Effect of Copper, Zinc and Potassium Phosphites on the Mycelium Growth of *Phytophthora nicotianae* in Olive Tree Dry Branch Disease

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Abstract

The genus *Phytophthora* is one of the most popular in plant pathology, because of the importance of the diseases it causes. In olive trees severe damages are caused by the disease known as "dry branch". Recently, our work team has identified as responsible of this disease the pathogens *Phytophthora palmivora*, *P. nicotianae* and *P.citrophthora*, being these the first report in Argentina for olive trees. *P. nicotianae* is one of the main responsible of yield losses in woody crops. It is why too much effort have been done to find efficient methods of control, with a low negative impact on environment and to avoid resistant strains selection. In this regard, treatment with phosphites could be a valid strategy. The aim of this work was to determine the fungistatic activity of copper, zinc and potassium phosphites on mycelium growth of *P. nicotianae*. The effect of phosphites at several concentrations was evaluated *in vitro*, through the supplemented culture medium technique. The colony diameter was recorded daily. The inhibition percentages were calculated and compared using analysis of variance. An inhibition curve was made and it was calculated by nonlinear regression the concentration required to inhibit 50 and 95% of mycelial growth. A completed inhibition of the mycelial growth was observed when copper and zinc phosphites were employed at 10 ppm. Copper, zinc and potassium phosphites concentration which inhibit the 50% of the mycelial growth of *P. nicotianae* was respectively 0,3; 0,6 and 0,7 ppm, whereas for the 95% inhibition was 1,7 ppm for the first two phosphites unfortunately, it couldn't be calculated for the potassium phosphite. It was concluded that all these phosphites have inhibited the growth of the mycelium of *P. nicotianae* and therefore could be considered as a viable alternative for its control.

INTRODUCTION

Diseases caused by species of the genus *Phytophthora* in general are among the most destructive and economically important agricultural problems world-wide (Matheron and Porchas, 2000; Portz *et al*., 2008). *Phytophthora* sp. is one of the most abundant soil fungi. It can infect both via the roots and the air, causing roots and the basal part of the stem to rot and also it wounds the stem cortex, especially in woody species such as olive (Del Río *et al*., 2003). In olive trees severe damages are caused by the disease known as "dry branch". The symptoms of the last one are not specific to a given disease, but are closely related to rootlets death of young olive trees (*Olea europaea L.*) (Sanchez-Hernández and Muñoz-García, 2000). Death of trees occurs rapidly, with or without previous yellowing or defoliation. The syndrome has been called "seca" (drying) to distinguish it from other diseases, such as *Verticillium* wilt or insect damage that can induce similar symptoms (Sanchez-Hernández et al., 1998). Recently, our work team has

identified as responsible one of the causal agent of this disease the pathogens *P.~~hytophthora~~ palmivora*, *P. nicotianae* and *P. citrophthora*, being these the first report in Argentina for olive trees. *P. nicotianae* is one of the most widespread and destructive soilborne plant pathogen associated to 301 host species (Erwin and Ribeiro, 1996), being responsible of important losses in woody crops. It causes root rots, stem necroses and crown decline, as well as fruit and foliar blights on many agronomic and horticultural plants in seed beds, nurseries, fields, and landscape plantings.

The control of diseases caused by species of the genus *Phytophthora* was even very limited and it is why too much effort have been done to find efficient methods of control, with a low negative impact on environment and to avoid resistant strains selection (Bock *et al*., 2012; Machinandiarena *et al*., 2012; Deliopoulos *et al*., 2010). Chemical control is the most effective measure used to protect crops against these pathogens, based on the use primarily upon copper and metalaxyl-based fungicides (Widmer and Laurent, 2006). However, this method increased production costs and generates environmental and health damage (Cooke *et al*., 2011; Widmer and Laurent, 2006). In this regard, treatment with phosphite salts could be a valid strategy for control of the diseases caused by *P. nicotianae* because reduces the intensive use of fungicides as well as production cost (Lobato *et al*., 2008).

