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Antifungal activity of methanolic extracts of *Jacquinia macrocarpa* and *Krameria erecta* on the growth of *Fusarium verticillioides* and effect on fumonisin production

Actividad antifúngica de extractos metanólicos de *Jacquinia macrocarpa* y *Krameria erecta* en el crecimiento de *Fusarium verticillioides* y su efecto en la producción de fumonisinas

Fabiola Fimbres-López [1], Ema Carina Rosas-Burgos [1], Armando Burgos-Hernández [1], Maribel Plascencia-Jatomea [1], María Lourdes Aldana-Madrid [1], Octavio Cota-Arriola [1], Eber Addi Quintana-Obregón [2], Mario Onofre Cortez-Rocha [1]

[1] Departamento de Investigación y Posgrado en Alimentos de la Universidad de Sonora. Rosales y Luis Encinas s/n. Col. Centro, C.P. 83000, Hermosillo, Sonora. México. [2] Universidad del Estado de Sonora, Hermosillo, Sonora, México

RESUMEN

Algunas plantas medicinales han sido estudiadas sobre hongos fitopatógenos para conocer si tienen propiedades antifúngicas, por ello en este estudio se evaluaron los extractos metanólicos de hojas de *Jacquinia macrocarpa* y *Krameria erecta* sobre el crecimiento radial, germinación de esporas y producción de biomasa por *Fusarium moniliforme* en medio agar papa dextrosa. El extracto de *J. macrocarpa* que presentó mejores resultados fue particionado con hexano, acetato de etilo y n-butanol. Solo la fracción butanólica presentó actividad, ya que retardó la germinación de esporas y el crecimiento de las colonias cambió de radial a apical, que indica que el hongo está estresado. La producción de fumonisina no fue afectada por el extracto. Se concluye que el extracto metanólico de *J. macrocarpa* y su fracción butanólica son capaces de retardar el crecimiento de *F. verticillioides in vitro* y no afectan la producción de fumonisinas.

PALABRAS CLAVE: germinación de esporas, crecimiento radial, micotoxinas.

ABSTRACT

Some medicinal plants have been studied on phytopathogenic fungi for their antifungal activity. For this reason the goal of this study was to evaluate methanolic extract of *Jacquinia macrocarpa* and *Krameria erecta* on radial growth, spore germination, biomass production of *Fusarium verticillioides*. Methanolic extract of *J. macrocarpa* which caused the best results was sequentially partitioned with hexane, ethyl acetate and n-butanol. Only the butanolic fraction was active. It delayed the spore germination and the colony growth changed from radial to apical, which is a way to express it is under pressure due to chemicals present in the fraction. Fumonisin production was not affected by the extract. We conclude that *J. macrocarpa* methanolic extract and its butanolic fraction are capable to delay the radial growth of *F. verticillioides* and the kinetic of spore germination and do not affect fumonisin production.

KEYWORDS: spore germination, radial growth, mycotoxins.

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Autor para correspondencia / Corresponding author:
Mario Onofre Cortez Rocha
mario.cortez@unison.mx

INTRODUCTION

Cereal grains and other commodities are commonly contaminated in the field and in the storage by fungi such as *Fusarium* and *Aspergillus* species. These molds produce secondary metabolites known as mycotoxins that reduce the commercial and nutritional grain quality (Doko *et al*., 1996; Tequida-Meneses *et al*., 2002). *Fusarium verticillioides* (Sacc.) Nirenberg (= *moniliforme*) and *Fusarium proliferatum* (Matsush.) Nirenberg have been reported as natural contaminants of cereals worldwide and are mainly found in corn and its by-products (Acuña *et al*., 2005; Marasas *et al*., 1996; Mazzani *et al*., 1999), sorghum and oat (Leslie *et al*., 1990; Bacon and Nelson, 1994), rice (Abbas *et al*., 1998), and wheat (Castoria *et al*., 2005; Shephard *et al*., 2005). *F. verticillioides* has also been isolated frequently from maize in several states of Mexico (Hernández-Delgado *et al*., 2007; Gallardo-Reyes *et al*., 2006; Cortez-Rocha *et al*., 2003; Robledo *et al*., 2001).

These fungi are important plant pathogens that produce a variety of mycotoxins, the major class of which is called fumonisin. Of the twenty-eight fumonisin analogues that have been currently described (Rheeder *et al*., 2002), three (FB_1, FB_2, and FB_3) have been reported to occur naturally at significant levels in corn and corn-based products (Sydenham *et al*. 1990, Doko *et al*. 1996). FB_1 is the most abundant and accounts for about 70% of the fumonisins in naturally contaminated corn samples. The presence of fumonisins in feeds has been implicated in outbreaks of equine leukoencephalomalacia, porcine pulmonary edema (Bezuidenhout *et al*., 1988; Norred and Voss, 1994). In humans *F. verticillioides* and fumonisins have been epidemiologically associated with esophageal cancer in areas of Transkei, South Africa (Sydenham *et al*., 1990; Marasas *et al*., 2001) and China (Yang, 1980), where FB_1-contaminated corn was consumed as a dietary staple.

