Allelopathic activity of *Piptadenia moniliformis* Benth. on the physiological potential of seeds of *Amburana cearensis* Allem.

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The adoption of agroforestry systems or reforestation programs contributes to the economy of a region in a sustainable way, since the adopted species can be exploited by the cosmeceutical and pharmaceutical industries. For the establishment of plant species in a given area, studies are needed on the interaction that can develop between them, with emphasis on research on allelopathy. Given the above, the aim of this study was to evaluate the allelopathic effect of the aqueous extract of leaves of the native species *Piptadenia moniliformis* on the initial development of the forest species *Amburana cearensis*. The aqueous extract was used at different concentrations. (0% - water, 25, 50, 75 and 100%), adopting a completely randomized design. The following characteristics were evaluated: emergence percentage, emergence speed index, mean emergence time, shoot and root length and total shoot, root and total dry part of normal seedlings. The 100% concentration of the aqueous extract of *P. moniliformis* leaves reduced the emergence percentage of *A. cearensis* seedlings. A smaller number of normal seedlings was observed at concentrations of 75 and 100%. The initial development of seedlings was hampered by the increase in extract concentrations. The aqueous extract of fresh leaves of *P. moniliformis* provides a negative allelopathic effect for both emergence and early development of *A. cearensis* seedlings, therefore intercropped cultivation among the forest species is not recommended.

**Keywords:** allelopathy, forest species, semi-arid.

A adoção de sistemas agroflorestais ou programas de reflorestamento contribui para a economia de uma região de forma sustentável, visto que as espécies adotadas podem ser exploradas pelas indústrias cosmeceutica e farmacêutica. Para o estabelecimento de espécies vegetais em uma determinada área, são necessários estudos sobre a interação que podem se desenvolver entre elas, com destaque para pesquisas sobre alelopatia. Diante do exposto, o objetivo do presente estudo foi avaliar o efeito alelopático do extrato aquoso de folhas da espécie nativa *Piptadenia moniliformis* sobre o desenvolvimento inicial da espécie florestal *Amburana cearensis*. O extrato aquoso foi utilizado em diferentes concentrações (0% - água, 25, 50, 75 e 100%), adotando um delineamento inteiramente casualizado. Foram avaliadas as seguintes características: porcentagem de emergência, índice de velocidade de emergência, tempo médio de emergência, comprimento da parte aérea e da raiz e parte seca total da parte aérea, raiz e total das plântulas normais. A concentração de 100% do extrato aquoso das folhas de *P. moniliformis* reduziu a porcentagem de emergência das plântulas de *A. cearensis*. Observou-se menor número de plântulas normais nas concentrações de 75 e 100%. O desenvolvimento inicial das plântulas foi prejudicado pelo aumento das concentrações do extrato. O extrato aquoso de folhas frescas de *P. moniliformis* proporciona um efeito alelopático negativo tanto para a emergência quanto para o desenvolvimento inicial de plântulas de *A. cearensis*, portanto, o cultivo consorciado entre as espécies florestais não é recomendado.

**Palavras-chave:** alelopatia, espécies florestais, semiárido.
1. INTRODUCTION

Natural products, especially those derived from plants, have been used in human health since the beginnings of medicine [1]. Plants are responsible for the biosynthesis of a wide variety of secondary metabolites, and these metabolites form the basis of many commercial pharmaceutical drugs, as well as herbal remedies derived from medicinal plants [2].

In the Caatinga region of northeastern Brazil, there are several species of plants with a medicinal use. The biological diversity of this biome houses species characterized by a multiplicity of uses, the Fabaceae family accounts for about a third of the richness of this biome [3]. Among plant species, Amburana cearensis (Allemão) A. C. Sm. stands out. It is popularly known as "cumaru," "amburana de cheiro" and "cumaru-do-Ceará." It may reach up to 10 m high in the Caatinga regions and up 20 m in forest areas. It occurs naturally from the Northeast to Central Brazil in Caatinga regions and in the rain forest of Minas Gerais in the Rio Doce valley [4].

Amburana cearensis is widely used in popular medicine, mainly for the treatment of diseases such as rheumatism, cough, bronchitis, asthma, and tummy ache. Stem barks and seeds are used for the production of syrup or tea for the treatment of colds, bronchitis, the flu, and asthma. The bath with stem bark is used against rheumatic pain, while seeds are used for symptomatic relief [5].

