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Research Article

Herbicidal activity of kidney leaf mud plantain leaves extracts on the germination of four species

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INFORMATION ARTICLE

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HIGHLIGHTS

- Plants are important sources of compounds that can be used for the development of herbicides.
- There is information related to the phytotoxic activity of extracts from the weed of irrigated rice known as kidney leaf mud plantain.
- Kidney leaf mud plantain proved to be promising for future studies that involve the fractionation of extracts and identification of compounds with biological activity.

ABSTRACT

Background: Plants synthesize compounds of specialized metabolism to defend themselves against biotic and abiotic stresses. These compounds could be used as models for pesticide development. Among the species that have not yet been studied for the potential to produce active compounds is the kidney leaf mud plantain (*Heteranthera reniformis*).
Objective: The goal was to evaluate the effect of hexanic, ethyl acetate and methanolic extracts of *H. reniformis* leaves on cress (*Lepidium sativum*) seed germination to determine which one have the highest biological activity; and to evaluate different concentrations of extract with the highest biological activity on lettuce (*Lactuca sativa*), barnyard grass (*Echinochloa* sp.) and giant arrowhead (*Sagittaria montevidensis*) germination.
Methods: The seeds were placed on petri dishes containing two sheets of germitest paper, and after the application of the extract, they were sealed and placed in growth chambers with controlled temperature and photoperiod. The variables analyzed were 1st and 2nd germination count, germination speed index and length of the aerial part and roots.
Results: The ethyl acetate extract provided greater phytotoxicity on cress than the other extracts. In general, the highest concentration of ethyl acetate extract was the most efficient in reducing variables for all species.
Conclusions: The ethyl acetate extract of *H. reniformis* presents inhibitory activity on the seeds of cress, lettuce, giant arrowhead and barnyard grass, but this activity was dependent on the concentration of the extract and the species studied. *H. reniformis* synthesizes compounds with phytotoxic activity and purification of extracts is required to isolate, identify and characterize the action mechanism of the compounds with herbicide activity, so that in the future these can be used as models for the development of herbicides.

1 Introduction

Weed management through herbicide utilization – the main control method used in several cultures – requires the discovery of molecules with new action mechanisms to complement or even replace the molecules that no longer have control efficiency. The emergence of cultures resistant to glyphosate in 1995 (Roundup Ready®), there was a decrease in the number of companies involved in the research and discovery of new herbicidal molecules, which contributed to the absence of launch of new mechanisms of action in the last 25 years (Duke, 2012; Dayan, 2019).

The structural diversity of natural phytotoxins is a source of compounds for the development of natural products or synthetic herbicides with new action mechanisms (Dayan and Duke, 2014). Of the 20 main mechanisms of herbicidal action, the glutamine synthetase inhibitors (GS) and hydroxyphenyl pyruvate dioxygenase (HPPD), whose herbicides present the active ingredient glufosinate and mesotrione, respectively, were derived from natural products (Leason et al., 1982; Lee et al., 1997). However, molecular biology studies and the research of natural products suggest that there are potentially many unknown herbicide action mechanisms to be explored (Duke and Dayan, 2015).

Among natural sources of molecules with herbicide activity, some plants stand out, which can synthesize compounds as a defense mechanism, aiming to inhibit the development of plants that are near (Dayan and Duke, 2014). These compounds are synthesized in routes of specialized metabolism and classified according to their chemical structure, in alkaloids, phenolic compounds, terpenoids, cyanogenic glycosides, among others (Li et al., 2010).

The identification of compounds with biological activity is initiated from their extraction in different parts of the plant, with the use of solvents of different polarities and the application of these extracts on target organisms (Macias et al., 2014). Besides, biological activity depends on the concentration of crude extract, because there are numerous compounds, and, with the increase of its concentration, it can be observed the concentration increase of the compounds separately.

Among the species with potential in the production of bioactive compounds is *Heteranthera reniformis*, one of the main weeds of irrigated rice crop, which is characterized by presenting fast growth in environments containing water layer, high luminosity and temperature, and, consequently, it has potential for rapid occupation of space (Rolon et al., 2018). The high invasive potential of the species can be related to the ability to synthesize specialized metabolites, among them the compounds with herbicide activity (Sharma et al., 2016).

Given the above, this study had as hypothesis the fact that the extracts of *H. reniformis* leaves present herbicide activity, which depends on the concentration of the extract. The objectives of this study were to evaluate (a) the effect of hexanic extracts, ethyl acetate and methanolic of *H. reniformis* leaves in the germination of crest seeds; and (b) evaluate different concentrations of the extract with great biological activity on the germination of lettuce, barnyard grass and giant arrowhead.

2 Material and Methods

H. reniformis plants were multiplied in boxes of 25 L capacity containing soil classified as Planosol of sandy texture from rice crops, in order to collect plant material for the extraction. The leaves of several plants of *H. reniformis* absent from injuries were collected, washed and dehydrated for seven days in a forced air oven at 40 ºC. The dry material was ground with the aid of a ball mill, adding liquid nitrogen during the grinding process, and subsequently stored in a freezer at -20 ºC until the extraction time.

The extraction of the ground dry plant material was performed with hexane, ethyl acetate and methanol solvents in the ratio of 1:10 (m/v). It was added 40 mL of each solvent in Erlenmeyer containing four grams of plant material, and the extraction was done with the aid of ultrasound bath for 30 minutes. After extraction, the centrifugation of the extract was performed in rotation of 4,000 *x g*, for 10 minutes. The supernatant was removed and added in volumetric balloon to subsequently perform the solvent evaporation in a Buchi model rotary evaporator until complete drying of the solvent. The remaining residue after evaporation of the solvent was weighed and resuspended with Dimethylsulphoxide (DMSO) in the proportion of 5 µL mL^{-1} of the buffered solution of 10 mM of 2-(*N*-morpholino) ethanesulfonic acid and 1 mM of NaOH, pH 5.6 (MES), seeking the concentration of 0.8 mg mL^{-1} or 1 mg mL^{-1}, depending on the experiment (Macias et al., 2000).

