ALLELOPATHIC POTENTIAL OF SERJANIA LETHALIS: EVIDENCE FROM SESAMUM INDICUM

Viviane De Cassia PEREIRA[1], Simoni ANESE[1], Maristela IMATOMI[1], Patrícia Umeda GRISI[1], Enzo MONTE CANEDO[2], Sonia Cristina JULIANO GUALTIERI[1], Edson RODRIGUES-FILHO[2].

[1] Universidade Federal de São Carlos, Departamento de Botânica, Rodovia Washington Luís, Km 235, São Carlos, Brasil.

[2] Universidade Federal de São Carlos, Departamento de Química, Rodovia Washington Luís, Km 235, São Carlos, Brasil.

For correspondence. vivicpereira@gmail.com

ABSTRACT

This study was designed to test the effect of different fractions of ethanolic extracts of young (y) and mature (m) leaves of *Serjania lethalis* A. St- Hil (Sapindaceae) on sesame (*Sesamum indicum* L.) seedling growth and metaxylem cells. Crude ethanolic extracts were prepared from the powder of young and mature *S. lethalis* leaves and fractionated by means of column chromatography. For the seedling growth bioassay, sesame seeds germinated at concentrations of 0.8, 0.4 and 0.2 mg mL^{-1} were used. After seven days, the lengths of the aerial part and of the primary root were measured. The sesame metaxylem cell growth bioassay was performed with seedlings grown in solutions containing the different fractions in the same concentrations as above. The tested fractions (Fy2; Fy3; Fy5 e Fm1) showed inhibitory activity on seedling growth, interfering mainly in root growth. The fraction Fy5 showed similar activity to the one caused by the herbicide Oxyfluorfen. This fraction was also responsible for causing the greatest inhibition of metaxylem cell growth in sesame roots at the concentration 0.8 mg mL^{-1}. The results permitted to conclude that the different fractions found in the ethanolic extract of *S. lethalis* young leaves are promising sources of substances with phytotoxic properties.

Keywords: Cerrado, phytotoxicity, Sapindaceae, sesame, xylem development.

INTRODUCTION

Allelopathy is an ecological process that involves the release, by plants, algae, bacteria or fungi, of secondary metabolism products capable of exerting positive or negative effects on plants in natural or agricultural systems (International Allelopathy Society, 2013). The plant metabolism compounds known as allelochemicals belong to several classes of chemicals, such as quinones, phenols, cinnamic acids, coumarins, flavonoids, tannins, terpenes and alkaloids (Rice, 1984). Some biological activities have been reported for different plant metabolites, including: antifungal (Tsuzuki *et al*., 2007), molluscicidal (Pires, 2008), antioxidant (Oliveira *et al*., 2007), antiparasitic (Traore *et al.*, 2000) and phytotoxic (Grisi *et al*., 2013; Kim *et al*., 2005).

Many studies have shown the phytotoxic activity of plant extracts on the germination and growth of other species (Alves *et al*., 2004; Gatti *et al*., 2010; Imatomi, 2010; Grisi *et al*., 2012), suggesting that these extracts may be used as natural herbicides (Grisi *et al*., 2011) by affecting the germination and growth rates of weed species. These allelochemicals cause biochemical and physiological changes that negatively affect the growth and development of seedlings (Weir *et al*., 2004). The natural herbicides have advantages when compared to the synthetic ones, being less harmful to the environment (Souza-Filho *et al*., 2006) and having a shorter half-life (Duke *et al*., 2000; Rimando and Duke, 2006). Inderjit and Dakshini (1995) state the importance of bioassays to test the activity of plant secondary metabolites, as it is by means of these bioassays that we may come to know the susceptibility of certain species to the allelochemicals and, consequently, the phytotoxic potential of the species studied.

Serjania lethalis A. St.-Hil., a member of the family Sapindaceae, is a liana typically found in the Brazilian cerrado (Fernandes and Negreiros, 2001). It is an ornamental plant that has narcotic activity (Correa, 1926) and pharmacological properties (Lima *et al*., 2006). The phytotoxic activity of ethanolic extracts of *Serjania lethalis* leaves is known, but the literature still lacks studies about the phytotoxic potential of the different fractions that can be extracted from this species (Grisi *et al.,* 2013).

