Original Research Article

Allelopathy of the *Melia azedarach* L. leaf extract on the germination of Atlantic Forest species

ABSTRACT

Allelopathy is a naturally occurring phenomenon that results in the release of chemicals capable of stimulating or inhibiting the development of other nearby plants. The objective of this work was to evaluate the allelopathic potential of *Melia azedarach* on the germination, speed index, length and dry mass of seedlings of three Atlantic Forest species. The treatments were distributed in a bi-factorial scheme consisting of 5 concentrations (0, 2.5, 5.0, 7.5 and 10%) and 3 species (*Ceiba speciosa*, *Leucaena leucocephala* and *Samanea tubulosa*), in four replicates of 25 seeds totaling 100 seeds for each treatment. The extracts were obtained from 100 mg of dehydrated and crushed leaves of *M. azedarach* in 1000 ml of distilled water. The sowing was carried out on germitest paper and the seeds kept germinating in germinators of the type (B. O. D) at 25 °C with photoperiod of 12 h. The germination, germination velocity index (GVI), length and dry mass of shoot and root were evaluated. The results obtained in the laboratory showed that the aqueous extract of leaves *M. azedarach* has an allelopathic effect on all studied species, reducing the percentage of germination, speed index, length and dry mass of seedlings.

Key words: Exotic species, ecophysiology, physiological quality.

1. INTRODUCTION

Allelopathy is a biological phenomenon in which a plant species produces chemical compounds of secondary metabolism that influence the growth, survival and development of other plant species [1]. The production of chemical compounds or allelochemicals can cause damage to the development of biological systems [2]. The allelochemicals exert ecological mechanisms in the adjacent plants, usually because they cause negative physiological effects such as the inhibition of the percentage and speed of the germination and the reduction of the initial growth of seedlings [3,4].

Organic acids, naphthoquinones, anthraquinones, quinones, phenols, flavonoids and tannins are chemical compounds recognized as allelochemicals [5,6], which are used for the development of natural herbicides or inhibitors for the germination, growth and/or development of plants [2], which is facilitated by the release of different forms, such as volatilization, root exudation, leaching [7,8].

Allelopathy is a practice also used in forestry for weed control (El Id et al., 2015) [9]. The adoption of new weed management strategies is important to replace or reduce the use of agrochemicals as they affect human health and the environment [2, 10]. On the other hand, the release of allelochemicals can affect the growth and development of other forest species, damaging the establishment of successor vegetation after [9]. Vegetation from afforestation areas is conditioned to a model of succession based on pre-existing plants and the chemicals released by them in the medium [11].

Considering the need to manage degraded areas, the establishment of successive vegetation and the efficiency of reforestation, it is essential to know the allelopathic potential of an exotic species, as well as the effects of its establishment in these ecosystems.

The objective of this work was to evaluate the influence of the concentrations of *Melia azedarach* L. leaf extract on seed germination and seedling development *Ceiba speciosa* (A. St.-Hil.) Ravenna, *Leucaena leucocephala* (Lam.) De Wit and *Samanea tubulosa* (Benth.) Barneby & JW Grimes.

2. MATERIAL E METHODS

The aqueous extract was obtained from young leaves *M. azedarach* L. which were placed in bags of Kraft paper and taken to the oven with forced circulation regulated at 65 °C until reaching constant weight (72 hours) which were cut into small pieces and mashed in a blender, then ground until a fine powder is obtained. In 1000 ml of water was added the 100 g of the powder obtained from the sheets, the mixture was allowed to stand for 24 hours at room temperature and then filtered through a cloth filter to give an extract in concentration of 10% (w/v). From this initial concentration, 2.5, 5.0 and 7.5% of extract and distilled water as control (0%) were diluted and other concentrations were obtained.

Seeds of *C. speciosa*, *L. leucocephala* and *S. tubulusa* obtained from fruits at the beginning of the natural dehiscence process, harvested in arrays in the city of Areia, Paraíba, Brazil were used as target species (allelochemical recipients).

The seeds *L. leucocephala* and *S. tubulusa* before sowing were scarified with sandpaper No. 80 in the region opposite the thread to overcome dormancy and subsequently disinfested with 2% aqueous solution of sodium hypochlorite for 3 minutes.

