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Article

Effectiveness of *Agave* genotype extracts applied alone or mixed with mineral oil against the cactus scale mealybug *Diaspis echinocacti* (Hemiptera: Diaspididae)

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ABSTRACT: Synthetic insecticides used in the management of the mealybug *Diaspis echinocacti* (Bouché, 1833) (Hemiptera: Diaspididae) can contaminate co-products from this plant. The use of *Agave* extracts and/or mineral oil is a sustainable alternative to reduce the residual effect of these chemicals in the palm production chain. The objective here was to determine the effectiveness of *Agave* genotype extracts applied alone or mixed with mineral oil against the cactus scale mealybug *D. echinocacti*. Two experiments were carried out to achieve this goal. The first one aimed to select the most promising *Agave* genotype extract concentration (CL_{80}) to kill 80% of the first instar mobile nymphs of *D. echinocacti* (Experiment 1), and the second to determine the effectiveness of the selected extract against nymphs, pupae, and adults of different ages of this mealybug in laboratory and greenhouse (Experiment 2). The extract of the genotype *Agave fourcroydes* cv. Cabinho with lethal concentration (LC_{80}) of 10.9 mL/10 mL is the most efficient in causing mortality of *D. echinocacti*. The lethal concentration of the *A. fourcroydes* cv. Cabinho killed 80% of the first instar motile nymphs and more than 74% of the nymphs, pupae, and adults of different ages of *D. echinocacti* in the laboratory and greenhouse followed by *Agave sisalana* cv. Tatuí 3. Furthermore, the mortality of this scale mealybug was higher with applications of mineral oil (Assist), alone or mixed with sisal extracts.

Key words: botanical insecticide, *Opuntia ficus-indica*, scale mealybug.

INTRODUCTION

The cactus pears *Opuntia ficus-indica* (L.) Mill and *Nopalea cochenillifera* (L.) Salm-Dyck are the most commercially cultivated forage plants and the main food source for ruminants in the semiarid northeastern region of Brazil (Arba et al. 2017, Bayar et al. 2018). Ants, caterpillars, and grasshoppers are insect pests of the cactus pears, but scale insects, such as *Diaspis echinocacti* (Bouché, 1833) (Hemiptera; Diaspididae) and the carmine insect *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) cause the greatest losses on these plants in the main producing regions of Brazil (Lopes et al. 2010).

The genetic homogeneity of the cactus pear plants and the high biotic potential and aggressiveness of the mealybug *D. echinocacti* facilitate severe damage on these plants in northeastern Brazil (Lopes et al. 2018). *Diaspis echinocacti*, known as scale mealybug-with-shield or with-carapace of the palm, is among the most evolved group of these insects because it is sessile and secretes a waxy carapace (Miller and Kosztarab 1979, Rafael et al. 2012). Chemical insecticides applied on cactus pear plants may select resistant populations *of D. echinocacti* and contaminate meat and milk of cattle, goats and sheep fed with cladodes of this plant (Ramdani et al. 2021). Therefore, alternatives to traditional pesticides are desirable, especially for small cactus pear producers.

The impact of botanical insecticides and surfactants on natural enemies is lower than that of synthetic insecticides while protecting crops (Mossa et al. 2018). These products are also used in agriculture to increase wettability, spread pesticides on plants and improving target pest control (Ngegba et al. 2022, Baliota and Athanassiou 2023). Botanical insecticides degrade quicker and are more selective and with lower impact on humans, domestic animals and to the environment besides having lower risks to induce resistance and may be highly toxic and repellent to pests (Tembo et al. 2018, Amoabeng et al. 2019). Extracts from *Agave* plants were toxic to the bovine tick *Boophilus microplus* (Cannestrini, 1887) (Broglio-Micheletti et al. 2009) and to larvae of the mosquitoes *Aedes aegypti* (Linnaeus, 1762) and *Culex quinquefasciatus* (Say, 1823) (Nunes et al. 2014, Oliveira et al. 2016). Those of the hybrid *Agave* (*Agave sisalana* Perrine) caused cytotoxicity in primary cells of *A. aegypti larvae*, and its formulation are deposited at the Instituto Nacional da Propriedade Industrial INPI BR1020180130056 with a description of the collection, stabilization and processing as a concentrated powder, soluble in water (Oliveira et al. 2016).

Mineral oils, petroleum distillates, are usually used with 1-2% of the active ingredient (McGraw et al. 2022), alone or combined with other insecticides to manage mealybugs (Donahue and Brewer 1998, Ramdani et al. 2021). Low residual activity, no relation to pest insect resistance, and reduced toxicity to mammals and beneficial insects are advantages of mineral oils (Baliota and Athanassiou 2023).

