Original Article

POTASSIUM PHOSPHITE: A PROMISING PRODUCT IN THE MANAGEMENT OF DISEASES CAUSED BY *Colletotrichum gloeosporioides* IN COFFEE PLANTS

FOSFITO DE POTÁSSIO: UM PRODUTO PROMISSOR NO MANEJO DE DOENÇAS CAUSADAS POR Colletotrichum gloeosporioides *EM CAFEEIRO*

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ABSTRACT: In recent years coffee productivity has been harmed by diseases caused by *Colletotrichum gloeosporioides* (*Cg*), for example, anthracnose, dieback and blister spot. Therefore, it has become necessary to develop alternative measures to control these diseases, since there are no fungicides registered in Brazil for their control. The aims of this work were: to evaluate the effect of potassium phosphite on the germination, fungal appressorium formation and mycelial growth of *Cg* and to verify this action in the reduction of disease severity of anthracnose in coffee leaves. The treatments used in an *in vitro* experiment were: potassium phosphite at doses of 1.25, 2.50, 5.0 and 10.0 $mL.L^{-1}$, Acibenzolar-S-methyl at dose $0.1g.L^{-1}$ and Chlorothalonil fungicide at dose $2.0 g.L^{-1}$. In an *in vivo* experiment, beyond the treatments used in the *in vitro* experiment, we used a control sprayed with water without inoculation and another inoculated with *Cg*. Potassium phosphite at doses of 5.0 $mL.L^{-1}$ and 10.0 $mL.L^{-1}$ and the Chlorotalonil fungicide showed greater inhibition of conidial germination, greater inhibition of appressorium formation and further reduction of mycelial growth of the pathogen. In the *in vivo* experiment, Potassium phosphite at a dose of 10.0 $mL.L^{-1}$ provided a greater reduction in disease severity, around 62.5%. This work demonstrated the potential of potassium phosphite in the management of diseases caused by fungi from the *Colletotrichum* complex in coffee plants.

KEYWORDS: Alternative control. *Coffea arabica* L. Acibenzolar-S-methyl. Chlorothalonil.

INTRODUCTION

Coffee growing is one of the main agricultural activities in Brazil, which is the largest producer and exporter of the crop worldwide. Substantial revenues are generated for the national economy, and particularly for the coffee producing municipalities of Minas Gerais state, responsible for more than half of national coffee production (CONAB, 2011).

However several problems affect coffee yield, mainly diseases caused by fungi. In recent years, it has been observed that coffee plantations have come under attack from diseases caused by *Colletotrichum*, such as anthracnoses in branches, leaves and fruits, blister spot and dieback. According to Paradela Filho et al. (2001) the main symptoms caused by *Colletotrichum* in coffee plants are: darkening and death of the stipule, necrotic spots on the edges of leaves, appearance of brown spots on the plant stem, brown necrotic injuries, turning black in buds, flowers and fruits, provoking death of the above mentioned as well as death of the branches. Beyond these symptoms, in some plants light-green spots have been noted on leaves, with an oily appearance, less bright than the rest of the surface, called blister spot (DIAS et al., 2005; FERREIRA et al., 2009; ARMESTO et al., 2011).

Despite their importance, studies directed to the management of coffee diseases caused by fungi of the *Colletotrichum* complex are scarce, and every day, more and more alternative methods are being used to control these diseases, such as the use of resistance inductors. One method that has stood out is phosphite, and so research is continuing in the pursuit of actions that can help in the control of diseases, but do not represent a risk to humans and the environment.

Phosphites are quickly absorbed by plants; therefore, they present a high degree of solubility and mobility. The systemic character (ascending and descending) and quick absorption by the roots, stems and leaves, allow various methods of application in accordance with the type of plant and characteristics of the pathogen to be controlled. The efficiency of phosphite application in certain pathosystems is due to the fact that the plant presents better assimilation in the presence of phosphorus, making it capable of activating defense

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mechanisms, such as pathogenesis-related proteins (PR-proteins), oxidative burst and production of phenolic compounds (NOJOSA et al., 2005).

Interest in phosphites appeared with the commercial product fosetyl-Al, because of its capacity to translocate from the leaves to the roots and to control some diseases of the root system (LOVATT & MIKKELSEN, 2006). Currently, most published works on phosphites are related to control of oomycetes; however, in some of these it has been found to be efficient in controlling other pathogens, for example Glomerella leaf spot (*Colletotrichum gloeosporioides*) (ARAÚJO et al., 2010) and apple scab (*Venturia inaequalis*) (BONETI & KATSURAYAMA, 2005), in post-harvest rot of apples (BRACKMANN et al., 2004) and in peaches against brown rot (*Monilinia fructicola*) (MOREIRA et al., 2002).

