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Table: 1, Figs : 4

SHORT COMMUNICATION

Allelopathic potential of *Aulonemia aristulata* (Döll) MacClure, a native bamboo of Atlantic Rain Forest

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ABSTRACT

The effects of aqueous and ethanolic extracts of *Aulonemia aristulata* plant material were investigated on the germination and growth of *Lactuca sativa* and *Sesbania virgata.* The aqueous extract of leaves inhibited the germination of lettuce seeds, but not of *Sesbania virgata.* The aqueous extract of leaves was partitioned affording an aqueous and n-butanolic fractions, which caused 17.3 ± 10.4% and 94.6 ± 1.3% germination inhibition of *Lactuca sativa* seeds. All extracts were monitored by TLC analysis using flavonoids, phenolic acid and giberellic acid compounds as standards. An HPLC profile of aqueous extracts of its leaves was prepared based on their retention time and increase of peaks after co-injection with a mixture of authentic samples of flavonoids and fenolics acids compounds. The HPLC data indicated that three compounds were involved in germination inhibitory effects based on retention time as quercetin, rutin and ferulic acid, corroborating with literature information's. These preliminaries results showed that allelopathic interactions, between the bamboos *A. aristulata* and natives species, have been proposed as an explanation to bamboo-dominance in disturbed areas in Atlantic Forest.

Key words: Allelopathic potential, Atlantic Rain Forest, *Aulonemia aristulata,* bamboo, disturbed areas

INTRODUCTION

Understory bamboos are very important components of many forest types in Brazil (10) but bamboo-dominance seems to negatively influence the forest dynamics and forest physiognomy, probably reducing recruitment of tree spp. (14,15,16,22). Anthropogenic and natural disturbances have been proposed in studies carried out in Atlantic Rain Forest to explain bamboo-dominance (9,22). Albeit this aggressive growth of bamboo capacity could explain the physical exclusion of juvenile trees (15,16), some studies have demonstrated that several subtropical bamboo species exhibited allelopathic potential (4,5) due to allelochemicals (terpenoids, phenolic compounds, long chain fatty acids, organic cyanides, alkaloids) (17). They are released into the environment as

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leachates, root exudates, volatiles and/ or products of biomass decomposition. Their toxicity depends on the concentration, release rates, age and stage of plant, climate, season and environmental conditions (12).

The bamboo-dominance in Atlantic Rain Forest changes the forest physiognomy and is the major factor to prevent the forest regeneration (21). *Aulonemia aristulata* (Döll) MacClure is a bamboo species belonging to the subtribe Bambuseae, [common in Parque Estadual das Fontes do Ipiranga (PEFI) (São Paulo-SP), Atlantic Rain Forest]. This woody species, which grows upto 1.0 to 4.0 m height, has decumbent culms (8), flowers and dies synchronously (Grombone-Guaratini, In press). The suppression of germination and growth by chemical compounds released from the bamboo leaves may be responsible for success and persistence of *A. aristulata*.

This study aimed to evaluate the potential effects of *A. aristulata* extracts on germination and growth of *Sesbania virgata,* a native shrub and *Lactuca sativa* as a preliminary attempt to explain the dominance phenomenon of bamboo species in Atlantic Rain Forest.

MATERIAL AND METHODS

The leaves, stems and subterraneous parts of *Aulonemia aristulata* were collected from the Parque Estadual das Fontes do Rio Ipiranga, São Paulo - Brazil, in October 2006. The voucher specimen of *Aulonemia aristulata* was deposited at the Herbarium of Instituto de Botânica of São Paulo Maria Eneida Kauffman Fidalgo (SP 398161). The plant material was dried (40ºC), then ground to a powder and extracted with H_2O in 1:20 ratio (w/v) for 3 h. The residues of aqueous extract were then extracted with ethanol 70% by stirring at room temperature for 48 h. The extracts were filtered, concentrated under vacuum in a rotary evaporator and dried in a steam bath at 50^{o}C yielding the crude aqueous extract (AE) and ethanolic extract (EE) from leaves, stems and subterraneous parts. The osmotic concentration and pH of extracts were determined using WP4 Dewpoint potentiometer (Decagon) and a pH meter (21 HANNA) respectively.