To reduce the associated problems with *Fusarium* and their toxins, it is necessary to prevent fungal growth on the grains, which can be achieved by the use of synthetic fungicides. The use of natural bioactive substances for the control of fungal infections has gained attention because of fungicide resistant strains, which increases food-borne pathogenic microorganisms, in addition to increasing the number of pesticides under observation or regulation (Rabea *et al*., 2003).

Plant extracts are generally assumed more acceptable and less hazardous than synthetic products and can be used as alternative antifungal treatment (Jobling, 2000, Ramírez-Chávez *et al*., 2000; Guerrero-Rodríguez *et al*., 2007). Aqueous plant extracts from garlic, creosote bush, and clove inhibited the growth of *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani*, and *Verticillium dahliae* (López-Benítez *et al*, 2005). According to Verastegui *et al*. (1996), alcoholic extracts from *Baccharis glutinosa* and *Larrea tridentata*, may act against the growth of fungi, yeast and bacteria. In addition, Sánchez-Rangel *et al*. (2005) reported the inhibition of both the growth and mycotoxin production by *Aspergillus flavus* and *Aspergillus parasiticus* when exposed to ethanolic, methanolic, and aqueous extracts of *Agave* species. Also, extracts of *Flourensia cernua* caused more than 91% of reduction in the colony growth of *Alternaria alternata*, *Penicillum digitatum* and *Colletrotichum gloeosporiodes*, but they don´t affected sporulation (Guerrero-Rodríguez *et al*., 2007). Methanolic extracts of *Baccharis glutinosa* have been reported to contain antifungal activity against phytophathogenic molds (Suárez-Jiménez *et al*., 2007; Tequida-Meneses *et al*., 2002). A study by Cespedes *et al* (2006) reported that chloroform/methanol extracts of *Tagetes lucida* inhibited 89% of the colony radial growth of *F. moniliforme*. *Krameria erecta* is one of 17 species belonging to the Family Krameriaceae. It has been reported for this genus the presence of phenolic compounds like tannins and lignans and various biological activities such as hepatoprotective effect, antioxidant and anti-inflammatory (Carini *et al*., 2002). Recently, Morán-Palacio *et al*. (2014) and Jimenez-Estrada *et al*. (2013) found that *K. erecta* has high antiproliferative activity, high flavonoids and total phenols content. In the study of Morán-Palacio *et al*. (2014), it was found that *K. erecta* possess five times the antioxidant activity of ascorbic acid and also they demonstrate high phenolic content that supports the beneficial properties attributed to these plants in traditional medicine. Torres-González *et al*. (2011) mentioned that *K. ramosissima* is used by traditional healers in the northeastern region of Mexico to protect against liver damage. The aim of this work was to study *J. macrocarpa* and *K. erecta* as a source of natural compounds for controlling *F. verticillioides* and its production of toxins. Effects of methanolic extracts were tested on spore germination, biomass produc-

tion, and radial growth. The best extract was fractionated and its effects on fumonisin production was measured.

MATERIALS AND METHODS

Aerial parts of *Jacquinia macrocarpa* and *Krameria erecta* were collected in the area of Los Arrieros, Sonora (Latitude N 28° 20.538' W 111° 08.911' altitude 280 feet and latitude N 28° 19.526' W 111° 08.828' altitude 227 feet) during august 2010. A voucher sample of each plant was deposited at the Herbarium of the Scientific Research and Technology Department of the University of Sonora (DICTUS) in Hermosillo, Sonora (Mexico) to confirm its identification. Plant material was dried at room temperature in the dark for 2 weeks and finely ground with a Wiley mill (200 µm mesh). Six grams of powdered aerial parts of each plant were extracted with 94 ml of 70% methanol, stirred during 1 h, and stored at room temperature for 10 days at darkness. The extracts were filtered first through Whatman filter paper No. 1. The methanolic extracts (crude extracts) were evaporated to dryness at 45 °C under reduced pressure. Crude extracts were evaluated for antifungal activity.

A strain of *Fusarium verticillioides* (ATCC 52539) was activated in PDA agar media (DIFCO, USA) and incubated at 25 ± 2 °C for 10 days using a 12 h light/dark cycle (Precision Low temperature Illuminated Incubator 818, USA). Spores were harvested by pouring a sterile solution of 0.1% (v/v) Tween 20 into the flask and stirring with a magnetic bar for 5 min. The spore concentration of the suspension was determined using a Neubauer chamber.

Kinetics of radial extension growth

Petri dishes of solid PDA media containing 500, 1000, and 2,000 mg/mL of each crude extract were centrally point-inoculated with $1x10^5$ spores/mL from 7-day-old cultures of *F. verticillioides* (ATCC 52539). Petri dishes with PDA and methanol and a blank with only PDA media were included as controls. All Petri dishes were incubated at 25 °C using a 12 h light/dark cycle. The colony diameters were measured with a caliper every 24 h and compared to the control media until the control reached the plate border. The extract concentration that delayed 50% of colony radial extension (CI_{50}) was determined at 95% of confidence intervals, using a Probit analysis with NCSS 97 statistical program (NCSS Inc., USA). All determinations were carried out in triplicate.

