Antifungal Activity of Plant Methanolic Extracts Against *Fusarium verticillioides* (Sacc.) Nirenb. and Fumonisin B1 Production

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Abstract. The aim of this work was to determine the inhibitory effect of methanolic extracts of *Ambrosia confertiflora*, *Azadirachta indica*, *Baccharis glutinosa*, and *Larrea tridentata* on spore germination and mycelial growth of *Fusarium verticillioides*. The spore germination percentage, radial growth extension, and biomass production were evaluated on potato-dextrose-agar (PDA) amended with 5.6, 11.0, 14.0, and 16.7% (v/v) of the extracts at 25°C. PDA medium with or without methanol was used as a control. Results showed that extracts of *B. glutinosa* and *L. tridentata* had the highest inhibitory effect ($P < 0.05$) on spore germination (> 92%), radial growth extension (> 90%), and biomass production (> 95%) at 100, 336, and 336 h after inoculation, respectively. On the other hand, extracts from *A. confertiflora* and *A. indica* were not significantly different ($P > 0.05$) from the control. These results indicate that the extracts of *B. glutinosa* and *L. tridentata* might be natural and effective alternatives for control of *F. verticillioides* during its different growth phases, spores germination, and mycelial growth.

Additional keywords: Antifungal properties, natural compounds, *Ambrosia confertiflora*, *Azadirachta indica*, *Baccharis glutinosa*, *Larrea tridentata*.

Resumen. El objetivo de este trabajo fue determinar el efecto inhibitorio de extractos metanólicos de *Ambrosia confertiflora*, *Azadirachta indica*, *Baccharis glutinosa* y *Larrea tridentata* en la germinación de esporas y en el crecimiento micelial de *Fusarium verticillioides*. El porcentaje de germinación de esporas, el crecimiento radial y la producción de biomasa se evaluaron usando papa-dextrosa-agar (PDA) con 5.6, 11.0, 14.0 y 16.7% (v/v) de los extractos a 25°C. Se utilizó medio PDA con y sin metanol como testigo. Los resultados indicaron que los extractos de *B. glutinosa* y *L. tridentata* presentaron el mayor efecto inhibitorio ($P < 0.05$) en la germinación de las esporas (> 92%), en el crecimiento radial (> 90%) y en la producción de biomasa (> 95%) a las 100, 336 y 336 h después de la inoculación, respectivamente. Por otro lado, los extractos de *A. confertiflora* y *A. indica* no fueron significativamente diferentes ($P > 0.05$) al testigo. Estos resultados indican que los extractos de *B. glutinosa* y *L. tridentata* pueden ser una fuente de productos naturales alternativos y efectivos para el control de *F. verticillioides* en sus diferentes fases de crecimiento, en la germinación de esporas, así como en el crecimiento micelial.

Palabras clave adicionales: Propiedades antifúngicas, compuestos naturales, *Ambrosia confertiflora*, *Azadirachta indica*, *Baccharis glutinosa*, *Larrea tridentata*.

The genus *Fusarium* is one of the most important pathogens in corn (*Zea mays* L.), being *F. graminearum* Schwabe , *F. verticillioides* (Sacc.) Nirenberg, and *F. subglutinans* (Wollenweb and Reinking) Nelson, Toussoun and Marasas the most persistent species (Marasas *et al*., 1984; Nelson, 1992; Pitt and Hocking, 1999). *F. verticillioides* is however, one of the predominant pathogens associated with corn worldwide. Besides the economic impact of corn diseases caused by *Fusarium*, some species are able to produce highly potent mycotoxins such as zearalenone, T-2 toxin, tricothecenes, vomitoxin and fumonisins, which may affect both animal and human health (Marasas *et al*., 1984; Moreno, 1988; Nelson *et al*., 1993). *F. verticillioides* is able to produce toxins such as fumonisins most frequently in corn (Blackwell *et al*., 1996; Marasas, 1996; Marasas *et al*., 2000; Nelson, 1992), sorghum [*Sorghum bicolor* (L.) Moench.] and oat (*Avena sativa* L.) (Acuña *et al*., 2005; Bacon and Nelson, 1994; Leslie *et al*., 1990), rice (*Oryza sativa* L.) (Abbas *et al*., 1998), and wheat (*Triticum aestivum* L.) (Castoria *et al*., 2005;

