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Prospection the action of *Eugenia uniflora L.*: Allelopathic effect on an agricultural species and an invasive species, and its antimicrobial potential

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ABSTRACT

Allelopathy is defined as the mechanism of action of the secondary metabolism of plants, which can affect other organisms in a beneficial or deleterious way. The aim of the present study is to test the allelopathic effect of the brazilian cherry tree (*Eugenia uniflora* L.), a native Brazilian tree, on the agricultural crop of maize (*Zea mays* L.) and on black-jack (*Bidens pilosa* L.). A phytochemical screening of the dried leaves was carried out to verify which metabolites are present. Experiments on allelopathic potential and effect were conducted in the laboratory with carrot seeds as bioindicators, using the aqueous extract of dried brazilian cherry leaves, in addition to the experiment in a greenhouse using the powder of dried leaves in the substrate. The results indicate that the leaves of the brazilian cherry tree have an allelopathic effect on the purple nutsedge, inhibiting its development, but do not affect the germination and growth of maize seedlings. The aqueous extract of the leaves exhibits an antimicrobial activity classified as low and very low.

RESUMO

A alelopatia é definida como o mecanismo de ação do metabolismo secundário das plantas, que pode afetar outros organismos de forma benéfica ou deletéria. O objetivo do presente trabalho é testar o efeito alelopático da pitangueira (*Eugenia uniflora* L.), árvore nativa do Brasil, sobre a cultura agrícola do milho (*Zea mays* L.) e sobre o picão-preto (*Bidens pilosa* L.). Foi realizada a prospecção fitoquímica das folhas secas para verificar quais são os metabólitos presentes. Os experimentos de potencial e efeito alelopático foram realizados em laboratório com sementes de cenouracomo bioindicadoras, utilizando o extrato aquoso das folhas secas de pitangueira, além do experimento em casa de vegetação utilizando o pó das folhas secas no substrato. Os resultados indicam que as folhas de pitangueira apresentam efeito alelopático sobre o picão-preto, inibindo seu desenvolvimento, porém não afetam a germinação e o crescimento das plântulas de milho. O extrato aquoso das folhas apresenta uma atividade antimicrobiana classificada como baixa e muito baixa.

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Introduction

Allelopathy is defined as the ability of interaction between organisms through the production and release of substances into the environment, which can positively or negatively influence the growth of other species (Molisch, 1937). In 1996, the International Allelopathy Society (IAS) expanded this definition to encompass processes related to the release of primary and secondary metabolites by plants, lichens, viruses, and fungi, which exert beneficial or harmful effects on the germination, growth, and development of biological systems (Silva et al., 2017).

This interaction can have several practical applications, such as in the pharmaceutical industry and especially in agriculture, since occupying a certain area without prior study, depending on the crop, the compounds (secondary metabolites) present in the location can cause damage to plant growth and productivity (Ferreira & Aquila, 2000). Considering this capacity, there are several studies in the literature about the potential for producing biological products for agriculture using allelopathic compounds as a substitute for chemical products (Stadink and Talamik, 2012).

In Brazil, about 41% of the territory is occupied by areas intended for agriculture, of which 77% corresponds to family farming (IBGE, 2017). Conventional and family agriculture are dependent on pesticides in our country and represent risks not only for the producers, but also for the nearby residents and for the final consumer (Yuantari et al., 2015; Souza et al., 2023).

The prolonged use of a single formulation can result in the development of resistance or tolerance of invasive plants to a certain chemical (Vargas, 2016). In Brazil, one of the main invasive field species is black-jack (*Bidens pilosa* L.), which has rapid and intense growth, in addition to being a host for fungi, nematodes, and viruses (Vallin, 2022), with the capacity to cause a loss of about 58% in crop yield (Rizzardi et al., 2003).

As an alternative to this problem, the possibility of using more natural products arises, obtained from plants that have secondary metabolites capable of inhibiting or slowing down the germination of invasive plants, as well as combating possible diseases caused by phytopathogenic bacteria. The brazilian cherry tree (*Eugenia uniflora L.*), species belonging to the Myrtaceae family (Carvalho, 2006) is the subject of study in several researches due to its compounds, which can significantly alter the germination and development of plants, as well as also having antimicrobial activity (Fiúza et al., 2009; Huller and Schok, 2011; Bastos et al., 2016).

From this, the need for studies that address sustainable ways of management within conventional agriculture is evident, which seek natural compounds with relevant biological activities.

