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Potential of phosphite products in the control of *Sclerotinia sclerotiorum in vitro*

[1]Stheffani Lucca, [2]Joanilson Vieira Prestes Junior, [3]Thayllane de Campos Siega, [4]Fabíola Mundstock, [5]Sérgio Miguel Mazaro

[1]Postgraduate Program in Agronomy (PPGAG), Federal Technological University of Paraná (UTFPR), Pato Branco, Paraná, Brazil.
[2]Postgraduate Program in Agronomy (PPGAG), Federal Technological University of Paraná (UTFPR), Pato Branco, Paraná, Brazil.
[3]Postgraduate Program in Agronomy (PPGAG), Federal Technological University of Paraná (UTFPR), Pato Branco, Paraná, Brazil.
[4]Postgraduate Program in Agronomy (PPGAG), Federal Technological University of Paraná (UTFPR), Pato Branco, Paraná, Brazil.
[5]Department of Agronomy, Federal Technological University of Paraná (UTFPR), Dois Vizinhos, Paraná, Brazil.

Correspondence Author: Thayllane de Campos, Federal Technological University of Paraná (UTFPR), Department of Agronomy, Estrada para Boa Esperança Km 4, Dois Vizinhos, PR. ZIP code: 85660-000.
E-mail: thayllanedecampos@hotmail.com.

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Abstract

Background: The pathogen *Sclerotinia sclerotiorum* is a soil fungus that causes serious damage to several crops of economic interest. **Objective:** The objective of this study was to evaluate the potential of phosphite products in the control of *Sclerotinia sclerotiorum* under *in vitro* conditions. The experiment was carried out in the Phytosanitary laboratory of the Universidade Tecnológica Federal do Paraná, Dois Vizinhos campus. The products used in the experiment were potassium phosphite, manganese phosphite and copper phosphite. The experimental design was completely randomized with four replicates, an experimental unit consisting of a Petri® plate. The mycelial growth assays were at 12, 24, 48, 72 and 96 hours after incubation of the plates in BOD, and the evaluations were stopped as soon as the control plate reached full growth. **Results:** The results showed that both products had potential to inhibit mycelial growth of the pathogen *S. sclerotiorum* and at the dose of 60 µL there was complete inhibition of fungus growth. **Conclusion:** At the end of the study, we concluded that there is potential of phosphite based products in the control of *S. sclerotiorum in vitro*. However future work should consider the pipeline(pathosystem) of interest, and field trials on the desired crops, to demonstrate the behavior of the plants in relation to the products.

Key words: Soil pathogen, Fungicide, Complementary control.

INTRODUCTION

The pathogen *Sclerotinia sclerotiorum* is a soil fungus that causes damage to several crops of economic interest, such as lettuce, soybean, beans and sunflower (DA SILVA, et.al., 2015). The control methods for this pathogen are very difficult, because it is a fungus that has a host range and also for developing resistance structures that can be viable in the soil for a long time, which makes it difficult to manage (KIN *et al*., 2011).

The transmission of the pathogen may be through mycelium dormant or the presence of sclerotia in seed lots (KAWASAKI & MACHADO, 2013), which is a method that infects areas over long distances. Another method of infection is through the release of ascospores through apothecia, which are structures formed from the germination of sclerotia, this is a method that infects areas at short distances, since it has been verified that ascospores remain in a range of 100 meters from the source of inoculum (BEN-YEPHET & BITTON 1985).

In view of this, it is a concern with the presence of *S. sclerotiorum* in the agricultural areas, and in addition to causing serious damage to plants has just been difficult to eradicate. In Brazil, it is estimated that about 6.3 million hectares planted with soybeans in the 2013/14 crop infested by disease (MAYER et.al., 2014).

The crop rotation, which is widely used for the management of the disease, presents good results considering the reduction of sclerotia and thus reducing initial inoculum pressure. However, considering the diversity of host plants and the susceptibility of several crops, it ends up requiring complementary methods to control this pathogen.

Thus, control methods that contribute to the elimination of this pathogen are of great value. One of the technologies being studied is the use of *Trichoderma.*

The inhibition of the mycelial growth of the pathogen with the use of *Trichoderma roseum in vivo* has been proven by Huang *et al*., (2000) in common bean plants. Another method of evaluation with the use of this antagonist is on the carpogenic germination of *S. sclerotiorum* (GERALDINE *et al*., 2013).

In addition to the increasing use of biological control, technicians have been recommending the use without chemical control of phosphites in association with fungicides. However, several studies have demonstrated their potential to inhibit an activity of pathogens on plants as well as to activate plant defense routes(SILVA, et.al., 2014).

Although there are already some promising works involving the use of phosphites in several pathosystem, as well as in the control of in vitro pathogens, on *S. sclerotiorum* there are no reports in the literature, becoming this unpublished work.

The goal of this work was to demonstrate the efficacy of potassium, manganese and copper phosphite based products in the control of *Sclerotinia sclerotiorum* under *in vitro* conditions.

MATERIAL AND METHODS

The experiment was conducted at the Phytosanitary Laboratory of the Universidade Tecnológica Federal do Paraná, Dois Vizinhos campus, and the sclerotia of *S. sclerotiorum* were obtained from this laboratory. These were grown in Petri® dishes containing PDA (potato, dextrose and agar) culture medium maintained

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in BOD at 25 °C ± 2 °C and photoperiod of 12 hours. Pure mycelial plates of the pathogen were obtained after 10 days, thus obtaining the mycelial discs for use in the experiment.

