

# Toxicity of four commercial insecticides on Dactylopius opuntiae (Hemiptera: Dactylopiidae)

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Abstract. Dactylopius opuntiae (Cockerell) (Hemiptera: Dactylopiidae) is a pest of Opuntia ficus-indica (L.) Miller (Caryophyllales: Cactaceae) in many regions around the world, and there are no formal insecticide toxicity assays. This study aimed to evaluate the lethal toxicity of malathion, lambda-cyhalothrin, spirotetramat, and potassium salts on D. opuntiae in laboratory and greenhouse assays. In the laboratory, using a potter tower, lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) were determined on adult females. Under greenhouse conditions, on O. ficus-indica infested plants, the LC<sub>90</sub> of those three conventional insecticides were evaluated, as well as the LC<sub>50</sub> of potassium salts; mortality was evaluated 48 and 144 h after application in laboratory and greenhouse assays, respectively. D. opuntiae was susceptible to all insecticides; malathion was the most toxic product, LC<sub>50</sub> 120.4 mg L<sup>-1</sup>, lambda-cyhalothrin (159.8 mg L<sup>-1</sup>) and spirotetramat (756.3 mg L<sup>-1</sup>) followed. Using potassium salts, 8,970.1 mg L<sup>-1</sup> were needed to achieve 50% mortality. The highest percentage of mortality (88.3%) in the greenhouse was obtained using the LC<sub>50</sub> (8,970.1 mg L<sup>-1</sup>) of potassium salts and the LC<sub>90</sub> of spirotetramat (11,567 mg L<sup>-1</sup>). Mortality caused by the LC90 of spirotetramat (77.3%) and malathion (66.8%) did not differ statistically; while the LC<sub>90</sub> of lambda-cyhalothrin (11,427 mg L<sup>-1</sup>) caused 45.4% mortality, and it was the least toxic insecticide in the greenhouse assay. In this work, the toxicity of insecticides to improve their use against D. opuntiae is discussed.

Keywords: Prickly pear, nopal, Opuntia ficus-indica, cochineal, chemical

control.

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# Introduction

Dactylopius The "wild cochineal", opuntiae (Cockerell) Dactylopiidae), is a key pest of *Opuntia ficus-indica* (L.) Miller (Caryophyllales: Cactaceae) and other Opuntia species in Mexico and around the world (Mann, 1969; Vanegas-Rico et al., 2010; Mazzeo et al., 2019). This cactus plant is used as forage, as fruit production for human consumption, vegetable, as ornamental, and as a host for insects (D. coccus Costa) to produce natural dyes (Hoffmann, 1995). It is also the cactus with the highest economic, ecological, and functional value in agroecosystems with limited rainfall in regions of America, Africa, and Asia (Le Houérou, 1996; Flores-Valdez and Olvera, 1995; Kiesling, 1999; Nobel, 2011). Dactylopius opuntiae produces a protective wax in the form of cotton fibers which covers its whole body, and carminic acid that has been characterized as an antipredation mechanism (Eisner et al., 1980; Barreto-García et al., 2020).

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These insects grow in colonies throughout the plant and mainly on the cladodes and fruits causing chlorosis; severe infestations (> 75% of cladode area covered) can cause fruit and young cladodes to drop and even death (Mann, 1969; Vanegas-Rico *et al.*, 2010). The species has worldwide attention for two different situations. Firstly, to be used as a biological control agent for invasive *Opuntia* spp. in Australia, South Africa, and some areas in other countries (Zimmermann and Moran, 1991); secondly, because is an invasive pest in thousands of hectares in at least 12 countries in three continents (Lopes *et al.*, 2009; Mazzeo *et al.*, 2019; Mendel *et al.*, 2020). In Brazil, is an exotic pest that caused losses in 100,000 ha of forage cactus (Lopes *et al.*, 2009; Torres and Giorgi, 2018). In Morocco, between 2015 and 2020, causing damage and destruction—to prevent the spread of the pest— in thousands of hectares of forage cactus (Bouharroud *et al.*, 2018; El Aalaoui *et al.*, 2019). In the Mediterranean basin, particularly North Africa and the Middle East, a pest invasion was recently recorded, and inestimable damage is expected (Mazzeo *et al.*, 2019; Mendel *et al.*, 2020). In Mexico, where *O. ficus-indica* and *D. opuntiae* are native (Griffith, 2004; Chávez-Moreno *et al.*, 2009), the insect is a key pest in commercial agroecosystems, but not in natural areas (Vanegas-Rico *et al.*, 2010, 2017; Barreto-García *et al.*, 2020).

The *Dactylopius opuntiae* is difficult to kill, its waxy fibers protect it most of its life as a resistant barrier to adverse weather conditions and chemical control (Longo and Rapisarda, 1995; Brito *et al.*, 2008; Torres and Giorgi, 2018). The management strategy to control *D. opuntiae* should include cultural and mechanical control; the use of vegetable oils, entomopathogenic fungi, and detergents (Palacios-Mendoza *et al.*, 2004; Vigueras *et al.*, 2009; de Santos *et al.*, 2011; Borges *et al.*, 2013; El Aalaoui *et al.*, 2019). There is also a proposal for biological control by conservation in Mexico, or a classic biological control program in Israel (Vanegas-Rico *et al.*, 2010, 2016, 2017; Mendel *et al.*, 2020). It is also known that there are selection programs of forage varieties of *O. ficus-indica* resistant to *D. opuntiae* in Brazil (Torres and Giorgi, 2018) and Morocco (Akroud *et al.*, 2021). Despite all this, chemical control has a relevant role in the integrated pest management (IPM) of *D. opuntiae*, particularly as a quick method to counteract the reproductive biology of the pest, and its ability to increase rapidly at high temperatures and low rainfall (Vanegas-Rico *et al.*, 2010; Palafox-Luna *et al.*, 2018; Torres and Giorgi, 2018).

