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Phytotoxic activity of extracts obtained from cagaita (*Eugenia dysenterica* DC. - Myrtaceae) on the growth of black-jack (*Bidens pilosa* L.)

Marlene Benedita Ribeiro[1], Rafael Soares Pozzi Malheiros[2*], Luciana Lucas Machado[1], Ana Maria Mapeli[1]

[1] Universidade Federal do Oeste da Bahia, Centro de Ciências Biológicas e da Saúde, Rua Professor José Seabra de Lemos, 316 - Recanto dos Pássaros. CEP 47808-021, Barreiras, Bahia, Brasil.
[2] Universidade Federal de Viçosa, Departamento de Biologia Vegetal, Avenida Purdue, s/nº Campus Universitário Edif. CCB II – Centro de Ciências Biológicas II, CEP 36570.900, Viçosa, Minas Gerais, Brasil.
* Autor para correspondência: rafaelpozzi@hotmail.com

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ABSTRACT – Cagaita (*Eugenia dysenterica*) is a native plant of the Cerrado with great economic, social and environmental importance. The objective of this work was to determine the phytotoxic potential of extracts of leaf and bark from the stem of *E. dysenterica* on the initial growth of *B. pilosa* (black-jack). The experiment was performed under laboratory conditions, and the extracts used were: leaf aqueous extract, leaf ethanolic extract, leaf hydroalcoholic extract 70:30, leaf hydroalcoholic extract 50:50, bark from the stem aqueous extract, bark from the stem ethanol extract, bark from the stem hydroalcoholic extract 70:30 and bark from the stem hydroalcoholic extract 50:50. All *E. dysenterica* leaf and bark from the stem extracts exerted inhibitory effects on the radicle and hypocotyl growth of black-jack seedlings. Thus, *E. dysenterica* leaf and bark from the stem extracts present high phytotoxic potential and may be useful in studies attempting to find new molecules with bioherbicidal function for controlling spontaneous plants.

Keywords: Allelopathy, growth inhibition, spontaneous plant.

RESUMO - Atividade fitotóxica de extratos obtidos de cagaita (*Eugenia dysenterica* DC.-Myrtaceae) sobre o crescimento de picão preto (*Bidens pilosa* L.). A cagaita (*Eugenia dysenterica*) é uma planta nativa do Cerrado com grande importância econômica, social e ambiental. O objetivo deste trabalho foi determinar o potencial fitotóxico de extratos de folhas e cascas do caule de *E. dysenterica* no crescimento inicial de *B. pilosa* (picão-preto). O experimento foi conduzido em condições de laboratório e os extratos utilizados foram: extrato aquoso foliar, extrato etanólico foliar, extrato hidroalcoólico foliar 70:30 extrato hidroalcoólico foliar 50:50, extrato aquoso do caule, extrato etanólico do caule, extrato hidroalcoólico do caule 70:30 e extrato hidroalcoólico do caule 50:50. Todos os extratos de folhas e caules de *E. dysenterica* exerceram efeitos inibitórios sobre o crescimento da radícula e do hipocótilo de plântulas de picão-preto. Assim, extratos foliares e de caules de *E. dysenterica* apresentam alto potencial fitotóxico e podem ser úteis em estudos que tentam encontrar novas moléculas com função bioerbicida para o controle de plantas espontâneas.

Palavras-chave: alelopatia, inibição do crescimento, plantas espontâneas

INTRODUCTION

The control processes for spontaneous plants in agricultural production increase the dependence of synthetic herbicides, causing degradation of ecosystems. In addition, an increase in herbicide-resistant invasive plants has been observed in many farming systems, indicating that new control strategies should emerge through developing natural products which have lower residence time and toxicity to an environment (Souza Filho & Alves 2002).

Secondary substances produced by plants, including terpenes, phenolic compounds and nitrogen compounds, provide a diversity of chemical structures, offering opportunities for producing new growth inhibitory biomolecules (Taiz *et al.* 2017). Studies in this area have been carried out based on allelopathy, a science which studies any process involving secondary compounds produced by plants, algae, bacteria and fungi, which positively or negatively influence the growth and development of biological systems (Harun *et al.* 2014).

