

Bioherbicidal activity of seed extract of *Campomanesia lineatifolia* on the weed *Sonchus oleraceus* L.

Actividad bioherbicida del extracto de semillas de *Campomanesia lineatifolia* sobre la maleza *Sonchus oleraceus* L.

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ABSTRACT

Sonchus oleraceus L. is a persistent weed in horticultural crops that generates high economic losses and its management is carried out with various chemical molecules. The objective of this research was to evaluate the bioherbicidal activity effect of the extract of *Campomanesia lineatifolia* seeds on the germination and growth of *S. oleraceus* L. seedlings. The study was carried out in two phases. In the first phase, germination was evaluated with two experiments, the first consisted of the addition of the ethanolic extract of *C. lineatifolia* every third day, in the second experiment the extract was only added at planting. In both cases, four concentrations of the extract (0%, 3%, 6%, and 9%) were evaluated. In the second phase, the same extracts were applied to plant leaves. *C. lineatifolia* extract inhibited the germination of *S. oleraceus*, when applied only once at sowing. As there was no germination with the extracts of *C. lineatifolia*, no direct inhibitory effect on the growth of the seedlings could be seen on the number of leaves, length of roots, and stem. With the foliar application, an incidence of 100% with symptoms of chlorosis and necrosis was observed using any of the concentrations evaluated, but the applications did not cause the plant death. The ethanolic extract from the seeds of *C. lineatifolia* has bioherbicide activity on *S. oleraceus*.

Key words: allelopathy, germination, growth, injury.

RESUMEN

Sonchus oleraceus L. es una maleza persistente en cultivos hortícolas que genera altas pérdidas económicas y su manejo se realiza con diversas moléculas químicas. El objetivo de este estudio fue evaluar la actividad bioherbicida del extracto de semillas de *Campomanesia lineatifolia* sobre la germinación y crecimiento de plántulas de *S. oleraceus* L. El estudio se llevó a cabo en dos fases. En la primera fase se evaluó la germinación con dos experimentos, el primero consistió en la adición del extracto etanólico de *C. lineatifolia* cada tercer día, en el segundo experimento, el extracto solo se adicionó en la siembra. En los dos casos se evaluaron cuatro concentraciones del extracto (0%, 3%, 6% y 9%). En la segunda fase se hizo la aplicación de los mismos extractos vía foliar a las plantas. El extracto de semillas de *C. lineatifolia* inhibió la germinación de las semillas de *S. oleraceus*, tanto con aplicación continua del extracto como aplicado sólo una vez a la siembra. Como no hubo germinación con los extractos de *C. lineatifolia* no se pudo ver efecto inhibitorio directo sobre el crecimiento de las plántulas a través del número de hojas, longitud de raíz y tallo. Con la aplicación foliar se observó incidencia del 100% con cualquiera de las concentraciones evaluadas con síntomas de clorosis y necrosis, pero no se causó la muerte de las plantas. El extracto etanólico de las semillas de *C. lineatifolia* presenta efecto como bioherbicida sobre *S. oleraceus*.

Palabras clave: alelopatía, germinación, crecimiento, daño.

Introduction

Sonchus oleraceus L. belongs to the Asteraceae family and has an annual life cycle, growing up to 1.5 m in height (Elkamali *et al.*, 2011). The plant is characterized by a whitish latex and has alternate simple leaves and inflorescences with yellow terminal capitulum (Fuentes *et al.*, 2011; Gámez *et al.*, 2018). This species has different common names in Spanish, among them is *cerraja*, *lechosa*

or *lechuguilla*. In English, it is known as dandelion sow thistle and hare's thistle.

S. oleraceus is widely distributed throughout the world and, in Colombia, it is present in the highlands of almost the entire territory (Fuentes *et al.*, 2011) and is considered invasive (Peerzada *et al.*, 2019). As a weed, it has been reported in corn, grasslands, oats, vegetables, asparagus, garlic, feijoa, pea, quinoa, apple, leek (Gámez *et al.*, 2018), among others.

