Capter 180

Effect of aqueous extract of leaves of *Piper aduncum* L. (PIPERACEAE) in the early development and cell cycle of Lactuca sativa L. (Lettuce)

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ABSTRACT
Piperaceae is a family of basal angiosperms comprising about 3,615 species distributed in a pantropical form. In Brazil, there are about 458 species distributed in four genera, *Piper* L. and *Peperomia* Ruiz, and Pav. the most representative. *Piper* comprises shrubs and subshrubs. The best-known species of the genus is the black pepper (*P. nigrum* L.), a spice used for many years. Representatives of the genus are often used in folk medicine and the manufacture of medicines, as well as in the production of insecticides due to the properties of their essential oils. Among the most varied uses, oils can play an allelopathic or mutagenic role, and these are the objects of recent studies in several aromatic plants. Certain doses of these oils can inhibit or accelerate the growth of other vegetables. In addition, they can cause cellular changes that can lead to death. Based on this, this study aims to determine the concentrations of P. *aduncum* L. leaf extract that plays an allelopathic role and toxic gene cite in a model organism. The extract of *P. aduncum* was prepared from the leaves of 3 individuals, prepared at concentrations of 100 mg mL-1, 50 mg mL-1, 25 mg mL-1, and 12.5 mg $^{mL-1}$. The design was completely randomized, with 5 treatments and 5 replications. Distilled water was used as a negative control. For allelopathy analysis, the following parameters were evaluated: germination speed index (GVI); germination percentage, root growth (CR), and aerial growth (AC). For the cytogenetic analysts, slides of the roots collected and fixed in ethyl alcohol: acetic acid by the crushing technique were prepared. The results show that the aqueous extract of *P. aduncum* leaves had an inhibitory effect on the germination and development of *Lactuca sativa* seeds, besides causing cellular changes such as bridges, C-metaphase, and chromosome loss.

Keywords: Allelopathy, Bioassay, Mutagenesis, Medicinal plant.

1 INTRODUCTION

Piperaceae Giseke is a family of basal angiosperms that gathers approximately 3,615 species (STEVENS, 2001). In Brazil, the occurrence of 458 species distributed in four genera is estimated; *Manekia* Trel. (1 ssp), *Peperomia* Ruiz & Pav. (166 ssp), *Piper* L. (290 ssp.) and *Ottonia* Spreng. (1 ssp.), (GUIMARÃES et al., 2015), and *Piper* and *Ottonia* are considered synonymous.

The representatives of the genus *Piper* L. are easily recognized in the field given their characteristic morphology. They have shrubby size, with the knot between the well-marked knot and hairiness in the branches (JUDD et al., 2009). Its leaves have a constantly asymmetrical base (SOUZA; LORENZI, 2012).

The flowers are tiny, acclaimed, and bisexual arranged on ears (SOUZA; LORENZI, 2012; JUDD et al., 2009).

The seeds are used as a condiment (MEDEIROS, 2006), while the leaves are used in the treatment of diarrhea, pain in general, fever, and stomach problems (SILVA, 2002). The leaves of *Piper marginatum* Jacq. and *P. peltatum* L. are attributed to liver properties (GUIMARÃES; GIORDANO, 2004). In vitro assays with extracts of *Piper tuberculatum* Jacq. demonstrated the efficiency of its active principles for the treatment of *Schistosoma mansoni* (SIMÕES, 2009). The extract of *Piper hispidum* Sw. demonstrates insecticidal activity against *Hypothenemus hampei* Ferrari 1867 popularly known as "coffee borer" (SANTOS et al., 2010). These effects are attributed to the presence of bioactive secondary metabolites found in essential oils or extracts of these plants.

Essential oils, in general, arouse great interest in the production of medicines, perfumes, foods, and folk medicine (SOUZA FILHO et al., 2009). There is also the possibility of use in agriculture, such as insecticides, fumigants, or fungicides (SOUZA FILHO et al., 2009). Among the genera of the family Piperaceae, *Piper* L. is one of the best known of the family given its economic and pharmacological properties (JUDD et al., 2009) attributed to the use of its essential oils.

Essential oils can still be used as potent inhibitors of seed germination and the development of plant species presenting phytotoxic effects, or allelopathic (SOUZA FILHO et al., 2009). The interest in allelopathy has been growing exponentially in recent decades (ALVES et al., 2018; ALVES et al., 2021; VASCONCELOS et al., 2022), due to the discovery of natural herbicides that act directly on weeds and do not cause damage to the environment (TAIZ & ZEIGER, 2004). It can be defined as a process where the metabolic products of a certain species influence the metabolism of another species, either in the germination or in the growth of the nearby plant (SOARES, 2000).

