

# Microwave-assisted extraction of phenolic compounds from *Canavalia ensiformis* leaves: preparation and evaluation of prospective bioherbicide on control of soybean weeds

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**Abstract**— Several chemical compounds are used for pest control in agriculture in order to increase productivity. It is scientific knowledge that such pesticides adversely affect human and animal health through contamination by these substances. Thus, the use of substances obtained from nature itself to combat pests and weeds is an alternative for the control of those and, consequently, prevent environmental contamination. This paper evaluated the aqueous extracts of jack-bean leaves (*Canavalia ensiformis*) as a prospective postemergent bioherbicide applied in the control of soybean weeds. The process of extraction of phenolic compounds was carried out in a digestion system by microwave, in vessels of 60 mL with approximately 2 g dry plant material and 30 mL of deionized water in pressurized system, followed by chromatographic injection. Optimal conditions for extraction were temperature of 100 °C, extraction time of 10 minutes and ramp of temperature of 5 minutes. Determinations were performed via HPLC-UV chromatography, based on peak ferulic acid compound in all assays. This study aimed to optimize a microwave-assisted extraction method and consequently evaluating these extracts as bioherbicide in soybean weeds.

**Index Terms**— bioherbicide, allelopathic effects, *Canavalia ensiformis*, weeds.

## I. INTRODUCTION

Organic farming involves a set of practices aimed at the production of environmentally friendly waste, avoiding the use of synthetic fertilizers and pesticides [1]. It has its emphasis on production for quality, sustainable practices and positive impacts on resource conservation, biodiversity and animal welfare [2].

According to EMBRAPA [3], there are 37 cataloged herbicides for the control in pre- and post-emergence of soybean weed, with toxicological classes varying from I to IV (extremely toxic to slightly toxic, respectively), besides many of these herbicides need to be prepared with adjuvants to increase their effectiveness, which in some cases can increase its deleterious effect.

Pesticides are one of the most representative groups of pollutants in the environment due to its heavy use in agriculture. The Brazilian Health Surveillance Agency (ANVISA) [4] shows out that Brazil is the largest consumer and producer of pesticides since 2009. Pesticides used in

agriculture to combat pests and diseases can cause contamination of water resources whose consequences are changes in the ecosystem and health damage [5].

The application of herbicides is usually a strategy to combat infestation by weeds, however, the continuous and intensive use, as in the case of Brazil, raises concern about the sustainability of soil fertility. The interaction of herbicides with various types of soil not only changes the effectiveness of herbicide against growth weed, but can also affect the cycle of various nutrients, resulting in terms of variation in the activity of soil enzymes [6]. When they reach the soil, such compounds are subjected to physical, chemical and biological processes that will influence their activity and persistence in environment. Some of these molecules can reach the soil because of the low volatilization and/or photodegradation. Other factors that favor its decomposition are the action of microorganisms in soil, humidity and high temperatures. If not absorbed by plants, the herbicides can be strongly adsorbed to organic matter in colloidal fraction of soil or be leached to groundwater [7].

The highest regard when using herbicides is sensitivity and danger to non-target species and organisms in the application area. Inadequate applications can cause damage to other species, thus affecting the quality of water, causing the accumulation of these compounds [8].

Phenolic compounds are secondary metabolites commonly found in many plants [9]. Among these compounds we can mention phenols, phenolic acids, coumarins, tannins and flavonoids that possess antioxidant activity, strongly related to the prevention of cancer, inflammatory disorders and cardiovascular diseases [10, 11, 12]. Among them, phenolic acids that can be found in both plants and soils, present allelopathic attributes [13, 14]. The mixture of phenolic acids increases synergistically inhibitory action [15].

Jack-bean (*Canavalia ensiformis*) is a fast-growing vegetable used as green cover, as well as consortium culture; it is a rustic plant tolerant to a wide pH range, as well as other environmental adversities. This species is able to associate with mycorrhizal fungi (beneficial fungi to the soil) and nitrogen-fixing bacteria, thus, ameliorate the soil, improving its fertility. It is native from South American and grows in the tropics and sub tropics, produced in large areas especially in low altitude regions, high temperatures and relative humidity [16, 17].

Previous work has confirmed the presence of allelopathic phenolic compounds in leaves, roots and seeds of *Canavalia ensiformis* and inhibitory effect on broad leaf weed

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development [18, 19].

