Screening Cactus Varieties Resistant to Cochineal Scale, *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) in Jordan

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Abstract: The cochineal scale, *Dactylopius opuntiae* (Cockerell) is a serious pest of cactus in many parts of the world. It was first recorded in the north of Jordan in 2018 and continued to spread in the country. To use environmentally safe control methods for this invasive pest, two experiments were conducted to find cactus accessions resistant to the cochineal scale. The first experiment was conducted on twenty-six accessions in a growth chamber at a temperature of 27°C ± 0.5°C and 70-80% of RH. The second experiment was conducted in the Jordan Valley and included ninety-nine accessions. All accessions were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA). Accessions from countries around the world were tested (Algeria, Argentina, Brazil, Italy, Jordan, Mexico, Morocco, South Africa, Syria, and Tunisia). All the first twenty-six accessions were found susceptible to the pest. The average duration of one generation of the insect ranged from thirty days to forty-one days with an average of 36.3 ± 2.59 days. On the other hand, out of the ninety-nine cactus accessions, four were found resistant and showed no infestation at all. These were Unknown 120 and Unknown 122 from Syria, *Opuntia robusta* 1280 from Argentina, and *Opuntia robusta* 200146 from Brazil. The most susceptible accessions were Zastron 4 and M3 Bianca di Macomer from Italy and 40-Tronzar and GS from Tunisia with an infestation rate of more than 90%.

Keywords: Cochineal, resistant varieties, prickly pear, Jordan.

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Introduction

In Jordan, *Opuntia ficus indica* is planted as a fence around farms, mainly for its fruits, which have a good market value in the country. It is sometimes used as animal feed. The cactus growing area in Jordan is estimated at 3,000,000 m², mainly in the Jordan Valley and Madaba, south of Amman, the capital city. However, the actual area is most likely larger than this. Some cactus farms have been established in Madaba, south of Amman, producing around 400 tons per year in this province. It is not easy to estimate the exact area, as cacti are sporadic in-home gardens and garden fences or are scattered in the wilderness and are not traditionally planted on large farms except occasionally. The cochineal scale, *Dactylopius opuntiae*, feeds on cacti causing serious damage to this crop in many parts of the world. Its current distribution includes thirty-one countries: Algeria, Australia, Brazil, Cape Verde, Chile, Cyprus, Egypt, France, Hawaiian Islands, India, Jamaica, Jordan, Kenya, Lebanon, Madagascar, Mauritius, Mexico, Morocco, New Caledonia, Pakistan, Palestine, Reunion, Saudi Arabia, South Africa, Spain, Sri Lanka, Syria, Tunisia, United Kingdom, United States, and Zimbabwe (García *et al*., 2016).

The cochineal scale, *D. opuntiae* attacks all parts of the cactus sucking the sap from cladodes causing yellowing at the beginning, and as the infestation proceeds it covers the entire cladodes and eventually kills the plant (Figure 1). Unfortunately, due to their short lifecycle and shortage of natural enemies in Jordan, farmers' initial control method

Figure 1. Destroyed cacti by *D. opuntiae* in Al-Rafeed in 2018, north of Jordan**.**

was to burn the infested plants. However, over the past decade, many integrated pest-management measures have been evaluated, whether mechanical (pruning), physical (fire), or biological through the multiplication and manipulation of predators and the usage of bio-degradable products and plant extracts such as Neem and Cassava (Borges *et al*., 2013). Vasconcelos *et al*. (2009) tested twenty forage cactus clones for resistance to the carmine cochineal in a greenhouse experiment in Brazil. Akroud *et al*. (2021) studied the mechanism of resistance of the Moroccan cactus ecotypes. Berhe *et al*. (2022) studied six *Opuntia* cultivars collected from different locations in Mexico. Then they studied the mechanism of resistance of the same six varieties (Berhe *et al*., 2023). Sabbahi and Hock (2022) discussed control measures against *D. opuntiae* (Cockerell) in Morocco which included natural extracts, biorational insecticides, natural enemies, pruning and uprooting heavy infestations, and planting resistant cactus varieties.

This research was conducted to screen diverse cactus varieties resistant to the cochineal scale, *D. opuntiae*, which will contribute to any integrated management program that adopts traditional pesticides-free control program for this pest.