Shephard *et al*., 2005). Fumonisins are stable during food processing: they are not degraded during corn fermentation (Scott and Lawrence, 1995); they are heat stable (Marasas *et al*., 2000) and resistant to canning and baking processes (Castelo *et al*., 1998), although in corn the nixtamalization process reduces fumonisin B_1 levels, a five-fold more toxic product with respect to the original level (Bullerman *et al*., 2002; Hendrich *et al*., 1993; Voss *et al*., 1996). To avoid effectively the associated problems with *Fusarium* and their toxins, it is necessary to prevent fungal growth on the substrate, which can be achieved by the use of chemical inhibitors (Moreno and Ramírez, 1985). Organic acids and their salts, like propionic and sorbic acids, have been added in corn destined for animal consumption (Marín *et al*., 1999a; 1999b). Recent studies have shown that growth of *Fusarium* species can be controlled by propionic acid and their commercial formulas, depending on water availability and temperature (Marín *et al*., 2000). The use of natural bioactive substances for control of postharvest fungal infections has gained attention due to problems associated with chemical agents. These include the development of fungal species resistant to chemical treatments, which increases food-borne pathogenic microorganisms, in addition to increasing the number of pesticides under observation or regulation (Rabea *et al*., 2003). Natural compounds such as essential oils of oregano (*Origanum vulgare* L.), lemon [*Citrus limon* (L.) N.L. Burm.], and palmarose [*Cymbopogon martinii* (Roxb.) J.F. Watson] (Vellunti *et al*., 2003), may constitute potential preservative compounds due to their capacity to inhibit fungal growth and toxin production of *Fusarium*. Also, essential oils of cinnamon (*Cinnamomum zeylanicum* Blume) and oregano have shown fungicidal activity *in vitro* against *Aspergillus flavus* Link: Fr. (García-Camarillo *et al*., 2006). Aqueous plant extracts from garlic (*Allium sativum* L.), creosote bush [*Larrea tridentata* (Seesé and Moc. ex D.C.) Coville], and clove [*Sycygium aromaticum* (L.) Merr. and Perry] inhibited the growth of *Fusarium oxysporum* Schlechtend:Fr. f. sp. *lycopersici* (Sacc.) Snyder and Hansen, *Rhizoctonia solani* Kühn, and *Verticillium dahliae* Kleb. (López-Benítez *et al*., 2005). According to Verastegui *et al*. (1996), alcoholic extracts from natural desert plants like *Baccharis glutinosa* William and Wilma and *Larrea tridentata*, may act against the growth of fungi, yeast, and bacteria. In addition, Sánchez *et al*. (2005) reported the inhibition of both growth and mycotoxin production by *Aspergillus flavu*s and *A. parasiticus* Speare when exposed to ethanolic, methanolic, and aqueous extracts of *Agave* species. For that reason, it is possible that native plants such as *L. tridentata*, *Baccharis glutinosa*, *Ambrosia confertiflora* DC, and *Azadirachta indica* A. Juss. can be used as source of natural preservative compounds for the control of filamentous fungi like *Fusarium verticillioides*. The aim of this work was to study the inhibitory effects of methanolic crude plant extracts (*Larrea tridentata*, *Baccharis glutinosa*, *Ambrosia confertiflora*, and *Azadirachta indica*), on spore germination, biomass production, and radial growth of *Fusarium verticillioides*. Also, to evaluate the effect of the extracts on fumonisin B_1 production.

MATERIALS AND METHODS

Plant extracts and media preparation. Stems and leaves of *Larrea tridentata*, *Baccharis glutinosa*, and *Ambrosia confertiflora* were collected from several regions of Sonora, Mexico. *Azadirachta indica* was obtained from Ciudad Obregon, Sonora. They were sun dried for 24 h and milled (Willey Laboratory Mill model 4, USA) to a particle size of 0.5-1.0 mm. Extracts were prepared by mixing 6 g of each plant powder with 94 mL of a 70% (v/v) methanol solution, with 15 min of stirring and incubated at 25 ± 2°C. Extracts were filtered through a Whatman No. 1 filter paper, then through a micropore glass fiber paper, and stored at 5°C in darkness until further use (Tequida-Meneses *et al*., 2002). Each methanolic plant extract was added to potato-dextrose-agar (PDA) nutrient medium (DIFCO, USA) at concentrations of 5.6, 11.1, 13.9, and 16.7% (v/v). PDA plates with 30% methanol and without methanol were used as controls.

Microorganism and growth conditions. A strain of *Fusarium verticillioides* isolated from natural contaminated corn, was selected due to its high fumonisin B_1 production (763.75 µg/g, detected by HPLC analysis) (Gallardo-Reyes *et al*., 2006). The strain was activated in PDA and incubated at 25 ± 2°C for 10 days. Spores were collected 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 1×10^5 was determined using a Neubauer chamber.

