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When the Enemy Becomes an ally: Allelopathic Action of Nut Grass on the Black-Jack

Quando o Inimigo se Torna um Aliado: Ação Alelopática de Tiririca Sobre o Picão Preto

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Abstract

Cyperus rotundus L, as it is a weed that is difficult to control and persistent in the agricultural system, has been investigated regarding its allelopathic potential compared to other weeds, such as Bidens pilosa L. considered as one of the weeds of annual crops that are difficult to control and with the highest incidence. in agricultural areas of central-southern Brazil. A completely randomized experimental design with three replicates, 2-factorial was performed, and evaluated under two contrasts: 1) aqueous extracts of C. rotundus (leaves and pseudo-tuber) and concentrations (0, 0.1, 1 and 10%); and 2) extracts (foliar or pseudo-tuber) and time (0-5 days after treatments).Foliar and pseudo-tuber extracts differ in their influences according to concentrations and time. Both extracts at the highest concentration (10%) reduced seed germination and seedling growth; pseudo-tuber 1% extract was negative to germination, but not to the growth; foliar 0.1% extract reduced germination; and foliar and pseudo-tuber 0.1% increased the root and hipocotyle length. It was concluded that Cyperus rotundus has chemical substances able to induce changes in the pattern of germination and seedling growth of Bidens pilosa. The source of extract produces no significant responses, but concentration and time had influence on germination and initial growth.

Keywords: Cyperus rotundus. Allelochemicals, Control of Bidens Pilosa.

Resumo

Cyperus rotundus L por ser uma planta daninha de difícil controle e persistente no sistema agrícola vem sendo investigada quanto seu potencial alelopático frente a outras plantas daninhas, como a Bidens pilosa L. considerada como uma das infestantes de culturas anuais de difícil controle e com maior incidência em áreas agrícolas do centro-sul do Brasil. Este trabalho avaliou a capacidade alelopática de extratos aquosos de C. rotundus sobre a germinação de sementes de B. pilosa. Um experimento com delineamento inteiramente ao acaso, com três repetições, bifatorial foi realizado e avaliado sob dois contrastes: 1) extratos (foliar ou de pseudotubérculo) e concentrações (0; 0,1; 1 e 10%); e 2) extratos (foliar ou de pseudotubérculo) e tempo (0-5 dias após o tratamento). A fonte do extrato não produziu qualquer resposta significativa, mas concentração e tempo tiveram influência na germinação e no crescimento inicial. Extratos da folha e do pseudotubérculo tiveram influências diferentes de acordo com as concentrações e o tempo. Ambos os extratos, a 10%, reduziram a germinação e o crescimento das plântulas; extrato de pseudotubérculo a 1% foi negativo para a germinação, mas não para o crescimento; extrato foliar a 0,1% reduziu a germinação; e extratos de folha e pseudotubérculo a 0,1% aumentaram o comprimento da raiz e do hipocótilo. Constatou-se que Cyperus rotundus apresenta substâncias químicas capazes de induzir modificações no padrão de germinação e crescimento de plântulas de Bidens pilosa.

Palavras-chave*: Cyperus rotundus. Aleloquímicos, Controle de Bidens Pilosa.*

1 Introduction

Food cultivation provides for a series of cultural practices, soil preparation, use of fertilizers and the use of chemical control products for invasive plants, pests and diseases.

The adoption of weed control practices involves a series of measures, but the use of herbicides is the most common and efficient.

Herbicides, are used to prevent the effects of competition for space and nutritional resources between cultivated plants and invasive plants (FONTES *et al*., 2003). Many species of invasive plants have shown resistance to the active principles of herbicides available on the market, not responding to treatments.

Alternative practices in the control of invasive plants have been adopted with the use of plants that produce allelopathic compounds (BLANCO, 2013; CREMONEZ *et al*., 2013).

Allelopathic compounds, such compounds can inhibit seed germination and/or impair the growth of target plants, by interfering with vital processes such as nutrient assimilation, photosynthesis, respiration and enzymatic protein synthesis (KOSTINA-BEDNARZ,*et al.,* 2023)

He nutsedge, *Cyperus rotundus* L. (*Cyperaceae*) and the black-jack, *Bidens pilosa* L. (Asteraceae) are two species with high potential for invasiveness (HOLM, *et al.,* 1977; BARTOLOME, *et al.,* 2013) and which cause considerable damage to agricultural production (BRIGHENTI, 2011).