The amount of extract was calculated by multiplying the weight of the germitest paper by 2.5 mass of the dry paper. Each treatment used 100 seeds, which were divided into four replicates of 25, distributed on two sheets of paper towel, covered with a third and organized in a roller form, placed inside a transparent plastic bag, avoiding the loss of the extract by evaporation. Biochemical Oxigen Demand (BOD) germination chambers were used in the germination and seedling development, respectively, and regulated to a constant temperature of 25 °C with a photoperiod of eight hours using daylight fluorescent lamps (4 x 20 W). The cotyledons and hypocotyl emergence were used as criteria for germination, and the germination percentage corresponded to the total percentage of germinated seeds until the tenth day after sowing, considering normal seedlings [12]. The percent germination was performed from daily counts, at the same time, from the fifth day to 10 days after sowing of normal seedlings, and the index was calculated using formula $GVI = \frac{G}{N_1} + \frac{G}{N_2} + ... + \frac{G}{N_n}$ [13].

Where GVI= rate of emergency speed; G1, G2 and Gn= number of seedlings emerged in the first, second and last counts; N1, N2 and Nn= number of days of sowing at the first, second and last count.

After the final germination test, at 10 days after sowing, the normal seedlings of each treatment and repetition were measured with a ruler in centimeters measured from the root region to the insertion point of the cotyledons, the results being expressed in cm seedlings^{-1}.

The seedlings of the previous evaluation were placed in bags of Kraft paper and taken to the oven set at 65 ° C until reaching constant weight (48 hours). After that period, the samples were weighed in an analytical balance with an accuracy of 0.001 g. The results expressed in g plantula^{-1}.

The experimental design used was entirely the case, with the treatments distributed in a bi-factorial scheme 5 × 3 (five concentrations and three species), in four replicates of 25 seeds for each treatment. For the quantitative effects, a polynomial regression analysis SISVAR for Windows version 4.6 software.

3. RESULTS AND DISCUSSION

Significant effects were observed in germination, germination speed index (IVG), seedling length and dry mass of seedlings for *L. leucocephala*, *S. tubulosa* and *C. speciosa*, except for *C. speciosa* dry matter.

The analysis of the results allowed to verify that there was a significant interaction for all analyzed variables, presenting a reduction as a function of the increase of the concentration of *M. azedarach* leaf extract on the seeds of *C. speciosa*, *L. leucocephala* and *S. tubulosa*. The species *L. leucocephala* showed to be the most sensitive species to increase the concentration of *M. azedarach* leaf extract, with germination significantly affected from the 2.5% concentration. In relation to *C. speciosa* and *S. tubulosa* there was a slight increase up to 5.0% concentration, and, from the 7.5% concentration of the extract, there were significant decreases (Figure 1).

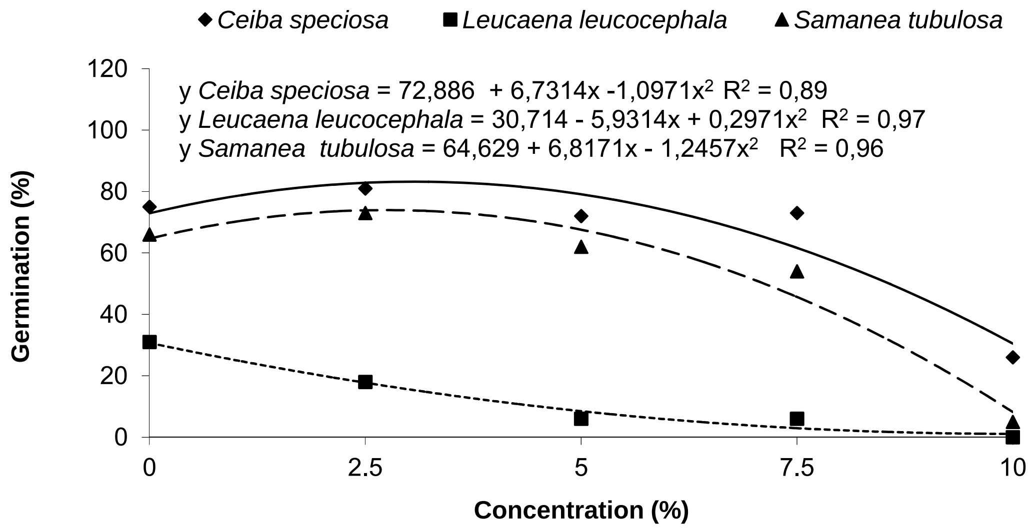

Figure 1. Seed germination of *Ceiba speciosa*, *Leucaena leucocephala* and *Samanea tubulosa*, submitted to different concentrations of aqueous extract of leaves of *Melia azedarach*.