Therefore, the present work aimed to evaluate the allelopathic effect on the agricultural species maize, the invasive plant Brazilian fleabane, and the antimicrobial potential, from the dried leaves of Surinam cherry (*Eugenia uniflora* L.), in order to verify the possibility of using the aqueous extract as a bioherbicide and natural antimicrobial.

Materials and Methods

The work was carried out at the Plant Physiology Laboratory (*Laboratório de Fisiologia Vegetal* - LAFEV) and at the Microbiology and Biotechnology Laboratory (*Laboratório de Microbiologia e Biotecnologia* - LAMIBI) of the State University of Western Paraná (*Universidade Estadual do Oeste do Paraná*), Cascavel campus, from August 2023 to March 2024. The leaves of the species *E. uniflora* used in the experiment were collected in July 2023, in the municipality of Medianeira (latitude: -25.2885, longitude: -54.1275), Paraná, and their processing and storage were carried out at LAFEV in Cascavel. The samples were transported from Medianeira to Cascavel in plastic bags without the need for refrigeration.

The collected leaves were dried in an oven with forced air circulation at a temperature of 40 degrees Celsius for 3 days. After drying, the leaves were ground in a Willey-type knife mill. The resulting powder was stored away from light and at room temperature, as in the methodology proposed by Mourão Junior and Souza Filho (2010).

Phytochemical prospecting

The tests related to the phytochemical screening of different plant extracts of *E. uniflora* were conducted according to the methodology described by Matos (1997). These tests were based on colorimetric visualization and/or the formation of a precipitate after the addition of specific reagents. The classes of secondary metabolites investigated were: Saponins through the reaction with distilled water and P.A. hydrochloric acid; steroids and triterpenoids through the Liebermann-Burchard reaction; alkaloids using Dragendorff's reagent, Mayer's reagent, and Bouchardat's reagent; anthocyanidins, anthocyanins, aurones, chalcones, flavones, flavonols, and xanthones based on pH changes in the medium; coumarins through a fluorescence reaction with potassium hydroxide; and tannins through the reaction with ferric chloride.

Potential and allelopathic effect

The preparation of the extract from dried brazilian cherry leaves was carried out at concentrations of 2.5%, 5%, 7.5%, and 10% (w/v). To prepare the extracts, 2.5, 5, 7.5, and 10 g of *E. uniflora* leaf powder were weighed for each extract concentration, which was then homogenized in 1 liter of water for one minute. The extract was left to rest protected from light for 4 hours, according to the methodology described by Carvalho et al. (2012). After the resting period, the extract was filtered with the aid of a cloth filter. For the control (extract at 0.0% concentration), only distilled water was used.

For the evaluation of allelopathic potential, carrot seeds (*Daucus carota* L.) purchased commercially were used, and they were used as bioindicator seeds, which are more sensitive to metabolites for this experiment and, therefore, give us a parameter of how the aqueous extract may work in other species.

For the allelopathic effect experiment, we used seeds of blackjack (*B. pilosa*) collected at the same location as the brazilian cherry leaves, transported and stored at LAFEV in a plastic bag, kept in an environment protected from light and moisture.

The seeds were placed in Petri dishes on three sheets of filter paper, which were moistened with 6 mL of water for the control and 6 mL of aqueous extract from dried *E. uniflora* leaves for each treatment (2.5%, 5%, 7.5%, and 10% w/v), totaling 5 treatments with 4 repetitions of 25 seeds for each experiment, with carrot and blackweed.

The experiments were kept in a B.O.D. type germination chamber, under constant temperature and controlled light, at 25°C and a 12-hour light/dark photoperiod. The experimental design used for the two experiments was completely randomized (CRD), and the experiments were evaluated for seven days, every day, counting as germinated those seeds that presented a 2mm primary root.

The variables analyzed were: Germination percentage (GP%), mean germination time (MGT), according to Edmond and Drapala (1958), and germination index (GI), according to Silva and Nakagawa (1995), and germination frequency (Labouriau and Agudo, 1987). The data were subjected to analysis of variance (ANOVA) and the means were compared using Tukey's test at 5% probability, using the RStudio program. (R Core Team, 2013).

Initial development in the greenhouse

The experiments regarding the initial growth of black-jack and maize were conducted separately, but the methodology applied is the same. It was necessary to use 144 plastic pots of size 2 with a capacity of 250 mL, filled with the HT® substrate that contained vermiculite, which were watered with three times the weight of the dry substrate.

