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Article

Testing the effect of chipilín extract and pyrrolizidine alkaloid in liquid diets on *Bactericera cockerelli*

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Abstract

Bactericera cockerelli is one of the most economically important and destructive pests in crops of the nightshade family, where chemical control has been the primary management strategy. The use of botanical extracts is a biorational alternative for managing this pest. The insecticidal activity of the genus *Crotalaria* is attributed to the presence of alkaloids, saponins, flavonoids and pyrrolizidine alkaloids. This study aimed to evaluate the insecticidal effect of *C. longirostrata* extract and the fraction of the pyrrolizidine alkaloid (1β,2β-Epoxy-1α-methoxymethyl-8α-pyrrolizidine) on *B. cockerelli* by supplying liquid diets. The feeding bioassays using liquid diets were conducted in the Toxicology Laboratory of the Antonio Narro Autonomous Agrarian University. A liquid diet supplemented with 5, 10, 15, 20, 30, 40 and 50 mg ml-1 of the methanolic extract of *C. longirostrata* and the fractionate of the pyrrolizidine alkaloid was implemented in plastic feeding chambers, where mortality was evaluated under a completely randomized design. The LC50 was determined for the 1β,2β-Epoxy-1α-methoxymethyl-8α-pyrrolizidine fraction and the methanolic extract of chipilín, obtaining a mortality in the methanolic extract between 42 and 78%, while the fraction of the pyrrolizidine alkaloid registered a mortality of 68-91%; the latter was the one that presented the lower LC50. The methanolic extract of chipilín and the fraction of the pyrrolizidine alkaloid showed insecticidal activity in liquid diets, demonstrating efficiency for their use in controlling *B. cockerelli*.

PKeywords:

Crotalaria longirostrata, feeding bioassays, pyrrolizidine alkaloid, tomato psyllid.

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Introduction

Tomatoes (*Solanum lycopersicum* L.) are a vegetable with a high nutritional contribution to the human diet. Mexico is one of the leading producers of this vegetable, with a production of 3 636 927 t (SIAP, 2024; Tamburino *et al*., 2020). However, production is limited by climate change (Mutale-joan *et al*., 2020), which has allowed the emergence of pests and diseases that limit yield and lead to the eventual loss of production (Liu and Wang, 2020).

The tomato psyllid *Bactericera cockerelli* Sulc. (Hemiptera: Triozidae) is one of the most economically important and most destructive pests in nightshades (Tang *et al*., 2020). *B. cockerelli* is native to North America, is distributed from Canada, the United States of America, and Mexico, but is also found in several Central American countries and has been reported in New Zealand and Australia (Walker *et al*., 2015; Olaniyan *et al*., 2020).

B. cockerelli is established and distributed in the main agricultural areas of Mexico, except for the states of Colima, Yucatán, Campeche, Tabasco, Quintana Roo and Guerrero (Cadena, 1993; Vega *et al*., 2008; Swisher *et al*., 2013; Cerna *et al*., 2021). *B. cockerelli* is responsible for the 60%decrease in tomato production (Rivera-Martínez *et al*., 2018), since, when feeding, it injects toxins that cause leaf yellowing, short and thickened internodes, retardation of plant growth, and reduction in fruit size.

High populations of *B. cockerelli* secrete sugars in the feces, where sooty mold develops. In addition, it is the primary transmitter of *Candidatus* Liberibacter solanacearum and other phytoplasmas (Prager *et al*., 2018; Swisher *et al*., 2018; Sumner *et al*., 2020; Gutiérrez-Ramírez *et al*., 2021). The main control strategy for the insect is based on the use of chemical insecticides, which has led to the need for up to 30 applications in potato crops for the management of this pest (Cerna *et al*., 2012; Tucuch-Haas *et al*., 2020).

This practice has generated resistance in *B. cockerelli* populations, the elimination of natural enemies, and phytotoxicity (Cerna *et al*., 2015; Kolomiiets *et al*., 2019), as well as economic losses due to the management of the insect and the diseases it causes in the plant (Gudmestad and Secor, 2007; Greenway and Rondon, 2018). A biorational alternative to this problem is the use of botanical extracts with insecticidal properties, which are safer and have a broader spectrum of activity compared to synthetic pesticides (Barrios-Díaz *et al*., 2016; Venkatesh and Arivudainambi, 2024).

