

Phytochemical screen of extracts *brachiaria brizantha* and *megathyrsus maximus* and their effects on germination and development of *parapiptadenia rigida* (benth.) Brenan

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Resumo

Alelopatia é reconhecida como um importante mecanismo ecológico, que influencia a dominância e a sucessão das plantas por meio da ação de substâncias químicas liberadas pelas plantas no ambiente. Este estudo teve como objetivo identificar grupos de aleloquímicos e a existência do potencial alelopático de *Brachiaria brizantha* e *Megathyrsus maximus* em sementes e mudas de *Parapiptadenia rigida*. Extratos hexânicos, acetato de etila e metanólicos foram preparados para a triagem fitoquímica e para bioensaios de germinação. Extratos aquosos nas concentrações de 10 %, 5 % e 2.5 % foram preparados e aplicados mensalmente em plantas de *P. rigida*. Embora grupos aleloquímicos tenham sido identificados nos extratos, eles não interferiram na germinação das sementes de *P. rigida*. Os extratos aquosos também não apresentaram potencial alelopático no desenvolvimento de *P. rigida*.

Palavras-chave: alelopatia; angico vermelho; gramíneas.

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Abstract

Allelopathy is recognized as an important ecological mechanism that influences plant dominance and succession through the action of chemicals released by plants into the environment. This study aimed to identify allelochemical groups and the existence of the allelopathic potential of *Brachiaria brizantha* and *Megathyrsus maximus* in *Parapiptadenia rigida* seeds and seedlings. Hexane, ethyl acetate and methanolic extracts were prepared for phytochemical screening and germination bioassays. Aqueous extracts at concentrations of 10 %, 5 % and 2.5 % were prepared and applied monthly to *P. rigida* plants. Although allelochemical groups were identified in the

extracts, they did not interfere with *P. rigida* seed germination. The aqueous extracts also did not show allelopathic potential in the development of *P. rigida*.

Keywords: allelopathy; red angico; grasses.

ALLELOPATHY OF INVASIVE GRASSES OVER A BRAZILIAN NATIVE TREE

1 INTRODUCTION

Allelopathy is a process of direct or indirect beneficial or harmful effects that one plant exerts on another through the production of chemical compounds that are released into the environment (RICE, 2013). These compounds are known as allelochemicals or secondary metabolites and, after its release, may influence the development of other plants. All plants produce allelochemicals throughout their life cycle, but the amount and type of metabolite produced varies according to environmental conditions, internal and external factors, plant age, species and organ where it is synthesized (GUSMAN et al., 2012). Secondary metabolites are usually classified according to their

biosynthesis route, and these are divided into three main chemically distinct groups: terpenes, phenolic compounds and nitrogenous compounds (TAIZ and ZEIGER, 2017). The action of secondary metabolites, known as allelopathic action, is an important ecological mechanism that influences plant dynamics in the process of natural regeneration, interfering with community formation (GATTI et al., 2004).

This natural regeneration is part of the forest growth cycle and refers to the early stages of its establishment and development. The use of native species in the recovery of degraded sites contributes to the conservation of regional biodiversity (MMA, 2012). Among these native species, a tree commonly found in southern Brazil is red angico. *Parapiptadenia rigida* (Benth.) Brenan, belonging to the Fabaceae family, naturally occurring in the semi-deciduous broadleaf forest. It is recommended for the recovery of degraded areas and for forest restoration, as it is an aggressive pioneer species, spreading very well naturally, with great capacity for development and adaptation (LORENZI, 2009).

However, in naturally regenerating sites, both invasive and opportunistic species

as well as native species may develop. Invasive species are plants that have passed through a biogeographic barrier with the help of humans, being classified as casual, naturalized and invasive species (KLEUNEN et al., 2018). Several African grasses brought to Brazil and used as fodder have spread over large areas and alter the evolution of native species by competition or allelopathy, becoming one of the main threats to biodiversity conservation in protected areas (MARTINS et al., 2006). Invasion by African grasses is considered one of the most serious threats to the country's biodiversity (DURIGAN et al., 2007). These invasive species, through allelopathic action, can drastically interfere with the emergence of native species during the natural regeneration process, as they can inhibit the germination of seeds present in the soil (NOVAIS et al., 2013) and after their naturalization, they begin to disperse and cause changes in the functioning of the biome, not allowing its natural recovery (ROCHA et al., 2017).