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In peach orchard under tropical conditions, it was reported as the species in fifth place with the highest importance value index (Moreno-Preciado & Balaguera-López, 2021). *S. oleraceus* is of great interest to cause a detrimental impact on agriculture through crop competition and allelopathic interference (Chauhan *et al.*, 2006; Elkhayat, 2009). It is a limiting species for crops because it has a great competitive capacity and produces a high number of most viable seeds (Peerzada *et al.*, 2019). It is also considered the host of different pests and diseases, such as aphids, fungi, nematodes, besides other organisms that affect crops of economic interest (Gámez *et al.*, 2018). Genetic diversity, efficient seed dispersal, resistance to some herbicides (groups M and B) are some of the factors that contribute to the successful distribution of *S. oleraceus* in the agroecosystems. In Australia, it is currently considered the second most important broadleaf weed (Peerzada *et al.*, 2019).

Herbicide use and herbicide resistance hinder weed management (Guglielmini *et al.*, 2007; Vencill *et al.*, 2012) and cause a high negative impact on crop yields (Menalled, 2010), environment, and human health (Böcker *et al.*, 2019). Therefore, new management alternatives must be found, within which biological management can be included (Peerzada *et al.*, 2019) based on the use of allelochemicals (Jabran, 2017). Allelopathy is a tool that allows for the sustainable management of weeds (Arafat & Ali, 2015), allowing the maintenance of a balance in the ecosystem by reducing the use of chemical herbicides (Nawaz *et al.*, 2014; Kaab *et al.*, 2020). Allelochemicals (including phenolic compounds, terpenoids, and alkaloids) can be formulated into bioherbicides, but many aspects that should be studied are still unknown, such as an understanding of their mode of action (Jabran, 2017).

The champa (in Spanish) or perfume guava (in English) (*Campomanesia lineatifolia* R. & P.) is a fruit tree of the Myrtaceae family. Its fruits are edible berries with a pleasant taste (Balaguera-López *et al.*, 2012; Porras *et al.*, 2020; González *et al.*, 2021), containing 6 to 8 seeds that represent an important percentage of the fruit volume (Balaguera-López *et al.*, 2012). The seeds appear to have a high and diverse content of secondary metabolites. Bonilla *et al.* (2005) found that the seed of *C. lineatifolia* has β -triketone-like components, which are characterized by the presence of several methyl groups, a flavonoid ring or chalcone, making it a relatively rare class of secondary metabolites. These compounds are known to have antimicrobial activity (Bonilla *et al.*, 2005; Osorio *et al.*, 2006). Muñoz *et al.* (2015) report in this species the existence mainly of diphenols and polyphenols, Madalosso

et al. (2012) mention the presence of flavonoids (catechin and quercitrin) and tannins, while Raphaelli *et al.* (2021) report that the *C. xanthocarpa* seeds contain phenolic compounds with different biological activities. The same authors also indicated that the species of the Myrtaceae family are recognized for having high levels of the bioactive compounds, mainly phenolics and carotenoids.

Some plants have biologically active compounds, whose crude, methanolic, and other allelopathic extracts inhibit the germination of weed seeds and affect plant growth causing necrosis or chlorosis (Kaab *et al.*, 2020). Several studies with allelopathic extracts have been carried out on Myrtaceae. Verdeguer *et al.* (2009) mentioned that the essential oil of *Eucalyptus camaldulensis* inhibits the germination and growth of *Amaranthus hybridus* L. and *Portulaca oleracea* L. Puig *et al.* (2018) observed that the aqueous extract of *Eucalyptus globulus* presents phytotoxic effects in *Amaranthus retroflexus* L. and *Echinochloa crus-galli* L. Recently, an allelopathic effect of *C. lineatifolia* seed extract was found on the germination and physiology of *Taraxacum officinale* L. (Cabeza-Cepeda *et al.*, 2021).

Therefore, the objective of this study was to evaluate the allelopathic activity of *C. lineatifolia* seed extract on the germination, seedling growth, and injury of *S. oleraceus* L. under laboratory conditions.