This study therefore aimed to evaluate the effect of potassium phosphite on germination, fungal appressorium formation and mycelial growth of *Colletotrichum gloeosporioides* and to verify this action in the reduction of the disease severity of anthracnose in coffee leaves.

MATERIAL AND METHODS

Colletotrichum gloeosporioides isolate.

The isolate was obtained from coffee plants that had branches with dry points and leaf symptoms of blister spot, from the experimental coffee field at the Federal University of Lavras. Infected tissues were disinfested with 50% alcohol for 30 seconds and 1% sodium hypochlorite, for 1 minute, washed in sterile distilled water and dried with sterilized filter paper. Then they were transferred to a Petri dish with 2% MEA medium (malt extract and agar) and kept in a growth chamber at 25°C and photoperiod of 12 hours for further work.

Potassium phosphite on germination and appressorium formation.

The experiment was mounted on microscope slides with a single cavity and placed in 9 cm diameter Petri dishes with filter paper disks at the bottom of the dish, sterilized and humidified with sterile distilled water. Each slide received an aliquot of 40µl of spore suspension (2 $x10^6$ $sporesmL^{-1}$), with quantification made in a Neubauer hemocytometer, and another aliquot of 40µl of the treatments mentioned in Table 1. After that, the dishes were kept in a humid chamber for 14 hours at 25ºC. After this period, germination was stopped with 10 µL of solution of lacto phenol.

The experimental design was completely randomized with five repetitions, where each repetition consisted of one microscope slide. To evaluate the average number of germinated spores, the abaxial surface of the microscope slides was divided into quadrants, where four areas for sampling had been determined. Twenty-five spores in each one of the areas, for a total of 100 spores had been counted. Spores were considered germinated when they presented any emission of the germination tube, independent of length.

Table 1. Specifications of the treatments used in the evaluation of the effect of potassium phosphite on the germination, appressorium formation and mycelial growth of *Colletotrichum gloeosporioides*.

Treatments	Commercial product	Doses of commercial product
Potassium Phosphite	Reforce ®	1.25 $mL.L^{-1}$
Potassium Phosphite	Reforce ®	2.5 $mL.L^{-1}$
Potassium Phosphite	Reforce ®	5.0 $mL.L^{-1}$
Potassium Phosphite	Reforce ®	10.0 $mL.L^{-1}$
Acibenzolar-S-methyl	Bion 500 WG ®	0.2 $g.L^{-1}$
Chlorotalonil	Bravonil 500 ®	2.0 $g.L^{-1}$
Control	Distilled water	----------

Reforce® = (25 % of K_2O and 35% de P_2O_5). Agrichem do Brasil Ltda; Bion 500 WG® = (50% of Acibenzolar-S-methyl). Syngenta Proteção de Cultivos Ltda; Bravonil 500® = (50% of Chlorotalonil). Syngenta Proteção de Cultivos Ltda.

Potassium phosphite on the inhibition of mycelia growth.

The treatments mentioned in Table 1 were individually mixed with 2% MEA, previously sterilized, and each Petri dish of 9.0 cm in diameter received 10 mL from this mixture, whose pH was calibrated to 7.0. Mycelial disks of 0.9 cm in diameter of the colonies of *Cg* were transferred to the center of the dishes and incubated in a growth chamber at 25°C, with a photoperiod of 12 hours. The evaluation of the mycelial growth was performed every 24 hours, measuring the diameter of the colonies, until the control occupied the entire dish.

The mycelial growth index was calculated by means of the formula IVCM= [Σ (D-Da)]/N (OLIVEIRA, 1992), where D is the current average diameter; Da corresponds to the average diameter of the previous day and N corresponds to the number of days after inoculation. The experimental design was completely randomized with five repetitions and the experimental parcel was constituted by one dish.

Potassium phosphite on the severity of the disease in coffee seedlings.

The seedlings were obtained from seeds of plants with blister spot symptoms from the cultivar Catuaí Vermelho, produced in the harvest of 2009/2010, collected at Fazenda da Laje, located in the municipality of Paraguaçu, Minas Gerais, because, according to Ferreira et al. (2010), these same plants present a susceptibility factor to the predisposition of the disease. After pulping, the seeds were sown in Styrofoam trays with 96 cells containing a commercial substrate, Vidaverde®, and kept in a greenhouse until inoculation.