Brachiaria brizantha (Hochst eg A. Rich) Stapf and *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs (colonist grass), are species of forage grasses widely used in Brazil. Both belong to the Poaceae family and were accidentally introduced to Brazil from slave ships, serving as beds for slaves (NAGANO et al., 2011). They are aggressive and resistant species, dispersing easily, significantly interfering with infested crops. Without control, they can replace native species and, with the soil covered, it is very difficult to grow new trees (OLIVEIRA JUNIOR et al., 2011).

Aiming at the importance of the development of native species during natural regeneration and the possible allelopathic intervention of invasive

species, this work aimed to perform the phytochemical screen of *B. brizantha* and *M. maximus* grass extracts in order to identify the respective groups of allelochemicals present and, consequently, the possibility of allelochemical suppression through germination tests. As well as to evaluate the possible interference of these extracts in the development of *P. rigida* plants through the allelopathic activity of these invasive grasses.

2 MATERIAL AND METHODS

The experiment was carried out in three steps divided into: 1) Phytochemical screen; 2) germination bioassay; 3) Plants development analysis. The invasive species *B. brizantha* and *M. maximus* were collected at a vegetative stage around the city of Cascavel (24° 57' 21" S; 52° 27' 19" W) and transported to the laboratory. The seeds and seedlings of the native species *P. rigida* were donated by the Paraná Environmental Institute – IAP of Cascavel - PR.

2.1 Phytochemical screen

To identify the presence of allelochemical groups in the different extracts of both invasive species, the phytochemical screen was performed. To perform these tests, the aerial part of the collected grasses was dried in an air circulation oven at 40 °C until its dry weight remained stable. Afterwards, the dry matter was ground in a Willey knife mill with 10 mm granulation sieve. This plant material (powder) of *B. brizantha* and *M. maximus* was subjected to exhaustive and successive cold extraction with solvents of increasing polarity: hexane, ethyl acetate and methanol.

The extraction was performed for a period of seven days (PARACAMPO, 2011). Each of the obtained solutions was filtered and distilled under reduced pressure using the TE-2II rotary evaporator to remove all solvents and obtain the respective crude extracts from each invasive species.

Phytochemical screening is a rapid assessment, where, by staining tests, simple reactions or precipitation reveal the presence or absence of secondary metabolites in an extract. The applied methodology was carried out according to the protocol proposed by Paracampo (2011) and conducted for each of the three hexanic, ethyl acetate and methanolic extracts of *B. brizantha* and *M. maximus*.

2.2 Germination bioassays

Methanol extracts from both invasive species did not have a good yield and were not sufficient. In this way the germination bioassays were performed only with the extracts ethyl acetate and hexanic.

In autoclaved and lined petri dishes with three sheets of filter paper, 3 mL of the extract was placed at 1% concentration; the filter paper was moistened with 3 mL of distilled water. On each plate were ten *P. rigida* seeds, disinfected with sodium hypochlorite (2 %). This procedure was performed for each extract of both invasive species in 4 repetitions. The plates were placed in germination chambers under controlled conditions of 25 °C constant temperature and 12 h photoperiod of light (MONDO, 2008). Germination was monitored for a period of seven days, and germinated seeds were those that had a primary root extension of 2 mm or more (HADAS, 1976).

2.3 Plant development analysis

The third part of the work was carried out in a greenhouse. For this test was used aqueous extracts of fresh leaves of the invasive species, which was prepared on the day of each application. Thus, the collected leaves were weighed, minced and crushed in a blender with distilled water, with 200 g of plant material per 1 L of water. The milled and processed product was filtered through fine mesh, considering the 10 % matrix extract and from it the following dilutions were made: 0, 2.5 and 5 % (w / v).

