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Efficacy of some biorational insecticides against the cactus scale pest *Diaspis echinocacti* (Bouché, 1833) (Hemiptera: Diaspididae) in Morocco

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Abstract

Diaspis echinocacti (Bouché) is a significant pest affecting various cactus pear species worldwide. Over the past three years, this scale insect was able to build up a considerable population and had now reached a pest status in Morocco. To control this pest, insecticides with different modes of action were evaluated in laboratory and greenhouse. In laboratory bioassays, d-limonene at 30-150 cc/hl, mineral oil at 700-2400 cc/hl, potassium salts of fatty acid at 15-60 cc/hl, and pyriproxyfen at 8-37 cc/hl were evaluated against first instar nymphs and adult females of the cochineal, while only d-limonene applied at 150 cc/hl, mineral oil applied at 2000 and 2400 cc/hl, and potassium salts of fatty acid applied at 60 cc/hl were used in greenhouse tests. In laboratory tests, mineral oil at 2400 cc/hl, d-limonene at 150 cc/hl, and potassium salts of fatty acid at 60 cc/hl were the most effective against nymphs and adult females of the cochineal 48 hours after exposure to insecticide treatments, resulting in LT_{50} values of 24, 24 and 48 h (nymphs), and 48 h (adult females), respectively. Under greenhouse conditions, mineral oil at 2400 cc/hl and d-limonene at 150 cc/hl showed significantly higher mortality rates [70 and 67% (nymph) and 45 and 42% mortality (adult female), respectively] and a high potential for degrading waxy carapace covering the body of the cochineal. Effective control of this harmful pest depends on a justified use of these two insecticides (mineral oil and d-limonene) and requires the development of an integrated management approach.

Keywords: *Diaspis echinocacti*, biorational insecticides, prickly pear, D-limonene, mineral oil

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Efficacité de certains insecticides biorationnels contre la cochenille de cactus *Diaspis echinocacti* (Bouché, 1833) (Hemiptera: Diaspididae) au Maroc

Résumé

Diaspis echinocacti (Bouché) est un ravageur important qui affecte différentes espèces de de cactus dans le monde entier. Au cours des trois dernières années, cet insecte a réussi à constituer une population considérable et est maintenant considéré comme un ravageur au Maroc. Afin de contrôler ce ravageur, des insecticides avec différents modes d'action ont été évalués au laboratoire et sous serre. Dans les essais du laboratoire, le d-limonène à des doses de 30 à 150 cc/hl, l'huile minérale à des doses de 700 à 2400 cc/hl, les sels de potassium d'acides gras à des doses de 15 à 60 cc/hl, et le pyriproxyfène à des doses de 8 à 37 cc/hl ont été évalués contre les nymphes du première stade et les femelles adultes de la cochenille. Sous serre, seules les doses de d-limonène à 150 cc/hl, d'huile minérale à 2000 et 2400 cc/hl, et de sels de potassium d'acides gras à 60 cc/hl ont été utilisées. Au laboratoire, l'huile minérale à 2400 cc/hl, le d-limonène à 150 cc/hl et les sels de potassium d'acides gras à 60 cc/hl se sont révélés les plus efficaces contre les nymphes et les femelles adultes de la cochenille, 48 heures après exposition aux traitements insecticides, résultant en des valeurs de LT_{50} de 24, 24 et 48 heures (pour les nymphes) et 48 heures (pour les femelles adultes), respectivement. Sous serre, l'huile minérale à 2400 cc/hl et le d-limonène à 150 cc/hl ont montré des taux de mortalité significativement plus élevés (70 et 67 % sur les nymphes et 45 et 42 % de mortalité sur les femelles adultes, respectivement) et un fort potentiel de dégradation de la carapace cireuse qui couvre le corps de la cochenille. La lutte efficace contre ce ravageur dépend de l'utilisation justifiée et judicieuse de ces deux insecticides (l'huile minérale et le d-limonène) et nécessite le développement d'une approche de gestion intégrée.

Mots-clés : *Diaspis echinocacti*, insecticides biorationnels, figuier de Barbarie, D-limonène, huile minérale.

