https://doi.org/10.26651/allelo.j/2024-62-1-1482 0971-4693/94 Euro 20.00
Allelopathy Journal 62 (1): 15-30 (May, 2024) *International Allelopathy Foundation* 2024
Table: 11, Figs: 5

Role of allelopathic potential of *Crotalaria* spp. L. to control *Panicum maximum* Jacq. weed

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(Received in revised form: April 7, 2024)

ABSTRACT

This study aimed to evaluate the allelopathic potential of *Crotalaria* spp. to control guinea grass (*Panicum maximum* Jacq.). Experiments were done on germination and development of *P. maximum* in Petri-plate Lab Bioassays with the seed exudates of germinating seeds germination water of seed and leaf extract of *C. juncea* and also in Pot Culture coexistence by sowing and transplantation of two test species. Among the species studied, *C. juncea* gave the best result to inhibit the germination of target plant, hence, it was selected for further studies. The germination results showed that the highest densities (5, 10 and 20) of *C. juncea* affect different stages of *P. maximum*. The higher the concentration of bioextract from *C. juncea*, the lower was development of *P. maximum* was decreased with doses from 25 to 100 % for its control. The greater was the density and concentration of *C. juncea* extracts, the greater was the decrease in germination speed, germination (%), root length, plant height and dry mass weight of *P. maximum*.

Keywords: Allelopathic potential, Crotalaria juncea, germination, guinea grass, herbicide, *Panicum maximum*, Phytochemical, seedling growth, weeds.

INTRODUCTION

Weed control in agricultural areas is an essential to obtain the desired productivity in qualitative and quantitative parameters (12,16) because the weeds compete with crops for light, nutrients and water (4). Understanding the interactions between cultivated plants and weeds is essential to develop cost-effective solutions and sustainable weed management practices (20).

The Guinea grass (*Panicum maximum* Jacq. Poaceae family) (Figure 1 C and D), is an important weed at different stages of sugarcane crops (9), thus justifying the need for its control. The *P. maximum* has great economic importance as a forage for livestock due to its high productivity, nutritional value and adaptability. It has characteristics of being highly adaptable to different soil and climate conditions and growing well in any type of soil, when found in annual crops of interest, it becomes a weed that interferes with production (12).

Brazilian farmers use synthetic herbicides to control weeds, but it is costly and causes environmental problems, besides altering the local flora and leading to herbicidal resistance in weeds (6). Resistance to herbicides has become a major problem. It restricts the application of certain types of herbicides in some crops (11), hence, there is an increasing demand for alternative weed control methods to synthetic herbicides. Among such methods, the use of plants with allelopathic potential stands out. Allelopathy is the ability to stimulate or inhibit the development of plants around the main crop (18). They are mainly used as

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natural herbicides (7) and can also be used to identify active molecules for the development of new synthetic herbicides (1,9).

Crotalaria juncea (Figure 1 A) is most frequently used as a green manure due to its biological nitrogen fixation, providing dry mass for soil cover and to control nematodes (8). Its ability to fix nitrogen in the soil, resistance to pests and diseases stand out. Its used as a cover crop to protect the soil erosion and improve its fertility. Its allelopathic capacity suppress inhibition of the development of plants in its surroundings, showing the alternate control of weeds and identify the new herbicidal molecules.

Figure 1. Population of (A) Sunn hemp (*Crotalaria juncea*) in the flowering phase and (B) Guinea grass plants.

This study aimed to evaluate the allelopathic potential of *Crotalaria* spp. to control Guinea grass (*Panicum maximum* Jacq.), testing the following hypotheses: (i) *Crotalaria juncea* L. has a greater allelopathic potential than *C. breviflora* DC., *C. ochreleuca* G. Don and *C. spectabilis* Roth; (ii) Higher the seed rate of *C. juncea*, greater the inhibitory effects on germination of Guinea grass; (iii) Greater the density of *Crotalaria juncea* L., the greater the suppression of the Guinea grass (*Panicum maximum*) when sown and (iv) Concentration of leaf aqueous extract of *C. juncea* decreased the germination and development of Guinea grass (*Panicum maximum*) depending on the concentration.

MATERIAL AND METHODS

The experiments were conducted between February and August 2021 in Experimental Area, Laboratory of Weed Plants, Department of Biology, the State University "Júlio de Mesquita Filho", Jaboticabal campus, São Paulo, Brazil, zone 22 K, longitude 780326.01 m E, latitude 7648405.18 m S, mean height above sea level 666 m. The region's climate, according to Alvarez (2014), is Cwa type, subtropical, dry in winter, with summer rains, with an average annual temperature of 22.7 °C and average rainfall of 1353 mm.

