

Control of Invasive Plants by the Phytotoxicity Effect of *Sorghum bicolor* [L.] Moench

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Abstract

Owing to an increasing demand for food, a constant agricultural production flow must be maintained. Further, for doing so, the use of pesticides is necessary. An alternative that results in less damage to the ecosystem and people themselves may be identified by studies on the allelopathic effect of weeds. Therefore, this work aimed to evaluate the allelopathic action of hexane, dichloromethane, butanol, and ethyl acetate fractions of sorghum (*Sorghum bicolor* [L.] Moench) on the initial growth of morning glory (*Ipomoea grandifolia* [Dammer] O'Donnell) and slim amaranth (*Amaranthus hybridus* L.), which was verified by the percentage of germination, speed of germination, seedling length, and fresh and dry biomass weight. The experiments were conducted in an incubation chamber at 25 °C for 7 and 14 days for morning glory and slim amaranth, respectively. The experimental design was completely randomized, with five replicates in Petri dishes. The data were evaluated by analysis of variance, and the averages between each treatment were compared using the Scott Knott test at a 5% significance level. The results indicated that the dichloromethane and ethyl acetate fractions decreased the initial growth of morning glory and slim amaranth more, when compared with the effects of hexane and butanol.

Keywords: secondary metabolism, sorgoleone, weeds

1. Introduction

The global population is expanding daily. In Brazil, the population increases on average by about one person every 20 s (Instituto Brasileiro de Geografia e Estatística [IBGE], 2016). As a result, there is an increasing demand for food, which can be addressed by developing agriculture and livestock technologies.

Because of the need for increased production, it is necessary for most farmers to use pesticides, which can cause an ecological imbalance, especially when used inconsistently and in excess. Because of this, there are more risks to consumer health and the environment (Pinto, 2015).

The considerable increase in the use of applied agrochemicals could bring about a series of disturbances and modifications in the environment, either by the contamination of the living communities that compose the environment or by the accumulation of the agrochemicals in the biotic and abiotic segments of the ecosystem (Ribas & Matsumura, 2009).

As an alternative against the incorrect and exacerbated use of agrochemicals, studies have been performed related to the allelopathic action of plants. These studies have searched for herbicidal or growth-regulating phytotoxins, since the synthetic versions of these products increase the proliferation of invasive, herbicide-resistant plants when used improperly. Phytotoxins generally belong to the class of secondary metabolites, which are less harmful to the environment when compared with agrochemicals (Magiero et al., 2009).

As such, it is extremely important to carry out research in this field to determine the mechanisms of action, production, and decomposition of allelopathic compounds (Rosado et al., 2009). The term allelopathy originated from a study on the chemical interference that one plant species exerts on another. The word unifies the terms “allelon” and “pathos,” which signify “mutual” and “injury,” respectively. It is known that allelopathic compounds can exert positive effects on the receiver or may exert negative effects by impairing some species, populations, or even surrounding communities (Brass et al., 2009). Furthermore, allelopathy occurs when a plant, through its living or decomposing tissue, interferes with the growth of another plant (Zimdahl, 2007).

Although allelopathy has been verified in all organisms, it is more common and evident in plants. It is considered as one of the defense mechanisms against pathogens, pests, herbivores, and other plants. Even after death, allelochemical substances can remain in plant tissues and be released through volatilization (for volatile products) or by leaching, through dew and rain (if soluble in the water), into the ground, thereby, when in sufficient concentration, they can influence the development of microorganisms and plants (Almeida, 1991). Therefore, the allelopathic effect can be pronounced both during the cropping cycle and in consecutive crops cultivated (Teixeira, Araújo, & Carvalho, 2004).

