Potential of the Dithiocarbimate Fungicides on the Control of Coffee Leaf Rust and Asian Soybean Rust

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Abstract

Coffee leaf rust (*Hemileia vastatrix*) and Asian soybean rust (*Phakopsora pachyrhizi*) are diseases that cause great losses in the productivity of these crops, not only in Brazil but on a global scale. Coffee and soybean varieties grown are susceptible to these diseases. Thus, it is necessary to search for efficient compounds for their chemical control, mainly from the group of protectors or residuals so that they can be formulated with systemic fungicides to control the diseases. This allows not only the efficient management of diseases but also the prevention of the emergence of resistant mutants in the populations of these pathogens. In this context, the present study aimed to evaluate the sensitivity and effect of *bis*(N-R-sulfonyldithiocarbimato)zincate(II) salts fungicides on the epidemiological components of pathosystems coffee × *H. vastatrix* and soybean × *P. pachyrhizi*. Initially, four zinc(II) complexes salts (1A, 2A, 1B, and 2B) with N-R-sulfonyldithiocarbimates were synthesized. In the first experiment, the *in vitro* sensitivity of *H. vastatrix* and *P. pachyrhizi* was studied for the four compounds synthesized and mancozeb at 0.5, 5.0, 50.0, 100.0 and 200.0 μmol L^{-1}. All the compounds synthetized in this study had inhibitory effects on *H. vastatrix and P. pachyrhizi.* In the greenhouse it was studied the effect of *bis*(N-R-sulfonyldithiocarbimato)zincate(II) salts on the epidemiological components of coffee leaf rust and Asian soybean rust. For the pathosystem coffee × *H. vastatrix*, there were no differences in the values obtained for the *bis*(N-R-sulfonyldithiocarbimato)zincate(II) salts and mancozeb for the latent period. For the sporulated lesion variable, the control treatment had a mean value of 149.0 lesions/leaf, differing significantly from the other treatments. The mean value of compound 2B was estimated as 25.0 lesions/leaf, differing significantly from treatments 1A, 1B, 2B, and mancozeb. Treatments 1A, 1B, 2B, and mancozeb did not differ significantly from each other. For the Asian soybean rust, the area under the disease progress curve had a mean value of 75.8 for the control, while for the 2A treatment the value was 4.1, differing from the other compounds. The treatments 1A, 1B, 2A, and mancozeb did not differ significantly from each other. In conclusion, compounds 1A, 2A, and 1B were more efficient in the control of the coffee leaf rust, while compound 2A was efficient in the control of the Asian soybean rust.

Keywords: *Hemileia vastatrix*, *Phakopsora pachyrhizi*, chemical control, multisite fungicides, dithiocarbimate group

1. Introduction

The increase in the world population has driven the search for new agrochemicals to ensure the sustainable production of food. Such agrochemicals aim to maintain the productivity potential of crops, not only in Brazil but on a global scale. In Brazilian agribusiness, the production of coffee (*Coffea arabica* L.) and soybean (*Glycine max* L. Merryl) correspond 45.3 milions bags and 120.3 milions tons, respectively (CONAB, 2019). Among the main factors capable of limiting coffee and soybean production in the Brazil and world, rust diseases stand out. The fungus *Hemileia vastatrix* Berk. & Br., the causal agent of coffee leaf rust, causes damage that can affect up to 50% of coffee production (Zambolim, 2016). Besides, the fungus *Phakopsora pachyrhizi*, the causal agent of Asian soybean rust, causes damage of up to 90% to soybean crops (Yorinori et al., 2005). Management strategies for these diseases are based on a combination of specific- site and multi-site fungicides (Godoy et al., 2016; Zambolim, 2016; Reis et al., 2021). Usually, for the coffee leaf rust, strategies are based on

the use of triazole fungicides formulated with strobilurins combined with multi-site copper fungicids. However, copper is a heavy metal that can accumulate in the environment, despite being crucial in the control not only of coffee leaf rust but also of other diseases and as a nutrient (Sousa et al., 2017). For the Asian soybean rust, chemical control strategies usually adopt triazoles, strobilurins, and dicarboxamides associated with multi-site chlorothalonil or mancozeb (Ponce et al., 2019). The use of multi-site fungicides has become indispensable to reduce the selection pressure on the pathogen population. Several studies of multiple resistance to triazole, strobilurin, and dicarboxamide groups are reported in the literature (Schmitz et al., 2014; Twizeyimana & Hartman, 2017; Simões et al., 2018). Currently, the companies have been using multi-site fungicides developed in the 1950-60s (Zambolim & Caixeta, 2018; Reis et al., 2021).

Several studies have shown, *in vitro*, the inhibition of mycelial growth of the fungi *Colletotrichum gloeosporioides* and *Botrytis cinerea* by new compounds belonging to the dithiocarbimate group (Bottega et al., 2013; Oliveira et al., 2015). The dithiocarbimate group is analogous to the dithiocarbamates but does not have a commercial formulation. The dithiocarbamates are widely used in agriculture, and their leading representative is mancozeb (Zambolim, 2008). Such groups differ chemically only by the existence of a double bond between the carbon and nitrogen atoms. In this context, researches seeking for new fungicide compounds that are highly efficient in disease control and have good acceptance by the society are vital. Dithiocarbimates are promising compounds since they have a multi-site action, either alone or as compound formulations with systemic fungicides, in the disease control of plants. The first study, involving this group of chemical compounds, in the disease control was conducted by Vidigal et al. (2019). The authors reported for the first time the effect of new heteroleptic nickel(II) complexes with aromatic and aliphatic sulfonyldithiocarbimates, triphenylphosphine and 1,2-*bis*(diphenylphosphine)ethane on the uredospore germination of *H. vastatrix* and *P. pachyrhizi*; however, their experiments were restricted to *in vitro* assays, and the control efficiency of this fungicide group in controlled conditions *in vivo* is unknown.

