

## Allelopathy of *Crotalaria juncea* L. aqueous extracts on germination and initial development of maize

*La alelopatía de extractos acuosos de Crotalaria juncea L. sobre la germinación de semillas y el crecimiento inicial del maíz*

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### ABSTRACT

The objective of this study was to evaluate the allelopathic potential of sunn hemp extracts on germination and initial development of maize. The trials were carried out in the laboratory and greenhouse with four concentrations (0; 7.5; 15 and 30%) of aqueous extracts obtained by grinding and leaching. In a germination chamber, more concentrated extracts (15% and 30%) inhibited maize germination, while shoot length (SL) was stimulated at 7.5% concentration. Ground extract stimulated root length (RL) at all tested concentrations. Root number (RN) was inhibited at higher concentrations of leached extract and stimulated for seeds exposed to sunn hemp ground extract. In the greenhouse, the longest root length (LRL) of maize was inhibited at the highest concentration (30%) of both extracts. The ground extract inhibited the LS in all concentrations and the 7.5% concentration stimulated the RN. It was concluded that sunn hemp extracts showed allelopathic effect on maize development, which varied according to how the extracts were obtained and concentrations used.

**Key words:** cover crop, secondary compounds, sunn hemp, *Zea mays* L.

### RESUMEN

*El objetivo de la investigación fue evaluar el potencial alelopático de los extractos de crotalaria sobre la germinación y el crecimiento inicial de maíz. Los ensayos se realizaron en el laboratorio y en el invernadero con cuatro concentraciones (0; 7,5; 15 y 30%) para los extractos acuosos obtenidos por molienda y lixiviación. En cámara de la germinación se observó que los extractos más concentrados (15 y 30%) inhibió la germinación de maíz y que la longitud de la parte aérea (LPA) se estimuló a una concentración de 7,5%. Para el extracto de molienda hubo estimulación en longitud de la raíz (LR) en todas las concentraciones ensayadas. El número de raíces (NR) se inhibió a concentraciones más altas de extracto de lixiviado y estimuló para las semillas expuestas a extracto machacado de crotalaria. En invernadero, la longitud de la raíz más larga (LRL) de maíz se inhibió a la mayor concentración (30%) de los extractos de ambas maneras obtenidos. El extracto machacado inhibió los LPA en todas las concentraciones y la concentración de 7,5% estimuló la NR. Se concluyó que los extractos de crotalaria mostraron efecto alelopático en el desarrollo de maíz, que variaba de acuerdo a cómo se obtuvieron los extractos y concentraciones utilizadas.*

**Palabras clave:** cultivo de la cobertura, compuestos secundario, crotalaria, *Zea mays* L.

### Introduction

Allelopathy is defined as the positive or negative interference of an individual, plant or microorganism on another, whose effect is mediated by compounds derived from secondary metabolism, named allelochemicals (Rizvi & Rizvi, 1992). Allelochemicals are released into the environment

and exert interference on growth and/or development of other plants directly and indirectly, through the transformation of these substances in soil by the activity of microorganisms (Ferreira & Aquila, 2000).

Plants produce and store a large number of secondary metabolites in their several parts (leaves, stems, roots, seeds), which are then released to the environment by leaching of organic and inorganic

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compounds by the action of rain, dew, volatilization, root exudation and plant residue decomposition (Almeida, 1991). According to Almeida (1991); even after the plant is dead the allelopathic substances still remain active in tissues and are released into soil. When they reach the required concentration, they can affect the development of microorganisms and plants found in soil. Some metabolites act only in the presence of others in synergism, because when isolated they do not reach the minimum required concentration to exert allelopathic effect (Almeida, 1988).

Many factors determine the toxicity of allelochemicals, such as environmental conditions, concentration, plant age and physiological state. Allelochemical production varies in quality and quantity with age, cultivar, plant organ and season (Singh *et al.*, 2003). According to Einhellig and Leather (1988), allelochemicals are also influenced by temperature, light intensity, nutrient availability, rhizosphere microbial activity and soil composition in which the roots are located.

The largest cropped area in Brazil uses no-tillage system, which aids weed management by allelopathy since one of the basic requirements for the system is the presence of mulch in the form of straw. The mulch acts as an insulating layer between the atmosphere and soil. It changes the temperature and soil moisture conditions, as well as decreasing their range variation. It also releases allelochemicals that inhibit seed germination and seedling development of certain species, depending on the mulch (Almeida, 1991), while it protects the soil surface, recycles nutrients and fixes nitrogen (Teixeira *et al.*, 2004).

The species most used as cover crops are Poaceae (grasses) and Fabaceae (legumes). Grasses produce residues with greater straw permanence and are used when the goal is to keep the soil covered. However, leguminous residues show higher quality and the best covering results. They are used to add residues to the soil (Menezes & Leandro, 2004).

