



ANTIFUNGAL AND ANTIOXIDANT ACTIVITY OF *Cupressus sempervirens* AND *Cupressus lusitanica* BOTANICAL FERMENTATES ON *Colletotrichum fructicola*: IN VITRO AND IN VIVO EVALUATION

Márcia Regina Pansera¹

Wendel Paulo Silvestre²

Luciana Bavaresco Andrade Touguinha³

Valdirene Camatti Sartori⁴

ABSTRACT

Purpose: The objective of this work was to verify the *in vitro* and *in vivo* antifungal activity of *Cupressus sempervirens* L. and *Cupressus lusitanica* Mill botanical fermentates on mycelial growth and germination of *Colletotrichum fructicola* conidia, as well as the antioxidant activity present in the compounds of the fermented botanicals using the DPPH[•] and ABTS^{•+} radical inhibition evaluation.

Theoretical framework: Plants are a promising source of several bioactive and multifunctional substances. To minimize the impacts of agrochemicals, which sometimes cause harmful environmental effects, alternative social technologies are being investigated more and more, such as the so-called botanical fermented products to control phytopathogenic fungal diseases of agricultural interest.

Methods: The antioxidant activity and contents of phenolic compounds, flavonoids, and individual phenolics were determined by spectrophotometry and high-performance liquid chromatography. The mycelial growth and conidia germination of *C. fructicola* were evaluated in a PDA medium containing the fermentates at zero, 10 % v/v, 20 % v/v, and 40 % v/v. *In vivo* tests were also conducted using *Acacia sellowiana* leaves inoculated with the phytopathogen.

Results and conclusion: The results showed that *C. sempervirens* fermentate controlled by 100 % the development of the fungal colony in the concentration of 40 vol.% by the dilution method in PDA medium. In the conidia germination, they showed strong antifungal activities against this important phytopathogenic fungus. The active compound identified in the highest concentration from the fermented *C. sempervirens* was gallic acid and naringin for the fermentate of *C. lusitanica*. Antioxidant activity varied in the range of 68 – 82 % sweeping depending on the evaluated radical.

Research implications: The results of this work show a promising potential for *C. sempervirens* fermentate to control *C. fructicola* in the culture of *A. sellowiana*.

Originality value: Assessing the potential use of botanical fermentates to help control fungal diseases in plants is a novel approach and is very important and interesting for organic farming practices, reducing the need for chemical synthetic molecules to control fungal phytopathogens.

Keywords: Organic Agriculture, Bioinputs, Botanical Fermentates, Phenolic Compounds.

¹ Universidade de Caxias do Sul, Caxias do Sul, Rio Grande do Sul, Brazil. E-mail: mrpancer@ucs.br
Orcid: <https://orcid.org/0000-0002-6868-829X>

² Universidade de Caxias do Sul, Caxias do Sul, Rio Grande do Sul, Brazil. E-mail: wpsilvestre@ucs.br
Orcid: <https://orcid.org/0000-0002-9376-6405>

³ Universidade de Caxias do Sul, Caxias do Sul, Rio Grande do Sul, Brazil. E-mail: lbatougu@ucs.br
Orcid: <https://orcid.org/0000-0003-0732-8714>

⁴ Universidade de Caxias do Sul, Caxias do Sul, Rio Grande do Sul, Brazil. E-mail: vcsartor@ucs.br
Orcid: <https://orcid.org/0000-0002-8107-5893>



ATIVIDADE ANTIFÚNGICA E ANTIOXIDANTE DOS FERMENTADOS BOTÂNICOS DE *Cupressus sempervirens* E *Cupressus lusitanica* SOBRE *Colletotrichum fructicola*: AVALIAÇÃO IN VITRO E IN VIVO

RESUMO

Objetivo: Este trabalho visou verificar o efeito *in vitro* e *in vivo* da atividade antifúngica dos fermentados botânicos de *Cupressus sempervirens* L. e *Cupressus lusitanica* Mill. sobre o crescimento micelial e a germinação de conídios de *Colletotrichum fructicola*, bem como avaliar a atividade antioxidante existente nos fermentados botânicos por meio da varredura dos radicais DPPH[•] e ABTS^{•+}.

