Biotecnia
Journal of biological and health sciences
http://biotecnia.unison.mx
Universidad de Sonora
ISSN: 1665-1456

Original Article

Seed coating with essential oils and hydrosols from *Lippia graveolens* enhances bio-protection against *Bemisia tabaci*

El recubrimiento de semillas con aceites esenciales e hidrolatos de *Lippia graveolens* mejora la bio-protección contra *Bemisia tabaci*

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ABSTRACT

Plant essential oils are gaining interest as biopesticides for crop protection. In this study, we evaluated the effect of seed coating with essential oils and hydrosol from *Lippia graveolens* as a bio-protection strategy against *Bemisia tabaci*, focusing on adult repellency and oviposition deterrence of whitefly. In addition, total phenol content in eggplant seedlings was measured to evaluate whether *L. graveolens* essential oil can induce plant defense responses by accumulating phenolic compounds. Essential oils and hydrosols from leaves, flowers, and a leaf–flower mixture were used as treatments. A completely randomized design was employed, and data were analyzed using one-way analysis of variance. Seed coatings with essential oil or hydrosol of *L. graveolens* did not produce adult repellency, however, they significantly deterred oviposition. Additionally, both essential oils and hydrosols significantly enhanced phenolic content in eggplant seedlings, suggesting activation of the plant defense system, which contributed to improved oviposition deterrence. Coating seeds with essential oils or hydrosols presents new possibilities for developing essential oil-based biopesticides. However, further research is required to understand the plant's specific response to the composition of essential oils and biotic stress.

Keywords: Mexican oregano, whitefly, oviposition deterrence, phenol content, plant defense inducers.

RESUMEN

Los aceites esenciales de plantas están ganando interés como bioplaguicidas para la protección de cultivos. En este estudio, se evaluó el efecto del recubrimiento de semillas con el aceite esencial y el hidrolato de *Lippia graveolens* como una estrategia de bioprotección contra *Bemisia tabaci*, enfocado en la repelencia de adultos y la disuasión de la oviposición de la mosca blanca. Además, se midió el contenido total de fenoles en plántulas de berenjena para evaluar si el aceite esencial de *Lippia graveolens* puede inducir respuestas de defensa en la planta mediante la acumulación de compuestos fenólicos. Se utilizaron aceites esenciales e hidrolatos obtenidos de hojas, flores y una mezcla de hojas y flores como tratamientos. Se empleó un diseño completamente al azar, y los datos se analizaron mediante un análisis de varianza de una vía. El recubrimiento de semillas con aceite esencial o hidrolato de *Lippia graveolens* no produjeron repelencia en adultos, sin embargo, disuadieron significativamente la oviposición. Además, tanto el aceite esencial como el hidrolato aumentaron significativamente el contenido de fenoles en las plántulas de berenjena, lo que sugiere una activación del sistema de defensa de la planta, contribuyendo así a una mayor disuasión de la oviposición. El recubrimiento de semillas con aceites esenciales o hidrolatos representa una nueva posibilidad para el desarrollo de bioplaguicidas a base de aceites esenciales. No obstante, se requiere más investigación para comprender la respuesta específica de la planta a la composición de los aceites esenciales y al estrés biótico.

Palabras clave: Orégano mexicano, mosca blanca, disuasión de oviposición, contenido de fenoles, inductores de defensa vegetal.

INTRODUCTION

Mexican oregano (*Lippia graveolens* Kunth) is a widely distributed aromatic plant species with a shrubby herbaceous habit. It is commonly used for culinary, cosmetic, and medicinal purposes in Mexico (Bautista-Hernández *et al*., 2021; Marin-Tinoco *et al*., 2023).