Microwave-assisted extraction (MAE) is a new extraction technique that can offer high reproducibility in less time, simplified handling, minimal sample manipulation, reduced solvent consumption and low energy used without reducing the yield of target compounds [20, 21]. In the bioherbicide preparation proposed here just water was used as solvent in the extraction, not generating toxic waste and thus avoiding contamination.

There is a debate about the adverse effects caused by herbicides. Thus, the production of possibly less toxic formulations (leaf aqueous extracts of *Canavalia ensiformis*) would be an environmentally friendly alternative to replacing synthetic compounds already used, to be a natural compound and possibly less toxic.

The objective of this study was to evaluate the allelopathic potential of the extracts obtained from *Canavalia ensiformis* leaves as a prospective bioherbicide postemergent applied on *Emilia sonchifolia* and *Sida spinosa* weeds in conventional soybeans cultivation after successive applications.

### II. EXPERIMENT

#### 2.1. Allelopathy assay in greenhouse

Soybean cultivar BRS-283, *Emilia sonchifolia* and *Sida spinosa* weeds were grown in plastic plots (10 liters) filled with silt-loam soil (58.7% clay, 29% silt and 11% sand) in greenhouse at a field of Campus 2 of University of São Paulo. Temperatures ranging from 25 to 30°C during the experiment. All assays were repeated three times. The jack-bean seeds were cultivated under no-tillage management, collected in area next to highway Luiz Augusto de Oliveira in the city of São Carlos, located at latitude 22°0'41.60"S, longitude 47°59'30.7"W.

#### 2.2. Microwave-assisted extraction

A Microwave Digestion System (Speedwave Four®, with built-in, non-contact, temperature and pressure measurement, BERGHOF, Germany) with capacity of 12 vessels of 60 mL and 1450 W maximum power was used. One hundred grams of the so dried and powdered jack-bean leaves, split into sub-samples of 2 g each were mixed with 30 mL of distilled water and submitted to microwave extraction. The optimization study was performed by a 23 factorial design. The extraction temperature ranged from 60 to 100 °C, the tested irradiation were from 10 to 40 min, using a power of 870 W (60%). The factorial design was randomly performed comprising 16 experiments in duplicate plus 7 additional experiments, also in duplicate.

Then, the vessels were cooled until room temperature before opening. Following, the extracts were put together and filtered through a 45 µm filter paper. An aliquot of the collected extract was further determined by high performance liquid chromatography. Another aliquot was concentrated under vacuum at 70 °C until obtaining a viscous liquid. The concentrated extract was weighed and a defined volume of water was added to prepare solutions with final concentrations of 100, 125, 150 and 200 g L<sup>-1</sup>. The concentrations of 100, 125, 150 and 200 g L<sup>-1</sup> with three replicates of each treatment were carried out on weeds, however, only the concentration of 200 g L<sup>-1</sup> led to phytotoxicity symptoms. Figure (1) summarizes the steps for obtaining the prospective bioherbicide.

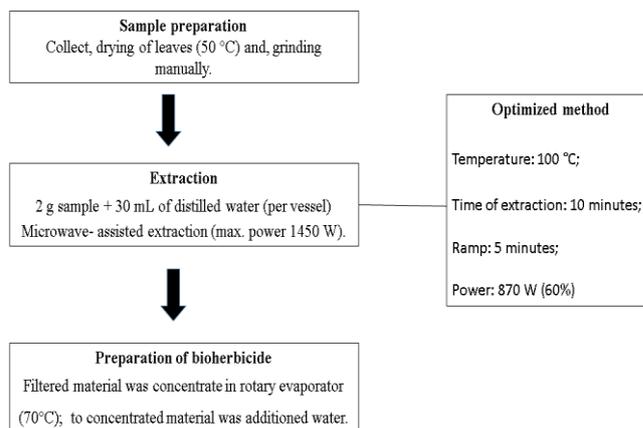


Fig. (1): Steps for obtaining the extracts containing the prospective bioherbicide

#### 2.3. Application of the extracts

Evaluations concerning injuries were performed in 'Days After Application' (DAA) of the prospective bioherbicide on the weeds counted from the first application.

