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Allelopathic Potential of *Bidens pilosa* L. Extracts on seed Germination and Seedling Development of Lettuce and Chicory

Potencial alelopático do extrato de Bidens. pilosa L sobre a germinação e desenvolvimento de plântulas de alface e almeirão

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ABSTRACT

Vegetable cultivation holds significant socioeconomic importance, while *Bidens pilosa* L., a weed with allelopathic effects, can negatively impact seed germination and early development in vegetables. This study aimed to determine the allelopathic potential of *B. pilosa* on the germination and initial development of lettuce and chicory seeds. Two separate trials were conducted, one with lettuce seeds and the other with chicory seeds. Germination and vigor tests were performed in petri dishes using a completely randomized design, with four concentrations of aqueous extract of *Bidens p.* (0, 20, 60, and 100%) and five replications, totaling 20 experimental plots. The parameters evaluated included: number of days to start germination, number of days to finish germination, germination percentage, germination speed index, average germination time, root length, and hypocotyl length. The number of days to start germination and the average germination time of lettuce and chicory seeds were greater as the concentration of aqueous extracts of *B. pilosa* increased. Conversely, with increasing extract concentrations, the germination percentage, germination speed index, root length, and hypocotyl length of lettuce and chicory seedlings were reduced. The aqueous extract of *B. pilosa* leaves exhibited inhibitory allelopathic activity on the physiology of germination and the development of lettuce and chicory seedlings.

KEYWORDS: *Cichorium intybus;* seed physiology; *Lactuca sativa;* weeds.

RESUMO

O cultivo de hortaliças tem grande importância socioeconômica, e a *Bidens pilosa* L. é uma planta daninha com efeito alelopático que pode diminuir a germinação de sementes de hortaliças e prejudicar seu desenvolvimento inicial. Com este estudo objetivou-se determinar a potencialidade alelopática de picão-preto sobre a germinação e desenvolvimento inicial de sementes de alface e almeirão. Foram conduzidos dois ensaios separadamente, um com sementes de alface e outro com sementes de almeirão. Os testes de germinação e vigor foram conduzidos em placas de petri e dispostos em delineamento inteiramente casualizado, com quatro concentrações de extrato aquoso de picão-preto (0, 20, 60 e 100%) e cinco repetições, totalizando 20 parcelas experimentais. As características avaliadas foram: número de dias para início de germinação, número de dias para finalizar a germinação, porcentagem de germinação, índice de velocidade de germinação, tempo médio de germinação, comprimento da raiz e do hipocótilo. O número de dias para iniciar a germinação e o tempo médio de germinação de sementes de alface e almeirão foram maiores, à medida que aumentou a concentração de extratos aquosos de picão-preto. Com o aumento das concentrações de extrato, o percentual germinativo, o índice de velocidade de germinação, o comprimento da raiz e do hipocótilo de plântulas de alface e almeirão foram reduzidos. O extrato aquoso de folhas de picão-preto apresentou atividade alelopática inibitória sobre a fisiologia da germinação e o desenvolvimento de plântulas de alface e almeirão.

PALAVRAS-CHAVE: *Cichorium intybus;* fisiologia de sementes; *Lactuca sativa;* plantas daninhas.

INTRODUCTION

The national vegetable growing area is 5.1 million hectares and has a production of 53 million tons, with socioeconomic importance (CARVALHO et al. 2019). However, these areas are managed with sequential crops throughout the year, intensifying land use (VENDRUSCOLO et al. 2023) and causing increased erosion processes and nutrient depletion (FERREIRA et al. 2019).

Vegetable cultivation is characterized by the need for fertile soil, high water availability (SILVA & CASTRO 2019), and significant use of chemical fertilizers, which favors the presence of weeds that compete directly with the main crop for resources such as water, light, and nutrients (SOARES et al. 2019). In addition, spontaneous plants release allelopathic substances that can negatively interfere with the culture of interest.

