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Allelopathic effect of *Azadirachta indica* fresh leaves on the germination of native plants in the Seasonally Dry Tropical Forest

Efeito alelopático das folhas frescas de *Azadirachta indica* na germinação de plantas nativas de floresta tropical sazonalmente seca

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Allelopathic potential, which is characterized by harmful or beneficial effects on a species, is a hypothesis that could explain the success of a vegetable species in process of invasion. For that reason, this study aims to investigate the allelopathic effect of the fresh leaf extract from *Azadirachta indica* on the germination and early growth of native species (*Mimosa caesalpiniifolia*, *Pityrocarpa moniliformis*, *Astronium urundeuva*, *Cenostigma pyramidale*, and *Libidibia ferrea*) in the seasonally dry tropical forest (caatinga). The raw extract was prepared with 200g of fresh leaves in 1L of distilled water, diluted in six treatments. The experiment followed a completely randomized design (CRD), with four repetitions of 25 seeds, and paper towel moistened with extract as substrate. The number of germinated seeds was counted daily. At the end of the experiment, radicle length (RL), germination percentage (GP), germination speed index (GSI), and average germination time (AGT) were evaluated. *P. stipulacea*'s GP and GSI averages and *A. urundeuva*'s AGT decreased as extract concentration increased. As far as RL is concerned, in intermediate concentrations the extract hindered *P. moniliformis* and *L. ferrea*'s growth but favored *C. pyramidale* and *A. urundeuva*'s. Results allow us to infer that *A. indica* has an allelopathic effect on native species and might interfere with their rooting in the field, which in turn would affect the succession and balance of the forest over time, since the community structure and the ecosystemic processes would be impacted.
Keywords: biological invasion, Caatinga, exotic species

O potencial alelopático, caracterizado pelos efeitos danosos ou benéficos sobre uma espécie, é uma hipótese para explicar o sucesso de uma espécie vegetal em processo de invasão. Deste modo, foi investigado o efeito alelopático do extrato de folhas frescas de *Azadirachta indica* sobre a germinação e o crescimento inicial de espécies nativas (*Mimosa caesalpiniifolia*, *Pityrocarpa moniliformis*, *Astronium urundeuva*, *Cenostigma pyramidale*, *Piptadenia stipulacea* e *Libidibia ferrea*) de Floresta Tropical Sazonalmente Seca (caatinga). O extrato bruto foi elaborado com 200g de folhas frescas em 1L de água destilada, diluído em seis tratamentos. O delineamento experimental foi inteiramente casualizado, com quatro repetições de 25 sementes, tendo como substrato papel-tolha umedecido com extrato. Contou-se diariamente a quantidade de sementes germinadas. Ao fim do experimento avaliou-se: comprimento da radícula (CR), porcentagem de germinação (PG), índice de velocidade de germinação (IVG) e tempo médio de germinação (TMG). Registrou-se uma redução das médias de PG e IVG, para *P. stipulacea*, e de TMG, para *A. urundeuva*, com o aumento da concentração de extrato. Nos valores de CR o extrato reduziu o crescimento de *P. moniliformis* e *L. ferrea* e favoreceu *A. urundeuva* e *C. pyramidale*, em concentrações intermediárias. Os resultados encontrados inferem que *A. indica* exerce efeito alelopático em espécies nativas, podendo interferir no estabelecimento dessas em campo, o que afetaria a sucessão e o equilíbrio da floresta ao longo do tempo, uma vez que a estrutura da comunidade e os processos ecossistêmicos seriam impactados.
Palavras-chave: invasão biológica, Caatinga, espécies exóticas

1. INTRODUCTION

Impacts caused by invasive exotic species are based on the disturbance or alteration of environmental variables involving mostly biodiversity loss and changes in the provision of some environmental services, especially those developed by native species [1, 2, 3, 4]. The presence of an invasive exotic species causes changes in structure and composition of vegetable communities, and thus impairs the functioning of ecosystems, representing a threat to natural environments [1, 2].

