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CONTENIDO

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Portada

Podopterus mexicanus Humb. & Bonpl. Polygonaceae. "Rompe capa". Árboles o arbustos de 1.5-6.0 m de altura, ramas con espina terminal, braquiblastos, hojas fasciculadas, flores blancas a verdosas en fascículos, y frutos cubiertos por el perianto externo que forma alas delgadas y largamente decurrentes hacia el pedicelo. Crece en bosques tropicales caducifolios y bosques espinosos, sobre suelo rocoso negro derivado de rocas ígneas. En elevaciones de 550-760 m. Florece de abril a mayo y fructifica de junio a septiembre. Se distribuye desde México hasta Centroamérica. En México se encuentra en los estados de Colima, Guerrero, Oaxaca, Puebla, Tamaulipas, Veracruz y Yucatán. Se utiliza como planta melífera y para leña.

Podopterus mexicanus *Humb.& Bonpl. Polygonaceae."Rompe capa". Trees or shrubs 1.5-6.0 m tall, branches with a terminal spine, brachyblasts, fasciculate leaves, white to greenish flowers in fascicles, and fruit covered by the external perianth that forms thin wings and longly decurrent towards the pedicel. It grows in tropical deciduous forests and thorny forests, on black, rocky soil derived from igneous rocks. At elevations of 550-760 m. Blossoms from April to May and bears fruit from June to September. It is distributed from Mexico to Central America. In Mexico it is found in the states of Colima, Guerrero, Oaxaca, Puebla, Tamaulipas, Veracruzand Yucatán. It is used as a melliferous plant and for firewood.*

por/by **Rafael Fernández Nava**

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RESUMEN: La plaga mosca de la fruta, ocasionada por Tefrítidos, afecta gravemente a los cultivos frutales en el mundo. El manejo integrado de la plaga incluye la aspersión de insecticidas sintéticos de amplio espectro. Debido a los efectos negativos a largo plazo del uso de insecticidas sintéticos, se han propuesto extractos botánicos como nuevas alternativas ecológicas. En este estudio, se evaluó el potencial insecticida de *Magnolia perezfarrerae*, *M. pugana* y *M. vovidesii* contra *Anastrepha ludens* y *A. obliqua* mediante bioensayos de alimentación en individuos adultos, mezcla de azúcar y extractos crudos de sarcotesta. Se realizaron perfiles químicos cualitativos para explorar la composición de los extractos crudos etanólicos de sarcotesta de cuatro especies de *Magnolia* con efectividad insecticida, mediante cromatografía en capa fina usando siete sistemas de polaridad. Además, se identificaron grupos de metabolitos secundarios mediante análisis cualitativos. La efectividad insecticida de los extractos de *Magnolia* fue mayor al 93% contra *A. ludens* en la primera dilución, por el contrario, la efectividad contra *A. obliqua* fluctuó desde el 66% de *M. perezfarrerae* hasta 92% de *M. vovidesii*. Los extractos de *Magnolia* mostraron una amplia diversidad de compuestos de diferentes polaridades. Además, se detectó la presencia de alcaloides, flavonoides y fenoles en todas las especies de *Magnolia*. Las propiedades insecticidas de *Magnolia* pueden contribuir al manejo integrado de *Anastrepha*.
Palabras clave: bioactividad, metabolitos secundarios, mosca mexicana de la fruta, Pesticidas botánicos.

ABSTRACT: The fruit fly pest caused by Tephritidae severely affects fruit crops in the world. Integrated pest management includes the spraying of synthetic broad-spectrum insecticides. Due to the long-term negative effects of the use of synthetic insecticides,

botanicals extracts have been proposed as new ecological alternatives. In the study, the insecticide potential of *Magnolia perezfarrerae*, *M. pugana* and *M. vovidesii* was tested against *Anastrepha ludens* and *A. obliqua* through feeding bioassays, mixture of sugar and crude extracts of sarcotesta. In addition, qualitative chemical profiles were carried out to explore the composition of insecticide-effectiveness ethanol crude extracts of sarcotesta of four species of *Magnolia*. Qualitative chemical profiles were performed using thin layer chromatography based on seven polarity systems. Moreover, secondary metabolite clusters were identified through qualitative analyses. The insecticide-effectiveness of *Magnolia* extracts was higher than 93% against *A. ludens* in the first dilution, whereas the effectiveness against *A. obliqua* ranged from 66% *M. perezfarrerae* to 92% *M. vovidesii*. The extracts of *Magnolia* showed a wide variety of compounds with different polarity. Furthermore, the presence of alkaloids, flavonoids and phenols was detected in all species of *Magnolia.* The insecticide properties of *Magnolia* can contribute to the integrated management of *Anastrepha*.
Key words: bioactivity, secondary metabolites, mexican fruit fly, botanical pesticides.

