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EFFECT OF THE ROOTS EXTRACT FROM *Heliopsis longipes* ON *Aspergillus parasiticus* GROWTH

EFECTO DEL EXTRACTO DE RAÍCES DE *Heliopsis longipes* SOBRE EL CRECIMIENTO DE *Aspergillus parasiticus*

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ABSTRACT

The organic extracts from *Heliopsis longipes* roots possess interesting biological and pharmacological activities. However, the effect on *Aspergillus parasiticus* has not been investigated so far. This study was aimed to evaluate the antifungal effect of the ethanolic extract from *H. longipes* roots against *A. parasiticus* growth. Four extract concentrations, 50, 75, 150 and 200 µg/mL were evaluated for antifungal effect, and the anti-aflatoxigenic assay was tested at 150 and 200 µg/mL. The minimal inhibitory concentration that inhibited 50 and 99% of growth (MIC50 and MIC99) were determined. Ethanolic extract was characterized by GC-EIMS analysis and its main bioactive compounds were identified. All tested concentrations inhibit the radial growth of *A. parasiticus* and the MIC50 and MIC99 were 116.94 and 1593.98 µg/mL, respectively. These results showed the first evidence of the antifungal effect of *H. longipes* on the radial growth and spore germination rate of this pathogenic fungus. The antifungal activity of *H. longipes* extract was attributed to affinin (7.24 ± 0.13 mg/g of fresh tissue), the most abundant alkamide detected by GC-EIMS analysis. Although, the extract did not inhibit the aflatoxins´ production, it can be used to prevent *A. parasiticus* growth before the mycotoxins production occur. Therefore, the extract has potential as natural antifungal agent against *A. parasiticus* contamination.

Keywords: *Aflatoxins production; Alkamide content; Antifungal effect; Aspergillus parasiticus; Heliopsis longipes roots.*

RESUMEN

Los extractos orgánicos de las raíces de *Heliopsis longipes* poseen actividades biológicas y farmacológicas interesantes. Sin embargo, el efecto sobre *Aspergillus parasiticus* no se ha investigado hasta el momento. Este estudio tuvo como objetivo evaluar la actividad antifúngica del extracto etanólico de las raíces de *H. longipes* contra el crecimiento de *A. parasiticus*. Las concentraciones de extracto de 50, 75, 150 y 200 µg/mL se evaluaron para determinar el efecto antifúngico y el ensayo anti-aflatoxigénico se evalúo a 150 y 200 µg/mL. La concentración inhibitoria mínima que inhibía el 50 y 99% de crecimiento (MIC50 y MIC99), también se determinaron. El extracto etanólico se caracterizó por análisis GC-EIMS y se identificaron sus principales compuestos bioactivos. Todas las concentraciones evaluadas inhibieron el crecimiento radial de *A. parasiticus* y los valores de MIC50 y MIC99 fueron 116.94 y 1593.98 µg/mL, respectivamente. Estos resultados mostraron la primera evidencia del efecto antifúngico de *H. longipes* sobre el crecimiento radial y la tasa de germinación de esporas de este hongo patógeno. La actividad antifúngica del extracto de *H. longipes* se atribuyó a la afinina (7,24 ± 0,13 mg / g de tejido fresco), la alcamida más abundante detectada por el análisis GC-EIMS. Aunque el extracto no inhibió la producción de aflatoxinas, puede usarse para prevenir el crecimiento de *A. parasiticus* antes de que ocurra la producción de micotoxinas. Por lo tanto, el extracto tiene potencial como agente antifúngico natural contra la contaminación por *A. parasiticus*.

Palabras clave: *Aspergillus parasiticus; Contenido de alcamidas; Efecto antifúngico; producción de aflatoxinas; Raíz de Heliopis longipes.*

INTRODUCTION

Heliopsis longipes (A. Gray) S. F. Blake (*H. longipes*) is a species belonging to the tribu Heliantheae of the family Asteraceae (Fisher, 1957). It is a medicinal plant endemic to Mexico, which grows particularly in the Sierra Gorda and Sierra de Alvarez in the states of Guanajuato, Querétaro and San Luis Potosí, where it is known by common names as "*Chilcuague", "Chilcuan"* and "*Chilmecatl"* from Náhuatl (Little, 1948; Martínez, 1989). The roots of this species have various medicinal and culinary applications, attributed to