Kinetics of spore germination

PDA plates centrally point-inoculated with $1x10^5$ spores/mL from 7-day-old cultures of *F. verticillioides* (ATCC 52539) were added with the estimated CMI (1, 882 mg/L) of *J. macrocarpa* crude extract and incubated at 25 °C using a 12 h light/dark cycle. Petri dishes with PDA and methanol and a blank with only PDA media were included as controls. Samples were taken at different times and 200 spores (germinated and non-germinated) were randomly counted using a light microscope. The number of germinated spores per plate was determined. A spore was considered germinated when the length of its germinal tube reached one-half of the spore-diameter (Plascencia-Jatomea *et al.*, 2003). All determinations were carried out in triplicate.

Biomass production

The biomass production was daily quantified as the mycelium dry weigh. Petri dishes of solid PDA media containing 1, 882 mg/L of *J. macrocarpa* crude extract were centrally point-inoculated with $1x10^5$ spores/mL from 7-day-old cultures of *F. verticillioides* (ATCC 52539). Petri dishes with PDA and methanol and a blank with only PDA media were included as controls. All Petri dishes were incubated at 25 °C using a 12 h light/dark cycle. The agar gel with the produced biomass was separated from the plate, poured into a glass beaker containing 200 mL of water, and heated until complete dissolution of the agar. The solution was vacuum filtered using a previously weighted Whatman No. 40 filter paper and washed with distilled water. Finally, the filter containing the mycelium was dried at 105 °C for 2 h and the colony dry weight was expressed in mg/cm^2, corresponding to mg of mycelium per plate area (Larralde *et al.*, 1997). All determinations were carried out in triplicate.

Partition of the crude methanolic Jacquinia macrocarpa extract

The crude extract of *J. macrocarpa* was evaporated to dryness at 40 °C, resuspended in water, and sequentially partitioned with hexane, ethyl acetate, and n-butanol. The crude extract and the partitioned extracts were evaluated for their antifungal

activity in the radial growth using 100, 500 and 1,000 mg/L as described previously. Controls were prepared using the different solvents except n-butanol because it totally inhibited the fungal growth.

Fumonisin B1 (FB1) production

The methanolic and butanolic extracts of *J. macrocarpa* were analyzed for their possible effects on FB_1 production in *F. verticillioides*-inoculated corn grain. Fifty mg of each extract were dissolved in 500 µL de MeOH and appraised to 10 mL with sterile water. FB_1 production was carried out using healthy maize as substratum. Corn grain (50 g) portions, free from FB_1, were placed in 500 mL Erlenmeyer flasks, adjusted at 40% humidity, and sterilized for two consecutive days in an autoclave for 15 minutes at 121 °C. Autoclaved maize was separately treated with the extracts. Control flasks were prepared following the same procedure with no extract added, only MeOH. Each treatment was inoculated with $1x10^6$ spores of *F. verticillioides*. Flasks were incubated for 30 days at 25 ± 2 °C using a 12 h light/dark cycle (Precision low temperature illuminated incubator 818, USA). Three replicates for each treatment were performed. For separation and purification of FB_1 the cultures were oven-dried overnight at 50 °C. Extraction procedure and quantification of FB_1 were based on the quantitative Fumonitest® Immunoaffinity Column method from VICAM (Fumonitest manual).

Statistical analysis

Statistics on a completely randomized design were determined using the one-way analysis of variance (ANOVA) procedure with JMP software (JMP version 5.0, SAS Institute Inc., USA), at a level of significance set at P = 0.050. Means for groups in homogeneous subsets were determined using the Tukey multiple comparisons test (Tukey's posthoctest), at 95% confidence interval. All data were presented as mean value with their standard error indicated (mean ±SE).

Results and discussion

Results from radial extension growth inhibition (%) are presented on Table 1. *K. erecta* crude extract at the three concentration tested showed low inhibition effect during 144 h. The application of 2,000 mg/L of the extract exhibited the higher inhibition (25%) at the first 24 h, however this effect diminished after this time, even more, the radial growth was higher than in the methanol control since the plate were completely covered with the mycelium after 144 h of incubation, except for the 2,000 mg/L treatment. *J. macrocarpa* crude extract did not affected mycelial growth during the first 24 h but after this time it hap-

Table 1. Inhibition of *Fusarium verticillioides* radial growth (%) after 264 hours of incubation in media with *Krameria erecta* and *Jacquinia macrocarpa* methanolic extracts