INTRODUCTION

The fruit fly pest from the Tephritidae family severely affects fruit crops all around the world. The Tephritids, called true fruit flies, consist of 4,700 species located throughout the temperate, tropical and subtropical regions of the world (Norrbom *et al*., 2012). There are specifically about 250 species in the Americas which are spread all the way from the south of the United States to the north of Argentina, including the Caribbean islands (Hernández-Ortiz *et al*., 2010). The genus *Anastrepha* Schiner is endemic to the Americas and four species are of economic and quarantine importance due to their high preferred range for cultivated and wild hosts; *e.g. A. ludens*, *A. obliqua*, *A. serpentina* y *A. striata* (Hernández-Ortiz *et al*., 2010; SENASICA, 2021). Their preferred hosts include up to 330 species belonging to 48 families, including Anacardiaceae, Cucurbitaceae, Myrtaceae, Rosaceae, Rutaceae, and Sapotaceae (Hernández-Ortiz, 1993; Hernández-Ortiz *et al*., 2010).

The integrated management of the fruit fly pest caused by *Anastrepha* has been implemented since 1992 through the National Program and the National Campaign Against Fruit Flies (NCFF), under an international agreement between Mexico, Guatemala, and the United States (Montoya *et al.*, 2010). The program is based on the implementation of phytosanitary measures to control, suppress, and eradicate fruit flies. The monitoring system consists of trapping and detecting larvae in fruits, and the control methods are based on the collection and destruction of infested fruits (mechanical control), spraying of specific baits (synthetic pesticides mixed with hydrolyzed protein; chemical control), and massive releases of sterile flies (sterile insect technique; autocidal control), and natural enemies in priority zones (biological control) (Miyatake, 2011; Montoya *et al*., 2010; SENASICA, 2021).

The national fruit fly campaign reported that 52% of Mexican territory was a fly free zone (SENASICA, 2021). In the remaining areas the pest persists, and the use of chemical control continues, based on organophosphate insecticides such as Malathion (2-[(dimethoxyphosphorothioyl)sulfanyl]butanedioate, diethyl), which has a broad-spectrum toxicity (SENASICA, 2019). This synthetic insecticide is highly neurotoxic, and its chronic exposure induces oxidative stress in mammals (Ali & Ibrahim, 2018; Delgado *et al.*, 2006) and morphological anomalies in early stages of amphibians (Krishnamurthy & Smith, 2011). Also, physiological alterations and protein reduction have been reported in fish (Singh *et al.*, 2004), imbalance in the abundance and composition of species in aquatic ecosystems (Smith *et al.*, 2018), as well as resistance in pest insects (Hsu & Feng, 2006; Jyoti *et al.*, 2014; Magaña *et al*., 2008).

Alternatives such as botanical pesticides have been suggested as a way of complementing integrated pest management and reducing the harmful effects of synthetic pesticides (Díaz-

Fleischer *et al*., 2017). Botanical pesticides derived from plants have agrochemical potential, because they are selective (*i.e*., for target insects), biodegradable and harmless to the environment (Amoabeng *et al*., 2019; Sarkar & Kshirsagar, 2014). In the case of fruit fly pest, various studies have focused on assessing insecticides botanicals. The ethanolic extracts of *Annona mucosa* had an 80% mortality against *Anastrepha fraterculus* adults a LC_{50} 728.36 mg L^{-1} (Stupp *et al*., 2020). Similarly, the extracts (MeOH-PE) of the fruit of *Citrus aurantium* had a 76% effectiveness in olive fruit fly adults (*Bactrocera oleae*) (Siskos *et al*., 2009).

The Magnoliaceae family is known worldwide for having bioactive compounds, secondary metabolites, with applications in the pharmaceutical, biotechnological, and agri-food industries (Chen *et al*., 2019; Lee *et al*., 2011; Poivre & Duez, 2017). Several species of this family have broad-spectrum inhibitory activity against viruses (Fang *et al*., 2015), bacteria (Jacobo-Salcedo *et al*., 2011; B. Wu *et al*., 2018), human pathogenic fungi (Bang *et al.*, 2000), fungi and plant pathogens (Lin *et al*., 2019; H. Wu *et al*., 2018), nematodes (Hong *et al*., 2007), and arthropods (Kelm *et al*., 1997; Miyazawa *et al*., 1994; Yang *et al*., 2015).

It has been shown that several species of *Magnolia* have bioinsecticidal effects on pest insects. The lignans of the *M. fargesii* flowers, which inhibit the larval growth of *Drosophila melanogaster*, stand out among the reported compounds (Miyazawa *et al*., 1994). Active and isolated compounds (Costunolide, geranial, methyl and isomethyl eugenol, neral, partenolide, and trans-anethole) from different vegetative structures of *Magnolia salicifolia*, induced 100% larval mortality of the *Aedes aegypti* (Kelm *et al*., 1997). In a similar way, crude ethanolic extracts from different vegetative structures of *M. dealbata*, and *M. schiedeana*, had insecticidal potential against the *Anastrepha ludens* fruit fly. In particular, sarcotesta showed the highest insecticidal potential, which reached 96% and 64%, respectively (Flores-Estévez *et al*., 2013; S. Vásquez-Morales *et al*., 2015). Therefore, it is essential to assess the insecticidal effect of a greater number of *Magnolia* species and to expand insecticide bioassays for a greater number of *Anastrepha* species. The objectives of the present study were: 1) To determine the insecticide-effectiveness of crude ethanolic extracts of sarcotesta of *Magnolia perezfarrerae*, *M. pugana* and *M. vovidesii* against adults of *Anastrepha ludens* and *A. obliqua*, 2) To identify the presence of secondary metabolite clusters through qualitative chemical analyses. To this aim, we focused on a system of experimentation of pest feeding assay to based extracts crude of sarcotesta of *Magnolia*, in addition identify the groups of secondary metabolites using thins layer chromatography.

