Ovicidal Effect of the Ethanolic Extract of *Moringa oleifera* Leaf on *Oligonychus punicae* (Trombidiformes: Tetranychidae) Eggs[1]

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Abstract Mexico is the leader in avocado (*Persea americana* Miller [Lauraceae]) production, with the United States being the principal consumer of avocados produced there. The avocado crop is susceptible to attacks by insect and mite pests, including the avocado brown mite, *Oligonychus punicae* (Hirst) (Acari: Tetranychidae), which is a serious economic pest of the crop in Mexico. This study evaluated the effects of different concentrations (0.1, 0.5, 1, 5, 10, 15, and 20% [v/v]) of the ethanolic extract from *Moringa oleifera* Lamarck (Moringaceae) leaves against *O. punicae* eggs and its residual effect on the density of larvae and nymphs emerging from treated eggs. Egg mortality ranged from 0.0 at the 0.1% concentration to 70.48% at the 20% concentration, compared with the control group. Egg mortality increased as extract concentrations increased. The density of larvae and nymphs that emerged from the treated eggs was significantly lower than the density in the control, indicating that both the number of living larvae and nymphs responded to extract concentration. Our results support the suggestion that the ethanolic extract of *M. oleifera* leaf has ovicidal and residual activity against *O. punicae* and can be incorporated into an integrated mite management program to control avocado brown mite populations. Further studies are needed to assess the efficiency and efficacy of *M. oleifera* extract against arthropod pests and natural enemies under greenhouse and field conditions.

Key Words biological control, survival, avocado brown mite, residual, acaricidal activity

Worldwide, Mexico is the main producer of avocado (*Persea americana* Miller [Lauraceae]), with the production of the avocado representing an annual economic benefit of approximately 50,538 million Mexican pesos. In 2021, exports of avocado were estimated at a value of US$3,546 million. The United States is the largest consumer of Mexican-produced avocados, importing 79.0% of the production in Mexico (SAGARPA-SIAP 2022).

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The Mexican avocado crop has at least 30 key pests that impact plant growth and yield (Peña and Wysoki 2008, Peña et al. 2013). In Mexico, the avocado brown mite, *Oligonychus punicae* (Hirst) (Acari: Tetranychidae), is one of the most economically important pests of avocado (Peña et al. 2013). It feeds on 37 plant species and is found in 22 countries, including Mexico and the United States (Migeon and Dorkeld 2023). *Oligonychus punicae* feeds on the upper leaf surface, and when its populations are high, it can be found on the abaxial leaf surface, causing leaf bronzing due to sap removal (McMurtry and Johnson 1996, Peña and Wysoki 2008, Peña et al. 2013). Densities of 300 mites (larvae, nymphs, and adults) per leaf or 70 adult females per leaf cause partial defoliation of the avocado tree (Peña and Wysoki 2008). In addition, the feeding damage of this mite reduces the chlorophyll content of the leaf that subsequently reduces the photosynthesis rate by >50%, thereby affecting avocado production by up to 20% (Castañeda-Cabrera et al. 2022, Maoz et al. 2011).

Because tetranychid mites have a short life cycle and high fecundity, the excessive use of chemical pesticides to keep their populations below economic thresholds creates ideal conditions for these mites to develop resistance to those chemicals (Dekeyser 2005, Knowles 1997, Van Leeuwen et al. 2015). In addition, the residues of chemical insecticides and acaricides can cause harmful effects on human health and have a high environmental impact. For these reasons, there is an urgent need to develop safe and ecological techniques to manage the mite pests. Therefore, botanical extracts with pesticidal activity have emerged as an ecological alternative for the management of pest arthropods, which has increased their recommendation as a strategy for the control of tetranychid mites, because plant-derived acaricides can control mites at low cost and with low risk to humans and the environment (Sivira et al. 2011).

The moringa tree (*Moringa oleifera* Lamarck [Moringaceae]) is native to India and is a most useful trees due to its medicinal and nutritional properties worldwide (Devkota et al. 2020, Kou et al. 2018). Secondary metabolites such as flavonoids or isothiocyanates, from different parts of the moringa tree, such as seeds, stem bark, leaves, and root bark, have beneficial biological functions, including anti-inflammatory, antioxidant, anticancer, hepatoprotective, neuroprotective, hypoglycemic, and blood lipid–reducing functions (Kou et al. 2018). Ethanolic extracts of the *M. oleifera* leaves can exert antifeeding, antioviposition, residual, and acaricidal potential against *Tetranychus merganser* Boudreaux and *O. punicae* adults (Heinz-Castro et al. 2021a,b) as well as reduce the hatching of *T. merganser* eggs and cause a residual effect on immature stages (larva and nymph) of *T. merganser* from eggs treated with the extract (Chacón-Hernández and Heinz-Castro 2023). However, until now, the ovicidal and residual effect of *M. oleifera* extracts on *O. punicae* eggs has not been reported.

Conversely, Moawad and Sadek (2018) found that *M. oleifera* oils affect the viability of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) eggs. Santos et al. (2012) reported that the water-soluble *M. oleifera* lectin (WSMoL) reduces hatching of *Aedes aegypti* (L.) (Diptera: Culicidae) eggs. Similarly, Coelho et al. (2009) and Agra-Neto et al. (2014) reported that WSMoL caused death of exposed *A. aegypti* larvae. The study reported herein aimed to evaluate the effects of the ethanolic extract of *M. oleifera* leaves at different concentrations on *O. punicae*

eggs and its postapplication effect (residuality) on the density of immature mites (larva and nymph) from the treated eggs.

