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Allelopathy of aqueous extracts from *Cyperus* species on the germination of *Tridax procumbens* L.

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Abstract

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Many weed species adversely affect agricultural productivity by releasing allelochemicals that inhibit the growth and development of surrounding plants. Members of the Cyperaceae family, in particular, are frequently associated with such allelopathic interference in cultivated areas. In this context, the present study aimed to evaluate the allelopathic potential of aqueous extracts derived from *Cyperus brevifolius* and *Cyperus sesquiflorus* on the germination of *Tridax procumbens* L. seeds. Plant materials from the donor species were collected and separated into aerial (aboveground) and underground parts. Aqueous extracts were prepared by mixing 1 g of powdered plant material with 10 mL of distilled water, yielding concentrations of 0, 20, 40, 60, 80, and 100%. The germination assay was conducted in quadruplicate, with 50 seeds per Petri dish. The treatments were incubated in a B.O.D. chamber under an 8-hour light photoperiod for seven days. Total flavonoid content was quantified using a spectrophotometric method based on a 5% aluminum chloride ($AlCl_3$) reaction in methanol. The results revealed that aqueous extracts from both *Cyperus* species significantly inhibited the germination of *T. procumbens*, with varying degrees of intensity depending on the species and plant part used. Notably, the aerial part extracts of *C. sesquiflorus* exhibited a stronger allelopathic effect compared to its underground parts, while the opposite trend was observed for *C. brevifolius*, where underground part extracts were more inhibitory. Furthermore, in both species, extracts from the aerial parts consistently displayed higher total flavonoid content across all tested concentrations. These findings underscore the potential of *Cyperus* species, particularly their flavonoid-rich aerial parts, as promising natural alternatives for weed management through allelopathic suppression.

Keywords: bioactivity; *Cyperaceae*; allelochemicals

Introduction

The family *Cyperaceae*, commonly referred to as the sedge family, is recognized as the third largest family among the monocotyledons, comprising over 5 000 species globally (Govaerts et al., 2020; Spalink et al., 2018). Members of this family are often herbaceous and predominantly found in moist or aquatic habitats. Despite their ecological importance, species within *Cyperaceae* are frequently categorized as spontaneous plants and are commonly labeled as weeds. This classification is largely due to their aggressive growth and adaptability, which allow them to colonize diverse environments, including agricultural systems (Brandão et al., 2012). As a result, they are often viewed negatively by farmers and agronomists, primarily because of the competitive pressure they exert on cultivated crops. These species compete intensely for essential resources such as light, water, and soil nutrients, which can significantly compromise crop yield and quality (Silva and Durigan, 2006).

Nevertheless, emerging studies challenge this conventional perception by highlighting the ecological and agronomic potential of certain sedge species. Among the most intriguing aspects is their allelopathic capacity – an ecological phenomenon in which plants release biochemicals (allelochemicals) into the environment that influence the growth and development of neighboring plants. Allelopathy is increasingly gaining attention as a sustainable and eco-friendly approach for managing weed populations, offering an alternative to the excessive use of synthetic herbicides, which are often associated with environmental contamination, herbicide resistance, and negative impacts on non-target organisms (Bezerra et al., 2018).

Allelochemicals can be present in various parts of the plant, including roots, leaves, stems, flowers, and seeds. When released into the surrounding soil or through leachates, they can interfere with critical physiological and biochemical processes in other plant species, such as seed germination, photosynthesis, enzyme activity, and nutrient uptake. Depending on the compound and concentration, these substances may exert either inhibitory or stimulatory effects. Therefore, identifying and characterizing the allelopathic potential of native or spontaneous plant species is of great importance for the development of biological control strategies that align with sustainable agriculture principles (Romeiro, 2019).

The use of plant extracts with allelopathic properties has shown promising results in biological weed management. According to Costa et al. (2018), this practice can be integrated as part of a preventive and environmentally sound weed control strategy. Among the *Cyperaceae*, *Cyperus rotundus* L., commonly known as nutgrass, is one of the most studied species with documented allelopathic effects. Several studies have reported that aqueous and methanolic extracts of *C. rotundus* are capable of inhibiting seed germination and early seedling development in various plant species (El-Rokiek et al., 2010; Miranda, 2019; Deomedesse et al., 2019). Given the allelopathic potential observed in *C. rotundus*, it is plausible to hypothesize that other species within the genus *Cyperus* may possess similar bioactive properties.

In this context, the present study investigates the allelopathic effects of aqueous extracts from *Cyperus brevifolius* and *Cyperus sesquiflorus* on the germination of *Tridax procumbens* L. – a weed species widely recognized for its prolific seed production, adaptability, and persistent presence in disturbed areas and croplands (Kissmann and Groth, 1991; Bacelar, 1994). *T. procumbens*, commonly known as coat buttons, poses a significant threat to agricultural systems due to its rapid spread and competitive behavior. Controlling its population through allelopathic methods could offer an innovative and low-impact solution, particularly in integrated weed management (IWM) programs.

