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Allelopathic effects of *Alternanthera tenella* over weeds*

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ABSTRACT – Allelopathy is defined as the interference of one plant in the other through the release of allelochemicals in the environment. This work evaluated the allelopathic influence of *Alternanthera tenella* on the germination and initial development of *Lactuca sativa*, *Digitaria insularis*, *Emilia fosbergii* and *Portulaca oleracea*, as well as to quantify and determine the phenolic compounds by High Performance Liquid Chromatography. Five leaf and root concentrations of the donor plant were tested using multi-well plates as containers in which 15 seeds were sown in six replications. The data obtained were subjected to analysis of variance and different inhibitory effects were observed, depending on the recipient species and the concentration used, with *Emilia fosbergii* and *Digitaria insularis* being the most sensitive species to leaf and root allelochemicals, respectively. Caffeic acid was identified in greater quantity, probably responsible for the effect on germination and initial development of recipient plants.

Keywords: Allelopathy, germination index, inhibition, weed management.

RESUMO – Efeitos allelopáticos de *alternanthera tenella* sobre ervas daninhas. Alelopatia é definida como a interferência de uma planta na outra, por meio da liberação de aleloquímicos no meio ambiente. Este trabalho avaliou a influência alelopática de *Alternanthera tenella* na germinação e desenvolvimento inicial de *Lactuca sativa*, *Digitaria insularis*, *Emilia fosbergii* e *Portulaca oleracea*, bem como quantificou e determinou os compostos fenólicos por Cromatografia Líquida de Alta Eficiência. Foram testadas cinco concentrações de folhas e raízes da planta doadora, utilizando placas multipoços como recipientes nos quais 15 sementes foram semeadas em seis repetições. Os dados obtidos foram submetidos à análise de variância e foram observados diferentes efeitos inibitórios, dependendo da espécie receptora e da concentração utilizada, sendo a *Emilia fosbergii* e *Digitaria insularis* as espécies mais sensíveis aos aleloquímicos de folhas e raízes, respectivamente. O ácido cafeico foi identificado em maior quantidade, sendo, provavelmente, o responsável pelo efeito na germinação e no desenvolvimento inicial das plantas receptoras.

Palavras-chave: Alelopatia, índice de germinação, inibição, plantas daninhas.

INTRODUCTION

The interference of some plants on others can occur through allelopathic processes and competition. Competition reduces or removes essential factors for plant development (water, nutrient, space) from the environment, but allelopathic effects occur through the addition of chemical compounds into the environment, interfering in the development of another species, and understanding these interactions is extremely important in natural environments and agroecosystems (Teasdale *et al.* 2012). Therefore, the vegetation of a given area can have a succession model conditioned to the plants which already existed there and to the chemical substances that they release into the environment. Therefore, previous occupation of the area can have a significant influence on the crops being installed for agricultural or forest management.

Among the wide variety of secondary metabolites found in plants, phenolic compounds and terpenoids present most of the compounds with identified allelopathic activities. The release of these compounds into the environment can occur by leaching, volatilization, root exudation, exudation after soaking the seeds and decomposition of plant tissues and still be increased or reduced by the age of the plant,

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type of plant organ, cultivar and various environmental factors (Souza Filho 2006).

These compounds can influence the growth and development of surrounding biological systems in nature and are an important ecological factor since they act in forming plant communities (Razavi 2011). Allelopathic studies have evaluated the action of several extracts and secondary bioactive metabolites extracted from plant material with promising results for weed control (Mendes & Rezende 2014, Grisi *et al.* 2015, Pereira *et al.* 2018, Ferreira *et al.* 2020). Species which are more sensitive to secondary metabolites such as indicator plants including lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum* Miller) are plants which indicate allelopathic activity and are often used in laboratory tests (Alves *et al.* 2004).

In this sense, it is of utmost importance to choose an appropriate methodology to identify allelopathic plants and/or released allelochemicals, since plants with compounds with inhibitory allelopathic activity can be used as efficient bio herbicides in controlling weeds, and also being indicative of new compounds with biocide action, contributing to increased productivity and better coexistence of weeds with cultivars, making the cultivation area more balanced (Zeng 2014).

The Amaranthaceae family belongs to the class of Magnoliopsida and the order Caryophyllales. It comprises 65 genera and approximately 1,000 species described, originating in tropical, subtropical and temperate zones of Africa, South America and Southeast Asia (Siqueira 1995). The Alternanthera Forsk. 1775 genus is located within the Gomphrineae tribe, and is composed of 80 species, 30 of which can be found in Brazilian territory.

