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Potential of phosphite-based products in the control of *Fusarium oxysporum in vitro*

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Abstract

Background: The fungus *Fusarium oxysporum* is a pathogen of difficult control, having resistance structures that remain viable for long periods of time, in addition, causes a great diversity of diseases in plants.
Objective: Therefore, the goal of this work was to evaluate the potential of phosphite products in the *in vitro* control of *F. oxysporum*. The experiment was conducted at the Phytosanitary Laboratory of the Universidade Tecnológica Federal do Paraná, Dois Vizinhos campus. The products were added at the doses of 10µL, 20µL, 40µL and 60µL in the PDA culture medium and then poured into the Petri® plates where they received a 5 mm disc containing the mycelium of the fungus. The mycelial growth evaluations were done at 12, 24, 36, 48 and 96 hours. The experimental design was completely randomized, with four replications.
Results: The results showed that the three products based on potassium phosphite, manganese phosphite and copper phosphite were effective in the control of *F. oxysporum* under *in vitro* conditions, and all three behaved in the same way, presenting fungicidal and fungistatic effect on the pathogen.
Conclusion: In view of the above, it is confirmed that the three products based on phosphite have potential to control *F. oxysporum* under *in vitro* conditions. However, more in-depth studies should be done to evaluate the potential of in vivo products seeking to elucidate their behavior on plants.

Key words: *Soil fungus, complementary control, fungicide*

1. INTRODUCTION

Fusarium oxysporum is a soil fungus that has resistance structures that remain viable for long periods of time, characterizing it as a pathogen of difficult control. The pathogen may survive in the form of mycelium on cultural remains or intermediate hosts. Its spread is made by contaminated implements, rainwater or irrigation, contaminated seedlings and by the soil revolving through conventional planting methods (AMORIN, 2011).

The incidence of the pathogen in the cultures depends on a series of factors, in this sense the triangle of diseases, where it is necessary the presence of the virulent pathogen, susceptible host and favorable environment. Thus, the complexity of the environmental system and the genetic variability of the genus Fusarium end up making it difficult to control, and in many cultures, making the use of genetic resistance unfeasible (MILANESE et.al, 2013).

In view of the complexity of the genus of the pathogen, traditional control with fungicides has been losing efficiency, thus seeking complementary and combined means that obtain positive results in relation to fungus control. Biological control with the use of microorganisms such as Trichoderma has the capacity to interfere in the survival of the fungus and its biological activities (BENITÉZ, 2004).

The use of phosphites appeared as a new alternative for the control of this fungus. These products are salts of phosphorous acid (H3PO3), which exhibit rapid absorption and translocation in plants via phloem and xylem (GUEST & GRANT, 1991). They also present a systemic action that reduces mycelial growth and the release of spores, besides having a toxic action for some species of fungi and having an important role in the activation of the plant defense mechanism (ALI et al, 1993; GUEST, 2005).

The use of phosphites can be done via seed treatment, foliar application separately or together with fungicides, aiming at increasing the effectiveness of the control, thus promoting additive or synergistic effect (MENEGHTTI et al, 2010). In addition, the use of phosphorous acid with a low dose of metalaxyl resulted in positive results in the control of *Sclerospora graminicola* and grain yield when compared to the dose recommended only with fungicide (CHALUVARAJU et al., 2004).

Thus, the use of phosphites can be used as a control medium for various plant diseases. The presence of potassium obtained positive results against pests and diseases, in addition, plants with adequate levels of this nutrient have the capacity to overcome the attack of diseases and to avoid the leakage of metabolites through membranes, being gateway for invading fungi (BASSETO, 2007).

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The use of manganese sources may be related to the greater resistance of plants against the attack of pathogens that occurs through lignification or direct inhibition (MALAVOLTA, 2006). The increase of the lignin content of the tissues may increase their impermeability, besides helping to obtain soybean seeds with high physiological potential (SILVA et al., 2008).

The use of phosphites on oomycetes is well known for the cultivation of potatoes, eucalyptus and avocados, but is still very small for the rest of the crops (JACKSON et al., 2000; MCDONALD et al., 2001). Thus the present work aims to evaluate the fungicidal, fungistatic and fungitoxic potential of the phosphites in the *in vitro* control of the pathogen *Fusarium oxysporum*.