Spore germination assays. Agar plates were inoculated by spreading the spore suspension onto the agar surface, and incubated at 25°C using a 12 h light/dark cycle (Precision low temperature illuminated incubator 818, USA). The number of germinated spores per plate was determined by taking at random 200 spores (germinated and non-germinated) at 4, 8, 12, 16, 20, 28, 48, 72, and 100 h after plating and using a light microscope. A spore was considered germinated when the length of its germinal tube reached one-half of the spore diameter (Paul *et al*., 1993; Plascencia-Jatomea *et al*., 2003; Smilanick *et al*., 1990). Each germination experiment was made in duplicate and the concentration that delayed 50% of spore germination (CC_{50}) was determined at 95% of confidence intervals using Probit analysis in the NCSS 2001 program (NCSS Inc., USA) (Finney, 1952; Infante and Calderón, 1994). For each inhibitor compound, the percentage of germinated spores of *F. verticillioides* was fitted to the logistic expression (Equation 1), where S was the percentage of germinated spores after time (t), S_0 was the initial percentage of germinated spores, S_{max} was the highest percentage of germinated spores

$$S = \frac{S_{max}}{1 + \left(\frac{S_{max} - S_0}{S_0}\right) e^{-kt}} \qquad \text{(Equation 1)}$$

as the time increases, and k was the spore germination rate. The S_0, S_{max} and k values were estimated using the NCSS 2001 program (NCSS Inc., USA). The inhibition of spore germination was calculated as a percentage of the control (Equation 2), in which S_e represented the percentage of spores germinating in the treated samples and S_c was the percentage of spores germinating in the control. The percentage of spore germination inhibition was calculated from data of 100 h old

$$\text{Inhibition (\%)} = \frac{(\bar{S}_c - S_e)}{\bar{S}_c} \times 100 \qquad \text{(Equation 2)}$$

cultures.

Radial extension growth. Puncture inoculation of *F. verticillioides* carried out by a point wise deposition of the inoculum in the center of the plate, was used to measure the radial growth of the colony which was measured daily and compared to the control media. The radial inhibition percentages were calculated using Equation 3: where R_c was the mean value of colony radius of control media and R_i was the colony radius of the inhibitor amended media (Holmes

$$\text{Radial inhibition (\%)} = \frac{(\bar{R}_c - R_i)}{\bar{R}_c} \times 100 \qquad \text{(Equation 3)}$$

and Eckert, 1999; Plascencia-Jatomea *et al*., 2003). The radial extension rate of the colony, U (µm/h), was determined from the slope resulting from the radio versus time graph. 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 2001 statistical program (NCSS Inc., USA). All the measurements or analysis were carried out in triplicate.

Biomass production. The biomass production was quantified daily as the mycelium dry weight during 2 weeks. The agar gel with the produced biomass was separated from the plate, poured into a glass beaker containing 200 mL of distilled 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 once 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; López *et al*., 1997). All determinations were carried out in triplicate. Growth curves were adjusted to the logistic expression (Equation 4), where X was the biomass density (mg/cm^2), X_{max} was the highest biomass production (when dX/dt = 0) and max was the specific growth rate (when $X<X_{max}$). Growth parameters were estimated using the

$$\frac{dX}{dt} = \mu_{max}\left(1 - \frac{X}{X_{max}}\right) X \qquad \text{(Equation 4)}$$

Statistica 6.0 program (Stat Soft, Inc., USA), which minimized the sum of the square of the error by comparing the experimental data to predicted data obtained by the resolution of Equation 4, given by Equation 5, where X_0 was the initial

$$X(t) = \frac{X_{max}}{1 + \left(\frac{X_{max} - X_0}{X_0}\right) e^{-\mu_{max} t}} \qquad \text{(Equation 5)}$$

value of biomass density.

Fumonisin B_1 production. The most effective extracts against fungal growth were selected to study their possible effects on fumonisin B_1 production in corn grain inoculated with *F. verticillioides*, using their CI_{50} on the radial growth. The extracts tested were *Baccharis glutinosa* and *Larrea tridentata* at concentrations of 7.4 and 4.0% (v/v), respectively. The FB_1 production was determined according to Castellá *et al*. (1999) 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 min at 121°C. Autoclaved maize was separately treated with extracts from *B. glutinosa* and *L. tridentata* at 7.4 and 4.0% (v/v), respectively. Control flasks were prepared following the same procedure with no extract added or methanol alone. Each treatment was inoculated with 1 x 10^7 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. Separation and purification of FB_1 was carried out according to Etcheverry *et al*. (2002). The cultures were oven-dried overnight at 50°C and the FB_1 was extracted with 150 mL of acetonitrile:water (1:1 v/v) by shaking the culture media and mycelia with the solvent for 30 min on an orbital shaker (150 rev min^{-1}). The extracts were filtered through a Whatman No. 4 filter paper (International Ltd, Maidstone, Kent, UK). An aliquot (1000 µL) was taken from the filtrate and diluted with 9 mL of acetonitrile:water. Diluted extracts were purified using a pre-conditioned strong anion exchange column (SAX) SPE (Bond Elut Sax, Varian, Inc., USA). FB_1 was eluted with 15 mL of methanol acetic acid 1% and evaporated to dryness under air flow.