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the high concentrations of alkamides which it produces and accumulates (Molina-Torres et al., 1996). The predominant bioactive alkamide reported in *H. longipes* roots is the affinin (*N*-isobutyl-2*E*,6*Z*,8*E*-decatrienamide), which represents ≥ 1% of dry weight of root tissue (Molina-Torres et al., 1996). This alkamide is also known as spilanthol when found in *Spilanthes* plant species (Paulraj et al., 2014). Its biological activities known so far, includes antimicrobial -bacteriostatic and fungistatic effects- against several phytopathogenic fungi, which have been attributed to this compound (Molina-Torres et al., 1999; Ramírez-Chavez et al., 2000; Molina-Torres et al., 2004). However, there are no current reports about the effect on the growth of *Aspergillus parasiticus,* a pathogenic and mycotoxigenic fungus that contaminates a great variety of cereals, predominantly maize (Ze*a mays* L.). This fungus is one of the main aflatoxin's producers (Dorner et al., 1984; Diener et al., 1987), a group of highly genotoxic and carcinogenic compounds to human and animals (Shephard, 2008). To prevent *A. parasiticus* contamination in cereals, and problems related to its aflatoxins, synthetic fungicides are currently used. In the last years, natural fungicides have been intensively investigated as new alternatives, especially, those obtained from plants, since they are considering less harmful to the organisms and the environment (El-Ghaouth, 1997; Wedge and Smith, 2006). To make full use of the resource of *H. longipes* and considering that foods and feeds contamination by *A. parasiticus* is a worldwide growing problem, the present study has been designed to assess the antifungal effect of the ethanolic extract from *H. longipes* roots against *A. parasiticus* growth, and aflatoxin's production in white maize (*Zea mays* L.).

MATERIALS AND METHODS

Aspergillus parasiticus growth conditions

Aspergillus parasiticus (ATCC 16992) strain was inoculated in potato dextrose agar (PDA, DIFCO, USA) and incubated at 27°C for 7 days. Spores were harvested by pouring a sterile Tween 20 (0.1 %) solution into a flask and stirred with a magnetic bar for 5 min at room temperature. The spore concentration was determined using a Neubauer chamber and adjusted to a final concentration of 1×10^5 spores/mL, this concentration was employed in all assays (Rosas-Burgos et al., 2009; 2011).

Plant material

Heliopsis longipes was grown under greenhouse conditions for one year at the Centro de Investigación y de Estudios Avanzados (CINVESTAV), Irapuato, Mexico. On December the 1st 2016, roots were collected, washed with distilled water, and subjected to extract preparation.

Preparation of ethanolic extract

Freshly *H. longipes* roots were ground to a fine powder in a mortar employing liquid nitrogen (-80 °C). For the preparation of *H. longipes* root extract, 100 g of ground plant material was subjected to maceration with absolute ethanol (Sigma-Aldrich) for 1 week in dark conditions in a 1:10 ratio (w/v). After that, the extract was filtered with the aid of a vacuum pump through Whatman filter paper No. 1 and the solvent removed by a rotary evaporator (Büchi R-121, 45 °C, 175 mBar) (Molina-Torres et al., 2004).

GC-EIMS analysis

Gas chromatography-electron impact mass spectrometry (GC-EIMS) was used to analyze and identify the alkamides in the ethanolic extract from *H. longipes* roots. The analysis was carried out in a gas-chromatograph (Agilent technologies, model 7890A) coupled to an electron impact ionization mass spectrometer (Agilent technologies, model 5975) using a pulsed split less injection mode. The chromatographic separation was carried out in an Agilent J&W DB-1MS UI capillary column (60 m × 320 µm × 1 µm) and helium was used as the carrier gas at a constant flow rate of 1 mL/min (Molina-Torres et al., 2004). The data obtained was collected with the software MassHunter Qualitative Analysis Version B.08.00 (Agilent Technologies, Inc.).

Alkamides quantification

Alkamide content was determined based on a standard curve of purified affinin at 0.1, 0.3, 1.0 and 3.0 mg/mL with ethanol absolute (Sigma-Aldrich) and expressed as mg/g of fresh tissue. Each point of the curve was obtained as an average of three injections. Quantification was performed from integrated peak area measurements observed in the chromatograms. Finally, the standard curve was produced using a linear regression $R^2 = 0.9903$ (Ramírez-Chávez et al., 2004).