These secondary compounds are distributed at varying concentrations in the different plant tissues and throughout their life cycle. These substances cause direct or indirect effects when they are released into the environment through leaching, root exudation, volatilization and/or organic matter decomposition (Borghetti *et al*. 2013), which may alter cell division and elongation processes, growth-inducing hormonal mechanisms, cell membrane

permeability, stomatal opening, photosynthesis, respiration, protein synthesis and lipid metabolism upon absorption by the plant (Einhellig 2004).

The *Eugenia* genus, representative of the Myrtaceae family, covers several species with potential for allelopathic studies, as they present a diversity of chemical compounds with proven allelopathic activity such as phenolics (Reynertson 2008, Jacques 2009, Imatomi *et al*. 2013, Malheiros *et al*. 2018). Few studies have been focused on the allelopathic potential of the Myrtaceae family in Brazil (Imatomi *et al*. 2013), and a lack of research on the allelopathic effect of native species of the Cerrado biome is noteworthy.

It is believed that extracts from *Eugenia dysenterica* (cagaita) present secondary bioactive metabolites which interfere in the growth of neighboring plants (Malheiros *et al*. 2018). After an *in loco* visit, it was verified that the occurrence of these specimens occurred through agglomerates, not coexisting with other plant species in the surroundings, which may be a strong indication of phytotoxic effect. Ethanolic and hydroalcoholic extracts of 70:30 and 50:50 ratios obtained from the leaves of this species inhibited the average growth of *Lactuca sativa* L. (lettuce) and *Zea mays* L. (corn) (Malheiros *et al*. 2016). Pina *et al*. (2009) reported the negative interference of aqueous extract from *E. dysenterica* leaves on *Sesamum indicum* L. (sesame) and *Raphanus sativus* L. (radish). However, there are no reports in the literature demonstrating the phytotoxic effect of cagaita on invasive species such as *Bidens Pilosa* (Asteraceae - black-jack), considered one of the most infesting plants found in corn and other annual crops, and which usually form dense infestations (Santos & Cury 2011).

Thus, the objective of this work was to evaluate the phytotoxic effect of aqueous, hydroalcoholic and ethanolic extracts from the leaves and stem bark of *E. dysenterica* on the germination and seed growth of *B. pilosa* (Asteraceae – black-jack).

MATERIAL AND METHODS

Fully expanded leaves and stem bark of ten adult *E. dysenterica* De Candolle plants were collected during the vegetative phase considering diameter at breast height, in December from inside the Serra da Bandeira (-12º04'48" S and -45º00'36" W) in the West region of Bahia, Brazil. The area has Cerrado vegetation, exhibiting *cerradão* and ciliary forest physiognomies.

The plant parts were immediately submitted to extract preparation after collection. The methodologies of Malheiros *et al*. (2016) were used to prepare the ethanolic, hydroalcoholic and aqueous extracts from leaves and stem bark, respectively. The extracts employed were leaf aqueous extract (LAE), leaf ethanolic extract (LEE), leaf hydroalcoholic extract 70:30 (LHE 70:30), leaf hydroalcoholic extract 50:50 (LHE 50:50), stem aqueous extract (SAE), stem ethanolic extract (SEE), stem hydroalcoholic extract 70:30 (SHE 70:30) and stem hydroalcoholic extract 50:50 (SHE 50:50). Leaf extracts and stem bark were used at concentrations 0, 250,500 and 1000mg L^{-1}, with the largest obtained by weighing and the others by dilution. The pH was adjusted to 6.5 after preparing the extract concentrations.

Bidens pilosa (black-jack) seeds were used as target species for preparing the experiments. Filter paper disks contained in previously autoclaved Petri dishes (9 cm in diameter) were impregnated with 2 ml (Brasil 2009) of extract concentrations in the bioassays, in addition to the control (distilled water), and then 50 seeds were seeded on each filter paper disc. The experiments were carried out during 2 months in which one type of plant extract was tested each week.

The experiment was maintained in BOD germination chambers with controlled temperature and light (25 ± 2 ºC, 230 μm m^{-2s-1}) under a light/dark photoperiod of 16/8 hours.

