

## Efficacy of *Libidibia ferrea* var. *ferrea* and *Agave sisalana* Extracts against *Dactylopius opuntiae* (Hemiptera: Coccoidea)

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### Abstract

The carmine cochineal (*Dactylopius opuntiae*) is an insect-plague of *Opuntia ficus-indica* palm crops, causing losses in the production of the vegetable used as forage for the Brazilian semiarid animals. The objective of this work was to analyze the efficacy of plant extracts, insecticides and their combination in the control of *D. opuntiae*. Leaf and pod extracts of *Libidibia ferrea* var. *ferrea*, *Agave sisalana* leaf at concentrations 10, 25, 50, 100 and 200 mg/mL, and insecticides (chlorpyrifos, acetamiprid, thiamethoxam and lambda-cyhalothrin) at concentrations minimum, median and maximum; were applied on cladodes infested by *D. opuntiae*. After 10 days of treatment, the percentages of mortality and LC<sub>50</sub> (concentration to kill 50% of cochineal) were obtained on the stages of nymphs II and adult females. The association between the LC<sub>50</sub> of the plant extracts with the insecticides on the cochineal it was tested. The results showed that *L. ferrea* extracts were more effective against cochineal, causing the 81% of nymphal II mortality (LC<sub>50</sub>: 20 to 160 mg/mL) and 97% of adult females (LC<sub>50</sub>: 43 to 50 mg/mL), whereas the extracts of *A. sisalana* only controlled adult females, causing the mortality from 51 to 97% (LC<sub>50</sub>: 17 to 46 mg/mL). The insecticide chlorpyrifos was the most efficient on nymphs and adult females cochineal (LC<sub>50</sub>: 0,084 mL/L). The combination of this insecticide with the extracts promoted a percentage of mortality above 90%. The extracts presented insecticidal potential on *D. opuntiae* and can be tested isolated or in combination with insecticides in control of cochineal.

**Keywords:** *Agave sisalana*, cochineal, insecticide, integrated pest control, *Libidibia ferrea*, plant extract

### 1. Introduction

The cactus *Opuntia* are used for various purposes such as food (fruit and fresh or dried cladodes) and forage for animals producing milk. They are also used as a substrate for the creation of the gender cochineal *Dactylopius*, producers of carminic acid (Medina, Rodríguez, & Romero, 2007). Among these, *Opuntia ficus-indica* (L.) Mill. (Caryophyllales: Cactaceae), a cactaceous of Mexican origin it is an important for the economic development of arid and semiarid areas, particularly in Latin America, mainly in Brazil and Mexico, where the palm is widely used as a food source, especially for animals of dairy production, because of their content water ( $\pm$  90%) and nutrients as soluble carbohydrates, calcium and vitamins (Ennouri, Fetoui, Bourret, Zeghal, & Attia, 2006; Vilela et al., 2010; Borges et al., 2013; Perez-Ramirez, Castrejón-Ayala, & Jiménez-Pérez, 2014). The nutritional significance of the palm fruit give the ascorbic acid, content of fibers and free amin acids (Stintzing, Schieber, & Carle, 2001), and the seed accumulates protein before fruit ripening (Walker, Famiani, Baldicchi, Cruz-Castillo, & Inglese, 2011), with palm potential in medicine due to its anti-inflammatory action, antioxidant and prevention

of ulcers (Galati, Mendello, Giuffrida, & Miceli, 2001; Park, Kahng, Lee, & Shin, 2001; Lee, H. R. Kim, J. Kim, & Jam, 2002).

Diverse species of *Opuntia* cactus are parasitized by *Dactylopius opuntiae* Cockerell (Hemiptera: Coccoidea), including *O. ficus-indica* and *O. cochenillifera* (L.) (Chaves-Moreno, Tecante, & Casas, 2009). In countries such as Peru, the Canary Islands, and Mexico, carmine cochineal is used to produce the natural coloring carmine that is used in the food industry; it can also be used in the biological control of some invader cactus species (Hoffmann, Moran, & Zimmermann, 1999; Volchanski, Hoffmann, & Zimmerman, 1999; Paterson et al., 2011). This insect attacks cultivated cacti in Brazil, Mexico, and other countries, causing severe losses of forage production and fruits, resulting in economic losses to farmers (Vigueras, Tovar, & Pelayo-Ortiz, 2009; Silva, Mergulhão, Medeiros, Figueiredo, & Burity, 2013; Bouharroud, Amarraque, & Qessaoui, 2016; Tiago et al., 2016).

The use of chemical insecticides in pest control presents immediate results, however, can cause environmental imbalance, toxic residues in foods, diseases in humans and other animals and development of resistance mechanisms in insects (Pourseyed, Tavassoli, Bermousi, & Mardani, 2010), and in the case of cochineal and the palm, there is not chemicals registered in Brazil. However, the use of chemical insecticides for the control of large outbreaks *D. opuntiae* infestation is recommended (Santos et al., 2006), and the use of resistant cultivars palms to keep the cochineal insect pest at levels which do not damage and increase the productivity of plantations (Borges et al., 2013; Fação, Oliveira, Mergulhão, Silva, & Santos, 2013). In addition, an alternative to be tested for the control of *D. opuntiae* is the use of plant extracts.

