

# Resistance of *Opuntia ficus-indica* cv 'Rojo Pelon' to *Dactylopius coccus* (Hemiptera: Dactylopiidae) under greenhouse condition

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**Abstract.** *Opuntia ficus-indica* (L.) Mill.), is called "beles" in the Tigray region of Ethiopia, where it is used for multiple purposes, such as food, fodder, and fences; however, in recent years the introduction of the cochineal (*Dactylopius coccus* Costa) to this region has caused it to become a plague that has affected thousands of hectares, causing an economic and social problem. Six cultivars: three from *Opuntia ficus-indica* ('Atlixco', 'Chicomostoc', and 'Rojo Pelón') and three others *O. cochenillifera* ('Nopalea' and 'Bioplástico') and *O. robusta* ('Robusta') were tested for resistance to *D. coccus* in completely randomized design (CRD) experiment with six replications under greenhouse conditions, for two seasons (cycles). Matured cladodes were infested by attaching a paper bag with ten ovipositing female *D. coccus*. The number of insects at different stages and yields were recorded, log transformed (insect count data), and subjected to statistical analysis. The number of nymphs (stages I and II) was significantly different in both cycles ( $P=0.0000$ ). The insects died at the nymph I stage at 'Rojo Pelón' and 'Robusta', in contrast, they completed their life cycle at 'Atlixco', 'Chicomostoc', and 'Nopalea' cultivars. Insects at 'Bioplástico' cultivar remained in the nymph I stage the whole experimental period; the molting was hampered. Although some crawlers started settling (nymph I) at 'Rojo Pelón', they couldn't survive and developed, and this confirms that this *O. ficus-indica* cultivar is resistant to *D. coccus*.

**Keywords:** Beles, cactus pear, cochineal, resistant cultivar

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## Introduction

*Opuntia ficus-indica* (L.) Miller (cactus pear) is among the Cactaceae family, which has about 127 genera of which 1750 are identified species of the order Caryophyllales (Christenhusz and Byng, 2016). During the XVI century, *O. ficus-indica* was an important commodity in Mexico (Reyes-Agüero *et al.*, 2005), and because of the different benefits this species had, like morphological peculiarities, anti-scurvy properties, sweet edible fruits, and ability to host the cochineal insect, from which an important commodity called cochineal dye was gained, the Spanish took it to their homeland. Later it was expanded to other regions of the world, including the Mediterranean area (Barbera *et al.*, 1992; Griffith, 2004). It was also introduced to Tigray, around the middle XVIII century (Neumann 1997; cited in Gebretsadik *et al.*, 2013). The cactus pear, locally called beles in the Tigrigna Language of Ethiopia, is a crop that is used in some parts of Tigray, as a source of human food for 4 or 5 months and for livestock feed, and it is considered an emergency crop. Although it can grow in different areas, it is obvious that beles can grow better in specific areas (Lemma *et al.*, 2010). It is appreciated for its various benefits, which include high biomass yield, growth in sandy soils, staying greenthroughout the year, and resistance to

drought; this suggests a high potential for use for different other purposes (Brutsch, 1997). In Mexico this plant is known as “nopal”, but in other countries is “tuna”, “cactus pear”, “prickly pear”, “green jewel” “green gold”, “fruit for the poor”, “treasure under its thorns”, “world plant dromedary”, “future plant”, “sacred plant”, and “monstrous tree” (Arias-Jimenez, 2013).

Cactus pear in North Africa, Brazil, and the Middle East is at risk from the wild cochineal (*Dactylopius opuntiae* (Cockerell)) (Bouharroud et al., 2016; Mazzeo et al., 2019; Torres and Giorgi, 2018). In Ethiopia, the fine cochineal insect (*D. coccus* Costa) has caused damage in several areas of the Tigray region (Belay, 2015; Berhe et al., 2020). Due to the aforementioned reasons, it is necessary to carry out research to combat this problem with some options including the utilization of resistant varieties.

*Dactylopius* species or biotypes prefer specific species of *Opuntia* as a host and parasitize only cacti (Volchansky et al., 1999; Hoffmann et al., 2002; Campana et al., 2015; Portillo and Viguera, 2006). The breeding of *D. coccus* is carried out in a small number of nopal species, it has been reported that only 10% of the total number of opuntias are used; among these species, *O. ficus-indica* is with its different cultivars, which is preferred by producers due to its ease of handling (few spines), larger cladodes, in addition to being used for other purposes such as the food and forage (Campana et al., 2015; Van Dam et al., 2015; Viguera and Portillo, 2014). *D. coccus* is a species cultivated worldwide (Portillo and Viguera, 2006; Van Dam and May, 2012), which produces the pigment called carminic acid and which is processed in different presentations such as lacquers and carmine (Piña, 1979; Viguera and Portillo, 2014). This dye is one of the most demanded and has replaced artificial dyes (red); it is used as an additive for food, medicines, cosmetics, and textiles (Arroyo-Figueroa et al., 2016; Torres-Ponce et al., 2015; Arroyo-Figueroa et al., 2009); and it is preferred for its high stability to light and temperature, in addition to being harmless to human health (Viguera and Portillo, 2014).

But it became invasive and a pest of the host *O. ficus-indica* in Tigray, because of different reasons; like the absence of natural enemies, dense plant populations, and in-proper management (Belay, 2015). Having long and short-term management plans are important to control insect and integrated pest management (IPM) gives more emphasis on environmental pollution and the well-being of human being. Resistant varieties are an important component of the IPM to manage the infestation in Tigray (Berhe et al., 2020).

Although the development or evaluation of resistant cultivars of *O. ficus-indica* to *D. coccus* is rarely reported, the utilization of pest resistant crop varieties is important for the economy, environment, and ecology. The economic advantages are reducing crop loss due to insects and minimizing the cost of insecticides. It is also easily available to small-scale growers, especially in developing countries. Mostly, the seed of insect-resistant varieties are a little more expensive than the cost of the susceptible varieties or sometimes can be equal (Teetes, 1996; Huang et al., 2013). More importantly, farmers don't require any special skills or techniques of application (Sharma and Ortiz, 2002). It also helps to reduce the use of chemicals and indirectly related risk of environmental pollution and human health (Munhame et al., 2021; Reinert et al., 2003; Viguera y Portillo, 2014). The genus *Opuntia* has higher diversity and distribution in the Americas than the other Cactaceae (Reyes-Agüero and Valiente-Banuet, 2006). There is a high genetic similarity between *O. ficus-indica* and *O. megacantha* and it indicates *O. ficus-indica* that could be a domesticated type of *O. megacantha* (Kiesling and Metzinger, 2017). *O. ficus-indica*, *O. albicarpa*, and *O. megacantha* possibly share an ancestry (Valadez-Moctezuma et al., 2015). Some of the wild species are resistant to *D. coccus* and the genetic similarity of *O. ficus-indica* with the different species

implies that; there could be genetic sharing hence some *O. ficus-indica* cultivars may have resistance capability. Plants have evolved a whole arsenal of defense strategies against pests, including morphological structures that form the first line of protection to capacity in synthesizing an enormous array of chemical compounds (War et al., 2020).

Tovar et al. (2005) reported that *D. coccus* yield obtained from var. Villanueva is higher than varieties tested in Mexico. Méndez-Gallegos et al. (2010) explained that 'Blanco Moscatel' and 'Colorado Moscatel' cultivars might have defense means for *D. coccus*, since the colony development on those hosts showed a high mortality rate and failed to reproduce. This behaviour may be associated with the presence of phytochemicals (such as terpenoids, flavonoids, tannins, and polyphenols) (Akroud et al., 2021; Guevara-Figueroa et al., 2010; Matos et al., 2021) and morphological-histological characteristics (epidermis and cuticle thickness and density of calcium oxalates) (da Silva et al., 2010). Host resistance should be considered in cochineal control (Vigueras and Portillo, 2014). Thus, it can be possible to find resistant cultivars which may have similar properties to resistant wild species, and exploration and verification are required. Identification of resistant varieties and a deeper understanding of the levels and mechanisms of resistance is vital for integrated pest management strategies (War et al., 2020). It is also imperative paramount to study the genetics of *O. ficus-indica* such as identifying the morphological resistance characteristics of genotypes and finding the genes that control resistance to cochineal (Felker and Inglese, 2003). Resistant varieties can be used either as the main method or as a supplement to other strategies of insect control, based on their resistance level (Jayaraj and Uthamassamy, 1990). Therefore, the research was conducted to assess the cultivar 'Rojo Pelon' of *O. ficus-indica* resistance to *D. coccus* for further utilization and breeding purposes.

