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PHYTOTOXIC POTENTIAL OF THE GEOPROPOLIS EXTRACTS OF THE JANDAIRA STINGLESS BEE (*Melipona subnitida*) IN WEEDS[1]

DANIEL SANTIAGO PEREIRA[2]*, JOÃO PAULO DE HOLANDA-NETO[3], MOZANIEL SANTANA DE OLIVEIRA[4], NATANAEL SANTIAGO PEREIRA[5], PATRÍCIO BORGES MARACAJÁ[6], ANTONIO PEDRO DA SILVA SOUZA FILHO[7]

ABSTRACT - The objective of the present research is to characterize the phytotoxic activity of geopropolis from the Jandaira bee (*Melipona subnitida* Ducke) in the state of Ceará in Northeast Brazil and to analyze its effects. Extracts were prepared in 80% v/v grain alcohol at 0.25, 0.5, 0.75, and 1.0%. Their effects were determined on seed germination, radicle elongation, and hypocotyl growth of the pasture weeds malícia (*Mimosa pudica*) and mata-pasto (*Senna obtusifolia*). Extract phytotoxicity varied as functions of plant species, application dosage, and plant organ. *M. pudica* was more sensitive to the inhibitory effects of geopropolis than *S. obtusifolia*. There was a phytotoxic effect of 50% (PE50) for *S. obtusifolia* in terms of seed germination and in rootlet development near the maximum applied concentrations. *M. pudica* had PE50 and PE90 at the minimum concentration (0.25%) and near the maximum (1.00%), respectively. Thus, geopropolis extracts from the Jandaira bee (*M. subnitida*) are potentially phytotoxic to certain plant species.

Keywords: Allelopathy. Wild bee propolis. Weed plants. Semiarid. Indigenous bees.

POTENCIAL FITÓXICO DE EXTRATOS DO GEOPROPOLIS DA ABELHA JANDAIRA (*M. subnitida*) EM PLANTAS DANINHAS

RESUMO - Este trabalho teve por objetivo caracterizar a atividade fitotóxica da geoprópolis de abelhas jandaira (*Melipona subnitida* Ducke), nativas do estado do Ceará, e analisar, comparativamente, seus efeitos. Os extratos obtidos com álcool cereal foram preparados em concentrações de 0,25, 0,5, 0,75 e 1,0%, tendo como eluente o etanol 80%, e testados sobre a germinação de sementes, alongamento da radícula e do hipocótilo das plantas daninhas de área de pastagens cultivadas, malícia (*Mimosa pudica*) e mata-pasto (*Senna obtusifolia*). Os extratos testados evidenciaram atividade fitotóxica em intensidades que variaram em função da planta receptora, concentração, e da parte da planta analisada. A espécie *M. pudica* apresentou maior sensibilidade aos efeitos inibitórios do que *S. obtusifolia*. A espécie *S. obtusifolia*, quando analisada isoladamente, apresentou Efeito Fitotóxico EF50 na germinação das sementes e no desenvolvimento da radícula em concentrações próximas à máxima utilizada, enquanto que a espécie *M. pudica* apresentou EF50 e EF90 nas concentrações mínimas e próximas à máxima, 0,25% e 1,00%. Os extratos da geoprópolis da abelha jandaira (*M. subnitida*) avaliados possuem em sua composição bioativos com potencial efeito fitotóxico às plantas daninhas estudadas.

Palavras-Chave: Alelopatia. Própolis de abelhas nativas. Plantas daninhas. Semiárido. Abelhas indígenas.

