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Insecticidal effect of the methanolic extract of *Argemone mexicana* for the control of *Bactericera cockerelli* (Sulc.) (Hemiptera: Triozidae)

Efecto insecticida del extracto metanólico de *Argemone mexicana* para el control de *Bactericera cockerelli* (Sulc.) (Hemiptera: Triozidae)

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ABSTRACT

Argemone mexicana L. is a weed, which is used as a medicinal plant. The biological activity of this plant has been observed on pathogens such as viruses, fungi, bacteria, protozoa, and agricultural crop pests; such activity is attributed to compounds such as flavonoids, glycosides, terpenoids, phenolic compounds, and alkaloids present in the plant. The study aimed to evaluate the effect of the methanolic extract of *A. mexicana* as an insecticide on *Bactericera cockerelli*. Thirteen metabolites were detected by gas chromatography coupled to mass spectrometry; six were reported to have biological activity; the compound identified as 5,7,8,15-Tetrahydro-3,4-dimethoxy-6-methyl[1,3]benzodioxolo[5,6-e][2] benzazecin-14(6H)-one is the second most abundant and is a benzylisoquinoline alkaloid. The observed mortality at 48 h was 83.6 and 83.9 % dependent on the high doses of 20 and 30 mg/mL; while at 72 h an increase in mortality up to 97.2 % was observed at concentrations of 8-30 mg/mL. LC_{50} was 7.63 mg/mL and an LC_{95} of 107.98 mg/mL. Analysis of the methanolic extract of *A. mexicana* leaves revealed that it can be used as a plant-derived insecticide by causing mortality in *B. cockerelli* nymphs.

KEY WORDS: Alkaloid; benzylisoquinoline; chicalote; mortality; tomato psyllid.

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RESUMEN

Argemone mexicana L. es una maleza, la cual es utilizada como planta medicinal. La actividad biológica de esta planta se ha observado en patógenos como virus, hongos, bacterias, protozoos y plagas de cultivos agrícolas; dicha actividad se atribuye a compuestos como flavonoides, glucósidos, terpenoides, compuestos fenólicos y alcaloides presentes en la planta. El objetivo del estudio fue evaluar el efecto del extracto metanólico de *A. mexicana* como insecticida sobre *Bactericera cockerelli*. Se detectaron 13 metabolitos por cromatografía de gases acoplada a espectrometría de masas; siendo seis los que tienen reporte de actividad biológica; el compuesto identificado como 5,7,8,15-Tetrahidro-3,4-dimetoxi-6-metil[1,3]benzodioxolo[5,6-e][2]benzazecin-14(6H)-ona es el segundo con mayor abundancia y es un alcaloide de bencilisoquinolina. La mortalidad observada a las 48 h fue de 83.6 y 83.9 % dependiente de las dosis altas de 20 y 30 mg/mL; mientras que a las 72 h se observó un aumento de la mortalidad hasta en un 97.2 % a las concentraciones de 8-30 mg/mL. La concentración letal media fue de 7.63 mg/mL y una LC_{95} de 107.98 mg/mL. El análisis del extracto metanólico de las hojas de *A. mexicana* reveló que puede ser empleado como insecticida de origen vegetal al causar mortalidad en ninfas de *B. cockerelli*.

PALABRAS CLAVE: Alcaloide, bencilisoquinolina, chicalote, mortalidad, psílido del tomate.

Introduction

Tomato (*Solanum lycopersicum* L.) is a widely cultivated vegetable in the world due to its nutritional and socioeconomic importance (Yu *et al.,* 2017; Tamburino *et al.,* 2020). A challenge sampled in tomato production is pest infestation in temperate, subtropical, and tropical regions worldwide (Fuentes *et al.,* 2017), which generates losses in yields and fruit quality (Liu and Wang, 2020).

The insect *Bactericera cockerelli* Sulc. (Hemiptera: Triozidae) known as tomato and potato psyllid is a pest of some Solanaceae such as eggplant, chili, potato, and husk tomato (Tang *et al.,* 2020). The main damage results from the transmission of toxins that affect plant growth (Sumner *et al.,* 2020), and indirectly it is a carrier of the bacterium *Candidatus* Liberibacter solanacearum associated with tomato perennial disease (García-Sánchez *et al.,* 2021, Roque-Enríquez *et al.,* 2021). The inadequate use of chemical insecticides has led to negative effects on *B. cockerelli* populations as they are used up to 12 times in the crop cycle; far from improving plant health, this leads to the potential selection of insect resistance and high costs for production (Gutiérrez-Ramírez *et al.,* 2021).

