

# The antifungal effect of *Jacquinia macrocarpa* plant extracts on the growth of *Aspergillus flavus*, *A. parasiticus* and *Fusarium verticillioides*

Daniel Fernando Valenzuela-Cota, Génesis Vidal Buitimea-Cantúa, Ema Carina Rosas-Burgos  
Francisco Javier Cinco-Moroyoqui, María Susana Yépez-Gómez, Mario Onofre Cortez-Rocha  
Maribel Plascencia-Jatomea, Armando Burgos-Hernández

Departamento de Investigación y Posgrado en Alimentos. Universidad de Sonora. Blvd. Luis Encinas y Rosales s/n. Col. Centro. C.P. 83000  
Hermosillo, Sonora, México

## Efecto antifúngico de los extractos de la planta *Jacquinia macrocarpa* en el desarrollo de *Aspergillus flavus*, *A. parasiticus* y *Fusarium verticillioides*

**Resumen.** El objetivo de este estudio fue evaluar el efecto de fracciones obtenidas de la planta *Jacquinia macrocarpa* contra los hongos fitopatógenos *Aspergillus flavus*, *A. parasiticus* y *Fusarium verticillioides*. Polvos de la planta deshidratada fueron extraídos con metanol (70 % v/v) y particionados con hexano, acetato de etilo y n-butanol. La fracción con mayor actividad contra el crecimiento radial de los hongos fue adicionalmente fraccionada por cromatografía en sílica gel. La fracción particionada en n-butanol (BF) mostró la mayor actividad antifúngica contra los tres hongos. La sub-fracción F5.4 inhibió el crecimiento radial de *F. verticillioides* completamente, mientras que el de *A. flavus* y *A. parasiticus* fue inhibido en 70 % y 64 %, respectivamente. La germinación de esporas de *A. parasiticus* y *F. verticillioides* fue inhibida en 7.8 % y 11.8 %, respectivamente. La longitud y el diámetro de las hifas de los tres hongos fueron menores que los de los controles. El diámetro de las esporas de las especies de *Aspergillus* fueron reducidas, mientras que las esporas de *F. verticillioides* no fueron afectadas. La viabilidad de *A. flavus*, *A. parasiticus* y *F. verticillioides* fue reducida en 40.7 %, 44.8 % y 46.3 %, respectivamente. La fracción F5.4 inhibió el desarrollo de los tres hongos significativamente, principalmente a *F. verticillioides*.

**Palabras clave:** actividad antifúngica, patógenos del maíz, extractos de plantas

**Abstract.** The aim of this study was to evaluate the effect of fractions obtained from the plant *Jacquinia macrocarpa* against phytopathogenic fungi *Aspergillus flavus*, *A. parasiticus* and *Fusarium verticillioides*. The powder of the dried plant materials was extracted with methanol (70 % v/v) and partitioned with hexane, ethyl acetate, and n-butanol. The most active fraction against the radial growth of the fungi was further fractionated by silica gel chromatography. The fraction from *J. macrocarpa* partitioned in n-butanol (BF) showed the highest antifungal activity against the three fungi. Sub-fraction F5.4 completely inhibited the growth of *F. verticillioides*, whereas the radial growth of *A. flavus* and *A. parasiticus* was inhibited 70 % and 64 %, respectively. Spores germination of *A. parasiticus* and *F. verticillioides* was inhibited 7.8 % and 11.8 %, respectively. The lengths and diameters of hyphae of the three fungi were smaller than those of controls. Spore diameters of the *Aspergillus* species were reduced, while spores of *F. verticillioides* were not affected. The spore viability of *A. flavus*, *A. parasiticus* and *F. verticillioides* was reduced 40.7 %, 44.8 % and 46.3 %, respectively. Fraction F5.4 significantly inhibited the development of the three fungi, mainly *F. verticillioides*.

**Keywords:** antifungal activity, maize pathogens, plant extracts

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Autor para correspondencia: Ema Carina Rosas-Burgos  
ecrosas@guayacan.uson.mx

## Introduction

The growth of mycotoxigenic fungi during culture and storage of corn causes serious economic losses and human and animal health problems in producer and consumer communities (Nelson, 1992; Nichols, 1983). Aflatoxins, produced mainly by *Aspergillus flavus* Link and *A. parasiticus* Speare (Dorner *et al.*, 1984) and fumonisins, produced mainly by *Fusarium verticillioides* (Sacc) Nirenberg (synonym: *Fusarium moniliforme* J. Sheldon) [teleomorph: *Gibberella fujikuroi* (Sawada) Wollenw or *Gibberella moniliformis* Wineland] (Nelson, 1982), cause acute and chronic toxicity in animals of economic importance and are associated to cancer in humans (Eaton and Groopman, 1994; Constable *et al.*, 2000; Marasas *et al.*, 1984). Aflatoxin B<sub>1</sub> is classified as potent carcinogenic agent, whereas fumonisin B<sub>1</sub> has been considered as possible carcinogenic to human (IARC, 2002).

