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PLANT PROTECTION
Note

Factors influencing the performance of phosphites on the control of coffee leaf rust

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ABSTRACT: This study investigated the effect of different phosphites on the control of coffee leaf rust (CLR) caused by *Hemileia vastatrix*, considered the major disease affecting coffee yield worldwide. Three-month-old coffee plants were sprayed with two doses each of the phosphite solutions (standard and double), as follows: K (40% P_2O_5; 1.5 and 3 mL·L^{-1}), K (30% P_2O_5; 3 and 6 mL·L^{-1}), Cu (2.5 and 5 mL·L^{-1}), Mn+Zn (3 and 6 mL·L^{-1}) and Na (3 and 6 mL·L^{-1}) 24 h before being inoculated with *H. vastatrix*. Leaves were not washed or washed with deionized water after the foliar phosphites spray. Plants sprayed with water served as the control treatment. Inoculation was performed on the abaxial side of the first pair of expanded leaves using a camel hairbrush with a suspension of urediniospores (1 mg per leaf). All phosphites applied at the standard doses (from 1.5 to 3 mL·L^{-1}) reduced the total number of pustules (TNP) per leaf by 28-69% regardless of washing treatment. The biggest reductions were observed for Cu phosphite, which decreased the TNP per leaf by 60 and 69% without and with leaf washing, respectively. A similar trend was observed when the double doses of phosphites (from 3 to 6 mL·L^{-1}) were used, though Na phosphite without leaf washing and Mn/Zn phosphite without and with leaf washing were not efficient to reduce the TNP per leaf. Without leaf washing, only Cu and Na phosphites decreased CLR severity at the standard doses (53-61%), whereas all phosphites, except the Mn/Zn phosphite, reduced CLR severity at the double dose. The CLR severity was decreased using K 30%, K 40% and Cu phosphites when applied at the standard doses with leaf washing (41-59%). With double doses, only Cu and Na phosphites decreased CLR severity by 55% for both treatments. In conclusion, the efficiency of the phosphites for CLR control varied according to the accompanying cation, dose and washing conditions. The Cu phosphite resulted in the best CLR control regardless of the dose used and the washing conditions.

Key words: *Coffea arabica* L., disease control, induced resistance.

Coffee is one of the most traded commodities worldwide (CrumLey 2013; Vega et al. 2003; Zambolim 2016). The occurrence of foliar and root diseases is one key factor limiting coffee yield. Coffee leaf rust (CLR), caused by the biotrophic fungus *Hemileia vastatrix* Berkeley & Broome, is among the most important foliar diseases affecting *Coffea arabica* globally (Waller et al. 2007). Yield reduction can range from 35 to 50% with the occurrence of premature leaf fall, the death of branches and low photosynthesis rates in the previous year (Zambolim 2016; Waller et al. 2007). Pale yellow spots on the abaxial side of the leaves that develop into orange-yellow pustules that often coalesce with each other are typical symptoms of CLR (Kushalappa and Eskes 1989; Zambolim 2016). Triazoles and strobilurins, sprayed on the leaves or applied into the soil, are the major fungicides used for CLR control (Zambolim 2016).

In the context of sustainable agriculture, the discovery of alternative methods for CLR control is urgently needed. Phosphite based compounds are marketed as foliar fertilizers and disease resistance inducers, their metal salts are effective in controlling diseases caused by oomycetes, including *Phytophthora* spp. (Machinandiarena et al. 2012; Dalio et al. 2014; Adaskaveg et al. 2015) and *Plasmopara viticola* (Pereira et al. 2012) as well as fungi such as *Rhizoctonia solani*, *Alternaria* *alternata*, *Fusarium* spp., and *Sclerotinia sclerotiorum* (Reuveni et al. 2003; Sharaf and Farrag 2004; Lobato et al. 2010; Cerqueira et al. 2017; Fagundes-Nacarath et al. 2018). The major action of phosphites is on the inhibition of pathogen mycelial growth through the rupture of their cell wall besides affecting the physiology of plants towards a potentiation of host defense responses, such as the production of phenolics, phytoalexins and lignin as well as high activities of chitinase, β-1,3-glucanase, peroxidase, polyphenoloxidase, and phenylalanine ammonia-lyase (Eshraghi et al. 2011; Jackson et al. 2000; Reuveni 1997; Fagundes-Nacarath et al. 2018; Novaes et al. 2019; Panicker and Gangadharan 1999; Daniel and Guest 2006; Dalio et al. 2014).