Referencial teórico: As plantas são uma fonte promissora de diversas substâncias bioativas e multifuncionais. Para minimizar os impactos do uso de agroquímicos, que por vezes causam impactos negativos ao meio ambiente, tecnologias sociais alternativas estão sendo cada vez mais investigadas, como os chamados produtos fermentados botânicos para controle de doenças fúngicas fitopatogênicas de interesse agrícola.

Métodos: A atividade antioxidante e os teores de compostos fenólicos, flavonóides e fenólicos individuais foram determinados por espectrofotometria e cromatografia líquida de alta eficiência. O crescimento micelial e a germinação de conídios de *C. fructicola* foram avaliados em meio BDA contendo os fermentados a zero, 10% v/v, 20% v/v e 40% v/v. Testes *in vivo* também foram realizados utilizando folhas de *Acca sellowiana* e inoculadas com o fitopatógeno.

Resultados e conclusão: Os resultados mostraram que o fermentado de *C. sempervirens* controlou em 100 % o desenvolvimento da colônia fúngica na concentração de 40 % v/v pelo método de diluição em meio BDA. Os fermentados apresentaram atividade antifúngica contra a germinação de conídios desse importante fungo fitopatogênico. O composto ativo identificado em maior concentração no fermentado de *C. sempervirens* foi o ácido gálico, enquanto a naringina foi o principal composto fenólico observado no fermentado de *C. lusitanica*. A atividade antioxidante dos fermentados variou na faixa de 68 – 82 %, dependendo do radical avaliado.

Implicações da pesquisa: Os resultados deste estudo mostram um potencial promissor do fermentado de *C. sempervirens* para o controle de *C. fructicola* na cultura de *A. sellowiana*.

Originalidade/valor: Avaliar o uso potencial de fermentados botânicos como ferramenta para ajudar no controle de doenças fúngicas em plantas é uma abordagem nova e muito importante e interessante para práticas de agricultura orgânica, reduzindo a necessidade de moléculas químicas sintéticas para controlar fitopatógenos fúngicos.

Palavras-chave: Agricultura Orgânica, Bioinsumos, Compostos Fenólicos, Fermentados Botânicos.

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1 INTRODUCTION

For the control of pathogens, the use of pesticides with lower residual power is essential; however, this is not always possible with the application of synthetic chemical products, with a concern with the quality of the food produced, aiming at a production system in compliance with the requirements of environmental sustainability, food safety, and economic viability, seeking less aggressive techniques to the environment and human health (Silva et al., 2017).

Sousa (2018) describes that natural pesticides, such as the use of plants, were gradually replaced by synthetic ones since they exposed problems such as variations in effectiveness due to differences in the concentration of the active ingredient between plants and low residual effect, which forced the need to make several applications in short periods. On the other hand, the interest in natural products to control pests and diseases is growing due to the growing



demand for healthy foods from organic agriculture (Menezes, 2012).

Products with less or no chemical residues are included in the natural pesticides or biocontrol agents category, such as the various liquid biofertilizers, lime sulfur, pheromones, and plant extracts (Silva et al., 2017). Alternative control has shown relevant results regarding disease management, aiming to minimize the impacts caused by the continuous use of chemical control products, avoiding the selection of resistant pathogens, and, in some cases, reducing production costs (Barboza et al., 2013).

The objective of this work was to evaluate the antioxidant activity and the use of fermented botanicals from the leaves of *Cupressus sempervirens* and *Cupressus lusitanica* in the control of *Colletotrichum fruticicola* *in vitro* and *in vivo*, using as a model plant the *Acca sellowiana* for the evaluation of *in vivo* antifungal activity.

2 THEORETICAL FRAMEWORK

The use of plants or extracts dates to ancient human history and has increasingly awakened research interest to interpret the potential of bioactive compounds more accurately from plants in various areas of interest, such as the agriculture, pharmaceutical, and cosmetics industries (Ishaq et al., 2019). The so-called botanical fermented products are mainly used by family farmers linked to agroecology and the production of organic products and in controlling insects and phytopathogenic fungi in cultivated plants (Costa et al., 2021; Pereira et al., 2023).

Fermented plants act as suppressive compounds with action in the control of phytopathogens and are a strategy to suppress phytopathogenic fungi (Bonanomi et al., 2017). The underlying mechanisms of the suppressive effect are not fully understood, although these mechanisms are mainly associated with the biological activity of microorganisms. A general suppression effect is attributed to consortia affecting more than one pathogen simultaneously, where diversified mechanisms offer protection against several pathogens (Cha et al., 2016).