Alternanthera tenella Colla is popularly known as "fire extinguisher" or "perpetual bush", belongs to the Amaranthaceae family and is not endemic to Brazil, but occurs in all regions of the country. It is an invasive species, common in wastelands, dry and humid fields, swamps, forests, anthropic areas and in mixed rainforest regions (Moreira 2011). It presents vigorous growth, always launching new branches on the existing vegetation and usually dominates the areas they infest, preventing the establishment of other plants (Teasdale *et al.* 2012). Studies on the chemical composition of this plant have indicated the presence of betacyanines, flavonoids and alkaloids (Brochado *etal.* 2003, Salvador & Dias 2004). Mehmood *et al.* (2017) also identified quercetin, chlorogenic acid, p-coumaric acid, cinnamic acid, caffeic acid, sirinic acid, synapic acid and vanillic acid in *Alternanthera philoxeroides* and *Alternanthera sessilis*.

Due to the agroecological importance of the species under study, this study aimed to investigate the allelopathic effects of *Alternanthera tenella* leaves and roots on seed germination and initial development of lettuce (*Lactuca sativa* L.), false-milkweed (*Emilia fosbergii* Nicolson) seedlings, purslane (*Portulaca oleracea* L.) and bitter grass (*Digitaria insularis* (L.) Fedde) as a promising option for future studies of isolation and identification of natural phytotoxins with herbicidal potential.

MATERIAL AND METHODS

The material from the donor plant (*Alternanthera tenella* Colla – Herbarium MAC 64852) was collected in the final vegetative stage at the premises of the Agricultural Sciences Center of the Federal University of Alagoas, separated into shoots (leaves + branches) and roots, washed under running water to remove impurities and then put into a forced air circulation oven for 96 hours (4 days) at a constant temperature of 40 °C until constant weight was obtained. It was subsequently crushed in a mill and packed in hermetically sealed and refrigerated plastic bags for later studies. Concentrations were obtained by weighing the crushed leaves and roots separately (0.01, 0.02, 0.04 and 0.08 g) on a Mettler Toledo scale.

In the allelopathic bioassays, the sandwich method used by Cândido *et al.* (2010) was employed using the agar-based culture medium prepared with seven grams of agar in one (1) liter of water and subjected to autoclaving at 121 ºC for 30 minutes. The experiment was set up in a completely randomized design (CRD) in multi-well plates separated into experimental groups and controls containing 15 seeds from each plant in each well, with six replicates for each experimental group (treated with the leaves and roots of the donor plant) and negative control (without plant material between the agar layers). A 5 ml agar solution was deposited in each well of the multi-well plates, and the dry matter of the leaves or roots of the donor plant was added in the tested concentrations to this first agar layer, and then it was covered with another 5 ml of agar on top of the first layer. After 24 h, 15 seeds of the plants under study were randomly sown in each well.

The seeds of the recipient plants – lettuce (*Lactuca sativa* L.), false milkweed (*Emilia fosbergii* Nicolson), purslane (*Portulaca oleracea* L.) and bitter grass (*Digitaria insularis* (L.) Fedde) – were also collected on the premises of the University and disinfected with sodium hypochlorite (2%) for approximately two minutes, after which they were washed with sterile water and introduced into the multi-well plates on top of the second agar layer. Then after seven days of setting up the experiment with a 12-hour photoperiod, the temperature germination percentage (GP), shoot length (SL) and primary root length (PRL) were evaluated.

Protrusion and geotropic curvature of the radicle were used as a criterion for germination evaluation according to the Rules for Seed Analysis (Brasil 2009). Seedling length was evaluated in conjunction with the germination test, in which six seedlings considered normal from each treatment were removed and shoot length (SL) and primary root length (PRL) were evaluated using a millimeter ruler.

After evaluating the germination percentage and initial development of the recipient plants, the cultivation medium, still with the plant material between the layers, was left in a methanol maceration process and the solvent was removed every 48 hours. This process was repeated three times and the material was evaporated and stored in amber glass. Then, the extract was submitted to a phytochemical analysis by High Performance Liquid Chromatography (HPLC) to identify and quantify the phenolic compounds. The compounds in the samples were identified by comparing the retention times with an appropriate standard by comparing the peaks obtained in the samples with those of the standards. All solvents used for chromatography were of analytical grade; methanol (panreac), formic acid (dinâmica) and ultrapure water obtained from a Milli-Q system. Samples and standards were eluted according to the gradient totaling an 80 minutes run. The wavelength used was 290 nm at a temperature of 33ºC, flow of 0.6 mL min^{-1} and injection volume of 20 µL.