MATERIALS AND METHODS

Plant material

Four endemic *Magnolia* species of Mexico were analyzed. *Magnolia perezfarrerae* is naturally distributed in state of Chiapas, *M. pugana* in state of Jalisco, *M. schiedeana* (Schltdl.) and *M. vovidesii* in state of Veracruz. Magnolias are evergreen trees, except *M. vovidesii* which is a deciduous tree. The tree height varies between 15 to 25 m, it has a rough cracked greyish bark covered with lichens; glabrous, elliptic, oblong or obovate leaves, with pubescence only on the underside. Flowers white or creamy; in particular, *M. vovidesii* have pink dots inside them, during the first hours of their opening. The fruit is a dehiscent ellipsoid polyfollicle, containing between 15 to 115 seeds, depending on the species. The physical and climate features of the collection sites are described in Table 1. The *M. perezfarrerae* was determinate in voucher No. 23948 of herbarium CH – El Colegio de la Frontera Sur. *M. pugana* and *M. vovidesii* in process of determination in herbarium XAL – Instituto de Ecología A.C. Further information on taxonomic aspects and collection sites are described in previous research (S. G. Vásquez-Morales *et al*., 2017; S. G. Vásquez-Morales & Ramírez-Marcial, 2019; Vázquez-García *et al*., 2002).

Table 1. Characteristics of collection sites in Mexico.

Species	*M. perezfarrerae*	*M. pugana*	*M. schiedeana*[b]	*M. vovidesii*
Polyfollicles Collection	March 3rd, 2018	Match 19th, 2018 and May 17th, 2019	July 10th, 2018	August 17th, 2018
Sites (Municipaly)	Ocuilapa de Juárez (Ocozocoautla de Espinosa)	CUCBA[a] (Zapopan)	La Martinica (Banderilla)	Coyopolan (Ixhuacán de los Reyes)
Latitude (N)	16º50´ 57´´	20º 44´51´´	19º 34´55´´	19º 21´59´´
Longitude (W)	93º 24´35´´	103º 30´46´´	96º56´55´´	97º04´05´´
Altitude (masl)	959	1 670	1 451	1 570
Mean annual temperature (ºC)	22	23.5	18	18
Mean annual rainfall (mm)	1 000	906	1 451	1 807

[a] Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) of University of Guadalajara.
[b] Due to the shortage of *M. schiedeana* seeds, only qualitative chemical tests were assessed.

Laboratory insects

Sterile laboratory flies *Anastrepha ludens* (Loew) and *Anastrepha obliqua* (Macquart), from 6 to 15 days of age were used in the bioassays. Flies are mass-produced at Planta MoscaFrut in Metapa de Domínguez, Chiapas, Mexico. These flies are irradiated with Cobalt 60 isotopes at a dose rate of 70 Gy/min (Montoya *et al*., 2010). Later, they are transferred by air, in pupal stage. The pupae were kept in wooden cages (approximately 25 g on cage) and covered with a cotton mesh of about 900 m^3, under laboratory conditions, that is, at a temperature of 25 ºC ± 1 ºC, a relative humidity of 70 ± 30% and a 12-h photoperiod. *Ad libitum*, they were given purified water (in a container with cotton to prevent them from drowning) and food (table sugar) until they underwent bioassays.

Crude extracts of *Magnolia*

The seeds of each *Magnolia* species were extracted from the polyfollicles; then, their sarcotesta (red outer seedcoat) was manually removed. The sarcotesta of each species was placed separately in paper bags and kept for 72 h in a drying oven (Mermmet Incubador IN30; Germany) at 40 ºC for total dehydration. Subsequently, it was grinded in a mortar until pulverization. The preparation of each *Magnolia* extract consisted of 50 g of pulverized sarcotesta and 250 mL of ethanol 96% (1:5 $w.v^{-1}$) were added. For each species, six crude *Magnolia* extracts were prepared and cold-stored (4 ºC) for 72 h. Afterwards, the solvent was removed from each extract and the solvent volume was concentrated in a rotary evaporator (Buchi, Model R-300; Switzerland), set at 40 ºC, with a 1.8 m^3/h final vacuum (absolute) and a 5 ± 2 mbar vacuum capacity, until a final extract volume of 10 mL to 22.5 mL was obtained, with an interval of yielder of 2 mg/mL^{-1} to 4.5 $mgmL^{-1}$.

