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Inhibitory action of fermented Cypress extracts on *Colletotrichum* fungi isolated from *Acca sellowiana*

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Abstract: This study aimed to evaluate the efficiency of fermented botanical extracts of *Chamaecyparis pisifera, Platycladus orientalis*, and *Cupressus sempervirens* in the *in vitro* control of *Colletotrichum fructicola, Colletotrichum theobromicola*, and *Colletotrichum pseudoacutatum* isolated from *Acca sellowiana*. The phenolic compounds of the different fermented botanical extracts were analyzed by high-performance liquid chromatography. In the *in vitro* evaluation, the different botanical extracts were incorporated into PDA medium at concentrations of zero, 10 %, 20 %, and 40 % v/v. Their effect on the mycelial growth of the pathogens was evaluated after 14 days, and the percentage of growth inhibition was determined. The *in vivo* test was only performed on the best results presented in the *in vitro* tests, that is, fermented *Chamaecyparis pisifera* against the phytopathogens *Colletotrichum fructicola* and *Colletotrichum theobromicola*. In the tests with *Colletotrichum fructicola*, the *Chamaecyparis pisifera extract* showed a 50 % inhibitory action at a concentration of 40 % v/v. For *Colletotrichum theobromicola, the Chamaecyparis pisifera* extract inhibited 48 % at the same concentration, although the other extracts did not show antifungal activity. In trials with *Colletotrichum pseudoacutatum*, fermented extracts of *Chamaecyparis pisifera, Cupressus sempervirens*, and *Platycladus orientalis* did not exhibit antifungal effects. *In vivo* trials, treatments T1 to T4 were effective in reducing the incidence and severity of anthracnose, with the best results in T2 (*Colletotrichum pisifera* with *Colletotrichum fructicola*) and T4 (*Chamaecyparis pisifera* with *Colletotrichum theobromicola*), reducing the incidence by 59 % and 54 %, respectively. The data suggest that the fermented extract of *Chamaecyparis pisifera* has potential as a protective agent against anthracnose.

Keywords: Regenerative agriculture, Botanical fermentations. Food security.

Resumo: Este estudo teve como objetivo avaliar a eficiência de extratos botânicos fermentados de *Chamaecyparis pisifera*, *Platycladus orientalis* e *Cupressus sempervirens* no controle *in vitro* de *Colletotrichum fructicola*, *Colletotrichum theobromicola* e *Colletotrichum pseudoacutatum*, isolados de *Acca sellowiana*. Os compostos fenólicos dos diferentes extratos botânicos fermentados foram analisados por cromatografia líquida de alta eficiência (HPLC). Na avaliação *in vitro*, os diferentes extratos botânicos foram incorporados ao meio BDA nas concentrações de zero, 10 %, 20 % e 40 % (v/v). O efeito sobre o crescimento micelial dos patógenos foi avaliado após 14 dias, e a percentagem de inibição do crescimento foi determinada. O teste *in vivo* foi realizado apenas com os melhores resultados obtidos nos testes *in vitro*, ou seja, com o extrato fermentado de *Chamaecyparis pisifera* contra os fitopatógenos *Colletotrichum fructicola e Colletotrichum theobromicola*. Nos testes com *Colletotrichum fructicola*, o extrato de *Chamaecyparis* pisifera apresentou 50 % de inibição na concentração de 40% (v/v). Para *Colletotrichum theobromicola*, o extrato de *Chamaecyparis pisifera* inibiu 48 % na mesma concentração, enquanto os outros extratos não apresentaram atividade antifúngica. Nos ensaios com *Colletotrichum pseudoacutatum*, os extratos fermentados de *Chamaecyparis pisifera*, *Cupressus sempervirens* e *Platycladus orientalis* não exibiram efeito antifúngico. Nos testes *in vivo*, os tratamentos T1 a T4 foram eficazes na redução da incidência e severidade da antracnose, com os melhores resultados observados em T2 (*Chamaecyparis pisifera* com *Colletotrichum fructicola*) e T4 (*Chamaecyparis pisifera* com *C. theobromicola*), reduzindo a incidência em 59 % e 54 %, respectivamente. Os dados sugerem que o extrato fermentado de *Chamaecyparis pisifera* tem potencial como agente protetor contra a antracnose.