Materials and Methods

A total of 125 cacti accessions were tested for resistance to *D. opuntiae.* The first group included twenty-six accessions and was conducted in a growth chamber at the Department of Plant Protection, School of Agriculture, the University of Jordan, while the second group included ninety-nine accessions and was conducted in the field in the Jordan Valley. All accessions were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA) located near Madaba city about twenty-five km south of the capital Amman. Accessions from various countries around the world were tested (Algeria, Argentina, Brazil, Italy, Jordan, Mexico, Morocco, South Africa, Syria, and Tunisia).

1. Assessment of twenty-six cactus accessions in a growth chamber

Three cladodes (two years old) from twenty-six cacti accessions from different countries worldwide (Table 1) were used. They were left to heal for two days before planting. Later, the three cladodes of each accession were planted in pots 20 cm in diameter and 18 cm in height, with a mixture of soil, sand, and manure (1:1:1 ratio) (Figure 2). The pots were placed in a controlled chamber at 27°C, 70 ± 10 % RH, and 12:12 (L:D). Baskets of medical cloth were hand-rolled, filled with fifteen gravid females, and then pinned on the top of each cladode (Figure 3). The movement of the first instar nymphs was observed daily as they crawled out of baskets and settled on cladodes. The life cycle was followed till the hatching and crawling of the first instar nymphs of the second generation. If one generation was completed on the cactus accession, it was considered susceptible.

Figure 2. Curing and planting of different cactus accessions in a growth chamber.

2. Assessment of ninety-nine cactus accessions in the field

This experiment was based on a research farm in the Deir Alla area in the Jordan Valley. It was conducted in an insect proof screen house (Figure 4). The temperature ranged from 35-45 °C, and relative humidity was 50-80% throughout the whole study period; both were recorded daily throughout the whole experiment via weather forecasts. Three cladodes of the ninety-nine accessions (Table 2) were planted in pots containing peat moss, sand, and clay soil mixed in a 1:1:1 ratio.

Figure 3. Hand-rolled infestation baskets containing gravid *D. opuntiae* females and crawlers.

Cladodes were infested as described in the first experiment. After infestation, observations on infestation occurrence and infestation percentage on each cladode were recorded throughout the weekly site visits, mainly focusing on the cacti accessions with the least *D. opuntiae* infestation. Thereafter, the accessions that showed no infestation were further assessed twice under the aforementioned growth-chamber conditions to make sure that no infestation occurred.

Figure 4. Cacti accessions planted in Deir Alla to assess their resistance to *D. opuntiae*.

Table 1. Names of cacti accessions used in the growth-chamber experiment and their source country.

No.	Accession name	Source country	No.	Accession name	Source country
1	9-FOZA9	Mexico	14.	46-Mornag B-74076	Tunisia
2	10-FOZA10	Mexico	15.	6-Ain Boudriess-96245	Tunisia
3	VN-Villanueva	Mexico	16.	48-Sefrou-74083	Morocco
4	2-25-15	Mexico	17.	32-Morocco-74001	Morocco
5	2-26-21	Mexico	18.	13-Bab Toza-74115	Morocco
6	Red san cono	Italy	19.	22-l Borouj-75018	Morocco
7	Trunzara yellow bronte	Italy	20.	32–Ain Jimaa-75019	Morocco
8	Trunzara yellow San Cono	Italy	21.	BAG-E.E. - Caruaru	Brazil
9	Mezzojuso	Italy	22.	IPA-90-73	Brazil
10	White Roccapalumba	Italy	23.	IPA-90-115	Brazil
11	33-Oueslatia-69246	Tunisia	24.	Additional-1258	Brazil
12	R	Tunisia	25.	Additional-1258	Brazil
13	20-Sbeitla-74071	Tunisia	26	Mucahqqer	Jordan

Table 2. Names of cactus accessions studied in field and their country of origin.

