

# Bioherbicidal action of *Phoma dimorpha* fermented broth on seeds and plants of *Senna obtusifolia*<sup>1</sup>

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## ABSTRACT

The weed *Senna obtusifolia* causes severe damages to pasture areas in Brazil, due to its high production and seed spread. This study aimed to evaluate the bioherbicidal action of *Phoma dimorpha* fermented broth in the pre-emergence and post-emergence of *S. obtusifolia*. The experimental design was completely randomized, with two treatments, one with and other without the application of the fermented broth. The bioherbicidal effects were measured in bioassays of pre-emergence (germination percentage), detached leaves (phytotoxicity) and post-emergence (phytotoxicity, plant height and fresh plant mass). The application of the fermented broth provided a pre-emergence bioherbicidal action, inhibiting the seed germination in 100 %. In detached leaves, it caused leaf necrosis and death on the ninth day after the application. In the post-emergence, this application caused moderate symptoms, such as leaf spots and reduction in the weed plant size. It was concluded that the *P. dimorpha* fermented broth has a potential herbicidal action and, therefore, represents an alternative in the development of bioproducts for a sustainable weed control in pastures.

**KEYWORDS:** *Senna obtusifolia*, biological control, phytotoxicity, weed.

## INTRODUCTION

The increasing weed infestation in growing areas may be responsible for significant reductions in crop yields. Several studies confirm the toxic potential and greater competitive power of weeds over cultivated plants, which have generated not only losses in the quality of agricultural products, but also in yield (28-68 % in large crops such as soybean

## RESUMO

Ação bioherbicida de caldo fermentado de *Phoma dimorpha* em sementes e plantas de *Senna obtusifolia*

A planta daninha *Senna obtusifolia* causa severos danos em áreas de pastagens no Brasil, devido à sua elevada produção e disseminação de sementes. Objetivou-se avaliar a ação bioherbicida do caldo fermentado de *Phoma dimorpha* na pré-emergência e pós-emergência de *S. obtusifolia*. O delineamento experimental utilizado foi inteiramente casualizado, com dois tratamentos, um com e outro sem aplicação do caldo fermentado. Os efeitos bioherbicidas foram mensurados em bioensaios de pré-emergência (porcentagem de germinação), folhas destacadas (fitotoxicidade) e pós-emergência (fitotoxicidade, altura de planta e massa fresca de planta). A aplicação do caldo fermentado proporcionou ação bioherbicida em pré-emergência, inibindo em 100 % a germinação das sementes. Em folhas destacadas, ocasionou necrose e morte das folhas no nono dia após a aplicação. Na pós-emergência, essa aplicação ocasionou sintomas moderados, como manchas nas folhas e redução no tamanho da planta daninha. Conclui-se que o caldo fermentado de *P. dimorpha* possui ação herbicida potencial e, por isso, representa uma alternativa no desenvolvimento de bioproductos para o controle sustentável de plantas daninhas em pastagens.

**PALAVRAS-CHAVE:** Fedegoso, controle biológico, fitotoxicidade, planta daninha.

and cotton), thus generating high economic losses (Pisula & Meiners 2010, Suriyagoda et al. 2014, Bajwa et al. 2016).

The weed species most frequently found in pastures, orchards, annual and perennial plants in Brazil, and also in other countries, is *Senna obtusifolia* (L.) H. S. Irwin & Barneby, popularly known in Brazil as “fedegoso”, due to its characteristic bad odor, and also known as “bush-pasture”, due to the

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damage it causes to pastures. Native of the American continent, it is classified as a bushy and woody plant, with approximately 70-160 cm of height (Lorenzi 2002).

Its potential as a weed is associated with its high seed production, as when the pods reach maturity, they spread their germinating seeds at the beginning of the rainy season. They are able to germinate and bloom over a wide temperature range (18-36 °C); therefore, the seeds are the main way of propagation. In addition to these problematic characteristics, its competitive ability and toxicity to domestic animals cause problems in agricultural and pasture areas (Walker & Oliver 2008, Peres et al. 2010).