1. PETRI-PLATE BIOASSAY

1.1. SEED EXUDATES:

Experiment 1. Effects of seeds exudate from different species of *Crotalaria* L. on germination of *P. maximum*

To obtain the germination exudate of crotalaria species, 25 seeds each of *C. juncea* L., *C. breviflora* DC., *C. ochreleuca* G. Don and *C. spectabilis* Roth were sown separately in Petri dishes (d = 9 cm and h = 2 cm) previously sterilized with 92.8 % hydrated alcohol containing germination paper hydrated with deionized water, plus the control (without the presence of seeds) and four replications for each treatment. The plates were moistened daily with deionized water and kept in a germination chamber at a constant temperature of 25 °C and a 12-h photoperiod.

After 7-days, the germinated seeds of *C. juncea* L., *C. breviflora*, *C. ochreleuca* and *C. spectabilis* were removed from the plates and discarded, keeping the liquid (exudate) from the Petri dishes. Then, 25 seeds of *Panicum maximum* Jacq. were sown in all plates containing the exudate from the germination (Table 1) of the 4-donor Crotalaria species and to control plates. The plates were placed in a germination chamber with the same conditions as previously in a completely randomized design.

Table 1. Values of pH, osmolality, soluble solids content (°Brix) and conductivity of shoots extracts of *Crotalaria juncea*.

Characteristic	**Concentration (%)**					
	0	**12.5**	**25**	**50**	**75**	**100**
pH	7.2	6.95	6.97	7	7.04	7.06
Osmolality (mmol/kg)	234	244	247	257	264	268
Soluble solids content (°Brix)	0	0	0	0.5	1	1
Conductivity (mS/cm)	0.19	0.62	0.99	1.78	2.41	3

Germination Speed Index: The numbers of seeds germinated on 2, 3, 4 and 7 days were counted and their germination speed index (GSI), was calculated with following formula (17):

$$\mathbf{GSI = (7*X_2+4*X_3+3*X_4+2*X_7)/7)}$$

Where, X: Number of seeds that germinated on the second, third, fourth and seventh day, respectively.

Experiment 2. Effects of seeds exudate of *C. juncea* on the seedling development of *P. maximum.*

To study the development of the radicle and the hypocotyl of *P. maximum*, the most inhibitory species of experiment 1 was selected, following the same protocol for obtaining the germination exudate.

Different densities of seeds of *Crotalaria juncea* L. were used in Petri dishes (Dia: 9 cm and Ht: 2 cm) previously sterilized with hydrated alcohol at 92.8 % and containing germination paper hydrated with deionized water. The densities were 1, 2, 5, 10, 15 and 20 seeds per plate, plus the control, with four replications for each treatment. The plates were moistened daily with 10 mL of deionized water and placed in a germination (Table 2) chamber with a constant temperature of 25 °C and a photoperiod of 12 h in a completely randomized design.

Table 2. Values of pH, osmolality and soluble solids content (°Brix) of the exudates from the seed germination of *Crotalaria juncea*.

Characteristic	**Seed density**						
	0	**1**	**2**	**5**	**10**	**15**	**20**
pH	7.16	7.96	7.94	7.86	8.34	8.23	8.36
Osmolality (mmol/kg)	233	247	240	241	249	250	250
Soluble solids content (°Brix)	0	0	0	0	0.1	0.1	0.3
Conductivity (mS/cm)	0.19	0.37	0.64	0.79	1.57	3.14	3.03

After discarding the seeds of *C. juncea* L., two seedlings of *Panicum maximum* Jacq. previously measured in terms of radicle and stem length were placed in the liquid exuded by the seeds of *C. juncea* L. on the plates. The seedlings remained in contact with the exudate for seven days in a germination chamber at a constant temperature of 25 °C and a photoperiod of 12 h and then new measurements of radicle and stem length were performed.

Experiment 3. Effects of seeds exudate of *C. juncea* L. on pre- and post-emergence of *P. maximum* Jacq.

To obtain the exudate from germination, 30 Petri dishes (Dia: 9 cm and Ht: 2 cm) were prepared by sterilizing them with 92.8 % hydrated alcohol and lining them with germination paper. The 30 Petri dishes were divided into seven treatments, where *C. juncea* was sown at densities of 1, 2, 5, 10, 15 and 20 seeds on plates, plus the control, all with five replications. The plates remained for seven days in the germination chamber set at 25 °C and a 12-h photoperiod, where they were daily moistened with 10 mL of deionized water. After this period, the seeds were discarded and the exudate from the germination of each density was submitted for pH determination (Quimis/Q-400BI), osmolality (Wescor, mod. 5500), soluble solids content (°Brix, RZ) and conductivity (Analion/C708-Plus).