*Corresponding author
[1]Received for publication in 07/04/2016; accepted in 02/02/2017.
[2]Botanic Departament, Empresa Brasileira de Pesquisa Agropecuária, Belém, PA, Brazil; daniel.pereira@embrapa.br.
[3]Center of Research and extension for bees, Instituto Federal de Educação Ciência e Tecnologia do Sertão Pernambucano, Ouricuri, PE, Brazil; jpholandaneto@gmail.com.
[4]Department of Food Science and Technology, Universidade Federal do Pará, Belém, PA, Brazil; mozaniel.oliveira@yahoo.com.br.
[5]Laboratory of soil water for irrigation purposes and vegetable tissues, Instituto Federal de Educação Ciência e Tecnologia do Ceará, Limoeiro do Norte, CE, Brazil; natanael@ifce.edu.br.
[6]Center for food science and Agrifood technology, Universidade Federal de Campina Grande, Pombal, PB, Brazil; patriciomaracaja@gmail.com.
[7]Departament of Agroindustry, Empresa Brasileira de Pesquisa Agropecuária, Belém, PA, Brazil; antonio-pedro.filho@embrapa.br.

INTRODUCTION

Bee propolis composition is complex due to genetic differences in the bees that collect the resin and the varying geographical origins of the substances in it (BARBOSA, 2009).

Meliponiculture is the development and maintenance of colonies of indigenous stingless Meliponini bees, which are important pollinators. It is estimated that there are 350–600 native bee species in Brazil. They produce geopropolis, which consists of a mixture of vegetable resins, soil, and/or clay (MICHENER, 2007).

The Jandaira (*Melipona subnitida* Ducke) is a typical stingless bee. It belongs to the subfamily Meliponinae found in semiarid regions of Brazil. This species can be raised easily, including by women and children, and this human activity contributes to the conservation of the bees and their habitats including through the planting and protection of trees that serve as bee nesting sites. In return, the bees pollinate local native flora (OLIVEIRA CRUZ et al., 2004).

The chemical composition of propolis varies qualitatively and quantitatively depending on the region in which it originates. Some of its constituents, like beeswax, are of animal origin. Others are derived from plants. In general, the biological activity of propolis is attributed to its constituents of plant origin (SALATINO; TEIXEIRA; NEGRI, 2005).

Allelopathy is the study of the interactions of plants, fungi, algae, and bacteria with other organisms in an ecosystem. Many of these interactions are mediated by secondary metabolites produced and released into the environment. Allelopathy is a multidisciplinary science involving ecologists, chemists, soil scientists, agronomists, biologists, plant physiologists, and molecular biologists who contribute to the understanding of the complex interactions in an ecosystem (MACÍAS et al. 2007).

The investigation of allelopathic properties in plants can help to elucidate these interactions (GUSMAN; VIEIRA; VESTENA, 2012; SOUZA FILHO, FONSECA, ARRUDA, 2005). Several recent studies have examined plant species with allelopathic potential (SOUZA FILHO; ALVES, 2000; MACÍAS et al. 2007; SOUZA FILHO et al., 2009a, b; ZOGHBI et al., 2009; FORTES et al., 2009; GAZIRI; CARVALHO, 2009; AZAMBUJA et al., 2010; REJILA; VIJAYAKUMAR, 2011; MOURA et al., 2015; BATISTA et al., 2016; OLIVEIRA et al., 2016).

Weeds severely limit tropical agriculture. Therefore, controlling these species is crucial to its success. Weed control represents the major maintenance cost component in cultivated pastures. Broadleaf weeds, especially those of the Fabaceae (Leguminosae) family such as *Mimosa pudica* and S*enna obtusifolia*, are among those that most commonly infest cultivation areas (OLIVEIRA et al., 2016).

Extracts of *Baccharis dracunculifolia*, the main botanical source of the "green" propolis produced in Southeastern Brazil, has shown phytotoxicity and allelopathic potential. It inhibited germination and early seedling in seven plant species. Green propolis also induced structural abnormalities in the seedlings (GUSMAN, BITTENCOURT, VESTENA, 2008).

Studies on the application of geo/propolis are ongoing because some of its plant-based constituents may, in fact, be bioherbicidal allelochemicals of agricultural interest.

The social, economic, and ecological importance of Brazilian meliponae (*M. subnitida*) are socially, economically, and ecologically important. There is the potential to develop an international market of bee products, including geopropolis from *M. subnitida* geopropolis. To this end, the present study investigated the allelopathic activity of an ethanolic extract of geopropolis derived from *M. subnitida.* Its effects were tested on the seed germination and seedling development of two weed species common to agricultural fields, namely, *Mimosa pudica* (malícia) and *Senna obtusifolia* (mata-pasto).