In this work, Pearson's correlation coefficients between characters were also estimated using the SAEG software program (Bernardes 2007) with the significance assessed by the Student's t-test (p> 0.05). Then, the effects were evaluated according to Lúcio *et al.* (2013), who evaluated the cause and effect relationships between the studied parameters: If the result was positive, the correlation was caused by the indirect effects; If the result was negative, the correlation was caused by the direct effects.

The obtained data were submitted to analysis of variance using the SISVAR software program (Ferreira 2011) according to that proposed by Pereira *et al.* (2009). In addition, the effect of the extract being significant was considered as the extract concentration which provides 50% control or growth reduction of the recipient species (CL_{50}), and the data were adjusted to the exponential decay non-linear regression model with two parameters (a – concentration x b – effect) using the SIGMAPLOT program (Streibig 1988). When the data did not fit, they were differentiated with linear polynomial regression[1].

RESULTS AND DISCUSSION

In the analysis of variance for germination percentage, shoot length and primary seedling root length, significant effects of the species were recorded for the tested concentrations. Table 1 shows the regressions for the parameters evaluated in this study adjusted to the exponential decay model, with the exception of the recipient *P. oleracea* species when the shoot length tested with leaves and the primary root length of *L. sativa* were evaluated when tested with roots, being differentiated with linear polynomial regression[1].

The substances released by the leaves and roots of *A. tenella* showed intense inhibitory effects on the germination of *E. fosbergii* and *D. insularis* seeds (Fig. 1A-B). It was also observed that the substances released in the highest concentration (0.08 g) were those which promoted the highest inhibition percentages. However, *P. oleracea* was less sensitive to the allelochemicals present in the roots of this donor plant, demonstrating the high resistance of this invasive species to adverse factors; this constitutes a fundamental characteristic to guarantee success in the occupation of new habitats, which explains its survival even in the most inhospitable places such as sewage, contaminated riversides, etc. (De Conti & Franco 2011). Differences in sensitivity between receptor species are common in studies verifying allelopathy and can be explained by the fact that the mechanisms of absorption, translocation and action of phytotoxins vary between species (Walsh *et al.* 2014, Anese *et al.* 2015).

There was no germination pattern, and a higher or lower concentration of materials often affects this parameter differently, depending on the species tested. For example, in tests with *Richardia grandiflora*, the germination test in *D. insularis, E. fosbergii* and *P. oleracea* was only negatively influenced in the highest concentrations (0.04 and 0.08 g), whereas lettuce germination inhibition occurred from the concentration of 0.01 g (Ferreira *et al.* 2020). In a test with *Scoparia dulcis*, it was found that there was a negative effect on the germination of all tested recipient species from the concentration of 0.02 g. Other authors have indicated that the ethanolic extracts of *Canavalia ensiformes* negatively influenced the germination of *D. insularis*, *E. fosbergii*, *P. oleracea* and *L. sativa*, regardless of the concentration used (Pereira *et al.* 2018).

Allelochemicals present in the leaves of *A. tenella* more significantly influenced the development of the radicle than the hypocotyl (Fig. 2C and 3E), with no relationship between the concentration or species in the hypocotyl length parameter, meaning that the different concentrations did not influence the development of the hypocotyl of the species tested (Fig. 2C). However, there was a stimulus in the hypocotyl growth in *L. sativa* in the 0.04 g concentration in relation to the control (Fig. 2C). This fact may be linked to the presence of some secondary metabolite acting as a growth hormone, which led to an increase in lengthening the hypocotyl instead of causing inhibition (Anese *et al.* 2015).

It was evidenced that the recipient plant roots were sensitive to the materials of the donor plant leaves, since the primary root length significantly contrasts with the control, especially in the highest concentration used, and specifically in *E. fosbergii* and *D. insularis* species (Fig. 3E). The specificity of extracts and allelochemicals from plants to more efficiently inhibit the root system of target species, particularly weeds, was recorded as phytotoxicity (Teerarak *et al.* 2012, Anese *et al.* 2015). The root growth is characterized by high rates in its metabolism, which make them highly susceptible to environmental stresses, such as allelochemicals in substrate (Walsh *et al.* 2014).