Weed plant control is reached by cultural, mechanical, chemical or biological activities. In the last five decades, the use of chemical herbicides is the main measure of weed control systems (Jabran et al. 2015). Despite the widespread use and effectiveness of herbicides in the weed control, in recent years, weeds with multiple resistance to the action of certain chemical molecules have occurred (Jabran et al. 2015, Meuller-Steover et al. 2016).

Because of the difficult control of certain weed species, as well as the potential negative impacts of chemicals on the environment and human health, alternative methods are being developed, which are environmentally safe and biodegradable in nature, in order to reduce the use of synthetic herbicides (Bajwa et al. 2016). The biological control is an alternative for the substitution of agrochemicals, favoring the reduction of their use and minimizing the damages caused to the environment (Schwan-Estrada et al. 2000).

Biologically active secondary metabolites coming from fermented media of microorganisms are used in the agricultural environment for biological control. These offer several benefits, such as a greater specificity to the target plant, rapid degradation and reduction in the use of synthetic herbicides, being considered economically and environmentally viable (Baque et al. 2012, Cordeau et al. 2016). Among the sources of secondary metabolites, fungi are the most studied microorganism producers, since several species of endophytic fungi are considered a potential source of natural products, being an important source for the selection of agents of interest to the biological control (Baque et al. 2012).

The *Phoma* fungi are a source of secondary metabolites with high biological activity, as some

species secrete secondary metabolites, which may be used for the manufacturing of products with bio-biocidal activity, as well as others, aimed at the biological control of phytopathogenic fungi and insects (Wijeratne et al. 2013). Several studies have shown that fungi of this genus produce a large variety of secondary metabolites with biological potential and they may be used for the formulation of bioproducts aimed at the agricultural environment (Hubbard et al. 2014, Brun et al. 2016, Todero et al. 2018b).

This study aimed to evaluate the bioherbicidal action of a fermented broth produced from *Phoma dimorpha* fungus in the pre-emergence and post-emergence of *S. obtusifolia*.

## MATERIAL AND METHODS

The bioassays were conducted at the Universidade Federal de Santa Maria (UFMS), in Santa Maria, Rio Grande do Sul state, Brazil, under laboratory (pre-emergence and detached leaf) and greenhouse (post-emergence) conditions, from May to July 2018. The microorganism used to obtain the fermented broth was a *P. dimorpha* (NRRL 43879) fungus strain originated from the collection of the Biotec Factory laboratory (UFMS).

The *S. obtusifolia* seeds used to perform the bioassays were collected in a pasture area located in Cuité de Mamanguape, Paraíba state, Brazil (06°55'15.4"S and 35°17'12.8"W).

The fungus strain was maintained in Petri dishes containing BDA medium at 8 °C, and subcultured every 15 days. For the fermentation process, Petri dishes containing the active growing fungus were kept in a bacteriological oven for 7 days, at 28 °C, from which 2 mycelium disks of 6 mm were removed and transferred to Erlenmeyer flasks with 130 mL of a liquid medium containing potato extract (200 g L<sup>-1</sup>), dextrose (20 g L<sup>-1</sup>), peptone (10 g L<sup>-1</sup>), yeast extract (7.5 g L<sup>-1</sup>), ammonium sulfate [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 2 g L<sup>-1</sup>], ferrous sulfate (FeSO<sub>4</sub>·7H<sub>2</sub>O; 1g L<sup>-1</sup>) and magnesium sulfate (MgSO<sub>4</sub>; 0.5 g L<sup>-1</sup> and MnSO<sub>4</sub>·H<sub>2</sub>O; 1g L<sup>-1</sup>) (Zhang et al. 2012), previously sterilized by autoclaving at 121 °C, for 30 min. The flasks were maintained at 28 °C, under agitation of 120 rpm, for 10 days (Innova 44R, New Brunswick) (Klaic et al. 2017). After this period, the cells were separated from the fermented broth through a filter paper with 14 µm pores in vacuum filtration with a

Büchner funnel. The cell free fermented broth was stored in a freezer at -20 °C, for 15 days. Then, three different bioassays were performed in order to verify the bioherbicidal action of the *P. dimorpha* fermented broth in pre-emergence, detached leaves and post-emergence of *S. obtusifolia*.

