Senecio filaginoides DC as a Source of Allelopathic Agents and Its Possible Use as a Bioherbicide

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors LAA and ABM designed the study, wrote the protocol and the first draft of the manuscript. Author AMH managed the analyses of the study. Authors ABM and AMH performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

In this work, we experimented with aqueous and ethanolic extracts obtained from the aerial parts of Senecio filaginoides DC to evaluate allelopathic interactions in vitro with possible application as bioherbicides. S. filaginoides aerial parts were collected in Comodoro Rivadavia city (province of Chubut, Argentina) in May 2017. A voucher specimen was authenticated and deposited in the Patagonia Regional Herbarium of Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB) under the following herbarium number: HRP N°6159-6170, the coordinates are South Latitude 45°50'17", West Longitude 67°30'49". Seeds of Solanum lycopersicum and Lolium multiflorum that were obtained in the commercial market were used. From the plant material of S. filaginoides, two extracts, aqueous and ethanolic, were obtained; dilutions were made at 2, 4, 10 and 20%. Common water was used as control. In vitro bioassays were carried out and it was observed: the germination time, the germination percentage, the root and the stem growth. The data obtained were statistically analyzed using an analysis of variance (ANOVA), compared by the Tukey test. It was observed that the aqueous extract did not present significant interaction with the species studied for the concentrations tested. The germination percentage showed an inhibition with the ethanolic extract for all the concentrations studied in L. multiflorum and from the 10% concentration for S. lycopersicum species. A similar behavior of these species were observed in root growth compared to the different doses tested. A significant interaction was observed in the study of stem growth, where the species S. lycopersicum shows a stimulus at a concentration of 2%. The other concentrations showed an inhibitory action for this species. Based on these results, the ethanolic extract of S. filaginoides could potentially be used as a safe and environmentally friendly herbicide to develop new weed management methods under organic farming systems.
Keywords: Senecio filaginoides; bioherbicide; allelopathy; germination; root growth; stem growth.

1. INTRODUCTION

At present, the control and fight against weeds and plant diseases are mainly done through the use of chemical pesticides. The massive use of chemical products has caused the appearance of various problems [1,2,3]. Against this, new crop protection techniques have been developed that seek to relate in a less harmful way with the environment. One of the strategies is the use of allelopathic techniques to produce bioherbicides, instead of chemical herbicides [4]. Allelopathy explains both beneficial and harmful biochemical relationships between plants [5]. This phenomenon has received great attention throughout the world since the 1980. Studies have been conducted with many crops, trees, shrubs and weeds both in vitro in the laboratory and in field trials to determine their allelopathic potential and their use for weed control [6].

The plant species belonging to the Asteraceae family are highly studied for their allelopathic potential to control weeds. These aromatic plants, known to be rich in active principles, can play an important role in plant-plant interactions and constitute a primary source of potential allelochemicals for the production of bioherbicides [7,8]. The production and accumulation of secondary metabolites is an important mechanism in the interactions between species, which inhibit and or stimulate the germination and development of other plants.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The genus Senecio (Asteraceae) is widely distributed all over the word (except Antarctica and Amazonas) and includes more than 2000 species. Senecio filaginoides De Candolle (DC) (Yuyo moro, Romerillo, Mata mora, Charcao plateado) is one of the 300 species which occur in Argentina and grows from Jujuy to Santa Cruz province [9] Fig. 1. The aerial parts from S. filaginoides were collected in Comodoro Rivadavia city (province of Chubut, Argentina) in May 2017. A voucher specimen was authenticated and deposited in the Patagonia Regional Herbarium of Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB) under the following herbarium number: HRP N°6159-6170, the coordinates are South Latitude 45°50’17”, West Longitude 67°30’ 49”. Plant material composed of young leaves, adult leaves and non-lignified branches was collected and were left at room temperature in order to obtain stabilized dry weight. Solanum lycopersicum and Lolium multiflorum seeds were obtained in the commercial market, they belong to: La Germinadora S.A. (www.lagerminadora.com.ar). They correspond to identified class seeds, with purity and germination established by law and cured Lot Number 92.12.06 and 87.90.11, respectively.

2.2 Obtaining the Alcoholic and Aqueous Extract

The extracts, alcoholic and aqueous, were obtained from the aerial parts of S. filaginoides plants, by macerating plant material at 15% by weight with 96% ethanol and common water respectively, for 72 hours in cold and darkness. The solvents were evaporated with a Yamato RE-300 brand vacuum rotatory at 40°C and 50 rpm, followed by 24 hours in an oven at 35°C. Subsequently, the dry extract as dissolved in common water up to a total volume of 100mL. Both extracts were considered as 100% solutions and were stored in the refrigerator and in the dark until use [10]. From the extracts obtained, dilutions at 2, 4, 10 and 20% were made.

