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COPPER FORMULATIONS IN BACTERIAL BLIGHT CONTROL AND TOXIC EFFECTS ON COFFEE SEEDLINGS

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Abstract

Bacterial blight of coffee (*Pseudomonas syringae* pv. *garcae)* is an important coffee disease and can be controlled using antibiotics and copper-based compounds. However, copper-based compounds raise doubts among coffee growers regarding bacterial blight control efficiency and phytotoxic potential. In this work, coffee plants were sprayed with different copper molecules in order to study their efficiency on bacterial blight control and the phytotoxic potential. Seven copper formulations, cuprous oxide, copper oxychloride, copper nitrate, copper hydroxide 1 (water-dispersible granules) and 2 (concentrated suspension), copper sulfate 1 (complexed with gluconic acid) and 2 (Bordeaux mixture) were studied. The copper formulations efficiency was compared with the antibiotic kasugamycin, saline solution, and control. In controlled environmental conditions of temperature, relative humidity, and photoperiod, coffee seedlings were sprayed with the treatments and after 24 hours they were inoculated with *Pseudomonas syringae* pv. *garcae* suspension. Disease incidence and severity assessments were performed in a 2-day interval during a 16-day period. Phytotoxicity incidence and severity, mapping, and quantification of copper on the leaf tissue surface, dried leaves weight, and total copper leaf content were assessed 16 days after pathogen inoculation. Data were submitted to the Scott-Knott test ($p < 0.05$). Cuprous oxide and copper sulfate 2 proved most efficient to bacterial blight control, causing lower phytotoxicity effect, best covering, and persistence on leaf tissues. Copper nitrate and copper sulfate complexed with gluconic acid were more phytotoxicity compared to other copper formulations.

Keywords: Antibiotic. Bacteria. Chemical Control. Incidence. Phytotoxicity. *Pseudomonas syringae* pv. *garcae*.

1. Introduction

Brazil is the largest coffee producer and exporter, with ~ 46.5% of world production of arabica coffee, equivalent to 48.2 million 60-kg coffee green bags in the 2018/2019 crop (USDA 2020). However, although the Brazilian coffee growers use high technological technics to achieve high yields, diseases still are a limiting factor to the quality and productivity of coffee plantations.

Bacterial blight of coffee is caused by *Pseudomonas syringae* pv. *garcae* (Amaral et al. 1956) an important disease in major coffee growing regions in Brazil and around the world (Pozza et al. 2010; Ithiru et al. 2013; MacieL et al. 2018). The disease outbreak is favored in cold *areas* with exposure to wind, and the predominant cultivation of susceptible cultivars and the losses have been potentialized with the absence of chemical efficient control (Petek et al. 2006; Pozza et al. 2010; Zoccoli et al. 2011; Rodrigues et al. 2013).

Bacterial blight symptoms are found on coffee leaves, flowers, fruits, and young branches (Costa and Silva 1960). The most characteristic symptom of the disease is the presence of brown necrotic lesions on leaves, surrounded by a chlorotic halo. Bacterial blight also affects seedlings in nurseries, causing lesions on leaves, die-back of the seedlings, and in many cases, the death of plants (Costa et al. 1957; Belan et al. 2014). In nurseries, the lack of control of the disease can cause damages in up to 100% of the seedlings, since the plants are more susceptible to the disease, they have tender tissues, still in formation, which facilitates the colonization by the pathogen, in addition, the density of seedlings facilitates the spread of the disease among the seedlings (Rodrigues et al. 2013).

In an attempt to contain the disease, coffee growers have used sprays with antibiotics and copper-based compounds. These products are applied mainly in nurseries when there is a critical phase to disease development and spread. Control measures aiming to reduce the disease in coffee seedlings nurseries can impact the spread and management of the disease in new coffee plantations (Maciel et al. 2018).

In general, copper-based compounds are the best foliar treatments to control bacterial diseases, they can be a source of Cu^{2+} to plant nutrition and have fungicidal and bacteriostatic effects. However, copper efficiency has been questioned by coffee growers regarding the difference among copper formulations. Some coffee growers have reported reduced efficacy and phytotoxicity caused by copper compounds. Besides, the use of copper may have long-term consequences due to its environmental animal and plant toxicity, which makes it necessary for the optimization of its use in agriculture (La Torre et al. 2018).

Copper compounds' efficiency depends on its metallic copper concentration, persistence on the surface of plants' tissue, and ease of absorption by plants. In this way, this work evaluated the efficiency of different copper formulations on bacterial blight control and their phytotoxic potential on coffee seedlings.

2. Material and Methods

Plant material and experimental conditions

The experiment was conducted in a plant growth chamber under controlled temperature 23 ± 2°C, relative humidity 70 ± 3%, and 12-hour photoperiod with 2000-Watt fluorescent bulbs.

Coffee seedlings from cv. Catuaí Vermelho IAC-99 with six pairs of leaves were cultivated in a substrate composed of soil, cattle manure, and sand in proportion 3:1:1, respectively. Water was provided daily on soil without hitting the plant leaves.