There are different hypotheses to explain the success of a plant species in an invasion process [5], such as propagule pressure [6], lack of enemies or predators [7, 8], evolution of increased competitive ability [9], and allelopathic advantage against native species [10, 11]. The latter hypothesis proposes that some exotic species develop or already have mechanisms for releasing unprecedented biochemicals – "unique weapons" – that are able to inhibit the germination and growth of neighboring native plants, which in turn impair essential processes for maintaining the natural community diversity [10, 12, 13, 14]. In this context, the exotic species *Azadirachta indica* A. Juss (popularly known as nim indiano), is considered invasive by several authors [15, 16, 17], but there is still a lot be studied in order to measure the potential and impacts of this species invasion in native plant communities [18].

Azadirachta indica is originated from India and was brought to Brazil in the 1980s for silvicultural studies on the use of its wood, leaves, and seeds [18]. Currently, it has been broadly used as an afforestation species in different areas of the country, particularly the Northeastern region [19, 20, 21, 22, 23], where there are favorable conditions for its establishment, growth, and reproduction [18, 24, 25].

Previous studies, such as Mondal et al. (2020) [26] and Xuan et al. (2004) [27], reported the allelopathic effect of *A. indica*. They identified the negative influence of this species through the decrease of germination, chlorophyll content, leaf humidity, and root length of *Oryza sativa* L. (rice); Rickli et al. (2011) [28] found similar results for the radicle growth of *Lactuca sativa* L. (lettuce) and *Phaseolus vulgaris* L. (common bean), in addition to germination speed and increase of average germination time of *L. sativa* and *Glycine max* (L.) Merr. (soybean); Hussain et al. (2019) [29] revealed decrease of morphological and physiological characteristics (dry weight, fresh weight, germination, plant total height, number of leaves, specific leaf area, root length, and chlorophyll content) of *Zea mays* L. (corn) individuals.

Similar studies investigated how the extract of exotic species affect native trees aiming to associate the allelopathic effect with the invasion potentiation. Araújo et al. (2017) [12] studied the effect of the exotic *Cryptostegia madagascariensis* Bojer ex Decne. (Madagascar rubber vine) on *Piptadenia stipulacea* (Benth.) Ducke, *Libidibia ferrea* (Mart. ex Tul.) LP Queiroz, and *Mimosa caesalpiniifolia* Benth.; Hartmann et al. (2017) [30] observed the effect of the exotic *Brachiaria brizantha* Stapf (palisade grass) on *Parapiptadenia rigida* (Benth.) Brenan; lastly, Costa and Freire (2018) [31] investigated the extract effect of the exotic *Prosopis juliflora* (Sw.) DC. (mesquite) on *Mimosa tenuiflora* (Willd.) Poir. Nevertheless, results show that the obtained effects depend on the species involved in the interaction, since contact with allelopathic compounds causes deleterious effects to seed emergence and to seedlings in the case of some species, increasing the invasive potential [12, 14]; but on other species there is not any negative allelopathic interference [13].

It is therefore expected that the aqueous extract of *A. indica* fresh leaves may have a significant influence on germination and early growth of native species in the seasonally dry tropical forest (caatinga). This influence shall affect germinability in a negative way, decreasing germination speed values, increasing germination time, and reducing radicle length, making it difficult or even impossible for native species to establish in new areas.

2. MATERIAL AND METHODS

2.1 Characterization and preparation of the samples

Seeds of six native species found in the caatinga were used in this research: *Mimosa caesalpiniifolia* Benth (sabiá), *Piptadenia stipulacea* (Benth.) Ducke (jurema branca), *Pityrocarpa moniliformis* (Benth.) Luckow & RWJobson (catanduva); *Astronium urundeuva* Fr. (M. Allemão) Engl. (urunday); *Cenostigma pyramidale* (Tul.) Gagnon & G.P. Lewis (catingueira); and *Libidibia ferrea* (Mart. ex Tul.) LPQueiroz (Brazilian ironwood). The first two were donated by the Embrapa Semiárido experimental farm, located in the city of Ibaretama, state of Ceará, Brazil, and kept in a plastic bag in a refrigerated environment, and the other ones were collected in the Serra das Almas Private Reserve of Natural Heritage, maintained by the Caatinga Association in Crateús, state of Ceará, which were also stored in plastic bags, but kept at ambient temperature and humidity conditions. Species selection was based upon their common presence in the seasonally dry tropical forest, in which the farm "Não me Deixes", in the city of Quixadá, is located [32], and also on their suitability for planting in these areas of Ceará [33].