The chemical control history against *D. opuntiae* includes the use of organophosphates and pyrethroids in South Africa (Pretorius and Van Ark, 1992); and neonicotinoids (thiamethoxam, imidacloprid) and pyrethroids (lambda-cyhalothrin and bifenthrin) in Brazil (Cavalcanti *et al.*, 2001; Torres and Giorgi, 2018). The organophosphates (malathion y chlorpyrifos), pyrethroids (alphacypermethrin), and pyridines (pyriproxyfen) in Morocco (El Aalaoui *et al.*, 2019) and carbamates (sevin) in the United States of America (Aldama-Aguilera, 2008). In Mexico, organophosphates, some detergents, and potassium salts of fatty acids are commonly used (Badii and Flores, 2001; Luna-Vázquez *et al.*, 2012; SENASICA, 2014). Nevertheless, there are no authorized insecticides for this pest in Mexico (CICOPLAFES, 2020; COFEPRIS, 2020).

The insecticide scientific papers on *D. opuntiae* established assays of biological effectiveness with slight variations, but lethal concentrations were not established (Palacios-Mendoza *et al.*, 2004; Vigueras *et al.*, 2009; de Santos *et al.*, 2011; Borges *et al.*, 2013; El Aalaoui *et al.*, 2019). Therefore, to provide information that serves as a basis for improving the chemical control tactic of *D. opuntiae*, the aim of this work was to evaluate the toxicity of four insecticides against *D. opuntiae* under laboratory and greenhouse conditions.

# **Material and Methods**

# Insect rearing

The *Dactylopius opuntiae* colony was established with insects collected in commercial plots of *O. ficus indica* from Tlalnepantla, Morelos, Mexico (19°00'28" N; 98°59'51" W). The cladodes used for the rearing were around ten months old (30-40 cm long and 4-6 cm thick). For the prevention of the incidence of fungi or bacteria, particularly at the base of cladodes, the cladodes were immersed in 0.2% ANIBAC® quaternary salts, allowed to dry, and then used for insect infestation. The *D. opuntiae* rearing was carried out following the "cladode cut" methodology (Palacios-Mendoza *et al.*, 2004), and it was kept at  $20 \pm 2$  °C,  $64 \pm 3$ % relative humidity (RH), and 14:10 L: D. In addition, the *D. opuntiae* colony was enriched every two weeks with field material from the same region. The insects came from fields that have used chemical control as one of the main control methods in the last 30 years (GIIN, 2007).

#### Insecticides

The formulations evaluated were four commercial insecticides and the criteria were different toxicological groups, commonly used in *O. ficus-indica* crops (Luna-Vázquez *et al.*, 2012; SENASICA, 2014; El Aalaoui *et al.*, 2021); in addition to commercial accessibility, and the inclusion of two products with the least impact cited on beneficial insects (Luna-Cruz *et al.*, 2015; Juarez-Maya *et al.*, 2021) (Table 1). The dilutions of insecticides were made with fresh water to obtain a stock concentration from which subsequent dilutions were made, including 0.1% Inex<sup>®</sup> adherent. The evaluation of the toxicity of insecticides was done through two independent experiments, one in the laboratory and the other in the greenhouse.

**Table 1.** Insecticides used in laboratory toxicity assays on *Dactylopius opuntiae*.

Active	Commercial	Toxicological	[a.i.]	Mode of action
ingredient (a.i.)	name (maker)	group (IRAC)	(%)	
Malathion	Malathion 1000 (Dragón)	Organophosphates (1B)	83.6	Affects synaptic transmission by inhibiting acetylcholinesterase
Lambda- cyhalothrin	Karate Zeon 5 (Syngenta)	Pyrethroid (3A)	5.15	Modulates sodium channels; acts on the nervous system
Spirotetramat	Movento 150 (Bayer)	Tetronic acid (23)	15.3	Inhibits lipid biosynthesis; regulates grow
Potassium salts of fatty acid	Impide (Gowan)	Unknown (UN)	49	Unknown

# Laboratory bioassays

The toxicity assays were performed on 40-50-day old adult females of *D. opuntiae* collected from the rearing. The females were separated into groups of 10 individuals inside Petri dishes (Ø 5.5 cm), containing a previously moistened filter paper base. The insecticides were applied using a Potter spray tower ( $60 \times 60 \times 120$  cm), with a pneumatic solid cone spray nozzle (Cat. 1 / 4J-SS + SU1A-SS, Spraying Systems, Wheaton, IL, USA), connected to a constant pressure air source. The system was calibrated to apply 2 mg cm<sup>-2</sup> of insecticide by introducing 10 mL of solution at 20 PSI pressure. The experimental units were placed 100 cm away from the spray source. The experimental units were closed after the application of each treatment and kept inside a bioclimatic chamber ( $20 \pm 2^{\circ}$ C,  $64 \pm 3\%$  RH, 14:10 h L: D). The mortality was recorded 48 h after application. The insects that did not react to being stimulated with the bristles of an entomological brush, or exhibited obvious signs of hardening, size reduction, and poor wax production were considered dead.

The biological response for each insecticide was first estimated. In this stage, the application of six serial concentrations of insecticide (1.0 - 1 × 10<sup>-5</sup> %), in each case a control and two replications were developed to identify those that caused between 10 and 100% mortality at 48 h. Subsequently, the bioassay was performed with at least seven doses; the bioassay design was completely random. The combination of each insecticide concentration was considered a replication, in which 10 individuals were used and four replications were performed. The control insects were treated with 10 mL of fresh water with 0.1% Inex®. The maximum acceptable mortality level in the control was 12%; if this happened, then, the mortality of the treatments was adjusted with the Abbott equation (Abbott, 1925).

