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Nematicidal activity of Brazilian honeys

Atividade nematicida de méis brasileiros

Actividad nematicida de las mieles brasileñas

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Abstract
In Brazil, there is a great cultural and social diversity, which leads to the use of several natural products due to their ethnopharmacological properties, incluing honey. Honey is a nutrient-rich food that has several beneficial activities, such as: anti-inflammatory, antioxidant, antimicrobial and nematicidal actions. However, there are no studies regarding the nematicidal effect of honeys from Brazil. Thus, the objective of this study was to evaluate the nematicidal action of honeys of *Apis mellifera* L. from orange, avocado, eucalyptus, multifloral and honey from *Tetragonisca angustula* species (known as Jataí) on *Panagrellus* sp. nematodes. In the nematicidal assays, solutions of these honeys were used in concentrations of 3; 1.5; 0.75 and 0.5% (w/v). The control group contained only distilled water. In assays with a concentration of 0.5% of avocado honey and *T. angustula* bee honey, a significant reduction was demonstrated, compared to the control group (respectively: 41.4% and 47.2%). The results obtained demonstrated, for the first time, that *A. mellifera* L. honey from avocado flowering and *T. angustula* honey showed significant nematicidal activity against juveniles of the free-living nematode model *Panagrellus* sp. Therefore, further studies on the compositions of these honeys must be developed.
Keywords: *Apis mellifera* L.; *Tetragonisca angustula*; Nematode; Honey.

Resumo
No Brasil há uma grande diversidade cultural e social, levando ao uso de diversos produtos naturais devido às suas propriedades etnofarmacológicas, incluindo o mel. O mel é um alimento rico em nutrientes que possui diversas atividades benéficas, tais como: ação anti-inflamatória, antioxidante, antimicrobiana e nematicida. No entanto, não existem estudos sobre o efeito nematicida dos méis do Brasil. Assim, o objetivo deste estudo foi avaliar a ação nematicida de méis de *Apis mellifera* L. de laranja, abacate, eucalipto, multifloral e mel da espécie *Tetragonisca angustula* (conhecida como Jataí) sobre nematóides *Panagrellus* sp. Nos ensaios nematicidas foram utilizadas soluções destes méis nas concentrações de 3; 1,5; 0,75 e 0,5% (m/v). O grupo controle continha apenas água destilada. Nos ensaios com concentração de 0,5% de mel de abacate e mel da abelha *T. angustula*, foi demonstrada redução significativa, em relação ao grupo controle (respectivamente: 41,4% e 47,2%). Os resultados obtidos demonstraram, pela primeira vez, que o mel de *A. mellifera* L. da floração do abacateiro e o mel de *T. angustula* apresentaram atividade nematicida significativa contra juvenis do nematóide de vida livre modelo *Panagrellus* sp. Portanto, mais estudos sobre as composições desses méis devem ser desenvolvidos.
Palavras-chave: *Apis mellifera* L.; *Tetragonisca angustula;* Nematoide; Mel.

Resumen
En Brasil, existe una gran diversidad cultural y social, lo que conduce al uso de varios productos naturales debido a sus propiedades etnofarmacológicas, entre ellos la miel. La miel es un alimento rico en nutrientes que tiene varias actividades beneficiosas, tales como: acción antiinflamatoria, antioxidante, antimicrobiana y nematicida. Sin embargo, no existen estudios sobre el efecto nematicida de las mieles de Brasil. Así, el objetivo de este estudio fue evaluar la acción nematicida de mieles de *Apis mellifera* L. de naranjo, aguacate, eucalipto, multifloral y miel de la especie *Tetragonisca angustula* (conocida como Jataí) sobre nematodos *Panagrellus* sp. En los ensayos de nematicidas se utilizaron soluciones de estas mieles en concentraciones de 3; 1,5; 0,75 y 0,5% (p/v). El grupo de control contenía solo agua destilada. En los ensayos con una concentración de 0,5% de miel de aguacate y miel de abeja *T. angustula*, se demostró una reducción significativa en relación al grupo control (respectivamente: 41,4% y 47,2%). Los resultados obtenidos demostraron, por primera vez, que la miel de *A. mellifera* L. de floración de aguacate y la miel de

T. angustula mostraron actividad nematicida significativa contra juveniles del nematodo de vida libre modelo *Panagrellus* sp. Por lo tanto, se deben desarrollar más estudios sobre las composiciones de estas mieles.
Palabras clave: *Apis mellifera* L.; *Tetragonisca angustula;* Nematodo; Miel.