Considering the importance of CLR to negatively impact coffee yield and the need of new control methods to reduce the amount of fungicide sprays, the present study aimed to examine the effect of potassium (K), copper (Cu), manganese (Mn) + zinc (Zn) and sodium (Na) phosphites on the control of CLR.

Three-month-old coffee plants with three pairs of leaves ('Catuaí Vermelho IAC 144', susceptible to *H. vastatrix*) grown in plastic pots containing 1 kg of substrate (soil, manure and sand in the proportion of 2.5:1:0.5) in a greenhouse (temperature of 30 ± 2 °C, relative humidity of 70 ± 5% and natural photosynthetically active radiation), were sprayed (20 mL per plant) with phosphite solutions as follows: K [Fitofos-K-Plus (40% P_2O_5 and 20% K_2O; 1.5 and 3 $mL{\cdot}L^{-1}$)] and Phosfik PK (30% P_2O_5 and 20% K_2O; 3 and 6 $mL{\cdot}L^{-1}$), Cu [Phosfik Cu4 (22% P_2O_5, 4% Cu, 1.76% S and 11% N; 2.5 and 5 $mL{\cdot}L^{-1}$)], Mn+Zn [Phosfik Mn+Zn [30% P_2O_5, 3% N, 5% Mn and 5% Zn; 3 and 6 $mL{\cdot}L^{-1}$]), and Na phosphite ($H_{10}Na_2O_8P^+$; 3 and 6 $mL{\cdot}L^{-1}$).The phosphite solutions were sprayed at the adaxial and abaxial leaf surfaces of each plant 24 h before inoculation with *H. vastatrix*. Plants were fertilized with a nutritive solution (Honorato Júnior et al., 2015) twice a week. Phosphite solutions, prepared using deionized water, were applied at standard (from 1.5 to 3 $mL{\cdot}L^{-1}$) and double (from 3 to 6 $mL{\cdot}L^{-1}$) doses. The standard doses used for each phosphite were based on the manufacturers' recommendations. A CO_2 pressurized backpack sprayer with a flat fan nozzle (XR 110 02, Teejet, Glendale Heights, IL, USA; 200,000-Pa pressure to reach a spray volume of 200 $L{\cdot}ha^{-1}$) was used to spray the plants with the phosphite solutions. Plants sprayed with deionized water served as the control treatment. Leaves were either not washed or washed with deionized water (5 mL per leaf during 10 min using a plastic syringe at 30 min) after the spray of phosphite solutions.

Plants were inoculated with *H. vastatrix* 24 h after washing or not the leaves with water. The inoculation was performed on the abaxial side of the first pair of expanded leaves per each plant by using a camel hairbrush with a suspension of urediniospores (1 mg per leaf) according to Honorato Júnior et al. (2015). After inoculation, plants were transferred to a moist growth chamber (temperature of 22 ± 1 °C and relative humidity of 90 ± 5%) in the dark for 48 h. After that period, plants were transferred to a growth chamber (temperature of 22 °C, relative humidity of 65 ± 5% and photoperiod of 12 h using fluorescent light [7.35/Wm^2]) until the end of the experiments.

The total number of pustules (TNP) per leaf and CLR severity were evaluated 30 days after inoculation. The CLR severity was evaluated in each leaf according to the diagrammatic scale proposed by Kushalappa (1978). This scale consists of three coffee leaves with 30, 50 and 70% of CLR severity on each leaf. Each leaf has a known area of 1, 3, 5, 7 and 10% occupied by individual pustules.