Materials and Methods

Mite colony. A colony of *O. punicae* was established with adult females and males obtained from a guamuchil tree, *Pithecellobium dulce* (Roxburgh) Bentham (Fabaceae) located in Ciudad Victoria (23°44′38.4′′N, 99°9′57.599′′W, 329 m above sea level), Tamaulipas, Mexico. Mites were reared on bean, *Phaseolus vulgaris* L. (Fabaceae), plants under greenhouse conditions of 30 ± 2°C and 70 ± 10% relative humidity (RH).

Preparation of plant material and extract. Leaves were collected from 15 2-yr-old moringa trees grown under field conditions (23°46′08.4720′′N, 99° 9′42.7680′′W, 308 m above sea level) to produce the extract. Visibly clean mature leaves of 10 moringa trees were collected and dried in a conventional oven (model 20GCE-LT, Quincy Lab, Chicago, IL) at 60°C for 3 d until a consistent weight was obtained. The sample was ground (model DBM-8 grinder, Cuisinart, Stamford, CT) to yield 1-mm particles (Castillo et al. 2010) that were stored in dark bottles at room temperature (27 ± 2°C) in preparation for extraction.

A sample of 14 g of dry powder from *M. oleifera* leaves was mixed with 200 ml of absolute ethanol at room temperature for 3 d with a magnetic stirrer in total darkness. Immediately, we filtered the mixture with using Whatman No. 1 filter paper. A rotary evaporator (IKA-RV 10 digital V, Staufen Baden, Württemberg, Germany) was used to remove the solvent under reduced pressure at temperatures <40°C. Finally, the flask was placed in the drying oven for 3 d to eliminate the remaining ethanol and obtain a consistent weight (Shami et al. 2013). The extract was scraped and stored in Eppendorf tubes and kept in a freezer at −10°C before performing the bioassays.

Phytochemical analysis of the extract. We quantified the total phenol concentration (TPC) with the Folin–Ciocalteu reagent according to Singleton et al. (1999). A 250-μl volume of each sample or standard was mixed with 125 μl of 1 N Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO) and incubated for 5 min. Next, we added 625 μl of 20% Na_2CO_3 (CTR, Monterrey, Nuevo León, Mexico) to each sample and the mixtures were incubated in the dark for 2 h. We recorded the absorbance of each reaction at 750 nm (UV-6000 spectrophotometer, Metash Instruments Co., Ltd., Shanghai, China). The concentration of the samples was determined according to a standard curve prepared with a 0.1-mg/ml solution of gallic acid (Sigma-Aldrich) in a range of 1–8 μg/ml. The concentrations were expressed in milligram equivalents of gallic acid per gram of dry weight.

We calculated the total flavonoid concentration (TFC) according to the methods of Chang et al. (2002). We mixed 1 ml of the sample, 1.5 ml of 95% ethanol (CTR), 0.1 ml of 10% aluminum chloride (Sigma-Aldrich), 0.1 ml of 1 M potassium acetate (Sigma-Aldrich), and 2.8 ml of sterilized distilled water. The mixtures were incubated for 40 min at room temperature. The absorbance obtained in each reaction was recorded at 415 nm and used for determination according to a standard curve prepared with quercetin as a standard (Sigma-Aldrich), at

concentrations of 10–100 μg/ml. The concentrations were expressed in milligrams of quercetin equivalents per gram of dry weight.

We determined the ferric ion reduction capacity of the samples by using a method adapted from Benzie and Strain (1996). An aliquot (10 μl) of the extract was added to 200 μl of ferric-reducing antioxidant power reagent (10 parts of 300 mM sodium acetate buffer, pH 3.6; 1 part of 10 mM TPTZ solution; and 1 part of 20 mM solution $FeCl_3 \bullet_6 H_2O$) and incubated the reaction mixture at 37°C. We measured the increase in absorbance at 595 nm after 60 min. The antioxidant capacity of the extract was expressed in equivalents of Trolox mM per gram of dry weight. We generated the standard curve with Trolox in the 100–1,200-μM range. We conducted the detections in triplicate.

Ovicidal effect. The experiment was conducted in a climatic chamber at 27 ± 1°C and 70–80% RH under a 16:8 (L:D) h photoperiod. To assess the ovicidal properties of the extract, we used Ahmadi's sand technique (Ahmadi 1983), with slight modifications that consisted of avocado leaf discs being 2.5 cm in diameter with the abaxial surface of the leaf facing up, placed on water-soaked cotton, and placed inside a 5-cm-diameter Petri dish with 20 *O. punicae* eggs per dish. We randomly divided bean leaf discs into eight groups: seven treated groups (e.g., one for each extract concentration) plus one control group. An avocado leaf disk with 20 eggs was the replicate with six replicates per group (120 eggs per treatment). In total, there were 48 replicates (960 eggs in total).

We placed 20 adult females and 10 adult males of *O. punicae* on each leaf disk to improve the probability of female reproduction and oviposition. After 24 h, males, females, and eggs were removed using a fine needle, leaving only 20 eggs on each disc. Twenty *O. punicae* eggs per disc were sprayed twice (0.7 ± 0.1 ml per spray) with each concentration, from a distance of 30 cm between the spray and the leaf discs. Manual sprayers, one for each group (model 14687, Truper®, Ciudad de Victoria, Mexico), were used to apply the concentrations of the extract. The spread amount of the extract was determined with the aid of a 2-ml sterile storage tube. It was sprayed 10 times into the storage tube to calculate the mean and SD of the amount sprayed.