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فعالية بعض المبيدات الحيوية ضد سوسة الصبار *Diaspis echinocacti (*Bouché, 1833) *(Hemiptera: Diaspididae)* في المغرب

ملخص

تعتبر(Bouché) *Diaspis echinocacti* آفة للعديد من أنواع الصبار في جميع أنحاء العالم. على مدى السنوات الثلاث الماضية، انتشرت هذه الحشرة المتقشرة بشكل كبير وتعتبر الآن آفة في المغرب. لمكافحة هذه الآفة، تم تقييم المبيدات الحشرية ذات أساليب عمل مختلفة في المختبر وفي البيوت الدفيئة. في الاختبارات المعملية، تم تقييم d-limonene بجرعات من 30 إلى 150 cc/hl، والزيت المعدني بجرعات من 700 إلى 2400 cc/hl، وأملاح البوتاسيوم للأحماض الدهنية بجرعات من 15 إلى 60 cc/hl، و pyriproxyfène بجرعات من 8 إلى 37 cc/hl. في اختبارات البيوت الدفيئة، تم استخدام فقط جرعات من d-limonene عند 150 cc/hl، والزيت المعدني عند 2000 و 2400 cc/hl، وأملاح البوتاسيوم للأحماض الدهنية عند 60 cc/hl. في الاختبارات المعملية، وجد أن الزيت المعدني عند 2400 cc/hl ، وd-limonene عند 150 cc/hl، وأملاح البوتاسيوم للأحماض الدهنية عند 60 cc/hl، أكثر فعالية ضد الحوريات والإناث البالغات للبق الدهني، بعد 48 ساعة من التعرض لعلاجات المبيدات الحشرية، بقيم LT_{50} تبلغ 24 و 24 و 48 ساعة (الحوريات)، و 48 ساعة (الإناث البالغات) على التوالي. في ظروف البيوت الدفيئة، أظهر الزيت المعدني عند 2400 cc/hl و d-limonene عند 150 cc/hl معدلات وفيات أعلى بشكل ملحوظ [70٪ و 67٪ (الحوريات) و 45٪ و 42٪ (الإناث البالغات)، على التوالي] وإمكانية تحلل عالية للقشرة الشمعية التي تغطي بطن الحشرة القشرية. تعتمد المكافحة الفعالة لهذه الآفة على الاستخدام المبرر والحكيم لهذين المبيدين (الزيت المعدني وd-limonène) وتتطلب تطوير نهج إدارة متكامل.

الكلمات المفتاحية: الحشرة القشرية المسلحة *Diaspis echinocacti* ، مبيدات حشرية حيوية، صبار، دي-ليمونين D-limonène، زيت معدني.

Introduction

The *Opuntia* cactus pear cochineal *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) is a cosmopolitan species present all over the world. This particular scale insect has been documented as a pest on 72 plant species belonging to Asteraceae, Cactaceae, Fabaceae, Portulacaceae, Grassulaceae, and Urticaceae (Ben-Dov, 2009 cited by Japoshvili et al., 2010). Among these species, 66 are classified under the family Cactaceae, with 19 specifically belonging to the genus Opuntia, and thus should be considered as a polyphagous species that prefers cactus pear species. The cochineal population dynamics generally differ according to seasons of the year and environmental conditions. This species have two generations per year in greenhouses in Moscow, Russia (Saakyan-Baranova, 1954). The life cycle of *D. echinocacti* from egg to adult requires 22.8 to 25.9 days under controlled (27°C) and greenhouse conditions (Oetting, 1984). The nymphs of the scale pest settle more vertically on the ground than horizontally, with males becoming first identifiable 2 to 3 days after settling, and female crawlers are more active than males (Oetting, 1984). The adult females have a pre-oviposition period of 12 to 23 days followed by 1 to 2 months of oviposition (Oetting, 1984). *Diaspis echinocacti* is frequently found on cladodes and fruits of its cactus host and occasionally on underground crowns and roots (Miller and Davidson, 2005). Like other scale species, *D. echinocacti* has the ability to spread rapidly to uninfected areas by natural means such as wind, surface water, and via birds and animals. The cochineal feeds directly on the *Opuntia* cactus pear plant, which reduces the vigor of the plant and the honeydew secreted promotes the growth of pathogens that interfere with photosynthesis and can lead to plant death (Japoshvili et al., 2010). The damage caused by *D. echinocacti* is related to its direct feeding and indirectly to pathogens and toxins that are introduced into its hosts while feeding (Miller and Kosziarab, 1979). Control strategies based on integrated pest management (IPM) have been used in the control of *D. echinocacti* in cactus pear plantations, which involves chemical, biological, mechanical and physical measures, to prevent the growth of the scale insect populations (Bergamin Filho and Amorim, 1999). Conventional insecticides used to control this cochineal may not only induce scale pest resistance but could also leave dangerous chemical residues affecting the environment, as well as animal and human health. Therefore, the use of alternative, less toxic insecticides and biological control to maintain the cochineal populations at low levels of infestation is encouraged (Lima, 1998) because these products do not interfere with their natural enemies. In the case of severe attacks of *D. echinocacti*, Brazilian farmers recommend a treatment with 1% mineral oil or a popular compound called "querobão" which is composed of soap, tobacco, kerosene and water (Santos et al., 1997). In some countries such as Brazil, extracts of seeds, branches, leaves and fruits of the neem tree (*Azadirachta indica* A. Juss) have also been used as alternatives to chemical pesticides to control this harmful scale pest (Moraes et al., 2007). The most successful biological control programs against scale insects worldwide have involved predators and parasitoid insects (Moore 1988). Natural enemies of *D. echinocacti* include predators of the families Coccinellidae and Nitidulidae (Coleoptera), Phlaeothripidae (Thysanoptera), Eupalopsellidae (Acari: Prostigmata), and parasitoid species of the families Aphelinidae and Encyrtidae (Hymenoptera) (Ben-Dov, 2009). In Greece, *D. echinocacti* and its principal parasitoid *Aphytis diaspidis* Howard (Hymenoptera: Aphelinidae) were recorded on *Opuntia spp*. (Argyriou et al., 1976 cited by Japoshvili et al., 2010).