Both produce a series of compounds with allelopathic biological activity, many with high potential for interfering with the development of other plants (CAMPBELL, *et al.*, 1982, QUAYYM, *et al.,* 2000, CONCI, 2004, SHARMA; GUPTA 2007, RABELLO *et al.,* 2008, LUCHMANN, *et al,* 2013, MELHORANÇA FILHO, *et al*., 2015, KHAMARE, *et al.,* 2022).

If a given invasive species, such as a black-jack, exposed to a source of phytotoxic allelochemicals, such as nutsedge, uses more energy in the detoxification process than in growth and reproduction, it will possibly become more apt to be excluded from the system (FORBES, 2000, IBRAHIM, *et al*., 2023).

Becoming less competitive and opening space for cultivars to develop without selective pressure, facilitating the final production of grains.

Given the potential for agricultural application of compounds produced by invasive plants, this work has as main objective determine if the aqueous extracts of sedge prepared with different parts of the plant (leaf or pseudotuber) with allelopathic biological activity of black-jack;

2 Material and Methods

In an agricultural area of conventional cultivation in the region of Guarapuava, Paraná (25°24'33.3"S 51°32'29.8"W), leaves and pseudotubers of sedge (*C. rotundus,*) were collected, which were packed in styrofoam boxes. In the laboratory, the materials were cleaned and taken to a drying oven, with forced air circulation at 40 °C, for 72 h. After drying, leaves and pseudotubers were separately ground in a knife mill.

Both the crushed leaves and the pseudo-tubers were prepared cold extracts, by immersion in distilled water at a concentration of 10% (mass/volume), which were kept at 8 ºC, in the dark, for 24 h. Subsequently, the extract was filtered and centrifuged, and the supernatant was diluted to obtain 0.1, 1 and 10% solutions, which were used in the allelopathy assay. The determination of the molar concentration of the extracts was carried out by the Chardakov method, and their values were transformed into MPa (SALISBURY; ROSS, 1992).

The assay was carried out in transparent gerbox boxes, lined with filter paper moistened with 6 mL of the extracts. In each box, 30 diaspores of black pica (*Bidens pilosa*) were distributed, previously disinfected with a 2% sodium hypochlorite solution for 10 minutes. The boxes were kept in a germination chamber, set at a constant temperature of 25 °C. The number of germinated seeds was counted at 24-hour intervals for five days (FERREIRA; BORGHETTI, 2004). Each gerbox box constituted a sample unit.

The statistical design used was completely randomized, with three replications. Two sources of plant material (leaf and pseudo-tuber), four concentrations of extract (0; 0.1; 1 and 10%) and five evaluation periods (five days after treatment) were evaluated under two contrasts, in order to clarify the objectives proposed in this study. Initially, source and concentration of extracts were evaluated in a two-factor assay. Subsequently, extracts based on leaves or pseudotubers were evaluated separately, in order to evidence the influence of concentrations throughout the germination process and initial growth of the seedlings.

Germination was evaluated from the following parameters: percentage of germinated seeds (G), being considered germinated the seeds that produced primary root with geotropic curvature; germination speed index (IVG), mean germination time (TMG) and mean germination speed (VMG), calculated according to Ferreira; Borghetti (2004).

The initial growth of the seedlings was evaluated by means of the primary root and hypocotyl lengths. The length of the primary root was determined by measuring the region between the meristematic apex and the collar, at 24-hour intervals, with the aid of a caliper. The same procedure was used to measure the hypocotyl of the seedlings, taking the measurement from the collar region to the point of insertion of the cotyledons (FERREIRA; BORGHETTI, 2004).

The data were initially evaluated by the Shapiro-Wilk, Levene and visual inspection tests, to verify the assumptions: normality, homoscedasticity and linearity. As these proved to be non-parametric, they were evaluated by means of two-way ANOVA, followed by Fisher's test, to evidence the least significant difference (DMS). The adopted significance level of 5%.

3 Results and Discussion

Aqueous extracts of nutsedge prepared with different parts of the plant (leaf or pseudotuber) did not produce different responses in germination and initial growth of black-jack, but the concentration of aqueous extracts did (Table 1).