Fumonisin B_1 quantification. The fumonisin B_1 content was determined by HPLC as described by Shephard *et al*. (1990). The dried eluate was re-dissolved with acetonitrile: water (1:1 v/v). For the analysis, 50 µL were transferred into a test tube, combined with 200 µL of o-phtaldialdehyde solution (OPA), shaken for 30 s, and a 20 µL aliquot was injected into the chromatography system consisting of a pump (9012, Varian, USA) connected to a fluorescence detector (9070, Varian, USA). Chromatographic separation was performed on a stainless steel reversed-phase column (Supercosil LC-ABZ, C18; 150 x 4.6 mm I.D., 5 µm particle size; Supelco, Sigma-Aldrich). Methanol:0.1 mol l^{-1} sodium dihydrogen phosphate

(75:25) solution, adjusted to pH 3.35 with orthophosphoric acid, was used as the mobile phase at a flow rate of 1.5 mL min^{-1}. The fluorescence of the fumonisin B_1-OPA derivative was recorded at excitation and emission wavelengths of 335 and 440 nm, respectively. Fumonisin B_1 quantification was performed by peak area measurements and compared with a reference standard solution. The standard solution was obtained by dissolving pure fumonisin FB_1 (Sigma-Aldrich) in acetonitrile:water (1:1 v/v) at a concentration of 100 µg/mL.

Statistical analysis. A completely randomized 3 factor design (plant, concentration, and time and their interactions) for the radial and germination percent inhibition was carried out. Samples were taken at random from the incubator in triplicate. Experiments on radial growth were done in triplicate while germination assays were done in duplicate. For the fumonisin B_1 production assay, 2 plants at two concentrations were applied in triplicate. The program JMP 2004 (JMP Inc., USA) computed the analysis of variance and the means were compared with the Tukey multiple range test ($p < 0.05$).

RESULTS

Spore germination. The spore germination percentage of *F. verticillioides* was calculated for each methanolic extract. The results showed that spores inoculated on PDA only germinated completely within 28 h of incubation, while spores placed on the control with methanol germinated after 100 h of incubation (Table 1). However, there was a significant difference ($p < 0.05$) in the percentage of spore germination inhibition between methanolic extract and the control with methanol (Table 2). Results showed that extracts from *Baccharis glutinosa* and *Larrea tridentata* at all concentrations, were the most effective in controlling spore germination of *F. verticillioides*. In the same way, in all concentrations tested, these extracts reduced the maximal percentage of germinated spores, S_{max} (< 12.4%). Extracts from *Azadirachta indica* and *Ambrosia confertiflora* significantly inhibited spore germination, only at two of the concentrations tested (11.1 and 16.7%, respectively). According to the adjusted logistic parameters, there was not an observed pattern that relates the spore germination rate (k) to the vegetal extract or concentration (Table 2).

Radial growth. The control with methanol showed an inhibitory effect on *F. verticillioides* since plates were completely covered with the mycelium after 336 h of incubation (Table 3), while control plates with PDA only, *F. verticillioides* completely covered the plate after 192 h. Due to its inhibitory effect, methanol was used as control to determine the radial inhibition percentage of extracts (Table 4). Extracts from *B. glutinosa* and *L. tridentata* were the most effective to control fungal colony growth at all concentrations. Extracts from *A. indica* and *A. confertiflora*, however, were not able to control effectively the colony growth at any concentration, even though fungi grew faster than the control with methanol. Compared with control, all extracts at all concentrations tested, significantly affected the radial extension rate, U (µm/h), of *F. verticillioides*. Extracts from *B.*

Table 1. Percentage spore germination of *Fusarium verticillioides* in potato-dextrose-agar containing methanolic extracts of plants at different concentrations at 25°C.

