

*Full Length Research Paper*

## **The lupeol content in *Machaerium* species by HPLC-APCI-MS/MS and the allelopathic action**

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The use of extracts from plants and/or allelopathic compounds from natural products can minimize the use of pesticides in agriculture. This is a chemical study of *Machaerium eriocarpum* branch extract and the evaluation of the allelopathic potential of *Machaerium hirtum* (Vell.) Steffed and *M. eriocarpum* Benth (Fabaceae) leaves and branches extracts. The triterpene lupeol was isolated from the hydroethanolic extract of *M. eriocarpum* branches. The quantification of this compound in all extracts was performed by HPLC-APCI-MS/MS and the highest amount of lupeol was observed in the leaves extract of *M. eriocarpum*. Allelopathy tests were carried out against seeds of *Sorghum bicolor* L. (sorghum), evaluating the final percentage of germination, germination speed index (GSI), root growth and number of lateral roots. The leaves extracts of both species were effective in the inhibition of the root growth as well as of the lateral roots. The branches extract of *M. hirtum* showed inhibitory effect in the root growth and lateral roots only in 50 and 100 mg.L<sup>-1</sup> doses. The lupeol showed mainly a strong inhibition of seed germination, root growth and lateral root appearance at 100 µM. This study showed the potentiality of *Machaerium* extracts and lupeol as bioherbicides.

**Key words:** Allelopathy, lupeol, *Machaerium*, Fabaceae, *Sorghum bicolor*, phytotoxicity

### **INTRODUCTION**

The continuous practice of agricultural synthetic herbicides can increase human health risks through the non-biodegradable compounds accumulation in the soil and the development of microorganisms resistance

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(Luz et al., 2010). Finding alternative strategies for weed management is now being focused on to reduce the dependence of farm chemicals by a sustainable agriculture based on plant derived materials, developing environmentally friendly alternatives approach for the weed control (Kuk et al., 2001). Substances produced by plants can offer new and excellent opportunities to diversify the control of invasive plants in agriculture, decreasing or eradicating environmental contamination, preserving natural resources and ensuring the availability of quality agricultural products. Theoretically, natural products and plant extracts with allelopathic activity with phytotoxic effect can be used directly in the formulation of bioherbicides or be modified in order to increase their biological activity (Yamagushi et al., 2011). According to Toderó (2017), until now there are thirteen bioherbicides originating from fungi, bacteria and plant, available on the international market. However, bioherbicides show a shorter half-life when compared with synthetic herbicides (Duke et al., 2000; Bailey, 2014; Toderó, 2017). Despite its natural origin, it is important to take into account the toxic potential for living beings when produced on a large scale, as well as the environmental impact of this production due to the demand for food for a growing population.

According to Abd-ElGawad et al. (2020) the essential oil of *Bassia muricata* reduces the germination and seedling development of the weed *Chenopodium murale*. The aqueous extract of *Sphenoclea zeylanica* Gaertn. had an inhibitory effect on rice germination and seedling growth at concentrations higher than 50 g.L<sup>-1</sup>, while completely inhibited rice seed germination at the concentration of 100 g.L<sup>-1</sup> (Krumisri et al., 2020). The flavonol quercetin and its derivatives from *Fagopyrum esculentum* root showed on haustorium induction in *Phelipanche ramosa* at concentration range 0.1 to 0.05 mM (Fernández-Aparicio et al., 2021). The methyl oleate, methyl stearate, methyl palmitate and methyl linoleate inhibited the seed germination of *Avena fatua*, *Chenopodium album*, *Euphorbia helioscopia*, *Rumex dentatus* and *Phalaris minor* in a dose-dependent manner (Anwar et al., 2021). The main compound of *Glycyrrhiza uralensis* Fisch, liquiritin displays inhibitory effects on the growth of lettuce (Yan et al., 2020).

Chou and Waller (1980), caffeine, theobromine, theophylline, paraxanthine, scopoletin, chlorogenic, vanillic, ferulic and *p*-hydroxybenzoic acids, which are present in the *Coffea arabica* extract showed allelopathic action on lettuce growth at the concentration of 100 ppm. Jose et al. (2016) described the allelopathic effect of *Merostachys riedeliana* extract, fractions and phenolic acids on *Mimosa bimucronata* seeds. The constituents present in the extract of *Cinnamomum septentrionale* leaves presented potent phytotoxic action on corn growth (Yang et al., 2017); while the naphthoquinones presented

potent allelopathic effects on the roots of *Echium plantagineum* (Zhu et al., 2016). According to De Oliveira et al. (2005) the leaf extract of *Machaerium scleroxylon* showed 100% of inhibitory effect in the length of the seedlings of *Lactuca sativa*.