The coffee seedlings were inoculated when they had presented four pairs of completely expanded true leaves, around nine months after sowing. Three days before inoculation, seedlings were put in a humid chamber, made from plastic bags, and then kept in a growth chamber at 25 ^{o}C. Treatments were sprayed seven days before inoculation with *Cg* with manual spraying of 0.5 L, always at the end of the afternoon.

The inoculation was performed with 10 µL of *Cg* suspension, on the abaxial face of the leaves, in places marked with self-adhering disks with orifices of 1.4 cm in diameter. Wounds were made in the areas of inoculation with a set of entomological needles to allow penetration and colonization of the fungi. Later, the inoculated area was covered with a 1.3 cm diameter semi-permeable paper disk, previously moistened, forming a micro-humidity chamber.

The treatments used were those mentioned in Table 1 with the addition of one control inoculated with the pathogen; all were sprayed seven days before inoculation with the pathogen. The evaluations of disease severity were carried out at 7, 14, 21, 28, 35 days after inoculation. For the evaluation of disease severity a scale adapted by Martins (2008) was used (Table 2).

Table 2. Criteria for evaluation of the spectrum of coffee leaf reaction to *Colletotrichum gloeosporioides.*

Grade (degree of symptoms)	Severity / Symptoms
0	Absence of visual reaction
1	Small and few (1 to 2) chlorotic or brownish lesions
2	More than 2 brownish lesions or coalescent lesions. The diameter of the lesion exceeding 0.5 mm
3	Extensive brownish lesions with numerous black points or dark lesions. Over 50% of area with lesions
4	Total area showing necrosis

From these data the disease index was determined (ID) (CIRULLI & ALEXANDER, cited by CARVALHO et al., 2005), applying the formula ID= ∑ (FxV)/(NxX) x 100, where F is the number of plants with a determined degree of symptoms; V represents the degree of symptoms; N is the total number of inoculated plants and X corresponds to the maximum degree of symptoms.

The area under the disease progress curve (AUDPC) was obtained on the basis of the indices of the disease and calculated in accordance with Shaner and Finney (1977), using the formula AACPD = ∑ [(Xi + X i+1) /2] (ti+1 -ti), where X is the intensity of the disease; t corresponds to the time of the evaluation and n represents the number of evaluations over time. The experimental design was a completely randomized block with four repetitions. The experimental plot was composed of a leaf with four self-adhering disks.

Statistical analyses

The data obtained for all the experiments were submitted to the analysis of variance and the means compared by Scott-Knott test at 5% of probability. The statistical analyses were carried out using statistical software Sisvar 5.1 (FERREIRA, 2011). All experiments were repeated twice.

RESULTS AND DISCUSSION

Direct effect *in vitro* of potassium phosphite on *Colletotrichum gloeosporioides* (*Cg*)

Chlorotalonil fungicide presented 100%

inhibition of germination of the *Cg* conidia, showing no consequent appressorium formation (Table 3). However, the doses 5.0 mL.L^{-1} and 10.0 mL.L^{-1} of potassium phosphite inhibited the germination of conidia by around 51.1% and 63.1%, respectively. Still, these conidia germinated, while practically no appressoria formed, which was interesting because, by means of these, the pathogen manages to breach the surface of the host and carry out the process of penetration and colonization (PASCHOLATI; LEITE, 1995). Although having presented inferior results in relation to the previous ones, the 2.5 mL.L^{-1} dose of potassium phosphite was statistically superior to the control, presenting 33% of the germinated conidia, of which only 4% had formed fungal pathogens (Table 3). Acibenzolar-S-methyl and the lower dose of potassium phosphite showed low toxicity for germination, but the phosphite at the lower dose provided low appressorium formation, at around 8%.

Table 3. Effect of potassium phosphite on germination of conidia, appressorium formation and mycelial growth index (MGI) of *Colletotrichum gloeosporioides.*

Treatments	Germinated conidia (%)	Appressorium formation (%)	Mycelial growth index (MGI)
Potassium Phosphite 1.25 mL.L^{-1}	47.6 d	8.40 b	2.97 c
Potassium Phosphite 2.5 mL.L^{-1}	37.8 c	4.00 b	2.88 c
Potassium Phosphite 5.0 mL.L^{-1}	27.6 b	0.60 a	2.35 b
Potassium Phosphite 10.0 mL.L^{-1}	20.8 b	0.00 a	2.39 b
Acibenzolar-S-methyl	52.8 d	33.8 c	2.74 c
Chlorotalonil	0,00 a	0.00 a	0.69 a
Control*	56.4 d	45.6 d	2.77 c

Values followed by the same letter in the column belong to the same group, by the Scott-Knott test (P ≤0.05).
* Control =distilled water.

