

# Allelopathic Potential of *Hesperozygis ringens* Extracts on Seed Germination of Soybeans and Beggarticks

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Received: July 27, 2020

Accepted: September 1, 2020

Online Published: October 15, 2020

doi:10.5539/jas.v12n11p1

URL: <https://doi.org/10.5539/jas.v12n11p1>

## Abstract

*Hesperozygis ringens* L., popularly known as *espanta-pulga*, is a native shrub found in rocky fields in Rio Grande do Sul. Its leaves produce a lot of essential oil, which contains pulegone as a main component that may be allelopathic. The objectives of this study were to analyze the allelopathic effects of *H. ringens* leaf extracts on the germination and initial development of soybean seeds and to evaluate the herbicidal potential of these extracts on beggarticks (*Bidens pilosa* L.). The seeds were placed on paper embedded in an aqueous extract of *H. ringens* leaves at concentrations of 0, 25, 50, 75 and 100% and maintained in a germination chamber at 25 °C and a photoperiod of 12 h. The tests included the following: germination, first germination count, germination speed index, and length and dry mass of seedlings. The experimental design was completely randomized. Based on the results, the aqueous extracts of *Hesperozygis ringens* leaves had a detrimental effect on the germination and seedling growth of the soybeans and beggarticks, evidencing their allelopathic action.

**Keywords:** allelopathy, weeds, germination process

## 1. Introduction

In Brazil, soybean is one of the main seed crops, represents a large part of the agricultural yield, and is linked to several production chains. Productivity is mainly limited by the effects of competing with weeds that can reduce seed productivity by up to 90%, depending on the weed species, population density and genotype used (Horneburg et al., 2017; Zandoná et al., 2018), which results in the chemical control of large agricultural areas. However, an increase in cases of resistance of beggarticks and other plants to herbicide inhibitors of 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), photosystem II (PS II) and acetolactate synthase (ALS) is a growing concern, making post-emergence control of soybeans more difficult and increasing control costs (Alcántara-De La Cruz et al., 2016; Takano et al., 2016; Mendes et al., 2019).

Thus, crop systems that use fewer chemical products have increased in recent years as a response to the social demand for agroecological or organic products. Associated to this, the use of plants with allelopathic potential can be an important integrated management strategy of weeds in production fields, is a cultural method, and can also be used to discover new herbicidal molecules, better understand the interaction of companion planting and adequate sowing systems among species, and control pests and diseases (Venzon et al., 2005; Jabran, 2017; Villa et al., 2019).

Studies show that allelopathic extracts interfere with the initial growth of invasive plants (Sartor et al., 2015). Additionally, the growing need to reduce the use of synthetic chemical inputs in agroecosystems makes studies linked to allelopathy a tool for the conservation of natural vegetation, and using allelopathy can improve the sustainability of production systems, since it is a biological alternative with a specific action that is less harmful to the environment (Tur et al., 2010). Allelopathy occurs from the liberation and action of compounds produced by the secondary metabolism of plants that possess the ability to inhibit the growth and germination of other species (Cruz-Silva et al., 2015).

Many Lamiaceae species have demonstrated allelopathic potential and the evaluation of this effect could lead to future applications (Kekeç et al., 2012). Recently, *Hesperozygis ringens* (*espanta-pulga*) was studied as a plant with high allelopathic potential capable of significantly affecting the initial growth of other plants (Pinheiro et al.,

2017). Chromatographic analyses report the presence of oxygenated monoterpenes (pulegone) that possibly affect plant development. Therefore, studies about the allelopathic action of *Hesperozygis ringens* extracts can contribute to the knowledge about interspecific relationships and establish alternatives for managing resistant weeds.

The objectives of this study were to analyze the allelopathic effects of *Hesperozygis ringens* leaf extracts on the germination and initial development of soybean seeds (*Glycine max* L. Merr) and to evaluate the herbicidal potential of these extracts on beggarticks (*Bidens pilosa* L.).

## 2. Material and methods

The experiment was conducted in the Plant Genetics Laboratory at the Biology Department of the Federal University of Santa Maria (UFSM), RS. Leaves of *Hesperozygis ringens* were ground in blender for approximately 2 minutes at the proportion of 100 g of fresh leaves to 1 liter of distilled water; this was considered the 100% (p/v) crude extract, according to Bonfim et al. (2011). Subsequently, the mixture was left to rest for 24 hours in the dark under refrigeration ( $\pm 10$  °C). After this, the extracts were filtered with a paper filter and concentrations of 25, 50, 75 and 100% were made. Distilled water was used as the control (0%).

