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ALTERNATIVE PRODUCTS FOR CONTROLLING *Sclerotinia sclerotiorum in vivo* AND *in vitro* IN CANOLA CROPS

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Abstract

Products that activate plant defense mechanisms, such as white mold, may increase the disease control spectrum in canola crops as an alternative to chemical control for disease management, aiming at lower environmental impacts and selection pressure on pathogens. This study evaluated *Sclerotinia sclerotiorum* control and resistance induction in canola crops with alternative products. *In vitro* and detached leaf experiments were conducted in a laboratory, and *in vivo* experiments occurred in a greenhouse. High Roots®; V6®; Maxi Flor®; Wert Plus®; potassium, manganese, and copper phosphites; manganese; procymidone fungicide; and *Ascophyllum nodosum*, *Bacillus subtilis,* and *Bacillus thuringiensis* extracts assessed *S. sclerotiorum* mycelial growth *in vitro* in detached leaves, white mold severity in a greenhouse, and enzymatic analysis in leaf tissues. The last two experiments also received the acibenzolar-S-methyl and *Trichoderma asperellum* treatments. *S. sclerotiorum* mycelial growth showed 100% inhibition under *B. subtilis* and Wert Plus® at 1000 ppm and procymidone at 100 and 1000 ppm *in vitro*. The procymidone and *B. subtilis* treatments in detached leaves inhibited 100% of fungus growth. The greenhouse experiments with lower severity were procymidone, copper phosphite, *B. thuringiensis*, *B. subtilis*, manganese phosphite, potassium phosphite, High Roots®, and V6®. Higher peroxidase enzyme activity occurred in plants treated with acibenzolar-S-methyl, copper phosphite, and fungicide 48 hours after pathogen inoculation. The polyphenol oxidase enzyme did not show activity. Alternative products were responsive and may aid the chemical control of white mold in canola crops.

Keywords: Biological control. *Brassica napus*. Enzymatic activity.

1. Introduction

Canola (*Brassica napus* L. var oleifera) is an oleaginous species of the cruciferous family susceptible to incorporation into grain production systems in Brazil, standing out as an excellent economic alternative and potential rotation crop (Tomm et al. 2009; Luz et al. 2012). The largest world producers are European Union countries, Canada, China, India, and Japan. Canola crops have been gaining prominence in Brazil, mainly in the south, with a planted area of 55.2 thousand hectares in the 2022/23 harvest, yielding 1,743 kg ha^{-1} (Conab 2023).

Phytosanitary problems focusing on diseases prevent crop expansion in national and international territories, and white mold (*Sclerotinia sclerotiorum* (Lib.) By Bary) is the most relevant crop disease (Solgi et al. 2015). It is a polyphagous fungus that may infect numerous hosts from at least 75 families, 278 genera, and 500 species of plants, including fruits, ornamentals, and weeds (Saharan and Mehta 2008; Cardoso et al. 2016). *Sclerotinia sclerotiorum* is among the more relevant pathogens worldwide, as it is distributed in all producing regions and is most common in temperate climates (Perveen et al. 2010).

Plant disease control is based on different measures, such as genetic, chemical, cultural, physical, and biological controls. However, the interest in alternative disease control methods has increased in recent decades due to low pesticide effectiveness and the environmental impacts of these products (Pozza Junior et al. 2021; Gabardo et al. 2022). Several studies have sought further information on plant resistance induction, seeking higher sustainability and lower impacts on plant disease control. Resistance induction or induced resistance occurs by promoting plant resistance against phytopathogens through localized infections, treatments with microbial components or products, or organic or inorganic compounds (Bonaldo et al. 2005). Alternative products were tested to reduce soybean (*Glycine max* (L.) Merril) downy mildew (*Peronospora manshurica* (Naumov) Syd) disease severity in the 2013/2014 and 2014/2015 growing seasons. The best result for downy mildew reduction was the application of acibenzolar-S-methyl, micronutrients (Cu, Mn, Zn), and *Ascophyllum nodosum* (Gabardo et al. 2020).

Using growth-promoting bacteria, such as *Bacillus subtilis*, increased the growth of tomato plants and the resistance to control soil pathogens (Samaras et al. 2021). *Bacillus thuringiensis* is another potentially viable bacterium because it induces systemic resistance and reduces damage from *S. sclerotiorum* when applied to *Brassica campestris* L. (Wang et al. 2023).

This study evaluated *in vitro* and *in vivo S. sclerotiorum* control in canola plants and resistance induction with biotic and abiotic product application.

2. Material and Methods

The experiments were conducted in a laboratory at the State University of Ponta Grossa, PR, Brazil. The used leaf fertilizer treatments were High Roots® (N - 18%, K_2O - 6%), V6® (Mn – 2.5%; Zn – 1.9%; Mo – 0.16%), Maxi Flor® (N - 14%; K_2O - 6%), Wert Plus® (Cu 4%; Mn 6%; Zn 3.9%), potassium phosphite (P_2O_5 26%; K_20 19%), manganese phosphite (P_2O_5 - 36%, Mn – 7.0%), copper phosphite (N 11%; P_2O_5 22%; S 1.76%; Cu 4%), manganese (Mn 10%; S 5.48%), fungicide (Sumilex® - procymidone, 50 w/w i.a), *Ascophyllum nodosum* seaweed extract (Acadian® - K_2O – 5.3%, total organic carbon – 6.0%), biological products based on *Bacillus subtilis* (Serenade® - 13.68 g L^{-1}; 1 x 10^9 CFU g^{-1}) and *Bacillus thuringiensis* (Dipel WP® - 32 g kg^{-1}; 25 billion viable spores g^{-1}), and the control.