With the exception of the *D. insularis* receiver*, A. tenella* roots did not present significant effects on the seedling shoot length of the studied recipients (2D). The radicle length was negatively affected by the higher concentrations of roots of the studied donor plant. The seedlings had a radicle length of less than 1.5 cm at a concentration of 0.08 g, differing from the control which had a length above 2 cm (Fig. 3F).

Table 1. Estimates of A and B parameters of the coefficient of determination (R^2) of the exponential decay model for germination (G), shoots length (SL) and primary root length (PRL) of seeds and seedlings of recipient species (*L. sativa*, *D. insularis*, *E. fosbergii* and *P. oleracea*), using leaves and roots of *A. tenella.*

		Germination (G)		
		Leaves		
		Parameters		
Recipient specie	A	B	R^2	F
L. sativa	97.93	8.07	0.53	1.1538
D. insularis	89.11	66.77	0.77	4.5391
E. fosbergii	83.53	42.01	0.64	2.1401
P. oleracea	87.65	27.59	0.86	8.5563
		Roots		
L. sativa	93.99	35.03	0.82	6.1274
D. insularis	101.46	91.27	0.93	19.9516
E. fosbergii	94.97	75.68	0.86	8.6373
P. oleracea	105.31	6.61	0.89	12.3203
		Shoot Length (SL)		
		Leaves		
L. sativa	0.82	1.79	0.12	0.0439
D. insularis	0.37	15.46	0.57	1.4971
E. fosbergii	0.51	29.04	0.61	1.7792
P. oleracea[1]	0.24	0.08	0.10	0.0330
		Roots		
L. sativa	0.45	6.38	0.97	46.5591
D. insularis	0.52	28.95	0.53	1.2250
E. fosbergii	0.76	5.07	0.61	1.7855
P. oleracea	0.21	2.37	0.41	0.6262
		Primary Root Length (PRL)		
Leaves				
L. sativa	2.04	11.29	0.81	5.7351
D. insularis	4.12	251.49	0.96	41.1715
E. fosbergii	1.63	1264.67	0.95	33.3228
P. oleracea	1.61	20.07	0.89	11.6912
		Roots		
L. sativa[1]	1.50	5.50	0.31	0.3295
D. insularis	4.25	85.75	0.96	35.1143
E. fosbergii	2.05	11.03	0.79	4.9521
P. oleracea	2.22	20.99	0.59	1.6578

[1] f = y0+a*x (regression linear)

The set of negative changes occurred simultaneously with the increase in the root concentration of the species evaluated. However, a stimulus in the root growth of *L. sativa* occurred at the concentration of 0.08 g of roots, which suggests that the allelochemicals released by the root system of this donor species have a favorable effect on growth and on the development of the radicle of this receptor, also featuring an allelopathic effect.

The toxic action of allelochemicals present in *A. tenella* leaves was also observed in the appearance of the seedlings. The highest concentrations caused darkening and atrophy in *P. oleracea* roots. According to Yamagushi *et al.* (2011), these symptoms resulted from the action of toxic substances present in the materials on the root meristem, leading to tissue death.

At the inhibitory concentration equivalent to 50 % effect (CL_{50}), it was observed that the *D. insularis* species was touched by the smallest CL_{50}, in the germination parameter.*E. fosbergii* had the lowest CL_{50} when the shoot length and the primary root length were evaluated when submitted to treatment with leaves (Tab. 2).

D. insularis was also more sensitive to the allelochemicals present in the growing concentrations of *A. tenella* roots because it had the lowest CL_{50} in all evaluated parameters