MATERIAL AND METHODS

Beehives inhabited by *M. subnitida* were selected for geopropolis collection and organized in a meliponary located at 3°55′37.6″S and 38°21′51.8″W on the bank of the Catú river in the municipal district of Aquiraz, 30 km from Fortaleza City, capital of the state of Ceará, Brazil.

Materials were collected and extracts prepared in 2013 and the bioassay performed in 2014 in Belém City, capital of the state of Pará, Brazil.

Extract preparation

Thirty-gram samples were weighed out, minced, and transferred to a 250 mL Erlenmeyer flask containing 100 mL 96% v/v grain alcohol. The mixture was set on a magnetic stirrer for 5 d. The extract was filtered through a No. 3 filter paper. The filtrate was then refrigerated and the residue washed, stirred, and filtered twice more.

The extracts were maintained at 0°C for 72 h then filtered through a No. 2 filter paper to remove waxes. Extraction solvents were removed using a rotation evaporator under low pressure at 40–50°C for up to 1 h (Figure 1).

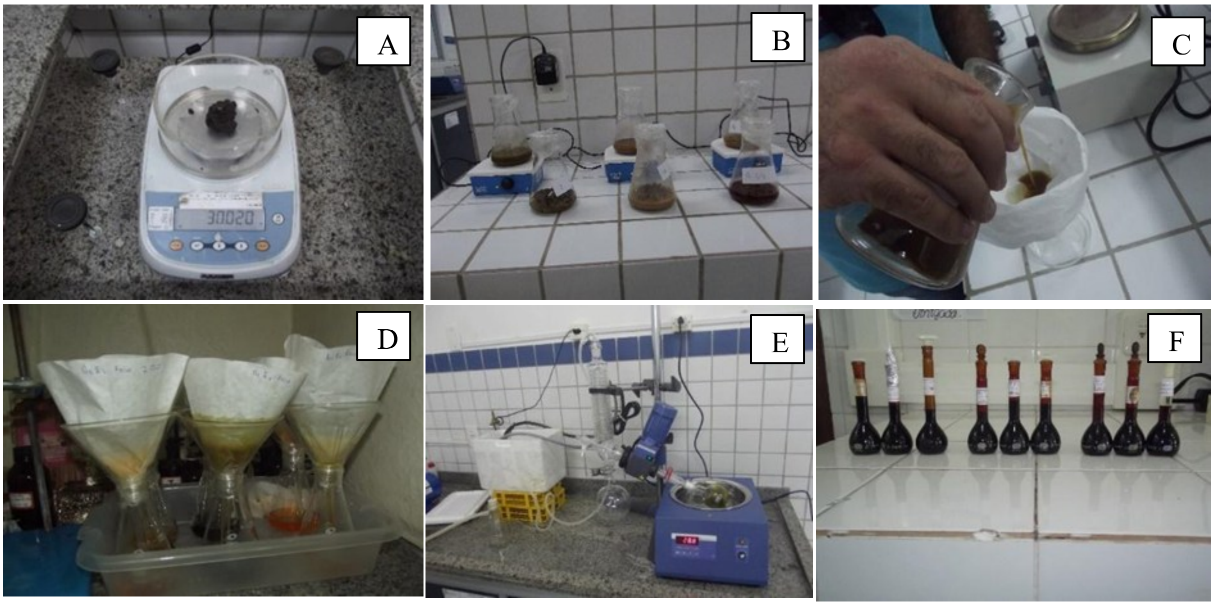

Figure 1. A) Weighing the crude propolis; B) Removal of the bioactive substances by shaking in grain alcohol; C) Filtration of the liquid phase; D) Filtered extracts; E) Concentration of the extracts in evaporator; F) Arrangement of the samples for extract color determination.
Photo: Daniel Santiago Pereira

The extracts were placed in a vacuum evaporator to remove any remaining solvent. One gram of the dried solid sample was collected and 100 mL 80% v/v ethanol was added to it, making up a concentration of 1% w/v (10,000 ppm).