The *S. sclerotiorum* pathogen was obtained from sclerotia collected in canola fields in Guaragi, PR, Brazil. They were subjected to asepsis with sodium hypochlorite (2.75%), dried under sterile filter paper, deposited in a Petri dish containing potato dextrose agar (PDA) culture medium, and maintained in a BOD growth chamber for fungus growth at 24 ± 2°C for a 12-hour photoperiod.

In vitro tests

The mycelial growth test incorporated all products into a melting PDA medium at 1, 10, 100, and 1,000 ppm. Additionally, biological products were combined with the culture medium after being sterilized in an autoclave. After incorporating the products and solidifying the culture medium in Petri dishes, 0.6cm mycelium discs of the fungus from a six-day-old colony plate were transferred to its center. The control corresponded to plates only with the PDA culture medium and a mycelium disc in the center without incorporating the products. The plates remained in a BOD chamber for pathogen development at 24 ± 2°C for a 12-hour photoperiod for five days. Fungus mycelial growth was evaluated daily for five days, and the mycelial growth speed index (MGSI) was calculated using the formula by Oliveira (1991).

The experimental design was completely randomized in a factorial scheme, represented by the products at different concentrations (14 treatments x five concentrations), with five replications for each treatment, and each Petri dish with colonies was considered a repetition.

Detached leaf tests

The plants used in the experiments were produced in a greenhouse of the State University of Ponta Grossa, PR, Brazil.

Ten Al High Tech M6 Clear Field (ALHT M6 CL) hybrid canola seeds were sown in three-liter containers with pine bark substrates. Three plants were cut and maintained after seedling emergence. The leaves were collected when reaching the stage of two true leaves, aided by disinfected scissors, and taken to the laboratory. Bradley et al. (2006) proposed the methodology used in the present study, in which canola leaves were packed in Gerbox boxes (disinfected in 70% alcohol and 5% sodium hypochlorite) containing two filter paper sheets previously sterilized and moistened with 20 mL of sterile water. Two microscopic slides prevented direct contact of the leaf with the wet paper, and a portion of cotton moistened with sterile distilled water placed in the leaf petiole ensured it remained alive. Absorbent paper protected half the leaf's surface to hinder contamination during product application, and the surface of the other half was sprayed (10 mL) until wet. The treatments were the same as for the *in vitro* test plus the synthetic acibenzolar-S-methyl resistance inducer - ASM (Bion®) (Table 1). The pathogen (*S. sclerotiorum*) was inoculated 24 hours after product application. Mycelium discs of 0.6cm diameter of the fungus grown in PDA and incubated for six days were placed on the leaf's central vein. Product reapplications occurred 72 hours after pathogen inoculation on the previously applied leaf half. The Gerbox boxes with the leaves were placed in a BOD chamber at 24 ± 2°C for a 12-hour photoperiod throughout the experiment.

Disease severity was analyzed daily, totaling six assessments, aided by the modified scale by Juliatti et al. (2013). An area under the disease progress curve (AUDPC) was calculated with the obtained data (Shaner and Finney 1977).

The experiment had a completely randomized design in a factorial scheme of 14 treatments x 2 (product application or not in the same leaf half) with five replications, and each leaf was a repetition. The control consisted of a leaf without product spraying, and the dose of each treatment was determined according to the manufacturer's recommendation.

Table 1. Products tested with the detached leaf method in canola (*Brassica napus*) and their respective doses for *Sclerotinia sclerotiorum* control.

Treatment	Concentration	Dosages ($0.2\ mL^{-1}$ water)
High Roots®	N 18%, K_2O 6%	400 µL
V6®	Mn 2.5%; Zn 1.9%; Mo 0.16%	400 µL
Maxi Flor®	Mn 2.5%; Zn 1.9%; Mo 0.16%	400 µL
Wert Plus®	Cu 4%; Mn 6%; Zn 3.9%	400 µL
Potassium phosphite	P_2O_5 26%; K_20 19%	6000 µL
Manganese phosphite	P_2O5 36%, Mn 7.0%	560 µL
Copper phosphite	N 11%; P_2O_5 22%; S 1.76%; Cu 4%	5000 µL
Manganese	Mn 10%; S 5.48%	3000 µL
Fungicide	Procymidone (50m/m i.a)	3 g
Ascophyllum nodosum	Acadian® - K_2O – 5.3%, total organic carbon – 6.0 %	900 µL
Bacillus subtillis	Serenade®-13,68 g L^{-1} (1.34%) - 1 x 10^9 CFU g^{-1}	8000 µL
Bacillus thuringiensis	Dipel WP® - 32 g Kg^{-1} (3.2%) - 25billions of viable spores g^{-1}	0.12 g
Trichoderma asperellum	Isolate SF (1.0 x 10 CFU g^{-1})	0.24 g
Bion®	Acibenzolar -S-methyl (ASM) (50m/m a.i.)	0.06 g

Greenhouse tests

The Al High Tech M6 Clear Field (ALHT M6 CL) hybrid canola plants were grown in three-liter plastic containers with pine bark substrates. Six seeds were sown per pot, and thinning was performed ten days after emergence, leaving two plants per pot. The treatments were the same as for the detached leaf experiments, and the control corresponded to plants sprayed only with sterile distilled water. Products were applied in the early flowering stage of plants with four expanded leaves. The first pair of true leaves

received the products, and a plastic cup protected the other leaves so they would not be contaminated by the products, thus allowing to verify resistance induction.