This work is justified due to the fact that, coffee leaf rust and Asian soybean rust can severely damage both crops. In addition, there is a great need to find new protective fungicide compounds to be used in mixtures with systemic and mesostemic fungicides, aiming to reduce the emergence of resistant mutants of specific mechanism of action fungicides, used in disease control.

Thus, this study aimed to evaluate the sensitivity and effect of *bis*(N-R-sulfonyldithiocarbimato)zincate(II) salts on the epidemiological components of the pathosystems coffee × *H. vastatrix* and soybean × *P. pachyrhizi*.

2. Material and Methods

2.1 Synthesis of N-R-Sulfonyldithiocarbimate Potassium Salts

The synthesis of the four N-R-sulfonyldithiocarbimate potassium salts consisted of two stages, following the procedures described by Amin (2011).

The first stage included the production of the precursors of potassium dithiocarbimates. In a round-bottom flask, 10,000 µmol of methanesulfonamide or 4-chlorobenzenesulfonamide; 20 mL of *N,N*-dimethylformamide; 0.60 mL of carbon disulfide (10,000 µmol) and 1.32 g of KOH (20,000 µmol) were added. The reaction mixture was kept under stirring until the consumption of KOH, indicating the end of the reaction. Then, 20 mL of absolute ethanol was added, and the round-bottom flask was placed in an ice bath.

The yellow precipitates resulting from the reaction were filtered and then washed with ethanol, ethyl acetate, and diethyl ether. Soon after, the precipitates were left in a desiccator for 24 hours. At the end of this reaction, the potassium methylsulfonyldithiocarbimate and the potassium 4-chlorophenylsulfonyldithiocarbimate were obtained as products.

The second stage consisted of the stabilization of the dithiocarbimates from the formation of zinc complexes. To perform the synthesis of the zinc complexes, 1,000 µmol of potassium dithiocarbimate, 20 mL of MeOH/H_2O solution (1:1 v/v), and 500 µmol of zinc sulfate dissolved in 4 mL of H_2O were added in a round-bottom flask. The reaction mixture remained under stirring for one hour. Then 1,000 µmol of tetraphenylphosphonium chloride or tetrabutylammonium bromide dissolved in water were added, drop by drop. The resulting precipitate was filtered in a funnel with a porous porcelain plate, washed five times, and then dried under reduced pressure for 24 hours. In this way, "1" is 4-chlorophenyl group and "2" is methyl group, white group "B" has the cation tetrabutylammonium and group "A" has the cation tetraphenylphosphonium. The four synthesized compounds were named as follows: tetraphenylphosphonium *bis*(4-chlorophenylsulfonyldithiocarbimate)zincate(II) (1A); tetraphenylphosphonium,   *bis*(methylsulfonyldithiocarbimate)zincate(II)   (2A);   tetrabutylammonium

bis(4-chlorophenylsulfonyldithiocarbamate)zincate(II)	(1B);	and	tetrabutylammonium *bis*(chlorophenylsulfonyldithiocarbimate)zincate(II) (2B).

2.2 Production of H. vastatrix and P. pachyrhizi Uredospores

Coffee seeds of the variety Caturra lineage IAC 144 were sown in the sand and kept at 28 °C. After thirty days, the seedlings were transplanted to 2.0 dm^3 vessels and cultivated for 45 days. Then, the third pair of leaves were inoculated with the *H. vastatrix uredospores race* II from the Plant Protection Laboratory, Department of Plant Pathology, Federal University of Viçosa, state of Minas Gerais, Brazil. The plants remained for 48 hours in a dew chamber in the dark at 22 °C during the entire experiment and were kept in a growth chamber with a 12-hour photoperiod for 35 days until the production of uredospores.

The uredospores *of P. pachyrhizi* from the Plant Protection Laboratory were inoculated at the V5 phenological stage in the soybean variety TMG 132. Subsequently, the plants in 2.0 cm^3 vessels were kept for 48 hours in the dew chamber, in the dark, and at 22 °C. After inoculation, the plants were placed in a growth chamber with a 12-hour photoperiod for 15 days to produce uredospores.

The produced uredospores were collected. One part was kept in a desiccator at 5 °C and 50% relative humidity and the other part in an ultra-freezer at -80 °C.

2.3 The Sensitivity of H. vastatrix and P. pachyrhizi to the bis(N-R-Sulfonyldithiocarbimato)Zincate(II) Salts

Two *in vitro* experiments were performed in a completely randomized design, with three replicates. The treatments were composed of mancozeb (Sigma Aldrich®) compounds 1A, 2A, 1B, and 2B at 0.5, 5.0, 50.0, 100.0, and 200.0 µmol L^{-1}, in addition to the controls with water agar, DMSO at 0.5% (v/v), and Tween 80 at 0.5% (v/v).