2.3 Bioassays

2.3.1 Experiment 1

The experimental units were 90 mm diameter Petri dishes, 25 seeds were placed per plate, with five repetitions per treatment for the selected species. A batch of seeds was considered as the control and was soaked with 16 mL of common water. The rest of the batches were treated with 16 mL of ethanolic extract of different concentrations: 2, 4, 10 and 20% [4].

The seeded plates were placed in a germination chamber at 20°C, and subjected to a photoperiod of 16 hours of light and 8 hours of darkness. Plates were checked daily. The data obtained at the end of the tests were the final germination percentage (%) and the length of the root and stem in millimeters (mm) [11]. For practical purposes, a seed was considered germinated when the protrusion of the radicle reached 5 mm [12]. The effects of radicle and stem elongation were determined by measuring their respective lengths after 14 days.
2.3.2 Experiment 2

An identical experiment to the previous one was carried out, substituting the ethanolic extract for the aqueous extract.

3. RESULTS AND DISCUSSION

3.1 Germination

To evaluate the possible allelopathic effects of the aqueous and ethanolic extracts of *Senecio filaginoides* on *S. lycopersicum* and *L. multiflorum*, the germination percentage was used. This is a parameter that shows the portion of seeds germinated in a given time and can be indicative of the inhibitory or stimulating power of the extract. In the germination tests carried out with the aqueous extract on *S. lycopersicum*, it was observed that there were no significant variations (P < .005) at concentrations of 2 and 4%. The germination percentages at those concentrations and the control were approximately 90% germination. In the plates soaked with the 10% extract, a clear decrease in the germination percentage was observed, which would indicate an inhibitory power of the extract on the seeds at this concentration. On the other hand, at a concentration of 20% the inhibition was total. In the bioassays carried out with the ethanolic extract; with dilutions at 2 and 4%, no significant variations were observed with regard to the control (P < .005). In the plates embedded with the 10% concentration, it was evidenced that the percentage of germination in these plates decreased compared to that of the more diluted concentrations, that is, the extract would influence germination when it is at 10 and 20% (P > .005).

These results can be seen in Fig. 2A.

In the case of ethanolic extract, a delay in germination time was also observed, the control began to show germinated seeds on the third day of the trial, while with concentrations of 2 and 4% this began to be evident after the fourth and fifth day respectively. The plates with concentration of 10 with germinated seeds began to be observed only after the tenth day, which could indicate that the beginning of the germination process is directly related to the dose used in the test. This trend was corroborated with the 20% extract, since no germination was observed under these conditions, with this concentration the inhibition in germination was total. Similar data on germination percentage were obtained by Tur et al. [13], using *Duranta repens* aqueous extracts on the germination of *S. lycopersicum* seeds and by Borella & Pastorini [14], using Ombú extracts, also on *S. lycopersicum* seeds. The germination...
of the *L. multiflorum* seeds in the treatment with the aqueous extract does not seem to modify their behavior. Both in the control plates and in those with concentrations of 2, 4 and 10%, the germination percentage remains between 82 and 88% (Fig. 2B). For the 20% solution, a decrease in the germination percentage was observed, which was 77.6%. It was observed that the interaction of the aqueous extract with the germination of *L. multiflorum* is not significant, according to the Tukey test (with a P < .005), for any of the concentrations studied. Siddiqui et al. [15] observed that the percentage of wheat germination decreased proportionally to the increase in the concentration of aqueous extracts of *Prosopis juliflora*. In the treatment with the ethanolic extract, it was observed that the germination decreased exponentially as the concentration of the ethanolic extract increased, this would show its inhibitory power even in the lowest concentrations until reaching total inhibition using the 20% extract (Fig. 2B). In this experiment, germination was evidenced from the third day for the control and the concentration of 2%, on the fourth day for the concentration of 4% and only after the twelfth day for the concentration of 10%. Germinated seeds were not recorded for concentrations of 20%.