Therefore, this research aims to contribute to the growing body of knowledge on plant interactions and provide insights into the potential use of native *Cyperus* species as natural agents in weed suppression. By evaluating the inhibitory effects of their extracts on *T. procumbens*, we seek to support the development of alternative, sustainable strategies for weed management that minimize chemical inputs and promote ecological balance in agroecosystems.

Methodology

Material collection

The botanical material was collected at the Campus of Engineering and Agricultural Sciences of the Federal University of Alagoas (CECA/UFAL). The species were properly identified by a specialist in the family and deposited in the Herbarium of the Institute of the Environment of the state of Alagoas (IMA) with the following registration numbers: MAC 64987 (*Cyperus brevifolius*) and MAC 64293 (*Cyperus sesquiflorus*).

Preparation of extracts

Initially, the botanical material from both species was divided into aerial part (AP) and underground part (UP), dehydrated in an oven at 42.5°C for 48 hours, and subsequently ground in a knife mill to obtain the powder. The extracts of each species were prepared using the ratio of 1 g of powder to 10 mL of distilled water. The solutions were kept at rest in the refrigerator (5° ± 1°C) for 48 hours. Subsequently, the supernatant was extracted and then filtered using qualitative filter paper, following the methodology proposed by Jacobi and Ferreira (1991).

Germination bioassays

A completely randomized design (CRD) was used, consisting of a 2 × 2 × 5 factorial arrangement: two species (*Cyperus brevifolius* and *C. sesquiflorus*), two plant parts (roots and shoots), and five extract concentrations (20%, 40%, 60%, 80%, and 100%). An additional treatment with distilled water was included as a control, resulting in a total of 21 treatment combinations. Each treatment consisted of four replications, with 50 *Tridax procumbens* seeds per replicate, totaling 200 seeds per treatment. The seeds were sown in Petri dishes with a diameter of 9 cm, each containing two sheets of autoclaved Germitest paper, following the procedures recommended by Brazil (2009). The Germitest papers were moistened with 4 mL of the respective aqueous extracts for each treatment. The dishes were then placed in a Biochemical Oxygen Demand (B.O.D.) germination chamber (SOLAR) set at 30°C, under an 8-hour daily photoperiod. Germination was monitored daily for seven days, and the following variables were assessed: first germination count, germination speed index (GSI), and the total number of germinated seeds at the end of the experimental period. The GSI was calculated using the formula described by Maguire (1962).

The formula used is as follows:

$$IVG = (G1/N1) + (G2/N2) +...+ (G/Nn).$$

where: G1, G2, Gn = number of seedlings in the first, second, and last count. N1, N2, Nn = number of days from sowing to the first, second, and last count.

The results were expressed in germination speed index, and the germination percentage was calculated based on the number of plants classified as normal, considering the Root Appearance Scale (RAS) (Brazil, 2009). The formula used for this experiment is as follows:

$$G\% = G25 \times 100$$

where: G (%) – germination percentage; A – number of germinated seeds; N – total number of seeds placed for germination in each repetition (Fanti and Perez, 1998).

Quantification of total flavonoids

In 5 mL volumetric flasks, 100 µL of 5% aluminum chloride (AlCl3) and 100 µg/mL of extracts from the aerial parts (APs) and underground parts (UPs) of *C. brevifolius* and *C. sesquiflorus* were added at corresponding concentrations of 20, 40, 60, 80, and 100%. Subsequently, the flasks were filled with methyl alcohol (methanol) and left to stand for 30 minutes. Then, spectrophotometer reading was performed at a wavelength of 425 nm (Alves and Kubota, 2013). The total content of flavonoids was expressed as µg of quercetin equivalents (QE/µg) per µg of extracts from *C. brevifolius* and *C. sesquiflorus*.

Statistical analysis

For statistical analyses, the Python programming language was used for data processing, statistical tests, and visualization.

Results and Discussion

Germination bioassay

This study demonstrates that aqueous extracts from both *Cyperus brevifolius* and *Cyperus sesquiflorus* exhibit allelopathic effects on the germination of *Tridax procumbens* L. seeds. Extracts from the aerial parts (AP) of *C. brevifolius* showed greater inhibitory activity at higher concentrations (80 – 100%), whereas *C. sesquiflorus* extracts were effective across a broader concentration range (20 – 100%). Notably, the Germination Speed Index (GSI) increased in seeds treated with the highest concentration (100%), particularly for *C. sesquiflorus* (GSI = 3.18), as shown in Table 1.

