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PRE- AND POST- EMERGENCE CONTROL OF *Hovenia dulcis* WITH EXTRACTS OBTAINED FROM PEPPER (*Capsicum baccatum*)

Mylena Gonçalves Anchieta<sup>1</sup>, Gianlucca Pigatto<sup>1</sup>, Jéssica Streck Baisch<sup>1</sup>, Bianca Motta Dolianitis<sup>1</sup>, Paulo Carteri Coradi<sup>2</sup>, Jerson Vanderlei Carús Guedes<sup>3</sup>, Marcio Antonio Mazutti<sup>4</sup>, Marcus Vinícius Tres<sup>1</sup>, Giovani Leone Zabot<sup>1</sup>\*

1 - Federal University of Santa Maria, Laboratory of Agroindustrial Processes Engineering, Cachoeira do Sul, Rio Grande do Sul, Brazil

2 - Federal University of Santa Maria, Department of Agricultural Engineering, Cachoeira do Sul, Rio Grande do Sul, Brazil

3 - Federal University of Santa Maria, Department of Plant Protection, Santa Maria, Rio Grande do Sul, Brazil

4 - Federal University of Santa Maria, Department of Chemical Engineering, Santa Maria, Rio Grande do Sul, Brazil

| Keywords:  | ABSTRACT  |  |  |  |  |
|--|---|--|--|--|--|
| Ultrasound-assisted extraction<br>Vegetable extracts<br>Germination inhibition | This study evaluated the efficiency of extracts obtained from <i>Capsicum baccatum</i> by ultrasound-<br>assisted extraction in the control of <i>Hovenia dulcis</i> , which is an invasive species popularly<br>known in Brazil as "uva-do-japão". The effects of extraction time $(20 - 60 \text{ min})$ , maximum<br>power fraction $(50 - 100\%)$ , and pulse cycle $(0.3 - 0.7)$ were investigated on the activities of<br>extracts. The pre-emergence assays demonstrated inhibition of 45-100% on the 14 <sup>th</sup> day. In<br>the post-emergence, the root and shoot lengths were 3.0 and 2.6 times smaller than the control<br>(without application of extract). The fresh mass and dry mass were 4.7 and 4.2 times lower than<br>the control. All these best results were found in the assay with higher extraction time (60 min)<br>and power fraction (100%), and lower pulse cycle (0.3). Chlorophyll a and b and carotenoids<br>ranged from 0.84 to 1.15, 0.19 to 0.35 and 0.32 to 0.40 mg/g fresh mass, respectively. Overall,<br>it is demonstrated that extracts from <i>Capsicum baccatum</i> presented an inhibitory effect on the<br>germination of <i>Hovenia dulcis</i> . Although the extract did not completely inhibit germination<br>or cause the death of seedlings, it presented an effect against the normal development of<br>seedlings, including the roots and shoots, thus allowing it to be used as a control strategy before<br>germination or for small plants of this invasive species. |  |  |  |  |
| Palavras-chave:<br>Plantas exóticas invasoras                                  | CONTROLE EM PRÉ- E PÓS-EMERGÊNCIA DE Hovenia dulcis COM EXTRATOS<br>OBTIDOS DE PIMENTA (Capsicum baccatum)  |  |  |  |  |
| Extração assistida por ultrassom   | RESUMO  |  |  |  |  |
| Extratos vegetais<br>Inibição da germinação                                    | Este estudo avaliou a eficiência de extratos obtidos de <i>Capsicum baccatum</i> por extração assistida<br>por ultrassom no controle de <i>Hovenia dulcis</i> , a qual é uma espécie invasora popularmente<br>conhecida no Brasil como "uva-do-japão". Os efeitos do tempo de extração ( $20 - 60 \text{ min}$ ),<br>fração de potência máxima ( $50 - 100\%$ ) e ciclo de pulso ( $0,3 - 0,7$ ) foram investigados nas<br>atividades dos extratos. Os ensaios de pré-emergência demonstraram inibição de 45-100%<br>no 14º dia. Na pós-emergência, os comprimentos de raiz e parte aérea foram 3,0 e 2,6 vezes<br>menores que o controle (sem aplicação de extrato). A massa fresca e a massa seca foram 4,7<br>e 4.2 vezes menores que o controle. Todos esses melhores resultados foram encontrados no   |  |  |  |  |