2. MATERIAL AND METHODS

The experiment was conducted at the Phytosanitary Laboratory of the Universidade Tecnológica Federal do Paraná (UTFPR), Dois Vizinhos campus.
The pathogen isolates were obtained from the collection of fungi from the phytosanitary laboratory of UTFPR – Dois Vizinhos. These were grown in Petri® dishes containing PDA culture medium (potato, dextrose and agar), maintained at 25 °C ± 2 °C and photoperiod of 12 hours. The use was made after 10 days of growth.
The phosphites used in the experiment were supplied by Spraytec Fertilizantes, being Ultra K10®, Ultra Mn10® and CUBO 700®.

The experimental plots consisted of glass Petri dishes, 9 cm in diameter. For all the phosphites studied (potassium, manganese and copper) the concentrations were standardized in 10 µL, 20 µL, 40 µL and 60 µL. For the control, only the PDA culture medium was used. The experimental design was completely randomized with 4 replicates per treatment.

The culture medium was prepared and was dispensed into the Erlenmayers in the amount of 100 ml for each. Thereafter, the medium were sterilized along with the Petri® plates, the tips, and also the distilled water.

All material was taken to the laminar flow chamber, and then the phosphites were incorporated in their proper concentrations into the culture medium within each well and manually stirred for better homogenization of the mixture.

20 ml of the culture medium was poured into the Petri® dishes. The plates without lid were then subjected to sterilization treatment within the laminar flow chamber through the use of ultra violet light during the 20 minute period.

After sterilization and solidification of the medium, the plates received a 5mm diameter disc containing the mycelium of the fungus *Fusarium oxyporum*.
The plates were then capped, sealed with film paper and transferred to a BOD incubator 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.

3. RESULTS AND DISCUSSION

The results observed (Table 1) demonstrated a high inhibition rate of mycelial growth of *F. oxysporum* by the products tested. The three products used showed similar behavior among themselves, inhibiting fungus development. It was also observed that all doses of the three products tested had fungicidal action under the pathogen.

Table 1. Means of mycelial growths of Fusarium oxysporum submitted to treatments with different phosphites

Treatment	Average mycelial growth
Copper Phosphite	0,325a
Potassium Phosphate	0,002a
Manganese phosphite	0,002a

This may be due to the direct action of the phosphite under the pathogen, as observed by McGrath (2004), where phosphorous acid acted in the process of oxidative phosphorylation in oomycetes, reducing mycelial growth.
Another hypothesis of the direct action is due to the presence of the products in the culture medium causing toxicity to the fungus, affecting its metabolism and consequently its growth.

The action of potassium phosphite under pathogens has been widely studied, as in the work conducted by Sobrinho (2016), where the action of potassium phosphite on passion fruit *F. solani* was tested *in vitro*. Significant inhibition of mycelial growth occurred from the 50 ppm dose, enhancing inhibition with increasing concentration tested.

The absence of sporulation in tomato leaves treated with potassium phosphite and inoculated with Phytophthora infestan indicates an important characteristic of the product, reducing the inoculum potential of the disease (TOFOLI, 2012).

Another action of potassium phosphite was to control mildew on the vine. The doses tested with a higher concentration of potassium phosphite provided a greater reduction in the incidence of mildew in the two harvests, with 60.5% control for phosphite A and 57.7 for phosphite B (PEREIRA, 2012).

For manganese and copper phosphites there are few reports in the literature of their direct action on pathogens. An experiment carried out with manganese through leaf spraying in four soybean cultivars showed an attack reduction of the pathogen *Fusarium spp.* with increased dose applied (CARVALHO, 2015).
For copper phosphite in disease control the product was tested in peach cultivars aiming at reducing the incidence of rust and brown rot. For rust in cultivar BR1 which was tested there was a reduction in disease severity in relation to the control treatment (KOWTA, 2012). Demonstrating the potential of the product for the treatment of pathogens.

4. CONCLUSION

In view of the above, it is confirmed that the three products based on phosphite have potential to control *F. oxysporum* under *in vitro* conditions. However, more in-depth studies should be done to evaluate the potential of *in vivo* products seeking to elucidate their behavior on plants.

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