The compounds 1A, 2A, 1B, and 2B (0.01 g) were dissolved into 10 mL of dichloromethane, and the volume corresponding to each concentration was transferred to 50 mL round-bottom flasks. The solvent was evaporated under reduced pressure. DMSO and Tween 80 were added to each round-bottom flask at 0.5% (v/v).

These solutions were homogenized in 2% water agar, and the mixtures were distributed in 60 × 15 mm Petri dishes. A 0.1 mL aliquot of 10^5 uredospores mL^{-1} from *H. vastatrix* or *P. pachyrhizi* was spread on each plate with a Drigalski spatula. The plates were kept at 25 °C under continuous darkness in a BOD Incubator for 24 hours. After this period, the germination of 100 uredospores per plate was randomly evaluated by optical microscopy at 400-fold magnification. The mean values of the *in vitro* sensitivity experiment were used to calculate the inhibition frequency of uredospore germination by employing the equation:

$$\text{Inhibition } (\%) = \frac{G_c - G_i}{G_c} \times 100 \tag{1}$$

where, G_c = Spores germinated in control treatment with DMSO and Tween 80; G_i = Total germination of each observation.

2.4 Effect of bis(N-R-Sulfonyldithiocarbimato)Zincate(II) Salts on the Epidemiological Components of the Coffee Leaf Rust and Asian Soybean Rust

For the tests of coffee leaf rust, coffee seedlings of the Caturra variety (susceptible) at four months of age for the Asian soybean rust, soybean seedlings at 40 days of age were used.

Two *in vivo* experiments were performed in a completely randomized design with three replicates, each including three 3.0 dm^3 vessels with three plants. The treatments consisted of compounds 1A, 2A, 1B, 2B and the fungicide mancozeb. To evaluate the effect of the compounds on *H. vastatrix* (coffee leaf rust), the mancozeb was used at 1,000 µmol L^{-1}; for *P. pachyrhizi* (Asian soybean rust), 50 µmol L^{-1} of water and Haiten adjuvant (Arysta ®) at 0.1 mL.L^{-1} were used as two negative controls. Therefore, in both experiments, seven treatments and three replicates were conducted.

In the experiment with coffee leaf rust, chemical compounds were applied to the abaxial face of coffee plants, on the third and fourth pairs of leaves, using a 100 mL manual atomizer. After 24 hours, the plants were inoculated by sprinkling, using a DeVilbiss No. 15, with a suspension at 10^5 uredospores mL^{-1}. They were kept for 48 hours in the dark, in a humid chamber at 22 °C. After this period, the plants were incubated in a chamber at 22 °C, with a 12-hour photoperiod. Evaluations were performed every two days, at the 18th day after inoculation. The evaluated response variables were as follows: incubation period (IP); latent period (LP); spore production (SP); number of lesions sporulation (NLS); area under the disease progress curve (AUDPC).

In the experiment with the Asian soybean rust, chemical compounds were applied in the third pair of soybean plants, using a 100 mL manual atomizer. After 24 hours, the plants were inoculated by sprinkling, using a DeVilbiss No. 15, with a suspension at 10^5 uredospores mL^{-1}. They were kept for 48 hours in the dark, in a

humid chamber at 22 °C. After this period, the plants were incubated in a chamber at 22 °C, with a 12-hour photoperiod. Evaluations were performed each day starting on the fourth day after inoculation. The response variables obtained included LP; pustules cm^{-2}; relative control efficiency (RCE) and AUDPC.

2.5 Statistical Analysis

The data obtained for the sensitivity experiment were transformed into log(x) and then submitted to logistic regression analysis to obtain the 90% inhibitory concentration (IC_{90}) of *H. vastatrix* or *P. pachyrhizi* uredospores. Data for the epidemiological components of coffee leaf rust and Asian soybean rust were submitted to analysis of variance. Then, the grouping of the mean values was performed through the Scott-Knott test. All analyses were performed on the R software using the DRC package (R Core Team, 2013; Ritz et al., 2015).

3. Results

All the '*in vitro*' and '*in vivo*' experiments were repeated at least three times with similar results.

3.1 Synthesis of N-R-Sulfonyldithiocarbimate Potassium Salts

The *N*-R-sulfonyldithiocarbimate potassium salts were characterized by infrared and the melting point and data are according to those found in the literature (Oliveira et al., 1999).