The objective of this work was to determine the effectiveness of *Agave* genotype extracts, applied alone or mixed with mineral oil, against the cactus scale mealybug *D. echinocacti*.

MATERIAL AND METHODS

Study location

The study was carried out in the entomology laboratory (7º13'32"S latitude and 35º54'19" W longitude) in a greenhouse (7º13'35"S latitude and 35º54'21"W longitude) and in the experimental field (7º13'50"S latitude and 35º52'52"W longitude) at the Empresa Brasileira de Pesquisa Agropecuária (Embrapa) Algodão in the municipality of Campina Grande, Paraíba state, Brazil, in 2021 with two experiments. In the first one, the most promising concentration of each sisal genotype to cause mortality of 80% (CL_{80}) of the first instar mobile nymphs of *D. echinocacti* was obtained, and in the second one, the effectiveness of this concentration, against nymphs, pupae, and adults of different ages of this scale mealybug was determined in laboratory and greenhouse.

Insects, plants, and plant extracts

Fifty cladodes of the cactus pear, from plants of the cultivar "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.] Haw., at 5 years old, were collected from experimental planting in the field of the Instituto Nacional do Semiárido (INSA) (07º14'00"S latitude and 35º57'00"W longitude), municipality of Campina Grande. Half of these cladodes was planted in the experimental field of Embrapa Algodão in an area of 54 m^2 (6 × 9 m) spaced by 2 × 1.5 m and the other half in 25 plastic pots with a volume of 20 L each and kept in a greenhouse of this institution. Ninety days after planting, ten cactus pear plants, cultivated in the field, were each one infested, with 20 first-instar nymphs of the scale mealybug *D. echinocacti*. Thirty days after the infestation, cladodes from this palm were collected, packed in Kraft paper bags, and taken to the Entomology Laboratory, where they were kept in a refrigerator at 8°C until the beginning of the bioassays. Sixty days before starting the bioassay, the plants grown in a greenhouse were infested.

Mature leaves of the six sisal genotypes with 3 years old were collected from the middle region of these plants and grinded in manual equipment until the complete extraction of their crude extracts. These extracts were filtered, transferred to plastic containers, and stored in a freezer at -10°C protected from light until use in the assay (Oliveira et al. 2016).

Experiment 1: selection of sisal genotype extracts based on their lethal concentrations

Six Agave extracts were tested, and one of those which lethal concentration caused 80% mortality (CL_{80}) of the *D. echinocacti* nymphs was selected. This selection was carried out in the laboratory by submerging intermediate cladode discs (rackets) from the palm "Orelha de Elefante Mexicana", *O. stricta*, collected 120 days after planting at Embrapa Algodão and after drying and infestation with *D. echinocacti* nymphs.

The experimental design was in randomized blocks with seven treatments and five replications per sisal extract concentration. The treatments were cactus pear cladodes submerged in five concentrations of *Agave* hybrid 22648 (T1), *A. fourcroydes* cv. Cabinho (T2), *A. fourcroydes* (T3), *A. sisalana* cv. Valente (T4), *A. sisalana* cv. Tatuí 3 (T5), *A. sisalana* cv. Tatuí 4 (T6) extracts in addition to the control (T7-distilled water). The concentrations were: pure *Agave* extract (EPDS), at the concentrations of 40 mg, 10 $m{\cdot}L^{-1}$, 10 $m{\cdot}L^{-2}$ and 10 $m{\cdot}L^{-3}$ mg i.a./100 mL for each genotype of this plant, in addition to the control treatment with 10 $m{\cdot}L^{-4}$ of distilled water. Each cladode disk, secondary and/or tertiary (3.5 cm in diameter × 1 cm thick), was cut from a non-infested pad by the scale mealybug using a steel pourer, submerged in the insecticide mixture and air dried for 1 hour. Then, ten mobile first instar *D. echinocacti* nymphs were transferred to each of the five discs per replication totaling 250 mobile nymphs per extract concentration. The cladodes discs were placed in Petri dishes and kept in a climate-controlled chamber at 25 ± 2°C, 60 ± 10% relative humidity and 12 hours photophase until the final evaluation.

Mortality of first-instar mobile *D. echinocacti* nymphs was determined daily for eight days under a stereomicroscope EL224 (BEL Engenharia, Monza, Milan, Italy) with a 20x magnification. Natural mortality data was corrected according to the control treatment with distilled water (Abbott 1925). Dead insects are dark in color and when dried they detach easily from the plant tissue when touched with the soft bristles of a fine brush (Zeitoun et al. 2020).