Time (h)	Control Colony diameter (cm)	*K. erecta* (mg/L)			*J. macrocarpa* (mg/L)		
		500	1,000	2,000	500	1, 000	2, 000
24	0.33	17.9±6.2^{b}	21.4±6.2^{b}	25.0±0^{b}	0.0±0a	0.0±0^{a}	0.0±0^{a}
48	0.7	5.9±3.9^{a}	5.9±3.9^{a}	23.5±0^{b}	0.0±0^{a}	2.0±3.4^{a}	9.8±6.8^{a}
120	2.1	8.4±2.5^{a}	5.3±2.8^{a}	15.1±11.6^{ab}	2.0±2.1^{a}	29.4±2.5^{b}	56.3±1.5^{c}
144	2.4	2.0±1.7^{a}	5.5±2.6^{ab}	16.5±6.8^{bc}	0.0±0^{a}	29.3±3.4^{c}	51.9±3.4^{d}
168	2.7	0.0±0^{a}	0.4±0.8^{a}	8.6±5.0^{a}	0.0±0^{a}	31.6±8.0^{b}	53.9±1.1^{c}
192	3.0	0.0±0^{a}	0.0±0^{a}	7.0±4.6^{a}	0.0±0^{a}	29.8±6.3^{b}	52.0±3.7^{c}
216	3.4	0.0±0^{a}	0.0±0^{a}	5.2±0.9^{a}	0.0±0^{a}	33.0±4.0^{b}	46.1±5.5^{c}
240	3.5	0.5±0.8^{a}	0.0±0^{a}	5.4±1.4^{a}	0.0±0^{a}	35.1±3.6^{b}	45.9±4.1^{c}
264	4.0	0.0±0^{a}	0.0±0^{a}	0.0±0^{a}	0.0±0^{a}	36.3±1.3^{b}	45.8±5.1^{c}

Values are the average of triplicates ± the standard error of the mean. Different letters mean different statistical groups. Tukey test $p < 0.05$.

pens. Radial growth was delayed for 264 h only with the 1,000 and 2,000 mg/L. An inhibition range from 29.4 - 36.3% was observed at 264 h of incubation when 1,000 mg/L was added to the medium. The highest reduction of radial growth (56.3%) was reached with 2,000 mg/L of crude extract at 120 h of incubation.

The radial extension rate, U (µm/h), of *F. verticillioides* was estimated from the radial growth results (Table 2). The lowest radial extension rate (2,010 µm/h) was observed with the 2,000 mg/L of *J. macrocarpa* crude extract and 2,350 µm/h with 1,000 mg/L. Both results agree to those of radial growth because they delayed it. The values from the radial extension rate of the PDA and PDA-methanol controls (4,320 µm/h and 4,280 µm/h, respectively) were higher than those from the *J. macrocarpa* methanolic extract exhibiting effects in *F. verticillioides*.

Krameria erecta at the low amount (500 and 1,000 mg/L) used in the study did not affect *F. verticillioides* development and weak effects were noticed when exposed to 2,000 mg/L. In accordance with our study, several authors have mentioned that fungi species reacts to plant extracts in different ways (Fokialakis *et al*., 2006; Hernández-Albiter *et al*., 2007; Tequida-Meneses *et al*., 2002). Jiménez-Estrada *et al*. (2013) reported that methanolic extract of *K. erecta* has high antioxidant and antiproliferative activities; however, it did not affect the energy process in the fungus development. Also, Morán-Palacio *et al*. (2014) reported the presence of polyphenols and terpenes in a methanolic extract of *K. erecta* from Sonora, Mexico. These authors found that *K. erecta* has five times greater than ascorbic acid and a high phenolic content. It has been reported that this kind of compounds have antimicrobial and antifungal activity, however they had no effect in fungus species treated. Due to the low growth inhibition of *F. verticillioides* exerted by *K. erecta*, we proceed to evaluate only the effects of *J. macrocarpa* extract on the spore germination, biomass and FB_1 production, using the estimated MIC (1,882 mg/L).

The spore germination inhibition percentages of *F. verticillioides* are shown in Table 3. We observed that *J. macrocarpa* extract delayed spore germination. The crude extract was most effective in controlling spore germination of *F. verticillioides* at the first hours after the treatment and this effect decreased as the time passed. This phenomenon might probably be due to presence of certain resistance compounds such as enzymes in the mold, or to mold adaptation to the extract present in the medium. This is in accordance with Trione (1981), who mentioned that it is possible that molds such *Aspergillus flavus* and *Fusarium moniliforme* have enzymatic mechanisms to inhibit the effects of plant metabolites and grow in their presence, which was observed in our study.

Table 2. Radial extension rate of *Fusarium verticillioides*

Treatment (mg/L)	Extension rate (µm/h)
Control PDA	4,320
Control MeOH	4,280
K. erecta	
500	4,600
1,000	4,690
2,000	4,430
Jacquinia macrocarpa	
500	4,270
1,000	2,350
2,000	2,010

Table 3. Inhibition of *Fusarium verticillioides* spores germination in media supplemented with *Jacquinia macrocarpa* (1,882 mg/L) methanolic extract

Time (h)	% Inhibition
4	100±0a
8	49±1.8b
12	38±1.1c
14	12±0.7d
16	9±1.8e
18	6±1.1f
20	6±1.1f

Values are the average of triplicates ± the standard error of the mean. Different letters mean different statistical groups.