The experimental design for this experiment was a randomized block design. Three treatments were carried out with 4 repetitions each, grown in substrate, with T1 watered only with distilled water, T2 supplemented with 5.57 g of *E. uniflora* leaf powder, and T3 supplemented with 11.15 g of the powder, both also watered with distilled water. The calculation of the required amount of powder was performed according to the methodology proposed by Rizzardi et al. (2008), which allows analyzing what amount of powder will be proportional to the leaf litter deposition in the natural environment. The pots used in this experiment have a diameter of 14 centimeters and an approximate area of 154 cm.

In the pots, 3 seeds of beggarweed and corn were placed, separately for the experiment, which were watered and evaluated daily, but for different periods. This occurs because the emergence time of corn and beggarweed is different, and environmental conditions also affect the species differently. The experiment with corn was concluded in 20 days, and with beggarweed in 48 days.

The variables analyzed were: emergency percentage (EP%), average emergency time (AET), according to Edmond and Drapala (1958), and emergency speed index (ESI), according to Silva and Nakagawa (1995), as well as root and shoot length and the dry mass of both parts, for corn and for black goona.

The data were subjected to analysis of variance (ANOVA) and the means were compared using Tukey's test at 5% probability, using the RStudio program (R Core Team, 2013).

Antimicrobial potential

The aqueous extract obtained from the dried leaves of *E. uniflora* used the same methodology of allelopathic potential, at a concentration of 200 mg/mL and filtered through a paper filter.

The antimicrobial activity of the plant extracts of E. uniflora was evaluated following the methodology proposed by Scur et al. (2014) with modifications. The microorganisms used are from the American Type Culture Collection (ATCC) and Cefar Diagnóstica (CCD), with the Gram-positive bacteria being: *Bacillus subtilis* (CCD-04), *Staphylococcus aureus* (ATCC 25923); and the Gram-negative bacteria: *Escherichia coli* (ATTCC 25922) and *Salmonella Tiphymurium* (ATCC 14028).

For the tests, the bacteria were recovered in Brain Heart Infusion (BHI) enrichment broth and incubated for 24 hours at 36±1° C. After this period, the strains were subcultured on plates containing Mueller Hinton Agar (MH) medium, incubated at 36±1° C for 24 hours. Shortly after, the inocula were prepared and standardized in saline solution (0.85%) according to the 0.5 McFarland scale.

Determination of the Minimum Inhibitory Concentration (MIC)

In 96-well microdilution plates, 150 µL of Mueller-Hinton broth (MHB) was added to all wells, 150 µL of the plant extract was added to the first well, with serial dilutions from 200 to 0.09 µL/mL in the subsequent wells. To each well, 10 µL of the microorganism inoculum (prepared previously) was added. The plates were gently homogenized and incubated for 24 hours at 36±1° C.

After this time interval, a 10 µL aliquot of 0.5% triphenyl tetrazolium chloride (TTC) solution was added to each well of the microplates, and they were reincubated for another three hours at 36±1° C. The presence of red coloration in the wells is interpreted as a negative proof of the extract's inhibitory effect, that is, the extract does not show inhibitory activity against the tested bacteria. As a positive control, 150 µL of Gentamicin solution at a concentration of 200 mg/mL was used for the bacteria, and 150 µL of MHB and 10 µL of the inoculum were added. For the negative control, only 150 µL of MHB and 10 µL of the inoculum were used. Sterility control of the extracts was also carried out. The MIC was performed in triplicate.

Determination of the Minimum Bactericidal Concentration (MBC)

Before adding TTC at 0.5% to the wells, 2 µL of the inoculum from each well was removed and added to Petri dishes containing Mueller-Hinton Agar (MHA). The plates were incubated for 24 hours at 36±1º C. To determine the MIC, it will be observed whether there was microbial growth on MHA. The lowest concentration at which there was an absence of microbial growth was considered the MIC. The MIC assays were also performed in triplicate.

The MIC and MBC of the plant extracts were classified according to Pandini et al. (2015); the activity will be categorized into one of the 4 classes: High (12.5 mg/mL), moderate (12.5 to 25 mg/mL), low (50 to 100 mg/mL), and very low (>100 mg/mL).

Results and Discussion

The phytochemical test of the aqueous extract of dried E. uniflora leaves confirmed the presence of compounds from the classes of saponins, terpenoids (triterpenoids), alkaloids, phenols, and flavonoids (flavones) (Table 1). Studies conducted by Zanusso et al. (2003), regarding the phytochemistry of *E. uniflora*, corroborate the results observed in the present work. Other research, however, with essential oil from the leaves and extracts from the barks of the genus Eugenia and the family Myrtaceae, also confirm the results obtained here (Marin et al., 2004; Ogunwande et al., 2005; Santos et al., 2021).

