Phytochemical Profile and Evaluation of the Allopathic Effect of Three Species of the Genus *Cyperus* (Cyperaceae)

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Abstract

The genus *Cyperus* is widely distributed worldwide and occurs in various regions of Brazil. Research has reported the allelopathic potential of species belonging to this genus. From this perspective, the present study analyzes the phytochemical profile and allelopathic effect of seven concentrations of the aqueous extracts of *Cyperus distans* L., *Cyperus laxus* Lam., and *Cyperus rotundus* L. The allelopathic potential was evaluated in triplicate from germination bioassays on seeds of two species, *Lactuca sativa* L. and *Emilia fosbergii* Nicolson. In addition, phytochemical analyses were performed to analyze possible allelochemicals present in aqueous extracts of three species by phytochemical screening and high-performance liquid.
chromatography (HPLC). The evaluated variables were: germination (G) and germination speed index (GSI). The data obtained were submitted to the F test, and regression analysis was performed to compare means. The results showed higher susceptibility of *E. fosbergii* to aqueous extracts of *C. distans*, *C. laxus*, and *C. rotundus* at all concentrations analyzed (0.94, 1.87, 3.75, 7.5, 15, and 30%). In turn, the germination rate of *L. sativa* seeds decreased only at the highest concentrations (between 15 and 30%) of the same extracts. This allelopathic potential may be directly associated with the biosynthesis of phenolic compounds by *Cyperus* spp., analyzed in this study by phytochemical prospecting and HPLC.

**Keywords:** allelochemicals, plant extracts, weeds, cyperaceae

1. Introduction

The term allelopathy was created by Hans Molisch in 1937, derived from the union of the words *allelon* (mutual) and *pathos* (injury) (Rice, 1984; Silva, 2012). It can be defined as a process where there is a positive or negative interference of secondary plant metabolites (Cremonez et al., 2013; Silva, 2012). Field suppression effects occur when allelopathic species are used as cover crops, mulch, or in crop rotation, waste incorporation, among others (Farooq et al., 2011). However, prior to the implementation of these tools, the development of bioassays is necessary to verify effects and analyze secondary metabolites involved with allelopathic effects (Pereira et al., 2018).

Over the years, different research has pointed out that some secondary plant metabolites impede the germination and development of other relatively closely related plant species (Cremonez et al., 2013; Salgado et al., 2013). In addition, these metabolites interfere with primary and secondary plant succession, the structure and composition of plant communities, the dynamics between different plant formations, and the dominance of certain plant species, affecting local biodiversity and agriculture (Oliveira et al., 2012).

The family Cyperaceae Juss. is represented by 109 genera and about 5000 species. They are included in the order Poales and are characterized by being cosmopolitan of herbaceous habit, occurring in flooded areas, or even in predominantly dry areas. It is the third most representative family among monocotyledons (Muasya et al., 2009; Rocha and Martins, 2011). It presents invasive weeds of economically important crops, with the following genera being the most frequent: *Cyperus* L., *Carex* L., *Eleocharis* R. Br., *Scirpus* Juss., and *Rhynchospora* Vahl (Oliveira, 1980). According to Alves et al. (2009), Brazil holds 678 Cyperaceae species. These are grouped into 42 genres and distributed into 1,700 names.

In taxonomic terms, Cyperaceae is the second most diverse tribe. Its largest genus, *Cyperus* (Linnaeus 1753: 44), includes about 600 species (Larridon et al., 2014; Reid et al., 2014). Some species of this genus are likely to have deleterious effects on the development of other plants that grow in nearby regions. The main example is *Cyperus rotundus* L., widely known for producing chemical constituents that confer a strong allelopathic effect, preventing seed germination and growth of other plant species (Ameena et al., 2015; Darmanti et al., 2015; Pego and Fialho et al., 2018).