### INTRODUCTION

Human exploitation of plant species and appropriation of land and water have significantly increased the rates of native species extinction. Human activities have increased immigration rates, deliberately or accidentally transporting and introducing many species into areas beyond normal biogeographical barriers to their spread, where they can establish viable populations (termed invasive aliens) (Bellard et al., 2016). Invasive species, once introduced from other environments, start to reproduce and use the space of native species. It can cause changes in natural ecological processes, becoming dominant after the time required for adaptation (Ziller et al., 2019). One example of invasive species is Hovenia dulcis, popularly known in Brazil as "uva-do-japão". It presents regeneration by seeds, and its pedicel (a pseudofruit) is consumed by animals, thus easily dispersing the seeds (Sehn et al., 2021). In this sense, the search for new natural herbicides has become crucial to overcome the growing resistance of invasive species, such as Hovenia dulcis.

It is necessary to further explore the phytotoxic effects of plant extracts and fungi broth against weeds and invasive species (Alsharekh et al., 2022; Brun et al., 2022; Portela et al., 2022). In this sense, active compounds found in the genus Capsicum can inhibit the development of invasive species (García-Mateos et al., 2013). Studies involving the action of allelopathic compounds contribute to the formulation of herbicides and the genetic improvement of cultivated plants that naturally synthesize substances with allelopathic potential (Chiapusio et al., 2004). The genus Capsicum has been reported as a source of bioactive compounds that are used to control Callosobruchus maculatus in stored cowpea and Sitophilus zeamais (Motsch) in stored maize (Oni, 2011). Capsicum baccatum is popularly known in Brazil as "dedo-de-moça" hot pepper and is one of the most consumed peppers in Brazil. Concerning its cultivation, it has allelopathic potential, which can interfere with the planting of other crops, especially during germination and initial seedling growth (Aquino et al., 2019).

Extraction techniques should be used to obtain the target compounds with biological activities found in allelopathic plants. One example is the ultrasound-assisted extraction technique, which is based on the action of low-frequency mechanical waves. The cavitation, a phenomenon responsible for the formation and collapse of bubbles, plays an important role in the process of extracting solutes. The mechanical effects of ultrasound allow the penetration of the solvent into cellular materials and improve mass transfer due to the effects of microextraction. Furthermore, the reduction of particle size, mass transfer across cell membranes, shear forces and microjets are reported effects (Shirsath *et al.*, 2012). This technique is an alternative to extracting bioactive compounds from plants, as it increases the extraction yield, working at lower temperatures and for a shorter time than in conventional techniques such as Soxhlet extraction (Confortin *et al.*, 2019; Pereira *et al.*, 2021).

Based on this background, the objective of this study was to evaluate the allelopathic effects of extracts obtained from *Capsicum baccatum* using ultrasound-assisted extraction with water as solvent. Firstly, *Cucumis sativus* was tested as a biomarker plant and, thereafter, *Hovenia dulcis* was tested in pre- and post-emergence using such extracts.

### MATERIAL AND METHODS

### Raw material

Seeds of *Hovenia dulcis* were collected in a forest fragment of the Federal University of Santa Maria (Santa Maria, Brazil). After collection, the seeds were taken to the laboratory and manually separated from the fruit. The drying was performed at room temperature (around 25°C). The seeds were immersed in 75% sulfuric acid for 15 minutes and washed in running water for the dormancy breaking. Afterward, the seeds were stored in an airy and clean place at room temperature (around 25°C) until performing the assays.

The pepper (*Capsicum baccatum*) fruits were obtained in the local market (Santa Maria, Brazil). The selection was carried out by criteria of integrity and visual quality. The seeds were manually removed from the fruit and a mechanical crushing was done using a domestic blender to decrease the size and obtain a material with an average particle size close to 1 mm. Afterward, the ground material was stored in a refrigerator (7°C) until performing the assays.

#### Ultrasound-assisted extraction

The extractions were performed using an ultrasonic probe (Hielscher, UP 400S, Germany) positioned in the center of the extraction vessel.