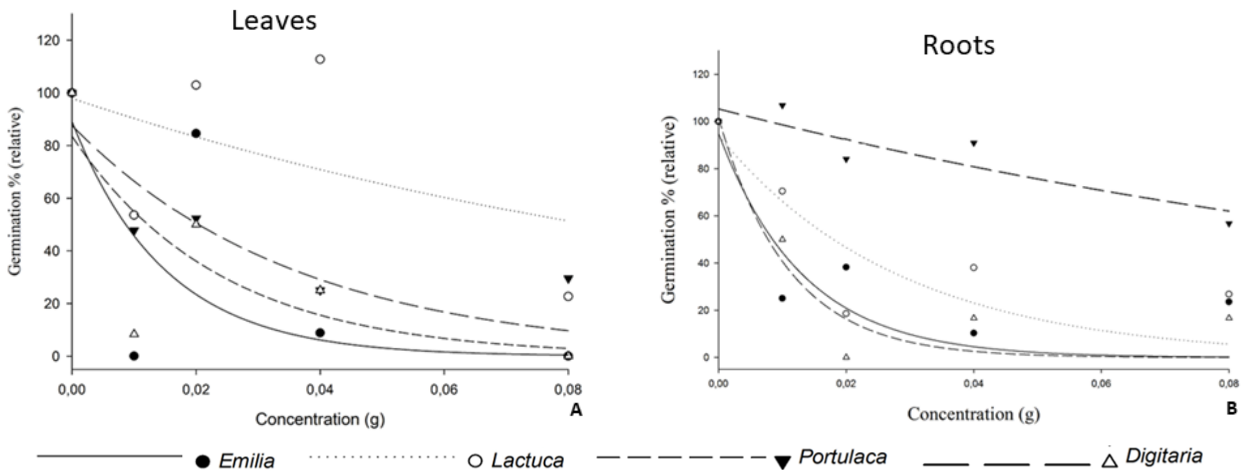


Figure 1. Seed Germination. **A.** Germination percentage of the recipient plants (*L. sativa, D. insularis, E. fosbergii* and *P. oleracea*) as a function of increasing concentrations of leaves of *A. tenella*; **B.** Germination percentage of the recipient plants (*L. sativa, D. insularis, E. fosbergii* and *P. oleracea*) as a function of increasing concentrations of roots of *A. tenella*.

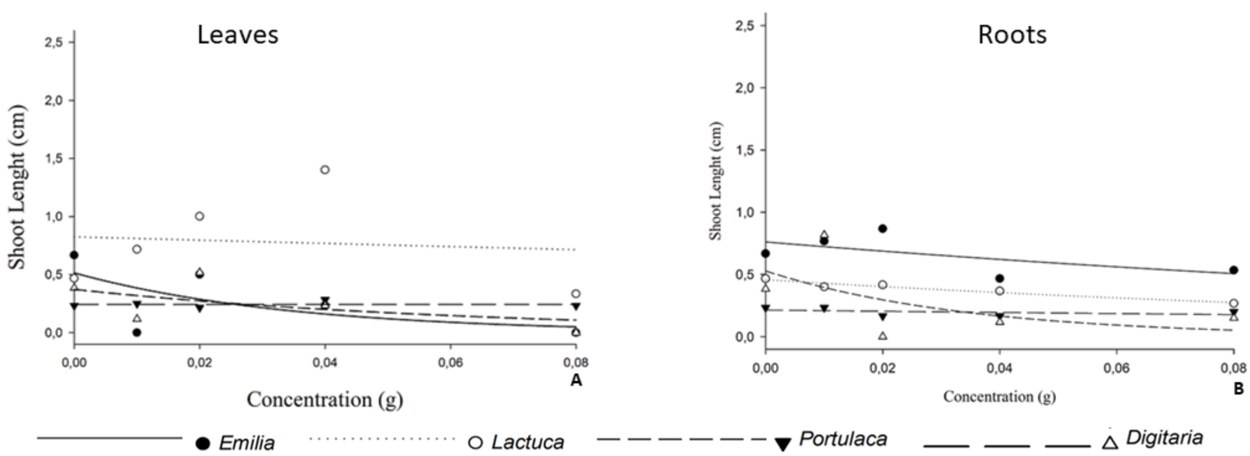


Figure 2. Size Of The Air Section. **A.** Shoot length of the recipient plants (*L. sativa, D. insularis, E. fosbergii* and *P. oleracea*) as a function of increasing concentrations of leaves of *A. tenella*; **B.** Shoot length of the recipient plants (*L. sativa, D. insularis, E. fosbergii* and *P. oleracea*) as a function of increasing concentrations of roots of *A. tenella*.