Synthetic fungicides have been employed for controlling pre- and postharvest diseases in plants (Sommer, 1985). However, in recent years, there has been a strong interest on biologically active plant compounds as potential alternatives to synthetic hazardous fungicides as that many microorganisms develop resistance to synthetic fungicide compounds (Cowen and Steinbach, 2008). In addition, use of synthetic fungicides has been increasingly restricted in many countries due to their entrance into the food chain (Soković and Van Griensven, 2006). Plants are generally assumed to be safer alternative than synthetic compounds (El-Ghaouth, 1997) and can be used as alternative antifungal treatments (Jobling, 2000; Rahman *et al.*, 2010).

*Jacquinia macrocarpa* Cav. (San Juanico) is a plant used in the traditional medicine by the native people of Mexico (López-Estudillo and Hinojosa, 1988). The antimicrobial activity of some species of *Jacquinia* genus has

been studied. An ethanolic extract of *Jacquinia ruscifolia* petals showed antifungal activity against ten of twelve pathogenic molds evaluated (Sharma *et al.*, 2008). The methanolic crude extract from roots of *Jacquinia flammea* showed moderate antifungal activity against dermatophytes and very strong antifungal activity against *Colletotrichum gloeosporioides*. In that study the compound sakurasosaponin was reported as the main metabolite responsible for the antifungal activity (García-Sosa *et al.*, 2011). Okunade and Wiemer (1985) found that the jacquinonic acid extracted from *Jacquinia pungens* showed ant repellent activity. Despite of all those studies, there is no research on the antimicrobial activity of *Jacquinia macrocarpa* specie and their affect against micotoxigenic fitopatogenic molds. The aim of this study was to evaluate the effect of *J. macrocarpa* crude and purified extracts against *A. flavus*, *A. parasiticus* and *F. verticillioides*.

## Materials and methods

### Plant materials

Aerial parts of the wild plant *Jacquinia macrocarpa* were collected in Los Arrieros Ranch, Guaymas, Sonora, México, a rural community located at the southwestern area of the state of Sonora (geographic coordinates: 28°17'00'' N, 11°02'00'' W). Identification of the plant species was carried out at the Herbarium of Departamento de Investigaciones Científicas y Tecnológicas de la Universidad de Sonora (DICTUS) in Hermosillo, Sonora, México (Voucher USON2008-6). Once in the laboratory, the plant samples were cut into small pieces and dried at room temperature (approximately 30 °C) during 15 days, in the dark.

### Fungal cultures

*Aspergillus flavus* Link (NRRL 55210) and *A. parasiticus* Speare, anamorph (ATCC 16992) were inoculated on potato

dextrose agar (PDA, Difco Laboratories; Detroit, MI) and incubated in the dark at 27 °C for 7 days until sporulation. *Fusarium verticillioides* (Sacc) Nirenberg (syn. *Fusarium moniliforme* J. Sheld, anamorph (ATCC 52539) was grown at the same conditions, except that the incubation temperature was 25 °C. Spores were harvested, counted using a Neubauer counting chamber and stored at 4 °C.

### Preparation of plant extracts

Sixty grams of powdered aerial parts of *J. macrocarpa* were extracted with 1 L of 70 % methanol, stirred for 1 h, and stored at room temperature for 72 h in the dark. After that, the extract was filtered first through Whatman filter paper No. 1 and then through micropore glass filter. The methanolic extract (crude extract) was evaporated to dryness at 40 °C under reduced pressure (19 % of yield was obtained), re-suspended in water, and sequentially partitioned with hexane, ethyl acetate and n-butanol (Koketsu *et al.*, 1996), yielding 48.0 %, 6.4 %, 5.0 % and 27.0 % of solids, respectively. The crude and partitioned extracts were evaluated in their antifungal activity.

### Chromatography in silica gel

The antifungal fraction obtained after partition with butanol (BF) was purified via column chromatography (2.5 × 100 cm) using silica gel 60 (Sigma-Aldrich Chemical Co., St. Louis, MO, particle size 70-230 mesh) and further fractionated by continuously washing the column with 500 mL of a solvent mixture containing butanol:acetic acid:water (4:1:5). Fractions of 5 mL were collected using a fraction collector. With the aim to identify and discard those fractions containing compounds of the preceding band, aliquots of 5 µL of every second fraction were analyzed by thin layer chromatography (TLC) using a precoated Kieselgel 60 F254 TLC plates (Merck Co., Darmstadt, Germany) and developed with the same solvent system used in the column separation. Spots in the TLC plates were visualized under UV light. The number of spots visualized on TLC indicated that five fractions eluted

from the column and were identified as F1 to F5 according to their order of elution. All these fractions were evaporated to dryness at 40 °C under reduced pressure, dissolved in methanol, and assayed for antifungal activity. Fraction F5, which showed the highest antifungal activity, was further fractionated by TLC using 2 mm thickness plates (DC-Fertigplatten Si G-200 UV254) using the same solvent system employed in the column separation (Buitimea-Cantúa *et al.*, 2010). Five fractions were obtained and identified as F5.1 to F5.5.

