

Allelopathic effect of *Sorghum bicolor* and *Digitaria insularis* on germination and initial development of Canola

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ABSTRACT

Agriculture depends on biotic and abiotic factors, and one of them is allelopathy, defined as the chemical interference that one plant has on others, which can negatively affect germination. The objective of this work is to evaluate the allelopathic effects of *Sorghum bicolor* and *Digitaria insularis* on seed germination and initial development of canola seedlings (*Brassica napus* L.). A completely randomized design was used in a 2x5 factorial scheme (two extracts: *D. insularis* and *S. bicolor*) and five concentrations (0, 25, 50, 75, and 100%), with four replicates of fifty seeds, the plot being constituted by a 'gerbox' box containing 50 seeds evenly distributed. The variables analyzed were germination percentage (G), abnormal seedlings (AS), dead seeds (DS), length of the shoot (SL) and primary root (RL), total length (TL) of the seedling, shoot ratio (S/T), and root ratio (R/T). The aqueous extracts of *D. insularis* interfere in all variables studied, which compromises the formation of normal seedlings, regardless of concentration. The extract of *S. bicolor* expressed allelopathic effects only in concentrations above 75%. Both species can inhibit the initial development of canola plants.

Keywords: allelochemicals, inhibition, *Brassica napus*.

Efeito alelopático de *Sorghum bicolor* e *Digitaria insularis* na germinação e desenvolvimento inicial da Canola

RESUMO

A agricultura é refém dos fatores bióticos e abióticos, e um desses fatores é a alelopatia, que se trata da interferência química que uma planta exerce sobre a outra, podendo provocar efeito negativo na germinação. O objetivo deste trabalho é avaliar os efeitos alelopáticos do *Sorghum bicolor* e *Digitaria insularis* na germinação de sementes e no desenvolvimento inicial de plântulas da canola (*Brassica napus* L.). Utilizou-se o delineamento inteiramente casualizado em esquema fatorial 2x5 (dois extratos: *D. insularis* e *S. bicolor*) e cinco concentrações (0, 25, 50, 75 e 100%), com quatro repetições de cinquenta sementes, sendo a parcela constituída por uma caixa 'gerbox' contendo 50 sementes uniformemente distribuídas. As análises realizadas foram: porcentagem de germinação (G), plântulas anormais (PA), sementes mortas (SM), comprimento da parte aérea (CA) e da raiz primária (CR), comprimento total (CT) da plântula, razão da parte aérea (AT) e razão da raiz (RT). Os extratos aquosos de *D. insularis* interferem em todas as variáveis estudadas, o que compromete a formação de plântulas normais, independente da concentração. O extrato de *S. bicolor* expressou efeitos alelopáticos apenas em concentrações acima de 75%. Ambas as espécies possuem capacidade de inibir o desenvolvimento inicial da cultura da canola.

Palavras-chave: aleloquímicos, inibição, *Brassica napus*.

1. Introduction

Canola (*Brassica napus* L.) is an agricultural product of great economic value due to its flexibility, being used both in human and animal consumption and biofuels production. It is the third oilseed most produced in the world, with around 67.8 million tons in the 2018/2019 harvest, having Canada as its main producer, with a production of around 28.5 million tons and in second position, the Union European Union, with a production of 20 million tons, being the largest consumer (24.3 million tons). Brazil has a low production, with 52 thousand tons in the 2019 harvest (EMBRAPA, 2018; USDA, 2019). Brazilian production is intended for oil production, derived from the genetic improvement of *Brassica napus* and *Brassica campestris*, to minimize the content of erucic acid and glucosinolates, enabling digestibility and palatability (Seabra Júnior et al., 2017).

Canola is used in Brazil as a winter crop and as an alternative in the crop rotation system, collaborating to reduce phytosanitary problems of crops of great economic weight such as soybeans, beans, corn, and wheat (Cargnelutti et al., 2015). It can also be used as a vegetal covering of the soil, being the species most cultivated in the country *Brassica napus* L. var. *oliferous*, where its late cultivation in winter could contribute to optimizing the use of agricultural resources further, also creating another income opportunity for farmers (Milciades et al., 2014).