Germination was evaluated daily considering a 2mm root protrusion. The analyzed variables were germination percentage (Labouriau 1983) and germination speed index (GSI), according to the formula GSI = (G1/N1) + (G2/N2) + (G3/N3) + ... + (Gn/Nn) (Maguire 1962). The Barnes & Soeiro (1981) method was employed for the radicle and hypocotyl growth bioassays. The experiment was terminated after three consecutive days of null germination.

The experimental design was completely randomized with five replications, employing 50 seeds as the experimental unit for the germination bioassays and 10 seedlings for growth evaluation. The data were submitted to analysis of variance, and tested for the assumptions of normality, randomness and homogeneity of variances. The treatments were compared by the Tukey test at 5% probability using the Sisvar® statistical program (Ferreira, 2000).

RESULTS

Leaf and stem hydroalcoholic extracts promoted inhibition in the germination percentage of *B. pilosa* at concentrations of 500 and 1000 mg L^{-1}, whereas the aqueous and ethanolic extracts did not cause significant changes in this variable at any concentration used (Tab. 1). The two highest concentrations of the LHE 70:30, LHE 50:50, SHE 70:30 and SHE 50:50 extracts reduced the germination percentage by 36, 35, 29 and 34%, respectively, compared to control (Tab. 1).

The GSI was inhibited by 17 and 35% by LAE at concentrations of 250 and 1000mg L^{-1}, respectively, while LEE caused a 28% mean reduction at concentrations of 250

and 500 mg L^{-1}, and a 58% reduction at a concentration of 1000 mg L^{-1} compared to the control. Regardless of the concentration, LHE 70:30, LHE 50:50 and SAE reduced the GSI of *B. pilosa* by 53, 51 and 46%, respectively. The SEE promoted an average reduction of 30% at concentrations of 250 and 500mg L^{-1}, while the reduction was 56% at the concentration of 1000mg L^{-1} in relation to the control. The SHE 70:30 reduced the GSI by 29% at the concentration of 250mg L^{-1}, and by 55% at the concentrations of 500 and 1000mg L^{-1}. The SHE 50:50 reduced the seeds GSI by 22% at the concentration of 250 mg L^{-1}, while the concentrations of 500 and 1000 mg L^{-1} provided a 46% reduction (Tab. 2).

All leaf and stem bark extracts had inhibitory effects on the radicle growth of black-jack seedlings. LAE caused an average reduction of 33% at all concentrations. The LEE caused inhibition of 34, 50 and 67% for the concentrations of 250, 500 and 1000mg L^{-1}, respectively. The LHE 70:30 promoted a reduction of 51% at the concentration of 250 mg L^{-1}, while the two higher concentrations reduced the radicle growth by 71% in relation to the control. In turn, LHE 50:50 (250, 500 and 1000mg L^{-1}) provided an average inhibition of 61%. The SAE caused an average reduction of 49% (250 and 500mg L^{-1}) and 67% (1000mg L^{-1}), while SEE inhibited the radicle growth at concentrations of 250 and 500mg L^{-1} by 38%, and by 62% at the concentration of 1000mg L^{-1}. Lastly, the SHE 70:30 and SHE 50:50 caused an average inhibition of 50%, regardless of concentration (Fig. 1).

In relation to the hypocotyl growth of black-jack seedlings, it was observed that the effect of cagaita extracts was less expressive regarding radicle growth, with the latter being more sensitive to secondary metabolites. The LAE only promoted a decrease at the concentration of 250mg L^{-1} (37%) in comparison to the control. On the other hand, the LEE only demonstrated an inhibitory effect (52%) in the two highest concentrations. As for the LHE 70:30, there was an average decrease of 55% in all concentrations. The LHE 50:50 caused 61% inhibition at the concentrations of 500 and 1000mg L^{-1} in relation to the control. Regarding SAE, no significant differences were observed in hypocotyl growth at any of the tested concentrations. In turn, the SEE only caused a reduction at the highest concentration (60%). The SHE 70:30 had a significant effect when used at 500 and 1000mg L^{-1}, with a mean hypocotyl reduction of 57%. However, the SHE 50:50 (250mg, 500 and 1000mg L^{-1}) promoted an average reduction of 49% in hypocotyl growth compared to control (Fig. 2).

Table 1. Effect of different leaf and stem bark extracts of *Eugenia dysenterica* DC. on the germination percentage (G%) of *Bidens pilosa* L. seeds.