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Only one percent of the total insecticides applied in agricultural areas attack the target pest, the rest is persistent in water, soil, and air; to reduce adverse effects, safer alternatives for pest and disease management should be considered (Koul *et al.,* 2004). The search for natural insecticides from wild plant species is promising for use as bioactive substances, but there is also a need for these tests to have more practical applications for production systems and bring socioeconomic benefits to producers (Tembo *et al.,* 2018). Dougoud *et al*. (2019) report that about 235 plant families (2,500 species) on the planet possess biological action against pests through plant extracts that exhibit active principles with repellent, antifeedant, and insecticidal properties.

The species *Argemone mexicana* L. considered a broadleaf weed, is called chicalote, belonging to the Papaveraceae family (Andleeb *et al.,* 2020, Manalil & Chauhan, 2019), and is one of the 25 species of the genus *Argemone* with high alkaloid content (Xool-Tamayo *et al.,* 2021). This species can be found widely distributed in open fields or along roadsides. Due to its high content of oils in the seed and alkaloids in the whole plant (*Martínez-Delgado et al., 2022*), in Mexico, it is used as a medicinal plant for the treatment of diseases such as asthma, ulcers, intestinal infections, and cancer (Das *et al.,* 2011; Elizondo-Luévano *et al.,* 2018; Datkhile *et al.,* 2021; Singh *et al.,* 2021).

The biological activity of this plant has been observed on pathogens such as viruses, fungi, bacteria, and parasitic protozoa (Elizondo-Luévano *et al.,* 2018; Andleeb *et al.,* 2020), such antimicrobial activity is attributed to compounds such as flavonoids, glycosides, terpenoids, phenolic compounds, and alkaloids (More *et al.,* 2017). Actions against agricultural crop pests such as *Bemisia tabaci, Spodoptera frugiperda, Aphis gossypii,* and *Tribolium castaneum* have also been reported (Granados-Echegoyen *et al.,* 2019; Miranda-Arámbula *et al.,* 2021; Martínez-Delgado *et al.,* 2022), however, evidence is limited due to the lack of trials on different pests and crops. Therefore, this study aimed to identify the compounds of the methanolic extract of the leaves of *A. mexicana* species, as well as to evaluate their biocidal activity on *B. cockerelli* nymphs.

Material and Methods

Bactericera cockerelli (Sulc.) colony

The insects came from potato and tomato crops collected in 2018 in Coahuila and Nuevo León (Mexican states). Colonies were established in a greenhouse from the Department of Parasitology, Universidad Autónoma Agraria Antonio Narro, and placed in wooden cages (50 cm long x 50 cm wide x 80 cm high) covered with organza fabric with 40 mm pore size, at 22 °C with a photoperiod of 14:10 h (Light/Dark) with tomato plants Rio Grande variety (Roque-Enríquez *et al.,* 2021).

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Sampling and obtaining an extract of *Argemone mexicana* L.

The collection was performed as described by Bobi *et al.* (2015), with modifications, in brief, whole plants were placed in paper bags for transport, and sampling was performed in the municipality of Saltillo, Coahuila, Mexico. The taxonomic identification of the plant was performed by Dr. José Ángel Villarreal Quintanilla and included in the ANSM herbarium of the Universidad Autónoma Agraria Antonio Narro with number 103807. Then, only leaves with the following characteristics of *A. mexicana* were selected: leaves with toothed margins, spine ends, intense green color, slightly glaucous tone, and whitish lines; and were left to dry in the shade for ten days, then ground in a blender (Waring Commercial, model 7011s) and immersed in 96 % methanol (Meyer, CDMX, Mexico) for seven days (at a ratio of 1 g dry matter mL^{-1} of solvent), with constant agitation at room temperature (yield 84 % v/v). The extract was vacuum filtered with Whatman No. 1 paper and stored in an amber bottle at 4 °C until use.

Gas Chromatography coupled to Mass Spectrometry (GC-MS) analysis.

