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Extracts of cinnamon (*Cinnamomum cassia*) and mint (*Mentha arvensis*) for the fungi control of *Sclerotinia sclerotiorum*

Laura Martins Ferreira[1], Murilo Gustavo Andrade Xavier[1], Paulo Ricardo Resende Dias[1], Gustavo Haralampidou da Costa Vieira[1]

[1] Universidade Estadual de Mato Grosso do Sul, Unidade Universitária de Cassilândia, Cassilândia, Mato Grosso do Sul, Brasil. E-mail: laura.mf99@hotmail.com, muri.gustavo@hotmail.com, pauloricardord@gmail.com, gcv@uems.br.

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ABSTRACT

Studies regarding vegetal extracts and essential oils from native plants have demonstrated efficiency in controlling phytopathogens of fungitoxic action, hampering the development of phytopathogenic fungi. This study aims to determine the fungicide effect of vegetal extracts during the mycelial growth of the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. The experiment was conducted in the phytosanitary laboratory of the UEMS in the unit of Cassilândia-MS, Brazil. A completely randomized design was used with a factorial scheme 4 x 5, comprising leaves, bark, and branches of cinnamon and mint leaves in the concentrations of 0, 2.5, 5.0, 10, and 20%, with six repetitions per treatment. The evaluations were made 24, 48, and 96 hours after the transplant, obtaining the diametrically opposite means with a caliper to determine the measures. The results indicate a higher inhibitory efficiency against fungi development using extracts of mint leaves and cinnamon leaves and branches.

Keywords: *Sclerotinia sclerotiorum*, Phytopathogen, Fungitoxic.

EXTRATOS DE CANELA (*Cinnamomum cassia*) E HORTELÃ (*Mentha arvensis*) NO CONTROLE FÚNGICO DE *Sclerotinia sclerotiorum*

RESUMO

Trabalhos realizados com extratos vegetais e óleos essenciais extraídos de plantas da flora nativa têm demonstrado eficiência no controle de fitopatógenos pela ação fungitóxica, impedindo o desenvolvimento de fungos fitopatogênicos. O presente trabalho objetivou determinar o efeito fungicida de extratos vegetais no crescimento micelial do fungo *Sclerotinia sclerotiorum* (Lib.) de Bary. O experimento foi conduzido no laboratório de fitossanidade da UEMS, unidade universitária de Cassilândia-MS. O delineamento experimental usado foi inteiramente casualizado em esquema fatorial 4 x 5 sendo (folhas, casca e ramos de canela e folha de hortelã) nas concentrações de 0; 2,5; 5,0; 10 e 20%, com seis repetições por tratamento. As avaliações foram 24, 48 e 96 horas após a repicagem, através da obtenção de médias diametralmente opostas, sendo usado um paquímetro para determinação das medidas. Os resultados indicaram maior eficiência inibitória contra o desenvolvimento do fungo com o uso de extratos de folhas de hortelã, folhas e ramos de canela.

Palavras-chave: *Sclerotinia sclerotiorum*, Fitopatopatógeno, Fungitóxico.

1. Introduction

White mold is a disease caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, which causes damages in many important crops with varied symptoms (Bardin & Huang, 2001; Bolton et al., 2006). Species attacked by the disease commonly known as white mold or white-rot present characteristic symptoms, such as soaked lesions of pale color, soft consistency, and cotton-like white mycelium covering the tissue affected. The symptoms are observed with higher frequency in the upper third of the plants, affecting organs such as leaves, petiole, main stem, and pods (Leite, 2005).

White mold presents a range of hosts. The disease affects soybean, lentil, common bean, canola, tomato, potato, sunflower, tobacco, and others. Due to the significant losses caused by this pathogen, adopting some measures to impede the succession of crops with susceptible species is essential. The avoidance of contaminated seeds is needed, and the use of plant species resistant to the pathogen, such as corn, white oat, or wheat, to degrade sclerotia using natural enemies (Leite, 2005).

The fungus has as a survival mechanism the production of sclerotia that resist different unfavorable environmental conditions, making the disease control more complex (Kamal et al., 2016). Chemical control is the most used by farmers; however, it has little efficiency due to the difficulty of controlling sclerotia present in the soil (Mueller et al., 2002; Alvarez et al., 2012).

The excessive use of fungicides worldwide in agriculture causes a series of environmental disorders and resistance of microorganisms to the active ingredient of these products when not applied appropriately. Thus, the study of alternative products, mainly plant extracts, is receiving more attention to avoid synthetic products (Silva et al., 2020).