The experimental unit consisted of vases of 10 L capacity cultivated with ten soybean seeds and same number of weed seeds, distributed in 5 triplicates and another vase used as reference. Thus, 16 vases were cultivated with soybean and *Emilia sonchifolia* and plus 16 vases with soybean and *Sida spinosa* weed. It is noteworthy that emerged eight soybean and 5-6 weed in each vase.

The extracts containing the prospective bioherbicides were applied in post-emergence (after germination of the seeds), i.e., after the onset 2 to 3 leaves of the weeds, which occurred after 31 days of planting (time 0).

The bioherbicide treatments were applied with the aid of a device that sprinkles the liquid like a spray, in order to reach the entire foliar surface of the plant. The total amount applied per plant was 3000 µL. The extracts were stored at 4 °C between application intervals.

The extracts were applied in a pre-defined time intervals (0, 15, 30, 45 and 60 days) for *Emilia sonchifolia* and (0, 15, 30 and 45 days) to *Sida spinosa*. The first assessment of phytotoxicity was held on the 15th, just before the second application of the extract. The second assessment of phytotoxicity was made on the 30th day, immediately before the third application. And so on, according to Table (1).

**Table (1):** Experimental design of application and evaluation of visual effects of the extract containing the prospective bioherbicide on weeds.

Time intervals	Applications*	<i>Emilia sonchifolia</i>	<i>Sida spinosa</i>
0	1 <sup>st</sup>		
15	1 <sup>st</sup> , <b>2<sup>nd</sup></b>	15th (1 <sup>st</sup> evaluation)	15th (1 <sup>st</sup> evaluation)
30	1 <sup>st</sup> , <b>2<sup>nd</sup></b> , <b>3<sup>th</sup></b>	30th (2 <sup>nd</sup> evaluation)	30th (2 <sup>nd</sup> evaluation)
45	1 <sup>st</sup> , <b>2<sup>nd</sup></b> , <b>3<sup>th</sup></b> , <b>4<sup>th</sup></b>	45th (3 <sup>th</sup> evaluation)	45th (3 <sup>th</sup> evaluation)
60	1 <sup>st</sup> , <b>2<sup>nd</sup></b> , <b>3<sup>th</sup></b> , <b>4<sup>th</sup></b> , <b>5<sup>th</sup></b>	60th (4 <sup>th</sup> evaluation)	60th (4 <sup>th</sup> evaluation)
75		75th (5 <sup>th</sup> evaluation)	

\*the bold indicates cummulative applications.

Apparent phytotoxicity evaluations of the bioherbicide so obtained were conducted in accordance with the scale of the Brazilian Society of Weed Science [22] in *Emilia sonchifolia* and *Sida spinosa* weeds, as well in soybeans, considering the visual changes compared to the control (no extracts application) as well as measurement of plant throughout the experiment.

#### 2.4. Chromatographic determinations by HPLC

An aliquot of 1 mL of the collected extract, obtained after microwave-assisted extraction, was diluted with 6 mL of methanol and determined by high-performance liquid chromatography. A HPLC system (Prominence LC-10AT, Shimadzu) equipped with detector UV/Vis (SPD-Prominence 20A), degasser (DGU-20A5 Prominence) and a reverse phase column (150 mm x 4.6 mm x 5 µm) Agilent XDB-C18 was used for separation of the compounds in isocratic mode. The mobile phase was 2% glacial acetic acid in water + methanol (75:25 in volume), and the LabSolutions software (Shimadzu) controlled and processed the data. The injection volume was 20 µL and all compounds were observed at 282 nm. Stock solutions of the compounds rutin, ferulic acid, chlorogenic acid and naringenin were prepared at various concentrations and determined via HPLC-UV. The extracts were determined by comparison to the retention times of the peak samples with standards. The ferulic acid was the phenolic compound highlighted in the optimization of methodology of extraction and in the chromatographic determinations.

### III. RESULTS AND DISCUSSION

#### 3.1. MAE: optimized methodology

The evaluated factors were extraction temperature ( $X_1$ ), irradiation time ( $X_2$ ), and ramp ( $X_3$ ). The P-values are used as a tool to check the significance of each parameter between the variables, i.e., the smaller the value of P, the more significant is the corresponding coefficient [23]. According to Table 2, the most significant factors in process were the temperature ( $X_1$ ) and the factor interactions between temperature and time of extraction ( $X_1X_2$ ). Thus, temperature of 100°C, extraction time of 10 minutes, and ramp time of the 5 minutes were optimal conditions defined.

