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Evaluation of the antifungal activity of propolis extracts from stingless bees on phytopathogenic fungi

Avaliação da atividade antifúngica de extratos de própolis de abelhas sem ferrão sobre fungos fitopatogênicos

Evaluación de la actividad antifúngica de extractos de propóleos de abejas sin aguijón sobre hongos fitopatógenos

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Clarice Rech Costantin
ORCID: https://orcid.org/0000-0003-0819-1457
Centro Tecnológico Universidade de Caxias do Sul, Brasil
E-mail: crcostantin@ucs.br
Helena Gil de Oliveira
ORCID: https://orcid.org/0000-0002-6393-9790
Centro Tecnológico Universidade de Caxias do Sul, Brasil
E-mail: hgoliveira@ucs.br
Luiza San Martins Roese
ORCID: https://orcid.org/0000-0002-5282-690X
Centro Tecnológico Universidade de Caxias do Sul, Brasil
E-mail: lsmroese@ucs.br
Márcia Regina Pansera
ORCID: https://orcid.org/0000-0002-6868-829X
Universidade de Caxias do Sul, Brasil
E-mail: mrpancer@ucs.br
Wendel Paulo Silvestre
ORCID: https://orcid.org/0000-0002-9376-6405
Universidade de Caxias do Sul, Brasil
E-mail: wpsilvestre@ucs.br
Gustavo Rubbo Siqueira
ORCID: https://orcid.org/0000-0002-5193-9451
Centro Tecnológico Universidade de Caxias do Sul, Brasil
E-mail: gsiqueir@ucs.br
Nadilson Roberto Ferreira
ORCID: https://orcid.org/0000-0003-4333-7968
Secretaria de Agricultura, Pecuária e Desenvolvimento Rural do Estado do Rio Grande do Sul, Brasil
E-mail: nadilson-ferreira@seapdr.rs.gov.br
Valdirene Camatti Sartori
ORCID: https://orcid.org/0000-0002-8107-5893
Universidade de Caxias do Sul, Brasil
E-mail: vcsartor@ucs.br

Abstract
The bees of the Meliponini tribe, known as stingless bees (SB), elaborate a distinct type of propolis, whose application potential is largely unexplored. Currently, there is some knowledge of the pharmacological activities of the propolis from *Apis mellifera*, however, little is known about the diversity and antimicrobial activity of Meliponini propolis, especially in relation to pathogens of agricultural importance. This study aimed to investigate the antimicrobial activity of the alcoholic extract of propolis from *T. clavipes* (Borá), *S. bipunctata* (Tubuna), and *T. angustula* (Jataí), comparing with the ethanolic extract from *A. mellifera* propolis. The autoclaved and non-autoclaved extracts were evaluated at the concentrations of zero, 1.6 %; 3.2 %; 6.4 %, and 12.0 % v/v. Fungal development was determined by measuring the mycelial diameter up to the fourteenth day of inoculation, with five replicates; the growth inhibition was calculated relative to the control. According to the results, the highest concentration of phenolic compounds and flavonoids was present in the ethanolic extract of *A. mellifera* propolis when compared to the SB extracts. The results indicated an inhibitory effect on the growth of the phytopathogens *Colletotrichum gloeosporioides*, *Fusarium* sp., *Botryosphaeria* sp., and *Botrytis* sp., mainly from 6.4 % and 12.0 % v/v when evaluated in the non-autoclaved medium, followed by the autoclaved medium. The fungus *Botrytis* sp. was the phytopathogen that suffered greater

inhibition from 3.2 mL·L^{-1}, with the *T. angustula* propolis extract. The obtained results demonstrate that the alcoholic extracts of propolis from SB have the potential to control phytopathogenic fungi of agricultural interest.
Keywords: Natural antifungals; Phenolic compounds; Total flavonoids; Meliponini; Propolis.