### Radial extension growth

Petri dishes with potato dextrose agar (PDA) media containing 5 mg mL<sup>-1</sup> of solids from plant extracts were centrally point-inoculated with 1 × 10<sup>4</sup> spores mL<sup>-1</sup> from 7-day-old cultures of *A. flavus*, *A. parasiticus*, and *F. verticillioides*. Two types of controls were prepared, one contained PDA media plus aliquots of each solvent used to dissolve the solids of the antifungal fraction, and the other one contained only PDA media. The inoculated Petri dishes were incubated in the darkness at 27 °C to grow *Aspergillus* species, or at 25 °C for *F. verticillioides*. The colony diameters were measured every 24 h during 336 h with the help of a caliper. The experiment was run in triplicate (Suárez-Jiménez *et al.*, 2007).

To determine the most appropriate concentration of the antifungal fractions to be used in subsequent analyses, the following experiment was made. The partitioned fraction that showed the highest inhibition activity (n-butanol fraction) was evaporated to dryness under vacuum at 45 °C and re-dissolved in methanol. Petri dishes containing PDA media plus 1.0, 2.0, 3.0, 4.0 and 5.0 mg mL<sup>-1</sup> of the extract were inoculated with 1 × 10<sup>4</sup> spores mL<sup>-1</sup> from 7-day-old cultures of each fungus. Measurement of colonies radius was carried out to determine the extract concentration that caused the maximum radial growth inhibition (MGI), the minimum inhibitory concentration that inhibited 50 % of radial growth

(MIC<sub>50</sub>), and the minimum lethal concentration (MLC) that inhibited 100 % of radial growth and that did not succeed when it was transferred to fresh culture media containing no antifungal fraction (Rosas-Burgos *et al.*, 2009). The experiment was run in triplicate.

The radial growth inhibition percentages were calculated using the following formula:

$$\text{Radial inhibition (\%)} = \frac{(R_c - R_i)}{R} \times 100$$

Where  $R_c$  is the mean value of colony radius of control PDA and  $R_i$  is the colony radius value of colonies grown in PDA media containing plant extracts taking into account the solvent effect (Plascencia-Jatomea *et al.*, 2003).

#### Kinetics of spores germination

PDA plates containing 5 mg mL<sup>-1</sup> of solids of the antifungal fraction F5.4 were inoculated by spreading 20 µL of a spore suspension containing 1 x 10<sup>5</sup> spores onto the agar surface. The plates were incubated at 27 °C to grow *Aspergillus* species or 25 °C to grow *F. verticillioides*. The number of germinated spores per plate was determined every 3 h by counting 200 spores (germinated and non-germinated) using a light microscope. A spore was considered as germinated when the length of its germinal tube reached one-half of the spore diameter (Paul *et al.*, 1993, Plascencia-Jatomea *et al.*, 2003). Each germination assay was made in duplicate. Two types of controls were prepared, one contained PDA media plus methanol, and the other one contained only PDA media. The inhibition of spore germination was calculated using the following equation:

$$\text{Germination inhibition (\%)} = \frac{(\bar{S}_c - S_e)}{\bar{S}_c} \times 100$$

Where  $S_e$  represents the percentage of germinated spores in samples treated with the antifungal fraction F5.4, and  $S_c$  is the percentage of germinated spores observed in the

control containing methanol.

#### Hyphal diameter and length

Petri dishes with potato dextrose agar (PDA) containing 3.0 mg mL<sup>-1</sup> of the antifungal fraction F5.4 was centrally point-inoculated with 1 x 10<sup>4</sup> spores mL<sup>-1</sup> from 7-day-old cultures of the fungi. After 72 h of incubation, one hundred measurements of diameters and lengths of apical hypha were made on mycelia. The measurements were carried out using the Image-Pro Plus version 6.3 software (Media Cybernetics, Inc.) as reported by Larralde *et al.* (1997) and Cox *et al.* (1998). One hundred measurements were also made on mycelia grown in the control plates. The experiment was repeated twice.

#### Spores diameter

Spores diameter was determined according to the procedure of Harris (1999). Coverslips were placed in Petri plates and covered with 10 mL of potato dextrose broth (PDB) containing 5.0 mg mL<sup>-1</sup> of F5.4. Two types of controls were prepared, one contained PDB plus a volume of methanol equal to that used to dissolve the solids of the antifungal fraction, and the other one contained only PDB. The plates were inoculated with 20 µL of a 1 x 10<sup>5</sup> spore suspension of *A. flavus*, *A. parasiticus*, or *F. verticillioides*, and their development was monitored until they germinated. One coverslip containing spores was removed at random every 3 h from the plates and 100 measurements of spore diameter were carried out using the Image-Pro Plus version 6.3 (Media Cybernetics, Inc.). For *F. verticillioides* spores, the measurements were made wide- and length-wise. The experiment was repeated twice.