In all experiments, the experimental design was completely randomized with four replications. The experimental units consisted of Petri plates containing two sheets of germitest paper, and 20 seeds of each species were distributed per plate.

In the first experiment, it was used to determine the most active extract, the treatments consisted of hexanic extracts, ethyl acetate and methanolic at concentration of 0.8 mg mL^{-1}; and of the negative controls: distilled water, MES buffered solution and MES+DMSO. In each Petri plate seeds of cress were added and, subsequently, the extract in volume corresponding to twice the weight of the germitest paper. Petri plates were sealed with Parafilm and led to the germination chamber BOD type, with temperature and photoperiod regulated according to the seeds' analysis rules (Brasil, 2009). For the first experiment, the cress species was chosen, since the seeds present rapid and uniform germination and are recommended for use in experiments with phytotoxic compounds presence in the extracts (Macias et al., 2000).

After determining the most active fraction, four other experiments were performed, where the concentrations of 0 mg mL^{-1}, 0.0001 mg mL^{-1}, 0.001 mg mL^{-1}, 0.01 mg mL^{-1}, 0.1 mg mL^{-1} and 1 mg mL^{-1} of ethyl acetate extract were evaluated on the seeds' germination of two lettuce cultivars (*Lactuca sativa*): Grand Rapids (experiment 2) and Grandes Lagos (experiment 3), barnyard grass *(Echinochloa crus galli*) (experiment 4) and giant arrowhead *(Sagittaria montevidensis*) (experiment 5). To obtain these concentrations, dilutions were performed from ethyl acetate extract solution of 1 mg mL^{-1}. Negative controls were the same described in experiment 1, and the positive controls consisted of herbicides imazapic+imazapyr (1 mg mL^{-1}), used in the lettuce experiments Grand Rapids cultivar and barnyard grass and oxyfluorfen (2.4 mg mL^{-1}), for lettuce Grandes Lagos cultivar and giant arrowhead. The temperature of the germination chamber ranged from 20 ºC to 25 ºC, depending on the species, and the photoperiod was 12 hours.

The variables analyzed were germination counts (%), germination speed index (GSI), root and the aerial part length (mm). Germination and GSI were determined by the daily count of germinated seeds and calculated by the formulas G% = N/A x 100, in which N = total of germinated seeds and A = total of seeds contained in the Petri plates; and GSI = G1/N1+G2/N2+... Gn/Nn, in which G means the number of germinated seeds in each day (I = 1, 2, 3, ..., 10) and N = number of days of counting (I = 1, 2, 3 ..., 10) (Brasil, 2009). In the germination evaluation it was considered the concept of physiology, described by Bewley and Black (1994), which define germination as the protrusion of the radicle. The GSI was not determined only for the lettuce Grandes Lagos cultivar.

The first germination count, also called vigor, was held in the fourth day for cress, lettuce and barnyard grass. The second germination count, called germination, was at the 7th day for cress, lettuce and giant arrowhead and at the 10th day for barnyard grass. For cress, the percentage of germination inhibition compared to the control was determined through the formula: Inhibition (%) = [(germination of the control distilled water – germination of treatment n)/germination control distilled water]*100. The root and aerial part lengths were measured for all species in the last germination count, through the measurement of the base until the apex with the aid of millimetric ruler.

Data were submitted to analysis of variance ($p \leq 0.05$) and, with statistical significance, the treatments of experiment 1 were compared by Duncan test ($p \leq 0.05$). As for the other experiments, the controls were compared with the concentrations through averages contrasts (Table 1), and among the concentrations, by confidence intervals (95%). For

Table 1 - Treatments and contrasts used in the germination experiments of lettuce, barnyard grass and giant arrowhead seeds

Treatment	Contrast							
	C1	C2	C3[4]	C4	C5	C6	C7	C8
T1- Distilled water	+[5]		+	+	+	+	+	
T2- MES[1]	-	+	+	+	+	+	+	
T3- MES+DMSO[2]		-	+	+	+	+	+	
T4- Herbicide[3]								+
T5- Ethyl acetate extract 0.0001 mg mL^{-1} [4]			-					
T6- Ethyl acetate extract 0.001 mg mL^{-1}				-				
T7- Ethyl acetate extract 0.01 mg mL^{-1}					-			
T8- Ethyl acetate extract 0.1 mg mL^{-1}						-		
T9- Ethyl acetate extract 1 mg mL^{-1}							-	-

[1] MES: 2-N-morpholino ethanesulfonic acid; [2] DMSO: dimethylsulfoxide; [3] 1 mg mL^{-1} of the herbicide imazapic+imazapyr used in the experiments of lettuce Grand Rapids cultivar and barnyard grass; 2.4 mg mL^{-1} of oxyfluorfen for lettuce Grandes Lagos and giant arrowhead cultivar. [4] Concentration or contrast 3 (C3) used only for the species giant arrowhead and lettuce Grandes Lagos cultivar. [5] + or - indicate the treatments that were used for the formation of the contrast.

the comparison among concentrations, the results were presented as percentage regarding the control, calculated through the formula: Inhibition (%) = [(mean of the control distilled water – mean of treatment n)/mean of the control distilled water]*100, in which zero represented the control; positive values represented stimulation; and negative represented inhibition of the variables.

3 Results and Discussion

In experiment 1, the extracts reduced the vigor, germination and GSI of the cress seeds compared to the controls, with the ethyl acetate extract showing the greatest inhibition (Table 2).

Table 2 - Vigor, germination inhibition compared to the control (%) and GSI of cress seeds after application of different extracts of *H. reniformis* leaves

Treatment	Vigor (%)	Germination inhibition (%)	GSI
Distilled water	36 a	0 d	2.9 a
MÊS[(1)]	30 a	2 d	2.7 a
MES+DMSO[(2)]	36 a	8 cd	2.7 a
Hexane extract	15 bc	32 b	1.7 b
Ethyl acetate extract	0 c	77 a	0.5 c
Methanol extract	15 bc	30 bc	1.8 b
CV (%)	44.5	20.7	20.9

[(1)] MES buffered solution with 2-N-morpholine ethanesulfonic acid; [(2)] Adjuvant dimethylsulfoxide. Means followed by the same letter do not differ among themselves by Duncan test (p≤0.05).

