



Research Paper

Bioherbicide action of aqueous extracts of *Sorghum* sp., *Dolichos* sp. and *Pachyrhizus* sp. in pre and post-emergence

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ABSTRACT

Plant extracts may contain substances with possible bioherbicidal effect. Determining the ideal method for preparing the extracts can optimize the results. This study aimed to evaluate the bioherbicide effect in weeds such as *Bidens pilosa*, *Euphorbia heterophylla* and *Digitaria insularis*, and extracts of *Dolichos lab lab*, *Pachyrhizus* sp. and *Sorghum bicolor* isolated and in a mixture, and submitted to different modes of preparation. In pre-emergence, it was evaluated the influence of aqueous extracts on germination and initial development of *B. pilosa*, *E. heterophylla* and *D. insularis*, in a completely randomized design, factorial 7x4+T, with four replicates. In post-emergence, the test was carried out in a completely randomized design with six treatments and six replicates. The bioherbicide effect varied in accordance with the preparation of the extract and the species evaluated. There was a reduction in the germination percentage with 80% for *B. pilosa* and 60% for *D. insularis*, besides the effects on the length of the hypocotyl with 44% for *E. heterophylla* and the length of the primary root. In post-emergence, *B. pilosa* showed increased accumulation of total dry mass, with phytotoxicity of up to 14 days after the application of the extracts. *D. lab lab*, *D. insularis* and *E. heterophylla* showed reduction in the accumulation of dry mass (30%). It can be concluded that the bioherbicide effect of extracts of *D. lab lab*, *Pachyrhizus* sp. and *S. bicolor* depends on the preparation of the extracts, the target species, and the period of application; however, the extracts can be explored on techniques for the production of compounds bioherbicide.

Barbosaja A^{1,2}, Canavessi H¹, Gibbert AM¹, Costa NV¹ and Stangarlin JR¹

¹State University of Western Paraná (UNIOESTE), Pernambuco Street, n. 1777, CEP 85960-000, Marechal Cândido Rondon, Paraná State, Brazil.

²Western Paraná Matology Study Group (GEMOP), Center of Agrarian Sciences, State University of Western Paraná (UNIOESTE), Marechal Cândido Rondon, Brazil.

*Corresponding author. E-mail: jaquelineabarbosa@hotmail.com. Tel: +55 45 9.9916-1143.

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INTRODUCTION

The weeds in the cultivation environment constitute a great threat to the good yield of cultivated plants. Although the chemicals are essential and have satisfactory results in the control of weed community, biological methods have become practices that are associated with management to reduce the environmental impacts caused by excessive and continuous use of herbicides (Farooq et al., 2017; Iqbal et al., 2019).

Allelopathy involves positive or negative effect of one plant on another plant released through the environment. *Sorghum bicolor* and *Dolichos lab lab* species

are endowed with biologically active compounds and used in techniques of green manure for the suppression of weed community in the agricultural environment (Fields et al., 2015; Chemma and Khaliq, 2000; Correa et al., 2005; Moreira et al., 2013). The biologically active compounds in plants can be exploited to produce bioherbicides (Carvalho et al., 2019).

Finding new plants with negative phytotoxic potential in weeds allows one to broaden the diversity of molecules with possible negative allelopathic effect and assist the handling of new substances with bioherbicide effect.

For that reason, we must consider that the extraction procedure influences the quantity and the phytotoxic effect of the substance (Scavo et al., 2019).

Although some reports suggest the presence of toxic compounds in plant organs of *Pachyrhizus* sp., until now, there is little evidence of a possible allelopathic effect of plant extracts of this plant species on weeds.

Therefore, this study aimed to evaluate the potential bioherbicide from the allelopathic effect of extracts of *S. bicolor*, *D. lab lab*, and *Pachyrhizus* sp., isolated and in a mixture, and submitted to different modes of preparation, under the conditions of pre and post-emergence of weeds *Bidens pilosa*, *Euphorbia heterophylla*, and *Digitaria insularis*.