Given that the use of allelochemical compounds can be an effective, economical, and natural
tool for weed management replacing the use of synthetic herbicides (Inoue et al., 2010), it is important to investigate new allelopathic plant extracts. Thus, knowing that the genus *Cyperus* contains species that are a potential source of allelochemicals, this research analyzes the phytochemical profile and allelopathic effect of aqueous extracts of *Cyperus distans* L., *Cyperus laxus* Lam., and *Cyperus rotundus* L. on seed germination of *Lactuca sativa* L. and *Emilia fosbergii* Nicolson.

2. Material and Methods

2.1 Plant Material

The species *Cyperus distans* L., *Cyperus laxus* Lam. and *Cyperus rotundus* L. were collected at the Center of Agricultural Sciences of the Federal University of Alagoas, from July/2016 to July/2017. The species were duly identified by the expert Dr. Ana Paula do Nascimento Prata and, subsequently, an *exsiccatae* of each specimen was deposited in the Herbarium of the Environment Institute of Alagoas under the numbers MAC-63603 (C. distans), MAC-62009 (C. laxus) and MAC-63547 (C. rotundus).

2.2 Obtaining Extracts

Aerial and underground parts of *C. distans*, *C. laxus* and *C. rotundus* were dried in a forced air circulation oven at 45 ºC and pulverized in a knife mill. The obtained powder was stored in plastic bags and frozen to -20ºC until the moment of its use for the preparation of aqueous extracts (Silva et al., 2020).

To obtain the aqueous extract, the *C. distans*, *C. laxus* and *C. rotundus* aerial and underground powder were separately used and a 30g 100 mL⁻¹ (30%) aqueous stock solution was prepared (weight/volume). This solution remained at rest for 24 hours. The extracts obtained were filtered and used for dilutions at concentrations of 0.94%, 1.87%, 3.75%, 7.5%, 15% and 30% (adapted from Pereira et al., 2018).

2.3 Evaluation of the Allelopathic Effect

The aqueous extracts of *C. distans*, *C. laxus* and *C. rotundus* (aerial and underground) were used in *Lactuca sativa* L. and *Emilia fosbergii* Nicolson seeds by controlling the germination of the seeds analyzed in seven different concentrations (0, 0.94, 1.87, 3.75, 7.5, 15 and 30%) as described by Silva et al. (2020). We used transparent plastic boxes with lid (11x11x4 cm) previously disinfected with alcohol and lined with two previously autoclaved germitest paper sheets at 120ºC, relative humidity, temperature (25ºC and photoperiod of 12 hours) and luminosity artificially controlled in BOD (Biochemical Oxygen Demand) germination chambers. Each plate received 30 lettuce seeds (*Lactuca sativa* L.) (locally sourced trade) and 50 emilia seeds (*Emilia fosbergii* Nicolson) (collected in the study area) moistened with 6 mL of the aqueous extracts. As negative control, 6 mL of distilled water was used. The experiment was conducted in triplicate. The evaluation was performed daily for 7 days for lettuce and 14 days for emilia, being the radicle emission the criterion for seed germination evaluation (Silva et al., 2020).
2.4 Phytochemical Screening

The phytochemical screening was based on the methodology proposed by Matos (1997). From the sample of aqueous extracts obtained from *C. distans*, *C. laxus* and *C. rotundus*, 35.0 mL were separated for phytochemical prospecting divided into seven 3.0 mL portions in test tubes numbered from “1” to “7” (Bezerra et al., 2019). Qualitative and semi-quantitative tests were performed for phenols and tannins (by reaction with ferric chloride), anthocyanins, catechins and flavonoids (by pH variation test with sodium hydroxide and hydrochloric acid), saponins (foam test), alkaloids (Dragendorff identification), and terpenoids (Silva et al., 2020).

2.5 High Performance Liquid Chromatography (HPLC)

For the separation and analysis of chemical compounds, the high performance liquid chromatography (HPLC) was used as described by Bezerra et al. (2019). 1 mL of the aqueous extract of the shoot of *C. distans*, *C. laxus* and *C. rotundus* was filtered through a 0.20 μm milipore filter using a syringe and transferred to appropriate vials for HPLC analysis. The chromatographic profile was performed on HPLC with ultraviolet detector (UV) and diode array (DAD), where the extracts were injected at a flow rate of 0.6 mL/min for 72 minutes using one phase column per stationary phase (Jupiter 5u reverse column C18 300A and a mixture of methanol, water and 0.1% trifluoroacetic acid per mobile phase) (Silva et al., 2020). Reference standards were used to identify possible chemical compounds (Table 1). Chromatograms were recorded at the wavelengths 254, 275 and 320 nm.