The maturation phase of the plant is related to the accumulation of different chemical components. Young leaves suffer high herbivory pressure, producing a greater variety of chemical components for defense (Lokvam and Kursar, 2005). Based on the observation that many species develop chemical defenses during their juvenile stages of development (Bryant and Julkunen-Tiitto, 1995), it can be assumed that there is a higher concentration of allelochemicals in young leaves than in mature leaves. Conversely, nutrients are reallocated from the leaves to developing organs or reserve tissues during senescence (Gan and Amasino, 1997). This reallocation may lead to low concentrations of phytotoxic compounds in senescent leaves, thus, we considered the hypothesis that mature leaves contain less phytotoxic compounds.

Based on this information, this study was designed to evaluate effect caused by the different fractions found in ethanolic extracts of young and mature leaves of *Serjania lethalis* on sesame (*Sesamum indicum* L.) seedling growth and cell elongation of root metaxylem.

MATERIAL AND METHODS

Plant material

Young and mature leaves of *Serjania lethalis* were collected from ten individuals, in a cerrado *sensu stricto* reserve, Federal University of São Carlos, São Carlos campus, São Paulo, Brazil (21° 58 'to 22° 00' S and 47° 51 'to 47° 52 'W). Were considered young the leaves that had a membranaceous texture and a light green color, and mature the leaves that had a leathery texture and a dark green color (Grisi *et al*., 2011). The plant material was dried in a forced circulation kiln at 40°C for 72 hours, ground in an electric mil, and stored in appropriately sealed plastic bags. A copy was deposited (voucher 8340) in the herbarium of UFSCar's Botany Department (HUFSCar).

Preparation of extracts

Ethanolic extracts were prepared with 1L of ethanol and 100 g of powder of young or mature leaves. The solutions were kept in a refrigerator at 4°C for 24h and, afterwards, filtered under vacuum. The ethanolic extracts were dried in a rotary evaporator under reduced pressure.

Fractionation of extracts

The ethanolic extracts of young and mature leaves were fractionated by normal phase column chromatography with silica gel (0.063-0.2 mm/70-230 mesh), with 900 mL of each of the following eluents, in this order: hexane:acetone (7:3), hexane:acetone (3:7), acetone, acetone:methanol (7:3), acetone:methanol (3:7), methanol, methanol:water (9:1) (Table 1).

Table 1. Fractions of aqueous and ethanolic extracts of young and mature leaves of *Serjania lethalis*.

Extract	Eluents	Fraction code
	Hex:Ac (7:3); Hex:Ac (3:7); Ac	**Fy1**
	Ac:MeOH (7:3)	**Fy2**
Young leaves	Ac:MeOH (7:3)	**Fy3**
ethanolic	Ac:MeOH (3:7)	**Fy4**
	MeOH	**Fy5**
	MeOH:H_20 (9:1)	**Fy6**
Mature leaves	Hex:Ac (7:3)	**Fm1**
ethanolic	Hex:Ac (3:7)	**Fm2**
	Hex:Ac (3:7)	**Fm3**
	Ac	**Fm4**

Fy (ethanolic extract of young leaves), **Fm** (ethanolic extract of mature leaves).

The solutions of each fraction used in the bioassays were solubilized in DMSO (5%) and buffer solution 2-[N-morpholino]ethanosulfonic acid (MES) and 1 M NaOH at pH 6, with concentrations of 0.8, 0.4 and 0.2 mg mL^{-1}.

Selection of fractions with phytotoxic activity

The selection of the active fractions was made by a bioassay with wheat (*Triticum aestivum* L.) coleoptiles according Macías *et al.*, (2010). The Fy2, Fy3, Fy5 and Fm1 fractions showed high inhibitory activity (data not shown) and were selected for testing for seedling growth and elongation of cells in the metaxylem of sesame roots.

