

Evaluation of Ethanol Extract of *Magnolia alejandrae* (Magnoliales: Magnoliaceae) against *Tetranychus merganser* (Acari: Tetranychidae)¹

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Abstract *Tetranychus merganser* Boudreaux (Acari: Tetranychidae) is one of the most economically important mite pests of papaya (*Carica papaya* L.) crops grown along the Gulf of México. Control of this mite depends mainly on chemical insecticides. This research aims to evaluate the effects of different concentrations (0.1, 0.5, 1, 5, 10, and 15% [v/v]) of the ethanolic extract of *Magnolia alejandrae* García-Morales and Iamónico (Magnoliaceae) leaves on *T. merganser* adult females, as well as to obtain information about the chemical composition of the extract. The ethanolic extracts contained some phytochemicals such as phenolic compounds, alkaloids, glycosides, ketones, terpenes, and quinones. Females treated with 0.1 and 15% (v/v) of the extract showed mortality of 0.0% and 33.3% at 72 h, respectively, as compared to the control treatment. The mites treated with 15% (v/v) of the extract decreased their oviposition rate (5.90, 5.35, and 4.77 eggs/female), compared to the control treatment (13.27, 13.95, and 14.39 eggs/female) at 24, 48, and 72 h, respectively, which led to a reduction in the growth rate. The feeding damage caused by *T. merganser* was reduced at high concentrations. *Magnolia alejandrae* leaf ethanolic extract has potential for development as a control agent for *T. merganser*.

Key Words biological control, botanical extract, residual effect, ovicidal

The demand for food to feed the human population is constantly growing, leading to the development and adoption of synthetic pesticides as a fast and effective strategy to control pests and crop diseases. However, the excessive use of synthetic insecticides causes detrimental effects on human and environmental health and causes pests and pathogens to develop resistance to these chemical compounds (Lengai et al. 2020). Therefore, identifying new alternatives is crucial to

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combat insect and mite resistance to agrochemicals. Plant-derived extracts are one option, as they offer a “green” alternative with minimal detrimental effects on the environment, humans, and other nontargets (Attia et al. 2013, Fathipour and Maleknia 2016, Lengai et al. 2020).

The magnolia tree, *Magnolia alejandrae* García-Morales and Iamónico (Magnoliaceae), is endemic to Mexico. This tree grows in cloud forests or humid forests of pines and oaks in the Sierra Madre Oriental (central-western state of Tamaulipas, Mexico) (García-Morales et al. 2017). It has been reported as host to *Leptoglossus dilaticollis* (Guérin-Ménéville) (Hemiptera: Coreidae) (Gallardo-Yobal et al. (2020). Those authors documented that of a mean (\pm SD) of 46.00 ± 6.55 seeds per cone, 35% of these seeds were damaged by *L. dilaticollis* feeding, which can reduce seedling emergence. *Magnolia alejandrae* is listed under the International Union for Conservation of Nature Red List criteria in the endangered category (Akande and Yobal 2021). In Mexico, some *Magnolia* species are important in traditional medicine due to their biological and pharmacological effects on various organisms. *Magnolia* spp. produce more than 255 metabolites, such as alkaloids, flavonoids, lignans, and terpenoids, from the leaves, flowers, bark, and seeds (Sarker et al. 2002, Vásquez-Morales et al. 2015). Several species of *Magnolia* (e.g., *Magnolia citrata*, *Magnolia dealbata*, *Magnolia fargesii*, *Magnolia kobus*, *Magnolia schiedeana*, and *Magnolia tamaulipana*) have insecticidal and acaricidal properties (Miyazawa et al. 1994, Park et al. 2002, Flores-Estévez et al. 2013, Vásquez-Morales et al. 2015, Chacón-Hernández et al. 2020, Luu-Dam et al. 2021). However, thus far, the chemical composition of *M. alejandrae* has not been determined and its application against any arthropod pest has not been reported.

Tetranychus merganser Boudreaux (Acari: Tetranychidae) is a major pest of papaya (*Carica papaya* L.) crops in several regions of the Gulf of México (Alfaro-Valle et al. 2022). The damage caused by the feeding activity of this mite is severe. These mites pierce the epidermis and parenchyma cells and absorb the cellular contents, which leads to reduced photosynthetic activity and transpiration rate. The damage manifests as small, white spots on the adaxial surface of the leaf, and with high infestations of mites, these spots can merge, causing the leaves to turn entirely white (Gutierrez 1994, Park et al. 2002, Bensoussan et al. 2016, López-Bautista et al. 2016). *Tetranychus merganser* is controlled by chemical pesticides. However, the short life cycle and high reproductive potential of these mites cause them to develop resistance to acaricides very quickly (Ullah et al. 2011a, 2011b). In addition, synthetic pesticide residues can cause harmful effects on human health and the environment. For these reasons, we urgently need to develop safe and ecological techniques. Here, we aimed to evaluate the effects of the different concentrations of the ethanolic extract of *M. alejandrae* leaves on adult females of *T. merganser*, as well as to obtain information about the chemical composition of the extract.

Materials and Methods

Mite colony. A colony of *T. merganser* was started with biological material obtained from the Population Ecology Laboratory at the Applied Ecology Institute at the Autonomous University of Tamaulipas (IEA-UAT). To increase the mite population, females and males of *T. merganser* were placed on bean plants (*Phaseolus*

vulgaris L.) under laboratory conditions at $27 \pm 1^\circ\text{C}$ and $60 \pm 10\%$ relative humidity (RH).

Preparation of plant material and extract. Mature leaves of *M. alejandrae* were collected in El Molino Canyon (N $23^\circ46'29.0''$, W $99^\circ19'23.4''$, Jaumave, Tamaulipas state, Mexico). The selected leaves were not contaminated (e.g., without external agents and physical contaminants). The samples were labeled and transported in brown paper bags inside coolers to the IEA-UAT Herbarium for their taxonomic identification (code 022755). Later, the samples were transferred to the IAE-UAT Phytochemistry Laboratory.

