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Potential of parasitoids to control *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) on *Opuntia* spp. cactus pear

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Abstract

Background *Diaspis echinocacti* (Bouché) is a major pest of cactus pear species worldwide. Over the past three years, this scale insect has seen increasing interest regarding its spread and importance, as well as the efficacy of its primary parasitoids *Aphytis debachi* Azim, and *Plagiomerus diaspidis* Crawford, as biocontrol agents. Infestation of cactus pear plants by *D. echinocacti* and parasitism rates of its two parasitoids were evaluated in fields in Zemamra-Morocco, for three years (2020–2022). Infested cladodes were collected and incubated in the laboratory (27 ± 1 °C) for parasitism study.

Results Infestation of cactus pear plants by the cochineal increased continuously during the study period to reach 66% in 2022. Parasitism rates were lowest in 2020 and increased thereafter to 74.10 and 38.38% in 2022 for *P. diaspidis*, and *A. debachi*, respectively. The most abundant parasitoid was *P. diaspidis*. The functional response and mutual interference of *P. diaspidis* and *A. debachi* attacking *D. echinocacti* second instar nymphs were studied in the laboratory. Logistic regression for both parasitoids tested had a negative linear parameter indicating a type II functional response. Attack rates were 0.73 and 0.60 and handling times were 0.09 and 0.06 h for *P. diaspidis* and *A. debachi*, respectively, were estimated using Holling's disk equation. Search efficiency decreased significantly from 0.060 to 0.002 for *P. diaspidis* and from 0.067 to 0.003 for *A. debachi* when the parasitoids density increased from 1 to 8.

Conclusions Parasitism potential, functional response and mutual interference of *P. diaspidis* and *A. debachi* attacking *D. echinocacti* were reported. The results of this investigation suggest that both *P. diaspidis* and *A. debachi* had the potential to effectively control infestations of *D. echinocacti* on cactus pear plantations.

Keywords *Diaspis echinocacti*, *Plagiomerus diaspidis*, *Aphytis debachi*, Cactus pear, Parasitism rate

Background

Cactus pear cultivation is an important component of agriculture in the arid areas due to its drought resistance. Although it is represented in the Moroccan rural landscape in anarchic plantations, it has become widely appreciated by farmers for its fruit and forage production

(El Aalaoui et al. 2019). A considerable effort to support the planting and development of cactus has been deployed by the Moroccan Ministry of Agriculture, and the goal of reaching an area of 160,000 ha planned for 2020 was already achieved in 2014 (El Aalaoui and Sbaghi 2022).

The invasive scale insect, *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae), is a major and specific pest of cactus pear species worldwide (Zamudio and Claps 2005), and recently it has seriously infested many cactus pear species in Morocco including *Opuntia dilenii* (Ker Gawl.) Haw. 1819 and *Opuntia ficus-indica* (L.) Mill. 1768. This cochineal attacks all parts of the

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host plant, however, it prefers old parts rather than young ones, probably due to the low activity of secondary metabolites in old segments (Qureshi 1963). *D. echinocacti* females have a dome-shaped carapace that protects them from natural enemies and insecticides. In addition, the high reproductive rate (150 individuals per female), and the propensity to spread quickly through natural supports (wind, water...) make the establishment and spread of the cochineal much easier than many other scale pest species (Qureshi 1963). *D. echinocacti* females have three biological developmental stages—egg, nymph (two stages), and adult—whereas males have egg, nymph (first, second, third), pre-pupa, pupa, and adult stages (Oetting 1984). Life cycle duration of the cochineal varied from 22.8 to 25.9 days between egg and adult stage under controlled (27 °C) and greenhouse conditions (Oetting 1984), and they live permanently attached to their host plants. The scale pest is reported to have two generations per year in greenhouse conditions and intense infestation can lead to the death of the attacked plant (Saakyan-Baranova 1954). To reduce the use of chemical insecticides, Integrated Pest Management (IPM) has been applied in many countries worldwide to control *D. echinocacti* in cactus pear plantations, which involves chemical, biological, mechanical and physical measures to prevent the insect to reach scale pest status (Bergamin and Amorim 1999). Natural enemies associated with *D. echinocacti* include parasitoids and predators. The ectoparasitoid, *Aphytis debachi* (Azim 1963) (Hymenoptera: Aphelinidae), and the endoparasitoid, *Plagiomerus diaspidis* (Crawford 1910) (Hymenoptera: Encyrtidae) are the most effective biological control agents used in IPM programs against this cochineal worldwide (Japoshvili et al. 2010).