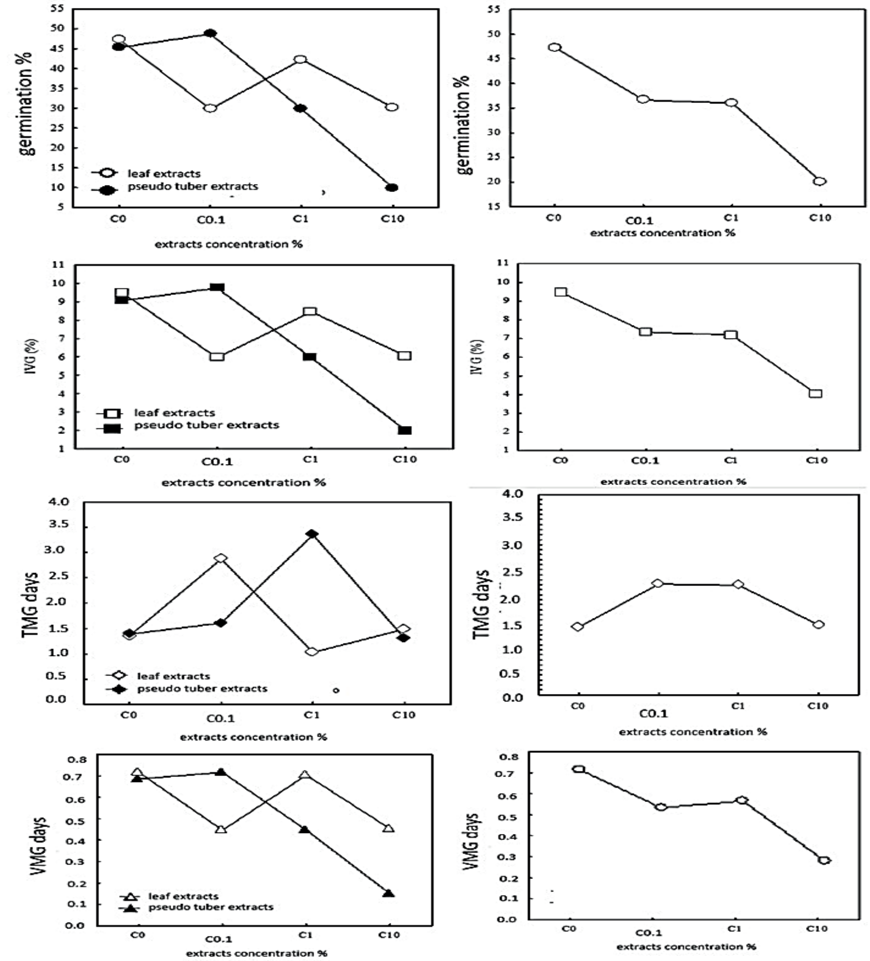
Table 1 - Significance levels for the effects of source (organ) and concentration of aqueous extracts of nutsedge on germination (G), germination speed index (IVG), mean germination time (AMR), mean germination speed of seeds (VMG), and lengths of the primary root (CRP) and of the hypocotyl (CHI) of seedlings of black-jack

Variables	F1 (organ)			F2 (concentration)			F1 x F2		
	SQ	F	P	SQ	F	P	SQ	F	P
G	468.2	0.51	0.48	10893.0	3.96	0.01*	6464.0	2.35	0.07
IVG	18.7	0.51	0.48	435.7	3.96	0.01*	258.6	2.35	0.07
TMG	1.5	0.19	0.67	20.7	0.86	0.46	51.4	2.13	0.10
VMG	0.2	0.96	0.33	2.6	4.27	0.01*	1.5	2.59	0.06
CRP	3.8	0.18	0.68	268.9	4.21	0.01*	104.6	1.64	0.85
CHIP	26.8	0.45	0.50	492.2	2.75	0.05*	133.3	0.74	0.53

Source: research data.