Table 4. Spore germination of *Fusarium verticillioides* with methanolic extract of *Jacquinia macrocarpa* (1,882 mg/L) and biomass production

Treatment	Germinated Spore (%)		Biomass (mg/cm2)	
	12 h	24 h	96 h	140 h
J. macrocarpa	62.25 ± 1.1a	94.25 ± 1.1a	0.0405 ± 1.9e-3a	0.0491 ± 5.2e-3a
Control MeOH	19.25 ± 1.1b	100 ± 0b	0.0539 ± 4.4e-3b	0.0586 ± 2.6e-3b
Control PDA	29.0±1.4c	100 ± 0b	0.0604 ± 3.5e-3b	0.0668 ± 2.3e-3b

Values are the average of triplicates ± the standard error of the mean. Different letters mean different statistical groups.

The percentage of spores germinated at 12 and 24 h are presented in Table 4. The spores placed on the control with methanol and PDA control germinated after 24 h of incubation. There was a significant difference ($p<0.05$) in the percentage of spore germination inhibition between those exposed to the *J. macrocarpa* extract and the controls. In addition, there was a statistical difference between the biomass produced by *F. verticillioides* in presence of *J. macrocarpa* crude extract and the control. Other authors have reported the effect of plant extracts on the germination process. Suárez-Jiménez *et al*., 2007 reported that 5.6% (v/v) methanolic extracts of *Larrea tridentata*, *Baccharis glutinosa*, *Ambrosia confertiflora*, and *Azadirachta indica* caused 68 - 88% inhibition of spore germination of *F. verticillioides* after 100 h of incubation, which agrees with our findings since they followed the same trends. In addition, Abou-Jawdah (2004) reported 90 to 100% of inhibition of spore germination of *Fusarium oxysporum* by the application of extracts of nine Lebanese wild plants. Hernández-Albíter *et al*. (2007) reported similar results when studied the effect of extracts from forty plants on the germination of *Colletotrichum gloeosporioides* spores. They found variation in the effects by the type of plant and place of plant collection.

Antifungal activity of partitioned extract

The hexane and ethyl acetate fraction of *J. macrocarpa* did not inhibit the mycelial radial growth of *F. verticillioides*. Ethyl acetate fraction promoted mycelial radial growth. This effect could be due to the presence of alellochemicals that stimulate the spore germination reported in other plant species (Montes-Belmont and García-Licona, 1997). Two concentration (500 and 1000 mg/mL) of the methanolic extract of *J. macrocarpa* and its butanolic fraction of *J. macrocarpa* highly affected negatively the radial mycelial growth (Table 5). Both fractions were more effective in inhibiting the growth of the fungus at both concentrations, causing growth delayed in the first 36 h. According to these results, we can assume the possible presence of flavonoids, phenols and alkaloids in both fractions of *J. macrocarpa*.

Table 5. Radial growth inhibition of *Fusarium verticillioides* with *Jacquinia macrocarpa* methanolic extract and its butanolic fraction

Time (h)	Butanolic (mg/L)			Methanol (mg/L)		
	100	500	1000	100	500	1000
24	100±0	100±0	100±0	100±0	100±0	100±0
36	57±0c	57±0c	66±0 c	9±4.9a	34±4.9b	40±0b
48	16±3.9b	25±0c	45±0d	7±3.9 a	39±0d	39±0d
60	19±0c	31±2.9d	54±0f	14±0bc	32±5.9d	44±0e
72	18±2.4b	30±2.4c	58±0e	18±2.4b	35±2.4c	49±0d
96	0±0 a	20±2.2d	48±0f	16±2.2d	36±0e	48±0f
120	17±2.3c	40±1.3e	62±1.3f	11±1.3b	35±0d	65±1.3f
144	17±1.2c	41±0d	62±1.2e	11±1.2b	39±0d	63±1.2e

Values are the average of triplicates ± the standard error of the mean. Different letters mean different statistical groups. Tukey test $p < 0.05$.

Spore germination in partitioned extracts

The results of the mycelial radial growth were used for Probit analysis to estimate the MIC of *J. macrocarpa* methanolic extract and its butanolic fraction. The MIC values were 1,408 and 1,883 mg/L, respectively) and were used for the spore germination analysis. Table 6 indicates that both fractions delayed the germination process compared to controls, which agrees to our results on radial growth. Also, both fractions exhibited the highest inhibition at 12 h of incubation (60 and 59%, respectively) but the effect decreases with the time.

Biomass production in partitioned extracts

The *F. verticillioides* mycelium production was low in plates where methanolic extract and the butanolic fraction of *J. macrocarpa* were added compared to those from the controls (Table 7). Also, after 48 h incubation the radial growth change to apical growth, probably due to presence of the plant extracts. Butanolic fraction had the same effects than the methanolic extract did. Gomez *et al*. (2007) found that *Fagara monophylla* methanolic extract had antifungal activity against nine fungi species whereas the butanolic fraction was only effective in three of them, *Aspergillus flavus, Penicillum digitatum*, and *Candida albicans*.

Fumonisin production

Results showed that the fumonisin production is not influenced by the *J. macrocarpa* extracts (Table 8). The obtained values were not significantly different (P> 0.05) among control and plant extract, ranging from 6.57 to 11.93 µg kg^{-1}. This results differs from those in study by Suárez-Jiménez *et al* (2007), they reported that *Baccharis glutinosa* and *Larrea tridentata* methanolic extracts increased the fumonisin B1 production in corn grain compared to methanolic control.