# Greenhouse insecticide evaluation

The *O. ficus-indica* plants were established in 15 L pots with compost as substrate and irrigated every three days. A continuous pruning was carried out to maintain two cladodes in each base cladode (mother plant). After 10 months of establishment, two cladodes (30-40 cm long and 3-5 cm thick) were infested with *D. opuntiae*. The infestation was carried out individually using the Vanegas-Rico *et al.* (2016) methodology. Briefly, a 9 cm<sup>2</sup> glassine paper bag with 30 *D. opuntiae* adult females was fixed in each cladode, the first instar nymphs colonized the cladodes for 72 h. After that, females and bags were removed from each cladode, and the plants were kept in the greenhouse at  $24 \pm 10^{\circ}$ C,  $54 \pm 11^{\circ}$  R.H., and the natural Spring photoperiod for Texcoco, Estado de Mexico (19°30'20" N; 98°52'55"W). After six weeks *O. ficus-indica* plants had 20 to 30 colonies of *D. opuntiae* per cladode with adult females around 40 days old.

The experiment consisted of determining the effectiveness of the baselines of each insecticide estimated in the lab assay. The  $LC_{90\text{'s}}$  of spirotetramat (11,567 mg  $L^{-1}$ ), malathion (3,209.4 mg  $L^{-1}$ ), and lambda-cyhalothrin (11,427 mg  $L^{-1}$ ) were evaluated. The  $LC_{50}$  (8,970.1 mg  $L^{-1}$ ) was evaluated for potassium salts, because the  $LC_{90}$  resulted in a viscous solution that made it difficult to apply with the spraying equipment. The two infested cladodes of each mother plant were considered as the experimental unit, there were at least 20 *D. opuntiae* colonies in each cladode. The experimental units were sprayed to runoff with the corresponding insecticide concentration using a 1/4 full cone stainless steel nozzle, and constant pressure of 30 PSI. For each treatment, eight replications were carried out, and for each one a control was included to which only fresh water and 0.1% Inex® were applied. The plants were randomly arranged inside the greenhouse; the mortality was recorded 144 h after insecticide application. As in the laboratory assays, insects that did not react when stimulated with the bristles of an entomological brush or that showed signs of hardening, reduction in size, and poor wax production, were considered dead.

# Analysis of data

For the laboratory bioassays insecticide concentrations, expressed in mg L<sup>-1</sup>, and *D. opuntiae* mortality were processed to estimate the LC<sub>50</sub> and LC<sub>90</sub> of each product and to perform a mean range test adjusted by Kruskal-Wallis ( $\alpha$  = 0.05). The fiducial limits (FL) at 95% reliability were also estimated, as well as the slope of the regression line with its associated standard error (b ± ee), and the chi-square value ( $\chi$ <sup>2</sup>) for the fit test to the Probit model (Yu, 2015; Robertson *et al.*, 2017). The mortality of *D. opuntiae* in the greenhouse was subjected to an analysis of variance (ANOVA), and a Tukey mean separation test ( $\alpha$  = 0.05). The data analysis was done with the statistical software R version 4.0.5.

# **Results and discussion**

# Laboratory bioassays

The *D. opuntiae* adult females were susceptible to all evaluated insecticides; these caused differential mortality in the laboratory (LC<sub>50</sub>, K= 12.72, df= 3, *p* <0.005; LC<sub>90</sub>, K= 8.66, df= 3, *p* <0.03). The toxicity depended on the insecticide and the applied concentration. The total mortality (100%) was obtained with 6,000 mg L<sup>-1</sup> of spirotetramat; malathion and lambda-cyhalothrin caused 90 and 92.5% mortality at 10,000 mg L<sup>-1</sup>, respectively. Using potassium salts, 100,000 mg L<sup>-1</sup> was needed to achieve 75% mortality, even at high doses affected the precise estimation of lethal concentrations in the product (Table 2).

**Table 2.** Mortality (%) of *Dactylopius opuntiae* adult females 48 h after insecticide application.

Concentration	Mortality (%)				
(mg L <sup>-1</sup> )	Malathion	Lambda-cyhalothrin	Spirotetramat	Potassium salts of fatty acid	
100,000	-	-	-	75 a	
35,000	-	-	-	70 a	
10,000	90 a*	92.5 a	100 a	57.5 b	
6,000	-	-	100 a	40 c	
3,500	-	85 ab	-	-	
2,000	-	-	55 b	27.5 d	
1,000	82.5 ab	80 b	47.5 bc	22.5 d	
600	72.5 bc	57.5 c	42.5 cd	-	
200	62.5 c	50 cd	35 d	-	
100	45 d	42.5 d	20 e	17.5 d	
35	30 e	-	-	-	
10	15 f	25 e	7.5 f	-	
Control	7.5 f	10 f	2.5 f	5 e	

<sup>\*</sup>Means within columns with different letters are significantly different according to the Kruskal-Wallis test (P ≤0.05).