Table 1. Standard used for identification of chemicals compounds by high performance liquid chromatography

| Retention time (min) | Compounds       | λ1 (nm) | λ2 (nm) | λ3 (nm) |
|---------------------|-----------------|---------|---------|---------|
| 11,17               | Gallic acid     | 195     | 218     | 283     |
| 27,41               | Chlorogenic acid| 250     | 288     | 315     |
| 33,62               | Epi-guaipyridine| -       | 276     | 315     |
| 34,00               | Guaipyridine    | -       | 276     | 315     |
| 50,68               | Luteolin        | -       | 259     | 332     |
| 52,12               | Apigenin        | 256     | 268     | 317     |
| 54,32               | Chrysin         | -       | 271     | -       |
| 55,78               | Acacetin        | 272     | 320     | 375     |
2.6 Statistical Analyses

The analyzed variables were germination percentage and germination speed index as described by Silva et al. (2020). The germination speed index (GSI) was calculated according to the Maguire equation (1962):

\[
\text{GSI} = (N1/E1) + (N2/E2) + \ldots + (Nn/En)
\]

Where: GSI = germination speed index; E1, E2, En = number of seedlings in the first, second and last counts. N1, N2, Nn = number of days of sowing to the first, second and last count.

The data were submitted to analysis of variance at 5 and 1% of probability by the F-test, and after the analysis of variance and a significant extract effect, data were adjusted according to the non-linear regression model, log-logistic type, with three parameters proposed by Seefeldt et al. (1995) to determine the LC50 (inhibitory concentration equivalent to 50% effect in relation to the control), using the Sigmaplot software:

\[
y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}
\]

where: y = percentage control; x = extract concentration; a, b, and c, estimated parameters of the equation, so that a = amplitude between the maximum point and the minimum point of the variable, b = concentration that provides 50% response of the variable and c = slope of the curve around b.

LC50 is the concentration of the extract in % that provides the value of 50% control or reduction of growth of the recipient species. The values of the control were considered as 100%, and the results of each variable of the other treatments were calculated in relation to the control using the following formula:

\[
RV (\%) = 100*V/Tm
\]

Where, RV = variable in relation to the control (%); V = analyzed variable (G, GSI); Tm = mean of the control (%).

3. Results

3.1 Allelopathic Activity

Aqueous extracts of aerial parts (AP) and underground parts (UP) of *Cyperus distans* showed significant allelopathic effect for the variables germination (G) and germination speed index (GSI) in *Lactuca sativa* seed tests. For the variable G (%) (Figure 1A), the aqueous extract of AP was superior to the extract of UP. For aerial parts, LC50 occurred at a concentration of 15%. In the extract of underground parts, it was not possible to determine the LC50. In Figure 1B (GSI), again the AP extract showed the best result, with LC50 at the same concentration (15%).
Figure 1A and 1B. Germination percentage (G) and germination speed index (GSI) of *Lactuca sativa* L. as a function of treatment (AP and UP) and increasing concentrations of the aqueous extract of *Cyperus distans* L.

When compared to *C. distans* extracts, aqueous extracts of *C. laxus* (AP and UP) showed lower allelopathic effect for the variables G and GSI in *L. sativa* seeds, and it was not possible to determine the LC₅₀ at the tested concentrations (Figure 2). The AP and UP extracts showed similar results (G), remaining stable with the initial concentrations, with reduction in germination at the highest tested concentration (30%) of the AP extract.

Figure 2A and 2B. Germination percentage (G) and germination speed index (GSI) of *Lactuca sativa* L. as a function of treatment (AP and UP) and increasing concentrations of the aqueous extract of *Cyperus laxus* Lam.