The *M. alejandrae* leaves were washed twice at room temperature, first with tap water and second with distilled water. Immediately, the plant tissue was dehydrated using a convectional laboratory oven at 45°C for 72 h. Later, dry leaf samples were ground by mortar and sieved (2.0-mm no. 10 sieve) until a fine and homogeneous powder was obtained, which was used for extraction.

We prepared the extract by the Soxhlet method (Yadav and Agarwala 2011, Thakur and Sidhu 2013). A total of 25 g of the fine powder was placed in the apparatus and extracted with 250 ml (1:10 w/v) of 95% ethyl alcohol. The solvent was evaporated at $30\text{--}40^\circ\text{C}$ in a rotary evaporator (RE100-PRO; DLAB Scientific Inc, Riverside, CA). Finally, the microtubes were stored at 4°C until used. A stock solution was prepared at a concentration of 10,000 ppm, namely, with 2 g in 200 ml (10 mg/ml).

Phytochemical analysis of the extract. Phytochemicals were extracted from the ethanol extract and then filtered through a $0.25\text{-}\mu\text{m}$ filter. After clarification, 0.5 μl of the sample was used in each analysis using an ultra-high-performance liquid chromatography system coupled with a time-of-flight/quadrupole time-of-flight mass spectrometer (Agilent G6545A; Agilent Technologies, Santa Clara, CA) equipped with a flux of 0.4 ml/min, high-pressure limit of 1,200 bar, 100% water in channel A, and 100% acetonitrile in channel B, both with 0.1% of formic acid. An Agilent InfinityLab Poroshell 120 EC-C18, $1.9\text{-}\mu\text{m}$ column was used at 40°C . Acquisition software was version 6200 series TOF/6500 series Q-TOF B.08.00 (B8058.3 SP1). Detection of the mass spectrum was completed in the positive ion mode with a mass range of $100\text{--}1,000$ m/z. The optimal values for the source parameters were as follows: dry gas temperature, 300°C ; gas flow, 10.0 L/min; nebulizer pressure, 45 psig; and spectrum velocity, 1 Hz. Automated tandem mass spectrometry assays were done using fixed collision energies of 15 and 20 V with argon as the collision gas and adjustment of the exploration time every 1 s. Tentative identity assignment was done by comparing mass fragmentation patterns (Montelongo-Ruíz et al. 2020, Olazarán-Santibañez et al. 2021). The comparison of the observed mass spectra was done with those found in the MassBank (<http://www.massbank.jp/>), ChemSpider (<http://www.chemspider.com>), and PubChem (<https://pubchem.ncbi.nlm.nih.gov>).

Experimental design. The experiment was evaluated in a bioclimatic chamber using a modified sand technique in a study by Ahmadi (1983), which consisted of bean leaf discs of 2.5 cm in diameter with the abaxial surface facing up that were placed on water-soaked cotton and placed inside a 5-cm-diameter Petri dish at a temperature of $28 \pm 1^\circ\text{C}$ with $60\text{--}70\%$ RH and a photoperiod 16:8 L:D. Each bean leaf disk had 10 1-day-old adult female *T. merganser*. To homogenize the age and

improve the reproduction and oviposition of *T. merganser* females, 10 teleiochrysalises were placed on each bean leaf disc (total discs = 30). When these mites emerged as female adults, 5 males were placed on each leaf disc (ratio 2:1, female:male). These individuals came from the stock colony. At 12 h, we removed the observed males and eggs, leaving only the females on each bean disc. The females of *T. merganser* begin to oviposit after 0.9 ± 0.04 (pre-oviposition period) days after having emerged to the adult stage, after which they were fed with beans and maintained at 27.5°C and 60–70% RH and on a photoperiod of 16:8 h L:D (Ullah et al. 2011a).

We randomly divided the bean leaf discs into a control group and seven treatment groups, one per extract concentration. A bean leaf disc served as a replicate with three repetitions per group. We placed 10 mite females on each bean leaf disc and sprayed twice (0.5 ± 0.1 ml) with each concentration. We used a manual sprayer applying concentrations of 0.1%, 0.5%, 1%, 2.5%, 5%, 10%, and 15% (v/v) of the extract. The control group was sprayed only with distilled water.

***Tetranychus merganser* mortality assay.** The number of dead mites was recorded at 72 h using a dissecting microscope (ZM180; UNICO Stereo and Zoom Microscopes, Princeton, NJ). Mites that presented ataxia (active movement, apparently messy) and the mites lying on their backs with their legs up or without moving were considered dead. We corrected the mortality data using Abbott's (1925) formula for control group mortality.

***Tetranychus merganser* oviposition.** We counted the number of eggs at 24, 48, and 72 h by using a dissecting microscope. The oviposition activity index (OAI) (Kramer and Mulla 1979) was calculated with the formula $OAI = [(NTG - NCG)/(NTG + NCG)]$, where NCG = the number of eggs in the control group and NTG = the number of eggs in the treated group. Values ranged from +1 to -1. Positive values indicate that more eggs laid per *T. merganser* female were observed in the treated group than in the control group, showing that the extract stimulated oviposition activity. In contrast, more eggs laid in the control group than in the extract-treated group resulted in a negative OAI, indicating that the extract inhibited the oviposition activity of *T. merganser* females.

***Tetranychus merganser* feeding.** We recorded the percentage of feeding damage of *T. merganser* at 72 h using a dissection microscope. Symptoms observed on bean discs were classified based on the ordinal scale and converted to percentages, as follows: 0 = 0% damage (no feeding damage), 1 = 1–20%, 2 = 21–40%, 3 = 41–60%, 4 = 61–80%, and 5 = 81–100% feeding damage (dense markings, or wilting, after the mite damaged the bean disc for its feeding) (Hussey and Parr 1963, Nachman and Zemek 2002). We used the feeding deterrence index (FDI) to determine the effect of *T. merganser* antifeeding. For this determination, we modified the criterion of Kramer and Mulla (1979), using the formula $FDI = [(\%DTG - \%DCG)/(\%DTG + \%DCG)]$, where %DCG = the percentage of damage caused by the feeding of *T. merganser* in the control group and %DTG = the percentage of damage caused by feeding in the treated group. Positive values show that there was more damage in the group treated with the extract than in the control group, indicating that the extract stimulates mite feeding. Negative values represent more severe damage to the control group than to the treated group, which suggests that the *M. alejandrae* extract inhibited mite feeding.