Resumo

As abelhas da tribo Meliponini, conhecidas como abelhas sem ferrão (ASF), elaboram um tipo distinto de própolis, cujo potencial de aplicação é pouco explorado. Atualmente, existe algum conhecimento sobre as atividades farmacológicas da própolis de *Apis mellifera*, entretanto, pouco se sabe sobre a diversidade e atividade antimicrobiana da própolis de Meliponini, principalmente em relação a patógenos de importância agrícola. Este estudo teve como objetivo investigar a atividade antimicrobiana do extrato alcoólico da própolis de *T. clavipes* (Borá), *S. bipunctata* (Tubuna) e *T. angustula* (Jataí), comparando com o extrato etanólico da própolis de *A. mellifera*. Os extratos autoclavados e não autoclavados foram avaliados nas concentrações de zero, 1,6 %; 3,2 %; 6,4 % e 12,0 % v/v. O desenvolvimento fúngico foi determinado medindo-se o diâmetro micelial até o décimo quarto dia de inoculação, com cinco repetições; a inibição do crescimento foi calculada em relação ao controle. De acordo com os resultados, a maior concentração de compostos fenólicos e flavonoides foi identificada no extrato etanólico da própolis de *A. mellifera* quando comparado aos extratos de ASF. Os resultados do presente estudo confirmaram o efeito inibitório sobre o crescimento dos fitopatógenos *Colletotrichum gloeosporioides*, *Fusarium* sp., *Botryosphaeria* sp. e *Botrytis* sp., principalmente a partir de 6,4 % e 12,0 % v/v quando avaliados em meio não autoclavado, seguido do meio autoclavado. O fungo *Botrytis* sp. foi o fitopatógeno que sofreu maior inibição a partir de 3,2 mL·L^{-1}, com o extrato de própolis de *T. angustula*. Os resultados obtidos demonstram que os extratos alcoólicos de própolis de ASF têm potencial para controlar fungos fitopatogênicos de interesse agrícola.
Palavras-chave: Antifúngicos naturais; Compostos fenólicos; Flavonoides totais; Meliponini; Própolis.

Resumen

Las abejas de la tribu Meliponini, conocidas como abejas sin aguijón (ASA), elaboran un tipo distinto de propóleos, cuyo potencial de aplicación está poco explorado. Actualmente, existe cierto conocimiento sobre las actividades farmacológicas del propóleo de *Apis mellifera*, sin embargo, poco se conoce sobre la diversidad y actividad antimicrobiana del propóleo de Meliponini, principalmente en relación con patógenos de importancia agrícola. El presente estudio tuvo como objetivo investigar la actividad antimicrobiana del extracto alcohólico de propóleo de *T. clavipes* (Borá), *S. bipunctata* (Tubuna) y *T. angustula* (Jataí), comparándolo con el extracto etanólico de propóleo de *A. mellifera*. Los extractos autoclavados y no autoclavados se evaluaron a concentraciones de cero, 1,6 %; 3,2 %; 6,4 % y 12,0 % v/v. El desarrollo fúngico se determinó midiendo el diámetro del micelio hasta el decimocuarto día de la inoculación, con cinco repeticiones; la inhibición del crecimiento se calculó en relación con el control. De acuerdo con los resultados, la mayor concentración de compuestos fenólicos y flavonoides se identificó en el extracto etanólico de propóleo de *A. mellifera* en comparación con los extractos de ASA. Los resultados del presente estudio confirmaron el efecto inhibitorio sobre el crecimiento de los fitopatógenos *Colletotrichum gloeosporioides*, *Fusarium* sp., *Botryosphaeria* sp. y *Botrytis* sp., principalmente a concentraciones de 6,4 % y 12,0 % v/v cuando se evaluó en medio no autoclavado, seguido de medio autoclavado. El hongo *Botrytis* sp. fue el fitopatógeno que sufrió mayor inhibición a partir de 3,2 mL·L^{-1}, con el extracto de propóleo de *T. angustula*. Los resultados obtenidos demuestran que los extractos alcohólicos de propóleo ASF tienen potencial para el control de hongos fitopatógenos de interés agrícola.
Palabras clave: Antifúngicos naturales; Compuestos fenólicos; Flavonoides totales; Meliponini; Propóleos.