These simple, inorganic salts have properties that make them desirable for inclusion in Integrated Disease Management (IDM) programmes. Their proposed advantages include low cost, favorable safety profile for humans and the environment and low mammalian toxicity (Olivier *et al*., 1999). Phosphites has a complex mode of action capable of controlling crop diseases caused by oomycetes through a direct effect on the pathogen and an indirect effect by stimulating host defense responses (Deliopoulos *et al*., 2010). Direct effects include the inhibition of mycelial growth and the reduction or alteration of the pathogen metabolism (King *et al*., 2010). The indirect effect involves the stimulation of plant defense mechanisms such as the enhanced production of phytoalexins and the reinforcement of the cell wall (Pilbeam *et al*., 2011; Eshraghi *et al*., 2011). This complexity of mechanisms involved in the prophylactic effects of phosphites has limited the development of pathogen resistance to these substances (Deliopoulos *et al*., 2010).

The aim of the present work was to determine the fungistatic activity of copper, zinc and potassium phosphites on mycelium growth of *Phytophthora nicotianae* in laboratory tests, and to establish the concentration of each of the phosphite causing 50% or 95% reduction in mycelial growth.

MATERIALS AND METHODS

Biological material and Chemicals

The isolate of *P.~~hytophthora~~ nicotianae* was obtained from the microorganisms collection of the Department of Plant Pathology at the Faculty of Agricultural Sciences in Luján de Cuyo, Mendoza. The isolate was recovered from necrotic roots of young olive trees in Mendoza and were maintained on clarified V8 agar. The phosphites salts used in the experiment were: copper, zinc and potassium phosphites.

Solid agar bioassay

Phytophthora nicotianae was grown on V8 juice agar (V8A; commercial V8 juice, 200 ml; CaCO3, 2 g; agar, 17 g; and distilled water, 800 ml) plates at 24°C for 5 days. Individual agar disks (4 mm in diameter) were removed from the edge of an actively growing culture of the pathogen and placed at the centre of each Petri dish (5,5 cm in

diameter) containing V8A amended with a test phosphite salts at concentrations 0,1; 1 and 10 ppm. The phosphites were added to V8A after autoclaving when the agar had cooled to approximately 60°C. Control plates were run simultaneously, using the growth medium without phosphites. Three replicate plates of each phosphites concentration as well as control were preparated. The experiment was repeated once. After a 4 day incubation period at 24°C in darkness, the test was considered concluded. In order to evaluate the mycelial growth inhibition, the mean mycelial growth of the pathogen was determined by measuring the colony diameter in two directions at right angles. These mean growth values were converted in to the inhibition percentage of mycelial growth in relation to the control treatment by using the formula of Mine Soylu and Kurt (2006):

$$Percentage\ inhibiton = \left(\frac{C-T}{T}\right) x\ 100;$$

where, "C" is control mean colony diameter, and "T" is treated mean colony diameter.

Data were subjected to analysis of variance (ANOVA). The mean comparisons were performed using Tukey's test to examine if differences between phosphites concentration and growth mycelial inhibition were significant (P<0,01). The analyses were performed with InfoStat-Statistical Software (version 2012 Add reference). The concentration of each of the phosphites required to inhibit 50 ($EC_{50\%}$) and 95% ($EC_{95\%}$) of mycelial growth was calculated by nonlinear regression (Logistic model) of the percent inhibition plotted against the phosphites salts concentration.

RESULTS AND DISCUSSION

The effects of different concentrations of copper, zinc and potassium phosphites on the mycelial growth of *P. nicotianae* are shown in *Fig. 1*. These data were recorded on the fourth day after inoculation of the agar medium. All phosphites evaluated were found to inhibit the growth of *P. nicotianae* in a dose-dependent manner. *P. nicotianae* was most sensitive to copper and zinc phosphites at a concentration of 10 ppm than that recorded for the same concentration of potassium phosphite. Mycelial growth of *P. nicotianae* was totally inhibited by copper and zinc phosphites at a concentration of 10 ppm, while for same concentration the potassium phosphite the percentage de inhibition of growth mycelial was 73%. At a concentration of 0,1 ppm of copper, zinc and potassium phosphites, inhibition of mycelial growth of *P. nicotianae* was 40; 24 and 31% respectively. While for the copper, zinc and potassium phosphites at 1 ppm the percentage of inhibition was 81; 75 and 58% respectively, showing no significant difference between treatments (*Fig. 1*).

