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Productive behavior of *Dactylopius coccus* in two confinement cultivars of *Opuntia ficus-indica* (L.) Mill

Zacarías-Alvarado, J. R.[1]**; Tovar-Robles, C. L.**[1]**; Aquino-Pérez, G.**[1]**; Magallanes-Quintanar, R.**[2]**; Méndez-Gallegos, S. de J.**[1*]

[1] Postgrado en Innovación en Manejo de Recursos Naturales, Colegio de Posgraduados, Campus San Luis Potosí, Iturbide #73, CP. 78622, Salinas de Hidalgo, San Luis Potosí, México.

[2] Universidad Autónoma de Zacatecas, Unidad Académica de Ingeniería Eléctrica. Av. Ramón López Velarde, 801, CP. 98064, Zacatecas, Zacatecas, México.

* Correspondence: jmendez@colpos.mx

ABSTRACT

Recently, the commercial breeding of *Dactylopius coccus* in confined environments has been developed not to depend on seasonal production and ensure the supply chain. Previous studies have shown the technical viability of their breeding of *Opuntia* in a repository also called "Nopaloteca". However, considering the genetic diversity of *Opuntia* in each region, it is necessary to evaluate alternative cultivars to maximize the efficiency of that production system. In this research, the evaluated cultivars were Esmeralda and Villanueva (*Opuntia ficus-indica* (L.) Mill.). Four height levels (m) within the "Nopaloteca" (N1: 0.5; N2: 1.0; N3: 1.5 and N4: 2.0) and two harvest indices (pre and post oviposition) were evaluated regarding their effects on the carminic acid concentration (CAC, %) and fresh weight (FW, g) of *D. coccus*. The results showed no significant difference in the CAC of the *D. coccus* colonies in both cultivars (18%); however, the highest FW was reached in the cv. Esmeralda with 6.3 g per cladode ($p \leq 0.05$). The highest CAC was found in the N4 treatment (2.0 m) with 18.6% ($p \leq 0.05$); while the highest average FW values ($p \leq 0.05$) were in the intermediate levels of the N2 (1.0 m) and N3 (1.5 m) treatments, with 6.4 and 6.1 g per cladode, each. The post oviposition phase harvest of *D. coccus* resulted in a higher CAC concentration (20.4%); meanwhile, in the pre-oviposition phase, 15.6% was harvested ($p \leq 0.05$). Due to the productivity and quality obtained, the Esmeralda cultivar could be used as an alternative host for the intensive breeding of *D. coccus* in confinement, in north-central Mexico.

Keywords: Reared, cochineal insects, quality, hosts, cactus pear.

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INTRODUCTION

One of the trends of consumers worldwide is the demand for natural, safe, and sustainable products, which has shown a greater preference towards natural colorants (Müller-Maatsch *et al.*, 2018), among which those obtained from the carmine cochineal *Dactylopius coccus* stand out. The females of the 11 species of *Dactylopius*, so far recognized, are characterized by containing a hydroxyanthraquinone linked to a glucose unit, called carminic acid (CA), mainly used in food, cosmetics, pharmaceuticals, and textiles (Cooksey, 2018), as an alternative to some synthetic dyes (González *et al.*, 2010). Both, the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) have allowed using cochineal extracts and carmines in food products (Rather *et al.*, 2020).

Derived from the above, *D. coccus* is one of the few insects domesticated since pre-Hispanic times by American cultures (Costa-Neto, 2015). It is currently breeded in several countries for commercial purposes. At the international level, its intensive breeding has been characterized by unarmed variants, locally or regionally available; although derived from this plant-insect interaction, differences in their quality and yield are generated. Campana *et al.* (2015) pointed out that *D. coccus* can survive in a wide range of host species, to our knowledge, there is little research where its productive behavior is assessed in different cultivars. For example, in southern Peru, the Amarilla, Blanca, and Morada cultivars are used (Anculle-Arenas *et al.*, 2017), although no evidence of their yield and quality is reported. In Mexico, alternative cultivars of fruit, forage, or horticultural importance have been evaluated to generate greater added value to be integrated into the value chain. Previous research indicates that the host used can influence both the development (Tovar *et al.*, 2005; Méndez-Gallegos *et al.*, 2010; Tovar and Moreno, 2010) and the quality of *D. coccus* (Coronado-Flores *et al.*, 2013). In this regard, Chávez-Moreno *et al.* (2011) recorded that in Mexico the predominant host is *Opuntia ficus-indica* (L.). Mill.; the cv Atlixco among those that stand out (Flores-Alatorre *et al.*, 2014) in the center and south of the country, and the cv Villanueva (Coronado-Flores *et al.*, 2013), mainly used in the north-central of Mexico.