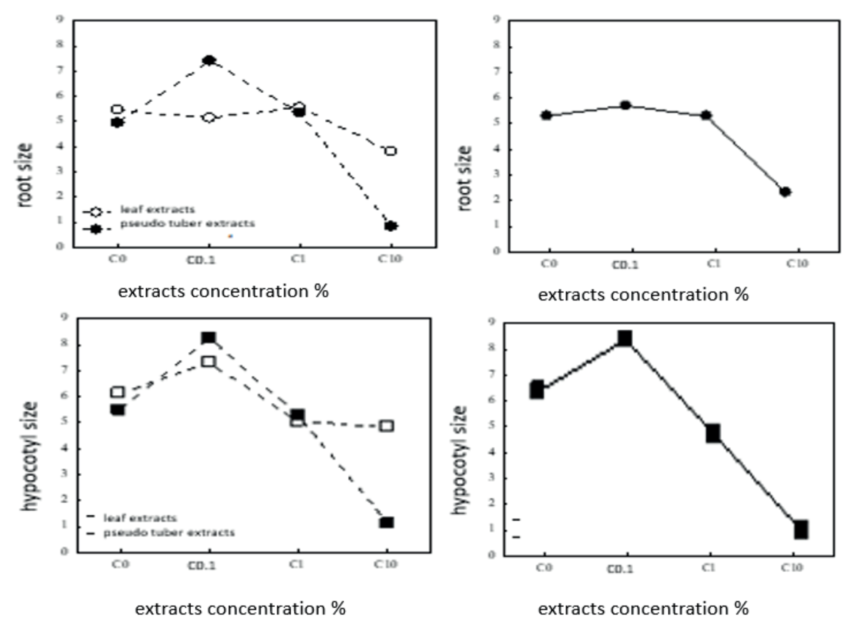
The highest concentration (10%) reduced G, IVG, VMG and CRAD (Figures 1 and 2).

Figure 1 - Germination, germination speed index (IVG), mean time to germination (AMT), mean speed of germination (VMG) of black-jack seeds treated with extracts of leaves and nutsedge pseudotuber at different concentrations. Graphs on the left show means of extracts separately, and graphs on the right, mean concentrations



Source: the authors.

Figure 2 - Means of primary root and hypocotyl lengths of black-jack seedlings treated with aqueous extracts of nutsedge leaf and pseudotuber at different concentrations. Graphs on the left show averages of the extracts separately, and those on the right show averages of concentration



Source: the authors.

Significant interactions of the factors (organ x concentration) were not evidenced, but G, IVG and VMG showed a strong tendency to different behaviors.

Variables related to seed germination, such as G, IVG and VMG did not respond equally to the extract source (organ). Leaf extracts at concentrations of 0.1 and 10% reduced PG, IVG and VMG, while extracts from pseudotubers produced these responses at 1 and 10% (Figure 1).

When the influence of the extracts is temporally evaluated, it is verified that the aqueous extracts of the leaves or of the nutsedge pseudotuber promoted differentiated responses in germination and in the initial growth of black-jack (Table 2),

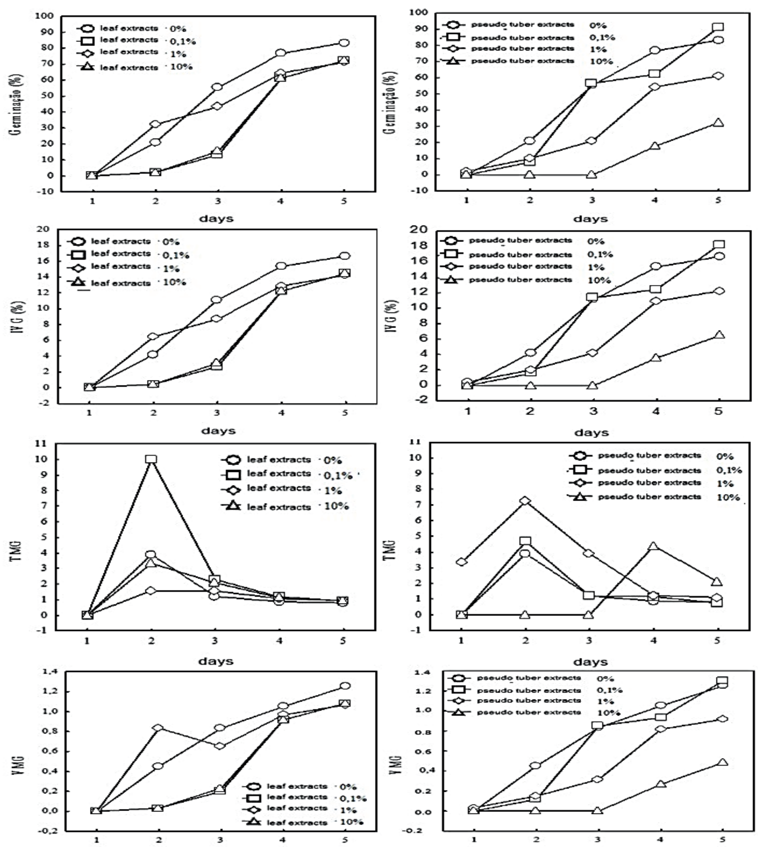
Table 2 - Significance levels for the effects of source (organ) and concentration of aqueous extracts of nutsedge on germination (G), germination speed index (IVG), mean germination time (AMR) and mean germination speed of seeds (VMG), and lengths of the primary root (CRP) and of the hypocotyl (CHIP) of seedlings of black-jack