### Materials and Methods

The experiment comprised six *Opuntia* cultivars collected from different production farms and locations in Mexico: three *O. ficus-indica* ('Rojo Pelón', 'Atlixco', and 'Chicomostoc'), two *O. cochenillifera* ('Nopalea' and 'Biolástico') and one *O. robusta* ('Robusta') (Table 1). For the infestation of the cladodes, females ovipositing was used (where nymphs were observed), this material was obtained from cochineal breeding in greenhouse. It was carried out at the University of Guadalajara, Center of Biological and Agricultural Sciences (CUCBA), Zapopan, Jalisco, Mexico, under greenhouse conditions and with a completely randomized design (CRD) replicated six times (cladodes as an experimental unit) in two seasons/cycles (Cycle 1: October-January 2020/2021 and Cycle 2: January-April 2021), since these two seasons have different climatic conditions. At Cycle 1 temperatures were 10 to 38.05°C and relative humidity between 36.95 to 56.77%; meanwhile, for Cycle 2 the records ranged from 12.3 to 45.82°C and relative humidity 10 to 66.95%.

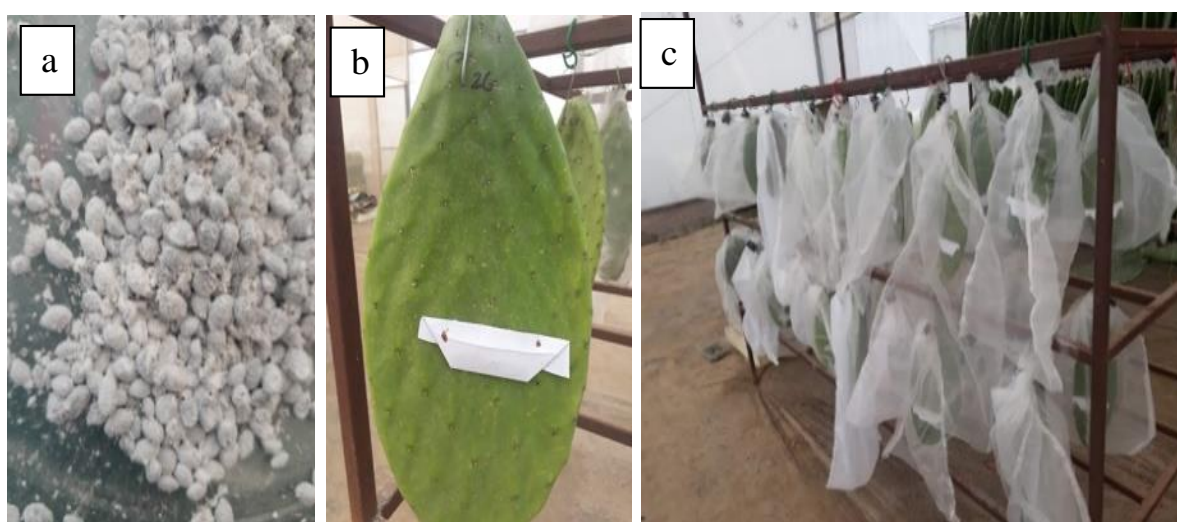
The climatic conditions from where the hosts were collected: Ojuelos, Jalisco has an average annual temperature of 13.9°C, and 594 mm rainfall (IIEG, 2021). Guadalajara has an average temperature of around 20.9°C and 900 mm rainfall; Villanueva, Zacatecas has an annual temperature of 17°C, annual mean rainfall of 510 mm (INEGI, 2022).

**Table 1.** Identity and cladode characteristics (cultivar name, species name, national ID, source, and presence of spines) of the six *Opuntia* cultivars evaluated.

Cultivar name	Species name	National ID	Source	Spinness
Rojo Pelón	<i>O. ficus-indica</i>	NOP-022-221104	OjJ	Spineless
Atlixco	<i>O. ficus-indica</i>	NOP- 018-221104	VNZ	Spineless
Chicomostoc	<i>O. ficus-indica</i>	NA	VNZ	Spineless
Bioplástico	<i>O. cochenillifera</i>	NA	VNZ	Very few
Nopalea	<i>O. cochenillifera</i>	NA	GDLJ	Spineless
Robusta	<i>O. robusta</i>	NOP-060-090617	OjJ	Spiny

Note: OjJ-Ojuelos, Jalisco, VNZ-Villanueva, Zacatecas, GDLJ-Guadalajara, Jalisco. Source: CNVV, 2020.

All selected matured cladodes were one-year-old, vigorous, and free of plagues and diseases. Cladodes were thoroughly washed with pure water for cleaning from any wild cochineal (*D. opuntiae*), their length, width, and thickness were measured with a vernier caliper and a weighing balance was used to measure their weight. Then, the cladodes were labelled and marked for repeat counts, hanged in reverse position, in such a way that they were pierced at their base with wire and placed on a metal barrel. For the infestation of the cladodes, females close to oviposition were used (when nymphs were observed), from this material was obtained from the cochineal breeding in the greenhouse. The adult females were collected (Figure 1a) from the source host cladodes with a fine brush and kept in small paper bags which were fixed with spines (Figure 1b) containing ten adult females each. Infested cladodes were covered with nylon clothing bags (60 cm x 30 cm size) to protect them from uncontrolled infestation and pests (Figure 1c). The paper bags remained attached to the cladodes for 7 days and then removed (Gusqui Mata, 2013). After the females started oviposition (matured), they were harvested with a fine brush to take the yield data.



**Figure 1.** Infestation of cladodes. a) female ovipositing cochineal ready for infestation, b) fixing paper bags that contain female ovipositing cochineal to the cladodes, c) infested cladodes covered with nylon clothing bags.

Data were collected on insect counts including the number of crawlers (mobile nymph I), nymph I established, nymphs II, cocoons, and female adults; total fresh and dry weight per cladode (g); the fresh and dry weight of individual adult female (mg) from each experimental unit (cladode). To take the individual weight, ten samples were randomly weighted, and the average was recorded. Dry weights were measured after the insects were dried in an oven at 60°C for 3 h. Data were checked for homogeneity, transformed with log (count data) and square root (number of cocoons), and an analysis of variance (ANOVA) was performed using the statistical package R.4.1.0. Tukey test at (0.05) was applied to compare means among treatments if significant differences were detected.

## Results and Discussion

### Cladode characteristics of the cultivars used

Cultivars used in cycle 1 and 2 had significant differences ( $P = 0.0000$  both) in cladode weight (g), width (cm), and thickness (cm), and ( $P = 0.0173$  and  $P = 0.0011$ ) in length (cm) (Table 2). In line with this finding, Peña-Valdivia *et al.* (2008) reported differences in morphological characteristics of *opuntias*, and presence of spines on the surface of cladodes, and cladodes' width and length. Adli *et al.* (2019) also observed significant differences in cladode length, width, and thickness among four *O. ficus-indica* accessions. Cladode shapes may be considered important parameters for taxonomic classification even to differentiate forage cacti (Lucena *et al.*, 2019). Morphological characteristics of cladodes (height, width, weight, and thickness) can have effects on the yield of cochineal (Arroyo-Figueroa *et al.*, 2020).

**Table 3.** Means of cladode weight (CWt) in g, length (CL) in cm, width (CW) in cm, and thickness (CT) in cm in cycle 1 (C1) and cycle 2 (C2).