Some species are extremely sensitive to allelopathy, such as *Lactuca sativa* L. (lettuce), *Lycopersicon esculentum* Miller (tomato), and *Cucumis sativus* L. (cucumber) (ALVES et al., 2004). These plants are considered indicators of allelopathic activities, being used as models in scientific experiments.

Alves et al. (2004) demonstrated the allelopathic potentialities of volatile extracts of essential oils of cinnamon, pepper rosemary, citronella grass, and carnation alfavaca under the germination and development of *Lactuca sativa* L., similar results were found for allelopathic analysis of *P. tenuistylum C.DC* EXTRACT (PAOLO, 2013). The allelopathic effects can still be reflected in the cells, where it is possible to see the action of the compounds directly in the cell cycle of the model species.

Piper aduncum L is a shrub with wide distribution in the Atlantic Forest (GUIMARAES et al., 2015). Its leaves are often used in traditional communities due to their pharmacological effects such as astringent, digestive stimulant, diuretic, sedative, laxative, antiseptic, and hemostatic (FAZOLIN et al., 2006). It also presents efficiency in the fight against *Aetalion* sp., inducing mortality of up to 80% of insects, probably due to the presence of phenylpropanoids (SILVA et al., 2007). Given the above, this work aimed to evaluate the

allelopathic and mutagenic effect of the aqueous extract of leaves of *Piper aduncum* L. (Piperaceae) using *Lactuca sativa* L. (lettuce) as a model for germination analysis, plant development, and cell cycle.

2 MATERIAL AND METHODS

Preparation of the extract

For the preparation of the extract, leaves of the species *Piper aduncum* L. (Piperaceae) (Figure 1) were collected in the Mata das Flores State Park. The leaves were placed in the oven at 60ºC for 48 hours. After this period, the leaves were crushed to prepare the aqueous extract at concentrations of 100 mg mL-1; 50 mg mL-1; 25 mg mL-1, and 12.5 mg $^{mL-1}$. Distilled water was used as a control.

Biological assay

Lactuca sativa L. seeds were used as a plant model. Germination was performed in Petri plates of 9 cm in diameter, lined with filter paper, and watered with *Piper aduncum extract*. Reverse osmosis water was used as a negative control. The plates were sealed with film paper and added to a germination chamber (BOD) at 24°C, where they were stored during the experiment (ALVES et al., 2021).

The experiment was conducted in a Completely Randomized Design, and each treatment consisted of five replications with 25 seeds each, totaling 25 Petri dishes and 625 seeds. The germination process was analyzed in periods of 8 every 8 hours for 48 hours. The germination speed index (GSI) and the germination percentage (Ger) were evaluated. After 48 hours, the root length of each seed was analyzed to determine root growth (GR). After 120h, the aerial parts of the seedlings were measured to determine the air growth (GA). The data were submitted for analysis of variance and the means compared with Dunnett's test at 5% significance (ALVES et al., 2021).

The roots were collected and fixed in ethyl alcohol: acetic acid (3:1), after 48h of exposure and stored at -4ºC for 24 hours. The roots were submitted to cytogenetic analysis through the crushing technique stained with acetic orcein 2% (ANDRADE-VIEIRA et al., 2014).

About 10,000 meristematic cells per treatment were analyzed, and the different phases of mitotic division, and possible chromosomal and nuclear alterations, were observed and quantified.

The mitotic index (MI) was obtained by dividing the number of dividing cells (prophase, metaphase, anaphase, and telophase) by the total number of cells analyzed in each treatment. The same process was done for the evaluation of chromosomal alterations. The frequency of nuclear changes and individual chromosomal changes obtained through the division of the number of cells with changes by the total of cells in division (ANDRADE-VIEIRA et al., 2014).

All data collected were submitted to ANOVA (analysis of variance) and Dunnett's Test ($p \leq 0.05$) by the Genes software (CRUZ, 2013).

Figure 1. *Piper aduncum* L. Field image and exsicata. Source: Guimarães et al (2015).