Keywords: Agricultura regenerativa. Fermentações botânicas. Segurança alimentar

1. Introduction

Acca sellowiana (O. Berg) Burret, popularly known as ‘mountain guava’ or ‘feijoa’, is a fruit tree belonging to the Myrtaceae family, native to the southern Brazilian plateau and northeastern Uruguay. It is adapted to cold climate conditions and frequently occurs in areas above 800 m in southern Brazil [1]. As a native species, it plays an important ecological and environmental role, in addition to having high fruit-bearing potential. Therefore, it represents a promising alternative for cultivation and income for family farming in these regions [2]. However, there are few commercial-scale crops in Brazil, with the largest producers and exporters of this species being New Zealand and Colombia [3].

In Brazil, there are no official statistics available on the production and consumption of feijoa, but some sources indicate that the country imports the fruit from Colombia, which held the largest world production, followed by New Zealand. Both countries developed varieties from seeds obtained in Brazil many decades ago, while only in recent decades has Brazilian cultivation been encouraged through genetic improvement and agricultural management. Feijoa cultivation is on a small scale in Brazil, largely due to a lack of good quality seedlings [4].

The genus *Colletotrichum* is considered one of the most important phytopathogenic fungi and can cause anthracnose in several crops, including *A. sellowiana* [5]. A antracnose está entre as infecções fúngicas pós-colheita mais comuns que afetam goiaba, manga e mamão [6].

C. gloeosporioides is a fungus that causes the most losses in the world, recognized as the cause of anthracnose disease. This disease

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causes darkening and deterioration of the fruit, generating large economic losses for producers and throughout the value chain [7].

The development of anthracnose in fruits has traditionally been controlled by the application of synthetic fungicides, such as thiophanate-methyl and other components of the benzimidazole and dithiocarbamate groups (e.g., mancozeb) [6].

Although these synthetic fungicides effectively suppress the development of anthracnose in fruits, their resistance-inducing effects in pathogenic fungi, which cause a progressive increase in effective antifungal doses in fruits, are a concern. The use of synthetic fungicides in fruits is also associated with increased risks to human health and environmental contamination [8].Consertar os números

Therefore, plant extracts can serve as a source of various bioactive compounds, such as alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glycosides, terpenes, phenylpropanes, and organic acids. These substances may exhibit antimicrobial properties and are of great biotechnological interest [9]. They represent a promising alternative for pathogen control due to the variety of substances that can exhibit different modes of action, allowing them to act against various agents and hinder the development of resistance in pathogens. Furthermore, they have low toxicity, rapid degradation, and are derived from renewable resources [10].

Some studies demonstrate that the fermentation process can decompose or convert complex substances into compounds of interest under the action of microbial agents and enzymes, enhancing the properties of the substrate by increasing the production and extraction of bioactive compounds [11]. Fermented botanical extracts, obtained through the spontaneous fermentation of microorganisms present in fresh plant material, have been investigated for the control of agricultural pests and diseases, with a focus on application primarily in organic, agroecological, and family farming production systems [12]. Recent studies demonstrate the inhibitory action of these extracts *Cupressus sempervirens* and *Cupressus lusitanica a*gainst various phytopathogens for exemple *Colletotrichum fructicola* [13,14].

Therefore, this investigation aimed to evaluate the antifungal potential of fermented extracts of *Chamaecyparis pisifera*, *Platycladus orientalis*, and *Cupressus sempervirens* on the mycelial growth of the phytopathogens that cause anthracnose, *Colletotrichum fructicola*, *Colletotrichum theobromicola*, and *Colletotrichum pseudoacutatum*, isolated from *Acca sellowiana*.

2. Material and methods

This work was developed at the Laboratory of Biological Control of Plant Diseases - Organic Agriculture Laboratory of the University of Caxias do Sul (UCS), Caxias do Sul, RS, Brazil.

2.1 Fungal isolates

The isolates of *C. theobromicola* (19207) and *C. pseudoacutatum* (19212) were donated by Federal University of Santa Maria (UFSM), RS, Brazil. The isolate *C. fructicola* (A004/19) is stored in the fungal collection at the Phytopathology Laboratory of the University of Caxias do Sul (UCS), RS. The multiplication of the isolates was performed by subculture on PDA medium. The Petri dishes were kept in a growth chamber at a temperature of 25 °C, relative humidity between 60 – 80 %, with a 12 h photoperiod, for 14 days.