1. Introduction

The growing global demand for foods with high-added biological value has driven many farmers to seek alternative methods to control diseases in cultivated plants. Fungal pathogens, one of the promoters of these diseases, are responsible for 30 % of all diseases in crops, with annual losses across the globe on the scale of billions of dollars (Shuping and Eloff, 2017).

In the search for an immediate solution came the massive use of pesticides, which consequently facilitated the emergence of multiresistant strains in the time scale and also promoted environmental contamination, which represents a serious problem for the quality of crops and human health. Such results cause frustration and high costs, requiring the stimulation and development of green biotechnology, especially in plant-pathogen interactions, for the production of food with quality (Amorim et al., 2018; Gomes et al., 2021). The use of products of natural origin presents itself as a promising alternative in the control of diseases in plants with care in maintaining the sustainability and resilience of production systems (Bankova et al., 2014; Campelo et al., 2015).

Among the alternative products, those derived from propolis stand out. Propolis is produced by eusocial bees, represented by *Apis mellifera* and the bees of the Meliponini tribe, known as stingless bees (SB). These insects collect resins from certain plants and in the nest add glandular substances to the processing resulting in propolis. SBs add a percentage of propolis to the wax to form the structures of the nest and, among them, some species still use a certain amount of clay, producing geopropolis or batume with a similar function, such as sealing and waterproofing the hive (Pasupuleti et al., 2017). Bees use propolis as a colony sanitizing and defense element due to its physical appearance with sticky and immobilizing characteristics, inactivating a possible enemy. Such attributes and active principles present in propolis are dependent, to a greater or lesser extent, on the bee species, resin-supplying plants, and seasonality of climatic factors, among others (Melo et al., 2014).

Propolis extract, as a derivative element, has antimicrobial, antifungal, or fungistatic activities, without promoting toxic risks (Maqbool et al., 2010; Cunha, et al 2013; Gama, 2015; Oliveira et al., 2017). These properties are linked to compounds identified in its constitution as anthocyanins, flavonoids, tannins, saponins, terpenoids, polypeptides, and lecithins, among others, hence its antibacterial, antifungal, antioxidant, and anti-inflammatory activities (Pazin et al., 2017). These pharmacological characteristics of propolis are so evident that they led to its use in folk medicine, with already proven applications as an antioxidant, anti-inflammatory, and antimicrobial agent (Pinto et al., 2011).

Much has been published about the composition of propolis from *A. mellifera* (European or Africanized bee), its botanical origin, and biological activity (Bankova, 2005; Farooqui and Farooqui, 2012; Silva-Carvalho et al., 2015). However, few studies address the composition, properties, and applications of propolis or geopropolis of the stingless bee group (Araujo et al., 2015; Cunha et al., 2015).

Thus, this study aimed to evaluate the chemical composition and the inhibitory/antifungal activity on phytopathogenic fungi of agricultural interest of the ethanolic extract of propolis from three species of SB (*Tetragona clavipes*, *Scaptotrigona bipunctata*, and *Tetragonisca angustula*), comparing them with propolis produced by *A. mellifera*.

2. Materials and Methods

2.1 Collection of propolis and extract preparation

Propolis samples from SBs and *A. mellifera* were collected in May 2022. Information regarding the species, common name, and sample collection site are presented in Table 1.

Table 1 – Information referring to the propolis samples studied in the present work.

Species	Common name	Municipality	Geographic Coordinates
Tetragone clavipes	Borá	Nova Petrópolis	29°22’45.9”S 52°18’37.2”W
Scaptotrigona bipunctata	Tubuna	Osório	29°47’48.9”S 50°21’28.8”W
Tetragonisca angustula	Jataí	Cambará	29°20’06.8”S 50°28’48.8”W
Apis mellifera	European bee	Cambará	29°20’06.8”S 50°28’48.8”W

Source: Authors (2022).

After collection, the materials were submitted to an extraction procedure, using a ratio of 30 g of sample to 70 mL of ethanol 96 % v/v (1:2), based on the procedure suggested by Garcia et al. (2004). It is noteworthy that ethanol was the solvent used in the production of propolis extract, as it is easily accessible and plays an important role in preserving the antimicrobial properties of propolis if it is used well for this purpose (Quiroga et al., 2006). The extracts were kept at room temperature and protected from sunlight for 60 days. The extracts were shaken manually once a day.