Experiment 2: effectiveness of the sisal extract and mineral oil in the laboratory

The effectiveness of the aqueous sisal extract, alone or in mixture with the mineral oil Assist (Basf S.A. Av. Brasil, 791, Guaratinguetá, SP, Brazil), in causing mortality of *D. echinocacti*, was determined in the laboratory. The sisal extract with the highest CL_{80} was selected and tested with the same methodology submerging the palm cladodes in the solutions. Assist oil is a mixture of paraffinic, paraffinic and aromatic saturated and unsaturated hydrocarbons from petroleum distillation and used as an emulsion in water at the concentration recommended by the manufacturer (10 $mL{\cdot}Liter^{-1}$).

The experimental design was in randomized blocks in a 2×5 factorial scheme represented by the forage cactus cladode discs artificially infested with first instar nymphs or naturally infested by nymphs, pupae, and adults of different ages of *D. echinocacti*. These discs were submerged in the *A. sisalana* extract (at the most efficient concentration determined in the laboratory bioassay) (T1), mineral oil (Assist) at a concentration of 10 mL/L (T2), Thiamethoxan (Actara 250 WG) at 0.2 $g{\cdot}L^{-1}$ (T3); *A. sisalana* extract (in the most efficient one determined in the laboratory bioassay) + (Assist) in the 10 $mL{\cdot}L^{-1}$ and distilled water (T5, control), with five treatments and four replications each. Discs (3.5 cm in diameter × 1 cm in thickness), naturally infested by *D. echinocacti*, were cut from 5-year-old palm cladodes in the experimental planting at INSA. These discs were transferred to Petri dishes, identified, and taken to the laboratory where live mealybugs were counted and, after that, submerged in the insecticide solution. The artificial infestation of palm disks, by the scale mealybug, followed the same methodology of exposing the individuals of this pest in natural infestation to the insecticide.

Mortality of first-instar nymphs and females and nymphs and pupae of *D. echinocacti*, at different ages, on palm cladode disks infested naturally or artificially, respectively, was evaluated every 24 hours for eight days using the same methodology and criteria, described, to confirm the death of this insect.

Effectiveness of sisal extract and mineral oil in a greenhouse

The effectiveness of the sisal extracts, alone or in mixture with mineral oil, against adults, nymphs, and pupae of different ages of *D. echinocacti*, in a greenhouse, was determined using the same concentration tested in the laboratory bioassay.

The experimental design was in randomized blocks, with five treatments and four replications each. The treatments were palm cladodes artificially infested with *D. echinocacti* and sprayed with sisal extract (at the most efficient concentration determined in the laboratory bioassay) (T1), mineral oil (Assist) at a concentration of 10 mL·L^{-1} (T2), Thiamethoxan (Actara 250 WG) at 0.2 g·L^{-1} (T3); *A. sisalana* extract at the most efficient concentration determined in the laboratory bioassay + Assist at 10 mL·L^{-1} (T4) and in the control (T5-distilled water). The experiment was carried out in duplicate.

The cactus pear cladodes were infested when the plants were 60 days old, and the bioassay started 60 days after infestation. Each cladode, from each of the 25 cactus pear plants, was artificially infested with 10 first-instar mobile nymphs of *D. echinocacti*, totaling 250 in the test. The cladodes of the apical region of the cactus pear plants were infested by fixing a disc of this plant (3.5 cm in diameter × 1 cm thick), with the scale mealybug, on one of the faces of each plant cladode grown in pots in the greenhouse using an entomological pin, previously disinfected with 70% alcohol.

Sixty days after infestation, a disk of the palm cladode, with a size similar to that used in the infestation, was cut from each plant where the number of adults, nymphs and pupae of different ages of *D. echinocacti* was counted. These discs were cut with the aid of a steel punch, and the samples placed in Petri dishes and identified in plastic trays by counting live scale mealybug specimens in the laboratory. After counting, the plants were sprayed with the respective treatments using a manual sprayer with a volume of 5 L of spray solution and a D2 empty cone nozzle. The spray tip was positioned laterally in relation to the cladodes at about 20 cm from the plants with a flow rate adjusted according to their growth stage.

The mortality of *D. echinocacti* was evaluated after 24, 72 and 120 hours of application, in each of the palm cladode discs sampled per period. Deaths of the insects were confirmed using the same methodology and criteria described. Scale mealybug males were not examined because they tend to cluster together, making them difficult to count.