To evaluate the allelopathic potential of the plant extracts on the seed germination of soybeans and beggarticks, we conducted the following tests:

*Germination*: This was conducted with four repetitions of 50 seeds distributed on three sheets of germitest paper moistened with distilled water or the respective extract. After sowing, the paper roles were maintained at 25 °C, with 12 hours of light, and each species was counted based on the Rules to Analyze Seeds (Brasil, 2009). The results are expressed as percentages of normal and abnormal plants and ungerminated seeds.

*First count*: conducted together with the germination test, where the percentage of normal seedlings was determined on day four of the test.

*Germination speed index (GSI)*: germinated seeds were counted daily at the same time. The criterion for germination was the protrusion of the radicle and the germination speed index was calculated based on the formula in Maguire (1962).

*Seedling length*: Ten seedlings of each repetition from the germination test of the different extracts were randomly measured with a millimeter ruler. The average length of the seedlings (total length, shoot length and root length) was obtained by adding the averages of each repetition and dividing by the number of seedlings measured, with the results expressed in centimeters (cm).

*Seedling dry mass*: This was conducted together with the germination test, where ten seedlings were weighted from each repetition. Then, the seedlings were placed in paper bags in an oven set at 60 °C until they reached a constant mass (24 h). Subsequently, the seedlings were reweighed on a precision balance (accuracy 0.001 g) and the results were expressed in milligrams (mg).

The experimental design was completely randomized, with treatments comprising the extract concentrations, and analyses were made with the program Sisvar (Ferreira, 2014).

## 3. Results and Discussion

The analysis of variance demonstrated the significant effect of the concentrations of the *Hesperozygis ringens* leaf extracts on the germination percentage, germination speed index (GSI), and dry mass of the seedlings, for both species, starting at a concentration of 25% compared to the control (Figure 1). However, these effects were more significant on the seed germination of beggarticks than the soybeans. High sensitivity of beggarticks to the extracts was observed, independent of the concentration used, with up to a 96% reduction in germination for the highest dose (Figure 1A). This behavior was not observed for the soybeans, which had germination values above 80%, except for the 25% concentration (Figure 1A).