Ethanol/water concentrations of 0.1, 0.5, 1, 5, 10, 15, and 20% (v/v) were prepared from an initial solution of 1,000-ppm powdered *M. oleifera* extract in ethanol. Immediately after preparation, we used a manual sprayer to apply the different concentrations of the extract. Control treatments were sprayed with water only. The numbers of eggs that were not hatched on the sixth day after treatment were recorded using a dissecting microscope (stereo and zoom microscopes ZM180, UNICO, Dayton, NJ). We based our decision to record unhatched eggs on the sixth day after treatment on the findings of Vásquez et al. (2008) and Ferraz et al. (2020). Vásquez et al. (2008) determined that the average hatching time of an *O. punicae* eggs on leaf discs of different grapevine cultivars (*Vitis vinifera* L. [Vitaceae]) ranges from 4.3 to 4.7 d at 27 ± 2°C, 80 ± 10% RH, and a 12:12 (L:D) h photoperiod. On leaf discs of *Eucalyptus pellita* F. Mueller, *E. tereticornis* Smith., *E. brassiana* S.T. Blake, *E. grandis* W. Hill Ex Maiden, and *Corymbia citriodora* L.A.S. Johnson (Myrtaceae), hatching time ranged from 5.03 to 5.27 d at 25 ± 2°C, 70 ± 10% RH, and a 12:12 (L:D) h photoperiod (Ferraz et al. 2020). Eggs that did not

hatch within those times were considered nonviable. We corrected the mortality data (unhatched eggs) using Abbott's formula (Abbott 1925).

Residual effect. We recorded the number of larvae and nymphs that did not progress to their next stage of development (nymph and adult, respectively). The developmental times of the larvae and nymphs (protochrysalis + protonymph + deutochrysalis + deutonymph + teliochrysalis) are 1.04 d for larvae and 4.04 d for nymphs when feeding at 22 − 26°C on avocado (*Persea indica* Sprengel) leaves (Tanigoshi and McMurtry 1977). Ferraz et al. (2020) documented that the developmental period of larvae and nymphs ranged from 1.24 to 1.94 d for larvae and from 4.61 to 5.03 d for nymphs when fed on leaves of six eucalyptus species at 25 ± 2°C, 70 ± 10% RH, and a 12"12 (L:D) h photoperiod. When *O. punicae* fed on the leaves of six grapevine cultivars, the development time was 2.82 d for larvae to 3.40 d for nymphs (Vásquez et al. 2008).

When assessing larval and nymphal mortality, we recorded the immature mite as dead when it exhibited disordered movement or was lying on its back or immobile. We used the Abbott's formula (Abbott 1925) to measure the effect residual of the extract on the larvae and nymphs with respect to the control group.

Statistical analysis. Data were analyzed using one-way analysis of variance or its homologous form, the Kruskal–Wallis test. Before analyzing the data, we checked the adjustment of residuals to the normal distribution and variance homogeneity according to the Shapiro–Wilk test ($P > 0.05$) and Bartlett test ($P > 0.05$), respectively. Significant differences were analyzed with Tukey's or Nemenyi's multiple comparison tests ($P < 0.05$) (Pohlert 2021, Wheater and Cook 2005). Probit analysis was used to estimate the lethal concentrations [$LC_{50(90)}$], which caused 50(90)% mortality of *O. punicae* eggs compared with the control on the sixth day after extract application, with 95% fiducial confidence limits (CL_{95}) for $LC_{50(90)}$ (Finney 1971). All data were analyzed using the R-project version 4.3.1 program (R Core Team 2023).

Results

Phytochemical analysis. The ethanolic extract of *M. oleifera* leaves showed a Total Phenol Content (TPC) of 885.56 ± 16.03 mg GAE/ml, Total Flavonoid Content (TFC) of 54.99 ± 0.86 mg Quantitative Estimation (QE)/ml, and the antioxidant activity (AC) of 4.07 ± 0.02 mM ET/ml.

Ovicidal effect. The ethanolic extract showed ovicidal activity at different concentrations against *O. punicae* eggs. Six days after applying the extract, there were significant effects on the number of unhatched eggs ($F = 139.26$; df = 6, 35; $P = 2.2 \times 10^{-16}$). The highest egg mortality rate (70.48%) was observed in the extract's 20% (v/v) concentration and the lowest (0%) was at a 0.1% (v/v) extract concentration. Egg mortality increased as concentrations increased (Fig. 1). The LC_{50} and LC_{90} values calculated for the ethanolic extract of *M. oleifera* leaf were 13.41% (fiducial confidence limits: $CL_{95} = 10.78–16.04$) for the LC_{50} and 60.92% (fiducial confidence limits: $CL_{95} = 19.71–30.57$) for the LC_{90} for *O. punicae* eggs 6 d after application of the extract. The slope (±SE) value of the regression line was 0.1093 (±0.0166) ($Z = 6.564$; $P = 5.24 \times 10^{-11}$).

Residual acaricidal effect. The number of *O. punicae* larvae that were dead upon emergence from the eggs treated with the *M. oleifera* extract differed

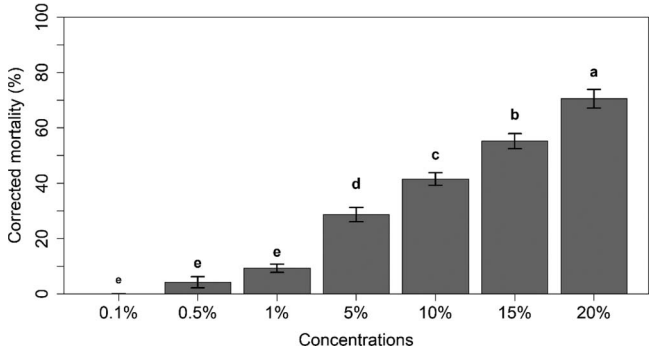

Fig. 1. Mean (±SE) percentage mortality of *Oligonychus punicae* eggs in response to different concentrations of the ethanolic extract of *Moringa oleifera* leaf under controlled laboratory conditions ($n = 6$, 20 eggs per repetition). Concentrations with different letters are significantly different (Tukey's test, $P < 0.05$).

significantly from the control group ($\chi^2 = 38.744$; df = 6; $P = 8.035 \times 10^{-07}$). Larval mortality ranged from 10.265 at the 0.1% concentration to 81.23% at the 20% concentration (Fig. 2).