In pre-emergence, the bioherbicidal action of the *P. dimorpha* fermented broth was evaluated through a germination test, with the seeds being initially submitted to the overcoming mechanical scarification dormancy method (Bortolini et al. 2011).

The germination test was carried out in a completely randomized design, with two treatments (with and without application of the fermented broth) and eight replicates of 25 seeds. For this, sheets of filter paper (Germitest®) moistened with 4 mL of the different treatments were used. Then, the boxes were conditioned in a BOD germination chamber at 25 °C, with photoperiod of 12/12 h of light/darkness. The evaluations were carried out daily until reaching stabilization, counting the number of seeds that germinated, having as a criterion the protrusion of the minimum radicle of 2 mm in length (Brasil 2009).

Another bioassay in a completely randomized design, with the same two treatments (without and with application of the fermented broth), was used to assess the bioherbicidal action in the leaf. Four replications of one leaf each were used. With this aim, *S. obtusifolia* leaves with 15 days of growth were collected. These leaves were arranged in gerbox boxes previously disinfected with 70 % ethanol and lined with two sheets of filter paper. Then, 3 mL of the fermented broth were applied and, for the control treatment, the same volume of distilled water was applied with the use of an automatic pipette on cotton wrapped in the leaf petiole. The gerbox boxes were conditioned in a BOD incubator chamber with temperature of 25 °C and photoperiod of 12/12 h of light/darkness. The evaluations were carried out

on the 3rd, 6th and 9th day after the application (Pedras & Ahiahonu 2004, Todero et al. 2018b) for the analysis of the appearance of symptoms, according to the scale presented in Table 1.

In post-emergence, the assay to evaluate the bioherbicidal action of the *P. dimorpha* fermented broth on the weed was carried out in a greenhouse. The *S. obtusifolia* culture was used for the subsequent application and evaluation of the treatments. The same two treatments were assigned completely randomized to ten replications each. Each experimental unit was composed of a polyethylene vessel with volume of 180 mL, filled with Macplant® commercial substrate. Initially, three seeds were sown in each vessel, and after the emergence of the seedlings, thinning was performed and only one plant was maintained per vessel, which was cultivated until the end of the experiment.

The application of the fermented broth was carried out at approximately 15 days after the emergence of the plants. For the application, a costal/manual, CO<sub>2</sub>-pressurized sprayer and 300 L ha<sup>-1</sup> of syrup volume (Brun et al. 2016), was used. Phytotoxicity evaluations were performed at 15 days after the treatment (Todero et al. 2018b) and the leaf damage to plants was estimated visually, based on the scale presented in Table 1. Then, the plant height (mm) and fresh plant mass (g) were also measured. Based on the plant height data, the growth reduction (%) of the treatment with application of the raw fungus fermented broth was calculated in relation to the control treatment (without fermented broth application).

The data were submitted to the normality and homogeneity errors tests, and then to the F-test of Anova, and the treatment means were compared by the Tukey test, adopting a significance level of 5 %. For this, the Sisvar software (Ferreira 2014) was used.

Table 1. Scale for evaluation of the phytotoxicity percentage in detached leaves and plants, developed based on the concepts applied to the toxicity assessments<sup>1</sup>.

Damage (%)	Description of the main categories
0-20	No injury or effect
20-40	Slight injury and/or growth reduction with rapid recovery
40-60	Moderate injury and/or growth reduction with slow or definitive recovery
60-80	Severe injury and/or reduction of non-recoverable growth and/or reduction of the stand
80-100	Complete destruction or just a few live plants

<sup>1</sup> Source: SBCPD (1995).

## RESULTS AND DISCUSSION

A bioherbicidal action of the *P. dimorpha* fermented broth on the germination percentage of *S. obtusifolia* seeds was observed (Table 2). The application of the fermented broth presented a complete inhibition of the germination, with effectiveness in the order of 100 % (Table 2 and Figure 1).