CV	CwtC1	CwtC2	CLC1	CLC2	CWC1	CWC2	CTC1	CTC2
RP	1128.33 <sup>d</sup>	932.5000 <sup>c</sup>	30.67 <sup>b</sup>	27.8333 <sup>bc</sup>	17.33 <sup>b</sup>	16.3333 <sup>bc</sup>	3.50 <sup>d</sup>	3.3333 <sup>c</sup>
A	467.50 <sup>a</sup>	705.0000 <sup>bc</sup>	30.67 <sup>b</sup>	31.3333 <sup>c</sup>	12.83 <sup>a</sup>	14.1666 <sup>ab</sup>	2.04 <sup>ab</sup>	2.2500 <sup>ab</sup>
C	741.67 <sup>b</sup>	591.6667 <sup>bc</sup>	31.33 <sup>b</sup>	29.5000 <sup>bc</sup>	18.50 <sup>b</sup>	17.8333 <sup>c</sup>	2.04 <sup>ab</sup>	1.9583 <sup>ab</sup>
B	476.67 <sup>ac</sup>	375.0000 <sup>ab</sup>	28.67 <sup>ab</sup>	26.3333 <sup>bc</sup>	12.50 <sup>a</sup>	11.0000 <sup>a</sup>	2.42 <sup>bc</sup>	2.2916 <sup>ab</sup>
N	247.50 <sup>a</sup>	238.3333 <sup>a</sup>	31.17 <sup>b</sup>	24.6666 <sup>ab</sup>	10.50 <sup>a</sup>	11.1666 <sup>a</sup>	1.46 <sup>a</sup>	1.8333 <sup>a</sup>
R	836.67 <sup>c</sup>	594.1667 <sup>bc</sup>	22.33 <sup>b</sup>	23.0000 <sup>a</sup>	19.33 <sup>b</sup>	17.1666 <sup>bc</sup>	2.96 <sup>cd</sup>	2.5416 <sup>b</sup>

Means sharing the same letter are not significantly different.

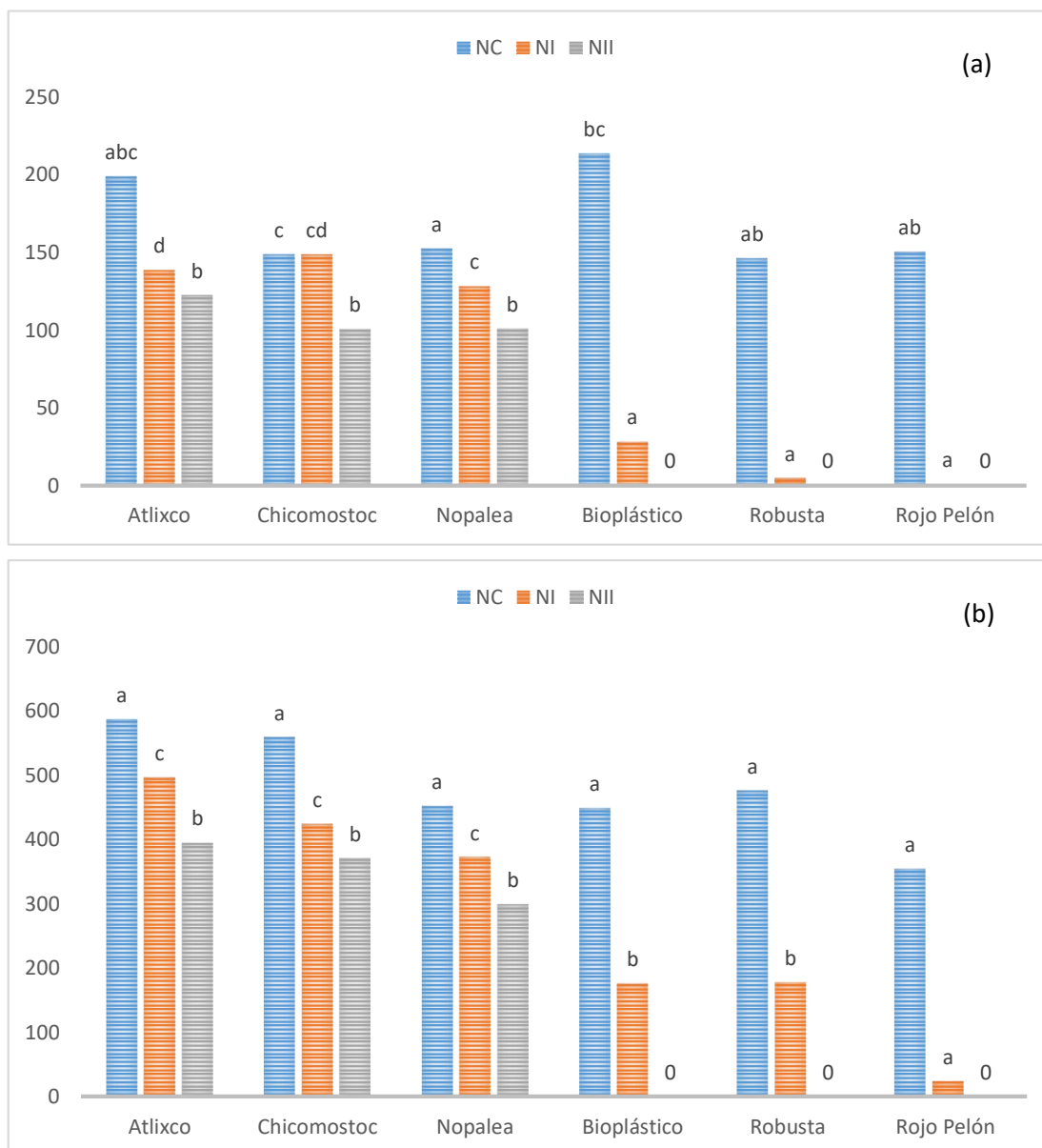
CV-cultivar, RP='Rojo Pelón', A='Atlixco', C='Chicomostoc', B='Biolástico', N='Nopalea', and R='Robusta'.

### D. coccus survival and development

Statistically, a significant difference was observed among the cultivars in the number of crawlers/mobile nymph I (NC) in cycle I ( $P = 0.0398$ ), but not in cycle 2 ( $P = 0.1520$ ), where the number was higher in 'Chicomostoc' but statistically similar with 'Atlixco' and 'Nopalea'. The number of nymphs I (NI) indicated a significant variation at both cycles ( $P=0.0000$ ). Additionally, for both cycles, there was a statistically significant difference in the number of nymph II (NII) ( $P=0.0000$ ). At cycle 2, a lower number of nymphs I was observed on 'Rojo Pelón', and a higher number was observed on 'Atlixco' (but statistically like 'Chicomostoc'). The number of nymphs I was significantly similar within the resistant ('Robusta', 'Rojo Pelón', and 'Biolástico'). The number of nymphs II was statistically similar among the susceptible cultivars, but there was no nymph II observed in the resistant cultivars (Figure 2).

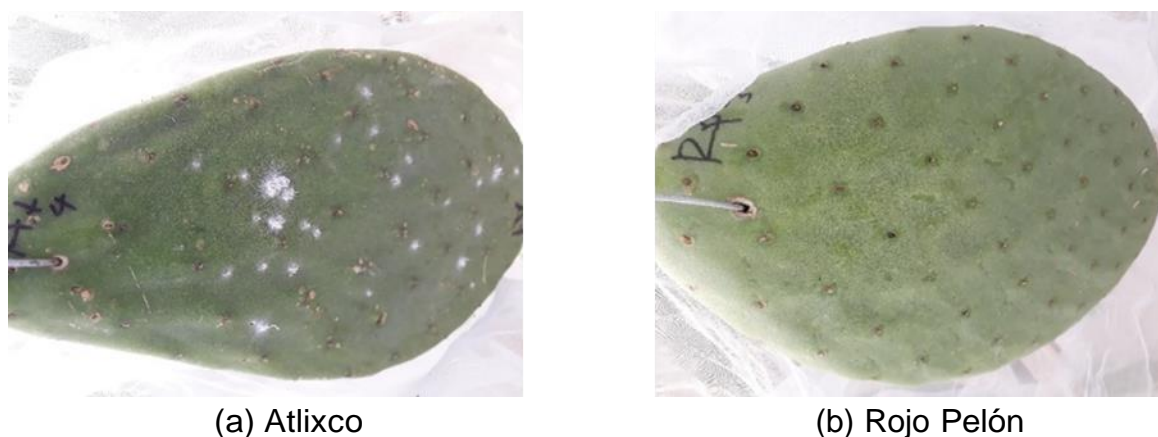
The similarity of *Opuntia* cultivars in the number of crawlers is an indication of the insects' non-preference for oviposition. The difference in development among different cultivars is similar to previous reports. Passos da Silva *et al.* (2007) found a difference in resistance among *Opuntia*

clones to *D. opuntiae*. Sbaghi et al. (2019) also reported that seven resistant ecotypes out of 241 tested proved to be resistant to *D. opuntiae* pest in Morocco.