The aerial part and root length of cress seedlings was also reduced by the application of the three extracts (Table 3). For both variables, the ethyl acetate treatment provided the shortest length, followed by hexanic and methanolic extracts. The buffered solution (MES) and the adjuvant DMSO, both used to resuspend the extracts after evaporation of the solvent, they did not interfere in the variables analyzed, except the root length, in which there was an increase in growth when the two were used in combination (MES+DMSO).

Table 3 - Aerial part and root length of cress at 7 days after application of different extracts of *H. reniformis l*eaves

Treatment	Aerial part (mm)	Root (mm)
Distilled water	4.4 ab[(3)]	8.9 ab
MÊS[(1)]	4.5 ab	8.3 bc
MES+DMSO[(2)]	4.9 a	10.7 a
Hexane extract	3.2 cd	3.3 d
Ethyl acetate extract	2.9 d	2.8 d
Methanol extract	3.8 bc	6.2 c
CV (%)	13.8	21.3

[(1)] MES buffered solution with 2-N-morpholine ethanesulfonic acid; [(2)] Adjuvant dimethylsulfoxide; [3]Means followed by the same letter do not differ among themselves by Duncan test (p≤0.05).

The MES buffered solution maintains the pH in appropriate value so that during the germination process the absorption of the extract solution containing the phytotoxic compounds occurs. Extreme pH values can act on the seeds and seedlings and mask the effect of phytotoxic compounds (Carmo et al., 2007). The DMSO, used in the removal of the extract adhered to the glassware after evaporation of the solvent, assisting in the dissolution of the compounds and miscibility in the MES aqueous solution.

According to the solvent chemical characteristics used in the extraction and the responses visualized in this experiment, it is possible that the same compound (s) responsible for the reduction of the variables is(are) present in more than one extract. Thus, the ethyl acetate extract was determined as the most active, choosing to test concentrations of this extract on the germination of species such as lettuce, barnyard grass and giant arrowhead.

In experiment 2, the data of vigor, germination, GSI, aerial part and root length of lettuce Grand Rapids cultivar presented statistical significance (Tables 4 and 5). The contrasts among the controls (T1xT2 and T2xT3) demonstrated that the use of DMSO and MES did not affect the germination process (Tables 4 and 5). In comparison among controls and concentrations, there was no difference among controls and treatments of 0.001 to 0.1 mg mL^{-1} for vigor, germination and GSI variables (contrasts 4, 5 and 6). The highest concentration of ethyl acetate extract of *H. reniformis* caused greater inhibition of vigor, germination and GSI of lettuce seeds of Grand Rapids cultivar compared to negative controls (contrast 7), as well as to positive control (contrast 9).

Table 4 - Vigor, germination and germination speed index (GSI) of lettuce seeds Grand Rapids cultivar and significance of the contrasts tested

Contrast	Vigor (%)	Germination (%)	GSI
C1- (T1) x (T2)	(67) x (59)^{ns}[(1)]	(68) x (59)^{ns}	(6) x (6)^{ns}
C2- (T2) x (T3)	(59) x (51)^{ns}	(59) x (59)^{ns}	(6) x (5)^{ns}
C4- (T1+T2+T3) x (T6)	(59) x (60)^{ns}	(62) x (64)^{ns}	(6) x (6)^{ns}
C5- (T1+T2+T3) x (T7)	(59) x (58)^{ns}	(62) x (60)^{ns}	(6) x (6)^{ns}
C6- (T1+T2+T3) x (T8)	(59) x (64)^{ns}	(62) x (65)^{ns}	(6) x (6)^{ns}
C7- (T1+T2+T3) x (T9)	(59) x (4)*	(62) x (10)*	(6) x (1)*
C8- (T4) x (T9)	(52) x (4)*	(53) x (10)*	(6) x (1)*
CV (%)	17.9	17.2	22.4

[(1)] * or ^{ns} significant and not significant contrasts (p≤0.05).

Table 5 - Aerial part and root length of lettuce Grand Rapids cultivar and significance of the contrasts tested

Contrast	Aerial part (mm)	Root (mm)
C1- (T1) x (T2)	(8.3) x (8.8)^{ns}[(1)]	(23.9) x (19.0)^{ns}
C2- (T2) x (T3)	(8.8) x (8.9)^{ns}	(19.0) x (15.7)^{ns}
C4- (T1+T2+T3) x (T6)	(8.7) x (8.1)^{ns}	(19.5) x (10.3)*
C5- (T1+T2+T3) x (T7)	(8.7) x (9.1)^{ns}	(19.5) x (15.8)^{ns}
C6- (T1+T2+T3) x (T8)	(8.7) x (5.2)*	(19.5) x (5.7)*
C7- (T1+T2+T3) x (T9)	(8.7) x (4.0)*	(19.5) x (2.5)*
C8- (T4) x (T9)	(6.7) x (4.0)*	(9.3) x (2.5)*
CV (%)	15.0	33.2

[(1)] * or ^{ns} significant and not significant contrasts (p≤0.05).