Morphological indicators of seedling growth

To evaluate the growth of sesame seedlings, the seeds were germinated in distilled water and, after achieving a primary root length of 2 mm, were transferred to transparent 500 mL (14 x 10 cm) plastic boxes. Each box contained two sheets of filter paper moistened with 13 mL of each fraction at concentrations of 0.8, 0.4 or 0.2 mg mL^{-1}. The boxes were placed in transparent plastic bags and kept in a growth chamber at 28°C, with a photoperiod of 12h (Carvalho *et al*., 2001). After seven days, shoot and primary root lengths were measured with a digital caliper. Seedlings that showed abnormal characteristics, as described by RAS (2009), were quantified and the types of abnormality were qualified.

Bioassay of sesame metaxylem cell growth

The anatomical study of root metaxylem cells was performed with sesame seeds germinated in distilled water and kept for 24h in a growth chamber at 28°C with a photoperiod of 12h (Carvalho *et al*., 2001). Were considered as germinated the seeds that had a primary root protrusion of at least 2 mm (RAS, 2009). The germinated seeds were placed in transparent 250 mL (13 x 8 x 3 cm) plastic boxes, each containing two sheets of filter paper moistened with 8 ml of each fraction in concentrations of 0.8, 0.4 and 0.2 mg mL^{-1}. Three replicates were used for each concentration, with ten seedlings in each box. The plastic boxes were placed in transparent bags and kept in a BOD at 28°C with a photoperiod of 12h. After four days the seedlings were removed from the boxes and a stylet was used to cut the primary root segment. This segment was then placed in 70% ethanol (Gatti *et al*., 2010) and incubated at 40°C for seven days. Subsequently, the roots were washed with distilled water, placed in a solution of 25% sodium hydroxide, and incubated at 40°C for two days for clearing. The root segments were then stained with Lacmóide dye for 24h, washed to remove excess dye, and each segment was placed on a microscope glass slide and covered with a drop of Apathy syrup and a cover slip. The whole root staining procedure was performed according to the modified Fuchs method (Kraus and Arduin, 1997).

The slides were observed under an optical microscope (Olympus-BX41) with an attached camera (Sony CCD-IRIS). Were photographed and half of length of each root from the central region upward of primary roots of sesame seedlings (Gatti *et al*., 2010; Grisi *et al*., 2013). From each photograph, 10 central cells of the metaxylem were measured at 20X magnification (ImageJ software) (Gatti *et al*., 2010).

Statistical Analysis

All results were tested for normality and homoscedasticity with the Shapiro-Wilk and Levene tests, respectively. Normal and homoscedastic data were analyzed with ANOVA followed by Tukey's test and non-normal and/or heteroscedastic data were analyzed with the non-parametric Kruskal-Wallis test, in the software Past 2.14. (Hammer *et al*., 2001).

RESULTS

Root length of sesame seedlings subjected to the fractions Fy2; Fy3; Fy5 and Fm1 differed significantly from the control at concentration 0.8 mg mL^{-1} (Fig. 1a), and the fraction Fy5 produced the lowest average length (1.16 cm), which was statistically similar to the herbicide effect. The aerial part of the seedlings was negatively affected only by fractions Fy3 and Fy5 at 0.8 mg mL^{-1} (Fig. 1a). Fractions Fy2, Fy3 and Fy5 caused inhibitory effects on root growth at 0.4 mg mL^{-1}, but no fraction inhibited the growth of shoots at the concentration of 0.4 mg mL^{-1} (Fig. 1b). At the concentration 0.2 mg mL^{-1} only fraction Fy5 inhibited the growth of roots and the shoot growth was not affected by any fraction (Fig. 1c). The positive control Oxyfluorfen differed significantly from the negative control in all concentrations.