Trials were conducted in randomized block design with 10 treatments and 4 replicates, three plants per replicate. The experiment was carried out two times to evaluate the result repetition. A combined analysis of variance of data was performed over time.

Treatments

Commercial products were used as copper sources. Efficiency was assessed using copper hydroxide, sulfate, oxychloride and nitrate, and cuprous oxide (Table 1). The trial used the highest doses of products registered at the Brazilian Ministry of Agriculture, according to laws and guidelines to evaluate efficiency and toxicity to animals and humans (Brasil 2020). Doses recommended by manufacturers ranged from 167.5 to 2500 ppm of copper. Antibiotic kasugamycin, registered at the Brazilian Ministry of Agriculture to control blister spot in coffee (Brasil 2020), was used for comparing the efficiency of *Pseudomonas syringae* pv. *garcae* control. Also, were used saline solution and control with sterilized distilled water (Table 1).

Two copper hydroxide formulations were used, a concentrated suspension (SC), and water-dispersible granules (WG). Foliar fertilizers had the lowest concentrations of metallic copper (Cu^{2+}).

Differently from fungicides, they presented formulations with other products. Copper is complexed with nitrate in a copper crop, and copper glucone contains gluconic acid and sulfur.

Treatments were sprayed on both sides of all seedling leaves to the point of runoff, using a Strong® sprayer with a cone nozzle filled at average pressure 30 lbs. After seven days the plants were inoculated with *P. syringae* pv. *garcae*.

Table 1. Treatments evaluated for control of bacterial blight of coffee.

Treatments/ active ingredient and controls	Commercial name	Formulation	Dose of commercial product	Quantity of Cu^{2+} in the commercial product (%)	Quantity of Cu^{2+} applied (ppm)[1]
Copper oxychloride	Recop®	Wettable powder	5 $g.L^{-1}$	50	2500
Cuprous oxide	Big red®	Wettable powder	5 $g.L^{-1}$	50	2500
Copper hydroxide 1	Kocide®	Water dispersible granules	4.72 $g.L^{-1}$	53	2500
Copper nitrate	Copper crop®	True solution	1.25 $mL.L^{-1}$	13.4	167.5
Copper sulfate 1	Copper glucone®	True solution	5 $mL.L^{-1}$	6	300
Copper hydroxide 2	Supera®	Concentrated suspension	7.15 $mL.L^{-1}$	35	2500
Copper sulfate 2	Copper sulfate	Soluble granules	10 $g.L^{-1}$	25	2500
Kasugamycin	Kasumin®	Concentrated solution	3 $mL.L^{-1}$	---	---
Saline solution (NaCl 0.85%)		---	---	---	---
Control	---	---	---	---	---

[1] based on 400 L/há.

Inoculum production and inoculation

Reference strain of *P. syringae* pv. *garcae* (CFPB1634) was used for inoculating seedlings. It was multiplied in 90 mm-Petri dishes with culture medium 523 Kado and Heskett (1970). Dishes were incubated at 28 ± 2°C and 12-hour photoperiod. After 48 hours, bacterial cells were suspended in sterilized saline solution (NaCl 0.85%). Bacterial concentration was determined using a spectrophotometer at 600 nm (OD600), according to Oliveira and Romeiro (1990). The bacterial cell suspension was prepared by dilution in sterilized saline solution at 1.1×10^9 CUF/mL concentration (absorbance 0.2). Inoculum suspension was sprayed on both sides to the point of runoff, using the same equipment and conditions described for spraying. To differentiate a possible phytotoxic effect caused by saline solution (NaCl 0.85%) from disease symptoms or copper application, was added a treatment with this solution without bacterial suspension inoculation.

Disease intensity assessment

After the first disease symptoms, disease incidence and severity assessments were performed on the first three pairs of leaves in seedlings, in a 2-day interval during a 16-day period. Incidence, was estimated from the relation between the total number of injured leaves and the total number of sampled leaves, was calculated the mean incidence per replicate. To disease severity assessment was used the diagrammatic scale of Belan et al. (2014); the mean disease severity was calculated for each plot.

In each experimental unit, incidence and mean severity rates over time were used to plot disease progress curves and calculate the area under the incidence progress curve (AUIPC) and area under severity progress curve (AUSPC), as proposed by Shaner and Finney (1977).

Weight of dried leaves and analysis of copper content in leaf tissue

After 30 days of application of treatments at the end of the experiment, leaves were collected, washed with deionized water to remove impurities and spray residues (Malavolta 1997), and dried in forced-air circulation oven at 60ºC. Dried leaves were then weighted (g) on a precision scale and analyzed for copper contents, according to the method by Malavolta (1997).

X-ray microanalysis of copper in outer leaf tissue

The spatial distribution of copper on the abaxial leaf surface was assessed using X-ray microanalysis mapping in scanning electron microscopy (SEM). Leaves were collected and washed in deionized water to remove impurities and copper residues. After washed, fragments of 5 cm^2 were randomly collected from leaf blades. These fragments were fixed in aluminum stubs with double adhesive carbon tape, they were identified and maintained for 24 hours in a desiccator with silica gel to dehydrate samples. Then, they were placed into sputter (MED 010, Balzer) for carbon coating. Samples were observed in scanning electron microscopy (Leo Evo 40 XVP) coupled to detection system MAX: EDS-X Flash Detector 5010 (Bruker) and analyzed with ESPIRIT 1.9 software (Bruker) (Belan et al. 2014).