The allelopathic potential of a cover crop depends on the amount of plant residue and weeds present in the soil (Tokura & Nóbrega, 2006). It also depends on the kinds of substances released into soil and the plants that grow there. This makes the choice of plant material to be used extremely important, since with similar amounts there are different responses among cover crops to the same plant species (Silva, 2012).

Based on the benefits that cover crops can add to a no-tillage system for plants of economic interest, some studies have investigated the development of crops such as *Avena sativa* and *Z. mays* (Schneider & Cruz-Silva, 2012); *Phaseolus vulgaris* (Carvalho *et al.*, 2012) and *Glycine max* (Mauli *et al.*, 2011), developed under mulch or extracts of these cover crops as well as *Z. mays* (Rosa *et al.*, 2011). According to Tokura & Nóbrega (2006), research concerning the allelopathic potential of crops has allowed reducing the costs of agricultural yield as well as the decreasing the environmental impact caused by the uncontrolled and increasing application of pesticides.

*Crotalaria juncea* L. (sunn hemp) is a shrub of the Fabaceae adapted to tropical conditions. It has erect and determinate growth, fast initial development and may reach up to 3.5 m height. In agriculture it is usually used as green manure in sugar cane soil recovery and as an alternative to crop rotation with grain crops (Moraes *et al.*, 2006). It is a well known botanical species because it provides a large amount of organic matter to soil and fixes nitrogen. However, its allelopathic effects are little known by farmers. This study aimed at evaluating, in laboratory and greenhouse, the allelopathic effect of sunn hemp aqueous extracts on seed germination and initial development of maize.

## Material and Methods

The trials were carried out in the Biochemistry, Botany and Plant Physiology laboratories as well as in greenhouses of Paranaense University (UNIPAR), Cascavel Campus, Paraná State. Fresh leaves of sunn hemp were collected in a private farm in the Capitão Leonidas Marques-PR municipality and used as donor plant material. The seeds were used as receivers for Agroeste 1570 hybrid maize, donated by the Cooperativa Agroindustrial (COOPAVEL) and treated with fungicide in order to reduce pathogen contamination.

In order to obtain the ground aqueous extract, fresh leaves were weighed, washed, chopped into pieces of about 1 cm<sup>2</sup> and then ground for three minutes. The extracts were prepared with 30 g fresh leaves per 100 ml distilled water. Extracts were filtered to produce 30% aqueous extract (crude extract). Dilutions (7.5% and 15%) were prepared up from the crude extract; distilled water was used as the control treatment.

The procedure and concentrations used to obtain the leachate aqueous extract were similar to those described above. The difference was that the plant material (fresh leaves) was soaked in water for a 48-hour period. All pH concentrations were recorded with a digital pHmeter for analysis of possible interference. Optimum pH values have been considered as 6 to 7 (Raven *et al.*, 2007).

Four experiment sets were carried out: two in laboratory (in a germination chamber) and two in greenhouse. For the laboratory trials 9 cm-diameter Petri dishes were used, with two filter paper sheets and 25 maize seeds per replication. To each dish 15 mL sunn hemp aqueous extract (by grinding or leaching) or distilled water was added.

Petri dishes were kept in a germination chamber at constant temperature ( $25 \pm 2$  °C), in dark for seven days. The germination chamber, counters and other materials were previously disinfected with 70% alcohol; Petri dishes and filter paper were autoclaved at 120 °C for 20 minutes.

All trials were composed of four treatments (0%, 7.5%, 15%, 30%) with four replications. The number of germinated seeds was counted daily to calculate the germination speed index (GSI). The final evaluation after seven days considered the variables germination percentage, root number, shoot and primary root length (cm).

Plastic containers with a 2.5 L capacity were used for greenhouse trials. They were filled with humus acquired in agricultural shops, in which ten maize seeds were sown. After sowing, the first watering was with 250 mL sunn hemp extracts (ground or leachate), while the second application of extracts occurred nine days after the first. The substrate moisture was maintained by watering when necessary. Five germinated seedlings per replicate were manually transplanted, with 20 seedlings per treatment. At 30 days the shoot and root length, numbers of leaves and roots were evaluated.

The experimental design was completely randomized. Statistical analyses were performed using JMP statistical software (Statistical System Analytis SAS Institute Inc. USA, 1989-200 4.0.0.). The data were submitted to analysis of variance (ANOVA) and treatment means comparisons used the Tukey test at 5% probability. Germination percentage data were transformed into arcsine  $\sqrt{x/100}$ .