This study aimed to investigate an effective and rapid control method against the cactus pear cochineal, D. echinocacti. The objective was to find suitable solutions to control this pest by using biorational insecticides recommended by the National Office of Food Safety (ONSSA), which are known for their safety and environmentally friendly properties. The study focused on testing different insecticides at various doses to determine their efficiency in controlling this scale pest, D. echinocacti. The evaluation was conducted under different conditions, including laboratory and greenhouse settings, to assess the performance of these insecticides in various environments.

Materials and methods

Cochineal rearing

The colony of *D. echinocacti* was established from infested cladodes of *Opuntia spp*. collected in the greenhouse, at the Agricultural Technical Institute of Khmiss Zemamra-Doukkala "CQA" (33°15' N, 8°30' W), Morocco. Infested cladodes were transferred to the entomology laboratory of the National Institute of Agricultural Research, Zemamra, and placed in transparent entomological cages (80 cm X 80 cm X 80 cm) covered with mesh fabric to allow ventilation. To increase the number and monitor the age of the cochineal insects, both infested and non-infested cladodes were introduced into the cages weekly. The cochineal colony was maintained at 25 ± 2 °C, 66 ± 5% relative humidity, and a photoperiod of 12:12h (L:D).

Laboratory experiments

The lethal effect of four biorational insecticides on the cactus pear cochineal *D. echinocacti* was evaluated. Limocide (60 g D-limonene/L) ("Vivagro, Martillac, France") applied at 30, 50, 100 and 150 cc/hl, Insecticide 101 (780 g mineral oil/L) ("UPL, Ankleshwar, India") applied at 700, 1000, 2000 and 2400 cc/hl, Hamper (500 g potassium salts of fatty acid/L) ("Gowan Crop Protection, Faenza, Italy") applied at 15, 20, 40 and 60 cc/hl, and Brai 10 EC (100 g of pyriproxyfen/L) ("Sociedad Lainco, Barcelona, Spain") applied at 8, 12, 25 and 37 cc/hl, and the Control (tap water).The selected insecticides have different modes of action (El Aalaoui et al., 2019).

The experiments were performed in Petri dishes (14.5 cm diameter). Pieces of infested *Opuntia spp.* cladodes (60 cm^2) containing ten *D. echinocacti* nymphs (10 days old) ("**Trial 1**") and ten adult females ("**Trial 2**") were transferred to each Petri dish. Briefly, a specified amount of each treatment was poured into one liter of tap water and sprayed using a Potter spray tower (Burkard Scientific Ltd, Uxbridge, UK) to apply the insecticidal solution of a prescribed active ingredient and the expected concentration as a mist over Petri dishes and insects at 150 kPa. Petri dishes were arranged in a completely randomized design with 5 replicates per treatment. Mortality and LC_{50} were recorded at 12, 24, and 48 hours post-treatment. To ensure the reproducibility of results, all experiments were repeated independently twice over time.

Greenhouse experiments

In 2021-2022, a trial was conducted at the Agricultural Technical Institute of Khmis Zemamra-Doukkala "CQA" in a greenhouse (11 m long x 7 m wide x 3 m high) at an average temperature of 28°C/17°C (day/night). The greenhouse contained *Opuntia*