Table (2) shows the analysis of variance of the experimental results with  $R^2 = 0.9992$  ( $R^2_{adj} = 0.9987$ ).

**Table (2):** Analysis of variance (ANOVA) for the experimental results.

Source	Sum of square	DF	Mean of square	F-value	P-value
Model	5.99E9	3	1.99E9	15.71661	1.85819E-4
$X_1$	3.31E9	1	3.31E9	11.01541	0.00507
$X_2$	6.93E8	1	6.93E8	1.42072	0.25310
$X_3$	1.49E9	1	1.49E9	3.45468	0.08422
$X_1X_2$	1.99E9	1	1.99E9	15.65401	0.00191
$X_1X_3$	4.99E6	1	4.99E6	0.02207	0.88436
$X_2X_3$	2.62E7	1	2.62E7	0.05926	0.81177
Error	1.52E9	12	1.27E8		
Total	7.52E9	15			

$X_1$  = temperature,  $X_2$  = time and  $X_3$  = ramp (time required for the equipment to reach the set extraction temperature). D.F (degree of freedom).

#### 3.2. Application of the extracts on soybean and weeds

Extracts of leaves from jack-bean were applied in conventional soybean plants (cultivar BRS-283) and in the *Emilia sonchifolia* and *Sida spinosa* weeds. All treatments were compared to reference vase (no extract application) along the evaluation steps.

The visual assessment of the prospective bioherbicide effects were carried out until 75 days after first application according conceptual scale of SBCPD [22] and weed control according to Latin American Weed Association [24]. In the soybean plants, the foliar application of extracts containing the prospective bioherbicide were enough to cause visible changes in approximately 24 hours after application (Figure 2).



**Fig. (2):** Phytotoxicity symptoms on the soybean plants 24 h after the application of the extract

The symptoms of phytotoxicity observed were the rapid change of color of leaves that became yellowish with some brown spots extending in the entire area of the leaf, which later resulted in the loss of them. However, it is noteworthy that the stem remains, which leads to the recovery and development of the plant.

#### 3.3. Evaluation of phytotoxicity of bioherbicide and control on the weeds

The weeds and conventional soybean seeds were cultivated together. The following tables and figures present the toxic effects and the control of the bioherbicide on *Emilia sonchifolia* and on *Sida spinosa* weeds.

**Table (3):** Results of visual evaluations of treatments at a concentration of 200 g L<sup>-1</sup> of the extract containing the prospective bioherbicide on the *Emilia sonchifolia* and on *Sida spinosa* weeds, after a single dose of 3.0 mL in 15, 30, 45, 60 and 75 days after germination.

Weeds	Phytotoxicity				
	Evaluation period (DAA)				
	15	30	45	60	75
<i>Sida spinosa</i>	a	d	d	e	N.E
<i>Emilia sonchifolia</i>	c	d	d	d	e

(a) No injury. (c) Moderate injury. (d) Severe injury. (e) Death. N.E (not evaluated).

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For treatments relating to concentrations of 100, 125 and 150 g L<sup>-1</sup> of the extracted material, up to 15 days, no injuries were observed. Deleterious effects began after application of 200 g L<sup>-1</sup>. Under this concentration, symptoms appeared after 24 hours: dark spots in various parts of the leaves of *Emilia sonchifolia* (Figure 3) and leaves with a slightly yellowish followed by a slight necrosis at the edges. For the *Sida spinosa* weed, the same symptoms appear in the 16th day, thus this symptoms indicates that the bioherbicide was working. Thus, the treatment at 200 g L<sup>-1</sup> concentration was chosen for further applications by presenting a detrimental effect within a few days after application.