Following the same method, six crude extracts of steam, leaves, and flowers of *Chrysanthemum grandiflorum* Dum. Cours., were obtained. These extracts were used as positive control for their insecticide activity as they contain pyrethroids (Haouas *et al*., 2012). The *C. grandiflorum* was purchased at "Mercado Embajadoras, Guanajuato City, Mexico". Concentrated crude extracts of *Magnolia* and *Chrysanthemum* were stored at 4 ºC, in the dark, until evaluation.

Treatments and bioassays

The experimental units were cages (wooden structures covered with cotton meshes of approximately 900 m^3) with fifty adult flies (25 females and 25 males) of *Anastrepha ludens* and *A. obliqua*. To ensure an adequate intake of the treatments, the flies were deprived of food (table sugar) and kept hydrated with purified water 24 h before each bioassay. For each *Magnolia* species, reduced crude extracts of sarcotesta were analyzed in three dilutions (0.2, 0.02, 0.002 mg/g) in three different cohorts of each species of *Anastrepha*. Each bioassay assessed five treatments: 1) 1 g of table sugar mixed with 2 mL of ethanol solution 96% (Negative Control), 2) 1 g of table sugar mixed with 2 mL of reduced crude extract of *Chrysanthemum grandiflorum* at 0.2 mg/g (Positive Control), 3) 1 g of table sugar mixed with 2 mL of reduced crude extract of sarcotesta of *Magnolia* at 0.2 mg/g (Dilution 1), 4) 1 g of table sugar mixed with 2 mL of reduced crude extract of sarcotesta of *Magnolia* at 0.02 mg/g (Dilution 2), and 5) 1 g of table sugar mixed with 2 mL of reduced crude extract of sarcotesta of *Magnolia* at 0.002 mg/g (Dilution 3). The treatments were applied on 0.07 g of cotton to reduce adherence and facilitate its consumption. For each *Magnolia* species, three bioassays were performed, on *A. ludens* and *A. obliqua*, with five replicates per treatment. For each bioassay, daily mortality was recorded for a period of five consecutive days.

Qualitative chemical profiles determination using TLC

Thin layer chromatography (TLC) experiments were performed using the following polarity systems (v/v); i) hexane (100%), ii) hexane-acetonitrile (75:25%), iii) hexane-acetonitrile (50:50%), iv) ethyl acetate (100%), v) acetonitrile-methanol (50:50%), vi) ethanol (100%), vii) methanol (100%). To this end, 1 mL of each crude extract of sarcotesta (*Magnolia perezfarrerae*, *M. vovidesii*, *M. pugana* and *M. schiedeana*) was dissolved in 1 mL of each solvent tested, and 10 µL of each sample was applied on silica gel aluminum TLC plates, coated with fluorescent indicator F254 (Merck KEGaA, 64271; Darmstadt Germany). The plates were developed in the different solvents systems for 10 minutes and finally were revealed using *p*-anisaldehyde (98%). The retention factor (Rf) was estimated for each visible spot, using the equation $Rf = dR/dFM$, where dR is the distance travelled by the extract and dFM is the distance travelled by the solvent.

For each qualitative test, 1 mL samples of ethanolic extracts of sarcotesta were used. Each test was performed in triplicate according to standard procedure (Domínguez, 1973; Zhang *et al*., 2019). In these assays, alkaloids, coumarins, flavonoids, phenols, saponins, steroids and terpenes were screened (Table 2).

Table 2. Methodology used to identify secondary metabolites groups by qualitative test.

Group	Methodology	Positive test	References
Alkaloids	Dragendorff's reagent of Merck	Turbidity or precipitate formation	Domínguez, 1973 Mora-Arango *et al*., 2012
Coumarins	Standard procedure with sodium hydroxide	Green, red, or yellow fluorescence	Domínguez, 1973 Mora-Arango *et al*., 2012
Flavonoids	Shinoda´s test	Orange, pink, red, or violet coloration	Mora-Arango *et al*., 2012 Zhang *et al*., 2019
Phenols	Standard procedure with iron chloride	Black, blue, or green coloration	Domínguez, 1973 Mora-Arango *et al*., 2012 Zhang *et al*., 2019
Saponins	Standard procedure with distilled water	Abundant foam was formed and remained stable at least for 5 min	Domínguez, 1973 Mora-Arango *et al*., 2012 Zhang *et al*., 2019
Steroids and terpenes	Standard procedure with acetic anhydride and sulfuric acid	Blue, green, red, or violet coloration	Domínguez, 1973 Mora-Arango *et al*., 2012 Zhang *et al*., 2019
Tannins	Gelatin-salt reagent	Turbidity or precipitate formation	Domínguez, 1973 Mora-Arango *et al*., 2012 Zhang *et al*., 2019

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Statistical Analysis

A completely randomized design was used in all bioassays. The data was analyzed with an analysis of variance (ANOVA, one-way) followed by a LS Means difference Tukey HSD post-hoc test in order to find the effect of the treatments in comparison with the controls in R package Version 3.3.1. (R Core Team, 2013). The natural mortality rate was corrected with the modified formula of Abbott $CM = (1 - (X - Y)/(50 - Z)\ x\ 100)$ (Abbott, 1925), where *CM* is the corrected mortality expressed as a percentage, *X* is the number of flies per experimental unit, *Y* is the average number of flies killed during treatment, and *Z* is the average number of dead flies in the negative control, which were later converted to square root. The survival analysis was performed under the Kaplan-Meier method followed by pairwise comparisons using Log-Rank test, in an R package Version 3.3.1 (R Core Team, 2013).