**Figure 2.** Development of *Dactylopius coccus* on six *Opuntia* cultivars. The number of crawlers (NC), nymphs I (NI), and nymphs II (NII) at cycle 1 (a) and cycle 2 (b). Means sharing the same letter are not significantly different.

It was noted that the insects died at the nymphs I stage, after 21 days of infestation on two resistant cultivars ('Robusta' and 'Rojo Pelón') (Figure 2). Growing nymphs II with white wax on 'Atlixco' (Figure 3a) and insect-free on 'Rojo Pelón' (Figure 3b).

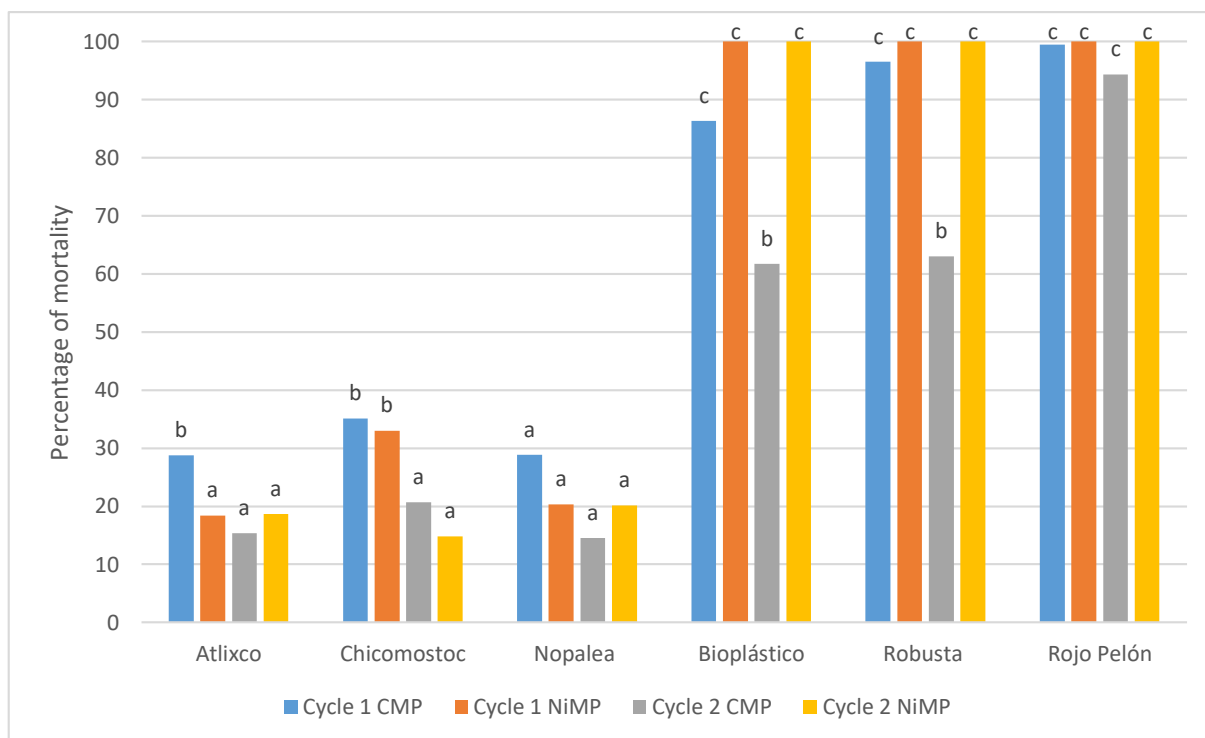


**Figure 3.** Status of insects in *Opuntia* cultivars after 21 days after infestation. a) susceptible cultivar ('Atlixco') with *Dactylopius coccus* showing covered with white wax, b) resistant cultivar ('Rojo Pelón') without *D. coccus*.

*Opuntia* cultivars also showed statistically significant differences ( $P=0.0000$ ) in the percentage of crawler mortality and nymph I mortality at both cycles at  $p\leq 0.05$ . Crawlers' mortality was higher in the resistant cultivars (86 % to 96%) than in susceptible cultivars (28% to 35% in cycle 1). In cycle 2, crawlers' mortality was higher at 'Rojo Pelón' (100%) followed by 'Robusta' (94%), and 'Bioplástico' (63%). Similarly, nymph I mortality was higher (100%) in resistant cultivars, 'Rojo Pelón', 'Bioplástico', and 'Robusta' (Figure 4). In agreement with this finding, nymph mortality of *D. opuntia* was high, ranging from 40 to 60% at different cultivars, during the first 24 days of development (Méndez-Gallegos *et al.*, 2010). Akroud *et al.* (2021) found that mortality of *D. opuntiae*, started on the 7<sup>th</sup> day after infestation and maximum mortality on the 28<sup>th</sup> day. The authors also discussed that the mortality might be due to the abnormalities that could be caused by the ingestion of compounds (secondary metabolites like alkaloids, carotenoids, diterpenes, amines, etc.), which are either insufficient or anti-nutrition for the insect's development. At the two resistant cultivars, all nymphs died at first instar which may be due to the cultivars possessing antibiosis mechanisms of resistance. This finding can suggest that 'Rojo Pelón' is a resistant cultivar to *D. coccus*. Similarly, Sbaghi *et al.* (2019) and Passos da Silva *et al.* (2007) concluded that five cultivars proved to be immune to the *D. opuntiae*, since there has been no insect development.

Adult and cocoon populations showed a significant difference across cultivars ( $P=0.0000$ ) at both testing cycles, but there was no statistical difference among susceptible cultivars (Table 3). Cultivars had no significant difference in total insect fresh weight at both cycles ( $P=0.5540$  and  $0.0744$ ), total dry weight at cycle 1 ( $P=0.7860$ ), individual fresh weight at cycle 2 ( $P=0.3680$ ), and individual dry weight at cycle 2 ( $P=0.3320$ ). However, there were statistically significant differences in individual insect fresh and dry weight ( $P=0.0003$  and  $P=0.0007$ ) at cycle 1 and total dry weight at cycle 2 ( $P=0.0475$ ) (Figure 5). 'Atlixco' followed by 'Chicomostoc' cultivars were superior to 'Nopalea' in insect performance, measured by the above parameters (Table 3 and Figure 5). These results support the conclusions made by Tovar *et al.* (2005) who noticed significant differences among cultivars. Similarly, Méndez-Gallegos *et al.* (2010) reported differences in the carminic acid percentage of *D. coccus* among different cultivars. Arroyo-Figueroa *et al.* (2020) also reported the effect of morphological characteristics in cladodes of different ages, such as length, width, and

weight affected *D. coccus* yield; which can be also different intra and inter-species (Peña-Valdivia et al., 2008; Adli et al., 2019).



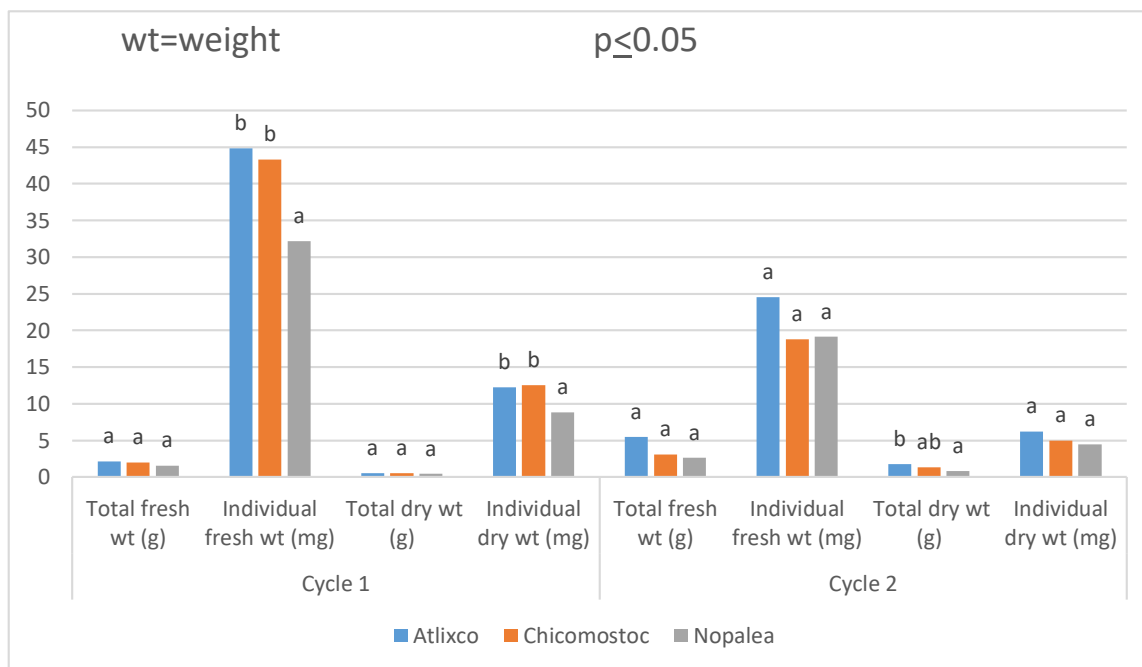
**Figure 4.** Mortality of nymphs (percentage) on six *Opuntia* cultivars. Mortality of crawlers at cycle 1 (cycle 1 CMP) and cycle 2 (Cycle 2 CMP), mortality of nymph I at cycle 1 (Cycle 1 NiMP) and cycle 2 (Cycle 2 NiMP) at  $p \leq 0.05$ . Means sharing the same letter are not significantly different.