Brazilian agriculture faces several biotic and abiotic factors that compromise the success of the crop; one of these factors is the competition between the establishment of the crop and the presence of weeds, highlighting the allelopathic effect as one of the limiting aspects of the presence of invasive plants, due to chemical interference that can negatively impact germination (Uhlmann et al., 2018). Lessa et al. (2019) define allelopathy as the interaction between chemical molecules produced by secondary metabolism and released into the environment, occurring changes that are generally harmful to the development of another plant. Allelochemical compounds can be produced in various parts of plants, from the root to the leaves, being released by living or dead tissues; the intensity by which the species are affected will depend on the quantity and concentration of these compounds in the environment (Souza Filho, 2014).

Allelopathy, as far as it is concerned, can occur due to the action of a weed exuding chemical substances harmful to the development of plants of the same or different species. Gonçalves et al. (2015) stated that several weeds have an allelopathic capacity that affects the development of commercial plants, such as rice grass (*Echinochloa crusgalli*), mattress grass (*Digitaria horizontalis*), and foxtail grass (*Setaria faberil*).

Among the species that have allelopathic potential, sorghum (*Sorghum bicolor*) stands out for the production of secondary compounds with allelopathic action, more specifically sorgoleone, a lipid benzoquinone produced in the shoot and in the root system of sorghum plants, which is phytotoxic to several plant species (Dayan et al., 2009). According to Dayan and Duke (2014), sorgoleone from the roots is diffused in the rhizosphere, reaching plants faster than the phytotoxins released by the shoot.

Digitaria insularis (sourgrass) is also classified as an allelopathic crop. The increase in agricultural areas demonstrates allelopathic effects on the corn crop, interfering in the development of the plant (Moreira and Mandrick, 2012). The potential for weed interference can vary according to climatic conditions and production systems.

Thus, this study aimed to evaluate the allelopathic effects of *Sorghum bicolor* and *Digitaria insularis* on seed germination and early development of Canola (*Brassica napus* L.).

2. Material and Methods

The experiment was conducted at the Seed Laboratory (LASEM-UEG) of the State University of Goiás, Southeast Campus, Ipameri Unit - Ipameri-GO. Plant materials of the two species, *S. bicolor* and *D. insularis*, were collected manually in sorghum production fields and areas with natural infestations of weeds in agricultural environments, specifically in the plant production sector of Ipameri Campus. The seeds of the cultivated species used - *Brassica napus* (Canola) - were of the Hyola 433 variety. A completely randomized design was used in a 2x5 factorial scheme (two extracts (*D. insularis* and *S. bicolor*) and five concentrations (0, 25, 50, 75, and 100%), with four replicates of 50 seeds each.

First, seed tests were performed to establish the initial germination pattern of the lot, represented by 75%. In order to do so, the canola seeds were placed in plastic boxes containing blotter paper moistened with distilled water with a volume of 2.5 times the weight of the paper and then conditioned in B.O.D chambers at 25 °C and a photoperiod of 12 h of light where germination percentage was evaluated seven days after the seeding. Seeds that produce seedlings with all the essential parts visible (root and plumule) were considered germinated, regarded normal those that formed a shoot with cotyledons, as a developed hypocotyl and primary root (Brasil, 2009).

The extracts were prepared with the species *S. bicolor* and *D. insularis*, where samples of these materials were collected in the field, washed, crushed using a blender, and taken to the air circulation oven at 50 °C for 24 hours. The solutions were prepared by adding deionized water

to 100 g.L⁻¹ of quantified material previously placed inside a container. The blend was stirred to obtain a homogeneous mixture, left to decant for 24 hours. Subsequently, filtering was performed using filter paper, and then solutions of 25, 50, 75, and 100 g.L⁻¹ were prepared by diluting the final extract (Zucareli et al., 2019).