The increase of the concentration of the extracts caused a significant reduction of the germination of the target species under study, possibly related to the increase in the quantity of allelochemicals of the solution. The intensity of the allelopathic effect caused by the extract depends on the extraction tissue and the concentration of the allelochemicals, which is related to the genotype and environment interaction [14]. In forest management, at the ecosystem level, they must be represented by relevant components that integrate the forest ecosystem in question, among these components, forest soil is the first to be influenced by silvicultural practices [9]. Studies on allelopathic relationships among forest species are scarce, so there is a need to include this information from forest ecosystems in research. The difference between the responses of the target species studied is caused by the concentration of the extract and the composition of the seeds [7].

The rate of germination (IVG) showed significant effects. Thus, as in the germination variable, a reduction was observed from the concentration of 7.5% in the *C. speciosa* target species; however, in *L. leucocephala* and *S. tubulosa* seeds, a reduction of 5.0% was observed (Figure 2). This fact demonstrates that IVG is a more sensitive variable to detect allelopathic effects than germination.

A higher germination speed index represents a greater vigor of the seeds and in the present research the aqueous extract of *M. azedarach* interfered, reducing the vigor of *L. leucocephala* seeds, used in the bioassay [15]. The allelopathic effect has no influence on the percentage of germination, but on the rate of germination or other physiological parameters of the seedling [9]. On the other hand, authors have reported that allelopathic compounds most often acted as inhibitors of germination and growth, some studies show that these can act as growth promoters when present in lower concentrations [15].

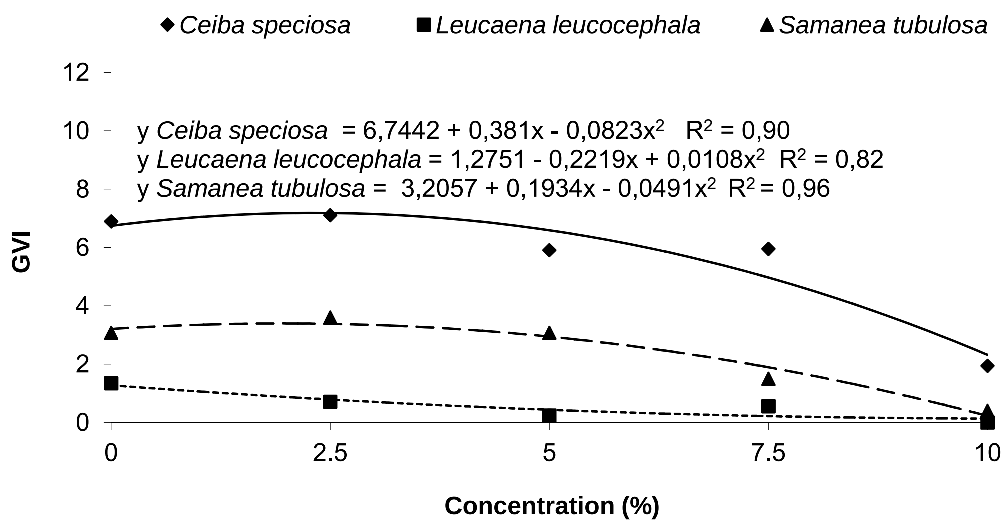

Figure 2. Germination speed index of *Ceiba speciosa, Leucaena leucocephala* and *Samanea tubulo*sa submitted to different concentrations of aqueous extract of young leaves of *Melia azedarach*.

The seedling length was the least sensitive variable to the concentrations of *M. azedarach* leaf extracts, where it was possible to detect a trend of significant reduction in the highest concentrations (7.5 and 10.0%), however, in this last concentration, the development was almost nil for all species tested.

The allelopathic effect of the *M. azedarach* extract adversely affected the seedling growth of the three species under study, being observed an abnormality mainly in the root system, where the primary roots were atrophied, defective and darkened and, in some cases, practically absent. The evaluation of seedling abnormality is a valuable tool in allelopathy experiments and root necrosis is the most common symptom of abnormality [16,17]. The initial growth of the seedlings is characterized by high metabolic activity, as well as, greater sensitivity to environmental stress being more sensitive than the phenomena observed in seeds [17].