The test was subdivided into pre- and post-emergence liquid application of *P. maximum* Jacq. For the pre-emergence stage, sterilized Petri dishes with a diameter of 3.5 cm contained hydrated alcohol 92.8 % and were lined with germination paper. The treatments were the volumes applied in 1 ml and 2 ml for each density (0, 1, 2, 5, 10, 15 and 20), with four replications, totaling 28 plates for each volume and 56 Petri plates in total, arranged in a completely randomized design. Then, all 56 plates were kept in germination chamber at 25 °C and a photoperiod of 12 h.

Germination Speed Index: It was calculated as per (17).

For the post-emergence test, 300-mL plastic pots containing a mixture of soil, sand and manure at the proportion (1:1:1) were used. Then, two seedlings of *P. maximum* Jacq. were transplanted 35 days after germination into each plastic cup. As a treatment, the application of 2 mL of exudate from the germination of different densities of *C. juncea* L.

(1, 2, 5, 10, 15 and 20), plus the control (2 mL of deionized water), was performed with five replications using a previously calibrated manual sprayer. The day after transplanting, the application was carried out. The temperature was 21°C, the relative humidity was 57 % and the wind speed was 14 km h^{-1}. After applying the exudate, the seedlings remained in plastic pots for 21 days. Three assessments were made (7, 14 and 21 days after application) as for the number of tillers, number of leaves and plant length. With these data, the absolute growth rates were calculated (1).

1.2. LEAF AQUEOUS EXTRACT

(i). Experiment 4. Effects of leaf aqueous extracts of *C. juncea* L. on *P. maximum* Jacq.

Plants of *C. juncea* L. were grown in pots with a capacity of 3 L filled with a mixture of soil, tanned manure and sand at a 2:1:1 (v/v/v) ratio. They were kept in an open area and moistened daily. When the plants were in full vegetative stage (33 days after sowing - DAS), the shoots were cut, washed under running water and placed in a cold chamber at 13 ºC until use. The extract was made with the aid of an industrial blender, to which 100 grams of green mass and 600 mL of deionized water were added. Then, two grinding cycles of three minutes each were performed, after which the extract was sieved and vacuum filtered. The extracts obtained were designated as 100 %; they were later diluted to concentrations of 12.5 %, 25 %, 50 % and 75 % using deionized water (0 %) as a control. The extracts obtained were characterized in terms of pH (Quimis/Q-400BI), osmolality (Wescor, mod. 5500), soluble solids content (°Brix, RZ) and conductivity (Analion/C708-Plus).

Petri dishes (Dia: 9 cm and Ht: 2 cm) were lined with germination paper on which 25 seeds of *P. maximum* were placed; subsequently, 15 mL of each extract were applied per plate (0 %, 12.5 %, 25 %, 50 %, 75 % and 100 %), with four repetitions for each concentration. Then, the plates were placed in germination chambers in a completely randomized design and adjusted to a constant temperature of 25 ºC and a photoperiod of 12 h, where they remained for seven days.

Germination Speed Index: It was calculated asper (17).

(ii). Experiment 5. Effects of post-emergence application of leaf extract of *C. juncea* on plants of *P. maximum*

To carry out the post-emergence application, 300 mL plastic cups containing washed sand as substrate were used, in which four *P. maximum* Jacq. seeds were sown. The material used in this step was processed following the procedures described for the Experiment 3.

The extract was made with the aid of an industrial blender, to which 100 g green mass and 600 mL of deionized water were added. Then, two grinding cycles of three minutes each were performed, after which the extract was sieved and vacuum filtered. The extracts obtained were designated as 100 %; they were later diluted to concentrations of 12.5 %, 25 %, 50 %, 75 % and 100 % using only deionized water as a control. The extracts thus obtained were characterized in terms of pH (Quimis/Q-400BI), osmolality (Wescor, mod. 5500), soluble solids content (°Brix, RZ) and conductivity (Analion/C708-Plus).

The application of the extracts was carried out 14 days after the emergence of *P. maximum* by applying 2 mL of the extract at concentrations of 0, 12.5, 25, 50, 75 and 100 % in each cup, arranged in a completely randomized design with five replications. The application was carried out using a previously calibrated sprayer and, on the day of application, it was verified that the temperature was 23 °C, the relative humidity was 51 % and the wind speed was 13 km h^{-1}. Four evaluations were carried out at intervals of seven days (7, 14, 21 and 28 days after application) as for the number of tillers, number of leaves and plant length. Absolute growth rates were calculated from this Data (1).