Five observations were performed per treatment. A fixed-size rectangular area was mapped for each examination, and the qualitative distribution of copper ions on the leaf abaxial surface was represented in images. These were generated at 20 Kv, distance 8.5 mm, increase ± 80 times, and Kcps ranging from 3 to 4.

Images were processed using Assess® software for the percentage of leaf area coated with copper. Four images per treatment were analyzed, corresponding to replicates.

Evaluation of phytotoxicity

By 21 days after inoculation, incidence, and severity of toxicity symptoms on leaves were assessed. The Cu toxicity symptoms on coffee leaves are characterized by brown color on the upper leaf surface and do not have a yellow halo. Toxicity incidence was based on the relation between the total number of leaves with symptoms and the total number of leaves sampled per plant. Severity was analyzed by digitized images of leaves and the percentage of injured leaf area in relation to total leaf area was quantified using Assess® software.

Data analysis

Data of variables AUIPC, AUSPC, incidence, and severity of toxicity, dried leaf weight, copper content in leaf tissue, and percentage of leaf area covered by copper were submitted to analysis of variance. As data did not meet ANOVA assumptions of normality (Shapiro Wilk), homogeneity (Bartlett), and independence (Durbin Watson) tests, they were transformed by $\sqrt{(x + 0.5)}$. Media treatments significant by F-test were grouping by the Scott-Knott test ($p < 0.05$). Statistical analyses were performed using R® software.

3. Results

The combined analysis of data over time showed no significant difference ($p < 0.05$) for both experiments. Thus, the results refer to the mean of experiments.

There was a difference ($p < 0.05$) between treatments for AUIPC and AUSPC in bacterial blight control on coffee leaves. All copper formulations and the antibiotic reduced AUIPC and AUSPC comparing with control (Table 2). However, copper molecules in the form of cuprous oxide, oxychloride, nitrate, and sulfate were more efficient than copper hydroxide and antibiotics regarding AUIPC (Table 2). To AUSPC, although all treatments differ from control, there was no difference between them (Table 2).

Table 2. Mean values of area under the disease incidence progress curve (AUIPC) and disease severity progress curve (AUSPC) of bacterial blight (*Pseudomonas syringae* pv. *Garcae*) in coffee leaves (*Coffea arabica*).

Treatments	AUIPC*	AUSPC*
Cuprous oxide	5.2^{C}	0.02^{B}
Copper oxychloride	12.5^{C}	0.06^{B}
Copper nitrate	17.7^{C}	0.08^{B}
Copper sulfate 2	37.5^{C}	0.17^{B}
Copper sulfate 1	37.5^{C}	0.18^{B}
Kasugamycin	40.6^{B}	0.2^{B}
Copper hydroxide 1	45.8^{B}	0.25^{B}
Copper hydroxide 2	111.4^{B}	0.62^{B}
Control	526.0^{A}	5.10^{A}
Saline solution	0 C	0^{B}

*Means followed by the same letter in the column do not differ by Scott-Knott test at 5% significance.

It was not observed difference ($p < 0.05$) to dry leaf weight among the treatments (Table 3).

There was a difference ($p < 0.05$) between treatments in relation to copper content in leaf tissue (Figure 1). The highest copper leaf content was observed on leaves of seedlings sprayed with copper sulfate 2 and the minimum values were observed in treatments with saline solution, kasugamycin, and control (Figure 1).

Table 3. Dry leaf weight.

Treatments	Dry leaf weight (g)
Saline solution	1,23^{A}
Control	1,21^{A}
Kasugamycin	1,28^{A}
Copper Hydroxide 2	1,31^{A}
Copper Nitrate	1,17^{A}
Copper Hydroide 1	1,28^{A}
Copper Oxychloride	1,19^{A}
Copper Sulfate 1	1,25^{A}
Cuprous Oxide	1,28^{A}
Copper Sulfate 2	1,3^{A}

*Means followed by the same letter in column do not differ by Scott-Knott test at 5% significance.

There was a difference between treatments to the percentage of foliar area covered with copper. The lowest percentages of leaf covered by copper occurred for saline solution, control, and kasugamycin. The highest percentage of leaf covered by copper was observed to cuprous oxide, copper hydroxide1 and 2, copper oxychloride, and copper sulfate 2 (Figure 2 and Table 4).

Table 4. Percentage of abaxial leaf area of coffee covered with copper, based on Assess® software processing of images generated by X-ray electron microscopy.

Treatment	Percentage of leaf area covered with copper*
Saline solution	0.6^{C}
Control	0.7^{C}
Kasugamycin	0.9^{C}
Copper nitrate	26.6^{B}
Copper sulfate 1	32.4^{B}
Copper hydroxide 1	94.6^{A}
Copper hydroxide 2	95.9^{A}
Copper oxychloride	96.8^{A}
Cuprous oxide	96.8^{A}
Copper sulfate 2	98.3^{A}

*Means followed by the same letter in column do not differ by Scott-Knott test at 5% significance.