**Table 3.** Means of the number of adults (females) and cocoons of *Dactylopius coccus* on six *Opuntia* cultivars at cycle 1 (C1) and cycle 2 (C2).

Cultivar	Adults C1	Cocoons C1	Adults C2	Cocoons C2
'Rojo Pelón'	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
'Atlixco'	49.17 <sup>b</sup>	14.83 <sup>b</sup>	255.83 <sup>b</sup>	66.33 <sup>b</sup>
'Chicomostoc'	49.00 <sup>b</sup>	18.00 <sup>b</sup>	201.50 <sup>b</sup>	54.17 <sup>b</sup>
'Bioplástico'	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
'Nopalea'	55.67 <sup>b</sup>	14.50 <sup>b</sup>	148.17 <sup>b</sup>	37.50 <sup>b</sup>
'Robusta'	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>

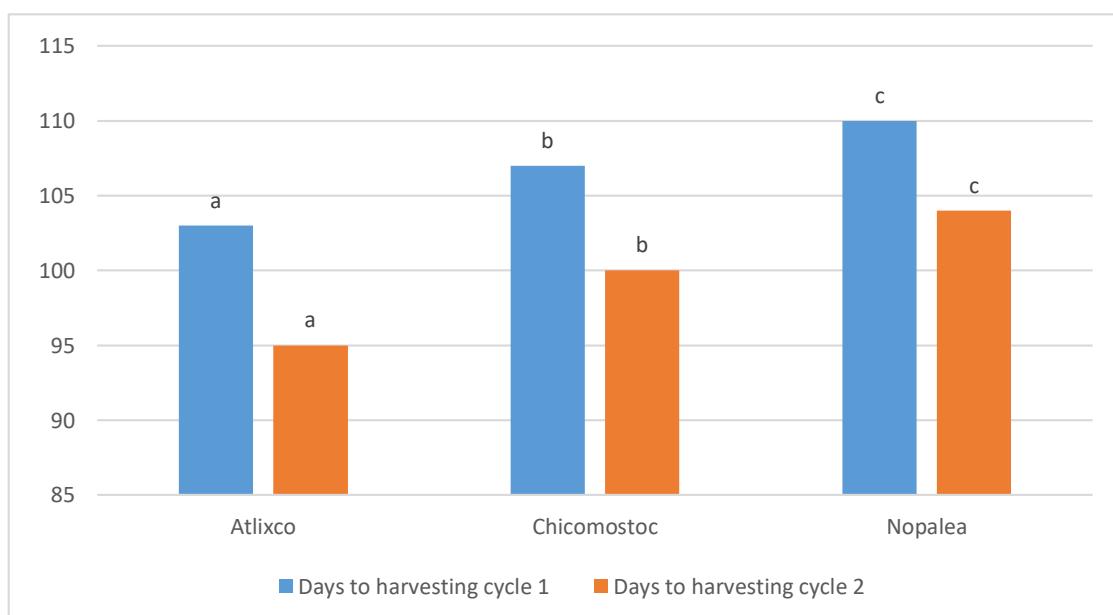
Note: Means sharing the same letter are not significantly different.





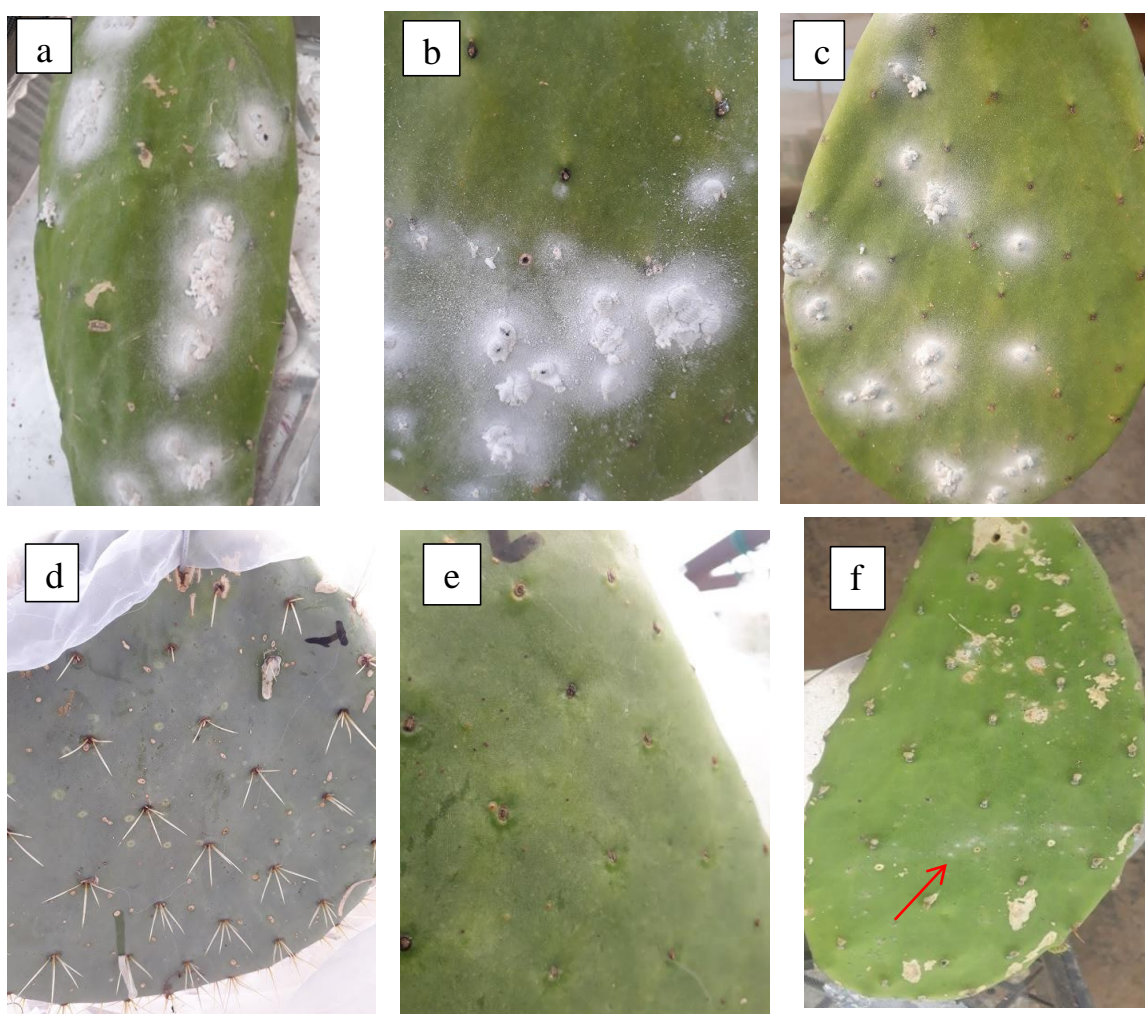
**Figure 5.** Insect fresh and dry weight total (g), fresh and dry weight individual (mg), at cycles 1 and 2.