2.2 Obtainment of plant material and extract preparation

The vegetal species used to produce fermented extracts were sent to the UCS Museum of Natural Sciences for identification and were identified as *Chamaecyparis pisifera* (Siebold & Zucc.) Endl. (HUCS 52422), *Platycladus orientalis* (L.) Franco (HUCS 51956) and *Cupressus sempervirens* L. (HUCS 51957).

The extracts were produced by adding 500 g of fresh plant material to 1.5 L of untreated water and blending until a homogeneous mixture was obtained. These extracts were stored in a glass container covered with gauze and kept in the dark for 15 days. After this period, the extracts were filtered through gauze [15].

2.3 High-performance liquid chromatography (HPLC)

A fraction of each extract was sent to the Analytical Chemistry Laboratory for analysis of phenolic compounds through HPLC in the University of Caxias do Sul [16]. The analyses were performed on HPLC equipment, model 1100, Lichrospher RP18 column (5 µm) equipped with a UV detector at 210 nm and a quaternary pump system. The reserve phase analysis consisted of solvent A - Milli-Q water with 1 % v/v phosphoric acid and solvent B - Acetonitrile. The mobile phase pumping system was gradient, with 90 % solvent A from zero to 5 min, 60 % A from 5 min to 40 min, and 90 % A from 45 min to 50 min. The standard flow rate was maintained at 0.5 $mL \cdot min^{-1}$, according to [19]. The samples were solubilized in Milli-Q water (5 $g \cdot L^{-1}$) and filtered through 0.45 µm pore diameter nylon membranes. The phenolic compounds were identified according to their elution order and by comparing their retention times with those of their pure standards. Quantification was performed by the external standardization method, by correlating the peak area (mAU·s) of the compound to the standard curve evaluated (gallic acid, epigallocatechin, catechin, epicatechin, epigallocatechin gallate, rutin, ferulic acid, narangin, hespiredin, myricetin, resveratrol, quercetin, apigenin, and kaempferol). The analysis was performed in duplicate, and the result was expressed in milligrams of compound per liter of extract ($mg \cdot L^{-1}$).

2.4 Action of extracts on mycelial growth

To evaluate the effect of fermented extracts on the mycelial development of pathogens, the pH was measured and corrected

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to a value of 6.0±0.2. These were then diluted in PDA medium at concentrations of zero, 10 %, 20 % and 40 % v/v, and autoclaved at 121 ˚C for 15 min. As a control, only the PDA medium was used.

The media were poured into Petri dishes, with five replicates per treatment. After the medium solidified, a 5 mm mycelial disk was placed in the center of each plate. The plates were sealed and placed in a BOD chamber for 14 days, with a 12 h photoperiod, at a temperature of 25 °C and a relative humidity of 60 – 80 %. The mycelial growth diameter was assessed on the 14th day after inoculation by measurements using a caliper. The percentage inhibition was calculated using equation 1.

$$I\ \% = 100 \times (1 - DT/DC) \quad (1)$$

Where 'I' is the percentage of inhibition (%), 'DT' is the colony diameter (mm), and 'DC' is the diameter of the control group (mm).

2.5 Evaluation of the antifungal activity of the fermented extract on conidial germination

The antifungal activity of the fermented products on conidial germination was evaluated according to Badawy et al. [17], with some modifications. Conidia of the three fungi were collected from colonies grown for 14 days on PDA medium at 25±2 °C under a 12 h photoperiod. Five milliliters (5 mL) of sterile water were added to a culture medium in Petri dishes. Conidia of the different *Colletotrichum* species were carefully removed from the colony surface using a sterile glass rod, and the suspension was filtered through three layers of cotton cloth to remove any mycelium fragments. The suspension was diluted with sterile water to obtain a suspension with $1 \cdot 10^6$ conidia·mL^{-1}. Aliquots of 50 µL of the suspension were placed in microtubes containing 500 µL of PDA medium containing the fermented products at concentrations of zero, 10%, 20%, and 40% v/v. The control treatment was only PDA medium.

The tubes were incubated at 25 °C for 18 h. The samples were placed in Neubauer chambers (10 µL), and conidial germination was observed by optical microscopy at tenfold magnification using a Primo Star optical microscope (Zeiss, Germany). All experiments were performed with five replicates, and 100 conidia were evaluated in each replicate. The conidia were considered germinated when the length of the germ tube was equal to or greater than the length of the conidium itself.

