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THE ALLELOPATHIC EFFECT OF SORGHUM AND ITS LINKS TO THE EXTRACT COMPOSITION AND THE SORGOLEONE

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ABSTRACT:
The use of sorghum on the no-tillage system has contributed to weed control but has generated problems in future soybean development. The objective of this work was to extract and quantify sorghum root exudate of three genotypes to determine the sorgoleone content, the chromatographic profile, and the relative toxicity of the root extracts through the evaluation of germination parameters in soybean (*Glycine max*), brachiaria grass (*Brachiaria decumbens*) and beggarticks (*Bidens subalternans*) seeds under laboratory conditions. Considering the average root density, BRS 716 presented 50% more numbers of hairs per mm² than the other two sorghum genotypes. Sorgoleone was found predominantly in the three evaluated sorghum extracts, and the chromatographic profile detected five other compounds for the BR 007, CMSXS 206 B, and 6 BRS 716 compounds. There was a difference between the dry mass of roots, dry mass of extract, and sorgoleone content among the three genotypes, with BRS 716 having the highest ratio of sorgoleone per dry mass of roots, followed by BR 007 B, and CMSXS 206 B. The BR 007 B extract was the one that reduced germination and the germination speed index (GSI) of soybean and hairy beggarticks, followed by BRS 716 and CMSXS 206 B. The results showed that sorgoleone is a potent inhibitor of plant germination, but the allelopathic action may be linked to the composition of each extract and not specifically to the sorgoleone.

Keywords: *Brachiaria decumbens*, *Bidens subalternans*, root extract, weed management

O EFEITO ALELOPÁTICO DE PLANTAS DE SORGO E SUA RELAÇÃO COM A COMPOSIÇÃO DO EXTRATO E AO SORGOLEONE

RESUMO:
A utilização do sorgo no sistema de plantio direto tem contribuído para o controle de plantas daninhas, mas tem gerado problemas no desenvolvimento da soja em sucessões. O objetivo deste trabalho foi extrair e quantificar o exsudato da raiz de sorgo de três genótipos, determinando o teor de sorgoleone e o perfil cromatográfico, e a toxicidade relativa dos extratos da raiz mediante a avaliação de parâmetros germinativos em sementes de soja (*Glycine max)*, brachiaria (*Brachiaria decumbens*) e picão-preto (*Bidens subalternans*) em condições de laboratório. Considerando a densidade média de raízes, o genótipo BRS 716 apresentou 50% mais pelos radiculares por mm² do que os outros dois genótipos de sorgo. O sorgoleone foi encontrado predominantemente nos três extratos de sorgo avaliados, e o perfil cromatográfico apresentou outros cinco compostos para os genótipos BR 007 e CMSXS 206 B e seis compostos BRS 716. Houve diferença entre a massa seca de raízes, massa seca de extrato e teor de sorgoleone entre os três genótipos, sendo o BRS 716 o que apresentou a maior proporção de sorgoleone por massa seca de raízes, seguido por BR 007 B e CMSXS 206 B. O extrato de BR 007 B foi o que reduziu a germinação e IVG (índice de velocidade de germinação) da soja e picão-preto, seguido pelo BRS 716 e CMSXS 206 B. Os resultados mostram que o sorgoleone é um

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potente inibidor da germinação, mas a ação alelopática pode estar relacionada a composição de cada extrato e não especificamente ao sorgoleone.

Palavras-chave: *Brachiaria decumbens, Bidens subalternans*, extrato radicular, controle de plantas daninhas.

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INTRODUCTION

The sorghum crop [*Sorghum bicolor* (L.) Moench] stands out in Brazilian agricultural systems in the second crop period, as a succession crop, especially in the summer season and in no-till systems, for leaving a large quantity of straw in the soil (Lima et al., 2017).

It is known that sorghum plants have the ability to exude chemical compounds of an allelopathic character, which can harm or stimulate nearby species and those that may be grown in the same area in succession (Peerzada et al., 2017; Farooq et al, 2020).

The allelopathic capacity of the genus Sorghum results mainly from substances exuded by their roots, which are hydrophobic in character, with golden colored droplets located near the apex of root hairs that can be extracted with chloroform (Weston e Mathesius, 2014; Pan et al., 2018). Quantifications and fractionation of sorghum root extracts concluded that about 90% of the composition of these extracts is represented by sorgoleone (Besançon et al., 2020).

Different methodologies have been studied to verify the allelopathic potential of plant species in laboratory conditions, and, for the most part, they are based primarily on obtaining plant extracts, identifying and quantifying the substances contained in these extracts (Allsadawi et al., 2015; Tiburagi et al., 2020).