3 RESULTS AND DISCUSSION

The aqueous extract of the leaves of *Piper aduncum* L. showed allelopathic and/or mutagenic effect in most treatments, which is dose-dependent (Figure 2), the higher the concentration of the extract, the greater the effect of this on the seeds of *Lactuca sativa* L. The treatment with 1 00 mg mL-1 of extract completely inhibited seed germination, making it impossible to evaluate the proposed variables and showing the strong effect of the extract (Figure 3).

Figure 2. Germination of seeds of *Lactuca sativa* L. submitted to the aqueous extract of *leaves of Piper aduncum* L. Where a – represents the treatment of 50 mg mL-1 of extract; b – represents the treatment of 25 mg mL-1 of extract; c – represents the treatment of 12.5 mg $^{mL-1}$ of extract; d – represents the negative control (water).

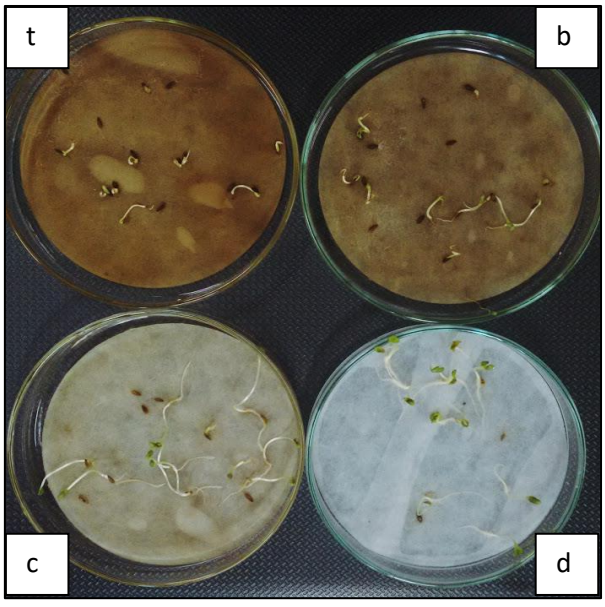
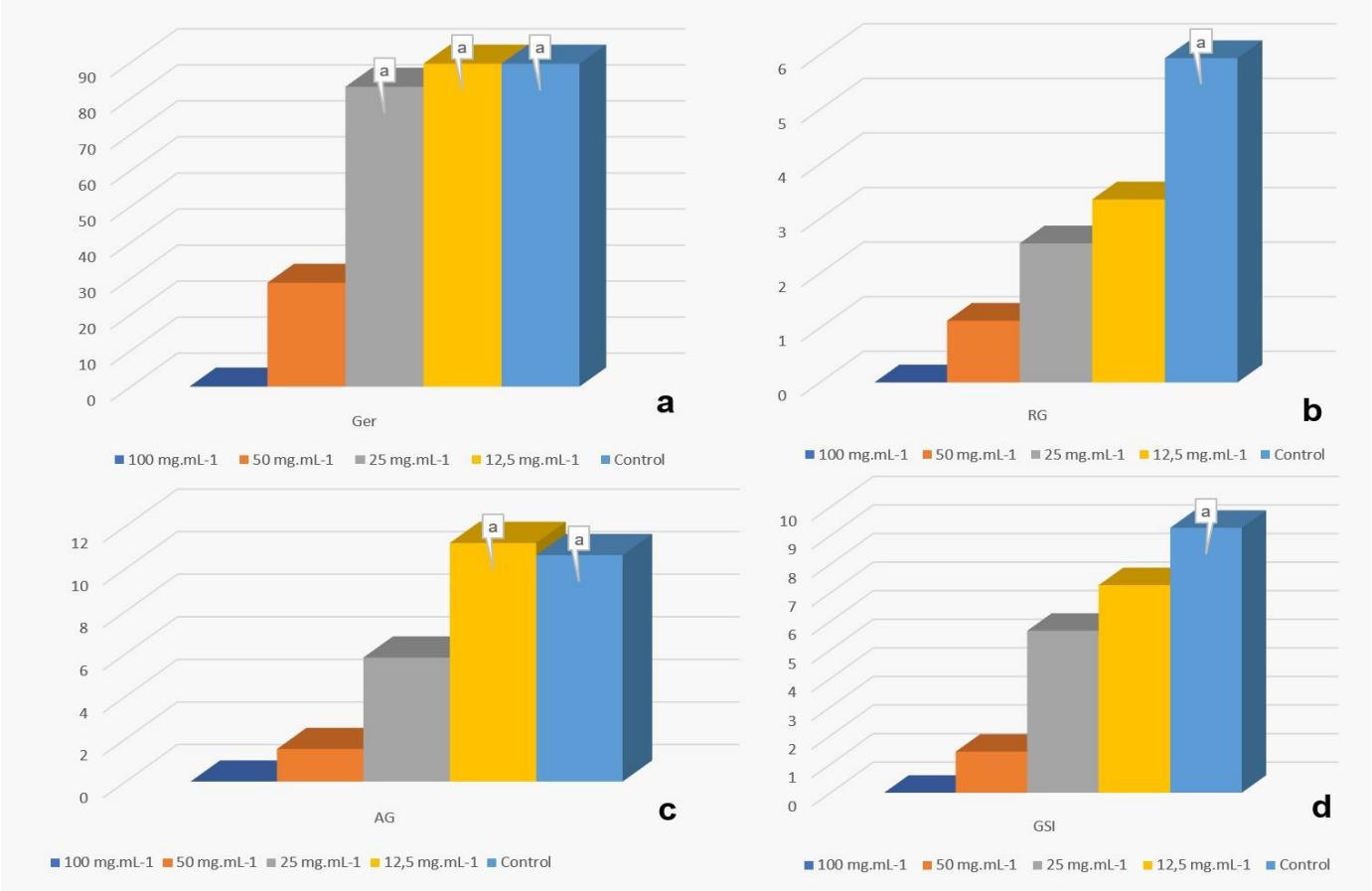