Table 1.

Phytochemical screening of the aqueous extract of dried leaves of *E. uniflora*.

Classes of metabolites	Aqueous extract of *E. uniflora*
Saponins	+
Steroids	-
Triterpenoids	+
Alkaloids	+
Phenols	+
Tannins	-
Coumarins	-
Anthocyanins	-
Flavonoids	+
Flavones	+

Note: The authors.

The literature highlights that the compounds present can vary depending on the extraction method. For example, in ethanolic extracts of leaves of the same species as the present study, the presence of cardiotonic glycosides, tannins, coumarins, and anthraquinones is observed (Silva and Lima, 2016; Zanusso et al., 2023). These differences are attributed to the use of different types of solvents, which have distinct molecular structures, polarities, and solubilities (Fernández-Agulló et al., 2013; Cabana et al., 2013). Furthermore, it is important to emphasize that the leaf collection period, temperature, soil type, and water availability can also alter the presence of secondary metabolites in the same species (Gobbo-Neto and Lopes, 2007), as also seen by Auricchio and Bacchi (2003), who compare the compositions of metabolites present in *E. uniflora* collected in different locations.

According to Table 2, it was found that the aqueous extract of dried *E. uniflora* leaves influenced all the variables analyzed in the allelopathic potential experiment. We observed that the application of the aqueous cherry extract at a concentration of 2.5% resulted in a reduction in the final germination percentage (FG%) of carrot seeds. At extract concentrations of 7.5% and 10%, germination was reduced to 17% and 12%, respectively, representing a reduction of more than 70% in germination in these treatments.

Table 2.

Germination (GP%), Mean Germination Time (MGT/days) and Germination Speed Index (GSI) of D. carota seeds submitted to the aqueous extract of E. uniflora at proportions of 0, 2.5, 5, 7.5 and 10% (w/v).

Concentration of the extract (%)	GP%, MGT and GSI of the allelopathic potential of dried *E. uniflora* leaves on D. carota.		
	GP%	MGT	GSI
0	57 a	3.63 c	5.58 a
2.5	30 b	3.84 bc	2.21 b
5	29 b	5.83 ab	1.45 bc
7.5	12 c	4.61 bc	0.73 c
10	17 bc	6.76 a	0.83 c
CV%	21.14%	19.21%	22.73%

Values followed by different letters differ significantly from each other according to Tukey's test at 5% probability.

Note: The authors.

Significant statistical differences were also observed for the MGT, while in the control the seeds took on average 3 days to germinate, the seeds in the 10% (w/v) treatment took an average of 6 days to complete the germination process. We can also observe that the GI showed the highest values for the control, and the application of all concentrations of the brazilian cherry aqueous extract reduced this variable (Table 2), a phenomenon that can be explained by the allelopathic action of the metabolites identified in the previously presented phytochemical screening table.