*Machaerium* is one of the largest tropical arboreal genera of Papilionoideae, with approximately 160 known taxa and popularly known as "jacarandás" (Plant, 2020; Bastos, 1987; Polido and Sartori, 2011). According to the literature, this genus is recognized by the occurrence of different class of natural products such as triterpenes, proanthocyanidins, cinnamylphenols, alkaloids, benzoquinones, steroids and the incisive presence of flavonoids (Ignoato et al., 2013; Kurosawa et al., 1978a, b; Ollis et al., 1978, 1968; Alves et al., 1966; Bento et al., 2018; Gregson et al., 1968; elSohly et al., 1999; Seo et al., 2001; Adrian et al., 2007; Santos et al., 2009; Oliveira et al., 1968; Braga et al., 1968). Based on previous studies involving the allelopathic activity of isovitexin and *Machaerium eriocarpum* leaf extract, associated with the absence of phytochemical studies of branch extract of this species, in this work the triterpene lupeol was isolated and its quantification was performed by HPLC-APCI-MS/MS and their allelopathic action.

## MATERIALS AND METHODS

### Plant material

The plant material *M. eriocarpum* Benth was collected in Porto Murtinho-MS, by A. L. B. Sartori, E. S. S. Lima and F. J. Kochanovski. An exsiccate of the species is deposited in the Herbarium of the Federal University of Mato Grosso do Sul, Campo Grande campus (CGMS411323). The branches and leaves of *Machaerium hirtum* (Vell.) Stellfeld were collected by Almeida, L. F. R. in Botucatu, São Paulo. The exsiccate is deposited in the Herbarium of the Department of Botany (BOTU 027643), Institute of Bioscience, Sao Paulo State University, Botucatu campus. The data collected was recorded in the SisGen platform (National System of Management of Genetic Heritage and Associated Traditional Knowledge) as genetic patrimony under the registration number A32A22B and A1BB684.

All material was dried at 40°C and ground using a knife mill with an average particle size of 1 to 3 mm.

### Extract fractionation and lupeol isolation

Separately, the plant materials of *M. eriocarpum* and *M. hirtum* were submitted to the percolation process in 70% ethanol. The material was weighed and swollen in 2 L of 70% (v/v) ethanol. The solvent was evaporated under reduced pressure at a temperature of 40°C and then stored in a sealed amber bottle (Table 1).

The branches extract of *M. eriocarpum* (1.54 g) was solubilized in 12 mL of methanol/H<sub>2</sub>O (8:2, v/v) and centrifuged at 10,000 rpm during 10 min. The supernatant was fractionated using a Sephadex LH-20 column (57 cm × 3.0 cm d.i) and eluted with methanol with a flow rate of 4 mL min<sup>-1</sup>. The 230 subfractions were analyzed by TLC using CHCl<sub>3</sub>/methanol/H<sub>2</sub>O 80:18:2 (v/v), which in turn, produced

**Table 1.** Yield of extract and the concentration of lupeol observed in the *Machaerium* extracts.

| Sample | Dried material (g) | Weight | Yield (%) | Peak area average | Amount (ppm) |
|--------|--------------------|--------|-----------|-------------------|--------------|
| MHL    | 1000               | 170    | 17        | 19568.5           | 0.23         |
| MHB    | 500                | 79.1   | 15.8      | 69794.0           | 0.79         |
| MEL    | 500                | 58.2   | 11.6      | 207040.3          | 1.51         |
| MEB    | 499.7              | 69.7   | 13.5      | 134966.7          | 2.31         |

MHL: *Machaerium hirtum* leaves extract; MHB: *M. hirtum* branches extract; MEL: *M. eriocarpum* leaves extract; MEB: *M. eriocarpum* branches extract.

11 groups of fractions (G1-G11). The G4 fraction group (50 mg) was applied on preparative TLC which was divided in two sides. On the left side, G4 was applied as a line (15 cm) and on the right side as a point using a capillary glass tube. After elution of the chromatographic plate using  $\text{CHCl}_3$ /methanol 98:2 (v/v) as mobile phase, the left side was covered with a glass plate and the right side sprayed with sulfuric anisaldehyde. After sprayed and heated with a thermic pistol the plate showed a pink spot on the right side. Then, a region on the left side with the same retention factor as the pink spot was removed using a spatula and extracted with methanol (subfraction 2). A TLC was made to verify the purity of the subfraction 2 (15 mg).

