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Fungitoxic effects of *Achyrocline satureioides* (marcela) on plant pathogens

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ABSTRACT

Achyrocline satureioides popularly known as "marcela" is a medicinal and aromatic plant native of Cordoba province in Argentina. In natural medicine is consumed as a tea and is recognized for anti-inflammatory, sedative, antispasmodic, emmenagogue and analgesic properties. In this study three extracts obtained from *A. satureioides* were evaluated in *in vitro* assay as an inhibitory agent of plant pathogenic fungi for it application in agricultural fields. Results showed greater inhibitory action on *Macrophomina phaseolina* growth with hexane and chloroform extracts (1000 µg mL^{-1}). Inhibitions were also observed with these extracts against *Fusarium graminearum*, *Fusarium verticillioides* and *Sclerotium rolfsii*. These results indicate that compounds with inhibitory activity of fungal growth are present in hexane and chloroform extracts of *A. satureioides*.

Keywords: Natural product, *Achyrocline satureioides*, Fungicidal activity, Phytopathogenic fungi.

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Introduction

The use of plant with therapeutic properties is as ancient as human civilization and for a long time, they were the main sources of drugs. In recent years, there has been a growing interest in alternative therapies especially those derived from plants (Rates, 2001).

At the present time, plant diseases control depend primary upon the application of chemical fungicides. However, these substances have the potential to exert toxic effects on humans and wildlife as well as to cause environmental pollution. Within this context, natural products from plants seem to be a good alternative since numerous plants have the potential to control phytopathogenic fungi, and have much prospect to be used as a fungicide. Additionally, natural products are generally easily biodegradable. In many countries there are now available in the market pesticides based on plant for the biological control of plant diseases. One example of those commercial products is developed with neem (*Azardirachta indica*) (Dubey *et al*., 2009).

In Argentina, there are numerous plants used in traditional medicine, one of this is *Achyrocline satureioides*, popularly known as "marcela" or "marcela del campo". This is a sub-bush that belongs to the family Asteraceae and is widely used in South America (Rivera *et al*., 2004). Experimental studies have shown hepatoprotection (Kadarian *et al*., 2002), antioxidant (Desmarchelier *et al*., 1998) antitumor and cytotoxic (Ruffa *et al*., 2002), antiviral (Zanon *et al*., 1999) and immunomodulatory properties (Cosentino *et al*., 2008). In spite of the widespread biological activities investigated for *A. satureioides* aerial part, there is no report of the activity on fungal plant pathogens growth.

The aim of the present study was to investigate the effect of different *A. satureioides* extracts on fungal plant pathogens growth.

Experimental

Plant extracts

Achyrocline satureoides was collected from their natural habitat in the mountainous region of the Córdoba province, Argentina in May 2008. The plant was identified by Ing. Luis del Vitto, professor in the Area of Botany of the Universidad Nacional de San Luis. A voucher specimen was deposited in the Herbarium of the same University (# 6362).

Aerial part were left to dried, powdered and successively extracted for 48 h at room temperature in n-Hexane (HE) and Chloroform (CE). Warm Aqueous Extract (WAE) was obtained when plant material was extracted with water at 70ºC for 48 h. Extracts were concentrated to dryness and dissolved in Dimethyl Sulfoxide (DMSO) to give a concentration of 100 mg mL^{-1}.

In vitro antifungal assay

The microorganisms used for the antifungal evaluation were the following plant pathogens of economical importance in agriculture: *Fusarium graminearum, Fusarium solani*, *Fusarium verticillioides, Macrophomina phaseolina* and *Sclerotium rolfsii*.

Agar dilution method

The extracts were added to molten Potato Dextrose Agar (PDA) to obtain a final concentration of 1000 µg mL^{-1} and then pour into the Petri dishes (9.0 cm in diameter). A 4 mm diameter plug of actively growing fungi, taken from PDA plates was placed onto the centre of Petri dishes; treatments were incubated at 30ºC. Each treatment was tested in triplicate; experiment was repeated 3 times. Parallel negative controls were included by mixing DMSO with PDA medium. Sensitivity of each fungal species to each tested extract was calculated as percentage of mycelial growth inhibition, according to the formula described by Pandey *et al*., (1982): (dc-dt)/dc x 100, where dc = average diameter of the fungal colony of the negative control and dt = average diameter of the fungal colony treated with the extract (Tegegne *et al*., 2008).

Microbroth dilution assay

The experiment was made following the guidelines of the National Committee for Clinical and Laboratory Standards (NCCLS) for filamentous fungi M-38-A2 in 96-well microplates (CLSI, 2008). The assay was made for *F. graminearum*, *F. solani*, *F. verticillioides* and *M. phaseolina*. Wells with different extracts concentration were inoculated with 100 µl of fungi inoculum. The plates were incubated at 30ºC for 48 h. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of extract at which no fungal growth was observed after incubation (Webster *et al*., 2008).