Radial growth inhibition

Antifungal effect was tested using the method of radial growth inhibition described by Rosas-Burgos et al. (2009). The ethanolic extract from *H. longipes* roots at 50, 75, 150, and 200 µg/mL was employed. These concentrations were previously used by others authors to evaluate its fungistatic activity (Molina-Torres et al., 1999; 2004). Petri dishes of PDA media containing the extract were centrally point-inoculated with *A. parasiticus* spores. Controls were PDA media and PDA media plus absolute ethanol (the solvent used to alkamides extraction). The inoculated Petri dishes were incubated at 27°C for fungus growth. The colony diameters were measured every 24 h for 72 h with the help of a caliper. The results were expressed as percentage (%) calculated following equation 1 (Plascencia-Jatomea et al., 2003).

$$\text{Radial growth inhibition (\%)} = [(Rc - Ri)/Rc] \times 100 \quad (1)$$

Where *Rc* is the value of colony radius in the control and *Ri*, the colony radius value of colonies grown in presence of the antifungal extract/compound.

Kinetics of spore germination

Microscope slides were placed at the bottom of plates; then, a solution containing potato dextrose broth (PDB, DIFCO, USA), and testing extract at 200 µg/mL was poured on each plate. Plates were inoculated with the fungi and incuba-

ted at 27 °C. The number of germinated spores per plate was determined every five hours (0, 5, 10, and 15 h) by counting 200 spores (germinated and non-germinated) using a light microscope and Image-Pro Plus version 6.3 software (Media Cybernetics, Inc.). The inhibition of spore germination was calculated following equation 2 (Rosas-Burgos et al., 2011).

$$\text{Germination inhibition (\%)} = [(Sc - Si)/Sc] \times 100 \quad (2)$$

Where *Sc* is the number of germinated spores in the control, and *Si,* the number of germinated spores presence of the antifungal extract/compound.

Morphometric analysis

Morphometric analysis included the measurements of spore diameter and hyphal width determined in 200 spores at 10 h. This analysis was performed using a light microscope, and the images were processed using the software Image-Pro Plus version 6.3 (Media Cybernetics, Inc.) (Larralde et al., 1997; Cox et al., 1998).

Anti-aflatoxigenic assay

The antifungal extract at 150 and 200 µg/mL was used to evaluate the effect on total aflatoxin production in white maize grains inoculated with *A. parasiticus*. Mycotoxins production was induced according to the method reported by Castellá et al. (1999) and most recently by Rosas-Burgos et al. (2011). Separation, purification and quantification of total aflatoxin was carried out by the VICAM procedure (Cota-Arriola *et al.,* 2011).

Statistical analysis

Results were presented as mean value ± standard error of three replicates. Analysis was carried out by one-way ANOVA and Tukey test using $p \leq 0.05$. IBM SPSS Statistic 21 program was employed.

RESULTS AND DISCUSSION

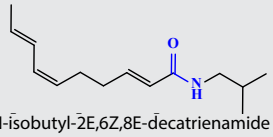
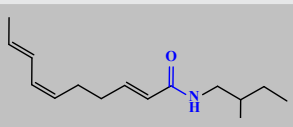
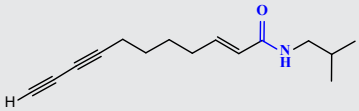
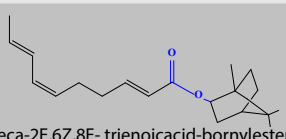
Characterization of ethanolic extract by GC-EIMS

The extraction yielded 1.64 g extract/g freshly roots. Solids obtained were re-suspended in absolute ethanol to obtain a final extract concentration of 5 mg/mL. The chromatographic analysis of the extract led to the identification of alkamides as the most abundat compounds. Analysis of alkamides in *H. longipes* roots revealed that the extract exhibit two major peaks with retention times (Rt) of 20.26 and 22.67 min, and two minor peaks at Rt= 24.01 and 27.24 min. Analysis of the compound at Rt = 20.26 min revealed a molecular ion [M+] of *m/z* 221 compatible to the molecular formula $C_{14}H_{23}NO$ and the fragmentation pattern *m/z* (relative intensity): 81(999), 141 (950), 41(345), 79(240), 126(239), 98(234), 53(178), 68(164), 69(159) and 86(156). Comparison of these data with the NIST database reveals 95 % similarities with *N*-isobutyl-2*E*,6*Z*,8*E*-decatrienamide or affinin, the main alkamide previously isolated and characterized from *H. longipes* roots (Molina-Torres et al., 1996). Analysis of the compound at Rt = 22.67 min with the NIST database reveals 93 % similarities with *N*-2-methylbutyl-2*E*,6*Z*,8*E*-decatrienamide, an alkamide with molecular formula $C_{15}H_{25}NO$ and molecular ion [M+] m/z 235. The other two compounds were identified as *N*-isobutyl-2*E*-en-8,10-diinamide and Deca-2*E*,6*Z*,8*E*-trienoicacid-bornylester (Table 1).