RESULTS

The ethanolic crude extracts of *Magnolia* presented a high mortality against *Anastrepha ludens* and *A. obliqua* adults. More precisely, the extract of sarcotesta of *M. perezfarrerae*, in the first dilution (0.2 mg/g), had an insecticide effectiveness of 95% against *A. ludens* (F= 12.24, df= 3, P<0.05) and of 66% against *A. obliqua* (F= 4.88, df= 3, P=0.03). These results did not show a significant difference with those shown by the extract prepared with *Chrysanthemum grandiflorum* (Table 3). Regarding the ethanolic extract of *M. pugana*, in the first dilution (0.2 mg/g), there was an effectiveness of 93% against *A. ludens* (F= 1.65, df= 3, P=0.25) and of 91% against *A. obliqua* with no significant difference between treatments (F= 0.80, df= 3, P=0.52). Regarding the extract from *M. vovidesii*, in this study it was only tested against *A. obliqua* and showed an effectiveness of 92% in the first dilution (0.2 mg/g) with no significant difference with the extract of *C. grandiflorum* (F= 13.75, df= 3, P<0.05; Table 3).

Table 3. Insecticide-effectiveness of crude extracts of sarcotesta of *Magnolia* against *Anastrepha* adults in three dilutions, 0.2 mg/g (D1), 0.02 mg/g (D2), and 0.002 mg/g (D3) with negative (NC= Ethanol 96%) and positive control (PC= *Chrysanthemum grandiflorum*). Mortality percentage and Abbott indices. Active extracts are presented in bold. Mean ± SD. Bars that do not share the same letter are significantly different from controls (P<0.05).

	A. ludens		***A. obliqua***	
Treatments	**% mortality**	**Abbott index**	**% mortality**	**Abbott index**
M. perezfarrerae				
NC	36.26 ± 20.5c		44.33 ± 10.8b	
PC	97.86 ± 3a	**97.37 ± 3.4a**	68.8 ± 39.3a	54.87 ± 48.3ab
D1	97.6 ± 1a	**95.69 ± 2.5a**	82.26 ± 11.4a	**66.09 ± 24.2a**
D2	67.6 ± 16.4b	48.92 ± 24.7ab	74.93 ± 10.3a	54.07 ± 15.7ab
D3	40.66 ± 24.4c	14.32 ± 15.2b	34 ± 13.2b	0 ± 0b
M. pugana				
NC	33.86 ± 11.5b		35.46 ± 10.5c	
PC	97.6 ± 2.8a	**93.91 ± 8a**	73.6 ± 43.3ab	65.01 ± 56.3a
D1	95.6 ± 5.1a	**93.73 ± 7.5a**	95.06 ± 5.9a	91.74 ± 10.8a
D2	63.6 ± 31.2b	44.62 ± 49.1a	78.8 ± 23.9ab	63.3 ± 43.0a
D3	66.4 ± 25.3b	44.12 ± 46.7a	60.8 ± 29.8bc	36.10 ± 50.0a
M. vovidesii				
NC			35.86 ± 2.2c	
PC			**98.26 ± 1.8a**	**97.31 ± 2.7a**
D1			**95.06 ± 3.2a**	**92.26 ± 5.1a**
D2			**92.13 ± 7.5a**	87.83 ± 11.3a
D3			55.73 ± 10.2b	30.53 ± 18.7b

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The Kaplan-Meier survival analysis demonstrated that crude extracts of *Magnolia* have a high insecticide-effectiveness from day two to five days of exposure and that there is a significant difference between treatments in *A. ludens* (*M. perezfarrerae* P<0.05; *M. pugana* P<0.05), and *A. obliqua* (*M. perezfarrerae* P<0.05; *M. pugana* P<0.05; *M. vovidesii* P<0.05) (Fig. S1). It is worth mentioning that the extracts of *M. perezfarrerae* and *M. pugana* in the first dilution (D1) showed lower survival of *A. obliqua* in comparison with *C. grandiflorum* (CP; Fig. S1A, B).

TLC profiling of *Magnolia* extract in the different solvent systems indicated the presence of diverse types of phytochemicals as a complex matrix. In general, the number of spots found in some of the mean polarity solvents was more varied than those spots observed in the low polar and polar solvent systems (Fig. 1).