The experiment was conducted with seven treatments: Treatment 1 (T1) or control where distilled water (0 %) was used; Treatment 2 (T2) 2.5 % *B. brizantha* extract; Treatment 3 (T3) 2.5 % *M. maximus* extract; Treatment 4 (T4) 5 % *B. brizantha* extract; Treatment 5 (T5) 5 % *M. maximus* extract; Treatment 6 (T6) 10 % *B. brizantha* extract; Treatment 7 (T7) 10 % *M. maximus* extract. Each extract had its pH measured and after six months an average of these values was calculated.

For the evaluation of the development of *P. rigida* seedlings under the effect of invasive plant extracts, we used plants with an average age of five months, which were transplanted to plastic pots (5 L) containing 4 kg of soil each and one plant. The plants were placed in a greenhouse, with irrigation (250 mL per pot) every two days, conditions of natural light (environment) and average temperature of 25 °C.

The soil used was Oxisols (Latossolo vermelho distroférico), analyzed in the laboratory and corrected with dolomitic limestone, 300 grams per pot and NPK (10-10-10) 100 grams per pot (SILVA et al., 2008). The pots with the seedlings were arranged in the greenhouse in four blocks. For each treatment (dilution)

four replicates were made with 10 plants each. These plants were evaluated for their development under the effect of the treatments, through the analysis of the parameters plant height: being measured with ruler / tape measure from the base of soil to the apical bud, stem diameter with the aid of a caliper and the counting of number of leaves per plant the evaluations were repeated for six months.

To simulate the release of allelochemicals in the soil by the invasive plants, the extracts were replenished monthly after each evaluation, following the pot field capacity calculation. Each pot was weighed, being kept contain 4 kg of soil plus 400 mL of water or extract in the proportions: Treatment 1: 400 mL of distilled water per pot; Treatments 2 and 3 (2.5 %): placed 390 mL water + 10 mL extract; Treatments 4 and 5 (5 %): placed 380 mL water + 20 mL extract per pot; Treatments 6 and 7 (10 %): 400 ml extract placed per pot.

2.4 Statistical analysis

For the germination laboratory bioassays was used the completely randomized

design (DIC), with four replications. The following parameters were analyzed: percentage germination (PG) and seed germination speed index (GSI) of *P. rigida*.

For the greenhouse development experiment it was used a completely randomized block design (DBC) in a 2 x 3 bifactorial scheme (two species X three dilutions) plus control addition, with split plots and four replications.

Data were analyzed for normality. The values expressed in percentage were transformed by arc sine $x / 100$ (BRAZIL, 2009). The results were submitted to analysis of variance (ANOVA) and the means of the treatments were compared by Tukey test, at 5 % probability, by the program GENES (CRUZ, 2016).

3 RESULTS AND DISCUSSION

The results obtained for *B. brizantha* extracts indicated the presence of allelochemicals of different classes, as shown in table 1. In the ethyl acetate extract were identified alkaloids, steroids and triterpenoids and for the

Table 1: Phytochemical screen of the hexanic, ethyl acetate and methanolic extracts of *Brachiaria brizantha* and *Megathyrsus maximus*. (A: absent and P: present)

ALLELOCHEMICAL CLASSES	PHYTOCHEMICAL SCREEN					
	HEXAN		ACETATE		METHANOL	
	B. brizantha	M. maximus	B. brizantha	M. maximus	B. brizantha	M. maximus
ALKALOIDS	A	P	P	A	A	P
CATECHINS	A	A	A	A	A	A
STEROIDS AND TRITERPENOIDS	A	A	P	P	P	P
FLAVONOIDS	A	A	A	A	P	P
FOAMED SAPONIN	A	A	A	A	P	P
TANNINS	A	P	A	A	P	P

Table 2: Variance analysis of a randomized block experiment for the variables: Germination percentage and germination speed index.

Causes of Variation	Middle Squares		
	GL	Germination Percentage	Germination Speed Index
Treatment	04	0,002 ^{ns}	69,50*
Residue	15	0,002	0,18
Overall Average		1,56	5,57
CV (%)		2,86	7,63

* Significant at 5% probability of error level. ns not significant at 5% probability of error level.

methanolic extract were identified steroids, triterpenoids, flavonoids, foam saponin and tannins. However, in the hexane extract no allelochemical groups were identified. In other studies, analyzes using *Brachiaria brizantha* cv. Marandu determined the presence of steroids and triterpenoids, alkaloids, tannins and foamed saponin (BARROS et al., 2005). In extracts of *B. humidicola* have been identified alkaloids, coumarins, foam saponin, tannins, saponin and flavonoids extracts and in *B. decumbens* extracts were found esters, triterpenes and aldehydes and saponin (BARBOSA et al., 2009).