The tests were carried out following the same procedure described above (germination test), except for the substrate moistening, where solutions of different concentrations (25, 50, 75, and 100%) were prepared from plant extracts, and a blank solution (0%), corresponding to the control, was made using deionized water, keeping a water volume of 2.5 times the weight of the paper. There was no need to change the substrate for further wetting, either with distilled water (control) or with plant extracts in their different concentrations during the tests; therefore, variations in extract concentration due to water addition on the same paper were avoided.

Variables evaluation, taken according to Brasil (2009), were reported as follows: percentage of germination (G), where the seeds that produced normal seedlings with all essential parts visible, shoot with cotyledons as hypocotyl and primary root developed were considered germinated; the other seedlings were separated into abnormal ones (PA) and dead seeds (SM) for analysis of behavior inherent to the concentrations of the employed solutions. The length of 20 seedlings was determined as a test with the aid of a millimeter ruler, measuring the shoot (SL) and the primary root (RL) and, subsequently, added to compose the total size (TL) of the seedling. The ratio of these variables was calculated in percentage by the ratio of the shoot (S/T) and the root (R/T) given by the formulas:

$$S/T = \frac{SL}{TL} \times 100$$

$$R/T = \frac{RL}{TL} \times 100$$

where: SL – shoot length (cm); RL – primary root length (cm); TL - total length of seedlings (cm).

The obtained data were initially subjected to normality tests (Kuiper and Watson), in which, when

presenting normal distribution, variance analysis (ANOVA) was performed under a 2x5 factorial scheme (two extracts, from *D. insularis* and *S. bicolor*) and five concentrations (0, 25, 50, 75 and 100%), with means compared by the Tukey test at 5% probability to differentiate the extracts and polynomial regression to study the behavior according to the concentrations of the plant extract. Sisvar 5.6 and SigmaPlot 10 software were used to assist the analysis.

3. Results and Discussion

Data analysis revealed a significant effect ($p \leq 0.01$) of two extracts studied for the different concentrations tested (Table 1). There was a significant interaction between treatments (Table 1), showing the influence of two extracts in different concentrations in canola cultivation.

Table 2 shows the differences between the average values between the studied species. It was observed that the *D. insularis* extract presented a greater interference, which was significant according to statistical analysis, where the variables of abnormal seedlings, shoot length and shoot ratio were more affected by this extract, compromising the seedlings' starting development. Thus, if applied on the field, these seedlings would suffer from the initial competition for light, so they would possibly be suppressed by weeds not being able to develop and reach adulthood.

For the evaluated germination parameters, dead seeds, primary root length, total length, and root ratio, it is noted that the *D. insularis* extract was highly harmful to the seed, interfering mainly on the root system formation, and difficulty in its settlement. This fact is shown in Figure 1, where it is observed a germination reduction of *Brassica napus* (Canola) at concentrations of 50, 75, and 100% of *S. bicolor* extracts, and even seeds death. In a study by Rizzardì et al. (2008) with *Brassica oleracea* cv. capitata and *Brassica rapa*, species of the same genus of canola, allelopathic effects were observed using aqueous extracts of *Bidens pilosa* leaves, both in germination and seedling growth, presenting similar results to those found in this work.

Table 1. Summary of the analysis of variance (ANOVA) for the physiological performance parameters of canola seeds, related to plant extracts and different concentrations, for germination (G), abnormal seedlings (AS), dead seeds (DS), shoot length (SL), primary root length (RL), total length (TL), shoot ratio (S/T) and root ratio (R/T). Ipameri-GO. LASEM / UEG, 2019

Source of variation	P-value								
	DF	G	AS	DS	SL	RL	TL	S/T	R/T
Extracts (E)	1	<0.01*	<0.01*	<0.01*	<0.05**	<0.01*	<0.05**	<0.01*	<0.01*
Concentration (C)	4	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*
E x C	4	<0.01*	<0.01*	<0.01*	<0.01*	<0.05**	<0.05**	<0.01*	<0.05*
Mean Squares									
Averages	-	29.3	39.25	31.45	0.33	0.05	0.60	3.72	32.85
CV (%)	-	13.73	25.41	29.34	16.73	32.9	20.34	12.17	17.47
LSD	-	5.83	6.47	5.98	0.03	0.27	0.07	47.14	3.72

