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Article

Allelopathic Potential of *Sorghum bicolor* at Different Phenological Stages

Potencial Alelopático da Parte Aérea de ***Sorghum bicolor*** *em Diferentes Fases Fenológicas*

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ABSTRACT **-** This study aimed to assess the allelopathic potential of the aqueous extract of the shoot of *Sorghum bicolor* at different phenological stages on seed germination and initial growth of kale (*Brassica oleracea* var. acephala) seedlings, which is considered a bioindicator species. The experiment was carried out at five development stages of *S. bicolor* (40, 50, 60, 70, and 80 days after emergence), which was used as aqueous extract at six concentrations (0, 20, 40, 60, 80, and 100%) applied in four replications per treatment in 10 or 50 kale seeds per plot. The percentage of germination, germination rate index, root growth, shoot length, and dry matter of kale seedlings were analyzed in the presence of this extract on different days of collection and concentrations. The aqueous extract of *S. bicolor* presented an allelopathic effect on germination and initial growth of kale seeds, with a higher inhibitory effect when more concentrated extracts from pre-flowering plants were used, which corresponds to 60 days after emergence. Thus, this stage should be recommended in the use of *S. bicolor* straw to help in controlling weeds.

Keywords: allelochemicals, aqueous extract of sorghum, bioindicator species.

*RESUMO - O objetivo deste estudo foi avaliar o potencial alelopático do extrato aquoso da parte aérea de S. bicolor, em diferentes fases fenológicas, sobre a germinação de sementes e crescimento inicial de plântulas de couve (**Brassica oleracea** var. acephala), que é considerada uma espécie bioindicadora. O experimento foi realizado em cinco fases de desenvolvimento de **S. bicolor** (40, 50, 60, 70 e 80 dias após emergência), sendo essa planta usada como extrato aquoso em seis concentrações (0, 20, 40, 60, 80 e 100%), para fazer aplicações de quatro repetições por tratamento em 10 ou 50 sementes de couve por parcela. Foram analisados a porcentagem de germinação, o índice de velocidade de germinação, o crescimento das raízes, o comprimento da parte aérea e a massa seca das plântulas de couve na presença desse extrato, em diferentes dias de coleta e diferentes concentrações. Assim, o extrato aquoso de **S. bicolor** apresentou efeito alelopático na germinação e crescimento inicial das sementes de couve, sendo o efeito inibitório maior quando utilizados extratos mais concentrados provenientes de plantas em pré-florescimento, que corresponde a 60 dias após emergência. Portanto, essa fase é a que deve ser recomendada na utilização da palhada de **S. bicolor** para auxiliar no controle de plantas daninhas.*

Palavras-chave: aleloquímicos, extrato aquoso de sorgo, espécie bioindicadora.

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INTRODUCTION

Allelopathy is defined as the direct or indirect inhibitory or beneficial effect of a plant on another living organism through the production of chemical compounds that are released into the environment. This phenomenon occurs in natural plant communities (Gressel and Holm, 1964) and may also interfere with the growth of agricultural crops (Muller, 1966; Bell and Koeppe, 1972). Probably the most significant consequence of allelopathy is the change in population density and plant development. Allelopathy has a great importance when plant residues are left on or embedded in the soil surface (Guenzi et al., 1967; Tukey Jr., 1969), indicating its great importance in no-tillage systems and green manure.

Sorghum (*Sorghum bicolor*) is known for its high allelopathic potential largely due to the production of a hydrophobic compound known as sorgoleone. Sorgoleone is one of the most studied allelochemicals since sorghum has a high concentration of this compound, which is released through ciliated cells of the root (Weston et al., 2001, 2012). Sorghum, in addition to the sorgoleone, which is a benzoquinone, has a diverse group of allelochemicals, including numerous phenolics and a cyanogenic glycoside (dhurrin), which have been isolated and identified recently from sorghum sprouts, roots, and root exudates (Weston et al., 2013).

The allelopathic activity of *S. bicolor* is dependent on several factors such as different cultivars, environmental conditions, and growth stages of the plant. In addition, the allelochemicals present in sorghum tissues may vary in different parts of the plant. The allelopathic potential of *S. bicolor* can be used to control weeds by means of the application of sorghum residues as mulch or including sorghum cultivars in a crop rotation (Jabran et al., 2015).