Materials and methods

The experiment was conducted in Tunja (Boyacá, Colombia), under laboratory conditions, with a mean temperature of 19°C and relative air humidity of 60.8%. *S. oleraceus* L. seeds were obtained from farms in the municipalities of Tunja and Paipa (Boyacá). *C. lineatifolia* seeds were collected from mature fruits in the Miraflores municipality (Boyacá) in “La playa” farm located at 1432 m a.s.l., 5°11'49" N and 73°08'47" W with a mean temperature of 24°C (González *et al.*, 2021).

The ethanolic extracts of the *C. lineatifolia* seeds were obtained using the following procedure. Initially, the seeds were dried at room temperature (18°C) for 24 h, then this material was grounded, and weighed to obtain the extract. A 400 g sample of the grounded material was immersed in 1 L of ethanol (96%), the mixture was shaken and then left to stand in a glass bottle and covered with aluminum foil for a period of 48 h. The bottle was shaken regularly to homogenize the sample. Subsequently, the liquid was separated from the solid part through a filtering process (Whatman 1 filter paper), then it was subjected to

a distillation process with a rotary evaporator to obtain the extract. From the concentrated extract, the different evaluated concentrations were prepared (% w/v) using distilled water as the final solvent. The study was carried out in two phases.

In the first phase, which corresponded to application on pre-emergent plants, germination was evaluated with two experiments. The first experiment consisted of the application of *C. lineatifolia* extract every third day. In the second experiment, the extract was only added at the time of sowing. In both experiments, a completely randomized design was used with four treatments corresponding to concentrations of *C. lineatifolia* seed extract (0%, 3%, 6%, and 9%), concentrations evaluated previously by Cabeza-Cepeda *et al.* (2021). Each treatment had four replicates and 16 experimental units; each unit consisted of a Petri dish with 100 *S. oleraceus* seeds.

In the second phase or the post-emergent application, the extracts were applied over the leaves of the plants. A completely randomized design with the same four concentrations of *C. lineatifolia* seed extract was used. Each of the 16 experimental units was composed of a pot with five *S. oleraceus* plants.

The seeds were sown in Petri dishes, on the base of which there was absorbent paper. Periodically the seeds were hydrated with the allelopathic extracts, except for the control that was hydrated with distilled water. In the second experiment, periodic hydration was done with distilled water. The Petri dishes were left at room temperature (19°C) and relative humidity (60.8%) with a 12 h photoperiod. Germination readings were carried out every third day from the moment the first seed germinated. It was considered as germination criterion that the radicle measured a minimum of 2 mm. From these data and with the formulas used by Carranza *et al.* (2016) and proposed by Ranal and Santana (2006) the percentage of germination and the mean germination speed (germinated seeds/d) were calculated. At the end of the experiment, the height (cm) and the length of the longest root (cm) of each seedling were determined by measurement with a Vernier caliper.

For the second phase, ten seeds of *S. oleraceus* were sown in pots with a volume of 500 ml, and peat moss was used as substrate. When the seeds germinated, 5 seedlings of similar size were chosen, and the rest were eliminated. Irrigation was carried out periodically, and fertilizer was applied weekly to avoid nutritional deficiencies. The fertilizer mixture was prepared by taking 20 ml of commercial fertilizer 12-4-8 (Follaje®, Davalia Fertilizantes, Colombia)

and dissolving it in 2 L of water. Of this solution, 40 ml per bottle was applied. When the plants had between 4 and 5 true leaves, the extracts were applied uniformly to the leaves of the entire foliage using a 10 ml atomizer. It was necessary to add an adjuvant (Cosmoflux® 411F, Cosmoagro, Colombia) to the extracts to achieve uniform coverage on the leaves.

At 2, 5, and 8 d after the application of the extracts, the following variables were determined: incidence of damage (%), defined as the number of plants with damage by the herbicide/total number of plants x 100. The severity of the damage (%) was determined with the scale used by Chaves Neto *et al.* (2020), where 0-20% indicates no injury or effect, 20-40% slight injury and/ or growth reduction with rapid recovery, 40-60% injury and/or growth reduction with slow or definitive recovery, 60-80% severe injury and/or reduction of non-recoverable growth and/or reduction of the stand, and 80-100% destruction or just a few live plants.