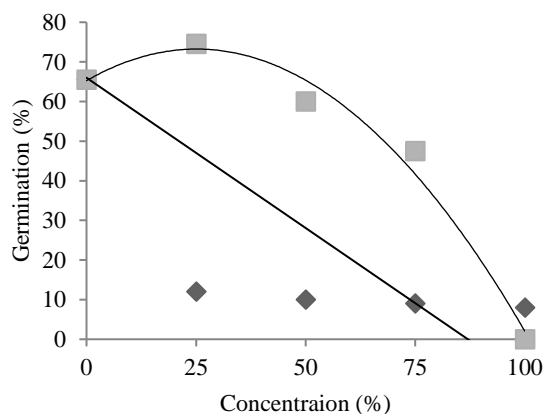
* Significant by the F-test ($P < 0.01$); ** Significant by the F-test ($P < 0.05$); ns not significant; DF: degrees of freedom.

Table 2. Mean values of germination (G), abnormal seedlings (AS), dead seeds (DS), shoot length (SL), primary root length (RL), total length (TL), shoot ratio (S/T), and root ratio (R/T) according to the extracts and its concentrations. Ipameri-GO. LASEM / UEG, 2019.

EXTRACTS	G	AS	DS	SL	RL	TL	S/T	R/T
	-----(-%)-----			-----(-cm)-----			-----(-%)-----	
<i>D. insularis</i>	21 B	66 B	13 A	3.5 A	2.1 B	5.6 B	54 A	26 B
<i>S. bicolor</i>	49 A	12 A	39 B	3.1 B	3.3 A	6.5 A	40 B	60 A
C.V. (%)	13.73	25.41	29.34	16.73	32.9	20.34	12.17	17.47
F-value	147.6	19.53	18.21	6.882	3.250	2.893	10.78	10.78

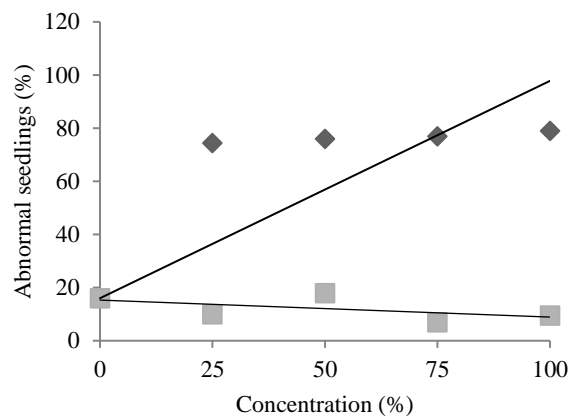
Means followed by a different letter in the column differ by the Tukey test, at 5% significance.

A.



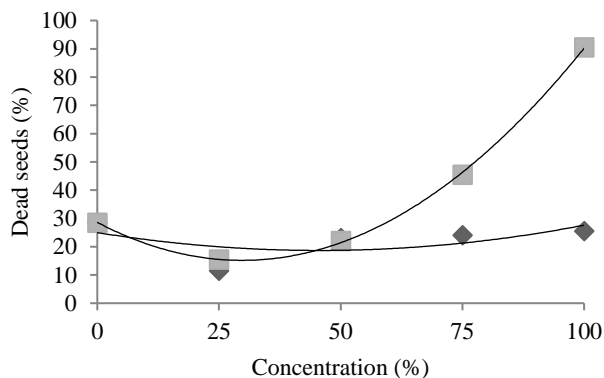
◆ *Digitaria insularis* $y = -0.472x + 44.5$ $R^2 = 0.65^*$
 ■ *Sorghum bicolor* $y = -0.0127x^2 + 0.6366x + 65.243$ $R^2 = 0.98$

B.