2. POT CULTURE

(i). Experiment 6. Effects of different densities of *C. juncea* L. sown on development of *P. maximum* in pots culture

To analyze the development of *P. maximum* Jacq. in concomitant sowing with *C. juncea*, pots with a capacity of 3 L were used. They were filled with a mixture of soil, tanned manure and sand at a 2:1:1 ratio (v/v/v), kept in an open area and moistened daily. One, two and three seeds of *C. juncea* and one seed of *P. maximum* were deposited per pot, with a control without sowing of *C. juncea*, totaling four treatments and five replications. The experimental design was completely randomized.

Thirty-three days after sowing, the number of tillers of Guinea grass was evaluated and then the shoot was cut and dried in an oven with forced air circulation at 60 ºC until constant mass.

(ii). Experiment 7. Effects of transplanting of *C. juncea* L. plants on development of *P. maximum* in pots.

To analyze the development of *P. maximum* Jacq. transplanted simultaneously with crotalaria, pots with a capacity of 3 L filled with a mixture of soil, manure and sand were used at a 2:1:1 ratio (v/v/v). They were kept in an open area and moistened daily. Seedlings of *C. juncea* L. and *P. maximum* Jacq. with 30 days were transplanted into the pots at densities of 3, 2 and 1 of *C. juncea* L. for one plant of *P. maximum* Jacq. During the experiment, 17 ml of 0.5 % urea were applied per pot. After 33 days of transplantation, the shoot was cut. The number of tillers of Guinea grass was evaluated and then the shoot was cut and dried in an oven with forced air circulation at 60 ºC until constant mass.

Data analysis

The normality of error distribution and the homogeneity of variance were tested. Data were subjected to analysis of variance by F test and means were compared by Tukey test at 5 % probability using the Statistica 12 software.

RESULTS AND DISCUSSION

1. PETRI-PLATE BIOASSAY

1.1. SEED EXUDATES:

Experiment 1. Can the seed exudates from different *Crotalaria* species inhibit the germination of *P. maximum*?

Analyzing the germination speed of *P. maximum* in contact with the exudate from the germination of the four crotalaria species (Table 3), there was a significant difference ($p < 0.05$) between the exudates of different species. There was a difference between

C. juncea ($p < 0.05$) and *C. spectabilis* and *C. breviflora*, but there was no difference in the GSI between *C. juncea* and *C. ochreleuca*. Regarding the GSI value, *C. spectabilis* was similar to control. However, other species differed from control with 89.8 % difference for *C. juncea*, 35.68 % for *C. breviflora* and 48.76 % for *C. ochreleuca*. This showed that among the treatments, the seed exudates of *C. juncea* drastically reduced the germination speed of *P. maximum*.

Table 3. Effects of seed exudates from the germination of seeds of *C. juncea*, *C. spectabilis*, *C. ochreleuca* and *C. breviflora* on the speed and germination (%) of *P. maximum*.

Species	**Variable**	
	GSI	**G %**
Control	37.94±7.21**a**	96.00±23.30**a**
C. spectabilis	25.54±3.70**ab**	65.25±8.22**b**
C. ochreleuca	19.44±4.00**bc**	54.00±10.60**b**
C. breviflora	24.40±5.00**b**	61.00±60**b**
C. juncea	3.87±3.26**c**	8.00±6.53**c**
CV (%)	36.19	37.07

Means followed by the same letter in columns do not differ by Tukey test at 5 % probability.
** = significant at 1 % probability by F test.

The seed exudates from the four Crotolaria species significantly decreased the germination (%) of *P. maximum* ($P < 0.005$) than control. The germination of control was 96 %, while the germination with seed exudates of *C. spectabilis*, *C. ochreleuca* and *C. breviflora*, was identical to each other and was 60.0 %, that is, these spp seed exudates reduced the seed germination of *P. maximum* by 37.4 %. *C. juncea* seed exudates caused maximum reduction of 91.7 % in germination of *P. maximum.* Given this highest allelopathic potential of *C. juncea* than other test Crotalaria spp. the other experiments were done only with *C. juncea*.

Experiment 2. Effects of seed exudates of *C. juncea* on the seedling development of *P. maximum*: Effects of density

The initial root length of plants before being placed in contact with the exudate did not differ. After 7-days of contact with the exudate from the germination of *C. juncea*, the root length and dry mass of *P. maximum* did not differ ($p > 0.05$) at the densities 1, 2 and 5 than control (Table 4). However, at densities of 10, 15 and 20, there was significant ($p < 0.05$) decrease in root length than control and lower densities 1, 2 and 5. In densities of 10, 15 and 20, it was possible to measure roots, as they were degraded by the *C. juncea* exudates, thus effectively in suppressed the root growth of *P. maximum*.

When evaluating the dry mass, the same situation described for root length was verified, with no significant differences ($p > 0.05$) between the control and the densities 1, 2 and 5. In densities 10, 15 and 20, the dry mass of seedlings was decreased by the exudates from the germination of 10, 15 and 20 seeds of *C. juncea* (shoot only). They decreased *P. maximum* seedlings dry mass by 64 % than control, showing that higher the density, the greater the inhibitory effects on *P. maximum* seedlings.