Part of the seeds of each species was placed in an oven at 105±3 °C for 24 hours to determine the moisture content (MC), following the guidelines for seed analysis [34]. A 0.001g precision scale was used to determine the weight of a thousand seeds (WTS). The seeds were counted manually and eight repetitions of 100 seeds were done for each species [34].

According to previous research, four of the species under study required dormancy breaking treatments. The seed dormancy of *M. caesalpiniifolia* was overcome with immersion in freshly boiled water for one minute [35], *P. moniliformis*'s with immersion in concentrated sulfuric acid for 20 minutes [36], *L. ferrea*'s with immersion in concentrated sulfuric acid for 10 minutes [37], and *P. stipulacea*'s with immersion in boiling water for two minutes [38]. The seeds of *A. urundeuva* [39] and *C. pyramidale* [40] had no dormancy.

2.2 Germination test

Germination tests were carried out in the Laboratory of Microbiology and Limnology (LAMILI), in the Laboratory of Wastes, Effluents and Biogas (LAREB), which is part of the Federal Institute of Education, Science and Technology (IFCE), in the city of Quixadá, and in the Biology Laboratory from the Educator Training Institute, located in the Federal University of Cariri (UFCA).

After breaking dormancy for those seeds that needed it, seeds of all species were washed in distilled water and then disinfected following the methods used by Costa, Nepomuceno and Santana (2010) [41], which consisted in immersion in 70% ethanol for one minute, followed by a 15-min immersion in a sodium hypochlorite solution (2.5% active chlorine) mixed with two drops of neutral detergent and four washes in distilled water.

Fresh leaves from 15 individual trees, collected from the afforestation area at IFCE, in Quixadá, were used to produce the aqueous extract of *A. indica* employed in tests. Then, the material was weighed to prepare the crude extract (100%), according to the ratio of 200g of leaves to each liter of distilled water, which were processed in a blender and filtered with a standard sieve [28]. Next, the crude extract was diluted to produce the other treatments, which were tested for pH in a bench pH-meter, stored in closed recipients, and kept at ambient conditions [42, 43].

All treatments were composed of an aqueous extract of *A. indica* fresh leaves at six concentrations: 0 (T1), 20 (T2), 40 (T3), 60 (T4), 80 (T5) and 100% (T6). T1 was the control treatment and consisted only of distilled water [28]. Germination assays of the species *M. caesalpiniifolia*, *P. moniliformis*, *A. urundeuva*, and *P. stipulacea* were carried out in a BOD Incubator at 25 ± 3°C, and the ones of *L. ferrea* and *C. pyramidale*, in an oven with air circulation at 30 ± 3°C, both in continuous darkness, assuming that the species are neutral photoblastic [44, 45]. The experiment followed a completely randomized design (CRD), with six treatments and four repetitions of 25 seeds, adding up to a 100 seeds per treatment.

As a germination substrate, a double layer of paper towel was cut to fit the size of 9-cm diameter Petri dishes and moistened with the extract of *A. indica* leaves, at the proportion of 2.5 times the weight of the paper, and, whenever necessary, moistened again in all replications of the test [34].

In order to maintain the sanitary conditions of materials and equipment defined by the Ministry of Agriculture, Livestock, and Supply in its guidelines for seed analysis [34], the germination incubator was washed with soap and water, and disinfected with 70% ethanol. The Petri dishes and paper were previously autoclaved at 121°C for 20 minutes.