Broth dilution method

Potato Dextrose Broth (PDB) was prepared for estimation of *M. phaseolina* mycelial yield at 1000, 500 and 100 µg mL^{-1} of hexane, chloroform and WAE. Flasks containing 20 mL of PDB with appropriate volume of extracts were inoculated with 3 agar blocks (each of 2 mm diam) taken from a PDA plate of actively growing fungi and

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were incubated at 30ºC for 3 days. Thereafter, cultures were filtered through pre-weighed Whatman filter paper No. 1. Mycelial yield was determined after drying the mycelial at 75ºC for 5 days. Percent loss/gain in mycelial dry weight was calculated by using the formula: 100 × (C-T)/C, where C = mycelial dry weight in control, T= mycelial dry weight in treatment (Dubey *et al*., 2009).

Results and discussion

The activity of *A. satureioides* hexane, chloroform and warm aqueous extracts on fungi plant pathogens growth was analyzed by different methodologies. **Figure 1** present the results of the agar dilution method.

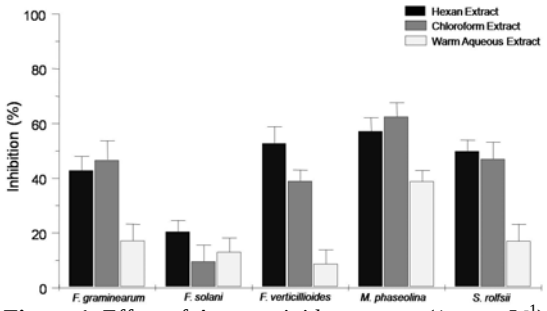

Figure 1. Effect of *A. satureioides* extracts (1 mg mL^{-1}) against *F. graminearum, F. solani, F. verticillioides, M. phaseolina* and *S. rolfsii* growth. Agar dilution method. Data are expressed as means (bars) ± S.E.M. (n = 9).

As shown in Figure 1, *A. satureioides* extracts exercised inhibitory action in 8-62% range against the phytopathogenic fungi tested. *Macrophomina phaseolina* was the most sensitive fungus and this result was observed with the 3 extracts. Radial mycelial growth inhibition were of 57, 62 y 39% for the EH, CE and WAE respectively. In addition, both EH and CE showed a good inhibition of *F. graminearum* (43 - 46%), *F. verticillioides* (52 - 39%) and *S. rolfsii* (49 - 47%). However, *F. solani* was lightly inhibited with all the extracts at the concentration tested (inhibition < 20%). On the other hand, the WAE was the least bioactive. The disc diffusion method was also assayed but no sensitiveness was observed (data not showed).

Table 1 present the results of the MIC obtained with the microbroth dilution method. The MIC for *M. phaseolina* with the HE and the CE was of 1000 µg mL^{-1}. For the other fungi tested the MIC was higher than 2000 µg mL^{-1}, except for *F. graminearum* (MIC of 2000 µg mL^{-1}).

Table 1: MIC (µg mL^{-1}) of *A. satureioides* extracts. Microbroth dilution method.

PLANT EXTRACTS	*Fungal strains*			
	Fg[a]	***Fs***[b]	***Fv***[c]	***Mp***[d]
Hexanic	>2000	>2000	>2000	1000
Chloroformic	2000	>2000	>2000	1000
Warm aqueous	>2000	>2000	>2000	>2000

Fg[a]*: F. graminearum;* ***Fs***[b]*: F. solani;* ***Fv***[c]*: F. verticillioides;* ***Mp***[d]*: M. phaseolina.*

The action of *A. satureioides* extracts at different concentrations in broth dilution method was evaluated against *M. phaseolina* because it was the most sensitive fungi in the previous methodologies. **Figure 2** shows the results.

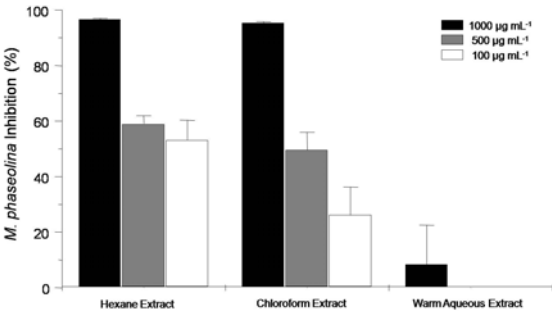

Figure 2. Effect of *A. satureioides* extracts against *M. phaseolina* growth. Broth dilution method. Data are expressed as means (bars) ± S.E.M. (n = 6)

Both HE and CE inhibited mycelia yield of *M. phaseolina* in 96 and 94% at 1000 µg mL^{-1} respectively. When the extracts were employed in 100 µg mL^{-1}, the inhibition exercised by of HE remains high, with a reduction in mycelia yield of 53%, while CE only inhibited 25%. WAE affect lightly the normal growth of *M. phaseolina* at the high concentration tested when compared to the negative control.

Other authors report antifungal activity of plants against plant pathogens in Argentina as Carpinella *et al*., in 2003 that publish a work were they study extracts of *Melia azedarach* L. (paraiso) as a potential antifungal agent against phytopathogenic fungi. They found 3 compounds isolated from the seed ethanolic extract active against *F. verticillioides.*

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Conclusions

Our results shows that *Achyrocline satureioides* hexane and chloroform extract inhibit fungal growth in *in vitro* assays and may have potential used as a fungicide in agriculture. It is important to continue investigating the biological properties of this specie and identify the active compounds that are present in the extracts.

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