MATERIALS AND METHODS

Leaves of *S. bicolor* and leaves and stems of *D. lab lab* and *Pachyrhizus* sp., were removed from the entire plant in the period prior to flowering. Part of the plant material was subjected to forced circulation oven at 65°C until constant mass, and part was subjected to dehydration in the Freeze Dryer (Liotop Model L101).

The preparation of the extracts was performed with the grinding in a blender industrial plant material dried or freeze-dried in distilled water, at a ratio of one gram of plant material for 20 ml of solvent. The solutions were kept at rest for 24 h on cooling (-10°C). After the rest period, the extracts to be subjected to high temperatures were kept in a water-bath (100°C ± 3) for 15 min, according to the methodology proposed by Arif et al. (2015).

The test was carried out in pre-emergence with all extracts, in a completely randomized design, and factorial 7x4+T, with four replications of 25 seeds. The first factor corresponded to the aqueous extracts (*D. lab lab*; *Pachyrhizus* sp.; *S. bicolor*; *D. lab lab* + *Pachyrhizus* sp.; *D. lab lab* + *S. bicolor*; *Pachyrhizus* sp. + *S. bicolor*; *D. lab lab* + *Pachyrhizus* sp. + *S. bicolor*). The second factor referred to preparation mode (freeze-dried plant material; freeze-dried plant material and submitted to the water-bath; dry plant material; dried plant material and submitted to the water-bath).

The seeds were conditioned in gerbox-type plastic boxes, with two sheets of paper towel previously sterilized in an autoclave (121°C ± 2°C/ 20 min) and imbibed with five milliliters of their treatment. The experiment was carried out in a germination chamber at 25 ± 3°C and photoperiod of 12 h light.

Daily monitoring of the number of germinated seeds from the day following sowing was carried out. It was considered the germinated seeds that presented emission of the primary root (Ferreira and Borghethi 2004, Lima and Moraes, 2008). For 14 days, the length of the primary root and hypocotyl of normal seedlings and 21 days to observe the stabilization of germination, gave up the ratings.

In the possession of the results, it was established the germination percentage, germination speed index - GSI (Maguire 1962), the average length of hypocotyl and primary root. The results were submitted to the Dunnett test at 1 and 5% of probability, using statistical software Genes (Cruz 2007).

For the test in post-emergence, five treatments were established considering the results obtained in the assessment in pre-emergence. The experiment was carried out in a completely randomized design with six treatments: *D. lab lab* + *Pachyrhizus* sp. + *S. bicolor*, prepared with dried plant material and subjected to a water-bath, *D. lab lab*, prepared with dried plant material, *Pachyrhizus* sp. + *S. bicolor* prepared with dried plant material, *D. lab lab* + *Pachyrhizus* sp., prepared with dried plant material and submitted the water-bath and *Pachyrhizus* sp. + *S. bicolor*, prepared with dried plant material and submitted the water-bath.

For each species (*B. pilosa*, *E. heterophylla*, *D. insularis*), were used six replicates, each one composed of a 2.5 L bottle containing oxisol and four plants. The sowing were performed in polyethylene trays and transplanted to pots when presented two true leaves. For 10 days after the transplant, was held the application of treatments with the aid of a backpack sprayer, pressurized CO₂, fitted with a single nozzle holder with tapered tip model (empty), with spray volume equivalent to 200 L ha⁻¹ using the pressure of 2.2 kgf cm⁻².

Visual assessment was performed at 7, 14 and 21 days after application (DAA), being assigned percentage notes of control, which ranged from 0% (lack of control) and 100% (plant death), according to the methodology proposed by the Brazilian Society of Weeds (SBPD 1995). At the end of 26 days, it was obtained the dry mass of plants, after the material will be subject to emissions of air circulation (65°C) until constant mass.

The results were submitted to analysis of variance and the averages were compared by the Tukey test at 1 and 5% of probability, using statistical software Sisvar (Ferreira 2011).