spp. cactus pear plants that were highly susceptible to *D. echinocacti*. These plants were planted in plastic pots with a diameter of 33 centimeters and a height of 12 centimeters. The pots were filled with a mixture of soil, sand, and peat in equal proportions (1/3 w/w). The greenhouse contained 140 two-year-old infested plants, arranged in completely random rows (spacing of 1.5 m between rows, and 5 cm between plants). The plants were irrigated as needed. Night temperature was determined from the 3 lowest daily values (Vinogradova and Reznik, 2013). Treatments with d-limonene applied at 150 cc/hl, mineral oil at 2000 and 2400 cc/hl, and potassium salts of fatty acid at 60 cc/hl providing the highest mortalities against *D. echinocacti* nymphs and adult females in laboratory bioassays were selected for greenhouse tests. To facilitate analysis of the results, only 100 individuals of nymphs and adult females of *D. echinocacti* identified with a hand loupe were kept on each plant while the additional insects were removed with a needle. The plants were covered with a mesh screen to limit the insects movement. For each plant, 100 ml of each evaluated treatment was applied using a laboratory sprayer to ensure complete coverage. A completely random rows design with twenty plants was adopted for each treatment. The control plants were sprayed with tap water. Each plant was considered as a replicate and all the experiment was repeated twice at different times (3 insecticides X 5 treatments X 20 replicates X 2 experiments performed in different times). Mortality of *D. echinocacti* nymphs and adult females was evaluated at 24-h intervals and continued until 72 h after treatment.

Statistical analysis

Prior to analysis, mortality data was corrected using Abbott's formula (Abbott 1925). Probit regression analysis and Finney's method (Finney 1971) were used to determine the LC_{50} and lethal time (LT_{50}), respectively. Before analysis, LC_{50} values were predicted from probit lines. To determine mean survival time and median lethal time (LT_{50}), survival analysis and the Kaplan-Meier method were adopted. Abbot-corrected mortalities under laboratory and greenhouse conditions were subjected to analysis of variance (ANOVA) followed by the Turkey's LSD test. All statistical analyses were performed using IBM SPSS 23.0 software (SPSS Inc., Chicago, Illinois, USA).

Results

Laboratory experiments

Firstly, we evaluated the lethal effect of four biorational insecticides at four different concentrations against nymphs (**Table 1**) and adult females (**Table 2**) of *D. echinocacti* using topical treatment application under laboratory conditions. The cochineal mortality was calculated from 12 h to 48 h after exposition. Results showed that mineral oil applied at 2000 and 2400 cc/hl, d-limonene applied at 150 cc/hl, and potassium salts of fatty acid applied at 60 cc/hl were the most efficient and caused 84, 97, 93, and 81% mortality, respectively against the cochineal nymphs and 79, 92, 88, and 76% mortality, respectively against the adult females at 48 h after exposure to insecticide treatments. The mortality noted at 48 h was much higher than after 12 and 24 h in all insecticide treatments tested. The lowest percentage of mortality after 48 h was observed with pyriproxyfen at 8 cc/hl (54% (nymph) and 47% mortality (female)). In addition, immediately after insecticide treatments, the waxy carapace covering the body of *D. echinocacti* became smaller in size and smoother, and began to dry out and disappear. The average mortality of the cochineal in the control treatment (tap water) was 1.0% and 0% for the nymphs and adult females respectively.

Table 1. Abbot-corrected mortality of nymphs of Opuntia cochineal scale pest, *D. echinocacti*, 12, 24 and 48 h of exposure to insecticides.

Treatment	Concentrations (cc hl^{-1})	Time (hours)			*P* value
		12	24	48	
D-limonene (60g/l)	30	41.0±7.4 ef	54.0±8.0 efg	61.0±9.4 ef	*P* < 0.0001
	50	46.0±8.8 def	57.0±8.2 defg	65.0±10.0 def	*P* < 0.0001
	100	54.0±6.0 bcd	69.0±5.6 bcd	80.0±10.0 bcd	*P* < 0.0001
	150	62.0±4.8 ab	79.0±3.6 ab	93.0±5.6 ab	*P* < 0.0001
Mineral oil (780 g/l)	700	41.0±9.2 ef	57.0±7.6 defg	70.0±8.0 cdef	*P* < 0.0001
	1000	48.0±6.8 def	63.0±6.2 cdef	76.0±8.0 cde	*P* < 0.0001
	2000	61.0±3.6 abc	75.0±6.0 abc	84.0±8.8 abc	*P* < 0.0001
	2400	70.0±4.0 a	85.0±7.0 a	97.0±4.2 a	*P* < 0.0001
Potassium salts of fatty acid (500g/l)	15	39.0±7.2 ef	50.0±6.0 eg	57.0±7.0 f	*P* < 0.0001
	20	41.0±5.4 ef	54.0±7.2 efg	63.0±9.6 ef	*P* < 0.0001
	40	48.9±7.2 cde	65.0±8.0 cde	74.0±8.8 cde	*P* < 0.0001
	60	58.0±6.4 abcd	74.0±8.0 abc	81.0±7.4 abcd	*P* < 0.0001
Pyriproxyfen (100 g/l)	8	36.0±8.0 f	48.0±6.4 g	54.0±10.0 f	*P* < 0.0001
	12	40.0±6.0 ef	54.0±8.8 efg	61.0±9.4 ef	*P* < 0.0001
	25	46.0±6.0 def	64.0±6.8 cde	69.0±7.4 cdef	*P* < 0.0001
	37	56.0±6.8 bcd	72.0±5.2 abc	77.0±8.4 bcde	*P* < 0.0001
Control (tap water)		0.0±0.0 g	0.0±0.0 h	1.0±1.8 g	*P* < 0.0001
Statistical analysis		F= 35.1 *P* < 0.0001	F= 48.8 *P* < 0.0001	F= 42.0 *P* < 0.0001	

Means within a column followed by the same letters are not significantly different according to Tukey's LSD test at α = 0.05.