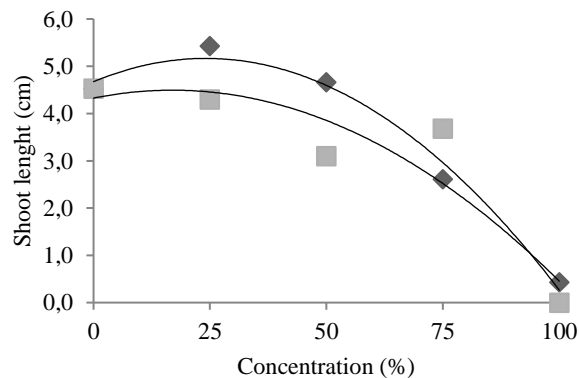
◆ *Digitaria insularis* $y = 0.818x + 16$ $R^2 = 0.56^*$
 ■ *Sorghum bicolor* $y = -0.064x + 15.3$ $R^2 = 0.89^*$

C.



◆ *Digitaria insularis* $y = 0.003x^2 - 0.2769x + 24.986$ $R^2 = 0.62^*$
 ■ *Sorghum bicolor* $y = 0.0152x^2 - 0.904x + 28.6$ $R^2 = 0.99^*$

D.



◆ *Digitaria insularis* $y = -0.0009x^2 + 0.0409x + 4.6731$ $R^2 = 0.98^*$
 ■ *Sorghum bicolor* $y = -0.0006x^2 + 0.0198x + 4.3266$ $R^2 = 0.83^*$

Figure 1. Germination (A), abnormal seedlings (B), dead seeds (C), and shoot length (D) of canola according to the aqueous extract obtained from plant materials of *Digitaria insularis* and *Sorghum bicolor*. * Significant at 5% probability by the t-test.

Germination results showed a quadratic increase of the means for aqueous extract of *S. bicolor*, where the point with the highest rate of normal seedlings (73%) is found in the concentration of 32%; for *D. insularis* extract, the linear adjustment of the data resulted in maximum germination of 66% (Figure 1A). The lowest estimates are found for the highest doses, with a concentration of 100% being critically harmful, regardless of the extract.

S. bicolor extract showed an influence on the germination rate of canola seeds, with a beneficial effect on the induction of germination in concentrations of 25%. In the case of *D. insularis* extract, there was a drastic reduction in the rates of normal seedlings (Figure 1A).

The results obtained by Rizzardi et al. (2008) agree with the present study, suggesting that the allelopathic effects are influenced by the concentration of the

metabolic substance present in the extract, negatively or positively interfering on germination. When evaluating the feasibility of using sorgoleone in the field, Uddin et al. (2013) were able to verify in their studies sorgoleone herbicidal activity against different weeds, as well as selectivity when applied in the form of wettable powder (WP); they also found germination inhibition of approximately 70% for gramineous species at a concentration of 0.2 g L⁻¹.

Abnormal seedlings percentage analysis revealed that the concentrations of *D. insularis* extract promoted a linear rising in the averages, with the highest value of 79% abnormality for 100% concentration dose; that is, the increase in concentrations drastically affects seedling development (Figure 1B). Allelochemicals have different allelopathic effects on the plant's life cycle, varying according to the concentration and donor or recipient species (Alves et al., 2014). The extract of *S. bicolor* obtained an average of 12% of abnormality regardless of the dose, with no variation in the development of canola.

Allelochemicals are natural herbicides and insecticides, produced by plants as their self-defense mechanism; these compounds, when isolated, are potential sources for molds of new structural arrangements and sustainable agricultural management, less invasive to the environment (Silva et al., 2017). As observed in *S. bicolor*, the number of dead seeds grow with increased extract concentrations, exhibiting a second-order dependence between these parameters (Figure 1C).

D. insularis extract has an average of 27% of dead seeds in the canola crop, regardless of the dose (Figure 1C). However, there is a high rate of abnormal seedlings, contributing to the failure development of the crops. Araújo et al. (2017) investigated the presence of allelopathic compounds extracted from the leaves of *C. madagascariensis* and their impact on the germination and initial growth of three native species of Brazil's semiarid region. It was reported that *C. madagascariensis* produces compounds with the potential to interfere in other plants under the evaluated conditions, due to its effects on *Lactuca sativa*. The authors indicated that the extracts contained allelopathic substances, which should be the cause of the negative effects on other species. However, this may be related to the greater sensitivity of the target plant.