#### Clean-up of fractions

The clean-up was performed with all leaves and branches extracts. Solid phase extraction (SPE) C18 cartridges (50 mg) were used. Each cartridge was previously activated in pure methanol and then set to 5.0 mL methanol/ $\text{H}_2\text{O}$  (8:2, v/v). The sample was filtered through the cartridge and eluted with 5.0 mL of the same methanol/ $\text{H}_2\text{O}$  mixture. The obtained fraction was dried at room temperature and the resulting solid was resuspended in methanol/ $\text{H}_2\text{O}$  (8:2, v/v), then filtered through a PTFE filter (0.22  $\mu\text{m}$ ) and stored in a glass vial.

#### Quantification of lupeol contained in the extracts of the branches and leaves

Analysis of lupeol was performed in a HPLC system Thermo Scientific® (Thermo Fischer Scientific Inc., Waltham, MA, USA) consisted of an Accela 600 pump, an autosampler, a PDA detector and a mass spectrometer LTQ XL equipped with an Atmospheric-pressure chemical ionization (APCI) source and a linear ion-trap analyzer. All ionization and chromatographic parameters showed here were specifically optimized for the quantification of lupeol. Source parameters were operated in positive mode and adjusted as follows: capillary temperature at 320°C, vaporizer temperature at 450°C, sheath gas flow 30 (arb), auxiliary gas flow 15 (arb), sweep gas flow 10 (arb), tube lens of 55 V, capillary voltage of 33.0 V and source voltage of 6.00 kV. Lupeol detection was made by single reaction monitoring (SRM), which detected transitions 409>257, 409>203 and 409>191 for confirmation. The parent ion for monitored lupeol was the  $[\text{M}-\text{H}_2\text{O}+\text{H}]^+$  adduct. Such water loss (due to the high temperature required in the APCI source) and product ions have been reported before in the literature (Mo et al., 2013). The product ions utilized were selected after direct injection of lupeol standard, followed by fragmentation in collision-induced dissociation (CID) mode against helium for ion activation at collision

energy of 20%, as well as activation time of 30 min/s, in the same source conditions as described earlier. Chromatographic method consisted of A (0.1% formic acid in  $\text{H}_2\text{O}$ ) and B (0.1% of formic acid in MeOH), in a gradient elution system (92-100%) of B in 6 min followed by a hold of 100% B for 3 min, where the first 0.8 min were discarded, flow of 0.4 mL  $\text{min}^{-1}$ , injection volume 10  $\mu\text{L}$ , column Waters® Acquity BEH C18 1.7  $\mu\text{m}$ , 2.1  $\times$  50 mm. For the direct infusion the software LTQ Tune Plus, version 2.7.0.1093 was utilized and for LC-MS analysis the Thermo Xcalibur 2.2 SP 1.48 was used.

Quantification was performed using the external standard method and the calibration curve was made with lupeol concentrations of 0.0781, 0.156, 0.313, 0.625, 1.25, 2.50, 5.00 and 10.0 ppm, in triplicate and linear regression. Extracts were injected at a concentration of 100 ppm each, in triplicate. Thus, for the quantification of this compound in the extracts data was obtained by taking into consideration the calibration curve ( $Y = -1021.06 + 90064.7x$ ),  $R^2 = 0.9999$ . The detection and quantification limits were determined, respectively as 0.11  $\mu\text{g mL}^{-1}$  and 0.037  $\text{mg mL}^{-1}$ , and then calculated according to the literature (Ribani et al., 2004).

#### NMR spectroscopy analysis

The data of  $^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) were obtained on a Bruker Avance III 600 spectrometer using  $\text{CDCl}_3$  as solvent.

#### Allelopathy assays

The seeds of *Sorghum bicolor* L. were commercially acquired and the effects of the extracts and germinating seeds were tested adopting a full randomized design with three repetitions for each treatment and control groups.