Treatment[z]	Time of incubation (h)								
	4	8	12	16	20	28	48	72	100
CPDA	7.75	8.75	35.5	49.75	95.5	100	100	100	100
CMeOH	7.25	5.5	7	13	18	20	50.25	58.25	100
BT1	2.75	4.25	4	4.25	4.75	6.25	13.25	9.25	11.5
BT2	3.5	4.5	4.5	6.0	6.8	7.8	8.3	11.0	9.8
BT3	2	2.25	2.5	4.0	4.5	5.0	12.5	12.5	11.3
BT4	0.5	1.25	1.8	3.8	3.5	5.3	6.5	10.5	10.3
NT1	5.5	6.5	7.5	7.3	9.3	8.5	36.0	28.0	100.0
NT2	8.75	8.75	8.0	7.0	12.3	5.5	31.3	50.3	17.3
NT3	3.75	17.25	9.5	10.0	7.5	7.8	33.5	22.8	100.0
NT4	4.75	12.75	14.3	13.0	11.5	8.8	32.0	98.8	100.0
ET1	2	5	9.0	8.5	6.8	5.8	15.3	36.0	32.0
ET2	2.75	5	6.0	6.0	5.3	7.0	14.5	71.5	95.0
ET3	2.5	5	5.8	8.0	8.8	5.3	7.3	23.0	26.8
ET4	1.75	5.75	5.8	6.3	6.8	6.0	6.5	9.5	12.5
GT1	1.5	2.5	5.5	5.3	2.5	3.8	5.8	5.3	10.0
GT2	1.25	2.75	3.5	2.0	5.0	2.8	11.0	7.3	7.3
GT3	0.25	3.75	3.3	3.5	4.5	4.3	5.3	5.5	4.5
GT4	1	4.5	6.3	3.3	5.8	2.0	11.5	5.5	6.8

[z]CPDA = Control with potato-dextrose-agar; CMeOH = Control with methanol (30% v/v); B = *Baccharis glutinosa*; N = *Azadirachta indica*; E = *Ambrosia confertiflora*; G = *Larrea tridentata*. T1 = 5.6 % concentration (v/v), T2 = 11.1%, T3 = 13.9%, T4 = 16.7%.

Table 2. Inhibition of spore germination[x] of *Fusarium verticillioides* and germination speed in media supplemented with plant extracts after 100 hours of incubation at 25°C.

Treatment[y]	Inhibition (%)	S_{max} (%)*	k (h^{-1})	R
CPDA	0.0 a	101.3 ± 8.70	0.321 ± 0.081	0.9864
CMeOH	0.0 a	25.5 ± 13.2	0.040 ± 0.005	0.9799
BT1	88.5 ± 0.50 de[z]	11.4 ± 0.84	0.080 ± 0.022	0.8994
BT2	90.3 ± 2.75 de	10.1 ± 1.91	0.067 ± 0.007	0.7089
BT3	88.8 ± 0.75 de	12.4 ± 1.30	0.091 ± 0.004	0.8776
BT4	89.8 ± 2.25 de	10.6 ± 1.47	0.063 ± 0.025	0.8994
NT1	0.0 a	0.0	0.030 ± 0.003	0.9680
NT2	82.8 ± 2.25 cd	34.1 ± 5.11	0.084 ± 0.039	0.7528
NT3	0.0 a	0.0	0.030 ± 0.004	0.9433
NT4	0.0 a	113.7 ± 16.40	0.062 ± 0.029	0.9636
ET1	68.0 ± 3.00 b	36.8 ± 4.59	0.056 ± 0.015	0.9259
ET2	5.00 ± 0.50 a	98.4 ± 2.56	0.097 ± 0.005	0.9906
ET3	73.3 ± 3.75 bc	39.9 ± 1.54	0.031 ± 0.001	0.8900
ET4	87.5 ± 1.50 de	50.2 ± 4.67	0.012 ± 0.003	0.8266
GT1	90.0 ± 1.00 de	0.0	0.011 ± 0.002	0.7668
GT2	92.8 ± 1.25 de	8.3 ± 1.07	0.089 ± 0.038	0.7622
GT3	95.5 ± 0.50 e	4.8 ± 0.52	0.237 ± 0.092	0.6484
GT4	93.3 ± 1.75 de	7.4 ± 1.45	0.075 ± 0.005	0.5254

[x]Values are the average of triplicates ± the standard error of the mean.
[y]CPDA = Control with potato-dextrose-agar; CMeOH = Control with methanol (30% v/v); B = *Baccharis glutinosa*; N = *Azadirachta indica*; E = *Ambrosia confertiflora*; G = *Larrea tridentata*. T1 = 5.6 % concentration (v/v), T2 = 11.1%, T3 = 13.9%, T4 = 16.7%.
[z]Values with different letters are statistically different (Tukey, $p < 0.05$) (SPSS 11.0).
*S_{max} = maximum percentage of germinated spores as the time increases; k = germination rate; R = correlation coefficient.