Cinnamomum cassia pertains to the Lauraceae family and is popularly known as Chinese cinnamon. It has an abundant distribution in Southeast Asia; it is a perennial and indigenous tree (Choi et al., 2001; Wang et al., 2008). Cinnamon antifungal activity (*C. cassia*) is due to a crucial component called (E)-cinnamaldehyde that reduces the severity of *S. sclerotiorum* (Ojaghian et al., 2015). (E)-cinnamaldehyde is abundant in the bark and the leaves of the genus *Cinnamomum* with a viscous aspect and characteristic smell (Yen & Chang, 2008).

Mint (*Mentha arvensis*) pertains to the Lamiaceae family and is widely used for medicinal and culinary purposes. Several studies associate it with antioxidant, antibacterial, and antifungal activity due to bioactive properties in its extract (Salin et al., 2011). The menthol present in mint species presents antibacterial and antifungal activity against Gram-positive and Gram-negative bacteria and fungi responsible for plant diseases (Iscan et al., 2002; Schelz et al., 2006; Trombetta et al., 2005).

Vegetal extracts used as alternatives to control pathogens of plant diseases have a range of chemical compounds with bioactive activity, present in different parts of the plants, such as stem, leaves, branches, bark, roots, tuber, flowers, among others (Das et al., 2010; Hao et al., 2015). The extracts are prepared by maceration or percolation of the material, which can be used green or dried in a forced-air ventilation greenhouse. Their bioactive properties are extracted from the vegetal tissue using solvents, such as water, ethanol, methanol, ethyl acetate, among others (Cos et al., 2006).

This study comprises data from studies about the chemical properties of different vegetal species and the increasing interest of society in alternative products for plant diseases control. Therefore, this study aims to determine the fungicide effect of vegetal extracts of cinnamon (*C. cassia*) and mint (*M. arvensis*) on the in vitro mycelial growth of the fungus *S. sclerotiorum*.

2. Material and Methods

The experiment was conducted in the phytosanitary laboratory of the Mato Grosso do Sul State University - UEMS (Universidade Estadual de Mato Grosso do Sul), in Cassilândia/MS, Brazil, from August 2021 to July 2022. Samples of cinnamon leaves, branches, and bark, and mint leaves were collected. After collection, the vegetal material asepsis was carried out with running water, and they were stored in a forced-air ventilation greenhouse at 40°C for 96 hours. Then, the samples were individually ground in a knife mill until they became powder.

The resultant powder was submitted to maceration with absolute ethanol at room temperature, kept at rest, and immersed in the solvent for three days. The solution obtained was duly filtered with a voile fabric to remove the solid material, and the solvent evaporated in controlled conditions. The different extract concentrations were obtained following Rodrigues et al. (2006).

The final extracts were stored in glass flasks at the temperature of 25 ±2 °C, protected from sunlight until their use in the experiment installation. The measurement of concentration and volume was conducted, weighted 2.5, 5.0, 10.0, and 20.0 g of the product obtained after grinding for each 110 mL of alcohol 98% to evaluate the in vitro antifungal activity of the vegetal extracts on the mycelial growth of *S. sclerotiorum*.

The medium used for fungi growth was PDA (potato dextrose agar) with the vegetal extracts from mint

leaves and cinnamon leaves, branches, and bark. 10 mL of the extract were incorporated into 90 mL of PDA medium still in flux, and 15 mL were poured in Petri dishes in a sterile environment. The mycelium disks of 5 mm in diameter were transferred for the Petri dishes containing the PDA medium with the extracts in different concentrations.

For each treatment, six repetitions were made, which were posteriorly sealed with plastic film and incubated in BOD at 25° C with a photoperiod of 12 hours. The evaluations of the antifungal potential for treatments in the concentrations of 2.5, 5.0, 10, and 20% were made using a caliper, where the mycelial growth was measured in millimeters. The evaluations were conducted 24, 48, and 96 hours after the experiment implementation.

3. Results and Discussion

For the first evaluation, conducted 24 hours after transplanting, no differences were observed between the treatments studied (Table 1). Nevertheless, 48 hours after transplanting, the extracts of cinnamon leaves and branches and mint leaves in the concentrations of 2.5, 5.0, and 20.0% presented the best inhibitory effects on mycelial growth (Table 1).

In the last evaluation period, corresponding to 96 hours after incubation, at the dosage of 2.5%, only extracts of cinnamon branches and mint leaves completely inhibited the fungus mycelial growth. In the following concentration, which corresponds to 5%, the total control of fungal growth was observed, except for the extract of cinnamon bark, whose colony growth was 13.33 mm. In the concentration of 10%, there were no significant differences between the results. In the higher concentration tested, of 20%, only the cinnamon bark was not efficient in the control of *S. sclerotiorum* (Table 1).