According to Santos et al. (2014), although *S. bicolor* is considered a species that has an allelopathic effect, its toxic effect is not the same for different crops because it depends on many factors such as genotype, concentration, plant density, fertility, and soil moisture. However, the effectiveness of control against some weeds may not occur immediately, such as that of commercial herbicides, but could be built over time, as with most natural products (Belz, 2007).

Oliveira et al. (2015) assessed the allelopathic potential of aqueous extracts of saccharine sorghum on germination and initial growth of lettuce (*Lactuca sativa*) and observed that the aqueous extracts affected germination, root system growth, and the formation of normal seedlings of lettuce. Lettuce seedlings also have their root development compromised due to root exudates of forage sorghum (Barbosa et al., 1998). Borges et al. (2014) verified that cover plants such as forage sorghum have a suppressive effect of more than 90% on weeds. Correia et al. (2005a, b) observed that forage sorghum straw increases the effectiveness of herbicides in controlling weeds, which have their emergence impaired due to phytotoxins released by sorghum straw.

In addition to these effects on lettuce and weeds, some studies have studied the allelopathic effect of sorghum on crops such as soybean. Olibone et al. (2006), for instance, observed that there was no nutrient reduction in the soil, but base saturation decreased due to soil cover with forage sorghum straw since its decomposition possibly releases compounds that inhibit the root growth of soybean. Costa et al. (2015) observed that soybean grain yield increases with the use of forage sorghum straw.

The allelopathic potential of *S. bicolor* and its implication in different systems in most of the studies, either in the laboratory or in the field, use the straw in a single sorghum development stage. However, there are few studies that show that the allelopathic effect of sorghum can vary with the development stage of this plant, i.e. at different plant development stages, the composition and amount of chemical substances, possibly allelochemicals, are variables mainly in the shoot (stem + leaf) (Marchi et al., 2008).

Plant age and its development, as well as its different organs, have also a considerable importance and may influence not only the total amount of produced metabolites but also the relative proportions of components of the mixture (Gobbo-Neto and Lopes, 2007).

Thus, the aim of this study was to assess the allelopathic potential of the aqueous extract of the shoot of *S. bicolor* at different phenological stages on seed germination and initial growth of kale (*Brassica oleracea* var. acephala) seedlings, which is considered a bioindicator species. The use of bioindicator species is one of the ways to verify the permanence of compounds in the soil.

These plants have a high sensitivity to allelochemicals, high growth rate, and genetic homogeneity (Nunes and Vidal, 2009).

MATERIAL AND METHODS

Obtaining the extract

Sorghum was sown in an experimental field and the shoot (stem + leaf) were collected from 20 plants at 40, 50, 60, 70, and 80 days after emergence (DAE), corresponding to the development stages 4, 5, 6, 7, and 8, respectively, according to Magalhães (2000). In stage 4 (visible flag leaf) occurs a rapid stem elongation. All leaves are fully developed, except for the last three or four. In stage 5 (booting), all leaves are fully developed, resulting in a maximum leaf area. The panicle reaches its maximum length, within the flag leaf sheath. In stage 6 (50% flowering), the period from emergence to 50% flowering (about 60 days) is of approximately two-thirds of the period from emergence to physiological maturation. In stage 7 (milky grain), about 50% of grain dry matter has already been accumulated (about 70 days after emergence) and the stem weight decreases. In stage 8 (pasty grain), about three-fourths of the grain dry matter has already been accumulated (about 85 days after emergence).

After collection, the plants were washed in running water, conditioned in a paper bag, and dried in a forced air circulation oven at 50 ^{o}C until constant weight.

The use of the aqueous extract was chosen since only soluble compounds, which are the main compounds released in the field from the plant in the leaching process, are extracted from it.

After the drying period, plant shoot was ground in a hammer mill and mixed with distilled water in a ratio of 100 g to 1,000 mL (w/v) of water. After 24 hours at rest, the mixture was filtered on filter paper to obtain a 100% extract (w/v). This most concentrated 100% extract (w/v) was diluted to obtain the other concentrations of 80, 60, 40, 20, and 0% (distilled water) (Oliveira et al., 2015).