Variables	**F1 (organ)**			**F2 (concentration)**			**F1xF2**		
leaf	SQ	F	P	SQ	F	P	SQ	F	P
G	3473.2	10.2	0.000*	49882.6	110.0	0.000*	3202.0	2.4	0.021*
IVG	138.9	10.2	0.000*	1995.3	110.0	0.000*	128.1	2.4	0.021*
TMG	30.0	1.3	0.274	155.4	5.22	0.002*	93.5	1.1	0.428
VMG	1.02	11.4	0.000*	10.1	85.61	0.000*	1.3	3.7	0.001*
CRP	29.0	7.6	0.000*	1125.0	219.4	0.000*	27.0	1.8	0.09
CHIP	78.6	63.0	0.000*	3506.9	2122.1	0.000*	221.0	45.0	0.000*
Pseudo tuber									
G	12837.0	85.9	0.000*	37649.2	188.9	0.000*	6694.4	11.2	0.000*
IVG	499.9	78.0	0.000*	1415.4	165.7	0.000*	257.3	10.0	0.000*
TMG	42.9	3.4	0.002*	69.3	4.1	0.007*	114.5	2.3	0.03*
VMG	2.8	54.0	0.000*	7.4	106.4	0.000*	1.34	6.4	0.000*
CRP	279.2	61.4	0.000*	880.6	145.1	0.000*	202.6	11.1	0.000*
CHIP	425.8	179.0	0.001*	2261.4	713.2	0.001*	532.1	55.9	0.001*

Source: research data.

With the effects produced by the extracts of the pseudotuber, more persistent (Figures 3 and 4).

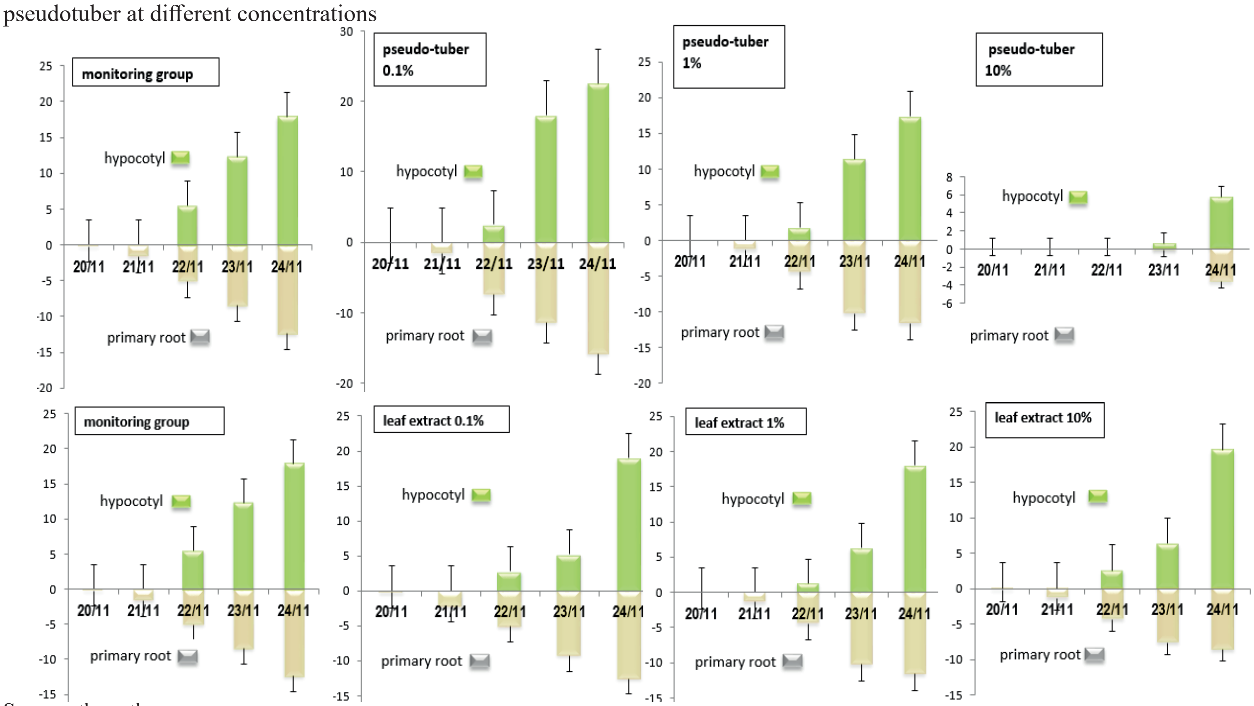
Figure 3 - Means of primary root and hypocotyl lengths of black-jack seedlings treated with aqueous extracts of nutsedge leaf and pseudotuber at different concentrations. Graphs on the left show averages of the extracts separately, and those on the right show averages of concentrate