The insecticide toxicity had the same trend at  $LC_{50}$  and  $LC_{90}$ . The malathion was the most toxic product for *D. opuntiae* ( $LC_{50}$  = 120.4 mg  $L^{-1}$ ;  $LC_{90}$  = 3,209.4 mg  $L^{-1}$ ), followed by lambda-cyhalothrin ( $LC_{50}$  = 159.8 mg  $L^{-1}$ ;  $LC_{90}$  = 11,427 mg  $L^{-1}$ ) and spirotetramat ( $LC_{50}$  = 756.3 mg  $L^{-1}$ ;  $LC_{90}$  =11,567 mg  $L^{-1}$ ). The potassium salts showed less toxicity ( $LC_{50}$  = 8,970.1 mg  $L^{-1}$ ;  $LC_{90}$  = 688,576 mg  $L^{-1}$ ); the low toxicity of the product on *D. opuntiae* did not allow for establishing a precise estimation. The value of the slope ranged between 0.61 and 1.72, which indicated heterogeneity of the population in its response to potassium salts and homogeneity for spirotetramat, respectively. The  $\chi^2$  values indicated that there was no difference between the observed and expected values (Table 3).

**Table 3.** The insecticide toxicity on *Dactylopius opuntiae* 48 h after application.

Variable	Malathion	Lambda- cyhalothrin	Spirotetramat	Potassium salts of fatty acid
Number of insects (n)	40	40	40	40
Slope ± standard error (b ± ee)	$0.8 \pm 0.07$	$0.72 \pm 0.07$	$1.72 \pm 0.43$	0.61 ± 0.08
† LC <sub>50</sub> (mg L <sup>-1</sup> )	120.4	159.8	756.3	8,970.1
Fiducial limits 95%	94.5-146.2	99.8-219.8	530.2-982.3	6,106-11,834
† LC <sub>90</sub> (mg L <sup>-1</sup> )	3,209.4	11,427	11,567	688,576
Fiducial limits 95%	1,382.5-5,036	1,819-21,035	42,92-18,842	-38,019-141,517
X <sup>2</sup>	0.011	0.017	0.33	0.024

† LC = Lethal concentration

<sup>-</sup>Concentration that was not assessed.

#### Greenhouse insecticide evaluation

The four insecticides were toxic to *D. opuntiae* at variable levels, depending on the product and their concentration; this mortality was different and slightly lower than in the laboratory. The highest percentages of mortality under greenhouse conditions (88.3% and 77.3%) were achieved using 8,970.1 mg L<sup>-1</sup> of potassium salts and 11,567 mg L<sup>-1</sup> of spirotetramat, respectively, while malathion at 3,209.4 mg L<sup>-1</sup> caused 66.8% mortality. The lambda-cyhalothrin was the least toxic insecticide, since the evaluated concentration (11,427 mg L<sup>-1</sup>) only caused 45.4% mortality (Table 4).

**Table 4.** Dactylopius opuntiae female adult mortality, 144 h after insecticide application inside a greenhouse.

Treatments	Doses (mg L <sup>-1</sup> )	Mortality (%)	
Potassium salts of fatty acid	8,970.1	88.34	a*
Spirotetramat	11,567	77.34	ab
Malathion	3,209.4	66.79	b
Lambda cyhalothrin	11,427	45.42	С

<sup>\*</sup>Means within columns with different letters are significantly different according to the Tukey test (P ≤0.05).

The wax that covers the body of *D. opuntiae* female adults is abundant and provides a protector barrier from adverse environmental conditions, including chemical control (Brito *et al.*, 2008; Vanegas-Rico *et al.*, 2010; Torres and Giorgi, 2018). Even so, the evaluated insecticides caused mortalities up to 100% in the laboratory, and from 45 to 88% in the greenhouse.

The malathion and lambda-cyhalothrin registered the highest toxicity on D. opuntiae in the laboratory,  $LC_{50}$  of 120.4 mg  $L^{-1}$  and 159.8 mg  $L^{-1}$ , respectively. The previously mentioned products must have contact with the insect body to be effective. The information about malathion translaminar properties is unknown, due to the barrier represented by the thick cuticle of the cladodes of O. ficus-indica (Nobel 2011). Although it has already been indicated that there are no assays that establish  $LC_{50}$  in D. opuntiae, there are some biological effectiveness field assays. For example, methyl parathion and dimethoate caused 90% mortality of D. opuntiae, and chlorpyrifos 82%, all in Brazil (Brito et al., 2008; Lopes et al., 2018). El Aalaoui et al. (2019) recorded more than 75% mortality after 96 h of malathion application at semi-field conditions (2 mL  $L^{-1}$ ).

In this study, lambda-cyhalothrin was toxic in laboratory ( $LC_{50}$  = 159.8 mg $L^{-1}$ ) but was ineffective in the greenhouse (45% mortality). This differed from field results reported by Lopes *et al.* (2018) in Brazil, who found 62 to 99% female mortality at 0.75 and 0.5 mL  $L^{-1}$  of the product, respectively. However, is not possible to compare those results because the lethal concentration was not established in the study, and the *D. opuntiae* colonies of Brazil and Mexico have been subjected to different insecticide pressure. Nevertheless, in Mexico, there are technical recommendations that do not approve pyrethroids against *D. opuntiae* because those insecticides are considered ineffective (Luna-Vázquez *et al.*, 2012; SENASICA, 2014). This situation and the 45% mortality achieved in greenhouse conditions, lead us to suppose that pyrethroids are not suitable candidates for the control of *D. opuntiae* populations in the study region; in addition to the fact that there are reports of null selectivity and low compatibility with natural enemies (El Aalaoui *et al.*, 2019, 2021; Juarez-Maya *et al.*, 2021).