2.6 In vivo *evaluation of* Acca sellowiana *plants*

One-year-old plants, grown from seeds, were maintained in a greenhouse at the University of Caxias do Sul. The best results identified *in vitro* were applied to the *in vivo* tests, that is, to the fermented extract of *C. pisifera*, at the highest concentrations. The *in vivo* experiments were conducted under controlled conditions of 28±2 °C, light intensity of 300 µE·m^{-2}·s^{-1} and 50 % relative humidity. The antifungal activity of *Chamaecyparis pisifera* extract on *A. sellowiana* leaves was evaluated according to the method described by Songwattana et al. [18], with modifications.

A curative assay was performed, in which both treated and untreated plants were inoculated with a 10 µL of the conidial suspension of the phytopathogens *C. fructicola* ($1 \cdot 10^5$ spores·mL^{-1}) and *C. theobromicola* ($3 \cdot 10^5$ spores·mL^{-1}) on each leaf. The inoculum was applied at the base of each leaf. Plants were irrigated one day prior to inoculation to maintain high humidity and promote disease development. Four hours after inoculation of conidial, the following treatments were applied by spraying: *C. pisifera* with *C. fructicola,* T1: concentration 20 % v/v; T2: concentration 40 % v/v; *C. pisifera* with *C. theobromicola,* T3: concentration 20 % v/v; T4: concentration 40 % v/v; T5: control with *C. fructicola;* T6: control with *C. theobromicola*; T7: control being water only.

The experimental design used was completely randomized, with five plants per treatment and 10 leaves per plant. Anthracnose symptoms on infected leaves were scored using a five-point scale, where 1: no visual disease infection, 2: 1 – 25 % of the leaf area infected, 3: 26 – 50 % of the leaf area infected, 4: 51 – 75 % of the leaf area infected, and 5: 76 – 100 % of the leaf area infected [19]. These scores were used in the assessments of the percentage of disease severity index (% DSI) using equation 2:

$$DSI\ (\%) = 100 \times \left(\frac{\text{sum of treatment scores}}{\text{total number of observations} \times \text{maximum score value}}\right) \quad (2)$$

2.7 Statistical analysis

The data were assessed for homoscedasticity (Levene's test) and normality of residuals (Shapiro-Wilk test). Subsequently, the data were subjected to analysis of variance (ANOVA), and means were compared using the Tukey test at a 5% significance level ($\alpha = 0.05$). The statistical analyses were performed using AgroEstat software (Brazil).

3. Results

The analysis of the phenolic compounds of the fermented extracts by HPLC showed the presence of epicatechin in the fermented extracts of *C. sempervirens*, *C. pisifera*, and *P. orientalis*, at values of 371.3 mg·L^{-1}, 365.9 mg·L^{-1}, and 326.3 mg·L^{-1}, respectively. Epicatechin (EC) derivatives have attracted considerable attention due to their powerful antioxidant, anti-inflammatory, anticancer, and antibacterial properties, all attributed to the phenolic hydroxyl groups in their structure [20].

In vitro tests with the pathogen *C. fructicola* (Table 1) demonstrated that the fermented extract of *C. pisifera* reached an inhibitory action around 31 % at a concentration of 10 % v/v, 43 % at a concentration of 20 % v/v, and 50 % at a concentration of 40 % v/v. The fermented extracts of *C. sempervirens* and *P. orientalis* showed reduced antifungal action against this pathogen but differed statistically from the control treatment (zero).

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Table 1. Percentage of inhibition of mycelial growth of *C. fructicola* at 14 days of inoculation, exposed to different concentrations of fermented botanical extracts.

Fermented	Control (0 % v/v)	10 % v/v	20 % v/v	40 % v/v
C. sempervirens	0.00±0.00 Aa	2.88±1.00 Aab	13.14±7.80 Ab	16.25±5.48 Bb
C. pisifera	0.00±0.00 Aa	30.95±12.05 Bb	43±15.29 Bc	50±11.5 Ac
P. orientalis	0.00±0.00 Aa	8.87±2.05 Aa	17.51±8.09 Ab	19.45±6.48 Bb

Means followed by the same letter, capitalized in a column (fermented) and lowercase in a row (concentration), do not differ statistically from each other by the Tukey test at the 5 % probability level. Source: Authors (2025)

Regarding the tests with *C. theobromicola* (Table 2), it can be observed that the fermented extract of *C. pisifera* reached an inhibitory action of 16 % at a concentration of 10 % v/v, 47 % at a concentration of 20 % v/v, and 49 % at a concentration of 40 % v/v, with no significant variation between the highest concentrations. The other extracts evaluated showed lower antifungal activity against this pathogen.