Table 3. Mycelial extension of *Fusarium verticillioides* on potato-dextrose-agar with methanolic extracts of plants at different concentrations at 25°C.

Treatment[z]	Time of incubation (h)												
	24	48	72	96	144	168	192	216	240	269	288	312	336
CPDA	0.0	0.6	1.2	1.8	2.9	3.5	3.9	3.9					
CMeOH	0.0	0.0	0.0	0.0	0.2	0.4	0.7	1.1	1.6	2.2	2.6	3.2	3.7
BT1	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.5	0.8	0.9	1.1	1.4
BT2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.3	0.4
BT3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.2	0.4	0.5
BT4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.5	0.6
NT1	0.0	0.0	0.0	0.0	0.3	0.4	0.7	1.0	1.5	2.0	2.5	3.0	3.2
NT2	0.0	0.0	0.0	0.0	0.2	0.4	0.6	0.9	1.4	1.7	2.1	2.5	2.8
NT3	0.0	0.0	0.0	0.0	0.2	0.3	0.6	0.7	1.0	1.5	1.9	2.3	2.8
NT4	0.0	0.0	0.0	0.0	0.2	0.4	0.6	1.0	1.4	1.9	2.3	2.8	3.2
ET1	0.0	0.0	0.0	0.0	0.2	0.3	0.6	0.9	1.2	1.7	2.0	2.5	2.8
ET2	0.0	0.0	0.0	0.0	0.2	0.4	0.6	0.9	1.3	1.7	2.0	2.4	2.7
ET3	0.0	0.0	0.0	0.0	0.1	0.4	0.6	0.9	1.3	1.7	2.1	2.5	2.9
ET4	0.0	0.0	0.0	0.0	0.2	0.4	0.8	1.0	1.4	1.8	2.2	2.6	3.0
GT1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.8
GT2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.2	0.3	0.4
GT3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2
GT4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.3

[z]CPDA = Control with potato-dextrose-agar; CMeOH = Control with methanol (30% v/v); B = *Baccharis glutinosa*; N = *Azadirachta indica*; E = *Ambrosia confertiflora*; G = *Larrea tridentata*. T1 = 5.6 % concentration (v/v), T2 = 11.1%, T3 = 13.9%, T4 = 16.7%.

Table 4. Inhibition of *Fusarium verticillioides* radial growth and velocity of growth[w] after 336 hours of incubation in media with plant extracts.

Treatment[x]	Inhibition (%)	Colony radius (cm)	U[y] (µm/h)
CPDA	0.0	3.90 ± 0.00 b	0.023
CMeOH	-0.0	3.73 ± 0.16 b	0.022
BT1	63.60 ± 3.97 b[z]	1.36 ± 0.15 a	0.008
BT2	89.93 ± 5.32 b	0.38 ± 0.19 a	0.004
BT3	87.07 ± 4.82 b	0.48 ± 0.18 a	0.004
BT4	84.40 ± 1.73 b	0.58 ± 0.06 a	0.006
NT1	14.27 ± 7.01 a	3.20 ± 0.26 b	0.018
NT2	25.67 ± 6.14 a	2.78 ± 0.60 b	0.015
NT3	24.57 ± 5.26 a	2.82 ± 0.19 b	0.018
NT4	13.83 ± 2.51 a	3.22 ± 0.09 b	0.017
ET1	26.13 ± 10.24 a	2.75 ± 0.38 b	0.015
ET2	26.80 ± 2.97 a	2.73 ± 0.48 b	0.014
ET3	23.63 ± 1.76 a	2.85 ± 0.06 b	0.015
ET4	20.30 ± 7.99 a	2.97 ± 0.29 b	0.015
GT1	79.23 ± 3.92 b	0.78 ± 0.14 a	0.005
GT2	88.83 ± 3.47 b	0.42 ± 0.13 a	0.004
GT3	95.97 ± 1.67 b	0.15 ± 0.06 a	0.003
GT4	92.60 ± 1.15 b	0.28 ± 0.04 a	0.004

[w]Values are the average of triplicates ± the standard error of the mean.