Plant species are resistant to insect attack and this resistance can be mediated by the production and action of primary and secondary metabolites, such as proteins (lectins), alkaloids, tannins, and terpenoids (Souza et al., 2011). These substances have insecticidal action more beneficial compared to chemicals because they are renewable, easily degradable and do not contaminate the environment (Oliveira, Lins-Neto, Araújo, & Albuquerque, 2007). Previous studies have reported the action the of extracts and vegetable oils and other bioactive substances in control of nymphs and adults of *D. opuntiae* (Vigueras et al., 2009; Vázquez-García, Garabito-Espinoza, Tabares-Vega, & Castillo-Herrera, 2011; Borges et al., 2013; Pérez-Ramírez et al., 2014; Santos, Oliveira, Costa, Tiago, & Oliveira, 2015).

*Libidibia ferrea* var. *ferrea* (Mart. Ex Tul.) L.P. Queiroz (= *Caesalpinia ferrea*) (Fabales: Fabaceae) is a legume tree distributed in the semiarid region of the North and Northeast of Brazil, known as ironwood or “jucá”, being used in the pharmaceuticals industry, in construction and in folk medicine (Lorenzi, 2002; Queiroz, 2010). The chemical components present in the leaves, fruits and roots of *L. ferrea* exhibit anti-inflammatory, analgesic, antibacterial and healing properties and may be insecticidal action (Trentin et al., 2011; Freitas et al., 2012; Araújo et al., 2014; Carvalho, Sampaio, Araújo, Pinto, & Rocha, 2016). In turn, the sisal, *Agave sisalana* Perrine ex Engelm (Asparagales: Agavaceae) is an herbaceous plant, native to Central America and Mexico and it is found in many tropical countries, such as Tanzania and Brazil (Chand, Tiwary, & Rohatgi, 1988). Liquid waste and secondary sisal metabolites (alkaloids, saponins and tannins) have insecticidal action, and this action proved by the use of raw juice and extracts of *A. sisalana* in larvae of mosquitoes *Aedes aegypti* (L.) and in the control of spider mite *Tetranychus urticae* (Koch.) (Barreto, Araújo, & Bonifácio, 2010; Nunes et al., 2015).

The need to minimize economic losses caused by the cochineal, especially in Northeastern Brazil, has encouraged researchers to seek new alternatives to control this insect. Therefore, this study aimed to investigate the potential of *L. ferrea* and *A. sisalana* extracts and, chlorpyrifos, acetamiprid, thiamethoxam, and lambda-cyhalothrin insecticides as well as the combination of extracts and insecticides in controlling *D. opuntiae*.

## 2. Material and Methods

### 2.1 Insect Collection and Rearing

The cochineal *D. opuntiae* was collected in plantations of *O. ficus-indica* in the municipalities of “Sertão do Moxotó and Pajeú”, Pernambuco State, Brazil. The insects were raised in culture chambers at 28±1 °C, with 37±3% relative humidity (RH), and 12 h photoperiod, on healthy cactus cladodes placed horizontally on wooden supports and infested with first stage nymphs. The biological trials used second stage nymphs and adult females of *D. opuntiae* after 12 and 40 days of infestation respectively, according to the durations of the biological cycle of *D. opuntiae* (Flores-Hernández et al., 2006). The cladodes with adult females were sprayed with a 2% solution of detergent before assay to improve contact of the suspensions with the insect bodies.

## 2.2 Preparation of Plant Extracts

The leaves and pods *L. ferrea* and leaves of *A. sisalana* specimens were collected in Recife (Pernambuco/Brazil). The species were identified in Botanic Department at Agronomic Institute of Pernambuco (8°03'50.2"S, 34°55'29.2"W), and after that the botanical material was deposited in Dárdano Andrade Lima Herbarium. After collection, the material was washed in distilled water to remove impurities, dried at room temperature and triturated. To obtain the aqueous extract of the leaves and pods of *L. ferrea* (AELLf and AEPLf), 20 g of plant material were mixed with 80 mL of 0.15 M NaCl solution to a final concentration of 200 mg/mL (w/v). The suspension was stirred 16 hours at 4 °C, then filtered and the extract was subjected to centrifugation at 10.000 rpm for 15 minutes at 4 °C. For the methanol extract of *L. ferrea* (MELLf and MEPLf), 20 g of plant material were subjected to infusion methanolic (Sigma-Aldrich®) (80 mL) with stirring for 24 hours, the suspension filtered and the extract subjected to a rotary evaporator. For *A. sisalana* were made the aqueous extracts (AELAs) and hydroethanolic extract (HELAs), the aqueous extract obtained according to the protocol used for *L. ferrea* extract. For hydroethanolic extract, 20 g of vegetable underwent ethanolic infusion (Sigma-Aldrich®) at 70% (80 mL) for two hours, and then filtered. Then, the alcohol was evaporated for 16 hours at 45 °C. The extracts at 200 mg/mL were diluted with Tween 80 (0.1%) solution to obtain concentrations of 100, 50, 25 and 10 mg/mL.

## 2.3 Chemical Insecticides Used in the Experiments

The chemical insecticides chlorpyrifos, acetamiprid, thiamethoxam, and lambda-cyhalothrin have been widely used for controlling Hemiptera insects such as cochineal, and are readily available commercially. These insecticides were tested at three different concentrations based on the recommendations of the manufacturers, with the median concentration (CMed) corresponding to that recommended for field use; the minimum concentrations (CMed/2) and maximum concentrations (CMedx2) are listed in Table 1.