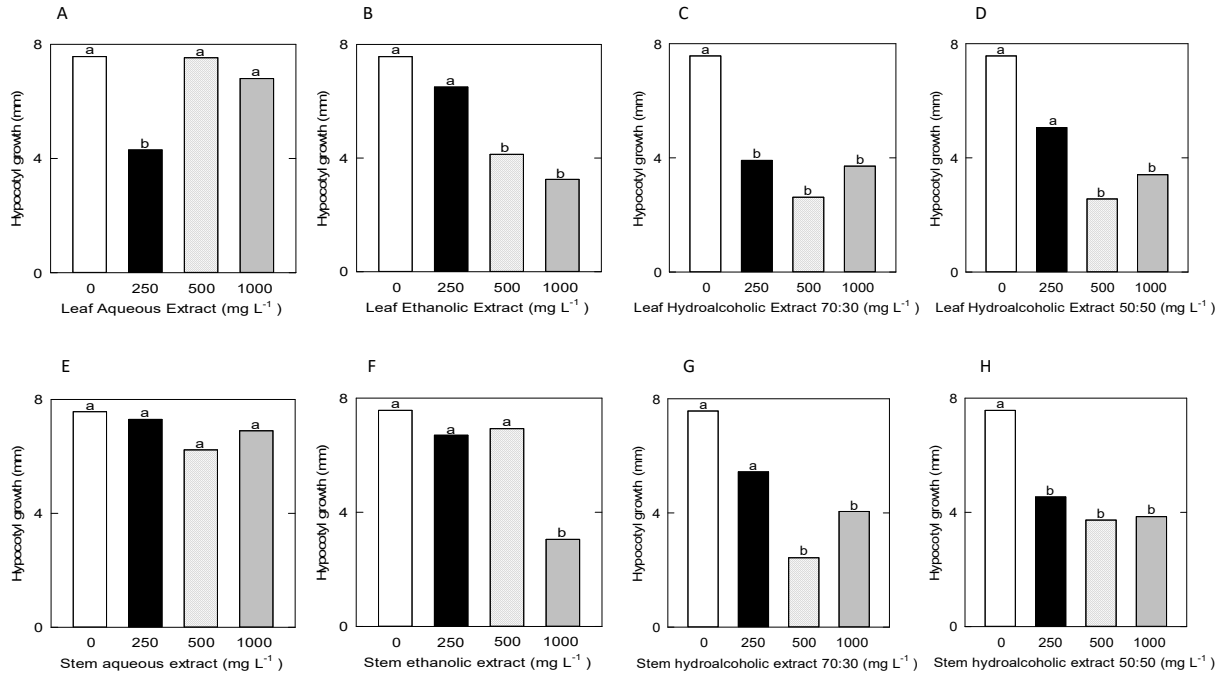
Concentration (mgL^{-1})	LAE	LEE	LHE 70:30	LHE 50:50	SAE	SEE	SHE 70:30	SHE 50:50
0	64.00 A	64.00 A	64.00 A	64.00 A	64.00 A	64.00 A	64.00 A	64.00 A
250	59.84 A	59.14 A	60.33 A	59.35 A	59.18 A	58.81 A	58.16 A	59.11 A
500	57.48 A	59.76 A	41.56 B	42.00 B	57.00 A	57.25 A	48.16 B	42.48 B
1000	58.75 A	57.70 A	40.18 B	41.14 B	59.40 A	58.14 A	42.12 B	41.18 B

Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability. Leaf Aqueous Extract (LAE), Leaf Ethanolic Extract (LEE), Leaf Hydroalcoholic Extract 70:30 (LHE 70:30), Leaf Hydroalcoholic Extract 50:50 (LHE 50:50), Stem aqueous extract (SAE), Stem ethanolic extract (SEE), Stem hydroalcoholic extract 70:30 (SHE 70:30), and Stem hydroalcoholic extract 50:50 (SHE 50:50).