2.2 Determination of the contents of phenolic compounds and flavonoids in the extracts

The contents of total phenolic compounds and flavonoids were determined by analyzing the extract after dilution with ethanol 96 % v/v, in the ratio of 5 mL of extract to 95 mL of ethanol (ratio of 1:19). The content of phenolic compounds was determined by the Folin-Ciocalteu method, according to the procedure described by Pereira et al. (2018). The total flavonoid content was determined by the aluminum chloride spectrophotometric method, according to the procedure proposed by Matic et al. (2017).

2.3 Antifungal activity of propolis extracts

The antifungal activity of the extracts was evaluated on the mycelial growth of the phytopathogenic fungi Colletotrichum gloeosporioides, Fusarium sp., Botryosphaeria sp., and Botrytis sp., all isolated from grapevine culture.

The test was carried out by mixing the extracts obtained with potato-dextrose-agar (PDA) medium, with and without sterilization by autoclaving. Concentrations of zero, 16, 32, 64, and 120 $mL \cdot L^{-1}$ (zero; 1.6 %; 3.2 %; 6.4 %, and 12.0 % v/v) were tested. The mixture was performed by adding the different extracts to the liquefied culture medium at a temperature of 40 °C. After solidification of the PDA medium, a 4 mm diameter disc of the mycelium of the fungus to be tested was placed in the center of the Petri dish.

Incubation was carried out in a BOD chamber at a temperature of 25 ºC and a photoperiod of 12 h for 14 days. The fungal mycelial growth was evaluated on the 14th day by measuring the orthogonal diameter of the colonies. The inhibition percentage was calculated following equation 1.

$$I\ (\%) = 100 \times \left(1 - \frac{D_T}{D_C}\right) \quad (1)$$

Being 'I' the inhibition percentage (%), 'D_T' the diameter of the colony (mm), and 'D_C' the diameter of the control group (mm).

2.4 Experimental design and statistical analysis

The contents of phenolic compounds and flavonoids were determined using triplicates. Antifungal activity tests followed a bifactorial design (factors: type of extract and concentration) and were performed in quintuplicate, with each replicate consisting of a Petri dish. Each fungus was isolated separately.

The results obtained were checked for homoscedasticity (Levene's test) and normality of the residues (Shapiro-Wilk test), being subsequently submitted to analysis of variance. The means were compared by Tukey's test at a 5 % error probability ($\alpha = 0.05$) with the aid of the AgroEstat software.

3. Results and Discussion

The results of the contents of total phenolic compounds and flavonoids for the studied extracts are compiled in Table 2. The highest concentration of phenolic and flavonoid compounds was identified in the extract of *A. mellifera* propolis, followed by *T. clavipes*, *T. angustula*, and *S. bipunctata* propolis extracts, which have not differed statistically among themselves. The purity content of A. mellifera propolis may have some interference in the differences since the European bee does not aggregate propolis with wax nor does it produce geopropolis.

The complex chemical composition of propolis and the influence that the flora, species of bee, location, and other geographic and climatic characteristics may interfere in the patterns and specificities of each propolis is also highlighted.

Studies already carried out on propolis from *A. mellifera* indicated a very large variation in the composition, both qualitative and quantitative, of the phenolic compounds (Silva et al., 2013; Cardozo et al., 2015; Sousa et al. 2019; Ferreira et al., 2020; Letullier et al., 2020; Ferreira et al., 2022).

Table 2 - Contents of total phenolic compounds and total flavonoids of different alcoholic extracts of propolis from stingless bees and *A. mellifera*.