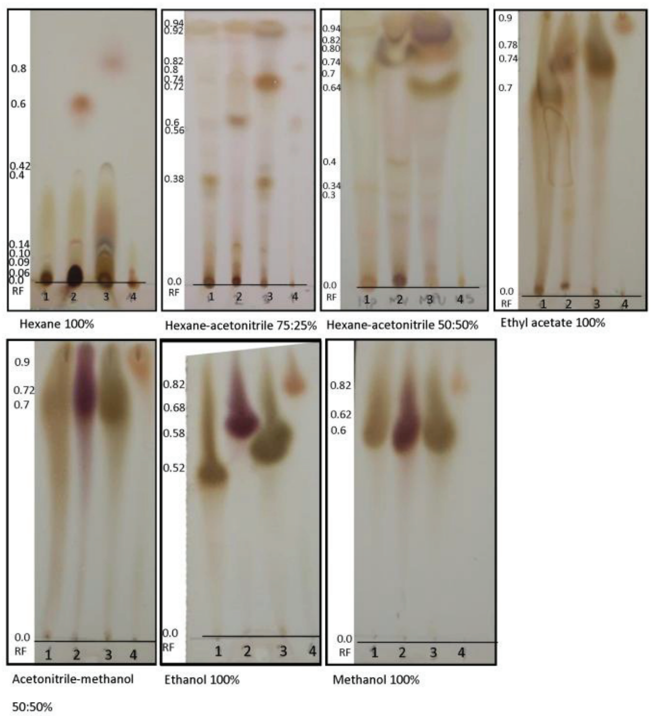

Fig. 1. Thin layer chromatography (TLC), revealed with p-anisaldehyde 98 %, of crude extracts of sarcotesta of *Magnolia* in seven solvent systems. *M. perezfarrerae* (1), *M. vovidesii* (2), *M. pugana* (3) and *M. schiedeana* (4). The distance travelled by the compound on the plate (RF).

We observed that diverse phytochemicals on samples traveled different distances up the TLC plate depending on the solvent system chosen. The retention factors (Rf) for each solvent system are detailed in Table S1, and solvent systems are sorted in ascending order. Variations in Rf values of the phytochemicals reflect an idea about their polarity. For example, compounds with high Rf values in less polar solvent have low polarity and with fewer Rf values have high polarity. On the TLC plate using hexane (100%) as the mobile phase was observed a good separation of low polarity compounds only for *M. vovidesii* (Rf=0.6) and *M. pugana* (Rf= 0.8) extracts.

In this study, sarcotesta extracts of the four *Magnolia* species present a greater amount of compounds of medium polarity. A solvent combination such as hexane-acetonitrile (75:25%) and hexane-acetonitrile (50:50%) were good solvent systems that moves different compounds of the mixture off the baseline compared to Ethyl acetate and Acetonitrile-methanol (50:50%).

Using hexane-acetonitrile (75:25%) were identified two compounds in *M. perezfarrerae* (Rf= 0.38, 0.92), in *M. vovidesii* were identified four compounds (Rf= 0.60, 0.74, 0.82, 0.94), in *M. pugana* were identified three compounds (Rf= 0.38, 0.72, 0.92), and were identified two compounds in *M. schiedeana* (Rf= 0.56, 0.80).

Using hexane-acetonitrile (50:50%) were identified three compounds in *M. perezfarrerae* (Rf= 0.34, 0.70, 0.94), in *M. vovidesii* were identified four compounds (Rf=0.30, 0.40, 0.74, 0.82), in *M. pugana* were identified four compounds (Rf= 0.30, 0.64, 0.74, 0.80), and were identified two compounds in *M. schiedeana* (Rf= 0.60, 0.82).

On the other hand, in the mobile phases of higher polarity with solvents such as ethanol and methanol, we do not observe a good separation of compounds that migrated upp the TLC plate, and some of them have similar Rf values. This information will drive future experiments in a selection of the appropriate solvent system for further separation, isolation, and identification of compounds from these plant extracts of *Magnolia* spp.

For the first time, the presence of alkaloids, flavonoids, and phenols in the four species of *Magnolia* endemic to Mexico is reported on qualitative phytochemical analyses. On the contrary, the tannin test was negative in all species. Extracts of *M. perezfarrerae* and *M. pugana* showed a high content of alkaloids, steroids, and terpenes. A medium amount of the three metabolites was detected in *M. vovidesii.* A low alkaloid content was detected in *M. schiedeana*; however, the test for steroids and terpenes was negative. A low content of coumarins, which were absent in the other analyzed plant species, was detected in the extracts of *M. perezfarrerae* and *M. pugana.* A high flavonoid content was found in *M. pugana*, a medium concentration in *M. vovidesii* and *M. schiedeana*, and low concentration in *M. perezfarrerae*. High levels of phenolic compounds were found in *M. schiedeana*, a medium amount in *M. perezfarrerae*, and a low amount in species of *M. pugana* and *M. vovidesii.* The test for saponins was positive only in extracts of *M. perezfarrerae*, but a low content of these compounds was found (Table 4).

