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ALLELOPATHIC ACTIVITY OF *Manihot esculenta* CRANTZ ON THE GERMINATION OF *Avena sativa* L. AND *Trifolium resupinatum* L. SEEDS

ATIVIDADE ALELOPÁTICA DE Manihot esculenta CRANTZ NA GERMINAÇÃO DE SEMENTES DE Avena sativa L. E Trifolium resupinatum L.

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ABSTRACT

Allelopathy can be considered a chemical interference of one or more substances from a plant species in the development of another species, encompassing both inhibition and stimulation effects. In this context, the goal of the research was to evaluate the allelopathic effect of Manihot esculenta (cassava) leaf extracts on white oat and Persian clover seed germination. The seeds were placed on germitest paper containing leaf extract at concentrations corresponding to 0, 25, 50, 75, and 100% and kept in a germination chamber at 20 °C in the presence of light. The results showed that M. esculenta extracts negatively affected the germination and initial growth of white oat and Persian clover seedlings. Thus, as an adequate management practice, it is recommended that M. esculenta leaves be removed from the fields before sowing in order to avoid a negative influence on the initial growth of these species.

Keywords: allelopathy, white oat, Persian clover, cassava, germination process.

RESUMO

A alelopatia pode ser considerada uma interferência química de uma ou mais substâncias de uma espécie vegetal no desenvolvimento de outra espécie, englobando tanto efeitos de inibição quanto de estimulação. Neste contexto, o objetivo da presente pesquisa foi avaliar o efeito alelopático de extratos de folhas de Manihot esculenta (mandioca) sobre a germinação de sementes de aveia branca e trevo Persa. As sementes foram dispostas em papel germitest contendo extrato de folhas nas concentrações correspondentes a 0, 25, 50, 75 e 100% e mantidas em câmara de germinação, a 20 °C em presença de luz. Os resultados evidenciaram que os extratos de M. esculenta afetaram negativamente a germinação e o crescimento inicial de plântulas de aveia branca e trevo Persa. Assim, como prática de manejo adequada recomenda-se que as folhas de M. esculenta sejam removidas dos campos antes da semeadura a fim de evitar influência negativa no crescimento inicial destas espécies.

Palavras-chave: *alelopatia, aveia, trevo Persa, mandioca, processo de germinação.*

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INTRODUCTION

Plants have the ability to release chemical substances (allelochemicals) into the environment that can positively or negatively interfere with the development of other plants (SILVA *et al*., 2022). These substances are mainly present in leaves and can be released during their decomposition process (BACHHETI *et al*., 2020) and used as bioherbicides, as they are less harmful to the ecosystem than synthetic agrochemicals (CHAUHAN; DHINGRA; KOUSARCHAUHAN, 2022).

The modes of action of allelochemicals may include inhibition of germination and cell division, reduction in mineral uptake, interference with enzyme activity, root growth, cellular respiration, and protein synthesis (JABRAN, 2017). In this sense, studies involving allelopathic activity are of great importance for the agricultural sector since many of the chemical substances can be used as an alternative in the control of weeds and, consequently, in the reduction of agrochemicals with the property to inhibit germination and growth underlying plant growth (SEMERDJIEVA *et al*., 2022). Furthermore, they constitute an option as natural herbicides because they have a specific effect and a lower environmental impact (PARRENO *et al*., 2020).

Thus, allelopathy emerges as a sustainable and ecologically correct alternative, in addition to being a natural technique that does not need the use of complex technology sophisticated equipment. It additionally offers a low-cost, non-polluting approach (OLIVEIRA *et al*., 2020). It can be used in different ways, alone or combined with doses of synthetic herbicides, soil incorporation, crop rotation, intercropping, or vegetation cover (ASLAM *et al*., 2017; MENNAN *et al*., 2020).