Population growth of *Tetranychus merganser*. We calculated the rate of increase (r , day^{-1}), the finite rate of increase (λ), and the population doubling time (DT) on the third day after the application of the extract. The demographic parameters (r , λ , and DT) indicate the growth of the *T. merganser* population. The r value was calculated as per Birch (1948) with $r = (1/t) \times \ln(Nt/N_0)$, where Nt = the number of live mites + the number of eggs at time t , N_0 = the number of mites at time 0 (initial cohort = 10 adult female mites), and t = the number of days from the start to the end of the bioassay (equal to 3 d). The finite growth rate (λ), that is, the number of times the population multiplied in a unit of time, was calculated as $\lambda = \text{antilog}_e r$. The doubling time of the *T. merganser* population was calculated by $DT = \ln(2)/r$.

Statistical analysis. The number of eggs laid; the percentage of feeding damage; the r , day^{-1} ; the λ ; and the DT were subjected to a one-way analysis of variance (ANOVA). We verified the assumptions of normality and heteroscedasticity of all the data before performing the ANOVA. If significant differences were detected, multiple comparisons of means were performed using Tukey's method ($P < 0.05$). Finally, we correlated the average number of eggs laid per female with the average percentage of feeding damage of *T. merganser* females. We used SAS/STAT software for all analyses (SAS 2002).

Results

Phytochemical composition. The gas chromatography-mass spectrometry analysis of the *M. alejandrae* leaf extract revealed the presence of 70 chemical components (Table 1). The chemical profile of the extract showed a greater amount of terpenes ($n = 26$), followed by phenolic compounds (14), and alkaloids (12).

Mite mortality. The ethanolic extract exhibited acaricidal activity against *T. merganser* females (Fig. 1). Concentrations of 0.1, 0.5, 1, 2.5, 5, 10, and 15% (v/v) caused acute toxicity to female mites at 72 h ($F = 13.50$; $df = 6, 14$; $P = 0.0001$). Female mite mortality had a positive linear trend, i.e., increasing mortality with increasing concentration.

Oviposition. The number of eggs oviposited per *T. merganser* female differed significantly among the concentrations at 24 ($F = 26.23$; $df = 7, 16$; $P = 0.0001$), 48 ($F = 132.81$; $df = 77, 16$; $P < 0.0001$), and 72 h ($F = 151.02$; $df = 7, 16$; $P = 0.0001$) (Table 2). At 24 h, the oviposition activity index ranged from 0.10 (0.1%) to 0.39 (15%) (Fig. 2). At 48 h, it ranged between 0.19 (0.1%) and 0.44 (15%) (Fig. 3), and at 72 h, the range was between 0.26 (0.1%) and 0.49 (15%), as compared to the control group (Fig. 4). In the three observation times (24, 48, and 72 h), the oviposition rate had a negative linear trend, i.e., decreasing rate with increasing concentration.

Feeding damage. The percentage of feeding damage was significantly different among the treatment concentrations ($F = 18.36$; $df = 7.16$; $P = 0.0001$) (Fig. 5A). The mean (\pm SD) percentage of damage in the control group was $66.67 \pm 7.64\%$, and in treated females, this percentage ranged from $52.67 \pm 6.43\%$ at 0.1% to $25.00 \pm 5.00\%$ at 15%. The feeding activity index of *T. merganser* females treated with 0.1 and 15% showed a decrease in their food intake of 0.12 and 0.45 at 72 h, compared to the control group, respectively (Fig. 5B), suggesting

Table 1. The chemical composition of ethanolic extract of *Magnolia alejandrae* leaves.

Cpd*	Retention time (min)	Name	Compound Type	Molecular Formula	m/z*
498	24.19	3-[[5-Methyl-2-(1-methylethyl)cyclohexyl]oxy]-1,2-propanediol	Alcohol	C ₁₃ H ₂₆ O ₃	253.17
512	24.67	Procyanidin B6	Alcohol	C ₃₀ H ₂₆ O ₁₂	579.15
497	24.18	Capillanol	Alcohol	C ₁₂ H ₁₄ O	175.11
407	20.61	Mecambrine	Alkaloid	C ₁₈ H ₁₇ NO ₃	296.12
862	38.66	Isopiperolein B	Alkaloid	C ₂₁ H ₂₉ NO ₃	366.20
51	2.21	3,6-Dihydroxynortropane	Alkaloid	C ₇ H ₁₃ NO ₂	144.10
52	2.29	3-Methyleneoxindole	Alkaloid	C ₉ H ₇ NO	146.06
107	5.08	Crotanecine	Alkaloid	C ₈ H ₁₃ NO ₃	172.09
199	11.42	Indole	Alkaloid	C ₈ H ₇ N	118.06
233	13.21	(-)-Caaverine	Alkaloid	C ₁₇ H ₁₇ NO ₂	268.13
344	18.02	(-)-Anonaine	Alkaloid	C ₁₇ H ₁₅ NO ₂	266.11
360	18.63	Floribundine	Alkaloid	C ₁₈ H ₁₉ NO ₂	282.14
454	22.47	Amabiline	Alkaloid	C ₁₅ H ₂₅ NO ₄	284.18
630	29.22	Mukoenine A	Alkaloid	C ₁₈ H ₁₉ NO	266.15
910	40.60	Dehydroemetine	Alkaloid	C ₂₉ H ₃₈ N ₂ O ₄	479.29
26	1.11	Carboxynorspermidine	Amine	C ₇ H ₁₇ N ₃ O ₂	176.13
690	31.67	1-Phenylethylamine	Amine	C ₈ H ₁₁ N	122.09
700	32.07	Phytosphingosine	Amine	C ₁₈ H ₃₉ NO ₃	318.29
665	30.63	Cinnamyl cinnamate	Carboxylic Acid	C ₁₈ H ₁₆ O ₂	265.12
81	3.65	Anthranilic acid	Carboxylic Acid	C ₇ H ₇ NO ₂	138.05
133	6.31	Phenylpyruvic acid	Carboxylic Acid	C ₉ H ₈ O ₃	165.05
339	17.82	2-Succinylbenzoate	Carboxylic Acid	C ₁₁ H ₁₀ O ₅	223.06
489	23.86	Cinnamyl isovalerate	Carboxylic Acid	C ₁₄ H ₁₈ O ₂	219.13
37	1.63	Salidroside	Glycoside	C ₁₄ H ₂₀ O ₇	323.10
140	6.61	5'-O-beta-D-Glucosylpyridoxine	Glycoside	C ₁₄ H ₂₁ NO ₈	332.13
301	16.40	Matsutakic acid A	Glycoside	C ₁₀ H ₁₆ O ₄	201.11
1053	46.51	Lividomycin B	Glycoside	C ₂₃ H ₄₅ N ₅ O ₁₃	600.31
462	22.80	(6S)-dehydrovomifoliol	Ketone	C ₁₃ H ₁₈ O ₃	223.13
29	1.25	1,2,3-Trihydroxybenzene	Phenolic compound	C ₆ H ₆ O ₃	127.03
213	12.27	Isoscopoletin	Phenolic compound	C ₁₀ H ₈ O ₄	193.04
235	13.39	2-Phenylethyl 3-methylbutanoate	Phenolic compound	C ₁₃ H ₁₈ O ₂	207.13
295	16.16	Obovatol	Phenolic compound	C ₁₈ H ₁₈ O ₃	284.18
326	17.34	3beta-Dihydroxymarasmene	Phenolic compound	C ₁₅ H ₂₂ O ₃	251.16
341	17.88	Lathodoratin	Phenolic compound	C ₁₁ H ₁₀ O ₄	207.06