For the variables G and CSI, *C. rotundus* AP and UP extracts showed a significant reduction in the development of *L. sativa* seedling with increasing concentrations (between 15 and 30%). Reduction was more significant for the extract of aerial parts, with LC₅₀ at the 15% concentration, coinciding with *C. distans*. It was not possible to determine the LC₅₀ for the aqueous extract of underground parts (Figure 3).
Figure 3A and 3B. Germination percentage (G) and germination speed index (GSI) of *Lactuca sativa* L. as a function of treatment (AP and UP) and increasing concentrations of the aqueous extract of *Cyperus rotundus* L.

Although the aqueous extracts of AP and UP of *C. distans* tested on *Emilia fosbergii* seeds had relevant allelopathic effect, there was no significant difference for the variables G and GSI, and it was not possible to determine the LC$_{50}$ (Figure 4). It is noteworthy that the aqueous extract (AP) of this species also presented allelopathic potential against *L. sativa* seeds.

Figure 4A and 4B. Germination percentage (G) and germination speed index (GSI) of *Emilia fosbergii* Nicolson as a function of treatment (AP and UP) and increasing concentrations of the aqueous extract of *Cyperus distans* L.

The aqueous extracts of *C. laxus* (AP and UP) showed relevant allelopathic effect when tested in *E. fosbergii* seeds. From the analyzed variables (G and GSI), it was possible to observe that *C. laxus* extracts inhibited the development of *E. fosbergii* seedlings at the tested concentrations (0, 0.94, 1.87, 3.75, 7.5, 15, and 30%) (Figure 5).
Figure 5A and 5B. Germination percentage (G) and germination speed index (GSI) of *Emilia fosbergii* Nicolson as a function of treatment (AP and UP) and increasing concentrations of the aqueous extract of *Cyperus laxus* Lam.

Species *C. rotundus* showed allelopathic effect in the two extracts (AP and UP) tested. The LC$_{50}$ of the aqueous extract of AP was reached when using the 15% concentration, reducing germination by 50%. For the variable GSI, the extracts of AP and UP reduced the germination speed by 50%, but there was no difference between the tested extracts. The best result was obtained at the 30% concentration with the AP extract (Figure 6).

Figure 6A and 6B. Germination percentage (G) and germination speed index (GSI) of *Emilia fosbergii* Nicolson as a function of treatment (AP and UP) and increasing concentrations of the aqueous extract of *Cyperus rotundus* L.

### 3.2 Phytochemical Screening

From the preliminary phytochemical analyses of the aqueous extracts of *C. distans*, *C. laxus*, and *C. rotundus*, it was possible to verify the occurrence of some classes of chemical compounds with allelopathic potential. The chemical reactions observed in the extracts of aerial parts (AP) and underground parts (UP) of the three studied species suggested the occurrence of phlobaphenic tannins, catechins, flavanones, flavones, flavonols, xanthones, and flavononols. Importantly, positive results were observed in all extracts for terpenoids and alkaloids (AP and UP) (Table 2).

Anthocyanin, anthocyanidin, and leucoanthocyanidins were not identified in these analyses. Similar data were observed by Bezerra et al. (2019) in Cyperaceae extracts. The absence of
these compounds in green (for leaves) and brown (for roots) extracts is justifiable because anthocyanins are pigments belonging to a subgroup of flavonoids that occur mainly in fruits, vegetables, and flowers, presenting a wide variety of colors from intense red to violet and blue (Patras et al., 2010; Petroni and Tonelli, 2011; Ribeiro et al., 2011; Bezerra et al., 2018).