In this context, this work aimed to quantify the sorgoleone in root exudates of three sorghum genotypes to obtain and compare the chromatographic profile of each exudate and to evaluate its suppressive potential for soybean, brachiaria grass (*Brachiara decumbens*), and beggarticks (*Subalternans bidens*) under laboratory conditions.

MATERIAL AND METHODS

The experiments were carried out with three sorghum genotypes: BRS 716 (hybrid, biomass), BR 007 B (strain, saccharine), and CMSXS 206 B (strain, forage). The seeds used in the bioassays, soybean KWS 262 10, beggarticks, and brachiaria grass were obtained from the seed bank of Embrapa Milho e Sorgo.

Obtaining the root exudate

The extraction of the root exudate was obtained by seeds germinated. Groups of 600 seeds of each sorghum genotype, were submitted by a disinfection procedure of 2.5% sodium hypochlorite solution for 10 minutes then washed three times with distilled water (Oliveira et al., 2021). Afterwards, the seeds were placed in acrylic boxes of 250 mL (11 x 11 x 3.5 cm), lined and covered with filter paper moistened with 3 mL of distilled water.

After seven days in constant darkness and at an average temperature of 27°C, the roots were detached from the seeds and merged by genotype in three groups of 200 roots and immersed in extracting solution of dichloromethane and glacial acetic acid 0.0025% (v/v) for 5 minutes (Oliveira et al., 2021). The extracts were filtered and concentrated on a rotary evaporator at 30°C. Subsequently, the dry mass of the roots, as well as the extract weight of each group, was obtained after drying them in an oven at 65°C. The variables evaluated were: the total production of the extract formed by the 200 radicles of the three sorghum genotypes, their relationship with the dry root mass, and the total production of sorgoleone.

Quantification and HPLC profile of extracts

The quantification of sorgoleone was performed by HPLC analysis (High Performance Liquid Chromatography) with Waters Alliance model under the following configurations: UV/Vis detector at 280 nm, Waters XBride C18 column (4.6 x 150 mm 3. 5 µm), injection volume of 20 µL, column temperature 30°C, mobile phase: acetonitrile and acetic acid (2.5%) 75:25 with flow 1.0 mL min^{-1} (isocratic) with a time of 25 min run, obtaining the chromatographic profile of each extract. These analyses were performed in triplicate. The fractions found in each extract profile were compared from the

retention time of each chromatographic peak. The amount of sorgoleone was calculated on the basis of a standard curve obtained from a purified sample. The sorgoleone standard was obtained as purposed by Barbosa et al. (2001).

Root hair quantification

Ten seeds of each sorghum genotype (BRS 716, BR 007 B, CMSXS 206 B) were germinated following the same methodology of the root exsudate. Seven days after germination, the roots were separated from the aerial part and placed in a 0.05% solution of trypan blue (w/v) in lactoglycerol (1:1:1 lactic acid, glycerol, and water) for 24 hours and then washed in 70% ethanol (v/v). The root hairs were photographed at about one inch below the root base with the Axio Zoom V16 (Zeiss) stereoscope. Length and density were measured with the free software ImageJ (http://rsbweb.nih.gov/ij/). The hair length was estimated by the average of ten hairs from each of the ten roots of each sorghum genotype. The density was determined by counting the number of hairs per mm^2 of the visualized segment. Photographs of the root hairs of the three sorghum genotypes were also taken for visual confirmation of the presence of sorgoleone.

Bioassays

Two experiments were carried out, both using extracts of sorghum roots from the three genotypes (CMSXS 206 B, BR 007 B, BRS 716), which were diluted in P.A. alcohol (99,5%). In the first experiment (Test 1), the concentrations used were 418.44; 209.22; 104.61; 52.3, and 26.15 µM of root extracts from the three sorghum genotypes. The second experiment (Test 2) tested standardized concentrations of 50, 25, 12.5, 6.25 and 3.125 µM of sorgoleone present in the extracts of the three genotypes. Both trials were controlled without the extracts (alcohol and water).

A 3 ml volume of each solution of the sorghum root extracts of each genotype was pipetted into acrylic gerboxes (11 x 11 x 3.5 cm), lined with autoclaved filter paper, and sat for 12 hours for complete evaporation of the solvent. In each gerbox, 20 seeds per species were placed, in a completely randomized design with 3 replications. This was the experimental plot. The experiments were carried out in a germination chamber for 7 days in the dark and with an average temperature of 27 °C.

The response variables evaluated were the germination percentage (G%) and the Germination Speed Index (GSI) using the formula (G / D1 + G / D2 + G / D3 ... G / DN), in which G is the number of seeds germinated and D is the day of counting the germinated seeds. The seeds were counted daily and those with root extension equal to or greater than 2 mm were considered germinated (Juntila, 1976).