Table 1. Continued.

Cpd*	Retention time (min)	Name	Compound Type	Molecular Formula	m/z*
352	18.40	N-Methyl-N-(2-phenylethenyl)-3-phenyl-2-oxiranecarboxamide	Phenolic compound	C ₁₈ H ₁₇ NO ₂	280.13
456	22.55	Murrayacinine	Phenolic compound	C ₂₃ H ₂₃ NO ₂	346.18
505	24.42	Isokaempferide	Phenolic compound	C ₁₆ H ₁₂ O ₆	301.06
542	25.74	Encecalin	Phenolic compound	C ₁₄ H ₁₆ O ₃	255.100
668	30.76	Zanthosimuline	Phenolic compound	C ₂₀ H ₂₃ NO ₂	310.18
709	32.42	6-Hydroxy-5,7,4'-trimethoxyflavone	Phenolic compound	C ₁₈ H ₁₆ O ₆	329.10
740	33.65	Chalcone	Phenolic compound	C ₁₅ H ₁₂ O	209.09
810	36.63	Magnolol	Phenolic compound	C ₁₈ H ₁₈ O ₂	267.13
423	21.21	Xanthorrhizol	Alcohol	C ₁₅ H ₂₂ O	219.17
485	23.71	Emodin	Quinone	C ₁₅ H ₁₀ O ₅	271.06
60	2.73	2-Propylthiophene	Terpene	C ₇ H ₁₀ S	127.05
205	11.83	4-Acetyl-1-methylcyclohexene	Terpene	C ₉ H ₁₄ O	139.11
225	12.89	(R)-p-Mentha-1,8-dien-7-ol	Terpene	C ₁₀ H ₁₆ O	153.12
241	13.62	Annuionone C	Terpene	C ₁₃ H ₂₀ O ₃	225.14
265	14.78	5,6-Dihydro-6-pentyl-2H-pyran-2-one	Terpene	C ₁₀ H ₁₆ O ₂	169.12
276	15.23	p-Cymene	Terpene	C ₁₀ H ₁₄	135.11
290	15.91	(S)-3-Butyl-1(3H)-isobenzofuranone	Terpene	C ₁₂ H ₁₄ O ₂	191.10
298	16.25	Heliannuol B	Terpene	C ₁₅ H ₂₀ O ₃	249.14
302	16.43	Iridotrial	Terpene	C ₁₀ H ₁₄ O ₃	183.10
316	16.98	5-Isopropyl-2-methylphenol acetate	Terpene	C ₁₂ H ₁₆ O ₂	193.12
317	17.01	Germacrone-13-al	Terpene	C ₁₅ H ₂₀ O ₂	233.15
328	17.42	4,5-Dihydrovomifoliol	Terpene	C ₁₃ H ₂₂ O ₃	227.16
405	20.56	Rishitin	Terpene	C ₁₄ H ₂₂ O ₂	223.16
426	21.38	Atractylone	Terpene	C ₁₅ H ₂₀ O	217.15
436	21.75	Geranyl acetoacetate	Terpene	C ₁₄ H ₂₂ O ₃	239.16
437	21.77	(3S,5R,6R,7E)-3,5,6-Trihydroxy-7-megastigmen-9-one	Terpene	C ₁₃ H ₂₂ O ₄	265.14
515	24.78	L-Menthyl acetoacetate	Terpene	C ₁₄ H ₂₄ O ₃	241.17
539	25.65	Spathulenol	Terpene	C ₁₅ H ₂₄ O	221.19
550	26.06	Anacyclin	Terpene	C ₁₈ H ₂₅ NO	294.18
561	26.53	Absinthin	Terpene	C ₃₀ H ₄₀ O ₆	519.27
577	27.20	alpha-Curcumene	Terpene	C ₁₅ H ₂₂	203.17

Table 1. Continued.

Cpd*	Retention time (min)	Name	Compound Type	Molecular Formula	m/z*
580	27.29	Icacine	Terpene	C ₂₂ H ₃₁ NO ₆	428.20
663	30.58	(R)-Hydnocarpic acid	Terpene	C ₁₆ H ₂₈ O ₂	275.19
684	31.40	4-(2,6,6-Trimethylcyclohex-1-enyl)but-2-en-4-one	Terpene	C ₁₃ H ₂₀ O	193.15
830	37.39	beta-Panasinsene	Terpene	C ₁₅ H ₂₄	205.19
854	38.32	Santene	Terpene	C ₉ H ₁₄	123.11

* Cpd, Compound label; m/z, mass-charge relationship.

that the feeding rate of *T. merganser* females depends on the concentrations applied.