Functional and numerical responses are the traditional primary analytical measures for assessing the impact of natural enemies as a function of varying prey or host density (Holling 1959) and are commonly used to predict the effectiveness of biological control agents on invasive pest populations (Umbanhowar et al. 2003). The functional response of a parasitoid refers to the number of host killed per parasitoid as a function of host density (Holling 1959), and the numerical response may refer to the number of its offspring as a function of increasing host density (Solomon 1949). Parasitoids can interfere with each other and affect the functional response of each individual parasitoid in a population (Abrams and Ginzburg 2000). To understand and therefore to improve the parasitism potential of *P. diaspidis* and *A. debachi* on *D. echinocacti* population, experiments measuring the functional and mutual interference of these species by altering the host or

parasitoid density while maintaining the other variables constant were conducted.

Methods

Rearing of cochineal and parasitoids

Colonies of parasitoids *P. diaspidis* and *A. debachi*, and cactus pear cochineal *D. echinocacti* were established from the cochineal-infested *O. ficus-indica* cladodes containing *P. diaspidis* adults and *A. debachi* larvae collected from a field in Zemamra, Morocco (32°37'48" N, 8°42'0" W, Elevation 165 m) (Fig. 1). These cladodes were placed in a cardboard box and brought to the entomology laboratory of the National Institute of Agricultural Research (INRA), Zemamara, Morocco and then placed in entomological cages (80×80×80 cm) covered with a mesh fabric to allow ventilation. The method described by Aguilera and Cázares (2003) was used to increase the number of insects and control their age. In order to maintain the colony for a long period of time, infested and uninfested cladodes were introduced into the cages weekly. The colonies of the cochineal and parasitoids were maintained at 25±2 °C, 66±5% RH, and a photoperiod of 12:12 h (L:D).

Recording of parasitization potential in the field

Surveys were conducted in Zemamra, Morocco fields for three years (2020–2022). Observations on about 150 *Opuntia* spp. cactus plants (including *O. dillenii* and *O. ficus-indica*) infested with *D. echinocacti* and the presence of its natural enemies were conducted between October and December of each year of the study, when cactus pear plantations were heavily infested by the scale pest. The average annual precipitation of the last three years (study period) was 330 mm and the average temperature varied between -1 °C (December–January) and 40–45 °C (July–August). For each year, a total of 150 cladodes infested by the cochineal and containing larvae and mummies of parasitoids were collected from different *Opuntia* spp. cactus plants randomly selected and transferred to the laboratory for examination. Infested cladodes were maintained individually in entomological cages covered with muslin cloth at 27±1 °C, 65% RH for the parasitoids' emergence. Infested cladodes were observed daily for emergence of parasitoids and were returned to the cage after each observation. Emergent parasitoids were identified by the authors as *A. debachi* Azim (Hymenoptera: Aphelinidae), and *P. diaspidis* Crawford (Hymenoptera: Encyrtidae) using keys by Rosen and DeBach (1979) and Trjapitzin (1989). Slides were mounted using the methodology developed by Noyes (2009). Voucher specimens were deposited at the Insectarium of the National Institute of Agricultural Research in Zemamra, Morocco. Based on the number



Fig. 1 Laboratory rearing of *D. echinocacti* and its parasitoids *P. diaspidis* and *A. debachi*. **A** the cochineal and parasitoids in the rearing cage, **B** different developmental stages of *D. echinocacti*, **C** *P. diaspidis* adults on cactus pear cladode, **D** *P. diaspidis* adult female, **E** *P. diaspidis* host mummy, **F** *A. debachi* adult

of emerged *P. diaspidis* adults and *A. debachi* larvae, the percentage of parasitism of *D. echinocacti* by these two potential parasitoid species was recorded throughout the study period. For results analysis, each cladode (weighing approximately 3–4 kg) was considered a replicate.

Functional response

All the laboratory experiments carried out in this study were conducted under similar laboratory conditions 26 ± 2 °C, $60 \pm 10\%$ RH, and 12:12 (L:D) h. *Opuntia* spp. cladodes containing *D. echinocacti* second instar nymphs at different densities (1, 5, 10, 20, 30, 40, or 50 nymphs) were exposed to a single two-day-old mated female of *P. diaspidis* (Trial 1) and *A. debachi* (Trial 2) in a plastic box ($40 \times 30 \times 30$ cm) for 24 h. Ten randomly distributed replicates were used for each combination of parasitoid and host density and all the experiment was repeated twice. A mixture of honey (40%) and brewer's yeast streak (40%) was used as food for the parasitoids. After 24 h, the introduced parasitoids were removed and the exposed infested cladodes were maintained under the same laboratory conditions until the emergence of *P. diaspidis* adults and *A. debachi* larvae. Dead nymphs from which parasitoids had emerged were counted and those that died for unknown reasons during the rearing period were

dissected under a binocular microscope to check for parasitism.