The plants were inoculated using the toothpick method 47 days after sowing (Klingelfuss et al. 2007). This technique prepares the inoculum by sterilizing toothpicks in an autoclave and placing them horizontally in Petri dishes with solidified PDA medium. A 0.6cm mycelium disc of the *S. sclerotiorum* fungus from a six-day-old fungus colony plate was transferred to the center of Petri dishes. The plates were incubated in a BOD chamber for six days at 20 ± 2°C for a 12-hour photoperiod to promote fungus colonization in the toothpicks, and they were completely colonized by the pathogen at inoculation. The toothpicks were inserted into the stems above the primary leaves. The control treatment perforated the stem only with sterile toothpicks. The inoculated plants were covered with a moistened transparent plastic bag to form a humid chamber where they remained for 36 hours. The experimental design was completely randomized with 14 treatments and 10 replications, and each plant was a repetition.

Disease severity assessments started by determining lesion size at wet chamber removal and every two days after that until completing ten evaluations (Klingelfuss et al. 2007). An area under the disease progress curve (AUDPC) was calculated with the obtained data.

Leaves were collected for biochemical analyses 24, 48, and 72 hours after pathogen inoculation. Each sample collected during the procedure was immediately packed in aluminum foil envelopes and frozen at -20°C. Samples were collected from treated true leaves (1st pair) and untreated true leaves (2nd pair) to potentially identify resistance induction in untreated plants (Stangarlin and Pascholati 2000).

Protein extract achievement

Resistance induction was assessed with the following greenhouse treatments: control with the fungus (the plant was inoculated with a pathogen without treatment application), control without the pathogen (plant without pathogen inoculation and treatment), *T. asperellum, B. subtilis, B. thuringiensis*, ASM, copper phosphite, and procymidone fungicide.

Leaf samples weighed 0.5 grams and were mechanically homogenized in 2 mL of a 0.01M sodium phosphate extraction buffer solution (pH of 6.0) in a porcelain mortar. The homogenate was centrifuged at 6,500 g (gravitational force) for ten minutes at 4°C, and the obtained supernatant was an enzymatic extract for later determining the protein content and peroxidase and polyphenol oxidase enzyme activities (Viecelli et al. 2010).

The Bradford method (1976) evaluated total protein content. Protein concentration was expressed as µg of bovine serum albumin (BSA) in 1 mL of the sample (µg protein mL^{-1}) and determined with the standard curve of BSA concentrations from 0 µg mL^{-1}.

Peroxidase activity (E.C. 1.11.1.7) was set at 30°C using a direct spectrophotometric method at 470 nm for 2.15 minutes (Lusso and Pascholati, 1999), measuring guaiacol conversion into tetraguaiacol. The reaction mixture contained 0.10 ml of protein extract and 2.9 ml of a solution with 12.5 ml of 2% guaiacol and 360 µL of hydrogen peroxide in 87.5 ml of 0.01 M sodium phosphate buffer (pH of 6.0). Peroxidase activity was expressed as absorbance min^{-1} g^{-1} of fresh weight.

The Duangmail and Apenten (1999) methodology defined polyphenol oxidase activity (E.C 1.10:3.2), measuring catechol conversion into a quinone. The substrate consisted of 20 mM catechol dissolved in 100 mM sodium phosphate buffer (pH of 6.8). The reaction occurred by mixing 950 µL of the substrate and 50 µL of the enzymatic extract. Enzyme activity occurred at 30°C using a direct spectrophotometric method at 420 nm for 2.15 minutes.

Polyphenol oxidase and peroxidase results were expressed as specific activities (absorbance min^{-1} mg protein^{-1}). The experimental design consisted of randomized blocks in an 8 x 2 factorial scheme (eight products with and without product application). The treatments consisted of control with the pathogen, control without the pathogen, *T. asperellum, B. subtilis, B. thuringiensis*, ASM, copper phosphite, and procymidone fungicide.

The data from all experiments underwent the analysis of variance by the F-test, and the Scott-Knott test compared means at 5% significance. The data were transformed into $\sqrt{(x+1)}$ for analyses in the R statistical system, version 2.13.2 (R Development Core Team, 2011).

3. Results

In vitro tests

Mycelial growth speed index

Treatment and dose significantly affected the mycelial growth index (Table 2). The first experiment analyzed each treatment at different concentrations, showing a significant difference between products at 1000 ppm (Table 2).