Extract	Phenolic compounds (mg/100 mL)[1]	Flavonoids (mg/100 mL)[2]
T. clavipes (bora)	63.5±4.4 b	171.9±8.4 b
S. bipunctata (tubuna)	54.8±1.5 b	51.8±4.7 d
T. angustula (jataí)	39.8±0.5 b	102.7±6.5 c
A. mellifera (European bee)	480.4±11.1 a	584.9±9.2 a
p-value	<0.0001	<0.0001
Coefficient of variation (%)	13.59	3.24

[1] – Content of phenolic compounds determined as milligrams-equivalent of gallic acid per 100 mL of extract. [2] – Flavonoid content determined as milligrams-equivalent of quercetin per 100 mL of extract. Column means followed by the same letter do not differ statistically by Tukey's test at 5 % error probability. Source: Authors (2022).

According to Silva et al. (2016), studying eight geopropolis samples collected from *Plebeia* Aff. *flavocincta*, the samples had high levels of phenolic compounds (using tannic acid as standard) and flavonoids (using quercetin as standard), relative to the others where, for total phenols, the values were (5.7±0.4 mg/100 g and 9.4±0.3 mg/100 g) and higher concentrations of total flavonoids (10.05 mg/100 g and 22.00 mg/100 g of geopropolis).

From the results observed, it was possible to observe that the inhibitory/antifungal activity increased with the increase of the concentration of alcoholic extract of propolis from stingless bees (SB) and alcoholic extract of *A. mellifera*, regardless of whether the extracts were autoclaved or not (Tables 3 to 6).

Table 3 shows that the phytopathogenic fungus Fusarium sp., suffered partial and total inhibition, from concentrations of 3.2 $mL \cdot L^{-1}$ and 12 $mL \cdot L^{-1}$ respectively, when not autoclaved. The opposite result occurred when the different SB and A. mellifera extracts were autoclaved, resulting in reduced inhibition of the phytopathogenic fungus *Fusarium* sp. Table 3.

Table 3 - Inhibition effect (%) of different concentrations of propolis extracts from stingless bees and *A. mellifera*, in autoclaved and non-autoclaved medium, on the mycelial growth of *Fusarium* sp., after 14 days of incubation.

Concentration (% v/v)	*T. clavipes*		*S. bipunctata*		*T. angustula*		*A. mellifera*	
	Non-autoclaved medium (%)	Autoclaved medium (%)	Non-autoclaved medium (%)	Autoclaved medium (%)	Non-autoclaved medium (%)	Autoclaved medium (%)	Non-autoclaved medium (%)	Autoclaved medium (%)
zero	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa
1.6	0.0Aa	0.0 Aa	29.0 Bb	0.0 Aa	16.6 Ba	15.3 Ba	47.7 Cb	34.0 Cb
3.2	31.8 Ab	0.0 Aa	32.8 Ab	77.1 Ab	60.2 Bb	17.6 Aab	50.3 Bb	35.3 Bb
6.4	80.3 Ac	0.0 Aa	80.3 Ac	52.0 BC	76.7 Ac	22.7 Cb	84.6 Ac	53.5 cc
12.0	100.0 Ad	56.5 Ab	100.0 Ad	46.2 BC	100.0 Ad	34.4 BC	100.0 Ad	75.5 Cd

Means followed by the same letter, uppercase in line and lowercase in column, do not differ statistically by Tukey's test at 5 % error probability. Source: Authors (2022).

According to Ozan et al. (2007), the concentration of 1.0 $mg \cdot L^{-1}$ of the ethanolic extract of *A. mellifera* propolis, caused the strongest inhibition growth on *Fusarium* sp.. The identified antifungal properties were mainly attributed to compounds from the flavonoid group. Davari and Ezazi (2022) also reported an inhibitory effect of propolis ethanol extract against *Fusarium* sp..

Propolis produced by Africanized bees is currently being investigated for its antimicrobial action. Several studies suggest that the main compounds responsible for its antimicrobial activity are flavonoids and phenolic acid esters (Sforcin et

al., 2000; Pietta et al., 2002). Other studies also addressed the antimicrobial and antifungal effects of propolis, such as those of Yang et al. (2011) and Curifuta et al. (2012).

Studies evaluating *A. melifera* propolis extracts have demonstrated promising antibacterial, antifungal, antiviral, and general antimicrobial properties (Kalogeropoulos et al., 2009). There are also literature reports on the antifungal activity of propolis against pathogenic fungi, such as *Candida albicans* and dermatophyte fungi. Other fungi species that are reported as susceptible to propolis extracts are *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Alternaria alternata*, and *Fusarium* sp., among others (Curifuta et al., 2012).