Logistic regression analysis of the proportion of hosts parasitized (N_e/N_0) versus the number of hosts offered (N_0) was used to determine the shape of the functional response (Trexler et al. 1988). The polynomial function that describes the relationship between N_e/N_0 and N_0 is as follows:

$$\frac{N_e}{N_0} = \left(\frac{\exp \left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3 \right)}{1 + \exp \left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3 \right)} \right),$$

where: N_e is the number of parasitized nymphs; N_0 is the initial density of nymphs; P_0 , P_1 , P_2 , and P_3 are the constant, linear, quadratic, and cubic parameters, respectively, related to the slope of the curve (SAS Institute 2004). A negative P_1 value indicates a type II functional response, and a positive P_1 value indicates host density-dependent parasitism and therefore represents a type III functional response.

The data indicate a type II functional response and were fitted to Holling's disk equation (Holling 1959) $N_e = aTN_0 / (1 + aN_0Th)$ - where: N_e is the number of parasitized hosts, (a) is the instantaneous rate of discovery, N_0 is the initial number of hosts offered, (T) is the duration

of the experiment (24 h), and (Th) is the handling time associated with each parasitized host. Significant differences in functional response model parameters for all the parasitoids tested were assayed using 95% confidence interval overlay criterion. Mean Th values were used to calculate the maximum attack rates T/Th (Hassell 2000), which is the maximum number of hosts that can be parasitized by a parasitoid during the time interval considered. The parasitoids searching efficiency (E) was calculated using the equation $E=N_e/N_0$ and the searching time was determined by the equation $Ts = T - ThN_e$ (Juliano and Williams 1987; Elliott 2003). Significance of the difference in number of hosts parasitized at different densities, searching efficiency, and searching time were determined, using the analysis of variance (ANOVA); Tukey's LSD test was used for multiple comparisons among significant treatment effects when they occurred (IBM SPSS19 statistics).

Mutual Interference

The parasitoid species tested were grouped into one, two, four, and eight individuals in four plastic boxes (40×30×30 cm) and provided with a mixture of water, honey, and brewer's yeast in a 20:40:40 proportion. *Opuntia* spp. cladodes containing 50 individuals of second instar nymphs of *D. echinocacti* were collected from the laboratory colony and added to each box. After 24 h, the parasitoids were removed and the infested cladodes were maintained under the same laboratory conditions until the parasitoids emerged. After eight days, the number of parasitized nymphs was recorded, and the dead scale nymphs were dissected to determine the total parasitism rate. The experiment was repeated ten times for each parasitoid density. The number of parasitized hosts per parasitoid in relation to parasitoid density was subjected to regression analysis using IBM SPSS Statistics ver.23. All experiments were performed twice over time.

The mutual interference (m) among parasitoids was estimated using Hassell and Varley (1969) model: $\log E = \log Q - m \log P$ by adjusting the data to the equation with a linear regression procedure (SAS Institute 2004).

Where: E is the parasitoid searching efficiency and was calculated for each host parasitoid density replicate as follows: $E = \left(\frac{1}{PT}\right) \ln \frac{N_0}{N_e}$, with P is the density of parasitoids, T is the duration of the experiment (24 h), N_0 is the number of the cochineal nymphs offered (i.e., 50) and N_e is the number of the cochineal nymphs parasitized, and Q is the quest constant (area of discovery when only one parasitoid was searching).

Statistical analysis

Data on cactus pear plants infestation and parasitism rates under field conditions, functional response, and mutual interference parameters were subjected to a one-way ANOVA, and means were separated by Tukey's LSD test. The mutual interference parameters of the two parasitoids tested, *P. diaspidis* and *A. debachi*, were compared using a t test ($P < 0.05$). Data measured in percentages were subjected to an arcsine transformation to approximate the normal distribution prior to analysis. All of these tests were performed using IBM SPSS statistics Ver. 23 software (Carver and Nash 2011).

Results

Recording of parasitization potential in the field

Percentages of 13.33, 49.33, and 66.00 *Opuntia* spp. cactus pear plants were found infested by *D. echinocacti* in Zemamra locality during the years 2020, 2021, and 2022, respectively. The parasitism rates by *P. diaspidis* and *A. debachi* in field-collected scale insects on *Opuntia* spp. cladodes were 70.58, 73.68, and 74.1% (*P. diaspidis*) and 29.42, 36.58, and 38.38% (*A. debachi*), in 2020, 2021, and 2022, respectively. Populations of the parasitoids and the cochineal tested showed significant differences among years, with a significant increase in infestation and parasitism in 2022 (cactus pear plants infestation $f=436,512.63$, $df=2, 8$, $P \leq 0.0001$; *P. diaspidis* $f=9.40$, $df=2, 149$, $P \leq 0.0001$; *A. debachi* $f=202.80$, $df=2, 149$, $P \leq 0.0001$) (Fig. 2). The abundance of the parasitoids was correlated with host plants infestation (*P. diaspidis* $r=0.84$, $P \leq 0.0001$; *A. debachi* $r=0.89$, $P \leq 0.0001$). In each year of the study, the most abundant parasitoid was found to be *P. diaspidis*.