Sorghum (*Sorghum bicolor* (L.) Moench) is considered a good alternative of cereals as a fall/winter crop, with the capability to tolerate water deficient conditions and a high capacity for water utilization and conversion to dry biomass. It should also be considered that sorghum straw has a high C/N ratio and, consequently, a higher soil persistence (Landau & Sans, 2008). Some studies have shown that the use of sorghum may allelopathically affect the development or germination of different plant species, such as wild poinsettia, morning glory, southern sandbur, and others (Carvalho, Teixeira, Neto, Moreira, & Cunha, 2016; Gomes, Bevilacqua, Silva, & Monquero, 2014; Oliveira, Peixoto, Poelking, & Almeida et al., 2015).

Owing to its recognized allelopathic potential, sorghum has been used in intercropping systems to reduce the use of chemical herbicides. This is only possible because of the production of compounds with biological activity, such as sorgoleone. This compound is naturally present in expressive quantities in the trichomes of sorghum roots and inhibits the growth of invading plants through contact (I. L. V. L. Santos, Silva, S. L. Santos, & Maia, 2012).

Morning glory (*Ipomoea grandifolia* [Dammer] O’Donell) is an annual angiosperm. Its best development occurs in tilled soil and under good humidity conditions. This plant has a round-branched stem with white hairs. The flowers emerge from the point where the leaves attach to the stem/branches. They are white in color at the base of the tube and pink at the top with a red center. The fruit is capsule-shaped and contains an average of four seeds (Gazziero et al., 2006). In addition, *Ipomoea grandifolia* (Dammer) O’Donell is a climber weed, characterized by the morphology of its stems and voluble branches that can get entangled with neighboring plants and grow over surfaces (Marques et al., 2005). Its presence in soybean fields can reduce crop productivity significantly, making harvesting, especially mechanical harvesting, difficult.

Slim amaranth (*Amaranthus hybridus* L.) is an annual, herbaceous, branched, erect, and pigmented angiosperm, which is about 40-100 cm high, native to tropical America, and a seed propagator. It is a weed plant of great importance and relatively high frequency in the south of the Brazilian country, mainly infesting cultivated soils of annual crops, orchards, coffee plantations, and vacant lots. It has a large reproductive capacity; a single plant can produce 117.000 seeds (Lorenzi, 2000).

Controlling invasive plants with a natural herbicide could eventually become a more economical, ecological, and sustainable alternative to chemical pesticides. Therefore, this study aimed to evaluate the allelopathic potential of sorghum, specifically on morning glory and slim amaranth weeds.

2 Material and Methods

The experiments were conducted from June 2016 to September 2018. Using a BOD (Biochemical Oxygen Demand) incubator (Logen), the germination and initial growth of the invading plants *Ipomoea grandifolia* (Dammer) O’Donell and *Amaranthus hybridus* L. were evaluated in the presence or absence of dichloromethane, hexane, butanol, and ethyl acetate fractions of *Sorghum bicolor* (L.) Moench.

2.1 Obtaining Sorghum Fractions by Liquid-Liquid Extraction

The dried and ground materials (3.75 kg) of the aerial parts of sorghum were extracted with cold methanol following maceration. After removal of the solvent under vacuum on a rotary evaporator at 33-35 °C, the crude methanolic extract (293.61 g) was obtained.

The crude extract was dissolved in 3:1 methanol-water (1.5 L) and partitioned with 3 × 300 mL of each of the organic solvents: hexane, dichloromethane, ethyl acetate, hydromethanol, and butanol. After removal of the

solvents using a rotary evaporator, the following fractions remained: hexane (32.33 g), dichloromethane (4.48 g), ethyl acetate (6.28 g), butanol (50.33 g), and hydromethanol (180.42 g) (Figure 1). The concentrations (0, 250, 500, 750, and 1000 $\mu\text{L}/\text{mL}$) of the hexane, dichloromethane, ethyl acetate, and butanol fractions were analyzed.