The number of days to complete the female life cycle of the insect (harvesting) showed a statistically significant difference among the susceptible cultivars ( $P=0.0000$ ). *D. coccus* completed its life cycle earlier at ‘Atlixco’ followed by ‘Chicomostoc’, and ‘Nopalea’ (Figure 6). Overall, the days taken to maturity are in the range of the life cycle of the insect. In accordance with this, Arroyo-Figueroa *et al.* (2020) explained that *D. coccus* needs 90 to 120 days to complete its life cycle. Méndez-Gallegos *et al.* (1993) also support that the period to maturity of *D. coccus* is around 90 days.



**Figure 6.** Days to harvesting of *Dactylopius coccus* under three susceptible cultivars (‘Atlixco’, ‘Chicomostoc’, and ‘Nopalea’). Means sharing the same letter are not significantly different.

Insects completed their life cycle on the three susceptible cultivars ('Atlixco', 'Chicomostoc', and 'Nopalea'). In 'Bioplástico' the insects remain in nymph I (Figure 7). At cultivars ('Robusta' and 'Rojo Pelón') the insects died during the first instar (nymph I) (Figures 4 and 7). The susceptible hosts are members of *O. ficus-indica* which are the commonly used hosts for cochineal production (Campana et al., 2015). The mechanism of resistance could be the presence of secondary metabolites (War et al., 2020) or physical barriers like calcium oxalates (Nakata, 2015; Molano-Flores, 2001). López-Palacios and Peña-Valdivia (2020) observed higher levels of hydroxycinnamic acid in the cladodes of wild species. Tovar-Puente and Pando-Moreno (2010) high concentration of calcium oxalate crystals stated that prevents the nymph from inserting the stylet and settling in the cladode. Oxalates are more concentrated on matured cladodes (Contreras-Padilla et al., 2011).



**Figure 7.** Infestation status of all six cultivars at the end of the experiment periods. a='Nopalea', b='Atlixco', c= 'Chicomostoc', d='Robusta', e='Rojo Pelón', and f= 'Bioplástico'. Susceptible cultivars a, b, and c with matured *Dactylopius coccus* and resistant cultivars d and e free of insects and f with nymphs (arrow).

Generally, the findings are supported by previous works. Tovar et al. (2005) and Méndez-Gallegos et al. (2010) reported differences in *D. coccus* performance among cultivars in Mexico and suggested the possibility of resistance. Sbaghi et al. (2019) also found seven ecotypes shown to be resistant to *D. opuntiae* in Morocco. Since there has been no pest establishment, a cultivar could be resistant; hence, our results confirm that 'Rojo Pelón' is a promising resistant cultivar of *O. ficus-indica* to *D. coccus*. The host and the insect have obviously been in

continuous adaptations and many wild species are resistant to these insects; so, it is inferred that this domesticated cultivar may share some genetic characteristics with some of the wild species.

The 'Rojo Pelon' cultivar probably originated from Northern Guanajuato and Southern San Luis Potosi (Central highlands of Mexico). Characterized by an elliptical shape, bright red fruit colour, and sweet export quality fruit that matures during the summer season. The tender and spineless cladodes it has been important for vegetable and livestock feed (Cervantes-Herrera *et al.*, 2006).

Remaining the insects in the nymph I stage throughout the experimental period of the complete life cycle on 'Bioplástico' cultivar (Figure 6) implies moulting was hindered, probably by some Phyto-ecdysones (terpenoids) (War *et al.*, 2020). Moulting growth can be continuous, as in hemimetabolous insects (e.g., aphids, cockroaches, grasshoppers, and locusts) or resting pupal stage, when the larval organs are completely changed to adult level (holometabolous insects, e.g., beetles, moths, flies, and ants) (Morgan and Poole, 1977). It is under the control of the brain (the corpus cardiacum) (Veelaert *et al.*, 1998) that stimulates the prothoracic gland to secrete moulting hormone or its precursor (Krishnakumaran and Schneiderman, 1968; Kobayashi and Yamazaki, 1974; Ishizaki and Suzuki, 2002).

### Conclusions

The evaluated cultivars belong to three species of *Opuntia*, three *O. ficus-indica* cultivars, one *O. robusta* cultivar, and two *O. cochenillifera* cultivars. These cultivars are different in resistance to *D. coccus*. It was also observed that there is sharing of characteristics among species in relation to resistance to *D. coccus*. Three cultivars from different species are resistant to the insect. The resistance characteristics could be shared among species since there is a genetically interlinked among the *Opuntia* spp. During the two cycles experiments, 'Rojo Pelón' was found to be resistant against *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. This is the first report on *D. coccus* resistance, which can be considered as an element of integrated pest management intervention or practice in Tigray and other areas having similar problems. It can be used for direct utilization and/or genetic improvements for the *D. coccus* resistance breeding programs for beles. Mechanisms of resistance or characteristics of the plants related to *D. coccus* resistance are also important subjects of study to use as a selection/improvement marker in resistant breeding. And it could be physical barriers, tissue structures, and secondary metabolites or combinations which lead to antibiosis (restricting growth or killing) resistance. Differently from the other cultivars, moulting of the insect was restricted in the 'Bioplástico' cultivar, this could be probably due to presence of unique secondary metabolites, and these phenomena of resistance are an important area of study.

### Ethics statement

Not applicable

### Consent for publication

Not applicable

### Disclosure statement

The authors declare that they have no conflict of interest.

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### Author contributions

All authors participated in the conceptualization of the project, made the investigation for the background of the manuscript, and wrote part of the original draft. The second and third authors participated in the application and development of the experiments; executed part of the administration of the project. They reviewed and edited all versions of the manuscript.

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### References

- Adli, B., Touati M., Yabrir B., Bakria T., Bezini, E., and Boutekrabt, A. 2019. Morphological characterization of some naturalized accessions of *Opuntia ficus-indica* (L.) Mill. in the Algerian steppe regions. *South African Journal of Botany*. 124: 211-217. <https://doi.org/10.1016/j.sajb.2019.04.017>.
- Akroud, H., Sbaghi M., Bouharroud R., Koussa T., Boujghagh M., and El Bouhssini, M. 2021. Antibiosis and antixenosis resistance to *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) in Moroccan cactus germplasm. *Phytoparasitica*. 49(4): 623-631. <https://doi.org/10.1007/s12600-021-00897-w>.
- Arias-Jimenez, E. 2013. Importancia de la tuna. *Cactusnet Newsletter*, 13:9-13.
- Arroyo-Figueroa, G., Méndez, C. H. H., Cauich, J. G. D., Rodríguez, L. V., and Caballero, V. P. 2016. Medición del color en productos cosméticos elaborados con subproductos de la grana cochinilla. *Acta Universitaria*. 26(1): 3-7. <https://doi.org/10.15174/au.2016.836>.
- Arroyo-Figueroa, G., Vargas-Rodríguez, L., and Gonzalez-Sánchez, G. 2009. Study of conditions for cotton dyeing with cochineal. *Acta Horticulturae*. 811: 241-246.
- Arroyo-Figueroa, G., Medina-Saavedra, T., Pérez-Sánchez, R. E., and Ortiz-Rodríguez, R. 2020. Morphology and age of the cactus pear *Opuntia ficus-indica* on the production of (*Dactylopius coccus* Costa) and carminic acid content. *Nova Scientia*. 12(25): 1-13. <https://doi.org/10.21640/ns.v12i25.2519>.
- Barbera, G., Carimi, F., and Inglese, P. 1992. Past and present role of the Indian-fig prickly-pear (*Opuntia ficus-indica* (L.) Miller, Cactaceae) in the agriculture of Sicily. *Economic Botany*. 46(1): 10-20.
- Belay, T. 2015. Carmine cochineal: fortune wasted in northern Ethiopia. *Journal of the Professional Association for Cactus Development*. 17: 61-80. <https://doi.org/10.56890/jpacd.v17i.62>.