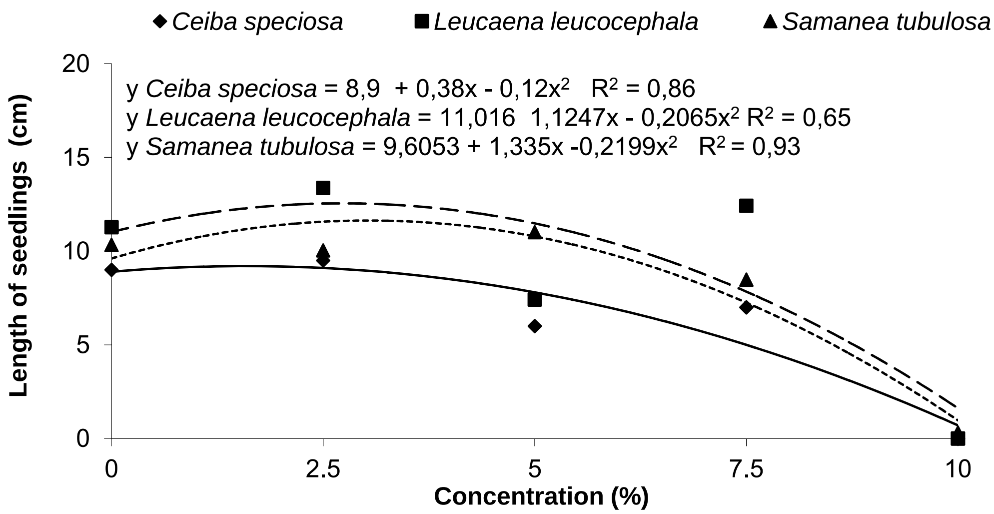

Figure 3. Length of seedlings of *Ceiba speciosa, Leucaena leucocephala* and *Samanea tubulosa* submitted to different concentrations of aqueous extract of young leaves of *Melia azedarach*.

Data on the dry mass of seedlings from *C. speciosa* seeds did not adjust to regression models presenting an average of 7.2 mg. The dry mass of *L. leucocephala* seedlings was reduced in all treatments containing extracts, indicating that the inhibitory effects of the allelochemicals present in the extracts are related to their concentration, while the dry mass of *S. tubulosa* presented the highest content (21.9 mg) at the concentration of 2.5%. Inhibition of seedling germination and seedling growth are often associated with allelopathic effects, being an important process in plant interactions in natural environments and agroecosystems [18].

The results obtained in the research show that the action of the allelochemicals had effects on the initial vegetative development, differentiating between the percentage of germination, length and dry mass of seedlings. This result may be a reflection of the distinct physiology of these different organs of the species under study.

The results allow to suggest that the allelopathic potential of the leaves of *M. azedarach* can be a strategy for their establishment and competition, delaying the establishment and growth of other plants and freeing them from their competition. Considering that *M. azedarach* is a deciduous species [19], it can produce a considerable amount of litter which, when covering the soil and decomposing, would release soluble substances with an inhibitory effect to the other species present. The inhibitory effect of *M. azedarach* is a robust competitive interference strategy that confers advantages to these individuals when found in natural environments [7].

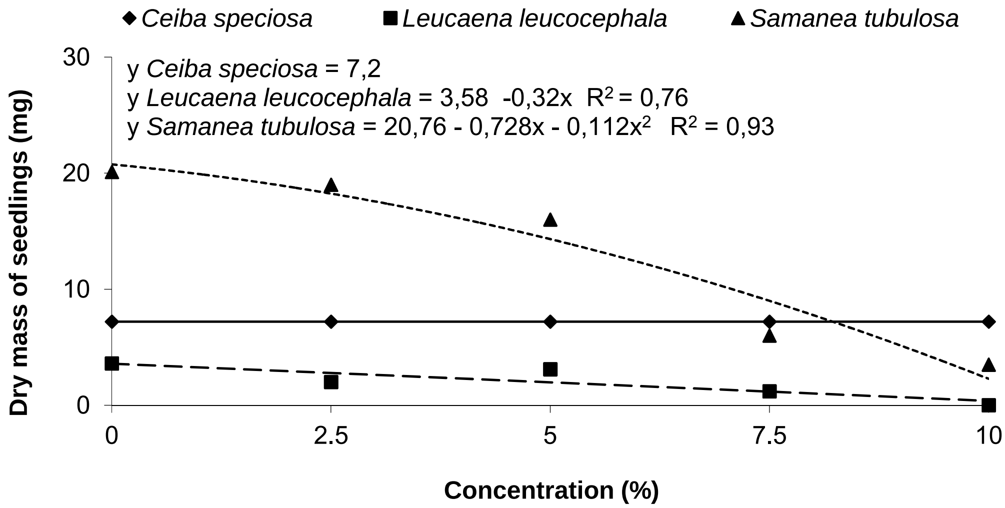

Figure 4. Dry mass of seedlings of *Ceiba speciosa, Leucaena leucocephala* and *Samanea tubulosa* seeds submitted to different concentrations of aqueous extract of young leaves of *Melia azedarach*.

4. CONCLUSION

The aqueous extract of *M. azedarach* leaves has an allelopathic effect on all species studied, reducing the percentage of germination, speed index, length and dry mass of seedlings.