The vessel contained 20 g of pepper pulp and 25.4 mL of distilled water. The temperature was adjusted to  $10^{\circ}C \pm 2^{\circ}C$  by circulating water through the extraction vessel jacket. The ultrasonic probe has a frequency of 40 kHz and a maximum power of 800 W. The effects of extraction time (20 - 60 min), the fraction of the maximum power (50 - 100%), and pulse cycle (0.3 - 0.7) (Confortin *et al.*, 2019) were evaluated through a 2<sup>3</sup> experimental design with triplicate at the central point on the performance of extraction of compounds with phytotoxic effects. After extraction, the samples were centrifuged at  $11.200 \times \text{g}$  for 5 min and the supernatant was carefully collected. The maximum ultrasound intensity was 85 W cm<sup>-2</sup>.

### Pre-emergence

Assays of pre-emergence were performed according to Confortin et al. (2017). Initially, the assays were performed in pre-emergence of Cucumis sativus. Afterward, when an effect on the biomarker plant was evidenced, the assays were performed in the pre-emergence of Hovenia dulcis. For the pre-germination tests, Gerbox boxes were used, which were previously disinfected with 70% ethanol. Two sheets of filter paper (Germitest®) moistened with the respective treatment were placed in each box to evaluate the effects of the extract on the seeds. The mass of the extract corresponded to a volume of 2.5 times the mass of the paper. Three replications were performed with 25 seeds for each treatment and placed in a BOD chamber (Solab, SL-2017, Brazil), with a constant temperature (25°C) and photoperiod of 12/12 hours of light/dark. A control assay (3 replications with 25 seeds) was performed with distilled water instead of extract.

Subsequently, these data were expressed as a percentage of germination inhibition or of abnormal plants (plants that did not have their normal growth) (Brun *et al.*, 2016). For *Cucumis sativus*, the first evaluation of the number of seeds germinated was carried out on the 4<sup>th</sup> day after the installation of the experiment (sowing date), followed by new assessments on the 7<sup>th</sup> and 10<sup>th</sup> days. For *Hovenia dulcis*, the evaluations were carried out on the 7<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> days because it is a specie that takes longer to germinate. All the seeds that germinated

and became normal, that is, the seeds that showed radicle growth higher than 2 mm, were counted as germinated.

### *Post-emergence*

Assays of post-emergence were carried out in a greenhouse in a controlled environment. Cultivations of *Hovenia dulcis* were carried out for subsequent application and evaluation of extracts of *Capsicum baccatum*. In each assay, 9 repetitions were done, which consisted of polyethylene pots of 200 mL filled with commercial Macplant substrate. One plant was transplanted per pot and large trays were used to accommodate the pots. Each tray was considered a treatment, totaling 12 trays (11 extracts + the control). The control assay consisted of pure distilled water instead of extract.

After 15 days of growth of *Hovenia dulcis*, the applications were done on the leaf surface using a manual sprayer with 50 mL of extract for each tray (Brun *et al.*, 2016). Nine quantitative response variables were evaluated: number of leaves, root length, shoot length, shoot fresh mass, shoot dry mass, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids.

### Photosynthetic pigments

The leaves of *Hovenia dulcis* used for the evaluation of photosynthetic pigments were collected 15 days after the application of the extract. The concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll, and carotenoids were evaluated according to the methodology described by Hendry and Price (1993) using 80% acetone with three samples per assay. The pigment concentrations were calculated after reading the absorbances at 480 nm (Chl a), 645 nm (Chl b), and 663 nm (carotenoids) (Lichtenthaler, 1987).

## Statistical analysis

Statistical analysis of a  $2^3$  experimental design with triplicate at the central point was performed in the Statistica 7.0 software (StatSoft, Inc.) with a confidence level of 95% to assess the main effects and interactions of extraction variables on the response variables. These variables are the germination percentage, root and shoot lengths, and the shoot fresh and dry mass. The analysis of variance (ANOVA) of the results was performed and the results are presented in Pareto chart form.