Table 2. Abbot-corrected mortality of adult females of *Opuntia* cochineal scale pest, *D. echinocacti*, 12, 24 and 48 h of exposure to insecticides.

Treatments	Concentrations (cc hl^{-1})	Time (hours)			*P* value
		12	24	48	
D-limonene (60g/l)	30	37.0±7.0 ef	50.0±6.0 ef	57.0±8.4 ghi	*P* < 0.0001
	50	41.0±5.4 def	52.0±6.8 ef	60.0±8.0 efghi	*P* < 0.0001
	100	49.0±5.4 bcd	64.0±6.0 bcd	75.0±8.0 bcd	*P* < 0.0001
	150	57.0±5.6 ab	74.0±6.0 ab	88.0±6.4 ab	*P* < 0.0001
Mineral oil (780 g/l)	700	36.0±7.2 ef	52.0±4.8 ef	65.0±9.0 defgh	*P* < 0.0001
	1000	42.0±6.4 cdef	58.0±6.4 de	71.0±5.6 cdef	*P* < 0.0001
	2000	56.0±6.0 ab	70.0±6.0 abc	79.0±7.4 abc	*P* < 0.0001
	2400	65.0±6.0 a	80.0±4.0 a	92.0±4.8 a	*P* < 0.0001
Potassium salts of fatty acid (500g/l)	15	34.0±5.6 ef	46.0±6.0 f	52.0±4.8 hi	*P* < 0.0001
	20	36.0±6.0 ef	49.0±5.4 ef	58.0±8.0 fghi	*P* < 0.0001
	40	44.0±6.8 cde	60.0±6.0 cde	69.0±5.4 cdefg	*P* < 0.0001
	60	53.0±5.6 bc	69.0±7.2 abcd	76.0±8.0 bcd	*P* < 0.0001
Pyriproxyfen (100 g/l)	8	31.0±5.4 f	44.0±4.8 f	47.0±5.6 i	*P* < 0.0001
	12	36.0±4.8 ef	50.0±6.0 ef	57.0±7.0 ghi	*P* < 0.0001
	25	41.0±5.4 def	59.0±7.2 cde	64.0±6.0 defgh	*P* < 0.0001
	37	51.0±7.2 bcd	67.0±7.0 bcd	72.0±8.8cde	*P* < 0.0001
Control (tap water)		0.0±0.0 g	0.0±0.0 g	0.0±0.0 g	-
Statistical analysis		F= 39.6 *P* < 0.0001	F= 56.2 *P* < 0.0001	F= 56.5 *P* < 0.0001	

Means within a column followed by the same letters are not significantly different according to Tukey's LSD test at α = 0.05.

Table 3 shows that mineral oil, d-limonene, and potassium salts of fatty acid had the lowest LC_{50} values of 375.2, 23.2, and 10.4 (nymph), and 407.7, 26.4, and 13.3 cc/hl (adult female), respectively, after 48 h of exposure, implying their efficacy against *D. echinocacti*.

Table 3. Median lethal concentration LC_{50} (cc hl^{-1}) of *D. echinocacti* treated by insecticides (ANOVA, α = 0.05).

Treatments	Cochineal stage	Time	Slope ± SE	LC%50	Chi-test (χ2) Sig	df	P-value
D-limonene (60g/l)	Nymph	12	3.2±0.7	64.9	11.4	38	*P* < 0.0001
		24	4.1±1.0	27.7	13.7	38	*P* < 0.0001
		48	5.7±1.5	23.2	30.1	38	*P* < 0.0001
	Adult female	12	3.0±0.2	95.8	8.6	38	P < 0.0001
		24	3.8±0.2	35.7	10.8	38	P < 0.0001
		48	5.2±0.3	26.4	19.1	38	P < 0.0001
Mineral oil (780 g/l)	Nymph	12	4.5±1.3	1076.1	11.3	38	P < 0.0001
		24	4.6±1.3	557.6	14.6	38	P < 0.0001
		48	4.9±1.8	375.2	31.7	38	P < 0.0001
	Adult female	12	4.5±0.3	1370	9.4	38	P < 0.0001
		24	4.4±0.3	676.8	10.4	38	P < 0.0001
		48	4.5±0.3	407.7	23.4	38	P < 0.0001
Potassium salts of fatty acid (500g/l)	Nymph	12	2.9±0.8	37.2	10.3	38	P < 0.0001
		24	3.8±1.0	15.7	13.2	38	P < 0.0001
		48	4.0±1.1	10.4	20.2	38	P < 0.0001
	Adult female	12	3.0±0.3	53.8	8.4	38	P < 0.0001
		24	3.7±0.3	20.2	9.7	38	P < 0.0001
		48	3.9±0.3	13.3	11.5	38	P < 0.0001
Pyriproxyfen (100 g/l)	Nymph	12	2.9±0.2	27.2	11.1	38	P < 0.0001
		24	3.7±0.2	9.3	12.1	38	P < 0.0001
		48	3.6±0.3	6.2	20.5	38	P < 0.0001
	Adult female	12	2.9±0.2	40.4	8.7	38	P < 0.0001
		24	3.5±0.2	12.2	9.1	38	P < 0.0001
		48	3.7±0.2	8.9	12.2	38	P < 0.0001