The gas chromatography process was performed in the Biogeochemistry laboratory (UBIPRO) at the Universidad Nacional Autónoma de México, Iztacala, México. The compounds present in the *A. mexicana* leaf extract were determined in a 6850 gas chromatograph (Aligent, Santa Clara, California), using an HP-5MS column (Aligent, Santa Clara, California) of 30 m × 250 µm diameter and 0.25 µm film; the oven was programmed for 2 min at 150 °C, then increased 10 °C per min until 300 °C was reached for 4 min; Helium was used in the mobile phase at a flow rate of 1 mL min^{-1}. It was coupled to a 5975C mass spectrometry detector (Aligent Technologies, USA) at 200 °C for 2 min, an ionization source was 230 °C and a quadrupole temperature of 150 °C (at 70 eV ionization); it was conditioned for a full scan over a mass range of 35 to 400 m z^{-1}. The identification of the compounds was established based on its mass spectra analysis using the National Institute of Standards and Technology (NIST version 08 MS) database.

Relative density

The present study was performed as described by López-López *et al.* (2022, with modifications), from the methanolic extract of *A. mexicana* leaves, with a 25 mL Gay-Lussac pycnometer (Brand 16038, Germany) at 22 °C, the density of the extract was calculated by the formula:

$$\text{Relative density} = \frac{(m_1 - m)}{m_2 - m} * d_{22}^{t}$$

Where: m is the mass of the empty pycnometer (g), m_1 is the mass of the pycnometer with the test sample (g), m_2 is the mass of the pycnometer with water (g), d_{22}^{t} is the density of water at 22 °C (0.997772 g cm^{-3}). Results are expressed in mg mL^{-1} for the preparation of concentrations from the extract.

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Bioassay against *B. cockerelli*

Using the methanolic extract of leaves of *A. mexicana*, a window of biological activity was obtained at concentrations of 0, 10, 20, 40, 40, 60, 80, and 100 mg/mL, an absolute control with distilled water. Susceptibility test number 032 version 1 of the Insecticide Resistance Action Committee (IRAC, 2014) with modifications was used. Due to the high mortality obtained in the biological window, seven concentrations were established at 2, 4, 8, 12, 16, 20, and 30 mg/mL; for which a leaf of saladette tomato Rio Grande variety with 11 nymphs of the 4th instar of *B. cockerelli* was submerged in each concentration; likewise, a leaf with nymphs was submerged in distilled water as absolute control for 5 s, allowed to dry and then placed in Petri dish with filter paper soaked with sterile distilled water. Four replicates were placed for each concentration and the absolute control. The test was kept with a 14:10 h photoperiod (light/dark) at 23 °C. The evaluation of mortality was carried out every 24 h after the application of the extract. A stereoscopic microscope (Carl Zeiss Stemi DV4) was used to verify the number of dead nymphs, considering the nymphs as dead when they did not show coordinated movement by physical stimulation with a brush.

Data analysis

Results were expressed as percentage mortality and mortality was adjusted using Abbott's formula (Abbott, 1925). A Probit analysis was performed with the mortality corrected for the concentration-mortality curve and the lethal concentrations (LC_{50} and LC_{95}) were recorded. Finally, the data were evaluated by an analysis of variance and the mean values were compared by Tukey's test ($p \leq 0.05$), under a completely randomized design. All the above analyses were performed using Statistical Analysis System (SAS) version 9.0 statistical software (SAS Institute, 2002).

Results and Discussion

Chromatographic analysis (GC-MS) of *A. mexicana* yielded 13 compounds (two saturated fatty acids, five unsaturated fatty acids, one terpene, two alcohols, and three alkaloids) (Table 1); seven of these compounds possess biological activity reported in the literature, which are 1, 2, 3, 4, 5, 6, and 12. Being the most abundant in the extract the 9,12,15-Octadecatrienoic acid and the second with 19.45 % was the alkaloid 5,7,8,15-Tetrahydro-3,4-dimethoxy-6-methyl[1,3] benzodioxolo[5,6-e][2]benzazecin-14(6H)-one.