1. Introduction

In Brazil, there is a great cultural and social diversity, which leads to the use of several natural products due to their ethnopharmacological properties. Among these natural products, honey stands out. Honey is a nutrient-rich food and has been used since ancient times in Egypt, Rome and Greece as a sweetener and also as a natural medicine (Kuropatnicki et al., 2018). Its composition varies according to the nectar of the plant species and the species of bee that produces it (Marcolin et al., 2021).

In addition to the honeybee (*Apis mellifera* L.), there are other stingless species that produce honey that are widely consumed in the world (Torres et al., 2003). One of these species is known in Brazil as "Jataí" (*Tetragonisca angustula)*, present in much of Latin America, which produces honey in smaller quantities compared to *A. mellifera* L., but with a much-appreciated sweetness and aroma (Anacleto et al., 2009).

Parasitic nematodes are responsible by several diseases that affects the health of humans, animals, and plants. In fact, soil-transmitted helminths infect approximately one billion people around the world, being the most neglected tropical disease (Loukas et al., 2021). Moreover, plant parasitic nematodes are another kind of nematodes that cause severe damage to agriculture (Pires et al., 2022).

Inadequate control of parasitic nematodes leads to the selection of resistant strains, which makes their control very difficult. Thus, the search for new drugs and alternatives to control these parasites is urgent and desired (Idris et al., 2019). Therefore, the use of natural products can be an interesting alternative control approach (Jayawardene et al., 2021).

There are several beneficial activities of honey already described around the world, such as: anti-inflammatory, antioxidant, antimicrobial and nematicidal actions (Bilal and Azim, 2018). Another bioactive products collected by honeybees (*Apis mellifera*) from plant sources already had their nematicidal action reported in literature, e.g., propolis (Salas et al. 2016).

In this context, the literature reports the presence of proteins and peptides from honeys with nematicidal action (Sajid and Azim, 2012; Bilal and Azim, 2018). However, there are still no studies on the action of honeys from Brazil on nematodes.

In previous papers, the authors Sajid and Azim (2012) and Bilal and Azim (2018) used the free-living nematode model nematode *Caenorhabditis elegans* as a model. However, there is no report in the literature on the use of the model nematode *Panagrellus* sp. to evaluate the nematicidal activity of honeys. This nematode is easily obtained and maintained, and is used in several studies as a target of nematicidal agents (Braga et al., 2012; Stefanello and Loreto, 2022).

Thus, the aim of this study was to evaluate the action of honeys of *A. mellifera* L. from different native flowering plants in Brazil and of *T. angustula* on the model nematodes *Panagrellus* sp.

2. Methodology

The study is quali-quantitative study (Pereira et al., 2018) and was carried out at the Department of Chemistry, Federal University of Lavras, located in the municipality of Lavras, Minas Gerais, Brazil. The honeys used in the present study were acquired from the local market. Honeys of *A. mellifera* L. from different flowering plants native to Brazil were used: avocado, orange, eucalyptus, multifloral. *T. angustula* honey was also purchased commercially and used.

The free-living nematodes *Panagrellus* sp. were purchased commercially and later cultivated following the methodology described by Sufiate et al. (2017), adding oatmeal with water in Petri dishes. Subsequently, they were kept in a dark environment at approximately 25 °C. Culture media were renewed weekly.

Nematicidal activities of the pure honeys were evaluated on juvenile nematodes in vitro, using 4 different concentrations: 3, 1.5, 0.75 and 0.5% (m/m) (Sajid and Azim, 2012). Dilutions were performed using distilled water. The control group contained only distilled water.

For the assays with nematodes, six groups were formed in sterile tubes, five groups were treated with the respective honeys (in each of the concentrations described above) and one control group. For each group, 6 replicates were performed. The whole experiment was repeated three times.