Amburana cearensis, according to the criteria of the International Union for the Conservation of Nature and Natural Resources - IUCN (2014), is an endangered species. Because this is of great importance, the development of studies aiming the elaboration of models of rational and self-sustaining exploitation to avoid harmful consequences is needed [6].

Piptadenia moniliformis Benth., popularly known as "angico-de-bezerro" or "rama-de-bezerro," is a pioneer, rustic and fast growing species suitable for heterogeneous reforestation for preservation purposes. It is common in the states of Maranhão, Piauí, Ceará and Bahia, Brazil. Because of its small size, its wood is used only locally in small construction works, light joinery, tool cables, and for firewood and coal. This species, besides producing an abundant quantity of seeds annually [7], presents medicinal properties, and provides forage for cattle breeding and goat breeding, and wood and firewood for the rural population. In the northeastern region of Brazil, where beekeeping has native plants as the source of flowers, this species stands out as a potential honey plant. Its flowers are appreciated by bees, providing excellent quality honey [8].

For the exploration to be sustainable it is necessary to understand the interactions established between plants grown in the same area, as this interaction can be unfavorable, this occurs when one species negatively affects the development of the other when competing for the same resource or through allelopathy [9]. Allelopathy is a biological phenomenon in which an organism (plant, bacteria, fungus, alga or virus) produces one or more secondary metabolites, these metabolites are called allelochemicals. Its effects on recipient organisms can occur directly or indirectly, being harmful or beneficial [10].

Because A. cearensis is used in traditional medicine for the treatment of several diseases and presents a potential to be used for the recovery of degraded areas, it is important to know the interaction of this species with other species. Thus, this study aims to evaluate the allelopathic effect of the aqueous extract of P. moniliformis leaves on the emergence and early development of seedlings of A. cearensis.

2. MATERIAL AND METHODS

Experiment location and experimental design

The experiment was conducted at the Federal Rural University of Pernambuco - Serra Talhada Academic Unit, Brazil. Green leaves of the species P. moniliformis were used, the native forest species analyzed for the allelopathic effect was A. cearensis. The seeds were donated by the Education and Environmental Monitoring Center (NEMA) of the city of Petrolina-PE, Brazil. They were collected in the municipality of Salgueiro - PE, where the seed lot consisted of 10
matrix trees. After collection and processing, the seeds were stored in a cold chamber set at 5-10°C and 24-30% relative humidity.

**Preparation and application of treatments**

The leaves of *P. moniliformis* were collected in the district of São Lourenço, municipality of Serra Talhada - PE, Brazil, in the morning, later placed in plastic bags and taken to the laboratory immediately.

To obtain the crude aqueous extract, the ratio between 250 grams of fresh leaves and 1,000 mL of distilled water was used according to Cruz et al. (2000) [11]. The grinding of leaves in a blender was previously performed, and then filtering using a 100% cotton cloth. To obtain concentrations, the crude extract (100%) was diluted at the concentrations 25, 50 and 75%; for control (0%), distilled water was used. The treatments were T1 - control (0%), T2 - extract at 25%, T3 - extract at 50%, T4 - extract at 75%, and T5 - extract at 100%.

The sowing of *A. cearensis* seeds was carried out in a greenhouse, using 128 cell polyethylene trays, filled with sterilized sand in an oven at 200°C for two hours [12]. Irrigation was performed daily using different concentrations considering the loss of substrate moisture.

**Variables analyzed**

The seedlings were counted daily for 25 days in order to obtain the percentage of emergence (PE), emergence speed index (ESI) according to Maguire (1962) [13], and mean emergence time (MET) according to Labouriau (1983) [14]. Considering the characterization of the initial development of normal seedlings, the emergence period was measured by calculating shoot length (SL) and root length (RL), and shoot dry matter (SDM), root dry matter (RDM), and total dry matter (TDM). The dry matter was obtained by drying in an oven at 80°C for 24 hours [15]. After drying, the shoot and root were weighed.

**Experimental design and statistical analysis**

The experimental design was completely randomized with five treatments (concentrations of aqueous extract), and five replicates with 20 seeds each. All data obtained in the evaluations were submitted to normality tests (Lillefors test) and homogeneity (Cochran test), which showed that it was not necessary to submit them to transformations. Data were subjected to analysis of variance, when significant the means were subjected to regression analysis. All statistical analysis was performed using the SISVAR software [16].