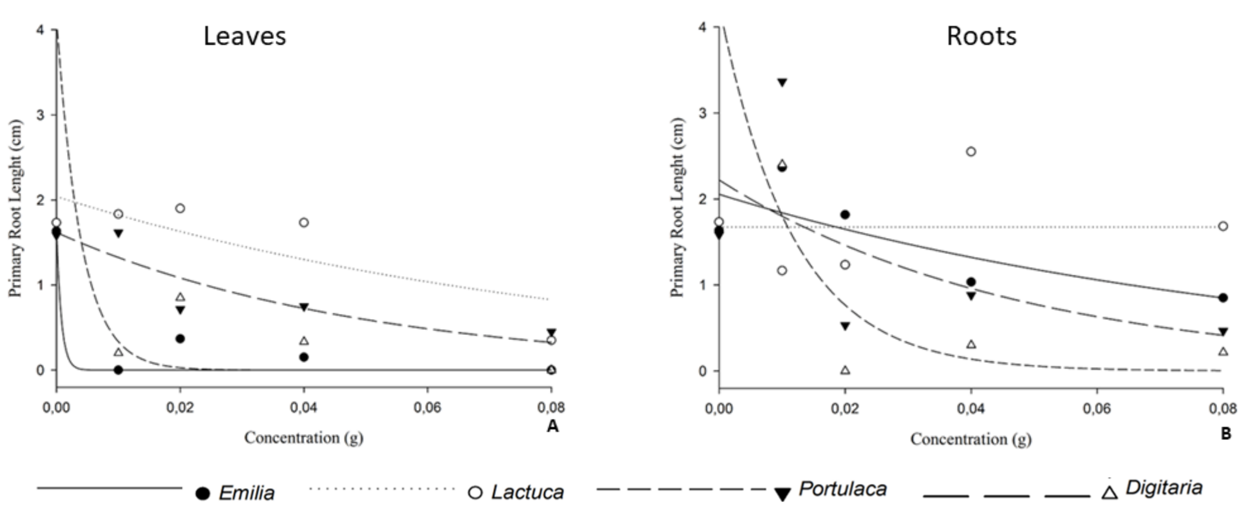


Figure 3. Primary Root Size. **A.** Primary root length of the recipient plants (*L. sativa, D. insularis, E. fosbergii* and *P. oleracea*) as a function of increasing concentrations of leaves of *A. tenella*; **B.** Primary root length of the recipient plants (*L. sativa, D. insularis, E. fosbergii* and *P. oleracea*) as a function of increasing concentrations of roots of *A. tenella*.

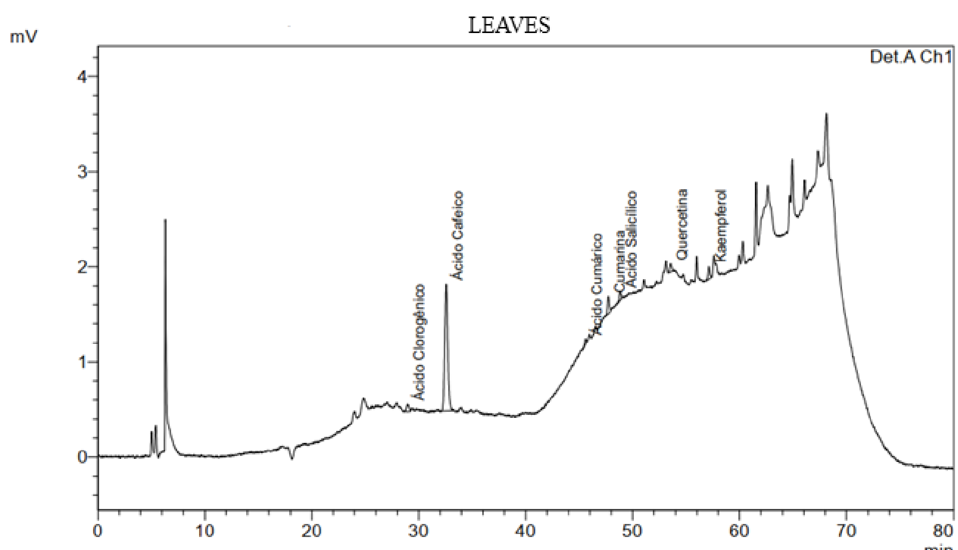
(Tab. 2). However, still regarding the treatment with roots, *P. oleracea* presented CL_{50} greater than that of the other recipient species for germination and shoot length (CL_{50} = 0.442 and 2.329, respectively), while it was less tolerant for the seedling root with CL_{50} = 0.148 g (Tab. 2). Corroborating the results observed for the *P. oleracea* species, the seedling root is generally more sensitive to allelochemicals when compared to other structures of the seedling due to the direct and prolonged contact of the roots with the extract, or from different physiological responses between structures (De Conti & Franco, 2011).