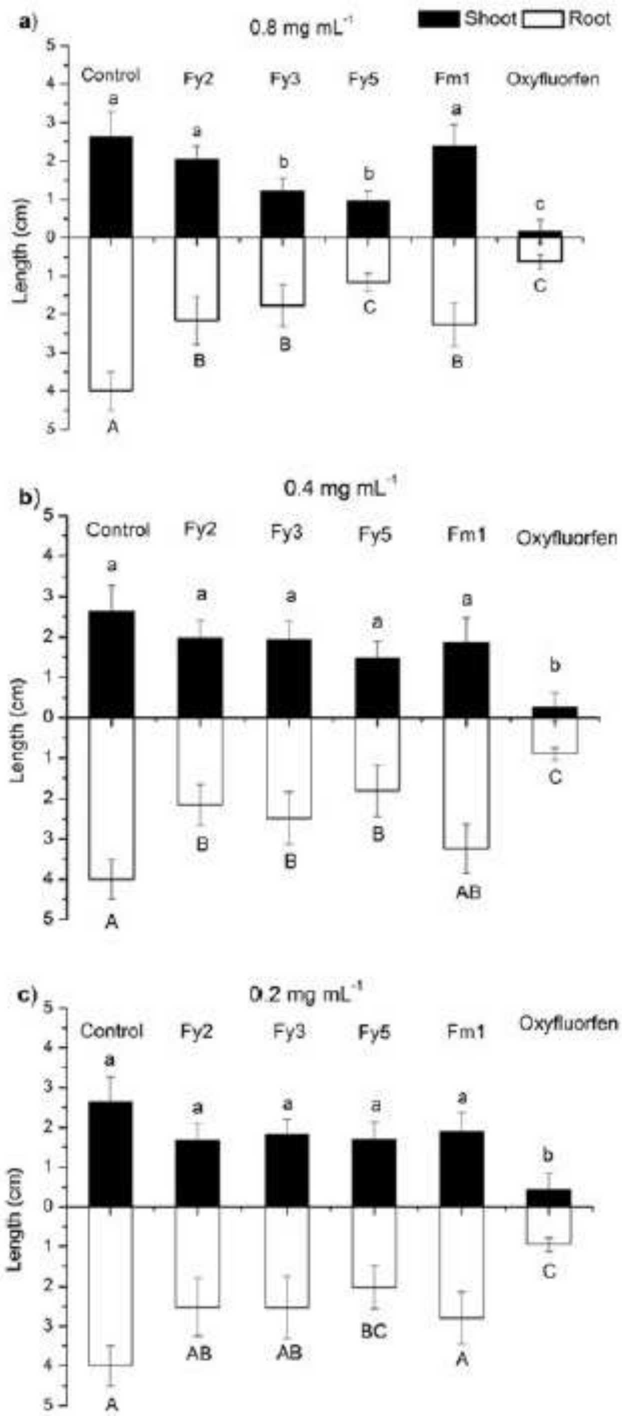

Figure 1. Length of roots and shoots of sesame (*Sesamum indicum*) seedlings subjected to the effects of different concentrations of fractions of *Serjania lethalis* leaf extracts. a) 0.8 mg mL^{-1} b) 0.4 mg mL^{-1} c) 0.2 mg mL^{-1}. Values with different letters indicate significant differences ($p < 0.05$) detected by ANOVA. The vertical bars indicate the standard deviation from the mean.

It can be seen that the percentage of abnormal seedlings was dependent on the concentration tested, with the higher concentrations showing the majors occurrence of abnormal seedlings. The fraction Fy3 was responsible for the largest percentage of abnormality in all concentrations, whereas the Fy2 fraction had the lowest incidence. The observed abnormalities were root necrosis, atrophy, and gravitropic reversal (table 2). The necrosis observed in seedlings of sesame caused their death due to rotting of plant tissue, whereas atrophy and gravitropic reversal caused physical deformities in seedlings, preventing them from growing upright and their roots from settling and developing. These observations corroborate the hypothesis that fractions obtained from extracts of *Serjania lethalis* young and mature leaves can impair growth and development of seedlings and that these fractions have characteristics that permit their use in the development of natural herbicides.

Table 2. Percentage and type of abnormality found at each concentration of each fraction of extracts of *Serjania lethalis* young and mature leaves: Necrosis (N), Atrophy (A), Gravitropic Reversal (GR).