Table 4 shows that the phytopathogenic fungus *Botryosphaeria* sp. was completely inhibited from non-autoclaved geopropolis alcoholic extracts of *S. bipunctata* and *T. angustula* at 3.2 mL·L^{-1} and 12 mL·L^{-1} and, *T. clavipes* showed a total inhibitory result at 12 mL·L^{-1}. The same was not observed with the autoclaved extracts (Table 4), whose total inhibition was identified only in the alcoholic extract of *A. mellifera* at 12 mL·L^{-1}.

Table 4 – Inhibition effect (%) of different concentrations of propolis extracts from stingless bees and *A. mellifera*, in autoclaved and non-autoclaved medium, on the mycelial growth of *Botryosphaeria* sp., after 14 days of incubation.

Concentration (% v/v)	*T. clavipes*		*S. bipunctata*		*T. angustula*		*A. mellifera*	
	Non-autoclaved medium (%)	*Autoclaved medium (%)*	*Non-autoclaved medium (%)*	*Autoclaved medium (%)*	*Non-autoclaved medium (%)*	*Autoclaved medium (%)*	*Non-autoclaved medium (%)*	*Autoclaved medium (%)*
zero	26.2 Aa	0.0 Aa	26.2 Aa	0.0 Aa	26.2 Aa	0.0 Aa	26.2 Aa	0.0 Aa
1.6	58.2 Ab	0.0 Aa	53.3 Abb	0.0 Aa	64.3 Abb	0.0 Aa	64.4 Bb	0.0 Aa
3.2	66.6 Ab	0.0 Aa	59.4 Ab	0.0 Aa	80.9 Bb	0.0 Aa	69.5 Abb	25.8 Bb
6.4	67.6 Ab	0.0 Aa	100.0 Bc	0.0 Aa	100.0 Bc	32.2 Bb	74.4 Abb	75.9 Cc
12.0	100.0 Ac	78.9 Cb	100.0 Ac	0.0 Aa	100.0 Ac	56.0 Bc	100.0 Ac	100.0 Dd

Means followed by the same letter, uppercase in line and lowercase in column, do not differ statistically by Tukey's test at 5 % error probability. Source: Authors (2022).

Similar results were observed for the fungus *C. gloeosporioides*, whose total inhibition occurred at 12 mL·L^{-1}, regardless of the evaluated extracts, when not autoclaved (Table 5). The autoclaved alcoholic extracts of *T. angustula* and *A. mellifera* at the dose of 12 mL·L^{-1} were 100 % efficient in controlling *C. gloeosporioides* (Table 5).

Table 5 – Inhibition effect (%) of different concentrations of propolis extracts from stingless bees and *A. mellifera*, in autoclaved and non-autoclaved medium, on the mycelial growth of *Colletotrichum gloeosporioides*, after 14 days of incubation.

Concentration (% v/v)	*T. clavipes*		*S. bipunctata*		*T. angustula*		*A. mellifera*	
	Non-autoclaved medium (%)	*Autoclaved medium (%)*	*Non-autoclaved medium (%)*	*Autoclaved medium (%)*	*Non-autoclaved medium (%)*	*Autoclaved medium (%)*	*Non-autoclaved medium (%)*	*Autoclaved medium (%)*
zero	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa
1.6	37.3 Bb	0.0 Aa	68.6 Db	0.0 Aa	0.0 Aa	0.0 Aa	50.3 Cb	47.6 Bb
3.2	61.5 cc	16.6 Aa	70.9 Cb	14.9 Aa	17.1 Aa	17.1 Aa	53.8 Bb	47.9 Bb
6.4	100.0 Cd	36.6 Ab	100.0 Cc	24.3 Aab	19.1 Aa	18.2 Aa	65.8 Bc	65.8 Bc
12.0	100.0 Ad	76.2 Bc	100.0 Ac	36.4 Ab	100.0 Ab	100.0 Cb	100.0 Ad	100.0 Cd

Means followed by the same letter, uppercase in line and lowercase in column, do not differ statistically by Tukey's test at 5 % error probability. Source: Authors (2022).