Bioassay of germination

The experiment was conducted in a 5 x 6 factorial scheme (five sorghum development stages x six extract concentrations), totaling 30 treatments with 4 replications of 10 or 50 kale (*Brassica oleracea*) seeds per plot. Plants were collected at 40, 50, 60, 70, and 80 days after emergence (DAE) and, after collection, the plants of each development period were used to prepare the 100% aqueous extract (w/v), which underwent several dilutions and reached the concentrations of 0, 20, 40, 60, and 80%.

The bioassay was carried out in a germination chamber with a constant temperature of 20 ^{o}C and 12 hour photoperiod for 12 days. The tests were conducted in transparent Gerbox acrylic boxes with two germitest paper sheets previously moistened with 10 mL of water or solution of aqueous extract of *S. bicolor*. For the germination test, 50 kale seeds were used per box. The germinated seeds were counted daily from the second day and after sowing, seeds with at least 2 mm of primary root were considered as germinated (Hadas, 1976).

The percentage of germination (Brasil, 2009) and germination rate index (GRI) (Maguire, 1962) were calculated. The GRI is given by the formula: $GRI = (G_1/N_1) + (G_2/N_2) + \ldots + (G_n/N_n)$, where G_1, G_2, and G_n are the number of seedlings in the first, second, and last count and N_1, N_2, and N_n are the number of days of sowing in the first, second, and last count.

Bioassay of growth

In the bioassay of seedling development, 10 kale seeds were sown per box, which was kept under the same conditions as the bioassays of germination. Primary root length, shoot length, and seedling dry matter were determined after 12 days. In order to obtain the seedling dry matter, seedlings were conditioned in paper bags and dried in a forced air circulation oven at 65 ^{o}C until constant weight. The result was obtained by dividing the mass by the number of seeds germinated in each replication, being expressed in grams.

Statistical analysis

The data were submitted to the analysis of variance at 5% significance. When the factors or the interaction between them was significant by the analysis of variance, a regression study was performed using the software SISVAR (Ferreira, 2014).

RESULTS AND DISCUSSION

The results of analysis of variance for the percentage of germination, germination rate index (GRI), root length (RL), shoot length (SL), and dry matter (DM) showed a significant difference with an interaction between the studied factors, except for DM. For this, the results of DM were not shown in this study.

Figure 1 shows that the aqueous extracts of the shoot of sorghum at 50 and 60 DAE (days after plant emergence) caused significant effects on the percentage of kale germination. In addition, the extract of 50 DAE decreased the percentage of germination in a dose-dependent manner. On the other hand, the extract of 60 DAE showed a double effect: a slight stimulus was observed in the percentage of germination at concentrations lower than 20 and 40% and an inhibition occurred at concentrations higher than 60, 80, and 100%. Thus, the results indicate that the most pronounced effect on the percentage of kale germination was that of the aqueous extract of sorghum at 60 DAE. In this extract, there may be compounds with stimulatory and inhibitory effects. These effects may be given by the same compound or by different compounds that have effects only at high concentrations. Apparently, in the other sorghum development stages, no significant amounts of these compounds are produced.

Regarding the germination rate index, all aqueous extracts had significant effects on kale germination, except for the aqueous extract of 40 DAE of sorghum, as shown in Figure 2. The extract of 60 DAE showed a similar effect to the percentage of germination, while the extracts of 50, 70, and 80 DAE showed dose-dependent effects. However, the extract of 50 DAE was the most potent, followed by those of 70 and 80 DAE. The results of the aqueous extract of 60 DAE complement the results of the percentage of germination because the higher the concentration of the extract is (80 and 100%), the lower the GRI.