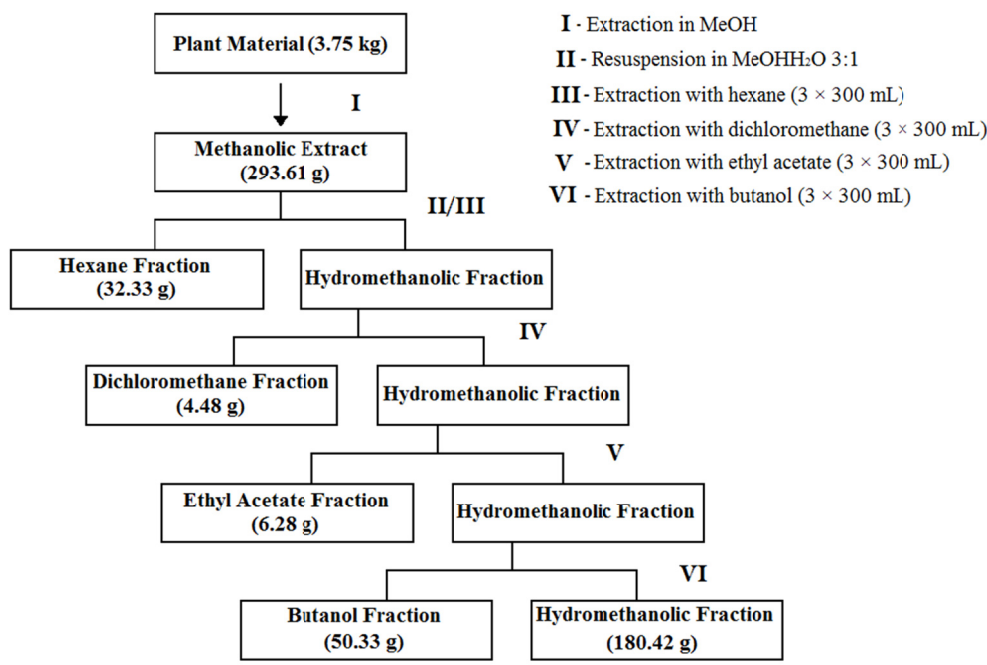


Figure 1. Fractionation of *Sorghum bicolor* (L.) Moench crude extract by liquid-liquid extraction

2.2 Biological Material

Morning glory and slim amaranth seeds were purchased from Cosmos Agrícola Produção e Serviços Rurais Ltda, a company that specializes in the production of weed seeds. The registration number on SisGen is AED2337.

2.3 Experimental Conduct

Before the implantation and evaluation of each experiment, the stand, germination chamber, experimenter's hands, and materials were disinfected. The seeds were then selected for size and shape according to their phenotypic characteristics, and immersed in a 2% sodium hypochlorite solution (NaClO) for 1-2 min. Finally, the seeds were washed extensively with distilled water.

2.4 Evaluation of Germination

The dichloromethane, hexane, butanol, and ethyl acetate fractions at different concentrations (0, 250, 500, 750, and 1000 $\mu\text{L}/\text{mL}$) were dissolved in 5 mL of 50% MeOH/H₂O and distributed in a 29 × 9 × 2 cm Petri dish, on two sheets of paper for germination. For 24 h, the fractions remained at rest for complete evaporation of methanol. In the control only, 5 mL of 50% MeOH/H₂O were added to the Petri dish. Five replicates of each treatment were performed, where each replicate contained 25 morning glory or slim amaranth seeds distributed in Petri dishes.

After sowing, the Petri dishes were conditioned in a BOD germination chamber at 25 °C, with a 12-h photoperiod, for 7 and 14 days for the morning glory and slim amaranth, respectively.

For the evaluation of germination of the seeds, daily counts of the morning glory and slim amaranth were carried out for 7 and 14 days, respectively. From the sowing, germinated seeds that presented root protrusion at about 2 mm were assessed, as described by Hartmann, Kester, Davies, and Geneve (2001).

2.4.1 Percentage of Germination (% G)

The percentage of germination (% G) was obtained by representing the percentage of seeds germinated in relation to the total number of seeds per plate under the given experimental conditions, determined as follows:

$$\% G = (\Sigma n_i \times N^{-1}) \times 100 \quad (1)$$

where, Σni = total number of germinated seeds; N^{-1} = number of seeds arranged to germinate.