## Results and Discussion

The pH of aqueous extracts of sunn hemp varied from 6.0 to 7.5, which probably did not affect maize development. According to Laynez-Garsaball & Méndez-Natera (2006) when pH values show little variation between control and extract concentrations, they presumably have no effect on the variables evaluated.

Maize seed germination showed significant differences for seeds submitted to extracts obtained both by grinding and by leaching sunn hemp leaves. The 15 and 30% concentrations inhibited maize germination compared to the control treatment, demonstrating the allelopathic effect (Table 1). Other studies also confirm the allelopathic effect of sunn hemp extracts. According to Teixeira *et al.* (2004), the shoot aqueous extract of this species also reduced *Lactuca sativa* L. and *Bidens pilosa* L. germination, with a lower percentage when exposed to extract compared to the control treatment. Araújo *et al.* (2010) found that *Ipomoea grandifolia* (Dammer) O'Donell was more sensitive than *B. pilosa* L. and *Euphorbia heterophylla* to the alkaloid extract effect of sunn hemp; increased extract concentration significantly delayed germination. Also, Adler & Chase (2007) reported that a 10% extract of sunn hemp fresh leaves inhibited *Lycopersicon esculentum* Mill. germination three and seven days after treatment, as well as *Amaranthus lividus* L. after three days; after seven, ten and fourteen days it also inhibited *Capsicum annuum* L germination.

According to Maraschin-Silva & Aquila (2006), allelopathic effects can vary in intensity; the allelochemical action is associated with several factors such as concentration, temperature and other environmental conditions. This behavior was observed in this study for the highest concentrations of both extracts, which inhibited maize germination.

The germination speed index (GSI) showed no significant differences for sunn hemp extract obtained by grinding. GSI reduction occurred with leached extract on maize seeds (66.66%) at the highest concentration (30%). A similar result was observed by Teixeira *et al.* (2004), who found that leguminous extracts including sunn hemp reduced the GSI for lettuce. According to Lima *et al.* (2007), 1:5 sunn hemp extract reduced the GSI of *B. pilosa*, meaning that the vigor was affected and that GSI reduction promoted an increase in the day number for germination. Fontanetti *et al.* (2007) pointed out

Table 1. Allelopathic effect of ground and leached aqueous extract of *Crotalaria juncea* L. fresh leaves on germination (G), germination speed index (GSI), shoot length (SL), root length (RL) and adventitious root number (ARN) of maize seedlings in germination chamber.

Extract (%)	G (%)	GSI (days)	SL (cm)	RL (cm)	ARN
Ground aqueous extract					
0	57 a	3.6 a	4.8 b	1.2 d	2.0 b
7.5	49 ab	3.5 a	6.7 a	3.0 b	3.7 a
15	25 b	3.3 a	3.9 b	4.4 a	3.7 a
30	37 b	3.0 a	3.8 b	2.3 c	4.1 a
Leached aqueous extract					
0	51 a	3.0 a	5.2 b	1.7 a	3.5 a
7.5	48 a	2.33 ab	6.5 a	1.8 a	3.9 a
15	28 b	1.6 ab	4.8 b	1.4 a	2.4 b
30	17 b	1.0 b	3.6 b	1.5 a	2.5 b

Different letters in columns indicate significant differences by the Tukey test at 5% probability.

that the treatment that was applied to sunn hemp in coverage decreased the emergence rate index of *Cyperus rotundus*.

Significant differences were observed in maize shoot length in the seedlings submitted to ground extract as well as leachate of sunn hemp leaf extract (Table 1). There was an allelopathic effect for this variable; a 7.5% extract concentration stimulated maize seedling development by 40 and 25%, respectively. For maize root length with ground extract, a 15% concentration provided longer seedling root length (4.4 cm); this was the extract concentration used that produced seedlings with the longest roots, statistically differing from other concentrations.

The root length of maize seedlings was not affected by the treatment with the leachate extract, while adventitious root number was significantly reduced with leachate extract at 15 and 30% concentrations (Table 1). Similarly, Carvalho *et al.* (2012), with another species of the same genus, did not observe any significant difference using 5% leached extract of *Crotalaria anagyroides*. However, when the concentration increased to 10%, the extracts of sunn hemp isolated or associated with other species (*C. juncea* + *Avena stringosa*, *C. juncea* + *Pennisetum glaucum*) affected the root system of *Phaseolus vulgaris* L. negatively, reducing its length.

Fontanétti *et al.* (2007) reported that allelochemicals may focus on different parts of plants such as roots, stems, fruits and leaves, thus the plant part used with allelopathic effect can define its purpose. Sunn hemp shoots with flowers

were used in this trial preparation; there appear to be allelochemicals in these parts, since sunn hemp interfered with maize germination and growth.