Mortality of *O. punicae* nymphs after emergence from eggs treated with *M. oleifera* extract differed significantly from those of the control group ($\chi^2 = 37.246$; df = 6; $P = 1.577 \times 10^{-5}$). Percentage mortality ranged from 7.68% at the 0.1% concentration to 100% at the 20% concentration (Fig. 3).

The residual effects of the leaf extract also was seen in the response of the density of adult mites that eventually developed from the treated eggs of *O. punicae*. Abundance of *O. punicae* adults differed significantly among the treatments ($F = 245.626$; df = 7, 40; $P < 2 \times 10^{-16}$). The mean (±SE) percentage of live adult mites was highest in the control group (89.17 ± 2.01), whereas the

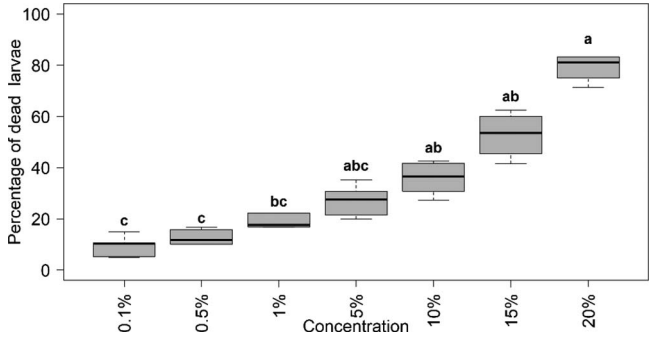

Fig. 2. Box-plot comparing the residual effect of *Moringa oleifera* leaves ethanolic extract on larvae that hatched from treated eggs of *Oligonychus punicae* compared with the control group. Concentrations with different letters are significantly different (Nemenyi's test, $P < 0.05$).

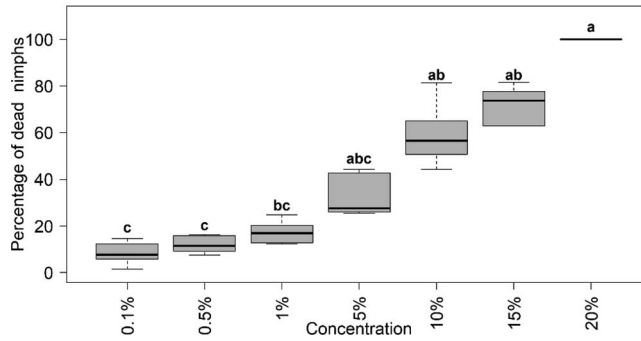

Fig. 3. Box-plot comparing the residual effect of *Moringa oleifera* leaves ethanolic extract on *Oligonychus punicae* nymphs. Concentrations with different letters are significantly different (Nemenyi's test, $P < 0.05$).

percentage of live mites decreased from 74.17 ± 2.71% at the 0.1% concentration to 0.83 ± 0.83% at the 20% concentration (Fig. 4).

Discussion

The ethanol extract of *M. oleifera* leaves showed a total flavonoid concentration of 54.99 mg QE/ml, total concentration of phenols of 885.56 mg GAE/ml, and an antioxidant capacity of 4.07 mM ET/ml. Based on reports from the literature, leaves of *M. oleifera* contain several secondary metabolites that are grouped as alkaloids, polyphenols, phenolic acids, flavonoids, tannins, saponins, vitamins, carotenoids, glucosinolates, isothiocyanates, and oxalates and phytates (Leone et al. 2015). Tlak Gajger and Dar (2021) reported that the bioactive compounds, such as flavonoids, alkaloids, phenols, and cyanogenic glycosides, possess pesticidal activity and are the most promising compounds for development in arthropod

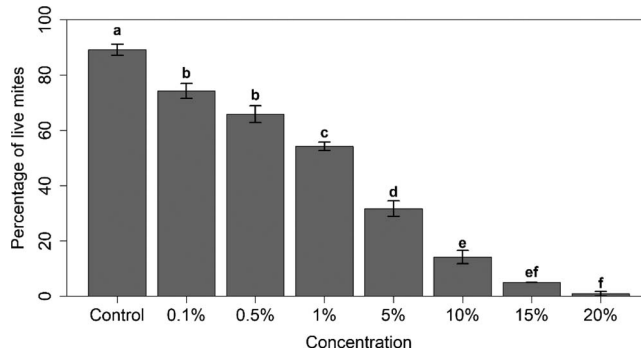

Fig. 4. Survival of *Oligonychus punicae* adults emerging from eggs treated with *Moringa oleifera* leaves ethanolic extract ($n = 6$, 20 mite eggs per replicate). Concentrations with different letters are significantly different (Tukey's test, $P < 0.05$).