2.4.2 Speed of Accumulated Germination

The speed of accumulated germination (AS) was obtained using the equation proposed by Ferreira and Borghetti (2004):

$$AS = G1/N1 + G2/N2 + \dots Gn/Nn \quad (2)$$

where, G = number of seeds; N = number of days after sowing.

2.5 Initial Growth Assessment

The initial growth of invasive plants was verified from the length of the seedling, as well as the respective fresh and dry biomass weights.

2.5.1 Seedling Length

The length of the seedlings was determined by the end of the main root to its stem apex, measured using a millimeter ruler. Only seedlings with developmental capacity were measured (Ministério da Agricultura e Reforma Agrária [MAPA], 2009).

2.5.2 Seedling Biomass

Afterwards, the fresh biomass weights of the morning glory and slim amaranth seedlings were obtained by weighing on an analytical balance. Subsequently, the samples were properly conditioned in paper bags and placed in an oven for drying at 60 °C to obtain the dry biomass weight (Borella & Pastorini, 2009).

2.6 Statistical Analysis

The experimental design was completely randomized with five replicates of each treatment. The data were evaluated by analysis of variance, and the means between treatments were compared by the Scott-Knott test at 5% significance (Scott & Knott, 1974). The data were transformed on a logarithmic scale. The software used was SISVAR of the Federal University of Lavras-UFLA (Ferreira, 2014).

3. Results

As shown in Table 1, the results indicated that the dichloromethane fraction of sorghum significantly inhibited in all variables analyzed, with the most significant effects being observed at the highest concentrations analyzed (750 and 1000 ppm) on morning glory seedlings (*Ipomoea grandifolia* [Dammer] O'Donell). The hexane fraction showed no significant difference in any of the analyses of the invasive plant.

Table 1. Effects of dichloromethane and hexane fractions of sorghum at different concentrations (250, 500, 750, and 1000 ppm) on the growth (length and fresh and dry biomass weights) and germination (% G and AS) of *Ipomoea grandifolia* (Dammer) O'Donell. Control experiments are indicated as water + methanol (met)

Treatment (ppm)	Length (cm)	Fresh Biomass Weight (g)	Dry Biomass Weight (g)	Germination (%)	AS
H ₂ O + MET	6.3572±0.2624Aa	0.702±0.0642Aa	0.049±0.0016Aa	82.40±1.4509Ba	19.58±0.446Aa
DIC 250	3.9597±0.1816B	0.701±0.0650A	0.050±0.0020A	88.00±1.2649A	17.26±0.974A
DIC 500	3.4775±0.3061B	0.580±0.0254A	0.042±0.0027A	86.40±3.2497A	15.19±0.703B
DIC 750	2.7120±0.2152C	0.337±0.0230B	0.030±0.0030B	76.80±1.9596B	11.02±0.977B
DIC 1000	2.6764±0.2128C	0.467±0.0320B	0.031±0.0030B	80.00±2.5299B	11.18±0.917C
HEX 250	5.5280±0.5135a	0.758±0.0780a	0.045±0.0040a	88.80±1.4967a	21.62±0.695a
HEX 500	6.6955±0.4629a	0.816±0.0632a	0.049±0.0030a	83.20±1.4967a	19.47±0.519a
HEX 750	6.6250±0.3120a	0.866±0.0730a	0.050±0.0040a	83.20±4.9640a	18.23±1.755a
hex 1000	6.2108±0.4122a	0.786±0.0780a	0.049±0.0030a	85.60±4.1183a	18.49±0.610a

Note. Upper- and lower-case letters represent the dichloromethane and hexane fractions, respectively. Statistically distinct results are represented by different letters according to the Scott-Knott test at 5% significance. Data are represented as averages±standard error of the mean (SEM).