Identification of the compounds present in the agar by High Performance Liquid Chromatography (HPLC) of *A. tenella* leaf and root extracts revealed the presence of eight polyphenols (Fig. 4 and 5). More significantly, caffeic acid, salicylic acid, coumarin and quercetin were

Table 2. Lethal concentration 50 % based on the A and B parameters of the exponential decay model for germination (G), shoot length (SL) and primary root length (PRL) of seeds and seedlings of recipient species (*L. sativa, D. insularis, E. fosbergii* and *P. oleracea*) subjected to *A. tenella* leaves and roots.

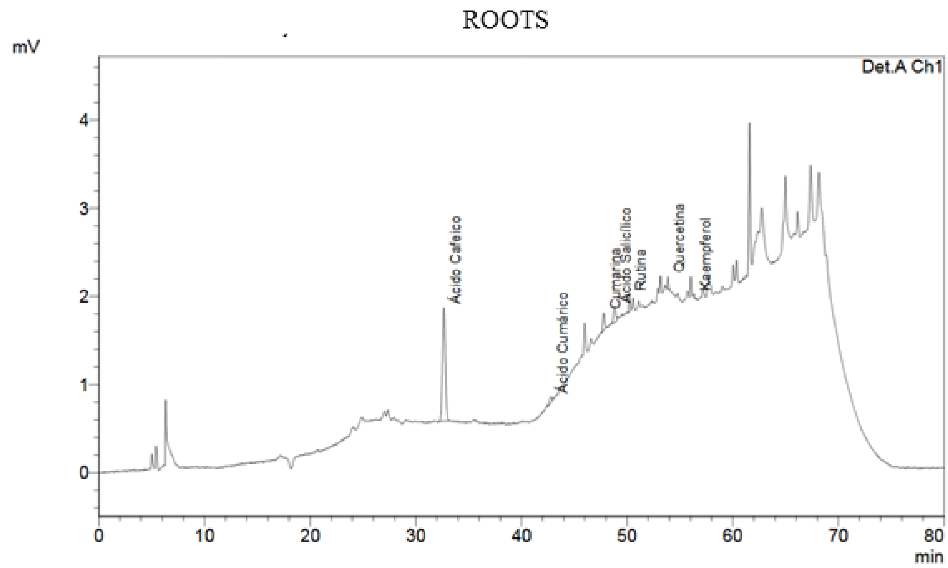
Donor specie (*A. tenella*)	*L. sativa*	*D. insularis*	*E. fosbergii*	*P. oleracea*
Leaves				
G	0.084	0.008	0.012	0.020
SL	2.283	0.317	0.158	599.51*
PRL	0.283	0.009	0.002	0.170
Roots				
G	0.018	0.007	0.008	0.442
SL	0.732	0.156	0.820	2.329
PRL	1.603*	0.028	0.289	0.148

*CL_{50} by linear regression parameters (f = y0+a*x).



POLYPHENOLS	CONCENTRATION (g L^{-})
Chlorogenic acid	0.412
Caffeic acid	2.699
Cumarico acid	0.234
Coumarina	0.499
Salicylic acid	0.619
Quercetin	0.447

Figure 4. Chromatogram and quantification of polyphenols present in the nutrient medium used in treatments with leaves of *A. tenella*



POLYPHENOLS	CONCENTRATION (g L^{-})
Caffeic acid	2.555
Coumarina	0.561
Salicylic acid	1.027
Rutin	1.345
Quercetin	0.685
Kaempferol	0.641

Figure 5. Chromatogram and quantification of polyphenols present in the nutrient medium used in treatments with roots of *A. tenella*.

detected in the leaf and root extracts of the donor plant. Chlorogenic acid and cumaric acid were only detected in the leaves, while rutin and kaempferol only in the roots; however, the extracts used in the bioassays in this study for preliminary diagnosis of allelopathy are mixtures of various substances, which can have additive or synergistic effects, making it important to analyze the action of each substance in isolation. Mehmood *et al.* (2017) also identified quercetin, chlorogenic acid, p-coumaric acid, cinnamic acid, caffeic acid, sirinic acid, synapic acid and vanillic acid in *Alternanthera philoxeroides* and *A. sessilis*, therefore corroborating the present study.