Bioassays

Mimosa pudica and *Senna obtusifolia* seeds were collected from fallow fields in the municipal district of Terra Alta-PA. They were mechanically cleaned then immersed in concentrated sulfuric acid for 15 min to break their dormancy (MOURÃO JÚNIOR; SOUZA FILHO, 2010).

The target plants were selected based on their fast germination, uniform and rapid development, and sensitivity to low concentrations of allelopathic substances (SOUZA FILHO; GUILHON; SANTOS, 2010).

The phytotoxicity tests were developed in the Laboratory of Agribusiness of Embrapa Amazonia Oriental, Belém, Pará, Brazil. Phytotoxicity was evaluated in *Mimosa pudica* (malícia - ML) and *Senna obtusifolia* (mata-pasto - MP). These are two weed species common to Amazonian pasture lands. Phytotoxicity was evaluated in terms of reductions in percentage germination, and growth and development of the radicle and the hypocotyl. The extract concentrations applied were 1%, 0.75%, 0.50%, and 0.25% (w/v) (BATISTA et al. 2016; OLIVEIRA et al., 2016).

Germination

Bioassays were conducted on germination at a constant 25°C and 12 h photoperiod. Germination was monitored for up to 10 d. Germinated seeds presenting radicles at least 2.0 mm in length were selected. Twenty-seed lots were placed on qualitative filter papers moistened with extract dilutions in Petri dishes 9.0 cm in diameter. The control consisted of twenty seeds placed on filter paper moistened with distilled water. All treatments were prepared in triplicate. Photographic images were taken (HADAS, 1976).

Radicle and hypocotyl elongation

The elongation of the radicle and the hypocotyl were monitored at a constant temperature of 25°C and photoperiod of 24 h.

Each Petri plate received 3.0 mL of the test solution and was lined with filter paper. After the evaporation of the solvent (80% ethanol), 5 ml of distilled water was added to maintain the original concentration. The test solutions were added only once at the beginning of the bioassays; subsequently, distilled water was added when necessary to maintain the seedlings.

In each plot, two three-day-old seedlings were allowed to grow for a period of 10 days, after which radicle and hypocotyl length were measured using a Vernier caliper. For the control, only distilled water was used.

Experimental Design and Statistical Analysis

The data were normalized by transformation into √x arcsine values. Normality was verified using Lilliefors and Shapiro-Wilk tests.

Statistical analyses were performed with SISVAR®. The experimental design was a fully randomized block (CRD). Factorial interactions with three replicates were evaluated. Tukey's means test ($\alpha = 0.5$) was applied when data were significant at a 1% probability. The reference control was distilled water.

The following factors were analyzed:

Target species (2) x extract concentrations (4).

The significance level was $\alpha = 0.05$. When the treatment effects were significantly different, the means were compared using a regression test.

RESULT AND DISCUSSION

The interaction between target species and extract concentration was significant only for hypocotyl length. Therefore, treatment effects were independent for all other variables (Table 1).

Table 1. Mean squares of ANOVA for germination rate, radicle growth, and hypocotyl growth with their respective significances and coefficients of variation (VC) in response to various concentrations of ethanol extracts of Jandaíra geopropolis (*Melipona subnitida* Ducke) in different target species[1, 2].

FV	GL	Germination	Radicle	Hipocotyl
Receiving species	1	3,059.14**	1,026.91**	2,252.54**
Concentration of the Extract	3	698.88**	270.39**	29.58$^{n.s.}$
Receiving Species x Extract Concentration	3	60.31$^{n.s.}$	10.63$^{n.s.}$	274.13**
Error	16	37.68	16.71	11.76
CV%		22.23	15.25	11.66

¹Data transformed into arcsines [(P%/100 +1)/2]. ²Original data were expressed in percentage inhibition relative to distilled water control. * and ** indicate significances of 5% and 1% probability, respectively, by the F-test.