Based on the laboratory results on D. opuntiae, spirotetramat (LC<sub>50</sub> = 756.3 mg L<sup>-1</sup>) could be classified as a toxicity intermediate. This product acts mainly by ingestion on some hemipteroid insects, and targets lipids and fatty acids, which are important constituents of the cuticle (Elizondo

and Murguido, 2010; Abdel-Fatah *et al.*, 2019). The spirotetramat is considered to inhibit lipid and fatty acid synthesis; then, the decrease of these can affect metabolic processes such as the constitution of the cuticle of insects, or in the biosynthesis of pheromones, waxes, and eicosanoids (lipid molecules); also, some energy sources and structural components of membranes and defensive secretions may be missing (Yu, 2015). The substantial differences in the LC<sub>50</sub> of *D. opuntiae* and other mealybug species to this product may be due to changes in the wax composition and structure of each species, and their role in protecting against insecticides. For example, spirotetramat had an LC<sub>50</sub> of 159.8 mg L<sup>-1</sup> on *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae) (Juarez-Maya *et al.*, 2021), and took only 1.2 mL L<sup>-1</sup> and three weeks to cause 95% mortality on *Planococcus citri* Risso (Hemiptera: Pseudococcidae) (Mansour *et al.*, 2010). The extra time to manifest effectiveness in the last mealybug species is important because, in this study, the contact effect was only evaluated at 48 h in the laboratory, and 144 h in the greenhouse, so is suggested to evaluate the effect at a longer exposure time to allow the product acting first on the waxy secretions, which later can lead to cuticle rupture and body deformation (Abdel-Fatah *et al.*, 2019).

The estimated LC<sub>50</sub> of potassium salts in the laboratory (8,970.1 mg L<sup>-1</sup>) indicates that they have lower toxicity on *D. opuntiae*. The most accepted mode of action of potassium salts and detergents is that they destroy the waxy layer (sometimes lacerate integument) that protects scale insects, which favors drying out due to solar radiation (Vavrina *et al.*, 1995; Toorani *et al.*, 2017). This is often the interpretation that was provided to explain its insecticidal effect on *D. opuntiae* (Palacios-Mendoza *et al.*, 2004; Brito *et al.*, 2008; El Aalaoui *et al.*, 2019).

The greenhouse assays provided additional information on the response of D. opuntiae to insecticides. The LC<sub>50</sub> of potassium salts caused a numerically different mortality (83.3%), but was statistically similar to the LC<sub>90</sub> of spirotetramat (77.3%). Furthermore, provided higher mortality than the CL<sub>90</sub> of malathion and lambda-cyhalothrin (66.7 and 45.4%, respectively). The potassium salts' effect on D. opuntiae wax, or its integument, was evidenced in the greenhouse because there was natural radiation and more probability of dehydration. If mortality limits for biological field tests of the Mexican regulates are considered, NOM-032-SAG/FITO 2014 (Anonymous, 2015), then potassium salts were effective in controlling D. opuntiae (they produced 88% mortality), but none of the conventional insecticides caused more than 85% mortality; therefore, they could be considered ineffective for controlling the pest.

During the greenhouse assay adult females were naturally grouped in compact colonies with abundant wax and were fixed and feeding on the cladodes. This condition probably allowed achieving mortalities close to what will happen in the field and could contribute to explaining differences in mortality from the artificial condition (laboratory test). In previous essays, insecticide evaluations were carried out including nymphs (first and second instar), and *D. opuntiae* adult females (Palacios-Mendoza *et al.*, 2004; El Aalaoui *et al.*, 2019; Rambdani *et al.*, 2020). The nymphs have less wax, and they are more susceptible to insecticides (Franco *et al.*, 2004), but in a natural condition can also remain under the body of adult females, or very close to them in compact colonies (Brito *et al.*, 2008; Vanegas-Rico *et al.*, 2010; Torres and Giorgi, 2018). This way, the influence of adult wax, and its protection under natural conditions can interfere with the precise determination of mortality. This condition, besides the high mortality of second-instar nymphs (12-16%) in less than 72 h, when were removed of cladodes, makes adult females recommended as the most convenient stage of development for insecticide evaluations.

The *D. opuntiae* is the main pest in *O. ficus-indica* agroecosystems around the world, and insecticides will be a control method within the IPM strategy. However, its misuse will lead to known problems in the environment, and damage to insects and non-target organisms (Pretorius and Van Ark, 1992; Pourseyed *et al.*, 2010; Vanegas-Rico *et al.*, 2010). Establishing insecticide toxicity (LC<sub>50</sub> and LC<sub>90</sub>) is important to demonstrate the effectiveness of products, and to start proposals on how to better use them. This control method applied in the opportune moments, in harmony with other control methods, the natural control, and monitoring population dynamics will provide better results for the management of *D. opuntiae*.

# Conclusion

The lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) of three conventional insecticides, and the LC<sub>50</sub> of potassium salts, were determined in adult females of *D. opuntiae* for the first time; so, this study could be a base for future development in the evaluation of insecticides on the pest. The *D. opuntiae* was susceptible to all insecticides in laboratory assays. The malathion was the most toxic product (LC<sub>50</sub> = 120.4 mg L<sup>-1</sup>) followed by lambda-cyhalothrin (159.8 mg L<sup>-1</sup>) and spirotetramat (756.3 mg L<sup>-1</sup>), and 8,970.1 mg L<sup>-1</sup> of potassium salts were needed to achieve its LC<sub>50</sub>. The highest percentages of *D. opuntiae* mortality under greenhouse conditions (88.3% and 77.3%) were obtained using the LC<sub>50</sub> of potassium salts and LC<sub>90</sub> of spirotetramat, respectively. The malathion mortality (66.8%) did not differ from spirotetramat, while lambda-cyhalothrin was the least toxic insecticide (45% mortality).

#### **ETHICS STATEMENT**

Not applicable

### **CONSENT FOR PUBLICATION**

This research study does not contain any person's data in any form (including any individual details, images, or videos).