In this context, the choice of solvents for the extraction of active compounds has an important role in the discovery of new compounds for the control of pests and diseases (Andleeb *et al.,* 2020). The techniques involved in the production of bioactive extracts or fractions from plants are usually the same, and the primary steps for this purpose are the selection of appropriate solvents (polar, intermediate polar, and non-polar), extraction procedures, and identification techniques (Abubakar and Haque, 2020). It has been observed that aqueous and ethanolic extracts have the function of protecting crops from pests (Tavares *et al.,* 2021). Furthermore, plant extracts based on ethanol, methanol, petroleum ether, ethyl acetate, dichloromethane, chloroform, and water protect plants from fungi (Choudhury *et al.,* 2018).

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Phytochemical compounds metabolized in plants are mainly stored in plant cell vacuoles, compounds such as steroids, terpenes, and in greater abundance phenolics (including alkaloids) have been recognized; depending on their concentration and extraction technique, excellent biopesticidal properties have been achieved (Jiménez-Reyes *et al.,* 2019). *A. mexicana* exhibits insecticidal activity from different extracts based on ethyl acetate, acetone, methanol, and hexane by direct extraction. Elango *et al.* (2012) report higher extraction yield in *A. mexicana* leaves with methanol (10.2 mg/g leaf) compared to acetone, ethyl acetate, and hexane (5.5, 5.3, and 3.1 mg/g leaf, respectively).

Table 1. Phytochemical compounds from methanolic extract of *A. mexicana.*

	Compound	CAS[1]	Reference	Rt[2]	Area %
1	Hexadecanoic acid, methyl ester	000112-39-0	113690	11.99	1.88
2	n-Hexadecanoic acid	000057-10-3	102726	12.78	12.35
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	000112-63-0	132273	14.61	1.65
4	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-)-	0007361-80-0	130794	14.70	4.08
5	Phytol	000150-86-7	133807	14.99	1.15
6	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	000463-40-1	119801	15.35	27.07
7	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	001191-41-9	141488	15.39	8.94
8	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	000463-40-1	119801	15.44	14.02
9	Erinine, 21-deoxo-23-hydroxy-, (23 alpha.)-	016843-68-8	188374	21.45	4.37
10	2,4-Cyclohexadien-1-one, 3,5-bis (1,1-dimethyl ethyl)-4-hydroxy-	054965-43-4	76340	22.09	0.75
11	(+)-Canadine	000522-97-4	164694	23.03	1.77
12	[1,3]Benzodioxolo[5,6-e] [2]benzazecin-14(6H)-one,5,7,8,15-tetrahydro-3,4-dimethoxy-6-methyl-	000485-91-6	182195	23.27	19.45
13	2,4-Cyclohexadien-1-one, 3,5-bis (1,1-dimethyl ethyl)-4-hydroxy-	054965-43-4	76340	23.72	1.82

[1] CAS: Chemical Abstracts Service; [2] RT: Retention time in minutes.

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The result of the metabolites identified in Table 1, helped to determine that certain compounds have been previously reported with the following biological activities: hexadecanoic acid methyl ester detected in the methanolic extract of *Azolla pinnata* showed an insecticidal effect on 4th instar larvae of *Aedes albopictus* (Ravi *et al*., 2018). The n-hexadecanoic acid, identified in the dichloromethane extract *of Ficus sycomorus*, was effective in repelling females of *Tetranychus urticae*, *Aphis craccivora,* and *Sitophilus oryzae* (Romeh, 2013). 9,12-octadecadienoic acid (Z,Z)- and 9,12,15-octadecatrienoic acid (Z,Z,Z,Z)- methyl esters present in the petroleum ether fraction of *Robinia pseudoacacia* manifested insecticidal effects on *Brevicoryne brassicae* and *Aphis gossypii* (Jiang *et al.,* 2018).

As for phytol, it has been reported in the ethanolic extract of *Petiveria alliacea* as the main metabolite with insecticidal activity against *Bemisia tabaci* nymphs (Cruz-Estrada *et al.,* 2013). The most biologically active compound in *A. mexicana* extract has been 9,12,15-Octadecatrienoic acid, (Z,Z,Z,Z)-, which is reported in ethyl acetate extract of *Moringa oleifera* as an inhibitor in egg hatching and mortality of 3rd instar nymphs of *Haemonchus contortus* and *Nacobbus aberrans* (Páez-León *et al.,* 2022). Finally, the alkaloid 5,7,8,15-Tetrahydro-3,4-dimethoxy-6-methyl[1,3] benzodioxolo[5,6-e][2]benzazecin-14(6H)-one obtained from the aqueous extract of *A. mexicana,* showed antiparasitic activity on *Plasmodium falciparum* (Simoes-Pires *et al.,* 2014). However, there is not enough information about the biological activity of this alkaloid against relevant agricultural pests including the orders Thysanoptera, Hemiptera, Homoptera, Coleoptera, Diptera, and Lepidoptera.