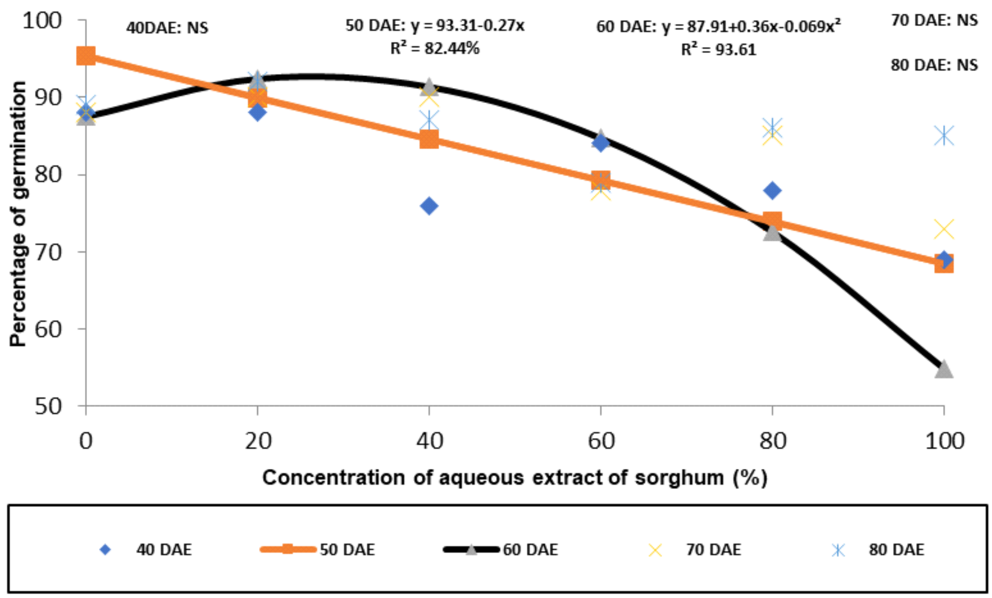

Figure 1 **-** Percentage of germination of kale (*Brassica oleracea*) seeds treated with different concentrations of aqueous extract of the shoot of sorghum (*Sorghum bicolor*) harvested at 40, 50, 60, 70, and 80 days after emergence (DAE).

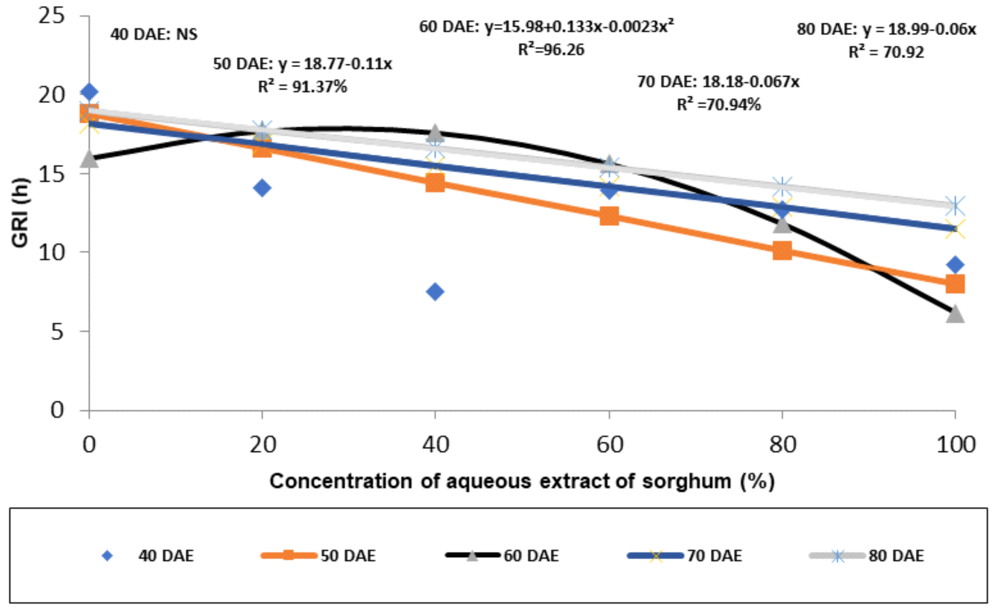

Figure 2 **-** Germination rate index (GRI) of kale (*Brassica oleracea*) seeds treated with different concentrations of aqueous extract of the shoot of sorghum (*Sorghum bicolor*) harvested at 40, 50, 60, 70, and 80 days after emergence (DAE).