Four zinc(II) complexes salts (1A, 2A, 1B, and 2B) with *N*-R-sulfonyldithiocarbimates were synthesized and characterized by infrared and the melting point and data are according to those found in the literature (Oliveira et al., 2007; Alves et al., 2009; Amin et al., 2011; Tavares et al., 2012).
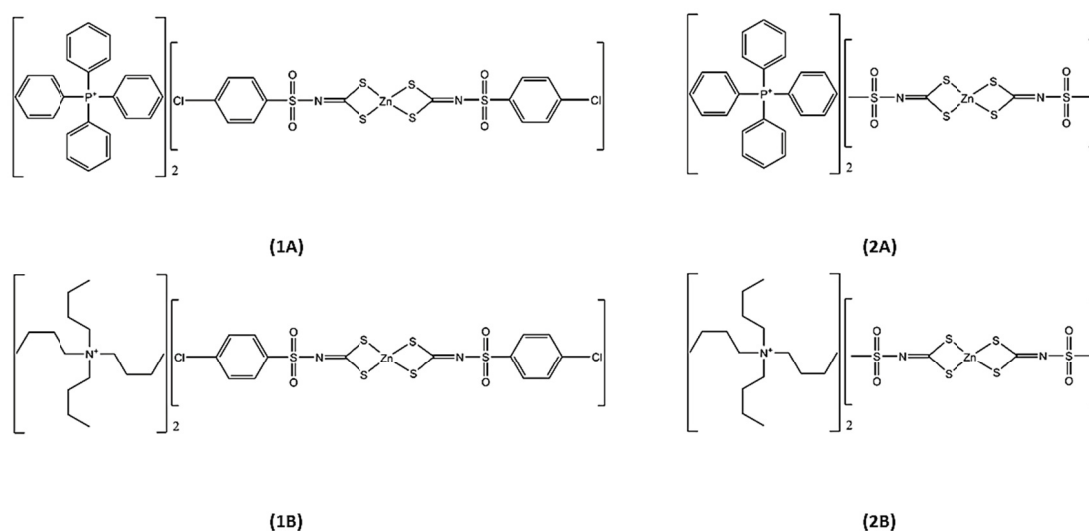

Figure 1. Structural formula of the four ditiocarbimates sintetized. Tetraphenylphosphonium *bis*(4-chlorophenylsulfonyldithiocarbimate)zincate(II) (1A); tetraphenylphosphonium *bis*(methylsulfonyldithiocarbimate)zincate(II) (2A); tetrabutylammonium *bis*(4-chlorophenylsulfonyldithiocarbamate)zincate(II) (1B); and tetrabutylammonium *bis*(chlorophenylsulfonyldithiocarbimate)zincate(II) (2B)

3.2 The Sensitivity of H. vastatrix and P. pachyrhizi to Zinc(II) Complexes Salts

The curves of the logistic model were adjusted, and then the IC_{90} values were calculated for each compound (Figures 2 and 3). All the compounds synthetized in this study had inhibitory effects on *H. vastatrix and P. pachyrhizi.*
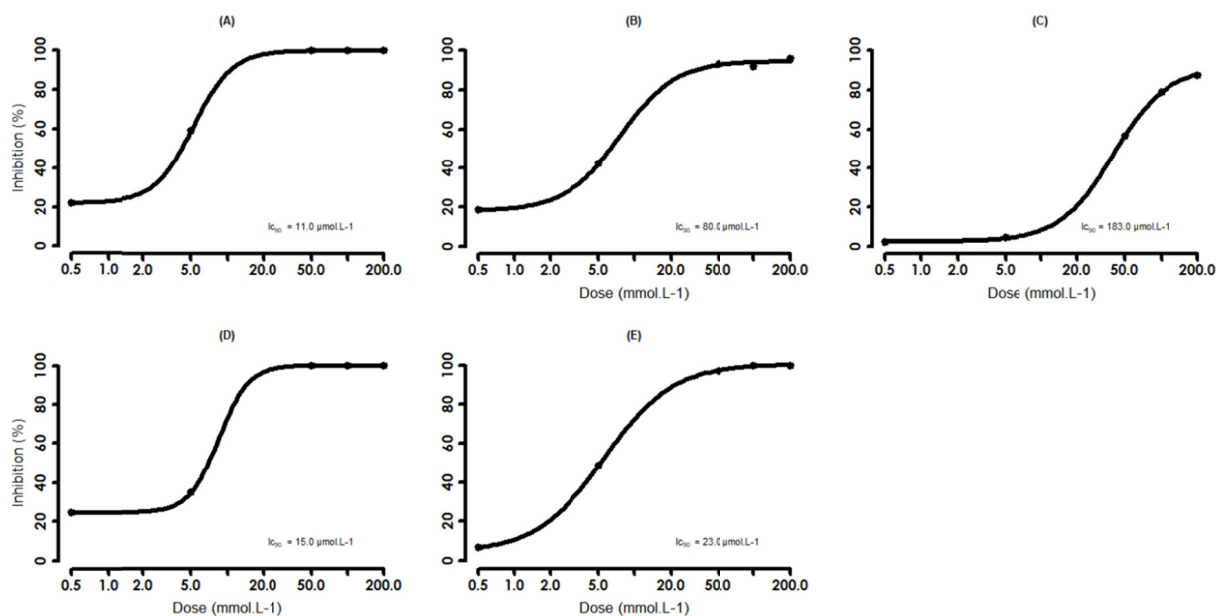
Figure 2. Inhibition of the germination of the uredopores of *H. vastatrix* and IC_{90}. A: Mancozeb; B: Compund 1A; C: Compund 2A; D: Compund 1B; E: Compund 2B
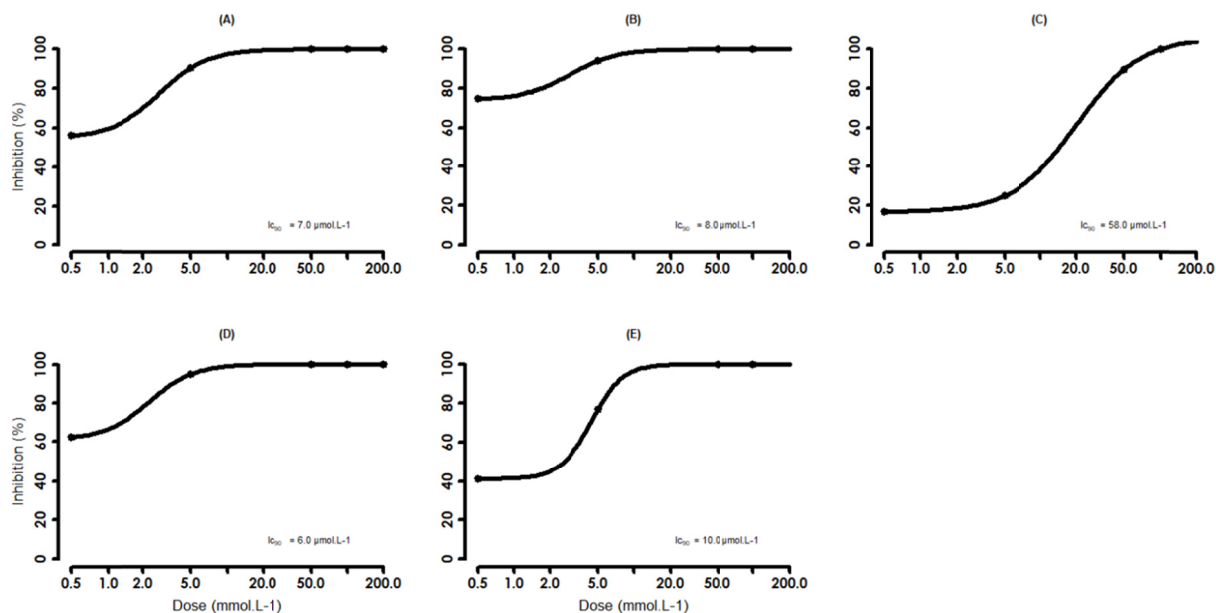