pest management strategies. Freeman and Beattie (2008) also noted that phenolic compounds are part of the secondary metabolites involved in plant defense against pathogens. Leone et al. (2015), in a review of the scientific literature on the secondary metabolites of *M. oleifera*, found that the total concentration of flavonoids in dried leaves varies from 5.059 to 12.16 mg/g of dry weight, whereas in freeze-dried leaves it ranges from 21.0 to 61.62 mg Rutin Equivalents (RE)/g of dry weight. The total concentration of phenols in dry leaves ranges between 2,090 and 12,200 mg GAE/100 g of dry weight (or between 1,600 and 3,400 mg Tannic Acid Equivalents (TAE)/100 g of dry weight). Vitamin C in the dry leaves of *M. oleifera* is lower than in the fresh leaves, decreasing from 18.7 to 140 mg/100 g of dry weight. Shanmugavel et al. (2018) reported 627 $\pm$ 12.26 mg GAE/100 g for TPC, 2.18 $\pm$ 0.89 mg Acetic Acid Equivalents (AAE)/g for vitamin C, and 22.16 $\pm$ 1.54 mg QE/g for TFC. Guzmán-Maldonado and Díaz-Fuentes (2017) reported a higher TPC from the leaves of *M. oleifera*, from 2,436.3 to 3,749.39 mg GAE/100 g. Cabrera-Carrión et al. (2017) reported total concentrations of phenols and flavonoids between 7.36 and 19.27 and 11.83 and 34.85 mg/g, respectively. These authors demonstrated that the concentrations of these secondary metabolites vary significantly depending on the age of the plant, the height of the plant, and the height of location of the leaf on the plant. These results were lower than those reported in this study. The high variations of the secondary metabolites can be due to the different environmental conditions, harvest times, plant genetics, drying methods, leaf maturities, and extraction methods or to the different sensitivity of the analytical methods (Leone et al. 2015).

In terms of using the leaf extract in pest management strategies, one approach in keeping *O. punicae* populations below economic thresholds is by reducing the hatch rate of the mite eggs. Our results demonstrate that egg mortality increases relative to higher concentrations of *M. oleifera* extract. Similar results were reported by Santos et al. (2012), Moawad and Sadek (2018), and Chacón-Hernández et al. (2023). Santos et al. (2012) documented that concentrations of 0.32 and 1.18 mg/ml of the *M. oleifera* seed extract killed 50 and 99%, respectively, of the *A. aegypti* eggs by 72 h after exposure. They also reported that concentrations of 0.10 and 0.30 mg/ml of water-soluble *M. oleifera* lectin caused 50 and 99% mortality, respectively, of *A. aegypti* eggs at 72 h. Moawad and Sadek (2018) found that the concentrations of 5–10% of *M. oleifera* oil caused 50.07–88.90% mortality of *S. littoralis* eggs. By contrast, Chacón-Hernández and Heinz-Castro (2023) reported an increase in mortality (3.11–72.58%) of *T. merganser* eggs in response to increasing concentrations (0.1–20%) of the ethanolic extract of *M. oleifera* leaves in 5 d. Similarly, the ovicidal effects of other plant extracts on *Oligonychus* spp. have been studied and these results are similar to the our results. Deka et al. (2022) reported an increase in mortality of *Oligonychus coffeae* Nietner (Acari: Tetranychidae) eggs with increasing concentrations (2–10 g/L) of an aqueous extract of *Murraya paniculata* (L.) Jack (Sapindales: Rutaceae), *Cassia tora* L. (Fabales: Fabaceae), *Amphineuron opulentum* (Kaulfuss) Holt (Polypodiales: Thelypteridaceae), *Tithonia diversifolia* (Hemsey) A. Gray (Asterales: Asteraceae), and *Cassia alata* L. (Fabales: Fabaceae) leaves at 12 d after treatment. Sarmah et al. (2009) documented that the concentrations (2.5–10.0%) of an aqueous extract of *Xanthium strumarium* L. (Compositae), *Acorus calamus* L. (Araceae), *Polygonum hydropiper* L. (Polygonaceae), and *Clerodendron infortunatum* L.

(Verbenaceae) caused egg mortality of *O. coffeae* in 12 d. Fetoh and Al-Shammery (2011) found that the ethanolic extracts of Duranta leaves (*Duranta plumeria* L. [Verbenaceae]), cumin seeds (*Cuminum cyminum* L. [Labiaceae]), and the entire Demsisa plant (*Ambrosia maritima* L. [Compositae]) caused 70.67, 30.67, and 87.33% mortality, respectively, of *Oligonychus afrasiaticus* McGregor (Acari: Tetranychidae) eggs at 72 h. Handique et al. (2017) reported that, at a concentration of 1.03, 4.75, and 5.47%, the aqueous extracts of *Phlogacanthus thyrsiformis* Nees (Acanthaceae), *Nyctanthes arbor-tristis* L. (Oleaceae), and *Sapindus mukorossi* L. (Sapindaceae) reduced the viability of *O. coffeae* eggs to 50%. Roy and Mukhopadhyay (2012) found that the aqueous seed extract of *Melia azedarach* L. (Meliaceae) at different concentrations (1–10%) reduced the egg hatchability of *O. coffeae* in a range of 1.70–64.41% in 12 d. By contrast, Carvalho et al. (2008) found that the extracts of leaves of *Ginkgo biloba* L. (Ginkgoaceae), *Nepeta cataria* L. (Lamiaceae), *Coffea arabica* L. (Rubiaceae), *Calendula officinalis* L. (Asteraceae), *Anonna squamosa* L. (Annonaceae), and *Recinus communis* L. (Euphorbiaceae) did not exert any ovicidal activity against *Oligonychus ilicis* McGregor (Acari: Tetranychidae) at 10 d.