Table 2. Phytochemical screening of the species Cyperaceae studied: A – *Cyperus rotundus* L. (AP); B – *Cyperus laxus* Lam. (AP); C – *Cyperus distans* L. (AP); D - *Cyperus rotundus* L. (UP); E – *Cyperus laxus* Lam. (UP); F – *Cyperus distans* L. (UP)

| Test               | A  | B  | C  | D  | E  | F  |
|--------------------|----|----|----|----|----|----|
| Phenols            | -  | -  | -  | -  | -  | -  |
| Tannins            | -  | -  | -  | -  | -  | -  |
| Tannins flobafenics| -  | -  | -  | +  | +  | +  |
| Anthocyanin        | -  | -  | -  | -  | -  | -  |
| Anthocyanidin      | -  | -  | -  | -  | -  | -  |
| Leuco anthocyanidin| -  | -  | -  | -  | -  | -  |
| Catechins          | ++ | ++ | +  | +  | +  | +  |
| Flavanones         | +  | +  | +  | -  | +++| -  |
| Flavonones         | +++| ++ | -  | -  | +++| -  |
| Flavonols          | +++| ++ | -  | -  | +++| -  |
| Flavononols        | and| +++| ++ | -  | -  | +++|
| xanthons           |    |    |    |    |    |    |
| Chalcones          | -  | -  | -  | -  | -  | -  |
| and                |    |    |    |    |    |    |
| Auranones          |    |    |    |    |    |    |
| Flavononols        | +  | +  | -  | +  | +  | -  |
| Steroids           | -  | -  | -  | -  | -  | -  |
| Terpenoid          | +++| ++ | +  | +++| +++| ++ |
| Saponins           | -  | -  | -  | -  | -  | -  |
| Alkaloids          | ++ | +  | ++ | +  | ++ | +  |

AP: Aerial part; (UP): Underground part. (+) weakly positive reaction, (++) positive reaction, (+++) reaction strongly positive, (-) absent.

3.3 High-Performance Liquid Chromatography (HPLC)

Considering that the aqueous extracts of underground parts (UP) of *C. distans*, *C. laxus*, and *C. rotundus* showed lower allelopathic effect when compared to the aqueous extracts of aerial parts (AP) of the three species analyzed, high-performance liquid chromatography (HPLC) analysis was performed only for aerial part extracts.

Using reference standards and 275 nm wavelength (Figure 7A), the chromatogram of the aqueous extract of *C. distans* showed a total of 5 chemical compounds (gallic acid, luteolin, apigenin, chrysin, and acacetin). In addition to flavonoids, two alkaloids were identified in
this chromatographic run (epi-guaipyridine and guaipyridine) (Figure 7B).

Figure 7A. Phenolic compounds identified in the chromatogram of the aqueous extract of *Cyperus distans* L. (aerial part) at a wavelength of 275nm; Figure 7B. Alkaloids identified in the same sample

Regarding the chromatogram of the aqueous extract of *C. laxus*, only four phenolic substances were identified by the techniques used (chlorogenic acid, luteolin, apigenin, and acacetin) (Figure 8A). It is noteworthy that major compounds were not identified. As in the *C. distans* chromatographic run, two alkaloids were also separated and identified in *C. laxus*: epi-guaipyridine and guaipyridine (Figure 8B).

Figure 8A. Phenolic compounds identified in the chromatogram of the aqueous extract of *Cyperus laxus* Lam. (aerial part) at a wavelength of 275nm; Figure 8B. Alkaloids identified in the same sample

The chromatographic run of the aqueous extract of aerial parts of *C. rotundus* showed only 3 phenolic compounds at 275 nm wavelength (chlorogenic acid, apigenin, and acacetin) (Figure 9A). *C. rotundus* also presented the alkaloids epi-guaipyridine and guaipyridine (Figure 9B), but the peak intensity for these substances was lower when compared to the *C. distans* (Figure 7B) and *C. laxus* chromatograms (Figure 8B).
4. Discussion