Figure 3. Inhibition of the germination of the uredopores of *P. pachyrhizi* and IC_{90}. A: Mancozeb; B: Compund 1A; C: Compund 2A; D: Compund 1B; E: Compund 2B

For *H. vastatrix*, the lowest IC_{90} values were for positive control with mancozeb (11.0 µmol L^{-1}). Among the compounds of group B, the lowest IC_{90} value was for the treatment 1B (15.0 µmol L^{-1}); for the compounds of group A, the lowest IC_{90} value was for treatment 1A (80.0 µmol. L^{-1}). For the variable IC_{90comb} (mean IC_{90} value of the two experiments), there was no significant difference between the treatments of group B compounds (1B, 2B) and mancozeb. However, the compounds of group B (1B, 2B) and mancozeb differed from those of the group A. There was a significant difference between compounds 1A and 2A (Table 1).

Table 1. Inibitory concentration [IC_{90} (μmol L^{-1})] for *H. vastatrix* and Logístic model f(y) = c \{d-c\}\{1exp(b(\log(x)-\log(e)))\}

Molecule	Experiment 1			Experiment 2			$IC_{90\ comb}$
	Parameters*	R^2	IC_{90}	Parameters*	R^2	IC_{90}	
Mancozeb	B = -2.7; c = 2.2; d = 100.0; e = 0.005	0.983	11.0	B = -2.7; c = 22.2; d = 100.0; e = 0.005	0.973	11.0	11.0 C
1 A	B = -2.0; c = 3.1; d = 100.0; e = 0.026	0.997	80.0	B = -1.2; c = 2.2; d = 100.0; e = 0.017	0.987	102.0	91.0 B
1 B	B = -3.5; c = 24.6; d = 100.0; e = 0.008	0.965	15.0	B = -3.5; c = 24.6; d = 100.0; e = 0.008	0.933	15.0	15.0 C
2 A	B = -1.9; c = 10.1; d = 100.0; e = 0.060	0.975	183.0	B = -1.9; c = 2.5; d = 100.0; e = 0.039	0.998	120.0	151.0 A
2 B	B = -1.5; c = 4.4; d = 100.0; e = 0.005	0.952	23.0	B = -1.3; c = 1.0; d = 100.0; e = 0.004	0.997	21.0	22.0 C

Note. IC_{90}: Inibitory concentration able to inhibit 90% of the germination of the uredospors of *H. vastatrix*; IC_{90}comb: Average values of the two replications of the experiments of IC_{90}; R²: Determination coeficiente; (*): Parameters different of 0 for the t test of Student (p $\leq$ 0.05). Similar letters in the column, do not differ significantly by the Scott-Knott test (p $\leq$ 0.05).

For *P. pachyrhizi*, the lowest IC_{90} value in experiment 1 was 6.0 μmol L^{-1} for compound 1B. In experiment 2, the lowest value was 6.0 μmol L^{-1} for mancozeb, followed by 8.0 μmol L^{-1} for the compound 1B (Table 2). The lowest doses of mancozeb, 1A, and 1B inhibited more than 50% of uredospore germination (Table 2; Figure 3). The analysis of the IC_{90comb} of the compounds showed no significant differences between the treatments 1A, 1B, 2B, and mancozeb (standard control) (Table 2). The lowest performance was observed for the compounds 2A, which differed significantly from other treatments.

