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XXIV SINAFERM • XV SHEB • XV ENZITEC FLORIANÓPOLIS • 2024
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August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
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PROSPECTING BIOMOLECULES FROM NATURAL EXTRACTS TO CONTROL *Bidens pilosa*

Bianca M. Dolianitis[1], Tainah R. Machado[1], Ricardo B. Rosa[1], João M. L. Bittencourt[1], Larissa Friedrich[1], Rafaela M. Assunção[1], Renan Pfeifenberg[1], Viviane D. S. Frescura[2], Marcus V. Tres[1] & Giovani L. Zabot[1]*

[1] Laboratory of Agroindustrial Processes Engineering, Federal University Santa Maria, Cachoeira do Sul, Brazil.
[2] Center of Natural and Exact Sciences, Federal University Santa Maria, Santa Maria, Brazil
** Corresponding author's email address: giovani.zabot@ufsm.br*

ABSTRACT

Natural extracts have been widely studied as potential bioherbicides due to various plants containing compounds that cause phytotoxicity in weeds. In this study, the phytotoxicity of extracts obtained from inflorescences of *Calliandra hamematocephala* Hassk, leaves of *Cymbopogon citratus* (DC) Stapf, and leaves of *Eucalyptus citriodora* were tested on the pre-emergence of the weed *Bidens pilosa*. Aqueous extracts were obtained by infusion, maceration, pressurized liquid extraction (PLE), and ultrasound-assisted extraction (UAE). The parameters evaluated were germination percentage, germination speed index (GSI), and root and shoot lengths. Among the plants used to prepare the extracts, *Eucalyptus citriodora* showed satisfactory results. When compared to control (only water), the extracts caused a reduction of 29.4% in germination, 48.1% in GSI, 64.3% in root length, and 36.8% in shoot length. The best results of control were achieved with extracts obtained by infusion and PLE. The potential of *Eucalyptus citriodora* as a bioherbicide is attributed to α-pinene, 1,8 cineole, and citronellol. These biomolecules should be further identified in the extracts by chromatographic analyses to explore their phytotoxic action on post-emergence assays.

Keywords: Weed. Biological Control. Extraction methods. Allelopathy. Bioherbicide.

1 INTRODUCTION

One of the main issues responsible for productivity loss in crops is the presence of weeds, with current estimated losses of approximately 34%.[1] *Bidens pilosa* is a difficult-to-control species found in various crops, with herbicide use being its primary and most effective form of control. In order to address this problem, farmers use chemical control methods with herbicides to manage weeds. Although this method is considered effective among available options, it poses several environmental challenges and can be toxic to humans and animals.[2] In order to mitigate the issues caused by the excessive use of herbicides in crops, bioherbicides, which are naturally derived products obtained from microorganisms such as fungi, bacteria, nematodes, or plants, are being developed and increasingly utilized worldwide.

Bioherbicides have the advantage of degrading rapidly in the environment and being less toxic due to their natural origin, which allows their use in organic farming. Additionally, they provide a solution to the problem of weeds resistant to herbicides. Plant-based bioherbicides are found in smaller quantities for commercialization in the global market. When compared to other bioinputs such as biofungicides and bioinsecticides, the number of registered bioherbicides worldwide is low, representing less than 10% of the registered bioinputs available in the market for commercialization.[3] Regarding the production and commercialization of bioherbicides, Brazil currently does not have any registered bioherbicides. But there is currently a growing body of research using plant extracts to test their phytotoxicity on weeds. Several studies indicate the biomolecules present in the extracts responsible for the phytotoxicity observed in weeds. Essential oils from *Eucalyptus citriodora* were tested for their bioherbicidal potential against the weeds *Portulaca oleracea*, *Lolium multiflorum*, and *Echinochloa crus-galli*, with the biomolecule citronellal identified as responsible for interfering with the germination and development of the weeds.[4] Additionally, *Eucalyptus grandis* inhibited the germination percentage of invasive species such as *Amaranthus hybridus* L. and *Portulaca oleracea* L., with α-pinene identified as the main biomolecule responsible for this inhibition.[5]

Therefore, the objective of the study was to test the allelopathic effect of extracts from plants against *Bidens pilosa* and prospect biomolecules that act in this biological control. Inflorescences of *Calliandra hamematocephala* Hassk, leaves of *Cymbopogon citratus* (DC) Stapf, and leaves from *Eucalyptus citriodora* were used to obtain extracts by four extraction methods, which were applied on the pre-emergence of the weed *Bidens pilosa*.

2 MATERIAL & METHODS

Leaves of eucalyptus (*Eucalyptus citriodora*), seeds of "picão-preto" (*Bidens pilosa*), inflorescences of calliandra (*Calliandra hamematocephala* Hassk), and leaves of lemongrass (*Cymbopogon citratus* (DC) Stapf) were collected in Cachoeira do Sul (Brazil). Part of the eucalyptus, calliandra, and lemongrass plant material was used fresh to obtain extracts by the maceration extraction method, while another part was dried in an oven at 40ºC for 7 days, except for the inflorescences, which were dried for 24 hours.

The plant extracts were obtained by four methods: maceration, infusion, pressurized liquid extraction (PLE), and ultrasound-assisted extraction (UAE). The extraction conditions were a 7-day extraction time for the maceration method, a temperature of 70ºC and an extraction time of 20 minutes for infusion, a power of 275 W, pulse 0 (continuous ultrasound) and an extraction time of 20 minutes for UAE (QR 550, Eco-sonics, Brazil), and a pressure of 10 MPa, a temperature of 50ºC and an extraction time of 20 minutes for PLE (homemade equipment). The solvent used in all methods was distilled water, and the biomass/solvent ratio was 1:10 (w/v). For all methods, the biomass used in each extraction was 5 g.