Quantitative analysis of alkamides

Chromatographic analysis of the extract led to determine that affinin (7.24 ± 0.13 mg/g) was the most

Table 1. Alkamide content in the ethanolic extract from *Heliopsis longipes* roots detected.
Tabla 1. Contenido de alcamidas en el extracto etanólico de las raíces de *Heliopsis longipes*

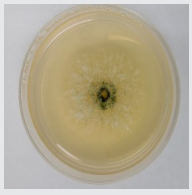
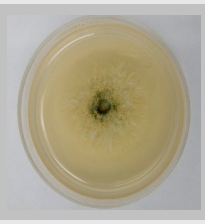
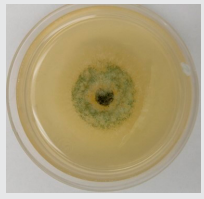
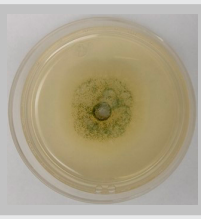
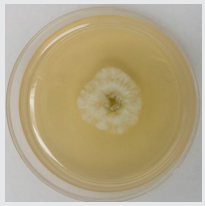
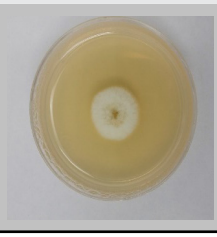
RT (min)	Compound structure	Molecular Ion [M+]	Concentration (mg g^{-1})	Molecular formula	Fragmentation patters *m/z*
20.26	 N-isobutyl-2E,6Z,8E-decatrienamide	221	7.24 ± 0.13	$C_{14}H_{23}NO$	81(999)*, 141 (950), 41(345), 79(240), 126(239), 98(234), 53(178), 68(164), 69(159), 86(156).
22.67	 N-2-methylutyl-2E,6Z,8E-decatrienamide	235	0.529 ± 0.16	$C_{15}H_{25}NO$	81(999)*, 155(850), 41(367), 86(299), 43(257), 84(248), 79(229), 69(196), 53(186), 98(175).
24.01	 N-isobutyl-2E-en-8,10-diin-undecamide	231	0.122 ± 0.03	$C_{15}H_{21}NO$	91(999)*, 116(490), 131(422), 103(409), 115(356), 63(311), 129(283), 55(245), 160(237), 188(237).
27.24	 Deca-2E,6Z,8E- trienoicacid-bornylester	302	0.110 ± 0.01	$C_{20}H_{30}O_2$	81(999)*, 137(599), 79(234), 93(200), 95(150), 109(150), 55(122) 121(109), 67(101), 53(100).

abundant alkamide detected, follow by the *N*-2-methylbutyl-2*E*,6*Z*,8*E*-decatrienamide (0.59 ± 0.16 mg/g) (Table 1). These results are similar to those reported by Molina-Torres et al. (1996), who isolated and characterized alkamides from *H. longipes* roots, and reported that affinin is present at a concentration of 7.3 mg/g dry underground tissue and the *N*-2- methylbutyl-2*E*,6*Z*,8*E*-decatrienamide at 0.5 mg/g dry underground tissue. Likewise, in *Acmella oppositifolia*, affinin has been quantified at a concentration of 0.351 mg/g in dry underground tissue (Molina-Torres et al., 1996). These results corroborate that *H. longipes* roots are a suitable source of alkamides especially affinin, in comparison with other plant species that produces them.

Antifungal effect of ethanolic extract from *H. longipes*

The four tested concentrations from *H. longipes* roots inhibit the radial growth of *A. parasiticus* (Table 2). According to literature, the *H. longipes* extract possess fungistatic activity (Molina-Torres et al., 2004). In this study, it was observed that after the lapse of 72 h, i.e. 96 h, the extract was capable

Table 2. Antifungal effect of ethanolic extract from *Heliopsis longipes* roots on *Aspergillus parasiticus* growth.
Tabla 2. Efecto antifúngico del extracto etanólico de las raíces de *Heliopsis longipes* sobre el crecimiento de *Aspergillus parasiticus*.