#### Spores viability

Spores viability analysis of the fungi was made according the methodology described by Granjo *et al.* (2007). Discs of sterilized cellophane measuring 2.5 cm in diameter were

distributed with sterile forceps on the surface of a Petri dish containing solidified PDA medium plus 5 mg mL<sup>-1</sup> of F5.4. Two types of controls with no cellophane discs were prepared, one contained PDA media plus a volume of methanol equal to that used to dissolve the solids of the antifungal fraction, whereas the other one contained only PDA media. The cellophane discs were inoculated with 50 µL of a 1 x 10<sup>5</sup> spore suspension of each fungus and incubated at 27 °C to grow *Aspergillus* species or at 25 °C to grow *F. verticillioides*. The cellophane discs were incubated by 0, 12, 18, 24, 48, 72 y 96 h. At the end of each incubation time, each cellophane disc was removed from the Petri dish and placed over a microscope slide and covered with equal volumes of final working solutions of FDA [3,6' diacetyl fluorescein-FDA (Sigma Chemical Co., St. Louis, MO, USA); 5 mg mL<sup>-1</sup> acetone and diluted 2500 times in phosphate-bufered saline (PBS), pH 7.4 at the time of use] and EB [2.7-diamino-10-ethyl-9-phenyl phenanthridine (Sigma Chemical Co.); 1 g mL<sup>-1</sup> in PBS and diluted 20 times in PBS at the time of use] (Granjo *et al.*, 2007). The treated slides were incubated at 25 °C for 30 min, covered with a coverslip, and observed under a fluorescence microscope (Leica DM 2500). One hundred spores were analyzed for each incubation time and the proportion of the green-colored viable spores and the red-colored non-viable spores was determined. The experiment was repeated twice.

#### Statistical analysis

Analysis of data was carried out by ANOVA using a factorial design (plant and extraction solvent). Comparison of means was performed by the Tukey test ( $P < 0.05$ ) using the SAS program (SAS, 2005).

## Results

### Radial extension growth

The crude methanolic extract from *J. macrocarpa* (CE) and every partitioned fraction showed radial growth inhibition against the three fungi ( $P < 0.05$ ), with major inhibition effect against *F. verticillioides* (Table 1). The n-butanol fraction (BF) showed the highest radial growth inhibition against the three fungi. *F. verticillioides* did not show statistical differences ( $P < 0.05$ ) in inhibition growth by BF and CE. Similarly, no statistical differences ( $P < 0.05$ ) were also observed in inhibition growth of *A. parasiticus* by AEF and WF, whereas the radial growth inhibition values of *A. flavus* by CE and all fractions differed significantly ( $P < 0.05$ ). The antifungal fractions obtained in the different stapes of purification (F5 and F5.4) showed high radial growth inhibition activity against the three fungi in comparison to those of the partitioned fractions and CE. The radial growth of *F. verticillioides* was totally inhibited when it was inoculated on PDA containing fractions F5 and F5.4.

### MGI, MIC<sub>50</sub> and MLC of BF

The three fungi showed gradual radial growth inhibition when the BF concentration in the PDA media was increased (Table 2). The MGI for *A. flavus* and *A. parasiticus* was 5.0 mg mL<sup>-1</sup>, which caused that the fungi did not reach 50 % of radial growth inhibition. *F. verticillioides* showed MGI and MIC<sub>50</sub> values of 4.0 mg mL<sup>-1</sup> and 1.0 mg mL<sup>-1</sup>, respectively. In the presence of BF, the radial growth inhibition of *A. flavus* and *A. parasiticus* were 70 % and 64 %, respectively. However, when *F. verticillioides* was inoculated on PDA media containing the fractions obtained from BF, its radial growth was completely inhibited (Table 1) although was able to grow when it was transferred to PDB containing no antifungal fraction. The influence of the variable concentration of the

Table 1. Inhibition of radial growth (%) of fungi by several *Jacquinia macrocarpa* fractions