Popularly known as “cassava”, “macaxeira” or “aipim”, *Manihot esculenta* Crantz is a species of the Euphorbiaceae family, originating in South America, and is one of the main energy foods for more than 700 million people, mainly in developing countries (EMBRAPA, 2023). Brazil contributes 5.7% of world production as the fourth largest producer in the world, preceded by Nigeria, Thailand, and Indonesia (ABAM, 2022). It is an important world agro-industrial crop, whose roots are used in the manufacture of food products. The leaves have phenolic compounds such as gallic acid, rutin, kaempferol, and apigenin (JAMPA *et al*., 2022) as well as other flavonoids such as myricetin-3-O-rutinoside, robinin, hyperoside, nicotiflorin, and narcissin (TAO *et al*., 2019).

Some studies have demonstrated the allelopathic activity of species of the Euphorbiaceae family, such as *Ricinus communis* L. (PARRENO *et al*., 2020; LOPES *et al*., 2022; FERRAZ *et al*., 2023), *Hevea brasiliensis* (ROCHA *et al*., 2021), *Euphorbia hypericifolia* L. (NDAM *et al*., 2021), *Croton heliotropiifolius* Kunth (OLIVEIRA *et al*., 2022), and *Manihot esculenta* Crantz (TAUPIK *et al*., 2022). However, information is still scarce and does not include studies on forage species used as a food supplement for animals or green manure (biological fixation). In this context, the objective was to evaluate the allelopathic activity of *Manihot esculenta* leaves on seed germination of two forage species of great economic importance (*Avena sativa* - white oat, and *Trifolium resupinatum* - Persian clover).

MATERIAL AND METHODS

To carry out the experiments, the leaves of *Manihot esculenta* collected in the afternoon were initially dried in an oven and ground in a blender for approximately 2 minutes at the rate of 10 g of dry leaves to 100 mL of distilled water, which was considered the 100% (p/v) crude extract, adapted from Lima *et al*. (2020). The mixture remained at rest for 24 hours (in the dark and refrigerated at 5 ºC) and was then filtered through filter paper. The extracts were composed of the following concentrations: T1: 0, T2: 25, T3: 50, T4: 75, and T5: 100%, prepared with distilled water (Figure 1). The pH of the aqueous extracts corresponded to 5.3 (T1), 5.2 (T2), 5.2 (T3), 5.1 (T4), and 5.1 (T5), with no significant difference in pH values between extracts.

Figure 1 - Schematic representation of the preparation of *Manihot esculenta* leaf extracts: filtering the extract diluted in water (A) and ready-made extracts (B)

Source: Authors

To evaluate the allelopathic effect on germination of white oat (*Avena sativa*) and Persian clover (*Trifolium resupinatum*), four repetitions of 50 seeds were distributed on germitest paper moistened with distilled water or extract, being made into rolls (or in gerbox boxes according to the species) that were stored in a BOD chamber (Biochemical Oxygen Demand) at a temperature of 20 °C, with a photoperiod of 12 hours. Counts were performed according to Brasil (2009).

The length and dry mass of seedlings were measured according to methodology proposed by Krzyzanowski et al. (2020), in which four replications of 20 seeds were sown in two rows in the upper third of the germitest paper and maintained under the same conditions as in the germination test. On the 5th (white oat) and 7th (Persian clover) days after sowing, the average lengths of 10 normal