### **RESULTS AND DISCUSSION**

# *Pre-emergence of Cucumis sativus and Hovenia dulcis*

According to Table 1, the maximum germination of *Cucumis sativus* using pepper extract did not exceed 38.7%. Assays 1 and 8 showed 100% inhibition both on the three evaluations. Otherwise, the control assay was not inhibited. These results indicated the "dedo-de-moça" hot pepper extracts contain phytotoxic compounds that inhibit the preemergence, thus justifying further assays with the target invasive plant: *Hovenia dulcis*. For *Hovenia dulcis*, the control presented germination of 65% on the 14<sup>th</sup> day (Table 2). For treatments with pepper extract, the most promising results were obtained in the assays 9 to 11 (central point), with germination lower than 3% on the 14<sup>th</sup> day. For the other treatments, the germination ranged from 8 to 55% on the 14<sup>th</sup> day. In terms of the effects of the variables studied in ultrasound-assisted extraction (time, power fraction, and pulse cycle), no significant effect was observed for any variable at 95% confidence. These results indicate that the extracts have compounds with inhibitory action, but no significant differentiation was seen in pre-emergence among the treatments with ultrasound.

As a result of the study for alternative products

 Table 1. Pre-emergence of seeds of Cucumis sativus with the application of extracts of Capsicum baccatum obtained by ultrasound-assisted extraction

| Assay   | Variables       |                    |                       | Germination (%)     |                     |                      |
|---------|-----------------|--------------------|-----------------------|---------------------|---------------------|----------------------|
|         | Pulse cycle (-) | Power fraction (%) | Extraction time (min) | 4 <sup>th</sup> day | 7 <sup>th</sup> day | 10 <sup>th</sup> day |
| 1       | 0.3 (-1)        | 50 (-1)            | 20 (-1)               | 0                   | 0                   | 0                    |
| 2       | 0.7 (+1)        | 50 (-1)            | 20 (-1)               | 0                   | 12.0                | 5.3                  |
| 3       | 0.3 (-1)        | 100 (+1)           | 20 (-1)               | 0                   | 12.0                | 9.3                  |
| 4       | 0.7 (+1)        | 100 (+1)           | 20 (-1)               | 0                   | 2.7                 | 10.7                 |
| 5       | 0.3 (-1)        | 50 (-1)            | 60 (+1)               | 0                   | 13.3                | 20.0                 |
| 6       | 0.7 (+1)        | 50 (-1)            | 60 (+1)               | 0                   | 33.3                | 38.7                 |
| 7       | 0.3 (-1)        | 100 (+1)           | 60 (+1)               | 0                   | 2.7                 | 5.3                  |
| 8       | 0.7 (+1)        | 100 (+1)           | 60 (+1)               | 0                   | 0                   | 0                    |
| 9       | 0.5 (0)         | 75 (0)             | 40 (0)                | 0                   | 2.7                 | 8.0                  |
| 10      | 0.5 (0)         | 75 (0)             | 40 (0)                | 0                   | 4.0                 | 5.3                  |
| 11      | 0.5 (0)         | 75 (0)             | 40 (0)                | 0                   | 8.0                 | 14.7                 |
| Control | -               | -                  | -                     | 100                 | 100                 | 100                  |

 Table 2. Pre-emergence of seeds of Hovenia dulcis with the application of extracts of Capsicum baccatum obtained by ultrasound-assisted extraction

| Assay   | Variables       |                    |                       | Germination (%)     |                      |                      |  |
|---------|-----------------|--------------------|-----------------------|---------------------|----------------------|----------------------|--|
|         | Pulse cycle (-) | Power fraction (%) | Extraction time (min) | 7 <sup>th</sup> day | 10 <sup>th</sup> day | 14 <sup>th</sup> day |  |
| 1       | 0.3 (-1)        | 50 (-1)            | 20 (-1)               | 43                  | 60                   | 29                   |  |
| 2       | 0.7 (+1)        | 50 (-1)            | 20 (-1)               | 41                  | 64                   | 52                   |  |
| 3       | 0.3 (-1)        | 100 (+1)           | 20 (-1)               | 35                  | 52                   | 40                   |  |
| 4       | 0.7 (+1)        | 100 (+1)           | 20 (-1)               | 43                  | 59                   | 55                   |  |
| 5       | 0.3 (-1)        | 50 (-1)            | 60 (+1)               | 12                  | 25                   | 19                   |  |
| 6       | 0.7 (+1)        | 50 (-1)            | 60 (+1)               | 16                  | 24                   | 13                   |  |
| 7       | 0.3 (-1)        | 100 (+1)           | 60 (+1)               | 36                  | 48                   | 35                   |  |
| 8       | 0.7 (+1)        | 100 (+1)           | 60 (+1)               | 0                   | 7                    | 8                    |  |
| 9       | 0.5 (0)         | 75 (0)             | 40 (0)                | 0                   | 3                    | 1                    |  |
| 10      | 0.5 (0)         | 75 (0)             | 40 (0)                | 0                   | 0                    | 0                    |  |
| 11      | 0.5 (0)         | 75 (0)             | 40 (0)                | 0                   | 1                    | 3                    |  |
| Control | -               | -                  | -                     | 63                  | 64                   | 65                   |  |