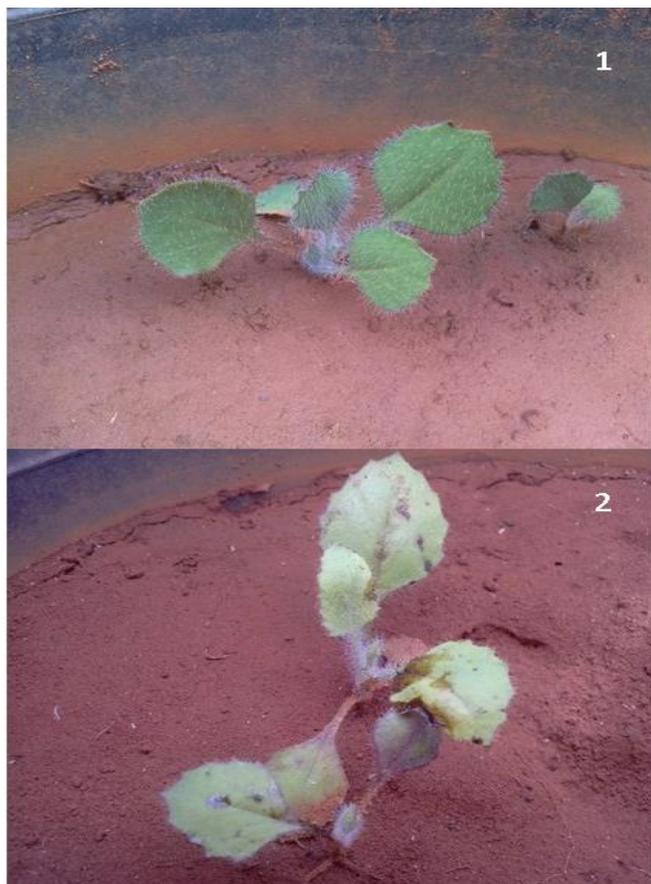


Fig. (3): Bioherbicide evaluation stages on *Emilia sonchifolia* weed. (1) Without application of bioherbicide; (2) 24 hours after application.

Table (4) shows the effects of bioherbicide on the growth of *Emilia sonchifolia* weed.

**Table (4):** Effects of bioherbicide on the growth of *Emilia sonchifolia* weed.

Evaluation period (DAA)	Weed height (cm)*	
	Bioherbicide	Control
15	5.28 ± 0.24 <b>a</b>	6.04 ± 0.28 <b>b</b>
30	12.26 ± 0.43 <b>a</b>	14.30 ± 0.33 <b>b</b>
45	13.22 ± 0.45 <b>a</b>	16.40 ± 0.68 <b>b</b>
60	14.08 ± 0.24 <b>a</b>	17.12 ± 0.50 <b>b</b>
75	14.30 ± 0.33 <b>a</b>	18.76 ± 0.46 <b>b</b>

\*(n=5). Means sharing the same letter in a line do not differ significantly by Tukey's test (P<0.05).

The weed submitted to bioherbicide contact reached a maximum height of approximately 14 cm. However, weeds used with control reached about 18 cm, showed a better growing.

Table (5) shows the concepts of percentage control of *Emilia sonchifolia* weed treated with the prospective bioherbicide

**Table (5):** Concepts of percentage control of *Emilia sonchifolia* weed treated with a single dose of 3.0 mL of *Canavalia ensiformis* extract at a concentration of 200 g L<sup>-1</sup>.

Evaluation period (DAA)	Concept*
15	Sufficient
30	Good
45	Good
60	Good
75	Very good

\*Scaling concepts of percentage used to assess the efficacy of weed control, according to ALAM (1974).

By applying 3.0 mL of the extract at 200 g L<sup>-1</sup> concentration, as mentioned earlier, the detrimental symptoms on the weed have been observed after 24 h, beginning with necrosis around the edges of the leaves followed by yellowing. In the 5th day after application, the plant exhibited brown spots and greater discoloration of the leaves. From the second evaluation (30th day), the symptoms of intoxication maintained a regularity until the 60th day, which we classified the weed control as good, according scaling of concepts of ALAM (1974). In the 60th day, a new application of the extracts was realized and a new evaluation done in the 75th day (last evaluation), showing plants totally wilt. The Figure 4 shows some of stages of evaluation of bioherbicide on *Sida spinosa* weed.