Souza Filho (2006) concluded that the inhibitory effects of *Paspalum* extracts are explained by either allelopathy or osmosis. The latter has an additive effect in combination with the former. When extract concentrations are high, the effects of the osmotic potential can supersede those of the allelopathic potential. In the present study, the concentration effects are attributable to the chemical composition of the extract since the maximum concentration tested was only 1% w/v.

The absence of any significant interaction between germination rate and radicle elongation indicates that the extract concentration affects these parameters independently in the target species studied here. It also suggests that there may be a wide-spectrum effect. On the other hand, the interaction observed for hypocotyl growth indicates that for each target species there is a different correlation between growth inhibition and extract concentration.

Germination

The curve plotting germination rate as a function of extract concentration indicates that the extract concentration effect accounts for >94% of the variation in germination rate (Figure 2).

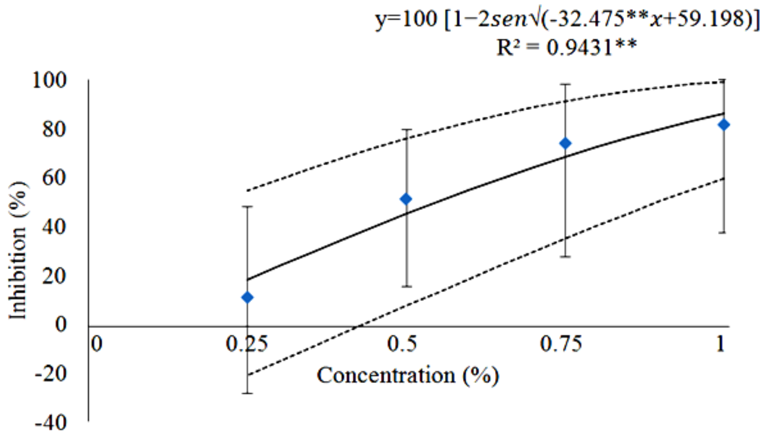

Figure 2. Average inhibitions of mata-pasto (ML) (*Senna. obtusifolia*) germination and malícia (ML) (*Mimosa pudica*) as functions of Jandaira bee (*M. subnitida*) geopropolis extract concentrations. Vertical charts represent upper- and lower limits corresponding to the standard deviation reverse-transformed into %. Upper- and lower stippled lines represent the respective estimates for malícia (ML) (*M. pudica*) and mata-pasto (MP) (*S. obtusifolia*), respectively. * and ** indicate 5% and 1% significance, respectively.

As a consequence of the interaction factor, the treatment effect was not significant ($p > 005$) for germination rate. The curves for (*M. pudica* and *S. obtusifolia*), denoted by the stippled lines (Figure 2), have the same angular coefficient and are parallel at the scale in which the data were analyzed. Therefore, they can be represented by an average curve. Nevertheless, reverse transformation of the original scale indicates that the differences in germination rate between the species decrease as the extract concentrations increase. There was a phytotoxic effect of 90% for *M. pudica* (0.73%) and 50% for *S. obtusifolia* (0.9%).

Based on the average curve shown in Figure 2, a 50% germination inhibition rate (PE50) is estimated for an extract concentration of 0.55%. The germination rate approaches PE90 when the extract concentration is 1%. It should be noted that an average for both target species was calculated because there was no species × concentration interaction and the mean germination inhibition rates differed significantly among the species (80.22% and 21.13% for malícia (*M. pudica*) and mata-pasto (*S. obtusifolia*), respectively).

Souza Filho, Pereira and Bayma (2005) reported that crude extracts were the most phytotoxic to seed germination in malícia (*M. pudica*): a 1% w/v concentration was 100% allelopathic. These results resemble those obtained with geopropolis extracts in the present study.

Souza Filho et al. (2009b) found that 2% w/v (200 mg/L) ethanol extract of grass-salsar (*Cymbopogon* sp.) was 30% phytotoxic to malícia and 18% phytotoxic to mata-pasto. Although these values are lower than those reported in the present study, once again ML was more sensitive to the allelopathic effects of the extract than MP.