Treatment	Photography	Mean colony radius (mm)	Growth (%)	Radial growth inhibition (%)
PDA		1.80 ± 0.41 e	100.00 ± 0.41 e	0.00 ± 0.41 e
PDA + solvent		1.75 ± 0.56 e	97.22 ± 0.56 e	2.78 ± 0.56 e
50 µg/mL		1.37 ± 0.43 d	76.11 ± 0.43 d	23.89 ± 0.43 d
75 µg/mL		1.20 ± 0.14 c	66.67 ± 0.14 c	33.33 ± 0.14 c
150 µg/mL		0.80 ± 0.30 b	44.44 ± 0.30 b	55.56± 0.30 b
200 µg/mL		0.53 ± 0.39 a	29.44 ± 0.39 a	70.56 ± 0.39 a

* Values are the average of three replicates ± standard error. Values with different letter in the same column are statistically different ($p \leq 0.05$).

of inhibit fungal growth, but when the fungus was retired from the system-growth, it was capable of develop. The most relevant inhibition ($p \leq 0.05$) was observed when *A. parasiticus* was incubated with the higher concentration of the extract tested of 200 µg/mL. At this concentration, growth was 29.44 ± 0.39 % and inhibition of 70.56 ± 0.39 %, suggesting that inhibition of the radial growth of *A. parasiticus* by the *H. longipes* extract is dose-dependent. The antifungal extract caused a considerable radial growth inhibition of *A. parasiticus* at very low concentrations, in comparison with other fungicidal plant extracts using higher concentrations. Vargas-Aispuro et al. (2005), evaluated the antifungal activity of stems and leaves crude extracts from *Larrea tridentata* at 500 µg/mL against *A. parasiticus.* They observed that crude extracts from leaves and stems of *L. tridentata* showed 53.37 and 68.7 % of mycelial growth inhibition, respectively. These inhibition results are lower than those obtained in our study with ethanolic extract from *H. longipes* roots at 200 µg/mL. In other study, the aqueous extracts of five medicinal plants were evaluated; *Cassia alata, Punica granatum, Polyanthia longifolia, Datura stramonium* and *Annona squamosa* were screened for antifungal activity against *A.parasiticus* (NCIM 898) at 5, 10, 15, 20 and 25% concentrations. It was observed that all aquous extracts present a maximum percentage of inhibition at 25% of extract concentration (Rajani et al., 2012). In recent years, Mahlo et al. (2016) investigated the activity of leaf extracts of *Bucida buceras, Breonadia salicina, Harpephyllum caffrum, Olinia ventosa, Vangueria infausta* and *Xylotheca kraussiana*, against *A. parasiticus* by the microdilution assay. All plant extracts presented antifungal activity, and the minimum inhibitory concentration (MIC) against the fungus was for *B. buceras* at 1.095 mg/mL, *B. salicina* 1.252 mg/mL, *H. caffrum* 1.407 mg/mL, *O. ventosa* 1.095 mg/mL, *V. infausta* 1.252 mg/mL, and *X. kraussiana* 0.358 mg/mL. This information supports the idea that *H. longipes* roots extract is bioactive against *A. parasiticus* and, for such activity, lower extract concentrations are required; about 2-3 times less than other extracts previously evaluated against the fungus.

Antifungal effect of ethanolic extract can be attributed to the high levels of alkamides, especially affinin, a compound with a wide variety of biological activities including the antifungal effect (Molina-Torres et al., 1999; Molina-Torres et al., 2004). Previous studies have described the antimicrobial activity of affinin isolated from *H. longipes* roots (Molina-Torres et al., 1999). This activity was assayed against *Escherichia coli, Pseudomonas solanacearum, Bacillus subtilis* and *Saccharomyces cerevisiae* suspension cultures. The authors reported that affinin inhibited *E. coli* and *S. cerevisiae* growth at 25 µg/mL, but higher concentrations of affinin (300 µg/mL) were necessary to inhibit growth of *P. solanacearum* and *B. subtilis*. These results demostrated that affinin affects mainly Gram-negative bacteria such as *E. coli* rather than Gram-positive such *B. subtilis,* probably due to its bacterial cell wall. Other studies were carried out to test the effect on the phytopathogens *Sclerotium cepivorum* and *Sclerotium rolfsii* (Ramírez-Chávez et al., 2000). These authors reported that at the highest concentration tested with purified affinin or crude extract, the development of mycelium was completely inhibited in both *Sclerotium* species. They reported that medium lethal dose for *S. rolfsii* was in the range of 15 to 20 µg/mL and that for the *S. cepivorum* was in the range of 5 to 10 µg/mL. That is to say, the general effect of the crude extract and the purified affinin was similar in both fungi. These results suggest that the use of the ethanolic extract is more suitable than the use of purified affinin. However, added to the aforementioned, the antifungal activity of the ethanolic extract from *H. longipes* roots against *A. parasiticus in vitro*, has not been previously reported.