Figure 3 shows that the aqueous extracts of sorghum obtained at all development stages had a dose-dependent inhibition on kale root length. At the highest concentration (100%), a more marked inhibition was observed in the extract of 40 DAE and a lower inhibition was observed by the extract of 60 DAE.

In relation to shoot length (Figure 4), the results showed effects different from those observed for kale root length. In this sense, all the aqueous extract had a double effect, except for that of 70 DAE. A stimulation was observed at lower doses (20 and 40%), followed by inhibition at higher doses (80 and 100%).

Thus, the extract collected after 60 DAE seems to have caused a higher effect on kale seed germination because close to the collection at 60 DAE (stage 6, i.e. 50% flowering) a higher concentration of allelopathic substances was observed in the plant, which corresponds to the period before flowering. This effect was also reported by Silva (2004), who observed that *Tithonia diversifolia* also showed a higher concentration of secondary metabolites in the pre-flowering period. In addition, the extracts had no significant effect on the percentage of kale germination after 60 DAE, indicating a lower allelopathic effect over time. This effect can be understood when considering the senescence of plants and translocation of photoassimilates and other substances to the fruits. According to Taiz and Zeiger (2017), a reduction in the metabolic activities and an increase in the expression of degradative enzymes occur during the senescence of plants, thus reducing the production routes of secondary metabolites.

The results also showed that the aqueous extracts of sorghum had effects predominantly of root inhibition regardless of the phenological stage, different from that observed in the germination. All aqueous extracts of sorghum inhibited kale root length as the extract concentration increased. This effect of root inhibition was also observed by Barbosa et al. (1998) in root exudates of forage sorghum on lettuce root, Correia et al. (2005) in extracts from different parts of forage sorghum on soybean root, and Olibone et al. (2006) in forage sorghum straw on the root growth of soybean cultivated in pots. These authors explained that the inhibition of root growth of soybean seedlings might be due to allelopathic substances present in plant tissues. Therefore, this reduction observed in the root system is due to the direct contact in which the root is exposed to the aqueous extract. Thus, due to the direct physical contact between root and substrate, the root is more affected by a high phytotoxin concentration of the extract when compared to the shoot.

Barbosa et al. (1998) also observed the effect of shoot growth stimulus in extracts of forage sorghum on lettuce germination. The effect of shoot stimulus and root suppression suggests a