Bioassays

The allelopathic potential of *Aulonemia aristulata* was tested on the seeds germination and seedling growth of *Lactuca sativa* and *Sesbania virgata* in Petri dish bioassays. This study consited of following four experiments (2 germination Bioassays, Growth assay and Chemical Screening).

Experiment 1. Germination Bioassay-I: The experimental treatments consisted of two factors: (I). *A. aristuculata* plants parts: 3 (leaves, stems, subterannean parts) and (II) Extracts: 2 (aqueous, ethanol). The aqueous extract (AE, 4.85 mg mL^{-1}) and ethanolic extract (EE, 7.8 mg mL^{-1}) of *A. aristulata* were tested on germination of *Lactuca sativa* and *Sesbania virgata* seeds. Fifty seeds of each test plant were sown per Petri dish in completely random design with four replicates. The filter paper (Whatman 42) was impregnated with 2.0 mL of solutions. The Petri plates were kept in the growth chamber (B.O.D.) for 48 h at 25°C and in constant light. Germinated seeds were counted 24 and 48

h after sowing. The AEL (Aqueous extract of leaves) of *A. aristulata* significantly inhibited the seeds germination of lettuce (84.0%) but not of *Sesbania virgata* seeds.

Experiment 2. Germination Bioassay-II: Its experimental treatments consisted of two factors: (I). Test plant spp. 2 (*Lactuca sativa, Sesbania virgata*) and (II). Aqueous extracts (AE, 4.85 mg mL^{-1}) of leaves of *A. aristulata* were tested. Fifty seeds of each test plant were sown per Petri dish in completely random design with four replicates. The filter paper (Whatman 42) was impregnated with 2.0 mL of the solutions. The Petri plates were kept in the growth chamber (B.O.D.) for 48 h at 25°C and constant light. Germinated seeds were counted 24 and 48 h after sowing. Since the highest inhibitory activity of *A. aristulata* was observed in leaves aqueous extract, this was further partitioned with H_2O/n-BuOH to obtain the aqueous fraction (FA) and n-butanolic fraction (F-but).

Experiment 3. Growth assay: Twenty five seeds of *Lactuca sativa* were placed in a Petri dish (9 cm diam) on Whatman filter paper soaked in water (control) or extract. After 24 h, the germinated seeds with radicles were shifted to Petri dishes moistened with 10 mL distilled water (control) or 10 mL AE (4.85 mg mL^{-1}) or EE (7.8 mg mL^{-1}) and were kept at 25°C, under constant light in growth chamber. The seedlings radicle and hypocotyls length were measured after 7 days. The effects of aqueous extract (AE, 4.85 mg mL^{-1}) of leaves (AEL), stems (AES) and subterraneous parts (AESP) and ethanolic extract (EE, 7.8 mg mL^{-1}) of leaves (EEL), stems (EES) and subterraneous part (EESP) of *Aulonemia aristulata* were tested on seeds germination of *Lactuca sativa*. Five concentrations of FA (3.8, 7.6, 15.2, 30.4 and 37.9 mg mL^{-1}) and F-but (0.24, 0.48, 0.97, 1.94 and 3.88 mg mL^{-1}) obtained fro Aqueous extract (AE) were used in growth assays. The experimental treatments consisted of three factors: (I). Extractants: 2 (Aqueous, Ethanol), (II). Plant parts: 3 (Leaves, stems, subterranneous parts) and (III). Fractions: 2 (Aqueous, Butanol). Test spp.: *Lactuca sativa.* Seeds were first germinated in Petri dishes and were later transplanted in Petri dishes for uniform growth in bioassay.