The exposure of *D. echinocacti* nymphs and adult females to selected insecticide doses during 48 h under laboratory conditions using the topical treatment method showed that mineral oil, d-limonene, and potassium salts of fatty acid provided the greatest amount of mortality (P ≤ 0.001) (**Tables 4** and **5**). For nymphs, greater than 70% mortality was observed with mineral oil applied at 2000 and 2400 cc/hl, d-limonene applied at 150 cc/hl, and potassium salts of fatty acid applied at 60 cc/hl after 48 h. Pyriproxyfen gave the lowest percentage of mortality. The same trend was obtained for adult females, with a mortality rate higher than 70% observed with mineral oil at 2400 cc/hl, and d-limonene applied at 150 cc/hl followed by mineral oil applied at 2000 (68.3% mortality), and potassium salts of fatty acid applied at 60 cc/hl (66% mortality).

There was a significant difference among the different insecticides tested in terms of the time required to kill the cochineal (**Tables 4** and **5**). Mineral oil applied at 2000 and 2400 cc/hl, and d-limonene applied at 150 cc/hl were the fastest killers of *D. echinocacti* nymphs, while mineral oil applied at 2400 cc/hl was the fastest killer of adult females of the cochineal (LT_{50} =24 h). The other insecticide treatments tested took about 48 h to achieve 50% mortality.

Table 4. Mortality (%), mean survival time, and LT_{50} (days) of *D. echinocacti* nymphs exposure to laboratory application rates of insecticides.

Treatments	Concentrations (cc hl^{-1})	Mortality (%)[a]	Mean survival time[b]	LT_{50} (95% CI)	N[c]
D-limonene (60g/l)	30	52.0	37.5	48.0	100
	50	56.0	36.7	48.0	100
	100	67.7	34.7	48.0	100
	150	78.0	33.0	24.0	100
Mineral oil (780 g/l)	700	56.0	37.2	48.0	100
	1000	62.3	35.9	48.0	100
	2000	73.3	33.5	24.0	100
	2400	84.0	31.8	24.0	100
Potassium salts of fatty acid (500g/l)	15	48.7	38.1	48.0	100
	20	52.7	37.5	48.0	100
	40	62.7	35.6	48.0	100
	60	71.0	33.9	48.0	100
Pyriproxyfen (100 g/l)	8	46.0	38.6	48.0	100
	12	51.7	37.6	48.0	100
	25	59.7	36.0	48.0	100
	37	68.3	34.3	48.0	100

[a] *Abbott-corrected percentage mortality of D. echinocacti first instar nymphs at the end of experiment;*
[b]*The mean survival time;*
[c]*Total number of scale insects in bioassay.*

Table 5. Mortality (%), mean survival time, and LT_{50} (days) of *D. echinocacti* adult females under laboratory conditions, after exposure to different rates of insecticides.

Treatments	Concentrations (cc hl^{-1})	Mortality (%)[a]	Mean survival time[b]	LT_{50} (95% CI)	N[c]
D-limonene (60g/l)	30	48.0	38.3	48.0	100
	50	51.0	37.7	48.0	100
	100	62.7	35.7	48.0	100
	150	73.0	34.0	48.0	100
Mineral oil (780 g/l)	700	51.0	38.2	48.0	100
	1000	57.0	37.0	48.0	100
	2000	68.3	34.4	48.0	100
	2400	79.0	32.7	24.0	100
Potassium salts of fatty acid (500g/l)	15	44.0	39.0	48.0	100
	20	47.7	38.5	48.0	100
	40	57.7	36.6	48.0	100
	60	66.0	34.8	48.0	100
Pyriproxyfen (100 g/l)	8	40.7	39.5	48.0	100
	12	47.7	38.4	48.0	100
	25	54.7	37.0	48.0	100
	37	63.3	35.2	48.0	100