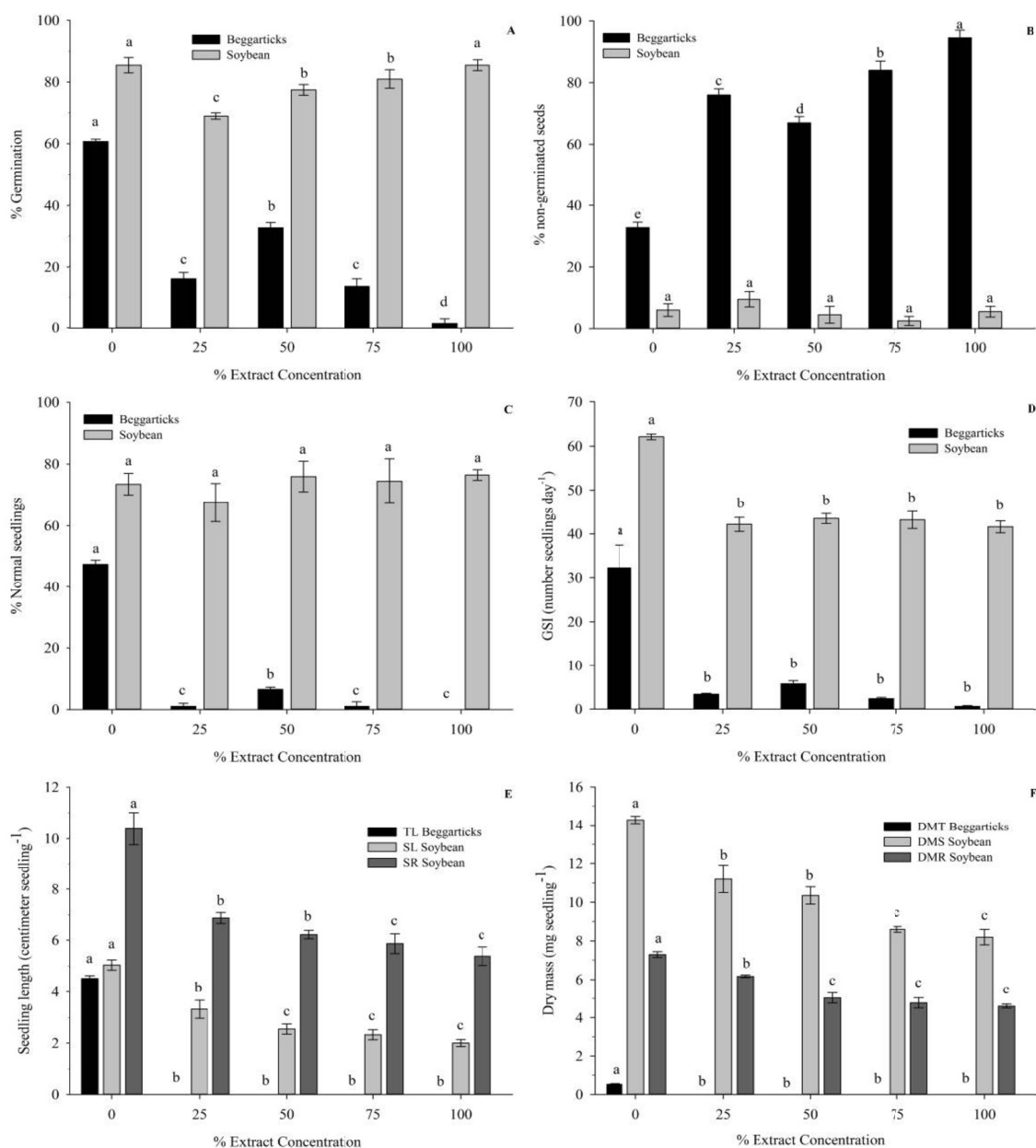


Figure 1. Effect of *Hesperozygis ringens* aqueous extracts on the seed germination of soybean and beggarticks seeds

Note. TL: total length, SL: shoot length, SR: root length, DMT: total dry mass, DMS: shoot dry mass, and DMR: root dry mass.

Similar results were observed by Pinheiro et al. (2017) using the essential oil of *Hesperozygis ringens*, which completely inhibited the germination of beggarticks at the highest concentration (5%) without significantly affecting the germination of rice and lettuce, although it delayed the emergence and harmed the root and shoot lengths of soybeans. Additionally, von Poser et al. (1996) observed the dominance of *H. ringens* in places where it was found and described the inhibition activity of its essential oil on the germination of lettuce seeds, which is considered a bioindicator species for allelopathic activity studies.

The number of ungerminated seeds, which were not dead or dormant, was also quantified for both species (Figure 1B). Apparently, these seeds had absorbed water and increased in size, but the radicle did not protrude during the seven-day period. Thus, the *Hesperozygis ringens* extract negatively affected the germination process, mainly for beggarticks (Figure 1B), probably because it interfered in the cellular division and elongation process that is essential in this initial phase, as well as modified the plant enzymatic system (Pourmorad et al., 2006; El

Shora, El Gawad, 2014). Inhibition of germination was observed in a study involving oxygenated monoterpenes isolated from allelopathic plants (the same category of compound found in the plant in the present study), with an increase in the number of ungerminated seeds and decrease in seedling growth due to multiple mechanisms of action, such as inhibition of DNA and RNA synthesis, damage to organelles (*e.g.*, the nucleus and mitochondria), inhibition of  $\alpha$ -amylase activity and accumulation of lipid bodies in the cytoplasm (Kato-Noguchi & Macías, 2006; Kordali et al., 2007).

In the first germination count (Figure 1C) a sharp decrease (an average of 90%) in the vigor of beggarticks was observed starting at the 25% extract concentration, which is evidence of the possible inhibitory action of the compound on the seed germination process. This result does not apply to the soybeans, demonstrating specificity between the compound/species tested.

For the germination speed index, the results demonstrate a significant reduction in the number germinated seeds per day in relation to the control, for both species (Figure 1D). Similar results were observed by Borella et al. (2012), who used extracts from *Piper mikanianum* (Kunth) Steudel leaves on the germination of radish seeds that influenced the GSI, a response that depended on the dose used.

Some authors note that delays in seed germination of any species can have relevant biological implications, since this can influence plant establishment under natural conditions and relationships with other nearby species (Escudero et al., 2000; Chaves et al., 2001). Among the possible changes in the germination process, effects on the permeability of membranes, RNA transcription and translocation, respiration, conformation of enzymes and receptors, and a combined action of these changes have been observed (Ferreira & Áquila, 2000).

The length (root and shoot) and dry mass of the seedlings of soybeans and beggarticks were significantly reduced with the increase in the concentration of the *Hesperozygis ringens* aqueous leaf extracts (Figures 1E and 1F). Additionally, the results indicate that beggarticks is more sensitive to the *H. ringens* aqueous extract compared to soybean, where starting at a concentration of 25% there was 100% reduction in length and dry mass compared to the control.

Studies of allelopathy of plants on *Bidens pilosa* have demonstrated that the impact on root and shoot growth and, consequently, dry mass, is directly linked to a lower rate of cellular division, and the respective change in the structure of cells, as well as a reduced ability to compete under field conditions (Abd El Gawad et al., 2015). In addition, Oliveira et al. (2019) report the negative effects of the development of *Bidens pilosa* seeds soaked in different sunflower, *Brachiaria brizantha* (Hochst.) and sorghum extracts, and note that the inhibition of root growth is one of the main methods to reduce the potential damage caused by weeds, since this affects the absorption of water and nutrients.