Correlation of the percentage of damage with the number of eggs of *T. merganser*. We found that the number of eggs oviposited per *T. merganser* female correlated with the feeding damage percentage caused by *T. merganser* females (Fig. 6). The R² of 0.7749 ($F = 20.65$; $df = 1.6$; $P = 0.0039$) indicated that 77.49% of the variation in the number of eggs laid was associated with the feeding damage. Our results also showed that the increase in extract concentration negatively influenced the relationship between the number of eggs laid per female and feeding damage of mite females.

Demographic parameters. The population growth rate of *T. merganser* also differed significantly among the treatments ($F = 104.45$; $df = 7, 16$; $P = 0.001$),

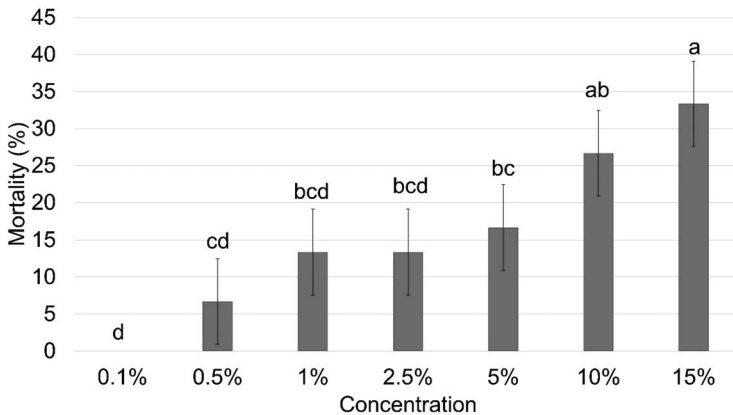


Fig. 1. Corrected mean (\pm SD) mortality of *T. merganser* females caused by exposure to different concentrations of *M. alejandrae* leaf ethanolic extract under controlled laboratory conditions ($n = 3$ with 10 mites per group). Bars topped with different letters indicate significant differences ($P < 0.05$; ANOVA and Tukey's honestly significant difference [HSD] test).

Table 2. Effect of ethanolic extract of *M. alejandrae* leaves on oviposition of *T. merganser* females.

Treatment	Eggs per female*		
	24 h	48 h	72 h
Control	13.27 ± 0.64 a	13.95 ± 0.83 a	14.39 ± 0.45 a
0.1%	10.83 ± 0.70 ab	9.52 ± 0.68 b	8.12 ± 0.73 b
0.5%	8.57 ± 0.90 bc	7.33 ± 0.52 c	6.31 ± 0.43 c
1%	6.73 ± 1.15 cd	6.0 ± 0.25 d	5.55 ± 0.25 cd
2.5%	6.70 ± 0.60 cd	5.92 ± 0.15 d	5.46 ± 0.27 cd
5%	6.57 ± 1.08 cd	5.86 ± 0.10 d	5.29 ± 0.53 cd
10%	5.90 ± 1.00 d	5.35 ± 0.22 d	4.77 ± 0.40 d
15%	5.90 ± 1.00 d	5.35 ± 0.22 d	4.77 ± 0.40 d

* Mean (± SD) number of eggs laid per female. Values within a column followed by different lowercase letters are significantly different ($P \leq 0.05$; ANOVA and Tukey's HSD test).

with rate decreasing as extract concentration increased. The highest mean (± SD) of the r of *T. merganser* was observed in the control treatment (1.2627 ± 0.01) and the lowest r of 0.8383 ± 0.01 with the 15% concentration treatment (Fig. 7). The finite growth rate significantly differed among the treatments ($F = 120.83$; $df = 7.16$; $P = 0.001$). With exposure to a concentration of 15%, the mean number of mites added to the population per female per day ($\lambda = 2.31 \pm 0.03$) was significantly lower than the control treatment (3.53 ± 0.04) (Fig. 8). The DT of the *T. merganser* population also was significantly different among the treatments ($F = 75.34$; $df = 7.16$; $P = 0.0001$).

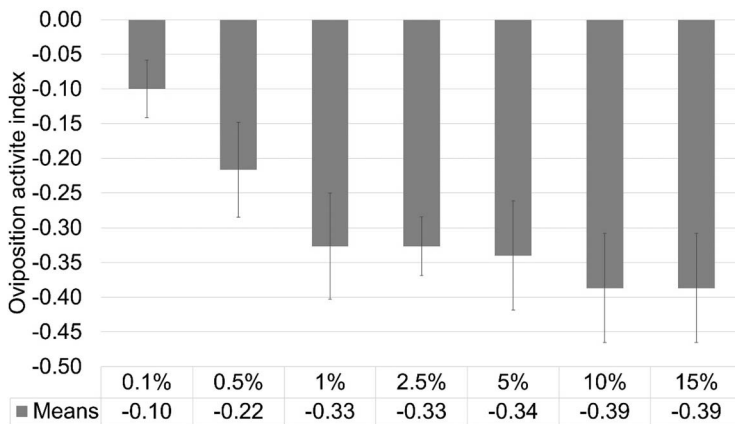


Fig. 2. Ovipositional activity index (mean ± SD) for *T. merganser* in response to different concentrations of *M. alejandrae* leaves ethanolic extract at (A) 24 h, (B) 48 h, and (C) 72 h.