Allelopathy is a common biological interference whereby an organism produces biochemical compounds that influence the growth, development, survival, and reproduction of other organisms (EL ID, 2020, HIERRO & CALLAWAY 2021). These compounds are known as allelochemical and can benefit or harm other organisms (SCAVO et al. 2019). In addition, they interfere with seed germination (ZHAO et al. 2022), which is a crucial stage in the development of a species.

Bidens pilosa L. is a weed with allelopathic effects that can reduce vegetable seed germination and hinder its initial development. Allelochemicals can affect plant metabolism and impair processes such as phytohormone synthesis, nutrient absorption, and enzymatic activities (COELHO et al. 2014), directly interfering with the productivity of the crop of interest, especially vegetables, which are more sensitive to competition between plants.

To overcome the effects of allelochemical in agriculture, the use of aqueous extracts has been studied to evaluate plant growth and production and control plant pathology. These studies are possible through the use of substances with high polarity and allelopathic activity that simulate the effects of certain weed species on plants of interest (SOUZA FILHO et al. 2010).

Vegetable crops such as lettuce (*Lactuca sativa* L.) and (*Cichorium intybus* L.) are cultivated on a large scale because they allow an increase in economic flow and the sustainable development of rural properties in southwestern Brazil. However, they are sensitive to the action of allelopathic substances, especially during seed germination, resulting in low productivity, non-uniformity in the field, and low productivity.

Understanding the different relationships and intensities between plants managed in the same space and time (FARIAS et al. 2020) is therefore indispensable for adopting the most appropriate crop management. Thus, this study aimed to identify the allelopathic effects of *B. pilosa* L. extract on the physiology of seed germination and development of lettuce and seedlings. This information will help optimize the management of spontaneous species, which can compete with the main crop, hindering their development and, consequently, causing losses to vegetable producers.

MATERIALS AND METHODS

The experimental tests were conducted at the Seed Technology and Production Laboratory of the Universidade Estadual do Sudoeste da Bahia (UESB), Vitória da Conquista-BA campus, in August 2018. The seeds were purchased from an agricultural store in Vitória da Conquista-Bahia. The studied species were lettuce (*L. sativa L.*) with a germination rate of 95% and purity of 99% and chicory (*C. intybus* L.) with a germination rate of 85% and purity of 99%.

The water content of lettuce and chicory seeds was obtained using the greenhouse method at a constant temperature of 105 ± 3 ºC for 24 h according to the Seed Analysis Rules (SAR), with four repetitions of 50 seeds each. The result was expressed as a percentage of the wet weight of the sample (BRASIL 2009).

To evaluate the germination characteristics and the initial development of the species (*L. sativa* and *C. intybus*), two experiments were carried out in a completely randomized design, with four concentrations of *B. pilosa* aqueous extract (0, 20, 60 and 100%) and five replicates, totaling 20 plots per trial.

The aqueous extract of *B. pilosa* was obtained from leaves collected in a UESB field. The leaves of *B. pilosa* were dried in a forced air circulation oven (65 ºC) until a constant weight was achieved. The material, once dried, was ground in a Willey mill to obtain a powder, which was then homogenized in deionized water at a ratio of 10% mass/volume (10 g of ground material for 100 ml of deionized water).

The aqueous extract of *B. pilosa* leaves was left to stand for 48 h in a refrigerator (5 ± 1 ºC), then filtered through a 2 mm mesh sieve and filter paper twice to obtain the crude extract (100%). From this, dilutions were made to the following concentrations: 20% and 60% (20 ml and 60 ml of the extract for 80 and 40 ml of deionized water, respectively), with the control (0%) consisting only of deionized water. The pH of the pure extract was measured using a pH meter.

Five replicates of 50 seeds were used (250 seeds per treatment. Sowing was performed in Petri dishes (12 cm in diameter) lined with two discs of autoclaved germiest paper (substrate). The substrates were moistened with deionized water (control) or the aqueous extract of *B. pilosa* leaves, according to each treatment, equivalent to 2.5 times the mass of dry paper in their respective concentrations. After seeding, the Petri dishes were placed in a Biochemical Oxygen Demand (B.O.D.) germination chamber at a constant temperature of 20 ºC.