Table 6. *Fusarium verticillioides* spores germination (%) in PDA medium amended with *Jacquinia macrocarpa* methanolic extract and its butanolic fraction

Time (h)	CPDA	CMeOH	CButanol	Methanolic Extract	Butanolic Fraction
4	18	15	13	21±4.5^{d}	32±6.7^{b}
8	28	28	25	32±1.3^{b}	41±3.8^{a}
12	77	75	78	40±1.9^{a}	41±2.4^{a}
16	94	89	95	35±1.2^{c}	35±2.0^{b}
20	100	100	100	22±0.4^{d}	21±0.4^{c}

Values are the average of triplicates ± the standard error of the mean. Different letters mean different statistical groups. Tukey test p < 0.05.

Table 7. *Fusarium verticillioides* biomass production (mg/cm^2) in PDA medium amended with *Jacquinia macrocarpa* methanolic extract and its butanolic fraction

Time (h)	cPDA*	cMethanol	Methanolic Extract	Butanolic Fraction
24	11.6±0.3^{a}	10.9±0.9^{a}	11.6±0.3^{a}	12.0±0.9^{a}
48	40.3±1.4^{b}	40.5±1.1^{b}	36.5±1.5^{a}	36.8±0.5^{a}
72	49.2±0.4^{b}	48.6±2.0^{b}	36.6±1.0^{a}	37.2±1.5^{a}
96	48.7±1.9^{b}	48.1±0.8^{b}	38.0±1.6^{a}	46.3±1.1^{b}
120	70.0±0.5^{c}	55.6±1.0^{b}	50.2±1.2^{a}	51.4±0.9$^{a\,b}$
144	71.1±0.3^{a}	56.3±0.9^{a}	50.5±0.8^{a}	52.2±0.7^{a}

Values are the average of triplicates ± the standard error of the mean. Different letters mean different statistical groups. Tukey test p < 0.05.

Also, Rosas-Burgos *et al* (2011) reported that one fraction from *B. glutinosa* (fraction F-6) obtained by chromatographic purification and dissolved in methanol considerably increased the production of mycotoxins such as fumonisin B_1 by *Fusarium verticillioides* and aflatoxins by *Aspergillus flavus* and *A. niger*.

Table 8. Fumonisin B1 produced by *Fusarium verticillioides* in corn grain treated with *Jacquinia macrocarpa* methanolic extract and its butanolic fraction

Treatment	Fumonisin B1 (µg kg-1)
Control water	11.9 ± 2.65 a
Control methanol	8.7 ± 3.25 a
Methanolic extract	6.57 ± 2.51 a
Butanolic extract	9.87 ± 1.96 a

Values are the average of triplicates ± the standard error of the mean. Different letters mean different statistical groups. Tukey test $p < 0.05$.

The results obtained from this study indicate that *K. erecta* do not have any antifungal activity against *F. verticillioides*. Butanolic fraction from methanolic extract of *J. macrocarpa* caused 66% of inhibition of radial growth and 41% reduction in spore germination. *J. macrocarpa* contain chemical constituents that inhibited radial growth, inhibit spore germination and reduction in the mycelium production of *F. verticillioides*. Fumonisin production was not affected by the methanolic and butanolic *J. macrocarpa* extracts. According to the polarity of the extraction solvents used sequentially, a diversity of polar compounds could be present, which will be under investigation.

References

Abbas, H.K., R.D, Carwrigth, W.T. Shier, M.M. Abouzied, C.B. Bird, L.G. Rice, P.F. Ross, G.L. Sciumbato, F.I. Meredith, 1998. Natural occurrence of fumonisins in rice with *Fusarium* sheat rots disease. Plant Disease 82: 22-25.

Abou-Jawdah, Y., R. Wardan, H. Sobh, A. Salameh, 2004. Antifungal activities of extracts from selected Lebanese wild plants against plant pathogenic fungi. Phytopathologia Mediterranea 43: 377-386.

Acuña, A., M.C. Lozano, M.C. de García, J.G. Díaz, 2005. Prevalence of *Fusarium* species of the *Liseola* section on selected Colombian animal feedstuffs and their ability to produce fumonisins. Mycopathologia 160: 63-66.

Bacon, C.W., P.E. Nelson, 1994. Fumonisins production in corn by toxigenic strains of *Fusarium moniliforme* and *Fusarium proliferatum*. Journal of Food Protection 57: 514-521.

Bezuidenhout, S.C., W.C.A. Gelderblom, C.P. Gorst-Allman, R.M. Horak, W.F.O. Marasas, G. Spiteller, R. Vleggaar, 1988. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. Journal of the Chemical Society, Chemical Communication 19: 743-745.

Carini, M., G. Aldini, M. Orioli, M. Facino, 2002. Antioxidant and photoprotective activity of a lipophilic extract containing neolignans from *Krameria triandra* roots. Planta Medica 68: 193-197.

Castoria, R., G. Lima, R. Ferracane, A. Ritieni, 2005. Occurrence of mycotoxin in Farro samples from Southern Italy. Journal of Food Protection 68: 416-420.