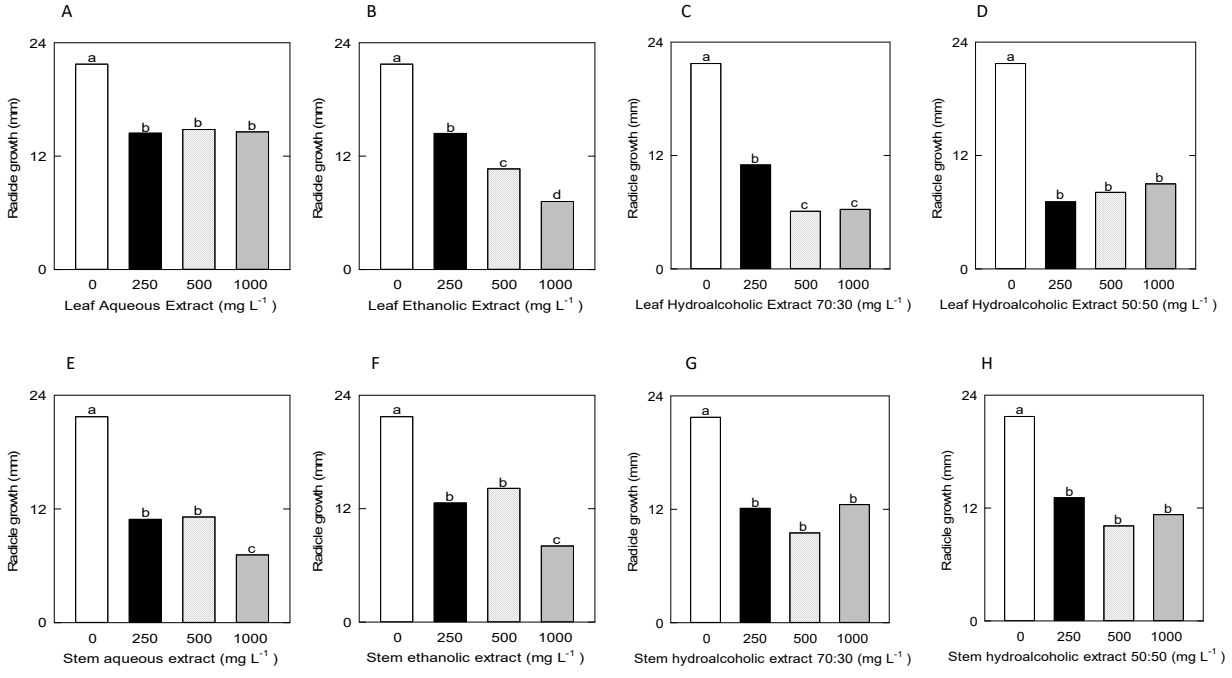
Table 2. Effect of different leaf and stem bark extracts of *Eugenia dysenterica* DC. on the germination speed index (GSI) of *Bidens pilosa* L. seeds.

Concentration (mgL^{-1})	LAE	LEE	LHE 70:30	LHE 50:50	SAE	SEE	SHE 70:30	SHE 50:50
0	18.20 A	18.20 A	18.20 A	18.20 A	18.20 A	18.20 A	18.20 A	18.20 A
250	15.10 B	13.60 B	9.95 B	7.30 B	10.60 B	13.30 B	12.90 B	14.20 B
500	19.61 A	12.20 B	8.18 B	9.60 B	9.65 B	12.20 B	8.41 C	9.79 C
1000	11.75 C	7.70 C	7.61 B	9.94 B	9.40 B	8.04 C	8.10 C	9.68 C

Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability. Leaf Aqueous Extract (LAE), Leaf Ethanolic Extract (LEE), Leaf Hydroalcoholic Extract 70:30 (LHE 70:30), Leaf Hydroalcoholic Extract 50:50 (LHE 50:50), Stem aqueous extract (SAE), Stem ethanolic extract (SEE), Stem hydroalcoholic extract 70:30 (SHE 70:30), and Stem hydroalcoholic extract 50:50 (SHE 50:50).



Figs. 1 A-H. Effect of different leaf and stem extracts of *Eugenia dysenterica* DC. on the radicle growth (mm) of *Bidens pilosa* L. seeds. Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability. **A.** Leaf Aqueous Extract (LAE); **B.** Leaf Ethanolic Extract (LEE); **C.** Leaf Hydroalcoholic Extract 70:30 (LHE 70:30); **D.** Leaf Hydroalcoholic Extract 50:50 (LHE 50:50), **E.** Stem aqueous extract (SAE); **F**. Stem ethanolic extract (SEE), **G**. Stem hydroalcoholic extract 70:30 (SHE 70:30); **H**. Stem hydroalcoholic extract 50:50 (SHE 50:50).