No.	Accession name	Source country	No.	Accession name	Source country
1	Mleeh	Jordan	**31**	M3 Bianca di Macomer	Italy
2	J-Jalpa	Mexico	**32**	M2 Rossa di macomer	Tunisia
3	V1-COPENA V1	Mexico	**33**	RSS Rossa San spate	Tunisia
4	VN-Villanueva	Mexico	**34**	RC Rossa di casttelsardo	Tunisia
5	F1-COPENA F1	Mexico	**35**	M1 Gialla di Macomer	Tunisia
6	Morado	Sicilia, Italy	**36**	GSH Gialla di sarroch	Italy
7	Zastron 4	Sicilia, Italy	**37**	8-Leavis	Tunisia
8	Trunzara Red San Cono	Sicilia, Italy	**38**	8-Algeria	Tunisia
9	Algerian 3/2	Sicilia, Italy	**39**	10-Bianca	Tunisia
10	Blue Motto	Sicilia, Italy	**40**	32-Matmata	Tunisia
11	Roly Poly	Sicilia, Italy	**41**	26-Montarnaud	Tunisia
12	Seedless Santa Margherita Belice	Sicilia, Italy	**42**	47-Mornag B	Tunisia
13	Red Santa Margherita Belice	Sicilia, Italy	**43**	34-Caref 58	Tunisia
14	Yellow Santa Margherita Belice	Sicilia, Italy	**44**	31-Burbank Azrou	Tunisia
15	White Santa Margherita Belice	Sicilia, Italy	**45**	15-Sicile Le folin	Tunisia
16	Yellow San Cono	Sicilia, Italy	**46**	1364	Tunisia
17	White San Cono	Sicilia, Italy	**47**	4-Mexico	Tunisia
18	Red Roccapalumba	Sicilia, Italy	**48**	N	Tunisia
19	Yellow Roccapalumba	Sicilia, Italy	**49**	2-17-25	Tunisia
20	Seedless Roccapalumba	Sicilia, Italy	**50**	2-11-85	Tunisia
21	Trunzara red Bronte	Sicilia, Italy	**51**	2-21-68	Tunisia
22	Yellow Belpasso	Sicilia, Italy	**52**	40-Tronzara	Tunisia
23	Tunzara Bianca bronte	Sicilia, Italy	**53**	17-Sanguinea	Tunisia
24	Trunzara yellow San Cono	Sicilia, Italy	**54**	22-El Borouj	Tunisia
25	Tunzara Bianca San Cono	Sicilia, Italy	**55**	Zelfeue	Tunisia
26	Red Roccapalumba/2	Sicilia, Italy	**56**	G	Tunisia
27	Seedless Margherita	Sicilia, Italy	**57**	GS	Tunisia
28	Spineless	Sicilia, Italy	**58**	2	Tunisia
29	Bari Gialla	Sicilia, Italy	**59**	10	Tunisia
30	BB Bianca de Bonacardo	Italy	**60**	24	Tunisia

61	26	Tunisia	**81**	100001	Brazil
62	29	Tunisia	**82**	200008	Brazil
63	38	Tunisia	**83**	*Opuntia robusta*	Brazil
64	37	Tunisia	**84**	200173	Brazil
65	41	Tunisia	**85**	100004	Brazil
66	42	Tunisia	**86**	100413	Brazil
67	B(6)1	Tunisia	**87**	100412	Brazil
68	43-Mexico	Tunisia	**88**	100408	Brazil
69	59-Unknown	Tunisia	**89**	100407	Brazil
70	60-Unknown	Tunisia	**90**	200002	Brazil
71	61-Unknown	Tunisia	**91**	200016	Brazil
72	20	Syria	**92**	100003	Brazil
73	21	Syria	**93**	20-Chico	South Africa
74	22	Syria	**94**	Unknown 120	Syria
75	25	Syria	**95**	Unknown 121	Syria
76	26	Syria	**96**	Unknown 122	Syria
77	27	Syria	**97**	*Opuntia robusta*-1020	Argentina
78	Mexican vegetable 1294	Brazil	**98**	ANV1-1.08	Argentina
79	100410	Brazil	**99**	*Opuntia robusta* 1280	Argentina
80	200001	Brazil			

Results

1. Assessment of twenty-six cactus accessions in a growth chamber

All the twenty-six cactus accessions obtained from ICARDA were susceptible to *D. opuntiae.* Figure (5) shows the development of the insect white colonies on some of the cactus accessions. The average duration of one generation (from 1st instar to 1st instar) ranged from thirty days to forty-one days with an average of 36.3 ± 2.59 days for all accessions at the temperature of 27°C ± 0.5°C and 70-80% of RH (Figure 6).

Figure 5. Some of the cacti accessions susceptible to *D. opuntiae* are covered with insects.