RESULTS AND DISCUSSION

The germination of the species *B. pilosa* was inhibited with the plant extracts. It was stressed the extract of *Pachyrhizus* sp + *S. bicolor* freeze-dried and subjected to a water-bath, which caused a reduction of 80% in the germination percentage in relation to witness, as can be observed in [Figure 1A](#).

For the species *E. heterophylla*, the plant extracts did not influence the percentage of germination in relation to witness ([Figure 1B](#)). However, for the species of *D. insularis*, the extracts of *S. bicolor* freeze-dried, *D. lab lab* + *Pachyrhizus* sp. dried and dried and submitted the water-bath, *Pachyrhizus* sp.+ *S. bicolor* dried, and the extract of *D.*

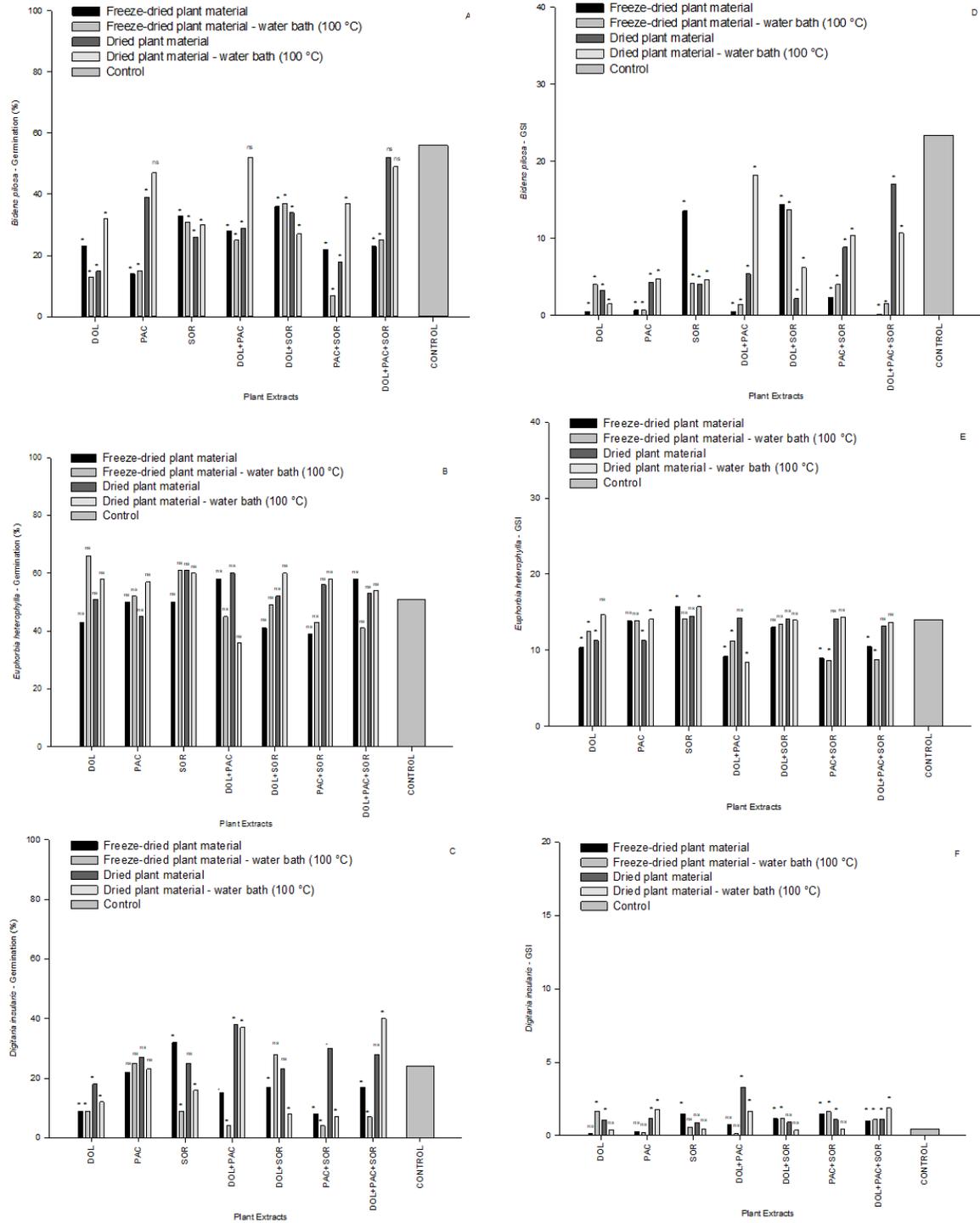


Figure 1: Percentage of germination and germination speed index of *B. pilosa*, *E. heterophylla* and *D. insularis* after application of extracts of green manures in pre-emergence.

lab lab + pachyrhizus sp.+ S. bicolor freeze-dried and subjected to a water-bath, stimulated the germination of the species up to 40% as compared with the control (Figure 1C).

It is interesting to observe that the same extracts that

provide the stimulus in germination percentage, to be changed its method of preparation, altered the allelopathic effect of stimulus for inhibiting the germination percentage. For example, for the extract of *D. lab lab + Pachyrhizus sp.* dried and submitted the water-bath stimulated

germination percentage of *D. insularis* up to 36% when used the plant material, and freeze-dried and subjected to a water-bath, it occurred the inhibition of germination of *D. insularis* in up to 60% as compared with the control.

The extracts can stimulate the germination percentage due to cellular changes that lead to an increase in the mitotic index and a significant increase in the frequency of mitotic cells with chromosomal alterations (Iganci et al., 2006; Carvalho et al., 2019). Santos et al. (2017), in verifying the allelopathic effects of the extracts of *Miconia albicans*, observed a significant increase in mitotic index of lettuce cells from the concentration of 25%, with the occurrence of chromosomal anomalies.