[x]CPDA = Control with potato-dextrose-agar; CMeOH = Control with methanol (30% v/v); B = *Baccharis glutinosa*; N = *Azadirachta indica*; E = *Ambrosia confertiflora*; G = *Larrea tridentata*. T1 = 5.6 % concentration (v/v), T2 = 11.1%, T3 = 13.9%, T4 = 16.7%.

[y]U = radial extension rate of the colony (determined from the slopes of the radial growth versus time).

[z]Values with different letters are statistically different (Tukey, $p < 0.05$) (SPSS 11.0).

glutinosa and *L. tridentata* presented the lowest radial extension rates (< 0.008 µm/h). The minimum inhibitory concentration that inhibited 50% of radial growth, CI_{50}, was determined to the extracts that showed the best inhibitory effects (*B. glutinosa* and *L. tridentata*). The CI_{50} for *B. glutinosa* and *L. tridentata* extracts were 7.4% and 4.0% (v/v), respectively.

Biomass production. Table 5 shows that extracts from *B. glutinosa* and *L. tridentata* were the most effective in inhibiting biomass production of *F. verticillioides* at all concentrations. These results were also inconsistent, because *F. verticillioides* was inhibited by more than 50% with the *A. confertiflora* extract at a 11.1% concentration, whereas the inhibition was lower at higher concentrations. *A. indica* was not effective in controlling biomass production at any concentration tested. For all treatments, a pattern which relates maximal biomass production (X_{max}) to the specific growth rate (μ_{max}) of the fungi in the presence of the methanolic extract was not observed.

Fumonisin production. The presence of fumonisin B_1 was detected in all treatments by HPLC analysis (Table 6). There were not significant differences ($p > 0.05$) among the treatments. Results showed that in the corn grain treated with either *B. glutinosa* and *L. tridentata* extracts at 7.4 and 4.0% concentration, respectively, there was no inhibition of fumonisin B_1 production. On the contrary, the fumonisin B_1 production was increased with the treatment compared with the control. Analysis determined that there was no difference ($p > 0.05$) between the control, and each of the treatments.

DISCUSSION

Baccharis glutinosa, *Larrea tridentata*, and *Azadirachta indica* extracts were effective in inhibiting spore germination and radial growth of *F. verticillioides*. The inhibitory effect of extracts did not increase when concentration increased. Indeed, these results were inconsistent, and in some cases the highest concentrations had no inhibition in some of the treatment, as observed with *A. indica* and *A. confertiflora* extracts. This agrees with the findings of *Juglal et al*. (2002), who also reported that neem oil did not affect mycelial growth of *Fusarium moniliforme* Sheld and *Aspergillus parasiticus*; however, fumonisin B1 production increased as in our study. The methanolic extracts with the highest fungistatic potential were *B. glutinosa* and *L. tridentata*, since they inhibited the radial and mycelial growth of *F. verticillioides*. This means that in order to inhibit 50% of *F. verticillioides* radial growth, 7.4 (v/v) of the *B. glutinosa* and 4.0% (v/v) of the *L. tridentata* extracts are required in the media. However, the same concentrations of these extracts were not effective in inhibiting the production of fumonisin B_1 in corn grain, whereas the extracts that can inhibit growth on synthetic media, are not effective on corn at the same concentrations. Nevertheless, further studies are necessary to evaluate the effectiveness of the extracts at other concentrations to inhibit fumonisin B_1 production by *F. verticillioides*. Similar results were reported by Vellunti *et al*. (2003), who found that essential oils from plants have an inhibitory effect on fumonisin B1 production by *Fusarium proliferatum* (Matsushima) Nirenberg, as well as in its growth. Bullerman (1974) and Bullerman *et al*. (1977) found that aflatoxin production was inhibited without affecting fungal growth. In contrast, López *et al*. (2004) reported that *Aloysia triphylla* (L´ Hér.) Britton and *Origanum vulgare* oils inhibited the mycelia growth of *F. verticillioides*, however, fumonisin B1 production was inhibited by *O. vulgare* while *A. triphylla* increased it. Cespedes *et al*. (2006) reported that *Tagetes lucida* Cav. methanolic extracts inhibited *F. moniliforme* growth, however, fumonisin production was not evaluated. Also, Cárdenas-Ortega *et al*. (2005) reported that essential oil of *Chrysactinia mexicana* Gray caused inhibition of *A. flavus* growth in liquid culture media. The *A. confertiflora* extract at a 11.1% concentration showed a similar effect ($p > 0.05$) to that observed when either *B. glutinosa*, or *L. tridentata* extract was used. The *A. indica* extract showed less than 50% inhibition, which is contrary to

Table 5. Inhibition of *Fusarium verticillioides* biomass production[w] after 336 hours of incubation in media with plant extracts.