Mexico is the second country with the highest worldwide production of oregano, producing 6,500 tons in 2022 (Cuevas-González *et al*., 2025). The primary purpose of cultivating this plant is to extract its essential oils, as they contain valuable compounds with economic value and potential use in various applications (Bautista-Hernández *et al*., 2021; Jyotsna *et al*., 2024). Commercial essential oils are primarily obtained through steam distillation, the most widely used and accepted process for large-scale essential oil production (Soto-Armenta *et al*., 2017). The water obtained after the distillation process is called hydrosol, containing a small amount of essential oil and metals, mucilages, and trace elements. Therefore, some hydrosols have therapeutic properties, such

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Received: March 8, 2025
Accepted: July 11, 2025
Published: August 23, 2025

as rosewater and orange blossom water, which are extensively used in the cosmetic and food industries (Cid-Pérez *et al*., 2019). However, recent research suggests their potential application in insect pest control (González-Güereca *et al*., 2023) and in inhibiting the growth of pathogenic and spoilage microorganisms in food products and work environments. Additionally, they may help in the removal of microbial biofilms (Proto *et al*., 2022; Rosińska, 2022). Plant-based essential oils form an excellent alternative source to synthetic pesticides and can reduce negative impacts on human health by decreasing food, water, and soil-borne pesticide residues that are otherwise toxic (Jyotsna *et al*., 2024). The essential oils of *Lippia graveolens* have shown great potential for pest management in agriculture, demonstrating strong toxic and repellent activity against a wide range of pest insects when applied directly to crops, in either a corrective or preventive manner (Ayllón-Gutiérrez *et al*., 2024). These oils are an important source of insecticidal compounds, mainly due to the presence of carvacrol and thymol, which are capable of providing the essential oil of *Lippia graveolens* with the potential to cause mortality, inhibit feeding, and prevent oviposition (Sombra *et al*., 2018; Herrera *et al*., 2023).

Among pest insects, the whitefly *Bemisia tabaci* is a highly harmful pest on tropical and subtropical agroecosystems. Whitefly infestations in horticultural crops result in crop yield losses of up to 100 % when severe transmission of *Begomovirus* occurs, and resistant populations to synthetic insecticides have been reported (Ruíz *et al*., 2019; Santana *et al*., 2022).

The essential oils of *Lippia graveolens* have shown enormous potential for pest management in agriculture (Herrera *et al*., 2023). These studies have demonstrated significant effects on egg and adult mortality, adult repellency, and a reduction in oviposition (Herrera *et al*., 2023; Ayllón-Gutiérrez *et al*., 2024). However, this study explored a different approach, evaluating the effect of seed coating with essential oils and hydrosols on plant defense. Seed coating is a process that involves the application of small quantities of external materials to cover the seeds (Ben-Jabeur *et al*., 2015). Seed coating with essential oils can act as priming molecules both in biotic and abiotic plant stress responses, and as an effective and sustainable tool for pest control (Kesraoui *et al*., 2022). Ben-Jabeur *et al*. (2015) suggest that coating seeds with essential oil presents a promising approach in crop production to enhance crop tolerance to biotic and abiotic stress, ultimately improving farmers' economic profitability. Also, seed coating with essential oils can provide effective bioprotection against pests by combining direct pest control with the activation of plant defense mechanisms (Kesraoui *et al*., 2022).

The metabolomic approach has been used recently for the characterization of metabolic pathways affected by plant priming (Kesraoui *et al*., 2022). Essential oils have also been shown to trigger induced resistance responses through complex hormonal interactions, involving the biosynthesis and signaling cascades of jasmonic acid (JA), ethylene (ET), and salicylic acid (SA) (Rienth *et al*., 2019). When applied to seeds, essential oils can stimulate the synthesis of phenolic compounds like flavonoids, tannins, and lignin, which strengthen the plant's physical and chemical barriers against pests (Ben-Jabeur *et al*., 2015). Phenolic compounds are primarily known for their role in plant defense against biotic and abiotic factors. Some phenolic compounds act as antimicrobials, inhibiting the growth of bacteria, fungi, and viruses (Moutassem *et al*., 2019; Marin-Tinoco *et al*., 2023). Others, such as tannins, have a bitter or astringent taste that discourages insects and other herbivores from feeding on the plant (Hosseini *et al*., 2020). Through seed coating, bioactive compounds are present from the earliest stages of plant development, protecting vulnerable young plants from pest attacks. This approach minimizes the need for repeated foliar sprays, saving time and labor (Kesraoui *et al*., 2022; Oğuz *et al*., 2023).

There is still a lack of information regarding the effects of seed coating with essential oils trials in field conditions. This information will be of great importance for scientific research and the needs of farmers. Furthermore, to date, only one study has investigated seed coating with essential oils in both laboratory and field conditions for sustainable wheat agriculture (Oğuz *et al*., 2023).