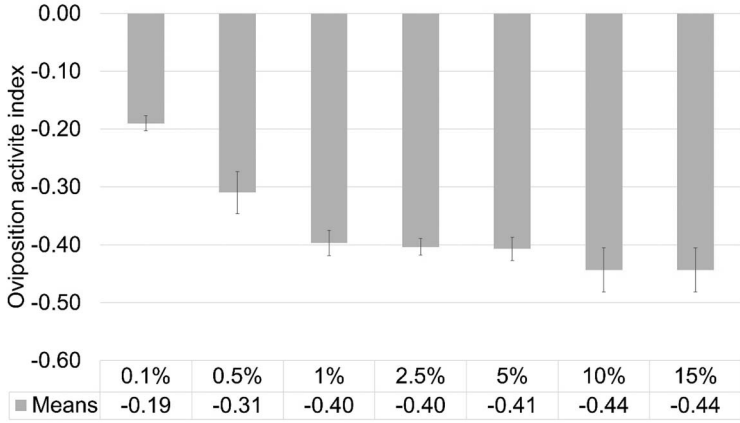


Fig. 3. Ovipositional activity index (mean ± SD) for *T. merganser* in response to different concentrations of *M. alejandrae* leaves ethanolic extract at 24 h.

The longest doubling time (0.8283 ± 0.01 d) was observed when females were treated with an extract concentration of 15%, and the shortest occurred with the control treatment (0.5493 ± 0.00 d) (Fig. 9).

Discussion

Much effort has been focused on the evaluation of *Magnolia* spp. as potential sources for the control of arthropod pests (Al-Alawi 2014, Chacón-Hernández et al. 2020, Luu-Dam et al. 2021, Reyes-Zepeda et al. 2022). The *Magnolia* species produce more than 255 kinds of secondary metabolites, mainly alkaloids, flavonoids, lignans,

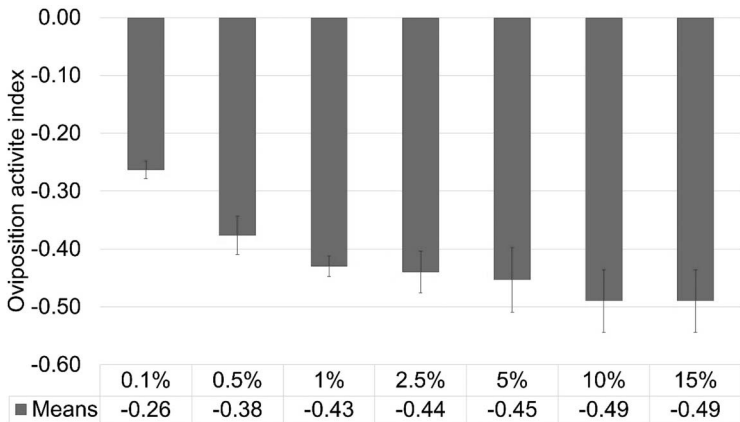


Fig. 4. Ovipositional activity index (mean ± SD) for *T. merganser* in response to different concentrations of *M. alejandrae* leaves ethanolic extract at 48 h.

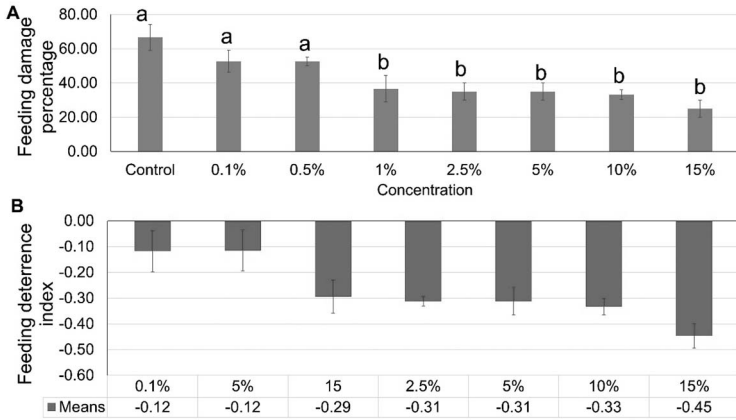


Fig. 5. Effect of *M. alejandrae* leaf ethanolic extract on the feeding of *T. merganser*. (A) Mean (\pm SD) percentage of feeding damage by females, (B) mean (\pm SD) index of feeding activity for *T. merganser* in response to different concentrations at 72 h. Bars topped with different letters indicate significant differences ($P < 0.05$; ANOVA and Tukey’s HSD test).

neolignans, and terpenoids, which have been isolated from the flowers, bark, cones, and leaves (Sarker et al. 2002). The phytochemical analysis results indicate that alkaloids, phenols, quinones, terpenes, ketones, and glycosides were present in the ethanolic extract of the *M. alejandrae* leaves (Table 1). Sarker et al. (2002) mentioned that the dicotyledonous family Magnoliaceae is well known for producing alkaloids and so is the genus *Magnolia*. The same authors documented that on approximately 40 *Magnolia* species, only 22, 21, and 15 species produce different alkaloids, flavonoids, and

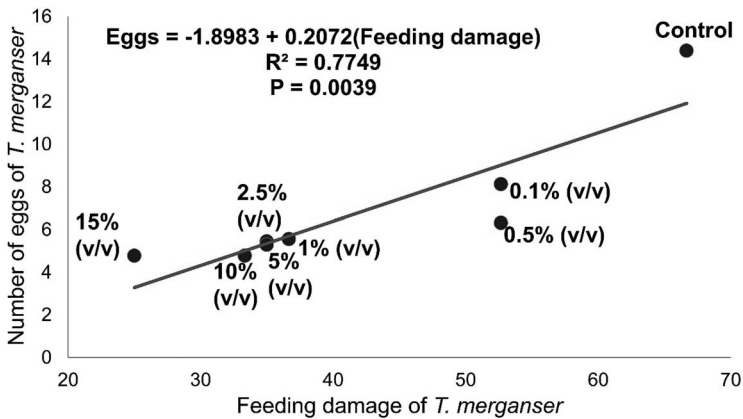


Fig. 6. Correlation of the number of eggs with the feeding damage of *T. merganser* females treated with different concentrations of *M. alejandrae* leaf ethanolic extract at 72 h.