## 2.4 Effect of Chemical Insecticides and Plant Extracts on *Dactylopius opuntiae*

For the *D. opuntiae* bioassays, cladodes infested with nymphs and adult females were sprayed with 10 mL of the extract concentrations, insecticides and with Tween 80 (0.1%) solution (control) using a De Vilbiss n° 15 manual sprayer. After spraying, the cladodes were placed in container (30 cm × 15 cm × 08 cm) and maintained at room temperature (28±1 °C), with 37±3% RH, for 10 days, then 50 adult females and 50 nymphs were collected from 64 cm<sup>2</sup> of each palm (8 cm × 8 cm). The experiments were performed in triplicate, totaling 150 females and 150 nymphs per treatment. The mortality counts were made using a stereo microscope, considering as dead those insects demonstrating color modifications, dehydrated or flaccid bodies, or without any movement. Then they determined the percentage of mortality and the Lethal Concentration (LC<sub>50</sub>).

## 2.5 Association Action between Plant Extracts and Pesticides on *Dactylopius opuntiae*

For this bioassay, the suspensions were made with the combination of LC<sub>50</sub> of each extract to LC<sub>50</sub> of insecticides (Table 3). Then palms infested with nymphs and adult females were sprayed with 10 mL of suspensions associated and for the treatment control, palms were sprayed with only the extracts, insecticides and Tween 80 (0.1%) solution (control). The palms were transferred to plastic containers and maintained at room temperature (28±1 °C), with 37±3% RH, for 10 days, then 50 adult females and 50 nymphs were collected from 64 cm<sup>2</sup> of each palm (8 cm × 8 cm), made three evaluations for treatment and mortality percentage nymphs and adult females was determined.

## 2.6 Statistical Analyses

Statistical analyses of the data were performed using the analysis of variance (ANOVA) and compared using the Tukey's test at a 5% probability level of significance, using the Proc ANOVA software from SAS (SAS Institute, 1999-2001). The mean lethal concentration (LC<sub>50</sub>) was determined by Proc Probit software (SAS Institute 1999-2001). The graphs were performed by the Prisma 6.0 software (Software GraphPad).

## 3. Results

All insecticides caused nymphal mortality and differ from the control treatment showed that mortality from 8% (p = 0.05). The insecticide chlorpyrifos was the most efficient, causing 93% nymphal mortality with the lowest concentration (0.75 mL/L), not differing from the other concentrations (1.5 and 3.0 mL/L). The other insecticides caused mortality from 53% to 80%, and these means differ between the tested and the control treatment concentrations, the effect being directly proportional to pesticide concentration increases (p = 0.05). The insecticides chlorpyrifos (82% to 97%) and lambda-cyhalothrin (62% to 99%) were the most effective against cochineal adult females, with means of mortality differing between concentrations and with control (p = 0.05), and the control treatment showed an average mortality of 15% (Table 2). The insecticides acetamiprid and

thiamethoxam caused mortality between 60% and 78% of adult females, with no statistical difference between the tested concentrations, but between these and the control there was statistical difference ( $p = 0.05$ ).

The extracts *L. ferrea* had a positive effect on nymphs and adult females of *D. opuntiae* (Figure 1), being more efficient with increasing concentration ( $p = 0.05$ ). The MEPLf and MELLf showed higher insecticidal potential the nymphs on with average mortality of 82% and 80% respectively at the concentration of 200 mg/mL, differing ( $p = 0.05$ ) from the control treatment (12%) (Figures 1c and 1d). The extracts from the leaves *L. ferrea* (AELLf and MELLf) exhibited greater insecticidal effect on adult females of *D. opuntiae*, because with the higher concentration (200 mg/mL) caused mortality of 97% and 81% respectively, and all treatments differed statistically ( $p = 0.05$ ) from the control (16% and 19%) (Figures 1b and 1d).

Table 1. Chemical pesticides utilized for controlling cochineal (order Hemiptera).

Product's name	Type of formulation	Chemical Type	Constituent Active	Concentration recommended by the manufacturer (L)		
				Minimum	Median	Maximum
Lorsban 480 BR	Emulsifiable concentrate	Organophosphate	Chlorpyrifos	0.75 mL/L	1.5mL/L	3.0 mL/L
Mospilan	Soluble powder	Neonicotinoid	Acetamiprid	0.125 g/L	0.25 g/L	0.5 g/L
Actara 250 WG	Dispersible granules	Neonicotinoid	Thiamethoxam	0.10 g/L	0.20 g/L	0.40 g/L
Karatê ZEON 250CS	Encapsulated suspension	Pyrethroid	lambda-cyhalothrin	0.50 mL/L	1.0 mL/L	2.0 mL/L

Table 2. Percentage mortality nymphs and adult females of *Dactylopius opuntiae* treated with insecticides, in different concentrations.

Insecticide	Mortality (%) <sup>1</sup>							
	Nymphs				Adult females			
	Concentration				Concentration			
	Control	Minimum	Median	Maximum	Control	Minimum	Median	Maximum
Chlorpyrifos	8b	93a	97a	100a	15c	82ab	92b	97a
Acetamiprid	8b	60a	70a	75a	15b	60a	68a	70a
Thiamethoxam	8c	64a	80ab	74b	15b	75a	77a	78a
lambda-cyhalothrin	8c	53a	67ab	77b	15d	62c	80b	99a
CV (%) <sup>2</sup>	15.45				5.58			

Note. <sup>1</sup>Means followed by the same letters do not significantly differ at a 5% probability level by the Tukey test. <sup>2</sup>CV: coefficient of variation.