Studies that corroborate the results obtained for *M. maximus* are scarce. After phytochemical analysis, the hexane extract showed alkaloids and tannins, the acetate extract identified steroids and triterpenoids and the methanolic extract identified alkaloids, steroids and triterpenoids and flavonoids (Table 1). Similar data were presented with the work done with *Megathyrsus* cv. Tobiata (SOUZA FILHO and ALVES, 2002).

The germination percentage (GP) of *P. rigida* seeds did not differ statistically at the significance level, as observed in figure 2. Under laboratory conditions the seeds of

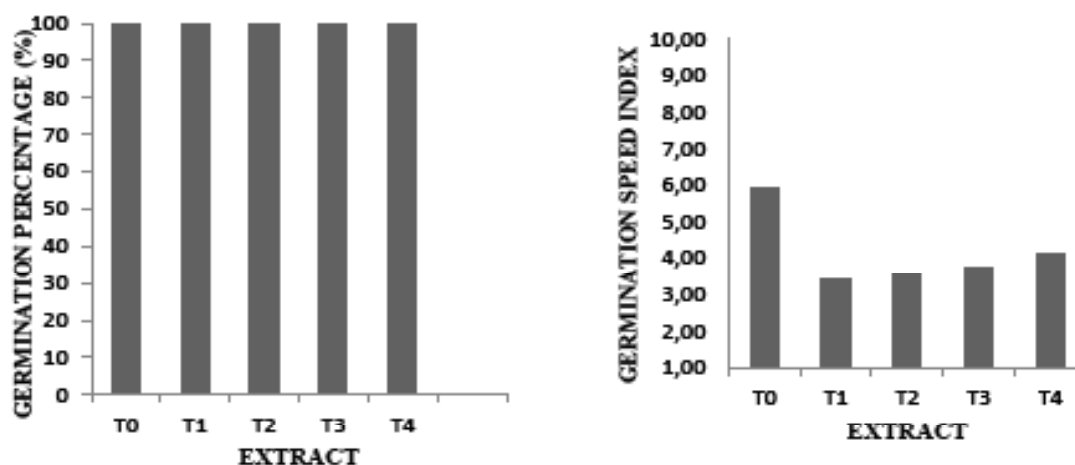


Figure 1: Germination Percentage (PG) and Germination Speed Index (IVG) of *P. rigida* seeds submitted to *B. brizantha* and *M. maximus* ethyl acetate and methanol extracts. T0: control; T1: *M. maximus* ethyl acetate extract; T2: *M. maximus* methanolic extract; T3: *B. brizantha* ethyl acetate extract; T4: *B. brizantha* methanolic extract.

the native species had 100 % germination when soaked with the different extracts. However, the germination speed index (GSI) was higher in the control seeds than in the seeds where it was applied dilutions of both invasive species. Seeds germinate faster in the absence of extracts (Figure 1). Both aqueous extracts applied to *P. rigida*

seedlings showed low variation between their pH values, remaining in a neutral range, which is considered ideal for the development of native species. *B. brizantha* extracts remained in the range of 6.92 - 5.95, and for *M. maximum* extracts the pH remained in the range of 7.05 - 6.92, both shown in figure 2. The mean values of the