Table 1. Allelopathic effect of aqueous extracts from the aerial parts of *Cyperus brevifolius* and *Cyperus sesquiflorus* on germination parameters of *Tridax procumbens* seeds

Species	Concentration (%)	First Germination Count (FCG) ± SE	Germination % (G) ± SE	Germination Speed Index (GSI) ± SE
C. brevifolius	0 (Distilled water)	7.75 ± 0.25	70 ± 2.5	6.30 ± 0.3
	20	9.00 ± 0.30	71 ± 2.0	6.75 ± 0.4
	40	8.00 ± 0.35	78 ± 3.0	6.76 ± 0.3
	60	8.25 ± 0.40	62 ± 2.8	5.91 ± 0.5
	80	4.75 ± 0.20	59 ± 2.6	5.09 ± 0.4
	100	5.75 ± 0.15	54 ± 3.1	4.87 ± 0.4
C. sesquiflorus	0 (Distilled water)	13.00 ± 0.50	90 ± 2.1	8.19 ± 0.6
	20	2.25 ± 0.10	54 ± 1.9	4.42 ± 0.3
	40	2.00 ± 0.12	71 ± 2.4	5.56 ± 0.4
	60	1.25 ± 0.08	60 ± 2.0	4.48 ± 0.3
	80	1.25 ± 0.06	70 ± 2.5	5.95 ± 0.5
	100	1.00 ± 0.05	52 ± 1.8	3.18 ± 0.2

Note: Values represent means ± standard error (SE) from four replicates.
Source: Authors' own elaboration

Statistical analysis (ANOVA, $p < 0.05$) revealed significant inhibitory effects on germination percentage and speed index with increasing extract concentrations for both species, with *C. sesquiflorus* exhibiting a stronger allelopathic impact at lower concentrations.

Aqueous extracts from the underground parts (UP) of *C. brevifolius* at 20 – 40% concentrations led to the lowest germination percentages (<60%), indicating stronger allelopathic activity compared to the aerial parts at similar concentrations. Interestingly, a 20% UP extract inhibited germination comparably to an 80% AP extract, suggesting the underground parts are more potent and cost-effective as an allelopathic source. Conversely, *C. sesquiflorus* showed the greatest inhibitory effect with the 100% UP extract.

Regarding GSI, the highest concentration (100%) of underground extracts resulted in the lowest values for both species, consistent with the aerial part results (Table 2).

The differential allelopathic effects observed between aerial and underground parts underscore the importance of considering plant organ-specific bioactivity when developing natural herbicide strategies. These results corroborate findings by El-Rokiek et al. (2010), who reported significant inhibitory effects of *Cyperus rotundus* extracts on weed species, with underground tuber extracts showing stronger effects than aerial parts.

Miranda (2019) further supports this by demonstrating stronger germination inhibition from leaf extracts than from tubers in several weed species, highlighting potential species- and organ-specific differences in allelopathic potency within the genus *Cyperus*. Similarly, Deomedesse (2019) found that tuber extracts from nutgrass delay seedling emergence and vigor, which may aid in managing weed competition under field conditions.

Together, these findings emphasize the potential of aqueous extracts from *Cyperus* species as eco-friendly bioherbicides with distinct bioactivity profiles depending on plant part and extract concentration.

Total flavonoid quantification

It was observed that the aerial parts of both species (*C. brevifolius* and *C. sesquiflorus*) contain similar levels of total flavonoids and exhibit higher concentrations compared to their respective underground parts. Specifically, the aerial parts consistently showed greater flavonoid content than the roots and rhizomes. Moreover, among the underground parts, *C. brevifolius* demonstrated a higher total flavonoid content than *C. sesquiflorus*. In this context, "better" refers to a higher concentration of total flavonoids, indicating a greater presence of these bioactive compounds in the underground parts of *C. brevifolius* compared to those of *C. sesquiflorus* (Graph. 1).