Osmotic potential

The osmotic potential of the extract solutions was estimated by comparison with sucrose solutions with known concentrations. A drop of the sample of each sucrose solution with known concentrations (0.0497; 0.0995; 0.1498; 0.1992; 0.2496 and 0.3M) were placed on the refractometer crystals for reading in °Bx and a calibration curve ($y = 0.0303x - 0.0089$) was obtained with R^2 0.992. This procedure was performed in triplicate. From this calibration curve, a drop of the sample from each solution of sorghum root extract from experiments 1 and 2 was read on a refractometer and each value in °Bx was converted into a molar concentration. From each molar concentration value of each solution of sorghum extracts, it was possible to estimate the osmotic potential of each solution using the Vant'off equation ($\Psi = -RTCi$), where Ψ is the osmotic potential (MPa), R is the perfect gas constant (0.00820574587 L atm^{-1} mol^{-1} K^{-1}), T is the temperature (K = °C + 273), C is the solution concentration, is the dissociation constant of the study molecule, which in the case of sucrose is 1. This procedure was performed in triplicate. The soybean, b*rachiaria* and beggarticks seeds were placed under sucrose solutions to evaluate their germinability and compared with a control sample.

Statistical analysis

The experiments were conducted in a completely randomized design in a 3x5 factorial (genotypes x concentrations). The variables were subjected to analysis of variance by the F test and when significant they were compared by the Tukey test at the level of 5% significance by the SYSTAT 2013 program. To verify the effect of the doses of sorghum root extracts, an analysis of non-linear regression by the Sigmaplot 2011 software. The germinability values were transformed by arc sen$\sqrt{x}$ / 100.

RESULTS

The images obtained show the droplets of sorgoleone exuded from the root hair (Figure 1). Note the golden yellow color described by Netzly and Butler (1986), which is a characteristic of the presence of sorgoleone.