Source: the authors.

Primary root growth (PRC) was more responsive to extracts based on pseudotubers, which increased its length at the 0.1% concentration, and reduced it at the 10% concentration. The negative response associated with the highest concentration was verified from the third day, while the positive response, from the fourth day (Figure 4).

Figure 4 - Means of primary root and hypocotyl lengths of black-jack seedlings treated with aqueous extracts of nutsedge leaf and pseudotuber at different concentrations



Source: the authors.

The hypocotyl responded similarly to aqueous extracts of leaves and pseudotubercles, its growth being increased by the 0.1% concentration, and reduced by the higher concentrations (1 and 10%). However, these responses occurred at different times. The positive influence of the 0.1% leaf extract on CHIP was verified from the third day onwards, and the negative influence (from the extracts with concentrations of 1 and 10%) from the fourth day onwards (Figure 4).

Pseudotuber extracts (regardless of concentration) were negative for hypocotyl growth on the third day (Figure 4). However, on the fourth day, it was found to be longer when treated with the 0.1% extract. On the fifth day of development, only the 10% concentration maintained the negative effect.

The lack of differences in the responses of black-jack to extracts prepared with leaves or pseudotubers could be related to two main facts: a possible similarity in the composition of the allelopathic compounds, or, more likely, a possible similarity in the mode of action of different allelochemicals. Several studies have shown that the composition of secondary compounds produced in the aerial part and in the root system of plants is not the same, considering the different functions and environments in which they develop.

In general, in a plant, the leaf is the most metabolically active organ, which according to Ribeiro *et al.* (2009) allows it to present a greater diversity of allelochemicals and phytotoxic potential. However, the answers obtained in this work show that leaf extracts were only negative when in low concentration (0.1%) and restricted only processes related to germination (decreases of 37% in the number of germinated seeds and in the germination speed). At the highest concentration, the extract based on pseudotubers was the one that presented the greatest restrictions on germination and initial growth of seedlings (decreases ranging from 79 to 85%). Possibly, the more accentuated responses of the extracts of higher concentration (10%) are due to the greater amount of allelopathic compounds.

Quayyum *et al.* (2000) identified the compounds present in extracts of leaves and tubers and reported that phenols and fatty acids are the most abundant. However, extracts from nutsedge tubers have high amounts and diversity of terpenoids (SONWA; KÖNIG, 2001, JAISWAL *et al*., 2014, ZHANG, *et al.,* 2022), and leaf extracts have higher levels of phenolic compounds (QUAYYUM, *et al*., 2000, ZHANG, *et al*., 2022). Terpenoids have general lipid properties (CASTRO, *et al*., 2004), being soluble in organic solvents and insoluble in water. Terpenoid toxicity results from several effects, the most common of which are: inhibition of ATP formation, disruption of hormonal activity and inhibition of respiration (PEÑUELAS, *et al.* 1995).Most phenolic compounds are not found in the free state in nature, they are present in the form of esters or heterosides and are therefore soluble in water. These are known for their great importance in the soil-plant system, as they act as inhibitors of various biological processes. At the cellular level, they interfere with cell division, lipid metabolism and the biochemical mechanism of respiration, inhibiting glucose transport and cellulose synthesis (CASTRO, *et al.,* 2004; MACÍAS *et al.,* 2019; PUTNAM; DUKE, 1978).