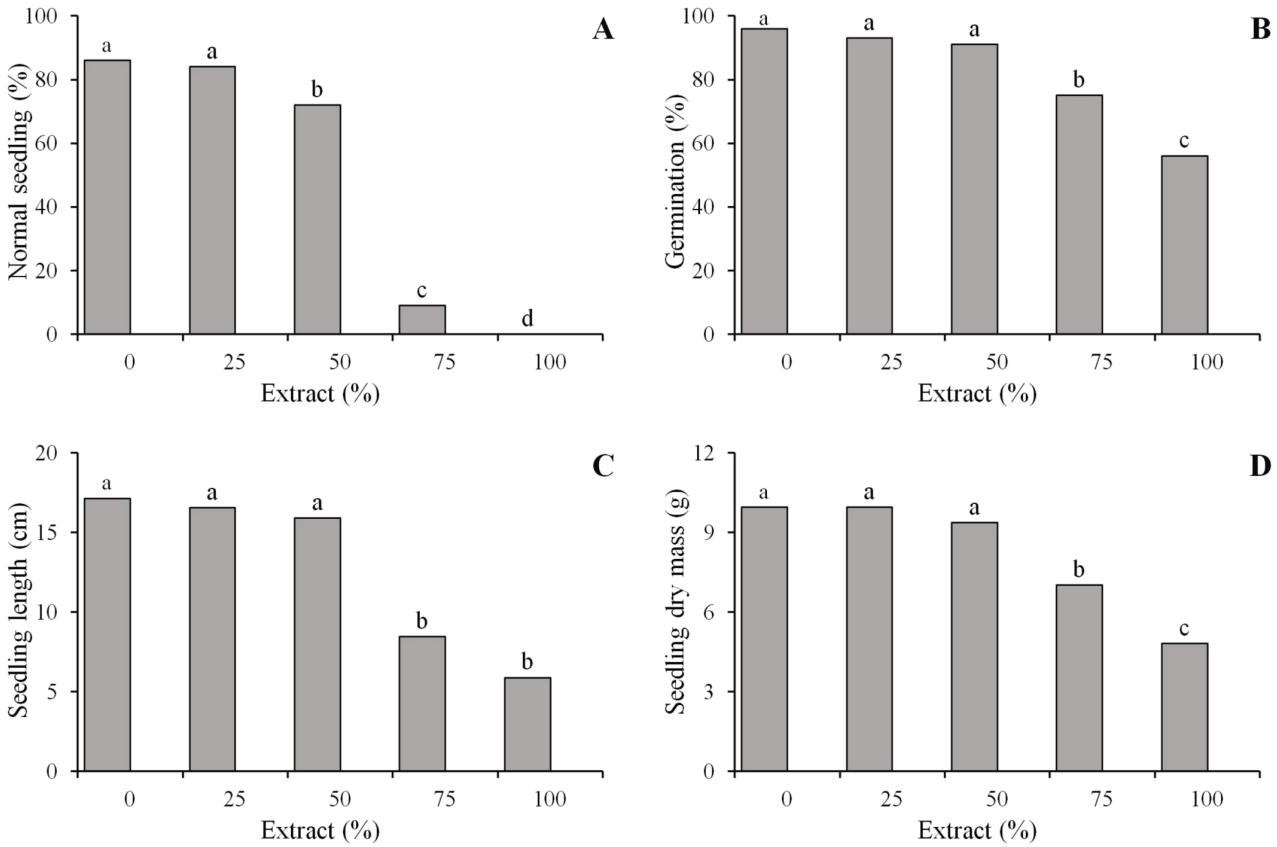
seedlings of each replication were measured. After drying the material in a forced ventilation oven at 60 ± 5 °C for 48 h, the seedlings were weighed on a precision scale (0.001 g), determining their dry mass.

The test was in a completely randomized design, with the treatment consisting of different extract concentrations (for each species), with the data analyzed using the Sisvar software (version 5.6).

RESULTS AND DISCUSSION

Analysis of variance showed that when white oat seeds were submitted to different extracts of *M. esculenta* leaves, there was a reduction in the percentage of normal seedlings from 25% (Figure 2A). Furthermore, a significant decrease in germination, length, and dry mass of seedlings was observed from 50% extract (Figures 2B-2D).