Gonçalves et al. (2001) mentioned that the death of the embryos in treated eggs is due to the penetration and toxicity of the secondary metabolites into the eggs. Tetranychid eggs have a unique respiration mechanism through embryonic stigmas connected both to the chorion and to a highly specialized region of the intermediate membrane that is enervated with perforations; this probably provides sites of gas exchange with the environment (Dittrich and Streibert 1963). These perforations are a route of entry for secondary metabolites, making the egg more susceptible to plant bioactive compounds, consistent with the high ovicidal activity of *M. oleifera* extract in eggs of *O. punicae*. Derbalah et al. (2013) postulated that plant bioactive compounds affect embryo formation within the egg, causing its death, and Fetoh and Al-Shammery (2011) mentioned that plant secondary metabolites cause mortality in mite eggs because these compounds block the micropyle region of the egg, thereby impairing gas exchange. In addition, secondary metabolites such as the phenolic compounds are strongly related to spider mites egg mortality (Tomczyk and Suszko 2011). Although more research is required, the presence of secondary metabolites in the *M. oleifera* extract, such as phenolic compounds, causes us to conclude that the *M. oleifera* leaf extract causes toxic effects inside the eggs of *O. punicae*.

We also observed a pronounced residual effect of the leaf extract after its application targeting *O. punicae* eggs on leaf surfaces. *Oligonychus punicae* larvae and nymphs emerging from treated eggs that were not killed by the extract often succumbed after exposure to the extract, with significantly higher mortality on treated leaves than on untreated controls. Chacón-Hernández and Heinz-Castro (2023) also reported residual activity by concentrations of ethanolic extracts of *M. oleifera* leaves when targeting *T. merganser* eggs. The ethanolic extract from *Tagetes patula* L. (Asteraceae) leaves targeting the eggs of *T. urticae* caused larval mortality reaching 100% by 6 d after application (Ismail et al. 2019). Moreover, they found that all larvae died before reaching the nymphal stage at the seventh, eighth, and ninth days after the application. Laborda et al. (2013) found that concentrations of 0.15 and 0.25% of *Rosmarinus officinalis* L. and *Salvia officinalis* L.

(Lamiaceae) essential oil had a residual effect on larvae that emerged from eggs oviposited by treated *T. urticae* females, with a documented decrease in the mean number of larvae per female compared with the control group.

The mortality of hatched larvae from mite eggs treated with botanical extracts can be attributed to direct contact of newly emerged larvae with the extract and toxic effects on egg development (Ismail et al. 2019). In addition, secondary metabolites can damage both the egg membrane before hatching and the embryo already formed within 1 d of age and therefore cause a sublethal effect that can be observed in larvae or nymphs from treated spider mite eggs (Derbalah et al. 2013). Bioactive plant compounds can act alone or in synergy to cause mortality of mite eggs and the larvae and nymphs hatching from treated eggs. A plant species with the potential to control insect and mite pest populations must synthesize a small amount of highly toxic secondary metabolites or a wide range of slightly toxic bioactive compounds (Rattan 2010). Understanding the mode of action, including the physical, biological, and chemical interactions between the pest and the botanical pesticide, is vital in pest management because it dictates the management strategy to be adopted (Lengaia et al. 2020).

In conclusion, this research demonstrates that the ethanolic extracts of *M. oleifera* leaves have potential as an ovicidal agent against *O. punicae* eggs. Moreover, the number of larvae and nymphs from treated eggs was reduced compared with that of the control group. Further detailed research needs to assess the efficacy and effects of *M. oleifera* extract on nontarget organisms, biological control agents, and yield without toxic consequences for crops and consumers.

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References Cited

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Agra-Neto, A.C., T.H., Napoleão, E.V. Pontual, N.D. Santos, L. de Andrade-Luz, C.M. de Oliveira, M.A. de Melo-Santos, L.C. Coelho, D.M. Navarro and P.M. Paiva. 2014. Effect of *Moringa oleifera* lectins on survival and enzyme activities of *Aedes aegypti* larvae susceptible and resistant to organophosphate. Parasitol. Res. 113: 175–184.

Ahmadi, A. 1983. Demographic toxicology as a method for studying the dicofol two spotted spider mite (Acari: Tetranychidae) system. J. Econ. Entomol. 76: 239–242.

Benzie, I.F.F. and J.J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. Anal. Biochem. 239: 70–76.

Cabrera-Carrión, J.L., C. Jaramillo-Jaramillo, F. Dután-Torres, J. Cun-Carrión, P.A. García and L. Rojas-de-Astudillo. 2017. Variación del contenido de alcaloides, fenoles, flavonoides y taninos en *Moringa oleifera* Lam. en función de su edad y altura. Bioagro 29: 53–60.

Carvalho, T.M.B., P.R. Reis, D.F. Oliveira, G.A. Carvalho and D.A. Carvalho. 2008. Evaluation of plant extracts in the control of *Oligonychus ilicis* (McGregor, 1917) (Acari: Tetranychidae) on coffee tree. Coffee Sci. 3: 94–103.

Castañeda-Cabrera, C., C. Perales-Segovia, H. Silos-Espino, I.G. Lopez-Muraira, M. Miranda-Salcedo and A. M. Garcia-Munguia. 2022. Evaluación de extractos etanólicos de plantas silvestres sobre *Oligonychus punicae* Hirst y *Oligonychus perseae* Tuttle,

Baker y Abbatiello, 1976 (Trombidiformes: Tetranychidae), en el cultivo de aguacate en México. J. Agric. Sci. Res. 2: 1–11.