The aqueous extracts of aerial parts of *C. distans*, *C. laxus*, and *C. rotundus* showed superior allelopathic effect when compared to the underground parts tested to verify the seed germination index of *L. sativa* and *E. fosbergii*. It was also observed that species *E. fosbergii* showed higher susceptibility to aqueous extracts of *Cyperus* spp. at all concentrations analyzed (0.94, 1.87, 3.75, 7.5, 15, and 30%). Unlike *E. fosbergii*, *L. sativa* seeds showed decreased germination rate only at the highest concentrations (between 15 and 30%) of aqueous extracts of *C. distans*, *C. laxus*, and *C. rotundus*. According to Darmanti et al. (2015), this effect is expected. These authors observed that the germination index of *Glycine max* L. cv. Grobogan decreased at the highest concentrations (25%) of aqueous extracts of *C. rotundus* tubers. Secondly, Dadar et al. (2014) observed that, at concentrations of 10, 20, 30, and 40%, aqueous extracts of aerial parts of *C. rotundus* decreased the germination percentage of *Lycopersicon esculentum* Mill seeds.

Investigations on the allelopathic effects of rhizome extracts of *C. distans* on seedling germination and growth of *Mimosa pudica* L., *Senna obtusifolia* (L.), and *Pueraria phaseoloides* (Roxb.) Benth indicated that scabequinone and other constituents such as the sesquiterpenes cyperotundone, α-cyperone, and cyperene contributed to phytotoxicity (Vilhena et al., 2014). The study developed by Belel and Rahimatu (2012) showed that aqueous extracts of leaves and seeds of *Cyperus tuberosus* Rottb. release allelochemicals that inhibit the development of *Arachis hypogaea* L. seedlings. The aqueous extract of *C. tuberosus* leaves also had an allelopathic effect on the germination and development of *Vigna unguiculata* (L.) (Belel and Belel, 2015).

The allelopathic activity identified in these species is directly associated with allelochemicals extracted from active plant raw material. Phenolic substances were widely identified in aqueous extracts of *C. distans*, *C. laxus*, and *C. rotundus*, both in phytochemical prospecting and in high-performance liquid chromatography (HPLC) analysis. Kamala et al. (2018) observed chemical classes such as phenols, flavonoids, and saponins in the phytochemical prospecting of the aqueous extract of *C. rotundus*. Hema et al. (2013) also verified the
occurrence of these substances, except flavonoids, in the aqueous extract of *C. rotundus* by preliminary phytochemical prospecting. In turn, Fiorenza et al. (2016) points out that some phenolic compounds may be directly related to allelopathic activity, which may inhibit the growth and even germination of other species belonging to the same environment.

In chromatographic analysis of the extracts of aerial parts of *C. rotundus* using HPLC, Alsaadawi and Salih (2009) revealed the presence of ferulic, caffeic, hydroxybenzoic, chlorogenic, and p-coumaric acids. According to these authors, these substances may have allelopathic effects. In the present research, chlorogenic acid was identified in aqueous extracts of aerial parts of *C. distans*, *C. laxus*, and *C. rotundus* analyzed by HPLC. No data were found in the literature proving the allelopathic effect of the alkaloids (epi-guaiapyridine and guaipyridine) identified in aqueous extracts of *C. distans*, *C. laxus*, and *C. rotundus* by HPLC. Notwithstanding, Clery et al. (2016) reported that a total of four guaipyridine-like compounds were isolated and identified from Cypriol oil (*Cyperus scariosus* R.Br.).

Although *C. rotundus* is reported by other authors as a well-known allelopathic plant, it is important to point out that results on the chemical composition of *C. distans* and *C. laxus* are scarce in the literature. There is a lack of reports on the allelopathic effect of these species, indicating that this study has an innovative character, providing new data on the topic.

5. Conclusion

The aqueous extracts of underground parts of *C. distans*, *C. laxus*, and *C. rotundus* showed low allelopathic potential, given that there was no significant interference in *L. sativa* and *E. fosbergii* seed germination. However, the aqueous extracts of aerial parts of the three species analyzed showed relevant allelopathic effect when evaluated in lettuce and *E. fosbergii* seed germination. Although this is a preliminary study, the results suggest that lettuce producers should be aware of the presence of *Cyperus* spp. in their properties. This allelopathic potential may be directly associated with the biosynthesized phenolic compounds of these plants, analyzed in this study by phytochemical prospecting and high-performance liquid chromatography (HPLC).

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