Investigation note

In vitro effect of potassium phosphite on *Athelia rolfsii* and *Pythium aphanidermatum*

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Abstract

The objective of this study was to determine the *in vitro* effect of potassium phosphite on the radial growth of the mycelium, the production of biomass and the production of sclerotia of *Athelia rolfsii* and oospores of *Pythium aphanidermatum*. Doses of 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mL L^{-1} of potassium phosphite and an absolute control (without potassium phosphite) were evaluated using PDA culture medium and distilled water. The radial growth of mycelium decreased due to potassium phosphite, so at the end of the evaluation and comparing it with the control, the radial growth of *P. aphanidermatum* decreased from 16.9 to 53.2% and from 15 to 21.3% for *A. rolfsii*. At 96 h after sowing, biomass production by *P. aphanidermatum* and *A. rolfsii* decreased from 16.9 to 53.2% and 58.3 to 63.4%, respectively. After 72 h of sowing, the formation of oospores of *P. aphanidermatum* on distilled water with potassium phosphite was not observed, which did occur in the control after 24 h. At 22 days after sowing, *A. rolfsii* produced an average of 30.2 sclerotia, whereas in PDA with potassium phosphite it did not form sclerotia. This indicates that potassium phosphite is an effective substance to reduce mycelial growth, the production of biomass, and inhibits the formation of oospores of *P. aphanidermatum* and of sclerotia of *A. rolfsii*.

Keywords: biomass production, mycelial growth, oospore formation, sclerotia formation.

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The main strategy used against fungi that parasitize cultivated plants is the chemical control, however, the indiscriminate use of conventional fungicide compounds contributes to environmental pollution, affects public health and reduces biodiversity in agroecosystems (Zavaleta-Mejía, 1999) in addition, the current food transaction markets demand products that are innocuous for consumers and that come from processes that are noble with the environment. Thus, there is an interest in alternatives to chemical control, one of which is the use of salts in order to conserve plant health (Homma *et al*., 1981).

In this regard, phosphites are salts derived from phosphorous acid, used in agriculture as sources of nutrition or as an alternative for the control of diseases in crops (Deliopoulos *et al*., 2010). Phosphites as an alternative for the control of parasitic organisms have been studied extensively and their effectiveness has been tested against oomycetes (Monsalve *et al*., 2012; Pinto *et al*., 2012; Akinsanmi and Dreth, 2013), fungi (Amiri and Bompeix, 2011; Costa *et al*., 2014; Yáñez *et al*., 2014), bacteria (Lobato *et al*., 2011; Monchiero *et al*., 2015) and phytoparasitic nematodes (Oka *et al*., 2007; Quintero and Castaño, 2012).

The mechanisms involved in the prophylactic effects of phosphites are diverse and include the stimulation of defense mechanisms in plants (Pilbeam *et al*., 2011; Lim *et al*., 2013) and direct action on phytoparasites by restricting growth and production of spores (Cerioni *et al*., 2013); however, there is an interspecific (Hofgaard *et al.*, 2010) and intraspecific difference (Wilkinson *et al.*, 2001) in the susceptibility to the phosphite ion, for all of the above, the objective of this investigation was to determine the *in vitro* effect of phosphite potassium on the radial growth of the mycelium, production of biomass, production of sclerotia of *Athelia rolfsii* and oospores of *Pythium aphanidermatum*.

During the period from October 2016 to February 2017 and through bioassays established under laboratory conditions, with a completely randomized experimental design and ten repetitions (one Petri dish per repetition), the *in vitro* effect of potassium phosphite (FP) on radial growth (CR), biomass production (PBM), production of sclorocios in *Athelia rolfsii* and production of oospores in *Pythium aphanidermatum* (organisms obtained from the collection of fungi of the laboratory of phytopathogenic fungi of the Faculty of Agronomy of the University Autonomous University of Sinaloa) was evaluated. Individually, different doses of FP were added: 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mL L^{-1} (Fosfimax 40.05%, Adama) to the potato dextrose agar culture medium (PDA, Bioxon) after the sterilization process.

Each mixture of PDA plus FP was emptied separately into Petri dishes (20 mL per box). One cylinder (0.5 cm diameter) with growth of each organism was deposited separately to the center of the Petri dishes and kept under laboratory conditions (28 ±1.8 °C). The CR was determined 36 hours after sowing (hds) for *P. aphanidermatum* and at 72 hds for *A. rolfsii*. The effect on the PBM was determined by growing the organisms for 96 h, after that time it was separated from the culture medium, deposited on filter paper (Whatman, 1100) and dried in an oven (80 °C) until constant weight was obtained. The effect on the production of oospores of *P. aphanidermatum* was determined by adding the different volumes of FP to distilled water; each dilution was poured into Petri dishes, immediately after four cylinders of PDA of 0.5 cm in diameter with growth of the pathogen were deposited. Observations were made to the compound microscope to determine the formation of oospores at 24, 48 and 72 h after having deposited the pathogen in the boxes.