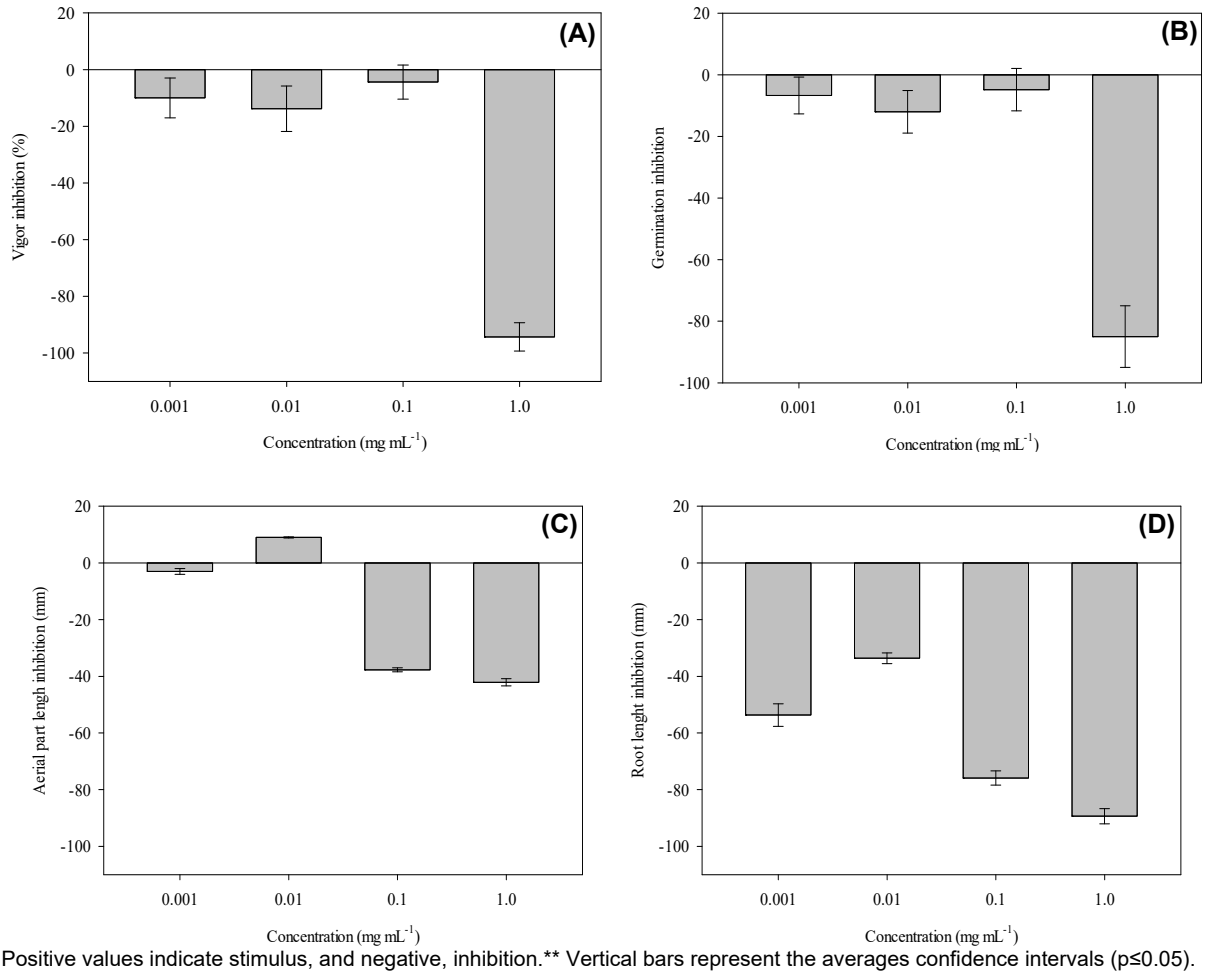
The lowest doses did not interfere with the growth of the lettuce aerial part and the root, except that of 0.001 mg mL^{-1}, which affected the root length (contrast 4) (Table 5). The concentrations of 0.1 and 1 mg mL^{-1} affected the aerial part and root length compared to the controls (contrasts 6 and 7). The highest concentration of ethyl acetate extract of *H. reniformis* leaves provided greater inhibition of the aerial part and root length compared to herbicide treatment with imazapic+imazapyr (contrast 8).

In the comparison among concentrations, for vigor and germination of lettuce seeds Grand Rapids cultivar, there was no difference among the concentrations of 0.001, 0.01 and 0.1 mg mL^{-1} (Figure 1A and B). The concentration of 1 mg mL^{-1} caused the greatest inhibition of vigor and germination, with reductions of 94% and 85% compared to control, respectively. High suppression levels occur when high phytotoxin concentrations coincide with initial stages of the seedlings' growth (Khanh et al., 2005).

Regarding the aerial part length of lettuce seedlings Grand Rapids, the treatments 0.001 and 0.01 mg mL^{-1} of ethyl acetate extract of *H. reniformis* leaves inhibited slightly and stimulated the variable, respectively (Figure 1C). Treatments 0.1 and 1 mg mL^{-1} caused greater inhibition of the aerial part length and differed from the other concentrations. Kato-Noguchi et al. (2014) verified that the extract of the plant *Eicchornia crassipes*, species of the same family as *H. reniformis*, caused phytotoxicity in dicotyledon and monocotyledon species, and phytotoxicity is also dependent on the concentration of the extract. According to these authors, from *E. crassipes* extract the loliolide substance was isolated, which causes phytotoxicity when applied on cress and ryegrass.

Considering the root length of lettuce seedlings Grand Rapids, all concentrations of ethyl acetate extract of *H. reniformis* leaves caused inhibition compared to the control (Figure 1D). The highest percentage of inhibition was caused by the concentration of 1 mg mL^{-1}, followed by treatments 0.1, 0.001 and 0.01 mg mL^{-1}.

In experiment 3, the lettuce Grandes Lagos cultivar was also subjected to different concentrations of



* Positive values indicate stimulus, and negative, inhibition.** Vertical bars represent the averages confidence intervals (p≤0.05).

Figure 1 - Inhibition regarding the control of vigor (A), germination (B), aerial part length (C) and root length (D) of lettuce Grand Rapids cultivar subjected to concentrations of ethyl acetate extract of *H. reniformis* leaves.

ethyl acetate extract of *H. reniformis* leaves observing significance for all variables analyzed (Tables 6 and 7). In the contrasts analysis, the controls (T1xT2 and T2xT3) presented high germination, and the addition of the MES buffered solution and the adjuvant DMSO did not interfere negatively in the variables analyzed. There was increase in the root length of lettuce when DMSO was added to the MES solution (contrast 2) (Table 7).