Given the currently implemented measures to ensure the safety of the insects and their derivatives for human consumption, mass-rearing systems under adequate sanitary conditions are required (Costa-Neto, 2015), to reduce the dependence on their recollection, or its production seasonality, as is the case of Peru, the world's leading producer of *D. coccus*. There, the agroclimatic conditions allow its cultivation in open-sky throughout the year (Anculle-Arenas *et al.*, 2017). However, in other countries, where they are not available, such as Mexico, intensive breeding has been used in confined systems such as the "Nopalotecas" (Campos-Figueroa and Llanderal-Cázares, 2003; Alonso *et al.*, 2013; Hernández *et al.*, 2017; Zacarías-Alvarado *et al.*, 2020), which ensures its quality, safety, and supply chain. Except for Alonso *et al.* (2013), in all these studies, only performance has been assessed based on the number of females, fresh weight, or dry weight per cladode. However, the final quality of the insect has not been evaluated, regard the carminic acid concentration (CAC) at the different levels of the "Nopaloteca".

Considering that the final price of this product is determined by the CAC, the methods and techniques that favor a higher CAC are required; therefore, the objective of this research was to determine the concentration of carminic acid (CAC, %) and the fresh weight (FW, g) of *D. coccus* in two *Opuntia ficus-indica* (L.) Mill., cultivars, collected in the pre and post oviposition phases in a confined "Nopaloteca".

MATERIALS AND METHODS

Study site

The experimental work was carried-out under semi-controlled conditions in a glass greenhouse, north-south oriented, from November 28, 2018, to May 8, 2019, at the facilities of the Colegio de Postgraduados, Campus San Luis Potosí, located at 22° 37’ 55.66” N, 101° 42’ 42.96” W and 2078 m altitude.

Characteristics of the breeding system used

The structure of the “Nopaloteca” was built with wood with a of 3×3 m (9 m^2) dimension; this was divided into four levels with a 0.50 m separation (Level 1=0.5 m, Level 2=1.0 m, Level 3=1.5 m and Level 4=2.0 m). This setting corresponds to the commonly used levels in the “Nopaloteca” production system in Mexico. Five horizontal lines of 12-gauge galvanized wire were included at each level, at a 0.20 m distance. The cladodes were suspended from the base of the cladode using a wire hook of the same gauge.

Infestation process

The used original seed of *D. coccus* was acquired from the GranaZac S.P.R.R.L. company, located at Villanueva, state of Zacatecas, Mexico. To carry out the artificial propagation of *D. coccus*, initially, the cladodes were carefully cleaned with a brush, to reduce predators and competitor’s incidence. Subsequently, these were horizontally placed on the floor and two sieves (40×40 cm) were placed on them, each containing 200 g of gravid females. After 48 h, the infested cladodes were removed and installed in the corresponding levels and rows in the “Nopaloteca”.

Experimental procedure

In order to determine if the cultivar used as a food substrate and the height levels within the “Nopaloteca” influence the carminic acid concentration (CAC, %) and the fresh weight (FW, g) of *D. coccus*, 120 whole cladodes were used, between 8 and 12 months old, from the Villanueva (60 cladodes) and Esmeralda (60 cladodes) cultivars, both *Opuntia ficus-indica* (L.) Mill.

In order to determine the biological stage for collecting that allows to obtain a higher CAC (%) and FW (g) of *D. coccus* two harvest indices were evaluated: pre and post oviposition. For the first phase, 60 cladodes from both cultivars were harvested from each row and level at 112 d after the infestation (prior to the egg deposition). In the second, the females of the remaining cladodes were collected at each level and line (60 cladodes in total). In this case, in particular, as soon as more than 50% of the females finished their reproduction was considered as a time index (169 d).

During the development cycle, the maximum, average and minimum temperature (°C) (TMAX, TPROM, TMIN) and the maximum, average and minimum relative humidity (%) (HRMAX, HRPROM, HRMIN) were recorded hourly using a HOBO Pro v2 temp / RH onset sensor, USA®, located at the center of each level. Also, illumination (lux) was quantified, once a week (8:00 a.m., 2:00 p.m. and 5:00 p.m.), at each level, as well as outside the greenhouse, with an EXTECH Instruments photometer (Nashua®, USA). The obtained values throughout the insect development cycle were added to obtain the final values. The exterior light was considered as the total illumination received (% IL), from which the proportion (%) of total illumination that affected each level (ILTOTAL) was acquired.