About 40 juveniles of *Panagrellus* sp. were poured into sterile tubes containing the honeys. Control groups had the same number of juveniles. Sterile tubes were incubated at 25°C, for 24 hours. After this time interval, the total number of live juveniles present in each tube of the groups was counted by means of optical microscopy (Braga et al., 2012). Juvenile mortality was verified by the completeness of the cuticle composition and body content of the nematode and also by means of movement of the same.

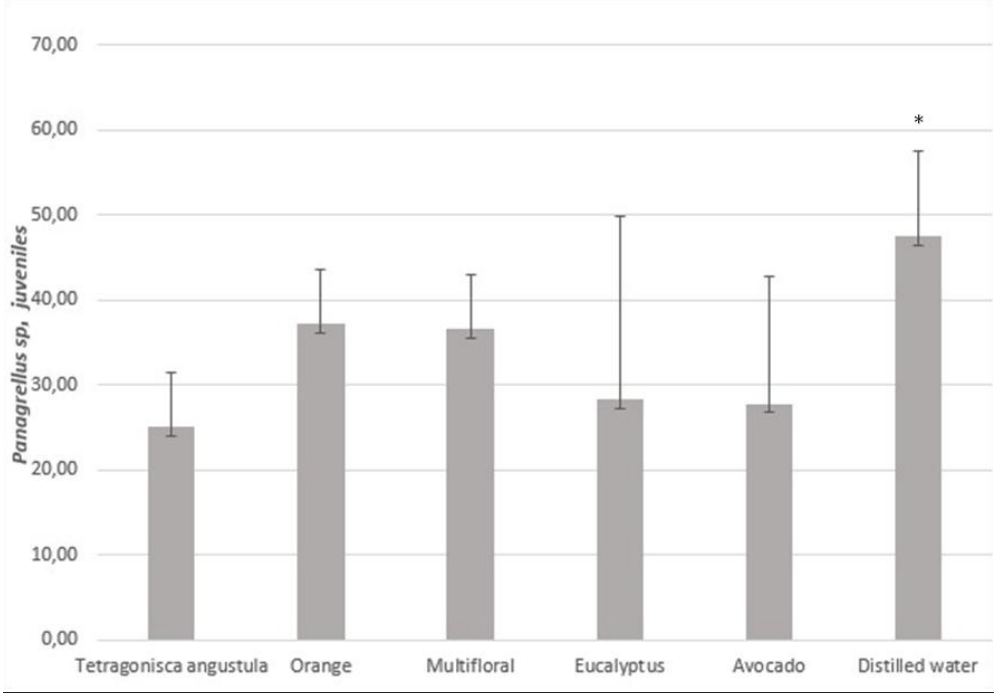
The data obtained in the assays underwent analysis of variance at significance levels of 5% probability and were evaluated by Tukey's test at the level of 5% probability (Ayres et al., 2003). Subsequently, the percentage of reduction in the mean number of juveniles of *Panagrellus* sp. was calculated according to the following equation:

$$\%\text{Reduction} = \left(\frac{\text{Average number of juveniles from control} - \text{Average number of juveniles from treatment}}{\text{Average number of juveniles from control}}\right) \times 100$$

3. Results

The results obtained in this study showed that there was a significant reduction ($p<0.05$) of juveniles of *Panagrellus* sp. treated with *A. mellifera* L. honey from avocado flowering and with *T. angustula* honey in relation to the control with distilled water, only when used at a concentration of 0.5%. For all other tested honeys (orange, eucalyptus and multifloral), and also *A. mellifera* L. honey from avocado flowering and with *T. angustula* honey, at concentrations of 3, 1.5 and 0.75% there was no significant difference ($p>0.05$) between the groups evaluated (Figure 1).

Figure 1 - Average number and standard deviation (bars) of *Panagrellus* sp. juveniles from assays. Five groups were treated with honeys of *Apis mellifera* L. from orange, avocado, eucalyptus, multifloral and honey from *Tetragonisca angustula* and one control group (distilled water) at the concentration of 0.5%. At concentrations of 3, 1.5 and 0.75% there was no significant difference between the groups evaluated. There was a significant difference ($p < 0.05$) between the treated groups with *A. mellifera* L. honey from avocado flowering and *T. angustula* honey and control group (asterisk) by Tukey's test.