Fermented products, such as the so-called biofertilizers, in addition to providing essential nutrients released gradually, also release phytohormones that have beneficial effects on crops and the soil, as they make the environment conducive to better plant development, making it possible to increase high productivity rates and quality of products. In addition, there is a resistance-inducing action with fungicidal, bacteriostatic, repellent, insecticide, and acaricide properties on various organisms (Fontenelle, 2017).

Cupressaceae is the most prominent family of coniferous plants, where species are known to have diverse biological activities, especially antimicrobial activity. Cypress is rich in secondary metabolites that lead to significant rates of antioxidant activity due to its content of phenolic compounds, flavonoids, and other compounds of bioactive capacity, such as tannins, saponins, terpenes, and essential oils. Cupresses have antibacterial, antifungal, antiviral, antioxidant, healing, anti-inflammatory, and insecticide activity, among others (Ibrahim et al., 2009; Sati; Joshi, 2010).

Among the species of phytopathogenic fungi of agricultural importance is the fungus *Colletotrichum* spp., which has been the target of many control strategies, such as the use of resistant cultivars and/or synthetic antimicrobials (Gomes et al., 2021). The *Colletotrichum* complex species is one of the ten most important pathogens parasitizing numerous plant species (Dean et al., 2012). Being the cause of anthracnose, it can affect woody to herbaceous plants, producing significant economic losses in tropical, subtropical, and temperate regions. Anthracnose can affect fruits, leaves, and stems and cause postharvest diseases (Damm et al., 2013; 2014).

The use of synthetic chemical products to control anthracnose, while not guaranteeing 100 % control, presents numerous environmental risks, such as contamination of surface and groundwater, toxicity problems due to the residues that these products can leave in the fruits



(Jardim et al., 2009). Misusing chemical fungicides can increase resistance in pathogen populations and have effects on non-target organisms. Furthermore, the continuing loss of biodiversity from traditional crops has favored renewed interest in developing green biotechnology products to study plant-pathogen interactions, improving crop production and food quality (Gomes et al., 2021).

3 MATERIALS AND METHODS

The fungus of the *Colletotrichum* genus was isolated from *A. sellowiana* fruits with disease symptoms and stored in a PDA culture medium. The isolate samples were sent for identification. The fungus was grown in potato dextrose broth for 48 h at 28 °C, 1.0 mL, centrifuged (10,000 g for 3 min), and the pellet was used for DNA extraction following the methodology of Tapia-Tussel et al. (2006).

The molecular identification of the fungus was carried out following the procedures described by Pansera et al. (2022). The generated sequences were compared with sequences deposited in GenBank.

3.1 Collection of Plant Material and Preparation of the Botanical Fermentates

The plant material was collected at the University of Caxias do Sul (geographical coordinates: 29.1630 S, 51.1454 W, and an altitude of 765 m above sea level). After collection, specimens of the material were sent for identification at the Herbarium of the University of Caxias do Sul and identified as *Cupressus Lusitanica* Mill. (HUCS 51244) and *Cupressus sempervirens* L. (HUCS 51957).

Cupressus fermented sp. were produced by adding 1.5 L of untreated water and 500 g of fresh plant (fine branches and leaves), liquefied until they were crushed. Fermentation occurred spontaneously and aerobically, kept in a dark environment until fermentation ceased and was stirred once a day. After production, the fermentates were kept from sunlight and stored at room temperature.

3.2 Determination of Physical-Chemical Properties, Bioactive Compounds, and Antioxidant Activity

The phenolic compounds of the fermented products were analyzed by High-Performance Liquid Chromatography (HPLC), following the procedures described by Morelli (2010). The analysis was performed in triplicate, with the results expressed in milligrams of compound per liter of sample ($\text{mg}\cdot\text{L}^{-1}$).

The flavonoid content of the extracts was determined by the aluminum chloride method, according to the methodology described by Matic et al. (2017). The results were expressed in milligrams equivalent of quercetin per 100 mL of sample. The pH and electrical conductivity of the extracts were determined by direct reading using a Digimed DM-22 pHmeter with an Ag/AgCl electrode and a Digimed DM-32 conductivity meter using a platinum electrode with a cell constant equal to 1.0 cm^{-1} , respectively.