Table 4. Effects of seed exudates from germination of 1, 2, 5, 10, 15 and 20 seeds of *C. juncea* on root length and seedling dry mass of *P. maximum*.

Seeds/Petriplate	**Variable**		
	Root length before test (cm)	**Root length after test (cm)**	**Dry matter (g)**
0	7.62±1.23**a**	8.10±1.83**a**	0.022±0.004**a**
1	7.06±2.30**a**	7.16±2.00**a**	0.019±0.005**a**
2	6.16±1.85**a**	6.60±1.30**a**	0.020±0.002**a**
5	7.75±3.30**a**	7.20±2.10**a**	0.012±0.004**a**
10	10.00±5.00**a**	0.00±0.00**b**	0.011±0.004**b**
15	6.75±2.28**a**	0.00±0.00**b**	0.008±0.001**b**
20	6.38±3.17**a**	0.00±0.00**b**	0.006±0.001**b**
C.D.	39.06	53.47	46.58

Means followed by the same lowercase letter in columns do not differ by Tukey test at 5 % probability. Ns = not significant by F test; ** = significant at 1 % probability by F test.

Experiment 3. Use of seed exudates of *C. juncea* in pre- and post-emergence of *P. maximum*

The application of exudates obtained from different densities of *C. juncea* did not (p >0.05) influence the germination speed and germination (%) of *P. maximum* (Table 5).

Table 5. Effects of exudates from the germination of *C. juncea*, in increasing densities, on the speed and percentage of germination of *P. maximum* seven days after sowing.

Density (seeds sown/plate)	Germination (%)		Speed of germination (GSI)	
	Seed Exudate 1.0 mL	Seed Exudate 2.0 mL	Seed Exudate 1.0 mL	Seed Exudate 2.0 mL
0	67.00±4.00**a**	65.00±11.50**a**	33.50±1.10**a**	34.30±5.20**a**
1	66.00±5.20**a**	51.00±9.50**a**	33.30±2.20**a**	24.40±3.00**a**
2	61.00±13.20**a**	49.0±5.00**a**	31.00±5.40**a**	23.60±3.60**a**
5	59.00±12.00**a**	61.00±6.00**a**	29.30±5.60**a**	30.00±2.20**a**
10	61.00±4.00**a**	56.00±9.80**a**	30.70±2.20**a**	27.60±5.00**a**
15	67.00±5.00**a**	57.00±10.50**a**	33.40±4.20**a**	33.30±10.00**a**
20	56.00±9.20**a**	52.00±12.60**a**	27.10±4.70**a**	24.70±4.00**a**
C.D.	13.43	16.74	15.39	33.57

The post-emergence application of the seed exudates from the increasing densities of *C. juncea* created a significantly influenced the number of tillers of *P. maximum* only at density of 20 seeds, 7th day after application (Figure 2G). However, the plants height and number of leaves did not show significant differences, when using the exudate from seed germination at different densities compared to control (Figure 2A-F, 2H-I).

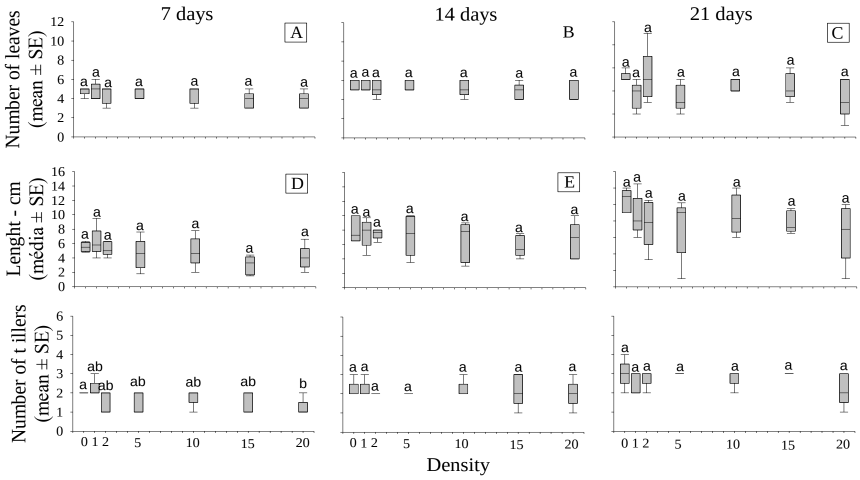

Figure 2. Effects of exudate from germination of increasing densities of *C. juncea* on the number of leaves, plant length and number of tillers of *P. maximum* at seven, 14 and 21 days after application. Means followed by the same lowercase letter do not differ by Tukey test at 5 % probability.