Data analysis

The lethal concentration (LC_{80}) and confidence intervals (CI_{95}) of the data for the aqueous extracts of the six *Agave* genotypes were calculated by Probit mortality regression analysis (Finney 1971) with the number of first-instar *D. echinocacti* nymphs versus log10 dose. Goodness of fit was assessed using Pearson's chi-square test. Estimates of lethal concentrations differed when their IC_{95} did not overlap. Data were analyzed using the R program (R Core Team 2017). The sisal aqueous extract selected was the one with the highest effectiveness and the lowest lethal concentration estimated to cause mortality of 80% (CL_{80}) of *D. echinocacti* nymphs. Additional replications or dilutions were performed whenever the regression line did not fit the model proposed (Fernandes et al. 2020). The adjustment of the extract concentration to a mortality efficiency of *D. echinocacti* ≥ 80% was made by multiplying per 0.5, one, 1.5 and two times the lethal dose obtained, initially, and the lethal concentration, again, recalculated by Probit analysis (Finney 1971).

The normality of the mortality data of *D. echinocacti* on the cactus pear, in the laboratory and greenhouse bioassays, was verified using the Shapiro-Wilk's test and the homoscedasticity of the residues with the Bartlett's test. The laboratory data were then submitted to two-way analysis of variance (ANOVA) and those from the greenhouse to ANOVA, with the means compared using the Student–Newman–Keuls' test ($P = 0.05$) with the statistical and genetic analysis system (SAEG) (Ribeiro Júnior 2001). The concentrations of *Agave* extract, used in the effectiveness bioassays, were calculated according to the CL_{80} estimated by Probit in the first bioassay.

RESULTS

Experiment 1: selection of *Agave* genotype extracts based on their lethal concentration

The lethal concentration of the extract from the sisal genotype *A. fourcroydes* cv. Cabinho (LC_{80} = 5.67 mL·100 L^{-1} and CI_{95} = 2.81–14.90) was the most efficient in causing mortality of 80% of *D. echinocacti* population (LC_{80}), followed by that of *A. sisalana* cv. Tatuí 3 (LC_{80} = 7.62 mL·100 L^{-1} and IC_{95} = 23.13–28.20) and those of the hybrid *Agave* 22648

(L_{C80} = 34 mL·100 L^{-1} and IC_{95} = 9.78–265) and from *A. sisalana* cv. Valente (LC_{80} = 30 mL·100 L^{-1} and IC_{95} = 10.30–159), the least efficient (Table 1). The lethal concentrations of the extracts from the sisal genotypes, estimated to cause mortality of 80% of the *D. echinocacti* population, except the *A. fourcroydes* cv. Cabinho, fitted the regression models, according to pearson's chi-square test (Table 1, P > 0.05), with the slope of the mortality lines, with values from 0.36 to 1.77 and from 0.40 to 1.27, respectively (Table 1).

Table 1. Estimation of the lethal concentration of aqueous extracts of six sisal genotypes to cause mortality of 80% of the population (LC_{80}) of *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae).

Sisal extract	N	LC_{80}	SL	χ^2	P	CI
Agave híbrido 22648	50	34.00	0.40	0.72	0.70	9.78; 265.00
Agave fourcroydes cv. Cabinho	50	5.67	1.27	4.69	0.03	2.81; 14.90
Agave fourcroydes	50	16.40	0.86	1.15	0.56	6.12; 73.00
Agave sisalana cv. Valente	50	30.00	0.82	0.36	0.88	10.30; 159.00
Agave sisalana cv. Tatuí 3	50	7.62	0.93	1.77	0.57	3.13; 28.20
Agave sisalana cv. Tatuí 4	50	23.70	0.88	1.70	0.59	8.76; 108.00

N: number of insects tested; Pearson's chi-square test (χ^2) to test the goodness of fitting to the Probit model; P > 0.05 indicated that the observed regression model did not differ from the expected one; LC_{80}: lethal concentration; SL: slope of the line; CI: confidence interval.

Experiment 2: laboratory mortality

The mortality of *D. echinocacti* after 10 days of contamination with extract from the *A. fourcroydes* cv. Cabinho sisal was 62.50 ± 3.83% (< 80%) (LC_{80} = 5.67 mL·10 L^{-1} and IC_{95} = 2.81–14.90) (Table 1).

The mortality data of newly hatched first-instar *D. echinocacti* nymphs on artificially infested palm cladodes were adjusted to the five new concentrations incorporated into the mortality curve, estimating a CL_{80} = 10.9 mL·10 L^{-1} and IC_{95} = 5.59–141.42 (Table 2).