Zoghbi et al. (2009) reported that the inhibitory allelopathic effect of long pepper oil was strongest on seed germination regardless of target species. The magnitude of this effect always surpassed 46% and reached maxima of 77% for *M. pudica* and 65% for *S. obtusifolia*. The maximum inhibition levels were always observed with 1% w/v extract (highest concentration). Malícia seed germination was more susceptible to the allelopathic effects than bush-pasture. This response was less evident with hypocotyl development.

Souza Filho, Cunha, and Vasconcelos (2009) tested the effects of *Azadirachta indica* oil on mata-pasto and malícia at concentrations up to 3% w/v. Contrary to the findings of the present study, the authors reported that forest pasture seeds were more susceptible to the allelopathic effects of the oil than the more resistant malícia seeds.

The extract was inhibitory even at low concentrations. Nevertheless, at 0.25% it may have actually enhanced seed germination rates in mata-pasto (*S. obtusifolia*) possibly by stimulating root development.

Radicle

Regression equations were adjusted to identify the relative differences in the phytotoxicity of the geopropolis on root elongation (that is, inhibition) in the target species (Figure 3).

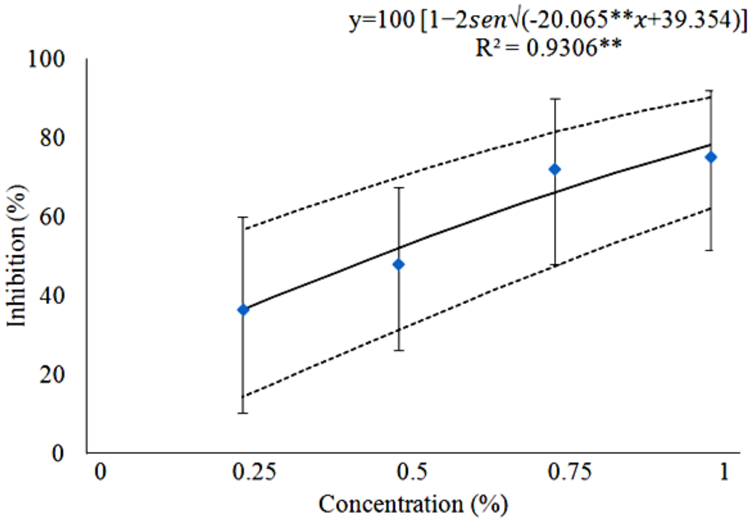

Figure 3. Average inhibition of mata-pasto (MP) (*S. obtusifolia*) and malícia (ML) (*M. pudica*) root development as functions of Jandaira bee (*M. subnitida*) geopropolis extract concentrations. Vertical bars represent the upper- and lower limits of the standard deviation reverse-transformed into %. Upper- and lower stippled lines represent the respective estimates for malícia and mata-pasto, respectively. * and * * indicate 5% and 1% significance, respectively.

The allelopathic effects of the extract on root development were stronger in *M. pudica* (malícia) than *S. obtusifolia* (mata-pasto). The average root elongation inhibitions were 36.45% and 18.23%, respectively. An adjusted regression model for the means indicated that PE50 was attained at an extract concentration of 0.45% (Figure 3). The stippled curves, drawn with the same regression coefficient for both species, estimated a PE50 at 0.8% for *S. obtusifolia* and only 0.25% for *M. pudica*, as was the case for seed germination. For *M. pudica*, PE90 occurred at a 1% extract concentration whereas no PE90 was determined for *S. obtusifolia*.

The phytotoxic effects observed in this study were stronger than those ones reported by Souza Filho, Cunha, and Vasconcelos (2009). They evaluated the effects of *Azadirachta* oil at concentrations of 0.5%, 1%, 1.5% and 3% and determined a maximum PE of 40% for root elongation in both *M. pudica* and *S. obtusifolia*. Souza Filho, Cunha, and Vasconcelos (2009) also reported that crude extracts inhibited pasture weed (*S. obtusifolia*) root elongation by up to 22% at 1% concentration (w/v). Similar results were reported for malícia (*M. pudica*).

Vilhena (2006) tested the phytotoxic effects of essential oils extracted from the rhizomes of *Cyperus articulatum* L. and *Cyperus giganteus* Vahl. They reported root growth inhibitions of 55% on malícia and 32% on mata-pasto at extract concentrations of only 100 mg L^{-1} (0.01%).