Statistical analysis

An analysis of variance was carried out with the data obtained to determine statistical differences. Where differences were observed, the Tukey's test ($P < 0.05$) was used. Variables that did not meet the assumptions of normality of the residuals (Shapiro-Wilk test) and homogeneity of variances (Levene test) were subjected to arcsine $\sqrt{x} + 1$ transformation and \sqrt{x} transformation. The analyses were carried out with the statistical program SPSS IBM v. 19.

Results and discussion

Phase 1. Pre-emergence treatments

Germination percentage: there were significant differences between the concentrations of the extract and the control (without the extract) in the two experiments. Germination of *S. oleraceus* seeds with *C. lineatifolia* extract was found to be inhibited (Fig. 1). In agreement with these results, this extract also inhibited germination in *T. officinale* (Cabeza-Cepeda *et al.*, 2021).

The total inhibition of germination obtained with *C. lineatifolia* extracts may be due to the presence of secondary metabolites that directly affect the cells and cause the death of the seeds. *C. lineatifolia* seeds contain several compounds, such as catechins, phenolic acids, chlorogenic acid, flavonoids and anthocyanins, mainly (data not shown). This composition is according with that reported in other studies previously. According to Catunda *et al.* (2002), germination can be inhibited by the secondary metabolites (phenols, terpenoids, saponins, etc.) by

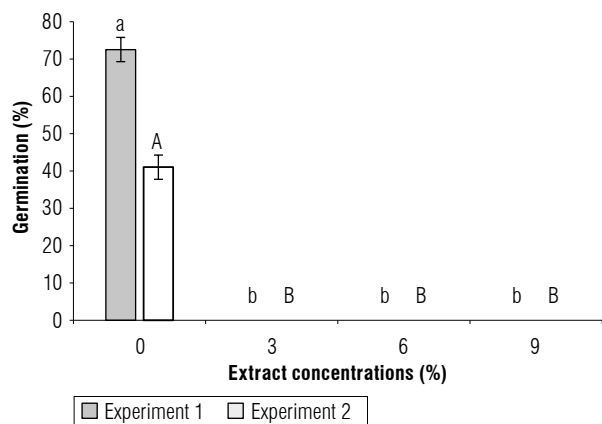


FIGURE 1. Bioherbicidal effect of the allelopathic extract of *C. lineatifolia* seeds on the germination percentage of *Sonchus oleraceus* seeds. Experiment 1: Continuous application of the extracts. Experiment 2: application of the extracts only at the sowing of the seeds. Means followed by different letters in each experiment indicate significant differences according to the Tukey test ($P < 0.05$). Vertical bars for each mean indicate the standard error ($n = 4$).

affecting several fundamental processes such as respiration, cell division, synthesis of gibberellins, permeability of the seed coat, and other effects. Carvalho *et al.* (2019) reported that allelochemicals affected germination by inhibition of cell cycle in meristematic cells. To clarify this situation, it is recommended to perform a seed viability test with triphenyl tetrazolium chloride on seeds that did not germinate; if the test is positive, germination would be inhibited but the embryo is still alive; if the test is negative, this would indicate that the extract of *C. lineatifolia* caused the death of the seeds.

Imatomi (2010) evaluated the allelopathic activity of aqueous extracts of leaves of 15 species belonging to the Myrtaceae family on the germination and growth of *Lactuca sativa*, *Solanum lycopersicum*, and *Allium cepa*, and found that the species *Myrcia multiflora*, *Myrcia splendens* and *Eugenia punicifolia* exhibited the highest inhibition of seed germination in bioassays, followed by the species *Myrcia bella*, *Psidium laruotteanum*, *Campomanesia pubescens*, *Psidium cenereum*, *Eugenia myrcianthes*, *Myrcia lingua*, and *Psidium rufum*. This supports the results obtained in this study where *C. lineatifolia* of Myrtaceae has an allelopathic effect that inhibits germination. However, knowing with certainty how the applied extract works is very difficult since the extract can have a significant number of different secondary metabolites that can have allelopathic action. According to Jabran (2017), the chemical inhibitors contained in plant extracts produced by allelopathic agents correspond to secondary metabolites and belong to various classes of compounds such as

phenols, aldehydes, glucosides, terpenes, and organic cyanogenic compounds.