2.3 Data collection and analysis

Germinated seeds were counted daily from the third day after the start of each test until stagnation in their number. Seeds were considered germinated if their radicle length was longer than 2 mm [42, 46, 47]. By counting germinated seeds, it is possible to determine the following variables:

Germination Percentage (GP), which considers the total number of germinated seeds according to the parameter stated previously, and the total number of plants sown. As the name suggests, it is expressed in percentage (%) [46];

Germination Speed Index (GSI), which considers the daily number of germinated seeds and is obtained through the formula of Maguire (1962) [48]. It lists the number of germinated seeds from the first to the umpteenth count (E_i) and the number of days elapsed from sowing also from the first to the umpteenth count (N_i). Results are then expressed in number of seedling emergences per day (N/d):

$$IVG = \sum_{i=1}^{n} \frac{Gi}{Ni}$$

Average Germination Time (AGT), which follows Baskin and Baskin formula [49], and corresponds to the average time (days) necessary to reach the maximum number of emergences, considering the number of seedlings emerged between each count (n_i), and the average time between the beginning of emergence and the umpteenth count (t_i).

Besides, in the last count for each species, the radicle length was measured with a 0.01-mm accuracy digital caliper [42, 69].

In order to evaluate if there were differences between treatments for each species, the obtained data for each parameter were submitted to Shapiro-Wilk test for normality. As they had normal distribution ($p > 0.05$), they went through one-way analysis of variance (ANOVA one-way) and the averages were compared using Turkey Test. When they did not have normal distribution ($p < 0.05$), the data were submitted to Kruskal-Wallis test. For both tests the significance level used was 5% of probability. Analyses took place in the R v3.3.1 environment [50].

3. RESULTS

Moisture content (MC) and weight of a thousand seeds (WTS) ranged from 5.67 to 9.39%, and from 11.28 to 191.95g, respectively. *Cenostigma pyramidale* had the lowest MC (5.67%) and the highest WTS (191.95g), while seeds from *A. urundeuva* were the most humid (9.39%) and the lightest (11.28g). For *P. moniliformis* and *L. ferrea*, MC and WTS were 7.73 and 7.57%, and 28.65 and 129.23g, respectively. In the case of *M. caesalpiniifolia* there were not enough seeds to calculate WTS, therefore 7.23% is its only figure, which corresponds to MC. Besides, pH values were also measured: they varied from 6.33 (T6) to 6.54 (T2) with an average of 6.47 ± 0.06.

Piptadenia stipulacea was the only species which presented meaningful GP and GSI differences between treatments. There was reduction in the total number of germinated seeds, and in the speed of germination (Table 1). Although allelochemical reaction is specific to each species, and the other species are more tolerant, results found for *P. stipulacea* corroborate our hypothesis that allelochemicals in extracts may cause negative effects on the germination of Caatinga species.

Table 1 – Average values of germination percentage (GP, in %), and germination speed index (GSI, in number of emergences per day) of six native species from the Seasonally Dry Tropical Forest submitted to different concentrations of an aqueous extract made of *Azadirachta indica* *leaves. Caption: TR = Treatment, T1 = 0%, T2 = 20%, T3 = 40%, T4 = 60%, T5 = 80%, and T6 = 100% of raw extract, SW = Shapiro-Wilk. Averages followed by the same small letter in the column do not differ from each other by the Turkey Test at 5% probability.*

TR	*Mimosa caesalpiniifolia*		*Astronium urundeuva*		*Pityrocarpa moniliformis*		*Cenostigma pyramidale*		*Libidibia Ferrea*		*Piptadenia stipulacea*	
	GP	GSI	GP	GSI	GP	GSI	GP	GSI	GP	GSI	GP	GSI
T1	26^{a}	8.48^{a}	55^{a}	13.16^{a}	34^{a}	7.86^{a}	55^{a}	8.91^{a}	75^{a}	20.11^{a}	91^{a}	3.85^{ab}
T2	30^{a}	10.49^{a}	66^{a}	14.55^{a}	43^{a}	10.13^{a}	74^{a}	16.01^{a}	80^{a}	24.50^{a}	85^{ab}	3.84^{ab}
T3	33^{a}	12.19^{a}	75^{a}	15.42^{a}	42^{a}	7.98^{a}	75^{a}	14.53^{a}	74^{a}	22.82^{a}	98^{a}	4.00^{ab}
T4	25^{a}	8.40^{a}	78^{a}	15.29^{a}	39^{a}	8.18^{a}	74^{a}	12.62^{a}	84^{a}	22.10^{a}	86^{ab}	4.91^{a}
T5	18^{a}	6.07^{a}	81^{a}	14.41^{a}	31^{a}	4.72^{a}	67^{a}	10.77^{a}	71^{a}	17.45^{a}	86^{ab}	4.08^{ab}
T6	25^{a}	7.26^{a}	77^{a}	14.31^{a}	18^{a}	3.05^{a}	48^{a}	8.76^{a}	67^{a}	15.88^{a}	77^{b}	3.39^{b}
SW (p-value)	0.93 (0.10)	0.96 (0.44)	0.97 (0.72)	0.98 (0.81)	0.95 (0.25)	0.90 (0.02)	0.96 (0.43)	0.97 (0.65)	0.94 (0.19)	0.95 (0.27)	0.92 (0.07)	0.94 (0.15)