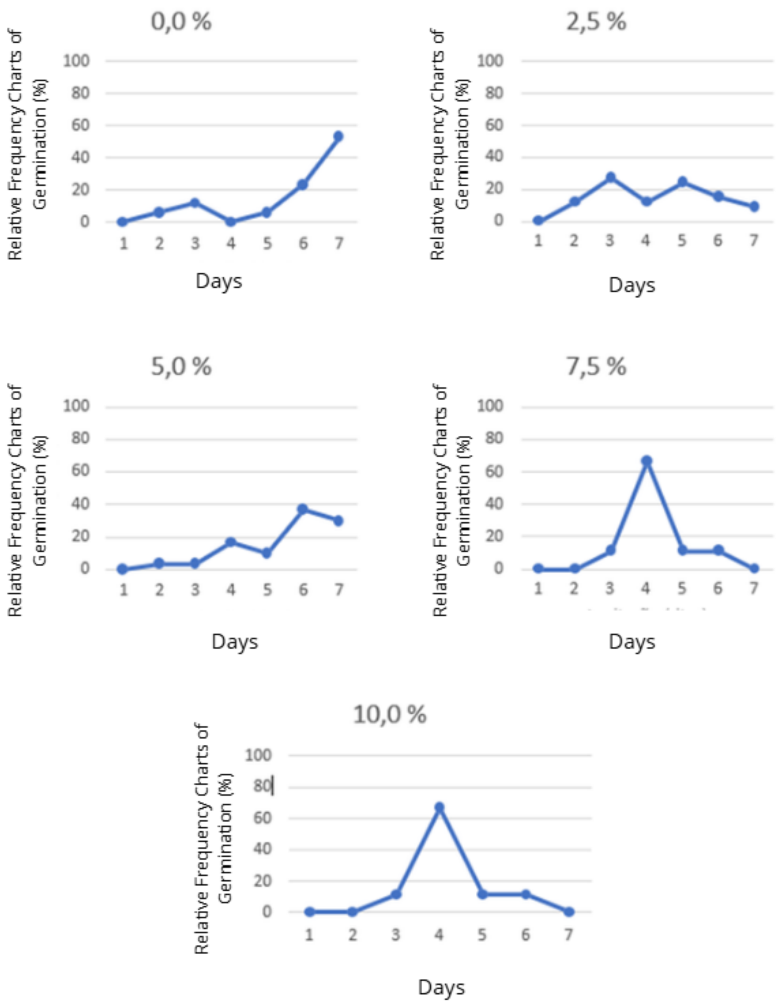
The results obtained by Boiago et al. (2018) corroborate those of this study, as the leaf extracts of *E. uniflora* influenced the germination of lettuce and corn seeds, concluding that the extracts from fresh brazilian cherry leaves were more phytotoxic than the extracts obtained through leaf infusion. The results of experiments using other species of the Myrtaceae family also validate those obtained in the present study, as seen by Giotto et al. (2007), who used the aqueous extract of cagaiteira leaves (*Eugenia dysenterica* Mart. Ex DC. Berg.), Sausen et al. (2009) working with aqueous extract of cherry leaves (*Eugenia involucrata* Dc.) and mountain guava tree (*Acca sellowiana* (O. Berg) Burret), besides Gusman et al. (2014), who used an aqueous extract of clove leaves (*Syzygium aromaticum* L.), thus confirming the allelopathic mechanism of action present in the Myrtaceae family.

One explanation for the increase in the average germination time may be due to the presence of triterpenoids, as this class of metabolites can lead to changes in the biochemical processes of plants, thus increasing the activity of enzymes such as peroxidases and polyphenol oxidases, interfering with the germination process of nearby species (Chowhan et al., 2011; Areco et al., 2014). The phenolic compounds, also identified in this study, can alter enzymatic reactions, induce stress, and inhibit the synthesis of photosynthesis enzymes and proteins (Mersie and Singh, 1993; Barkosky and Einhellig, 2003; Zhang et al., 2013), affecting the species as observed in this experiment.

When examining the germination behavior of carrot seeds, according to the germination frequency (%) graphs (Figure 1), it is observed that germination is distributed throughout the experimental period, showing a polymodal pattern for the control and at 2.5 and 5.0% extract concentrations. At 7.5 and 10% concentrations, a unimodal pattern is observed with a shift to the right, indicating that the identified metabolites were able to delay the germination of carrot seeds and corroborating the MGT and GSI results presented in Table 2.

Figure 1.

Relative Frequency Charts of Germination of carrot seeds (*D. carota*) submitted to the aqueous extract of *E. uniflora* in the proportions of 0, 2.5, 5, 7.5, and 10% (w/v).



Note: The authors.

The experiment to evaluate the allelopathic effect of *E. uniflora* extract on *B. pilosa* seeds showed that the germination percentage did not present statistically significant differences between the control and the different concentrations of extract applied (Table 3). The literature often highlights that the allelopathic effect does not affect the germination process itself, but rather the speed of germination, which is why it is important to monitor germination daily (Ferreira and Áquila, 2000).

Table 3.

Germination Percentage (GP%), Mean Germination Time (MGT/days), and Germination Speed Index (GSI) of black-jack seeds (*B. pilosa*) subjected to *E. uniflora* aqueous extract at proportions of 0, 2.5, 5, 7.5, and 10% (w/v).

Concentration of the extract (%)	GP%, MGT e GSI do potencial alelopático de folhas secas de *E. uniflora* sobre *B. pilosa*.		
	GP%	MGT	GSI
0	44	3.35 b	3.45 a
2.5	42	4.90 a	2.41 ab
5	47	4.64 a	2.7 ab
7.5	34	4.36 a	1.76 ab
10	22	6.80 a	0.97 b
CV%	35.81%	11.93%	37.07%

Values followed by different letters differ significantly from each other according to Tukey's test at 5% probability.

Note: The authors.

For the average germination time of *B. pilosa*, it is observed that the control differs from all treatments with aqueous extract of *E. uniflora* leaves, with the treatment at 10% extract concentration requiring twice as many days for germination to occur compared to the control. Furthermore, in the GSI we also observed a difference between the control and the 10% treatment, showing that allelopathy significantly interfered with germination.