The antioxidant activity of the fermentates was evaluated by the DPPH[•] and ABTS^{•+} inhibition methods using the methods proposed by Yamaguchi et al. (1998) and Rufino et al. (2007), respectively. The results for the two assays of antioxidant activity were expressed as a percentage of inhibition of radical DPPH[•] and ABTS^{•+} radicals. All tests were carried out in triplicate.



3.3 Evaluation of the *In Vitro* Antifungal Effect of the Fermentates on the Mycelial Growth and Conidia Germination of *C. fruticicola*

To verify the antifungal activity of the fermented botanicals, PDA (Potato-Dextrose-Agar) culture media were prepared and autoclaved at 121°C for 15 min. After autoclaving with the medium still melting, the fermented botanicals were added at concentrations of zero, 10 vol.%, 20 vol.%, and 40 vol.%. After medium solidification, a 5 mm mycelial disc of a 7-day-grown fungus *Colletotrichum fruticicola* was deposited in the center of the plate. The plates were sealed and incubated in BOD-type chambers at 25 °C and a 12 h photoperiod. To evaluate the mycelial growth, diameter measurements were performed using a digital caliper on the 3rd, 7th, and 14th day after disc inoculation, and with the data obtained, the percentage of growth inhibition (PIC) of the treatments relative to the control.

The effect of the botanical fermentates on conidia germination was assessed according to the method proposed by Pansera et al. (2022), with the difference that the aliquots of the conidia suspension (50 µL) were placed in microtubes containing 500 µL of PDA medium (Potato Dextrose Agar) treated with the fermented product at concentrations of zero, 10 vol.%, 20 vol.%, and 40 vol.%, respectively.

3.4 Evaluation of the *In Vivo* Antifungal Effect of the Fermentates on the Development of *C. Fruticicola* in *A. Sellowiana* Leaves

Fermented cypresses were selected based on their efficiency in controlling mycelial growth and conidial germination and were evaluated on *A. sellowiana* leaves *in vivo*. Leaves without application of fungicides and free of lesions were incubated in gerbox boxes previously sanitized with 70 vol.% ethanol solution, and a layer of cotton moistened with autoclaved distilled water was placed to ensure the necessary humidity in both tests. The leaves were washed in a 20 vol.% sodium hypochlorite P.A. solution. The preventive test was carried out by adding 50 µL of the fermented product (20 vol.% and 40 vol.%) in three leaf points, and 24 h later, the fungus disk was inoculated. Twenty leaves were used for each treatment. The evaluation was performed after 21 days of inoculation.

3.5 Experimental Design and Statistical Analysis

The experiment followed a completely randomized design. Data were analyzed for homoscedasticity and homogeneity of residuals by Levene's test and Shapiro-Wilk test, respectively. The results underwent one-way analysis of variance (one-way ANOVA), and the means were compared by Tukey's test at a 5 % probability of error using the AgroEstat® software (Brazil).

4 RESULTS AND DISCUSSION

Initially, the chemical parameters of pH, electrical conductivity, and content of phenolic compounds and flavonoids of the produced fermentates were characterized. The results are compiled in Table 1.



Table 1. Chemical parameters of *Cupressus sempervirens* and *Cupressus lusitanica* botanical fermentates.

Parameter	<i>C. sempervirens</i>	<i>C. lusitanica</i>
pH	4.87±0.02	4.82±0.04
Electrical conductivity (dS·cm ⁻¹)	2.43±0.44	1.76±0.31
Total phenolic compounds (mg·100 mL ⁻¹)	1094.91±12.50	1382.97±17.11
Total flavonoids (mg·100 mL ⁻¹)	83.50±2.25	124.61±3.67

Source: Authors (2023).

Phenolic compounds are abundant in the plant kingdom, and the ability of this class of substances to inhibit the germination of spores of pathogenic fungi is recognized (Cushnie; Lamb, 2005). The antimicrobial properties of the compounds are associated, in plants, with protective action against pathogenic microorganisms (Sanchez-Rangel et al., 2013). These antimicrobial secondary metabolites are found among various substances and compounds extracted from plants. The variability of its efficiency would not only be connected to the different types of secondary metabolites (alkaloids, terpenoids, polyphenols, quinones, and coumarins, among others) that plants produce but also to the effect of these biomolecules on microorganisms (Karou et al., 2007).