When it comes to average germination time (AGT), *A. urundeuva* was also the only species that showed significant variation between treatments, which is in consonance with the hypothesis raised. It had the lowest AGT when submitted to the control treatment (4.22 days), and got higher values as concentration increased. The top AGT (5.33 days) happened under an 80% dilution of *A. indica*'s crude extract (Table 2).

Results obtained for radicle length (RL) were different between species, for some even the opposite of what was expected. This scenario allows the separation of species into three distinct groups: one in which the aqueous extract of *A. indica* leaves had negative allelopathic effect (RL reduction), one in which the effect was neutral (no influence on RL), and one in which the effect was positive (RL increase).

The negative effect was observed in *P. moniliformis* and *L. ferrea*. These two tended to have RL reduction in elevated concentrations of extract, in which the lowest values match seeds submitted to T5 (16.94 and 60.89mm, respectively). In the neutral group, constituted by *M. caesalpiniifolia* and *P. stipulacea*, the aqueous extract of *A. indica* leaves did not have any influence on RL (Table 2).

The positive effect group is formed by *A. urundeuva* and *C. pyramidale*. They had their RL favored by the aqueous extract of *A. indica*. Seeds of *A. urundeuva* submitted to the control treatment had shorter radicles (14.26mm), while longer ones were obtained when the seeds were submitted to intermediate treatments (the values were between 25 and 27mm between T3 and T5). In the case of *C. pyramidale*, the lowest value was found in the control treatment (29.19mm) and the highest in T4 (65.47mm). Both species suffered negative effect in relation to previous treatments when submitted to T5 and T6 (Table 2).

Table 2 – Average values of average germination time (AGT) and radicle length (RL) of seeds from six native species found in the Seasonally Dry Tropical Forest submitted to different concentrations of an aqueous extract of Azadirachta indica leaves. Caption: TR = Treatment, T1 = 0%, T2 = 20%, T3 = 40%, T4 = 60%, T5 = 80%, and T6 = 100% of raw extract, SW = Shapiro-Wilk. Averages followed by the same small letter in the column do not differ from each other by the Turkey Test at 5% probability.

TR	*Mimosa caesalpiniifolia*		*Astronium urundeuva*		*Pityrocarpa moniliformis*		*Cenostigma pyramidale*		*Libidibia ferrea*		*Piptadenia stipulacea*	
	AGT	RL	AGT	RL	AGT	RL	AGT	RL	AGT	RL	AGT	RL
T1	4.03^{a}	63.58^{a}	4.22^{c}	14.26^{c}	1.75^{a}	28.67^{a}	5.30^{a}	29.19^{c}	4.55^{a}	62.12^{ab}	6.25^{a}	43.28^{a}
T2	3.85^{a}	75.65^{a}	4.57^{bc}	23.56^{ab}	2.14^{a}	28.46^{a}	6.54^{a}	55.49^{b}	4.67^{a}	70.91^{a}	6.03^{a}	47.44^{a}
T3	3.43^{a}	78.52^{a}	4.75^{abc}	27.55^{a}	1.91^{a}	23.00^{ab}	4.87^{a}	56.85^{b}	3.93^{a}	70.95^{a}	6.07^{a}	41.18^{a}
T4	3.95^{a}	82.70^{a}	5.02^{ab}	26.31^{ab}	1.82^{a}	23.84^{ab}	7.17^{a}	65.47^{a}	5.05^{a}	70.81^{a}	5.02^{a}	44.71^{a}
T5	4.00^{a}	78.10^{a}	5.33^{a}	25.10^{ab}	1.29^{a}	16.94^{b}	7.64^{a}	51.74^{b}	5.50^{a}	60.89^{b}	5.83^{a}	44.75^{a}
T6	5.60^{a}	75.80^{a}	5.22^{ab}	23.05^{b}	0.77^{a}	19.12^{ab}	9.78^{a}	45.72^{bc}	5.83^{a}	65.48^{ab}	5.92^{a}	43.20^{a}
SW (p-value)	0.89 (0.01)	0.94 (0.10)	0.97 (0.74)	0.99 (0.72)	0.89 (0.01)	0.96 (0.25)	0.91 (0.03)	0.98 (0.43)	0.97 (0.71)	0.99 (0.20)	0.95 (0.25)	0.84 (0.002)