Table 4. Qualitative analysis of secondary metabolites in ethanolic extracts of sarcotesta of *Magnolia*. Symbols (+), (++), (+++), indicate a low, medium, or high content or the absence (-) of this type of metabolites.

Species	Alkaloids	Coumarins	Flavonoids	Phenols	Tannins	Saponins	Steroids and terpenes
M. perezfarrerae	+++	+	+	++	-	+	+++
M. pugana	+++	+	+++	+	-	-	+++
M. vovidesii	++	-	++	+	-	-	++
M. schiedeana	+	-	++	+++	-	-	-

DISCUSSION

Magnolias have a high insecticide potential against Tephritidae. Among botanical pesticides there is a wide range of effectiveness that is determined by the botanical species, its vegetative structures, and the target pest species (Haouas *et al*., 2012; Hernández-Carlos & Gamboa-Angulo, 2019). In this study, it was observed that extracts of sarcotesta of *M. perezfarrerae* and *M. pugana* had more than 93% of insecticide-effectiveness against *A. ludens* and up to 91% against *A. obliqua.* This corresponds to the effectiveness shown by other species of Magnolias located in Mexican territory. In a preliminary study the insecticidal potential of *M. dealbata* (currently *M. vovidesii*) against *A. ludens* adults was recorded, with an effectiveness range of 19% to 96%, dry sarcotesta manifested itself as the vegetative structure with the highest effectiveness (Flores-Estévez *et al*., 2013). Likewise, the vegetative structures of *M. schiedeana* showed an effectiveness of 0.08 % for the flower and up to 64 % for the sarcotesta of its seeds against *A. ludens* adults (S. Vásquez-Morales *et al*., 2015).

Magnolias are known to have secondary metabolites with multiple biological effects (Lee *et al*., 2011; Sarker *et al*., 2002). This study confirmed qualitative that the assessed endemic species of Mexico contained alkaloids, flavonoids, phenols, and steroids or terpenes, consistent with chemical profiles reported for the Magnoliaceae family (Sánchez-Velásquez *et al*., 2016; Sarker *et al*., 2002). For example, the bark of *M. officinalis* has been reported to be a rich source of alkaloids (Yan *et al*., 2013), and the seeds of *M. grandiflora* contain alkaloids, saponins, and terpenes (Thakur & Sidhu, 2013). It is interesting to mention that *M. perezfarrerae, M. pugana* and *M. vovidesii* stood out for their high toxicity against fruit flies, *Anastrepha* spp; *M. perezfarrerae* distinguished itself for its high content of alkaloids, steroids, and terpenes, whereas *M. pugana* and *M. vovidesii* stood out for their high content of alkaloids, steroids, terpenes, and flavonoids (Table 4).

In nature terpenes are important compounds for plant defense mechanisms against herbivores and it has been suggested that they can be developed as biopesticides (Isman, 2000). Besides their effect on Diptera such as mosquitoes (Maheswaran & Ignacimuthu, 2012) and houseflies (Rossi & Palacios, 2013) has been reported. Several alkaloids have been reported as highly toxic to insects due to their effect on acetylcholinesterase receptors and sodium channels (Albuquerque *et al*., 2009; Crossthwaite *et al*., 2017). Our results preliminary suggest that the metabolites groups identified in the assayed Magnolias may contribute to the insecticidal effects reported for the fruit fly; nevertheless, studies of structure elucidation and chemical quantification of the major compounds in the extracts of sarcotesta of *Magnolia* spp are required.

Likewise, plants produce phenolic and flavonoid compounds as response mechanisms against herbivorous insects and plant pathogens (Ahmed *et al*., 2019; Bhattacharya *et al*., 2010). Polyphenolic compounds derived from the phenylpropanoid pathway, such as lignans, honokiol and magnolol, are the main components of *Magnolia* species; they possess antiviral (Amblard *et al*., 2006), antibacterial (Jacobo-Salcedo *et al*., 2011; B. Wu *et al*., 2018), fungicide (Chen *et al*., 2019) and insecticide properties (Wang *et al*., 2019; Yang *et al*., 2015). Honokiol, magnolol, 5-arylbenzofuran and their derivatives showed insecticidal activity against the black bean aphid (*Aphis fabae*), the fall webworm (*Hyphantria cunea*), the moth (*Mythimna separata*), and the swallowtail butterfly (*Papilio palamedes* y *P. troilus*) (Lin *et al*., 2019; Nitao *et al.*, 1992). Likewise, the active compounds of *M. denudata* seeds (palmitic acid, linoleic acid and honokiol) showed potent larvicidal effects in *Culex pipiens pallens* y *Aedes aegypti* (Wang *et al.*, 2019).

Alonso-Castro *et al*., (2014) determined the presence of honokiol and magnolol in the seeds of *M. dealbata* (currently *M. vovidesii*), so it can be inferred that the ethanolic extracts of sarcotesta of *M. vovidesii,* used in this study, contain these active insecticidal compounds, because the seeds come from the same location (Table 1). In addition, phenylpropanoid (iso-methyl eugenol) and sesquiterpene lactone (Costunolide) were isolated from hexane extracts of mature fruits of *M. salicifolia*, both compounds have insecticide activity against *Aedes aegypti*. Iso-methyl eugenol and costunolide have 0.13 and 0.14 Rf, respectively, in TLC with hexane (Kelm *et al.*, 1997), hence we can extrapolate that both compounds or their derivatives may be present in the sarcotesta of *M. vovidesii* and *M. pugana* species (Fig. 1).