to replace or combine with traditional pesticides of chemical origin in agriculture, plant extracts have shown promising results. According to the scientific literature, pepper extracts have been applied for different purposes, such as repellent and insecticidal agents for pests. In addition, antifungal, antioxidant and anti-inflammatory activities have been reported (Allemand *et al.*, 2016; Batiha *et al.*, 2020; Nidiry, 2022).

Elsewhere, alcoholic extracts of long pepper (*Piper hispidinervum*) were evaluated on the germination and initial growth of lettuce. The inhibition of germination of most all lettuce seeds in higher extract concentrations was reported (Da Silva *et al.*, 2012). Also, both the fruit and the leaves of *Capsicum baccatum* were used in the preparation of extracts to verify the allelopathic effects on the germination of corn and lettuce seeds. Both extracts showed inhibition of the germination of the species, thus indicating that the leaves also have phytotoxic compounds (Garilio *et al.*, 2019).

Other plants have been tested for allelopathic effects. Aqueous and ethanolic extracts of cultivated cardoon (Cynara cardunculus L.) leaves were applied to the germination of invasive plants such as Amaranthus retroflexus L., Portulaca oleracea L., Stellaria media (L.) Vill., and Anagallis arvensis L., commonly found in Mediterranean Basin, causing a significant reduction in their germination (up to 95%) (Scavo et al., 2020). Extracts from Leucaena leucocephala were obtained and applied to control seeds of Bidens pilosa L., which a germination inhibition of 40% was found (Peron and Bonini, 2012). In the same trend, extracts of leaves and bark of Haldina cordifolia caused a total reduction in the number of germinated seeds of two invasive plants known as giant mimosa (Mimosa *pigra*) and southern sandbur (*Cenchrus echinatus*), and 25% inhibition of mung bean (Vigna radiata) (Suksungworna et al., 2016).

## Post-emergence of Hovenia dulcis

Post-emergence assays consisted of visual assessments of inhibition using the scale adapted from Frans *et al.* (1986), which indicated that most

of the samples are classified as "light effect". In terms of light effects, slight discoloration (slight change from green to yellow color) was obtained, which characterizes percentage damage below the 30% range. In visual terms, it cannot be inferred that differences exist or not among the assays with pepper extract and compared with the control. Therefore, other responses were analyzed, such as quantitative analyses of root and shoot lengths and fresh and dry mass (Figure 1). The experimental levels of assays 1-11 for the variables time, power fraction, and pulse cycle are those presented in Tables 1 and 2.

While the control presented an average root length of 3.6 cm, the other assays (except for assay 6) presented shorter average lengths. The lowest value of root length occurred in assay 7, with 1.2 cm, which is 3 times smaller than the control. The mode of action of compounds present in vegetable extracts is based on their contact with the plants, mainly leaves and stems. Thus, direct interference in the root system does not necessarily exist in a short time. Some studies corroborate this statement, such as Bastos et al. (2017), which evaluated the length of Cucumis sativus roots and reported that the root length was not significantly influenced by the treatments evaluated, ranging from 14.4 to 18.3 cm. Brun et al. (2020) reported the root dry mass of Amaranthus viridis ranging from 10 mg to 16 mg with a coefficient of variation of 2.51% among seven treatments with spray-dried powder containing metabolites from Diaporthe schini. The control presented a mass of 20 mg, but it was not significantly different from the other assays.

In the statistical approach (Figure 2), it can be observed that the variables power fraction, pulse cycle and the time  $\times$  power fraction interaction were significant at 95% confidence over the root length response. As the objective is to inhibit the post-emergence of *Hovenia dulcis*, these results indicate that the interaction between time and power fraction is positive to cause a shorter root length. In an integrated assessment, the appropriate ultrasound-assisted extraction condition would be 0.3 pulse cycle and a combination of time  $\times$ 



Figure 1. Root and shoot lengths and fresh and dry mass of *Hovenia dulcis* after the application of *Capsicum* baccatum extracts and comparison with the control assay

positive power fraction, i.e., 20 min of extraction and 50% power or 60 min of extraction and 100% power fraction.