Coffey and Joseph (1985) found that phosphite acid (4,1 a 6,2 ppm) inhibited the mycelial growth of *P. cinnamomi* in *in vitro* studies. Fenn and Coffey (1985) suggested that phosphite metabolism may be one target of toxicity in Oomycetes. Other researchers (Cookey and Little, 2001) found Phytophthora was controlled significantly by phosphites on potato. In natural ecosystem, it was also reported that phosphite salts have been successful protecting native plants from *P. cinnamomi* in Western Australian forest (Komorek and Shearer, 1995Add reference).

In our *in vitro* test; the potassium phosphite inhibited the mycelial growth of *P. nicotianae*. The same effect was also found on *P. palmivora* by Grant *et al*., 1990, and on *P. cryptogea* and *P. capsici* by Perez *et al*., 1996. Marks and Smith (1992) found that phosphites salts were also effective against *P. cinnamomi, P. nicotianae* and *P. palmivora*.

Copper, zinc and potassium phosphites concentration which inhibit the 50% ($EC_{50\%}$) of the mycelial growth of *P. nicotianae* was respectively 0,3; 0,6 and 0,7 ppm, whereas for the 95% ($EC_{5095\%}$) inhibition was 1,7 ppm for the first two phosphites unfortunately, it couldn't be calculated for the potassium phosphite (*Fig. 2*).

CONCLUSIONS

All the phosphate salts tested on this study inhibited the mycelial growth of *P. nicotiane*. Thus it could be proposed that they should be considered as potentially good tools for the management of *P. nicotianae*.