Regarding our research, based on the antifungal effect, we selected the ethanolic extract of *H. longipes* roots to carry out the study in order to demostrate that the extract is a good source of alkamides and antifungal compounds. Finding that ethanolic extract significantly inhibits the radial growth of *A. parasticus* was of great interest. This finding represents the first demonstration that affinin is capable of inhibit *A. parasticus* radial growth. Considering that affinin was the most abundant constituent of the *H. longipes* ethanolic extract; this compound is more likely responsible for the antifungal effect by the ethanolic extract.

Additional to radial growth inhibition, *A. parasiticus* conidia in presence of *H. longipes* roots extract showed a smooth surface morphology, and an effect on the green pigment formation in the fungal spores was observed (Table 2). This effect depends on the concentration of the antifungal extract. It was observed that at low concentrations, the green pigment increased, but decreased at high concentrations leading to the development of pale colonies (not quantified data). This indicates that the extract has an inhibitory effect on the secondary metabolism for the fungal pigment formation. Kim et al. (2011), report similar results by salicylaldehyde at 9.5 mM. In addition, green pigment is synthesized by dihydroxynaphthalene (DHN)-melanin pathway, and when conidia lacking pigmentation is due to the inhibition of polyketide synthase (PKS) pathway (Tsai et al., 1997; Langfelder et al., 1998). This suggest that *H. longipes* extract might be inhibiting the PKS pathway in *A. parasiticus.*

Kinetics of spore germination

Spore germination is an essential stage in the development of filamentous fungi. The results showed that spores germinated similarly in the controls median, and was considerably less in the treatments in comparison with controls. At 15 h of incubation, on culture PDA control 100 % of spores are germinated, while that on culture PDA with extract has 40 ± 0.8 % of germination, suggested that extract reduced the 60 ± 0.8 % of spore germination rate (Figure 1). This probably originated by denaturation of the enzymes responsible for spore germination or interference with the use of *L*-alanine and other amino acids necessary for the initiation of this process (Nychas, 1995).

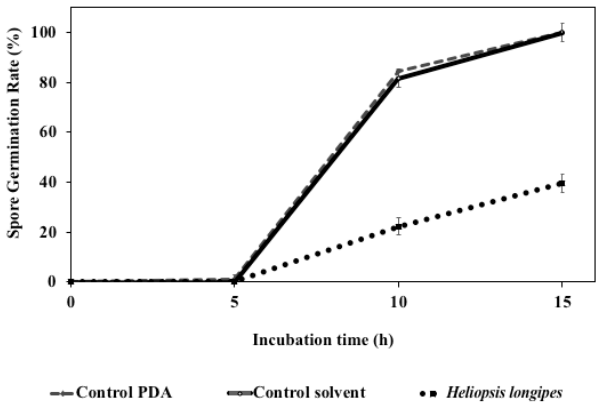


Figure 1. Effect of *Heliopsis longipes* root extract on the *Aspergillus parasiticus* kinetics of spore germination. Error bars represent standard error for n= 3 ($p \leq 0.05$).
Figura 1. Efecto del extracto de las raíces de *Heliopsis longipes* sobre la cinética de germinación de las esporas de *Aspergillus parasiticus.* Las barras de error representan el error estándar para n=3, ($p \leq 0.05$).