Treatments with the fungicide, non-autoclaved and autoclaved *B. subtilis*, and the Wert Plus® nutrient inhibited pathogen growth at 1000 ppm. The fungicide also controlled 100% of fungus growth at 100 ppm but without a statistical difference from autoclaved *B. subtilis*. The treatments with lower pathogen control at this concentration were *B. thuringiensis* (autoclaved and non-autoclaved), manganese and potassium phosphites, and manganese, Wert plus®, High Roots®, and Maxi Flor® nutrients. The fungicide showed higher pathogen development control at 10 ppm, standing out among other treatments, but not statistically differing from autoclaved *B. thuringiensis* and *B. subtilis*, copper phosphite, and V6®. The treatment with the highest fungus control, superior even to the fungicide, at the lowest tested concentration (1ppm) was autoclaved *B. thuringiensis*, followed by autoclaved *B. subtilis*, without statistically differing from copper phosphite and V6®.

B. subtilis controlled 100% of fungus growth at all tested concentrations in the second experiment (Table 2). The procymidone fungicide inhibited 100% of mycelial growth at 100 and 1000 ppm. It did not statistically differ from *B. subtilis* (autoclaved and non-autoclaved), autoclaved *B. thuringiensis*, copper and manganese phosphites, and manganese and Wert Plus® nutrients at 1000 ppm. The treatments showing lower control over fungus mycelial growth were the *A. nodosum* and *B. thuringiensis* (non-autoclaved) biological controls and the High Roots® nutrient. They showed higher mycelial growth control at 100 ppm without statistical differences for the fungicide, *B. subtilis* (autoclaved and non-autoclaved), copper phosphite, manganese, and V6®. Only *B. subtilis* (non-autoclaved) efficiently inhibited fungus mycelial growth at 10 ppm and 1 ppm, followed by *B. thuringiensis* and *B. subtilis* (autoclaved), manganese phosphite, and Wert Plus®.

The comparison of products separately for each dose showed that treatments in the first experiment with the fungicide, *A. nodosum*, Maxi Flor®, and *B. subtilis* (non-autoclaved) reduced fungus mycelial growth at higher concentrations, with *B. subtilis* showing 100% of fungus control at 1000 ppm. Treatments with copper phosphite and Wert Plus® showed a significant reduction between 100 and 1000 ppm, with Wert Plus® demonstrating 100% pathogen development control at the latter concentration.

B. subtilis controlled fungus mycelial growth at all concentrations when evaluating the treatments separately in the second experiment (Table 2). The fungicide, copper phosphite, manganese, and V6® showed higher fungus development control as product concentrations increased from 1 to 1000 ppm.

Detached leaf tests

The AUDPC in detached canola leaves did not significantly differ regarding application factors (the left-side product was not applied, and the right-side product was), indicating no systemic resistance induction from the products tested for the pathogen (Table 3). The fungicide and *B. subtilis* controlled 100% of pathogen development on both leaf sides but did not statistically differ from the control (Table 3).

Greenhouse tests

Plants treated with copper phosphite, *B. thuringiensis*, and *B. subtilis* in a greenhouse reduced the area under the disease progress curve (AUDPC) relative to the control, not statistically differing from the fungicide (Table 4). The other treatments did not differ from the control. *B. thuringiensis* and *B. subtilis* did not statistically differ from the fungicide and copper phosphite (Table 4), also showing smaller lesions on canola stems, providing higher disease control.

The other treatments (ASM, *T. asperellum*, and manganese and potassium phosphites) did not statistically differ from the control, even though the lesion was smaller. ASM inducer was expected to reduce disease severity, but the literature reports that the inductor does not always protect against some pathogens, as in the present study.

The treatments in the second experiment with lower AUDPC (Table 5) were the chemical control with the fungicide and the biological control with *B. subtilis*, manganese, potassium, and copper phosphites, and High Roots® and V6® micronutrients. The other treatments did not statistically differ from the control.

The V6® treatment (Table 5) responded similarly to manganese phosphite, which formulation had manganese (2.5%), zinc (1.9%), and molybdenum (0.16%).

The other treatments (Table 5) with *B. thuringiensis, T. asperellum, A. nodosum*, ASM, Wert Plus®, manganese, and Maxi Flor® presented higher lesion values. Manganese was higher than the control, indicating that it stimulated pathogen growth instead of inhibiting it, which was confirmed in the mycelial growth evaluation by *in vitro* tests.

Enzymatic activity

Peroxidase

Peroxidase activity changed between treated and untreated leaves according to the used products. Sample collection intervals of 24, 48, and 72 hours after pathogen inoculation also showed significant statistical differences (Figure 1). Treatment comparisons at each collection time showed lower peroxidase activity in 24-hour collections for leaves treated with *B. subtilis*, the control (without fungus inoculation), and sprayed and non-sprayed leaves with fungicide. The 48-hour collection presented lower peroxidase activity in some treatments (Figure 1).

Higher enzymatic activity occurred in leaves sprayed with the ASM inductor and non-sprayed leaves with copper phosphite and fungicide. The 72-hour collection did not show significant differences between treatments, but peroxidase activity decreased in non-sprayed leaves with copper phosphite, differing from the 48-hour collection (Figure 1). This low activity in sprayed leaves may have been due to the phytotoxicity observed in plants, especially those treated with potassium and copper phosphites. There were no statistical differences for the three collection times in most treatments. Copper phosphite and fungicide (untreated leaf) showed lower enzymatic activity changes in 24 hours. These treatments had increased activities in the 48-hour collection, which reduced again in 72 hours compared to other treatments.