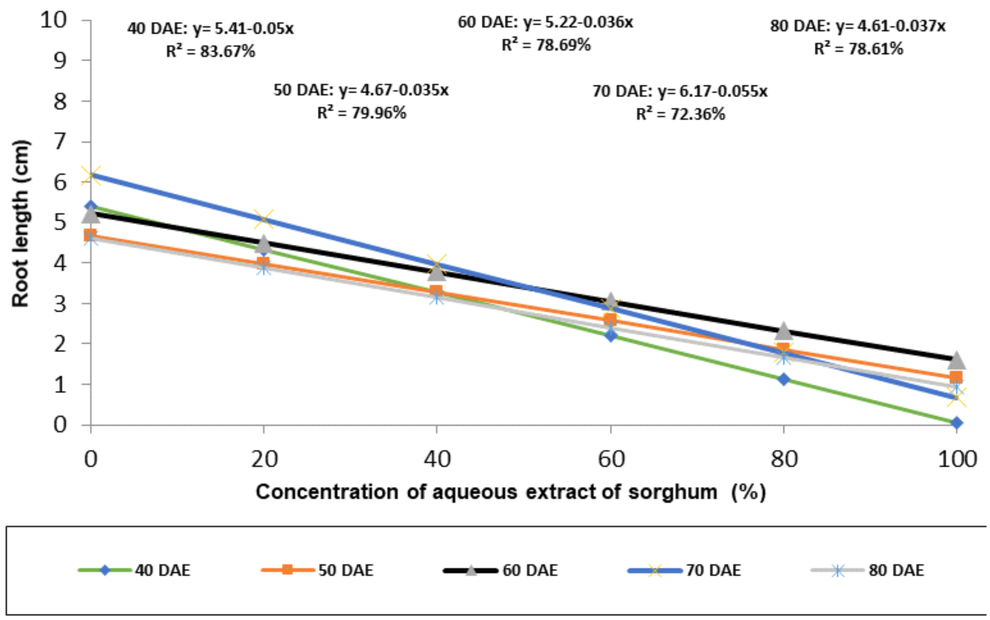

Figure 3 - Root length (RL) of kale (*Brassica oleracea*) seedlings treated with different concentrations of aqueous extract of the shoot of sorghum (*Sorghum bicolor*) harvested at 40, 50, 60, 70, and 80 days after emergence (DAE).

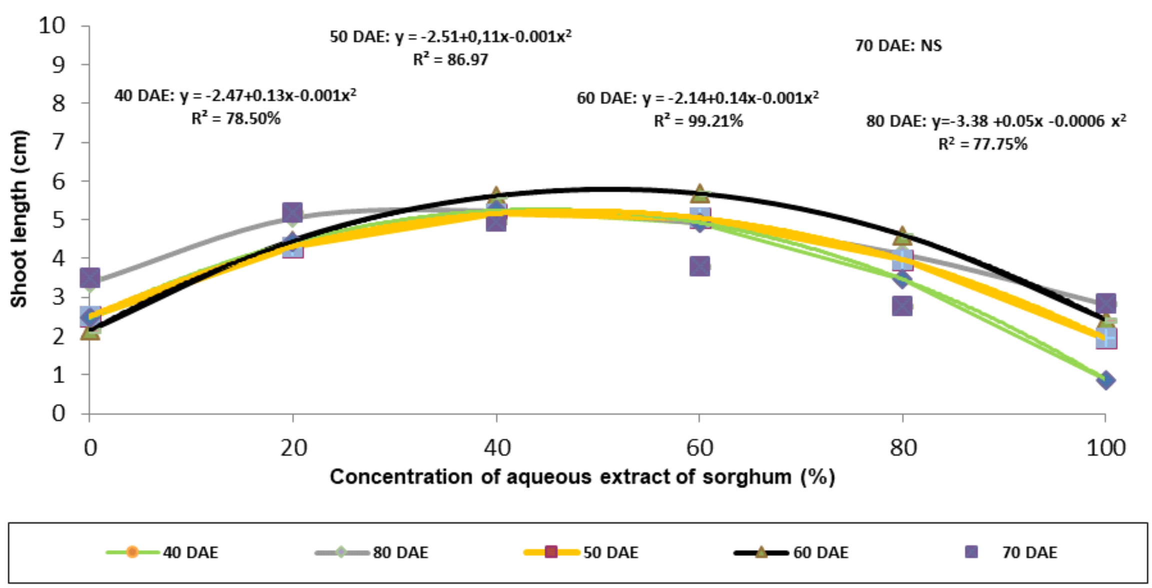

Figure 4 - Shoot length (SL) of kale (*Brassica oleracea*) seedlings treated with different concentrations of aqueous extract of the shoot of sorghum (*Sorghum bicolor*) harvested at 40, 50, 60, 70, and 80 days after emergence (DAE).

hormonal imbalance. Allelochemicals may alter the level of indoleacetic acid (AIA), and this alteration may have raised the hormone to an optimal concentration for shoot growth. However, root growth is strongly inhibited by concentrations that promote stem and coleoptile growth, as mentioned by Taiz and Zeiger (2017). The same behavior was not observed in the 100% extract, which probably kept the concentration of allelochemicals high enough throughout the time, thus providing a lower shoot growth when compared to the others.

Therefore, the shoot of *S. bicolor* has hydrophilic compounds that can contribute to the allelopathic effect of sorghum and this effect, as shown by the germination and initial growth of kale, is predominantly inhibitory and more pronounced when more concentrated extracts from pre-flowering plants are used. Probably this aqueous extract has compounds that act interfering with the balance of phytohormones necessary for plant development. Therefore, this study shows that the pre-flowering period is the recommended stage for using *S. bicolor* straw to assist in weed control.

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