The characteristics evaluated were: number of days to start germination (NSG), number of days to finish germination (NFG), recorded daily from the start of germination to full germination; germination percentage (%GER); germination speed index (GSI) (MAGUIRE 1962); average germination time (AGT) (LABOURIAU & VALADARES 1976); root length (RL); and hypocotyl length (HIPL).

Evaluations were performed daily, with seeds considered germinated when they had a primary root of 2 mm. At the end of the experiment, the percentage of total germination was obtained from the formation of normal seedlings, with the results expressed as percentages (BRAZIL 2009). Seven and fourteen days after the formation of lettuce and chicory seedlings, the average root and hypocotyl lengths were measured using a digital caliper, taking 10 seedlings at random from each repetition. Between the reading days, the Petri dishes were moistened with standard amounts of deionized water and extract concentrations for the respective treatments.

The data were submitted for analysis of variance using the F test ($p \leq 0.05$). Subsequently, the concentrations of the aqueous extract were submitted for polynomial regression analysis, with regression equations adjusted to the third degree. The analyses were performed using SISVAR 5.4 statistical software (FERREIRA 2019).

RESULTS AND DISCUSSION

The initial water contents of lettuce and chicory seeds were 5.0% and 5.8%, respectively. These values are within the expected standards for vegetable seeds stored in airtight containers.

The aqueous extract of *B. pilosa* leaves had a pH of 6.0, which is within the neutral range, thereby reducing the possibility of pH interference with the results. This supports the hypothesis that compounds in *B. pilosa* extracts have phytotoxic effects on the germination of lettuce and chicory seeds. Similar values (6.29, 6.32 and 6.95) were obtained by BORELLA & OLIVEIRA (2023) when evaluating different B. pilosa extraction solutions.

The concentrations of the aqueous extract of *B. pilosa* influenced the characteristics observed for lettuce and chicory species (Tables 1 and 2).

Table 1. *Summary of the analysis of variance and coefficient of variation (CV) for the number of days to start germination (NSG), number of days to finish germination (NFG), germination percentage (%GER), germination speed index (GSI), average germination time (AGT), root length (RL), and hypocotyl length (HIPL) of lettuce (A) and chicory (B) seedlings as a function of the concentration of the aqueous extract of B. pilosa.*

(A)

FV	GL	Mean squares						
		NGS	NFG	%GER	GSI	AGT	RL	HIPL
Concentration of the extract	3	18,38**	7,53*	5822,85**	1416,60**	20,22**	3,97**	11,33**
Residual	16	0,05	1,58	30,90	10,56	0,11	0,03	0,07
CV (%)		10,91	21,27	7,36	14,75	10,28	20,30	12,92

(B)

FV	GL	Mean squares						
		NGS	NFG	%GER	GSI	AGT	RL	HIPL
Concentration of the extract	3	1,20**	5,20	1172,45**	932,84**	19,11**	2,46**	6,52**
Residual	16	0,08	13,33	53,35	10,28	0,35	0,03	0,37
CV (%)		19,56	38,02	8,70	14,76	17,69	21,09	22,88

Significant ($p \leq 0.01$) by the F test; *significant ($p \leq 0.05$) by the F test.