The average values of the densities described in Figure 2 showed that the Guinea grass plants continued to grow, 7 to 21 days after application, showing an increase in the length and number of leaves (Table 6).

Table 6. Effects of seeds exudates from germinating of seeds of increasing densities of *C. juncea* (0, 1, 2, 5, 10, 15 and 20 seeds) on the number of leaves, plant height and number of tillers of *P. maximum* at seven, 14 and 21 days after application.

DAA	**Density Mean Values**		
	No. of leaves	**Height (cm)**	**No. of Tillers**
7	4.37±0.74**c**	4.58±1.70**c**	2.00±0.52**b**
14	5.25±0.73**b**	7.06±2.15**b**	2.00±0.55**b**
21	5.82±1.23**a**	10.06±2.53**a**	3.00±0.75**a**
C.D.	20.28	39.73	20.20

Means followed by the same letter in columns do not differ by Tukey test at 5 % probability. *,** = significant at 5 % and 1 % probability by F test.

Taking into account the low values of osmolality, pH, soluble solids content and electrical conductivity of extracts and the seed exudates from germinating seeds, it is possible to assume that the suppressive action may be due to the presence of allelochemicals. *Crotalaria juncea* plants has allelopathic compounds from the phenol and terpene groups. They act directly on the breakdown of metabolic enzymes involved in glycolysis and the oxidative pathway of pentose phosphates. Also, terpenes are the main players in inhibiting adenosine triphosphate (ATP), which interrupts the plant's metabolism by forming a complex with proteins (22). Also, its presence is verified through the inhibition of respiration, a situation observed in this work, when the interruption of germination and development of seedlings and plants occurred in experiments 1, 2, 3 and 4. This happened mainly in the tests done to verify the root development of seedlings of *P. maximum* in contact with the seed exudates from the *C. juncea* (3,5), especially at higher densities, in which root degradation occurs due to the action of the exudate, evidencing its allelopathic potential. According to studies, extracts produced from leaves are more efficient than extracts produced from roots and stems (19). This may explain the results obtained here in experiments in which the materials were leaves. These experiments compared the level of suppression of *C. juncea* referring to the extracts produced from the exudate arising from germination.

1.2. LEAF AQUEOUS EXTRACT

Experiment 4. Bioassay with leaf aqueous extract of *C. juncea* on *P. maximum*

The leaf aqueous extract of *C. juncea* significantly (p <0.05) Influenced germination speed index and germination (%) of *P. maximum* (Table 7) than control except 12.5 % concentration. The GSI for the control was 35.75, it decreased with increasing concentrations and 75 % and 100 % concentration were the most efficient, these inhibited the germination by 100 %. The germination in control was 48 %, similar to 12.5 % concentration, afterwards the reduction was gradual, reaching 0 % at the 75 % and 100 % concentrations. By analyzing the values for each treatment, the higher the concentration, the lower the germination speed and germination (%) up to the point where the total suppression of *P. maximum* occurred.

Table 7. Effects of concentrations of the aqueous extract of the leaf of *C. juncea* on the speed and percentage of germination of *P. maximum* seven days after sowing

Leaf Aqueous Extract Conc (%)	**Variable**	
	Germination (%)	**GSI**
0	48.00±19.00**a**	35.75±2.36**a**
12.5	30.50±2.00**ab**	23.25±2.74**b**
25	27.00±7.00**bc**	17.60±4.76**c**
50	8.50±1.00**cd**	6.20±1.24**d**
75	0.00±0.00**d**	0.00±0.00**e**
100	0.00±0.00**d**	0.00±0.00**e**
C.D.	45.69	66.35

Means followed by the same letter in columns do not differ by Tukey test at 5% probability. ** = significant at 1% probability by F test. GSI = Germination speed index.

Experiment 5. Post-emergence application of leaf extract of *C. juncea* on plants of *P. maximum*

The leaf extracts of 50 % concentration of *C. juncea* significantly inhibited the plant height of *P. maximum* than control. The effect was more accentuated with the concentration of 75 % and 100 % throughout the period evaluated (Figures 3, 4 and 5). However, the 75 % concentration caused greater decrease in length throughout the period. However, the extracts did not affect the number of tillers per plant (Figure 5).

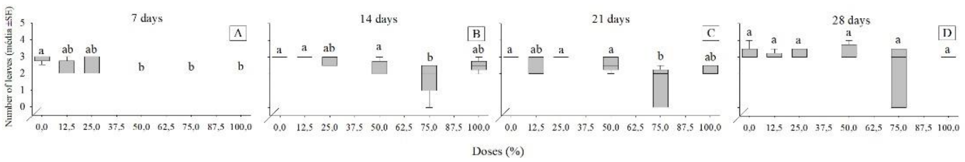

Figure 3. Effects of concentrations of the aqueous extract of the leaf of *C. juncea* on the number of leaves of *P. maximum* at seven, 14, 21 and 28 days after application. Means followed by the same letter do not differ by Tukey test at 5 % probability.