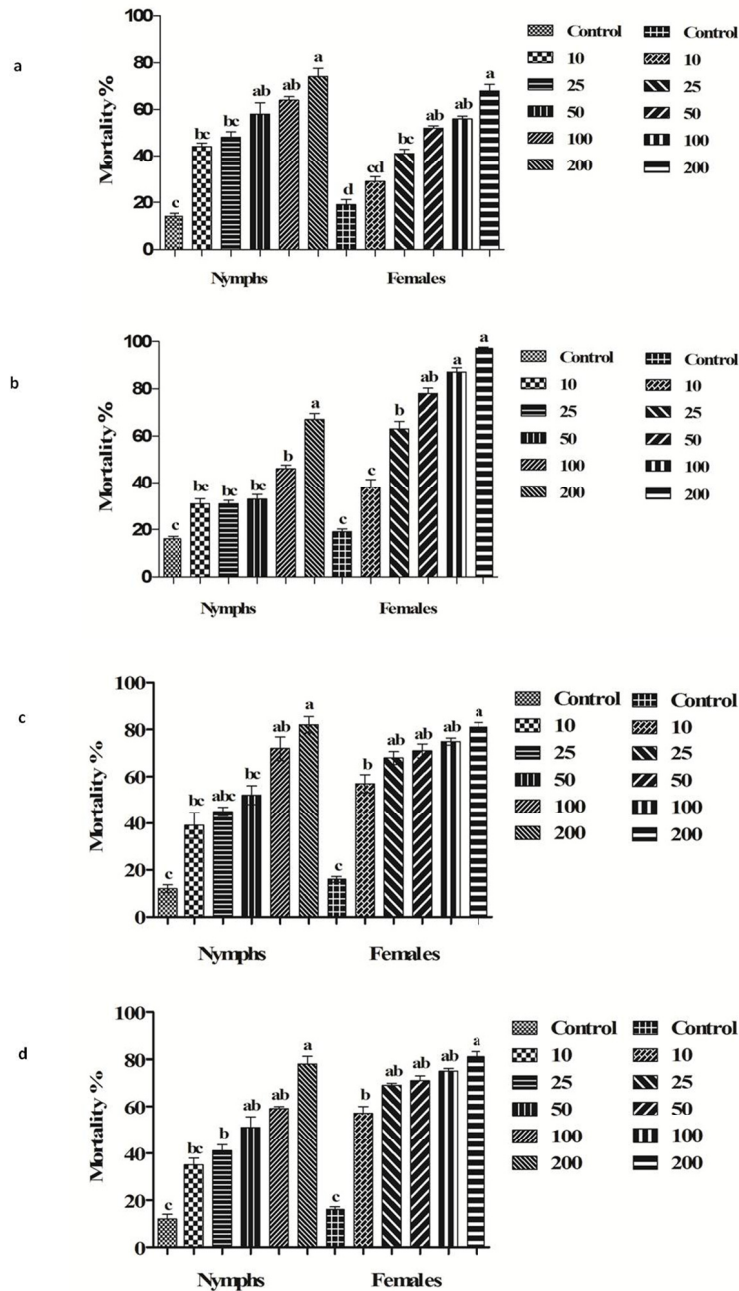


Figure 1. Mortality of nymphs and adult females of *Dactylopius opuntiae* treated by *Libidibia ferrea* extracts (mg/mL). AEPLf (a); AELLf (b) MEPLf (c); MELLf (d)

Note. Different letters in bars differ statistically at a 5% probability level by the Tukey test.

The AELAs and HELAs were not efficient in the control of nymphs from the carmine cochineal, showing average mortality ranging from 24% to 48% at the concentrations tested ( $p = 0.05$ ) (Figures 2a and 2b). On the other hand, the HELAs was more efficient in the control of adult females, causing mortality of 51% to 95%. The HELAs got greater potential insecticide on adult females with increasing concentrations and was statistical difference ( $p = 0.05$ ) between the mean concentrations and the control treatment (10%) (Figure 2b).

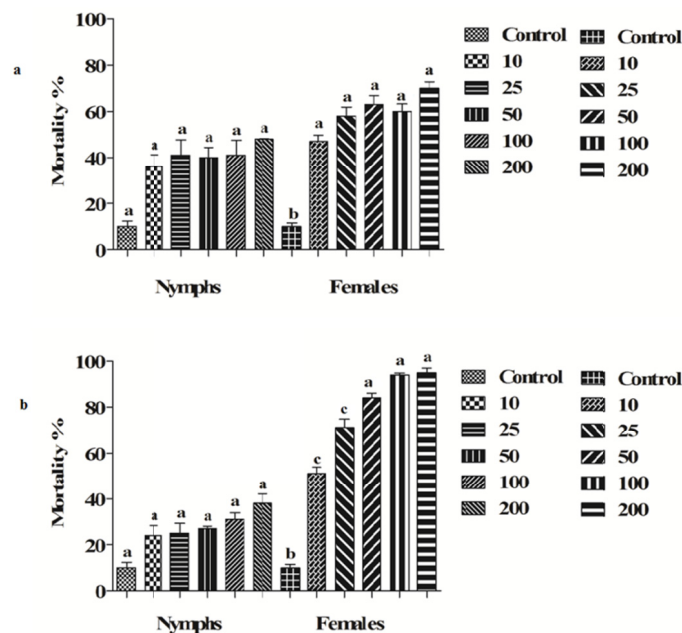


Figure 2. Mortality of nymphs and adult females of *Dactylopius opuntiae* treated *Agave sisalana* extracts (mg/mL). AELAs (a); HELAs (b)

Note. Different letters in bars differ statistically at a 5% probability level by the Tukey test.