Functional response study

The linear coefficient $P1$ value was significantly negative ($P < 0.0001$) for both parasitoids tested, indicating a type II functional response (Table 1). The mean numbers of *D. echinocacti* second instar nymphs parasitized by *P. diaspidis* and *A. debachi* at the different densities tested were nonsignificantly different between the two parasitoid species ($t=0.78$, $df=138$, $P=0.438$, Fig. 3). However, a greater number were parasitized by *P. diaspidis* than by *A. debachi* at all the host densities tested. In general, for both parasitoids, the number of parasitized hosts increased with increasing density of *D. echinocacti* second instar nymphs from 1 to 50.

The estimates of attack rate (a) and handling time (Th) for *P. diaspidis* and *A. debachi* on *D. echinocacti* second instar nymphs are shown in (Table 2). The parasitoids' attack rates were significantly higher for *P. diaspidis*

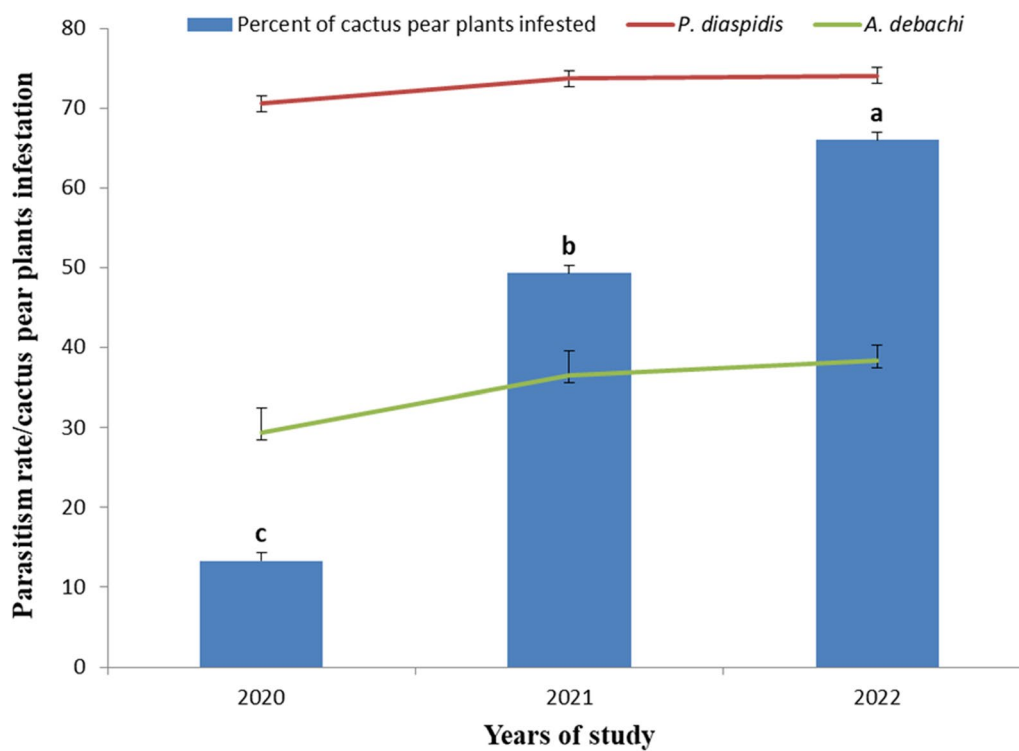


Fig. 2 Population trends of *D. echinocacti* and its potential parasitoids *P. diaspidis* and *A. debachi* on *Opuntia spp.* cactus pear plants during 2020–2022. Bars with same small letters indicate that there is no significant difference in percentage cactus pear plants infestation by *D. echinocacti* according to Tukey's LSD test ($P > 0.0001$)

Table 1 Estimates of the parameters P_0 , P_1 , P_2 and P_3 of the proportion of *D. echinocacti* host parasitized by *P. diaspidis* and *A. debachi*