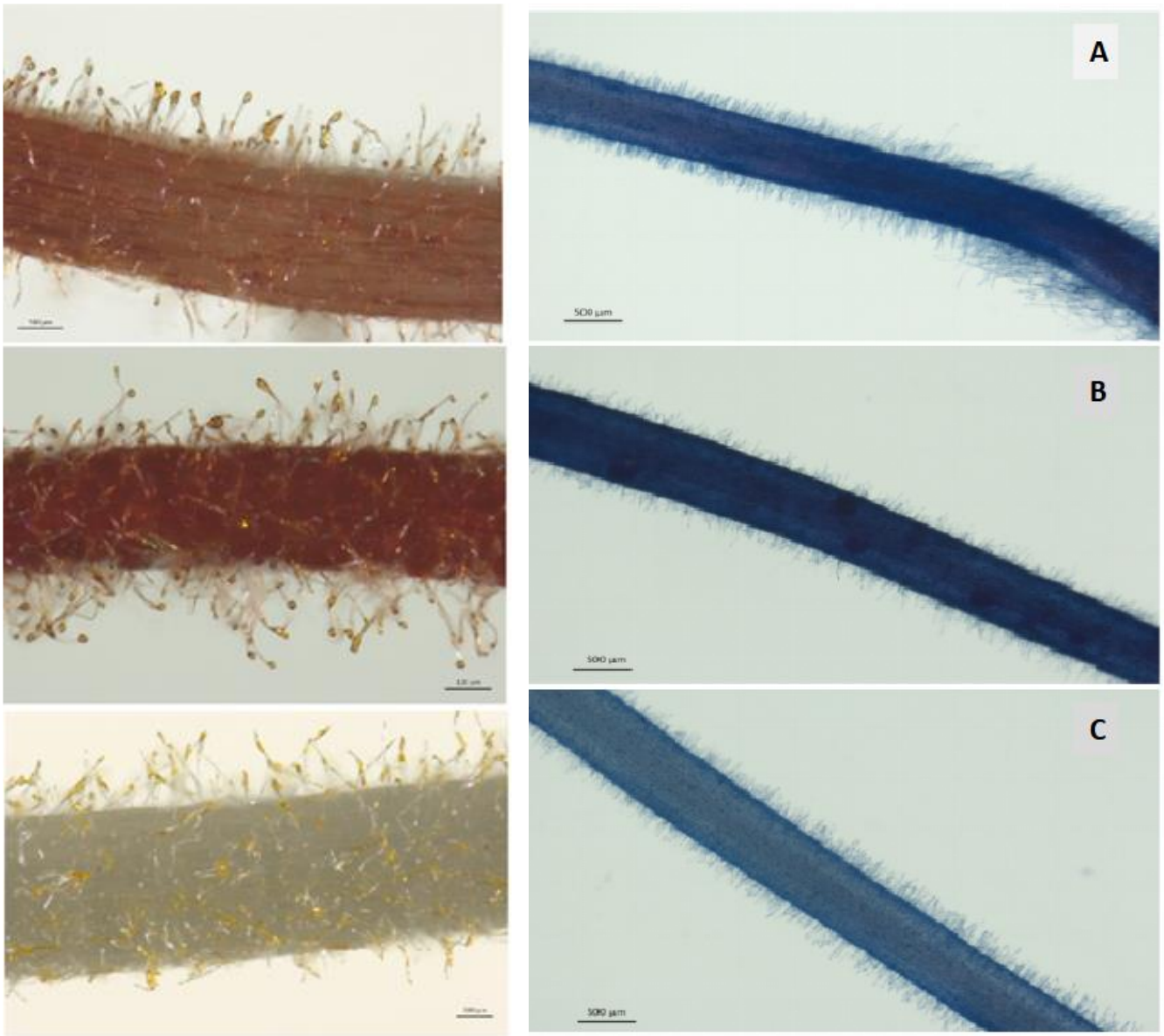

Figure 1. Sorgoleone exudation in the root of the genotype BRS 716, BR 007 B, and CMSXS 206 B. 80x enlarged photo on the Carl Zeiss equipment. Root hairs of the genotypes BR 007 B (A), BRS 716 (B) and, CMSXS 206 B (C). 16X enlarged photo on Axio Zoom V16 (Zeiss) stereoscope equipment.

The average root hair length of BRS 716 was significantly 30% greater than BR 007 B and 40% greater than CMSXS 206 B (Figure 1). Considering the average density of root hairs, BRS 716 showed 50% more hair numbers per mm² than the other two sorghum genotypes (BR 007 B and CMSXS 206 B), which showed statistically similar values.

The sorgoleone content of each extract was determined based on a calibration curve obtained using a standard. Chromatographic analyzes of the crude extracts obtained from the three evaluated genotypes detected six compounds for the CMSXS 206 B and BR 007 B genotypes, and seven compounds for the BRS 716 genotype (Table 1).

In this work, the substance with the largest relative area in the three extracts was sorgoleone, representing an average of 80% of the composition of the evaluated crude extracts (Table 1). In the extracts of the genotypes CMSXS 206 B and BR 007 B, in addition to sorgoleona, 5 other compounds were observed, and in the BRS 716 genotype, in addition to these 5 compounds, the compound with 0,95 % was also observed (Table 1).

Table 1. Mean percentage of chromatographic peak area relative to the total compounds present in sorghum root exudates

Genotypes	Relative area (%)							
	Compounds							
	SGL	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
BR 007 B	79,84 a	2,67 a	4,88 b	3,44 a	0,58 b		8,59 a	100
BRS 716	77,51 b	1,79 b	6,26 a	0,83 c	3,05 a	0,95	9,61 a	100
CMSXS 206 B	79,50 a	2,36 a	6,24 a	2,44 b	0,45 c		9,00 a	100

The same letters in the column do not differ by Tukey's test a 5% significance

Regarding RDW (roots dry weight), BRS 716 and BR 007 B were similar, and CMSXS 206 B was 20% higher. The EM (extract mass) of BRS 716 and CMSXS 206 B did not differ, and were about 29% lower than BR 007 B. CMSXS 206 B and BRS 716 had the same extract production by RDW, being 37% higher than BR 007 B. (Table 2).

There was no significant difference between the sorgoleone amount (mg) found between the genotypes BR 007 B and CMSXS 206 B. However, both differed from BRS 716 by approximately 30% less (Table 2). The same occurred with the amount of sorgoleone (mg) per dry root mass (g) in the BRS 716 which was about 22% higher than the other two genotypes.

Table 2. Sorghum genotypes used, roots dry weight (RDW), extracted mass (ME), mg of extract per gram of RDW, sorgoleone (SGL) found in the extract produced by the roots of each genotype, and sorgoleone found per gram of dry root mass.

Genotypes	RDW (g)	ME (mg)	mg extract	Sorgoleone	mg g^{-1}
BR 007 B	0,152 ± 0,01 a	6,75 b	43,36 b	3,29 b	20,56 b
BRS 716	0,154 ± 0,01 a	10,05 a	69,46 a	4,55 a	28,67 a
CMSXS 206 B	0,122 ± 0,01 b	8,9 a	67,8 a	3,17 b	23,97 b

The same letters in the column do not differ by Tukey's test a 5% significance.

The test that verified the germinability of soybean, brachiaria grass, and Bidens *subalternans* seeds revealed that there was no statistical difference between the germination of the seeds under sucrose solutions and the control. The estimation of the osmotic potential revealed, for all extract concentrations, values greater than - 0.11 MPa. According to Trezzi et al. (2005), these levels are

unable to generate osmotic effects harmful to seed germination.