Céspedes, C.L., J.G. Ávila, A. Martínez, B. Serrato, J.C. Calderón-Mugica, R. Salgado-Garciglia, 2006. Antifungal and antibacterial activities of Mexican Tarragon (*Tagetes lucida*). Journal of Agriculture and Food Chemistry 54: 3521-3527.

Cortez-Rocha, M.O., W.R. Ramírez-Astudillo, R.I. Sánchez-Mariñez, E.C. Rosas-Burgos, F.J. Wong-Corral, J. Borboa-Flores, L.G. Castillón-Campaña, M. Tequida-Meneses, 2003. Fumonisins and fungal species in corn from Sonora, México. Bulletin of Environmental Contamination and Toxicology 70: 668-673.

Doko, M.B., C. Canet, N. Brown, E.W. Syndenham, S. Mpuchane, B.A. Siame, 1996. Natural co-occurrence of fumonisins and zearalenone in cereals and cereal-based foods from Eastern and Southern Africa. Journal of Agriculture and Food Chemistry 44: 3240-3243.

Fokialakis, N., C.L. Cantrell, S.O. Duke, A.L. Skaltsounis, D.E. Wedge, 2006. Antifungal activity of thiophenes from *Echinops ritro*. Journal of Agriculture and Food Chemistry 54: 1651-1655.

Gallardo-Reyes E.D., G.M. Ibarra-Moreno, R.I. Sánchez-Mariñez, G. Cuamea-Cruz, D. Molina-Gil, N.V. Parra-Vergara, E.C. Rosas-Burgos, M.O. Cortez-Rocha, 2006. Micobiota del maíz (*Zea mays* L.) recién cosechado y producción de Fumonisina B1 por cepas de *Fusarium verticilliodes* (Sacc.) Nirenb. Revista Mexicana de Fitopatología 24: 27-34.

Gómez, Y., K. Gil, E. González, L.M. Farías, 2007. Actividad antifúngica de extractos del árbol *Fagara monophylla* (Rutaceae) en Venezuela. Revista de Biología Tropical 55: 767-775.

Guerrero-Rodríguez, E., S. Solís-Gaona, F.D. Hernández-Castillo, A. Flore-Olivas, V. Sandoval-López, D. Jasso-Cantú, 2007. Actividad biológica *in vitro* de extractos de *Fluorensia cernua* D.C. en patógenos de postcosecha: *Alternaria alternata* (Fr.: Fr.) Keissl., *Colletotrichum gloeosporioides* (Penz.) Penz. y Sacc. y *Penicillium digitatum* (Pers.: Fr.) Sacc. Revista Mexicana de Fitopatología 25: 48-53.

Hernández-Albíter, R.C., L.L. Barrera-Necha, S. Bautista-Baños, L. Bravo-Luna, 2007. Antifungal potential of crude plant extracts on conidial germination of two isolates of *Colletotrichum gloeosporioides* (Penz) Penz. y Sacc. Revista Mexicana de Fitopatología 25: 180-185.

Hernández-Delgado, S., M.A. Reyes-López, J.G. García-Olivares, N. Mayek-Pérez, 2007. Incidencia de hongos potencialmente toxígenos en maíz (*Zea mays* L.) almacenado y cultivado en el norte de Tamaulipas, México. Revista Mexicana de Fitopatología 25: 127-133.

Jiménez-Estrada, M., C. Velázquez-Contreras, A. Garibay-Escobar, D. Sierras-Canchola, R. Lapizco-Vázquez, C. Ortiz-Sandoval, A. Burgos-Hernández, R.E. Robles-Zepeda, 2013. *In vitro* antioxidant and antiproliferative activities of plants of the ethnopharmacopeia from northwest of Mexico. BMC Complementary and Alternative Medicine 13: 329.

Jobling, J., 2000. Essential oils: A new idea for postharvest disease control. Good Fruit and Vegetables Magazine, Sydney Postharvest Laboratory Information Sheet. 3p.

Larralde, C.C., L.F. López, G.G. Viniegra, 1997. Morphometric evaluation of the specific growth rate of *Aspergillus niger* grown in agar plates at high glucose levels. Biotechnology and Bioengineering 56: 287-294.

Leslie, J.F., C.A.S. Pearson, P.E. Nelson, T.A. Toussoun, 1990. *Fusarium* spp. from corn, sorghum, and soybean fields in the Central and Eastern United States. Phytophatology 80: 343-350.

López-Benítez, A., S.R. López-Betancourt, M.E. Vázquez-Badilio, S.A. Rodríguez-Herrera, M. Mendoza-Elos, E. Padrón-Corral, 2005. Inhibition of mycelial growth of *Fusarium oxysporum* Schlechtend. f. sp. *lycopersici* (Sacc.) Snyder and Hansen, *Rhizoctonia solani* Kühn, and *Verticilllium dahliae* Kleb. by aqueous plant extracts. Revista Mexicana Fitopatologia 23: 183-190.

Marasas, W.F.O, 2001. Discovery and occurrence of the fumonisins: A historical perspective. Environmental Health Perspective 109 (suppl. 2): 239-243.