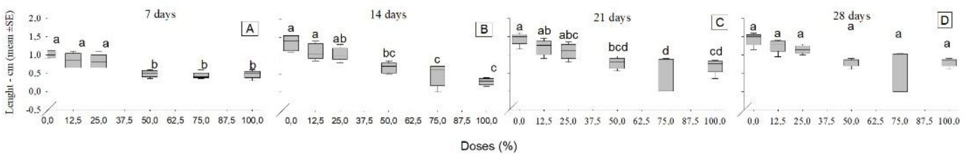

Figure 4. Effects of concentrations of the aqueous extract of the leaf of *C. juncea* on the length of *P. maximum* at seven, 14, 21 and 28 days after application. Means followed by the same lowercase letter do not differ by Tukey test at 5 % probability

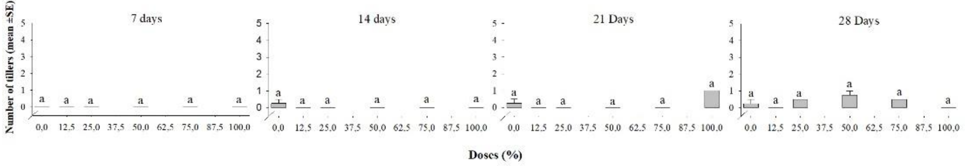

Figure 5. Effects of different concentrations of the aqueous extract of the leaf of *C. juncea* on the number of tillers of *P. maximum* at seven, 14, 21 and 28 days after application.

The number of leaves of *P. maximum* were reduced with the application of leaf extract to shoots of *C. juncea* at 50, 75 and 100 % concentrations at seven DAA (Figure 4). The effect was more accentuated at 75 % concentration and at 14 and 21 DAA. The effects of 50 % concentration were observed only at 14 DAA. On the other hand, the effects of the 12.5 % concentration were statistically close to 100 % concentration at 28 DAA; however, there was no difference for 75 % concentration in any evaluated period.

Table 8. Effects of the leaf aqueous extract of *C. juncea* on the number of leaves, length and number of tillers of *P. maximum* from seven to 28 days after application (mean values of concentrations).

DAA	**Mean values of variables**		
	No. of leaves	**Shoot length (cm)**	**No. of Tillers**
7	2.00**b**	0.68**c**	0.00**b**
14	2.60**ab**	0.82**bc**	0.02**ab**
21	2.00**b**	0.94**ab**	0.08**ab**
28	3.00**a**	0.99**a**	0.10**a**
C.D.	28.91	40.21	36.74

Means followed by the same letter in columns do not differ by Tukey test at 5 % probability.
** = significant at 1 % probability by F test. DAA: Days after application

The analysis over time showed that there was significant growth of plants (number of leaves, plant length and number of tillers), at 7 days after application than at 28 days (Tables 8 and 9).

Table 9. Absolute growth rate (AGR) of *C. juncea* on the number of leaves, plant height and number of tillers of *P. maximum* at 21 and 28 days after application.

Growth (Number of leaves, plant height and number of tillers) of *P. maximum*						
Variable	**No. of leaves/Plant**		**Height (cm)**		**No. of Tillers/Plant**	
	21 DAE	**28 DAE**	**21 DAE**	**28 DAE**	**21 DAE**	**28 DAE**
AGR (%)	0.276	0.50	0.776	0.33	0.50	1.2

This may indicate that the same happened here, when *C. juncea* did not control *P. maximum* in the period when both coexisted, as the allelopathic action would be more intense at the initial stage of *P. maximum*. This is in agreement with the tests of application of extract and seed exudate from germination (1, 2 and 3) to seeds and seedlings. In these tests, there was a greater suppression of *P. maximum*, also combining data from the experiment in which coexistence occurs since sowing (4) there was a decrease in dry mass accumulation.

Another aspect that must be taken into account is that, during the experiment with transplants into pots, urea was applied to supply nitrogen. *P. maximum* responds well to nitrogen fertilization (5), which is closely related to dry mass and tillering. Assessing that there was no marked interference due to allelopathy and the supply of urea, it is inferred that *P. maximum* was in full development and even shaded *C. juncea*.