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Figures

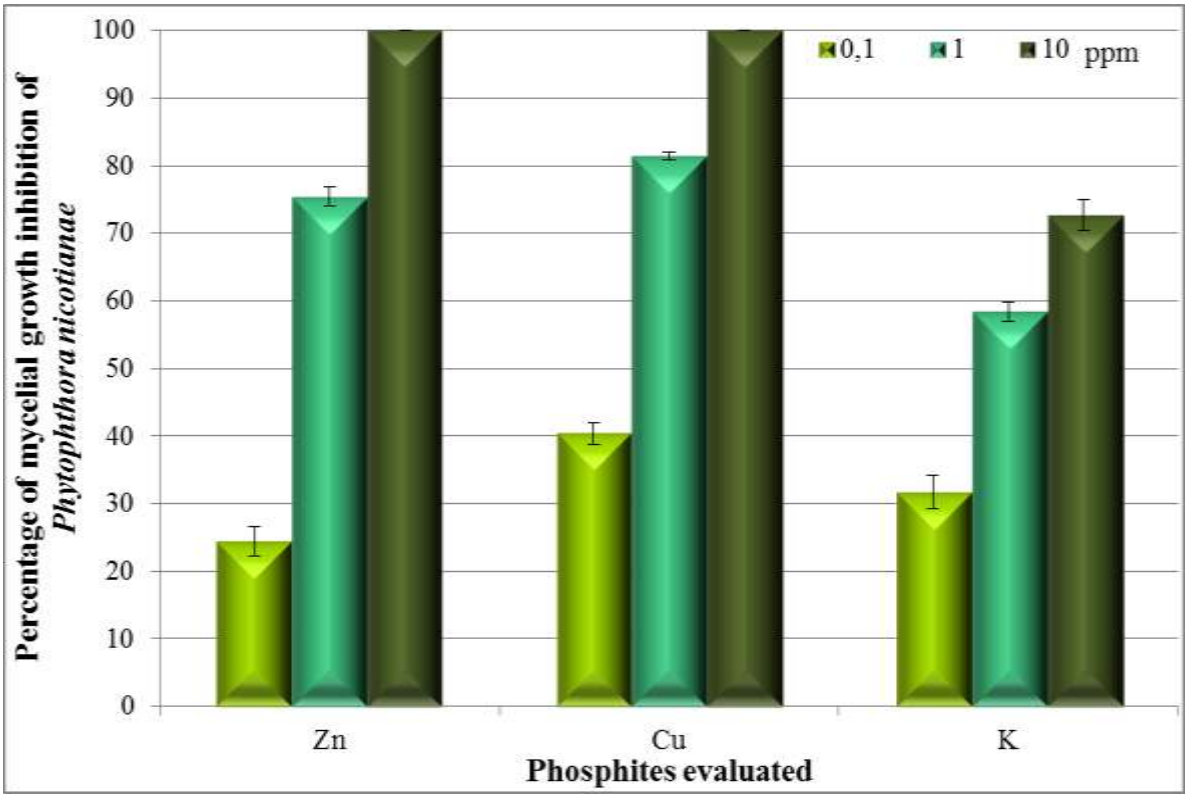

Fig. 1. Influence of dose of copper, zinc and potassium phosphites on mycelial growth of *Phytophthora nicotianae.* Columns with diverse letters differ significantly according to Tuckey's test, P<0,01.

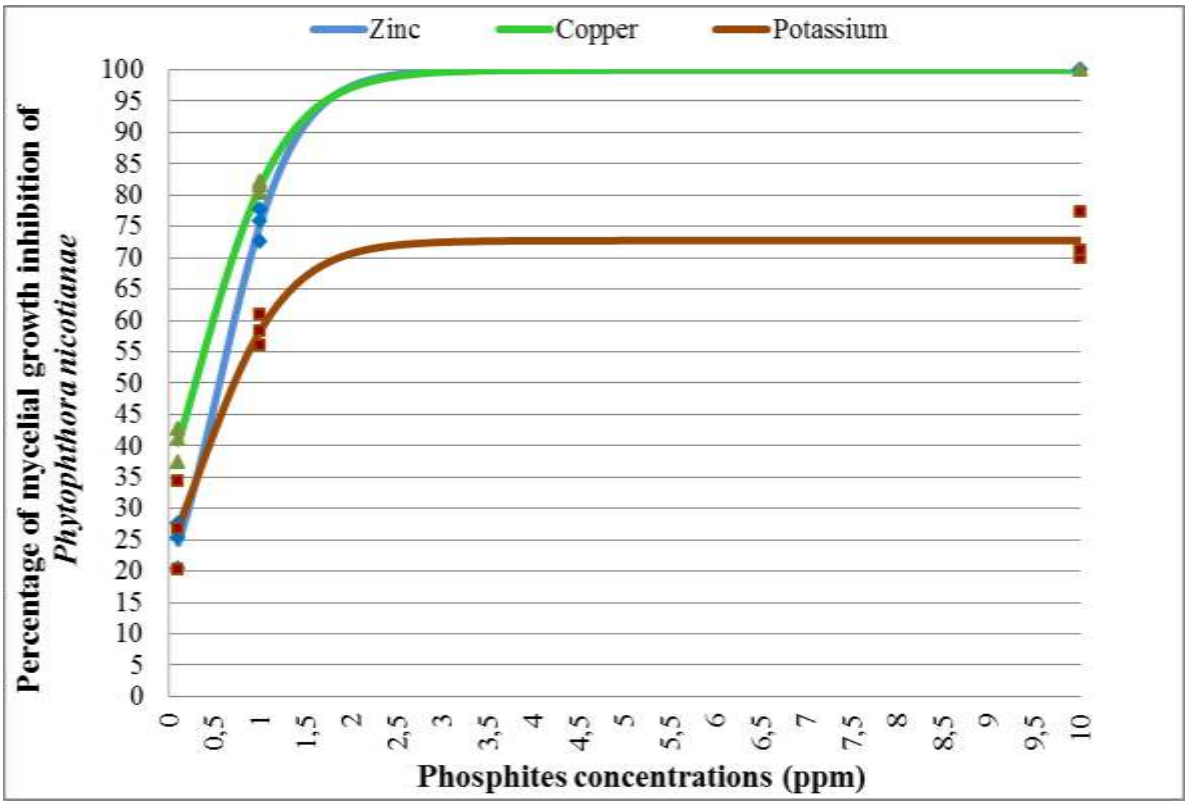

Fig. 2. Logistic Model Adjustment of the dose-influence of copper, zinc and potassium phosphites on the inhibition of *P. nicotianae* mycelium growth.