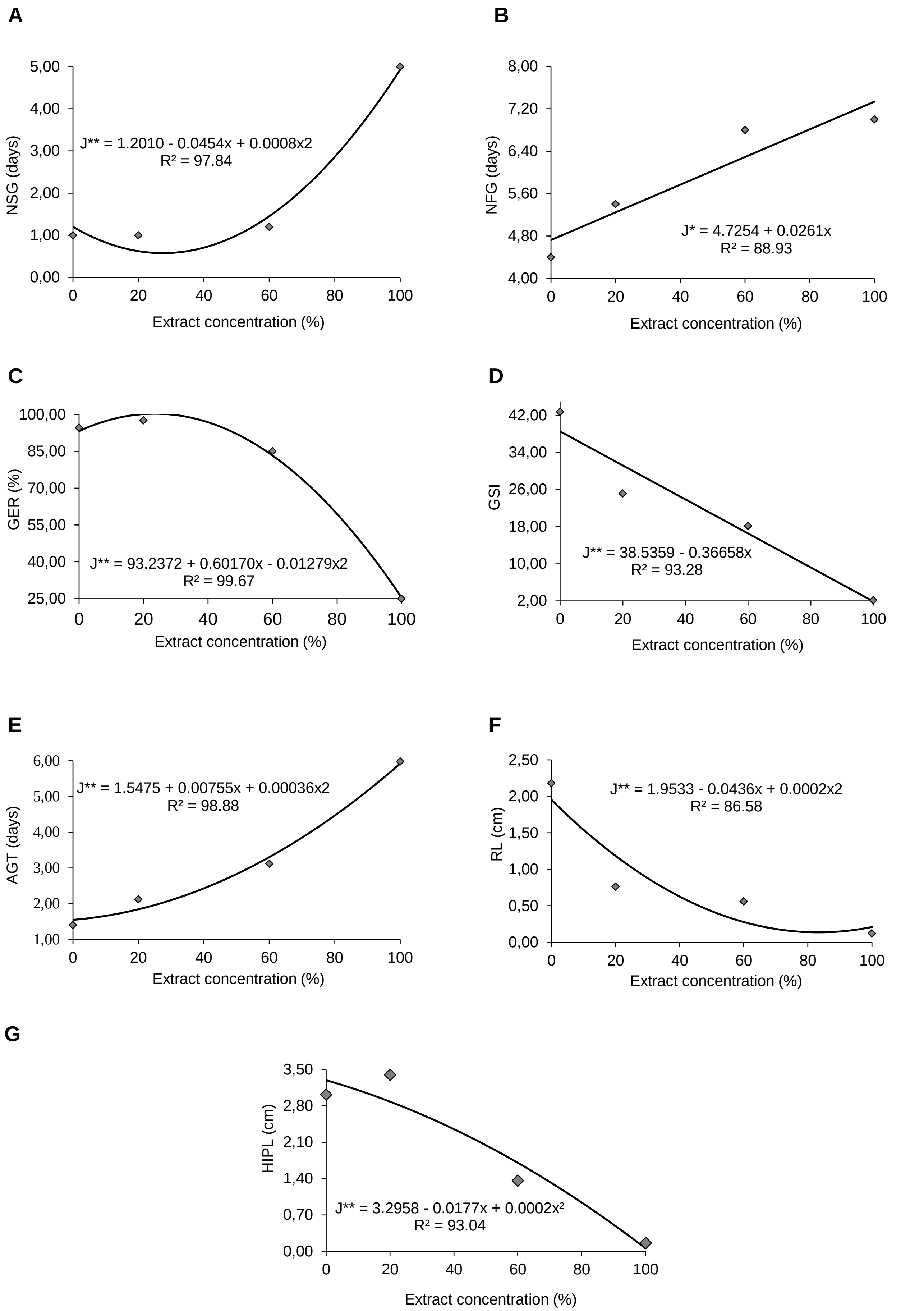


Figure 1. *Number of days to start germination (NSG) (A); number of days to finish germination (NFG) (B); germination percentage (%GER) (C); germination speed index (GSI) (D); average germination time (AGT) (E); root length (RL) (F); and hypocotyl length (HIPL) (G) of lettuce seedlings as a function of the concentration of the aqueous extract of B. pilosa.*

The extract concentration data in relation to NSG fit the decreasing quadratic model (Figure 1A). The seeds showed the shortest time (0.58 days) to start germination at a minimum concentration of 26%. In addition to this concentration, the NSG content increased as the extract concentration increased. On the other hand, it was observed that the NFG (Figure 1B) adjusted to the increasing linear equation, indicating that higher extract concentrations may promote slower germination in lettuce seeds.