The seed germination inhibition observed in this study characterizes that the fungus fermented broth presents a bioherbicidal action and potential for the development of bioherbicides in the agricultural environment. Researches focused on weed biological control by application of secondary metabolites from fungi of the *Phoma* genus were also conducted by Cimmino et al. (2013), Hubbard et al. (2014), Evidente et al. (2015) and Todero et al. (2018b). These authors characterized phytotoxic effects in pre-emergence (germination reduction) and post-emergence (spots, chlorosis, deformations and collapse of leaves, besides necrosis in plants) of several weed species.

Table 2. Mean germination percentage of *Senna obtusifolia* seeds resulting in normal seedlings after to the application of *Phoma dimorpha* fermented broth.

Treatment	Germination (%) <sup>1</sup>
Control (without fermented broth)	99.00 ± 1.51 a
With fermented broth	0.00 ± 0.00 b
MSD	1.15
CV (%)	2.16

<sup>1</sup> Means with distinct letters are statistically different by the Tukey test at 5 % of probability. MSD: minimum significant difference; CV: coefficient of variation.

Similar results were reported by Brun et al. (2016), when evaluating the herbicide potential of raw fermented broth from *Phoma* sp. submerged in bioreactor on the seed germination of *Cucumis sativus* and *Sorghum bicolor*. They found that the application of this fermented broth caused a reduction in the germination, with percentages of 100 % and 84 % of inhibition, respectively. These authors suggest that a compound named Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-3- (2-methylpropyl), found as a major compound in the chemical characterization of fermented extracts, has a herbicidal effect.

Todero et al. (2018a), when evaluating the effectiveness of the bioherbicidal activity of *Phoma* sp. fermented broth concentrated by membrane from submerged fermentation, found that a 30 % concentrate in the pre-emergence of *Bidens pilosa* and *Amaranthus retroflexus* seeds reached an inhibition of 100 % in all the dilutions used. These results are in agreement with those found in the present research, confirming the bioherbicidal action of the fermented broth produced by representatives of this fungus genus.

The percentage of phytotoxicity, as a function of the application of *P. dimorpha* fermented broth, on leaves of *S. obtusifolia* is shown in Table 3. In the detached leaves of *S. obtusifolia*, there was yellowing (40 % of phytotoxicity) since the first evaluation (72 h after the application), intensifying up to the last evaluation, performed at 9 days after the application of the raw fermented broth, when there were severe lesions with yellowing and marked necrosis, characterizing 90 % of phytotoxicity (Table 3 and Figure 2).

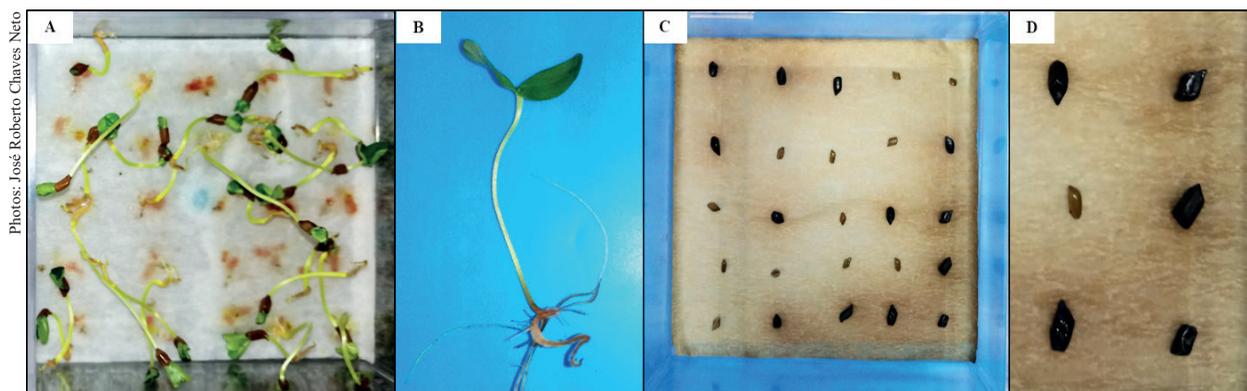


Figure 1. Phytotoxic test on seeds of *Senna obtusifolia* submitted to the application of *Phoma dimorpha* fermented broth. A) Control treatment (without fermented broth); B) seedling of the control treatment; C) treatment with application of the fermented broth; D) detail of the seed when the fermented broth was applied.