A 6 × 2 × 2 factorial experiment consisting of six foliar treatments (plants sprayed with water [control] or with solutions of K 40%, K 30%, Cu, Mn+Zn, and Na), washed and nonwashed leaves and phosphite doses (standard and double) was arranged in a completely randomized design with ten replications. Each replication corresponded to a plastic pot containing one plant. The experiment was repeated. Data from TNP per leaf and CLR severity from the two experiments were analyzed using the MIXED procedure of the SAS software (Release 8.02 Level 02 M0 for Windows, SAS Institute) to determine if data from the experiments could be combined (Moore and Dixon 2015). Data was checked for normality and homogeneity of variance and subjected to analysis of variance (ANOVA) using the generalized linear model and the Minitab software (version 18; Minitab Corporation).

Based on ANOVA, the foliar treatments were the most significant factor ($p < 0.001$) influencing the TNP per leaf and CLR severity (Table 1). The interaction among washing, foliar treatments and doses was significant for both TNP per leaf and CLR severity ($p < 0.01$), indicating that the performance of phosphites for CLR control was affected by the doses and washing factors.

All phosphites applied at the standard doses reduced the TNP per leaf by 28-69%, regardless of the washing treatment (Fig. 1a and b). The greatest reductions were observed for Cu phosphite, which decreased the TNP per leaf by 60 and 69% without and with leaf washing, respectively. When applied the double dose, only Na phosphite without leaf washing and Mn/Zn phosphite with and without leaf washing did not reduce the TNP. The TNP decreased for K phosphite 40% applied at the double dose, relative to the standard dose without leaf washing. Leaf washing decreased the performance of K phosphite 40% at the double dose and Mn/Zn Phi at the standard dose.

Table 1. Analysis of variance for the effects of washing, foliar treatments, doses, and their interactions for the total number of pustules per leaf and coffee leaf rust (CLR) severity.

Sources of variation	*F* values	
	Total number of pustules per leaf	CLR severity
Washing (W)	0.28^{ns}	0.34^{ns}
Foliar treatments (FT)	35.60**	31.67**
Doses (D)	0.14^{ns}	< 0.01^{ns}
W × FT	1.18^{ns}	2.97*
W × D	< 0.01^{ns}	1.66^{ns}
FT × D	7.87**	2.56*
W × FT × D	4.23**	7.78**

$^{*, **, \text{ and } ns}$ = significant at the levels of probability of 1 and 5% and nonsignificant, respectively.

In general, Cu and Na phosphites were more efficient in reducing CLR severity. The K phosphites applied at the double dose (without leaf washing) and at standard dose (with leaf washing) also decreased CLR severity, which was reduced by doubling the dose of K phosphite 40% without leaf washing. The performance of K 30% and 40% phosphites were generally decreased by leaf washing.

The present study brings new pieces of evidence on the efficacy of different phosphites in the CLR control. The decrease in CLR symptoms herein reported is consistent with other studies that investigated the potential of phosphites in controlling coffee diseases. The control of CLR and brown eye spot by Cu, K and Mn phosphites in field conditions was greater than 45% (Costa et al. 2014). Similarly, the area under the severity of coffee rust progress curve was decreased by 63% due to Mn Phi in a greenhouse study (Monteiro et al. 2016). However, results from the present study indicate that the effectiveness of phosphites in CLR control was largely dependent on the accompanying cation of the phosphite formulation, dose and washing.

The dependence of the accompanying cation in the efficiency of phosphite in disease control has been recorded in previous studies. While Cu and Zn phosphites were effective in controlling white mold in common bean plants (Fagundes-Nacarath et al. 2018) and powdery mildew in eucalyptus seedlings (Silva et al. 2013), K phosphite was ineffective in the control of anthracnose in apple trees (Araujo et al. 2010), Verticillium wilt of cacao trees (Ribeiro Júnior et al. 2006), pink rot in potatoes (Al-Mughrabi et al. 2007) and late blight in tomatoes (Nascimento et al. 2008). In the present study, Cu phosphite provided the most consistent results in terms of CLR control since this phosphite was the most efficient in decreasing both TNP per leaf and CLR, regardless of the dose and washing treatments. The similar performance obtained between washed and nonwashed conditions indicates the higher tenacity of phosphite in the presence of Cu. The fungitoxicity of Cu against *H. vastatrix* is well documented and cupric fungicides are recommended for CLR control (Costa et al. 2019). Besides its direct activity against plant pathogens, Cu makes up of regulatory proteins and plays a role in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling (Zambolim 2016). In addition, Cu is a cofactor of many enzymes such as Cu/Zn superoxide dismutase (SOD), cytochrome *c* oxidase, amine oxidase, laccase, plastocyanin and polyphenol oxidase (Yruela 2005). Therefore, Cu may have an additive effect in the phosphite performance by its fungitoxicity or activating host defense responses against infection by *H. vastatrix*.