In relation to the germination speed index, the variable differed from the witness for all species. For the species of *B. pilosa*, all extracts, in addition to causing a reduction in the germination speed index, delayed the germination of the species. Gulzar and Siddiqui (2017) observed a delay in germination and low rate of germination of the species after treatment with aqueous extracts, and describe that this result is related to the fact that the extracts damaged the membrane system of seed.

However, for the species *E. heterophylla*, there was no difference in the percentage of germination in relation to witness, but there was a delay in the speed of germination. For the species *D. insularis*, there was a reduction in the percentage of germination, however, the extracts that differed from the control, increased the speed of germination index.

The results are in line with those of Silva et al. (2015) and Braine et al. (2011) who also observed changes in the index of germination speed, without effect on the total percentage of germination, and therefore the need to maintain daily evaluations since the very first day after sowing.

The influence of aqueous extracts in the initial periods of germination slows the time of germination of the species. The delay in beginning the process of germination of weeds favors the cultivation of interest, because it reduces weed competition and over time, you can change the seed bank of the cultivation environment (Silveira et al., 2012 Souza and Zvr, 2016)

It has been observed that for each plant species used in the preparation of aqueous extracts, there is a way of preparing, amending its action and protocols should be established when seeking a new compound in a plant material. Scavo et al. (2019) also observed that the extraction procedure improved the allelopathic activity of leaf allelochemicals of *Cynara cardunculus* var. *Altilis*.

Lessa et al. (2017), using the extract of *Plectranthus barbatus* subjected the infusion, observed a reduction in the germination percentage at 86% of seeds of *Amaranthus deflexus*. Already in the absence of infusion, the reduction was 66% as compared to control. The freeze-dried method allows you to dehydrate and maintain the characteristics of the original material and can be a practice to be adopted in

procedures of extraction, because the dehydration process occurs without the loss of the substances present in the material. According to Marques and Costa (2015), the freeze-dried process causes the loss of water, but keeps the properties and characteristics of the original product after rehydration.