Works published on the use of potassium phosphite with other pathogens of coffee plants are scarce. However, satisfactory results in this work was observed in other pathosystems, where potassium phosphite in 2.5 and 5.0 mL.L^{-1} doses reduced the germination of conidia in *Verticillium dahliae* to 100 and 99%, whereas in the 0.62 and 1.25 mL.L^{-1} doses germination fell by 65% and 89% respectively (RIBEIRO JÚNIOR et al., 2006). Moreover, these authors had related that Acibenzolar-S-methyl (0.1 g.L^{-1}) did not present a fungicidal effect, reducing only 40% of conidial germination.

In the present work the results showed that potassium phosphite has direct toxic activity against *Cg* conidia, that is, it presents a fungicidal effect. As in relation to low germination and appressorium formation of fungal pathogens caused by Acibenzolar-S-methyl, this was expected, because it is registered as a resistance inductor, not presenting toxicity to most fungal conidia.

As for inhibition of germination and appressorium formation, the treatments that stood out in the reduction of mycelial growth were Chlorotalonil fungicide, with inhibition around 75% in relation to the control, and the two highest doses of potassium phosphite, that is, 5.0 mL.L^{-1} and 10.0 mL.L^{-1}, with inhibition of 20.9% and 19.5% respectively. Regarding the mycelial growth index, the two lower doses of potassium phosphite were statistically equal to that of Acibenzolar-S-methyl and the standard control (Table 3).

Nojosa et al. (2009) reported that potassium phosphite at 10.0 mL.L^{-1} provided inhibition of 62.3% of mycelial growth of *Phoma costarricensis*, standing out compared to the other treatments tested. This direct effect of potassium phosphite on mycelial growth of fungi has also been verified for other pathosystems, such as for *Colletotrichum gloeosporioides*, the causal agent of gala leaf spot and Glomerella leaf spot in apple (ARAÚJO et al., 2010), a fact also observed in this work.

Potassium phosphite in the control of *Colletotrichum gloeosporioides* in coffee seedlings.

The control treatment did not present any symptom of disease, that is, leaf necrosis. As for potassium phosphite, the dose of 10 mL.L^{-1} was the best treatment, presenting the least area under the disease progress curve (AUDPC), itself differing statistically from the inoculated control and the other treatments. Following potassium phosphite at doses of 1.25, 2.5 and 5.0 mL.L^{-1}, the fungicide Chlorotalonil and ASM presented intermediate AUDPC with no statistical differences between the two and differing statistically from the inoculated control (Table 4).

Table 4. Area under the disease progress curve (AUDPC), in coffee leaves inoculated with *Colletotrichum gloeosporioides*.

Treatments	Area under the disease progress curve (AUDPC)
Potassium Phosphite 1.25 mL.L^{-1}	608.35 c
Potassium Phosphite 2.5 mL.L^{-1}	638.02 c
Potassium Phosphite 5.0 mL.L^{-1}	674.48 c
Potassium Phosphite 10 mL.L^{-1}	336.56 b
Acibenzolar-S-methyl	612.50 c
Chlorotalonil	670.83 c
Control*	0.00 a
Inoculated control	897.39 d

Values followed by the same letter belong to the same group, by the Scott-knott test (P ≤0.05). CV= 29.46%; * Control = sprayed with distilled water

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Nojosa et al. (2009), working with the pathosystem *Phoma costarricensis* x Coffee, observed that potassium phosphite at 2.5 and 5.0 mL.L^{-1} concentrations and Acibenzolar-S-methyl at 0.1 g.L^{-1} concentration, when applied seven days before the inoculation of the pathogen, presented the smallest areas under the disease progress curve, having greater control than the fungicide tebuconazol.

Araújo et al. (2010) tested potassium phosphite in the control of Glomerella leaf spot, caused by the fungi *Colletotrichum gloeosporioides*, in apples, and observed the curative effect of the product sprayed 48 hrs after infection, reducing the severity of the disease by 62%.