Based on the above, in this study we evaluated the effect of seed coating with essential oils and hydrosols from *Lippia graveolens* as a bio-protection strategy against *Bemisia tabaci* in eggplant (*Solanum melongena*) seedlings.

MATERIAL AND METHODS

Plant material and extraction of essential oils and hydrosols

Plant material of wild oregano was obtained from El Polvo, located in the municipality of Valle de Zaragoza, Chihuahua, Mexico. Plants were harvested at the flowering stage in November 2022. Plant samples were dried in the shade and had a moisture content of 6 %. The oregano plant was subjected to steam distillation with a 2 L glass apparatus for 3 hours to separate the essential oil from leaf, flower, and mixture (leaf + flower) (Soto-Armenta *et al*., 2017). The extraction yields of essential oil from leaf, flower, and mixture were 2.15 %, 2.14 %, and 2.24 % on a dry basis, respectively, with essential oil densities of 0.923 g mL^{-1} for leaf, 0.926 g mL^{-1} for flower, and 0.903 g mL^{-1} for the mixture (leaf + flower) essential oil. The hydrosols from leaf and flower were obtained from the residual process of the essential oil extraction. Both essential oils and hydrosols were stored in amber-colored tubes at 4 - 6 °C until used.

Preparation of essential oil and hydrosol solutions.

Three treatments were prepared with 0.1 % leaf essential oil, flower essential oil, and a leaf-flower mixture (1:1). These were emulsified using Tween 80, ethanol, and distilled water (0.3 % v/v) (Herrera *et al*., 2023). The hydrosols of leaf, flower, and the mixture of both were prepared at a 10 % concentration (Rosińska, 2022). Control treatments included Tween 80 with ethanol and distilled water (0.3 % v/v) for the essential oils and only distilled water for the hydrosols. In total, eight treatments were prepared and stored in dark conditions at 4°C until used.

Breeding colony of *Bemisia tabaci*

The *Bemisia tabaci* biotype B insects used were obtained from a colony established in a greenhouse at the Instituto Tecnológico de Conkal, in Conkal (21º4'N, 89º31'W, 10), Yucatan, Mexico. The *Bemisia tabaci* were obtained originally from habanero pepper crops (*Capsicum chinense*) in the municipality of Conkal, Yucatan (Ruíz *et al*., 2019). The colony has been sustained on host eggplant plants (*Solanum melongena* L.) in a greenhouse, with environmental conditions set to a temperature range of 25-37°C, relative humidity between 70-90 %, and a photoperiod of 14 hours of light and 10 hours of darkness.

Repellency and oviposition deterrence against Bemisia tabaci whitefly

Eggplant seedlings were used, as this species is highly susceptible to *Bemisia tabaci* and serves as an excellent host plant in the whitefly breeding colony, making it a suitable model for studying induced defense responses. The eggplant seeds were soaked for 24 hours in their respective treatments, then sown in 2 L plastic pots filled with soil, where the seedlings were grown until they reached 45 days of age. At this age, eggplants are highly susceptible to *Bemisia tabaci*. Then, 10 pots per treatment were transferred to the greenhouse breeding colony of *Bemisia tabaci* under the same environmental conditions and arranged in rows at a 40 cm distance from the infested eggplant plants.

Repellency and oviposition deterrence were assessed 5 days after the plants were exposed to infestation. On each plant, the number of adult whiteflies on the abaxial side of two leaves in the upper third was counted. Immediately, the leaves were cut and transported to the laboratory for observation under an SMZ-161-BL stereoscope to determine the number of eggs. The leaf area was calculated using Image J software. One leaf corresponded to one repetition, and 10 repetitions were used per treatment (Herrera *et al*., 2023). The adult repellency index was estimated using the following formula:

$$RI = [2T/(T + C)]$$

where T is the number of insects attracted to the treated leaf, and C is the number of insects attracted to the control leaf. Values of RI lower than 1 indicate repellency (Ruíz *et al*., 2019). The oviposition deterrence index (ODI) was calculated by the formula:

$$ODI = \frac{T - C}{T + C} * 100$$

Where *T*= Number of eggs counted on the leaves of plant treatments, and *C*= Number of eggs counted on the leaves of the controls. Values of ODI from 0 to −100 indicate complete deterrence (Ruíz *et al*., 2019).