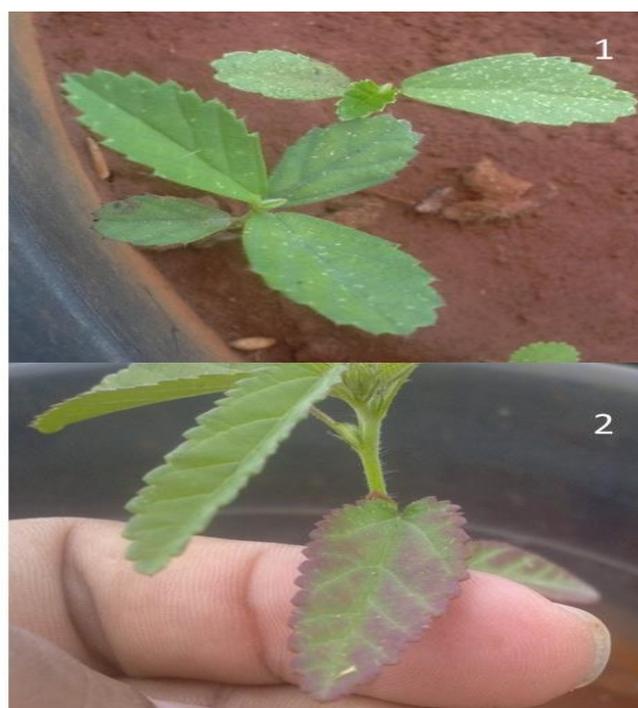


Fig. (4): Bioherbicide evaluation stages in *Sida spinosa* weed. (1) Without application of bioherbicide; (2) 16 days after application.

Assessing the injury caused after extract application, visible symptoms appear in the 16th day, so in the following step of evaluation (30th day), the injuries observed increased significantly. From the third step of evaluation (45th day) the symptoms of intoxication maintained a regularity, i.e., characteristics as necrosis of the tissue, color of leaves and plant size not shown significant differences until the 60th day (last evaluation). The evaluation to the 60th day was the step where the total wilting and death of the plants were reached, defining the end of experiment.

Table (6) shows the effects of bioherbicide on the growth of *Sida spinosa* weed.

**Table (6):** Effects of bioherbicide on the growth of *Sida spinosa* weed.

Evaluation period (DAA)	Weed height (cm)*	
	Bioherbicide	Control
15	1.88 ± 0.21 <b>a</b>	2.48 ± 0.37 <b>b</b>
30	4.38 ± 0.25 <b>a</b>	6.68 ± 0.39 <b>b</b>
45	7.18 ± 0.14 <b>a</b>	15.54 ± 0.37 <b>b</b>
60	10.10 ± 0.55 <b>a</b>	21.56 ± 0.82 <b>b</b>

\*(n=5). Means sharing the same letter in a line do not differ significantly by Tukey's test (P<0.05).

Statistically, the measurements of the control weeds were different from the weeds under application of bioherbicide. *Sida spinosa* weed reached a size of approximately 21 cm, with twice the size of those submitted to the application of the bioherbicide. Table (7) shows the concepts of percentage control of *Emilia sonchifolia* weed treated with the prospective bioherbicide.

**Table (7):** Concepts of percentage control of *Sida spinosa* weed treated with a single dose of 3.0 mL of *Canavalia ensiformis* extract at a concentration of 200 g L<sup>-1</sup>.

Evaluation period (DAA)	Concept*
15	None
30	Sufficient
45	Very Good
60	Very Good

\*Scaling concepts of percentage used to assess the efficacy of weed control, according to ALAM (1974).

Until the evaluation regarding the 15th day, the extracts did not provide any injury visible, and the first symptom of damage to the plants was observed one day later, after second application of the extracts. Based in the injuries caused and signs of bad development on the weed, presents in the 30th day, consider the action of extracts sufficient statements regarding the control of weed. In the days following evaluation 45 to 60th day, the concept of control was classified as very good, consider the inhibiting of development of weed and especially the phytotoxicity symptoms in the weed that already were in a stage well advanced. Therefore, the bioherbicide tested showed an effective control considered very good.

The bioherbicide was not selective to the soybean crop. The treatment under 100 g L<sup>-1</sup> concentration caused injury against conventional soybean, causing visible changes after 24 h of contact with the herbicide, however, 7 to 8 days later the plant is able to recover and continue to develop.

Due to these results, the extract containing the prospective bioherbicide was not applied directly to soybeans, just over the weed to be inhibited. The application followed a similar procedure to that used with application of commercial post-emergence herbicides. Such herbicides are applied with a jet directed to the weeds.