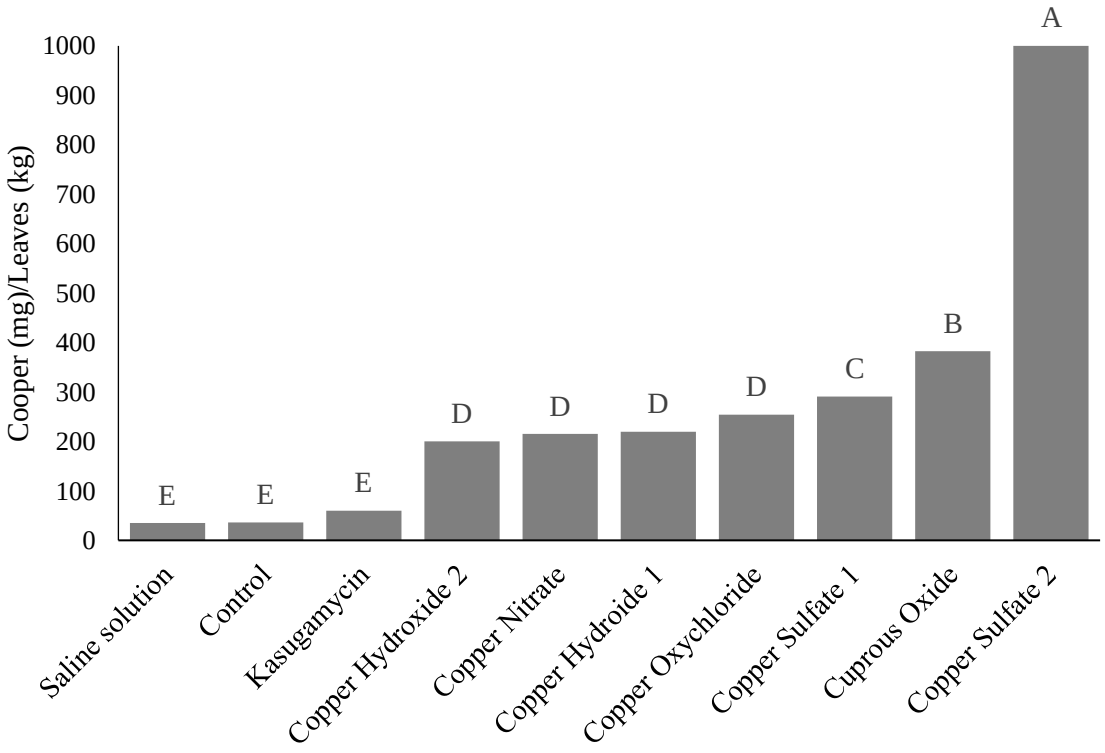

Figure 1. Mean values of copper contents in mg.kg^{-1} of the dry weight of leaves in coffee seedlings sprayed with different copper formulations. Bars with the same letter do not differ by the Scott-Knott test at 5% significance.

