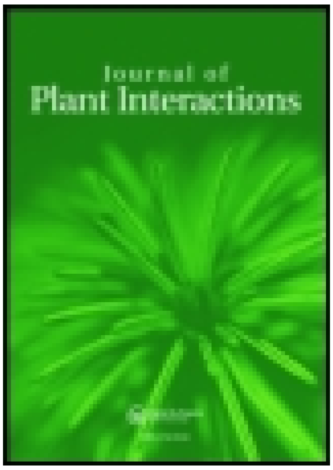

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In vitro allelopathic effects of extracts and amenthoflavone from Byrsonima crassa (Malpighiaceae)

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ORIGINAL ARTICLE

In vitro allelopathic effects of extracts and amenthoflavone from *Byrsonima crassa* (Malpighiaceae)

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Abstract
Extracts and pure amenthoflavone isolated from *Byrsonima crassa* (Malpighiaceae), a shrub growing in the semi-arid region of Brazil Cerrado, were evaluated *in vitro*, at different doses, for their effects on tomato seed germination and subsequent growth of seedlings. A hydromethanolic extract showed general stimulatory effects. The EtOAc extract stimulated root elongation and root weight of tomato; shoot elongation was inhibited, while shoot weight was not altered. The pure amenthoflavone isolated from the plant, stimulated shoot elongation at concentrations ranging between 10^{-4} M and 10^{-6} M.

Keywords: Byrsonima crassa*, amenthoflavone, tomato seeds, germination, shoot weight and elongation, root weight and elongation*

Introduction

Allelopathy plays an important role in agro-ecosystems leading to a wide range of influences and interactions in biotic communities. Effects can be direct or indirect on plants, including microorganisms, through the production of natural products released into the environment (Rice 1984). Researchers and farmers worldwide have recognized allelochemicals as a viable alternative to synthetic pesticides, in agro-ecosystems and the research in allelopathy is increasing in the fields of agriculture and forestry in order to reduce environmental pollution and increase agricultural production (Qasem & Foy 2001). Different strategies for allelochemical discovery have been proposed. Some authors have proposed approaches analogous to those for the discovery of lead compounds in the pharmaceutical industry involving the screening of crude extracts and purified compounds for biological activities (e.g., Macías et al. 2002). On the other hand, the observation in the field of chemical response(s) to environmental stresses to which plants are subject in ecosystems appears important.

The Cerrado is a semi-arid region of Brazil, in which plants are submitted to metabolic stress resulting in defense mechanism(s) activated when plants are confronted with unfavorable environmental conditions. As the plants from Cerrado produce a wide range of secondary metabolites, these species are used as natural medicines by people living in the area to treat several diseases (Almeida et al. 1998, Silva et al. 2001). Despite the chemical richness of these plants, and the fact that aromatic and medicinal plants are regarded as good sources of allelochemicals (Mathela 1994), there are no studies on the potential allelochemical properties of Cerrado species.

Byrsonima crassa Niedenzu (Malpighiceae) is a native species from the Brazilian savanna, popularly known as *murici-cascudo* or *murici-vermelho*. Its fruits are used as a food and the bark of the plant is used in traditional medicine as an antiemetic, a diuretic, a febrifuge and to treat ulcers, gastritis and diarrhea (Silva et al. 2001). We have recently reported the isolation from the plant of flavonoid derivatives, with the biflavonoid amenthoflavone as one of the major compounds (Sannomiya et al. 2004, 2005). No other phytochemical studies have been carried out on this plant.

As phenolics constitute an important group of allelochemicals, the present study has been

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conducted to evaluate the allelopathic potential of extracts and pure amenthoflavone from *B. crassa* on seed germination and seedling growth of tomato.

Materials and methods

Plant material

Byrsonima crassa was collected at Porto Nacional, Tocantins State, Brazil, and authenticated by Eduardo Ribeiro dos Santos. A voucher specimen of the plant (nr. 3377) is deposited at the Herbarium of the Tocantins University.