Table 6 - Vigor and germination of lettuce Grandes Lagos cultivar and significance of the contrasts tested

Contrast	Vigor (%)	Germination (%)
C1- (T1) x (T2)	(98) x (96)$^{ns(1)}$	(98) x (98)^{ns}
C2- (T2) x (T3)	(96) x (98)^{ns}	(98) x (100)^{ns}
C3- (T1+T2+T3) x (T5)	(97) x (99)^{ns}	(99) x (99)^{ns}
C4- (T1+T2+T3) x (T6)	(97) x (99)^{ns}	(99) x (100)^{ns}
C5- (T1+T2+T3) x (T7)	(97) x (91)*	(99) x (95)^{ns}
C6- (T1+T2+T3) x (T8)	(97) x (91)*	(99) x (95)^{ns}
C7- (T1+T2+3) x (T9)	(97) x (10)*	(99) x (47)*
C8- (T4) x (T9)	(0) x (10)*	(0) x (47)*
CV (%)	5.5	4.6

(1) * or ^{ns} significant and not significant contrasts (p≤0.05).

Table 7 - Aerial part and root length of lettuce seedlings Grandes Lagos cultivar and significance of the contrasts tested

Contrast	Aerial part (mm)	Root (mm)
C1- (T1) x (T2)	(7.5) x (7.3)$^{ns(1)}$	(8.4) x (6.3)^{ns}
C2- (T2) x (T3)	(7.3) x (8.1)^{ns}	(6.3) x (11.8)*
C3- (T1+T2+T3) x (T5)	(7.6) x (7.4)^{ns}	(8.8) x (8.1)^{ns}
C4- (T1+T2+T3) x (T6)	(7.6) x (7.1)^{ns}	(8.8) x (8.9)^{ns}
C5- (T1+T2+T3) x (T7)	(7.6) x (7.1)^{ns}	(8.8) x (7.3)^{ns}
C6- (T1+T2+T3) x (T8)	(7.6) x (8.9)*	(8.8) x (7.0)^{ns}
C7- (T1+T2+3) x (T9)	(7.6) x (3.3)*	(8.8) x (2.7)*
C8- (T4) x (T9)	(0.0) x (3.3)*	(0.0) x (2.7)*
CV (%)	13.6	24.2

(1) * or ^{ns} significant and not significant contrasts (p≤0.05).

The vigor of lettuce seeds was affected from the concentration of 0.01 mg mL^{-1} (contrast 5), with the highest effect for 1 mg mL^{-1} (contrast 7) (Table 6). As for the seeds' germination, it was reduced only in the highest concentration of the extract (contrast 7), while in the other concentrations there was no difference of the control treatments. In the highest concentration of the extract (contrast 7), it was verified a reduction of 90% and 52% of the vigor and germination of lettuce seeds, respectively.

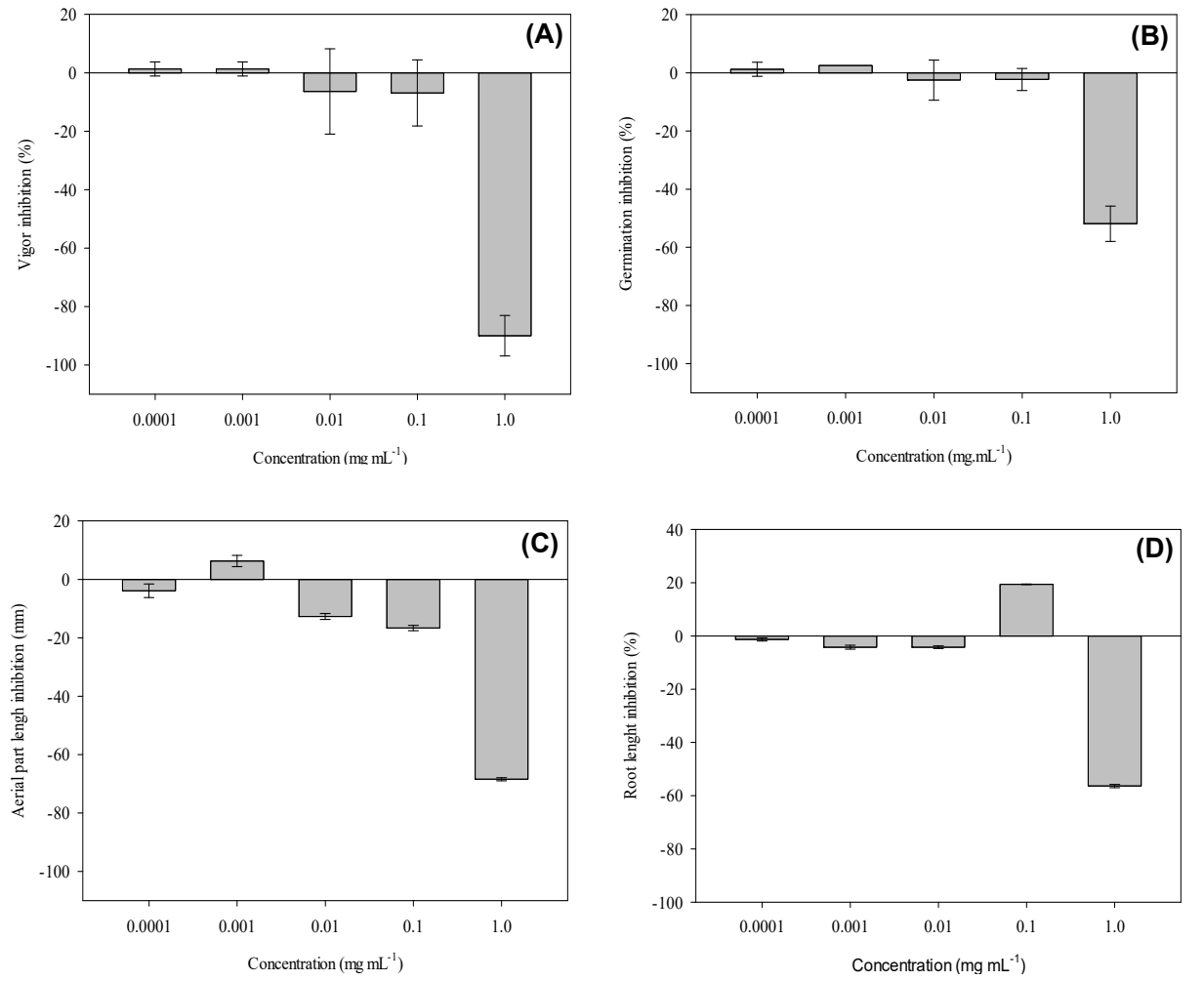
Regarding the aerial part and root length of lettuce seedlings Grandes Lagos, it was verified that only the concentration of 1 mg mL^{-1} of the ethyl acetate extract of *H. reniformis* leaves (contrast 7) negatively affected the variables and differed from the control treatments (Table 7). The concentration of 0.1 mg mL^{-1} (contrast 6) stimulated the aerial part growth of lettuce seedlings, compared to controls. The lettuce seeds Grandes Lagos showed extreme sensitivity to the herbicide oxyfluorfen (contrast 8), and the vigor, germination and aerial part and root length were totally inhibited (Tables 6 and 7). Similarly, to germination, the other concentrations did not change the aerial part and root length.