Parasitoid	Parameter	Estimate	SE	t-test	P value
<i>P. diaspidis</i>	P_0	0.991	0.075	13.297	$P < 0.0001$
	P_1	-0.013*	0.003	-4.816	$P < 0.0001$
	P_2	-0.034	0.010	-3.357	$P = 0.001$
	P_3	-0.104	0.024	-4.400	$P < 0.0001$
<i>A. debachi</i>	P_0	0.746	0.048	15.400	$P < 0.0001$
	P_1	-0.007*	0.002	-4.158	$P < 0.0001$
	P_2	-0.016	0.007	-2.362	$P = 0.021$
	P_3	-0.064	0.016	-4.123	$P < 0.0001$

* A significant negative value for the parameter P_1 indicates that the slope of the functional response curve is declining, thus a type II functional response

than for *A. debachi* ($t = 2.19$, $df = 138$, $P = 0.030$). Also, the model estimated that the handling time was significantly higher for *P. diaspidis* than for *A. debachi* ($t = 5.47$, $df = 138$, $P < 0.0001$). Maximum attack rate values were significantly higher for *A. debachi* than for *P. diaspidis* ($t = -4.55$, $df = 138$, $P < 0.0001$) (Table 2). Searching efficiency values of *P. diaspidis* and *A. debachi* on second instar nymphs of *D. echinocacti* were not significantly

different ($t = 1.94$, $df = 138$, $P = 0.054$). Also, searching efficiencies of the two parasitoids decreased as the host density increased from 1 to 50 reflecting a direct increase in the probability of finding the host at higher densities. The total searching time was significantly lower for *P. diaspidis* than for *A. debachi* ($t = -4.15$, $df = 138$, $P < 0.0001$).

Mutual interference

For the two parasitoid species tested, the parasitization efficiency of a single female parasitoid on *D. echinocacti* second instar nymphs decreased with increasing parasitoid density. The average number of *D. echinocacti* nymphs parasitized by a single adult female of *P. diaspidis* and *A. debachi* decreased significantly from 11.80 to 4.16 and from 10.10 to 3.76, respectively, when parasitoid density increased from 1 to 8 (*P. diaspidis* $f = 131.8$, $df = 3$, 39 , $P \leq 0.0001$; *A. debachi* $f = 104.0$, $df = 3$, 39 , $P \leq 0.0001$) (Table 3). This indicates that mutual interference occurred when multiple females of the parasitoids forage simultaneously in the same limited arena. After fitting the experimental data to the Hassell and Varley model equation, the resulting linear equation for *P. diaspidis* was $\lg E = -0.14 - 2.98 \lg P$ ($r = 0.87^{**}$, $P < 0.0001$).