[a] *Abbott-corrected percentage mortality of D. echinocacti first instar nymphs at the end of experiment;*
[b]*The mean survival time;*
[c]*Total number of scale insects in bioassay.*

Greenhouse experiments

The exposure of *D. echinocacti* nymphs to insecticide treatments , tested in the greenhouse for 24 h, showed that mineral oil applied at 2400 cc/hl, d-limonene applied at 150 cc/hl, and potassium salts of fatty acid applied at 60 cc/hl provided the highest mortality (**Fig.1**). After 48 and 72 hours, mineral oil applied at 2400 cc/hl, d-limonene applied at 150 cc/hl showed significantly higher mortality rates (70 and 67%, respectively) against the cochineal nymphs. A mortality of almost 62% was observed with mineral oil applied at 2000 cc/hl after 72 hours. The control treatment gave the least percent of mortality (F = 621.1, df = 4, P < 0.0001). For adult females, it was observed that mortality rates were lower compared to nymphs in all the treatments tested (**Fig.1**). Mineral oil applied at 2400 cc/hl (45% mortality), d-limonene applied at 150 cc/hl (42% mortality), and potassium salts of fatty acid applied at 60 cc/hl (42% mortality) provided significantly higher mortality under greenhouse conditions than the other insecticide treatments after 24 h. This suggests that these treatments act more quickly against adult females of *D. echinocacti*, making them the most effective options in terms of speed of action. Mortality by mineral oil applied at 2400 cc/hl, and d-limonene applied at 150 cc/hl was remarkably high until 48 h (60 and 57% mortality,

respectively) and then reached its maximum after 72 h (65 and 62% mortality, respectively) (F = 954.3, df = 4, P < 0.0001). The least efficient insecticide treatment after 72 h was found to be the mineral oil applied at 2000 cc/hl. These results clearly indicate that all the insecticide treatments tested can provide a good control of cactus cochineal at high level of infestation when environmental conditions are favorable.

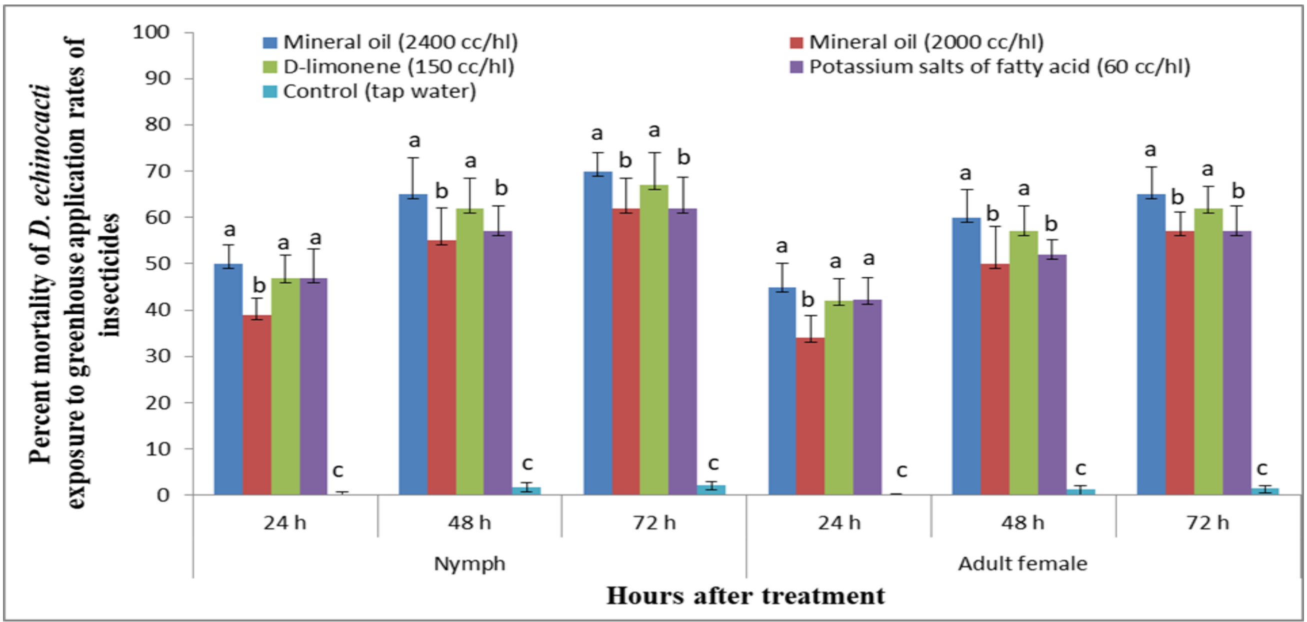

Fig 1. Percent mortality of *D. echinocacti* nymphs and adult females after 24, 48 and 72 h exposure to insecticides on *Opuntia spp*. cactus pear plants.