The allelopathic potential of leaf and branch extracts of *M. eriocarpum* and *M. hirtum* as well as lupeol compound were observed on sorghum (*S. bicolor* L.) seed germination and seedlings growth. The seeds were previously treated against contamination in 2% sodium hypochlorite solution for 2 min and then washed in distilled water. Stock solutions were prepared for each extract tested, and then diluted for application in assays at the following concentrations: 30, 50, 80, 100 and 1000  $\text{g L}^{-1}$ . The lupeol was diluted in the concentration of 100  $\mu\text{M}$ . The treatments were carried out in 9 cm Petri dishes, with 10 sorghum seeds and 10 mL of solution. A control (conventional sorghum without *M. eriocarpum* or *M. hirtum* extract) was performed using only deionized water. The treatments were carried out in triplicates. The seeds were kept

in a germination chamber programmed with photoperiod of 12/12 h light/dark at 25°C. The number of germinated seeds was counted after 24, 48, 72, 96 and 120 h after application of the treatments. After 120 h the following parameters were analyzed: final germination percentage, germination speed index (GSI), root growth and number of lateral roots. The GSI was obtained with the equation (Al-Mudaris, 1998):

$$GSI = (G1/N1) + (G2/N2) + (G3/N3) + L + (Gn/Nn)$$

where G1, G2, G3, ..., Gn = number of seedlings computed in the first, second, third, and last count.

N1, N2, N3, ..., Nn = number of days from seeding to first, second, third, and last count.

### Statistical analysis

The data were subjected to analysis of variance (ANOVA) with significant differences in  $p < 0.001$ .

## RESULTS AND DISCUSSION

In order to obtain the extracts, the leaves and branches of *M. eriocarpum* and *M. hirtum* were submitted to the extraction process by percolation. The yield of each extract obtained in this process is shown in Table 1. Highest percentage of yield was found in *M. hirtum* leaf extract (17% w/w), while *M. eriocarpum* leaf extract was slightly lower (11.6% w/w) (Table 1).

The fractionation of the hydroethanolic extract of *M. eriocarpum* branches by gel permeation chromatography followed by a preparative thin layer chromatography led to isolation of the lupeol. In the  $^1\text{H}$  NMR spectrum (300 MHz,  $\text{CDCl}_3$ ) two broad singlets, were observed at  $\delta$  4.65 and 4.55 ppm, referring to the two hydrogens of the terminal double bond (H-29). The double doublet at  $\delta$  3.18 ( $J = 5.8$  and  $8.6$  Hz) refers to H-3. In the region between  $\delta$  0.8 and 1.8 of the spectrum, the presence of the signals related to the methyl and methylene hydrogens were detected. The structural identification was established by  $^1\text{H}$  NMR and mass spectrometry data analysis compared with the literature (Jácome et al., 2004; Branco and Pizzolletti, 2002; Magalhães et al., 2011).

The lupeol was used as TLC standard to evaluate the presence of this metabolite in the other extracts studied. Using the solvent mixture of chloroform/methanol/water (80:18:2, v/v) as mobile phase, the lupeol presented the RF value of 0.8 and pink coloration after using anisaldehyde as spray reagent. After comparison, the lupeol was detected in all evaluated *Machaerium* extracts (Wagner and Bladt, 1996).

This triterpene is commonly found in many plants and it was previously identified in the extract of *M. hirtum*

(Ignoato et al., 2013). The literature reports numerous biological activities associated with lupeol, such as glucose absorption, insulin secretion, vascular diabetic dysfunction, nephropathy, retinopathy, as well as anti-inflammatory, antimalarial and antiarthritic (Chaturvedi et al., 2008; Hamid et al., 2015). These data showed the potentiality of this natural product.

Resistance or tolerance to secondary metabolites is a species-specific characteristic, with the most sensitive species being *S. bicolor* L. (sorghum), *Lactuca sativa* L. (lettuce), *Lycopersicon esculentum* Miller (tomato) and *Cucumis sativus* L. (cucumber), which are considered to be indicative plants of allelopathic activity. According to Wang et al. (2014), the ursolic acid identified in the *Alstonia scholaris* leaf, impact the growth of neighboring weeds by inhibiting seed germination and radicle growth of *Bidens pilosa*. Experimental trials have shown phytotoxic effects of lupeol and lupenone applied on the weeds of *Mimosa pudica* and *Senna obtusifolia* (Luz et al., 2010). The lupeol completely inhibited the etiolated wheat coleoptile elongation at  $10^{-3}$  M and the bioactivity was retained upon dilution (Nebo et al., 2015).