Similar to these results, Santos *et al.* (2011) observed an increase in germination inhibition of all seeds (pasture, malice) when using solutions with mixtures of phenolic acids, flavonoids and terpenoids when compared to bioassays in which solutions containing only one of the compounds identified in the extracts were used.

The compounds identified in the *A. tenella* extracts are well-known allele chemicals which have been identified in other studies (Franco *et al.* 2016, Pereira *et al.* 2018, Ferreira *et al.* 2020) with plants considered allelopathic, because they correspond to the class of secondary metabolites in which most of the compounds to have allelopathic activity are found (Rice 1984), affecting the elasticity of the cell wall, in addition to blocking mitochondrial breathing (Oliveira *et al.* 2014).

The extracts prepared from different organs of the same plant with allelopathic potential were able to suppress the germination or growth of test plants (Linhares Neto *et al.* 2014, Habermann *et al.* 2015). *A. tenella* leaves and roots showed allelopathic effects on the weed species investigated. In this sense, both organs of the donor species are a promising option for further studies of isolation and identification of natural phytotoxins with potential herbicide.

The phytotoxic effect of the extracts observed in the bioassays was probably due to the allelochemical caffeic acid (Franco *et al.* 2016, Pereira *et al.* 2018). According to Lúcio *et al.* (2013), this allelochemical showed a direct association of cause and effect (with the negative value closer to zero – Tab. 3), being considered the main determining effect in the allelopathy of the donor plant studied.

In using phenolic acids (chlorogenic, p-anisic, ferulic and caffeic) and flavonoids (genistein, quercetin, naringenin, kaempferol and rutin) extracted from *Calopogonium mucunoides* (Fabaceae), Santos *et al.* (2011) observed inhibition values which varied from 5.1 % for forest pasture seeds to 53.4 % for malice seeds submitted to the solution of these compounds, therefore confirming the allelopathic potential of these compounds already described in the literature and present in the leaves and roots of the donor plant in this study.

Table 3. Estimates of Pearson's correlations (*Significant at 5 % probability by the Student's t-test) between the secondary metabolites (independent variables) of *A. tenella* leaves and roots and the dependent variables (germination percentage, shoot length and primary root length).

Independent variables	Dependent variables
Chlorogenic Acid	-0.7065
Caffeic acid	-0.7944*
Cumaric Acid	-0.7070
Coumarina	0.7491*
Salicylic acid	0.8967
Quercetin	0.4698
Rutin	0.9999
Kaempferol	0.9317

In similar studies, Chuah *et al.* (2013) demonstrated that seed germination, water absorption, electrolyte retention capacity and O_2 consumption were significantly reduced in durum wheat (*Triticum turgidum* ssp. Durum Desf.) after coumarin treatment. Franco *et al.* (2016) identified the presence of flavonoids (kaempferol and quercetin) in *Copaifera langsdorffii* (Fabaceae) extract, and there was a reduction in *Sorghum bicolor* seed germination and seedling growth when submitted to the extract containing these compounds. Pereira *et al.* (2018) also identified caffeic acid, chlorogenic acid, ferulic acid, naringin, rutin and kaempferol compounds in *C. ensiformis* leaves, interfering with germination and initial weed growth. These studies corroborate the results of the present work, since *A. tenella* leaves and roots presented these polyphenols in their composition and interfered, in a certain way, in the germination and initial development of the recipient plants.

According to Nuria *et al.* (2014), studies such as this on allelopathic substances and identifying plants which have active principles capable of causing some effect is a matter of great importance, both in the use of extracts capable of inhibiting weeds in an attempt to decrease the use of commercial herbicides. Furthermore, in determining cultural practices and more adequate managements which avoid the interference of these substances in the growth of others. Thus, *A. tenella* presented itself as a promising source for future studies in the bioherbicide line due to its chemical composition and remarkable presence in the environments in which it is established.

CONCLUSION

The application of the sandwich method allowed to efficiently determine the allelopathic activity of *A. tenella* leaves and roots in the germination and primary root length of the receiving plants; however, the same behavior did not occur for the shoot length, regardless of the part of the tested plant.*E. fosbergii* and *D. insularis* were the specie receptors which were most sensitized with allelochemicals in the highest concentrations, regardless of the parameters evaluated and the part of the plant tested, which demonstrated the specificity of the plant studied.

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