The test with a methanolic extract of *A. mexicana* leaves on *B. cockerelli* nymphs showed a 67% of mortality at the concentration of 20 mg/mL in 24 h (Table 2); at the concentrations of 20 and 30 mg/mL, the maximum mortality was observed at 48 and 72 h up to 97.3%. The mean lethal concentration (LC_{50}) indicated by the mortality data for the methanolic extract of *A. mexicana* at 48 h was 7.63 mg/mL and LC_{95} was 107.98 mg/mL with $p \leq 0.05$ (Table 3).

Authors such as Danga *et al.* (2015) and Kosini *et al.* (2021) suggested that the presence of alkaloids and other secondary metabolites in plant extracts could lead to loss of extract activity during fractionation. This leads to the need to combine solvents, resulting in high production costs and potentially harmful effects on humans and the environment.

Within the genus *Argemone*, several alkaloids have been recognized in different plant tissues as frequent metabolites with medicinal and cytotoxic properties. Synthesized from amino acids, the alkaloids are highly reactive in nature and biologically active in a wide variety of organisms (Brahmachari *et al.,* 2013; Dey *et al.,* 2020).

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Table 2. Effect of methanolic extract of *A. mexicana* on mortality of *B. cockerelli* nymphs.

Treatments (mg/mL)	Hours after the application[1]		
	24	48	72
Control	0 ± 0 d	0 ± 0d	0 ± 0 c
2	16.6 ± 8.67 cd	29.9 ± 17.06 c	55.5 ± 15.72 b
4	16.6 ± 4.48 cd	26.4 ± 18.91cd	55.6 ± 31.43 b
8	28.9 ± 21.92 bcd	46.1 ± 14.45 bc	75 ± 16.66 ab
12	23.9 ± 23.29 cd	54.2 ± 9.49 bc	86.1 ± 10.64 ab
16	45.5 ± 20.45 abc	61.9 ± 14.86 ab	88.9 ± 9.07 ab
20	67.05 ± 15.75 a	83.6 ± 6.75 a	94.4 ± 6.41 a
30	64.3 ± 12.27 ab	83.9 ± 5.84 a	97.2 ± 5.55 a
p valor	0.0001	0.0001	0.0001

Data represent mean mortality per treatment. [1] Lowercase letters in each column indicate significant differences, according to Tukey's test ($p \leq 0.05$).

Table 3. The lethal concentration of methanolic extract of *A. mexicana* on nymphs of *B. cockerelli.*

Hours after the application	Median lethal dose (LC) (mg/mL)		Regression equation	Coefficient of determination
	LC_{50}	LC_{95}		
24	17.96	309.63	Y =-1.6690x + 1.3304	0.841
48	7.63	107.98	Y=-1.2608x + 1.4291	0.897
72	2.25	28.97	Y=-0.5230x + 1.4828	0.819

The median lethal dose in mg/mL.

The process of benzylisoquinoline alkaloid (BIA) biosynthesis in the genus *Argemone*, begins with enzymatic reactions that are first regulated by cytochrome P450 (CYP450) proteins of the CYP719 family; which catalyze tyrosine with tyrosine/DOPA decarboxylase (TYDC) to form dopamine, (S)-norcoclaurine synthase (NCS) and monophenol oxygenase condense to yield 4-hydroxyphenyl acetaldehyde (4HPPA) (Liscombe & Facchini, 2008; Rubio-Pina & Vázquez-Flota, 2013). From 4HPPA the compound (S)-norcoclaurin is formed by interference of norcoclaurin synthase (NCS), then, by methylation and hydroxylation, (S)-reticulin is obtained by the participation of (RS)-norcoclaurin 6-O-methyltransferase (6OMT), (S)-coclaurin-N-methyltransferase (CNMT),

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3'-hydroxy-N-methyl-(S)-coclaurin 4'-O-methyltransferase (4OMT) and N-methylcoclaurin 3'-hydroxylase (NMCH) (Rubio-Pina & Vázquez-Flota, 2013; Takemura *et al.,* 2013).