	Percentage of abnormality in each concentration (mg mL^{-1})			Abnormality		
Fraction	0.8	0.4	0.2	0.8	0.4	0.2
Fy2	65.0	62.5	50.0	N/A/GR	N/A/GR	N/A
Fy3	92.5	75.0	57.5	N/A/GR	N/A/GR	N/A/GR
Fy5	80.0	63.7	52.5	N/A/GR	N/A/GR	N/A/GR
Fm1	75.0	63.7	52.5	N/A/GR	N/A/GR	N/A/GR

The same fractions were subjected to a bioassay to measure the metaxylem cell growth of the sesame root. The results indicate that, at both concentration 0.8 and 0.4 mg mL^{-1}, all fractions had an inhibitory effect on elongation, when compared to the negative control, and sesame metaxylem cells exposed to the Fy5 and Fy2 fractions at 0.8mg mL^{-1} were similar to the effect of the herbicide (Fig. 2 and table 3). At the concentration of 0.2 mg mL^{-1}, inhibition of cell growth was observed for the fractions Fy2, Fy3 and Fy5, whereas the last two fractions were significantly similar to the herbicide activity (table 3). The effects of the positive control with Oxyfluorfen were significantly different from those of the fractions and of the negative control.

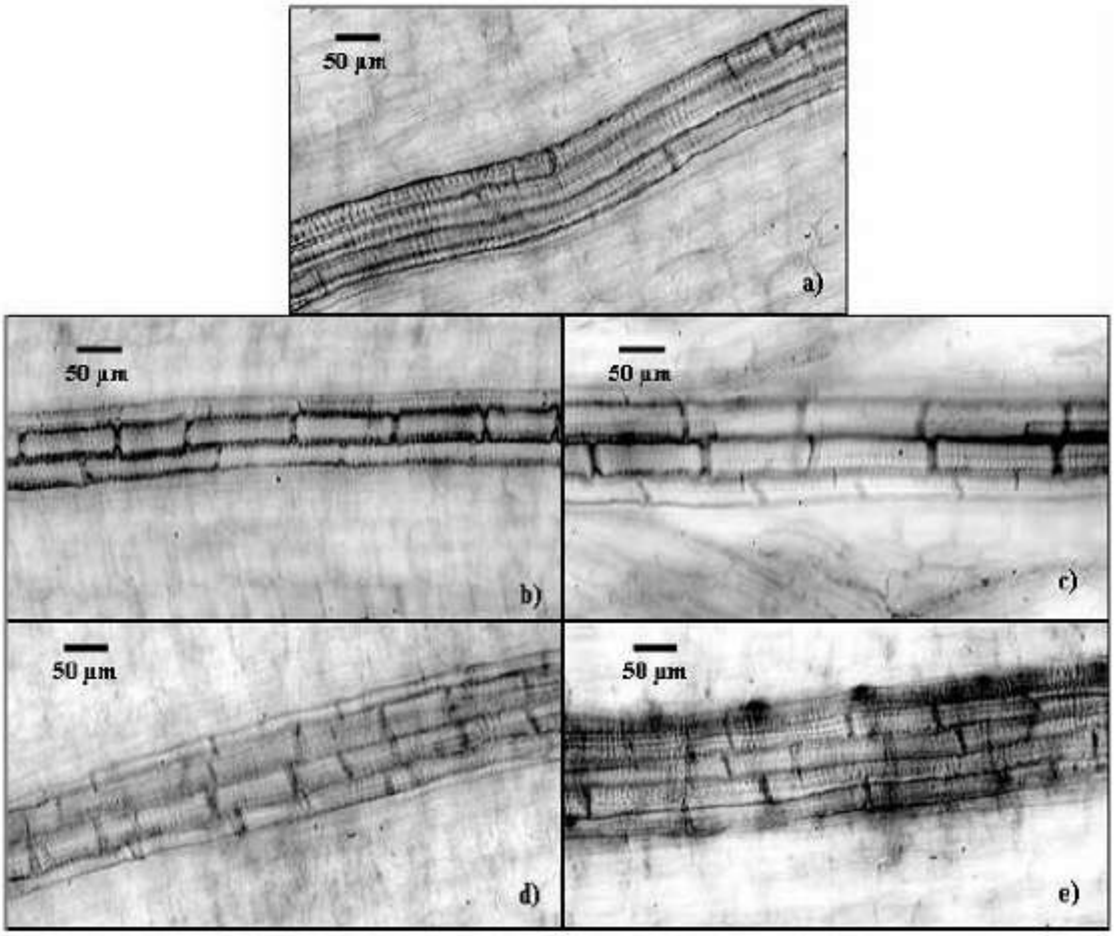

Figure 2. Metaxylem cells of sesame (*Sesamum indicum*) root grown in negative control (a) and with **Fy2** (b), **Fy3** (c), **Fy5** (d) and **Fm1** (e) at a concentration of 0.8 mg mL^{-1}. (Photo: Pereira, 2012).