*Canavalia ensiformis*, due of its robustness, rapid growth and capacity to promote population growth of nitrogen-fixing bacteria, among other properties, stands out as a plant of interest for recovery and conservation of soils. The use of jack-bean leaves for the production of bioherbicide keeps the plant under constant development, contributing to the maintenance of the organic matter cycle.

#### IV. CONCLUSION

Microwave-assisted extraction was suitable for extracting phenolic compounds present in the leaves of *Canavalia ensiformis*, being the process realized in an extraction time of 10 minutes and 100°C.

Compounds extracted from jack-bean leaves have herbicide capacity against *Emilia sonchifolia* and *Sida spinosa* weeds. The application of these extracts after the emergence of weeds ensures control of these in about 15 days to the *Emilia sonchifolia* and 30 days to the *Sida spinosa* as mentioned. The herbicide effect is fast acting. The treatment in the concentration of 200 g L<sup>-1</sup> was sufficient to cause visible and severe injuries within 1 and 16 days on the *Emilia sonchifolia* and *Sida spinosa* weeds, respectively, when the first symptoms appear.

The application of the bioherbicide requires monitoring of any weeds, so that the application can be made immediately in emergency stage of the seeds and the inhibitory effect of the extracts can be effective. For being characterized as a postemergence bioherbicide, in large cultivated areas, chemical control should be done by means of directed jet which makes it wearily job and consequently disadvantageous. Besides, significantly more, very well designed research is required to access the bioherbicide potential of the extracts from *Canavalia ensiformis* leaves: regulatory, production, formulation, economics and other issues will challenge the development and commercialization of a *Canavalia* bioherbicide.

However, *Canavalia ensiformis* cultivation contributes to the maintenance of soil fertility, what must be taken into account for a full analysis of costs and benefits of agricultural production.

This work complements the allelopathic studies of phenolic compounds present in *Canavalia ensiformis*.