Table 2. Adjusted estimate of the lethal concentration of the aqueous extract of *Agave fourcroydes* cv. Cabinho to cause mortality of 80% of the population (LC_{80}) of *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae).

Sisal extract	N	LC_{80}	SL	χ^2	CI
A. fourcroydes cv. Cabinho	250	10.9	0.47	0.056	(5.59; 141.42)

N: number of insects tested; Pearson's chi-square test (χ^2) to test the goodness of fitting to the Probit model; P > 0.05 indicated that the observed regression model did not differ from the expected one; LC_{80}: lethal concentration; SL: slope of the line; CI: confidence interval.

The interaction between the percentage of adjusted mortality of *D. echinocacti* with the biological target and the insecticide 10 days after contamination was significant ($F_{4.36}$ = 2.64; P < 0.05) (Table 3). Mortality of newly hatched first-instar nymphs in artificial infestation and adults + immature stages, predominantly sessile forms (> 90%) of this scale mealybug in natural infestation, ranged from 79.17 ± 1.65 to 100 ± 0 and from 74.51 ± 0.93 to 100 ± 0, respectively, depending on the insecticide used (Table 4).

Table 3. Summary of analysis of variance for the percentage of mortality of *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) on discs of cactus pear cladodes "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.] Haw. cv. per stage (nymphs and adults) and insecticide in the laboratory.

Source of variation	DF	MS	*F*	*P*
Insecticide (I)	4	16,513.56	3,182.601	< 0,001
Stages (S)	1	72.70254	14.012	< 0,001
I × S	4	13.69573	2.64	= 0,049
Residue	36	5.188698	-	-

DF: degrees of freedom; MS: mean square.

Table 4. Mortality percentage (average ± standard error) of newly hatched first-instar nymphs in artificial infestation (Nymphs) and of adults and immature stages (adults and immature) with different ages, of the scale mealybug *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) in natural infestation in the laboratory on cladode discs of the cactus pear "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.] Haw. cv., after immersion in a solution with sisal extract (value adjusted to the proposed model), mineral oil (Assist), Thiamethoxan (Actara 250 WG), mixture of sisal extract + mineral oil in different concentrations and control (distilled water)*.

Treatments	Conc.	Nymphs	Adults and immature
Sisal extract	1,200 mL/L	79.17 ± 1.65 dA	74.51 ± 0.93 dB
Mineral oil	10 mL/L	94.79 ± 1.65 bA	90.11 ± 0.50 bB
Thiamethoxan	0.2 g/L	87.50 ± 2.08 cA	84.78 ± 0.67 cA
Sisal extract + mineral oil	1,200 + 1 mL/L	100.00 ± 0.00 aA	100.00 ± 0.00 aA
Control	-	0.00 ± 0.00 eA	0.00 ± 0.00 eA

*Means followed by the same lowercase letter per column or uppercase per line do not differ by the Student–Newman–Keuls' test (P = 0.05).

The mortality of *D. echinocacti* in the laboratory was higher with the mixture of the *Agave* extract with mineral oil followed by this material and thiamethoxan with lower values. Mortality of first-instar mobile nymphs in artificially infested palm discs was higher with *A. fourcroydes* cv. Cabinho extract and mineral oil, separately, and that of adults and immatures (nymphs of other instars and pupae) of different ages of *D. echinocacti* in naturally infested palm discs was lower. Mortality of first-instar mobile nymphs under artificial infestation and of nymphs, pupae and adults under natural infestation was similar with thiamethoxan, sisal extract + mineral oil and distilled water (Table 4).

Mortality in greenhouse

The cladode areas of the forage cactus Orelha de Elefante Mexicana and the previous numbers of *D. echinocacti* individuals, estimated by disc and cladode of this plant, were similar between treatments (Table 5).

Table 5. Cladode and disc areas of the cactus pear "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.], and initial numbers of individuals of the scale mealybug *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) estimated per disc (NISED) and cladode (NIEC) of this plant in a greenhouse per treatment*.

Treatments	Cladode (cm^2)	Disc (cm^2)	NISED	NIEC
T1 Sisal extract	292.02 ± 25.49a	3.14	91.80 ± 3.32a	8,489.40 ± 685.32a
T2 Mineral oil	262.98 ± 23.48a	3.14	92.00 ± 4.11a	7,708.78 ± 737.01a
T3 Thiamethoxan	345.77 ± 77.87a	3.14	87.20 ± 3.43a	9,650.42 ± 2,355.37a
T4 Sisal extract + mineral oil	303.17 ± 24.47a	3.14	83.00 ± 3.77a	8,055.93 ± 820.61a
T5 Control	308.98 ± 27.68a	3.14	84.60 ± 3.94a	8,303.60 ± 768.08a
F	0.58	-	1.15	0.42
P	> 0.05	-	= 0.37	> 0.05
C.V.	29.14	-	9.8	30.25

*Means followed by the same letter per column do not differ by the Student–Newman–Keuls' test (P = 0.05); C.V.: coefficient of variation.