REFERENCES

1. Cheng F, Cheng Z. Research Progress on the use of Plant Allelopathy in Agriculture and the Physiological and Ecological Mechanisms of Allelopathy. Frontiers in Plant Science. 2015; 6(1):1-16.
2. Ben Ghnaya A, Hamrouni L, Amri I, Ahoues H, Hanana M, Romane A. Study of allelopathic effects of Eucalyptus erythrocorys L. crude extracts against germination and seedling growth of weeds and wheat. Natural product research. 2016; 30(18): 2058-2064.
3. Macías FA, Molinillo JMG, Varela RM, Galindo JCG Allelopathy - a natural alternative for weed Control. Pest Management Science. 2007; 63(4):327-348.
4. Li J, Lin S, Zhang Q, Zhang Q, Hu W, He H. 2019. Fine-root traits of allelopathic rice at the seedling stage and their relationship with allelopathic potential. PeerJ. 2019; 7(1): e7006.
5. Zhao W, Zheng Z, Zhang J, Roger SF, Luo X. Allelopathically inhibitory effects of eucalyptus extracts on the growth of *Microcystis aeruginosa*. Chemosphere. 2019; 225(1): 424-433.
6. Jiang P, Xiong J, Wang F, Grace MH, Lila MA, Xu R. α-Amylase and α-Glucisidase inhibitory activities of phenolic extracts from *Eucalyptus grandis x E. urophylla* Bark. Journal of Chemistry. 2017; 2017(1): 1-7.
7. Albuquerque MB, Santos RC, Lima LM, Melo Filho PA., Nogueira RJMC, Camara CAG et al. Allelopathy, an alternative tool to improve cropping systems. A review. Agronomy for Sustainable Development. 2011; 31(2): 379-395.

8. Puldeko K, Majchrzak L, Narozna D. 2014. Allelopathic effect of fibre hemp (*Cannabis sativa* L.) on monocot and dicot plant species. Industrial Crops and Product. 2014; 56(2): 191-199.
9. El Id VL, Costa BV, Mignoni DSB, Veronesi MB, Simões K, Braga MR et al. Phytotoxic effect of Sesbania virgata (Cav.) Pers. on seeds of agronomic and forestry species. Journal of Forestry Research. 2015; 26(2): 339-346.
10. Ackerman F., Whited M, Knight P. Would banning atrazine benefit farmers?. International journal of occupational and environmental health. 2014; 20(1): 61-70.
11. Li, ZH., Wang Q, Ruan X, Pan CD, Jiang DA. 2010. Phenolics and plant allelopathy. Molecules. 2010; 15(12): 8933-8952.
12. Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Rules for seed analysis. Secretaria Nacional de Defesa Agropecuária. Brasília: 2009. MAPA/ACS, 395p. Brazilian
13. Maguire, JD. Speed of germination aid in selection and evaluation for seedling emergence and vigor. Crop Science. 1962; 2(1): 176-177.
14. Wu AP, Yu H, Gao SQ, Huang ZY, He WM, Miao SL et al. Differential belowground allelopathic effects of leaf and root of *Mikania micrantha*. Trees Structure and function. 2009; 23(1):11-17.
15. Masum SM, Hossain MA, Akamine H, Sakagami JI, Ishii T, Gima S et al. Isolation and characterization of allelopathic compounds from the indigenous rice variety 'Boterswar'and their biological activity against *Echinochloa crus-galli* L. Allelopathy Journal. 2018; 43(1): 31-42.
16. Oliveira APP, Pereira SR, Cândido ACS., Laura VA, Peres MTLP. Can allelopathic grasses limit seed germination and seedling growth of mutambo? A test with two species of *Brachiaria* grasses. Planta Daninha. 2016; 34(4): 639-648. Brazilian.
17. Masum SM, Hossain MA, Akamine H, Sakagami JI, Ishii T, Konno T et al. Comparison Study of Allelochemicals and Bispyribac-Sodium on the Germination and Growth Response of *Echinochloa crus-galli* L. Journal of Plant Growth Regulation. 2019; 38(2): 501-512.
18. Piršelová B, Lengyelová L, Galuščáková Ľ, Kuna R. Impact of Sugar Beet Shoot Exudates on Germination and Root Growth of Wheat, Barley and Maize. Listy Cukrovarnické a Reparské. 2019; 135(3):112.
19. Lorenzi, H. 2009. Brazilian trees: identification and cultivation of tree plants in Brazil.3.ed. Nova Odessa: Instituto Plantarum, 368p. Brazilian.