Treatment <sup>1</sup>	<i>A. flavus</i>	<i>A. parasiticus</i>	<i>F. verticillioides</i>
CE	36.0±0.06 <sup>b</sup>	19.0±0.06 <sup>c</sup>	94.4±0.07 <sup>a</sup>
HF	34.4±0.05 <sup>c</sup>	14.0±0.07 <sup>d</sup>	66.8±0.05 <sup>e</sup>
BF	40.0±0.10 <sup>a</sup>	38.0±0.32 <sup>a</sup>	94.4±0.10 <sup>a</sup>
AEF	23.2±0.13 <sup>e</sup>	24.8±0.06 <sup>b</sup>	58.8±0.13 <sup>d</sup>
WF	33.2±1.06 <sup>d</sup>	25.2±0.05 <sup>b</sup>	72.0±1.06 <sup>b</sup>
F5	62.0±0.0	48.0±0.0	100.0±0.0
F5.4	70.0±0.0	64.0±0.0	100.0±0.0

<sup>1</sup>CE=crude extract, HF=Hexan fraction, BF=n-butanol fraction, AEF=ethyl acetate fraction, WF=water fraction, F5=fraction obtained by column chromatography in silica gel from BF, F5.4=fraction obtained by thin layer chromatography from F5. Values are the average of three replicates ± standard error. All values were corrected considering the solvent effect. Values with different letter in the same column are different ( $P<0.05$ ).

Table 2. Inhibition of radial growth (%) of fungi after 336 h of incubation in different concentrations of the n-butanol fraction of *Jacquinia macrocarpa*

Solids (mg/mL)	<i>A. flavus</i>	<i>A. parasiticus</i>	<i>F. verticillioides</i>
1.0	22.7±0.0 <sup>d</sup>	0.0±0.0 <sup>e</sup>	63.6±0.0 <sup>d</sup>
2.0	31.8±0.0 <sup>e</sup>	13.6±0.0 <sup>d</sup>	77.3±0.0 <sup>e</sup>
3.0	36.4±0.0 <sup>b</sup>	22.7±0.0 <sup>c</sup>	81.5±0.0 <sup>b</sup>
4.0	36.4±0.0 <sup>b</sup>	31.8±0.0 <sup>b</sup>	86.4±0.0 <sup>a</sup>
5.0	40.9±0.7 <sup>a</sup>	36.4±0.0 <sup>a</sup>	86.4±0.0 <sup>a</sup>

Values are the average of three replicates ± standard error. Values with different letter in the same column are different ( $P<0.05$ ).

antifungal fraction BF over the three fungi is shown in Figure 1. respectively).

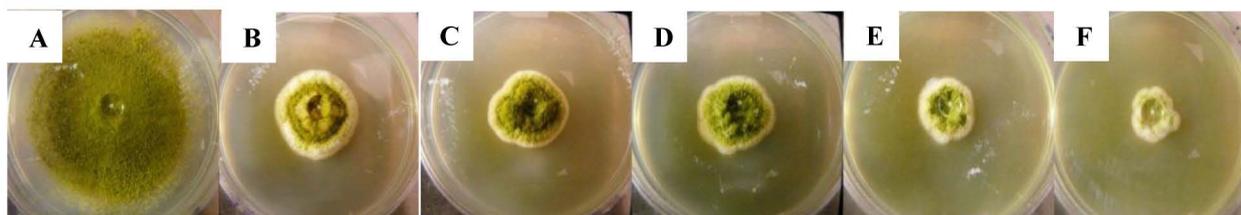
1.

#### Kinetics of spores germination

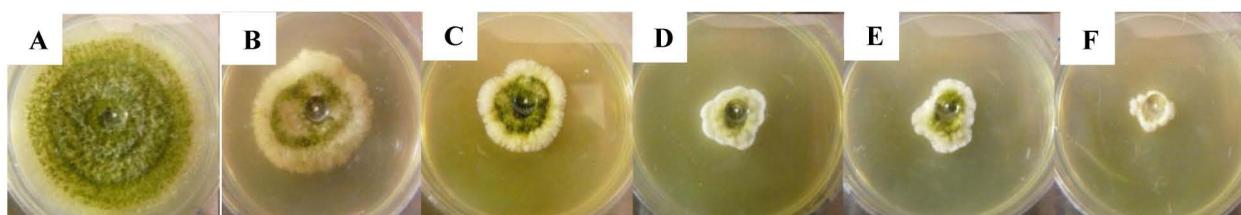
The spores of the three fungi inoculated on the PDB controls completely germinated within 30 h. Although spores germination of *A. flavus* was delayed when they were grown on PDA containing the antifungal F5.4 fraction, all spores were able to germinate at the end of the experiment (Figure 2). Similar results were observed for *A. flavus*, *A. parasiticus* and *F. verticillioides* spores, which were delayed in growth on PDA containing the F5.4 fraction (Figure 2). However, the spores of the two last fungi showed significant ( $P<0.05$ ) germination inhibition at the end of the incubation time (7.8 % and 11.8 % for *A. parasiticus* and *F. verticillioides*,