Gil *et al.* (2010) reported that the application of the extracts of *Swinglea glutinosa* and *Lantana camara* inhibited the germination of *Senna obtusifolia*, *Amaranthus dubius*, *Rumex crispus*, *Brassica rapa*, and *Poligonum segetum*. In the case of *Swinglea*, the extracts inhibit germination with the concentration 0.5% and *Lantana* inhibits germination with an extract of 2.0% (Gil *et al.*, 2010). Seabra *et al.* (2017) evaluated allelopathic concentrations of *Canavalia ensiformis* on the seed germination of *Carthamus tinctorius*, where the inhibition was obtained with an extract concentration of 75% indicating that the extract of *C. lineatifolia* is very efficient because it needed only 3% to inhibit germination.

For mean germination speed (MGS), in the two experiments, statistical differences were found between *C. lineatifolia* extracts. Any concentration inhibited the germination of *S. oleraceus*; and, therefore, there was no germination speed. With the control (0 % of the extract) the seeds had a MGS of 5 germinated seeds/d for experiment 1 and 3 germinated seeds/d for experiment 2 (Fig. 2).

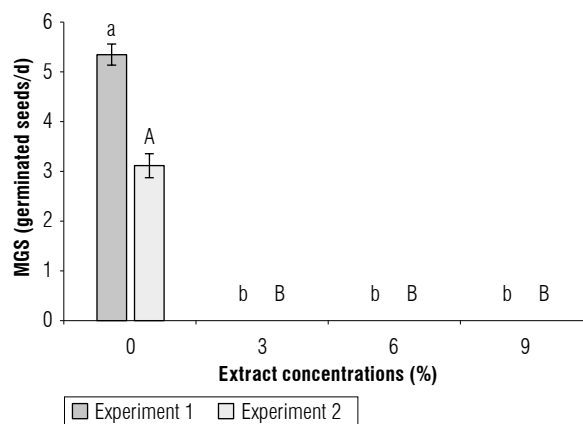


FIGURE 2. Bioherbicidal effect of the allelopathic extract of *C. lineatifolia* seeds on the mean germination speed (MGS) of *Sonchus oleraceus* seeds. Experiment 1: Continuous application of the extracts. Experiment 2: application of the extracts only at the sowing of the seeds. Averages followed by different letters in each experiment indicate significant differences according to the Tukey test ($P < 0.05$). Vertical bars in each mean indicate the standard error ($n = 4$).

This shows that the extract of *C. lineatifolia* caused phytotoxicity in the seeds of *S. oleareaceus*, which (as explained above) could be due to compounds derived from secondary metabolism such as phenolic compounds. That affect the growth and development of plants (Jabran, 2017), which, in this case, are the seeds.

Seabra *et al.* (2017) indicate that the allelopathic effects of plants can cause inhibition of the percentage and the germination speed and can reduce initial growth. This agrees with this study since the evaluated extract negatively affected germination. For their part, Gindri *et al.* (2020) found that the aqueous extract of *Lantana camara* causes inhibition of the germination speed index in *Bidens pilosa* seeds.

Length of roots and stems: since the extract of *C. lineatifolia* inhibited the germination of *S. oleraceus*, growth in seedlings that could only be measured in the control treatment, significant differences were observed concerning the other concentrations of the extract. On average, the seedlings had 2 leaves (Fig. 3A), root length was 1.98 cm and stem length were 0.18 cm (Fig. 3B). The number of leaves was 2 because only the cotyledonal leaves were present at the time of the experiment. The stem was very short at that time, the longest organ was the root. In addition, the stem had a reddish-green color and the leaves were completely green. The allelopathic effect of the extract on seedling growth cannot be evaluated because the effect was 100% inhibition in the germination process. In another study, the phenolic compounds were responsible for inhibiting the growth of the *Echinochloa crus-galli* root (Chon & Kim, 2004), while Gindri *et al.* (2020) indicate that the triterpenes lantadene A and B from the aqueous extract of *Lantana camara* causes growth inhibition in epicotyl and root of *Bidens pilosa*.