Another important phytopathogenic fungus, *Botrytis* sp., evaluated in this work, was completely inhibited from 3.2 mL·L^{-1} with all non-autoclaved SB propolis extracts. The alcoholic extract of *A. mellifera* was efficient at 12 mL·L^{-1}. (Table 6). The autoclaved extracts that showed an inhibitory effect on the mycelial growth of *Botrytis* sp. were *T. angustula* from 3.2 of mL·L^{-1}, *T. clavipes* and *S. bipunctata* from in 6.4 mL·L^{-1}, and *A. mellifera* with 12 mL·L^{-1} (Table 6).

Table 6 - Inhibition effect (%) of different concentrations of propolis extracts from stingless bees and A. mellifera, in autoclaved and non-autoclaved medium, on the mycelial growth (mm) of Botrytis sp., after 14 days of incubation.

Concentration (% v/v)	*T. clavipes*		*S. bipunctata*		*T. angustula*		*A. mellifera*	
	Non-autoclaved medium (%)	*Autoclaved medium (%)*	*Non-autoclaved medium (%)*	*Autoclaved medium (%)*	*Non-autoclaved medium (%)*	*Autoclaved medium (%)*	*Non-autoclaved medium (%)*	*Autoclaved medium (%)*
zero	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa	0.0 Aa
1.6	73.4 Bb	45.3 Bb	81.0 Cb	29.0 Ab	83.4 Cb	68.3 Cb	55.4 Cb	74.4 Bb
3.2	100.0 Bc	52.7 Ab	100.0 Bc	57.2 Ac	100.0 Bc	100.0 Bc	57.0 Ab	51.8 Ab
6.4	100.0 Bc	100.0 Bc	100.0 Bc	100.0 Bd	100.0 Bc	100.0 Bc	74.4 Ac	73.5 Ac
12.0	100.0 Ac	100.0 Ac	100.0 Ac	100.0 Aa	100.0 Ac	100.0 Ac	100.0 Ad	100.0 Ad

Means followed by the same letter, uppercase in line and lowercase in column, do not differ statistically by Tukey's test at 5 % error probability. Source: Authors (2022).

Mechanisms of antifungal activity using propolis extracts are addressed in the literature. The fungal cell membrane may be a possible target of a propolis extract; another possibility is the triggering of apoptosis (Gucwa et al., 2018; Corrêa et al., 2020). Other studies also showed that propolis extract can inhibit extracellular phospholipase activity, leading to attenuation of cell adhesion to the epithelium (D'Auria et al., 2003).

The antimicrobial activity verified in this work is consistent with the evolutionary heritage of these insects, as propolis is characterized by inhibiting the growth of undesirable microorganisms in the hive, producing substances rich in polyphenols, with bactericidal and fungicidal functions (Viuda-Martos et al., 2008; Bankova et al., 2014; Massaro et al., 2014; Campelo et al., 2015; Silva et al., 2016; Anjum et al., 2019; Lavinas et al., 2019; Silva et al., 2019; Ali and Kunugi, 2020; Ferreira et al., 2020; Tran et al., 2020; Fernández-Calderón et al., 2020). According to some works carried out by Dutra (2012), Araújo (2013), Batista (2016), and Cunha (2017), propolis/geopropolis from *Melipona fasciculata* is composed of some classes of chemical compounds, such as phenolic acids, hydrolyzable tannins, triterpenes, among others. These compounds have biological activities such as antioxidant, antileishmanial, anthelmintic, antimicrobial, and antitumoral, acting as a defense resource of the hive against these classes of pathogens.

4. Conclusion

By the results obtained for antifungal activity, the ethanolic extracts of propolis from the three species of bees evaluated showed promising inhibitory activity as a potential alternative to the indiscriminate use of pesticides. Other studies to test the stability and practicality of propolis-based compounds need to be stimulated, to make the use of propolis and its derivatives more widespread and easier for use in agriculture, especially small producers and organic production systems.

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