The allelopathic inhibitory effects were observed in bioassays with different responses. In test 1 for soybean, there was an interaction between sorghum genotypes and variation in doses and extracts for GSI. The models of non-linear equations allowed to estimate the maximum percentage of germination reduction of each extract of sorghum genotypes for the tested species. The extracts of the genotypes BR 007 B, BRS 716, and CMSXS 206 B, suppressed 60%, 44%, and 29%, respectively, the germination of soybean seeds (Figure 2A). The reduction in the GSI of soybean seeds was estimated through the equations in 93%, 87% and 68% of the extracts of BR 007 B, BRS 716, and CMSXS 206 B, respectively (Figure 2B).

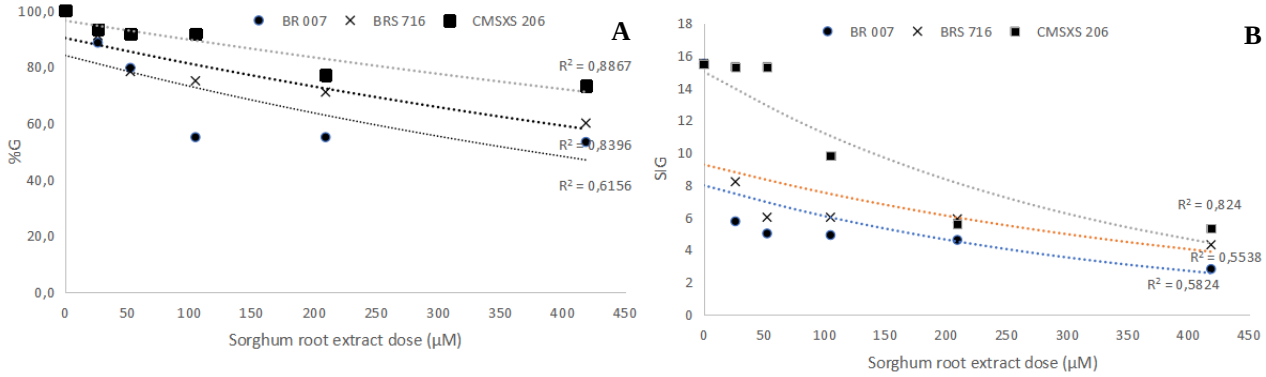

Figure 2. A - Germination percentage of soybean seeds under doses of root extracts of different sorghum genotypes. B – Germination Speed Index of soybean seeds under doses of root extracts of different sorghum genotypes.

In general averages, both the soybean seeds germination percentage and the GSI were affected by the three sorghum genotypes. BR 007 B had the greatest potential to inhibit germination among the three genotypes, about 18% to CMSXS 206 B and 10% to BRS 716. The soybean seeds GSI was also more affected by the genotype BR 007 B than the other two genotypes. The soybean seeds germination under the effect of BR 007 B was 75% slower than CMSXS 206 B and 19% than BRS 716.

In test 2 for soybean, there was no effect between the three sorghum genotypes or between the interaction of the three genotypes and the variation in doses of sorgoleone for germination percentage. There was only an effect on the variation of the average doses with a maximum percentage of germination reduction of about 40%. The soybean seeds GSI was affected by the interaction between the three sorghum genotypes and the variation in the doses of sorghum root extracts in terms of sorgoleone (Figure 3). According to the models of non-linear equations, the standardized sorgoleone doses reduced the soybean seeds GSI by 86%, 89%, and 79% by genotypes BR 007 B, BRS 716, and CMSXS 206 B, respectively. The soybean seeds GSI average showed a statistical difference between sorghum genotypes in the cultivar CMSXS 206B for BR 007 and BRS 716, which were similar.

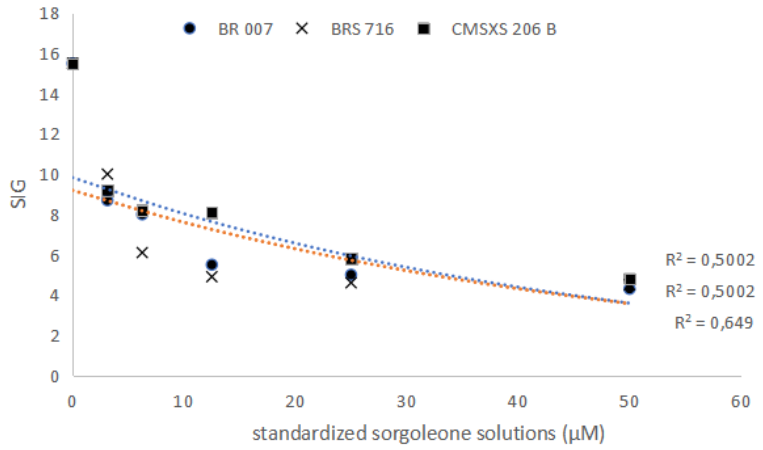

Figure 3. Germination Speed Index of soybean seeds under standardized doses of sorgoleone solutions on different sorghum genotypes.