Polyphenol oxidase

The present study did not report polyphenol oxidase enzyme activity in canola plants with any tested product, without significant differences for the three collection periods (24, 48, and 72 hours).

4. Discussion

The *B. subtilis* bacterium inhibited *S. sclerotiorum* mycelial growth because it produces volatile and non-volatile metabolites (Kamal 2015). This bacterium changes the cell wall structure of phytopathogenic fungi. Muis and Quimio (2014) observed the diffusion and volatility of *Bacillus* spp. toxic metabolites in a culture medium in *Rhizoctonia solani* fungus development. The authors confirmed the effectiveness of toxic compound production by the bacterium through an inhibition halo formation during fungus mycelial growth and volatile compounds inhibiting fungus mycelial growth.

Among the compounds produced by *B. subtilis* are lytic enzymes (chitinases, proteases, and cellulases), antibiotics, and volatile compounds that change the structure of several phytopathogenic fungi. Examples of antibiotics are iturin A and surfactin (effective in controlling *Botrytis cinerea* and *R. solani*), bacillomycin D (*Aspergillus flavus*), and mycosubtilin (*Pythium aphanidermatum*). Hydrogen cyanide,

aldehydes, alcohols, chitons, and sulfides represent volatile compounds produced by the bacterium, causing structural deformation in several phytopathogenic fungi (Maheshwari 2013).

Table 2. Mycelial growth speed index (MGSI) of *Sclerotinia sclerotiorum* submitted to alternative product application at different concentrations *in vitro*.

1st Experiment - Dose (ppm)					
Treatment	0	1	10	100	1000
Procymidone fungicide	17.75^{aB}	25.47^{dA}	13.50^{aC}	0.00^{aD}	0.00^{aD}
Ascophyllum nodosum	17.75^{aA}	20.47^{cA}	19.83^{bA}	16.36^{cA}	15.50^{dA}
Bacillus thuringiensis	17.75^{aB}	22.42^{dA}	25.11^{cA}	24.83^{dA}	24.67^{fA}
Bacillus subtillis	17.75^{aB}	23.33^{dA}	21.44^{bA}	6.42^{bC}	0.00^{aD}
Bacillus thuringiensis autoclaved	17.75^{aB}	9.03^{aC}	16.03^{aB}	21.31^{dA}	1.89^{bD}
Bacillus subtillis autoclaved	17.75^{aA}	15.33^{bB}	18.28^{aA}	0.11^{aC}	0.00^{aC}
Copper phosphite	17.75^{aA}	17.75^{cA}	16.11^{aA}	16.44^{cA}	4.11^{bB}
Manganese phosphite	17.75^{aB}	22.50^{dA}	22.31^{cA}	22.69^{dA}	1.97^{bC}
Potassium	17.75^{aA}	21.78^{dA}	21.47^{bA}	21.75^{dA}	8.81^{cB}
Manganese	17.75^{aA}	21.56^{dA}	19.19^{bA}	20.44^{dA}	3.89^{bB}
V6®	17.75^{aA}	19.92^{cA}	15.75^{aA}	16.33^{cA}	8.33^{cB}
Wert Plus®	17.75^{aA}	21.81^{dA}	20.94^{bA}	20.94^{dA}	0.00^{aB}
Hight Roots®	17.75^{aB}	22.61^{dA}	19.14^{bB}	22.33^{dA}	17.83^{dB}
Maxi Flor®	17.75^{aA}	23.92^{dA}	23.67^{cA}	23.28^{dA}	20.78^{eA}
C.V. (%)	15.82				
2nd Experiment – Dose (ppm)					
Treatment	0	1	10	100	1000
Procymidone fungicide	15.39^{aB}	25.47^{dA}	13.50^{bB}	0.00^{aC}	0.00^{aC}
Ascophyllum nodosum	15.39^{aA}	18.81^{cA}	19.61^{cA}	19.89^{eA}	21.53^{dA}
Bacillus thuringiensis	15.39^{aB}	23.39^{dA}	25.67^{dA}	22.81^{eA}	23.81^{dA}
Bacillus subtillis	15.39^{aA}	0.00^{aB}	0.00^{aB}	0.00^{aB}	0.00^{aB}
Bacillus thuringiensis autoclaved	15.39^{aB}	9.03^{bC}	16.03^{bB}	21.31^{eA}	1.89^{aD}
Bacillus subtillis autoclaved	15.39^{aA}	18.97^{cA}	18.71^{cA}	11,22^{bB}	8.92^{aB}
Copper phosphite	15.39^{aA}	17.19^{cA}	14.50^{bA}	14.11^{cA}	4.11^{aB}
Manganese phosphite	15.39^{aA}	14.98^{cA}	17.47^{cA}	18.14^{dA}	3.25^{aB}
Potassium	15.39^{aA}	18.56^{cA}	13.61^{bA}	16.81^{dA}	11.67^{bA}
Manganese	15.39^{aA}	16.19^{cA}	15.36^{bA}	13.19^{cA}	3.31^{aB}
V6®	15.39^{aA}	17.78^{cA}	13.75^{bA}	9.47^{dB}	9.50^{bB}
Wert Plus®	15.39^{aB}	9.47^{bC}	23.97^{dA}	23.61^{eA}	0.33^{aD}
Hight Roots®	15.39^{aB}	22.61^{dA}	19.14^{cA}	22.33^{eA}	17.83^{cA}
Maxi Flor®	15.39^{aA}	18.69^{cA}	18.53^{cA}	21.97^{eA}	10.56^{bA}
C.V. (%)	25.23				

* Averages followed by the same lowercase letter in the columns and uppercase in the lines do not differ by the Scott-Knott test at 5% significance; C.V. = coefficient of variation.