2. Assessment of ninety-nine cactus accessions in the field

The average infestation % ranged from 0% to 96.67% with an average of 34.7% for all the accessions tested (Figure 7). Four accessions were resistant (0% infestation) to *D. opuntiae*: Unknown 120 and Unknown 122 from Syria, *O. robusta 1280* from Argentina and *O. robusta 200146* from Brazil (Figure 8). Their cladodes showed no attack by nymphs or establishment of a colony formation from the beginning of infestation till the end of the experiment after six weeks. Another twenty-seven cactus accessions had an average infestation % of ten or less. These accessions may be considered more resistant to the scale than the rest of the accessions. These accessions were from countries in the five continents from which the accessions were originally collected. The most susceptible accessions were M3 Bianca di Macomer from Italy and Zastron from Sicilia, Italy. Both accessions had an average infestation% of 96.67. These two accessions were followed by Zelfeue, 40-Tronzara, and GS all from Tunisia with

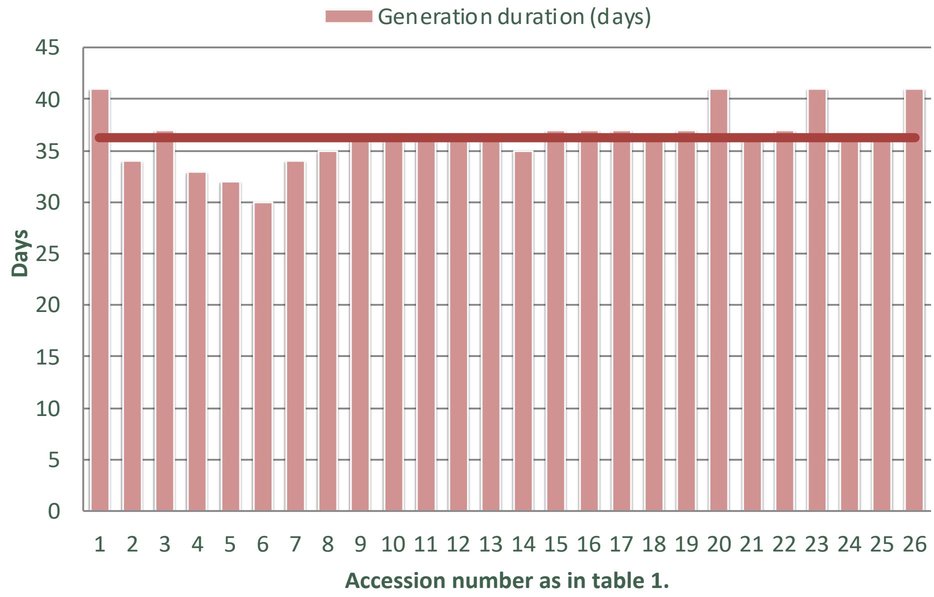

Figure 6. Generation duration per accession.

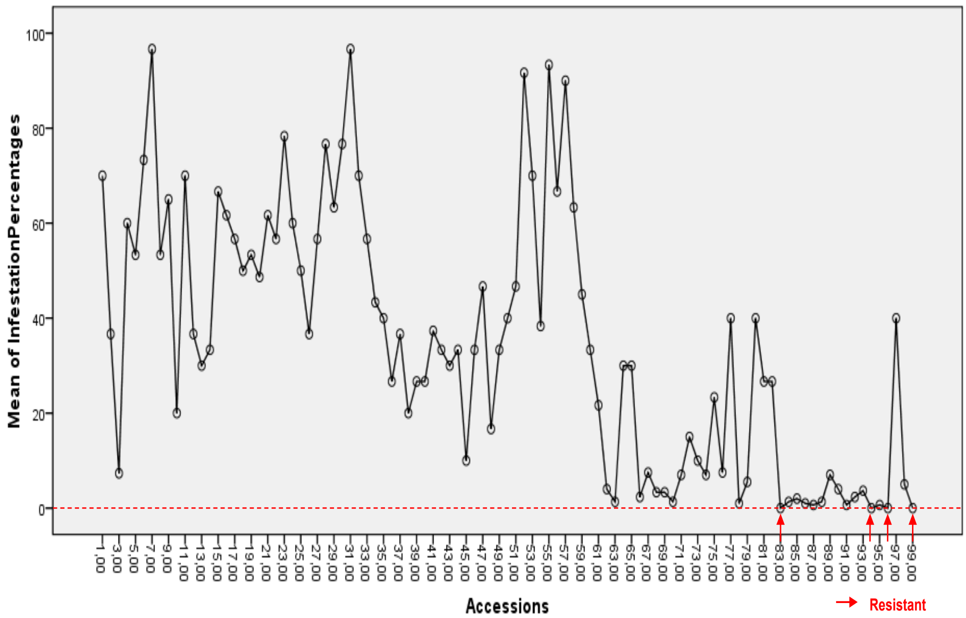

Figure 7. Mean percentage of infestation in the ninety-nine cactus accessions.

an average infestation% of 93.33, 91.67, and 90.00, respectively. These four accessions may be considered as highly susceptible (with 90% infestation or more). The re-infestation of the four resistant accessions under controlled conditions showed no infestation for a period of thirty days.