Gil *et al.* (2012) found that with *Swinglea* extracts there is complete root inhibition in the five weeds that they evaluated, which is consistent with the negative effect on

germination. If there is no germination, there is no radicle protrusion and growth, as occurred in the present study.

Phase 2. Post-emergence treatments

Incidence (%): from 2 d on it was evident that all the plants showed symptoms of damage from the extract of *C. lineatifolia*, which is why 100% of incidence was evident. At 5 and 8 d, 100% incidence of damage continued to be observed (Fig. 4). The same values were found in *T. officinale* with applications of *C. lineatifolia* extract (Cabeza-Cepeda *et al.*, 2021). As seen in Figure 5, the symptoms were localized yellowing and necrotic spots, showing that *C. lineatifolia* has potential as a bioherbicide. In this regard, Khan and Khan (2015) found that the extracts of several plants decrease the density of weeds to one-third about control in a wheat crop with the application of *Ammi visnaga* and *Convolvulus arvensis*.

For severity (%), significant differences were found throughout the study and the damage was greater as the concentration of the extract increased. At 2 d after application (first reading) the damage with the extracts was over $40.0 \pm 0.4\%$; at 5 d (second reading) with 9% of the extract, $63.0 \pm 2.0\%$ damage was reached, and at 8 d (third reading) the damage with this treatment was $74.5 \pm 1.7\%$. The other extracts produced lower effects but showed representative damage (Fig. 6). Although the plants did not die and some even tended to start flowering, perhaps due to the stress produced by the extract, this shows that *C. lineatifolia* exerts an allelopathic effect on *S. oleraceus*, apparently, due to the presence of phenolic compounds, therefore, it can be considered as a bioherbicide with great potential.

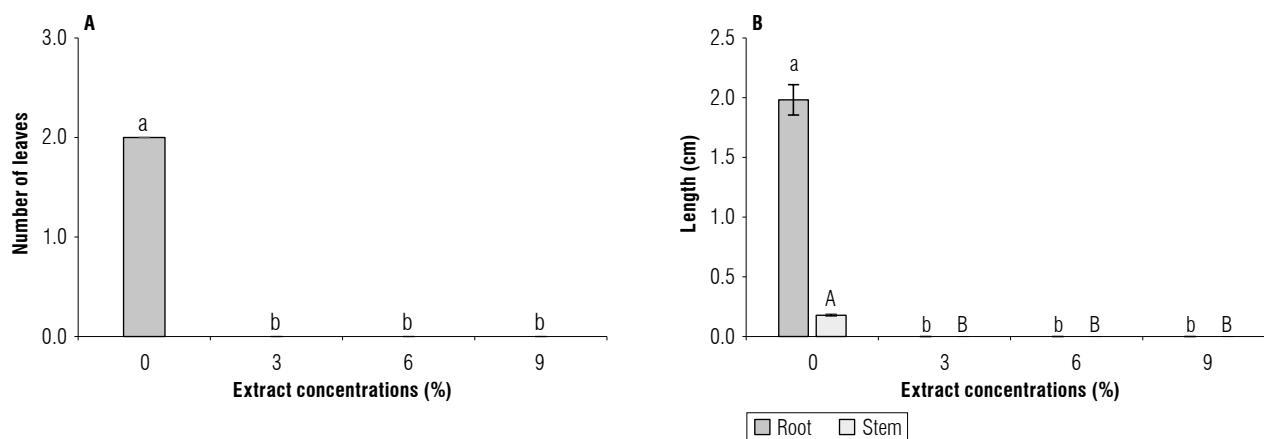


FIGURE 3. Bioherbicidal effect of the allelopathic extract of *C. lineatifolia* seeds on the number of leaves (A) and the stem and root length (B) of *Sonchus oleraceus* seedlings. Means followed by different letters indicate significant differences according to the Tukey test ($P < 0.05$). Vertical bars for each mean indicate the standard error ($n=4$).