Phosphites yielded satisfactory results at higher product concentrations, highlighting copper and manganese phosphites. A report showed their toxic effect on mycelial growth, sporulation, and spore germination of some fungi, demonstrating the direct or indirect fungistatic activity by resistance induction (Guest and Grant 1991).

The treatment with autoclaved *B. thuringiensis* in the present study also had positive results for *S. sclerotiorum* mycelial growth control. Remuska and Dalla Pria (2007) reported a similar finding in controlling the same fungus. Maheshwari (2013) stated that *B. thuringiensis*, like other bacteria of the same genus, produces the zwittermicin A antibiotic and toxins, such as butyric acid, cyanide, exotoxins, and hydrogen sulfate but without the same effectiveness in controlling phytopathogenic fungi.

Pathogen control with nutrients has also been tested for some years. Chahal and Rawla (1980) evaluated the influence of micronutrients and organic compounds on *Penicillium crustosum* Thorn fungus growth *in vitro*, concluding that compounds such as Fe, Zn, Cu, Mo, and other organic composites are essential for fungus development at given concentrations that, as they increase, progressively inhibit hyphae growth and their deficiency. The authors also found that Mn, Ca, and Co nutrients are not required for normal fungus development.

Table 3. Area under the disease progress curve (AUDPC) of white mold (*Sclerotinia sclerotiorum*) disease in the detached canola leaf (*Brassica napus*) assay without product application (left side) and with product application (right side).

Treatment	Left side	Right side
Control	64.0^{bA}*	72.8^{bA}
Procymidone fungicide	0.0^{bA}	0.0^{bA}
Bacillus thuringiensis	92.7^{aA}	90.0^{bA}
Ascophyllum nodosum	45.8^{bA}	45.2^{bA}
Bacillus subtilis	0.0^{bA}	0.0^{bA}
Trichoderma spp.	131.6^{aA}	144.8^{aA}
Potassium phosphite	20.1^{bA}	19.5^{bA}
Manganese phosphite	78.8^{bA}	65.4^{bA}
Copper phosphite	100.3^{aA}	100.5^{bA}
Acibenzolar-S-methyl	150.4^{aA}	137.0^{aA}
High Roots®	60.8^{bA}	70.6^{bA}
V6®	177.4^{aA}	203.6^{aA}
Maxi Flor®	156.1^{aA}	143.7^{aA}
Wert Plus®	55.8^{bA}	56.0^{bA}
Manganese	65.7^{bA}	61.1^{bA}
C. V. (%)	112.48	

* Averages followed by the same lowercase letter in the columns and uppercase in the lines do not differ by the Scott-Knott test at 5% significance; C.V. = coefficient of variation.

Table 4. Area under the disease progress curve (AUDPC) of *Sclerotinia sclerotiorum* in canola plants (*Brassica napus*) inoculated with the toothpick method in a greenhouse.

Treatament	AUDPG
Control	8.92^{a}*
Procymidone fungicide	2.37^{b}
Bacillus thuringiensis	4.70^{b}
Bacillus subtillis	4.66^{b}
Trichoderma spp.	6.74^{a}
Acibenzolar-S-methyl	7.01^{a}
Manganese phosphite	7.75^{a}
Potassium phosphite	7.96^{a}
Copper phosphite	3.52^{b}
C. V. (%)	56.53%

* Averages followed by the same lowercase letter in the columns do not differ by the Scott-Knott test at 5% significance; C.V. = coefficient of variation.

Table 5. Area under the disease progress curve (AUDPC) of *Sclerotinia sclerotiorum* in canola plants (*Brassica napus*) inoculated with the toothpick method in a greenhouse. Ponta Grossa – PR, Brazil, 2017.

Treataments	AUDPG
Control	114.58^{a}*
Procymidone fungicide	81.04^{b}
Bacillus thuringiensis	107.29^{a}
Bacillus subtilis	44.1^{b}
Trichoderma	108.82^{a}
Ascophyllum nodosum	95.35^{a}
Acibenzolar-S-methyl	119.52^{a}
Manganese phosphite	64.50^{b}
Potassium phosphite	46.57^{b}
Copper phosphite	41.22^{b}
Maxi Flor®	105.01^{a}
Wert Plus®	151.19^{a}
Manganese	122.09^{a}
High Roots®	81.48^{b}
V6®	64.63^{b}
C.V. (%)	55.64%

* Averages followed by the same lowercase letter in the columns do not differ by the Scott-Knott test at 5% significance; C.V. = coefficient of variation.