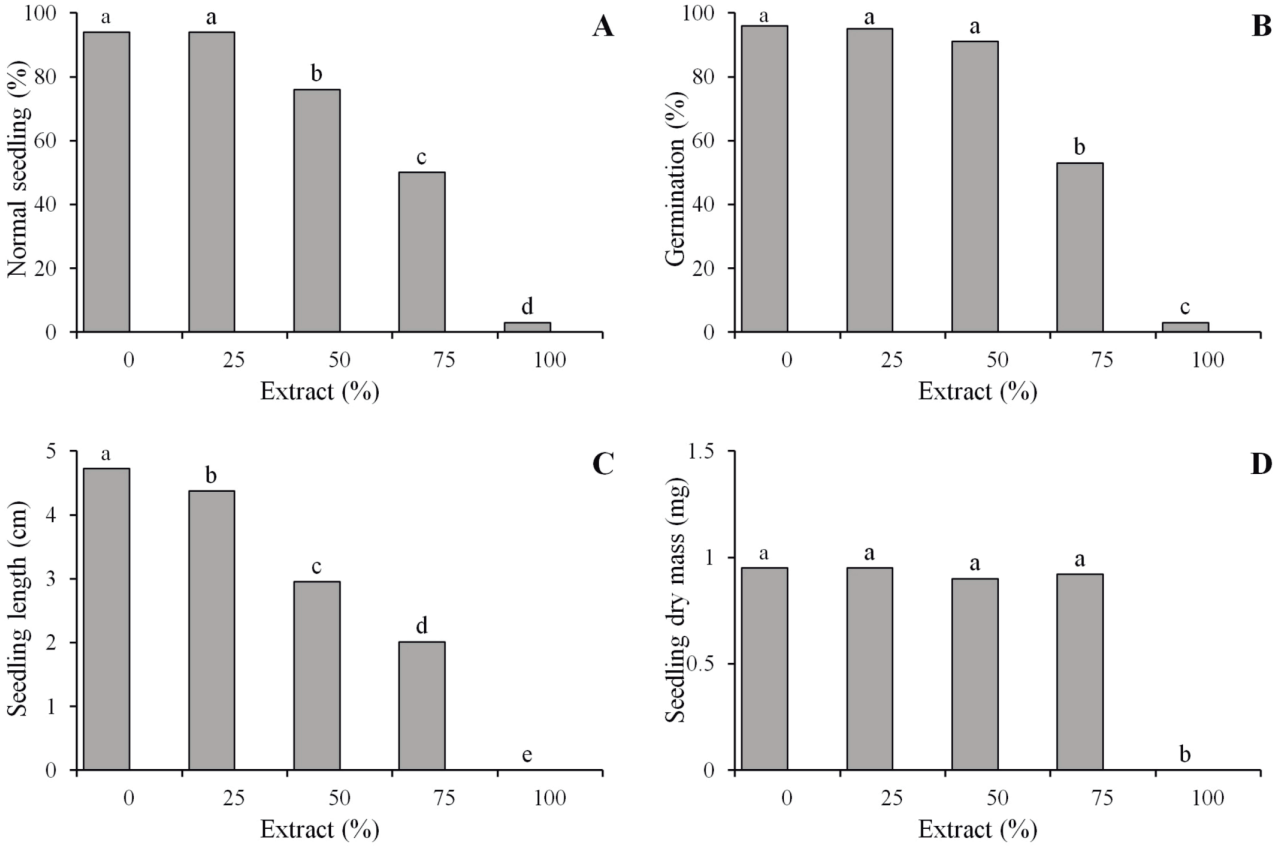
Figure 2 - First germination count (A), germination (B), total length (C), and dry mass (D) of white oat seedlings under different concentrations of *Manihot esculenta* extracts. Means followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability



Source: Authors

For Persian clover, exposure to cassava extract resulted in reduced germination and growth parameters (Figures 3A-3D), with effects that depend on concentration. The null values obtained in the treatment with 100% extract indicate that the seedling stopped growing, which may have led to impairment of the seedling's metabolic activities, resulting in a decrease in its total length (Figure 3C).

Figure 3 - First germination count (A), germination (B), total length (C), and dry mass (D) of Persian clover seedlings under different concentrations of *Manihot esculenta* extracts. Means followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability



Source: Authors

The results of this study confirmed that *M. esculenta* extract has a negative effect on germination and initial growth of white oats and Persian clover, and the allelopathic effects are concentration-dependent (Figure 4).