The qualitative analyses by HPLC-APCI-MS/MS showed the presence of this compound in all extracts studied. In order to quantify this metabolite in all extracts, the quantitative analysis was performed on lupeol, under analytical conditions using HPLC-APCI-MS/MS (Table 2). The calibration curve was obtained using standard solutions of lupeol in eight different concentrations (0.0781, 0.156, 0.313, 0.625, 1.25, 2.50, 5.00 and 10.0 ppm).

The results showed a higher content of lupeol in *M. eriocarpum* species and the highest value was observed in the branches extract with 2.31 ppm while in leaves, was 1.51 ppm. Comparatively, a higher lupeol content was detected in the branch extracts, when compared with the leaves in both species studied (Table 2). The obtained results indicate that HPLC-APCI-MS/MS is a rapid and valid method for lupeol quantification.

All extracts of the two *Machaerium* spp. were submitted to the allelopathy tests with *Sorghum bicolor* L. seeds for the evaluation of the final percentage of germination, germination speed index (GSI), root growth and number of lateral roots.

All extracts did not show statistical differences compared to the control in the *S. bicolor* germination seeds (Table 2). The *Machaerium* branches extract show a significant phytotoxic effect in the three doses (30, 50 and  $100 \text{ mg L}^{-1}$ ) tested during the evaluation of the germination speed index (Table 2). The reduction of the germination speed investigated by IVG indicated the allelochemical activity affected by the mechanisms of stretching and cell division. Even with the reduced germination speed, the final count of germinated seeds

**Table 2.** Biological assays with branches and leaves extracts of *Machaerium eriocarpum* and *M. hirtum* in sorghum seeds and seedlings.

| Extract | Concentration<br>(mg L <sup>-1</sup> ) | Seed germination<br>(%) | GSI<br>(relative)     | Root growth<br>(mm)      | Lateral roots<br>(absolute) |
|---------|--|-------------------------|-----------------------|--------------------------|-----------------------------|
| Control | 0                                      | 90 ± 10 <sup>A</sup>    | 126 ± 25 <sup>A</sup> | 26.1 ± 7.3 <sup>A</sup>  | 3.2 ± 1.3 <sup>A</sup>      |
|         | 30                                     | 90 ± 10 <sup>A</sup>    | 78 ± 9 <sup>B</sup>   | 20.9 ± 5.3 <sup>A</sup>  | 1.1 ± 0.7 <sup>B</sup>      |
| MEB     | 50                                     | 77 ± 25 <sup>A</sup>    | 63 ± 17 <sup>B</sup>  | 10.3 ± 10 <sup>A</sup>   | 0.2 ± 0.3 <sup>B</sup>      |
|         | 100                                    | 80 ± 10 <sup>A</sup>    | 64 ± 9 <sup>B</sup>   | 12.7 ± 8.2 <sup>A</sup>  | 0.03 ± 0.06 <sup>B</sup>    |
| MEL     | 30                                     | 87 ± 15 <sup>A</sup>    | 66 ± 17 <sup>A</sup>  | 12.3 ± 5.1 <sup>B</sup>  | 1.2 ± 0.7 <sup>B</sup>      |
|         | 50                                     | 73 ± 6 <sup>A</sup>     | 93 ± 19 <sup>A</sup>  | 9.2 ± 7.1 <sup>B</sup>   | 0.3 ± 0.4 <sup>B</sup>      |
|         | 100                                    | 83 ± 21 <sup>A</sup>    | 69 ± 15 <sup>A</sup>  | 3.5 ± 1.8 <sup>B</sup>   | 0 ± 0 <sup>B</sup>          |
| MHB     | 30                                     | 80 ± 17 <sup>A</sup>    | 70 ± 9 <sup>B</sup>   | 17.8 ± 8.3 <sup>A</sup>  | 1.4 ± 0.9 <sup>A</sup>      |
|         | 50                                     | 87 ± 6 <sup>A</sup>     | 68 ± 6 <sup>B</sup>   | 8.2 ± 7.4 <sup>B</sup>   | 0.4 ± 0.3 <sup>B</sup>      |
|         | 100                                    | 87 ± 6 <sup>A</sup>     | 71 ± 9 <sup>B</sup>   | 8.3 ± 2.4 <sup>B</sup>   | 0.03 ± 0.05 <sup>B</sup>    |
| MHL     | 30                                     | 83 ± 15 <sup>A</sup>    | 82 ± 8 <sup>A</sup>   | 18.5 ± 11.5 <sup>A</sup> | 1.5 ± 0.9 <sup>A</sup>      |
|         | 50                                     | 70 ± 17 <sup>A</sup>    | 86 ± 32 <sup>A</sup>  | 1.5 ± 0.3 <sup>B</sup>   | 0 ± 0 <sup>B</sup>          |
|         | 100                                    | 80 ± 10 <sup>A</sup>    | 67 ± 9 <sup>B</sup>   | 6 ± 2.1 <sup>B</sup>     | 0.06 ± 0.1 <sup>B</sup>     |
| Lupeol  | 100 (µM)                               | 86 ± 15 <sup>A</sup>    | 55 ± 29 <sup>B</sup>  | 3.9 ± 0.5 <sup>B</sup>   | 0 ± 0 <sup>B</sup>          |