The %GER response was adjusted to the increasing quadratic model with an estimated maximum of 100% to 16% extract concentration. With the increase in the concentrations used, the germination percentage and the GSI were reduced, and in the treatment of 100%, the GER and GSI were 26% and 1.88%, respectively (Figures 1C and 1D). This was also observed by BITENCOURT et al. (2021), who reported that the germination percentage of lettuce seeds decreased with increasing concentrations of *Schinus terebinthifolius* Raddi extract.

Regarding the GSI, AZAMBUJA et al. (2010) stated that the presence of allelochemical in infusions promotes the inhibition of the rate of development and translocation of nutritional elements from the endosperm to the embryo, thereby reducing this index. Other authors, such as FIORESI et al. (2021), observed a decrease in GSI in lettuce seeds exposed to *Solanum pimpinellifolium* extracts.

The increase in extract concentrations also resulted in an increase in AGT, with an increasing quadratic effect, and shorter AGT (1.55 days) at the 0% concentration (Figure 1E). Overall, the 0% concentration (control) promoted the highest germination speed and, consequently, the seeds germinated in a shorter time. However, as the extract concentrations increased, the germination speed decreased and the average germination time increased. This delay in germination due to increased aqueous extract concentrations occurs because of allelochemical actions that influence plant physiological processes (GARCIA et al. 2022).

Higher extract concentrations also influenced the root and hypocotyl lengths of lettuce seedlings, with a decreasing quadratic effect and minimum lengths of 0.14 cm and 0.6 cm at concentrations of 78% and 100%, respectively. This was possibly due to allelopathic compounds in *B. pilosa* leaves, which affect the metabolism and growth of roots and other structures.

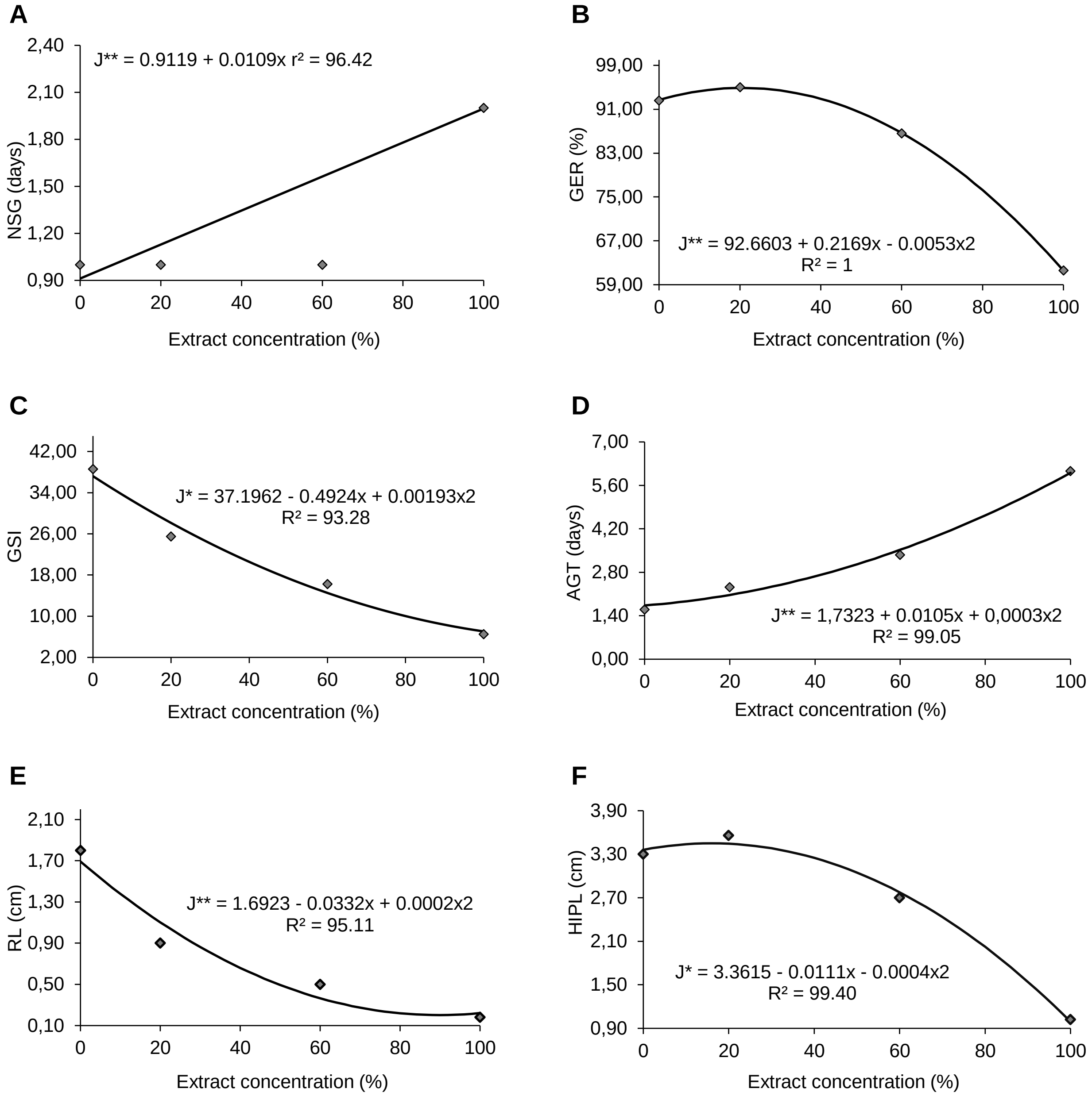
For chicory seed analysis, data referring to NSG, GER%, GSI, AGT, RL, and HIPL fits linear and quadratic models. The NFG responses to extract concentrations did not differ; therefore, the regression equations were not adjusted. For the quadratic models, the maximum or minimum concentration of *B. pilosa* extract in the seeds was calculated (Figure 2).