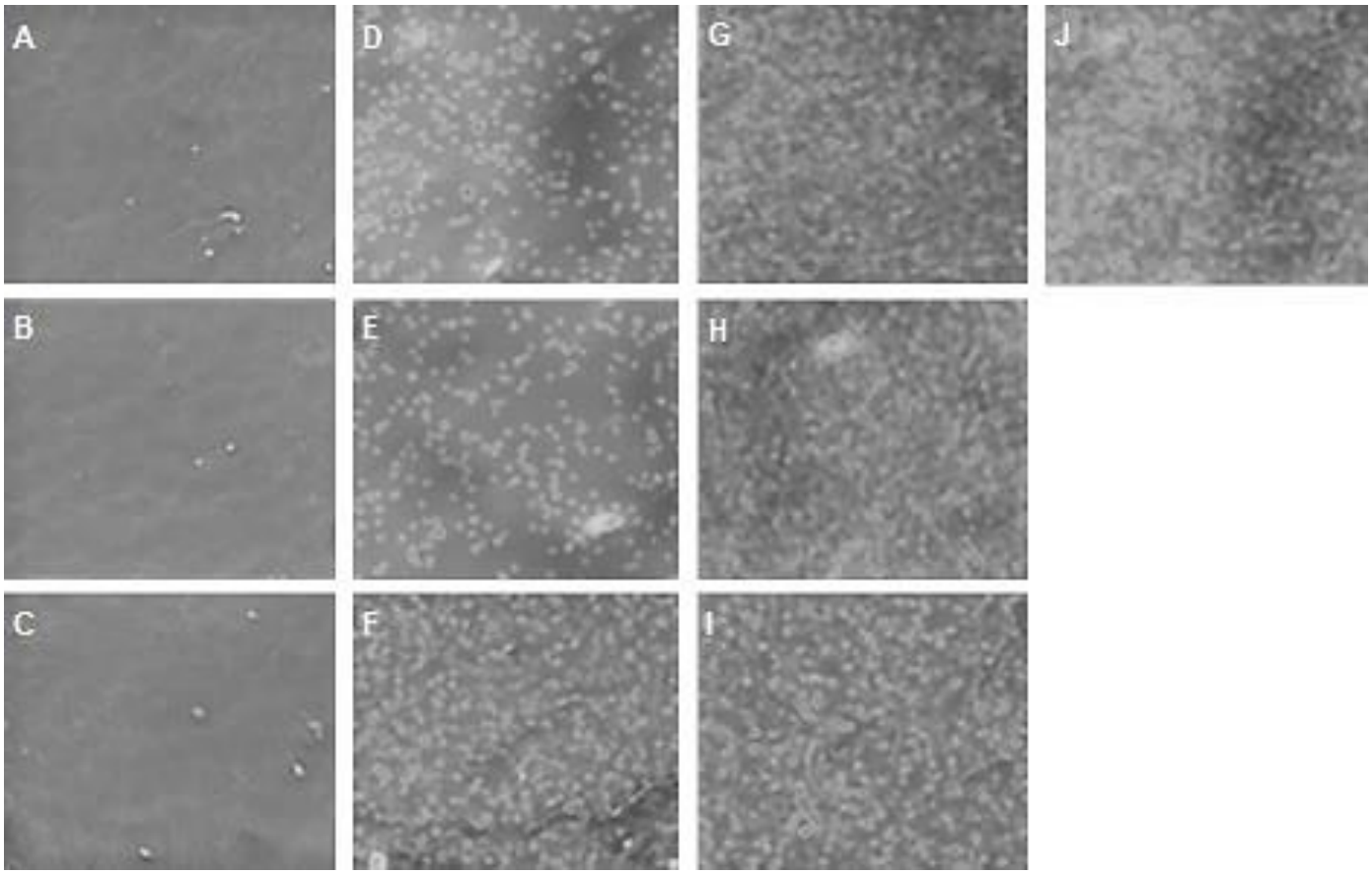

Figure 2. X-ray microanalysis for mapping copper (white color) on the abaxial surface of coffee leaves in seedlings sprayed with the following treatments: A - saline solution; B - Control; C - kasugamycin; D - copper sulfate1; E - copper nitrate; F - copper hydroxide 1; G - copper oxychloride; H - copper hydroxide 2; I - cuprous oxide; J - copper sulfate 2.

Symptoms of toxicity in leaves were tanning followed by necrosis of the affected area. All treatments caused toxicity in leaves of coffee seedlings; however, there were differences ($p < 0.05$) in the incidence and severity of toxicity. The treatment containing copper nitrate showed higher incidence and severity of toxicity symptoms in leaves, although be the lowest dose of Cu^{2+} applied (Table 5).

Table 5. Mean values of toxicity incidence and severity on coffee seedlings sprayed with different copper formulations.

Treatment	Toxicity	
	Incidence[1]	Severity [2]
Kasugamycin	4.17[D]	0.03[C]
Copper sulfate 2	9.03[C]	0.05[C]
Saline solution	13.2[C]	0.06[C]
Copper hydroxide 1	14.58[C]	0.07[C]
Control	14.59[C]	0.07[C]
Cuprous oxide	15.97[C]	0.07[C]
Copper hydroxide 2	18.06[C]	0.09[C]
Copper oxychloride	16.67[C]	0.09[C]
Copper sulfate 1	39.58[B]	0.21[B]
Copper nitrate	63.20[A]	0.35[A]

*Means followed by the same letter in the column do not differ by the Scott-Knott test ($p < 0.05$). [1]Percentage of injured leaves. [2]Percentage of injured leaf area.

4. Discussion

All copper treatments reduced disease severity greater than 87%, mainly in concentrations of 2500 ppm. Copper products form a layer of copper on the plant surface playing a protective role (Gisi and Sierotzki 2008). Therefore, during the infectious process on the leaf surface, copper is absorbed by bacterial cells and becomes free in the cytoplasm to catalyze reactions involving reactive oxygen. The oxygen molecules in the cytoplasm cause lipid peroxidation and protein oxidation (Santo et al. 2011) leading to a bacteriostatic effect. Copper also forms complexes with sulfhydryl groups of enzymes (Chillappagari et al. 2010), causing generalized metabolic disorder, which can lead to cell rupture and death (Gisi and Sierotzki 2008).

However, bacterial death is not widespread, and part of the population still survives on the leaf surface due to the bacteriostatic effect. In addition, when copper concentration is not sufficient to cause death, bacterial cells protected by mucilaginous capsules still maintain their integrity, thus being able to infect the host (Ordax et al. 2006; Marcuzzo et al. 2009).

Patrício et al. (2012) observed reduced severity of bacterial blight of coffee using copper oxychloride and hydroxide at 550 ppm. Tomato (*Solanum lycopersicum* L.) in treatments with 200 to 550 ppm of different copper formulations showed the lower intensity of leaf spot caused by bacteria of the same species (*P. syringae* pv. *syringae*) (Gilardi et al. 2010). That is, copper-based products in different concentrations can reduce the severity of bacterial diseases.