A completely randomized design was used, containing four replicates in each treatment. The sample unit consisted of a 9 cm diameter Petri Glass plate. The concentrations used were standardized in 10µL, 20µL, 40µL and 60µL for all the products in question. The control treatment consisted only of PDA. In the laminar flow chamber the phosphites were incorporated into the culture media in their proper concentrations, then they were poured into the appropriately sterilized Petri® plates.

Subsequent to the solidification of the medium, the plates received a 5mm diameter disc containing the mycelium of the *S. sclerotiorum* fungus. The plates were then capped, sealed with film paper and conditioned to incubator BOD which maintained them at 25 °C ± 2 °C and 12-hour photoperiod. Mycelial growth evaluations were done 24, 48,72 and 96 hours after incubation, until the control plate is completely filled by the mycelium of the pathogen. The data were evaluated for normality and submitted to analysis of variance, then evaluated by regression using the statistical program Genes (Cruz, C.D GENES, 2013).

RESULTS AND DISCUSSION

The observed results showed a significant and inversely proportional effect between the concentration factors and the mycelial growth of the fungus *Sclerotinia sclerotiorum*, *in vitro* culture. That is, with the increase of the concentration, there was reduction of mycelial growth of the pathogen.

For the product and concentration factors there was no significant difference, demonstrating that both products behaved in the same way, having an inhibitory effect of mycelial growth. As can be seen in the chart below.

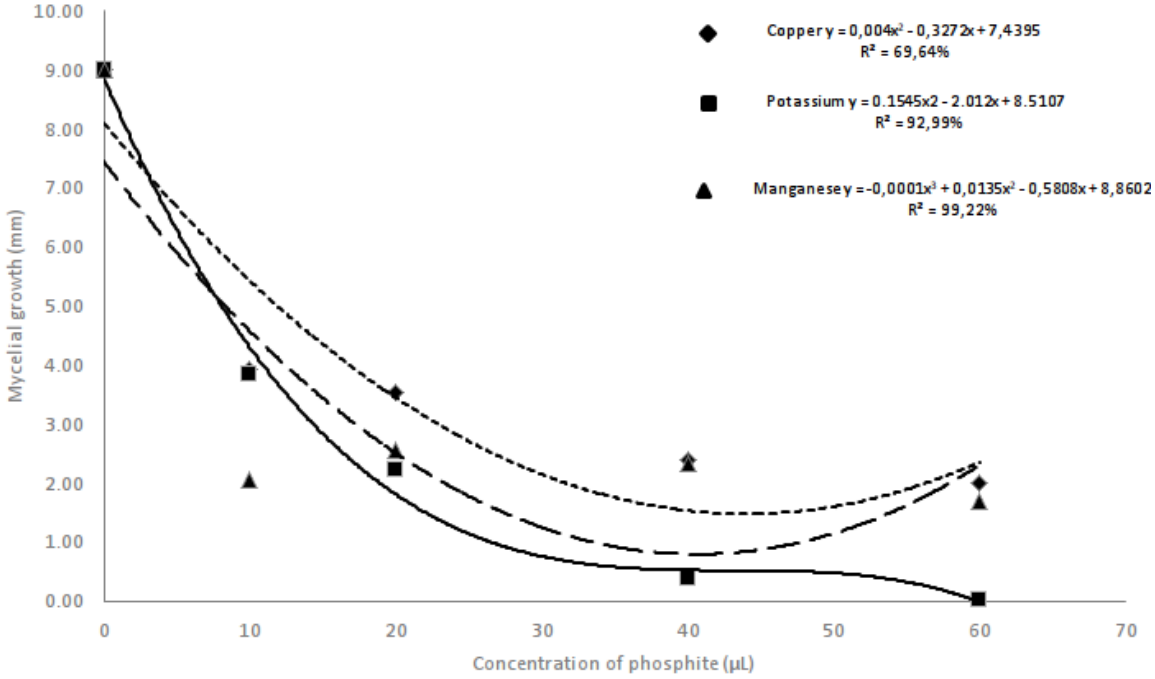

Fig. 1: Mycelial growth as a function of product doses.

In the regression analysis, for the concentration factor, the regression curve was quadratic, with a maximum efficiency point of 8.35%, and the concentration at which the highest inhibition of mycelial growth was obtained was 60 µL.

For potassium phosphite there are several works proving its efficiency. As reported by Araujo (2010), where potassium phosphite was used in the control of *Colletotrichum gloesporiodes*, direct action of the product on the growth of the pathogen was obtained at the doses tested.

Nephew (2016) tested potassium phosphite in the control of passionflower *Fusarium solani*, and obtained positive results in the inhibition of the pathogen at concentrations of 50 ppm *in vitro* culture.

For products based on copper phosphite and manganese there are still no reports of their direct action on the growth of pathogens. Thus, it can be inferred that the mode of action of these products on the pathogens was fungitoxic and fungicidal, since the products may have acted unspecific ally on the membrane of the fungus, inhibiting the protein and enzymatic action, or may have occurred due to the excess of the products in the culture medium, even in low concentrations (JULIATTI).

Conclusion:

In view of this, the work in question demonstrates the potential of phosphite based products in the control of *S. sclerotiorum in vitro*. However future work should consider the pipeline of interest, and field trials on the desired crops, to demonstrate the behavior of the plants in relation to the products.

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