Fonte: Autores.

The reduction percentages of *A. mellifera* L. honeys from avocado flowering and *T. angustula* in relation to the control with distilled water, after 24 hours of incubation, were respectively: 41.4% and 47.2%.

4. Discussion

Natural honey has been used in traditional medicine around the world for millennia. There are several therapeutic properties associated with its consumption, such as antimicrobial, antioxidant, anticancer, wound healing and nematicidal activities (Bogdanov et al., 2008; Azim & Sajid, 2009; Pena-júnior et al., 2022). In relation to this last action, to the best of our knowledge, there are no scientific publications regarding the honeys of *A. mellifera* L. and *T. angustula* native to Brazil. Therefore, the present study is a pioneer, the first study to evaluate the nematicidal action of honeys of *A. mellifera* L. from different native flowering plants in Brazil and of *T. angustula* against nematodes.

In this context, it is important to highlight that parasitic nematodes cause a number of health problems for animals and humans, affecting billions of people worldwide (Jourdan et al., 2018; Pires et al., 2022). In addition, plant parasitic nematodes generate immense losses in agriculture, leading to the loss of billions of dollars, as well as affecting productivity and food production around the world (Kumar et al., 2020; Kantor et al., 2022). Thus, the search for alternatives to control these parasites is extremely important. Here, we demonstrate that c and honey from *T. angustula* have potential for use in nematode control, since they showed in vitro nematicidal action on *Panagrellus sp.* These free-living nematodes are excellent models as they are similar to parasitic species (Srinivasan et al., 2013; Burns et al. 2015). In previous studies, nematodes of the genus *Panagrellus* were successfully used as a model for evaluating nematicidal activity (Soares et al., 2015; Gomes et al., 2018; Ferreira et al., 2019). Thus, a significant nematicidal activity against these organisms points to a great potential for use on

parasitic nematodes.

Avocado honey contains perseitol, a sugar-alcohol that was exclusive identified in it. In addition, this honey has a high antioxidant power (Bonvehi et al., 2019). Regarding *T. angustula* honey, Pucciarelli et al. (2014) demonstrated that it's microbiological and physicochemical properties are different from those of *A. mellifera* honeys. However, other studies must be carried out to find out which substance or substances were responsible for the nematicidal action observed in the present study.

Nematicidal action of honeys has already been described in the literature by Azim and Sajid (2009), Sajid and Azim (2012) and Bilal and Azim (2018). However, those authors only used honey from honeybees (*A. mellifera* L.) and from native flowers of Pakistan. On the other hand, they demonstrated that the nematicidal action of honeys was linked to their protein portions. Sajid and Azim (2012) reported that a 5.5 kDa glycoconjugate was the main nematicidal component of the evaluated honey. However, Bilal and Azim (2018) observed that honey glycoprotein complexes (with molecular masses of 260 kDa and 160 kDa) constituted by aggregates containing the main royal jelly protein 1, showed strong nematicidal activity. Those glycoproteins caused regulation of gene transcripts involved in the primary metabolism and physiology from the nematode. Thus, glycoproteins may be one of the possible actors responsible for the nematicidal action observed in the present study.

Stingless bee honeys are appreciated and consumed all over the world (Torres et al., 2003). However, these honeys had not yet had their nematicidal potential investigated, until the present study. Here, we demonstrate that the honey of *T. angustula* showed significant nematicidal activity, with a percentage reduction, in relation to the control group, of 47.2%. Thus, new possibilities for research involving this kind of honey derived from a stingless bee are opened.

5. Conclusion

The results obtained demonstrated, for the first time, that *A. mellifera* L. honey from avocado flowering and *T. angustula* honey showed significant nematicidal activity against juveniles of the free-living nematode model *Panagrellus* sp. Therefore, with the need for further studies on which compositions of these honeys are responsible for the nematicide action, new experiments will be conducted by our research group.

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