Table 2. Inibitory concentration [IC_{90} (μmol L^{-1})] of *P. pachyrhizi* uredospores and Logístic model f(y) = c\{d-c\}\{1exp(b(\log(x)-\log(e)))\}

Molecule	Experiment 1			Experiment 2			$IC_{90\ comb}$
	Parameters*	R^2	IC_{90}	Parameters*	R^2	IC_{90}	
Mancozeb	B = -2.1; c = 54.8; d = 100.0; e = 0.002	0.987	7.0	B = -2.0; c = 57.0; d = 100.0; e = 0.002	0.995	6.0	6.5 C
1 A	B = -1.6; c = 72.4; d = 100.0; e = 0.022	0.976	8.0	B = -1.5; c = 81.9; d = 100.0; e = 0.002	0.994	9.0	8.5 B
1 B	B = -2.1; c = 60.3; d = 100.0; e = 0.002	0.923	6.0	B = -2.1; c = 60.3; d = 100.0; e = 0.002	0.854	8.0	7.0 C
2 A	B = -1.4; c = 22.9; d = 100.0; e = 0.012	0.998	62.0	B = -1.8; c = 16.8; d = 100.0; e = 0.017	0.969	58.0	60.0 A
2 B	B = -2.8; c = 41.2; d = 100.0; e = 0.004	0.936	10.0	B = -3.0; c = 29.89; d = 100.0; e = 0.004	0.893	9.0	9.5 C

Note. IC_{90}: Inibitory concentration able to inhibit 90% of *P. pachyrhizi* uredospores germination; $IC_{90\ comb}$: average values of the two replications of the experiments of IC_{90}; R²: Determination coeficiente; (*): Logistic model parameters different from 0 for the t Student test (p $\leq$ 0.05). Similar letters in the column, do not differ significantly by the Scott-Knott test (p $\leq$ 0.05).

3.3 Effect of Zinc(II) Complexes Salts on the Epidemiological Components of H. vastatrix

Figure 4 shows the result of the epidemiological components when Caturra coffee plants were inoculated with *H. vastatrix* uredospores. The lowest PI for the control with water was 16 days; the highest values were achieved for the compounds mancozeb, 2A, and 2B, which lead to expressed symptoms at 24 days. All treatments differed from the control with water. The PI of the compounds did not differ significantly from the mancozeb (standard control) (Figure 4).
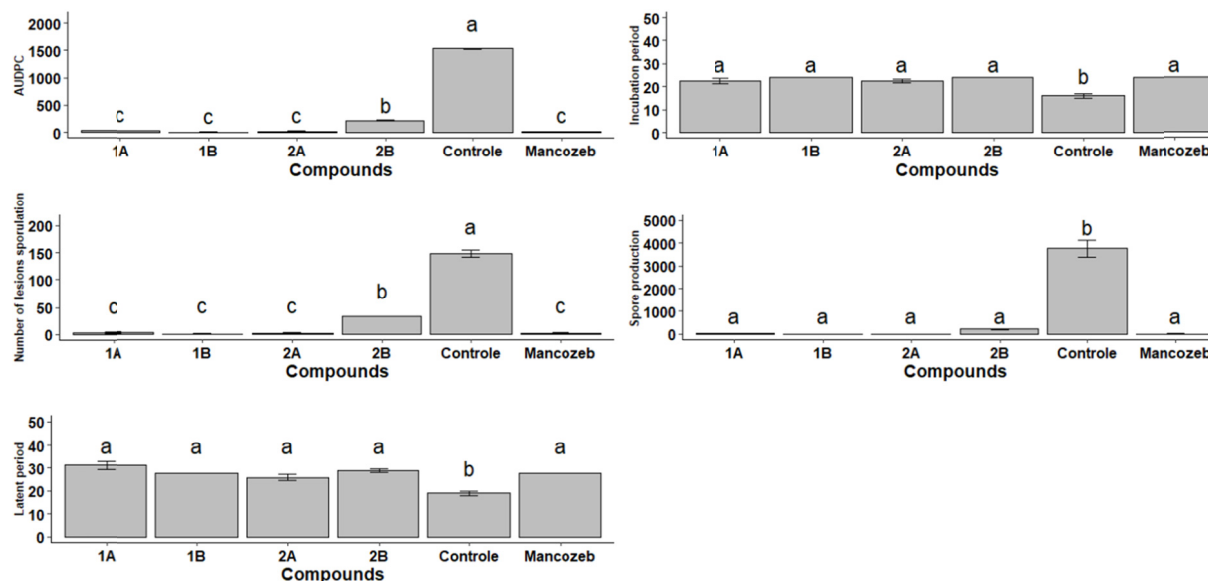
Figure 4. Epidemiological components of the Coffee leaf rust. Compounds "1" is 4-chlorophenyl group and "2" is methyl group. Compounds group "B" has the cation tetrabutylammonium and group "A" has the cation tetraphenylphosphonium. Similar letters do not differ significantly by the Scott-Knott (p ≤ 0.05) test

For the LP, the lowest value (19 days) was achieved for the control with water, differing significantly from the other treatments. There were no differences in LP values between the compounds and the mancozeb (Figure 4).