Figure 3. Phytotoxicity of the aqueous extract of *Piper aduncum* on the initial development of *Lactuca sativa* L. Where a – are the data for the germination variable (Ger); b – are the data for the variable root growth (GR); c – are the data for the air growth variable (AG); d – are the data for the variable germination speed index (GSI). The médias followed by the same letter do not demonstrate significant differences by Dunnett's test at 5% significance.



For the effect on the germination of *Lactuca sativa L. seeds*, it is observed that the extract of leaves of *Piper aduncum* L. presented a significant allelopathic effect on lettuce seeds when submitted to treatment with 50 mg $^{mL-1}$ presenting 28.8% germination (Figure 3). For the GR variable, all treatments showed significant differences when compared to the control treatment.

For the variable AG, the treatment with 12.5 mg $^{mL-1}$ of aqueous extract did not differ from the control, presenting 5.4% growth, while the two major treatments showed significant differences when compared to it. For the GSI variable, all treatments differed significantly from the control (Figure 3).

The effects of the components of secondary plant metabolism on seed germination and early development are often explained in individual terms of some of the main constituents (SOUZA FILHO et al., 2009). The phytochemistry of the genus *Piper* L. presents more than 600 chemical constituents of the class of bioactive components, such as alkaloids, amides, and propenylphenols (FAZOLIN et al., 2006).