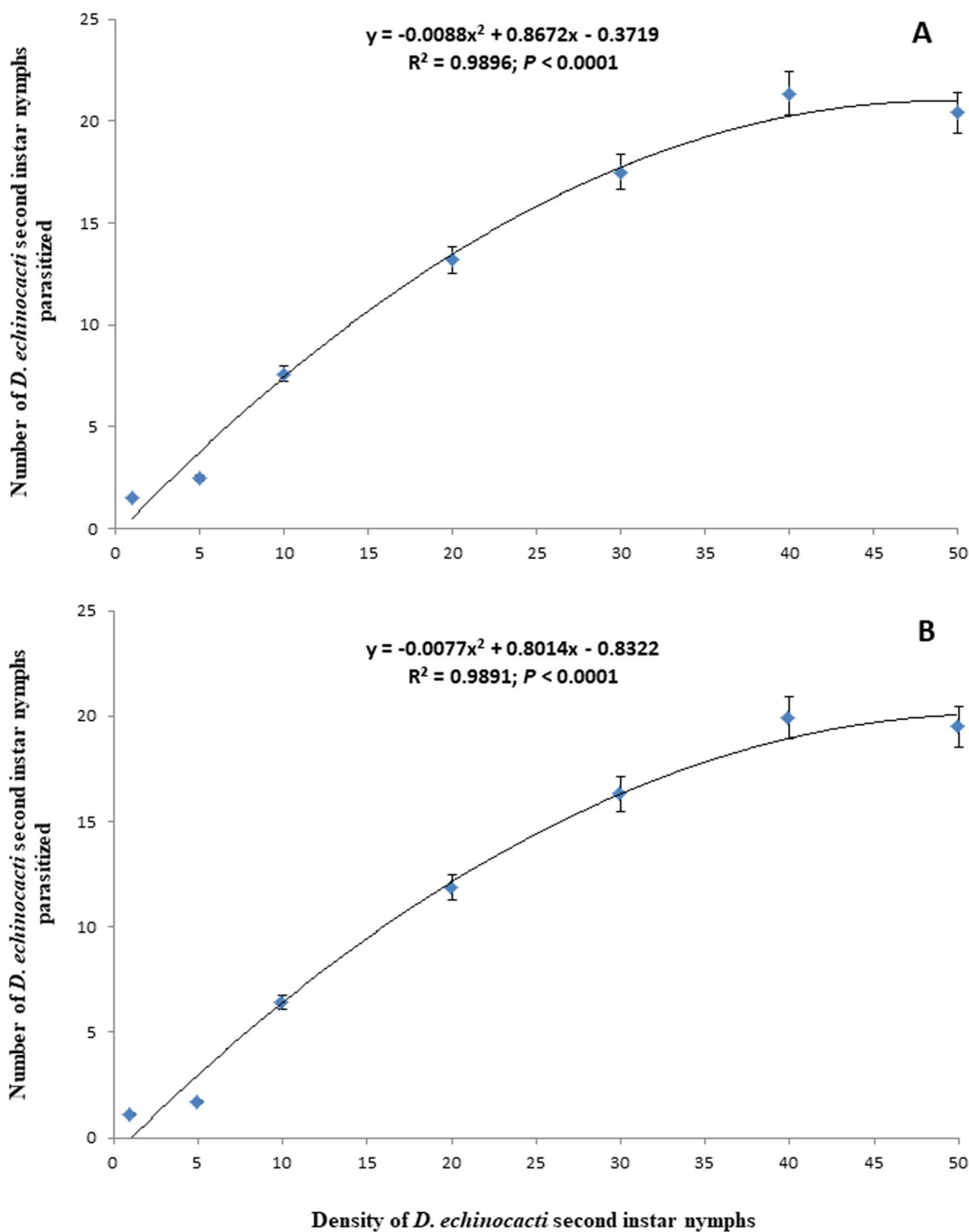


Fig. 3 Functional response of *P. diaspidis* (A) and *A. debachi* (B) to variable host densities of *D. echinocacti* second instar nymphs

The mutual interference constant (*m*) was estimated to be 2.98. For *A. debachi*, the resulting linear equation using the same model was found to be $\lg E = -0.22 - 2.89 \lg P$ ($r = 0.85^{**}$, $P < 0.0001$). The mutual interference constant

(*m*) was estimated to be 2.89. Due to increasing mutual interference with increasing parasitoid density, the area of discovery (*a*) (*P. diaspidis* $f = 39.3$, $df = 3, 39$, $P \leq 0.0001$; *A. debachi* $f = 40.0$, $df = 3, 39$, $P \leq 0.0001$) and searching

Table 2 Functional response parameters estimated for a single female of *P. diaspidis* and *A. debachi* parasitizing *D. echinocacti* second instar nymphs

Parasitoid	Parameter	Estimate	SE	95% CI	
				Lower	Upper
<i>P. diaspidis</i>	Attack rate (h^{-1}) (<i>a</i>)	0.73	0.25	0.63	0.84
	Handling time (h) (Th)	0.09	0.02	0.08	0.10
	Max. attack rate (<i>T</i> /Th)	323.59	109.45	278.44	368.74
	Searching efficiency (<i>E</i>)	0.71	0.26	0.60	0.81
	Searching time (<i>T</i> - Th <i>N₀</i>)	22.88	0.74	22.68	23.08
<i>A. debachi</i>	Attack rate (h^{-1}) (<i>a</i>)	0.60	0.18	0.54	0.67
	Handling time (h) (Th)	0.06	0.02	0.05	0.07
	Max. attack rate (<i>T</i> /Th)	480.21	146.78	428.40	532.02
	Searching efficiency (<i>E</i>)	0.59	0.19	0.52	0.68
	Searching time (<i>T</i> - Th <i>N₀</i>)	23.36	0.43	23.24	23.48

efficiency (*E*) (*P. diaspidis* $f=1002.4$, $df=3$, 39 , $P\leq 0.0001$; *A. debachi* $f=951.2$, $df=3$, 39 , $P\leq 0.0001$) of the tested parasitoids decreased significantly with increasing parasitoid density (Table 3). When *P. diaspidis* and *A. debachi* were compared regarding the number of hosts parasitized per parasitoid female (parasitoids density=1 $t=-3.021$, $df=18$, $P=0.753$; parasitoids density=2 $t=-2.111$, $df=18$, $P=0.258$; parasitoids density=4 $t=-3.122$, $df=18$, $P=0.803$; parasitoids density=8 $t=-3.765$, $df=18$, $P=0.191$), the area of discovery (parasitoids density=1 $t=-3.025$, $df=18$, $P=0.684$; parasitoids density=2 $t=-2.187$, $df=18$, $P=0.179$; parasitoids density=4 $t=-3.127$, $df=18$, $P=0.594$; parasitoids density=8 $t=-3.772$, $df=18$, $P=0.080$), and the

searching efficiency (parasitoids density=1 $t=2.995$, $df=18$, $P=0.996$; parasitoids density=2 $t=1.936$, $df=18$, $P=0.512$; parasitoids density=4 $t=3.101$, $df=18$, $P=0.992$; parasitoids density=8 $t=3.743$, $df=18$, $P=0.310$) at different parasitoid densities tested, non-significant difference was recorded between the two parasitoids (Table 3).