The chemical control of white mold in canola crops is performed with procymidone, a systemic fungicide from the dicarboximide group with protective and curative action (Tornincasa et al. 2002). The plants in the present study treated with this fungicide in a greenhouse showed smaller lesions. Considering this is a systemic fungicide with protective and curative action, the roots absorb it with translocation to leaves and flowers, inhibiting spore germination, promoting hyphae branching, swelling, and lysis, and inhibiting fungus mycelial growth (Tornincasa et al. 2002).

According to Tomlin (2002) and cited by Rodrigues (2006), procymidone controls fungi of the genus *Botrytis, Sclerotinia*, and *Helminthosporium* spp. in fruits, grapevines, vegetables, ornamentals, cereals, sunflower, canola, soybeans, peanuts, and tobacco. Cardoso et al. (2015) evaluated fungicide efficiency in controlling white mold in soybean crops, achieving satisfactory results in reducing disease severity with thiophanate-methyl fungicides, procymidone, and fluazinam, as verified by Venegas and Saad (2010) in bean crops.

Copper phosphite also had a low AUDPC value as the fungicide (Table 3). These compounds directly inhibit mycelial growth and sporulation of the pathogen and indirectly activate the plant's defense mechanism with phytoalexin production (Jackson et al. 2001; Mcgrath 2004). Töfoli et al. (2012) demonstrated that phosphite works directly on the pathogen, and its fungicidal potential is probably due to phosphite toxicity in essential parts of fungi, such as hyphae, mycelia, and spores. Studies show that copper phosphite prevents and cures diseases caused by fungi and improves the nutritional status of plants, thus reducing the predisposition to pathogen attacks (Nojosa et al. 2005; Melo et al. 2016).

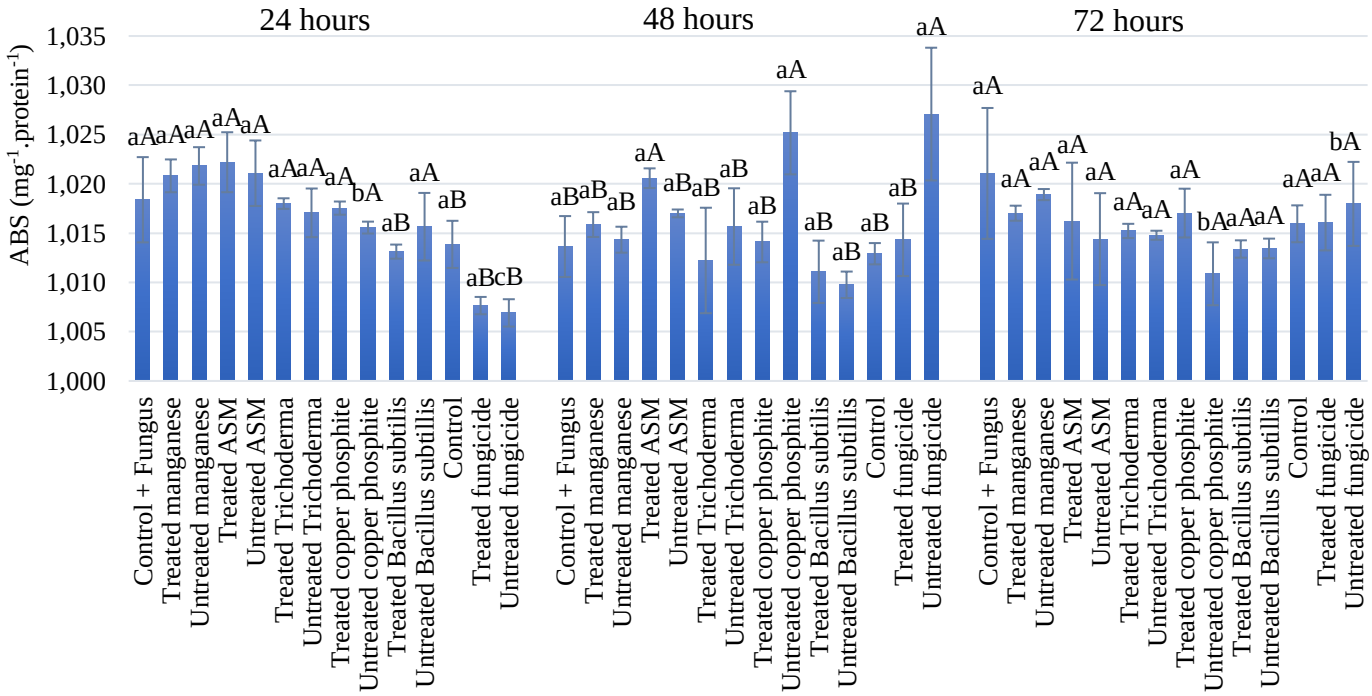

Figure 1. Original analysis data transformed into $\sqrt{(x+1)}$. Specific peroxidase activity in canola plants (*Brassica napus*) treated with different products and inoculated with *Sclerotinia sclerotiorum* 24 hours (A), 48 hours (B), and 72 hours (C) after pathogen inoculation. *Means followed by the same letter do not differ by the Scott-Knott test (P ≤ 0.05). Lowercase letters compared the same treatment with collection times, and uppercase letters compared treatments with the same collection time.