It was observed that the insecticides and plant extracts caused degradation of the protective wax of the body of nymphs and adult females of *D. opuntiae* and subsequent dehydration and their mortality (Figure 3).

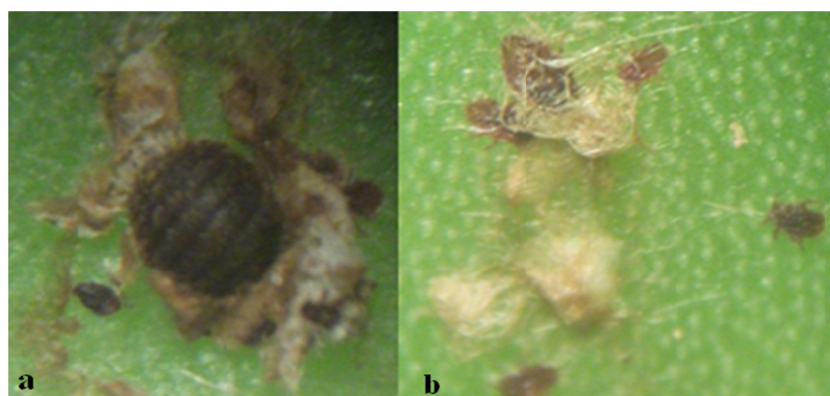


Figure 3. Effect methanolic leaves extract of *Libidibia ferrea* on adult female (a) and nymphs (b) of *Dactylopius opuntiae*

The values of lethal concentrations of plant extracts and pesticides on the nymphs and adult females of *D. opuntiae* are shown in Table 3. The LC<sub>50</sub> values presented by the insecticide were much lower than the concentrations (minimum, median and maximum) of these tested on nymphs and adult females. The MEPLf and MELLf showed lower LC<sub>50</sub> values for nymphs (20 mg/mL and 32 mg/mL, respectively), while the lowest LC<sub>50</sub> values for adult females were 43 mg/mL (AELLf) and 17 mg/mL (HELAs).

Table 3. Lethal concentration (LC<sub>50</sub>) of chemical insecticides and plant extracts on *Dactylopius opuntiae*.

	Nymphs			Adult females		
	LC <sub>50</sub> (CI) <sup>1</sup>	Regression Equation	(χ <sup>2</sup> ) <sup>2</sup>	LC <sub>50</sub> (CI)	Regression Equation	(χ <sup>2</sup> )
Chlorpyrifos	0.084 mL/L (0.011-0.062)	Y=6.69896+1.58005*logX	13.30	0.084 mL/L (0.115-0.0598)	Y=6.14107+1.06547*logX	34.83
Acetamiprid	0.883 g/L (0.108-0.070)	Y=6.37602+1.30568*logX	40.66	0.054g/L (0.067-0.038)	Y=6.32907+1.03806*logX	40.66
Thiamethoxam	0.089 g/L (0.108-0.724)	Y=6.39356+1.32660*logX	28.82	0.072 g/L (0.094-0.54)	Y=6.04968+0.92081 *logX	14.67
Lambda-Cyhalothrin	0.348 mL/L (0.464-0.256)	Y=5.40824+0.89150*logX	40.51	0.127 mL/L (0.169-0.092)	Y=5.93508+1.04520*logX	29.30

Plants extracts	Nymphs			Adults females		
	LC <sub>50</sub> mg/mL (CI)	Regression Equation	(χ <sup>2</sup> )	LC <sub>50</sub> mg/mL (CI)	Regression Equation	(χ <sup>2</sup> )
AEPLf	150 (240-9.15)	Y=4.92593+0.41688*logX	38.36	46 (8.15-2.80)	Y=4.73885+0.39348*logX	29.05
AELLf	160 (38.36-8.54)	Y=4.56417+0.36212*logX	40.17	43 (0.61-0.28)	Y=5.24981+0.68520*logX	77.36
MEPLf	20 (2.82-1.48)	Y=4.79742+0.64363*logX	67.97	50 (0.75-0.33)	Y=5.16841+0.58266 *logX	25.34
MELLf	32 (4.74-2.24)	Y=5.40824+0.89150*logX	45.64	47 (0.71-0.29)	Y=5.17571+0.25889*logX	21.65
AELAs	-	-	-	17 (2.51-1.17)	Y=4.87128+0.37508*logX	36.42
HELAs	-	-	-	46(0.62-0.33)	Y=5.29697+0.90229*logX	47.96

Note. <sup>1</sup> 95% Confidence interval. <sup>2</sup> Chi-square test, as calculated by Probit.

The Figures 4, 5 and 6 show the potential involvement of the LC<sub>50</sub> extracts of *L. ferrea* and *A. sisalana* with the LC<sub>50</sub> of chlorpyrifos insecticides, acetamiprid, thiamethoxam and lambda-cyhalothrin on *D. opuntiae*. The associations of the extracts with insecticides were efficient on the nymphs and adult females, occurring a synergism in the association, in general, with the increase in the percentage of insect mortality compared with the products tested alone, with the highest mortality *D. opuntiae* was caused by the combination of extracts with insecticide chlorpyrifos (above 90%), which differed from other treatments and control (p = 0.05).