Discussion

Diaspis echinocacti attained scale status in Morocco during the study period (2020–2022). In the study site (Zem-amra, Morocco), infestation of *Opuntia* spp. cactus pears by this scale pest increased each year from 2020 to 2022. The average percentage of infestation increased from 13.33% in 2020 to 66% in 2022, a level that could cause significant damage to cactus pears. The relatively high susceptibility of *D. echinocacti* to insecticides, especially mineral oil (de Souza Born et al. 2007), and the identification of its natural enemies (predators and parasitoids) in many countries worldwide where the cochineal has attained pest status make this cochineal manageable and, therefore, of less economic importance. In Morocco, populations of *D. echinocacti* increases between October and December of each year when the air temperature ranges between 10 and 28 °C and relative humidity between 50 and 60%; at this time of the year, the cochineal becomes an economically important pest requiring efficient and effective control. Hence, the development of *D. echinocacti* populations had taken longer in Morocco than in other countries such as Brazil (Dantas et al. 2019). This increase in infestation of cactus pears by *D. echinocacti* in Morocco in recent years may be due to a variety of factors, such as increasing temperatures and scarcity of rainfall (climate change), which play an important role in enhancing the survival and reproduction of the scale pest. Climate change, especially increasing temperatures,

Table 3 Mutual Interference parameters estimated by Hassell and Varley model equation at various densities of parasitoids and constant density (50) of *D. echinocacti* second instar nymphs

Parasitoid	Parasitoids density	Host density	Host parasitized per parasitoid female (mean ± SE)	Area of discovery (<i>a</i>)	Searching efficiency (<i>E</i>)
<i>P. diaspidis</i>	1	50	11.80 ± 1.04 A	0.12 A	0.060 A
	2	50	8.75 ± 0.9 B	0.09 B	0.022 B
	4	50	7.15 ± 0.37 C	0.09 B	0.005 C
	8	50	4.16 ± 0.23 D	0.06 C	0.002 D
<i>A. debachi</i>	1	50	10.10 ± 0.94 A	0.10 A	0.067 A
	2	50	7.80 ± 0.63 B	0.08 B	0.024 B
	4	50	6.48 ± 0.34 C	0.08 B	0.007 C
	8	50	3.76 ± 0.16 D	0.05 C	0.003 D

Means within a column followed by the same letter are not significantly different (Tukey's test, $P>0.05$)

plays an important role in the development and reproduction of all insect species, affecting indirectly the trophic interactions (host and natural enemies) (Stange and Ayres 2010). In this context, scale pests such as *D. echinocacti* pose a serious menace to cactus pear crop in countries that have not yet been infested.

Populations of the two *D. echinocacti* primary parasitoids, the endoparasitoid, *P. diaspidis* and the ectoparasitoid, *A. debachi* increased each year from 2020 to 2022 to make use of the more abundant hosts available to them. Parasitism rates increased from 70.58 and 29.42% in 2020 to 74.1 and 38.38% in 2022 for *P. diaspidis*, and *A. debachi*, respectively. This may be due to the low host density in 2020–2021 than in 2022, although other studies have reported that parasitism rates can be high even at low host densities (Williams 2003). *P. diaspidis* and *A. debachi* are the most widespread and common parasitoid species attacking *D. echinocacti* worldwide (Japoshvili et al. 2010). The dominant species in Morocco was *P. diaspidis*; this species is also the most common and potential endoparasitoid found associated with *D. echinocacti* in Greece and many other European countries (Japoshvili et al. 2010).

Functional response is an important tool for obtaining basic information regarding the biological control potential and efficacy of different parasitoid and predator species (Overholt and Smith 1990). In this study, Holling's disk equation (Holling 1959) indicated that *P. diaspidis* and *A. debachi* exhibited functional response type II to *D. echinocacti*. *P. diaspidis* had a higher parasitism rates than *A. debachi* at all densities of *D. echinocacti* second instar nymphs tested. This suggests that *P. diaspidis* may be a more effective parasitoid against *D. echinocacti* than *A. debachi* in Morocco. *P. diaspidis* is a primary solitary endoparasitoid of *D. echinocacti* that can kill more than 80% of the second instar nymphs of the scale pest (Japoshvili et al. 2010). The parasitism potential of this endoparasitoid against *D. echinocacti* has also been reported in many other studies (Panis and Pinet 1999). *A. debachi* is a primary solitary ectoparasitoid of *D. echinocacti*, which parasitizes both second instar nymphs and adult females of the scale insect in Greece (Japoshvili et al. 2010). The same authors reported that its parasitism rate reached 9.3% in March and 12% in April 2009. The effect of host density on parasitoid efficacy can be attributed to the simultaneous action of various phenomena, including the nature of the experimental arena, attack rate and handling time (O'Neil and Stimac 1988). Attack rates were significantly higher for *P. diaspidis* than for *A. debachi* at all host densities tested, this may be related to the high size of *P. diaspidis* than *A. debachi*.