Extraction and isolation

The air-dried and powdered leaves (2.0 kg) of *B. crassa* were extracted successively with chloroform, methanol, and a methanol-water mixture (80:20) at room temperature (48 h for each solvent). Solvents were evaporated at 60°C under reduced pressure affording the $CHCl_3$ (53.8 g), MeOH (158.3 g) and MeOH-H_2O (95.7 g) extracts. A portion (10.0 g) of the MeOH-H_2O biologically active extract was partitioned with a mixture of EtOAc/H_2O (1:1, v/v), to obtain two portions. Amenthoflavone was isolated from the EtOAc portion according to methodology described by Sannomiya et al. (2004).

HPLC analysis of amenthoflavone

An aliquot of the MeOH-H_2O extract, its EtOAc soluble fraction and pure amenthoflavone were analyzed using a Varian ProStar HPLC system equipped with a RP-18 column (250 × 4.60 mm i.d., 5 μm, Phenomenex Luna). The mobile phase used a linear gradient of 32–38% (20 min), increasing 38–80% (40 min) maintained 80% (5 min) acetonitrile in water over 65 min eluted at flow rate of 0.8 ml min^{-1}, and the effluent was monitored using a ProStar 330 photodiode-array ultraviolet detection (DAD) system at 254 nm. The pure amenthoflavone was also identified by ^1H and ^{13}C NMR analyses obtained using a Varian INOVA 500 Spectrometer and by comparison with literature data (Castaneda et al. 1992).

Bioassay

A bioassay was carried out in order to evaluate some parameters of the germination of tomato seeds. Twenty-five seeds of tomato (*Lycopersicon esculentum* Mill. 'Rapid Growth') were grown for 7 days in continuous light at 25°C in 6 cm plastic Petri dishes containing a 5 cm sheet of Whatman No. 1 filter paper or 5 ml of tested extract or pure compound solution. At the seventh day, percentage of germination, and the length and weight of roots and shoots were measured. For EtOAc portion, amounts of 40, 100, 200, 300 mg were diluted in deionized H_2O. For amenthoflavone, a standard solution (10^{-4} M) was prepared and solutions of different concentratrions (10^{-5} to 10^{-7} M) were obtained by diluting the standard solution. pH values were adjusted to 6.0 before bioassay. Each experiment was carried out five times.

Statistical analyses

Data were subjected to analysis of variance (ANOVA) with significant differences between mean ($p < 0.001$).

Results and discussion

The hydromethanolic extract of *B. crassa* showed no effects on the germination of tomato seeds (data not shown). At the lower doses tested (40 and 100 mg/ml), hydromethanolic extract of *B. crassa* exerted stimulatory effects on the weight of both aerial parts and roots of tomato seedlings and on their root lenght. At higher doses, these parameters are inhibited. All doses tested showed stimulatory effects on root length and inhibitory effects on aerial parts length of tomato (Table I).

Both EtOAc extract and pure amenthoflavone did not show any effect on the germination of tomato (data not shown). At doses of 40 and 100 mg/l, the EtOAc portion stimulated root length (26.8% and 41.3%, respectively) and root weight (31.7% and 41.3%, respectively). Aerial parts length was inhibited at 200–300 mg/l (24.5% and 32.1%, respectively), but the weight of the same plant parts showed no statistical difference (Table II). Amenthoflavone

Table I. Effects of MeOH-H_2O extract of *B. crassa* on tomato growth, after 7 days from sowing. Data are expressed (mean of five replicates ± SD) as percentage of inhibition (−) or stimulation (+) respect to control.

	APW	RW	APL	RL
40 mg/l	+2.65 ± 0.11	+28.29 ± 0.08	−9.26 ± 0.03	+14.52 ± 0.25
100 mg/l	+5.70 ± 0.07	+45.85* ± 0.23	−22.36* ± 0.05	+26.92* ± 0.12
200 mg/l	−12.29 ± 0.120,01	+12.68 ± 0.11	−35.39* ± 0.04	+18.98 ± 0.10
300 mg/l	−16.23* ± 0.22	−27.80* ± 0.22	−29.39* ± 0.05	+9.26 ± 0.31

APW, aerial parts weight; RW, roots weight; APL, aerial parts length; RL, roots length.
*($p < 0.001$).