Brun et al. (2016) and Toderó et al. (2018a) reported similar phytotoxicity percentages, when evaluating the phytotoxic effect of *Phoma* sp. raw fermented broth on leaves of *C. sativus*. Yellowing and necrosis occurred on detached leaves of this species after 72 h of application (30 % of phytotoxicity), leading to the total leaf death in the last evaluation (80 % of phytotoxicity).

Similar results were also described by Graupner et al. (2003), Vikrant et al. (2006) and Bailey et al. (2011a), when evaluating the application

effectiveness of raw fermented broth of different *Phoma* species obtained by submerged fermentation on leaves of *Cirsium arvense* and *Parthenium hysterophorus* (white losna) plants. They observed a fast yellowing and chlorosis, evolving for leaf necrosis and death, as well as growth reduction.

The application of *P. dimorpha* fermented broth resulted in leaf necrosis (30 % of phytotoxicity) of *S. obtusifolia* plants at 15 days after the application (Table 4 and Figure 3). According to Mello et al. (2003), substances which are present in fermented

Table 3. Phytotoxicity on leaves of *Senna obtusifolia* at 3, 6 and 9 days after the application (DAP) of *Phoma dimorpha* fermented broth.

Treatment	Phytotoxicity (%) <sup>1</sup>		
	3 DAP	6 DAP	9 DAP
Control (without fermented broth)	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b
With fermented broth	40.00 ± 8.16 a	70.00 ± 8.16 a	90.00 ± 8.16 a
MSD	9.99	9.99	9.99
CV (%)	28.87	16.50	12.83

<sup>1</sup> Means with distinct letters are statistically different by the Tukey test at 5 % of probability. MSD: minimum significant difference; CV: coefficient of variation.

Table 4. Means<sup>1</sup> of plant phytotoxicity, plant height, growth reduction and fresh mass of *Senna obtusifolia* plants at 15 days after the application of *Phoma dimorpha* fermented broth.

Treatment	Phytotoxicity (%)	Plant height (cm)	Growth reduction (%)	Fresh mass (g)
Control (without broth)	0.00 ± 0.00 b	8.43 ± 0.76 a	0.00 ± 0.00 b	1.97 ± 0.45 a
With fermented broth	30.00 ± 4.08 a	6.93 ± 0.70 b	20.80 ± 2.07 a	1.20 ± 0.14 b
MSD	2.71	0.69	8.48	0.31
CV (%)	19.25	10.06	26.68	21.15

<sup>1</sup> Means with distinct letters are statistically different by the Tukey test at 5 % of probability. MSD: minimum significant difference; CV: coefficient of variation.