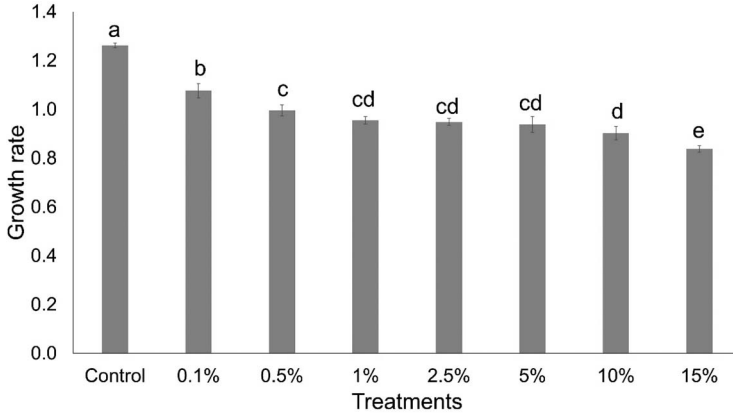


Fig. 7. Effects of *M. alejandrae* leaf ethanolic extract on the growth rate of *T. merganser*. Bars topped with different letters indicate significant differences ($P < 0.05$; ANOVA and Tukey's HSD test).

terpenoids, respectively. Furthermore, they reported that *M. grandiflora* is the one that produces more secondary metabolites such as alkaloids, coumarins, flavonoids, lignans, neolignans, phenylpropanes, terpenoids, and other secondary metabolites. From this information, we can infer that the different species of *Magnolias* can show different effects on the biology of insects and mites, with the help of extracts obtained from flowers, bark, cones, and leaves and different polarities of solvents.

The ultra-high-performance liquid chromatography–mass spectrometry analysis of the *M. alejandrae* leaf ethanolic extract revealed the presence of chemical components that have insecticidal activity such as p-cymine (Regnault-Roger and Hamraoui 1995, Zhang et al. 2017, Saad et al. 2019), emodin (Shang et al. 2019),

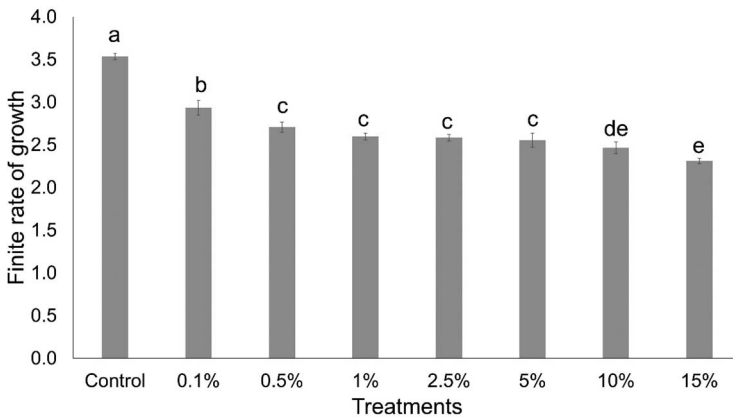


Fig. 8. Effects of *M. alejandrae* leaf ethanolic extract on the finite rate of growth of *T. merganser*. Bars topped with different letters indicate significant differences ($P < 0.05$; ANOVA and Tukey's HSD test).

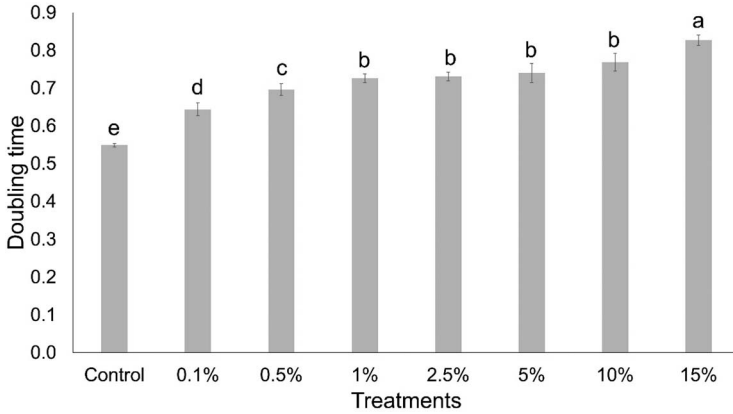


Fig. 9. Effects of *M. alejandrae* leaf ethanolic extract on the population doubling time of *T. merganser*. Bars topped with different letters indicate significant differences ($P < 0.05$; ANOVA and Tukey's HSD test).

magnolol (Ye et al. 2015), and anonaine (Bezerra et al. 2022, Ngegba et al. 2022). Regnault-Roger and Hamraoui (1995) found that different concentrations (1.5×10^{-6} to 3×10^{-5} M) of the p-cymene compound inhibit fecundity/female/day, oviposition, larval development, and adult emergence of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). Zhang et al. (2017) documented that the concentration of 5 μL of p-cymene causes 100% mortality of *Musca domestica* L. (Diptera: Muscidae). Saad et al. (2019) found that p-cymene caused a repellent effect at a concentration of 0.001 mg/cm² and contact low toxicity ($\text{LC}_{50} > 0.5$ mg/cm²) against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Shang et al. (2019) reported that the compound emodin caused mortality in *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) ($\text{LC}_{50} = 84.30$ $\mu\text{g/ml}$) and *Mythimna separata* Walker (Lepidoptera: Noctuidae) ($\text{LC}_{50} = 548.74$ $\mu\text{g/ml}$). Ye et al. (2015) reported that a concentration of 0.324 mM of magnolol killed 50% of an *N. lugens* population. Ngegba et al. (2022) mentioned that anonaine is a bioactive ingredient in *Anona squamosa* L. (Annonaceae), which shows feeding deterrent activity against pests. Moreover, Bezerra et al. (2022) found that anonaine isolated from *Anona crassiflora* (Martius) is a potential candidate for the control of *Rhipicephalus (Boophilus) microplus* Canestrini (Acari: Ixodidae) through the inhibition of the tick's enzyme glutathione S-transferase.