Castillo, F., G. Gallegos, M. Mendez, R. Rodríguez, A. Reyes and C. Aguilar. 2010. In vitro antifungal activity of plant extracts obtained with alternative organic solvents against *Rhizoctonia solani* Kühn. Ind. Crop Prod. 32: 324–328.

Chacón-Hernández, J.C. and R.T.Q. Heinz-Castro. 2023. Ovicidal and residual effects of *Moringa oleifera* leaf ethanol extract on *Tetranychus merganser* (Trombidiformes: Tetranychidae). J. Plant Dis. Prot. 130: 519–528.

Chang, C.-C., M.-H. Yang, H.-M. Wen and J.-C. Chern. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 10: 176–182.

Coelho, J. S., N.D.L. Santos, T.H. Napoleão, F.S. Gomes, R.S. Ferreira, R.B. Zingali, L.C.B.B. Coelho and S.P. Leite, Navarro, D.M.A.F.; Paiva, P.M.G.2009. Effect of *Moringa oleifera* lectin on development and mortality of *Aedes aegypti* larvae. Chemosphere 77: 934–938.

Deka, B., A. Babu, C. Baruah and S. Sarkar. 2022. Plant extracts as potential acaricides for the management of red spider mite, *Oligonychus coffeae* Nietner (Acarina: Tetranychidae), in the tea ecosystem: An eco-friendly strategy. Front. Agron. 4: 685568.

Dekeyser, M.A. 2005. Acaricide mode of action. Pest Manag. Sci. 61: 103–110.

Derbalah, A.S., A.Y. Keratrum, M.E. El-Dewy and E.H. El-Shamy. 2013. Efficacy of some insecticides and plant extracts against *Tetranychus urticae* under laboratory conditions. Egypt. J. Plant Prot. Res. 1: 47–70.

Devkota, S., K.K. Bhusa and F. Yildiz. 2020. *Moringa oleifera*: A miracle multipurpose tree for agroforestry and climate change mitigation from the Himalayas. A review. Cogent Food Agric. 6: 1805951.

Dittrich, V. and P. Streibert. 1963. The respiratory mechanism of spider mite eggs. Zeitschrift. Angew. Entomol. 63: 200–211.

Ferraz, L.C., P.B. Ramalho-Silva, M. Pinheiro-Amaranes, J.W. Da Silva-Melo, D. Barbosa-De-Lima and S.M. De França. 2020. Biology and fertility life table of *Oligonychus punicae* Hirst (Acari: Tetranychidae) associated with eucalyptus in a clonal minigarden. Syst. Appl. Acarol. 25: 103–112.

Fetoh, B.E.A. and K.A. Al-Shammery. 2011. Acaricidal ovicidal and repellent activities of some plant extracts on the date palm dust mite, *Oligonychus afrasiaticus* Meg. (Acari: Tetranychidae). Int. J. Environ. Sci. Eng. 2: 45–52.

Finney, D.J. 1971. Probit Analysis. Third Edition. Cambridge Univ. Press, Cambridge, United Kingdom.

Freeman, B.C. and G.A. Beattie. 2008. An overview of plant defenses against pathogens and herbivores. The Plant Health Instructor 194.

Guzmán-Maldonado, S.H. and V.H. Díaz-Fuentes. 2017. Diversity in the phenolic composition and antioxidant capacity of moringa collections in the state of Chiapas. Rev. Mex. Cienc. Agric. 8: 1641–1645.

Handique, G., S. Roy, A. Rahman, F. Rahman, and A. Barua. 2017. Use of some plant extracts for management of red spider mite, *Oligonychus coffeae* (Acarina: Tetranychidae) in tea plantations. Int. J. Trop. Insect Sci. 37: 234–242.

Heinz-Castro, R.T.Q., R. Arredondo-Valdés, S. Ordaz-Silva, H. Méndez-Cortés, A. Hernández-Juárez and J.C. Chacón-Hernández. 2021a. Bioacaricidal potential of *Moringa oleifera* ethanol extract for *Tetranychus merganser* Boudreaux (Acari: Tetranychidae) control. Plants 10: 1034.

Heinz-Castro, R.T.Q., R. Arredondo-Valdés, S. Ordaz-Silva, H. Méndez-Cortés, A. Hernández-Juárez and J.C. Chacón-Hernández. 2021b. Evaluation of ethanol extract of *Moringa oleifera* Lam. as acaricide against *Oligonychus punicae* Hirst (Trombidiformes: Tetranychidae). Insects 12: 476.

Ismail, M.S., M.H. Tag and M.A. Rizk. 2019. Acaricidal, ovicidal, and repellent effects of *Tagetes patula* leaf extract against *Tetranychus urticae* Koch (Acari: Tetranychidae). J. Plant Prot. Res. 59: 151–159.

Knowles, C.O. 1997. Mechanisms of resistance to acaricides, Pp. 57–77. *In* Sjut, V. (ed.), Molecular Mechanisms of Resistance to Agrochemicals. Chemistry of Plant Protection. Vol. 13. Springer, Berlin, Germany.

Kou, X., B. Li, J.B. Olayanju, J.M. Drake and N. Chen. 2018. Nutraceutical or pharmacological potential of *Moringa oleifera* Lam. Nutrients 10: 343.

Laborda, R., I. Manzano, M. Gamón, I. Gavidia, P. Pérez-Bermúdez and R. Boluda. 2013. Effects of *Rosmarinus officinalis* and *Salvia officinalis* essential oils on *Tetranychus urticae* Koch (Acari: Tetranychidae). Ind. Crops Prod. 48: 106–110.

Lengaia, G.M.W., J.W. Muthomi and E.R. Mbega. 2020. Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. Sci. Afr. 7: e00239.