The genus *Crotalaria* includes about 600 species, distributed in the tropical and subtropical regions of the world. Species in this genus contain alkaloids, saponins, flavonoids, and pyrrolizidine alkaloids, to which some biological activity is attributed (Morton, 1994; Thoden *et al*., 2009; Miranda-Granados *et al*., 2018). A high content of pyrrolizidine alkaloids has been identified in this genus, such as monocrotaline, a compound that inhibits proteases in generalist herbivores associated with agricultural crops (Rech *et al*., 2022). These pyrrolizidine alkaloids have exhibited various biological activities in nematodes, including nematicides, ovicides or repelentes (Thoden *et al*., 2009).

In recent years, there have been reports of the insecticidal effect of the crude methanolic extract of *C. longirostrata* on *B. cockerelli* nymphs (López-López *et al*., 2022), as well as the effect of the acetone extract of *C. paniculata*, which has a potent insecticidal activity (60-73.33% mortality) and antifeedant activity against *Spodoptera litura*, where the insecticidal effect is attributed to the presence of flavonoids (Venkatesh and Arivudainambi, 2024). The species *C. retusa* has an insecticidal effect on *Callosobruchus maculatus*, with mortalities of 54% and a reduction in adult emergence of up to 62% (Obembe and Kayode, 2013). The dichloromethane extract from *C. pallida* seeds exhibits insecticidal activity on pupae and larvae of *Drosophila melanogaster* associated with the usaramine content (Peñaloza y Peláez, 2014).

This study aimed to evaluate the insecticidal effect of the methanolic extract of *C. longirostrata* and the fraction of the pyrrolizidine alkaloid (1β,2β-Epoxy-1α-methoxymethyl-8α-pyrrolizidine) supplied in liquid diets to *B. cockerelli*.

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Materials and methods

B. cockerelli colony

The insects were collected at the Autonomous University of Aguascalientes (UAA, by its Spanish acronym) in March 2024, in Jesús María, Aguascalientes. The maintenance, development and increase of the insect colony were carried out in entomological cages with tomato plants of the Río Grande variety, under a photoperiod of 14:10 h (light/dark) and 25 ±1 ° C (Roque-Enríquez *et al*., 2021).

Collection and preparation of *C. longirostrata* extract

Collection of *C. longirostrata* was performed in July 2023 in Chiapa de Corzo, Chiapas, Mexico, as described by Miranda-Granados *et al*. (2018), where stems with leaves were collected, and only the leaves were selected to be left to dry in the shade for seven days. The dehydrated leaves were pulverized in a laboratory blender (Waring Commercial, model 7011s), and the resulting powder was macerated in 96% methanol at a ratio of 0.2 g dry matter ml of solvent for 30 days. After 30 days, the macerate was vacuum filtered with Whatman No. 1 paper and stored at 4 °C until use (López-López *et al*., 2022).

Gas chromatography coupled to mass spectrometry (GC-MS

The determination of the metabolites in the methanolic extract was carried out by the Biogeochemistry Laboratory (UBIPRO) of the National Autonomous University of Mexico (UNAM), Iztacala Faculty of Higher Studies (FES, by its Spanish acronym), Mexico, where the metabolites present in the extract were identified in a gas chromatograph, model 6850 (Agilent Technologies, USA), using an HP-5MS column (Agilent) (30 mm, Ø= 25 mm) with a 0.25 µm film. The oven was programmed to 150 °C for 2 min, then increased by 10 °C min to 300 °C for 4 min. In the mobile phase, helium was used with a flow rate of 1 ml min^{-1}. The 5975C mass spectrometry detector (Agilent Technologies, USA) was set to full scan mode at a mass range of 35 to 400 m z^{-1}, with an ionization of 70 eV, at 230 °C as the ionization source temperature and 150 °C as the quadrupole temperature. The identification of the compounds was carried out with the National Institute of Standards and Technology (NIST) database version 08 MS.

Alkaloid fractionation

The methanolic extract was dehydrated in a drying oven at 50 °C until it maintained a stable weight. The oleoresin obtained was placed in a borosilicate glass column (Ø= 30 mm; 60 cm in length) in aliquots of 30 g added with 20 g of coarse silica, 60-200 mesh (Davisil®, grade 22, Sigma-Aldrich) to form the head of the column, 200 ml of dichloromethane:methanol (4:6) eluent was added, and these fractions were subjected to Dragendorff's, Wagner's, and Mayer's tests (Hernández *et al*., 2017) to corroborate the presence of alkaloids. The determination of the pyrrolizidine alkaloid in the fractionate was corroborated with the aforementioned GC-MS methodology.