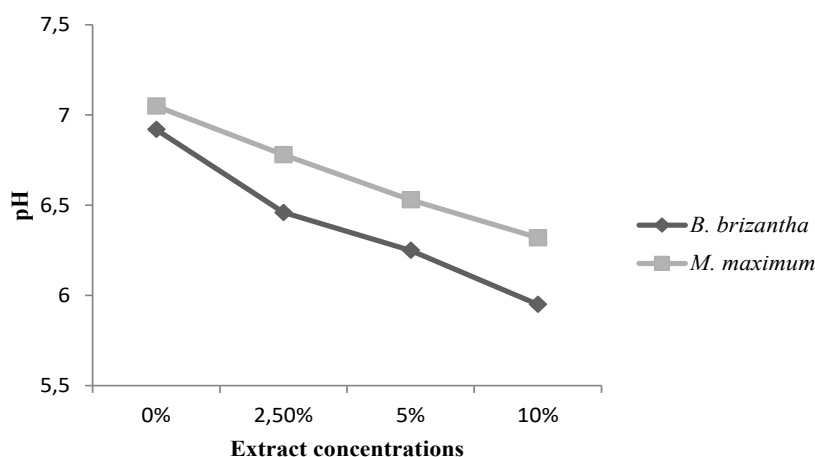


Figure 2: Average pH levels of *B. brizantha* and *M. maximum* aqueous extracts

six months of pH decreased with increasing dilutions of the extracts.

Even though the results of the phytochemical screen indicated the presence of allelochemicals in the extracts of the invasive species, germination bioassays did not show their allelopathic interference in *P. rigida* seeds. Apparently, the allelopathic substances present in the grasses were at levels lower than those required to promoting inhibition of seed germination of this specie, or *P. rigida* may have shown mechanical or physiological

resistance to these substances.

Corroborating these results, Souza Filho et al. (2005) isolated chemical groups from *B. humidicola* aerial parts, however, the analysis of the inhibitory allelopathic activity of germination of these substances revealed low activity, not exceeding 0.5 %. Little is known about the production of substances with allelopathic potential in invasive plants used as fodder, especially about the likely variations in the intensity of allelochemical production during the plant cycle, however, Wardle (1987)

Table 3: Means of the variables plant height, stem diameter and number of leaves of *P. rigida* plants submitted to *B. brizantha* and *M. maximum* extracts.

VARIABLE	SQUARE MEAN	<i>B. brizantha</i>	<i>M. maximum</i>
HEIGHT	0.00001	0.02199 a	0.02211 a
DIAMETER	0.00051	0.64938 a	0.63188 a
N° of LEAVES	1.10	23.02375 a	0. 22.3025 a

Means followed by the same lowercase letters in horizontal do not differ statistically from each other by the Tukey test at 5% significance.

mentions that pasture plants are probably allelopathic only at certain stages of their life cycle.

After the analysis of the growth parameters, no statistically significant difference was observed, as provided for in table 3, in any of the percentages of the different extracts of both weeds. *P. rigida* plants developed over 6 months increasing their height, stem thickness and leaf number (Figure 3). When *P. rigida* was used as target species, no interaction was identified significant allelopathic. Secondary metabolites produced by plants are not always active. Sometimes these compounds need to be activated as they

are stored as nontoxic products in the cells themselves or specifically in their cell vacuoles and subsequently take the active form to be used when needed (stress, herbivory or pathogens, UV, temperature, weather, etc.) (SANTOS et al., 2016).

On the other hand, in bioassays using other target plants, was observed existence of allelopathic action of *B. brizantha* and *M. maximus* in bioindicator species such as lettuce, carrot and eucalyptus species (BOCCHESE et al., 2007). When analyzing the germination percentage of *Centrosema pubescens*, *Stylosanthes guianensis*, *Calopogonium mucunoides* and *Macrotyloma axillare* seeds irrigated with