Abnormalities were recorded for the soybean seedlings, mainly in the root system (Figure 2). The most common symptoms were much shorter roots and a decrease in secondary roots, as well as oxidation and necrosis on the root tips. Seedling abnormality is an important instrument in trials of allelopathy, in which root necrosis is the most common symptom (Ferreira & Áquila, 2000). According to Carvalho et al. (2014), the root system of plants is most sensitive to the action of allelopathic substances, since elongation depends on cellular divisions that, if inhibited, can compromise normal development.

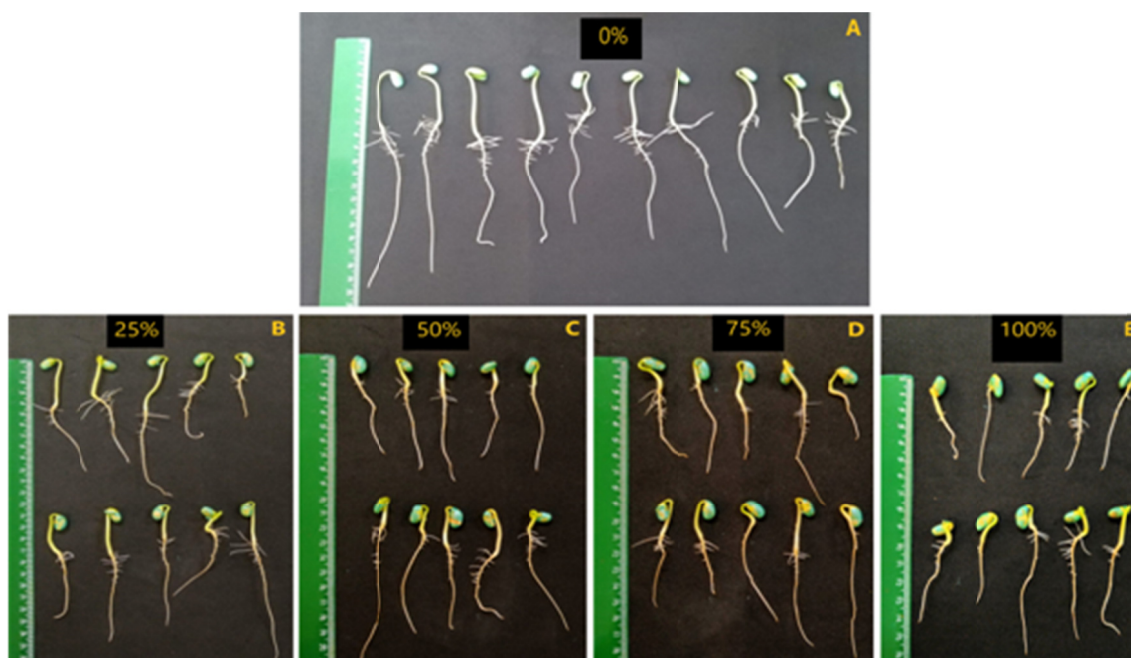


Figure 2. Soybean seedlings under aqueous leaf extracts of *Hesperozygis ringens*. (A) Control. (B) 25% extract. (C) 50% extract. (D) 75% extract. (E) 100% extract.

Studies conducted by von Poser et al. (1996) show that for all concentrations tested, alcoholic extracts of *Hesperozygis ringens* exhibit a significant inhibitory effect on the seed germination of lettuce and, at a higher dilution (1:64), the root tips exhibited some necrosis. Similarly, using an extract of *Artemisia artemisiifolia* L., Formigheiri et al. (2018) verified that a concentration of 75% significantly reduced the number of normal soybean seedlings and observed atrophy in the stems and delayed growth of seedlings. In this case, the soybean seedlings remained with the seed coat adhered to the cotyledons, preventing the release of the leaflets.

Considering the greater significant effects on beggarticks, the results of this work suggest the possible use of *Hesperozygis ringens* leaf extracts to control this weed in soybean crops, as well as the possibility of creating new herbicidal molecules as an alternative to control resistant plants. However, to compliment this idea, future studies are needed because allelopathy is a dynamic process that involves the influence of environmental factors (e.g., allelopathic behavior in different soil types), and age and organ of the plant used. Further, the discovery of the allelopathic effect of *Hesperozygis ringens* and its uses should stimulate the preservation and multiplication of this species.

#### 4. Conclusion

The aqueous extracts of the *Hesperozygis ringens* leaves had a detrimental effect on the germination and seedling growth of the soybeans and beggarticks, evidencing their allelopathic action.

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