, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Geider, K. (1999). ´Interference of copper sulphate in growth of *Erwinia amylovora*.´ *J. Phytopathol.* 147: 521-526.

Hausbeck, M.K., J. Bell, C.M. Medina-Mora, R. Podolsky & D.W. Fulbright (2000). ´Effect of bactericides on population sizes and spread of *Clavibacter michiganensis* subsp. *michiganensis* on tomatoes in the greenhouse and on disease development and crop yield in the field.´ *Phytopath*. 90, 38-44.

Klement, Z., K. Rudolph & D.C. Sands (1990). ´Methods in Phytobacteriology.´ Budapest: Akademiai, Kiado.

Marco, G.M. & R.E. Stall **(**1983). ´Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that differ in sensitivity to copper.´ *Plant Dis.* 67: 779-781.

Mappes, D., W. Porreye & T. Deckers (1984). ´Trial results with a new copper formulation for the control of fire blight.´ *Acta Hortic*. 151: 173-177.

Popović, T., Z. Milićević, N. Trkulja, A. Milosavljević, P. Milovanović, G. Aleksić & Ž. Ivanović, (2012). ´Cu-Citrate, a new source of Cu ion as a fungicide.´ Proceedings of the International Symposium: Curent trends in Plant Protection, September 25th-28th, Belgrade, Serbia, 363-366.

Rózycki, H. (1992). ´A rapid agar-diffusion test for quantifying the toxic effects of copper on microorganisms.´ *Acta Microbiol Pol.* 41(1-2): 57-64.

Savary, S., P.S. Teng, L. Willocquet & F.W. Nutter Jr. (2006). ´Quantification and modeling of crop losses: a review of purposes.´ *Annu. Rev. Phytopathol*. 44: 89-112.

Scheck, H. & J.W. Pscheidt (1998). ´Effect of copper bactericides on copper resistant and copper sensitive strains of *Pseudomonas syringae* pv. *syringae*.´ *Plant Dis.* 82: 397-406.

Strange, R.N. & P.R. Scott (2005). ´Plant disease: a threat to global food security.´ *Annu. Rev. Phytopathol*. 43: 83-116.

Thirumalesh, B.V., Thippeswamy, B., Banakar, S.P., Naveenkumar, K.J. & M. Krishnappa (2011). ´Assessment of Chemical Compounds for in vitro and in vivo Activity against Bacterial Black Spot of Mango.´ *Recent Research in Science and Technology* 3(9): 57-61.

Vidaver, A.K. & P.A. Lambrecht (2004). ´Bacteria as plant pathogens.´ *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2004-0809-01.

Voloudakis,A.E., T.M. Reignier & D.A. Cooksey (2005). ´Regulation of Resistance to Copper in *Xanthomonas axonopodis* pv. *vesicatoria*.´ *Applied and environmental microbiology* 71(2): 782–789.