In relation to the length of hypocotyl, for the species of *B. pilosa* and *D. insularis*, the extracts did not differ in relation to witness. But for the species of *E. heterophylla*, the allelopathic effect of inhibition in the hypocotyl length was observed and were used the extracts of *D. lab lab* dried, *D. lab lab* + *Pachyrhizus* sp. dried and subjected to a water-bath, *Pachyrhizus* sp. + *S. bicolor* freeze-dried and subjected to a water-bath, *D. lab lab* + *Pachyrhizus* sp. + *S. bicolor* freeze-dried and subjected the water-bath, which reached 44% inhibition of the growth of the hypocotyl (Figure 2A B and C).

The hypocotyl presents itself as a structure that is not in direct contact with the aqueous extract, which minimizes the effects of this component to treatment. Borella et al. (2009) also observed that the length of the hypocotyl becomes less influenced by aqueous extracts than the other variables.

The length of the primary root of species of *B. pilosa*, the extracts that caused inhibition and different from the witness, *D. lab lab* in all modes of preparation, and the extract of *Pachyrhizus* sp. + *S. bicolor* freeze-dried and subjected to a water-bath, reached 61% inhibition of primary root (Figure 2D).

For the species *E. heterophylla*, there was also a reduction in the length of the primary root. The extracts caused inhibition in the development up to 63% when used the extract of *Pachyrhizus* sp. + *S. bicolor* dried. However, for the weed plant species *B. Bilosa*, the allelopathic effect was stimulated, mainly by the extract of *S. bicolor* freeze-dried and subjected to a water-bath, with stimulus of 68% in the length of the primary root (Figure 2E). In *D. insularis*, the length of the primary root was stimulated in relation to witness (Figure 2F).

Without the proper development of the primary root at the beginning of the development, all subsequent growth becomes compromised, and to make this plant less aggressive in the environment of cultivation, favors the cultivation of interest (Souza and Zambar, 2016).

It was not observed predominance in plant extracts when all the variables were evaluated. Similarly, Carvalho et al., (2011) also used a mixture of extracts of green manures to verify the allelopathic effects on seeds of *L. sativa*. Thus predominance was not observed in the treatments that were mixtures or in the plant species used isolated.

In post-emergence, the extracts that differed from the control, allowed greater accumulation of dry mass of the species of *B. pilosa* at 26%, a result which occurred after the implementation of the extract of *D. lab lab* dried, as can be observed in Table 1.