The mortality of nymphs, pupae, and adults with different ages of *D. echinocacti* on cladodes of the forage palm Orelha de Elefante Mexicana artificially infested in a greenhouse was higher with mineral oil, alone or in mixture with *Agave* extract in the four first assessments (Table 6). On the tenth day after application, the mortality of nymphs, pupae, and adults, with different ages, of *D. echinocacti* was higher with the mixture of mineral oil and *Agave* extract.

Mortality of *D. echinocacti* was lower in the first, third, eighth and tenth day after application of *Agave* extracts and similar at five days after application of sisal extracts and thiamethoxan (Table 6).

Table 6. Mortality (%) of the scale mealybug *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) on the cactus pear "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.], cladodes in greenhouse after one, three, five, eight and 10 days of the immersion (DAP) of their immersion in solutions of the sisal extract (value adjusted to the proposed model), mineral oil (Assist), Thiamethoxan (Actara 250 WG), mixture of extract of sisal + mineral oil and control (distilled water)*.

Treatments	Mortality (%, mean ± standard error)				
	1 DAP	3 DAP	5 DAP	8 DAP	10 DAP
T1 Sisal extract	14.99 ± 1.03c	34.99 ± 2.00c	56.19 ± 0.43b	67.11 ± 0.74c	74.72 ± 0.55d
T2 Mineral oil	57.81 ± 1.08a	71.15 ± 1.74a	86.99 ± 0.63a	92.16 ± 1.99a	96.09 ± 1.09b
T3 Thiamethoxan	34.16 ± 1.83b	49.23 ± 0.83b	61.21 ± 2.84b	76.53 ± 0.48b	81.75 ± 0.45c
T4 Sisal extract + mineral oil	57.49 ± 0.49a	72.97 ± 1.28a	88.47 ± 0.44a	95.33 ± 1.98a	98.40 ± 0.98a
T5 Control	00.00 ± 0.00d	00.00 ± 0.00d	00.00 ± 0.00c	00.00 ± 0.00d	00.00 ± 0.00e
F	778.18	389.74	744.25	824.38	4,281.68
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
C.V.	6.26	7.45	5.02	4.56	1.97

*Means followed by the same letter per column do not differ by the Student–Newman–Keuls' (P = 0.05); C.V.: coefficient of variation.

DISCUSSION

Experiment 1: selection of *Agave* genotype extracts based on their lethal concentration

The greatest efficiency of the lethal concentration of extracts from the sisal genotype *A. fourcroydes* cv. Cabinho to cause 80% mortality of the *D. echinocacti* population followed by that of *A. sisalana* cv. Tatuí 3 and with lower values for the *Agave* hybrid 22648 and *A. sisalana* cv. Valente extracts confirms the greatest toxicity of the first two (Pereira et al. 2019). The variations in the composition of secondary metabolites such as phenols, flavonoids, phytosterols and saponins between agave genotypes can explain these results (Zykin et al. 2018, Bermúdez-Bazán et al. 2021). These chemical constituents affect insects in different ways. Tannins can access the hemolymph of the gypsy moth through its intestinal peritrophic membrane reducing the growth of this insect (Rossiter et al. 1988). Flavonoids are the main chemicals of 5–10% of secondary metabolites involved in plant defense mechanisms with toxicity to insects (Upasani et al. 2003). Sterols, like Δ7 and Δ8, cannot be converted by insects into cholesterol and, when fed diets rich in these compounds, they have high mortality and reduced reproduction (Behmer 2017). Saponins with deterrent properties interfere with the permeability of the intestinal mucosal cells, reducing the digestibility and absorption of nutrients and, consequently, insect growth (Abubakar et al. 2021).

The quality of fitting of the CL_{80} data of the *D. echinocacti* population mortality with extracts of the genotypes *Agave* hybrid 22648, *A. fourcroydes, A. sisalana* cv Valente, *A. sisalana* cv Tatuí 3 and *A. sisalana* cv Tatuí 4 was significant comparing the observed and expected regression models, but not for the *A. fourcroydes* cv. Cabinho.