In greenhouse experiments, 30 days after emergence the root length of maize seedlings was inhibited by a 30% concentration of sunn hemp ground extract. There was a significant difference compared to seedlings with concentrations of 7.5 and 15%. In the leachate extract, the longest root length was also inhibited at 30% concentration compared to 7.5% concentration (Table 2). Araújo *et al.* (2011) tested sunn hemp aqueous extract in different spacings and densities. The authors reported that maize root length was negatively influenced by a density of 40 plants m<sup>-1</sup>, but it was more evident with 50 plants m<sup>-1</sup>. At low density (25 plants m<sup>-1</sup>), root length hardly differed from the control treatment. According to the authors, this was probably due to the fact that the metabolite concentration produced by sunn hemp was not enough to cause interference. These data corroborate those observed in this trial at the highest concentration of the studied extracts (30%), which induced the least root growth of maize.

Maize shoot length was inhibited at all concentrations of ground extract in relation to the control treatment, with 32% growth reduction at both 7.5 and 15% concentrations and 47.5% at 30% concentration (Table 2). Leachate extract did not influence the shoot development of maize seedlings, which may be related to the way extracts were obtained and their biochemical characteristics. Resende *et al.* (2003) highlighted that sunn hemp presence as green manure showed a negative allelopathic effect on *Saccharum officinarum* L.

Table 2. Allelopathic effect of ground and leached aqueous extract of *Crotalaria juncea* L. fresh leaves on the longest root length (LRL), shoot length (SL), leaf number (LN) and root number (NR) of maize seedlings, 30 days after emergence in a greenhouse.

Extract (%)	LRL (cm)	SL (cm)	LN	RN
Ground aqueous extract				
0	26.2 ab	8.2 a	4.9 a	7.0 a
7.5	28.6 a	5.5 b	5.1 a	8.7 b
15	26.9 a	5.5 b	5.2 a	8.3 ab
30	19.9 b	4.3 b	5.2 a	8.0 ab
Leached aqueous extract				
0	28.1 ab	5.77 a	5.15 a	7.25 a
7.5	31.7 a	6.10 a	4.90 a	7.25 a
15	29.8 ab	4.92 a	4.90 a	6.45 a
30	24.0 b	4.77 a	4.90 a	7.35 a

Different letters in columns indicate significant differences by the Tukey test at 5% probability.

growth. The density increase of sunn hemp resulted in a progressive reduction of weed biomass.

The extracts tested in greenhouse showed no effect for some variables, and depending on the tested concentrations, influenced others. Similar to the observed data in this trial, Tokura & Nobrega (2005) tested the allelopathic potential of cover crop aqueous extracts (*Triticum aestivum*, *A. stringosa*, *P. glaucum*, *Raphanus sativus* L. and *Brassica napus*) and observed that the extract presence did not affect maize germination; however, it affected root and shoot growth and seedling dry weight.

There was no influence on leaf number with both extracts, and root number with leachate extract (Table 2). According to Carvalho *et al.* (2012), typically the seedling shoot is less responsive to the allelochemical action than the root system, which is more sensitive to its action and is directly in contact with the extract. However, root number was influenced by a 7.5% concentration of ground extract, which stimulated their development in relation to the control treatment. Araújo *et al.* (2011) found that *P. vulgaris* L. showed the highest susceptibility to sunn hemp extract compared to maize. According to Silva (2012), all plants produce secondary metabolites that vary in quality and quantity from species to species. The resistance or tolerance to allelochemicals is more or less specific, but some species are more sensitive than others (Ferreira & Aquila, 2000).

According to Almeida's (1988) point of view, leachates contain organic and inorganic substances which are either toxic, harmless or stimulants. The activities of these products on soil are usually temporary, since they are subject to degradation

and adsorption inactivation and conversion by microorganisms. This might explain why the allelopathic effect was not observed in most variables in the leachate extract tested in the greenhouse.

Maize seedlings were influenced by the two methods of sunn hemp aqueous extract in the evaluated concentrations. It is emphasized that the results obtained in the germination chamber were more significant and the extracts influenced all variables in at least one way. Both extracts inhibited germination at the highest concentrations (15% and 30%) and stimulated shoot length at 7.5% concentration. The leachate extract inhibited the GSI at 30% concentration. The root length was stimulated by all concentrations of ground extract, while root number was positively influenced by ground extract and negatively by leachate extract. In the greenhouse, the ground extract showed more significant effect than the leachate extract. It also reduced the shoot length at all concentrations and stimulated root number at 7.5% concentration.

It is noteworthy that, apart from the different form of seedlings grown in a greenhouse, there are factors that can influence their development such as temperature, action of microorganisms in soil, insects and light that are controlled in germination chamber.

## Conclusion

The sunn hemp extracts showed allelopathic effect on maize development, which varied according to how the extracts were obtained and concentrations used.

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