For the species *E. heterophylla*, there were both stimulus

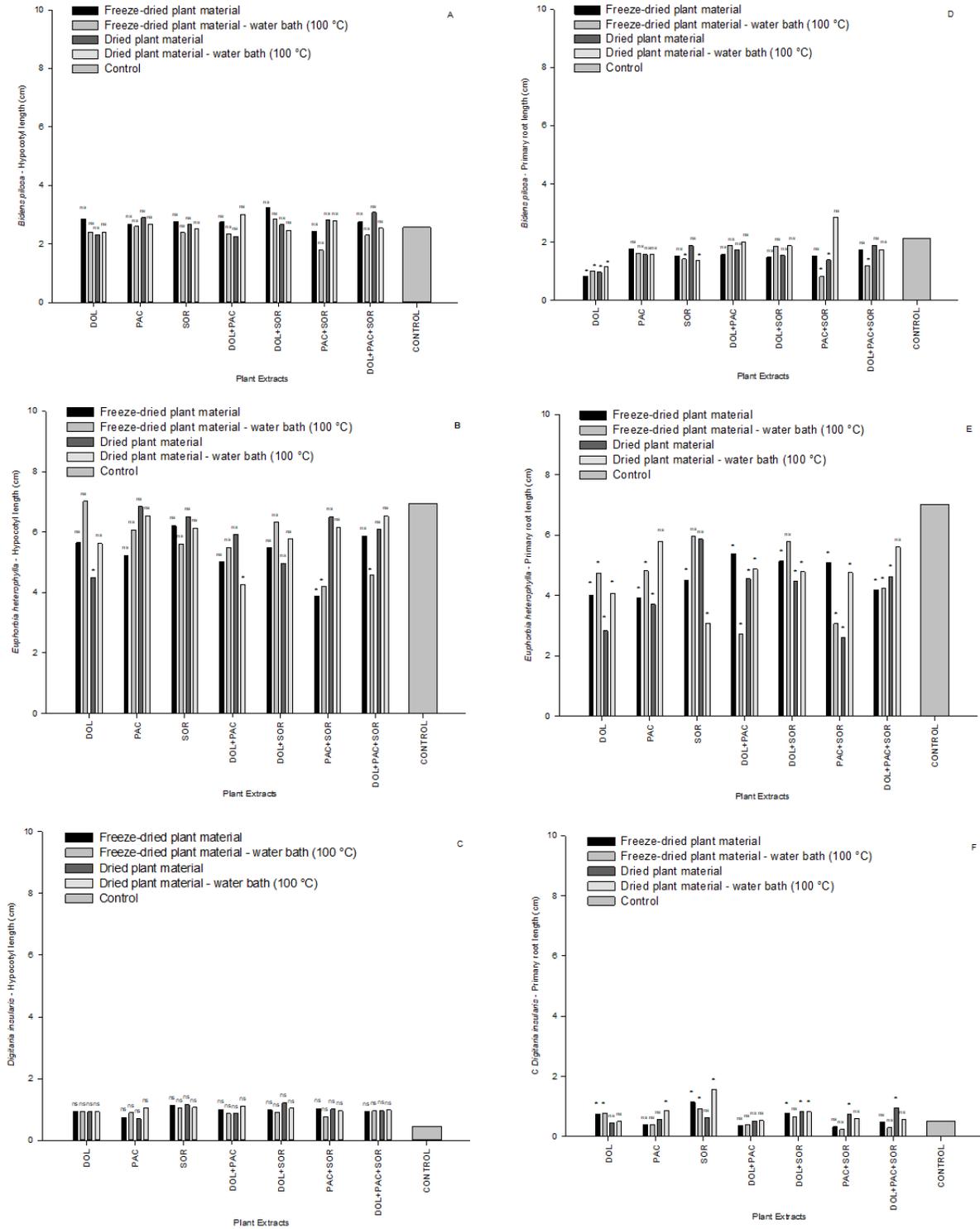


Figure 2: Length of hypocotyl and primary root of *B. pilosa*, *E. heterophylla* and *D. insularis*

and inhibition, as the accumulation of dry mass was higher at 17% when used the extract of *D. lab lab* + *Pachyrhizus* sp. Freeze-dried and subjected the water-bath. In using the extracts of *Pachyrhizus* sp. + *S. bicolor* freeze-

dried and subjected the water-bath and the extract of *D. lab lab* + *Pachyrhizus* sp. + *S. bicolor* freeze-dried and subjected to a water-bath, there was a reduction in the accumulation of total dry mass by 25 and 30%,

Table 1: Average values for total dry mass of *B. pilosa*, *E. heterophylla* and *D. insularis*, submitted to the aqueous extracts of green manure in different modes of prepared, isolated and in a mixture.

Treatments	<i>B. pilosa</i>	<i>E. heterophylla</i>	<i>D. insularis</i>
	MST (g)	MST (g)	MST (g)
Control	3.04 bc	1.95 b	1.80 c
<i>Pachyrhizus</i> sp. + <i>S. bicolor</i> -Freeze-dried	2.85 c	2.15 ab	2.32 a
<i>Pachyrhizus</i> sp. + <i>S. bicolor</i> - Freeze-dried - water bath	2.80 c	1.46 c	1.20 e
<i>D. lab lab</i> + <i>Pachyrhizus</i> sp. - Freeze-dried - water bath	2.93 c	2.35 a	1.37 de
<i>D. lab lab</i> + <i>Pachyrhizus</i> sp. + <i>S. bicolor</i> - Freeze-dried - water bath	3.68 ab	1.35 c	2.05 b
<i>Dolichos lab lab</i> - Dried	4.14 a	1.92 b	1.49 d
Means	3.24	1.86	1.71
CV (%)	11.94	7.93	7.65

Medium followed by the same letter in column do not differ by Tukey test at 1% and 5% of probability. * and ** significant at the level of 5 and 1% probability, respectively.

respectively.