Experiment 2: laboratory mortality

Mortality lower than 80% of *D. echinocacti* with the sisal extracts *A. fourcroydes* cv. Cabinho indicates that their CL_{80} estimated by Probit did not correspond to the actual observed values, which may be related to the distance between the dilution intervals (Fernandes et al. 2020). Therefore, the mortality curve was adjusted with the inclusion of five additional concentrations, besides the lethal one, and the data resubmitted to Probit analysis to estimate the LC_{80} again.

The adjustment of mortality data of newly hatched first-instar *D. echinocacti* nymphs on artificially infested palm cladodes, with the five concentrations incorporated into the mortality curve, is due to the larger sample size (dilutions) improving the quality of the parameters of binary regression obtained from the logit and Probit link functions (Fernandes et al. 2020). This is important because the new estimated CL_{80} corresponded to the observed mortality, complying with Brazilian legislation requiring minimum values of 80% to select a potential insecticide molecule (Brasil 2004).

The significant interaction between the percentage of the adjusted *D. echinocacti* mortality at 10 days after immersion of the cladode into insecticide mixtures and the stage of this scale mealybug indicates variations with extract and insecticides. This may be due to the different action modes of these products (Martín-López et al. 2006) and the greatest susceptibility of mobile nymphs compared with the sessile ones due to the chemical composition of the tegument of the former (Quesada et al. 2018). Botanical insecticides and/or mineral oils have ovicidal action and affect insect behavior, physiology, morphology, and metabolism, inhibiting their growth and oviposition (Acheuk et al. 2022, Baliota and Athanassiou 2023). In addition, many of them exhibit neurotoxic mechanisms through interference with the neuromodulator octopamine or GABA-gated chloride channels (Taverner et al. 2001, Isman 2006).

The highest mortality of *D. echinocacti* with the mixture of sisal extract and mineral oil indicates an additive effect of the latter as found for different oils, alone or combined with imidacloprid or pirimicarb against *Myzus persicae* Sulzer (Hemiptera: Aphididae) in the laboratory (Martín-López et al. 2006) and imidacloprid against *Bemisia tabaci* (Hemiptera: Aleyrodidae) in laboratory and field (Ismail 2021). This can facilitate the use of biopesticides from agave plants, reducing that of synthetic products. The mortality of adult females of the carmine scale mealybug, *D. opuntiae* was higher with the hydroalcoholic extract of the *A. sisalana* leaves mixed with the synthetic insecticide chlorpyrifos than with these products alone, which was attributed to the synergic effect between the insecticide and the extract (Lopes et al. 2018). On the other hand, the high mortality of *D. echinocacti* with mineral oil, alone, is due to the action Mode of this material, covering the waxy carapace of scale insects with a thin film and killing them by asphyxiation (Martín-López et al. 2006). Furthermore, mineral oils are non-polar and therefore more likely to penetrate the hydrophobic integument of the scale mealybug (McGraw et al. 2022) as shown for eggs, nymphs, and adults of the mealybugs *Lepidosaphes beckii* (Newman) and *Parlatoria ziziphus* (Lucas) (Hemiptera: Diaspididae) on citrus plants treated with mineral oil in Sheben El-Kanater, Caliubia province, Egypt (Helmy et al. 2012).

The lower *D. echinocacti* mortality with the sisal extract in the laboratory alone can be attributed to its total contents of phenolics and flavonoids and other secondary metabolites, which vary with extraction method, agave species, plant tissues used, age of the plant, geographical origin, and solvent (Gurjar et al. 2012, Ramdani et al. 2021, Soto-Castro et al. 2021). The high concentration of sisal extracts, to cause great mortality of the different immature stages and females of *D. echinocacti*, confirms the need to improve the extraction methods for these secondary metabolites and to develop products based on pure, stable, and bioactive compounds. However, high temperatures and pressures and ultrasound monitoring improved the yield of phenols and flavonoids by reducing the extraction period of these metabolites (Ben Hamissa et al. 2012, López-Romero et al. 2018) and the rupture of vacuoles, by cavitation, increased the release of aglycones and the yield of phenols extracted from agave (Ameer et al. 2017).

The lower accumulation of wax on the bodies of first-instar mobile nymphs of *D. echinocacti* can explain their greater vulnerability and higher mortality on artificially infested palm disks after application of *Agave* extracts and mineral oil alone compared to those of other instars and pupae and adults of different ages of this insect in naturally infested palm discs (Quesada et al. 2018). Sessile nymphs of the scale mealybugs, predominant in natural infestations, feed on the cell plant contents without producing honeydew and are characterized by a waxy covering, formed 24 to 48 hours after its fixation on the leaf tissue with 50% of wax in its weight and rather hydrophobic (Quesada et al. 2018). The effectiveness of insecticides may vary with the chemical composition of the integuments of mobile and sessile nymphs.