The Root development inhibition levels resembled those observed for seed germination. Nevertheless, mata-pasto (*S. obtusifolia*) was slightly less affected by the extract (produced longer roots) than malícia (*M. pudica*). For these characteristics, then, malícia was more sensitive to geopropolis extract phytotoxicity than mata-pasto.

Souza Filho, Cunha, and Vasconcelos (2009) also reported that malícia root development was more sensitive to the allelopathic effects of *O. americanum* essential oils than that of mata-pasto.

Pereira (2013) verified the allelopathic effects of ethanol extracts of four types of propolis on flax (*Linum usitatissimum* L.) plantlets. The author reported that flax root growth was much more severely inhibited by the propolis extracts than was hypocotyl length. Relative to the control, the PE was 75.9% at 200 mg/L.

According to Pereira (2013) and Cheng, Ruyter-Spira and Bouwmeester (2013), the strong PE on root development can be explained by cytotoxicity inhibiting the apical transport of auxins, which promote the development of lateral or adventitious roots.

It is known that many plant exudates and secondary metabolites, such as terpenes, flavonoids, and alkaloids, are commonly found in propolis (MACÍAS et al., 2007; GUSMAN; BITTENCOURT; VESTENA, 2008; FALCÃO et al. 2010). Some of these substances possess allelopathic properties. Gusman, Bittencourt and Vestena et al. (2008) tested and confirmed the allelopathic effects of *Baccharis dracunculifolia* (a plant source of the green propolis produced by bees in west central Brazil) on cultivated plants.

Hypocotyl

ANOVA and the F-test verified a significance of 1% probability for the interaction between target species and extract concentrations for the hypocotyl characteristic. The same statistical design was applied to all other traits but no interactions were identified (Table 1). Figure 4 shows the linear adjustments of the inhibition of hypocotyl development in mata-pasto and malícia as a function of geopropolis extract concentration.

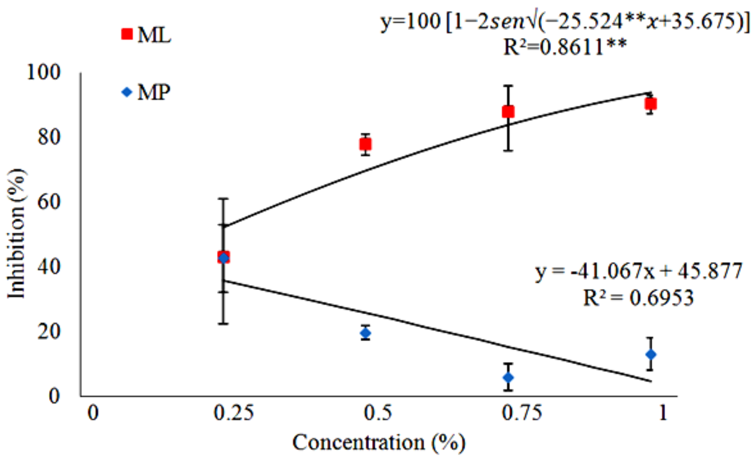

Figure 4. Percentage hypocotyl growth inhibition of mata-pasto (MP) (*Senna obtusifolia*) and malícia (ML) (*Mimosa pudica*) as functions of Jandaira bee (*M. subnitida*) geopropolis extract concentrations. Vertical bars represent the upper- and lower limits relative to 1 standard deviation, reverse-transformed in %. * and ** indicate significance at 5% and 1% probabilities, respectively.

Figure 4 indicates that malícia (*M. pudica*) hypocotyl elongation was more susceptible to *M. subnitida* geopropolis phytotoxic effects (PE) than that of mata-pasto (*S. obtusifolia*). In the latter case, there was 36% hypocotyl growth inhibition at an extract concentration of 0.25%. For malícia, the PE on hypocotyl elongation was approximately 52% at the same dosage and reached 94% at 1% extract concentration.