Experiment 4. Chemical Screening

Thin-Layer Chromatographic (TLC) Analysis: The extracts (200 µg), fractions (100 µg) and standard compounds (100 µg) were applied on TLC silica gel 60 F_{254} (Merck) eluted with up phase of n-butanol-glacial acetic-acid-water (BAW, 40:10:50, v:v) phase and observed under ultraviolet light (UV) in 254 nm and 366 nm, without chemical treatment. The flavonoids and phenolic compounds were detected with the spray reagents NP/PEG (2-(diphenylboryloxy)-ethylamine/ 1 polyethylene glycol reagent, Merck), vanillin-hydrochlo-ric acid reagent and aluminum chloride (1%, $AlCl_3$, Synth) and visualized under λ= 366 nm in system CAMAG-REPROSTAR 3. Quercetin dihydrate, rutin hydrate, ferulic acid and chlorogenic acid (Sigma-Aldrich Company) were used as standard compounds (29).

HPLC Analysis: The aqueous extract was analyzed in HPLC Varian Pro Star 310 system with a 20 µL loop using a Phenomenex C-18 (250 x 4.6 mm) column maintained at 20 °C. 20 µL of the sample (200 µg) was injected using as mobile phase water (0,1% TFA): ACN

(95:5 increase gradient until 30:70) and flow rate (0.6 mL mL^{-1}). Sample standards used were quercetin dihydrate, rutin hydrate, ferulic acid, chlorogenic acid, syringic acid, *p*-coumaric acid, 4-hydroxybenzoic acid and vanillic acid (Sigma).

Statistical analysis

The differences in the percentages of germination, radicle length and hypocotyl elongation were subjected to statistical analysis by Kruskal-Wallis test in PAST (2008). HPLC Data analysis was in retention time values (R*t*=min) of peak observed in the chromatograms and TLC in factor retention values (R*f*) of spots of extracts and standards compounds.

RESULTS AND DISCUSSION

The yield of AE of *A. aristulata* was 8.8% relative to the dried leaves (1.212 g), 6.37% (0.955 g) to the stems and 7.65% (1.147 g) to subterraneous parts (Table 1). The yield of EE was 12.74% (1.911 g) to leaves, 5.37% (0.806 g) to stems and 13.10% (1.965 g) to subterraneous parts. The *A. aristulata* extracts pH was 5.2 to 7.2 and the osmotic concentration was low – 0.26 MPa for aqueous extract and – 0.30 MPa for ethanolic extract. This water potential is considered low to cause inhibition of germination (2).

Table 1. Effects of aqueous (4.85 mg mL^{-1}) and ethanolic (7.8 mg mL^{-1}) extracts of leaves, stems, subterraneous parts and aqueous (3.88 mg mL^{-1}) and n-butanolic (0.97 mg mL^{-1}) fractions of *Aulonemia aristulata* on germination inhibition of *Lactuca sativa* seeds.

Treatment	Germination inhibition (%)
Distilled water	1.0 ± 0.6 a
AEL (Aqueous extract of leaves)	84.0 ± 5.7 b
AESP (Aqueous extract of subterraneous part)	11.5 ± 4.5 a
AES (Aqueous extract of stems)	19.5 ± 6.7 a
EEL (Ethanolic extract of leaves)	55.5 ± 7.0 c
EESP (Ethanolic extract of subterraneous part)	5.0 ± 3.1 a
EES (Ethanolic extract of stems)	6.0 ± 2.4 a
AF (Aqueous fraction)	17.3 ± 10.4 a
F-but (n-butanolic fraction)	94.6 ± 1.3 b

The AEL (4.85 mg mL^{-1}) and EEL (7.8 mg mL^{-1}) of *Aulonemia aristulata* significantly ($p < 0,05$) inhibited the germination of lettuce by 84.0% and 55.5%, respectively (Table 1), but did not inhibit the germination of *Sesbania virgata* seeds (data not shown). The lack of germination inhibition of *S. virgata* with the same extract showed that this native species can tolerate the leaching metabolites of *A. aristulata. Sesbania virgata* contains high amounts of (+)-catechin and quercetin in the seeds coat (24). However, its anatomical features makes rapid mobilization of its own chemical compounds (3). Our data suggest that this species can tolerate the same allelochemicals released by others species. Additional experiments are needed to investigate this mechanism.