The similar mortality of first-instar mobile nymphs, with artificial infestation, and that of nymphs, pupae, and adults, with natural infestation, with thiamethoxan and sisal extract + mineral oil may be related to the formulation type of the first two insecticides. Thiamethoxan is formulated in water-dispersible granules with the active ingredient combined with a larger particle (granule), but without the suffocating effect of mineral oil on the scale mealybug, as in the mixture of sisal extract + oil (Martín-López et al. 2006). The mortality of the scale mealybug in different stages reaching 100% confirms the synergistic effect of the mixture of sisal extract + oil as reported for the synthetic insecticide Eforia 043 ZK (thiamethoxam + lambdacyhalothrin), applied alone and in reduced doses of 1/5 and 2/5 in mixture with Akarzin mineral oil, on *Tychius flavus* (Beck) (Coleoptera: Curculionidae), *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae), *Acyrthosiphon pisum*

(Harr.) (Hemiptera: Aphididae), harmful thrips (Thysanoptera) and cicadas (Hemiptera: suborder Auchenorrhyncha) (Nikolova and Georgieva 2018).

The highest mortality of immature stages and females of *D. echinocacti* with the *A. fourcroydes* cv. Cabinho extract and that of 16.7, 3.3 and 3.3% of females of this scale mealybug with *Stryphnodendron adstringens* (Fabaceae) extract at the concentration of 10% and of *Annona crassiflora* (Annonaceae) at 10 and 20%, respectively, is important, as values equal to or greater than 80% are satisfactory for using plant extracts in pest management (Potenza et al. 2005).

Mortality in greenhouse

The cladode areas and the initial numbers of individuals of *D. echinocacti*, per disc of the cactus pear cladode, in different treatments, indicate a similar population density of this scale mealybug in the initial count, reducing the variability of these data (Taylor 1987, Sudo et al. 2019).

The highest mortality of *D. echinocacti*, in the tenth day after the application of the mixture of mineral oil and *Agave* extract, confirms the additive effect of the first one. This is important, as the effect of mineral oils, pure or mixed with chemical insecticides, varies with their type, insecticide group, insect or host plant species, and abiotic factors (Nikolova and Georgieva 2018).

The extraction methodology and seasonal collection variations explain the lower *D. echinocacti* mortality with the *Agave* extract, alone, in a greenhouse. The mortality of nymphs, pupae, and adults of different ages of *D. echinocacti*, with the agave genotype *A. fourcroydes* cv. Cabinho extract was lower than the minimum required by the Brazilian legislation to select potential insecticidal molecules (Brasil 2004). However, this does not exclude the potential of this *Agave* extract against this scale mealybug, as the addition of mineral oil and the extraction and drying process can increase its concentration, stability, and efficiency (Purkait et al. 2019, Bermúdez-Bazán et al. 2021). In addition, obtaining sisal extract is inexpensive, with the residue from defibration being the main part of this plant. This material is not normally used in the production chain of this plant, facilitating its use against insects (Mishra et al. 2004). These actions can ease the obtention of molecules with selectivity and greater efficiency to manage the scale mealybug.

CONCLUSION

The extract of the genotype *A. fourcroydes* cv. Cabinho caused higher mortality of *D. echinocacti*.

The ideal concentration of the aqueous extract of *A. fourcroydes* cv. Cabinho to cause mortality in 80% of the population (LC_{80}) of *D. echinocacti* was 10.9 $mL \cdot 10\ L^{-1}$.

The mineral oil + sisal extract showed an additive effect against the scale mealybug *D. echinocacti* in greenhouse.

Mortality of nymphs, pupae, and adults of different ages of *D. echinocacti* in laboratory and in the greenhouse was greater than 74% with the extract of the sisal genotype *A. fourcroydes* cv. Cabinho.

AUTHORS' CONTRIBUTION

Conceptualization: Albuquerque Junior, P. S., Silva, C. A. D. and Zanuncio, J. C.; **Methodology:** Albuquerque Junior, P. S., Silva, C. A. D., Medeiros, E. P. and Zanuncio, J. C.; **Investigation:** Albuquerque Junior, P. S. and Silva, C. A. D.; **Writing – Original Draft:** Albuquerque Junior, P. S., Silva, C. A. D., Medeiros, E. P. and Zanuncio, J. C.; **Writing – Review and Editing:** Silva, C. A. D., Medeiros, E. P. and Zanuncio, J. C.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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