In this research, the root length of lettuce Grand Rapids cultivar was affected by all concentrations tested, unlike the Grandes Lagos cultivar, which was affected only by the highest concentration, highlighting the importance of using different concentrations of extracts, cultivars and species. In a study conducted by Novaes et al. (2013), the variables of lettuce aerial part and root length were more affected by the ethyl acetate extract of *Rapanea umbelata* leaves than the germination variable, regardless of the concentration used. In another research, lettuce did not prove as sensitive to the application of the extracts of wolf apple's leaves *(Solanum lycocarpum*) as the tomato and cress species, but the root was the most affected organ (Oliveira et al., 2012).

In the comparison among concentrations, for the variables such as vigor and germination of lettuce Grandes Lagos, it was observed similarity among the concentrations of 0.0001, 0.001, 0.01 and 0.1 mg mL^{-1} (Figure 2A and B). The extract in the concentration of 1 mg mL^{-1} caused greater inhibition for all variables (Figure 2A to D). In the aerial part length, there was stimulus when the concentration of 0.001 mg mL^{-1} was used and inhibitions of up to 20% for concentrations below 1 mg mL^{-1} (Figure 2C). For the root length, all concentrations caused inhibition of the variable, except for concentration of 0.1 mg mL^{-1} of the ethyl acetate extract of *H. reniformis (*Figure 2D). The changes in the seedlings' growth are reflection of the metabolism change by the presence of some interferent in the extract (Ranal and Santana, 2006), suggesting the presence of compounds with herbicide activity in the ethyl acetate extract of *H. reniformis* leaves.

In experiment 4, when analyzing the contrasts of vigor and GSI of barnyard grass seeds, it was verified that the addition of DMSO to the MES solution (contrast 2) negatively affected the two variables (Table 8). The highest concentration of the extract (T9) reduced the vigor and the germination speed index of barnyard grass seeds (contrast 7). In contrast 8, it was also verified reduction of the GSI variable by the extract action. However, the reduction in the treatment with the highest concentration of the extract may not be the result of the extract action but of the adjuvant DMSO.

The adjuvant and the buffered solution did not negatively interfere in the germination result, as verified for vigor and GSI (Table 8). The highest



* Positive values indicate stimulus, and negative, inhibition.** Vertical Bars represent the averages confidence intervals (p≤0.05).

Figure 2 - Inhibition regarding the control (%) of vigor (A), germination (B), aerial part length (C) and root length (D) of lettuce Grandes Lagos cultivar subjected to concentrations of ethyl acetate extract of *H. reniformis* leaves.

concentration of the extract (T9) reduced the germination of the seeds compared to the controls (T1+T2+T3) and also to the herbicide imazapic+imazapyr (T4). The other concentrations did not differ from the controls. In the experiment with lettuce Grand Rapids and barnyard grass, this herbicide did not affect the germination and vigor, since during the evaluations were considered germinated seeds when they issued 2 mm of root, and at that time they were not affected by the herbicide.

The adjuvant and the buffered solution did not interfere in the aerial part and root length of barnyard grass seedlings (contrasts 1 and 2) (Table 9). The extracts in the concentrations of 0.1 and 1 mg mL^{-1} reduced the aerial part length compared to the controls (contrasts 6 and 7). In this case, the herbicide imazapic+imazapyr was more effective in the reduction of the aerial part than the concentration of 1 mg mL^{-1} of the ethyl acetate extract of *H. reniformis* leaves (contrast 8).

Table 8 - Vigor, germination and germination speed index (GSI) of barnyard grass seeds and significance of the contrasts tested