- Berhe, Y., Aymut K. M., Gebremariam, B. L., Gebreziher, H. G., and Siyum, Z. H. 2020. Introduction of carmine cochineal to northern Ethiopia, current status of infestation on cactus pear, and control measures. *International Journal of Botany Studies*. 5(1): 32-38.
- Bouharroud, R., Amarraque, A., and Qessaoui, R. 2016. First report of the *Opuntia* cochineal scale *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) in Morocco. *EPPO Bulletin*. 46(2): 308-310. <https://doi.org/10.1111/epp.12298>.
- Brutsch, M. O. 1997. The beles or cactus pear (*Opuntia ficus-indica*) in Tigray, Ethiopia. *Journal of the Professional Association for Cactus Development*. 2: 130-141.
- Campana, M. G., Robles Garcia, N. M., and Tuross, N. 2015. America's red gold: multiple lineages of cultivated cochineal in Mexico. *Ecology and Evolution*. 5(3): 607–617. <https://doi.org/10.1002/ece3.1398>.
- Cervantes–Herrera, J., Gallegos–Vázquez, C., Reyes–Agüero, J. A., Fernández–Montes, R., Mondragón–Jacobó, C., Martínez, J. C., and Luna–Vázquez, J. 2006. Mexican cultivars of *O. ficus indica* (L.) Mill. with economic importance. *Acta Horticulturae*. 728: 29-33.
- Christenhusz, M. J., and Byng, J. W. 2016. The number of known plants species in the world and its annual increase. *Phytotaxa*. 261(3): 201-217. <https://doi.org/10.11646/phytotaxa.261.3.1>.
- CNVV. Catálogo Nacional de Variedades Vegetales. 2020. Servicio Nacional de Inspección y Certificación de Semillas. Edición: Anual (octubre 2020) Núm. 17. [https://www.gob.mx/cms/uploads/attachment/file/606027/CNVV\\_2020\\_OK.pdf](https://www.gob.mx/cms/uploads/attachment/file/606027/CNVV_2020_OK.pdf)
- Contreras-Padilla, M., Perez-Torrero, E., Hernández-Urbiola, M. I., Hernández-Quevedo, G., del Real, A., Rivera-Muñoz, E. M., and Rodríguez-García, M. E. 2011. Evaluation of oxalates and calcium in nopal pads (*Opuntia ficus-indica* var. redonda) at different maturity stages. *Journal of Food Composition and Analysis*. 24(1): 38-43. <https://doi.org/10.1016/j.jfca.2010.03.028>.
- da Silva, M. G. S., Dubeux, Jr J. C. B., Cortes, L. C. D. S. L., Mota, D. L., da Silva, L. L. S., dos Santos, M. V. F., and dos Santos, D. C. 2010. Anatomy of different forage cacti with contrasting insect resistance. *Journal of Arid Environments*. 74(6): 718-722. <https://doi.org/10.1016/j.jaridenv.2009.11.003>.
- Felker, P., and Inglese, P. 2003. Short-term and long-term research needs for *Opuntia ficus-indica* (L.) Mill. utilization in arid areas. *Journal of the Professional Association for Cactus Development*. 5: 131-151.
- Gebretsadik, G., Animut G., and Tegegne, F. 2013. Assessment of the potential of cactus pear (*Opuntia ficus indica*) as livestock feed in Northern Ethiopia. *Livestock Research for Rural Development*. 25(2): 1-10.
- Guevara-Figueroa, T., Jiménez-Islas, H., Reyes-Escogido, M. L., Mortensen, A. G., Laursen, B. B., Lin, L. W., and de la Rosa, A. P. B. 2010. Proximate composition, phenolic acids, and

- flavonoids characterization of commercial and wild nopal (*Opuntia* spp.). *Journal of Food Composition and Analysis*. 23(6): 525-532. <https://doi.org/10.1016/j.jfca.2009.12.003>.
- Griffith, M. P. 2004. The origins of an important cactus crop, *Opuntia ficus-indica* (Cactaceae): new molecular evidence. *American Journal of Botany*. 91(11): 1915-1921. <https://doi.org/10.3732/ajb.91.11.1915>.
- Gusqui Mata, R. C. 2013. Eficacia de los métodos de infestación y el número óptimo de hembras madre por cladodio de tuna (*Opuntia ficus-indica*), para la producción de grana cochinilla (*Dactylopius coccus* Costa), bajo invernadero en la comunidad Chingazo Alto del Cantón Guano, Provincia de Chimborazo (B.S. thesis). Escuela Superior Politécnica de Chimborazo, Ecuador. <http://dspace.esPOCH.edu.ec/handle/123456789/2232>.
- Hoffmann, J. H., Impson, F. A. C., and Volchansky, C. R. 2002. Biological control of cactus weeds: implications of hybridization between control agent biotypes. *Journal of Applied Ecology*. 39(6): 900-908. <https://doi.org/10.1046/j.1365-2664.2002.00766.x>.
- Huang, Y., Sharma, H. C., and Dhillon, M. K. 2013. Bridging conventional and molecular genetics of sorghum insect resistance. pp. 367-389. In Paterson, A.H. *Genomics of the Saccharinae. Plant Genetics and Genomics: Crops and Models*. Springer, New York, NY.
- IIEG, 2021. Ojuelos de Jalisco, diagnóstico del municipio. Instituto de Información Estadística y Geográfica de Jalisco. pp.8-9.
- INEGI, 2022. Cuentame de México. <https://cuentame.inegi.org.mx/default.aspx>
- Ishizaki, H., and Suzuki, A. 2002. The brain secretory peptides that control moulting and metamorphosis of the silkworm, *Bombyx mori*. *International Journal of Developmental Biology*. 38(2): 301-310.
- Jayaraj, S., and Uthamassamy, S. 1990. Aspects of insect resistance in crop plants. *Proceedings: Animal Sciences*. 99(3): 211-224. <https://doi.org/10.1007/BF03186391>.
- Kiesling, R., and Metzger, D. 2017. *Origin and taxonomy of Opuntia ficus-indica*. pp. 13-19 In: Inglese P., Mondragón C., Nefzaoui A., and Sáenz C. (eds.). Crop ecology, cultivation, and uses of cactus pear. Food and Agriculture Organization of the United Nations. <http://www.fao.org/3/a-i7628e.pdf>.
- Kobayashi, M., and Yamazaki, M. 1974. Bioassay for brain hormone. pp 113–120. In: Burdette W.J. (Ed.). *Invertebrate Endocrinology and Hormonal Heterophyly*. Springer, Berlin, Heidelberg. [https://doi.org/10.1007/978-3-642-65769-6\\_8](https://doi.org/10.1007/978-3-642-65769-6_8).
- Krishnakumar, A., and Schneiderman, H.A. 1968. Chemical control of moulting in arthropods. *Nature*. 220(5167): 601-603. <https://doi.org/10.1038/220601a0>.
- Lemma, H., Haile M., Fetene, M., and Belay, T. 2010. Cactus in southern Tigray: Current status, potential use, utilization and threats. pp. 135-156. Nefzaoui, A., P. Inglese, and T. Belay (Eds.). *Improved utilization of cactus pear for food, feed, soil and water conservation and*