### 3.2 Root Growth

The root growth of *S. lycopersicum* subjected to the treatment with the aqueous extract does not show significant variations in the tests with concentrations of 2 and 4% (according to Tukey’s test for P < .005), with regard to the control. On the other hand, root growth noticeably decreases when treated with the 10% extract. The aqueous extract of *Duranta repens* caused significant reductions on the growth of the radicle of *S. lycopersicum*, in concentrations of 1, 2 and 4%, Tur et al. [12]. Qasem & Hill [16] worked with leachates from the root of *Senecio vulgaris* and found that they produced notable reductions in root growth of *S. lycopersicum*. In this case of treatment with the ethanolic extract, it is observed that the root growth of the *S. lycopersicum* seedling decreases proportionally to the increase in the concentration of the extract. This would show that the growth inhibitory interaction is directly proportional to the dose of the solution used, decreasing the root elongation process (Fig. 3A). In Fig. 3B it is observed that the root growth in *L. multiflorum* under the influence of the aqueous extract showed a tendency to slightly decrease its growth when the concentration increases. In the treatment of these seedlings with the ethanolic extract, a decrease in root growth was observed as the concentration increased and it turned out to be more pronounced and significant for the Tukey test with P < .005. The data obtained in this study coincide with those obtained in the work of Siddiqui et al. [15], where it was shown that aqueous extracts of *Prosopis juliflora* reduced the root growth of *Triticum aestivum* (wheat), as the concentration was increased. These data also coincide with those obtained by Butnariu [17], in his work with alcoholic extracts of *Pteridium aquilinum* on seeds of *Poa pratensis*. The more pronounced results when inhibiting root length than germination could be explained from the basis that the initial growth of the seedlings is more sensitive than their germination, since for each seed the phenomenon is discrete; germinating or not [18].

**Fig. 2. Mean ± Standard Deviation of the final germination percentages of *S. lycopersicum* and *L. multiflorum* seeds with different concentrations of aqueous and ethanolic extracts of *S. filaginoides*. Equal letters do not differ in the Tukey test (with a P <0.005). (-) Not analyzed**
Data for the concentration at 20% was excluded from the analysis, taking into account that, at this concentration, there were only germinated seeds of *L. multiflorum* and only with the aqueous extract. This would make comparison difficult and would not add relevant information to the issue raised. The same determination was made for the growth of the aerial part.

### 3.3 Growth of the Aerial Part

In the study of the aerial part (stem and leaf) of the *S. lycopersicum* seedlings, the aqueous extract would slightly stimulate their growth at concentrations of 2 and 4%, with regard to the control, and would inhibit it, also slightly, at the concentration of 10%, without showing a significant difference for any of the concentrations tested. The ethanolic extract produces, first, a significant stimulation (P < .005) in the growth in the concentration of 2% and a later and clear decrease in the growth in the highest concentrations (4 and 10%), being in this case all significant variations (P < .005) (Fig. 4A).

Borella and Pastorini [14] observed that the growth of the aerial part of *S. lycopersicum*, in contact with aqueous extracts derived from ombú leaves, was stimulated when it was found to be concentrated at 1%. Likewise, at higher concentrations of this extract, progressive decreases in growth occurred. In the study of the growth of the aerial part of the *L. multiflorum* seedlings, differences were observed in the results between the test treated with the aqueous extract and that carried out with the ethanolic extract (Fig. 4B). In the case of the treatment with the aqueous extract, no significant variations were observed for any of the concentrations studied with regard to the control, so an interaction between the aqueous extract and the initial growth of the aerial part of the seedlings could not be established. In the measurements made on the seedlings treated with the ethanolic extract, a decrease in the length of its aerial parts was observed proportional to the increase in the concentration of the extract, being significant (P < .005) from the concentration of 4%, with regard to the control (Fig. 4B).
4. CONCLUSIONS

In this study, in the extracts studied were observed that the aqueous extract does not present significant interaction for any of the parameters measured, neither with the L. multiflorum nor with S. lycopersicum the for the concentrations tested. It was observed that the ethanolic extract significantly inhibited the germination of L. multiflorum seeds at all concentrations, but not for S. lycopersicum, where inhibition was observed only at concentrations of 10 and 20%. In addition, the effect on the length of the root was observed more sustained in the seeds of the species L. multiflorum depending on the dose tested. A singular interaction was observed in the study of the growth of the aerial part, where the species S. lycopersicum shows a stimulus at the concentration of 2%. The other concentrations showed inhibition for the two species. The final conclusion is that the germination percentages is dependent on the doses used for the ethanolic extract. This information could benefit future studies in search of environmentally friendly herbicides. In contrast, a priori, the aqueous extract would not provide encouraging results to be used as a bioherbicide.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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