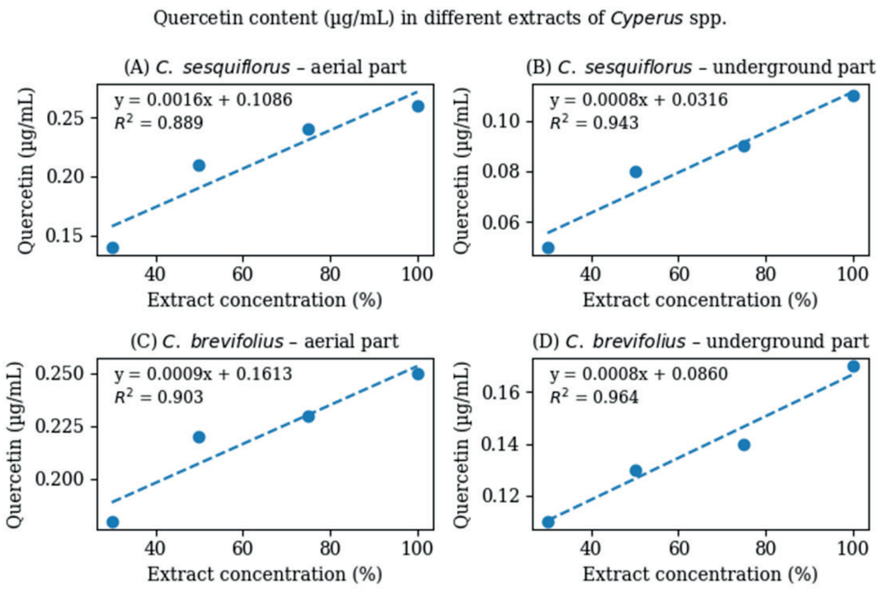
Bezerra et al. (2018) quantified total flavonoids in Cyperus iria and *Cyperus articulatus*, identifying approximately 52% total flavonoids in the aerial parts (AP), and a content of 14.2% in the underground parts (UP) of C. iria, and approximately 22.6% in the AP and 4.3% in the UP of *C. articulatus*. Vicensi et al. (2021) and Oliveira and de Melo (2018) also found positive results when quantifying total flavonoids in the species *Cyperus rotundus*.

Based on this, it is valid to affirm that the results of this study underscore the importance of species from the genus *Cyperus L*. due to the presence of total flavonoids as identi-

Table 2. Allelopathic effect of aqueous extracts from the underground parts of *Cyperus brevifolius* and *Cyperus sesquiflorus* on germination parameters of *Tridax procumbens* seeds

Species	Concentration (%)	First Germination Count (FCG) ± SE	Germination % (G) ± SE	Germination Speed Index (GSI) ± SE
C. brevifolius	0 (Distilled water)	7.75 ± 0.25	70 ± 2.5	6.30 ± 0.3
	20	7.00 ± 0.20	59 ± 2.1	5.34 ± 0.4
	40	7.25 ± 0.22	53 ± 1.8	5.05 ± 0.3
	60	4.00 ± 0.18	67 ± 2.3	5.07 ± 0.3
	80	2.00 ± 0.10	63 ± 2.2	4.98 ± 0.4
	100	4.50 ± 0.15	61 ± 2.0	4.87 ± 0.3
C. sesquiflorus	0 (Distilled water)	13.00 ± 0.50	90 ± 2.1	8.19 ± 0.6
	20	16.75 ± 0.45	96 ± 1.8	9.78 ± 0.5
	40	15.75 ± 0.35	83 ± 2.0	8.78 ± 0.5
	60	11.25 ± 0.30	63 ± 2.4	6.46 ± 0.4
	80	9.25 ± 0.25	72 ± 1.9	6.23 ± 0.3
	100	5.25 ± 0.15	47 ± 2.1	4.15 ± 0.2

Note: Values represent means ± standard error (SE) from four replicates.
Source: Authors' own elaboration



Graph 1. A: Quercetin values in µg/mL found per percentage in extracts of the aerial part of Cyperus sesquiflorus (Torr.) Mattf. & Kük. on Tridax procumbens L. B: Quercetin values in µg/mL found per percentage in extracts of the underground part of Cyperus sesquiflorus (Torr.) Mattf. & Kük. on Tridax procumbens L. C: Quercetin values in µg/mL found per percentage in extracts of the aerial part of Cyperus brevifolius (Torr.) Mattf. & Kük. on Tridax procumbens L. D: Quercetin values in µg/mL found per percentage in extracts of the underground part of Cyperus brevifolius (Torr.) Mattf. & Kük. on Tridax procumbens L.
Source: Authors' own elaboration

fied in this study and others cited. Overall, the importance of all the species studied in ecology was highlighted. These findings encourage further research and reinforce the need for conservation and study of Cyperaceae species as a whole, contributing to a better understanding and sustainable utilization of plant biodiversity.

Conclusion

It can be concluded that both *Cyperus* species studied exhibit significant allelopathic activity on the germination of *Tridax procumbens* seeds. Notably, the aqueous extracts from the aerial parts of *C. sesquiflorus* demonstrated greater allelopathic potential than those from its underground parts. In contrast, for *C. brevifolius*, the extracts from the underground parts showed a more pronounced inhibitory effect compared to those from the aerial parts. Additionally, aerial part extracts of both species consistently presented higher total flavonoid content across all tested concentrations. These findings highlight the potential of *Cyperus* extracts particularly those rich in flavonoids as sustainable and natural alternatives to synthetic herbicides in weed management strategies.

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