Starting from the precursor (S)-reticulin, allocryptopin (AL) is synthesized by the berberine bridge enzyme (BBE), CYP719A2 (S)-escoulerin 9-O-methyltransferase (SMT), tetrahydro protoberberine-N-methyltransferase (TNMT) and methyltetrahydroprotoberberine 14-monooxygenase (MTMO) (De-La-Cruz Chacón *et al*., 2012; Takemura *et al*., 2013). The structural core of AL is a protopin, which is derived from the protoberberine metabolic pathway (Marek *et al*., 1998; Beaudoin & Facchini, 2014).

Compound 12 is 5,7,8,15-Tetrahydro-3,4-dimethoxy-6-methyl[1,3]benzodioxolo[5,6-e][2] benzazecin-14(6H)-one, a compound described under the common name allocrytopin (Sakai *et al.,* 1988; Brahmachari *et al.,* 2013; Nigdelioglu-Dolanbay *et al.,* 2021) present in the genus *Argemone* (Elizondo-Luévano *et al*., 2018) and in the *A. mexicana* species (Vacek *et al.,* 2010; Simoes-Pires *et al.,* 2014; Gobato *et al.,* 2015) where up to 25 BIA have been identified (Elizondo-Luévano *et al.,* 2018). AL belongs to the group of true isoquinoline alkaloids, in the classification of BIAs derived from the aminoacids phenylalanine and tyrosine (Dey *et al.,* 2020). It is found with higher accumulation in leaves of *A. mexicana* (Diaz-Chavez *et al.,* 2011; Martinez-Delgado *et al.,* 2022) and about 2,500 ABIs have been identified so far in various families, including Papaveraceae (Vacek *et al.,* 2010).

The mechanisms described for BIAs involve inhibition of DNA replication by generating double-strand breaks at DNA replication sites, causing cytotoxicity (Inoue *et al.,* 2021), inhibition of acetylcholinesterase (Houghton *et al.,* 2006), inhibition of cytochrome P450 enzymes (Menéndez-Perdomo & Facchini, 2018) and anti-herbivory due to low nutritional input to the insect (Lee *et al.,* 2013).

Despite generalist and specialist insects have developed detoxification mechanisms on secondary metabolites or foreign substances, through specific enzymes produced by cytochrome P450, esterases, and glutathione S-transferases in the insect midgut, to achieve immediate suppression of toxic substances (Schuler, 1996). BIAs possess certain properties that limit their detoxification in insects as mentioned above.

In addition, organic solvents are effective for diffusion in plant tissues and entrain aromatic secondary metabolites of similar polarity; which have the potential to interact in alteration or disruption of the cell membrane, inactivation or deprivation of enzyme-substrate, interaction with DNA, insect nerve cells, inhibition in oviposition, egg hatching, and form disulfide bridges in secretory proteins which help insects in oxidative stress (Gurjar *et al.,* 2012; Lengai *et al.,* 2020). This may explain the effect on mortality mentioned below.

The methanolic extract based on leaves of *A. mexicana* caused mortality of *Coptotermes formosanus* larvae at 24 and 48 h (58 and 71 %, respectively) from 2 mg/mL (Elango *et al.,* 2012). Petroleum ether extract of *A. mexicana* leaves exhibited insecticidal activity with an LC_{50} of 48.89 ppm at 48 h on third instar larvae of *Culex quinquefasciatus* (Sakthivadivel *et al.,* 2012). It was also demonstrated that leaves of *A. mexicana* in 5 % (w/v) aqueous solution generated on *B. tabaci* the reduction of the population at the end of the experiment by 20.57 %, in tomatoes

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under greenhouse (Martínez-Tomás *et al.,* 2015). These results demonstrate that polar solvents extract secondary metabolites from *A. mexicana* leaves and are biologically active against insects. Similarly, the effect of AL on *T. cruzi* was demonstrated with an LC_{50} of 32 mg/mL (Simoes-Pires *et al.,* 2014). This indicates that the biological activity of *A. mexicana* reported as an organic extract has the potential for agricultural pest control.