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## REFERENCES

- [1] Leifeld, J. (2012). How sustainable is organic farming? *Agriculture, Ecosystems and Environment*, 150, 121-122. doi:10.1016/j.agee.2012.01.020.
- [2] Dinis, I.; Ortolani, L.; Bocci, R.; Brites, C. (2015). Organic agriculture values and practices in Portugal and Italy. *Agricultural System*, 136, 39-45. <http://dx.doi.org/10.1016/j.agsy.2015.01.007>
- [3] EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. (2011). *Tecnologias de produção da soja - região central do Brasil 2012 e 2013*, Londrina, Embrapa Soja.
- [4] ANVISA – Agência Nacional de Vigilância Sanitária. <http://portal.anvisa.gov.br/wps/portal/anvisa/home>. 20 Out. 2013
- [5] Dellamatrice, P. M.; Monteiro, R. T. R. (2014). Principais aspectos da poluição de rios brasileiros por pesticidas. *Revista Brasileira de Engenharia Agrícola e Ambiental*. 18 (12),1296-1301.
- [6] Singh, A.; Ghoshal, N. (2013). Impact of herbicides and various soil amendments on soil enzymes activities in a tropical rainfed agroecosystem. *European Journal of Soil Biology*, 54, 56-62. doi: 10.1016/j.ejsobi.2012.10.003
- [7] Moura, M. A. M.; Franco, D. A.; Matallo, M. B. (2008). Impactos de herbicidas sobre os recursos hídricos. *Revista Tecnologia & Inovação Agropecuária*, 142-151.
- [8] Callihan, B.; Smith, L.; McCaffrey, J.; Michalson, E. (1995). *Yellow starthistle management for small acreages*. University of Idaho, Cooperative Extension System. Agricultural Experiment Station, CIS 1025, p.8.
- [9] Bernal, J.; Mendiola, J.A.; Ibáñez, E.; Cifuentes, A. (2011) Advanced analysis of nutraceuticals. *Journal of Pharmaceutical and Biomedical Analysis*, 55, 758-774. doi:10.1016/j.jpba.2010.11.033
- [10] Cirale, S.; Lucas, L. & Keast, R. (2010). Biological activities of phenolics compounds present in virgin olive oil. *Internacional Journal of Molecular Science*, 11, 458-479. doi:10.3390/ijms11020458
- [11] Omar, S. H. (2010). Oleuropein in olive and its pharmacological effects. *Scientia Pharmaceutica*, 78, 133-154. doi:10.3797/scipharm.0912-18
- [12] Servili, M.; Esposito, S.; Fabiani, R.; Urbani, S.; Taticchi, A.; Mariucci, F.; et al. (2009). Phenolics compounds in olive oil: Antioxidant, health and organoleptic activities according to their chemical structure. *Inflammopharmacology*, 17, 76-84. doi: 10.1007/s10787-008-8014-y
- [13] Inderjit, I. (1996). Plant phenolics in Allelopathy. *The Botanical Review*, 62 (2), 186-202.
- [14] Inderjit, I.; Nishimura, H. (1999). Plant phenolics and terpenoid: transformation, degradation, and potential of allelopathic interactions. In: INDERJIT, I.; DAKSHINI, K. M. M.; FOY, C. L. (Ed.). *Principles and practices in plant ecology: allelochemical interactions*. Boca Raton: CRC Press, 255-266.
- [15] Einhellig, F. A. (1999). An integrated view of allelochemicals amid multiple stresses. In: INDERJIT, I.; DAKSHINI, K. M. M.; FOY, C. L. (Ed.). *Principles and practices in plant ecology: allelochemical interactions*. Boca Raton: CRC Press, 479-494.
- [16] Molina, M. R.; Argueta, C. E.; Bressani, R. (1974). Extraction of nitrogenous constituents from the Jack-bean (*Canavalia ensiformis*). *Journal of Agricultural and Food Chemistry*, 22, 309-312.
- [17] Andrade, S. A. L.; Gratão, P. L.; Azevedo, R. A.; Silveira, A. P. D.; Schiavinato, M. A.; Mazzafera, P. (2010). Biochemical and physiological changes in Jack-bean under mycorrhizal symbiosis growing in soil with increasing Cu concentrations. *Environmental and Experimental Botany*, 68, 198-207. doi:10.1016/j.envexpbot.2009.11.009
- [18] Santos, S.; Moraes, M. L. L.; Rezende, M. O. O. (2007). "Allelopathic potential and systematic evaluation of secondary compounds in extracts from roots of *Canavalia ensiformis* by capillary electrophoresis". *Eclética Química (Araraquara)*, 32, 13-18. <http://dx.doi.org/10.1590/S0100-46702007000400002>
- [19] Mendes, I. S.; REZENDE, M. O. O. (2014). Assessment of the allelopathic effect of leaf and seed extracts of as postemergent bioherbicides: A green alternative for sustainable agriculture. *Journal of Environmental Science and Health. Part B. Pesticides, Food Contaminants, and Agricultural Wastes*, 49, 374-380. doi: 10.1080/03601234.2014.882179.
- [20] Li, J.; Zu, Y. G.; Fu, Y. J.; Yang, Y. C.; Li, Z. N.; Wink, M. (2010). Optimization of microwave- assisted extraction of triterpene saponins from defatted residue of yellow horn (*Xanthoceras sorbifolia* Bunge) kernel and evaluation of it antioxidant activity. *Innovative Food Science Emerging technologies*, 11, 637-643. doi: 10.1016/j.ifset.2010.06.004
- [21] Silva, D. F.; Azevedo, E. B.; Rezende, M. O. O (2016). Optimization of microwave-assisted extraction of a bioherbicide from *Canavalia ensiformis* leaves. *American Journal of Environmental Sciences*, 12 (1), 27-32. doi: 10.3844/ajessp.2016.27.32.
- [22] SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS- SBPCD. *Procedimento para instalação e análise de experimentos com herbicidas*. Londrina: 1995, 42 p.
- [23] Song, J.; Li, D.; Liu, C.; Zhang, Y. (2011). Optimized microwave-assisted extraction of total phenolics (TP) from *Ipomoea batatas* leaves and its antioxidant activity. *Innovative Food Science and Emerging Technologies*, 12, 282-287. doi:10.1016/j.ifset.2011.03.001
- [24] ASOCIACIÓN LATINOAMERICANA DE MALEZAS (ALAM). (1974). *Recomendaciones sobre unificación de los sistema de evaluación em ensayos de control de malezas*, ALAM, 1 (1), 35-38.