Contrasting with Cu phosphite, in some cases K phosphites 30% and 40% had reductions in their performance against CLR due to washing. It is the first evidence that phosphites are differentially affected by washing. Despite the absence of literature dealing with the effect of washing on phosphite's performance, the efficiency of pesticides is known to be affected by leaf washing. In soybeans, the rainfall simulation, even 120 min after fungicide application, decreased the Asian soybean rust control effectiveness (Stefanello et al. 2016). It is important to highlight that different factors may impact the effect of products employed in disease control, including plant genotype, product, adjuvant, leaf age, drop size and time of application (Stefanello et al. 2016). The results indicate that Cu afforded a better tenacity to phosphite than K. Therefore, less removal of Cu phosphite deposits on coffee leaves should be expected due to rainfall under field conditions.

Dose stood out as a key factor influencing CLR control due to phosphite treatment. Interestingly, the efficiency of CLR control was sharply affected by the dose for some phosphites, however it did not affect others. The K phosphite 40% was particularly

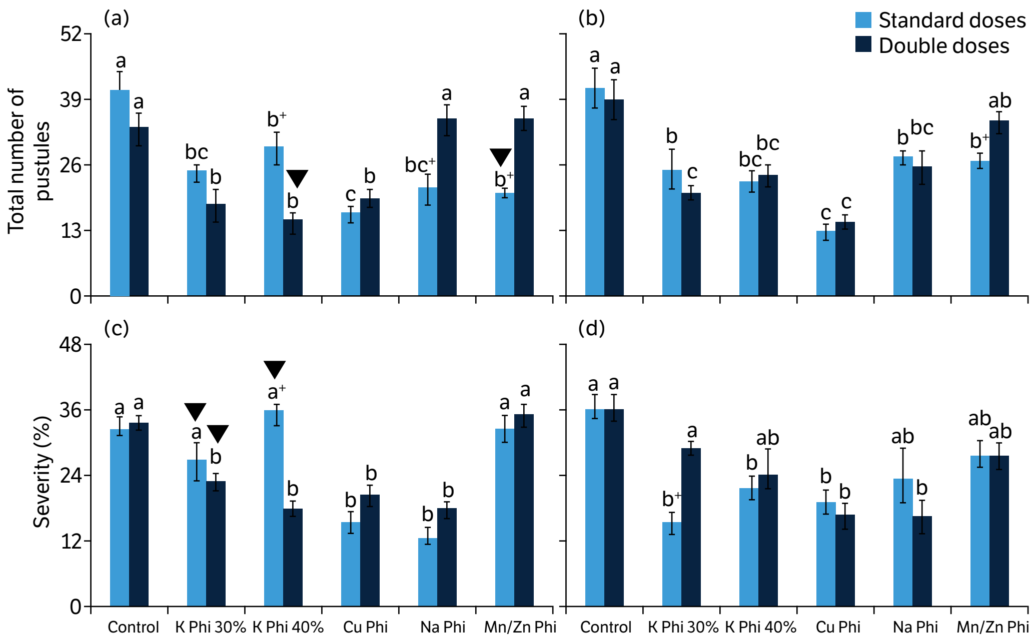

Figure 1. Total number of pustules per leaf and leaf rust severity (%) determined in the leaves of coffee plants nonsprayed (control) or sprayed with phosphites (Phi) containing potassium (K) with 30 and 40% of P_2O_5 (K Phi 30% and K Phi 40%, respectively), copper (Cu Phi), sodium (Na Phi) or manganese + zinc (Mn/Zn Phi). Phosphite solutions were applied at the standard and double doses. Leaves were not washed (a and c) or washed (b and d) with deionized water after foliar treatment. For each dose, means followed by different letters are significantly different ($p \leq 0.05$) according to Tukey's test. Means followed by asterisk (*) for standard and double doses and inverted triangle (▼) for nonwashed and washed leaves are significantly different ($p \leq 0.05$) according to t-test. The bars represent the standard error of the means.