#### Hyphal diameter and length

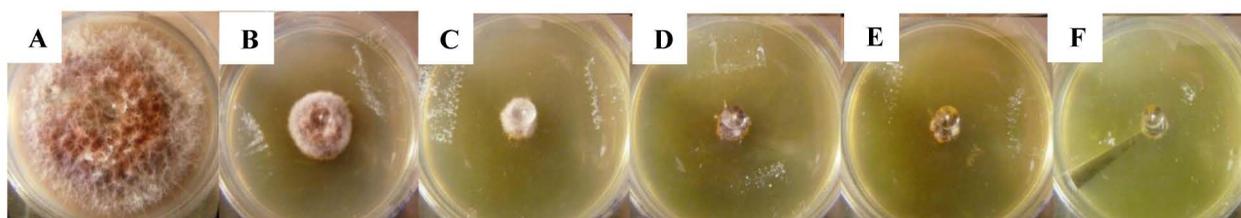
The three fungi significantly ( $P<0.05$ ) underwent a reduction in diameter and length of apical hyphae when they were grown on PDA agar media containing the F5.4 fraction in respect to the PDA and methanol controls. *A. flavus* apical hyphae showed a reduction in diameter and length in 20.8±1.8 % and 43.8±2.1 %, whereas those for *A. parasiticus* were reduced in 18.5±0.8 % and 40.1±1.7 %, respectively. The decrease in diameter and length of *F. verticillioides* apical hyphae was of 18.2±1.6 % and 41.4±2.6 %, respectively. The three fungi showed the higher ( $P<0.05$ ) reduction percentage in length than in diameter.



*Aspergillus flavus*



*Aspergillus parasiticus*



*Fusarium verticillioides*

Figure 1. Radial growth of *Aspergillus flavus*, *A. parasiticus* and *Fusarium verticillioides* over potato dextrose agar media (PDA) containing n-butanol partitioned fraction of *Jacquinia macrocarpa* (BF) at concentrations of: A) 0 mg/mL, B) 1 mg/mL, C) 2 mg/mL, D) 3 mg/mL, E) 4 mg/mL, and F) 5 mg/mL.

#### Spores diameter

Diameter of spores of each fungi were measured every 3 h of incubation. In both *Aspergillus* species, the spores diameter increased when they were incubated in PDB and PDB containing methanol, however the diameter of spores of *Aspergillus* species were reduced in PDB containing the antifungal fraction F5.4 (Figure 3). Spores of *F. verticillioides* showed no significant differences ( $P < 0.05$ ) in length or diameter throughout the incubation time (Figure 3).

#### Spores viability

Spores of three fungi treated with the antifungal fraction F5.4 showed a significant viability reduction ( $P < 0.05$ ) after 96 h of incubation. The spores viability of *A. flavus*, *A. parasiticus*

and *F. verticillioides* was reduced in  $40.7 \pm 1.8\%$ ,  $44.8 \pm 1.9\%$  and  $46.3 \pm 0.9\%$ , respectively.

## Discussion

Fungal growth inhibition of few *Jacquinia* species has been reported. Sharma *et al.* (2008) studied an ethanolic extract of petals of *J. ruscifolia* and reported growth inhibition of some fungi such as *A. flavus* and *F. moniliforme* (syn. *F. verticillioides*). García-Sosa *et al.* (2011) reported antifungal activity of a methanolic extract of *J. flammea* against phytopathogenic fungi different to those evaluated in the present study. Although, extracts from other plants have been