Differences in germination response (G, IVG, VMG) at 0.1% and 10% (leaf) and 1% and 10% (pseudotuber) concentrations may be due to the different concentration of compounds in the organs, as previously reported. The collection period and the phenological phase of the plant can promote changes in the production and accumulation of compounds, and the use of fresh or dry parts can also infer differences in the concentration of compounds accumulated in plant tissues, causing significant variations in the concentration of extracts. It is known that the production of allelochemicals is not constant, varying with the age of the tissues and with external factors, such as intensity, duration and amount of light, availability and quality of nutrients and water, temperature, attack by pests and diseases (ALMEIDA, 1990).

C. rotundus has substances capable of inhibiting the germination and growth of several cultivated plants, such as rice, corn, cucumber, tomato, sorghum and onion (DROST; DOLL, 1980; QUAYYUM *et al.,* 2000). However, some plants may have different responses. For example Castro *et al.* (1983), found that aqueous extracts of *C. rotundus* inhibited tomato (*Lycopersicon esculentum*) germination and growth, while Andrade *et al.* (2009), found that tomato and lettuce (*Lactuca sativa*) did not show decreases or delays in the germination of their seeds. Gusman *et al.* (2011) found that sedge extracts were only negative for lettuce and tomato seed germination when they were not diluted (100%).

Although lettuce seeds, a plant belonging to the same family as black-jack (Asteraceae), did not respond to extracts of Nutsedge, the initial development of seedlings was impaired (ANDRADE *et al*., 2009), similarly to what was observed with black-jack. These had the root length reduced by extracts with concentrations from 10%, and the stem length reduced only in extracts with concentrations above 70% (ANDRADE *et al*., 2009; GUSMAN *et al.,* 2011).

The positive responses of the low concentration of the extracts (0.1%) on the growth of the radicle and hypocotyl of Black-jack may be related to a possible hormetic effect of the allelochemicals, or to the action of auxins and/or allelochemicals that act as synergists of the indole acetic acid (QUAYYUM, *et al.,* 2000), which are produced in large quantities by nutsedge.

Hormesis (CHAPMAN, 2002; CALABRESE, 2018, 2022; JALAL *et al.,* 2021, STEBBIN, 1998) corresponds to a stimulatory effect caused by toxic substances or conditions that generate stress in organisms, and which is directly related to the maintenance of homeostasis. The loss of homeostasis induces the organism to a biphasic dose-response behavior, in which positive responses are triggered at low concentrations, or intensities, and inhibitory responses are observed at high concentrations, or intensities, (CALABRESE; BLAIN, 2002; CALABRESE, 2018, 2022). In the study by Calabrese (2002), the hormetic effect was associated with increments of 30 to 60%, values close to those observed in this study (20% in the CHIP of seedlings treated with extracts of leaves or pseudo-tuber, and 56% in the CRP of seedlings treated with with pseudo-tuber extract.

If the levels of auxinic substances are responsible for the increases in the length of these organs, then it is possible that these were insufficient to induce changes in the growth of the hypocotyl (in the extracts with a concentration of 0.1%), and even though the leaf and pseudotuber had differences. Quantitative measurements of these phytohormones, these would not cause differences in responses. As the root is more sensitive to auxins and responds to lower concentrations (TOOLE; TOOLE, 1999), a difference in the amount accumulated in leaves and pseudotubers could generate inhibition if above the optimal level or increase if at the ideal level. Thus, the data from this study suggest that the leaves have higher amounts of growth-inducing substances, as its extract was associated with roots 7% smaller than those of 0% concentration were, while the pseudotuber extract was associated with 56% longer roots.

4 Conclusions

The data from this work allow us to conclude that. The aqueous extracts of nutsedge influence the germination and growth of seedlings of black-jack woodpecker;

The concentration of the extract is more important in modulating the responses than the source of the plant material (leaf or pseudotuber); The responses are the same in the extracts with the highest concentration (10%), but they differ between the sources (plant organs) in the concentrations of 0.1 and 1%. Extracts with the lowest concentration (0.1%) had a positive influence on root and hypocotyl growth, but showed different responses in germination according to the source (leaf promoted decreases and pseudotuber did not influence). Pseudotuber extracts produced more persistent effects than leaf extracts. It is important to report that future studies on the potential of *Cyperus rotundus* as a source of non-toxic herbicides must be evaluated from both a technological and environmental point of view.

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