Relative density of the extract

The relative density of the methanolic extract was determined as described by López-López *et al*. (2022) with a Gay-Lussac pycnometer (Glascco). The relative density of the extract was calculated with formula 1:

$$Relativedensity = \frac{m1 - m}{m2 - m} * d24t$$

1). Where: m= mass of the empty pycnometer (g); m1= mass of the pycnometer with the sample (g); m2= mass of the pycnometer with water (g); and *d24t* = the density of water at 24 °C (0.997299 g cm^{-3}). The relative density was expressed in mg ml^{-1}.

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Feeding bioassays

Feeding bioassays were performed in the Toxicology Laboratory of the Department of Parasitology of the Antonio Narro Autonomous Agrarian University, Mexico. The liquid diet was composed of 15% sucrose and phosphate buffer [1x] (Oh and Tamborindeguy, 2023), supplemented with 5, 10, 15, 20, 30, 40 and 50 mg ml^{-1} of *C. longirostrata* extract, preparing the same concentrations for the fraction of the pyrrolizidine alkaloid (López-López *et al*., 2022) and a control was included only with the liquid diet.

For the bioassay, newly hatched adult females were collected from the established colonies and placed in plastic chambers (height= 5 cm, Ø= 3 cm) that facilitated their feeding. The chambers were covered with a parafilm sheet, onto which 60 ml of the liquid diet described above was placed; this was replaced as required. Feeding bioassays were performed in triplicate with 22-25 individuals per replication and treatment, where insect mortality was counted every 24 h.

Data analysis

Mortality was corrected with the formula of Henderson and Tilton (1955). The Probit analysis was performed with mortality corrected for the concentration-mortality curve (LC_{50}). Mortality data were analyzed using an analysis of variance (Anova) and the comparison of means using Tukey's test ($p = 0.05$), under a completely randomized design, using the SAS statistical program, version 9.0.

Results and discussion

The detection of alkaloids in the fractionate yielded positive results in Dragendorff's, Wagner's and Mayer's tests. Gas chromatography indicated that the extract contained nine main compounds: 1β,2β-Epoxy-1α-methoxymethyl-8α-pyrrolizidine; 11-Tridecen-1-ol; Phenol, 4-(1-phenylethyl); Phenol, 2,4-bis(1-phenylethyl); Hexadecanoic acid methyl ester; n-Hexadecanoic acid; 9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z); 9,12,15-Octadecatrienoic acid, (Z,Z,Z), and Hexanedioic acid, bis(2-ethylhexyl) ester. These nine compounds represent 65.91% of the extract's composition; for its part, the pyrrolizidine alkaloid 11β,2β-Epoxy-1α-methoxymethyl-8α-pyrrolizidine was the most abundant, with 10%.

The detection of the pyrrolizidine alkaloid in the extract and in the fraction showed a retention time of 4 793 min in the CG-MS. The results of the feeding bioassay with liquid diets showed that, after four days of the psyllids feeding on the diet supplemented with *C. longirostrata* extract, mortality was significant ($p < 0.0001$) at the 50 mg ml^{-1} concentration with a mortality of 78.16% (Table 1) and for the pyrrolizidine alkaloid fraction bioassay, mortalities greater than 80% were obtained with the application of 10-50 mg ml^{-1}.

Table 1. Mortality of *B. cockerelli* in feeding bioassays.

Concentration (mg ml^{-1})	Mortality ± standard deviation	
	Extract	Pyrrolizidine
50	78.16 ±7.89 a	91.85 ±7.69 a
40	70.9 ±4.07 a	90.10 ±8.89 a
30	73.54 ±5.5 a	88.38 ±6.72 a
20	73.15 ±7.82 a	87.58 ±11.98 a
15	69.85 ±4.17 a	84.95 ±6.42 a
10	58.02 ±11.21 ab	80.95 ±8.05 a
5	42.17 ±11.33 bc	68.80 ±26.17 a
0	29.52 ±2.16 c	19.89 ±8.73 b
p -value	<0.0001	

Means followed by the same letter in each column show no statistical difference (Tukey, $p > 0.05$).