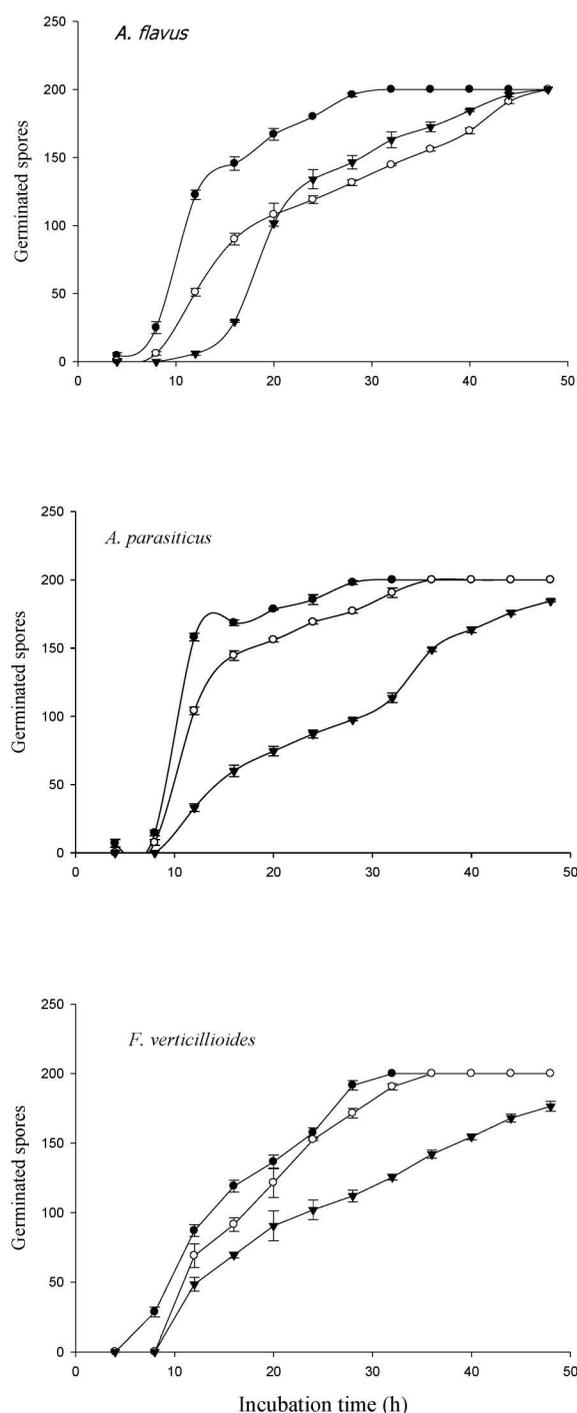


Figure 2. Spore germination kinetics of *A. flavus*, *A. parasiticus* and *F. verticillioides*, grown on PDA (●), PDA plus methanol (○), and PDA plus F5.4 (▼).

tested in their antifungal activity against *A. flavus*, *A. parasiticus* and *F. verticillioides* (Dabur *et al.*, 2005; Rosas-Burgos *et al.*, 2009; Sánchez *et al.*, 2005; Satish *et al.*, 2007; Suárez-Jiménez *et al.*, 2007; Tequida-Meneses *et al.*, 2002; Vargas-Arispuro *et al.*, 2005), no studies on the antifungal activity of *J. macrocarpa* have not been reported before. Results of the radial growth inhibition test for *A. flavus* were similar to those reported by Tequida-Meneses *et al.* (2002), although they differed to those reported by other authors (Cowen and Steinbach, 2008; Rosas-Burgos *et al.*, 2009; Sánchez *et al.*, 2005; Satish *et al.*, 2007; Vargas-Arispuro *et al.*, 2005).

Radial growth inhibition of *A. parasiticus* and *F. verticillioides* by methanolic plant extracts have also been observed by Sánchez *et al.* (2005) and Suárez-Jiménez *et al.* (2007), respectively. The radial growth inhibition value of *F. verticillioides* was the highest observed in all fungi, which is in agreement with other authors (Tequida-Meneses *et al.*, 2002; Rosas-Burgos *et al.*, 2009). Sharma *et al.* (2008) reported a similar inhibition effect against *F. moniliforme* and *A. flavus* of ethanolic petals extracts of *J. ruscifolia*. In the present study, the fractions obtained after the chromatographic purification steps (F5 and F5.4) from BF, showed the higher antifungal activity over the three fungi than that of the crude extracts and partitioned fractions. It was observed that *F. verticillioides* was the most inhibited mold when it was inoculated on PDA containing the fraction F5 or F5.4. The concentration of BF that caused the maximum radial growth inhibition of both *Aspergillus* species was significantly higher ( $P < 0.05$ ) than that for *F. verticillioides*, which confirms that this last fungus is very sensible to the antifungal fraction of *J. macrocarpa*. The results of this study indicate that *J. macrocarpa* contains antifungal compounds that are active against the growth of the fungi tested in the present study, especially against *F. verticillioides*.

Retarded spore germination observed in the present study was also reported in *F. verticillioides* by methanolic