The seeds of the weed *Bidens pilosa* were sanitized with sodium hypochlorite and distilled water. Subsequently, 25 seeds were placed in a Petri dish containing 2 filter paper sheets, which were moistened with an amount of extract equivalent to 2.5 times the mass of the filter paper. The Petri dishes were placed in a germination chamber (MA402, Marconi, Brazil) at 25ºC and a photoperiod of 12 hours to assess the influence of the extracts on the germination of *Bidens pilosa*. The assay lasted for 15 days, and the variables analyzed were germination percentage, root length and shoot length, and germination speed index (GSI) (Equation 1).

$$\text{GSI} = \Sigma \left(\frac{ti}{ni}\right) \quad (1)$$

Where: ni = number of seeds that germinated at time "i"; ti = time after the test setup (day).

The experiment was conducted in a completely randomized design (CRD) with two factors and an additional control treatment (3x4+1), with 3 replications. Factor A corresponded to the different plants used in the preparation of the extracts, factor D corresponded to the different extraction methods, and the additional control treatment corresponded to the use of distilled water. The results were analyzed using the Sisvar software and subjected to analysis of variance (ANOVA), with means compared using the Tukey test at a 5% probability of error.

3 RESULTS & DISCUSSION

After conducting the pre-emergence experiment with the weed *Bidens pilosa*, the extracts obtained by PLE and infusion from leaves of *Eucalyptus citriodora* exhibited allelopathy, reducing the GSI, germination percentage, and root and shoot lengths. The other plants tested for extract preparation did not show satisfactory results for controlling *Bidens pilosa* (Table 1).

Table 1 Germination, GSI, root length, and shoot length of *Bidens pilosa* after application of aqueous extracts from *Calliandra hamematocephala* Hassk *Cymbopogon citratus* (DC) Stapf, and *Eucalyptus citriodora.*

Plant	Method	Germination (%)	GSI	Root length (cm)	Shoot length (cm)
Calliandra hamematocephala Hassk	Infusion	89.33 a	31.33 a	4.3 a	2.8 a
	Maceration	88.00 a	24.57 b	4.6 a	2.4 a
	PLE	88.00 a	26.93 ab	2.5 b	1.6 b
	UAE	90.67 a	31.05 a	4.3 a	2.6 a
	Control	90.67 a	23.68 b	5.6 a	1.9 b
Cymbopogon citratus (DC) Stapf	Infusion	85.33 a	18.04 c	1.9 b	1.5 d
	Maceration	93.33 a	31.89 a	4.7 a	2.5 b
	PLE	82.67 a	27.03 a	3.4 a	3.1 a
	UAE	90.67 a	20.12 bc	3.5 a	2.2 b
	Control	90.67 a	23.68 b	5.6 a	1.9 c
Eucalyptus citriodora	Infusion	64.00 b	12.28 b	2.0 c	1.4 b
	Maceration	86.67 a	19.29 a	4.5 a	2.3 a
	PLE	68.00 b	12.47 b	3.0 bc	1.2 b
	UAE	89.33 a	18.40 a	3.8 ab	1.9 a
	Control	90.67 a	23.68 a	5.6 a	1.9 ac

Means followed by the same letter in each column for each plant do not differ statistically according to Tukey's test at a 5% level of probability of error.

The potential of *Eucalyptus citriodora* leaf ethanolic extracts obtained by maceration was investigated elsewhere on the germination and initial growth of *Bidens pilosa*, also concluding that the extract reduced the GSI of the weed.[6] Other eucalyptus species, such as *Eucalyptus grandis* and *Eucalyptus camaldulensis*, also exhibited allelopathy against *Bidens pilosa*, reducing the germination percentage of the seeds.[7,8] This demonstrates that eucalyptus species have potential for controlling the weed *Bidens pilosa*. The bioherbicidal potential exhibited by eucalyptus species against weeds is possibly due to compounds present in the species such as α-pinene, 1,8 cineole, and citronellol.[9] The next steps of the work will attest such inferences based on post-emergence assays and chromatographic analyses.

The allelopathy of *Eucalyptus tereticornis* was tested on the germination and development of the weed *Amaranthus viridis* using essential oils obtained from the *Eucalyptus* species. Furthermore, the oils were analyzed for their chemical composition, revealing a significant reduction in the initial growth and vigor of both the seedlings and the weed. Additionally, there was a decrease in the content of photosynthetic pigments and in the cellular respiration of the seedlings. The main components responsible for these damages were identified as α-pinene (32.5%) and 1,8-cineole (22.4%). Other compounds were also identified in the oil in smaller quantities, such as citronellol (1.40%).[10] In other *Eucalyptus* species, the presence of compounds such as 1,8-cineole and citronellol has been identified, which were responsible for phytotoxicity in *Ageratum conyzoides* and *Amaranthus retroflexus* L.

There are also reports of the identification of α-pinene in the *Eucalyptus grandis* species, which is responsible for inhibiting the germination of weeds such as *Amaranthus hybridus* L. and *Portulaca oleracea* L.[2]

4 CONCLUSION

The aqueous extracts obtained from *Eucalyptus citriodora* leaves showed the highest allelopathic effect on the weed *Bidens pilosa*, indicating the potential of this species of eucalyptus to be used in the production of bioherbicide formulations. However, further studies in pre- and post-emergence should be conducted to evaluate the bioactivities and to identify the biomolecules present in these extracts.

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ACKNOWLEDGEMENTS

This work was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES), the National Council of Technological and Scientific Development (CNPq: 404308/2023-6), and the Research Support Foundation of the State of Rio Grande do Sul (FAPERGS). G. L. Zabot (308067/2021-5) and M. V. Tres wish to thank CNPq for the productivity grants.