The negative allelopathic effect of the dichloromethane fraction on the length of the morning glory seedlings was verified at all analyzed length concentrations, and a reduction of up to 57.9% (1000 ppm) was observed when compared to that in the control experiment (water + methanol).

Regarding biomass weight, dry and fresh, both were reduced at concentrations of 750 and 1000 ppm of the dichloromethane fraction. A concentration of 750 ppm showed a greater reduction, decreasing the fresh biomass

weight by 51.99% and dry biomass weight by 38.78%.

Considering germination, the dichloromethane fraction of sorghum increased the percentage of germination (% G) at concentrations of 250 and 500 ppm, with stimuli of 6.8% and 4.85%, respectively. As for the speed of accumulated germination (AS), there was a reduction in concentrations higher or equal than 500 ppm, and an inhibition of 42.9% was observed at the highest concentration (1000 ppm).

Table 2 illustrates the ethyl acetate and butanol data on sorghum fractions, considering *Ipomoea grandifolia* (Dammer) O'Donell seedlings.

Table 2. Effects of ethyl acetate and butanol fractions of sorghum at different concentrations (250, 500, 750, and 1000 ppm) on the growth (length and fresh and dry biomass weight) and germination (% G and AS) of *Ipomoea grandifolia* (Dammer) O'Donell. Control experiments are indicated as water + methanol (met)

Treatment (ppm)	Length (cm)	Fresh Biomass Weight (g)	Dry Biomass Weight (g)	Germination (%)	AS
H ₂ O + MET	7.8511±0.4904Aa	1.052±0.0693Aa	0.046±0.0032Ab	85.33±4.8075Aa	21.42±0.939Aa
ACET 250	5.4116±0.2040A	1.086±0.0392A	0.060±0.0023A	88.80±0.8000A	20.12±0.361A
ACET 500	5.0696±0.1445B	1.102±0.0449A	0.054±0.0004A	82.40±2.0397A	15.42±0.546B
ACET 750	4.8255±0.1635B	1.107±0.0550A	0.059±0.0030A	83.20±1.4967A	15.43±0.135B
ACET 1000	4.5095±0.1979B	0.851±0.0532B	0.052±0.0022A	76.80±3.6662A	14.56±0.364B
BUT 250	6.9077±0.3366b	1.270±0.1082a	0.057±0.0041a	88.00±4.1954a	22.44±0.649a
BUT 500	7.5924±0.0770a	1.201±0.1100a	0.056±0.0017a	82.40±3.7096a	19.73±0.433a
BUT 750	7.7337±0.0958a	1.134±0.1122a	0.063±0.0041a	74.40±6.4002a	17.84±1.739b
BUT 1000	6.2307±0.2868b	0.948±0.0187a	0.049±0.0027b	75.20±3.4410a	16.50±0.382b

Note. Upper- and lower-case letters represent the ethyl acetate and butanol fractions, respectively. Statistically distinct results were represented by different letters according to the Scott-Knott test at 5% significance. Data are represented as averages±standard error of the mean (SEM).

It was found that the ethyl acetate fraction reduced seedling length at concentrations of 500, 750 and 1000 ppm, while the butanol fraction decreased seedling size at concentrations of 250 and 1000 ppm. The highest reductions were observed at 1000 ppm in both fractions, decreasing by 42.56% and 20.64% in the ethyl acetate and butanol fractions, respectively.

The fresh biomass weight was inhibited (19.11%) only in the ethyl acetate fraction of 1000 ppm. Regarding dry biomass weight, a positive influence was found in the butanol fraction, with a stimulus mostly at 750 ppm, increasing by 36.96% compared to that in the control.

In relation to germination, there were no statistical differences in the germination percentage. However, the speed of accumulated germination of *Ipomoea grandifolia* (Dammer) O'Donell was observed in both fractions, with the highest inhibitions at 1000 ppm, reduced by 32.03% and 22.97% in the ethyl acetate and butanol fractions, respectively.