When the shoot length was analyzed, a quadratic increase in averages was observed for the two extracts, with a smaller value of 4.49 cm for *S. bicolor* at a concentration of 17%, and a higher value of 5.14 cm for *D. insularis* at 23% of the extract concentration (Figure 1D). It can be assumed that very low doses of these extracts can act as shoot stimulants; nevertheless, high doses inhibit the initial development of canola. Carvalho et al. (2014) stated that allelopathic compounds, in most

cases, act as germination and growth inhibitors; however, some researches demonstrate their performance as growth agents when present in lower concentrations.

The reduction in the length of the primary root is associated with morphological changes when compared to the control seedlings (Figure 3 A, B), with deformities in the epicotyl and the primary root of the seedlings exposed to the *D. insularis* extract, with twisted and disproportionate parts, and greater appearance of secondary roots. These characteristics are accentuated in higher extract concentrations (Figure 3), to the point that there are no longer normal seedlings in the germination test when 25% concentration is used, being statistically confirmed in Figure 2 A. According to Brasil (2009), seedlings with deformations are considered within the category of abnormal seedlings; that is, those that do not show potential to continue their development and give rise to normal plants.

For total length, the effect of the two extracts is analogous (Figure 2), in which a negative regression is observed, that is, the increase in concentrations reduced the potential for crops development in all cases, as seen in Figure 3, except for the control and Figure 3B, in which its development did not undergo major changes. Sorgoleone, a compound present in the extract of *S. bicolor*, possibly acts as a plastoquinone equivalent, interfering in plastoquinone binding to proteins in photosystem II (Santos et al., 2012), thus acting in the photochemical phase of photosynthesis and reducing the production of assimilates, resulting in less growth.

In the shoot ratio variable, is observed a quadratic increase for the averages of *D. insularis* and *S. bicolor* with the highest percentages of 88% and 60%, respectively, with a dose of 43%, that is, with low concentrations, the development of the epicotyl increased, as seen in Figure 3, for seedlings resulting from treatments with intermediate doses (Figure 3 C and D). Carvalho et al. (2014) also observed the growth-regulating effect in low concentration of the extracts when evaluating the effect of different extracts concentrations obtained from the straw of three gramineous: black oats (*Avena strigosa* Schieb), sorghum (*Sorghum bicolor* (L.) Moench) and millet (*Pennisetum glaucum* (L.) R. Brown) on the germination and development of lettuce seedlings.

In the root ratio variable, a quadratic reduction was observed for the averages of *D. insularis* and *S. bicolor*; that is, at the highest concentrations, there is less root development, as shown in Figure 3 for seedlings resulting from treatments with higher doses (Figure 2C). The disproportion between the roots and the shoot of the seedlings is due to the situation in which there is greater contact between the roots and the extract (phytotoxins) than with other structures of the seedlings (Chung et al., 2001).