Leone, A., A. Spada, A. Battezzati, A. Schiraldi, J. Aristil and D. Bertoli. 2015. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. Int. J. Mol. Sci 2015. 16: 12791–12835.

Maoz, Y., S. Gal, M. Zilberstein, Y. Izhar, V. Alchanatis, M. Coll and E. Palesvsky. 2011. Determining an economic injury level for persea mite, *Oligonychus perseae*, a new pest on avocado in Israel. Entomol. Exp. Appl. 138: 110–116.

McMurtry, J.A. and H.G. Johnson. 1996. An ecological study of the spider mite *Oligonychus punicae* (Hirst) and its natural enemies. Hilgardia 37: 363–402.

Migeon, A. and F. Dorkeld. 2023. Spider Mites Web: A comprehensive database for the Tetranychidae (http://www1.montpellier.inra.fr/CBGP/spmweb).

Moawad, S.S. and H.E. Sadek. 2018. Evaluation of two eco-friendly botanical oils on cotton leafworm, *Spodoptera littoralis* (Boisd) (Lepidoptera/Noctuidae). Ann. Agric. Sci. 63: 141–144.

Peña, J. and M. Wysoki. 2008. Plagas en Israel, México, Estados Unidos, Centroamérica y Perú. Plagas del palto en México, Pp. 303–309. *In* Ripa, R. and P. Larral (eds.), Manejo de Plagas en Paltos y Cítricos. Instituto de Investigaciones Agropecuarias, Santiago, Chile.

Peña, J. E., M.S. Hoddle, M. Aluja, E. Palevsky, R. Ripa and M. Wysoki. 2013. Insect and mite pests, Pp. 423–488. *In* Schaffer, B., B.N. Wolstenholme and A.W. Whiley (eds.), The Avocado: Botany, Production and Uses. Second Edition. CAB International, Oxfordshire, United Kingdom.

Pohlert, T. 2021. The pairwise multiple comparison of mean ranks package (PM-CMR). R package (https://rdrr.io/cran/PMCMR/f/inst/doc/PMCMR.pdf).

Rattan, R.S. 2010. Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Prot. 29: 913–920.

R Core Team. 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (https://www.R-project.org/).

Roy, S. and A. Mukhopadhyay. 2012. Bioefficacy assessment of Melia azedarach (L.) seed extract on tea red spider mite, *Oligonychus coffeae* (Nietner) (Acari: Tetranychidae). Int. J. Acarol. 38: 79–86.

(SAGARPA-SIAP) Secretaría de Agricultura Ganadería, Desarrollo Rural, Pesca y Alimentación-Servicio de Información Agroalimentaria y Pesquera. Panorama Agroalimentario. 2022. Mexico City, Distrito Federal, Mexico (https://drive.google.com/file/d/1jVWS4EFKK7HGwQOBpGeljUyaDT8X8Iyz/view).

Santos, N.D., K.S. de Moura, T.H. Napoleão, G.K. Santos, L.C. Coelho, D.M. Navarro and P.M. Paiva. 2012. Oviposition-stimulant and ovicidal activities of *Moringa oleifera* lectin on *Aedes aegypti*. PLoS One 7: e44840.

Sarmah, M., A. Rahman, A.K. Phukan and G. Gurusubramanian. 2009. Effect of aqueous plant extracts on tea red spider mite, *Oligonychus coffeae*, Nietner (Tetranychidae: Acarina) and *Stethorus gilvifrons* Mulsant. Afr. J. Biotechnol. 8: 417–423.

Shami, A.M.M., K. Philip and S. Muniady. 2013. Synergy of antibacterial and antioxidant activities from crude extracts and peptides of selected plant mixture. BMC Complement. Altern. Med. 13: 360.

Shanmugavel, G., K. Prabakaran and B. George. 2018. Evaluation of phytochemical constituents of *Moringa oleifera* (Lam.) leaves collected from Puducherry region, south India. Int. J. Zool. Appl. Biosci. 3: 1–8.

Singleton, V. L., R. Orthofer and R.M. Lamuela-Raventos. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299: 152–179.

Sivira, A., M.E. Sanabria, N. Valera and C. Vásquez. 2011. Toxicity of ethanolic extracts from *Lippia origanoides* and *Gliricidia sepium to Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae). Neotrop. Entomol. 40: 375–379.

Tanigoshi, L.K. and J.A. McMurtry. 1977. The dynamics of predation of *Stethorus picipes* (Coleoptera: Coccinellidae) and *Typhlodromus floridanus* on the prey *Oligonychus punicae* (Acarina: Phytoseiidae, Tetranychidae). Part II. Effects of initial prey-predator ratios and prey distribution. Hilgardia 45: 262–288.

Tlak Gajger, I. and S.A. Dar. 2021. Plant allelochemicals as sources of insecticides. Insects 12: 189.

Tomczyk, A. and M. Suszko. 2011. The role of phenols in the influence of herbal extracts from *Salvia officinalis* L. and *Matricaria chamomilla* L. on two-spotted spider mite *Tetranychus urticae* Koch. Biol. Lett. 48: 193–205.

Van Leeuwen, T., L. Tirry, A. Yamamoto, R. Nauen and W. Dermauw. 2015. The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. Pestic. Biochem. Physiol. 121: 12–21.

Vásquez, C. O. Aponte, J. Morales, M.E. Sanabria and G. García. 2008. Biological studies of *Oligonychus punicae* (Acari: Tetranychidae) on grapevine cultivars. Exp. App. Acarol. 45: 59–69.

Wheater, C.P. and P.A. Cook. 2005. Using statistics to understand the environment. Taylor & Francis: New York.