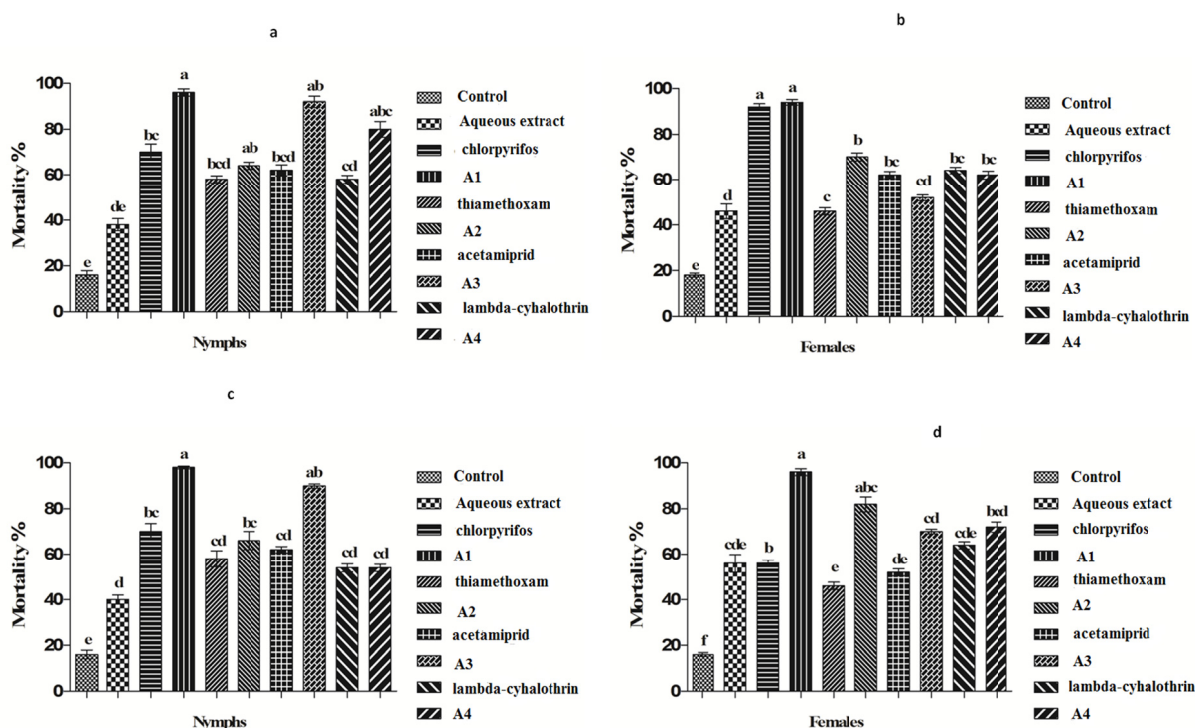


Figure 4. Effect of insecticides and aqueous extracts of *Libidibia ferrea* association on nymphs and adult females of *Dactylopius opuntiae*: AELLf (a and b) and AEPLf (c and d). Treatments: Control, LC<sub>50</sub> of extracts, LC<sub>50</sub> and LC<sub>50</sub> of insecticides associations (A1: extract + chlorpyrifos, A2: thiamethoxam+extract, A3: extract + acetamiprid, A4: extract + lambda-cyhalothrin)

Note. Different letters in bars differ statistically at a 5% probability level by the Tukey test.

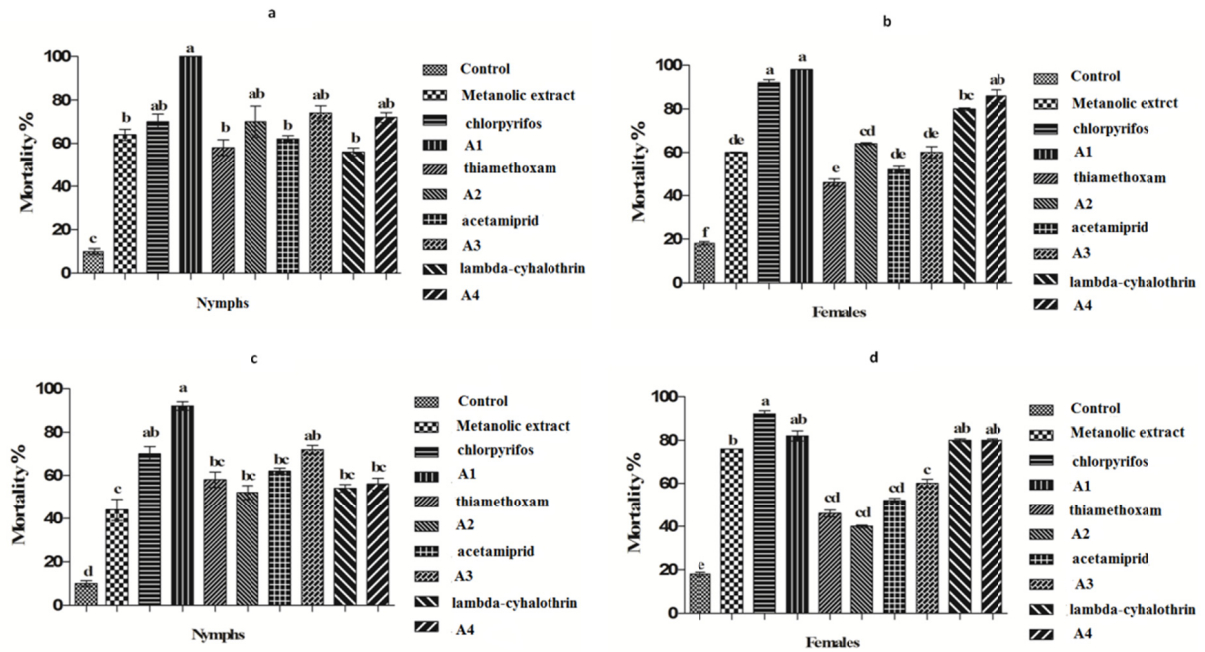


Figure 5. Effect of insecticides and methanol extracts of *Libidibia ferrea* association on nymphs and adult females of *Dactylopius opuntiae*: MELLf (a and b) and MEPLf (c and d). Treatments: Control, LC<sub>50</sub> of extracts and insecticides, LC<sub>50</sub> and LC<sub>50</sub> of insecticides associations (A1: extract + chlorpyrifos, A2: thiamethoxam + extract, A3: extract + acetamiprid, A4: extract + lambda-cyhalothrin)

Note. Different letters in bars differ statistically at a 5% probability level by the Tukey test.