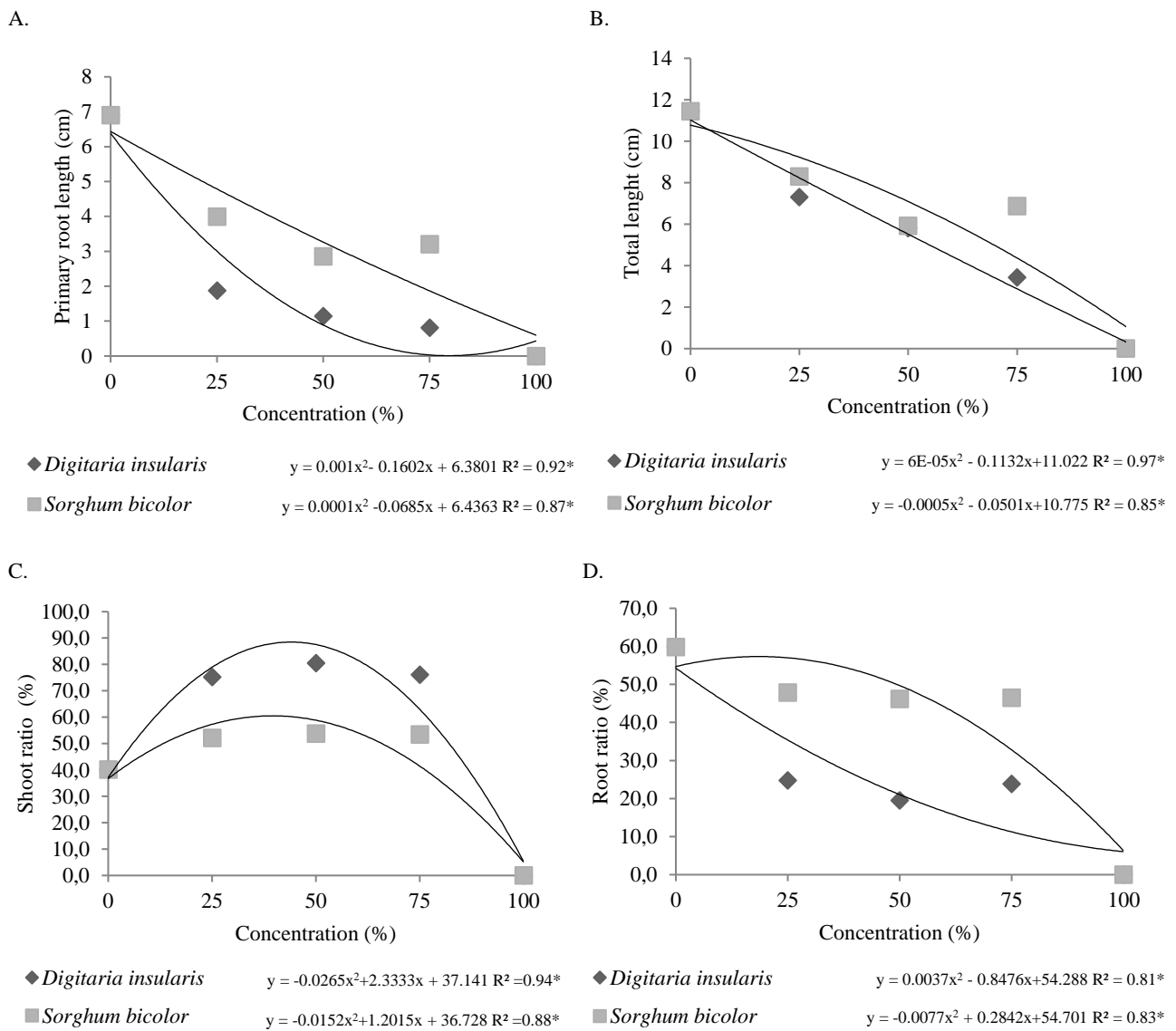
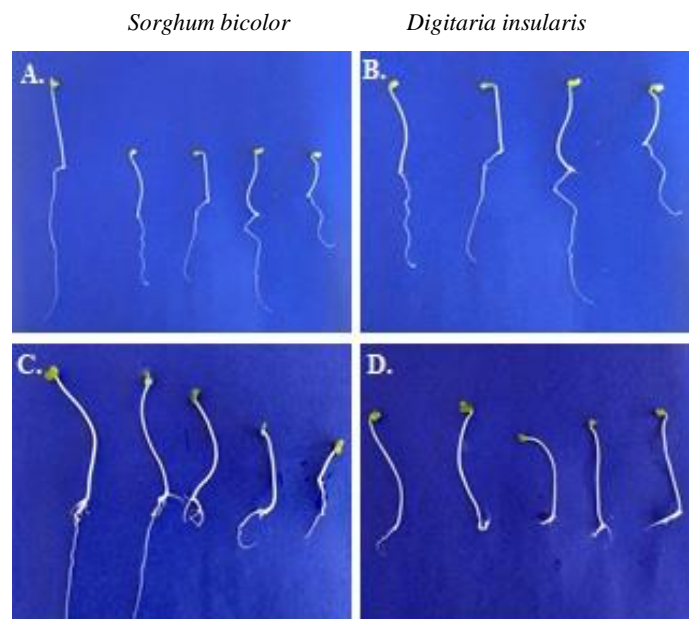


Figure 2. Primary root length (A), total length (B), shoot ratio – S/T (C), and root ratio – R/T (D) of canola according to the aqueous extract obtained from plant materials of *Digitaria insularis* and *Sorghum bicolor*. * Significant at 5% probability by the t-test.