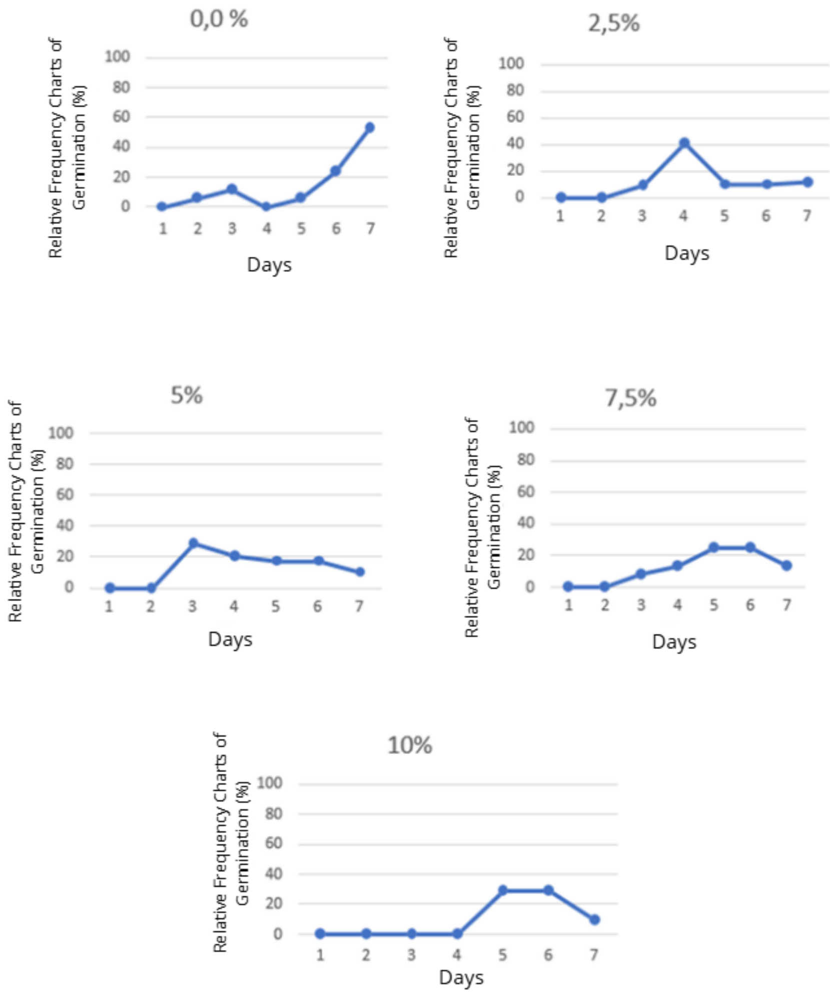
Huller and Schok (2011), comparing the extract of three Eugenia species, observed that only the mean germination time (MGT) of lettuce seeds showed a statistical difference when subjected to the extract of *E. uniflora* and *E. involucrata*, while the application of the extract of *E. pyriformes* affected the germination percentage and MGT of this species, indicating an allelopathic effect of the species belonging to the genus Eugenia.

The delay in the germination of *B. pilosa* seeds subjected to different concentrations of *E. uniflora* extract is evident in the germination frequency graphs (Figure 2). In the treatments with the presence of extract, it is possible to observe that the curves shifted considerably to the right. Furthermore, the seeds only began to germinate after the second day of the experiment, in contrast to the control, whose germination started on the first day. The start of germination only on the fourth day for the *B. pilosa* seeds subjected to a 10% extract concentration is also related to the previously reported MGT and GSI values.

The experiments conducted by Ribeiro et al. (2020), in which different solvents were used to extract *Eugenia dysenterica* DC. extract on *B. pilosa* seeds, validate the results obtained in this study, which observed statistical differences in germination rate values with the aqueous extract and differences in germination percentage with the hydroalcoholic extract. Similar to *E. uniflora*, *E. dysenterica* also contains flavonoids and terpenoids in its leaves (Couto et al., 2009), confirming the potential of these compounds to influence the effects observed on the germination of *B. pilosa*.

Figure 2.

Relative Frequency Charts of Germination of B. pilosa seeds subjected to the aqueous extract of E. uniflora at proportions of 0, 2.5, 5, 7.5, and 10% (p/v).



Note: The authors.