The effect on sclerotia production of *A. rolfsii* was determined by growing the organism in PDA plus FP and 22 days after sowing (dds) the amount of sclerotia produced was counted. Each test was performed twice and with the data obtained from the variables studied, analysis of variance and multiple comparison of means were made with the Tukey test ($p \leq 0.05$) and simple linear regression analysis.

None of the tested concentrations of FP prevented the growth of *P. aphanidermatum* and *A. rolfsii*; however, it significantly restricted ($p \leq 0.05$) the CR and PBM of both organisms (Table 1). The susceptibility of microorganisms to the phosphite ion was reported by Wong *et al*. (2009), who determined the negative effect of phosphite and positive phosphate on the growth of *Phytophthora cinnamomi*, when grown in culture medium enriched with salts containing each ion individually.

Table 1. Effect of potassium phosphite on the radial growth of mycelium and the biomass production of *Pythium aphanidermatum* and *Athelia rolfsii*.

Treatments	Radial growth of mycelium (cm)		Biomass production (mg)	
Potassium phosphite (mL L^{-1})	*Pythium aphanidermatum*	*Athelia rolfsii*	*Pythium aphanidermatum*	*Athelia rolfsii*
0 (control)	8 a[1]	8 a	77 a	235 a
0.05	4.58 b	6.8 b	64 b	98 b
0.1	3.37 c	6.3 c	51 c	90 b
0.15	2.79 d	6.3 c	52 c	88 b
0.2	2.54 e	6.3 c	50 c	88 b
0.25	2.33 f	6.3 c	41 d	88 b
0.3	1.86 g	6.3 c	36 d	86 b

[1]= means with different literals in the same column are statistically different ($p \leq 0.05$), according to the Tukey test. Each figure represents the average of ten repetitions.

With FP the CR of *P. aphanidermatum* (36 hds) was lower than that observed in the control culture medium, in 42.8, 57.9, 65.1, 68.3, 70.9 and 76.8% with 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mL L^{-1}, respectively. The PBM was significantly restricted ($p \leq 0.05$) by the effect of the FP, in such a way that in relation to the biomass produced in the control (77 mg), it was lower in 16.9, 33.8, 32.5, 25, 46.8 and 53.2% with the respective doses mentioned above. In addition, oospore formation was not observed in the presence of FP (72 hds), while 24 hours showed abundant oospore formation in the control (without FP).

At 72 hds, FP resulted in a significant decrease ($p \leq 0.05$) in the CR of *A. rolfsii*. The decrease fluctuated between 15 and 21.3%, in comparison with the CR of the fungus in culture medium without FP. The PBM was significantly restricted ($p \leq 0.05$) with FP, in such a way that in relation to the biomass produced in the control (235 mg), it was lower in 98.0, 61.7, 62.6, 62.6, 62.6 and 63.4% with the respective 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mL L^{-1} of FP. In addition, with FP in the culture medium no mature sclerotia formation was observed (22 dds); however, in the PDA culture medium without FP, *A. rolfssi* formed 30.2 sclerotia on average.

The results obtained in this investigation, in relation to the interspecific susceptibility to FP, are similar to those previously described by Hofgaard *et al*. (2010), who reported restriction of 60, 80 and 90% in the CR of the mycelium of *Fusarium culmorum*, *F. graminearum* and *Microdochium majus*, respectively. Also, with those reported by Lobato *et al*. (2010), since they reported that the degree of restriction of CR, as a consequence of the phosphite ion, was determined by the organism tested and the amount of phosphite added to the culture medium.

The simple linear regression analysis between the FP, CR and PBM concentration variables in the two fungal species indicated a negative relationship (Figure 1), in such a way that the progressive decreases of the CR and PBM of *P. aphanidermatum* are explained in the respective 54.3 and 43.2% due to the effect caused by the increases in FP doses (Figure 1A and 1C). While for *A. rolfsii* they are explained in 75.2 and 90.2%, respectively (Figure 1B and 1D). These results coincide with that reported by Smillie *et al*. (1989), who determined that *Phytophthora cinnamomi*, *P. palmivora* and *P. nicotiana* are susceptible to FP, explaining further that as the concentration of phosphite in the culture medium increased, the weight of the biomass produced by the three species of *Phytophthora*.