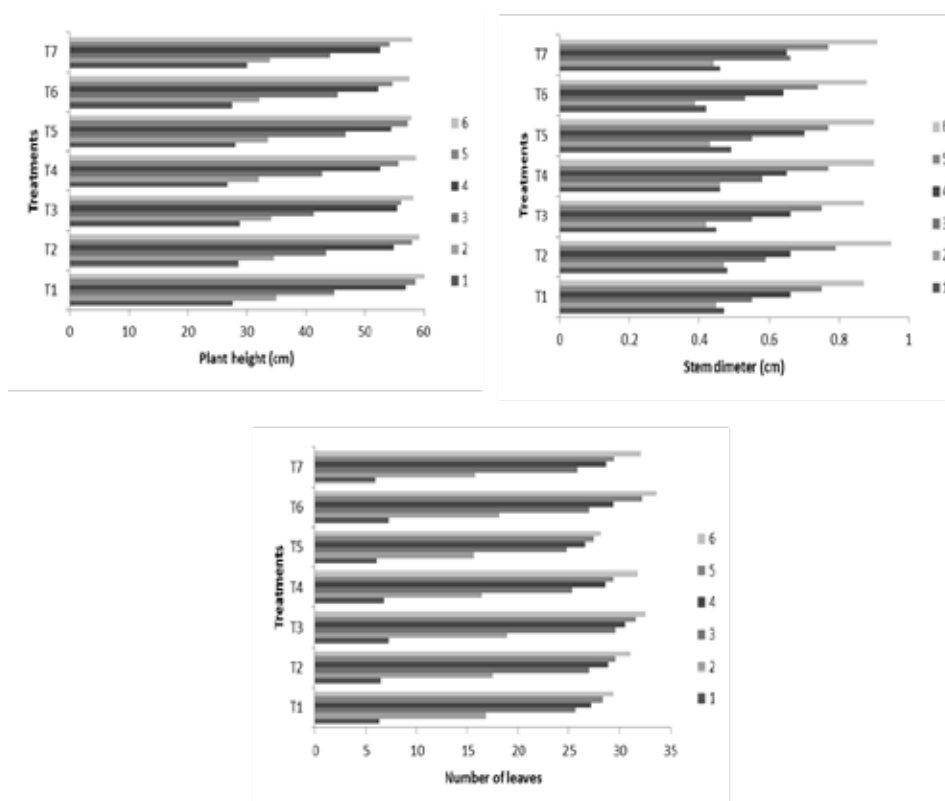


Figure 3: Monthly development of height, stem diameter and leaf number (1-6) of *P. rigida* seedlings submitted to different concentrations of aqueous extracts of *B. Brizantha* and *M. maximus*: Treatment 1 (T1) or control where distilled water (0%) was used; Treatment 2 (T2) 2.5% *B. brizantha* extract; Treatment 3 (T3) 2.5% *M. maximus* extract; Treatment 4 (T4) 5% *B. brizantha* extract; Treatment 5 (T5) 5% *M. maximus* extract; Treatment 6 (T6) 10% *B. brizantha* extract; Treatment 7 (T7) 10% *M. maximus* extract.

aqueous extracts of *Brachiaria decumbens*, *B. brizantha* cv. Marandu and *B. humidicola*, Almeida and collaborators (1997) found a difference in this percentage. Evaluating the effect of aqueous extracts of three cultivars of *M. maximus*, found that there was a difference in germination percentage when irrigated *Leucaena leucocephala* and *Cajanus cajan* seeds with extracts at 10 % and 20 % (ALMEIDA et al., 2000).

Lactuca sativa seed is considered a bioindicator of phytotoxicity. When extracts of *M. maximus* (1 L water / 200 g leaves) were tested, the root length was changed with a concentration of 20 %, being halved. At 100 % fresh extract concentration, germination was completely inhibited. However, in the seeds of native species studied *Peltophorum dubium* (Spreng.) Taub and *Parapiptadenia rigida* (Benth.) Brenam, the only effect observed was stimulatory (ROSA et al., 2011). In another germination bioassay whose extracts were obtained from leaves and roots by immersing 2, 10, 25 and 40 g (dry weight) of leaf or root materials in 100 mL of distilled water, *M. maximus* leaf extract at 40 % caused complete inhibition of *L. sativa* germination.

In situ studies on the presence and effect of allelochemicals in forest soil are very problematic due to low concentrations, low persistence and the possibility of chemical changes due to soil microorganisms. The influence of soil on the action of allelochemicals, in turn, is little discussed in the literature, especially in native tree species. The effect of the extract depends on the sensitivity of the target plant to the allelochemical, and in some species an allelopathic substance present may be a germination or growth inhibitor, and in another it may be harmless or stimulating or not yet present no effect on the target

plant in question (ALMEIDA, 1988). The effects of allelochemicals may depend on climatic conditions and the type of soil in which they are found, and may turn into other compounds and some metabolites only act in presence of others, acting in synergism, as they do not reach the minimum concentration required to exercise the allelopathic effect (ALMEIDA, 1988).