In test 1 for beggarticks, there was an interaction between the three sorghum genotypes and the variation in the root extract doses for seeds germination percentage and GSI. Following the estimate of the non-linear equations models adjusted for each genotype, the extracts of the genotypes BR 007 B, BRS 716, and CMSXS 206 B, suppress, respectively, 73%, 64% and, 63% of the beggarticks seeds germination (Figure 4A). And the reduction in the GSI was estimated at 73%, 71%, and 54% of the extracts of BR 007 B, BRS 716, and CMSXS 206 B, respectively (Figure 4B). In this same trial, in general averages, the three genotypes responded statistically similarly to a general average of germination inhibition of 48%. The GSI average between the three genotypes differed statistically, with the beggarticks seeds GSI average being less affected by the genotype CMSXS 206 B in 18% to BR 007 B and 11% to BRS 716.

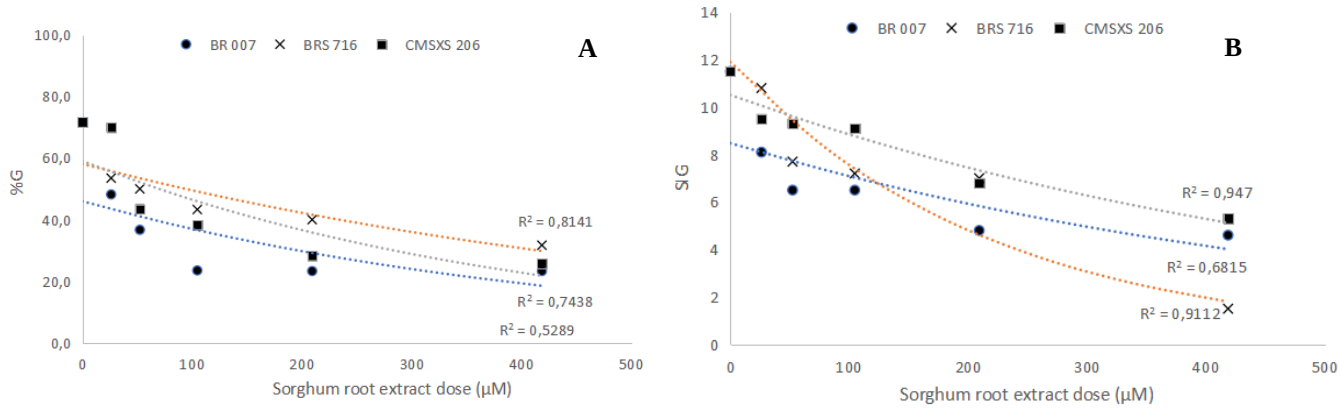

Figure 4. A - Germination percentage of beggarticks seeds under doses of root extracts of different sorghum genotypes. B – Germination Speed Index of beggarticks seeds under doses of root extracts of different sorghum genotypes.

The analysis of variance found a significant effect of the interaction between the three sorghum genotypes and the variation in the doses of extracts in the beggarticks seeds germination percentage and GSI from test 2. In the second experiment, the standardized doses of 50 µM of sorgoleone, using non-linear equations, estimated a reduction in the beggarticks seeds germination percentage of 89.5%, 80%, and 73% of the extracts of BR 007 B, BRS 716, and CMSXS 206 B, respectively (Figure 5A). The

beggarticks seeds GSI was reduced by 96%, 86% and 69% by genotypes BR 007 B, BRS 716, and CMSXS 206 B, respectively (Figure 5B).

The average doses of sorgoleone significantly affected the germination among the three sorghum genotypes, with BR 007 B inhibiting about 34% of the beggarticks seeds germination, about 22% more than the other two genotypes (BRS 716 and CMSXS 206 B), which were statistically equal. There was also an effect between the three sorghum genotypes regarding the beggarticks seeds GSI, with BR 007 B reducing by 80% than BRS 716 and 46% than CMSXS 206 B.