Figure 4 - Seedlings of white oat (A) and Persian clover (B) in different concentrations of *Manihot esculenta* extract (0, 25, 50, 75, and 100%)

Source: Authors

Similar results were observed in *Eleusine indica*, *Ageratum conyzoides*, and *Cyperus distans*, where the aqueous extract of cassava leaves inhibited germination and seedling growth (TAUPIK *et al*., 2022). In addition, in other studies, *Hevea brasiliensis* extract affected the number of germinated seeds and cellular respiration in lettuce (ROCHA *et al*., 2021), and castor bean extracts (seeds, fresh and dry leaves) showed allelopathic effects on germination and inhibition of root length in tomato and maize (PARRENO *et al*., 2020).

Additionally, aqueous extracts of *Ricinus communis* inhibited seed germination and seedling growth of *Bidens bipinnata* (LOPES *et al*., 2022). The germination and the germination speed index were affected by the extracts, and the hypocotyl length showed an exponential decline, with total inhibition from 15 g L^{-1}. In other studies, emergence and shoot and root length of *Cyperus rotundus* were reduced in the presence of *R. communis* leaf extracts (FERRAZ *et al*., 2023).

Analyzing leaf, root, and stem extracts of *Euphorbia hypericifolia* (100%), Ndam *et al*. (2021) observed inhibitory effects on germination, shoot, and root lengths of *Zea mays*, *Bidens pilosa*, *Lactuca sativa*, *Lycopersicon esculentum*, and *Amaranthus spinosus*. Nonetheless, Santos *et al*. (2023) verified that the allelopathic potential of *Portulaca oleracea* and *Raphanus raphanistrum* extracts (0 to 10 g L^{-1}) on lettuce seed germination was dependent on the concentration and type of extract. On the other hand, *Croton heliotropiifolius* Kunth leaf extract (0.1 to 1 mg mL^{-1}) demonstrated a beneficial allelopathic effect, as it stimulated radicle development and did not delay the germination process of lettuce seeds (OLIVEIRA *et al*., 2022).

It is assumed that allelopathy is related to a set of allelochemicals and not to a single compound (SZWED *et al*., 2020). These compounds interfere with neighboring plants and may cause positive physiological effects when in lower concentrations or negative ones such as germination inhibition and growth reduction (MASUM *et al*., 2018; LI *et al*., 2019). Furthermore, the compounds present in the extracts decrease the percentage of germinated seeds because they have an osmotic potential in the absorption range, which affects germination and cell division, with the most pronounced effects in treatments with the highest concentrations (EL-MERGAWI; AL-HUMAID, 2019*; ŠOLN; KLEMENČIČ; KOCE*, 2022).

The chemical compounds present in plant extracts, mainly phenolic compounds and flavonoids (YI *et al*., 2011; GAZOLA *et al*., 2019), are absorbed by the roots, affecting processes such as stomatal opening and closing, respiration, and photosynthesis, resulting in wilting and stunted growth (KANNAN; PALAYIAN, 2022). Root growth is more vulnerable than other organs as it is the first to come into contact with the allelochemical (ISLAM *et al*., 2019). The emergence of shorter roots may be a consequence of direct growth inhibition, slow germination, and developmental delay due to the presence of allelochemicals (*ŠOLN; KLEMENČIČ; KOCE*, 2022).

Finally, the present study revealed that increasing concentrations of *M. esculenta* extracts inhibited germination and seedling growth of white oat and Persian clover, suggesting that these

allelopathic effects are concentration-dependent. This information is extremely important for decision-making regarding the cultivation of these species in environments where there are plant remains of cassava. Thus, as an adequate management practice, it is recommended that cassava leaves be removed from the field before sowing, in order to avoid a negative influence on growth. Beyond that, more studies are needed to verify the efficiency of these extracts in the emergence of seeds in the field and to evaluate the viability of their use as raw materials for the production of natural herbicides to be introduced in the management of unwanted plants. A better understanding of allelopathic effects will help in choosing the appropriate management, helping agricultural production, and preventing future damage.

CONCLUSION

Manihot esculenta extracts negatively influence the seed germination and the initial growth of white oat and Persian clover. Thus, it is recommended that cassava leaves be removed from the field before sowing in order to avoid a negative influence on the initial growth of these species.

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