Total phenols determination

Eggplant seedlings (45-days old) were used in the experiment. Polyphenol content was expressed as milligrams of gallic acid equivalents per milliliter of extract (mg eq GAE/mL extract) and determined by the Folin-Ciocalteu method (Singleton *et al*., 1999). For this, 1.5 mL of calcium carbonate (Na_2NO_3), 0.5 mL of 50 % Folin-Ciocalteu reagent, 2.75 mL of deionized water, and 0.5 mL of sample extract were mixed in a test tube. After incubating the sample for one hour at room temperature, a calibration curve was established using gallic acid as a reference for the quantification of total phenols, measuring their absorbance level in a Thermo Scientific Genesys 10s UV/Vis spectrophotometer at 760 nm.

Experimental design and statistical analysis

In the repellency and oviposition deterrence against whitefly assay, a completely randomized experimental design with a total of 10 replications was used. Before performing the analysis of variance (ANOVA), all data were tested for normality and homogeneity. A one-way analysis of variance was used to analyze the data, with Duncan's mean comparison test ($p < 0.05$) (Ruíz *et al*., 2019). For the total phenol analysis, a completely randomized experimental design with three replications was used. The data were subjected to ANOVA, followed by Tukey's test for pairwise mean comparisons, with a significance level of $p < 0.05$. All statistical analyses were performed using the Infostat 2020 software (Di Rienzo *et al*., 2020).

RESULTS AND DISCUSSION

Effect of seed coating with essential oils and hydrosols on adult repellency and oviposition deterrence against Bemisia tabaci whitefly

Seed coatings with essential oils (Figure 1A) or hydrosols (Figure 1B) of *L. graveolens* did not produce any repellent effect on adult *B. tabaci*.

The oviposition deterrence against whiteflies is shown in Figure 2. Both essential oils and hydrosols exhibited oviposition deterrent effects compared to the control (Figure 2A and 2B). However, essential oils showed a greater deterrent effect, reducing oviposition by 66 % to 75 % relative to the control (Figure 2A). Among hydrosols treatments, significant differences were observed, with HH showing an oviposition deterrence of 59 % compared to the control (Figure 2B).

Several species of the genus *Lippia* have been evaluated as repellents and oviposition deterrence, primarily through spray application or direct contact with the essential oils. Herrera *et al*. (2023) evaluated the effect of *Lippia origanoides* essential oil and two of its volatile organic compounds (carvacrol and thymol) sprayed on eggplant leaves infested with *Bemisia tabaci*. The authors reported a significant effect on adult repellency and oviposition deterrence of *Bemisia tabaci*. In other species, such as the cattle tick *Rhipicephalus microplus*, Flores-Fernández *et al*. (2016) observed oviposition inhibition with higher concentrations (10 %) of Mexican oregano essential oil. Furthermore, Nerio *et al*. (2009) found that *Lippia origanoides* essential oil exhibited high repellency aga-