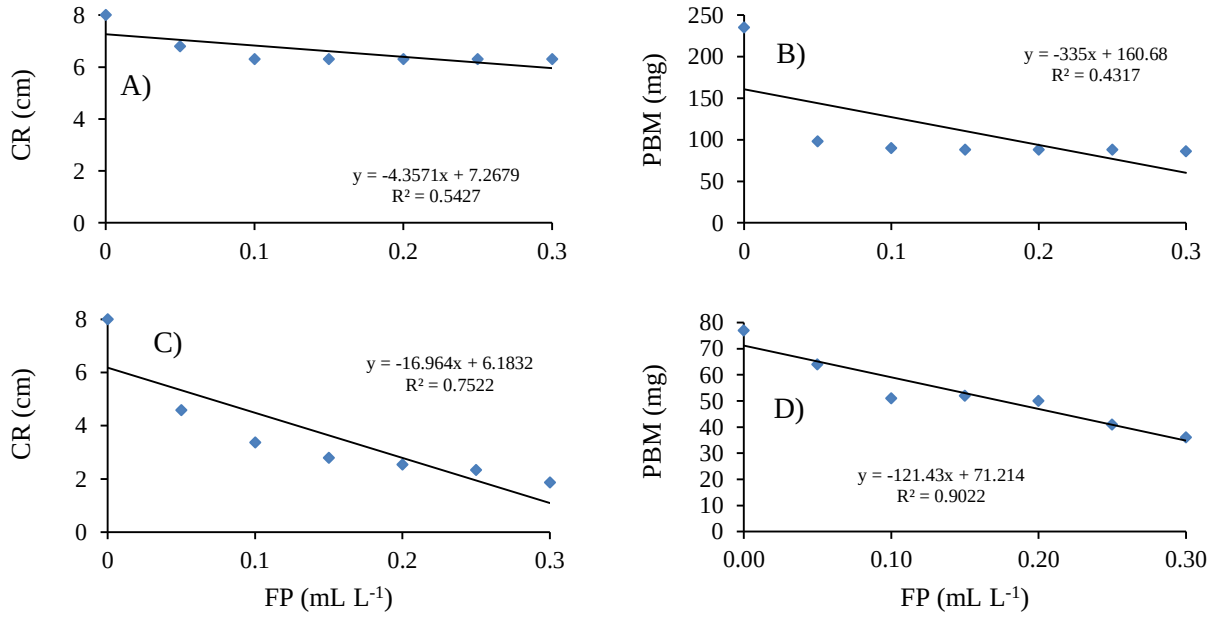

Figure 1. Association between radial growth of mycelium (CR) and biomass production (PBM) of *Pythium aphanidermatum* (A-B) or *Athelia rolfsii* (C-D) and concentration of potassium phosphite (FP).

However, the growth of *P. aphanidermatum* was not completely inhibited at 24 hours, 0.05 mL L^{-1} of FP was sufficient to inhibit the formation of oospores, while in the control there was abundant formation of these structures. reproduction. These results agree with those reported by Davis and Grant (1996) regarding the production of spores in *Fusarium oxysporum* f sp. *cubense*; likewise, with those published in relation to *Mycosphaerella fijiensis* (Mogollon and Castaño, 2012), *Peronospora sparsa* (Hukkanen *et al*., 2008), *Phytophthora plurivora* (Dalio *et al*., 2014) and *P. cinnamomi* (Wong *et al*., 2009). In the species *P. plurivora*, Dalio *et al*. (2014) also recorded a significant decrease of the mycelium CR and zoospore production when cultured on PDA culture medium enriched with the phosphite ion.

The FP also originated restriction results in the CR, PBM and prevented the formation of sclerotia in *A. rolfsii*, which also agree with those reported in *Sclerotium cepivorum* by Ortega *et al*. (2011), who added potassium bicarbonate in the PDA culture medium and observed that with 50 mM the growth of the mycelium decreased significantly and the formation of sclerotia was avoided.

According to King *et al*. (2010), the FP added to the culture medium inhibits the expression of genes related to the synthesis of proteins that constitute the wall and the cytoskeleton of the cells of *P. cinnamomi*, reason why the formation of mycelium and reproductive structures in the microorganisms are diminished when they are grown in culture media mixed with said salt.

Conclusions

Potassium phosphite was effective in decreasing the radial growth of the mycelium and the production of biomass in phytopathogenic organisms, and preventing the production of oospores in *Pythium aphanidermatum* and sclerotia of *Athelia rolfsii*.

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