Table 2. Percentage of inhibition of mycelial growth of *C. theobromicola* at 14 days of inoculation, exposed to different concentrations of fermented botanical extracts.

Fermented	Control (0 % v/v)	10 % v/v	20 % v/v	40 % v/v
C. sempervirens	0.00±0.00 Aa	0.67±0.02 Aa	5.94±2.03 Ab	7.89±1.89 Cb
C. pisifera	0.00±0.00 Aa	15.86±5.26 Bb	47.32±13.52 Cc	49.8±10.5 Ac
P. orientalis	0.00±0.00 Aa	13.08±6.54 Bb	17.92±1.59 Bb	25.52±2.48 Bc

Means followed by the same letter, capitalized in a column (fermented) and lowercase in a row (concentration), do not differ statistically from each other by the Tukey test at the 5 % probability level. Source: Authors (2025)

In tests with the fungus *C. pseudoacutatum*, the fermented extracts of *C. pisifera*, *C. sempervirens*, and *P. orientalis* showed significant antifungal activity when compared to the control (Table 3). At the highest concentration, 40 % v/v, for both *C. sempervirens* and *C. pisifera,* there was 27 % control of the phytopathogen.

Table 3. Percentage of inhibition of mycelial growth of *C. pseudoacutatum* at 14 days of inoculation, exposed to different concentrations of fermented botanical extracts.

Fermented	Control (0 % v/v)	10 % v/v	20 % v/v	40 % v/v
C. sempervirens	0.00±0.00 Aa	15.64±8.21 Bb	16.6±8.56 Ab	17.7±7.4 Ab
C. pisifera	0.00±0.00 Aa	8.04±5.23 Ab	14.54±7.16 Ab	27.5±9.8 Bc
P. orientalis	0.00±0.00 Aa	0.00±0.00 Aa	16.75±9.25 Ab	18.75±2.4 Ab

Means followed by the same letter, capitalized in a column (fermented) and lowercase in a row (concentration), do not differ statistically from each other by the Tukey test at the 5 % probability level. Source: Authors (2025)

As shown in Table 4, for *C. fructicola*, the fermented extract of *C. pisifera* differed significantly from the control at all concentrations evaluated, with only 13% of the conidia showing germination at the highest concentration. Additionally, it was observed that the fermented extracts of *C. sempervirens* and *P. orientalis*, when applied to *C. fructicola* at a concentration of 40% (v/v), also reduced conidia production (33% and 60%, respectively), showing a statistically significant difference compared to the control. Assays conducted with the phytopathogen *Colletotrichum pseudoacutatum* showed a slight statistical difference in conidia production compared to the control group.

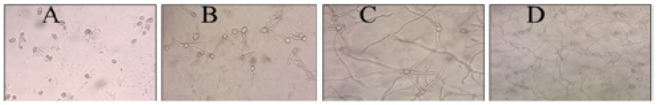
Table 4. Effect of different concentrations of fermented extracts of *C. sempervirens*, *C. pisifera*, and *P. orientalis* on the germination of conidia of *C. fructicola*, *C. theobromicola*, and *C. pseudoacutatum*.

Concentration (% v/v)	Conidia germination (%)		
	C. sempervirens	*C. pisifera*	*P. orientalis*
C. fructicola			
zero	100±0.00 Aa	100±0.00 Aa	100±0.00 Aa
10	67±2.12 Bb	100±0.00 Aa	100±0.00 Aa
20	40±3.09 Bc	70±2.05 Ab	80±3.56 Ab
40	33±3.40 Ac	13±5.15 Bc	60±3.88 Ac
C. theobromicola			
zero	100±0.00 Aa	100±0.00 Aa	100±0.00 Aa
10	100±0.00 Aa	30±4.14 Bb	85±2.55 Aa
20	90±2.15 Aa	10±3.52 Bb	80±2.88 Aa
40	90±2.52 Aa	0±0.00 Bc	75±2.94 Aa
C. pseudoacutatum			
zero	100±0.00 Aa	100±0.00 Aa	100±0.00 Aa
10	100±0.00 Aa	90±0.00 Bb	100±0.00 Aa
20	90±3.08 Ab	88±2.65 Ab	95±1.45 Ab
40	87±2.83 Ab	85±2.89 Ab	90±1.89 Ab

Means followed by the same letter, capitalized in a row (fermented) and lowercase in a column (concentration), do not differ statistically from each other by the Tukey test at the 5 % probability level. Source: Authors (2025)