In the experiments conducted in the greenhouse with *B. pilosa*, it was also possible to observe that the dried leaves of *E. uniflora* influenced the germination process, indicating once again the allelopathic effect of this species (Table 4). The presence of phenolic compounds, triterpenoids, and saponins in the leaf may explain the results obtained, as they are related to the inhibition of germination, are capable of altering the cell cycle and causing chromosomal changes, in addition to being associated with generalized cytotoxicity (Rice, 2012; Latif et al., 2017; Bezerra et al., 2020).

Table 4.

Emergency Percentage (EP%), Average Emergency Time (AET/days) and Emergency Speed Index (ESI) of *B. pilosa* seeds submitted to the powder of dry leaves of *E. uniflora*, in a greenhouse.

Amount of leaf powder (g)	Allelopathic effect of dry leaves of *E. uniflora* on *B. pilosa* in a greenhouse		
	EP (%)	AET	ESI
0	23.61 ab	7.24 b	0.69 a
5.57	26.38 a	9.9 a	0.57 ab
11.15	19.44 b	8.01 ab	0.51 b
CV%	31.86%	31.75%	32.47%

Values followed by different letters differ significantly from each other according to Tukey's test at 5% probability.

Note: The authors.

The low values of the emergence percentage in both the control and the treatments with the aqueous extract of brazilian cherry may be associated with the dormancy phenomenon, a common defense mechanism in most invasive species. This mechanism allows seeds to remain in the soil for months or years, waiting for ideal environmental conditions to trigger their germination (Vivian et al., 2008). Even with the possible dormancy of the seeds, it was possible to observe a statistical difference between the treatments, mainly in the treatment with 11.15 g of powder, which inhibited the germination of about 27% of the seeds and showed the lowest ESI value among all treatments, indicating a delay in germination.

There are several studies in the literature related to the use of plant extracts aiming to inhibit the germination of invasive species such as Brazilian cress (Corsato et al., 2010; Krenchinski et al., 2017; Kapoor et al., 2019; Mousavi et al., 2021). As in the case of the brazilian cherry tree, phenolic compounds are also present in the species used in these studies and are associated as the class in which most of the compounds related to allelopathic activity are found (Rice, 1984), which can mainly affect mitochondrial activity and the elasticity of the plant cell wall (Weir et al., 2004). Also present in the leaves, alkaloids are known to be germination inhibitors, as they accumulate in the soil through the process of leaching and make the environment toxic for seeds to germinate (Levitt and Lovett, 1985; Rice, 2012).

No differences were identified in the measurements of aerial part length (cm), root length (cm), and dry mass (mg) of both parts of *B. pilosa* seedlings (Table 5). These results differ from those obtained by Pires et al. (2001), who observed a reduction in growth and deformation in the leaf blade of black weed with the application of aqueous extract of leucaena (*Leucaena leucocephala* (Lam.) de Wit.) in the pot soil in a greenhouse, with this reduction and deformation directly affecting its length and mass measurements. This phytotoxic effect can be explained by the presence of alkaloids, a class of allelochemicals common to both leucaena and brazilian cherry, as reported by Pires et al. (2001) and in Table 1 of this study.

Table 5.

Shoot length (cm), root length (cm), shoot dry mass (g) and root dry mass (g) of *B. pilosa* seedlings subjected to the powder of *E. uniflora* dried leaves, in a greenhouse.

Amount of leaf powder (g)	Allelopathic effect of dry leaves of *E. uniflora* on *B. pilosa* in a greenhouse			
	Part of the área P.A (cm)	Roots (cm)	Dry mass P.A (mg)	Root dry mass (mg)
0	3.47 a	7.74 a	5.00 a	4.00 a
5.57	4.22 a	8.03 a	1.00 a	3.00 a
11.15	5.07 a	14.57 a	8.00 a	3.00 a
CV%	35.95%	70,46%	70.45%	99.08%

Values followed by different letters differ significantly from each other according to Tukey's test at 5% probability.

Note: The authors.

For the experiments conducted with corn seeds in a greenhouse, it was observed that there was no statistical difference between the analyzed variables (Table 6). In intercropping systems such as the brazilian cherry tree with agricultural crops like corn, it is important that the compounds do not affect productivity, avoiding losses.

Table 6.

Emergency Percentage (EP%), Average Emergency Time (AET/days) and Emergency Speed Index (ESI) of corn seeds (*Zea mays*) subjected to the powder of *E. uniflora* dried leaves at proportions of 0, 2.5, 5, 7.5 and 10% (w/v), in a greenhouse.