Both concentrated suspension and dispersible granules of copper hydroxide were less efficient in reducing disease incidence in nursery seedlings. Yamanda et al. (2014) observed a lower efficiency of copper hydroxide in relation to cuprous oxide at dose 2500 ppm in the same pathosystem. Menkissoglu and Lindow (1991) and Gilardi et al. (2010) also observed this result to other pathovars of *Pseudomonas syringae* species. The low efficiency of kasugamycin compared to other treatments may be due to the isolate resistance. Mello et al. (2011) found *Pectobacterium carotovorum* subsp. carotovorum resistance to kasugamycin in Chinese cabbage crop.

Different levels of disease and phytotoxicity did not influence the weight of dried leaves, the period between inoculation and leaves collection to determine the weight was short and not enough to grow, besides, there was not seedling defoliation during the experimental period.

Even leaves had been washed with deionized water to remove impurities before being analyzed, copper content in leaves sprayed with cupric molecules ranged from 200 to 1000 $mg.kg^{-1}$ (Figure 1), which are far above the tolerable limit for coffee, 13 to 55 $mg.kg^{-1}$according Carmo et al. (2012). Copper sulfate 2 and cuprous oxide presented the highest leaf contents, however with low phytotoxicity levels (Table 5).

Treatments sprayed at the same concentration presented diferentt results, this high copper leaf content can be due to strong fixation of copper not absorbed outside of the leaf, with a low amount absorbed and high resistance to washing, as analysis of leaf content requires previous washing with deionized water to remove impurities (Malavolta 1997). Tecchio et al. (2015) also found strong copper fixation on leaves in lemon seedlings sprayed with copper sulfate and cuprous oxide under sprinkler irrigation. This greater persistence of products on the leaf can increase the plant protection period. Oliveira et al. (2002) found higher control of rust in coffee sprayed with cuprous oxide even by 60 days after application under rain simulation.

Copper covering on the abaxial surface of leaves varied according to the presence of copper in treatment and type of cupric molecule (Table 4). In samples from treatments with kasugamycin, control, and saline solution, copper was detected on the leaf surface by mapping close to zero (Table 4), as treatments contained no copper (Table 1). Amount applied (Table 1) and copper covering in treatments nitrate and copper sulfate 1 (Table 4) were smaller than in other copper treatments; however, copper leaf content found in these treatments was similar to the other treatments (Figure 1). Thus, copper content detected in these treatments was mostly absorbed in leaf, as they were foliar fertilizers (Fageria et al. 2009). In the other treatments, the leaf surface was evenly covered by copper, as it is desirable for protecting leaf against bacteria.

Copper is essential for coffee plant nutrition, as it activates enzymes and provides higher plant growth (Dias et al. 2015). However, copper excess may cause phytotoxicity since its active sites in plant pathogens are proteins and membranes, which are also present in plants (Chillappagari et al. 2010; Santo et al. 2011). Copper toxicity symptoms in leaves are tanning followed by necrosis of the affected area (Paradela et al. 2006).

All copper treatments used in this study caused phytotoxicity varying in intensity according to the molecule. Although most of the products were sprayed at 2500 ppm, copper nitrate and copper sulfate 1 were applied at 167.5 and 300 ppm respectively and presented the highest toxicity rates (Table 5). Thus, phytotoxicity is not necessarily linked to the amount of Cu^{+2} but rather to properties of other product constituents, which influence the foliar absorption of copper.

Nitrate molecules are highly dissociable in water (Aghaie et al. 2007), providing higher plant uptake of Cu^{+2} and accompanied ion (Peyvast et al. 2009). Brunetto et al. (2008) found increased cation Ca^{+2} absorption in peach leaves treated with calcium nitrate, thus confirming the role of nitrate in the absorption of the accompanied cation.

Copper sulfate 1 contains gluconic acid, which decreases pH on the leaf surface and changes cuticular permeability, thus increasing the absorption rate of Cu^{+2} ions in solution (Marschner 2012) and consequently rising toxicity in plants. The acid pH of the mixture promotes high absorption of Cu^{+2} in coffee leaf (Dias et al. 2015). Thus, copper absorbed in treatments can exceed 55 ppm within the tissue, which is the toxicity limit for coffee plant cells (Carmo et al. 2012). Saline solution (0.85% NaCl) atomized in seedlings also caused toxicity. Symptoms were initially gray-brown spots progressing to tissue necrosis mainly in young tender leaves and leaf margins, where suspension concentrated after atomization. Thus, although the saline solution is necessary to calibrate concentration of bacterial cell suspension (Lyon et al. 2005; Jiang et al. 2009), it may cause toxicity symptoms which must be distinguished from disease symptoms during disease assessment.

All copper molecules were efficient in disease control. Foliar fertilizers copper sulfate 1 and copper nitrate provided less tissue covering and more absorption, causing phytotoxicity when compared with other formulations. Conversely, copper fungicides provided more leaf tissue covering and remained on the outer side of leaves without causing toxicity. In addition, copper sulfate 2 and cuprous oxide molecules were more resistant to removal by washing.