4. DISCUSSION

4.1 Characterization of botanical material

Moisture content values ranged from 5.67 to 9.39%, which are considered low but within the expected for seeds from caatinga species [51]. Other germination studies also registered similar humidity for *M. caesalpiniifolia*, 8% [52], *A. urundeuva*, 10% [53], *P. moniliformis*, 7-9% [54], *C. pyramidale*, 6.22% [55] and *L. ferrea*, 7.46% [56].

Obtaining the weight of a thousand seeds was important to assemble germination tests, which allowed the separation of a mass containing the necessary amount (600 seeds) for each test, and hence reducing waste in the case of seeds that went through dormancy break. In addition, such values indicate that the state of seed maturity and health is close to the ones found in previous successful studies which involved the species [57, 58, 59]. The highest values of WTS were found for *L. ferrea* and *C. pyramidale*, a fact generally associated with bigger seeds [34], which implicate higher water absorption mainly in the beginning of germination [49]. Because of that, rewetting was necessary as early as the third day of experiment.

Controlling pH and concentration of extracts is essential because they might contain substances such as sugars, amino acids, and organic acids which influence ionic concentration and are osmotically active [60]. The average pH value (6.47 ± 0.06) registered in this paper was close to neutral and within the limits of 6 to 7.5, which are ideal for germination [49], so this parameter probably did not interfere with the experiments performed, corroborating the observations of Rosa et al. (2011) [42].

4.2 Germination speed index and germination percentage

Allelopathic compounds do not always interfere in a significative way with all species and variables analyzed [61, 62], which explains why each germination variable (GP, GSI, and AGT) shows a meaningful difference in one species only. Ferreira and Borguetti (2004) [60] state that germination is less sensitive to allelochemicals, while length is the variable which best expresses the effects of allelochemical compounds on seedlings, for they are sensitive to toxicity and stay in direct contact with extracts.

It should be noted that GP values for *M. caesalpiniifolia*, besides being the lowest in the whole experiment, were also lower than values from other germination tests involving the species under the same conditions of substrate and temperature [52]. It is thought that seed viability was impaired under storage conditions in a low temperature fridge, and also due to a curculionid proliferation which precluded WTS counting and harmed seed vigor [63, 64].

Non-significative variation in GP and GSI values was also observed in tests with the native *M. caesalpiniifolia*, *L. ferrea*, and *P. stipulacea* when under the effect of *C. madagascariensis*' extracts. This species, which comes from Madagascar, was introduced into the semiarid region and has a high invasive potential [12]. *M. caesalpiniifolia* showed no GP effect when submitted to the leaf or fruit extracts of *P. moniliformis* either [65]. As for the rest of the species (*A. urundeuva*, *P. moniliformis*, and *C. pyramidale*) there could not be found any studies which investigated the allelopathic effect on GP and GSI so that their values could be used for comparison. Therefore, our work is the first one to test the influence of allelopathic compounds of exotic species on GP and GSI of these native species, giving its contribution so that the impacts they suffer and the possible consequences to the natural community are known.

In principle, the small variation between the averages found for GP and GSI represents the influence of the studied extract's allelopathic effect, but it is important to take other germination variables into account [12, 28, 49].