Table 3. Length of sesame (*Sesamum indicum*) metaxylem cells (µm), subjected to the effect of different concentrations of fractions of *Serjania lethalis* leaf extracts.

Fraction / Control	Cell length (µm) compared with the control a)		
	Concentration (mg mL^{-1})		
	0.8	0.4	0.2
Fy2	113.50 cd	156.25 b	187.00 b
Fy3	165.25 b	144.50 b	149.00 bc
Fy5	95.50 d	144.25 b	159.50 bc
Fm1	141.25 bc	164.50 b	297.75 a
Oxyfluorfen	84.50 d	71.00 c	133.25 c

a) The average value of the control was 287.25 µm. Means followed by the same letter in the column do not differ by Tukey test at 0.05% probability.

DISCUSSION

The allelochemicals are capable of interfering with biochemical and physiological processes related to seedling growth and development (Weir *et al*., 2004). The absorption of water and minerals by the root is altered, enzymes in the plasma membrane are compromised, and protein synthesis, respiration and photosynthesis are impaired, leading to reduction in the size of the seedlings (Bogatek *et al*, 2005). Ethanolic extracts of *Serjania lethalis* leaves and stems were responsible for the inhibition of the growth of seedlings of weed species, especially in their roots, and in some cases the effect caused by the ethanolic extract was equal or superior to that caused by the herbicide used for positive control (Grisi *et al.*, 2013). These results corroborate those found in this study, in which the effect on root length caused by the fraction Fy5 at 0.8 and 0.2 mg mL^{-1} was statistically similar to that caused by the herbicide, but the same effect was not observed for shoot growth. The changes induced by the fractions prevent the seedlings from developing normally, compromising the seedlings' establishment in the environment.

Gatti *et al.*, (2010) obtained similar results with cells of sesame root metaxylem grown in aqueous extracts of *Aristolochia esperanzae* Kuntze. Cell growth is stopped under stressful conditions, with auxin being the agent responsible for the regulation of cell size, auxin controls shoot growth and root architecture, tropic responses to light, among other processes (Tamimoto, 2005). The fractions tested compromised the normal growth of metaxylem cells, thus affecting the development of tissues and, consequently, the plant's growth. The inhibition of seedling growth may be directly associated with inhibition of sesame metaxylem cell growth, as had been observed in the work of Grisi *et al.*, (2013), in which inhibition of seedling growth of wild poinsettia (*Euphorbia heterophyla*) was associated with the inhibition of sesame metaxylem cell growth. According to Al-Wakeel *et al.*, (2007), plant secondary metabolic compounds act directly on the process of cell division, as well as on the hormonal balance of the plant. The phytotoxic potential of young leaves is known, young leaves have chemical defenses against herbivory, so concentrate more secondary metabolites (Novaes, 2011). In addition, extracts from young leaves of sorghum (*Sorghum bicolor* L.) were more phytotoxic to the germination of lettuce (*Lactuca sativa* L.) and tomato (*Solanum lycopersicum* L.) that extracts of mature leaves (Marchi *et al.*, 2008). These results corroborate those found in this work, where the young leaves extracts of *S. lethalis* were more phytotoxic than extracts of mature leaves.

CONCLUSIONS

The fractions of ethanolic extracts of *Serjania lethalis* young and mature leaves were phytotoxic to the growth of sesame seedlings, however the fraction Fy5 of the ethanol extract of young leaves showed more pronounced effects. The reduction in the root growth of sesame seedlings seems to be associated with the inhibition of metaxylem cell elongation.

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