- other products in Africa. Proceedings of International Workshopo, Mekelle (Ethiopia), 19-21 october, 2009. 224 p..
- López-Palacios, C., and Peña-Valdivia, C. B. 2020. Screening of secondary metabolites in cladodes to further decode the domestication process in the genus *Opuntia* (Cactaceae). *Planta*. 251(4): 1-14. <https://doi.org/10.1007/s00425-020-03371-9>.
- Lucena, L., Leite M., Simões V., Izidro J., and Simplício, J. 2019. Cladode shape analysis of *Nopalea cochenillifera* (forage cactus giant sweet clone) using anatomical landmarks. *Cuban Journal of Agricultural Science*. 53(3): 331-338.
- Matos, T. K. B., Guedes, J. A., Alves Filho, E. G., Luz, L. R., Lopes, G. S., Nascimento, R. F. D., and Zocolo, G. J. 2021. Integrated UPLC-HRMS, chemometric tools, and metabolomic analysis of forage palm (*Opuntia* spp. and *Nopalea* spp.) to define biomarkers associated with non-susceptibility to carmine cochineal (*Dactylopius opuntiae*). *Journal of the Brazilian Chemical Society*. (32): 1617-1627.
- Mazzeo, G., Nucifora S., Russo A., and Suma, P. 2019. *Dactylopius opuntiae*, a new prickly pear cactus pest in the Mediterranean: an overview. *Entomologia Experimentalis et Applicata* 167(1): 59-72. <https://doi.org/10.1111/eea.12756>.
- Méndez-Gallegos, S.J., Vera-Graziano J., Bravo-Mojica H., and López-Collado, J. 1993. Tasas de supervivencia y reproducción de la grana cochinilla *Dactylopius coccus* (Homoptera: Dactylopidae) a diferentes temperaturas. *Agrociencia Serie Protección Vegetal*. 4(1): 7-22.
- Méndez-Gallegos, S., Tarango-Arámbula L.A., Carnero A., Tiberi R., and Díaz-Gómez, O. 2010. Growth parameters of cochineal *Dactylopius coccus* Costa reared in five cactus pear cultivars *Opuntia ficus-indica*. *Agrociencia*. 44(2): 225-234.
- Molano-Flores, B. 2001. Herbivory and calcium concentrations affect calcium oxalate crystal formation in leaves of sida (Malvaceae). *Annals of Botany*. 88(3): 387-391. <https://doi.org/10.1006/anbo.2001.1492>.
- Morgan, E., and Poole, C. 1977. Chemical control of insect moulting. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*. 57(2): 99-109. [https://doi.org/10.1016/0305-0491\(77\)90157-2](https://doi.org/10.1016/0305-0491(77)90157-2).
- Munhame, J. A., Batista, Â., Monnerat, J. P. I. S., Maciel, M. V., Lopes, L. A., Silva, T. G. P., and Carvalho, F. F. R. 2021. Intake, digestibility, ingestive behavior and performance of goats fed spineless cactus genotypes resistant to carmine cochineal. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 73: 1209-1216.
- Nakata, P. A. 2015. An assessment of engineered calcium oxalate crystal formation on plant growth and development as a step toward evaluating its use to enhance plant defense. *Plos one*. 10(10): 1-15. e0141982. <https://doi.org/10.1371/journal.pone.0141982>.
- Passos da Silva, D. M., Houllou-Kido, L. M., Cordeiro dos Santos, D., Gonçalves Ferreira, R., Felipe dos Santos, V., Melo Ferreira, W., and Silva de Lima, M., Marinho Falcão, H., and de Sena Tabosa, F. 2007. Resistance of *in vitro* grown forage cactus clones to

- Dactylopius opuntiae* (Hemiptera: Dactylopiidae). *Acta Horticulturae*. 811:299-302. <https://doi.org/10.17660/ActaHortic.2009.811.40>.
- Peña-Valdivia, C. B., Luna-Cavazos, M., Carranza-Sabas, J. A., Reyes-Agüero, J. A., and Flores, A. 2008. Morphological characterization of *Opuntia* spp.: a multivariate analysis. *Journal of the Professional Association for Cactus Development*. 10: 1-21.
- Peterson, P. M., Arrieta, Y. H., & Romaschenko, K. (2018). Phylogeny of *Muhlenbergia* subg. *Pseudosporobolus*, including *M. spatha* (Poaceae, Chloridoideae, Cynodonteae, Muhlenbergiinae) now found in Zacatecas, Mexico. *PhytoKeys*, (103), 83.
- Piña, L. 1979. Principales países productores de grana fina y algunos aspectos biológicos sobre la producción de este colorante. *Revista de los Laboratorios Nacionales de Fomento Industrial* 5(3): 14-16.
- Portillo, L., and Viguera, A.L. 2006. A review on the cochineal species in Mexico, hosts and natural enemies. *Act. Hort*. 728: 249-256. <https://doi.org/10.17660/ActaHortic.2006.728.35>.
- Reinert, J. A., Engelke, M. C., and Read, J. C. 2003. Host resistance to insects and mites, a review- A major IPM strategy in turfgrass culture. In: I International Conference on Turfgrass Management and Science for Sports Fields 661. pp. 463-486.
- Reyes-Agüero, J. A., Aguirre-Rivera, J. R., and Hernández, H. M. 2005. Systematic notes and a detailed description of *Opuntia ficus-indica* (L.) Mill. (Cactaceae). *Agrociencia*. 39(4): 395-408.
- Reyes-Agüero, J. A., and Valiente-Banuet, A. 2006. Reproductive biology of *Opuntia*: A review. *Journal of Arid Environments*. 64(4): 549-585. <https://doi.org/10.1016/j.jaridenv.2005.06.018>.
- Sbaghi, M., Bouharroud, R., Boujghagh, M., and Bouhssini, M. E. 2019. Sources de résistance d'*Opuntia* spp. contre la cochenille à carmin, *Dactylopius opuntiae*, au Maroc. *EPPO Bulletin*. 49(3): 585-592. <https://doi.org/10.1111/epp.12606>.
- Sharma, H. C., and Ortiz, R. 2002. Host plant resistance to insects: an eco-friendly approach for pest management and environment conservation. *Journal of Environmental Biology*. 23(2): 111-135.
- Teetes, G. L. (1996). Plant resistance to insects: a fundamental component of IPM. In: *Radcliffe's IPM world textbook*. Radcliffe, E. B., Hutchison, W. D., and Cancelado, R. E. (Eds.) (University of Minnesota: St Paul) Available at <http://ipmworld.umn.edu/chapters/teetes.htm> [Verified 7 October 2008].
- Torres, J. B., and Giorgi, J. A. 2018. Management of the false carmine cochineal *Dactylopius opuntiae* (Cockerell): perspective from Pernambuco state, Brazil. *Phytoparasitica*. 46(3): 331-340. <https://doi.org/10.1007/s12600-018-0664-8>.
- Torres-Ponce, R.L., Morales-Corral, D., Ballinas-Casarrubias, M. D. L., and Nevárez-Moorillón, G. V. 2015. Nopal: semi-desert plant with applications in pharmaceuticals, food



- and animal nutrition. *Revista Mexicana de Ciencias Agrícolas*. 6(5): 1129-1142.
- Tovar, A., Pando-Moreno, M., and Garza, C. 2005. Evaluation of three varieties of *Opuntia ficus-indica* (L.) Miller as hosts of the cochineal insect *Dactylopius coccus* Costa (Homoptera: Dactylopiidae) in a semiarid area of northeastern Mexico. *Economic Botany*. 59(1): 3-7.
- Tovar-Puente, A., and Pando-Moreno, M. 2010. Factores físicos y químicos del nopalque inciden en la producción de grana fina (*Dactylopius coccus* Costa) (Homoptera: Dactylopiidae). pp 113-122. *In: Conocimiento y Aprovechamiento de la Grana Cochinilla*. Guadalajara, México. Universidad de Guadalajara. 228 p.
- Valadez-Moctezuma, E., Samah, S., and Luna-Paez, A. 2015. Genetic diversity of *Opuntia* spp. varieties assessed by classical marker tools (RAPD and ISSR). *Plant systematics and evolution*. 301(2): 737-747. <https://doi.org/10.1007/s00606-014-1112->.
- Van Dam, A. R., and May, P. B. 2012. A new species of *Dactylopius coccus* Costa (*Dactylopius gracilipilus* sp. nov.) (Hemiptera: Coccoidea: Dactylopiidae) from the Chihuahuan desert, Texas, USA. *Zootaxa*. 3573(1): 33-39.
- Van Dam, A. R., Portillo, L., Jeri, C. A., and May, P. B. 2015. Range wide phylogeography of *Dactylopius coccus* (Hemiptera:Dactylopiidae). *Annals of the Entomological Society of America*. 108(3): 299-310.
- Veelaert, D., Schoofs L., and De Loof, A. 1998. Peptidergic control of the corpus cardiacum-corpora allata complex of locusts. *International review of cytology*. 182: 249-302.
- Vigueras, A. L., and Portillo, L. 2014. Control de cochinilla Silvestre y Cría de Grana Cochinilla. Consejo Estatal de Ciencia y Tecnología del Estado de Jalisco. 66 p.
- Volchansky, C. R., Hoffmann, J. H., and Zimmermann, H. G. 1999. Host-plant affinities of two biotypes of *Dactylopius opuntiae* (Homoptera: Dactylopiidae): enhanced prospects for biological control of *Opuntia stricta* (Cactaceae) in South Africa. *Journal of Applied Ecology*. 36(1): 85-91.
- War, A. R., Buhroo, A. A., Hussain, B., Ahmad, T., Nair, R. M., and Sharma, H.C. 2020. Plant defense and insect adaptation with reference to secondary metabolites. *Co-Evolution of Secondary Metabolites*. 795-822. [https://doi.org/10.1007/978-3-319-96397-6\\_60](https://doi.org/10.1007/978-3-319-96397-6_60).