The evaluation of the performance of the investigated fermented products showed statistically significant differences between treatments. The fermented extract of *C. pisifera*, compared to *C. theobromicola*, showed a more pronounced effect on conidia at all tested concentrations, promoting complete inhibition of germination at the maximum concentration evaluated (40% v/v), in relation to the other fermented products. These results are illustrated in Figure 1. Optical microscopy analyses corroborate the high inhibitory activity of the fermented extracts on the reproductive structures of phytopathogenic fungi. Based on this parameter, significant reductions in conidia density were observed in colonies treated with the fermented extract of *C. pisifera* at different concentrations, when compared to the control (Figure 1).

Figure 1: Optical microscopy images (100 µm) demonstrating changes in the structure and germination of *C. theobromicola conidia* after exposure to fermented *C. pisifera* at concentrations of zero (D), 10 % v/v (C), 20 % v/v (B), and 40 % v/v (A), during incubation for 18 h in a BOD chamber.



Source: Authors (2025).

In vivo tests performed with the phytopathogens *C. fructicola* and *C. theobromicola*, all treatments differed from the control. Treatments T1 to T4 were effective in reducing the incidence and severity of anthracnose, with the best results in T2 and T4 (40 % v/v concentration of fermented *C. pisifera* extract), with incidences of 48 % and 45 %, respectively. These results can be seen in Table 5 and better demonstrated in Figure 2.

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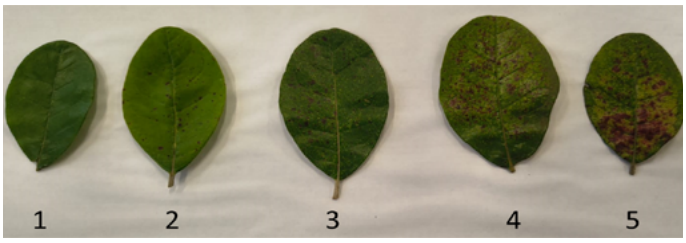
Table 5. Incidence (%) in *A. sellowiana* plants treated with different concentrations of fermented *C. pisifera* extracts against the phytopathogen *C. fructicola* and *C. theobromicola*.

Treatment	Incidence (%)	Severity/Grading Scale
T1	59.5±4.5 B	3.0±1.2 B
T2	48.3±10.4 C	2.0±3.5 C
T3	54.2±5.1 B	3.0±2.5 B
T4	45.9±2.2 C	2.0±1.9 C
T5	100±0.00 A	5.0±0.0 A
T6	100±0.00 A	5.0±0.0 A
T7	0±0.00 D	0.0±0.0 D
Coefficient of variation (%)	20.42	18.89

Chamaecyparis pisifera with *C. fructicola* T1: concentration 20 % v/v; T2: concentration 40 % v/v; *C. pisifera* with *C. theobromicola,* T3: concentration 20 % v/v; T4: concentration 40 % v/v; T5: control only *C. fructicola;* T6: control only *C. theobromicola*; T7: control with water only. Means followed by the same uppercase letter, in the column, do not differ from each other by the Tukey test at a 5 % probability of error.

Figure 2: Diagrammatic scale for measuring disease severity with anthracnose symptoms on leaves.



Grading scale: from 1 (completely healthy leaves), 2: 1-25%, 3: 26 - 50% a, 4: 51 - 75 %, 5: 76 - 100 % leaf damage. Source: Authors (2025)

4. Discussion

Epicatechin is a phenolic compound of the flavonoid group [20]. Its antimicrobial properties have been observed in studies evaluating its potential direct action on microorganisms, as well as its presence in extracts with antimicrobial properties [21]. Triaca et al. [22] observed that epicatechin can inhibit the appressorial melanization of *C. kahawae*, preventing the disease that attacks coffee fruits. Ullah et al. [23] observed the antifungal action of epicatechin in *Populus nigra* (poplar) against the poplar rust agent (*Melampsora* spp.), inhibiting spore germination and reducing the mycelial growth of the pathogen. The same authors considered that the presence of this substance constitutes one of the plant's antifungal chemical defenses.

In the study by Zhang et al. [24], the inhibition of gray mold of apple (*Botrytis cinerea*) by epicatechin was verified through the effective activation of different branches of the phenylpropanoid metabolism pathway. Borges et al. [25] demonstrated that the production of epicatechin, induced by *Ectropis grisescens* damage in *Camellia sinensis*, plays an important role in the defense against this pest.