According to Romani et al. (2002), the main chemotaxonomic chemical components identified in the genus *Cupressus* are biflavonoids and flavonoid dimers, with a variety of chemical structures and different levels of biological activity (Kim et al., 2008), such as anti-inflammatory, antimicrobial, antioxidant, among others. According to Omafuvbe and Akanbi (2009), the antimicrobial and antifungal potential of the extract of fermented plants is due to bioactive compounds such as tannins, saponins, phenolic compounds, and flavonoids.

Traditional fungicides have been widely applied to manage these fungal plant pathogens. Still, the extensive use of chemical fungicides causes direct and indirect deleterious effects on soil biodiversity, human health, and the environment (Samet et al., 2018). Consequently, researchers and growers seek biodegradable, non-toxic, low-cost, and broad antifungal agents to contain fungal plant diseases without deleterious effects (Nicolopoulou-Stamati et al., 2016). In the literature, records of the efficiency of plant extracts obtained from different botanical species of other genera have been verified.

According to Parvez et al. (2006), the plant fermentation technique decomposes or converts substrates into compatible components under the action of microbial enzymes, improving substrate properties through production and increasing the extraction of bioactive compounds. Zhang et al. (2012) comment that many changes that occur during fermentation lead to changes in the properties of the mixture, such as the development of bioactivity. Therefore, new compounds can be generated from the fermentation of existing precursors in plant tissue.

According to some research, it was verified that in the beverage fermentation process, specific organic acids such as lactic acid and acetic acid are important in the antibacterial and antifungal activity of this fermentation process (Velicanski et al., 2014). In recent years, many researchers have evaluated the antioxidant activity of phenolic compounds of *Cupressus* species due to biological efficacy (Senol et al., 2015).

4.1 Individual Contents of Phenolic Compounds

Table 2 compiles the levels of individual phenolic compounds in the produced and evaluated botanical extracts.



Table 2. Main phenolic compounds identified from *Cupressus sempervirens* and *Cupressus lusitanica* botanical fermentates.

Fermentate	Gallic acid ($\mu\text{g}\cdot\text{mL}^{-1}$)	Naringin ($\mu\text{g}\cdot\text{mL}^{-1}$)	Rutin ($\mu\text{g}\cdot\text{mL}^{-1}$)	Epigallocatechin gallate ($\mu\text{g}\cdot\text{mL}^{-1}$)	Quercetin ($\mu\text{g}\cdot\text{mL}^{-1}$)
<i>C. sempervirens</i>	228.8 $\pm$ 13.5	7.2 $\pm$ 2.1	1.1 $\pm$ 0.1	nd	nd
<i>C. lusitanica</i>	40.4 $\pm$ 6.9	144.3 $\pm$ 10.6	nd	73.6 $\pm$ 5.4	37.2 $\pm$ 3.3

nd: not detected.

Source: Authors (2023).

The result of the HPLC evaluation made it possible to identify that the main phenolic compound present in the fermented *C. sempervirens* was gallic acid. Gallic acid consists of a tri-hydroxylated phenolic structure and is an intermediate of plant secondary metabolism, being a component of hydrolyzable tannins (Grundhofer et al., 2001). Studies show that gallic acid and its derivatives have biological activity, such as antimicrobial and antiviral potential, in addition to acting as scavengers of reactive oxygen species (Li et al., 2017) and inhibition of sporulation/germination of saprophytic fungi (Lattanzio et al., 2006).

The results and potential referring to the botanical fermented leaves of *C. sempervirens* are largely unexplored since the primary studies with representatives of the genus *Cupressus* are based on essential oils and extracts (Selim et al., 2014).

4.2 Antioxidant Activity of the Botanical Fermentates

The antioxidant activity of the produced fermentates, assessed by the sweeping of DPPH[•] and ABTS^{•+} radicals, are shown in Table 3.

Table 3. Results of antioxidant activity of the botanical fermentates of *Cupressus sempervirens* and *Cupressus lusitanica* based on the inhibition of DPPH[•] and ABTS^{•+} radicals.

Fermentate	DPPH [•]		ABTS ^{•+}	
	Sweeping percentage	mM equiv. Trolox [®]	Sweeping percentage	mM equiv. Trolox [®]
<i>C. sempervirens</i>	68.22 $\pm$ 0.41	1.04 $\pm$ 0.013	81.37 $\pm$ 0.73	1.24 $\pm$ 0.011
<i>C. lusitanica</i>	69.52 $\pm$ 0.96	1.06 $\pm$ 0.013	81.52 $\pm$ 1.15	1.25 $\pm$ 0.018

Source: Authors (2023).