Field conditions may present different results than those observed in the laboratory or greenhouse, as the allelopathic activity of a plant does not depend solely on the presence of chemical compounds in its extract, this action depends on numerous environmental, physiological, morphological factors, besides the action of other organisms, which act triggering its allelopathic potentiality on other species (BRAZIL, 2009).

4 CONCLUSION

The phytochemical screen of the hexanic, ethyl acetate and methanolic extracts of *M. maximus* and *B. brizantha* resulted in the identification of different groups' allelochemicals. *P. rigida* seeds germinate without visible interference of extracts, just as their plants developed even subjected to the extracts mentioned above. Under the conditions in which this experiment was carried out, no allelopathic action of *B. brizantha* and *M. maximus* extracts was observed on germination and development of *P. rigida*.

REFERENCES

- ALMEIDA, F. S. **Allelopathy and plants**. Londrina: IAPAR. p. 60, 1998.

- ALMEIDA, A. R. P.; LUCCHESI, A. A.; ABBADO, M. R. Allelopathic effect of *Brachiaria grisea* species about some tropical forage legumes. II. Greenhouse Reviews. **Revista Boletim da Indústria Animal**. v. 54, n. 2, p. 45-54, 1997.
- ALMEIDA, A. R. P.; RODRIGUES, T. de J. D.; SANTOS, J. M. Allelopathy of *Panicum maximum* JACQ. cultivars on shrub and tree forage legumes. I. Laboratory Evaluation. **Revista Boletim da Indústria Animal**. v.57, n.2, p.113-127, 2000.
- BOCCHESE, R. A.; MELOTTO, A. M.; CÉSAR FILHO, L. C. C.; FERNANDES V. M.; FRANCESCHI, M. L.; LAURAS, A. V. Evaluation of competition between *Brachiaria brizantha* cv Marandu, native tree species from Cerrado and *Eucalyptus citriodora*. **Revista Brasileira de Biociências**. v.5, n. 2, p.153-155, 2007.
- BARBOSA-FERREIRA, M.; BRUM, K. B. B.; FERNANDES, C. E; PINTO, G. S.; MARTINS, C. F.; CASTRO, V. S.; REZENDE, K.G.; RIET-CORREA, F.; HARAGUCHI, M.; WYSOCKI, H. L. J.; LEMOS, R. A. A. **Variations of saponin level x maturation in *Brachiaria brizantha* leaves**. In: 8th International Symposium on Poisonous Plants, 2009, João Pessoa, Paraíba. Program and Abstracts. Campina Grande, UFPB, 2009, p.13.
- BARROS, A. P. Q.; SOUZA FILHO, A. P.; MORAIS, R. C. **Production pattern of allelopathic chemical compounds in two *Brachiarias***. In: SEMINÁRIO DE INICIAÇÃO CIENTÍFICA DA UFRA, 2005, Belém, PA. Ciência e tecnologia com inclusão social: anais. Belém, PA: UFRA: Embrapa Amazônia Oriental, 2005. 1 CD-ROM.
- BRAZIL. **Rules for seed analysis**. Ministry of Agriculture and Agrarian Reform. SNDA / DNDV / CLAV, Brasília, 2009.
- CRUZ, C. D. Genes Software – extended and integrated with the R, Matlab and Selegen. **Acta Scientiarum**. v.38, n.4, p.547-552, 2016.
- DURIGAN, G.; SIQUEIRA, M. F.; FRANCO, G. A. D. C. Threats to the Cerrado remnants of the state of São Paulo, Brazil. **Scientia Agricola**. v. 64, n.4, p. 355-363, 2007.
- GATTI, A. B.; PEREZ, S. C. J. G. A.; LIMA, M. I. S. Allelopathic activity of aqueous extracts of *Aristolochia esperanzae* O. Kuntze on the germination and growth of *Lactucasativa* L. and *Raphanus sativus* L. **Acta Botânica Brasílica**. v.18, n. 3, p. 459-472, 2004.
- GUSMAN, G. S; VIEIRA, L. R; VESTENA, S. Allelopathy of plant species with pharmaceutical importance for cultivated species. **Revista Biotemas**. v. 4, n. 25, p. 37-48, 2012.
- HADAS, A. Water uptake and germination of leguminous seeds under changing external water potential in osmotic solutions. **Journal of Experimental Botany**. v. 27, n. 98, p. 480-489, 1976.
- KLEUNEN, M.; BOSSDORF, O.; DAWSON, W. The Ecology and Evolution of Alien Plants. **Annual Review of Ecology, Evolution, and Systematics**. v.49, p.25-47, 2018.
- LORENZI, H. **Brazilian trees: manual for**