affected by it. At the standard dose without leaf washing, CLR severity was like the control treatment. In contrast, both TNP per leaf and CLR were reduced by 50% by doubling the dose of K phosphite 40%. This may explain the contradictory results observed across different studies. Accordingly, K phosphite applied at the dose of 0.2 $mL \cdot L^{-1}$ did not control powdery mildew in eucalyptus (Bizi et al. 2008), whereas no symptoms and no plant mortality caused by *Phytophthora plurivora* were recorded in plants of common beech treated with K phosphite at the dose of 5 $mL \cdot L^{-1}$ (Dalio et al. 2014). However, the TNP increased when doubling the dose of Na and Mn/Zn phosphites, which may be linked to the saline alkaline stress. The activity of photosystem II reaction center in plant leaves is decreased by salt stress, especially due to Na, through reduced activity of the oxygen-evolving complex at the donor side of PSII and degradation of D_1 protein on the acceptor side of the PSII (Zhang et al. 2018). Thus, phosphite formulations containing Na and Mn/Zn may have decreased plant photosynthesis, which reduced carbohydrate supply for host defense reactions activated by phosphites, therefore nullifying the benefit of phosphite in CLR control.

A complex mode of action of phosphites in disease control can occur. They can act indirectly by inducing resistance in plants and directly by its fungitoxic activity against the pathogens (Dalio et al. 2014). Both mechanisms were found to operate in the phosphite afforded protection in coffee plants against CLR. *In vitro* trials revealed that the germination of urediniospores of *H. vastatrix* was completely inhibited by Cu, K and Mn phosphites (Costa et al. 2014), indicating a direct effect of phosphite against the fungus. However, foliar sprays of Mn phosphite induced defenses in coffee seedlings, such as increased activities of ascorbate peroxidase, SOD and polyphenol oxidase in response to infection by *H. vastarix* (Monteiro et al. 2016). The last finding is somehow contrasting with the results of this work, in which Mn/Zn did not decrease CLR severity. It is speculated that Zn could have counteracted the effect of Mn in disease suppression. Accordingly, Zn supplied as a soil drench increased the severity of powdery mildew in wheat through a combination of pathogen stimulation and host predisposition (Meyer 1950). The severity of aerial blight caused by *R. solani* in the leaves of soybean plants was greater as the Zn rates increased in the soil (Silva et al. 2012). Zinc can improve mycelial growth of several fungi species by increasing nitrogen uptake (Duffy 2007), therefore enhancing pathogen's aggressiveness. Phosphites, which were effective in CLR control, particularly Cu phosphite, may have reduced TNP per leaf and CLR severity in the present study by having both direct and indirect mechanisms against fungal infection.

In conclusion, different formulations of phosphites were uncovered to control CLR, though their efficiency varied according to the accompanying cation, dose and washing conditions. The Cu phosphite was found to have a better performance on CLR control regardless of the dose used and the washing conditions. Washing had a negative effect in the performance of some phosphites, notably for K 30 and 40% phosphites. The CLR control provided by K 30% phosphite increased by doubling its dose, whereas Na and Mn/Zn phosphites had a worse performance when their dose increased. Therefore, Cu phosphite may represent a feasible tool to be included in the integrated management of CLR and further studies should be performed under field conditions to validate the findings herein reported.

AUTHORS' CONTRIBUTION

Conceptualization: Honorato Júnior J. and Zambolim L.; Methodology: Honorato Júnior J., Zambolim L. and Rodrigues F. A.; Investigation: Honorato Júnior J.; Writing – Original Draft: Honorato Júnior J., Debona D. and Rodrigues, F. A.; Writing – Review and Editing: Debona D. and Rodrigues F. A.; Funding Acquisition: Zambolim L.; Resources: Zambolim L.; Supervision: Zambolim L. and Rodrigues F. A.

DATA AVAILABILITY STATEMENT

Data will be available upon request

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