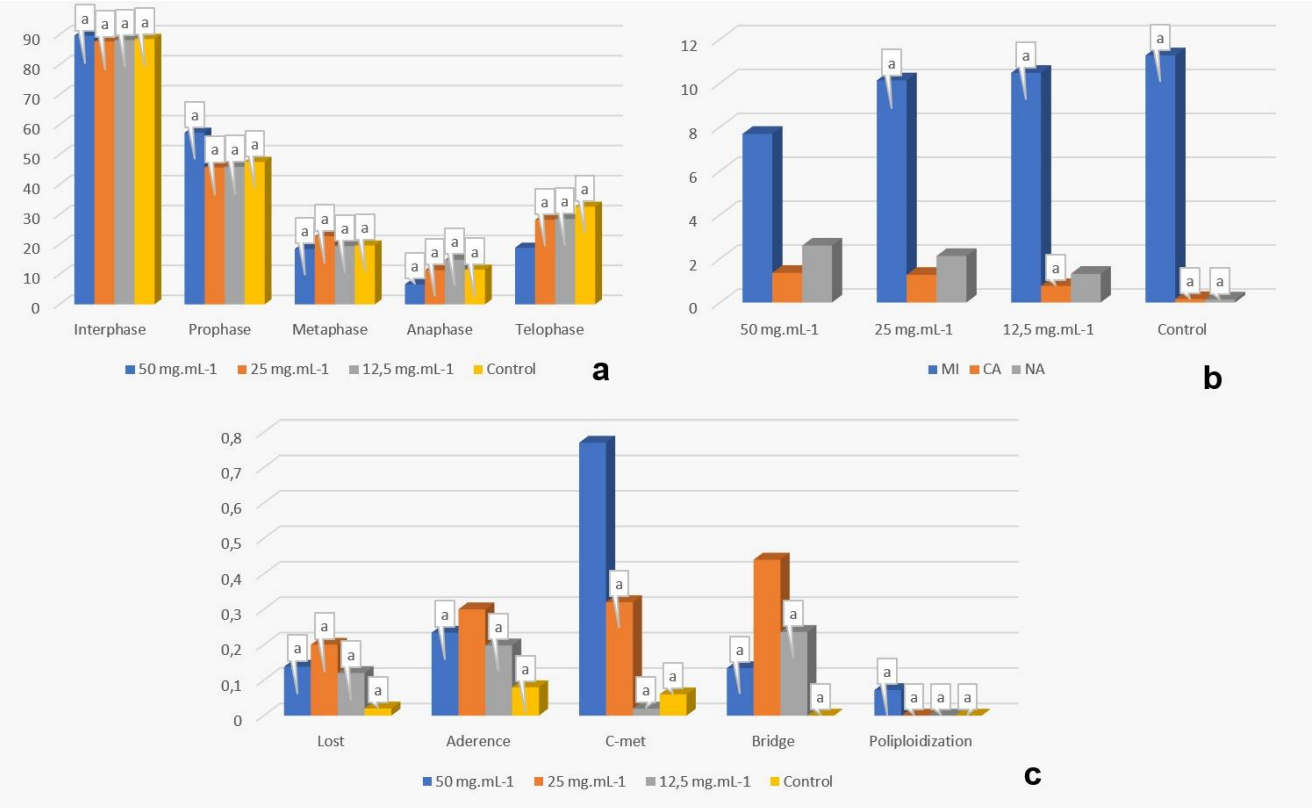
For *Piper aduncum*, several classes of compounds are characterized, such as benzoic acid prenilates, oxygenated monoterpenes and sesquiterpenes, chromenes or benzopyrans, flavonoids, alkaloids and phenylpropanoids (SOUZA FILHO et al., 2009). Souza-Filho et al., (2009), suggest that the allelopathic activity of *Piper* species extract is related to a large number of monoterpenes, oxygenated monoterpenes, and sesquiterpenes.

This species is also effective in controlling animal diseases, such as bovine mastitis, and vegetables, such as bacterial burning, the rot of stems and various roots, and passion fruit warts, among others (FAZOLIN et al., 2006). However, little is known about the beneficial effects of this extract given its lethal activity in most cases of pest insect affection (FAZOLIN et al., 2006).

Results similar to those found in the present study were found by Lustosa et al., (2007) and Machado et al. (2012), evaluating the extract of *P. aduncum* on the seeds of *Mimosa pudica*, *Senna obtusifolia* (Mata pasto) and *Lactuca sativa* L. (lettuce), and the higher the concentration of the extract, the greater the number of ungerminated seeds. Silva et al. (2012) determined the allelopathic effect of *P. hispidinervum* C.DC. on lettuce seeds, data that corroborate with Souza-Filho et al. (2009), evaluating the extract of the same species on the growth and germination of weeds.

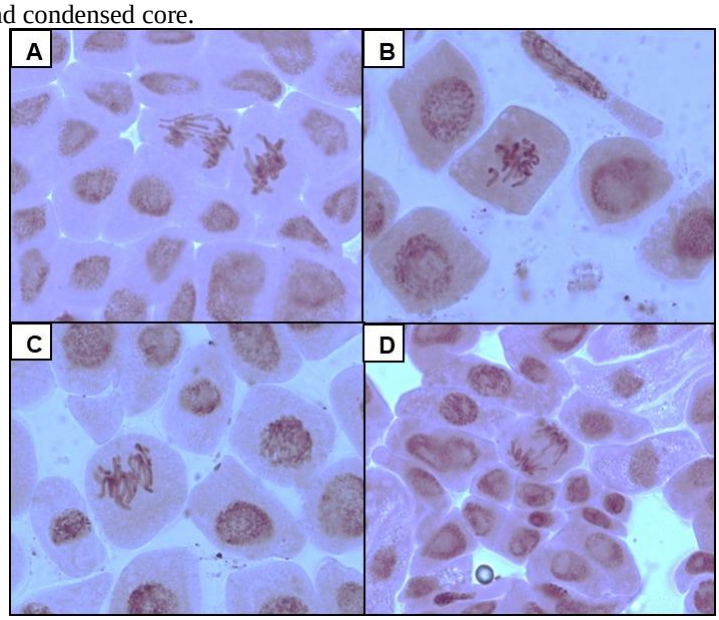
Regarding the cytogenetic analyses,p ara the treatment with 100 mg $^{mL-1}$ of the extract is not possible to perform analysis due to the absence of germination in the fit of the toxic experiment. The results are shown in Figure 4.