The shoot length (Figure 1) showed a behavior similar to that one of the roots. Only samples from two assays (3 and 6) presented an average length longer than the control (4.2 cm). The samples from the other tests showed shoot lengths varying between 1.6 and 4.1 cm. The statistical analysis (Figure 2) indicated only the time × power fraction interaction as significant at 95% confidence for this response variable. This represents that the positive combination of the two independent variables, both at their higher or lower levels, results in a lower average shoot length, which is of interest in terms of controlling this invasive exotic plant under study.

The shoot length has been studied in other works. Spiassi *et al.* (2011) evaluated the allelopathic effects of straw used as ground cover during winter on the initial growth of maize and concluded that there was a reduction in shoot and root lengths, as well as a loss of shoot dry mass of seedlings of maize. This infers that maize growth is sensitive to straw (*Crambe abyssinica*), which may be related to the allelopathic effect of straw on maize plants. Brun *et al.* (2022) reported a growth inhibition and shoot lengths 15%, 28%,



Figure 2. Pareto chart of effects of extraction variables applied in ultrasound-assisted extraction of compounds from *Capsicum baccatum* on post-emergence responses of *Hovenia dulcis* 

71% and 36% smaller than the control by applying fermented broth from *Diaporthe schini* on *Bidens pilosa*, *Amaranthus viridis*, *Lollium multiflorum* and *Echinocloa crusgalli*, respectively.

The results of fresh and dry mass of *Hovenia dulcis* plants are also shown in Figure 1. Again, assay 6 (60 min time, 50% power fraction, and 0.7 pulse cycle) showed higher results than the control (117 mg). However, assay 2 (20 min time, 50% power fraction, and 0.7 pulse cycle) showed the highest fresh mass value: 168 mg. In contrast, assay 7 (60 min time, 100% power fraction, and 0.3 pulse cycle) presented fresh and dry mass of only 25 and 7 mg, respectively.

For fresh mass, only the pulse cycle was significant at 95% confidence (Figure 2), indicating that it is preferable to apply a pulse cycle of 0.3 instead of 0.7. Pulse cycles close to 1.0 in ultrasound-assisted extraction may cause a partial loss of bioactivity of the compounds present

in extracts of Capsicum baccatum. Consequently, the less inhibitory effect is verified in phytotoxicity analysis parameters. In a study developed with Mortierella isabellina, to optimize the extraction process, an influence on the variation of the pulse cycle in the extract obtained by ultrasound was reported, where the pulse factor was one of the optimized variables in the central composite design (Sallet et al., 2019). The effect of cavitation allows the breakdown of the cell walls of the plant matrix, improving solvent penetration and facilitating the release of extractable compounds (Pereira et al., 2021). Confortin et al. (2019) showed the variation of extraction yields with a variation of intensity and pulse cycle. The increase in intensity and pulse cycle favored the recovery of extracts from the matrices (roots and fruits) of Solanum viarum Dunal.

For dry mass, all interactions were significant (Figure 2). When considering the time × pulse cycle,

an inversely proportional relationship is observed, that is, in short times (20 min) a longer pulse cycle is indicated (0.7) and in longer times (60 min) a smaller cycle pulse (0.3) is indicated. However, when considering the time  $\times$  power fraction or power fraction  $\times$  pulse cycle, proportional relationships are observed, that is, both independent variables of each double interaction must be together at their lowest or highest levels.

The number of leaves of the *Hovenia dulcis* plants with application of pepper extract was also evaluated. This response variable ranged from 1 to 3 leaves, which the lowest value (1 leaf) was obtained for the assay 7 (time of 60 min, power fraction of 100%, and pulse cycle of 0.3) and the highest values (3 leaves) were obtained for the assays 3. 5 and 6, in addition to the control. The other assays (1, 2, 4, 8-11) presented 2 leaves. In terms of assay 7, the number of leaves is corroborated by the results presented in Figure 1, where the smallest values of lengths and mass were also obtained in that assay.