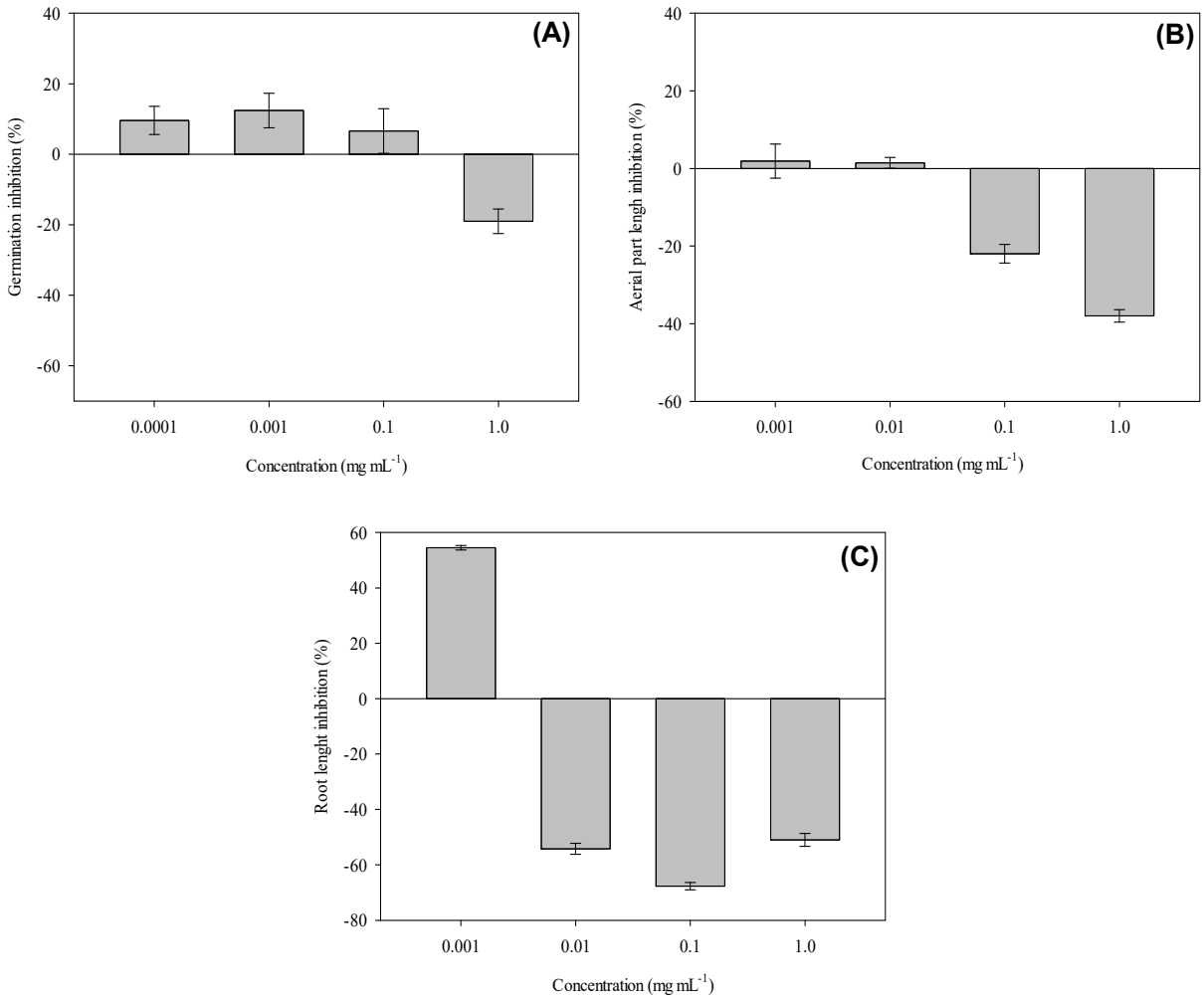
Contrast	Vigor (%)	Germination (%)	GSI
C1- (T1) x (T2)	(53) x (45)$^{ns(1)}$	(84) x (90)^{ns}	(4.0) x (4.2)^{ns}
C2- (T2) x (T3)	(45) x (21)*	(90) x (85)^{ns}	(4.2) x (3.3)*
C4- (T1+T2+T3) x (T6)	(40) x (33)^{ns}	(88) x (95)^{ns}	(3.8) x (4.2)^{ns}
C5- (T1+T2+T3) x (T7)	(40) x (45)^{ns}	(88) x (98)*	(3.8) x (4.6)*
C6- (T1+T2+T3) x (T8)	(40) x (46)^{ns}	(88) x (93)^{ns}	(3.8) x (4.2)^{ns}
C7- (T1+T2+T3) x (T9)	(40) x (20)*	(88) x (70)*	(3.8) x (2.8)*
C8- (T4) x (T9)	(37) x (20) ^{ns}	(86) x (70)*	(4.1) x (2.6)*
CV (%)	31.5	8.9	11.9

(1) * or ^{ns} significant and not significant contrasts (p≤0.05).

Table 9 - Aerial part and root length of barnyard grass seedlings and significance of the contrasts tested

Contrast	Aerial part (mm)	Root (mm)
C1- (T1) x (T2)	(15.4) x (15.5)$^{ns(1)}$	(11.7) x (11.5)^{ns}
C2- (T2) x (T3)	(15.5) x (15.0)^{ns}	(11.5) x (10.1)^{ns}
C4- (T1+T2+T3) x (T6)	(15.3) x (15.7)^{ns}	(11.1) x (18.1)*
C5- (T1+T2+T3) x (T7)	(15.3) x (15.6)^{ns}	(11.1) x (5.4)*
C6- (T1+T2+T3) x (T8)	(15.3) x (11.9)*	(11.1) x (3.8)*
C7- (T1+T2+T3) x (T9)	(15.3) x (9.5)*	(11.1) x (5.7)*
C8- (T4) x (T9)	(6.0) x (9.5)*	(4.3) x (5.7)^{ns}
CV (%)	10.4	31.2

(1) * or ^{ns} significant and not significant contrasts (p≤0.05).



* Positive values indicate stimulus, and negative, inhibition.** Vertical bars represent the averages confidence intervals (p≤0.05).

Figure 3 - Inhibition regarding the control (%) of germination (A), aerial part length (B) and root length (C) of barnyard grass subjected to concentrations of ethyl acetate extract of *H. reniformis* leaves.

Root length was reduced in all concentrations of the extract, except 0.001 mg mL^{-1} (contrast 4), where there was stimulus (Table 9). When comparing the concentration of 1 mg mL^{-1} (T9) of the *H. reniformis* extract with the herbicide imazapic+imazapyr (T4), there was no difference. The roots, the first vegetable organs to emerge, come in contact with the extracts and absorb directly the active compounds, which explains its sensitivity to the extract (Tanveer et al., 2012).

In comparison among the concentrations for the barnyard grass species, the vigor was not analyzed, since DMSO negatively affected the variable (Figure 3). The germination data of barnyard grass in different concentrations of the extract demonstrated that there was stimulus to germination when the extract concentrations of 0.001 to 0.1 mg mL^{-1} was used (Figure 3A). The germination of the barnyard grass in the concentration of 1 mg mL^{-1} has reduced compared to the other concentrations, and this reduction is of about 17% when compared to the control treatment.