The data is shown as average ± standard deviation. The statistical analysis is shown as A equals to the control and B different to the control.

MHL: *Machaerium hirtum* leaves extract; MHB: *M. hirtum* branches extract; MEL: *M. eriocarpum* leaves extract; MEB: *M. eriocarpum* branches extract.

does not differ from the control. This process can occur by the activation of cellular detoxification mechanisms that prevents the substances from inhibiting germination through enzymes of oxidative metabolism (Hoagland and Williams, 2004; Martinez et al., 2018).

According to the data obtained in Table 2, the leaves and branches extract of *M. hirtum* had a inhibitory effect on the growth of the root, when compared with the control. *M. hirtum* extracts showing inhibitory effects for this parameter only at doses of 50 and 100 mg L<sup>-1</sup>. An increase in the inhibitory effect on the growth of the root is observed with an increase in the dose of *M. eriocarpum* leaf extract.

*Machaerium* leaf extracts show lower values at root growth parameters than branch extracts. The root growth showed more sensibility to the effect of allelochemicals when compared with the germination process. According to Suzuki et al. (2009), the root growth reduction can be one of the first evident effects of allelochemical exhibition probably by the premature lignification of cell walls. The inhibition of root system leads to a reduction in the competitive pressure of the plant favoring neighboring species (Belter and Cahill, 2015; Jacob et al., 2017). All extracts were significantly relevant in the inhibition of

lateral root growth at 50 and 100 mg L<sup>-1</sup> (Table 2). Only, *M. eriocarpum* extracts showed inhibitory effect of this parameter in the three doses tested; thus, as the observation of an increase of the inhibitory effect with the dose increment. The inhibitory effect of two *M. hirtum* extracts in this parameter was restricted to 50 and 100 mg L<sup>-1</sup> doses.

The lupeol was evaluated by these parameters only at 100 µM. According to Table 2, this compound showed the GSI value of 55 ± 29 mm, 3.9 ± 0.5 mm for the growth root and 0 ± 0 mm value for lateral roots. The response was effective for the four parameters evaluated against sorghum seed. It is important to emphasize the inhibition of seed germination, root growth and lateral root appearance. The lupeol content in *M. eriocarpum* extracts (0.23 to 2.31 ppm, Table 2) may have an influence on phytotoxic activities of extracts. At low concentrations it can promote cell growth by increasing the efficiency of enzymes and proteins; but at high concentrations, it can hyperpolarize membranes, altering the functioning of ATP pumps, promoting toxicity to the cells and causing growth reduction (Arora et al., 2015; de Martino et al., 2012). Inhibition of root growth and lateral root development may be directly related to the presence

of lupeol, which in turn, may modulate auxin transport by altering membrane polarization patterns, and promoting the cells homeostasis of the root tissue. The stress caused by the presence of allelochemicals can divert the auxin pathway to cell division and become a secondary marker for the expression of stress tolerance genes, reducing root development (Berleth et al., 2007).

## Conclusion

All extracts had an allelopathic effect on sorghum seeds, mainly in relation to root growth and lateral root growth parameters. The *Machaerium* leaves extract presents inhibitory effect to root growth and lateral roots parameters. The *M. hirtum* branches extract shows inhibitory activity at root growth at 50 and 100 mg L<sup>-1</sup> doses. Apparently, there is no difference in the inhibitory results obtained for lateral roots. A potent allelopathic effect was observed for lupeol, through the process of seed germination and, therefore, of root growth and appearance of lateral roots. The allelopathic action observed in extracts of the two species studied is directly associated with the content of lupeol associated to the synergistic effect of other natural products present in the extract. Further studies were necessary for the possible application of *M. hirtum* leaves as bioherbicide, showing the potential sustainable use of Brazilian biodiversity.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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