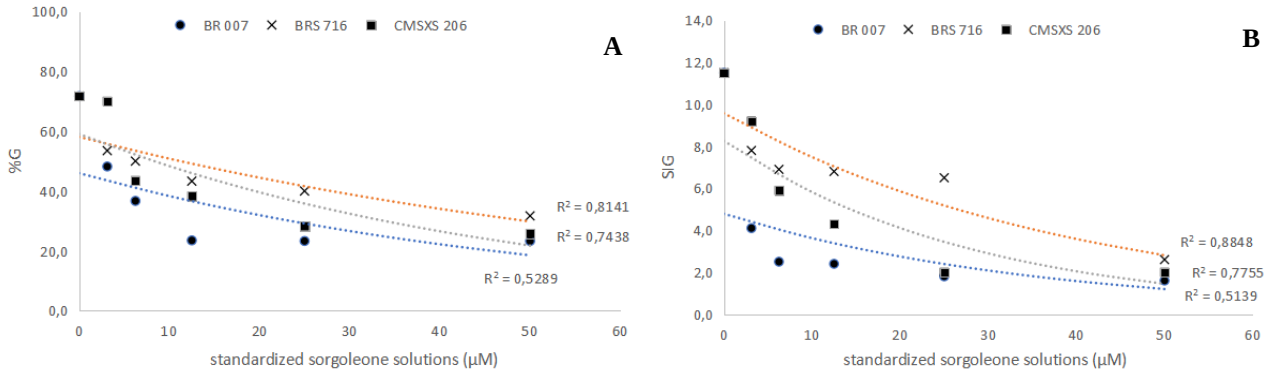

Figure 5. A- Germination percentage of beggarticks seeds under standardized doses of sorgoleone solutions on different sorghum genotypes. B – Germination Speed Index of beggarticks seeds under standardized doses of sorgoleone solutions on different sorghum genotypes.

In test 1 for brachiaria grass, there was an effect on the interaction between the three sorghum genotypes and the variation in extract doses for both the germination percentage and the GSI. Applying the models of non-linear equations, the maximum percentage of germination reduction of each extract of genotypes, BR 007 B, BRS 716, and CMSXS 206 B managed to suppress, respectively, 53%, 53%, and 60% of the brachiaria grass seeds germination (Figure 6A). The reduction in the GSI was estimated at 39%, 33%, and 48% of the extracts of BR 007 B, BRS 716, and CMSXS 206 B, respectively (Figure 6B).

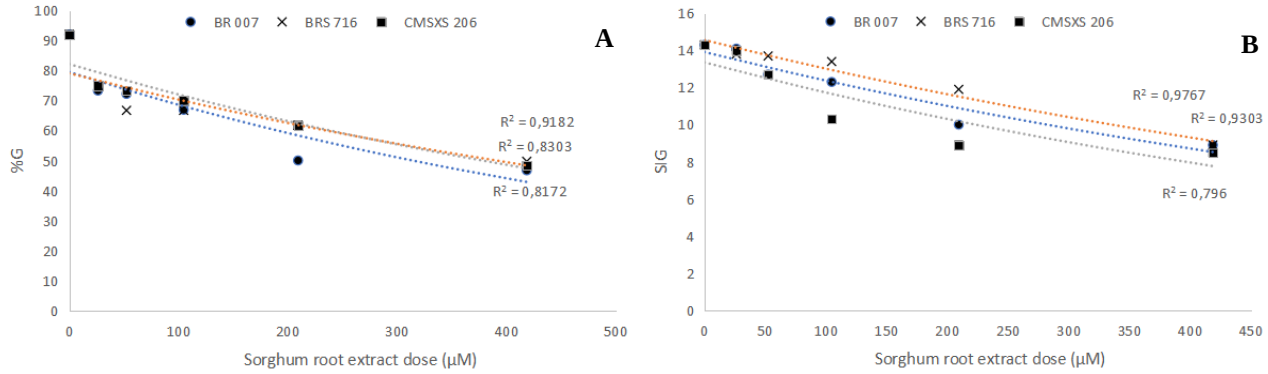

Figure 6. A - Germination percentage of brachiaria grass seeds under doses of root extracts of different sorghum genotypes. B – Germination Speed Index of brachiaria grass seeds under doses of root extracts of different sorghum genotypes.

The interaction between the three sorghum genotypes and the variation between doses of sorghum root extracts in terms of sorgoleone was significant for the germination percentage and GSI of the brachiaria grass seeds. According to the equations that were adjusted, in the second experiment, the

standardized doses of sorgoleone through non-linear equations estimated a reduction in the germination percentage of the seeds of 58%, 43.0%, and 64% of BR 007 B, BRS 716, and CMSXS 206 B extracts, respectively.

Standardized doses of sorgoleone reduced the brachiaria grass seeds GSI by 41%, 40% and 45% by genotypes BR 007 B, BRS 716 and CMSXS 206 B. When testing the extracts in terms of sorgoleone, of the three sorghum genotypes, there was a difference in the germination percentage and GSI of the brachiaria grass seeds. CMSXS 206 B inhibited the germination percentage about 41% more than BRS 716 and 8% more than BR 007 B (Figure 7A). The brachiaria grass seeds GSI under the extract of CMSXS 206 B was 34% less than BRS 716 and 17% less than BR 007 B (Figure 7B).