# **AVAILABILITY OF SUPPORTING DATA**

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

# **COMPETING INTERESTS**

The research has no financial or commercial purpose that must be interpreted as a potential conflict of interest. The authors declare that they have no competing interests.

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# **AUTHOR CONTRIBUTIONS**

Conceptualization, EHE, LDOA, and ERL; methodology, EHE, LDOA, ERL; formal analysis EHE, LSR, LDOA; investigation, EHE, LDOA, ERL, JRLF, LSR and ALJ; writing—original draft preparation, EHE, LDOA, LSR and ERL; writing—review and editing, EHE, LDOA, ERL, JRLF, LSR and ALJ; supervision, EHE, LDOA, ERL, JRLF and LSR.

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#### References

- Abdel-Fatah, R.M., Mohamed, S.M., Aly, A.A., and Sabry, A-K.H. 2019. Biochemical characterization of spiromesifen and spirotetramat as lipid synthesis inhibitors on cotton leaf worm, *Spodoptera littoralis*. *Bulletin of the National Research Centre*. *43*: 65. https://doi.org/10.1186/s42269-019-0107-9
- Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265-267.
- Akroud, H., Sbaghi, M., Bouharroud, R., Koussa, T., Boujghagh, M. and El Bouhssini, M. 2021. Antibiosis and antixenosis resistance to *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) in Moroccan cactus germplasm. *Phytoparasitica.* 49: 623-631. https://doi.org/10.1007/s12600-021-00897-w
- Aldama-Aguilera, C. 2008. Límites máximos de residuos e intervalos de seguridad de plaguicidas en tuna, *Opuntia ficus-indica*. Tesis de Doctorado en Ciencias, Colegio de Postgraduados, Montecillo, Texcoco, Estado de México, México. 77 p.
- Anonymous. 2015. Modificación a la Norma Oficial Mexicana NOM-032-FITO-1995. Por la que se establecen los requisitos y especificaciones fitosanitarios para la realización de estudios de efectividad biológica de plaguicidas agrícolas y su dictamen técnico. DOF 11 de agosto de 2015. Consulted: September 15, 2021. Source: https://www.dof.gob.mx/nota\_detalle.php?codigo=5403310&fecha=11/08/2015
- Badii, M.H. and Flores, A.E. 2001. Prickly pear cacti pests and their control in Mexico. *Florida Entomologist. 84*: 503-505.
- Barreto-García, O.A., Rodríguez-Leyva, E., Lomeli-Flores, J.R., Vanegas-Rico, J.M, Vigueras, A.L. and Portillo, L. 2020. *Laetilia coccidivora* feeding on two cochineal insect species, Does the prey affect the fitness of the predator? *Biocontrol.* 65: 1-10. https://doi.org/10.1007/s10526-020-10047-6
- Borges, L.R., Santos, D.C., Falcão, H.M. and da Silva, D.M. 2013. Use of biodegradable products for the control of *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) in cactus pear. *Acta Horticulturae.* 995: 379-386. https://doi.org/10.17660/ActaHortic.2013.995.49
- Bouharroud, R., Sbaghi, M., Boujghagh, M. and El Bouhssini, M. 2018. Biological control of the prickly pear cochineal *Dactylopius opuntiae* Cockerell (Hemiptera: Dactylopiidae). *EPPO Bulletin. 48*: 300-306. https://doi.org/10.1111/epp.12471

- Brito, C.H., Batista, E., de Albuquerque, I.C. and de Luna-Batista, J. 2008. Avaliação da produtos alternativos e pesticidas no controle da cochonilha-docarmim na Paraíba. *Revista de Biologia e Ciencias da Terra. 8*: 1-5.
- Cavalcanti, V.A.L.B., Sena, R.C., Coutinho, J.L.B., Arruda, G.P. and Rodrigues, F.B. 2001. Controle das cochinilhas da palma forrageira. *Buletim IPA Responde. 39*: 1-2.
- Chávez-Moreno, C.K., Tecante, A. and Casas, A. 2009. The *Opuntiae* (Cactaceae) and *Dactylopius* (Hemiptera: Dactylopiidae) in Mexico: a historical perspective of use, interaction and distribution. *Biodiversity and Conservation*. 18: 3337-3355. https://doi.org/10.1007/s10531-009-9647-x
- CICOPLAFEST. Comisión Intersecretarial para el Control del Proceso y Uso de Plaguicidas y Sustancias Tóxicas. 2020. México. Consulted: August 12, 2020. Source: http://www.siicex.gob.mx/portalSiicex/SICETECA/Acuerdos/Regulaciones/SSA/cicop lafest.htm
- COFEPRIS. Comisión Federal para la Protección contra Riesgos Sanitarios. 2020. México. Consulted: May 12, 2020. Source: http://siipris03.cofepris.gob.mx/Resoluciones/Consultas/ConWebRegPlaguicida.asp
- de Santos, P.S., da Silva, M.A.Q., Monteiro, A.C. and Gava, C.A.T. 2011. Improving photoprotection of *Beauveria bassiana* conidia for biological control of the cactus pest *Dactylopius opuntiae* in the semiarid region northeast of Brazil. *Biocontrol Science and Technology*. 21: 893-902. https://doi.org/10.1080/09583157.2011.586022
- Eisner, T., Nowicki, S., Goetz, M. and Meinwald, J. 1980. Red cochineal dye (carminic acid): its role in nature. *Science.* 208: 1039-1042. https://doi.org/10.1126/science.208.4447.1039
- Elizondo, S.A.I., and Murguido, M.C.A. 2010. Spirotetramat, nuevo insecticida para el control de insectos chupadores en el cultivo de la papa. *Fitosanidad. 14*: 229-234.
- El Aalaoui, M., Bouharround, R., Sbaghi, M., El Bouhhssini, M., Hilali, L. and Khadija, D. 2019. Comparative toxicity of different chemical and biological insects against the scale insect *Dactylopius opuntiae* and their side effects on the predator *Cryptolaemus montrouzieri*. *Archives of Phytopathology and Plant Protection*. *52*: 155-169. https://doi.org/10.1080/03235408.2019.1589909
- El Aalaoui, M., El Bouhssini, M., Bouharroud, R. and Sbaghi, M. 2021. Lethal and sublethal effects of the insecticides d-limonene, mineral oil, and potassium salts of fatty acid on *Dactylopius Opuntiae* potential predator *Cryptolaemus montrouzieri*. *International Journal of Tropical Insect Science*. https://doi.org/10.1007/s42690-021-00473-z.