The AES, AESP, EES and EESP did not present significantly activity in germination bioassay (Table 1). The aqueous extract (4.85 mg mL^{-1}) of leaves inhibited the

germination (Table 1); but stimulates the radicle length (134.5%) and hypocotyl elongation (41.2%). The inhibitory effects of aqueous extract of leaves of *A. aristulata* on seeds germination and growth of *Latuca sativa* may be due to high contents of phenolic compounds in its leaves. Thus we did the HPLC analyses of aqueous extract of leaves.

The AEL was partitioned into aqueous (3.88 mg mL^{-1}) and n-butanolic (0.97 mg mL^{-1}) fractions, which caused 17.3 ± 10.4% and 94.6 ± 1.3% germination inhibition of *Lactuca sativa* seeds (Table 1). The inhibitory potential of F-but was enhanced with an increase in its concentration (Fig. 1). The F-but concentrations (0.24, 0.48, 0.97, 1.94 and 3.88 mg mL^{-1}) inhibited the lettuce germination. The F-but fraction (0.24 to 1.94 mg mL^{-1}) was inhibitory to the radicle length (20% to 31%) and hypocotyl elongation (50%) when compared to the control (Fig. 3). On the other hand, FA fraction inhibited the germination only at higher concentrations (30.4 mg mL^{-1} inhibited 72%) (Fig. 2). The radicle length was stimulated by 211% and 100%, at lower concentrations of 1.94 and 3.88 mg mL^{-1} respectively. While higher concentrations of 7.6 and 15.2 mg mL^{-1} caused inhibition of 83% and 87%, respectively. The FA at 1.94 mg mL^{-1} increased the hypocotyl elongation by 21% and but its higher concentration (15.2 mg mL^{-1}) caused 52% reduction (Fig. 4). At higher concentrations (15.2 and 30.4 mg mL^{-1}) this fraction killed 25 and 100% seedlings, respectively. In all concentrations, the cotyledons showed some necrosis.

Seed germination, radicle growth and hypocotyl elongation assays are common response parameters in allelopathy tests (5,6). The radicle length is very sensitive to the allelochemicals (19). The inhibitory effects of aqueous extract and F-but on germination and growth of *Latuca sativa* could be related to the presence of higher concentrations of phenolic compounds in the complex mixture.

Chemical study of AEL of *A. aristulata* by TLC (366 nm) showed two orange spots with R_f 0.52 and 0.47, which are characteristics to flavonoids and three light blue fluorescence spots (R*f* 0.59; 0.65 and 0.74) probably to phenolic acid compounds. The analysis of extract by TLC using vanillin (1% HCl) and standard (+) catechin did not show their characteristic red colour in this extract. The spray reagent $AlCl_3$ showed a tail of light blue fluorescence from the beginning onwards. The compounds identified in AEL by HPLC were: ferulic acid (R*t* 27.09 min, 0.26 µg), quercetin dihydrate (R*t* 26.33 min, 41.59 µg), rutin (R*t* 23.11 min, 10.65 µg) and giberellic acid (R*t* 22.05 min, 17.89 µg). Other compounds were detected based in R*t* as chlorogenic acid (R*t* 20.40 min), syringic acid (R*t* 20.87 min), 4-hydroxybenzoic acid (R*t* 20.49 min) and vanillic acid (R*t* 22.16 min). Detection of flavonoids by TLC and HPLC in *A. aristulata* extracts are an indication that these compounds are involved in these effects.