Table II. Effects of EtOAc extract of *B. crassa* on tomato growth, after 7 days from sowing. Data are expressed (mean of five replicates ± SD) as percentage of inhibition (−) or stimulation (+) respect to control.

	APW	RW	APL	RL
40 mg/l	+16.23 ± 0.05	+31.76* ± 0.03	−5.97 ± 0.35	+26.80* ± 0.34
100 mg/l	+27.43 ± 0.01	+41.39* ± 0.11	−6.28 ± 0.03	+41.32* ± 0.19
200 mg/l	−0.27 ± 0.01	−9.76 ± 0.22	−24.55* ± 0.04	+9.33 ± 0.59
300 mg/l	+10.52 ± 0.01	+6.34 ± 0.01	−32.13* ± 0.08	+4.14 ± 0.49

APW, aerial parts weight; RW, roots weight; APL, aerial parts length; RL, roots length.
*(p <0.001).

Table III. Effects of amenthoflavone from *B. crassa* on tomato growth, after 7 days from sowing. Data are expressed (mean of five replicates ± SD) as percentage of inhibition (−) or stimulation (+) respect to control.

	APW	RW	APL	RL
10^{-4} M	+0.13 ± 0.12	−6.05 ± 0.03	+19.34 ± 0.34	+4.53 ± 0.77
10^{-5} M	+5.47 ± 0.09	−16.98 ± 0.04	+35.81* ± 0.25	−9.66 ± 0.69
10^{-6} M	−3.31 ± 0.15	−25.81* ± 0.05	+31.26* ± 0.30	−18.02 ± 0.19
10^{-7} M	+9.85 ± 0.11	−36.05* ± 0.02	+26.84* ± 0.34	−8.29 ± 0.22

APW, aerial parts weight; RW, roots weight; APL, aerial parts length; RL, roots length.
*(p <0.001).

did not affect root length, but root weight was reduced at concentrations of 10^{-7} and 10^{-6} M (36.0% and 25.8%, respectively). Shoot length was stimulated at concentrations of 10^{-7}, 10^{-6} and 10^{-5} M (26.8%; 31.2% and 35.8%, respectively), without affecting the shoot weight of tomato plants (Table III).

These different responses to different concentrations of extracts or amenthoflavone agree with others which are available in allelopathic literature: Several inhibitory allelochemicals are known for their stimulatory activity on growth at low levels (Stebbing 1982). As far as our knowledge to date, there is no information available focusing on the allelopathic properties of *B. crassa*. The biflavonoid amenthoflavone was reported as an allelopathic compound from the Euphorbiaceous *Celaenodendron mexicanum* Standl with inhibitory effects on germination of seeds of *Amaranthus leucocarpus* S. Wats. (Amaranthaceae) and *Echinochloa crus-galli* P. Beauv. (Poaceae) (Castaneda et al. 1992). The same compound showed different activities on some phyotopathogenic Fungi growth: Stimulatory activity on *Helminthosporium* sp. and inhibitory effects against *Alternaria* sp. and *Fusarium* sp. (Castaneda et al. 1992).

Herbicides and agrochemicals based on natural products are attractive for a variety of reasons. Allelochemicals that suppress or eliminate interfering plant species near the source plant have received special attention due to their agricultural potential as selective natural herbicides (Rizvi & Rizvi 1992, Duke et al. 2002), and compounds responsible for the stimulation of germination and growth of other plants are also an important field of research (Mazzafera 2003).

Flavonoids are secondary metabolites with a wide range of biological activity and have attracted the attention of many researchers (Harborne 1999). Their physiological targets in plants include mitochondrial oxygen uptake, electron transport and the reduction in photosystem II efficiency (Moreland & Novitzky 1987a, 1987b, 1988). These multiple activities result in a generalized cytotoxicity (Einhellig 2004). Tomato plants were found to be more sensitive to flavonoids than other crop species. Macías et al. (1997) have shown that effects of flavanones and flavonols on tomato seeds depend on their stereochemistry and concentration, and that seedling growth is influenced, but germination and radical length are frequently not affected. Our results can contribute to address a sustainable agricultural approach on possible allelopathic properties of *B. crassa*.

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