The antioxidant evaluation showed similar percentages of activity considering the comparison between species within the same radical both in sweeping percentage and in millimolar of Trolox[®] equivalents, but different indices when comparing the methods.

The antioxidant activity values for the DPPH[•] radical of both fermented products were like those found by Senol et al. (2015), who evaluated the antioxidant activity and *in vitro* cholinesterase inhibitory effect of *C. sempervirens* using different solvents (acetone, ethanol, and ethyl acetate) where obtaining scanning percentages in the range of 47 – 77% for the radical DPPH[•]. Plant fermentates typically contain active components, such as saccharides, oligosaccharides, and peptides, with potential antioxidant properties and antibacterial action (Ghosh et al., 2015).

The antifungal activity of botanical fermentates of *C. lusitanica* and *C. sempervirens* was evaluated in controlling the mycelial growth of *Colletotrichum fructicola*, whose results are compiled in Table 4



Table 4. Antifungal activity results (percentage of control) on *Colletotrichum fructicola* depending on the type of fermentate and the concentration used.

Concentration	<i>C. sempervirens</i>	<i>C. lusitanica</i>
zero	0.0±0.0 Ac	0.0±0.0 Ab
10 vol.%	0.0±0.0 Ac	0.0±0.0 Ab
20 vol.%	8.7±1.2 Ab	2.3±0.7 Bb
40 vol.%	100.0±0.5 Aa	10.3±1.4 Ba
Coefficient of variation	15.31 %	

Means followed by the same letter, uppercase in line (type of fermentate) and lowercase in column (concentration), do not differ by Tukey's test at 5 % probability of error.

Source: Authors (2023).

The fermentate of *C. sempervirens*, at a concentration of 40 vol.%, completely inhibited (100 %) the mycelial growth of *Colletotrichum fructicola*. On the other hand, this fermented product was inefficient at concentrations of 10 vol.% and 20 vol.%. At none of the concentrations tested, the *C. lusitanica* fermentate effectively controlled the growth of *C. fructicola*. According to Liu et al. (2018) and Ricci et al. (2019), the fermentation process produces compounds that inhibit the growth of microorganisms.

This phytopathogenic fungus has broad importance on *Acca sellowiana* and species of economic importance. In this sense, studies of the effects of plant extracts against fungal diseases have revealed that they can stimulate or inhibit the growth of some species of fungi (Costa et al., 2021). In the present study, it was noted that the fermentate of one species (*C. sempervirens*) promoted growth inhibition. In contrast, that of the other species (*C. lusitanica*) did not affect the phytopathogen.

Some authors have reported the biological activity of some species of the Cupressaceae family in controlling bacteria and fungi (Sati; Joshi, 2010). The antifungal activity of the conifer *Chamaecyparis obtusa* was reported by Hong et al. (2004). The result of the present work demonstrates that the botanical fermented product of *C. sempervirens* can be an alternative for the control of phytopathogenic fungi aiming at the protection of cultivated plants. Other botanical fermented products have been investigated for managing fungi and have shown promising results (Triaca et al., 2018; Costa et al., 2021).

The alternative control of anthracnose with botanical fermentates in mountain guava orchards is crucial for family farming in southern Brazil, especially regarding management in areas of organic agriculture. It is also important to point out that this species of native fruit, known as feijoa, *Acca sellowiana*, is of great economic interest and is in the process of being domesticated, mainly in areas of family farming. Fungi of the genus *Colletotrichum* are responsible for peduncle rot and can cause losses of up to 100 % in plants in full fruiting (Araújo; Pinto, 2018; Duarte et al., 2023).

The results referring to the antifungal activity of fermented *Cupressus* sp. on the germination of *C. fructicola* conidia are shown in Table 5.

Table 5. Antifungal activity (percentage of control) of *C. sempervirens* and *C. lusitanica* botanical fermentates on the germination of *Colletotrichum conidia fructicola*.