For the species of *D. insularis*, the allelopathic effect of stimulation or inhibition in accordance with the extract used also occurred. The extracts *Pachyrhizus* sp. + *S. bicolor* freeze-dried and *D. lab lab* + *Pachyrhizus* sp. + *S. bicolor* freeze-dried and subjected to a water-bath, accumulated more dry matter of about 22 and 12%, respectively while the extracts *Pachyrhizus* sp. + *S. bicolor* freeze-dried and subjected to a water-bath, *D. lab lab* + *Pachyrhizus* sp. freeze-dried and subjected to a water-bath, *D. lab lab* dried, reduced the accumulation of dry matter by 33%, 24% and 17%, respectively.

The substances present in aqueous extracts can act as growth regulators, interfering in metabolic pathways, or in the production of chlorophyll, having effects on the uptake of luminous energy, and changing the pattern of growth, multiplication and maintenance of cells (Carmo et al., 2007; Steffen et al., 2010). Pires et al. (2001), in assessing the extract of *Leucaena leucocephala* in post-emergency, observed a reduction in growth and leaf deformation of *B. pilosa*.

Khaliq et al. (2012) evaluated the toxic action of aqueous extracts of wheat plants (*Triticum aestivum*) in morphological and biochemical attributes of horse purslane and observed a reduction of 67% in the dry mass of roots. Allelopathic effect of stimulus in the variables assessed in this study was not observed; however, the high concentrated extract used in this study may have influenced the results.

The extracts caused phytotoxicity symptoms only in the species *B. pilosa*, which are shown in Figure 3.

According to Braz et al. (2011), the highest levels of control occur when management is carried out in the early stages of development. They observed phytotoxic effect on the species *B. pilosa* only in the first evaluation, but this effect was absent for 28 days after the application. This is similar to the result of the present study, in which for the 21 days of evaluation, the phytotoxicity decreased for all aqueous extracts used.

Arif et al. (2015), by using the extracts of *Sorghum* sp., *Brassica* sp. and *Helianthus* sp. in the management of weeds in the field, observed a reduction of the population density of weeds up to 59%. Iqbal et al. (2019) also evaluated the effect of *Sorghum* in combination with brassica and/or with sunflowers in weed control, in a mixture of extracts, and obtained a reduction of 60% in total density of weeds, however, after 8 applications.

The substance sorgolene is present in plants of *S. bicolor*, and according to Dayan et al. (2009), it inhibits photosynthesis in young plants, which may explain the mixtures present in the species of sorghum, applied in post emergence and exhibited phytotoxicity in the first week.

The mixture of Sorghum with extracts of oleifera Lam resulted in an increase of 35% in the income of the population control of weeds as compared with the sorgaab alone (Kamran et al., 2016; Khan et al., 2012). Similarly, the combination of the extract of sunflower and sorghum sprayed at 6 L ha⁻¹ had the greatest negative impact on wild oat and canary grass in field tests of wheat (Jamil et al., 2009). Such benefits were also observed in other trials with combinations of extracts of sorghum, brassica and sunflower, where the dual applications of concentrated extracts of three species at 45 and 75 days after sowing provided good weed control and higher yield of wheat (Awan et al., 2012). When compared to the conventional treatments with herbicides (iodosulfuron more Mesosulfuron), doubles foliar sprays of sorghum, sunflower and *Brassica* extract combinations at 18 L ha⁻¹ provided economic alternatives to herbicides that have resulted in a reduction of 48 to 58% of weeds in wheat production system (Mahmood et al., 2015).