Treatment[x]	Inhibition (%)	BM production[y] (mg/cm^2)	μ_{max} (mg/cm^2)	R
CPDA	-----	2.92 ± 0.36 c		
CMeOH	-----	1.95 ± 0.15 bc	0.015	0.9119
BT1	90.12 ± 7.84 cd[z]	0.16 ± 0.16 a	-----	-----
BT2	96.39 ± 0.70 cd	0.05 ± 0.02 a	-----	-----
BT3	100 ± 0.00 d	0.02 ± 0.001 a	-----	-----
BT4	98.05 ± 0.97 cd	0.02 ± 0.008 a	-----	-----
NT1	0.00 ± 0.00 a	2.63 ± 0.31 c	0.016	0.9284
NT2	3.66 ± 0.06 a	2.36 ± 0.31 bc	0.038	0.9313
NT3	0.00 ± 0.00 a	2.40 ± 0.03 bc	0.036	0.9198
NT4	0.00 ± 0.00 a	2.36 ± 0.17 bc	0.014	0.9216
ET1	39.12 ± 7.97 abc	1.52 ± 0.81 abc	0.012	0.6208
ET2	66.67 ± 3.33 bcd	0.82 ± 0.08 ab	-----	-----
ET3	29.32 ± 10.65 ab	1.37 ± 0.20 abc	0.008	0.8869
ET4	22.25 ± 3.18 ab	1.51 ± 0.06 abc	0.001	0.9641
GT1	95.74 ± 2.24 cd	0.08 ± 0.04 a	-----	-----
GT2	100 ± 0.00 d	0.02 ± 0.007 a	-----	-----
GT3	95.15 ± 0.30 cd	0.10 ± 0.008 a	-----	-----
GT4	99.20 ± 0.80 cd	0.00 ± 0.00 a	-----	-----

[w]Values are the average of triplicates ± the standard error of the mean.
[x]CPDA = Control with potato-dextrose-agar; CMeOH = Control with methanol (30% v/v); B = *Baccharis glutinosa*; N = *Azadirachta indica*; E = *Ambrosia confertiflora*; G = *Larrea tridentata*. T1 = 5.6 % concentration (v/v), T2 = 11.1%, T3 = 13.9%, T4 = 16.7%.
[y]BM = Biomass; μ_{max} = specific growth rate (when $X<X_{max}$); R = correlation coefficient.
[z]Values with different letters are statistically different (Tukey, $p < 0.05$) (SPSS 11.0).

that reported in a previous study in which an ethanolic extract from *A. indica* inhibited the incidence of *Colletotrichum glocosporioides* (Penz.) Penz. and Sacc. in mango (*Mangifera indica* L.) (Bommarito *et al*., 1998). This might suggest that the extract effectiveness may depend on both the target organism and the substrate. The inhibitory activity of *L. tridentata* extract on growth of *F. verticillioides* found in the present study agrees with its effect against the growth of *A. flavus* and *A. parasiticus* reported previously by Vargas-Arispuro *et al*. (2005). They analyzed the composition of the ethanolic extract of *L. tridentata* leaves, finding norhydroguaiaretic acid (NDGA) and methyl-norhydroguaiaretic (methyl-NDGA) as the main components responsible for the antifungal effect. Fumonisin production was not inhibited with the two extracts at the tested concentrations, even though when they reduced the radial growth of *F. verticillioides*. Although when radial growth was restringed by the plant extracts, we observed atypical apical growth and the aerial mycelium reached the cover of the Petri dishes. These could be due to the stressful environment generated by the plant extracts in the Petri dishes and physiological responses to overcome these conditions may result in over expression of mycotoxin production. The use of these plant extracts alone does not appear feasible to control fumonisin production by *F. verticillioides*. Our findings evidence that the use of methanolic crude plant extracts of *Baccharis glutinosa* and *Larrea tridentata* inhibited fungal growth, but they did not affect fumonisin production.

Table 6. Fumonisin B_1 production[y] in different treatments by *Fusarium verticillioides* on corn (*Zea mays*) grain after 30 days of incubation.

Treatment	Fumonisin B_1 (µg/g)
Baccharis glutinosa 7.4%	1602.0 ± 524.34 a[z]
Larrea tridentata 4.0%	1110.4± 350.35 a
Control with PDA	585.8 ± 351.34 a
Control with MeOH	417.3 ± 110.37 a

[y]Values are the average of triplicates ± the standard error of the mean.
[z]Values with different letters are statistically different (Tukey, $p < 0.05$) (SPSS 11.0).

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