The phytotoxic effects of the geopropolis extract on hypocotyl development in malícia followed the same trend as that seen for seed germination and radicle growth but at a lower intensity. The lowest level of inhibition in mata-pasto hypocotyl development may have been the result of radicle growth inhibition. Seed storage reserves may have been mobilized to the hypocotyl and the radicle was affected by high geopropolis concentrations. This mechanism could be adaptive in this species and requires further study.

Souza Filho et al. (2009a) reported similar effects in mata-pasto and malícia exposed to essential oils of *O. americanum.* Nevertheless, Souza Filho, Cunha and Vasconcelos (2009) reported that there was a PE of 22% in for mata-pasto and <10% for malícia exposed to *Azadirachta indica* essential oils at concentrations of ≤3%.

Pereira et al (2007) evaluated the growth of the seedlings of the coffee cultivar Thick Acaiá MG-1474 subjected to varying doses of ethanol propolis extracts (EEP). They found a quadratic correlation between plant height and EEP dosage. The maximum seedling height occurred at 7.30% v/v EEP concentration. It was proposed that ethanol intoxication may have contributed to the growth inhibition in the coffee seedlings. In the present study, the ethanol solvent was evaporated before the propolis was applied to the seedlings.

According to Souza Filho, Cunha, and Vasconcelos (2009), the biological activity of an allelochemical depends on its concentration and the sensitivity of the target species. The inhibitory limit of a substance is not constant but rather it is strongly correlated with the sensitivity of the target species, plant metabolism, and environmental conditions.

Overall, the allelopathic effects of the propolis extracts were positively correlated with extract concentration and with the target species. *M. pudica* tended to be more susceptible to the inhibitory effects of propolis than *S. obtusifolia* and these effects were more noticeable in the seed germination and in radicle development but less evident in hypocotyl development.

The propolis extracts effects are frequently described in an individual way in some of their main constituents. Nevertheless, propolis extract is a mixture of different constituents in varying proportions and, in general, it is unknown how they interact with each other or affect the organisms exposed to them. There is also considerable variability in the composition of the propolis extracts.

Souza Filho, Cunha, and Vasconcelos (2009) stated that the biological activity of an allelochemical depends on its concentration and the relative sensitivity of the target species to it. The limit of efficacy for a certain chemical is not constant; it is strongly correlated to the susceptibility of the target species, plant metabolism, and environmental conditions.

The present study showed that geopropolis inhibited seed germination, hypocotyl growth, and radicle development in malícia (*M. pudica*). These effects can be attributed to the various constituents in the geopropolis extract, their concentrations, and the susceptibilities of the target plant species. For example, Vilhena (2006) compared two *Cyperus* species attributed the divergences in the allelopathic intensity to the ones verified in the chemical composition of the oils.

Overall, geopropolis extracts are phytotoxic to seed germination, radicle development and hypocotyl growth in both weed species tested. Although preliminarily, these findings indicate the feasibility of using geopropolis in weed management especially on cultivated pasture lands.

According to Oliveira et al. (2016), the strong inhibitory effect of geopropolis on germination hinder new plant pest growth in cultivated areas and reduces their density there. The reductions in radicle and hypocotyl elongation impede weed competition for light, space, etc. As a result, a cultivated area treated with geopropolis has a low weed density and growth of the desired plant species is favored.

According to Pereira et. al (2007), the use of natural products (such as propolis) on cultivated plants has several advantages: (a) accessibility; (b) ease of handling; (c) low health risk to worker and consumer; and (d) minimal environmental impact.

CONCLUSIONS

Mimosa pudica germination was sensitive to the inhibitory effects of geopropolis extract. Radicle elongation in *S. obtusifolia* was the most severely affected in higher concentrations of geopropolis. In contrast, however, hypocotyl growth inhibition was inversely proportional to the tested geopropolis concentrations. The geopropolis extract of jandaira bees (*Melipona subnitida*) demonstrated an inhibitory effect on both the seeds and seedlings of *M. pudica* and *Senna obtusifolia.* Therefore, geopropolis may have an allelopathic effect on these plant species and may be a biological weed control strategy.

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