For the variable NLS, the control treatment with water had a mean value of 149.0 lesions/leaf, differing significantly from the other treatments. The mean value for the compound 2B was estimated at 25.0 lesions/leaf, differing significantly from the treatments 1A, 1B, 2B, and mancozeb. The treatments 1A, 1B, 2B, and mancozeb did not differ significantly from each other (Figure 4).

Estimates for AUDPC showed that control with water differed significantly from the other treatments, with a mean value of 1,843.5. Treatment 2B had a mean value of 175.33 and differed significantly from the other compounds. The compounds1A, 1B, 2A, and mancozeb did not differ significantly from each other (Figure 4).

The results regarding the SP variable were significantly different between the control with water (mean of 3,770 uredospores mL^{-1}) and the other treatments. The 2B treatment had an estimated mean value of 145.60 uredospores mL^{-1} and differed significantly from the other compounds. Treatments 1A, 1B, 2A, and mancozeb did not differ significantly from each other (Figure 4).

3.4 Effect of Zinc(II) Complexes Salts on the Epidemiological Components of P. pachyrhizi

Figure 5 shows the results of the epidemiological components when Caturra coffee plants were inoculated with *P. pachyrhizi* uredospores. The results of treatments 1B and 2B were not considered. Data from treatments 1B and 2B were impaired by the expression of phytotoxicity symptoms in soybean leaflets, 24 hours after applying the treatments, at 50 µmol L^{-1}. The treatments 1A, 2A, and mancozeb showed no damage to the soybean leaflets at the concentrations used (Figure 5).

The LP for the control with water was nine days. The appearance of symptoms was later in the 2A treatment (mean of 21 days), and the estimate for compound 1A was 12 days. Since lesions were not observed in plants treated with mancozeb, the LP was not estimated.

For the variable pustules per cm^2, the control with water had a mean value of 18.30 pustules/cm^2, differing significantly from the other treatments. The mean value for the compound 2A was estimated at 0.8 pustules/cm^2 and for mancozeb, 0.0; these treatments did not differ significantly from each other (Figure 5).

Regarding the AUDPC variable, the control with water had a mean value of 75.8 and differed significantly from the other treatments. The compound 1A had an estimated mean value of 43.50 and was significantly different between the treatments 2A (4.16) and mancozeb (0.0). Compound 2A and mancozeb did not differ significantly from each other (Figure 5).
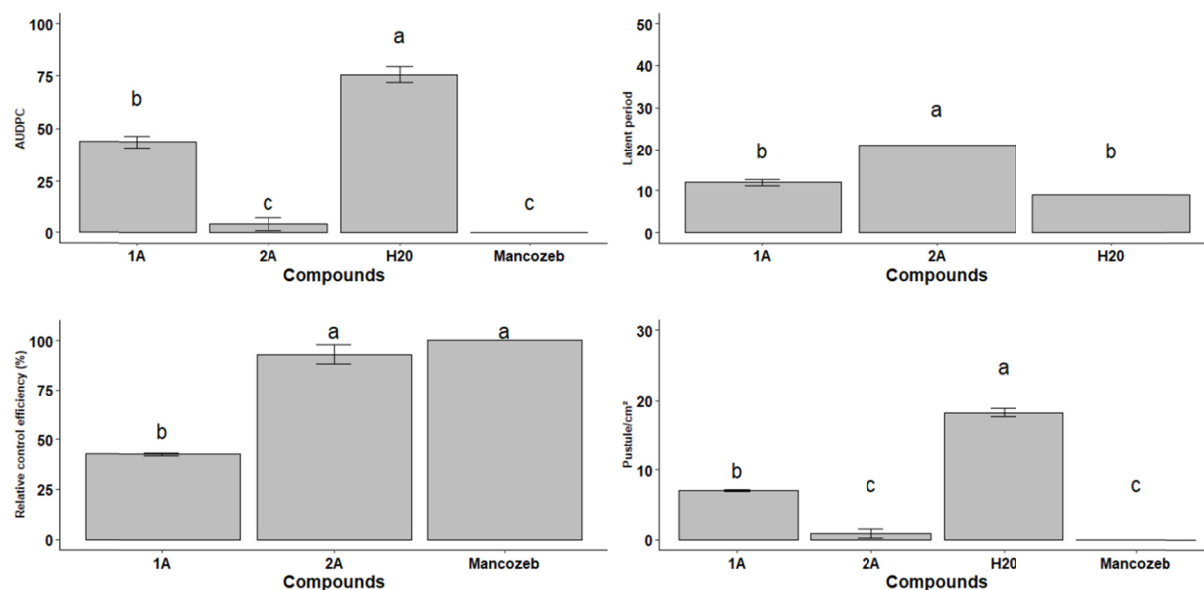
Figure 5. Epidemiological components of the Asian soybean rust. Compounds "1" is 4-chlorophenyl group and "2" is methyl group. Compounds group "B" has the cation tetrabutylammonium and group "A" has the cation tetraphenylphosphonium. Similar letters do not differ significantly by the Scott-Knott ($p \leq 0.05$) test

The compound 1A had the smallest RCE, with values lower than the control treatment (42.7% pustule/cm^2). There was a significant difference between the compound 2A (92.9%) and mancozeb (100.0%) in the disease control. However, compound 2A and mancozeb did not differ significantly from each other (Figure 5).