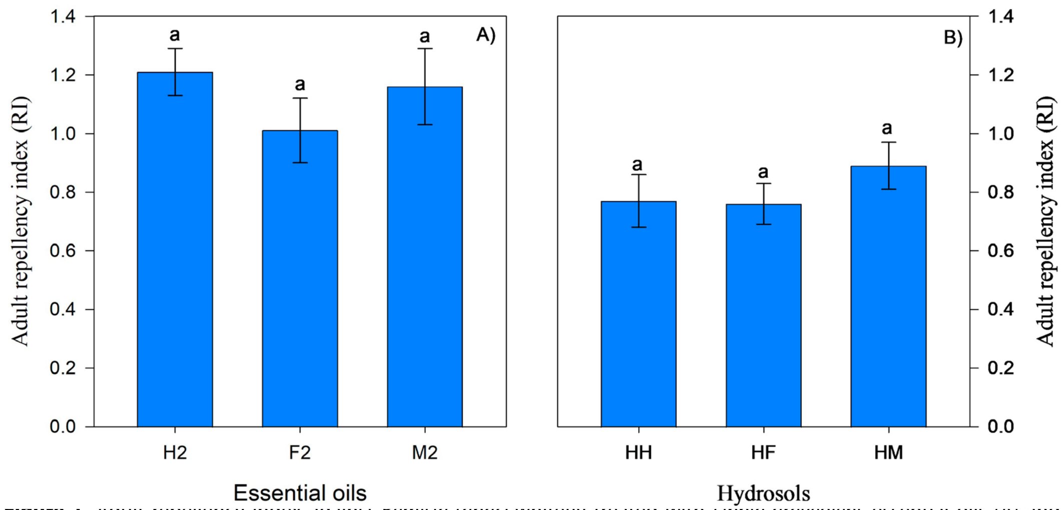

Figure 1. Adult repellency index against *Bemisia tabaci* whitefly treated with *Lippia graveolens* essential oils (A) and hydrosols (B) seed coating. H2: leaf, F2: flower, and M2: mixture (leaf + flower) essential oils, HH: Leaf hydrosol, HF: Flower hydrosol, HM: Mixture (leaf + flower) hydrosol. The values represent means and standard error. Different letters indicate statistically significant differences between treatments ($p < 0.05$, Duncan's test).

Figura 1. Índice de repelencia de adultos contra la mosca blanca *Bemisia tabaci* tratadas con aceites esenciales (A) e hidrolatos (B) de *Lippia graveolens* en el recubrimiento de semillas. H2: hoja, F2: flor y M2: mezcla (hoja + flor) de aceites esenciales; HH: hidrolato de hoja, HF: hidrolato de flor, HM: mezcla (hoja + flor) de hidrolato. Los valores representados muestran las medias y el error estándar. Letras diferentes indican diferencias estadísticamente significativas entre tratamientos ($p < 0.05$, prueba de Duncan).

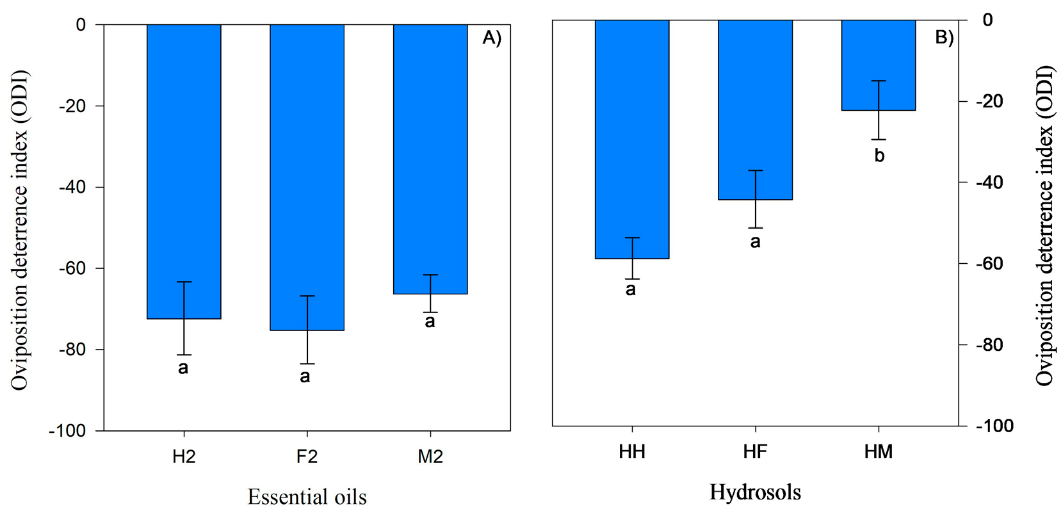

Figure 2. Oviposition deterrence index against *Bemisia tabaci* whitefly treated with *Lippia graveolens* essential oils (A) and hydrosols (B) seed coating. H2: leaf, F2: flower and M2: mixture (leaf + flower) essential oils, HH: Leaf hydrosol, HF: Flower hydrosol, HM: Mixture (leaf + flower) hydrosol. The values represent means and standard error. Different letters indicate statistically significant differences between treatments ($p < 0.05$, Duncan's test).