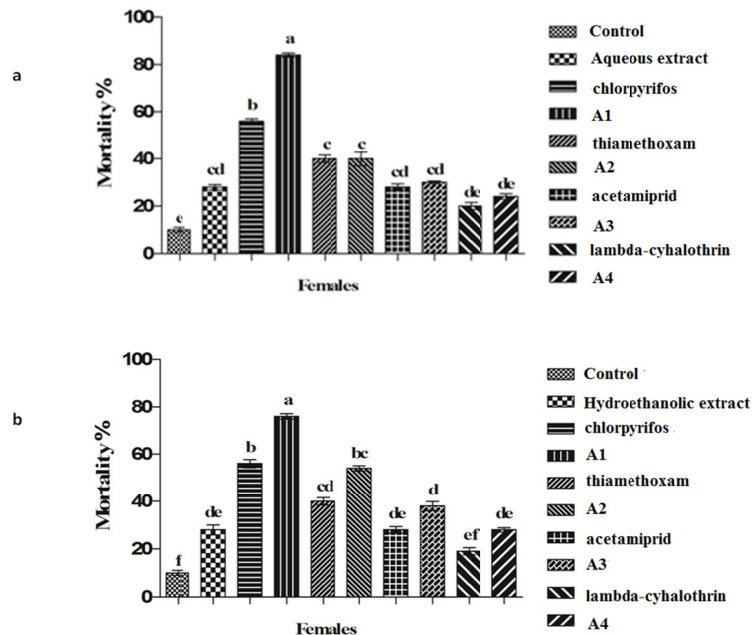


Figure 6. Effect of combination of insecticides with the aqueous extract (a) and hydroethanolic (b) of *Agave sisalana* on adult females of *Dactylopius opuntiae*. Treatments: Control, LC<sub>50</sub> of extracts, LC<sub>50</sub> and LC<sub>50</sub> of insecticides associations (A1: extract + chlorpyrifos, A2: thiamethoxam+extract, A3: extract + acetamiprid, A4: extract + lambda-cyhalothrin)

Note. Different letters in bars differ statistically at a 5% probability level by the Tukey test.



#### 4. Discussion

Pesticides and plant extracts caused the nymphs and adult females of *D. opuntiae* mortalities, at the concentrations tested. Palacios-Mendonza, Nieto-Hernández, LlanderaL-Cázares, and Gonzáles-Hernández (2004) and Viguera et al. (2009) have reported the importance of degradation of the wax covering the body of the nymphs and adults female cochineal carmine of biodegradable products such as detergents and plant extracts, which cause dehydration and subsequent death of *D. opuntiae*. It was observed the degradation of the wax that covers the body of nymphs and adult females of *D. opuntiae*, after application of insecticides and especially of *L. ferrea* and *A. sisalana* extracts, and therefore, dehydration and death of the insects. These data are similar to those reported by Nobel (2001) and Flores-Hernández et al. (2006) which reported the products tested on the control of *D. opuntiae* present substances that degrade the wax layer, which is able to repel aqueous solutions and protect the insect. Santos et al. (2006) recommended for the control of large focus of *D. opuntiae* infestation, first cutting and incineration of cladodes contaminated, and the use of chemical products such as imidacloprid, triacloprid, thiamethoxam, chlorpyrifos, acetamiprid, carbaryl and lambda-cyhalothrin. Thus, it becomes necessary to establish a minimum concentration of these insecticides to minimize the impending risks of these products to the environment, or to use them in combination with other forms of control.

The thiamethoxam, chlorpyrifos, acetamiprid and lambda-cyhalothrin insecticides were effective on nymphs and adult females. The chlorpyrifos showed more potential of control carmine cochineal, promoting the death of more than 90% of the insects with the lower concentration (LC<sub>50</sub> 0.084 mg/mL) for nymphs and adult females. The insecticide efficiency is determined by the minimum chemical concentration necessary to cause insect death and this variable concentration according to existing products, various physiological reactions of insects, among other causes. There are few studies on the effects of pesticides and the minimum concentration to control *D. opuntiae*, and the data found in this research are important for the development of efficient techniques to control this insect. Vigueiras et al. (2009) reported that the insecticide Agrobion® 400 at 3% promoted the death of 85% and 100% of nymphs and adult females of *D. opuntiae*, respectively.

With the exception of the AELAs, and extracts HELAs to the nymphs, all other extracts showed insecticidal activity against nymphs and adult females of *D. opuntiae*, suggesting that these are formed by primary or secondary metabolites that have biopesticide potential against insect since caused the death of nymphs and adult females at the concentrations tested. The vegetables produce bioactive substances that are harmless to man and the environment and are considered alternative sources in the discovery of natural insecticides (Luna et al., 2005; Omena et al., 2007). These substances, such as lectins, alkaloids, tannins, terpenoids, glycosides, phenolic, phenylpropanoids must have attractive properties, deterrents and insecticide (Cheng, Chang, Wu, & Chang, 2007; Melo-Santos, Araújo, Rios, & Regis, 2009; Souza et al., 2011). The MEPLf and MELLf extracts were the most efficient in the control of nymphs, and this action possibly mediated by secondary metabolites of lipid origin, not found in the aqueous extracts tested, because these substances are extracted only by organic solvents such as methanol.