The time of seeds exudate application and the treatment directly interfere with the suppression of the development of *P. maximum* (Table 6). The late experiment in which the seed exudate from different densities of *C. juncea* seeds as applied did not differ over control with both volumes tested and at the different densities. This shows that the amount of allelochemicals present in the seed exudate is not enough to suppress a plant that is already at an advanced vegetative growth (35 DAE). This did not happen in the experiment with initial post-emergence application with application of shoot extract (10), as the plant was still at the beginning of its vegetative development (14 DAE) and the shoot extract proved more effective than control. This corroborates what was described here and also with Rugare and collaborators (16), who reported a greater efficiency of leaf extracts. However, the extract applied early to plants of *P. maximum* not only delayed the development, but also caused complete suppression. This indicated that, the allelopathic compounds in seed of *P. maximum* plants were more effective.

2. POT CULTURE

Experiment 6. Effects of sowing of different densities of *C. juncea* on development of *P. maximum* in pots

Analyzing the development of *P. maximum* in coexistence since sowing with increasing densities of *C. juncea* (Table 10), there was no difference ($p > 0.05$) for the number of tillers.

Table 10. Effects of density of *C. juncea* plants on the number of tillers and shoot dry mass of *P. maximum* seeded.

Density	**Variable – 33 days of coexistence**	
Plants/pot	**No. of Tillers**	**Dry biomass of leaves (g)**
0	12.00±2.80**a**	24.00±9.30**a**
1	8.00±1.50**a**	16.80±4.10**ab**
2	8.00±2.80**a**	9.80±1.70**b**
3	8.00±4.10**a**	10.60±5.40**b**
C.D.	30.35	42.74

Means followed by the same letter in columns do not differ by Tukey test at 5 % probability. ns = not significant by F test; ** = significant at 1 % probability by F test

However, the dry mass of *P. maximum*, significantly decreased ($p < 0.05$) compared to control with the densities of two and three plants. A value of 24 g was verified, being 59.2 % and 55.8 % greater than densities 2 and 3, respectively, which, did not differ from each other or from the density of a *C. juncea* plant.

Experiment 7. Effects of transplanting of different densities of *C. juncea* on development of *P. maximum* I pots

The development of *P. maximum* 33 days after transplanting (coexistence) did not show significant differences ($p > 0.05$) for the number of tillers and for shoot dry mass (Table 11).

Table 11. Effects of *C. juncea* plant density on the number of tillers and dry mass of the aboveground part of transplanted *P. maximum*.

Density	**Variable – 33 days of coexistence**	
Plants/pot	**No. of Tillers**	**Dry biomass of leaves (g)**
0	11.00±4.10**a**	28.80±11.40**a**
1	11.00±1.40**a**	31.50±6.00**a**
2	9.00±3.30**a**	20.00±6.60**a**
3	10.00±3.30**a**	22.50±4.80**a**
C.D.	36.34	41.78

Means followed by the same letter in columns do not differ from each other by F test at 5 % probability. ns = not significant.

The experiment conducted with sowing in pots (4) resembles the same situation as that Timossi (21) described. The authors verified that green manure suppressed weeds and decreased the accumulation of dry biomass. The studies reported a suppressive effect of green manures on weeds, showing that *C. juncea* has the potential to decrease the dry biomass accumulation of *P. maximum* (10,14,15).

Even if *C. juncea* has an allelopathic potential in controlling weeds and reducing the accumulation of biomass, according to Blaise (3) the amount of allelochemicals released effectively controlled weeds. This showed that, although it has an effect on the decrease in dry mass, in the Pot experiments the density of *C. juncea* was not enough to effectively control *P. maximum*. It was possible to observe the effect of different treatments to develop the plants and to tiller in a similar way to control.

Regarding transplantation into pots (5), a factor that may have contributed to the lack of suppression of *P. maximum* could be that, despite its slow initial growth (23), seedlings were transplanted together with *C .juncea* 30 days after sowing, which nullified the effect of the accelerated growth of *C. juncea* that would generate competition for resources with *P. maximum*, also interfering in the allelopathic action since they did not coexist since sowing. These data are corroborated by Weston and Duke (24), who reported that the allelopathic compounds released by sorghum inhibit weed development when applied at an early stage of development.

CONCLUSIONS

Among the different *Crotalaria* species tested (*C. juncea*, *C. breviflora*, *C. ochreleuca* and *C. spectabilis*), *C. juncea* had the highest allelopathic potential. Its seed exudates were more inhibitory to germination of *P. maximum* than *C. breviflora*, *C. ochreleuca* and *C. spectabilis*. The higher the sowing density of *C. juncea*, the greater were the inhibitory effects of seeds exudates on the germination of *P. maximum*. When *C. juncea* and *P. maximum* were grown together the higher the density of *C. juncea*, the greater were the inhibitory effects on *P. maximum*. The post-emergence application of the leaf aqueous extract of *C. juncea* decreased the development of *P. maximum*.

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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