Recent research, such as by Simon et al. (2016), Schurt et al. (2013), and Silva et al. (2014), also report the action of phosphites in the mycelial growth of phytopathogenic fungi, such as *Diplocarpon rosae* and *Rhizoctonia solani*, and the *Erwinia carotovora* bacterium. Gadaga et al. (2017) and Hennipman et al. (2019) reported lower anthracnose and angular spot severity with copper phosphite application on bean crops. However, Christmann et al. (2019) and Assunção et al. (2020) did not find the same result for anthracnose control in the same crop.

Using biological agents for controlling *Sclerotinia*, such as the genera *Pseudomonas, Agrobacterium*, and *Bacillus*, has been reported (Fernando et al. 2004). The species of the genus *Bacillus* are potential biocontrol agents for leaf and soil diseases (Jacobsen et al. 2004). Using this bacterium to control *S. sclerotiorum* in canola crops has also inhibited hyphae growth and sclerotia germination in the pathogen (Fernando et al. 2004; Kamal 2015). Kamal (2015) found vacuolated hyphae of the fungus via electron microscopy, with cytoplasm losses and a fully broken sclerotium layer with bacterial colonization, indicating these bacteria as possible microbial biopesticides. These bacteria produce numerous bioactive lipopeptides, compounds that suppress plant pathogens through antibiosis (Chen et al. 2014).

Chen et al. (2014) observed via scanning electron microscopy the vacuolization and disintegration in the fungus hyphae cytoplasm where the bacteria developed, and there was a delay in the host's pathogen infection. However, the literature does not indicate the *B. thuringiensis* species for controlling *S. sclerotiorum* in canola crops.

The treatment with the *T. asperellum* fungus in the greenhouse experiment did not statistically differ from the control (Tables 4 and 5), but it was a relevant agent of biological pathogen control. Dildey et al. (2016) experimented in a greenhouse to control anthracnose in beans using 21 *Trichoderma* isolates, verifying that all controlled the disease and differed from the control. Mehta, Hi, and Sangwan (2012) obtained satisfactory white mold control with a product based on *Trichoderma harzianum*. The authors reported a linear reduction in *S. sclerotiorum* mycelial growth and apothecium production and a decreased lesion length and disease incidence when applied simultaneously or seven days before pathogen inoculation under greenhouse conditions. The antagonism occurred in response to chemical stimuli by the host (Chet et al. 1998). The mechanisms used by *Trichoderma* are antibiotic production, competition, and mycoparasitism. Howell (2003) showed that chitinases and β-1,3-glucanases produced by some *Trichoderma* species are the main enzymes in cell wall lysis during mycoparasitic action against pathogenic fungi.

Several studies report the use of resistance inducers, such as ASM. Although many authors present positive results, others do not confirm the same success, as in the present study (Tables 4 and 5), showing varied efficiency in different crops. Walters, Ratsep, and Havis (2013) did not achieve *Ascochyta* control with ASM in coffee crops. Barilli, Prats, and Rubiales (2010) found a similar effect, with ASM not controlling the infection with rust (*Uromyces pisi*) in pea plants. However, Zanatta (2019) obtained satisfactory results by using this inducer for controlling white mold in soybean crops.

As in the described treatments, using nutrients to induce plant resistance to diseases has been reported. High Roots®, a nutrient applied in the present study, consists of the potassium nutrient, and it reduces the susceptibility of plants to diseases, as verified by Huber and Graham (1999). This treatment and V6® showed lower disease severity in canola than other foliar fertilizers in the present study.

Resistance induction requires activating enzymes related to this process. Several studies have demonstrated the positive effects of peroxidase on plant resistance induction (Campos et al. 2004; Cavalcanti et al. 2008). Copper phosphite increased peroxidase enzyme production in the present investigation. That corroborates Ávila (2011), who evaluated the effect of phosphite supply on growth, phosphate nutrition, and resistance induction in maize, reporting an increase in lignin levels in the plant's leaf tissues and the same effect for peroxidase activity. However, these findings differ from Ribeiro Júnior et al. (2006), who did not verify the influence of phosphite on peroxidase activity in cocoa plants.

The ASM inducer is used in several crops and increases the peroxidase enzyme, similar to the findings by Kuhn and Pascholati (2010). The authors reported an increase in this enzyme, activated by ASM and *B. cereus* treatments for controlling *Xanthomonas axonopodis* pv. *phaseoli*. Zanata (2019) found the same result, reporting an increase in this enzyme 24 hours after ASM application for white mold control in soybean crops.

5. Conclusions

The *in vitro* evaluation showed lower *S. sclerotiorum* mycelial growth with *B. subtilis*, fungicide, and the Wert Plus® micronutrient.

Fungicide and *B. subtilis* inhibited 100% fungus growth in detached leaf tests.

In a greenhouse, the treatments with the lowest white mold severity were procymidone fungicide, copper phosphite, *B. thuringiensis*, *B. subtilis*, manganese and potassium phosphites, High Roots®, and V6®.

The biochemical analysis did not show polyphenol oxidase enzyme activity. Higher peroxidase activity occurred only in the collection performed 48 hours after pathogen inoculation for the acibenzolar-S-methyl, copper phosphite, and fungicide treatments.

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