The insecticidal activity of *L. ferrea* extracts can be explained by the toxicity presented by their primary and secondary compounds. Previous chemicals studies leaf extract, stem and bark *L. ferrea* reported the presence of flavonoids, saponines, tannins, gallic acid, coumarins, steroids and phenolic compounds (Gonzales, Barros, & Bach, 2004; Wyrepkowski et al., 2014). According Vázquez-García et al. (2011) the toxicity of oils essential and plant extracts of *Ocimum basilicum* L., *Mentha spicata* L., *Cymbopogon winterianus* (Jowitt) and *Lippia graveolens* Kunth on first instars *D. opuntiae*, *M. spicata* were the most efficient. Due to its characteristics of toxicity and repellency of insects, commercial terpenoids Egenol 99% (Across Organics, Mexico), 1.8-Cineol 99% and 99% Menthol (Sigma Aldrich, Mexico) were analyzed on *D. opuntiae*, and products significantly reduced the insect fixation on the palm of cladodes (Pérez-Ramírez et al., 2014).

The extracts *L. ferrea* leaves (AELLf and MELLf) and HELAs were more bioactive against adult females, at the concentrations tested. Vigueiras et al. (2009) found similar results when they tested the *Chenopodium ambrosioides* L., *Mentha piperita* L., *M. viridis* L., *Tagetes erecta* L. and *T. florida* Sweet extract on *D. opuntiae*, with the mortality rate of between 35% nymphs and 98% for adult females, suggesting that the terpenoids present in extracts may be responsible for toxicity. Similarly, Santos et al. (2015) observed the effect of aqueous and hydroethanolic extracts *Ricinus communis* L. and *Poincianella pyramidalis* (Tul.) LP Queiroz (5, 10 and 20%) on the females of *D. opuntiae*, and observed for mortality 61.23% to 100%. The neem oil action was tested on colonies of *D. opuntiae*, in concentrations of 1%, 2% and 3% causing significant reduction of the insects, with the elimination of most colonies of *D. opuntiae* on cladode (Gorlach-Lira & Lira, 2011). Also, Borges et al. (2013) verified the effect of neutral detergent, cassava starch, of liquid waste generated from the processing of cassava, mineral oil and neem the control of cochineal, neem oil being the most efficient. The extracts of *L.*

*ferrea* and *A. sisalana* can be tested for cochineal control program in palm plantations in semiarid areas, alone or with other control methods, such as traditional control-based chemicals. Previous studies demonstrate the importance of using alternative products, replacing or linking to chemical insecticides, because of low toxicity to humans and the environment (Borges et al., 2013; Silva et al., 2015).

The association of *L. ferrea* and *A. sisalana* extracts with chlorpyrifos, acetamiprid, thiamethoxam and lambda-cyhalothrin insecticides was efficient in controlling *D. opuntiae* in particular the combination of extracts with chlorpyrifos insecticide, killing more than 90% of adult females and nymphs cochineal. The results show positive synergism of products in insect control, it is possible to decrease the concentrations of these, and especially pesticides, aiming thus a smaller impact on the environment when the products are tested on infested palm plantations by *D. opuntiae*. Likewise, Santos et al. (2015) verified the insecticidal action of the *R. communis* and *P. pyramidalis* extracts together with *Fusarium incarnatum-equiseti* species complex (FIESC) on *D. opuntiae*, and found more efficient fungus association with the *R. communis* aqueous extract, with mortality percentage of 100% *D. opuntiae*. The insecticides with the active principles pyrethroid, organophosphate and neonicotinoid were effective against nymphs and adult females of cochineal citrus, *Praelongorthezia praelonga* Douglas. The pesticides associations (bifenthrin chlorpyrifos + mineral oil and lambda-cyhalothrin methidathion + mineral oil) caused mortality of 91.50% and 95.60%, respectively, after 14 days of treatment, which shows the synergistic effect of the tested products (Schinor, Martelli, Pacheco, & Azevedo, 2011). Therefore, the association of LC<sub>50</sub> extracts and LC<sub>50</sub> pesticides, in particular with the insecticide chlorpyrifos could replace the pesticide use in the control of large infestations of *D. opuntiae* in field testing, which is a suggested option for employment in the environment. The combination of entomopathogenic agents with chemical insecticides or plant extracts can extend the action of these in pest control and reduce the damage caused to the environment (Amjad, Bashir, Afzal, Sabri, & Javed, 2012; Silva, Alves, E. A. L. A. Lima, & V. L. M. Lima, 2015; Santos et al., 2015).

## 5. Conclusion

The *D. opuntiae* has become an important pest for palm plantations in northeastern Brazil, and other countries like Mexico. Therefore, it is essential to use efficient methods to be used in the control of the cochineal, in order to minimize the damage of this pest in the plantations of *O. ficus-indica*.

The results show the efficacy of *L. ferrea* and *A. sisalana* extracts and the insecticides chlorpyrifos, acetamiprid, thiamethoxan and lambda-cyhalothrin in the control of *D. opuntiae*.

The extracts can be tested alone or in combination in the control of carmine cochineal, palm plantations.

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