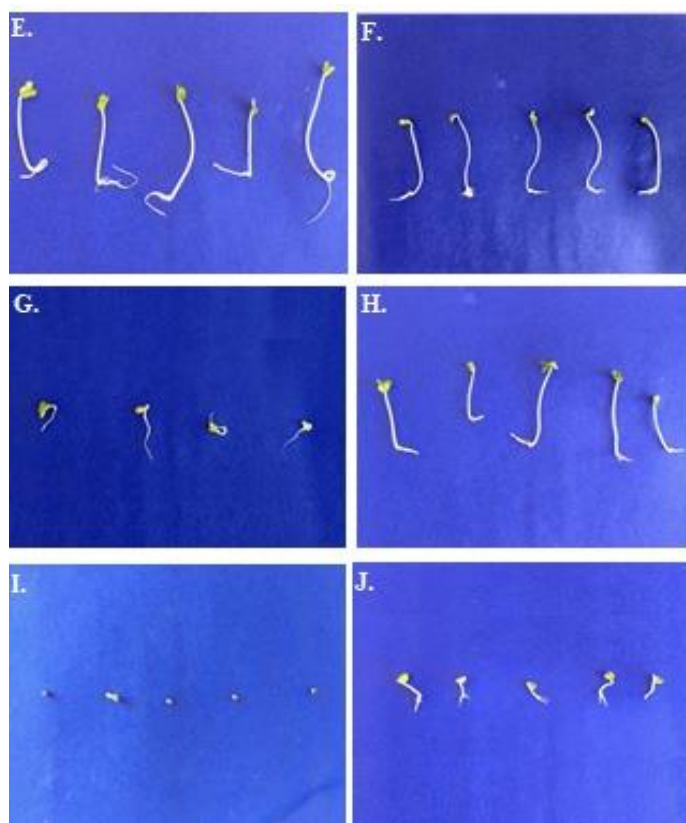


Figure 3. Canola seedlings from germinating tests using aqueous extracts of *Sorghum bicolor* and *Digitaria insularis* with concentrations of 0% (A, B), 25% (C, D), 50% (E, F), 75% (G, H), and 100% (I, J). Ipameri-GO. LASEM/UEG, 2019.

Visual evaluation enables a more detailed perception of the harmful effects of the extracts, allowing even a visual diagnosis of the statistical results of the variables. This facilitates the identification of root malformation due to direct contact between root and substrate, becoming clearer when the development of the control parcels (Figure 3 A, B) is compared with other submitted to different extracts and concentrations (Figure 3 C, D, E, F, G, H, I and J). These findings can be partially explained by the interactions of allelochemicals present in the extract of *D. insularis* and *S. bicolor*, with plant hormones in the meristematic cells of canola seedlings. In lettuce seed bioassays, when the shoot of the seedlings is evaluated, assimilation and, consequently, the concentration of allelopathic compounds in the root system are favored due to the physical contact of the root with the filter paper, exposed directly to the aqueous extract (Gonçalves et al., 2016).

Thereby, a relationship with auxin is suggested, as this regulator is associated with plant growth and playing a role in practically all aspects of development that includes the phenomenon of tropism when considered that its functions are regulated by the flow of auxin hormone in the root cap and apex of the coleoptile (Taiz et al., 2017). Therefore, plant extracts in different concentrations affected canola seeds germination, and the presence of allelopathic potential was verified in the seedling developmental characteristics.

4. Conclusions

D. insularis aqueous extracts interfere in all variables studied, which compromises the formation of normal seedlings, regardless of concentration. *S. bicolor* extract expressed allelopathic effects only in concentrations above 75%. Both species can inhibit the initial development of canola plants.

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