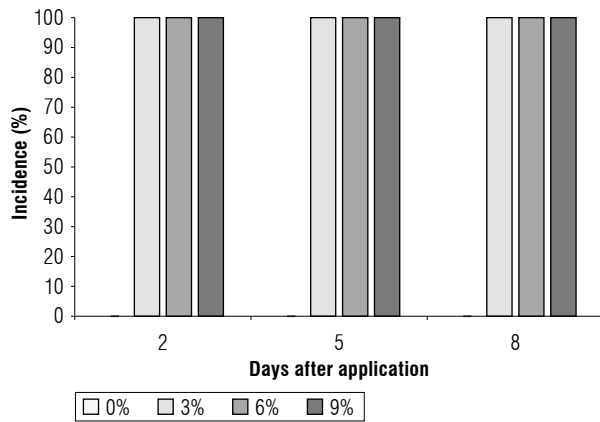


FIGURE 4. Bioherbicidal effect of the allelopathic extract of *C. lineatifolia* seeds on the incidence of damage in *Sonchus oleraceus* plants.

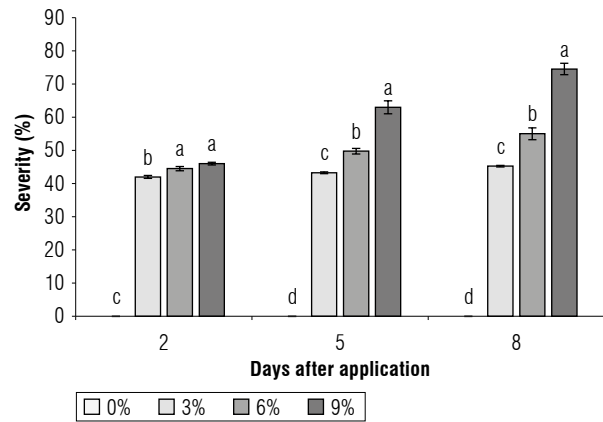


FIGURE 6. Bioherbicidal effect of the allelopathic extract of *C. lineatifolia* seeds on the severity of damage in *Sonchus oleraceus* plants. Means followed by different letters on each evaluation day indicate significant differences according to the Tukey test ($P < 0.05$). Vertical bars of each mean indicate the standard error ($n = 4$).