Marasas, W.F.O, 1996. Fumonisins: history, world-wide occurrence and impact. In: Jackson, L.S., J.W. DeVries, L.B. Bullerman (eds.), Fumonisins in food. Plennum Press, New York. Pp. 1-16.

Mazzani, C., O. Borges, O. Luzon, V. Barrientos, P. Quijada, 1999. Incidence of *Aspergillus flavus*, *Fusarium moniliforme*, aflatoxins and fumonisins in trial of corn hybrid in Venezuela. Fitopatología Venezolana 12: 9-13.

Montes-Belmont, R., R. García-Licona, 1997. Efecto de extractos vegetales en la germinación de esporas y en los niveles de daño de *Alternaria solani* en tomate. Fitopatología 32: 52-57.

Morán-Palacio, E.F., L.A. Zamora-Álvarez, N.A. Stephens-Camacho, G.A. Yáñez-Farías, A. Virgen-Ortiz, O. Martínez-Cruz, J.A. Rosas-Rodríguez, 2014. Antioxidant capacity, radical scavenging kinetics and phenolic profile of methanol extracts of wild plants of Southern Sonora, Mexico. Tropical Journal of Pharmaceutical Research 13: 1487-1493.

Norred, W.P., K.A. Voss, 1994. Toxicity and role of fumonisins in animal diseases and human esophageal cancer. Journal of Food Protection 57: 522-527.

Plascencia-Jatomea, M., G. Viniegra, R. Olayo, M.M. Castillo-Ortega, K. Shirai, 2003. Effect of chitosan and temperature on spore germination of *Aspergillus niger*. Macromolecular Bioscience 3: 582-586.

Rabea, E.I., M.E.T. Badawy, C.V. Stevens, G. Smagghe, W. Steurbaut, 2003. Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 4: 1457-1465.

Ramírez-Chávez, E., L. Lucas-Valdez, G. Virgen-Calleros, J. Molina-Torres, 2000. Actividad fungicida de la afinina y del extracto crudo de raíces de *Heliopsis longipes* en dos especies de *Sclerotium*. Agrociencia 34: 207-215.

Rheeder, J.P., W.F.O. Marasas, H.F. Vismer, 2002. Production of fumonisin analogs by *Fusarium* species. Applied and Environmental Microbiology 68: 2101-2105.

Robledo, M.L., S. Marín, A.J. Ramos, 2001. Contaminación natural con micotoxinas en maíz forrajero y granos de café verde en el Estado de Nayarit (México). Revista Iberoamericana de Micología 18: 141-144.

Sánchez-Rangel, D., A. Sanjuan-Badillo, J. Plasencia, 2005. Fumonisin production by *Fusarium verticillioides* strains isolated from maize in Mexico and development of a polymerase chain reaction to detect potential toxigenic strains in grains. Journal of Agriculture and Food Chemistry 53: 8565-8571.

Shephard, G.S., L. Van der Westhuizen, G.M. Gatyeni, D.R. Katerere, W.F.O. Marasas, 2005. Do fumonisin mycotoxins occur in wheat? Journal of Agriculture and Food Chemistry 53: 9293-9296.

Suárez-Jiménez, G.M., M.O. Cortez-Rocha, E.C. Rosas-Burgos, A. Burgos-Hernández, M. Plasencia-Jatomea, F.J. Cinco-Moroyoqui, 2007. Antifungal activity of plant methanolic extracts against *Fusarium verticillioides* (Sacc.) Nirenb. and fumonisin B1 production. Revista Mexicana de Fitopatología 25: 134-142.

Sydenham, E.W., P.G. Thiel, W.F.O. Marasas, G.S. Shephard, D.J. Van Schalkwyk, K.R. Koch, 1990. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, southern Africa. Journal of Agriculture and Food Chemistry 38: 1900-1903.

Tequida-Meneses M, M. Cortez-Rocha, E.C. Rosas-Burgos, S. López-Sandoval, C. Corrales-Maldonado, 2002. Efecto de extractos alcohólicos de plantas silvestres sobre la inhibición de crecimiento de *Aspergillus flavus, Aspergillus niger, Penicillium chrysogenum, Penicillium expansum, Fusarium moniliforme* y *Fusarium poae*. Revista Iberoamericana de Micología 19: 84-88.

Torres-González, L., L.E. Muñoz-Espinosa, A.M. Rivas-Estilla, K. Trujillo-Murillo, R. Salazar-Aranda, N. Waksman de Torres, P. Cordero-Pérez, 2011. Protective effect of four Mexican plants against CCl_4-induced damage on the Huh7 human hepatoma cell line. Annals of Hepatology 10: 73-79.

Trione, E.J., 1981. Natural Regulators of fungal development. In: R.C. Staples, G.H. Toenniessen (eds.), Plant Disease Control. Resistance and susceptibility. John Wiley and Sons. New York. Pp. 85-102.

Verástegui, M.A., C.A. Sánchez, N.L. Heredia, J.S. García-Alvarado, 1996. Antimicrobial activity of extracts of three major plants from the Chihuahua desert. Journal of Ethnopharmacology 52: 175-177.

Yang, S., 1980. Research on esophageal cancer in China: a review. Cancer Research 40: 2633-2644.