4.3 Average germination time

AGT is taken as a great indicator to assess how quickly a species occupies an area, that is the lower the AGT, the bigger the odds a species settles. It also indicates environment noise or interference in the metabolic processes of germination [46, 47]. *A. urundeuva*, the only species which had significative AGT difference, distinguishes itself by rapid germination (four days) without any pre-germination treatments [39, 68]. Nevertheless, our results showed that the aqueous extract of *A. indica* increased *A. urundeuva*'s AGT gradually, which suggests that in natural conditions the native species may delay establishing its seeds, while the exotic one may favor its offspring and have an advantage in competition scenarios. This prediction is worrisome specially when it comes to a species like *A. urundeuva*, which has been explored in multiple ways and had its natural populations reduced [66].

4.4 Radicle length

The negative effect observed for *P. moniliformis* and *L. ferrea* confirms the results found in other experiments which used extracts of exotic species, *A. indica* inclusive. Araújo et al. (2017) [12] found out that seeds of *L. ferrea* had their radicle length shortened under the effect of a fresh leaf and litter extract made from *C. madagascariensis*. Something similar was also noticed by Rickli et al. (2011) [28] concerning the radicle length of *Lactuva sativa L.* (lettuce), *Glycine max* L., and *Bidens pilosa* L. (hairy beggarticks) under different extract concentrations of *A. indica*. It is worth highlighting that the root system is important for absorbing nutrients and water, and for supporting the plant, therefore malformation problems like radicle length reduction interfere with the establishment of individuals, as Carvalho et al. (2016) [67] took notice. Such characteristic is pointed out in the allelopathy literature as one of the main indicators of the phenomenon [12, 28, 47].

The radicle length neutrality observed for *M. caesalpiniifolia* when submitted to the aqueous extract of *A. indica* leaves is different from the negative reaction found by Pacheco et al. (2017) [65] and Araújo et al. (2017) [12], who evaluated the RL of the same species submitted to the extracts of *P. moniliformis* and *C. madagascariensis*, respectively. Positive results found for *A. urundeuva* and *C. pyramidale* demonstrated that some of the native species tested tolerate low extract concentrations, which allows us to infer that the presence of fresh leaves of *A. indica* in the field does not interfere with the germination of those species in a negative way. Hartmann et al. (2017) [30] also reported a positive effect on seedling growth for the native species *Parapiptadenia rigida* (Benth.) Brenan when submitted to leaf extracts made from the exotic *Megathyrsus maximus* (Jacq.) BKSimon & SWLJacobs. The same authors suggest that it is not possible to state categorically which physiological process influenced this growth, although it can be a consequence of the phenomenon known as hormesis, i.e., when a toxic substance in much lower doses than those which have toxicity stimulates the plant development. Such results may be useful for the production of forest seedlings of *A. urundeuva* and *C. pyramidale*, since the use of the extract in an adequate dose stimulated the longest radicle length. Nonetheless, it is important that the variables are evaluated together, and that studies which assess the initial development of these species submitted to different extract concentrations are accomplished.

5. CONCLUSION

The results found allow us to conclude that *A. indica* in fact has an allelopathic effect on germination and early stages of growth in five out of the six species studied. Germination percentage and germination speed index were affected by the leaf extract only for *P. stipulacea*. When average germination time is regarded, the species *A. urundeuva* was the only one to show a significative difference, increasing the average number of days till germination at the end of the experiment. Now, as far as radicle length is concerned, extract concentrations of *A. indica* harmed *P. moniliformis* and *L. ferrea* at the same time that favored *A. urundeuva* and *C. pyramidale* in intermediate concentrations. It should be noticed that these last two species had their RL average values reduced when they were submitted to higher extract concentrations. The only species which remained indifferent to the aqueous extract of *A. indica* fresh leaves was *M. caesalpiniifolia*.

We believe that results such as the ones found in this study contribute to a better understanding of the dynamics in an environment influenced by the presence of the exotic species investigated, and draw attention of managers and society in general to planning the protection and preservation of native species; and although laboratory tests such as this one in fact prove that the allelopathic effect exists, it is necessary to encourage and develop field and laboratory experiments that assess other characteristics, like using other plant parts to contain allelopathic compounds – roots, branches, dry leaves or leaf litter, for instance or testing the effect on more native species and how they establish in the field.

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