5. Conclusions

Cuprous oxide and copper sulfate 2 at 2500 ppm proved most efficient for bacterial blight control, causing lower toxicity and more covering of leaf tissue in coffee seedlings.

Micronutrients copper nitrate and copper sulfate complexed with gluconic acid should be used with caution because they are able to promote plant toxicity even in lower concentrations.

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References

AGHAIE, M., AGHAIE, H. and EBRAHIMI, A. Thermodynamics of the solubility of barium nitrate in the mixed solvent, ethanol+ water, and the related ion-association. *Journal of molecular liquids*. 2007, **135**(1), 72-74. http://dx.doi.org/10.1016/j.molliq.2006.10.005

AMARAL, J.D., TEIXEIRA, C. and PINHEIRO, E. A bactéria causadora da mancha aureolada do cafeeiro. *Arquivo do Instituto Biológico*. 1956, **23**, 151.

BELAN, L.L., et al. Diagrammatic scale for assessment of bacterial blight in coffee leaves. *Journal of Phytopathology*. 2014, **162**, 801-810. https://doi.org/10.1111/jph.12272

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *AGROFIT: Sistema de Agrotóxicos Fitossanitários*. 2020. Available from: http://agrofit.agricultura.gov.br/agrofit_cons/!ap_produto_form_detalhe_cons?p_id_produto_formulado_tecnico=5369&p_tipo_janela=NEW

BRUNETTO, G., MELO, W. and KAMINSKI, J. Foliar application of calcium in peach in Serra Gaúcha: evaluation of content of nutrients in the leaf, fruit, and yield. *Revista Brasileira de Fruticultura*. 2008, **30**(2), 528-533. http://dx.doi.org/10.1590/S0100-29452008000200045

CARMO, D.L., et al. Micronutrientes em solo e folha de cafeeiro sob sistema agroflorestal no sul de minas gerais. *Coffee Science*. 2012, **7**(1), 76-83.

CHILLAPPAGARI, S., et al. Copper stress affects iron homeostasis by destabilizing iron-sulfur cluster formation in Bacillus subtilis. *Journal of bacteriology*. 2010, **192**, 2512-2524. http://dx.doi.org/10.1128/JB.00058-10

COSTA, A.S., et al. Bacterial halo blight of coffee in Brazil. *Phytopathologische Zeitschrift*. 1957, **28**, 427-444.

COSTA, A.S. and SILVA, D.M.A. Mancha aureolada do cafeeiro. *Bragantia*. 1960, **19**, 62-68.

DIAS, K.G.L., POZZA, A.A.A. and POZZA, E.A. Cobre via foliar na nutrição e na produção de mudas de cafeeiro. *Coffee Science*. 2015, **10**(4), 516-526.

FAGERIA, N.K., et al. Foliar fertilization of crop plants. *Journal of Plant Nutrition*. 2009, **32**(6), 1044-1064. http://dx.doi.org/10.1080/01904160902872826

GILARDI, G., GULLINO, M. and GARIBALDI, A. Evaluation of spray programmes for the management of leaf spot incited by Pseudomonas syringae pv. syringae on tomato cv. Cuore di bue. *Crop protection*. 2010, **29**, 330-335. http://dx.doi.org/10.1016/j.cropro.2009.11.010

GISI, U. and SIEROTZKI, H. Fungicide modes of action and resistance in downy mildews. *European Journal of Plant Pathology*. 2008, **122**, 157-167. http://dx.doi.org/10.1007/s10658-008-9290-5

ITHIRU, J.M., et al. Methods for early evaluation for resistance to bacterial blight of coffee. *African Journal of Agriculture Research*. 2013, **8**(21), 2450-2454. http://dx.doi.org/10.5897/AJAR2013.6717

JIANG, W., MASHAYEKHI, H. and XING, B. Bacterial toxicity comparison between nano-and micro-scaled oxide particles. *Environmental Pollution*. 2009, 157, 1619-1625. http://dx.doi.org/10.1016/j.envpol.2008.12.025

KADO, C. and HESKETT, M. Selective media for isolation of Agrobacterium, Corynebacterium, Erwinia, Pseudomonas, and Xanthomonas. *Phytopathology*. 1970, **60**, 969-976.

LA TORRE, A., IOVINO, V. and CARADONIA, F. Copper in plant protection: current situation and prospects. *Phytopathologia Mediterranea.* 2018, **57**(2), 201-236. https://doi.org/10.14601/Phytopathol_Mediterr-23407

LYON, D.Y., et al. Bacterial cell association and antimicrobial activity of a C60 water suspension. *Environmental toxicology and chemistry*. 2005, **24**, 2757-2762. http://dx.doi.org/10.1897/04-649R.1

MACIEL, K.W., et al. Bacterial halo blight of coffee crop: aggressiveness and genetic diversity of strains. *Bragantia*. 2018, **77**(1), 96-106. http://dx.doi.org/10.1590/1678-4499.2016267

MALAVOLTA, E., VITTI, G.C. and OLIVEIRA, S.A. *Avaliação do estado nutricional das plantas: princípios e aplicações*. 2 ed. Piracicaba: Potafos, 1997.