Among the flavonoids presents in these bamboo species, rutin, quercetin and ferulic acid as well as syringic, 4-hydroxybenzoic and vanillic acids and a diterpenoid gibberellic acid could be involved in the inhibition of germination and growth. Potential allelopathic chemicals (*p*-coumaric acid, vanillic acid, and ferulic acid) have been isolated from *Phyllostachys edulis* leaves, which suppresses or eliminates the understory plants (5). Flavonoids compounds have shown allelopathic activity in other plants (7,11,13). The chemical and biological results suggest a probable synergistic or additive effects of compounds present in the aqueous extract that was partitioned in two fractions. On the oner hand, the aqueous fraction increased the radicle length and hypocotyl elongation at low concentrations, indicating some stimulatory activity of certain constituents. This effect

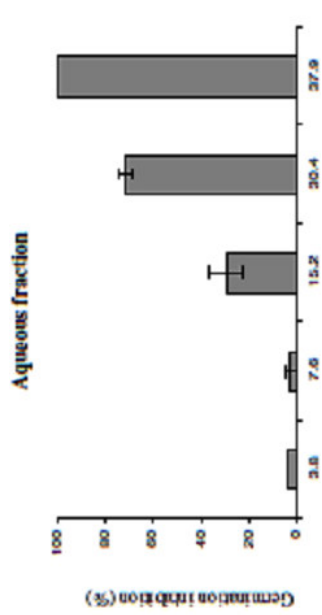

Figure 1. Curve concentration-effects of n-butanolic fraction of *Aulonemia aristulata* on germination of *Lactuca sativa* seeds.

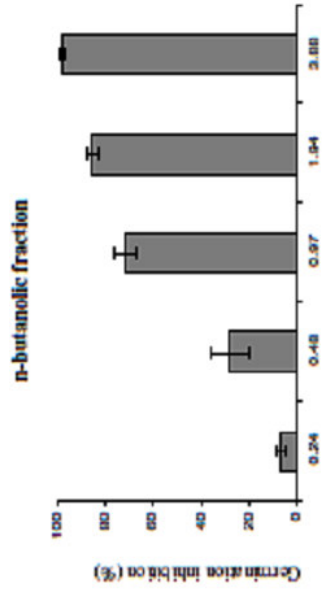

Figure 3. Changes in the radicle length and hypocotyl elongation of *Lactuca sativa* in response to different concentrations of n-buthanolic fraction of *Aulonemia aristulata*. Bars marked the same letter do not differ significantly at $p< 0.05$.

Figure 2. Curve concentration-effects of aqueous fraction of *Aulonemia aristulata* on germination of *Lactuca sativa* seeds.

Figure 4. Changes in the radicle length and hypocotyl elongation of *Lactuca sativa* in response to different concentrations of the aqueous fraction of *Aulonemia aristulata*. Bars marked the same letter do not differ significantly at $p< 0.05$.

can be attributed to giberellic acid (20) detected in the AEL. Several bamboo species (*Phyllostachys edulis*, *P. bambusoides* and *Sasa kurilensis*) contains the endogenous gibberellins (GAs) in young vegetative tissues (23).

One of the suggested explanations for the allelopathic effect of flavonoids is the modification in mitochondrial respiration after the decreased supply of ATP for all processes, which reduced the seedling growth (12). Quercetin may act on an electron transfer steps between quinone pool and oxygen (25). Likewise, the ferulic acid inhibits or delays the seed germination by reducing isocitrate lyase enzyme activity and to act on isocitrate lyase gene expression (18).

The phenomenon of allelopathy includes all types of chemical interactions among the plants and could account for the observation that certain plants sometimes do not grow in places where other plants grow (12). In *Phyllostachys edulis*, another bamboo species, the continuous release of water soluble phytotoxins and accumulation of these compounds in the soil suppresses the growth of understory or death of seedlings (5). The rapid dominance of *A. aristulata* and the growth suppression of tree species in disturbed areas in Atlantic forest are probably due to physical factors and allelopathic substances act synergistically as suggest in *Phyllostachys*.

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