Discussion

In the present study, the effectiveness of four biorational insecticides within different modes of action (d-limonene, mineral oil, potassium salts of fatty acid and pyriproxyfen), was evaluated against the first instar nymphs and adult females of *D. echinocacti* under laboratory and greenhouse conditions. These insecticides, recommended by the National Office of Food Safety (ONSSA), were assessed by considering different concentrations for each treatment and the calculation of the required time to effectively kill the scale pest (*D. echinocacti*). Our findings indicate that mineral oil applied at 2400 cc/hl, d-limonene applied at 150 cc/hl, and potassium salts of fatty acid applied at 60 cc/hl gave better control of nymphs and adult females of *D. echinocacti* in laboratory and greenhouse experiments. Santos et al. (1997) reported that 1% mineral oil provided good control of this cochineal in case of heavy infestation. In our study, the same treatments above caused 97, 93, and 81% mortality, respectively against the cochineal nymphs and 92, 88, and 76% mortality, respectively against the adult females at 48 h after laboratory treatment. A good control of *D. echinocacti* was achieved by the same insecticide treatments in the greenhouse. The use of d-limonene in pest control was initiated in 1974 by Taylor and Vickery; who introduced this insecticide as a reproductive inhibitor and growth regulator on many insect species, however, the work of Brennan et al. in 2013 reported that d-limonene causes chitin degradation in pests. Also, mineral oils are commonly used in crop protection to combat various pests and scale insects, they block the spiracles of pest adults and nymphs and prevent gas exchange in eggs which causes their asphyxiation

and death (Cranshaw and Baxendale, 2011; Helmy et al., 2012). Potassium salts of fatty acids are contact agents that act by penetrating the interior of the integument of arthropods, obstructing cell membranes and causing their dehydration and death (Tsolakis and Ragusa, 2008). An effective control of the cactus-specific harmful scale pest, *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) was obtained by d-limonene and mineral oil (El Aalaoui et al. 2019).The least effective insecticide treatment against *D. echinocacti* under laboratory and greenhouse conditions was found with pyriproxyfen. Generally, treatment with pyriproxyfen results in suppression of insect embryogenesis, metamorphosis, and pupal and adult development (Rimoldi et al., 2017). However, the mode of action of pyriproxyfen is more specific and depends on the presence of insecticide-specific endocrine receptors (Sullivan and Goh 2008). This may be implicated in the low efficacy of this insecticide, which is an analog of the insect juvenile hormone, against *D. echinocacti* compared to the other applied insecticidal treatments. *Diaspis echinocacti* adult females have a waxy carapace covering the body, which provides protection against direct contact with insecticides. This explains the higher percentage of mortality observed in *D. echinocacti* nymphs compared to adult females in this study.

Bergamin Filho and Amorim (1999) recommend Integrated Pest Management (IPM) for the control of *D. echinocacti* in cactus plantations, which includes chemical, mechanical, physical and biological methods to prevent the growth and spread of the insect population. Integrated pest management strategies against cochineals have largely applied the combined use of biological control agents and safe insecticides (Elzen, 2001), but it is important to note that the most widely used chemical pesticides in the world (e.g., neonicotinoids, organophosphates, carbamates, pyrethroids) lead to environmental pollution risks and are expensive (Mazzeo et al., 2019). In each country, regulations govern the use of insecticides on crops, and sometimes the most effective ones are not approved for use on cactus pear. This situation is evident in Brazil, where certain insecticides are not authorized for cactus pear crops; similarly, in Italy, only a limited number of insecticides are registered for use on cactus pear crops, and paraffinic oils are the only option employed against cochineals (Mazzeo et al., 2019). Other substances (e.g. mineral oil, vegetable oil, neutral detergents) are effective in controlling scale pests and simultaneously preserve ladybeetles and hoverfly larvae in treated fields (Torres and Giorgi, 2018). In Morocco, mineral oil (780 g/l) and d-limonene (60 g/l), could be used in integrated management strategies of cactus pear pests in combination with other biocontrol agents especially ladybirds (El Aalaoui et al., 2021).

Conclusion

Due to the lack of previous complete studies regarding the control of cactus pear cochineal *D. echinocacti* with biorational insecticides, our results clearly identified the potential of mineral oil applied at 2400 cc/hl, and d-limonene applied at 150 cc/hl for this pest's control and propose them as viable alternatives to other high-risk chemical pesticides currently used worldwide to control this harmful scale pest. However, further research is needed, especially if these insecticides are used in combination with other biological control agents, such as predators, parasitoids, and entomopathogens of this scale pest in an integrated pest management program under field conditions.

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