Figura 2. Índice de disuasión de oviposición contra la mosca blanca *Bemisia tabaci* tratadas con aceites esenciales (A) e hidrolatos (B) de *Lippia graveolens* en el recubrimiento de semillas. H2: hoja, F2: flor y M2: mezcla (hoja + flor) de aceites esenciales; HH: hidrolato de hoja, HF: hidrolato de flor, HM: mezcla (hoja + flor) de hidrolato. Los valores representados muestran las medias y el error estándar. Letras diferentes indican diferencias estadísticamente significativas entre tratamientos ($p < 0.05$, prueba de Duncan).

inst *Sitophilus zeamais*, likely due to the action of thymol, the primary component identified. In this regard, the chemical composition of *L. graveolens* essential oil includes flavonoids and terpenes, mainly thymol and carvacrol (Bautista-Hernández *et al*., 2021; Marin-Tinoco *et al*., 2023). However, this study showed that seed coating with *L. graveolens* essential oil and hydrosols effectively deterred oviposition. This oviposition deterrence may be related to the bio-protective priming effects triggered by the essential oil and hydrosols of *Lippia graveolens*, which can induce plant defense mechanisms by activating hormonal signaling pathways and promoting the accumulation of phenolic compounds and defense-related proteins. Proto *et al*. (2022) evaluated induced resistance in tomato plants against bacterial leafspots via soaked roots with essential oil and hydrosols of different plants. Results showed that tomato plants treated with *Citrus aurantium* hydrosol at 4.5 % and *Origanum compactum* essential oil at 0.03 % and 0.015 % showed significantly reduced disease severity concerning the negative control.

Essential oil has an activity directly on the plant and indirectly on pathogens. A more in-depth study has been conducted by Rienth *et al*. (2019), providing insight into the underlying mechanisms of the host-pathogen essential oil interaction and demonstrated that *Oregano vulgare* essential oil treatment activated a multilayered immune response in plants. This response was mediated through the increased transcription of genes involved in the salicylic acid, jasmonic acid, and ethylene pathways, the activation of PR protein gene transcription, and the synthesis of phytoalexins (Rienth *et al*., 2019). This response may be linked to the action of compounds that stimulate systemic resistance through the activation of innate immune pathways in plants (Kant *et al*., 2015). The induction of the plant's innate immune system is closely linked to signaling pathways mediated by salicylic acid, jasmonic acid, and ethylene, which regulate the expression of defense-related genes, including those involved in the biosynthesis of phenolic compounds via the shikimate and phenylpropanoid pathways. A key enzyme in this process is phenylalanine ammonia-lyase (PAL), which catalyzes the first step in the phenylpropanoid pathway, converting L-phenylalanine into trans-cinnamic acid. The accumulation of phenolics contributes to plant resistance against insects through structural reinforcement, feeding deterrence, repellency, or toxic effects (Delgado-Oramas *et al*., 2020).

Effect of seed coating with essential oils and hydrosols on phenol content of eggplant seedling.

To determine whether seed coating with *Lippia graveolens* essential oil has the potential to induce the plant defense system, the content of phenolic compounds, which are known to be involved in plant defense responses, was examined. Results showed that seed coating with essential oils and hydrosols caused a significant effect on phenol content in eggplant seedlings (Figure 3A and 3B). The phenol content of plants treated with *Lippia graveolens* essential oil was five times higher than their respective control (CTE) nevertheless, no significant differences were observed between the essential oil of leaves, flowers, and their mixture (Figure 3A). Similarly, plants treated with hydrosols also exhibited a higher amount of phenols compared to their control group (Figure 3B). The treatment HM had the highest amount of phenols with 10.2 mg mL^{-1} extract (Figure 3B). These results showed that seed coating with essential oils and hydrosols had a positive effect on the phenol content of eggplant seedlings, which strengthened the plant's physical and chemical barriers against *Bemisia tabaci* whitefly. In a study conducted by Ben-Jabeur *et al*. (2015), the authors investigated the potential of thyme essential oil as a defense inducer to protect tomato seedlings against gray mold and Fusarium wilt. They observed an accumulation of phenolic compounds in leaves and roots with values from 4.8 mg g^{-1} DW in the leaves and 7.8 mg g^{-1} DW in the roots, five days after treating the seedlings, likely resulting from the direct interaction between the oil and the roots. Similarly, Moutassem *et al*. (2019) investigated the effects of essential oils on reducing Fusarium wilt severity in chickpeas and their role in inducing systemic resistance by stimulating the accumulation of phenolic and flavonoid compounds. The highest polyphenol content was recorded with *Thymus pallescens*, ranging from 22.7 to 57.5 mg g^{-1}.