Amount of leaf powder (g)	Allelopathic effect of dry leaves of *E. uniflora* on *Z. mays* in a greenhouse		
	EP (%)	AET	ESI
0	70.83 a	2.44 a	6.18 a
5.57	84.72 a	2.93 a	7.31 a
11.15	81.94 a	2.85 a	6.97 a
CV%	17.03%	16.28%	18.37%

Values followed by different letters differ significantly from each other according to Tukey's test at 5% probability.

Note: The authors.

As in Table 6, for the emergency variables, the powder of dried *E. uniflora* leaves also did not interfere with the average length and dry mass of the aerial part and root of the maize seedlings (Table 7).

The results obtained here can be considered positive in the context of a consortium between agricultural crops and native trees in an Agroforestry System (AFS). These systems aim for a more sustainable agricultural practice, interacting with native trees that promote improvements in soil fertility and recovery, reduction of dependence on external inputs, as well as generating socioeconomic benefits for the producers (Abdo et al., 2008; Carvalho, 2020; Maceno et al., 2021).

In the same way observed in this study, in an experiment conducted in the laboratory, Menegon et al. (2023) also found the allelopathic effect of sibipiruna (*Poincianella pluviosa* DC.) on lettuce (*Lactuca sativa* L.) and tomato (*Solanum lycopersicum* L.) seeds as bioindicators, but the metabolites did not affect the germination and the aerial part length of corn, which may suggest a consortium between the species in an agroforestry system context.

Table 7.

Length of the aerial part (cm), root length (cm), dry mass of the aerial part (g), and dry mass of the root (g) of maize (*Zea mays*) seedlings subjected to the powder of dry *E. uniflora* leaves, in a greenhouse.

Amount of leaf powder (g)	Allelopathic effect of dry leaves of *E. uniflora* on *Z. mays* in a greenhouse			
	Part of the área P.A (cm)	Roots (cm)	Dry mass P.A (mg)	Root dry mass (mg)
0	30.82 a	42.38 a	510.0 a	370.0 a
5.57	28.94 a	41.28 a	590.0 a	410.0 a
11.15	21.72 a	32.97 a	590.0 a	430.0 a
CV%	33.19%	36.61%	19.27%	17.5%

Values followed by different letters differ significantly from each other according to Tukey's test at 5% probability.

Note: The authors.

In addition to the results obtained through the interaction between plants, the present study also verified the allelopathic activity by inhibiting the growth of bacteria, highlighting the possibility of using this plant defense mechanism not only as a bioherbicide, but also as natural antibiotics that can assist both in the pharmaceutical industry and in inhibiting phytopathogenic diseases.

Table 8 shows the values of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the aqueous extract of dried brazilian cherry leaves, indicating antimicrobial activity both in MIC and MBC, in low and very low amounts of efficiency according to Pandini et al. (2015).

Table 8.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the aqueous extract of E. uniflora against standard microorganisms.

	MIC (mg/mL)	MBC (mg/mL)
Gram (+)		
B. subtilis	200	100
S. aureus	100	50
Gram (-)		
E. coli	100	-
S. Tiphymurium	100	-

Note: The authors.

The antimicrobial activity of *E. uniflora* has also been tested in other studies using the extract with different solvents. Wuerges and Gandra (2016) identified the antimicrobial activity of *E. uniflora* extract against microorganisms such as *Candida albicans*, *Salmonella spp.*, *Streptococcus salivarius*, as did Fiúza et al. (2009), who observed growth inhibition of *Enterobacter cloacae*, *E. coli*, and *Pseudomonas aeruginosa* using the ethanolic extract. Oliveira et al. (2008) also identified antimicrobial activity of the extract obtained by infusion of fresh brazilian cherry leaves, which complements the results obtained here.

Furthermore, the literature presents several studies related to the activities of the essential oil of brazilian cherry leaves, as seen by Ogunwande (2005), Alves et al. (2010), and Souza et al. (2018), which confirms the antimicrobial activity of brazilian cherry leaves just as the experiments in the present study confirmed.

Based on the results presented, therefore, more studies are needed related to the allelopathic effect of the brazilian cherry tree both on other plants and on other bacteria. A deeper understanding of these effects may contribute to practical applications and the development of new products based on the compounds present in the brazilian cherry tree.

Conclusion

It was observed that the aqueous extract of dried *E. uniflora* leaves showed allelopathic activity on Bidens pilosa in a greenhouse, but did not affect corn germination, in addition to presenting antimicrobial activity against the tested bacteria, which suggests the possibility of using this species as a bioherbicide and natural antibiotic.

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