- identification and cultivation of native Brazilian tree plants.** New Odessa: Plantarum Institute, 2009.
- MARTINS, D; MARTINS, C. C; COSTA, N. V. Allelopathic potential of *Brachiaria brizantha* cultivated soil solutions: effects on forage grass germination and pasture weeds. **Weed Plant.** v. 24, n. 1, p. 61-70, 2006.
- Ministry of the Environment Plants for the future. Available at: <http://www.mma.gov.br/biodiversidade/conservacao-e-promocao-do-uso-da-genetica-plantas/plantas-para-o-futuro>. Visited in August 2014.
- MONDO, V. H. V; BRANCALION, P. H. S; CICERO, S. M; NOVENBRE, A. D. L; DOURADO NETO, D. *Parapiptadeni rigida* (BRENTH.) BRENAN (FABACEAE) seed germination test. **Revista brasileira de sementes.** v. 30, n. 2, p. 77-183, 2008.
- NAGANO, N. R.; ZANET, C.; TIRITAN, C. S.; SANTOS, D. H. Effect of nitrogen fertilization and cutting height on Tanzania grass. **Scientia Agraria Paranaensis.** v.10, n.1, p.100-112, 2011.
- NOVAES, P; IMATOMI, M.; MIRANDA, M. A. F. M.; GUALTIER, S. C. J. Phytotoxicity of leaf aqueous extract of *Rapanea umbellata* (Mart.) Mez (Primulaceae) on weeds. **Acta Scientiarum Agronomy.** v. 35, n. 02, p. 231-239, 2013.
- OLIVEIRA JUNIOR, R. S; CONSTANTIN, J; INOUE, M. H. **Weed biology and management.** Curitiba: Omnipax, 2011.
- PARACAMPO, N. E. N. P. **Phytochemical prospecting of medicinal plants.** Bethlehem: Embrapa Eastern Amazon, 2011.
- RICE, E. L. **Allelopathy.** New York: Academic Press, 2013.
- ROCHA, L. F; LIMA, G. S; MARTINS, S. V; TORRES, F. T. P; REIS, C. R. Evaluation of the presence of exotic species in state protected areas of Minas Gerais, Brazil. **Journal of Agro-Environmental Sciences.** v.15, n.2, p. 240-248, 2017.
- ROSA, D. M.; FORTES, A. M. T.; MAULI, M. M.; MARQUES, D. S.; PALMA, D. *Panicum maximum* JACQ Allelopathic Potential on Seed Germination of Native Species. **Floresta e Ambiente.** v.18, n.2, p.198-203, 2011.
- SANTOS, V. H. M.; MECINA, G. F.; FERREIRA, P. C.; DOKKEDAL, A. L. SALDANHA, L. L.; PIGATTO, G.; SILVA, L. P.; SILVA, R. M. G. Allelochemicals of *Neea theifera* Oerst. (Nyctaginaceae) with phytotoxic potential on plant germination and growth. **International Journal of Environmental & Agriculture Research.** v.2, p.124-131, 2016.
- SILVA, J. de C.; CASTRO, V. R. de.; XAVIER, B. A. **Forest Farmer's Primer.** Viçosa, Minas Gerais, 2008.
- SOUZA FILHO, A. P. S; ALVES, S. M. **Allelopathy: basic principles and general aspects.** Embrapa, Belém-Pa, 2002.
- SOUZA FILHO, A. P. S; PEREIRA, A. A. G.; BAYMA, J. C. Allelochemical produced by the forage grass *Brachiaria humidicola*. **Weed.** n. 23, v.1, p. 25-3, 2005.