In the study by Sardi et al. [26], seed and leaf extracts of *Eugenia* spp. showed intense antifungal action against mature *Candida albicans* biofilms, and the phenolic compounds epicatechin and gallic acid were the main compounds present in the extracts. Girardi et al. [27] tested the action of *Lotus* spp. extracts against *Alternaria* sp. and *Fusarium* sp. and attributed the antifungal properties to the presence of catechin and epicatechin. In the study by Castro et al. [28], the essential oil of *Psidium cattleianum* showed antifungal activity, and the main compounds found were phenolic compounds, where epicatechin was identified as one of the major components present in the material.

Some authors have reported the biological activity of extracts from species of the Cupressaceae family in the control of bacteria and fungi [16, 29]. Furthermore, studies using conifer exudates have demonstrated this same type of action [30]. The genus *Chamaecyparis*, which makes up this botanical family, has already been cited in several studies that demonstrate the antimicrobial activity of some of its species [31].

The antifungal activity of *C. pisifera* was observed in the work of [32], in which terpenes from this species were isolated and their action against the fungus *Seiridium unicorne*, the causative agent of cypress cortical canker, was evaluated. Seven of the diterpenes isolated from the bark were shown to be effective in controlling this pathogen. Ignea et al. [33] observed antibacterial activity of diterpenes derived from the abietane skeleton isolated from *C. pisifera* against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.

Studies regarding the chemical characterization of *C. pisifera* report the presence of several types of terpenes in its composition. Lasram et al. [34] analyzed the essential oils of two varieties of *C. pisifera*. The main components observed in *C. pisifera* Endl. were α-pinene and bornyl acetate, and in *C. pisifera* var. filifera, 3-carene, (-)-bornyl acetate, and α-pinene. In addition to these compounds, pisiferic acid, a diterpenic acid isolated from the leaves and twigs of *C. pisifera*, showed antibacterial and antifungal activity in some studies [35, 36].

Azzaz et al. [37] mentioned the inhibitory potential of cypress essential oil against two fungi, *Verticillium* spp. and *Aspergillus* spp. Different microorganisms were exposed to *C. sempervirens* essential oil. Some of these, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger*, and *Candida albicans*, were inhibited, with varying degrees of inhibition. Notably, *B. subtilis* was found to be the most susceptible. *E. coli* was resistant, and a moderate effect was observed in *S. aureus*, *C. albicans*, and *A. niger*.

Recently, Galovičová et al. [38] reported different types of biological activity of the essential oil of *C. sempervirens* as antibacterial against *Pseudomonas aeruginosa, Salmonella enterica, Yersinia enterocolitica, Bacillus subtilis, Enterococcus faecalis*, and *Staphylococcus aureus* and antifungal against *Candida krusei, C. albicans, C. tropicalis, C. glabrata, Penicillium citrinum, Aspergillus flavus*, and *Botrytis cinerea,* as well as

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antioxidant and anticancer activities. Computational approaches are currently an essential tool for providing information on the mechanisms of action of biologically active compounds [39].

Bae et al. [40] reported that, when searching for plant extracts that are active in controlling plant diseases such as rice blast (*Magnaporthe grisea*), tomato gray mold (*Botrytis cinerea*), wheat leaf rust (*Puccinia triticina*), and pepper anthracnose (*Colletotrichum gloeosporioides*). The authors observed that the methanolic (MeOH) extract of *Platycladus orientalis* exhibited high efficacy in disease control against rice blast. The plant species *P. orientalis* (L.) Franco (= *Thuja orientalis* L.; *Biota orientalis* (L.) Endl.) is an evergreen coniferous tree belonging to the Cupressaceae family. Because *P. orientalis* can grow in various climates and soil environments, this plant species is widely distributed throughout the world, including India, China, Japan, and Korea. It has been reported that *P. orientalis* extracts exhibited various types of activity, such as antioxidant, anticancer, and anti-inflammatory activity, and the terpene compounds identified in *P. orientalis extracts* showed pharmacological activity. However, little is known about *P. orientalis extracts* and their active compounds against fungal plant diseases. Bae et al. [40] suggested that the antifungal effect exerted by *P. orientalis extracts* depends on the synergism of many compounds, and that a single component of *P. orientalis* has limited roles in the overall antifungal activity.