Our study presented here showed that the mortality of *T. merganser* females increases as the concentration of *M. alejandrae* extract increases. The corrected mortality rate of the mites ranged between 0.00 (0.1%) and 33.33% (15%), as compared to the control group. Likewise, the oviposition, growth, and feeding rates decreased as extract concentration increased. The literature does not refer to other studies on different extracts of *Magnolia* spp. causing death to *Tetranychus* spp. However, other studies have reported the mortality of insects caused by different polarities of solvents of *Magnolia* spp. (Nitao et al. 1992, Vásquez-Morales et al. 2015, Ali et al. 2020, Luu-Dam et al. 2021, Vásquez-Morales et al. 2022). Vásquez-Morales et al. (2015) found that the proportion of 1:5 w v⁻¹ of the

ethanolic extract of mature leaves, young leaves, flowers, and bark of *M. schiedeana* caused mortality in *Anastrepha ludens* Loew (Diptera: Tephritidae) (30.8%, 31.2%, 0.08%, and 0.54%, respectively) to the fifth day. Luu-Dam et al. (2021) reported that the concentrations (1.0, 0.5, 0.25, and 0.1 $\mu\text{g}/\mu\text{L}$) of *M. citrata* leaf essential oil caused *Aedes aegypti* L. (Diptera: Culicidae) mortality (100, 40, 20, and 0%, respectively). Nitao et al. (1992) documented that aqueous extract of *Magnolia virginiana* killed 92.00% of first-instar *Papilio palamedes* Drury (Lepidoptera: Papilionidae). Ali et al. (2020) found that 125 ppm of *Magnolia grandiflora* essential oil of immature and mature fruit caused 100% mortality in *A. aegypti* larvae. In contrast, leaf, flower, and seed essential oils gave only 20%, 0%, and 50% mortality, respectively. On the other hand, Vásquez-Morales et al. (2022) documented that *Magnolia perezfarrerae*, *Magnolia pugana*, and *Magnolia vovidesii* crude extracts of sarcotesta increased the mortality of *A. ludens* (14.32 to 95.69%, 44.12 to 93.73%, and 0.00%, respectively) and *A. obliqua* Macquart (Diptera: Tephritidae) adults (0.00 to 66.09%, 36.10 to 91.74%, and 30.53 to 92.26%, respectively) on the fifth day at concentrations ranging between 0.002 mg/g and 0.2 mg/g.

We further determined in this research that the oviposition of *T. merganser* females was affected by the different concentrations of the ethanolic extract of *M. alejandrae*. The number of eggs laid per female decreased as the extract concentration increased. Our results are similar to those reported by Chacón-Hernández et al. (2020) who reported oviposition inhibition rates of *Tetranychus urticae* Koch (Acarina: Tetranychidae) at concentrations of 5, 10, 50, 100, 250, 500, and 1,000 $\mu\text{g}/\text{mL}$ of *M. tamaulipana* ethanolic powdered extract. The oviposition inhibition rate increased at 24 h (18.18% to 95.56%), 48 h (7.69% to 95.83%), and 72 h (11.74% to 95.39%), as compared to the control. Reyes-Zepeda et al. (2022) documented that *M. tamaulipana* leaf ethanolic powdered extract reduces the egg-laying activity (4.97 to 0.80 eggs/female) of *Oligonychus punicae* in all the concentrations (5 to 1,000 $\mu\text{g}/\text{ml}$) at 72 h, as compared to the control (6.87 eggs/female). Hussein et al. (2023) reported that at a 10 mg/L concentration, the *M. grandiflora* leaf water extract reduced the egg-laying capacity of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (196.5 ± 5.3 eggs/female), as compared to the control (216.2 ± 5.2 eggs/female).

The antioviposition of *T. merganser* could be due to the presence of different phenolic compounds in the *M. alejandrae* leaf ethanolic extract. Several studies have reported that phenolic compounds present in plants decrease the number of eggs laid per female of different pest arthropods, e.g., the twospotted spider mite, *T. urticae* (Larzon and Berry 1984); carmine spider mite, *Tetranychus cinnabarinus* Boisduval (Acarina: Tetranychidae) (Chen and Dai 2015); *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) (Sharma and Sohal 2016), and the melon fly, *Zeugodacus cucurbitae* Coquillett (Diptera: Tephritidae) (Puri et al. 2022). Although more research is required, the antioviposition effect caused by the ethanolic extract of the *M. alejandrae* leaf may be the result of the secondary metabolites present in the extract that altered the production of pheromones or directly affected the female ovaries (Dimetry et al. 1990, 2003), which can cause partial or temporary sterilization of the females, and, thus, reduce the number of eggs per female compared to the control group (Hosny 2010).

In this research, the damage percentage of *T. merganser* decreased as the concentration of *M. alejandrae* extract increased, indicating that the extract inhibited the feeding of *T. merganser*. A similar result was obtained by Chacón-Hernández et al. (2020), who found that different concentrations (5 to 1,000 µg/ml) of *M. tamaulipana* ethanolic powdered extract have antifeedant effects. They reported a feeding inhibition rate increase between 29.11 and 69.89% compared to the control treatment. Reyes-Zepeda et al. (2022) found that the concentrations (5, 10, 50, 100, 250, 500, and 1,000 µg/ml) of *M. tamaulipana* ethanolic powdered extract inhibited feeding of *O. punicae* females (16, 18, 19, 31, 40, 54, and 67%, respectively), as compared to the control at 72 h. The inhibition of the food intake of *T. merganser* can be due to the presence of secondary metabolites, such as terpenes and alkaloids, in the *M. alejandrae* leaf ethanolic extract. In this regard, Koul (2016) and Singh et al. (2021) mentioned that secondary metabolites such as alkaloids, coumarins, terpenes, and phenols have antifeeding effects, but the most potent antifeedants belong to the terpenoid group (Koul 2016). Although more research is required, the antifeeding effect of *T. merganser* females may be because some secondary metabolites, such as phenols, terpenes, and alkaloids present in the *M. alejandrae* extract, entered into contact with its gustatory and olfactory sensory systems so that its palatability was altered, reducing its food intake.

In conclusion, our results show ethanolic extract of *M. alejandrae* leaves can be promising in *T. merganser* control. The extract caused mortality, antifeeding, and antioviposition in females, leading to lower population growth rates. Further research is necessary, including the assessment of different polarities of solvents of *M. alejandrae* against *T. merganser* and other species of pest mites, as well as studying its effects on the biology and physiology of natural enemies and the residual of the extract under greenhouse and field conditions.

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