Figure 4. I cite the generous toxicity of the aqueous extract of *Piper aduncum* in meristematic cells of *Lactuca sativa* (lettuce). Where: a are the variables of the cell cycle phases (inertia, prophase, metaphase, anaphase, and telophase); b – are the variables mitotic index (MI), chromosomal changes (CA), and nuclear changes (NA); c – are the frequencies of each type of AC (lost, adherence, c-metaphase, bridge, and polyploidization). Which followed by the same letter do not demonstrate significant differences by Dunnett's test at 5% significance.



For the treatment with 50 mg mL-1 of extract analysis of meristematic cells of the roots of *Lactuca sativa* L. demonstrated that the variables telophase, MI, C A, NA, and C-met (Figure 4c) showed significance concerning the control. The MI decreased significantly in the control, that is, the roots treated with an aqueous extract of *P. aduncum* showed a decrease in dividing cells.

Figure 5. Cellular changes caused by *Piper aduncum* extract on *Lactuca sativa* cells. A- C. adherent and bridge; B- C. lost; C- adhesion points; D- Bridge and condensed core.



MI is an important parameter in the study of cytotoxicity. Its inhibition means the existence of a smaller amount of cells in mitosis. This reduction may be due to changes arising from contact with the test agent, being a way for the individual to minimize contact with the substance (ALVES et al., 2021).

Evaluating the effect of *Hymenea coubaril* L. extract on the growth of *Allium cepa* and *Lactuca sativa*, Solano et al. (2015) found irregularities in the stages of mitosis, considering the genotoxic species, as it presented irregularities in the process of cell division in the different phases in the different concentrations.

For the treatment with 25 mg mL-1, the variables CA, NA, adhesion, and bridge (Figure 4a)d were significant concerning the control, while for the treatment with 12.5 mg mL-1, only the variable NA showed significance.

Similar results were found for the species Peperomia pellucida, Piper marginatum, and *Piper hispidum* suggesting that the extracts of all species are considered toxic, being *Peperomia pellucida* the most toxic when compared to the others (DANTAS and PEREIRA n.d. Evaluating the mutagenicity of *Piper methysticum* L., Palioto and Rocha (2013) found cellular changes in *Aspergillus nidulans* when exposed to the extract of the species. The author noted that there was a reduction in the mitotic index about the control in all treatments with the extracts of the plants tested.

NA are changes that occur in the cell nucleus, resulting from changes in its morphology and biochemistry. These changes can represent different actions of the test agent. The presence of micronucleus-type NA, for example, represents a direct action on the DNA molecule that ultimately results in the

breakdown of the molecule causing the formation of micronuclei. On the other hand, the observation of condensed nucleus NA is the cytological proof that the cell is in the process of cell death (ALVES et al., 2018).

On the other hand, AC represents alterations: (a) directly in the DNA, when of the clastogenic type, such as the AC of the bridge type; (b) in the mitotic spindle formation machinery, when anegenic, such as c-metaphase and adherence CAs (VASCONCELOS et al., 2022).

Thus, the aqueous extract of *P. aduncum* has allelopathic activity, determining cytological changes. It has both aneugenic and clastogenic mechanisms of action since it has determined changes in spindle formation and DNA molecules.

4 CONCLUSION

It was found that the concentrations of *Piper aduncum* L extract. negatively influenced the germination rate of *Lactuca sativa* L seeds. in all concentrations, and as the concentration of the aqueous extract increases, the percentage of non-germinated cells increases. However, the extract of *Piper aduncum* has a direct influence on seed germination.

Cellular changes were found in the cells treated with *P. aduncum* extract, and the treatment with 50 mg $^{mL-1}$ of extract differed the most from the control treatment.

Due to the wide use of *Piper* L. species in folk medicine, this work suggests the determination of mutagenic effects for the main species of the genus. In addition to the regularized implementation of the use of these species with competent bodies.

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