However, there is no information available on alocriptopine BIA assays with insecticidal activity; however, the effects on humans have been studied as hepatoprotective, antithrombotic, anti-inflammatory, antitussive, anticancer, antiparasitic, and antibacterial activity (Beaudoin & Facchini, 2014; Huang *et al.,* 2018).

In addition, it was reported that tylamosin an BIA isolated from *Tiliacora acuminata,* affected *Culex quinquefasciatus* eggs by up to 91 % at 120 h and in larvae presented significant midgut cell damage; as well as several different movements of larvae compared to the control. This was caused by the inhibition of acetylcholinesterase (AChE), increasing acetylcholine in the insect synapse, leading to decreased electrical impulses and activation of excitatory postsynaptic receptors resulting in paralysis and death (Matsuura & Fett-Neto, 2015; Sandhanam *et al.,* 2019). *Argemone platyceras* plant expresses the compound munitagin, an BIA that inhibits AChE, butyrylcholinesterase (BChE) and prolyl oligopeptidase (POP), and AL exhibited weak activity on AChE and POP (Siatka *et al.,* 2017). Also, BIA sanguinarine was shown to block the transcription of α-amylase, serine protease, and lipase enzymes in the midgut of *Lymantria dispar* larvae, reflecting an antifeedant effect and decreased insect survival (Zou *et al.,* 2019).

It has been recognized that BIAs such as sanguinarine, protoberine, benzophenanthridine, berberine, phenanthridine, and ungeremine can inhibit nucleic acid synthesis by targeting dihydrofolate reductase and Z-ring proteins involved in cell division (Matsuura & Fett-Neto, 2015). Likewise, the assay performed by Quiroz-Carreño *et al.* (2020) on *Drosophila melanogaster* and *Cydia pomonella* larvae, with four ABIs (boldine, coclaurin, pukatein, and laurolitsin) showed a mortality of 80 %; possibly caused by the coupling of the heterodimer of the ecdysone and octapamine receptor, which interacts with the hormonal system of insects to modulate in an agonistic way the BIAs with selective insecticidal action.

As shown in Table 3, the LC_{50} decreased from 57.5 % to 87.5 % at 48 and 72 h, respectively. This indicates that the 13 compounds identified in the methanolic extract of *A. mexicana* leaves could be interacting at sensitive sites in the insect body as the concentration of the extract increases and accumulates with exposure time; through the decrease of enzymes secreted by the insect as a protective mechanism and growth regulators (Al-Rashidi *et al.,* 2022).

In the mortality study of Anon & Adday (2020), using an alkaloid-based extract of *Datura inoxia* leaves on 4th instar larvae of *C. quinquefasciatus*, the mean inhibitory concentration was 31.4 % and 62.4 % higher compared to extracts with turbine compounds and phenols. However, exposure at 24 h resulted in very similar mortality rates from 60 % to 70 %, with no statistically significant differences observed. This is possible since alkaloids also act on tubulin polymerization during mitosis, causing toxicity, a disturbance of sugar-metabolizing enzymes, and generating alterations in the physiological processes of the insect (Bhambhani *et al.,* 2021). As well as a

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specific blockade of calcium channels of the sarcoplasmic reticulum in muscle cells causing paralysis, cardiac arrhythmia, and death of the insect (Alves *et al.,* 2019).

Conclusions

The results of the present GC-MS study showed that compound 12 (belonging to the BIA group), is the second most abundant compound in the methanolic extract of *A. mexicana* leaves. Seven secondary metabolites (1, 2, 3, 4, 5, 6, and 12) were identified in the methanolic extract of *A. mexicana* leaves with biological activity. The methanolic extract of *A. mexicana* leaves exhibited 83.9 % of mortality at 48 h in *B. cockerelli* nymphs at concentrations 20 and 30 mg/mL, with an LC_{50} of 7.63 mg/mL and an LC_{95} of 107.98 mg/mL. The leaf extract of *A. mexicana* showed an insecticidal effect to be used as biocontrol of *B. cockerelli.*

Contribution of the authors

JCDO and HLL conceived, designed, and conducted the investigation. JCDO, HLL and ECA; in participation with MBB, performed the data analysis and interpretation. HLL prepared the manuscript with the support of JCDO, YMOF, and ECC. All authors contributed to the discussion, revision, and acceptance of the final manuscript.

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Conflict of interest

The authors declare having no conflict of interest.

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