In the aerial part length of barnyard grass, there was no difference among the concentrations of 0.001 and 0.01 mg mL^{-1} and the control treatment (Figure 3B). There was growth reduction of the aerial part from 0.1 mg mL^{-1}, with the highest effect on the concentration of 1 mg mL^{-1}. For the root length of barnyard grass, all concentrations presented shorter root length than the control treatment, except the 0.001 mg mL^{-1}.

According to research results, the sensitivity to the extracts depends on the species and also on the concentration of the foliar extract (Bari and Kato-Noguchi, 2017). The seedlings' growth can be stimulated by compounds in low concentration and, on the contrary, can be reduced or inhibited in high concentrations (Kato-Noguchi et al., 2016), a fact observed in this research for the two lettuce cultivars and barnyard grass. The responses dependent on

the concentration, where low concentrations are stimulants and high concentrations are inhibitory, are called hormesis (Belz et al., 2011).

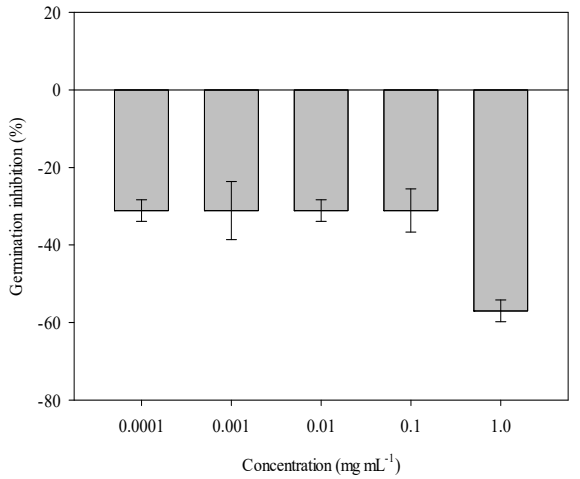
In experiment 5, the germination data and GSI of giant arrowhead presented statistical significance (Table 10). The vigor variable was not evaluated, because there is no information for the species in the seeds analysis rules, and the germination counts and GSI were performed until the moment they stabilized (Brasil, 2009). The aerial part and root length data did not show statistical significance (data not presented).

Table 10 - Germination and germination speed index (GSI) of giant arrowhead seeds and significance of the contrasts tested

Contrast	Germination (%)	GSI
C1- (T1) x (T2)	(39) x (43)^{ns}	(1.9) x (1.8)^{ns}
C2- (T2) x (T3)	(43) x (34)^{ns}	(1.8) x (1.6)^{ns}
C3- (T1+T2+T3) x (T5)	(39) x (27)*	(1.8) x (1.2)*
C4- (T1+T2+T3) x (T6)	(39) x (27)*	(1.8) x (1.2)*
C5- (T1+T2+T3) x (T7)	(39) x (27)*	(1.8) x (1.4)^{ns}
C6- (T1+T2+T3) x (T8)	(39) x (27)*	(1.8) x (1.2)*
C7- (T1+T2+T3) x (T9)	(39) x (17)*	(1.8) x (0.7)*
C8- (T4) x (T9)	(14) x (17)*	(0.6) x (0.7)^{ns}
CV (%)	27.5	30.6

(1) * or ^{ns} significant and not significant contrasts (p≤0.05).

For the germination variable of giant arrowhead, it was verified that the percentage of the controls treatments was low (T1xT2 and T2xT3), not exceeding 43% (Table 10). Just as for the lettuce species (experiments 2 and 3) and barnyard grass (experiment 4), the addition of MES (contrast 1) and DMSO (contrast 2) did not affect the germination and GSI of giant arrowhead seeds.



* Positive values indicate stimulus, and negative, inhibition.** Vertical bars represent the averages confidence intervals (p≤0.05).

Figure 4 - Inhibition regarding the control (%) of germination of giant arrowhead seeds submitted to concentrations of ethyl acetate extract *of H. reniformis* leaves.

All concentrations of the extract reduced the germination and GSI of giant arrowhead seeds compared to the controls, except the dose of 0.01 mg mL^{-1} (contrast 5) for the GSI variable. The contrast between the herbicide oxyfluorfen (T4) and the highest concentration of the extract (T9) demonstrated that the herbicide was more efficient in the germination reduction (contrast 8).

In comparison among concentrations for the germination variable of giant arrowhead seeds, it was verified that all concentrations of the ethyl acetate extract *of H. reniformis* caused inhibition (Figure 4). The concentrations of 0.0001, 0.001, 0.01 and 0.1 mg mL^{-1} differed only from the highest concentration of the ethyl acetate extract (1 mg mL^{-1}), where the germination was reduced by around 56%.

The seeds' germination is less sensitive to the action of the compounds from the extracts than the seedlings' growth, but significant reductions in the germination or in GSI indicate that the compounds present in the plant extract act negatively on the target species (Borghetti and Ferreira, 2004), as seen in this research. The inhibitory effect of the ethyl acetate extract of *H. reniformis* leaves on the variables: germination, aerial part and root length is an indication that the compound (s) present in the extracts modifies different physiological processes.

4 Conclusions

The ethyl acetate extract of *H. reniformis* presents inhibitory activity on the seeds of cress, lettuce, giant arrowhead and barnyard grass, but this activity was dependent on the concentration of the extract and the species studied.

In general, the aerial part and root length variables demonstrate to be more sensitive to the action of the ethyl acetate extract of *H. reniformis* leaves compared to the germination.

H. reniformis synthesizes compounds with phytotoxic activity and purification of extracts is required to isolate, identify and characterize the action mechanism of the compounds with herbicide activity, so that in the future these can be used as models for the development of herbicides.

5 Contributions

LTP: conduction of the experiments, tabulation of the data, statistical analysis and writing of the manuscript; DA: statistical analysis and writing of the

manuscript; Other authors: conduction of the experiments and writing of the manuscript.

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