4. Discussion

All the zinc(II) complexes salts (1A, 2A, 1B, and 2B) were able to inhibit the uredospore germination of *H. vastatrix* and *P. pachyrhizi* when compared with the standard treatment mancozeb and were more efficient when compared to the results obtained by Vidigal et al. (2019). In this study, the lowest IC_{90} values for the compound 1B were 15 μmol L^{-1} for *H. vastatrix* and 6.0 μmol L^{-1} for *P. pachyrhizi*. The lowest IC_{90} values found by Vidigal et al. (2019) for the *bis*(triphenylphosphino)(4-isopropylphenylsulfonyldithiocarbimate)nickel(II) were 405 μmol L^{-1} for *H. vastatrix* and for the *bis*(triphenylphosphino)(butylsulfonyldithiocarbimate)nickel(II) were 280 μmol L^{-1} for *P. pachyrhizi*.

For *H. vastatrix*, the IC_{90comb} of the curves of the two experiments showed that the treatments 1B and 2B had a performance similar to the control with mancozeb. However, compounds group A were less effective in inhibiting *H. vastatrix* germination, when compared with the compounds of group B and mancozeb. Analysis of the IC_{90comb} values showed that the compounds 1A, 1B, and 2B were capable of inhibiting *P. pachyrhizi* sporulation in a similar way to the standard control with mancozeb. Treatment with the compound 2A differed from the others and was the least active. However, 100% of spores were inhibited from 100 μmol L^{-1}. The fungitoxic action can explain the performance variation of the zinc(II) complexes salts in the spore germination. Physiological and genetic factors may influence the ability of the fungus to respond to stresses caused by fungicides. Usually, fungi respond to the stress caused by fungicides from the altered target site, detoxification of fungicide, over-expression of the target with increased fungicide compounds in the cytosol, and efflux of fungicides from the target site (Kretschmer et al., 2009).

Having the ability to inhibit the germination of a fungus *in vitro* assays does not mean that the compound is promising for controlling a disease. A hydrophobicity of the compounds diffused in agar may affect performance. The physical-chemical compound interactions with plant cells may also present phytotoxic effects. In this sense, the selection of new compounds using epidemiological components is a useful approach in the early stages of development of new fungicides because the new compounds are challenged at the time of interaction with the host. The selection of new fungicides based on the analysis of epidemiological components may contribute to the decision about application dose, application methods, mobility in plant, role in crop protection, breadth of activity, interactions with adjuvants, and control efficiency disease (Zambolim et al., 2014).

The results of the epidemiological components for both diseases showed that *bis*(N-R-sulfonyldithiocarbimato)zincate(II) salts had an efficacy similar to the positive control with mancozeb. The compounds 2A, 1B, and 1A stood out in the analyses. The highest PI and LP values and lowest SP values were obtained for these compounds, suggesting that these compounds can efficiently reduce disease cycles (Leclerc et al., 2014).

The higher estimated values of the epidemiological components IP and LP concomitantly with lower SP value found in this study suggest that the use of that *bis*(N-R-sulfonyldithiocarbimato)zincate(II) salts may reduce the progress rate (r) of the coffee leaf rust epidemic, and most likely have a lower impact on coffee productivity (Zambolim, 2016). On the other hand, the 2B compound had lower estimated LP values combined with higher SP values for coffee leaf rust. Thus, the compound 2B should be disregarded in new studies since the epidemiological components indicate risks of inefficiency in the control of coffee leaf rust. When chemical control is inefficient, the disease causes defoliation in coffee plants and consequently reduces the yields of crops in the following year (Souza et al., 2011; Talhinhas et al., 2017).

The results of the variables LP, pustules/cm^2, RCE, and AUDPC showed that treatment with compound 1A was not efficient to control the Asian soybean rust. On the other hand compound 2A and mancozeb were efficient and did not differ significantly from each other. However, the efficiency of compound 1A may increase at higher doses. In the present study, the *bis*(N-R-sulfonyldithiocarbimato)zincate(II) salts were used in the molar ratio to standardize the number of compounds in each treatment. Thus, the molar concentration of each compound used in the experiment (50 µmol L^{-1}) was equivalent to a reduction of 175 times concerning the recommendation of 3.75 kg of the active ingredient mancozeb per hectare.

Compounds 1B and 2B caused phytotoxicity in soybean leaflets. However, there were no visible anomalies in the leaf tissues submitted to treatment with compounds 1A and 2A. The phytotoxicity property of the compounds group B may be related to the presence of different counter-ions. Group A contains tetraphenylphosphonium, and group B contains tetrabutylammonium. Besides, phytotoxicity may be related to the interaction of ammonium ion in leaf tissues. The plant cells absorb the ammonium ion passively; however, if the absorption levels are higher than those of metabolization, the intoxication of the leaf tissues may occur (Barker, 1999). Usually, damage caused by the ammonium ion affects photosynthetic processes, ATP synthesis, and the electron transport chain (Opanasenko & Vasyukhina, 2009).

5. Conclusion

The salts "*bis*(N-R-sulfonyldithiocarbimato)zincate(II)" are an option for the control of coffee leaf rust and Asian soybean rust; their efficiency is comparable to the standard product mancozeb. For Asian soybean rust, only the compound 2A was efficient for the control. Compounds 1A, 2A, and 1B were adequate for the control of coffee leaf rust.

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