Table 3 shows that the hexane fraction did not alter growth (length and fresh biomass weight) of slim amaranth seedlings. Data on dry biomass weight, as well as on the other fractions analyzed, were not presented because they were not detected on the balance owing to the reduced size of the species.

Table 3. Effect of the hexane fraction of sorghum at different concentrations (250 and 500 ppm) on growth (length and fresh biomass weight) and germination (% G and AS) of *Amaranthus hybridus* L. Control experiments are indicated as water + methanol (met)

Treatment (ppm)	Length (cm)	Fresh Biomass Weight (g)	Germination (%)	AS
H ₂ O + MET	0.7486±0.0696a	0.0246±0.0022a	40.80±2.9395a	4.197±0.238a
HEX 250	0.6992±0.0488a	0.0481±0.0055a	29.33±1.3334b	2.690±0.369b
HEX 500	0.6292±0.0426a	0.0199±0.0024a	24.00±2.3095b	1.893±0.260b
HEX 750	-	-	-	-
HEX 1000	-	-	-	-

Note. Different letters indicate statistically distinct results, according to the Scott-Knott test at 5% significance. Data are represented as averages±standard error of the mean (SEM).

The percentage and speed of accumulated germination were inhibited at concentrations of 250 and 500 ppm. The 500 ppm concentration demonstrated greater effects, indicating reductions of 41.18% in the % G and 54.9% in the AS.

Experiments were also performed using the dichloromethane and ethyl acetate fractions on *Amaranthus hybridus* L. However, these fractions drastically inhibited the initial germination and development of this plant, making it impossible to verify the parameters proposed in this work. Similar results were found with the highest concentrations (750 and 1000 ppm) of the hexane fraction.

The butanol fraction promoted a significant increase in the length of *Amaranthus hybridus* L. at a concentration of 1000 ppm, with an increase of 33.53% in relation to the control, as shown in Table 4.

Table 4. Effect of the butanol fraction of sorghum at different concentrations (250, 500, 750, and 1000 ppm) on the growth (length and fresh biomass weight) and germination (% G and AS) of *Amaranthus hybridus* L. Control experiments are indicated as water + methanol (met)

Treatment (ppm)	Length (cm)	Fresh Biomass Weight (g)	Germination (%)	AS
H ₂ O + MET	0.7777±0.0326b	0.0426±0.0006a	58.40±3.4872a	4.1364±0.182a
BUT 250	0.7685±0.0523b	0.0230±0.0017b	66.67±5.8120a	4.1962±0.342a
BUT 500	0.8531±0.0463b	0.0270±0.0013a	61.60±4.1184a	4.2926±0.148a
BUT 750	0.8293±0.0669b	0.0369±0.0012a	55.20±3.4410a	4.4207±0.165a
BUT 1000	1.0385±0.0494a	0.0345±0.0009a	45.60±3.2497a	2.9617±0.117b

Note. Different letters indicate statistically distinct results, according to the Scott-Knott test at 5% significance. Data are represented as averages±standard error of the mean (SEM).

In contrast, the fresh biomass weight was inhibited at 250 ppm, reduced by 46.01% compared to that of the control. However, no differences were observed in the percentage of germination of *Amaranthus hybridus* L. when the seeds were exposed to the butanol fraction. The speed of accumulated germination was inhibited only at a concentration of 1000 ppm, with a reduction of 28.4% compared to that of the control.

4. Discussion

Allelopathic substances are capable of inhibiting germination and growth, as they interfere with cell division, membrane permeability, enzyme activation, and hormone production in plants (Rodrigues, Rodrigues, & Reis, 1992). Allelopathic compounds may be a form of communication because they allow plants to distinguish between organisms that are harmful or beneficial (Almeida, Lucchesi, & Abbado, 1997).

Germination is a sequence of physiological events that can be influenced by factors external and internal to the seeds, in which each factor can act independently or interact with others to optimize the percentage, uniformity, and speed of germination. The germination speed is affected mainly by controllable and manipulable external (environmental) factors and may undergo changes because of compounds present in the substrate (Nassif, Vieira, & Fernandes, 1998).