- Flores-Valdez, C.A. and Olvera, J. 1995. La producción de nopal verdura en México. Memorias del VI congreso nacional y IV congreso internacional sobre el conocimiento y aprovechamiento del nopal. Universidad de Guadalajara, Guadalajara, Jalisco, México. 282-289 p.
- Franco, J., Suma, P., da Silva, E., Blumberg, D. and Mendel, Z. 2004. Management strategies of mealybug pests of citrus in Mediterranean countries. *Phytoparasitica*. 32: 507-522. https://doi.org/10.1007/BF02980445
- GIIN. Grupo Interdisciplinario de Investigación del Nopal. 2007. Memorias del II taller de avances de investigación del Grupo Interdisciplinario de Investigación del Nopal. Rodríguez-Leyva, E.; Lomeli-Flores, J.R; López-Jiménez A. (Eds.). Texcoco, Estado de México, México. 69 p.
- Griffith, M.P. 2004. The origin of an important cactus crop, *Opuntia ficus-indica* (Cactaceae): new molecular evidence. *American Journal of Botany.* 91: 1915-1921. https://doi.org/10.3732/ajb.91.11.1915
- Hoffmann, W. 1995. Etnobotany. *In*: Barbera G.; Inglese, P; Pimienta-Barrios, E. (Eds.). FAO Plant Production and Protection Paper 132. Rome, FAO, pp.12-19.
- Juarez-Maya, M.A., Ortega-Arenas, L.D., González-Hernández, H., Lagunes-Tejeda, A., Solís-Aguilar, J.F. and García-Méndez, V.H. 2021. Toxicidad y selectividad de insecticidas sobre cochinilla rosada del hibisco y sus enemigos naturales. *Southwestern Entomologist.* 46: 479-496. https://doi.org/10.3958/059.046.0217
- Kiesling, R. 1999. Domesticación y distribución de *Opuntia ficus-indica*. *Journal of Professional Association for Cactus Development*. *3*: 50-59.
- Le Houérou, H.N. 1996. The role of cacti (*Opuntia* spp.) in erosion control, land reclamation, rehabilitation and agricultural development in the Mediterranean Basin. *Journal of Arid Environments. 33*: 135-159. https://doi.org/10.1006/jare.1996.0053
- Longo, S., and Rapisarda, C. 1995. Pests of cactus pear. Agro-ecology, cultivation and uses of cactus pear. FAO. Rome, Italy, 219 p.
- Lopes, E.B., Albuquerque, I.C., Brito, C.H., and Santos, D.C. 2009. Velocidade de dispersao de *Dactylopius opuntiae* em palma gigante (*Opuntia ficus-indica*). *Engenharia Ambiental.* 6: 644-649.
- Lopes, R.S., Oliveira, L.G., Costa, A.F., Correira, S.T.M., Luna-Alvez, L.E.A., and Lima, V.L.M. 2018. Efficacy of *Libidibia férrea* var. *férrea* and *Agave sisalana* extracts against *Dactylopius opuntiae* (Hemiptera: Coccoidea). *Journal of Agricultural Science*. 10: 255-267.