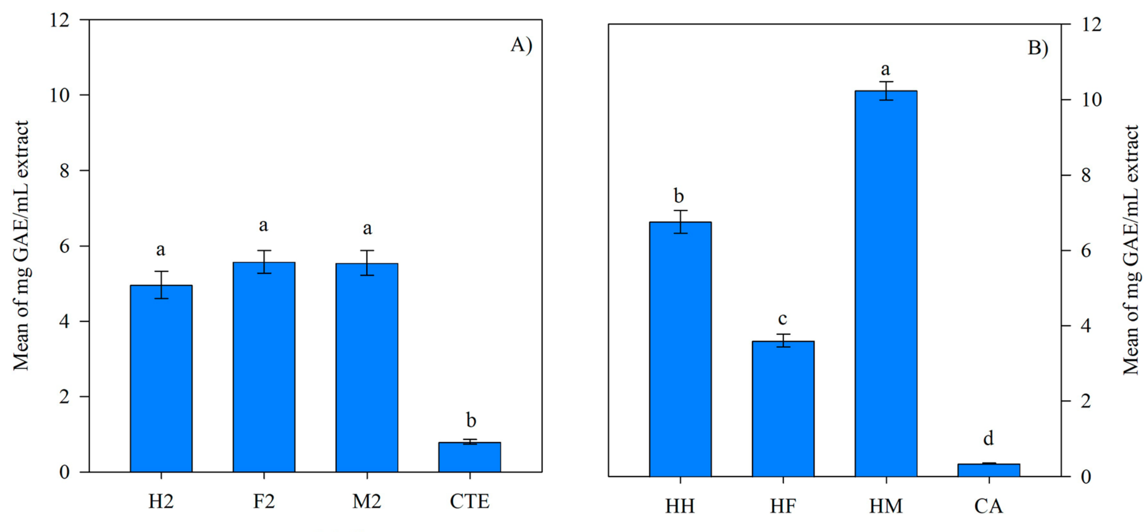

Figure 3. Effect of seed coating with essential oils of *Lippia graveolens* (A) and hydrosols (B) on total phenols in eggplant seedlings. H2: leaf, F2: flower and M2: mixture (leaf + flower) essential oils, CTE: Control, HH: Leaf hydrosol, HF: Flower hydrosol, HM: Mixture hydrosol mixture, CA: Control. The values represent means and standard error. Different letters indicate statistically significant differences between treatments ($p < 0.05$, Tukey´s test).

Figura 3. Efecto del recubrimiento de semillas con aceites esenciales de *Lippia graveolens* (A) e hidrolatos (B) sobre los fenoles totales en plántulas de berenjena. H2: hoja, F2: flor y M2: mezcla (hoja + flor) de aceites esenciales, CTE: Control; HH: hidrolato de hoja, HF: hidrolato de flor, HM: mezcla de hidrolato; CA: Control. Los valores representados muestran las medias y el error estándar. Las letras diferentes indican diferencias estadísticamente significativas entre tratamientos ($p < 0.05$, prueba de Tukey).

These compounds are recognized for their role in cell wall strengthening and lignification processes. Likewise, *Allium sativum* and *Rosmarinus officinalis* essential oils preserved the quality parameters in treated strawberry fruits against *Colletotrichum nymphaeae* due to an increase in their phenolic content and the activity of defense-related enzymes such as peroxidase (Hosseini *et al*., 2020).

CONCLUSION

Based on our results and supporting scientific evidence, essential oils and hydrosols from Mexican oregano (*Lippia graveolens*) enhance bio-protection against *Bemisia tabaci* whitefly by inducing the plant's defense system, increasing total phenolic content, and improving oviposition deterrence. Seed coating with essential oils and hydrosols opens new opportunities for the development of essential oil-based biopesticides. However, more research is needed to determine the specificity of the plant response to the essential oil composition and the biotic stress.

ACKNOWLEDGMENTS

The authors would like to thank SECIHTI Mexico for awarding a scholarship to Laura Catalina Soto Armenta. We also acknowledge the TecNM campus Conkal for granting access to the *Bemisia tabaci* breeding colony for the bioassays.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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