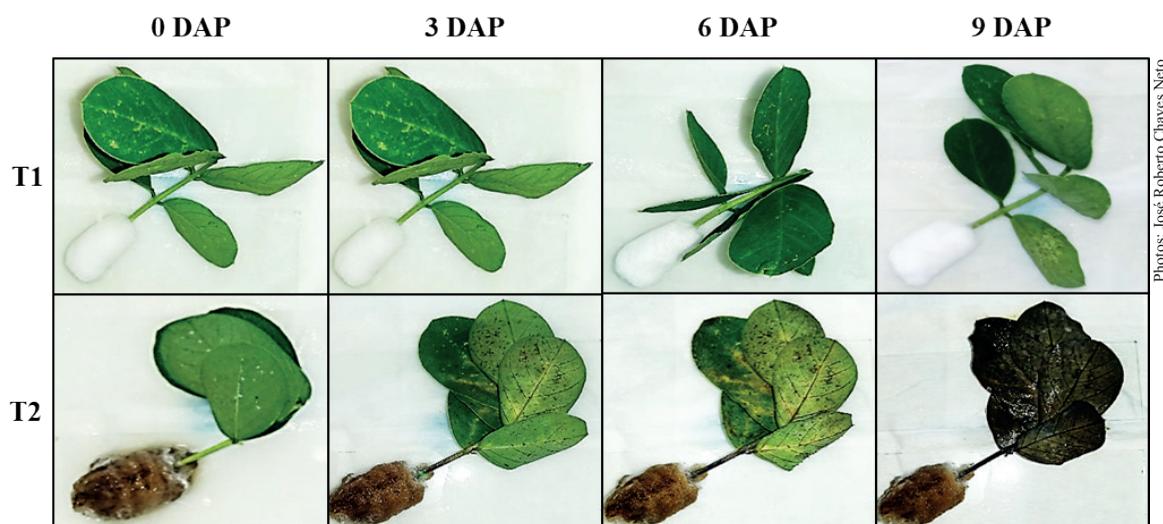


Figure 2. Phytotoxic effects on leaves of *Senna obtusifolia* after 3, 6 and 9 days after the application (DAP) of *Phoma dimorpha* fermented broth (T1: control treatment without the fermented broth; T2: treatment with the fermented broth).



Figure 3. Phytotoxic effect on *Senna obtusifolia* plants at 15 days after the application of *Phoma dimorpha* fermented broth (T1: control treatment without the fermented broth; T2: treatment with the fermented broth).

fungi may cause symptoms such as chlorosis, wilt, waterlogging and alteration in plant growth.

According to Bailey et al. (2011b), the macrocadin A and Z compounds produced by the *Phoma macrostoma* fungus have as their mode of action the inhibition of the carotenoid biosynthesis, inducing chlorosis, bleaching and eventual necrosis in susceptible plants. These authors observed that compounds applications produce symptoms of photodegradation in broadleaf weeds such as *Cirsium arvense*, *Taraxacum officinale* and *Stellaria media*.

Similar results were found by Cimmino et al. (2013), who evaluated the isolated potential of the chenopodoline metabolite produced by the *P. chenopodolica* fungus in leaves of *Cirsium arvense* and *Setaria viridis*. They reported symptoms such as necrosis, wilt and foliar tissue degradation. Todero et al. (2018a) reported that the application of fermented *Phoma* sp. in post-emergence of *C. sativus* plants caused yellowing at 7 days after the application, evolving up to the occurrence of wilting and necrotic lesions. In addition, these authors observed alterations in plant growth.

A significant difference ( $p < 0.05$ ) was observed for plant height with the application of the *P. dimorpha* fermented broth on *S. obtusifolia* plants, resulting in a lower height (Table 4). Similar results were observed by Klaic et al. (2017), who found a reduction of 30 % in the plant height of the bioindicator *C. sativus*, when submitted to the same fermented broth.

The influence of the *P. dimorpha* fermented broth on the plant height of *S. obtusifolia* was confirmed by the reduction in the growth percentage (Table 4). Hence, it was possible to conclude that the

application of this fermented broth causes a significant growth reduction in plants of *S. obtusifolia*. Similar results were found by Todero et al. (2018b), when evaluating formulations with secondary metabolites from submerged *Phoma* sp. with herbicidal action against the weed species *Bidens pilosa*, *Amaranthus retroflexus* and *Conyza canadensis*. These authors observed a reduction of 31 % in plant height. According to Gronwald et al. (2002), the weed growth reduction is a factor to determine the bioherbicide potential.

The fresh mass of *S. obtusifolia* plants was also reduced with the application of the *P. dimorpha* fermented broth (Table 4). This reduction, as a function of the fermented broth, was also observed by Brun et al. (2016) on *C. sativus* plants at 7 days after the application of *Phoma* sp. Similarly, Klaic et al. (2017), after optimizing the solid state fermentation process for the production of the bioherbicide through *Phoma* sp. secondary metabolites, also observed its phytotoxicity on *C. sativus* plants, with a reduction of 20 % in the fresh mass, in comparison to the control treatment.

## CONCLUSION

The application of *Phoma dimorpha* fermented broth provides a pre-emergence bioherbicidal action on *Senna obtusifolia*. This treatment inhibited the seed germination in 100 %. On detached leaves, it caused leaf necrosis and death at the ninth day after the application. In the post-emergence, the application caused leaf spots, as well as reduced the size and fresh mass of this weed. Thus, the *P. dimorpha* fermented broth has proved to be an alternative for the development of weed management

bioproducts, with a view of reducing the use of the synthetic herbicides and preventing pollution in pasture ecosystems.

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