In the literature, there are registers of the efficiency of vegetal extracts in the inhibitory control of phytopathogenic fungi, among them cinnamon and mint extracts. Ojaghian et al. (2014), while using extracts of *C. cassia* Presl. (cinnamon) observed satisfactory results for the control of the fungus *S. sclerotiorum*. A study conducted by Venturoso et al. (2011) showed that the cinnamon extract has antifungal activity but is unexpressive. Cinnamon bark and mint extracts presented intermediate antifungal activity compared with other vegetal extracts. In carrot crops, Ojaghian et al. (2014, 2019) observed that vegetal extracts and essential oils of medicinal plants, such as cinnamon and rosemary, were responsible for reducing the severity of rotting in carrots caused by *S. sclerotiorum*.

The cinnamon bark extract did not present good antifungal activity after 48 hours of incubation in any dosage tested. On the other hand, the cinnamon leaves at the concentrations of 5% and 20% were responsible for the total inhibition of the mycelial growth of the fungus *S. sclerotiorum*. Thus, the concentration of 5% ethanolic extract can be used. However, mycelial growth inhibition was not perceived at the concentration of 10% of the extract, which may be related to alterations during preparation since there are no results in the literature that explain such results. The extracts of mint leaves and cinnamon branches presented inhibitory activity in all dosages tested, inhibiting the mycelial growth of pathogens by 100% (Figure 1).

Table 1- Mycelial growth of *S. sclerotiorum* in PDA growth medium with ethanolic extracts of cinnamon leaves (CL), cinnamon branches (CB), cinnamon bark (CK), mint leaves (ML) in the periods of 24, 48, and 96 hours of evaluation.

				Concentration		
Treatments		**0.0**	**2.5**	**5.0**	**10.0**	**20.0**
		Mm				
	CL	0a	0a	0a	0a	0a
24 hours	CK	0a	0a	0a	0a	0a
	CB	0a	0a	0a	0a	0a
	ML	0a	0a	0a	0a	0a
	CV%			0		
				Mm		
	CL	37.833[a]	4.833b	0.0b	4.92a	0.0b
48 hours	CK	37.833[a]	14.733a	12.00a	4.50a	10.833a
	CB	37.833[a]	0b	0.0b	0.0a	1.50b
	ML	37.833[a]	0b	0.0b	0.0a	0.0b
	CV%			41.8		
				Mm		
	CL	100.0a	11.833a	0.0b	5.833a	0.0b
96 hours	CK	100.0a	15.333a	13.333[a]	4.92a	11.77a
	CB	100.0a	0.0b	0.0b	0.0a	4.0b
	ML	100.0a	0.0b	0.0b	0.0a	0.0b
	CV%			21.64		

Equal letters in the column did not differ according to the Tukey test at 5% probability.