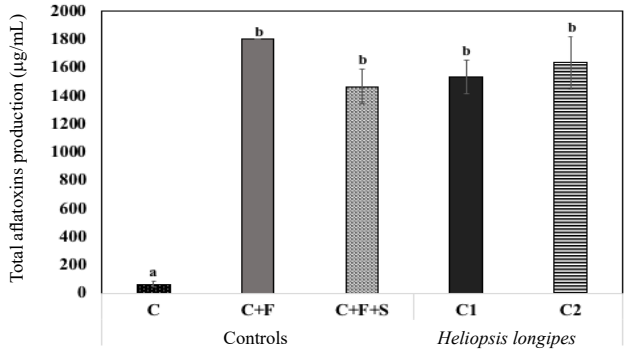


Figure 2. Effect of *Heliopsis longipes* root extract on total aflatoxins production by *Aspergillus parasiticus*. Controls: C = corn grains, C + F = corn grains inoculated with fungus, C + S + F = corn grains inoculated with fungus plus solvent. Bars represent standard error for n= 3. Bars followed with different letter are significantly different ($p \leq 0.05$).
Figura 2. Efecto del extracto de raíces de *Heliopsis longipes* sobre la producción total de aflatoxinas por *Aspergillus parasiticus.* Las barras de error representan el error estándar para n=3, ($p \leq 0.05$). Control: C = granos de maíz, C + F = granos de maíz inoculados con el hongo, C + S + F = granos de maíz inoculados con el hongo más solvente. Las barras de error representan el error estándar para n=3, ($p \leq 0.05$).

Morphometric parameters of spores and hyphae

In presence of the antifungal extract exist an increment of the spores diameter and hyphal width of *A parasiticus,* and some structural alterations apparently at 10 h post-inoculation (Table 3). Those effects have been reported when there is a damage in the fungal cell wall integrity by some antifungal extracts plants (Buitimea-Cantúa et al., 2013). Consequently, plasma membrane permeability might be affected, which could explain the changes in morphology and size of spores and hyphae (Rosas-Burgos et al., 2011), so that extract may be acting in the main structural cell wall components.

Anti-aflatoxigenic assay

Anti-aflatoxigenic properties of the ethanolic extract from *H. longipes* roots at 150 and 200 µg/mL were evaluated upon *A. parasiticus* (Figure 2). Data analysis found that extract from *H. longipes* roots do not have anti-aflatoxigenic activity. Similar results have been reported by other authors, who have found an inhibitory effect on the fungus growth, but the mycotoxins production has not been inhibited (Fandohan et al., 2004; López et al., 2004; Rosas-Burgos et al., 2011). It is important to point out that aflatoxins are another fungal metabolite PKS-derived; however, the concentrations tested of the ethanolic extract from *H. longipes* roots were not capable of inhibit their production *in vitro.* Therefore, the extract can be used to prevent *A. parasiticus* growth before the mycotoxins' production occurs, or well, investigate the effect of higher concentrations.

CONCLUSIONS

The present work comprises the first report on the antifungal activity of the ethanolic extract from *Heliopsis longipes* roots against *Aspergillus parasiticus.* It was shown that ethanolic extract at 200 µg/mL displayed a significant inhibition of fungal radial growth, and spore germination rate of A. parasiticus. The *H. longipes* extract was found to be a good source of alkamides such as affinin, and these compounds may be able to provide protection against this fungus. The result derived from this study suggest that ethanolic extract from *H. longipes* roots is a potent antifungal and an suitable natural alternative to prevent *A. parasiticus* growth before the mycotoxins' production occurs.

Table 3. The effect of the ethanolic extract from *Heliopsis longipes* roots on *Aspergillus parasiticus* spore diameter and hyphal width.
Tabla 3. El efecto del extracto etanólico de las raíces de *Heliopsis longipes* sobre el diámetro de la espora y el ancho de la hifa de *Aspergillus parasiticus.*

Treatment	Spore diameter germinated (µm)	Increment (%)	Spore diameter non-germinated (µm)	Increment (%)	Hyphal width (µm)	Increment (%)
PDB	9.87 ± 0.98 a	0.0 ± 0.98 a	7.06 ± 1.94 a	0.0± 1.94 a	4.65 ± 0.44 a	0.0± 0.44 a
PDB+ Solvent	9.86 ± 0.81 a	0.1± 0.81 a	7.08 ± 1.61 a	0.3± 1.61 a	4.66 ± 0.48 a	0.3± 0.48 a
Heliopsis longipes	10.92 ± 0.81 b	10.64± 0.8[1] b	9.35 ± 1.77 b	32.44± 1.77 b	5.42 ± 0.45 b	16.56± 0.45 b

[*] Values are the average of three replicates ± standard error. Values with different letter in the same column are different ($p \leq 0.05$)

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