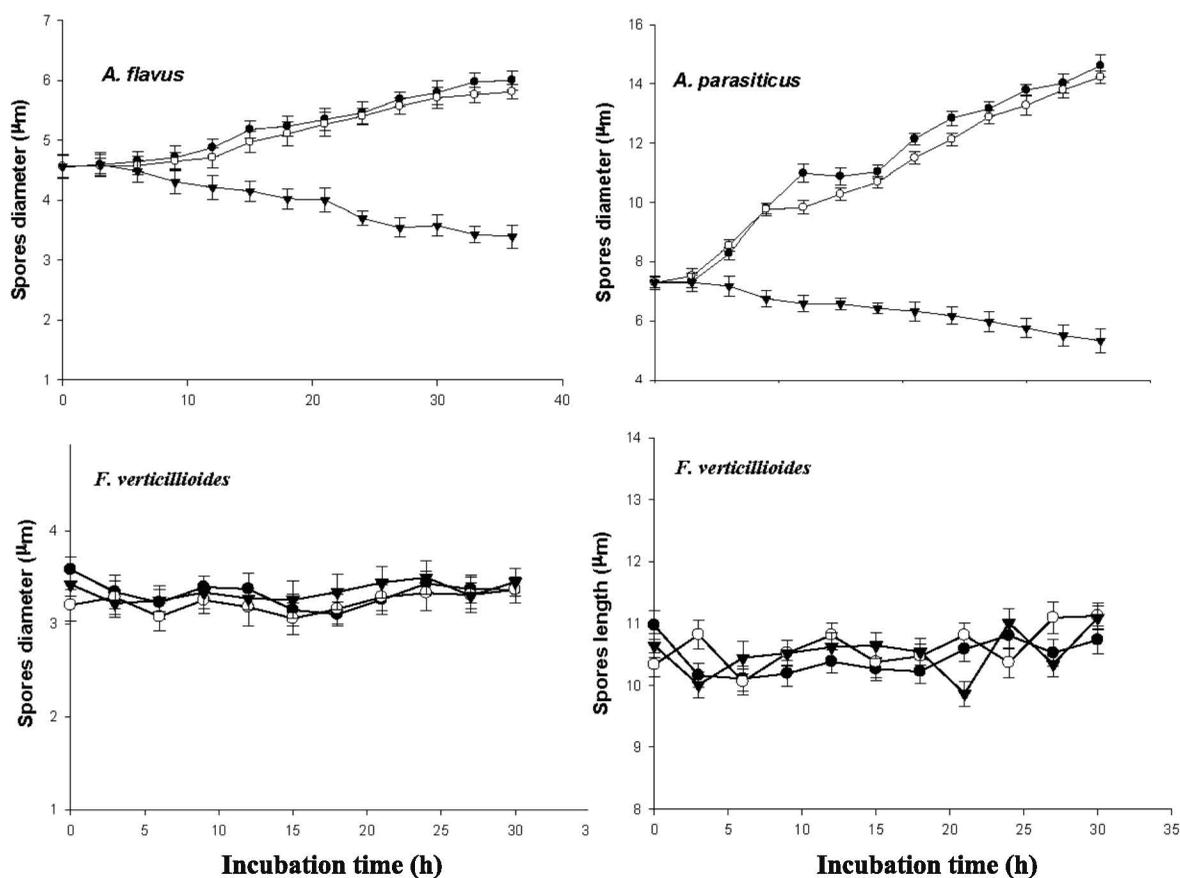


Figure 3. Spore size of *A. flavus*, *A. parasiticus* and *F. verticillioides*, grown on potato dextrose agar (PDA) (●), PDA plus methanol (○), and PDA plus F5.4 (▼).

extracts of *Baccharis glutinosa* (Suárez-Jiménez *et al.*, 2007). More recently, Rosas-Burgos *et al.* (2011) reported retarded germination of spores of *A. flavus*, *A. parasiticus* and *F. verticillioides* by a purified fraction of *B. glutinosa*. Rosas-Burgos *et al.* (2011) observed lower spore germination inhibition in *A. parasiticus* than those observed in the present study. However, those authors reported higher inhibition of *F. verticillioides* spores, which was not observed in this work. The high spore viability inhibition of *A. parasiticus* and *F. verticillioides* ( $P < 0.05$ ) coincided with high spore germination inhibition, whereas the opposite was observed in *A. flavus*, that is, at lower number of viable spores, the lower spores number with germination capacity.

Reduction in diameter and length of apical hyphae observed in the three fungi treated with a purified fraction of

*B. glutinosa* was previously observed by Rosas-Burgos *et al.* (2011), although these authors observed a more pronounced antifungal effect of the plant extract. In the present study, spores of *Aspergillus* species incubated in PDA controls increased in size, while the spores incubated in the presence of the antifungal F5.4 fraction showed a reduction. Rosas-Burgos *et al.* (2011) reported that in *A. flavus* and *A. parasiticus* the spores diameter increased when they were incubated in media controls and media containing the *B. glutinosa* antifungal fraction, although the increase observed was smaller with the antifungal treatment in comparison of that of the controls.

A study reported by Ha *et al.* (2006) indicated that *Fusarium solani* f. sp. *pisi*, resistant to caspofungin acetate, which target is the fungal wall, decreased its resistance when

the expression of the gene *FsFKS1* that encodes the  $\beta(1,3)$ -D-glucan synthase was reduced, leading to a reduction in spore viability and causing lysis of spores and hyphae. The radial growth inhibition, delay of spores germination, size reduction of both hyphae and spores and spores and viability observed in the present study, might be due to the loss of cell wall integrity.

It is possible that some compounds such as sakurasaponin and jacquinonic acid obtained from other *Jacquinia* species (*J. flammea* and *J. pungens*, respectively), which have been reported to show antifungal or repellent activity (García-Sosa *et al.*, 2011; Okunade and Wiemer, 1985), might be responsible of the antifungal activity of *J. macrocarpa* fractions used in this study.

The results of this study demonstrated that *J. macrocarpa* contains antifungal compounds that could be used as an alternative method to treat corn and other cereals grains to control phytopathogenic molds, especially *F. verticillioides*.

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