Figs. 2 A-H. Effect of different leaf and stem extracts of *Eugenia dysenterica* DC. on the hypocotyl growth (mm) of *Bidens pilosa* L. seeds. Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability. **A.** Leaf Aqueous Extract (LAE); **B.** Leaf Ethanolic Extract (LEE); **C**. Leaf Hydroalcoholic Extract 70:30 (LHE 70:30); **D.** Leaf Hydroalcoholic Extract 50:50 (LHE 50:50); **E.** Stem aqueous extract (SAE); **F**. Stem ethanolic extract (SEE); **G**. Stem hydroalcoholic extract 70:30 (SHE 70:30); **H.** Stem hydroalcoholic extract 50:50 (SHE 50:50).

DISCUSSION

The target species in the present study demonstrated a delay in germination and germination speed index (Tables 1 and 2). The effect promoted by ethanolic and aqueous extracts obtained from *E. dysenterica* leaves may be associated with the presence of several secondary compounds, including phenols, hydrolysable tannins, flavonols, flavanones, flavanones, xanthones, flavones, free steroids and saponins (Malheiros *et al.* 2019). The most expressive results were promoted by hydroalcoholic extracts, agreeing with the study by Lousada *et al*. (2012) which showed that the black-jack germination was also inhibited by lemongrass hydroalcoholic extracts. Allelopathic compounds alter seed germination through a multiplicity of effects on physiological and biochemical processes, since there are hundreds of different structures upon which they exhibit multiple phytotoxic effects (Imatomi *et al*. 2013), as is the case with phenolic compounds which affect cell wall elasticity and block mitochondrial respiration (Weir *et al.* 2004), and saponins which showed action on the cell membrane, modifying cell permeability (Alves & Santos, 2002).

Considering weeds, the delay in seed germination may be favorable because the longer the seeds remain in the field without germination, the longer they will be exposed to pathogens, environmental factors and insect predation (Aires *et al*. 2005). In addition, some authors report that plant extracts may present greater inhibitory potential in seed germination than the synthetic herbicide itself, with the advantage of being biodegradable, thereby reducing soil and aquifer contamination (Silva *et al*. 2011).

Black-jack radicle growth was inhibited by all tested extracts, and the most pronounced effects were generally observed at higher concentrations (Fig. 1). This is favorable because it makes it difficult to establish the plant, since the root is essential for support and absorption of water and nutrients. The inhibitory effect observed for radicle growth was similar to that observed for hypocotyl growth (Fig. 2). This is possible since the same damaged radicles can continue to absorb solutes, which eventually affects the shoot (Burgos *et al*. 2004). In addition, allelopathic effects are mainly observed in seedling development as a whole, and plant length is the parameter most commonly used to evaluate allelopathic action (Souza Filho & Alves 2002).

Leaf and stem hydroalcoholic extracts were the treatments which had greater inhibitory potential on the black-jack growth (Figs. 1 and 2). The plants produce numerous chemically diverse compounds with wide variation in polarity and distribution in the plant, with it being recognized that polar extracts can provide high expression of bioactive compounds, especially those related to phenolic compounds (Chon 2002, Leu 2002). The most expressive extracts on *B. pilosa* in this work have intermediate polarity (hydroalcoholic extract), indicating that extraction of the secondary compounds present in cagaita can be related to the balance between the polarities. In addition, the leaf hydroalcoholic extracts (LHE 70:30 and LHE 50:50) showed the highest inhibition percentage for radicle and hypocotyl growth, respectively. This fact suggests that these extracts could have greater extraction capacity for inhibitory compounds on growth. A recent study has shown that the phytotoxic potential of *E. dysenterica* in model species is possibly associated with the presence of phenolic compounds, tannins, flavonoids and saponins present in its leaves (Malheiros *et al*. 2018). Thus, *E. dysenterica* leaf and stem extracts present high phytotoxic potential and may be useful in studies attempting to find new molecules with bioherbicidal function to control spontaneous plants. However, field tests and a phytochemical analysis of the extracts are necessary to provide effective proof of the phytotoxic effects observed in this work.

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