Figure 8. The four resistant accessions (A: *O. robusta* 1280, B: Unknown 122, C: Unknown 120, D: *O. robusta* 200146) at the end of the experiment, free of infestation.

Discussion

Since the cochineal scale was able to develop for an entire generation on all tested cactus accessions in the first experiment, all twenty-six accessions were considered susceptible. The average duration of one generation of *D. opuntiae* on the first twenty-six cactus accessions ranged from thirty days to forty-one days with an average of 36.3 ± 2.59 days. According to Flores Hernandez *et al*. (2006), the ideal temperature and relative humidity for the development of *D. opuntiae* are 26 ° C and 60%, respectively. Palafox-Luna *et al*. (2018) found that females took 16.7 ± 2.69 days to complete development, while males took up to 24.48 ± 2.23 days under a temperature of 25 ± 1 °C and 40 ± 10% of RH. Whereas in this study, the insects took a longer period (an average of 36.3 ± 2.59 days for all accessions) at a temperature of 27°C ± 0.5°C and 70-80% of RH. This difference in the generation time could be attributed to the different cactus accessions used in both experiments or is due to the difference in external environmental factors such as RH, temperature, and light.

When a larger number of cactus accessions were studied (the second patch of ninety-nine accessions), four cactus accessions did not show any signs of infestation and were later proven to be resistant to *D. opuntiae* in growth-chamber conditions (Unknown 120 and Unknown 122 from Syria, *O. robusta* 1280 from Argentina and *O. robusta* 200146 from Brazil). No previous studies were found in the literature related to these specific accessions. However, Vasconcelos *et al*. (2009) tested twenty forage cactus clones for resistance to the carmine cochineal in a greenhouse experiment in Brazil from September 2001 to January 2002. The clones that showed greater resistance to the pest attack were Miúda and Orelha de Elefante, while the Redonda clone was highly susceptible. Berhe *et al*. (2022) studied six *Opuntia* cultivars collected from different locations in Mexico: three *O. ficus-indica* ('Rojo Pelón', 'Atlixco', and 'Chicomostoc'), two *O. cochenillifera* ('Nopalea'and 'Bioplástico') and one *O. robusta* ('Robusta'). Their results showed that the 'Rojo Pelón' variety was resistant to *D. coccus* among the *O. ficus-indica* cultivars because the insects could not develop and complete the life cycle but died at the nymph I stage. Berhe *et al*. (2023) studied six cultivars, those were three *O. ficus-indica* (resistant 'Rojo Pelón' and susceptible 'Atlixco' and 'Chicomostoc'), two *O. cochenillifera* (resistant 'Bioplástico' and susceptible 'Nopalea') and one *O. robusta* (resistant 'Robusta'). They found that cladode thickness, calcium oxalate number, and epidermis thickness had positive correlations with resistance. These results demonstrate that calcium oxalate number and epidermis thickness might have a positive role in *Dactylopius coccus* Costa resistance in *O. ficus-indica*. Akroud *et al*. (2021) showed that antibiosis and antixenosis (nonpreference), played a role in the resistance of the Moroccan cactus ecotypes. Eight cultivars resistant to *D. opuntiae* were identified, registered, multiplied, and planted on a large scale in Morocco (Sabbahi and Hock, 2022).

The authors believe that the four resistant cactus accession found in this study are potential tools in developing an integrated pest management program against the cochineal scale. Further research is needed to study the sources of resistance in these accessions whether they are chemical, physical or both. In addition, the suitability of these accessions for the farmers and consumers should be studied. It is important to emphasize the benefits of using resistant varieties by farmers and plant protection specialists as well. Using resistant varieties is considered an ideal pest control strategy since it reduces the insect population in the area and shows permanent effects. This strategy is also safe for humans and the environment and does not require advanced knowledge on the part of farmers when it comes to implementation.

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