Several botanical and synthetic insecticides are inhibitors of acetylcholinesterase, an enzyme that inactivates the neurotransmitter excitation of acetylcholine during synapses, which leads to hyperexcitation in the insect (Hernández-Carlos & Gamboa-Angulo, 2019). It was demonstrated that the magnaldehyde B isolated from the bark of *M. officinalis* has a potent inhibitory activity against acetylcholinesterase at IC_{50} values of 12.63 ± 0.51 (Zhang *et al*., 2019). Likewise, guaiacol and caffeic acid (structurally related phenol compounds of honokiol) inhibit acetylcholinesterase in *Aedes aegypti* larvae and those treated with honokiol and magnolol showed spots all over their bodies due to damage and rupture of the middle intestine with no nucleus cell organelles and severely damaged mitochondria and plasma organelles with indiscernible appearances (Nitao *et al*., 1992).

It has been reported that the highest impact of the pest is caused by adult insects, especially females who oviposit their eggs on the fruits (Hernández-Ortiz, 1993; Montoya *et al*., 2010). However, the control of *A. ludens* flies at third-instar larval was also studied using the aqueous extract of *Annona lutescens* stem, obtaining a 95% effectiveness at 72 h of exposure (González-Esquinca *et al*., 2012).

CONCLUSIONS

Our study confirms the insecticide effectiveness of extracts of sarcotesta of *M. perezfarrerae, M. pugana* and *M. vovidesii* species against *Anastrepha ludens* and *A. obliqua* fruit fly species. The three *Magnolia* spp investigated showed differences in their phytochemical profile. The insecticide properties of *Magnolia* can contribute to the integrated management of Tephritids.

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Supplementary material

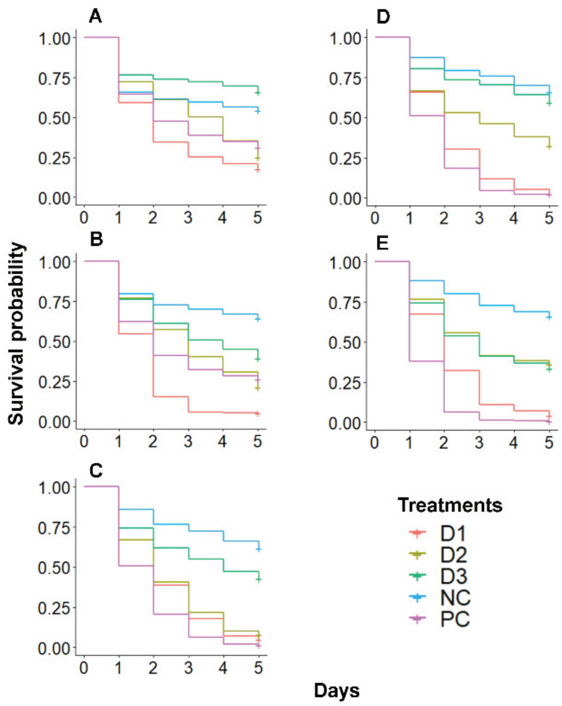

Fig. S1. Kaplan-Meier survival plot for *A. ludens* (right) and *A. obliqua* (left) exposed to ethanolic extracts of *M. perezfarrerae* (A, D), *M. pugana* (B, E) and *M. vovidesii* (C) in three dilutions, 0.1 mgmL^{-1} (D1), 0.01 mgmL^{-1} (D2) and 0.001 mgmL^{-1} (D3) with negative (NC= Ethanol 96%) and positive control (PC= *C. grandiflorum*).

Table S1. The retention factor (Rf) for each of *Magnolia* spp in different solvent systems, solvents are sorted in ascending order.

Polarity	Solvent	Proportion (%)	Retention factor (Rf)			
			M. perezfarrerae	***M. vovidesii***	***M. pugana***	***M. schiedeana***
Low polarity	Hexane	100	0.09	0.14 0.40 0.60	0.10 0.14 0.42 0.80	0.06
Mean polarity	Hexane-acetonitrile	75:25	0.38 0.92	0.60 0.74 0.82 0.94	0.38 0.72 0.92	0.56 0.80
	Hexane-acetonitrile	50:50	0.34 0.70 0.94	0.30 0.40 0.74 0.82	0.30 0.64 0.74 0.80	0.60 0.82
	Ethyl acetate	100	0.70	0.74	0.78	0.90
	Acetonitrile-methanol	50:50	0.70	0.72	0.72	0.90
High polarity	Ethanol	100	0.52	0.68	0.58	0.82
	Methanol	100	0.60	0.60	0.62	0.82