Boneti e Katsurayama (2005) found satisfactory results in the control of apple scab (*Ventura inaequalis*) by spraying potassium phosphite at the dose of 3 mL.L^{-1} seven days before inoculation of the pathogen, showing that the product can act indirectly in inducing resistance to the disease. In another work, the application of the product associated with $CaCl_2$ (2%) significantly reduced the percentage of rotten injuries in apples cv. Fuji inoculated with *Penicillium* spp., *Botrytis* spp. and *Rhizopus* spp., showing the same results as the standard fungicide iprodione (BRACKMANN et al., 2004).

The use of potassium phosphite in the control of the severity of plant diseases has shown satisfactory results in diverse pathosystems, mainly those phytopathogens of the oomycota class, such as grape downy mildew, whose etiologic agent is *Plasmopara viticola* (PEREIRA et al., 2010) and root rot caused by *Pythium* and *Phythophtora* (DANIEL; GUEST, 2006; LOBATO et al., 2008). In these, the action of the phosphites seems to be related to the accumulation of phosphorus in the forms of polyphosphate and pyrophosphate, intervening with fundamental metabolic routes (NIERE et al., 1994). Moreover, potassium phosphite seems to have a similar effect to that of the fungicide fosetyl-Al, favoring the protection of the plants against fungal pathogens, with descending translocation in the plant through the phloem, from the leaves to the roots (MCDONALD et al., 2001).

It is important to mention that in this work, although good results were seen for Chlorotalonil fungicide *in vitro*, the same was not observed for the reduction in the severity of the disease. On the other hand, potassium phosphite showed itself to be efficient for both situations, where, beyond the toxic effect on the pathogen, it possibly presented another form of action. In the literature it is evidenced that phosphites occur in a direct form, being toxic to the pathogen, or indirect, by means of activating plan resitance mechanisms (WILKINSON et al., 2001; JACKSON et al., 2000).

As there are few articles published on potassium phosphite in the management of diseases in coffee plants, this work supplied important information that must be taken advantage of in field studies for the management of important diseases, including those caused by the pathogen *Cg*. Moreover, as there is strong evidence that the product also acted to induce resistance, other studies are necessary, such as the analysis of molecular and biochemical mechanisms involved in coffee plant defense against the pathogen.

CONCLUSIONS

Potassium phosphite at 5.0 mL.L^{-1} and 10.0 mL.L^{-1} doses and Chlorotalonil fungicide were most efficient in the *in vitro* control of *Colletotrichum gloeosporioides*.

The area under the disease progress curve (AUDPC) was smaller for the 10.0 mL.L^{-1} dose of potassium phosphite.

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RESUMO: Nos últimos anos a produtividade de café tem sido prejudicada por doenças causadas por *Colletotrichum gloeosporioides* (*Cg*), como por exemplo, antracnose, seca de ponteiros e mancha manteigosa. Portanto torna-se necessário desenvolver medidas alternativas de controle para essas doenças, visto que não existem fungicidas registrados no Brasil para seu controle. O presente trabalho teve como objetivos: avaliar o efeito do fosfito de potássio na germinação, formação de apressórios e no crescimento micelial de *Cg*e verificar a ação do mesmo na redução da severidade da antracnose em folhas de cafeeiro. Os tratamentos utilizados no experimento *in vitro* foram: fosfito de potássio nas doses de 1,25; 2,50; 5,0 e 10,0 $mL.L^{-1}$; acibenzolar-S-metil 0,1$g.L^{-1}$; fungicida clorotalonil 2,0 $g.L^{-1}$. Já *in vivo*, além dos tratamentos anteriores, foi utilizada uma testemunha pulverizada com água sem inoculação e outra inoculada com *Cg*.O fosfito de potássio nas doses de 5,0 $mL.L^{-1}$ e 10,0 $mL.L^{-1}$ e o fungicida clorotalonil proporcionaram maior inibição da germinação de conídios, maior inibição da formação de apressórios e maior redução do índice de velocidade de crescimento micelial do patógeno. No experimento *in vivo,* o fosfito de potássio na dose de 10,0$mL.L^{-1}$ proporcionou maior redução na severidade da antracnose, em torno de 62,5 %.Este trabalho demonstrou o potencial do fosfito de potássio no manejo de doenças causadas por fungos do complexo *Colletotrichum* em cafeeiro.

PALAVRAS-CHAVE: Controle alternativo. *Coffea arabica* L. Acibenzolar-S-metil. Clorotalonil.

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