One important aspect to be highlighted when working with natural and biological compounds against invasive and undesirable species is the fact that the death of such species is not necessarily needed to increase crop productivity or reforestation. Suppressing invasive species populations is also a desirable outcome and comprises part of this reduced-risk strategy in comparison with the application of traditional chemical products for attaining sufficient control. It is acceptable to have a reduction in efficiency when the integrated target is to cause low harm to the environment and people because biological/natural extracts present a short-lived environmental fate and low toxicity (Bailey, 2014). Overall, according to Bergamin et al. (2022), many worldwide regions will remain suitable for the occurrence of Hovenia dulcis, and an expansion trend is foreseen in the higher latitudes of the Northern Hemisphere. From a biodiversity conservation perspective, efforts should be put to avoid the introduction or dissemination of this species. The authors suggest that all local conservation authorities should prioritize this action, especially in the overlap zones between *Hovenia dulcis* suitable areas and the Global Biodiversity Hotspots. Therefore, the strategy shown in this work could contribute with the environmental conservation.

# Photosynthetic pigments

The chlorophyll and carotenoid contents of the *Hovenia dulcis* plants with the application of pepper extract are shown in Figure 3. Except for assay 3, where it was not possible to perform the analysis, the values of Chl a, Chl b, and total chlorophyll ranged from 0.82 to 1.15, 0.19 to 0.35, and 1.93 to 2.71 mg g<sup>-1</sup> fresh mass, respectively. For the control, the values of Chl a, Chl b, and total chlorophyll were 1.02, 0.31, and 2.39 mg g<sup>-1</sup> of fresh mass, respectively.

Therefore, based on a direct comparison, 7 assays showed lower Chl a, Chl b, and total chlorophyll than the control. The slight yellowing of the *Hovenia dulcis* plants may have occurred due to the reduction of nutrient absorption inhibited by the pepper extract, leading to a slight reduction in the chlorophyll content in the leaves. Bastos *et al.* (2017) reported that fungal fermented broth affected the photosynthesis of *Cucumis sativus*, leading to a reduction in the chlorophyll content and also interfering with other parameters, such as root length, height, and plant mass.

Regarding the carotenoid content, except for assay 3, this response variable ranged from 0.319 to 0.398 mg g<sup>-1</sup> fresh mass, while the control showed an average value of 0.301 mg g<sup>-1</sup> fresh mass (Figure 3). Carotenoids are pigments that, during photosynthesis, can perform two distinct functions: absorption of light in the light-harvesting complexes acting as accessory pigments and exerting a photoprotective action of the photochemical apparatus, preventing photo-oxidative damage to chlorophyll molecules (Kerbauy, 2004). The application of treatments did not have sufficient effects to cause changes in the structural pigments of carotenoids, which are involved in the protection of chlorophyll (Munns and Tester, 2008).



Figure 3. Chlorophyll and carotenoid content of *Hovenia dulcis* after the application of *Capsicum baccatum* extracts and comparison with the control assay

### CONCLUSION

• The extracts obtained by ultrasound-assisted extraction from *Capsicum baccatum* showed an inhibitory effect in the pre-emergence of *Cucumis sativus* and *Hovenia dulcis*. In post-emergence, the results in *Hovenia dulcis* showed slight yellowing of the plants. The extracts obtained by ultrasound, for the most part, influenced the plant development, reducing root and shoot lengths. Consequently, this inhibitory effect was also observed in the reductions of the fresh and dry mass of samples submitted to the application of pepper extracts, with a significant effect of process variables, predominantly the ultrasound power fraction. The extracts of pepper may become an alternative for the control of invasive plants, with the need for further studies in the area.

# AUTHORSHIP CONTRIBUTION STATEMENT

ANCHIETA, M.G.: Conceptualization, Formal Analysis, Investigation, Methodology, Writing

-original draft; **PIGATTO**, **G.:** Investigation; **BAISCH**, **J.S.:** Methodology; **DOLIANITIS**, **B.M.:** Writing – review & editing; **CORADI**, **P.C.:** Methodology; **GUEDES**, **J.V.C.:** Methodology; **MAZUTTI**, **M.A.:** Data curation, Resources; **TRES**, **M.V.:** Data curation, Resources; **ZABOT**, **G.L.:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

# **DECLARATION OF INTERESTS**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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