Khaliq et al. (2012) also observed phytotoxic effect after the application of aqueous extracts of wheat on the weed plant *Portulaca oleracea*. According to the researchers, the phytotoxicity observed can be attributed to the phytotoxin present in the aqueous extract or its effect due to the combination with other substances present in the plant material.

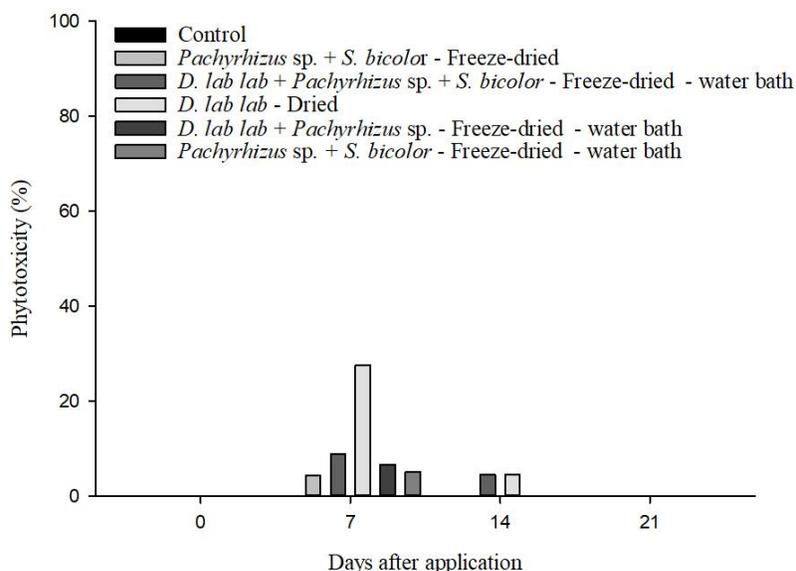


Figure 3: Phytotoxicity of plants of *Bidens pilosa* subjected to aqueous extracts.

It must be considered that the effect of plant extracts is not immediate, and only one application will not provide the effect of an herbicide chemical, hence the ecological and environmental advantages in using alternative methods.

CONCLUSIONS

The bioherbicide effect of aqueous extracts of *D. lab lab*, *Pachyrhizus* sp. and *S. bicolor* depends on the mode of preparation of aqueous extract of weed species targets and the time of application.

In *Bidens pilosa*, germination was inhibited by 80% and the length of the primary root was inhibited by 61% when the extract of *Pachyrhizus* sp + *S. bicolor* freeze-dried and subjected the water-bath was used. In post-emergence, the ethanolic extract of *D. lab lab* dried allowed greater accumulation of dry mass (26%); however the species presented phytotoxicity to the extract for up to 14 days after the application.

In *E. heterophylla*, there was no effect on germination percentage; however, the extracts delayed the beginning of the germination process, and reduced the length of the hypocotyl by 40% using the extracts of *D. lab lab* dried, in addition to reduction of 63% in the length of the primary root. There was a reduction in the accumulation of total dry mass by 25 and 30% when used the extracts of *Pachyrhizus* sp. + *S. bicolor* freeze-dried and subjected the water-bath and the extract of *D. lab lab* + *Pachyrhizus* sp. + *S. bicolor* freeze-dried and subjected the water-bath.

In *D. insularis*, the extract of *D. lab lab* + *Pachyrhizus* sp. freeze-dried and freeze-dried and subjected the water-bath inhibited up to 60% the percentage of germination. While the extracts *Pachyrhizus* sp. + *S.*

bicolor freeze-dried and subjected to a water-bath, *D. lab lab* + *Pachyrhizus* sp. freeze-dried and subjected to a water-bath, *D. lab lab* dried, reduced the accumulation of dry matter by 33, 24 and 17%, respectively.

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