The attack rate relates the functional response curve to increasing densities of host. The results showed that the slope of the curve was not much differ between the two parasitoids, meaning that *P. diaspidis* and *A. debachi* had similar abilities to respond to increasing host densities. The handling time of *P. diaspidis* was significantly higher than that of *A. debachi*; this may be due in part to the long handling time for host parasitization required by *P. diaspidis*. Also, the higher Th for *P. diaspidis* and *A. debachi* in the present study compared to previous reports for other parasitoid species parasitizing different scale insects (Kaçar et al. 2017) may, in part, be due to the reduced accessibility to *D. echinocacti* compared to other host species because this cochineal has a waxy carapace covering the body, which provides protection from direct contact with insecticides and natural enemies. The maximum attack rate and searching time were significantly lower for *P. diaspidis*, but nonsignificant difference was observed between the two parasitoids for searching efficiency parameter; this suggests that both *P. diaspidis* and *A. debachi* had the potential to control *D. echinocacti* at the same population level (Byeon et al. 2011).

Regarding mutual interference study, the present investigation showed that for the two tested parasitoids, the average number of parasitized *D. echinocacti* second instar nymphs increased with parasitoid density; however, increasing the density of parasitoids resulted in a decrease in the average number of parasitized hosts per parasitoid. Doubling the number of parasitoids used to parasitize hosts did not result in a doubling of the number of parasitized hosts, due to mutual interference (Omkar 2004). Comparison of the average number of parasitized hosts per parasitoid with increasing parasitoid density indicated that interference between parasitoids was much greater when parasitoid density was high. In addition, the area of discovery (a) and searching efficiency (E) of the tested parasitoids decreased with increasing parasitoid density. The results of this study are in agreement with those of other studies (Fathipour et al. 2006), using different parasitoids and host species. Area of discovery and mutual interference are good indicators for estimating the effectiveness of natural enemies under field conditions (Hassell and Varley 1969). In addition, the high mutual interference constant recorded for *P. diaspidis* (2.98) compared to *A. debachi* (2.89) predicts the tendency of *P. diaspidis* to interact with its host and become more stable in nature (Hassell and Varley 1969). Mutual interference can affect the biological and reproductive performance of parasitoids and plays an important role in parasitoids mass rearing and release projects (Fathipour et al. 2006).

Conclusion

During the years (2020–2023), the cactus pear cochineal *D. echinocacti* was able to build up a considerable population and had now reached a pest status in Morocco. Concurrently, two parasitoid species, the endoparasitoid, *P. diaspidis* and the ectoparasitoid, *A. debachi*, were found in association with the pest populations and the parasitism rate increased to reach 74.1 and 38.38% in 2022 for *P. diaspidis*, and *A. debachi*, respectively. This important parasitism rates suggests that parasitoids can contribute significantly in the biological control of this scale pest in Morocco. The two parasitoids exhibited a Holling's type II functional response on *D. echinocacti* second instar nymphs, with *P. diaspidis* being the most efficient in detecting and attacking the host. Host handling time by the two parasitoids was relatively higher (between 0.06 and 0.09 h) at higher host densities (50) and most of this handling time was due to the fact that the parasitoids spent a lot of time removing the carapace that protects *D. echinocacti*. The parasitoids searching efficiency and area of discovery decreased with host density and parasitoid aggregation. Finally, there is always a relationship between parasitism and host density; however, the density and diversity of other non-host species and parasitoids may alter the functional response of the parasitoids. Therefore, further field experiments are needed for a clear estimation of the parasitism potential of *P. diaspidis* and *A. debachi* and their efficacy as biological control agents against the cactus pear scale pest *D. echinocacti*.

Abbreviations

IPM	Integrated Pest Management
RH	Relative humidity
Th	Handling time
<i>a</i>	Instantaneous rate of discovery
<i>E</i>	Searching efficiency
<i>m</i>	Mutual interference
<i>Q</i>	The quest constant

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MEA conceptualization, methodology, formal analysis, prepared figures, and wrote the main manuscript text; MS supervision, visualization, and review of the article. All authors reviewed the manuscript. Both authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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