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The mean lethal concentrations (LC_{50}) obtained in the evaluations of the liquid diet for both the methanolic extract and the fractionate were 3.8 and 1.14 mg ml^{-1}, respectively, presenting greater toxicity in insects that fed on the liquid diet supplemented with the alkaloid fraction (Table 2).

Table 2. Estimation of LC_{50} in *B. cockerelli* in feeding bioassays.

Treatment	LC_{50}	Fiducial limits		Prediction equation	Coefficient of correlation
		Lower	Upper		
Extract	3.77	3.09	8.56	y= -0.675x + 0.87	0.649
Fraction	1.14	0.19	2.53	y= -0.50x + 0.87	0.674

In recent research, other pyrrolizidine alkaloids have been detected, such as trachelanthamidine and 1β,2β-Epoxy-1α-methoxymethyl-8α-pyrrolizidine as the most abundant compound in aqueous, ethanolic, and methanolic extracts in *C. longirostrata* leaves (López-López *et al*., 2022; Hernández-Reyes *et al*., 2024). The alkaloid 1β,2β-Epoxy-1α-methoxymethyl-8α-pyrrolizidine is classified as an iminosugar, which is a hydrophilic compound easily administered orally that acts as an inhibitor of carbohydrate-modifying glucosidase enzymes in the absorption and assimilation in the digestive tract, and as an inhibitor in the synthesis of polysaccharides (glycosyltransferases). This inhibitor interferes with the hydrolysis processes of glycosidic bonds in humans and insects, so its potential as an insecticide is high (Ramesh, 2020).

The results obtained with the liquid diet supplemented with the methanolic extract were inferior to those obtained by López-López *et al*. (2022), who reported a mortality of 24-100% at concentrations of 2-30 mg ml^{-1} 72 h after applying the chipilín extract; in contrast, the fraction demonstrated a toxicity similar to that reported by López-López *et al*. (2022); these same authors established an LC_{50} of 4.78 mg ml^{-1} in the leaf immersion bioassays; on the other hand, in the present research, for the administration of the crude extract, the LC_{50} obtained was 3.77 mg ml^{-1} and for the fraction, it was 1.14 mg ml^{-1} (Table 2).

The biocidal effect of pyrrolizidine alkaloids, such as monocrotaline, in various nematode species has been effective, 0.01-10 mg ml^{-1} concentrations with a MIC50 of 0.4-5.8 mg m^{-1} (Thoden *et al*., 2009). According to assessments by Hernández-Reyes *et al*. (2024), who determined the dose-response curves in zebrafish (*Danio rerio*), the water extract of *C. longirostrata* leaves showed toxicity with an LC_{50} of 2.41 µg ml^{-1}, followed by the EtOH-water extract with an LC_{50} of 2.49 µg ml^{-1} and the EtOH extract with an LC_{50} of 3.41 µg ml^{-1}, while oleoresin presented the lowest toxicity in the evaluation with an LC_{50} of 4.99 µg ml^{-1}. These authors have attributed the toxic effect to the presence of the pyrrolizidine alkaloid trachelanthamidine. The lethal concentrations obtained for the ethanolic extract are similar to those observed in this research.

The insecticidal effect of *C. longirostrata* extracts and fractionates is attributed to the ability of pyrrolizidine alkaloids to interact with microorganisms in the digestive tract of insects, causing the insect's gut to become alkaline, killing the insect (Fürstenberg-Hägg *et al*., 2013; Tlak Gajger and Dar, 2021). They are also associated with the ability of pyrrolizidine alkaloids to inhibit the trehalase enzyme in the small intestine of the insect, which can cause a decrease in energy generation through the hydrolysis of trehalose and affect the biological processes of growth, chitin synthesis, metamorphosis, and energy for flight; therefore, pyrrolizidine alkaloids may be a promising option as insecticides or larvicides (Shukla *et al*., 2015).

Conclusions

The application of the methanolic extract of *C. longirostrata* and the fraction of the pyrrolizidine alkaloid (1β,2β-Epoxy-1α-methoxymethyl-8α-pyrrolizidine) in liquid diets was efficient in the mortality of *B. cockerelli* with 78.1 and 91.85%, respectively. The greatest toxicity was recorded with the application of the fractionate (LC_{50} of 1.14 mg ml^{-1}) of the pyrrolizidine alkaloid through feeding bioassays.

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