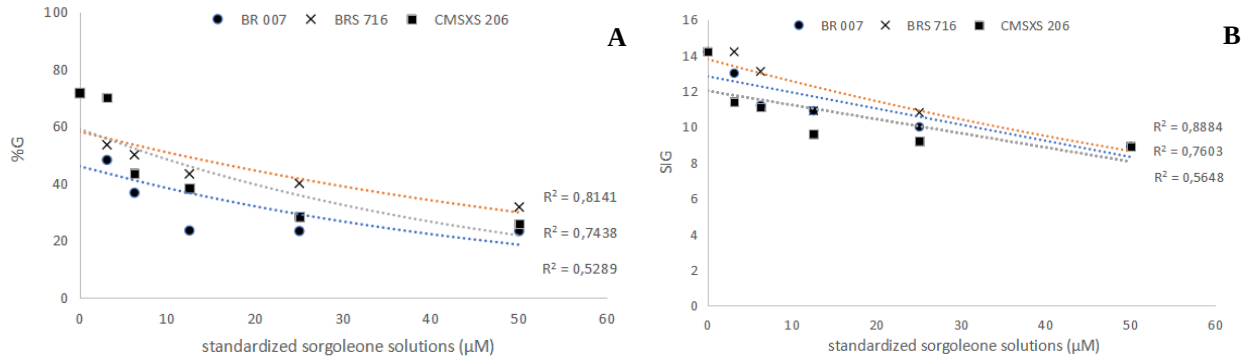

Figure 7. A - Germination percentage of brachiaria grass seeds under standardized doses of sorgoleone solutions on different sorghum genotypes. B – Germination Speed Index of brachiaria grass seeds under standardized doses of sorgoleone solutions on different sorghum genotypes.

DISCUSSION

In this work, we show that the allelopathic effects of sorghum are not confirmed by sorgoleon alone. The profile of phenolic compounds exudate by roots showed its most importance in the allelopathic effects, as well the phenolic profile and the botanical family.

Sorgoleone is the predominant compound in the composition of extracts from the roots of sorghum species, representing up to 90% of the composition of crude extracts of sorghum roots (Allsadawi et al, 2015; Rab et al., 2016). However, allelopathic activity is not always related to a higher sorgoleone concentration.

In standardized sorgoleone solutions, soybean seeds are more sensitive to the compounds of the BR 007 B extract than the other extracts (BRS 716 and CMSXS 206 B). The same response was observed in the beggarticks seeds, which were more sensitive to the compounds of the extract of BR 007 B than of the others. However, brachiaria grass seeds showed a totally divergent behavior, with the CMSXS 206 B extract inhibiting the germination percentage more than BRS 716 and BR 007 B, and that the allelopathic action may be linked to the composition of each extract and not specifically to the sorgoleone.

The difference in the content of each substance found in the extracts of the sorghum genotypes indicates a chemical variation in the content of root exudate that probably has a genetic basis, since the conditions of temperature, water, and humidity were the same for the three evaluated genotypes (Besançon et al., 2020).

Sorghum have a broad spectrum of polyphenols. These have a many functions in ecological relations such as antiproliferative properties associated with the prevention of certain types of cancer, antimicrobial and anti-inflammatory properties in addition to antioxidants. They could also be used to develop bioactive food ingredients and

green pesticides (Espitia-Hernández et al., 2020; Uchimiya, 2020).

Recently works have shown the relation of sorgoleone and mycorrhizal colonization and nitrification activity (Sarr et al., 2020; Wang et al., 2020; Oliveira et al., 2021; Sarr et al., 2021). The ecological function of sorgoleone and the combination of the other phenolic compounds should be an interesting aspect to plant biotic interaction (Mareya et al., 2020). This interaction should affect the nutritional availability to other plants in a positive or negative relation, like the observed in this study with soybean, beggarticks, and brachiaria grass.

The results revealed that sorgoleone is a potent inhibitor of plant germination and growth, but the allelopathic action may be linked to the composition of each extract and not specifically to the sorgoleone.

CONCLUSION

Low doses of sorgoleone are necessary to cause a phytotoxic effect on the germination percentage and GSI of soybean, beggarticks and, brachiaria grass seeds under laboratory conditions.
BR 007 B extracts caused a greater allelopathic effect for soybean and beggarticks seeds, while CMSXS 206 B extracts caused negative allelopathic effect for the brachiaria grass species, so further studies are needed to understand if there is any relationship between the chemical composition of sorghum genotypes and the characteristics of the botanical species treated.

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