**3. RESULTS AND DISCUSSION**

**Influence of the aqueous extract of leaves of *Piptadenia moniliformis* on the emergence of seedlings of *Amburana cearensis***

The analysis of variance of the emergence percentage and emergence speed index (Table 1) of *A. cearensis* seeds indicated a significant effect (p <0.05) when they were submitted to different concentrations of the aqueous extract of fresh leaves of *P. moniliformis*, demonstrating that different concentrations interfered in the emergence of this species. For the mean time of emergence, the different concentrations of the extract had no effect.
Table 1. Analysis of variance of emergence percentage (EP), emergence speed index (ESI) and mean emergence time (MET) of Amburana cearensis seeds submitted to different concentrations of aqueous extract of leaves of Piptadenia moniliformis.

|          | FV  | DF | EP (%) | ESI   | MET (days) |
|----------|-----|----|--------|-------|------------|
| Treatment| 4   |    | 978,5**| 0,165**| 0,213ns    |
| Residue  | 20  |    | 111,5  | 0,016 | 0,758      |
| CV%      | 20,38 | 19,64 | 25,52 |

Significant effect at 1% (**), at 5% (*) and not significant effect (ns); variation factor (FV); degree of freedom (DF); coefficient of variation (CV).

For a percentage of emergence of *A. cearensis* seedlings (Figure 1A), as the concentration of the aqueous extract of the leaves of *P. moniliformis* increased, there was a reduction for this variable. The leaves and fruits of *P. moniliformis* present saponins, flavonoids, triterpenes, and gallic tannins [17]. Triterpenic saponins are able to inhibit chlorophyll production by acting on etioplasts. They cause loss of electrolytes in cells of plant tissue fragments, inhibit germination and growth, and inhibit nodulation in certain legumes, elongation of the primary root and secondary root development of certain plants [18]. This explains why the emergence was affected by the concentration 100% of the extract.

By submitting *A. cearensis* seeds to different concentrations of *Cynophalla hastata*, Silva et al. (2021) [19], observed that regardless of the concentration of the aqueous extract there was a significant reduction in the percentage of emergence in relation to the control (concentration 0%), the following allelopathic compounds were found in the aqueous extract of *C. hastata* leaves: alkaloids, saponins, phenolic compounds and flavonoids. Inhibitory action on germination and initial development of *Mimosa hostilis* seedlings was verified by Silva et al. (2020) [20], when aqueous extracts of fresh leaves of *Piptadenia moniliformis* and *Capparis hastata* were used; the authors attributed the results to the presence of chemical substances in the leaves with an allelopathic effect.

Figure 1. Effects of different concentrations of aqueous extract of leaves of *Piptadenia moniliformis* on the emergence of *Amburana cearensis* seedlings.

The concentrations zero, 25 and 50% provided the highest rate of emergence. The values were between 0.84 and 0.71, and the highest concentrations (75 and 100%) resulted in a reduction in ESI (Figure 1B). Pacheco et al. (2017) [21], using aqueous extract of *Pityrocarpa moniliformis* observed that although there was no influence of extract concentrations on the germination percentage of *Mimosa caesalpinifolia*, observed that the germination process speed decreased sharply as the concentration of leaf extract increased, while the extracts of the fruits provided little significant reduction. Furthermore, Araújo et al. (2021) [22] observed deleterious effects of *Cenostigma bracteosum* leaf extracts, from the concentration of 5%. on seed germination and
seedling vigor of *Mimosa caesalpiniifolia*. The reduction in germination capacity is a reflection of the phytotoxic effect, which according to Azambuja et al. (2010) [23] would be due to the lower mobilization of nutritional reserves, thus affecting the formation of the seedling.

**Influence of the aqueous extract of leaves of *Piptadenia moniliformis* on the initial development of seedlings of *Amburana cearensis***

For all variables related to the initial seedling development from *A. cearensis* seeds, there was a significant effect (p <0.05), when submitted to different concentrations of the aqueous extract of fresh leaves of *P. moniliformis* (Table 2).

**Table 2 - Analysis of variance for shoot length (SL), root length (RL), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM) of seedlings of *Amburana cearensis* submitted to different concentrations of aqueous extract of leaves of *Piptadenia moniliformis***.

|                | FV | DF   | Mean Square |
|----------------|----|------|-------------|
| SL (cm)        | 12,004* | 4  | 1,544*      | 0,470** | 0,262** | 0,718** |
| RL (cm)        | 3,003 | 20 | 0,476       | 0,038  | 0,002  | 0,058  |
| SDM (g)        | 12,70 | 17,14 | 25,03      | 24,55  | 24,16  |
| RDM (g)        | 25,03 | 17,14 | 25,03      | 24,55  | 24,16  |
| TDM (g)        | 25,03 | 17,14 | 25,03      | 24,55  | 24,16  |

Significant effect at 1% (**), at 5% (*) and not significant effect (ns); variation factor (FV); degree of freedom (DF); coefficient of variation (CV).