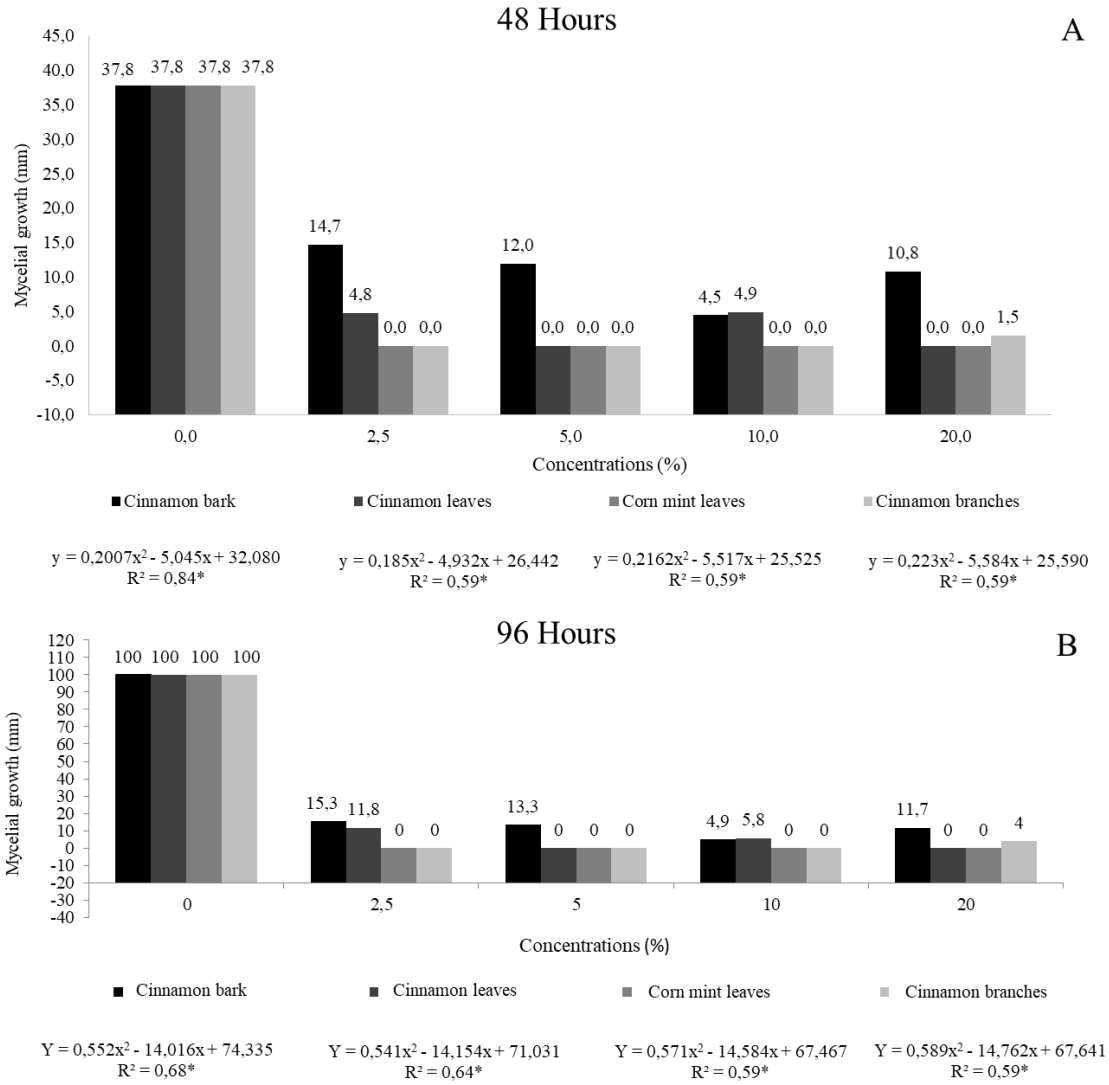

Figure 1. Mycelial growth of S. sclerotiorum after 48 and 96 hours of incubation in Petri dishes with ethanolic extracts of cinnamon leaves, cinnamon branches, cinnamon bark, and mint leaves at different concentrations in direct contact with the fungus S. sclerotiorum.

The ethanolic extract of cinnamon bark did not present antifungal action after 96 hours of incubation, permitting the mycelial growth of *S. sclerotiorum*. On the other hand, the cinnamon leaves at 5 and 20% concentrations inhibited the fungus growth by 100%. Hence, a concentration of 5% is recommended since there were no differences between both concentrations. The extracts of mint leaves and cinnamon branches inhibited mycelial growth at all concentrations tested. Thus, the lowest concentration (2.5%) can be used for the fungus control.

The family Lamiaceae stand out by presenting a compound called menthol ($C_{10}H_{20}O$), which by its turn is a terpenoid, a secondary compound found in some vegetal species used for antifungal control (Al-Bayati, 2009). Concerning the cinnamon extract, Venturoso et al. (2011) verified an inhibition higher than 50% of the mycelial growth of some fungi that cause significant damages in soybean seeds, which demonstrates the cinnamon extract efficiency. The antifungal property of cinnamon branches, leaves, and bark in the experiment is due to the (E)-cinnamaldehyde present in the genus *Cinnamomum*. However, the cinnamon bark extract presented low antifungal action for controlling *S. sclerotiorum*, possibly due to the low compound concentrations in the bark.

4. Conclusions

Based on the results, extracts of mint leaves and cinnamon leaves and branches presented antifungal action on *S. sclerotiorum*. Thus, they can be used as alternatives for the control of pathogens.

Cinnamon leaves extract inhibited the activity of pathogens by 100% at the concentration of 5%, which is recommended.

Extracts of cinnamon branches and mint leave inhibited the activity of pathogens, and they can be used

at the minimum concentration of 2.5%. The cinnamon bark extract presented low antifungal activity on *S. sclerotiorum*. Therefore, it is not considered a viable alternative for the control of pathogens.

Authors' Contribution

Laura Martins Ferreira contributed to the conduct, data collection of the experiment and writing of the manuscript. Murilo Gustavo Xavier contributed to creating the figures and writing the manuscript. Paulo Ricardo Resende Dias contributed to the statistical analysis and writing of the manuscript. Gustavo Haralampidou da Costa Vieira contributed to the statistical analysis and writing of the manuscript.

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