The response of extract concentrations in %GER fits the increasing quadratic model, with the maximum germination (95%) at 12% concentration. The GSI fits the descending quadratic model, with the lowest index (7.0) at 100% concentration (Figures 2B and 2C). NIG (Figure 2A) and AGT (Figure 2D) fit increasing linear and quadratic models, respectively, indicating that higher extract concentrations impair the start time and total germination time, with the shortest AGT (1.7 days) at 0% concentration.

The use of *B. pilosa* plant extract interferes with seed germination speed by inhibiting or delaying the absorption of water and allelochemical during the imbibition phase, indicating that the presence of *B. pilosa* in the field can also impair chicory germination. In addition, increasing concentrations of the B. pilosa aqueous extract have negative effects on the physiology of chicory seeds.

The root and hypocotyl lengths fit decreasing and increasing quadratic models, respectively (Figures 2E and 2F). The greatest reduction in root length was 86% with a concentration of only 0.20 cm, and the greatest hypocotyl length (3.5 cm) was 16% in concentration. Thus, higher extract concentration impair root and hypocotyl growth. Similar results were obtained from SANTOS et al. (2023), who observed 90% root length inhibition in lettuce seeds with increasing concentrations of Portulaca oleracea extract. According to the authors, allelopathic effects depend on the concentration and type of extract used.

Therefore, this study highlighted the importance of investigating the interactions between agricultural crops and weeds, which may affect their development, especially during the initial growth phase of seedlings. We emphasize the need for future research, particularly under field conditions, to explore the behavior of these vegetables in areas dominated by *B. pilosa*.



**, * Significant ($p \leq 0.01$ and 0.05, respectively) by regression of variance analysis.

Figure 2. *Number of days to start germination (NSG) (A); number of days to finish germination (NFG) (B); germination percentage (%GER) (C); germination velocity index (GSI) (D); average germination time (AGT) (E); root length (RL) (F); and hypocotyl length (HIPL) (G) of chicory seedlings as a function of the concentration of the aqueous extract of B. pilosa.*

CONCLUSION

The aqueous extract of *B. pilosa* leaves showed inhibitory allelopathic potential in the physiology of seed germination and in the development of lettuce and chicory seedlings, indicating the need to control this invader, especially during the seeding and emergence of vegetables studied in the field.

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