Fungitoxic activity of *Zuccagnia punctata* extracts

C. M. Jiménez¹, D. A. Sampietro¹, V. González²; J. R. Soberón¹, M. A. Sgariglia¹ and M. A. Vattuone¹

¹Cátedra de Fitoquímica, Instituto de Estudios Vegetales “Dr. A. R. Sampietro”, Facultad de Bioquímica., Química y Farmacia, Universidad Nacional de Tucumán, España 2903 (4000), S. M. Tucumán, Argentina. dasampietro2006@yahoo.com.ar. ²Sección Fitopatología, Estación Experimental Agroindustrial Obispo Colombres. Willian Cross 3150 (T4101XAC), Las Talitas, Tucumán, Argentina. laboratoriofito@eeaoc.org.ar.

ABSTRACT

The antifungal activities of aqueous (infusion and decoction) and ethanolic (tincture) extracts from a medicinal plant, *Z. punctata*, were evaluated on important fungal pathogens isolated from major crops of Argentina. Fungal species were *Fusarium oxysporum*, *F. thapsinum*, *F. verticillioides*, *Macrophomina phaseolina* and *Rhizoctonia solani*. Percentage of hyphal growth inhibition was evaluated by agar dilution method. Tincture was the most fungitoxic extractive form assayed. Inhibition of mycelial growth increases with concentration. Our results suggest that *Z. punctata* has a broader fungicidal activity than that previously showed and most fungicidal principles were in the tincture. Further research is needed to identify and characterize these bioactive compounds.

Keywords: *Z. punctata*, fungal pathogens, extract and crops in Argentina

Corresponding author: D. A. Sampietro: Cátedra de Fitoquímica, Instituto de Estudios Vegetales “Dr. A. R. Sampietro”, Facultad de Bioquímica., Química y Farmacia, Universidad Nacional de Tucumán. España 2903 (4000), S. M. Tucumán, Argentina. dasampietro2006@yahoo.com.ar

Received: February 22, 2010. Accepted: March 10, 2010
Introduction
Phytopathogenic fungi reduce 20% harvest yields in Argentina and are often controlled using synthetic fungicides. Nevertheless, these compounds suffer a loss of biocide effectiveness and have a slow biodegradation (Brady, 1984). Plants can provide more environmentally friendly fungitoxic compounds (Quiroga et al., 2001). One example is Zuccagnia punctata (Cav.) which showed antifungal activity (Quiroga et al., 2001). This medicinal plant is used as antiseptic pedic, and to treat arthritis, rheumatism, fever, edema, infections and tumors (Ratera et al., 1980). The aim of this study was to evaluate the antifungal activity of aqueous and ethanolic extracts of Z. punctata on pathogens affecting production and marketing of major crops in Argentina.

Experimental
Zuccagnia punctata Cav. was collected in Tucuman province (Argentina) and identified at Instituto de Estudios Vegetales "Dr. A. R. Sampietro" (FBQF, UNT, Tucumán, Argentina). Phytopathogenic fungi were isolated by INTA (Pergamino-Leales) and Sección Fitopatología of EEAOC (Tucumán): Rhizoctonia solani, Fusarium oxysporum, F. verticillioides, F. thapsinum and Macrophomina phaseolina. They were cultured and maintained in modified rose bengal agar (RBA) and modified peptone malt agar (PMA).

Aerial parts of Zuccagnia punctata were dried at 45 °C for 5 days. The dry material was powdered and extracted by: 1) Decoction: 5 g of powder in 50 ml of boiling distilled water, boiled for 20 min. 2) Infusion: 5 g of powder in 50 ml of boiling water. Both decoction and infusion were filtered through Whatman No. 1 filter paper and lyophilized. 3) Tincture: 10 g of powder in 100 ml ethanol 96%. After incubation at 37°C (40 cycles/min) for 7 days, suspension was filtered through Whatman No. 1 filter paper and dried at 30 °C.

Dry residues were weighed, dissolved in methanol (concentrated extract, CE) and kept at -20°C. An aliquot of each CE was evaporated at 40°C. Dry weight was extracted material (EM) of each extracting form. Extraction efficiency was calculated.

Each CE was diluted with ethanol 96% to 4, 8, and 16 mg of EM/ml. 0.5 ml of each dilution was incorporated into 4.5 ml of warm PMA medium obtaining final concentrations of 0.4, 0.8 and 1.6 mg EM/ml. Controls were 0.5 ml of ethanol 96% in 4.5 ml of PMA. 3 mm diameter-mycelial plugs were placed in the center of PMA plates. Then, they were incubated at 30°C for 3 to 4 days in a moist environment. Average diameter of mycelial growth was determined. Percent of growth inhibition was calculated: % inhibition = [(MGC-MGE)x100]/MGC. Where MGC is average diameter of mycelial growth in the control and MGE is average diameter of mycelial growth in the presence of a tested extract dilution.

Results and discussion
Zuccagnia punctata inhibited growth of the phytopathogenic fungi tested. Antifungal activity was higher at increasing concentrations of assayed extracts (Fig 1,2 and 3).

Tincture had a higher extraction yield (35.7%) than infusion (21.2%) and decoction (24.5%). Tincture exerted a stronger growth inhibition than the aqueous extractive forms. This situation was also observed for other folk argentinian plants (Davicino et al., 2007).

In our work, decoction inhibited the growth of F. thapsinum, M. phaseolina and R. solani more than infusion. Conversely, F. oxysporum was more sensitive to infusion. In the case of F. verticillioides, the inhibitory effect of decoction and infusion was similar.
Figure 3. Antifungal activity of tincture obtained from *Z. punctata*.

**Conclusions**

Our results suggest that *Z. punctata* has a broader fungicidal activity than that previously showed (Quiroga *et al.*, 2001). Most fungicidal principles were in the tincture. Further research is needed to identify and characterize these bioactive compounds.

**Acknowledgements**

This work was supported by grants CIUNT 26 D455-2, PICT 2006-850 and PICT - PAE 077/07. Lic. Marisol Jimenez wants to thank to CIUNT (UNT) for her doctoral fellowship.

Note: Part of this study was presented at the ‘II Reunión de Biotecnología aplicada a plantas medicinales y aromáticas’ (Second Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2009.

**References**