MARCUZZO, L.L. Importance of epiphytic populations in the epidemiology of bacterial diseases. *Revista de Ciências Agroveterinárias*. 2009, **8**(2), 146-151.

MARSCHNER, P. *Marschner's mineral nutrition of higher plants.* 3 ed. Adelaide: Academic Press, 2012.

MELLO, M., et al. Use of antibiotics and yeasts for controlling Chinese cabbage soft rot. *Horticultura Brasileira*. 2011, **29**(1), 78. http://dx.doi.org/10.1590/S0102-05362011000100013

MENKISSOGLU, O. and LINDOW, S.E. Chemical forms of copper on leaves in relation to the bactericidal activity of cupric hydroxide deposits on plants**.** *Phytopathology*. 1991, **81**(10), 1263-1270.

OLIVEIRA, J.R. and ROMEIRO, R.S. Reação de folhas novas e velhas de cafeeiro a Pseudomonas cichorii e Pseudomonas syringae pv. garcae. *Fitopatologia Brasileira*. 1990, **15**(4), 355-357.

OLIVEIRA, S.H., SANTOS, J.M.F. and GUZZO, S.D. Effect of rain on tenacity and efficiency of fungicides associated with vegetable oil in the control of rust coffee disease. *Fitopatologia Brasileira*. 2002, 27(6), 581-585. http://dx.doi.org/10.1590/S0100-41582002000600004

ORDAX, M., et al. Survival strategy of Erwinia amylovora against copper: induction of the viable-but-nonculturable state. *Applied and environmental microbiology*. 2006, **72**, 3482-3488. http://dx.doi.org/10.1128/AEM.72.5.3482-3488.2006

PARADELA, A.L., et al. Avaliação do índice de fitotoxidez de triazóis em mudas de café e eficiência dos triazóis aplicados via foliar no controle da ferrugem (Hemileia vastatrix) do cafeeiro (*Coffea arabica*). *Fitopatologia Brasileira*. 2006, **32**(2), 72-81.

PATRÍCIO, F.R.A., et al. Avaliação da eficiência de fungicidas cúpricos no controle da mancha aureolada (*Pseudomonas syringae* pv. *garcae*) em mudas de cafeeiro. *Anais do Congresso Brasileiro de pesquisas cafeeiras*. 2012, **38**, 1-2.

PETEK, M.R., et al. Selection of progenies of Coffea arabica with simultaneous resistance to bacterial blight and leaf rust. *Bragantia*. 2006, **65**(1), 65-73. http://dx.doi.org/10.1590/S0006-87052006000100009

PEYVAST, G., et al. Uptake of calcium nitrate and potassium phosphate from foliar fertilization by tomato. *Journal of Horticulture and Forestry*. 2009, **1**(1), 7-13.

POZZA E.A, CARVALHO V.L and CHALFOUN, S.M. 2010. Sintomas de injurias causadas por doenças do cafeeiro. In: GUIMARÃES, R.J., MENDES, A.N.G. and BALIZA, D.P. (Eds.). *Semiologia do cafeeiro*. 1st ed. Lavras: Editora UFLA, pp. 69-101.

RODRIGUES, L.M.R., et al. *Mancha aureolada do cafeeiro causada por Pseudomonas syringae pv. garcae*. Campinas: IAC, 2013.

SANTO, C.E., et al. Bacterial killing by dry metallic copper surfaces. *Applied and environmental microbiology*. 2011, **77**, 794-802. http://dx.doi.org/10.1128/AEM.01599-10

SHANER, G. and FINNEY, R.E. The effect of nitrogen fertilization on the expression of slow-milde wing resistance in Knox wheat. *Phytopathology*. 1977, **67**(8), 1051-1056.

TECCHIO, M.A., MERLIM, T.P.D.A., LEONEL, S. and GRASSI FILHO, H. Copper fertilization in citric seedlings. *Irriga*. 2015, **1**(1), 87. http://dx.doi.org/10.15809/irriga.2015v1n1p87

USDA. *Coffee: World Markets and Trade. Foreign Agricultural Service*. 2020. Available from: https://downloads.usda.library.cornell.edu/usdaesmis/files/m900nt40f/6m3129089/r494w654j/coffee.pdf

YAMANDA, J.K. *Resistência de isolados de Pseudomonas syringae pv. garcae*. 2014. Dissertação de Mestrado, Universidade Federal de Lavras, 2014. Available from: http://repositorio.ufla.br/jspui/bitstream/1/4791/1/DISSERTA%C3%87%C3%83O%20Resist%C3%AAncia%20de%20isolados%20de%20Pseudomonas%20syringae%20pv.%20garcae%20ao%20cobre.pdf

ZOCCOLI, D.M., TAKATSU, A. and UESUGI, C.H. Ocorrência de mancha aureolada em cafeeiros na Região do Triângulo Mineiro e Alto Paranaíba. *Bragantia*. 2011, **70**(4), 843-849. https://doi.org/10.1590/s0006-87052011000400017

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