- Luna-Cruz, A., Rodríguez-Leyva, E., Lomeli-Flores, J.R., Ortega-Arenas L.D., Bautista-Martínez, N. and Pineda, S. 2015. Toxicity and residual activity of insecticides against *Tamarixia triozae* (Hymenoptera: Eulophidae), a parasitoid of *Bactericera cockerelli* (Hemiptera: Triozidae). *Journal of Economic Entomology.* 108: 2289-2295. https://doi.org/10.1093/jee/tov206
- Luna-Vázquez, J., Zegbe-Domínguez, J.A., Mena-Covarrubias, J., Rivera-Lozano, R.M. 2012. Manejo de plantaciones de nopal tunero en el altiplano potosino. Folleto para productores INIFAP, San Luis Potosí, México. 23 p.
- Mann, J. 1969. Cactus-feeding insects and mites. Smithsonian Institution Government Printing Office Washington. *United States National Museum Bulletin.* 256: 1-158.
- Mansour, R., Grissa, L.K., and Rezgui, S. 2010. Assessment of the performance of some new insecticides for the control of the vine mealybug *Planococcus ficus* in a Tunisian vineyard. *Entomologia Hellenica*. *19*: 21-33. https://doi.org/10.12681/eh.11591
- Mazzeo, G., Nucifora, S., Russo, A. and Suma, P. 2019. *Dactylopius opuntiae*, a new prickly pear cactus pest in the Mediterranean: an overview. *Entomolologia Experimentalis et Applicata*. *167*: 59-72. https://doi.org/10.1111/eea.12756
- Mendel, Z., Protasov, A., Vanegas-Rico, J.M., Lomeli-Flores, J.R., Suma, P. and Rodríguez-Leyva, E. 2020. Classical and fortuitous biological control of the prickly pear cochineal *Dactylopius opuntiae* in Israel. *Biological Control.* 142: 104157. https://doi.org/10.1016/j.biocontrol.2019.104157
- Nobel, P.S. 2011. Sabiduría del desierto, agaves y cactos: CO2, agua, cambio climático. Biblioteca básica de agricultura. Texcoco, México, 160 p.
- Palacios-Mendoza, C., Nieto-Hernández, R., Llanderal-Cázares, C. and González-Hernández, H. 2004. Efectividad biológica de productos biodegradables para el control de la cochinilla silvestre *Dactylopius opuntiae* (Cockerell) (Homoptera: Dactylopiidae). *Acta Zoológica Mexicana. 20*: 99-106.
- Palafox-Luna, J.A., Rodríguez-Leyva, E., Lomeli-Flores, J.R., Vigueras-Guzmán, A.L. and Vanegas-Rico, J.M. 2018. Life cycle and fecundity of *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) in *Opuntia ficus-indica* (Caryophyllales: Cactaceae). *Agrociencia*. *52*: 103-114.
- Pourseyed, S.H., Tvassoli, M., Bermousi, I. and Mardani, K. 2010. *Metarhizium anisopliae* (Ascomycota: Hypocreales): An effective alternative to chemical acaricides against different developmental stages of fowl tick *Argas persicus* (Acari: Argasidae). *Veterinary Parasitology.* 172: 305-310. https://doi.org/10.1016/j.vetpar.2010.05.014

- Pretorius, M.W. and Van Ark, H. 1992. Further insecticide trials for the control of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) as well as *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) on spineless cactus. *Phytophylactica*. 24: 229-233.
- Ramdani, C., Bouharroud, R., Sbaghi, M., Mesfioui, A. and El Bouhssini, M. 2020. Field and laboratory evaluation of different botanical insecticides for the control of *Dactylopius opuntiae* (Cockerell) on cactus pear in Morocco. *International Journal of Tropical Insect Science*. 41: 1623-1632.
- Robertson, J.L., Jones, M.M., Olguin, E., and Alberts, B. 2017. Bioassays with arthropods. Third edition. CRC Press. USA. 212 p.
- SENASICA. Servicio Nacional de Sanidad Inocuidad y Calidad Alimentaria. 2014. Plan de acción preventivo nopal. Acciones para reducir riesgos de contaminación por plaguicidas en empacado, transporte y distribución de nopal dentro y fuera del país. Secretaría de Agricultura y Desarrollo Rural (SADER), México. Consulted: March 5, 2021.

  Source: https://www.gob.mx/cms/uploads/attachment/file/257726/PlandeAccionNopal2014.p
- Toorani, A.H., Abbasipour, H. and Kalkenari, L.D. 2017. Toxicity of selected biorational insecticides to *Pulvinaria aurantii* Cockerell and its predator, *Cryptolaemus montrouzieri* Mulsant in citrus field. *Acta Agriculturae Scandinavica*. 67: 723-729. https://doi.org/10.1080/09064710.2017.1338745
- Torres, J.B. and Giorgi, J.A. 2018. Management of the false carmine cochineal *Dactylopius opuntiae* (Cockerell): perspective from Pernambuco state, Brazil. *Phytoparasitica.* 46: 331-340. https://doi.org/10.1007/s12600-018-0664-8
- Vanegas-Rico, J.M., Lomeli-Flores, J.R., Rodríguez-Leyva, E., Mora-Aguilera, G. and Valdez, J.M. 2010. Enemigos naturales de *Dactylopius opuntiae* (Cockerell) en *Opuntia ficus-indica* (L.) Miller en el centro de México. *Acta Zoológica Mexicana*. 26: 415-433.
- Vanegas-Rico, J.M., Rodríguez-Leyva, E., Lomeli-Flores, J.R., González-Hernández, H., Pérez-Panduro, A., and Mora-Aguilera, G. 2016. Biology and life history of *Hyperaspis trifurcata* feeding on *Dactylopius opuntiae*. *BioControl*. 61: 691-701. https://doi.org/10.1007/s10526-016-9753-0
- Vanegas-Rico, J.M., Pérez-Panduro, A., Lomeli-Flores, J.R., Rodríguez-Leyva, E., Valdez-Carrasco, J. and Mora-Aguilera, G. 2017. *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) population fluctuations and predators in Tlalnepantla, Morelos, Mexico. *Folia Entomológica Mexicana.* 3: 23-31.

- Vavrina, C.S., Stansly, P.A. and Liu, T.X. 1995. Household detergent on tomato: phytotoxicity and toxicity to silverleaf whitefly. *HortScience.* 30: 1406-1409. https://doi.org/10.21273/HORTSCI.30.7.1406
- Vigueras, A.L., Cibrián-Tovar, J. and Pelayo-Ortiz, C. 2009. Use of botanical extracts to control wild cochineal (*Dactylopius opuntiae* Cockerell) on cactus pear. *In*: Proceedings of the VI International Congress on cactus and cochineal. *Acta Horticulturae*. *811*: 229-234. https://doi.org/10.17660/ActaHortic.2009.811.28
- Yu, S.J. 2015. The toxicology and biochemistry of insecticides. Second Edition. CRC Press. USA. 380 p.
- Zimmermann, H.G. and Moran, V.C. 1991. Biological control of prickly pear, *Opuntia ficus-indica* (Cactaceae), in South Africa. *Agriculture, Ecosystems and Environment.* 37: 29-35.