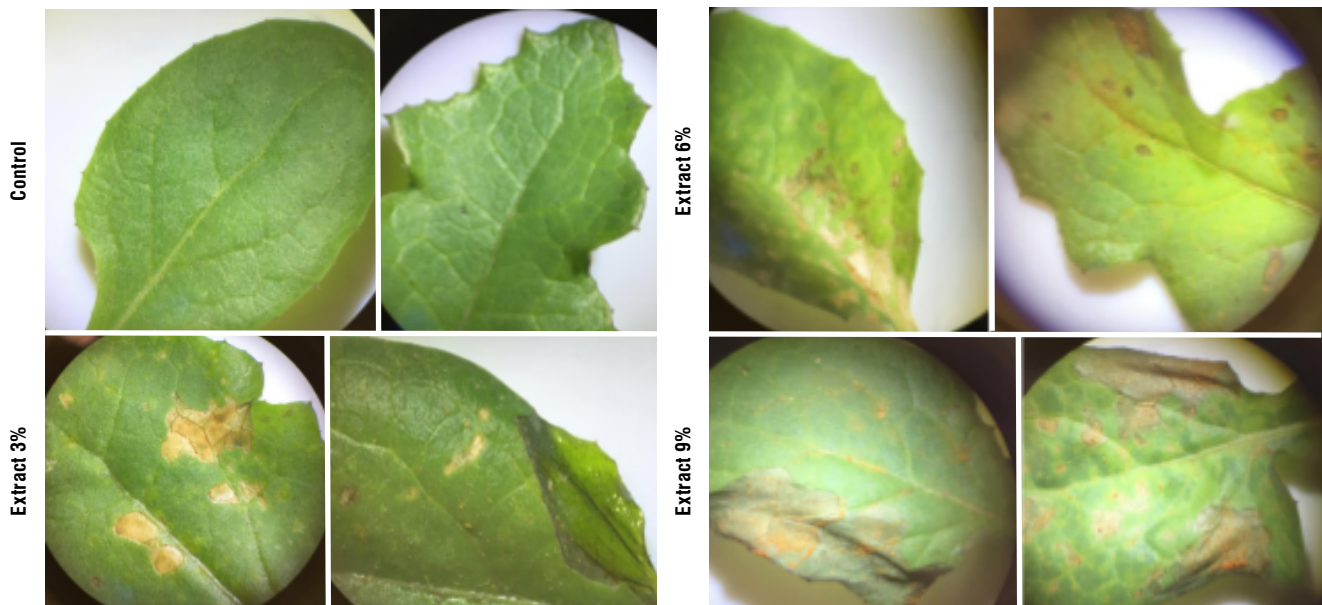


FIGURE 5. Symptoms of damage in *S. oleraceus* leaves caused by application of *C. lineatifolia* ethanolic extract.

In agreement with these findings, Gil *et al.* (2010) report from slightly visible toxic symptoms up to death for different weeds (*Senna obtusifolia*, *Amaranthus dubius*, *Rumex crispus*, *Brassica rapa*, and *Polygonum segetum*) with the application of *Swinglea glutinosa* and *Lantana camara* extracts. It is important to highlight that, in the study by Gil *et al.* (2010), the extracts were at the maximum of 5% concentration. Blanco (2006) mentions various mechanisms of action of allelopathic agents, for example, by stimulating stomatal closure, inhibiting electron transport in photosystem II and mitochondria, decreasing ATP synthesis, affecting cell membranes, and

degrading auxins, among other effects. In *C. lineatifolia*, the effects are unknown. It is not known how the application affects the plant. Possibly, the application directly affects chlorophylls because it generates yellowing or chlorosis. It could also greatly affect cell membranes because the effects it generates appear very fast with typical symptoms such as necrosis. Among the chemical herbicides, those that cause these symptoms, include membrane disruptors and photosynthesis inhibitors (Zimdahl, 2018). In addition, it is still not known if the effects are selective, but perhaps it is contact because the damage was localized (Fig. 5). In *T. officinale*, the extract

of *C. lineatifolia* affected parameters of chlorophyll fluorescence (Cabeza-Cepeda *et al.*, 2021) indicating that this bioherbicide can affect the photosynthetic process. In turn, the allelochemicals can be more biodegradable than traditional herbicides, but they can also have harmful effects on crops. For this reason, it is necessary to carry out more studies before adopting commercial use (Singh *et al.*, 2003; Ma *et al.*, 2006).

The results obtained are consistent with Stefanello (2016), in which allelopathic substances have been studied to minimize the application of agrochemicals, such as herbicides, that in the long term can minimize the negative effects on the environment and human health. Thus, Oliveros-Bastida (2008) mentioned a great diversity of substances with allelopathic potential. For phytotoxic evaluation, interest can be focused on the base molecules for the development of herbicides having around 100 molecules from each of the following groups or substances: terpenes, coumarin, benzoquinones, and alkaloids. This same author writes that the development of the use of herbicides from natural sources in the agrochemical industry has been very limited. With *C. lineatifolia* the first step is being taken, and other subsequent studies are necessary to make it viable as a bioherbicide on a commercial level.

Conclusions

The ethanolic extract of *Campomanesia lineatifolia* seeds inhibited the germination of *Sonchus oleraceus* seeds. With the foliar application, an incidence of 100% control was observed with any of the concentrations evaluated showed symptoms of chlorosis and necrosis. The severity of damage was observed starting from d 2 after application, indicating that the bioherbicidal effect is rapid. At any concentration of the extract, symptoms of damage were observed in the *Sonchus oleraceus* plants, and, although it was not possible to cause the death of the plants, the ethanolic extract from the seeds of *C. lineatifolia* has bioherbicide activity on *S. oleraceus*.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

CAMC and JAF designed the experiments, CAMC carried out experiments, CAMC, HEB and JAF contributed to the data analysis, and CAMC, HEB wrote the article. All authors reviewed the manuscript.

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