As for shoot length (Figure 2A), decreasing linear behavior was observed for the concentrations of the aqueous extract of *P. moniliformis* leaves, in which the use of the concentrations zero and 25% led to the highest averages (15.38 and 14.76 cm, respectively), whereas the use of the crude extract resulted in a lower shoot length (11.43 cm), with a 25.7% reduction in length in relation to the concentration zero.

According to Cheng and Cheng (2015) [24], allelochemicals may alter the contents of growth regulators or induce hormonal imbalances, resulting in reduced plant growth and development. Oliveira et al. (2015) [25] reported that the seedling length of lettuce was significantly affected as the concentrations of aqueous extracts of fresh leaves of *Ziziphus joazeiro* was increased compared to the control. The aqueous extracts of leaves and fruits of *P. moniliformis* interfered negatively with the initial growth of seedlings of *M. caesalpiniifolia* [21], corroborating the results found in this work.

![Figure 2. Effects of different concentrations of aqueous extract of leaves of *Piptadenia moniliformis* on the development of seedlings of *Amburana cearensis*.](image-url)
Regarding root length (Figure 2B), the concentration 25% resulted in the longest length. As concentrations increased, there was also a decrease in root growth. By using the highest concentration, there was a reduction of 32.9% when compared to the concentration of 25%. In general, the roots are sensitive to the substances present in the extracts compared with the other structures of the seedlings [26]. This is because roots are in direct and prolonged contact with the extract (allelochemicals) in relation to the other structures of the seedlings [27]. Changes in hormonal balance resulting from allelopathy change the length of seedling organs according to Alves and Santos (2002) [28], as the roots are in direct contact with the plant extract or because they are more sensitive to its action in relation to the shoot, they are verified more abnormalities in the root system, in addition to shorter length. Also, studies by Pereira et al. (2008) [29] indicated the greater sensitivity of the root system to the phytotoxic effects of compounds at low concentrations, in relation to hypocotyl growth or germination.

Among the compounds found in the leaves and fruits of *P. moniliformis*, there are flavonoids [21]. These substances may inhibit the germination and root growth of several angiosperm species, it may affect mitochondria respiration of plants. Araújo et al. (2017) [30], applying three extracts (control, fresh leaves and leaves of the litter of the invasive species (*Cryptostegia madagascariensis*) to native species of the Brazilian semiarid (*Piptadenia stipulacea*, *Libidibia ferrea* and *Mimosa caesalpinifolia*, in addition to the bioindicator species *Lactuca sativa*), observed that *L. sativa* had its germination and seedling growth reduced by about 50% or inhibited in the presence of the extracts. For the native species, the extracts had a negative effect only on the growth of seedlings, reducing by 30% the root growth of *M. caesalpinifolia* and *L. ferrea*.

As for the accumulation of dry matter (shoot, roots and total) (Figures 3A, 3B, 3C), quadratic behavior was verified, in which the concentrations zero and 25% provided the highest values. The concentrations 75 and 100% caused a reduction in the accumulation of dry matter.

![Figure 3. Effects of different concentrations of aqueous extract of leaves of *Piptadenia moniliformis* on the development of seedlings of *Amburana cearensis*.](image-url)
As the green economy is a reality, the use of reforestation programs, recovery of degraded areas and agroforestry systems requires research to study possible interactions between plants, such as allelopathy. Including, not only the negative effects of allelopathy, but also the beneficial ones, such as stimulating the germination process and/or the establishment of seedlings. Within this context, it was observed that the germination and vigor of *Mimosa caesalpinifolia* seedlings were favored by the extracts of leaves of *Sarcomphalus joazeiro* [22], denoting a positive influence of allelopathy. Silva et al. (2021) [31] highlight the importance of developing research on allelopathic interactions between native tree species in the Caatinga (forest and/or fruit trees), aiming to determine synergistic species that can favor the recovery of degraded areas and sustainable management.

4. CONCLUSION

The aqueous extract of fresh leaves of *Piptadenia moniliformis* exerts a negative allelopathic effect for both emergence and early development of seedlings of *Amburana cearensis*, therefore intercropped cultivation among the forest species is not recommended.

5. ACKNOWLEDGEMENTS

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