These secondary compounds comprise a diverse group of natural compounds that play a crucial role in protecting plants from biotic and abiotic stress, serving as a defense against pests and diseases [41]. Furthermore, they have medicinal and antimicrobial properties [42].

Considering that fungal spore germination is the key process necessary to initiate vegetative growth and ultimately cause parasitism, plant compounds can be used in the development of novel antifungal agents targeting the fungal spore germination process. According to Bae et al. [40], the main active compounds are probably more effective on germination than when compared to mycelial growth.

Phenolic compounds are abundant in the plant kingdom, and the ability of this class of substances to inhibit the germination of pathogenic fungal spores is recognized [43].

The antimicrobial properties of these compounds are associated, in plants, with protective action against pathogenic microorganisms [44]. These antimicrobial secondary metabolites are found among various substances and compounds extracted from plants. The variability in their effectiveness is linked not only 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, both isolated and synergistically [16].

Such a behavior indicates that the overall strength of a terpene's antimicrobial activity depends on the intrinsic resistance of the microorganism, the terpene's toxic potential, and interactions with other substances. (synergistic and antagonistic effects). However, as noted by Tariq et al. [45], the antimicrobial or antifungal activity of essential oils and extracts may be the result of the physicochemical properties of terpenes, which, due to their lipophilic nature and small molecular weight, can interact deleteriously with the cell wall and membrane, causing cell death or inhibiting the spread of fungi and bacteria.

Plant fermentations that act as suppressive compounds in the control of plant pathogens are an interesting strategy, although the biological effect of the mixture is often inconsistent or unpredictable [46]. The mechanisms underlying this biological effect are not fully understood. However, these mechanisms are primarily associated with the biological activity of microbiomes, which interact with the soil and the host plant. A general suppressive effect is attributed to consortia that affect more than one pathogen simultaneously, where diverse mechanisms offer basal protection against a broad range of pathogens [47].

In vivo plant assessments result in a potential reduction in fungicide use and can therefore reduce economic costs and ecological impacts in agricultural crop production systems. These reliable and timely assessments of the occurrence and spread of plant diseases are the basis for planning targeted plant protection activities in field or greenhouse production and for predicting the temporal and spatial spread of diseases in specific growing regions [16, 48].

Anthracnose severity is lower in plants that are not yet in the reproductive phase [6], as observed in this study. Likely, plant exposure to the pathogen at an earlier stage of development promotes the formation of physical barriers that result in better responses to pathogen attack [49] After absorption, the plant extract may form a protective barrier, leading to a reduction in infection by plant pathogens.

Plants respond to pathogen attack by activating many defense mechanisms. These mechanisms include the production of antimicrobial metabolites and proteins, the physical reinforcement of cell walls through the production of callose and lignin, and the induction of hypersensitivity reactions. These changes occur at the tissue and cellular level after the first contact with a pathogen and are important for subsequent compatible or incompatible interactions, or, at the plant level, for the susceptibility or resistance of a genotype [50].

According to Oerke et al. [51], disease symptoms result from physiological changes in plant metabolism caused by the pathogen. The change in membrane semipermeability is one of the host's first reactions to the pathogen's attack. Changes in the plant's primary and secondary metabolism result from this change.

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Barley leaves infected with *P. hordei* export less sucrose to other plant organs, and diseased plants exhibit reduced growth [52]. The reduction in carbohydrate accumulation in *V. labrusca* roots is significant even in plants with low levels of rust severity (*Puccinia* spp.). Because grapevines use starch stored in their roots for growth in the early stages of development, from budbreak to the beginning of flowering, the occurrence of grapevine rust in one season can reduce vine vigor in the subsequent season [53]. Therefore, evaluating the effectiveness of the extracts reinforces the importance of seeking natural and sustainable solutions for controlling plant pathogens, especially in organic farming systems and small-scale agriculture.

The in vivo assay was conducted using exclusively the treatments that showed the greatest efficacy in the in vitro tests, specifically the fermented extract of Chamaecyparis pisifera against the phytopathogens *Colletotrichum fructicola* and *Colletotrichum theobromicola*. The results obtained show that the fermented extract of *Chamaecyparis pisifera* exhibits antagonistic activity both in vitro and *in vivo* against C. theobromicola and *C. fructicola*, indicating its potential as a protective agent in the control of anthracnose. Additionally, these findings contribute to the advancement of the understanding of the interactions between phenolic compounds and phytopathogenic fungi, providing support for the development of alternative control strategies based on the use of substances of plant origin.

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