Ferreira and Áquila (2000) stated that changes in the germination pattern may be due to effects on enzyme and receptor conformations, membrane permeability, DNA transcription and translation, respiration, oxygen sequestration, secondary messenger function, or a combination of the mentioned factors.

Research conducted in the field by Trezzi and Vidal (2004) showed that sorghum straw, when increased in terms of its soil cover, reduced infestations of *Brachiaria. plantaginea* and *Sida. rhombifolia* weeds. Ferreira, Medeiros, and Soares (2008), using allelopathic compounds, verified the inhibition of AS of *Eragrostis plana* Nees over Bahia grass and kazungula grass, which was in accordance with the findings of Gomes et al. (2018), in which the AS of lettuce was affected by extracts with sorgoleone.

Many studies related to allelopathy have only evaluated the effects exerted by allelochemicals on the germination and growth of the test plant and have not investigated the cellular events related to physiological changes in the plant system or plant growth (Pires et al., 2001).

Sorghum is an important cereal that can assist in the control of invasive plants. This is possibly owing to its ability to produce substances located in the trichomes of its roots, including sorgoleone, capable of persisting in the soil for extended periods. Sorgoleone is a proven allelopathic compound and is capable of inhibiting the

development of certain plants by primarily inhibiting the photosynthetic pathway (I. L. V. L. Santos, Silva, S. L. Santos, & Maia, 2012).

The alternative control of weeds through the phytotoxics properties of sorghum could be a useful tool to enable the productive systems of conservation, taking advantage of the use of agrochemicals (Karam, Silva, Vasconcelos, Rodrigues, Mourão, 2011). Sorghum plants release both hydrophobic and hydrophilic chemicals into the environment. The action of these substances on the growth and development of weeds depends, especially, on the genotype used, part of the plant considered, and environmental conditions (Trezzi, Vidal, & Kruse, 2005).

T. M. L. Barbosa, Ferreira, Souza, L. C. A. Barbosa, and Casali (1998) also observed a phytotoxic effect of allelochemicals from the root exudates of sorghum seedlings, in which growth was inhibited and the dry matter and leaf area of lettuce were decreased. Other authors, such as Navas, Monteiro, Medeiros, and Pereira (2018) and Pires, Prates, Filho, Oliveira, and Faria (2001), have found some inhibitory effects of allelopathic compounds on weed length.

The results of this research indicate that the dichloromethane and ethyl acetate fraction extracts of sorghum are phytotoxics allelochemicals, which promote a high bioherbicidal effect. Similar findings occurred at high concentrations of the hexane fraction, presenting a phytotoxic effect on *Amaranthus hybridus* L. Poor development and certain characteristics of the radicle can be considered as factors that indicate plant susceptibility to phytotoxics effects (Souza-Filho & Duarte, 2007). However, it is necessary to identify these secondary metabolites extracted in each fraction and investigate parameters, such as the osmotic potential, enzymatic activities, lipid peroxidation, and cell viability, to elucidate the mechanism of action.

Identifying phytotoxics allelochemicals can lead to the discovery of natural substances with inhibitory characteristics, since some herbicides can cause cell death and induce oxidative stress (Silva et al., 2016). It is worth mentioning that a large part of laboratory findings on allelopathy possibly remain unconfirmed under natural conditions, since the simultaneous occurrence of biotic and abiotic factors may interfere with the final results (Tur, Borella, & Pastorini, 2010).

5. Conclusion

It was concluded that the dichloromethane and ethyl acetate fractions had a phytotoxic effect on the morning glory and slim amaranth varieties, which indicated that isolated compounds in these fractions could inhibit the initial growth of these invasive plants, thereby acting as bioherbicides.

The butanol fraction had no inhibitory effects on the species studied. The hexane fraction did not show significant effects on morning glory; however, it had extensive phytotoxics effects on slim amaranth.

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