Concentration	<i>C. sempervirens</i>	<i>C. lusitanica</i>
zero	0.0±0.0 Ad	0.0±0.0 Ab
10 vol.%	19.3±2.7 AC	5.0±1.3 Bb
20 vol.%	33.0±3.6 Ab	15.0±3.4 Ba
40 vol.%	100.0±0.0 Aa	16.7±4.2 Ba
Coefficient of variation	22.15 %	

Means followed by the same letter, uppercase in line (type of fermentate) and lowercase in column (concentration), do not differ by Tukey's test at 5% probability of error.

Source: Authors (2023).

In evaluating the germination of *C. fructicola* conidia, it was verified that the botanical



fermentate of *C. sempervirens* completely inhibited its development at a concentration of 40 vol.%. At 20 vol.%, the inhibition was 33 % compared to the control. For the botanical fermentate of *C. lusitanica*, the highest inhibition was 17 % at the concentration of 40 vol.%.

Synthetic chemicals are extensively used to control fungi and other plant diseases. However, resistance problems caused by synthetic control agents are widespread. Meanwhile, synthetic control agents lack selectivity and are toxic to non-target organisms. Public awareness of the environment, food security, and pathogen resistance to many fungicides have led to a need to develop alternative control strategies (Li et al., 2017).

Fermentates from plants that act as suppressive compounds in the control of phytopathogens is an interesting strategy, although the biological effect of the mixture is often inconsistent or unpredictable (Bonanomi et al., 2017). The mechanisms underlying the biological effect are not fully understood. However, these mechanisms are mainly associated with the biological activity of the microbiomes, which interact with the soil and the host plant. A general suppression effect is attributed to consortia affecting more than one pathogen simultaneously, where diversified mechanisms offer basal protection against a wide range of pathogens (Cha et al., 2016).

In the present study, the fermented extract of *C. sempervirens* showed an antifungal effect, a behavior like those reported by Jiang et al. (2021). Fermentation technology based on lactic fermentation and medicinal plants can be used as a solution for pathogenic bacteria and fungi. It has been proposed that the antimicrobial effects involve the inhibition of different cellular mechanisms, followed by an increase in the permeability of the plasma membrane, resulting in the leakage of ions from the cells, which causes an imbalance in the cytoplasmic pH (Kang et al., 2006).

Considering that only the botanical fermented product of *C. sempervirens* promoted total control of *C. fructicola* *in vitro*, only this fermented product was tested for *in vivo* control of the fungus on the leaves of *A. sellowiana*. The results are shown in Table 6.

Table 6. *In vivo* antifungal activity of *Cupressus sempervirens* botanical fermentate on *Colletotrichum fructicola* inoculated on *Acacia* leaves *sellowiana*.

Concentration	Control (%)
zero	10.0±3.2 c
20 vol.%	57.5±8.7b
40 vol.%	82.3±12.1 a
Coefficient of variation	30.28 %

Means in a column followed by the same letter do not differ statistically by Tukey's test at a 5 % error probability.

Source: Authors (2023).

It can be observed that the botanical fermented product of *C. sempervirens* at a concentration of 40 vol.% had a control percentage in the range of 80 % of *C. fructicola*. The lower control efficiency observed in the *in vivo* test compared to the *in vitro* test can be related to the effects of chemical interaction between the fungus and the leaf and between the substances present in the fermented product and the *A. sellowiana* leaves.

As noted by Bonanomi et al. (2017), the biological effect of botanical extracts and fermented products is highly variable and dependent on several factors, such as the way the plant material is obtained and prepared, the microbiota responsible for the fermentation process, and environmental effects on the fermented product after field application. Even so, this level of control makes this fermented product interesting for application as a biological input, acting as a potential tool to aid in managing phytopathogens of agronomic importance.



5 CONCLUSION

The botanical fermentate of the leaves of *C. sempervirens* completely inhibited the development of hyphae and the germination of conidia of *C. fructicola* at a concentration of 40 vol.%. These results may contribute to understanding the reactions between terpenes and other metabolites with phytopathogenic fungi, aiming to improve the alternative control of these fungi using substances of plant origin. *In vitro* bioassays showed that the botanical fermentate of *C. sempervirens* remarkably inhibited the germination of conidia and mycelia of *C. fructicola*. Therefore, in the current context, the search for alternative bioinputs, such as the use of fermentation of plant species, can be a valid strategy as a potential source of low-cost substrates for controlling phytopathogenic fungi of agricultural interest.

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