Carminic acid concentration and fresh weight of *D. coccus* assessment

The dry bodies of the females from the cladodes of both cultivars located in each level and developmental phase under study were pooled in a composite sample. The CAC (%) was determined following the methods by Briseño-Garzón and Llanderal (2008) using a GENESYS 10S VIS Thermo Scientific® spectrophotometer at a 494 nm absorbance. The boiling time established in the mentioned above methodology was increased from 10 to 15 min because it was previously verified that the CAC is maximized in laboratory conditions in which the procedure was carried out. The evaluations were made in triplicate and 10 readings were considered for each sample. The CAC was acquired by substituting the absorbance values in the equation generated from a calibration curve with 90% carminic acid (SIGMA®).

To determine the FW (g) of both evaluated phases, pre and post oviposition, after harvesting the females of the cladodes of both cultivars and each level, the weight of each sample was recorded in an analytical balance Explorer Pro (NJ, USA®) d=1/0.1 mg. Subsequently, the product was placed in a Petri dish and placed in a Maytag® freezer at −10 °C, for 3 days, for its sacrifice. Afterward, the samples were subjected to environmental drying, inside the greenhouse, for 10 d. Finally, each sample was pulverized in a Wiley Mini Mill® (3383-L10 115 V, 60 Hz, Thomas Scientific Swedesboro, NJ 08085-0099-U.S.A.) Reducing it to particles of 40 mesh, for the CAC assesing.

Statistical analysis

A completely randomized design was used, with 15 replicates consisting of a cladode as an experimental unit. To determine the statistical difference among means, the Tukey test was used ($p \leq 0.05$). Also, a simple correlation analysis (Pearson's r correlation coefficient) was carried out between the climatic variables and the evaluated response parameters. For the statistical processing the R Version 3.5.1 software (R Core Team, 2018) was used.

RESULTS AND DISCUSSION

Carminic acid concentration in *D. coccus*

The carminic acid concentration (CAC, %) was statistically influenced by the height at which the level was located within the "Nopaloteca" (gl=3; Pr>F=0.001) and by the developmental phase in which the insects were (gl=1, Pr>F=0.001). The magnitude of the response due to the cultivar effect was reduced since they generated similar values of 18.0% (gl=1, Pr>F=0.9464).

In this research, the CAC in *D. coccus* consistently increased, as the height of the level at which the cladodes were located raised with respect to the ground level. For example, 1% increase in the CAC was registered, when the level height increased from 0.5 m (17.6%) to

2 m (18.6%), these values were statistically different, as well as the obtained values in the intermediate levels 2 and 3, located at 1.0 and 1.5 m (Figure 1).

During the crop cycle in which the research was carried out (autumn-winter) the average values of CAC increased, as the height rises from the floor where the level was located. Similar behavior was recorded by Alonso *et al.* (2013) when evaluating three height levels under the same system, since the CAC, also increased from 16.6% at 0.50 m to 17.5% at 1.5 m. Likewise, the average values of CAC obtained in this research were higher than those reported in previous studies such as those reported by Méndez-Gallegos *et al.* (2014), who obtained average values of 11.5%, but in brood chambers and under different photoperiods. On the contrary, Méndez-Gallegos *et al.* (2010) and Coronado-Flores *et al.* (2015) determined average values in the CAC higher than those registered in this work, with 19.1% and 21.5%, respectively. Although in this last study, even though the breeding was developed under the same system, the CAC was not differentiated between levels.

Regard the biological phase in which *D. coccus* females were collected, there was a statistical difference ($p \leq 0.05$) between both evaluated indices. When these were harvested in the post-oviposition phase, the highest CAC was acquired, with average values of 20.5%, while the CAC in the pre-oviposition phase was only 15.7%.

Consistently, in both cultivars and in the four height levels of the evaluated "Nopaloteca", when the females were collected in post-oviposition, it was possible to achieve a 5% CAC. This trend is comparable with that reported by Flores-Alatorre *et al.* (2014) who determined a higher CAC (23.8%) in the post-reproductive stage. Also, Aldama-Aguilera *et al.* (2005) acquired between 19.4 and 22.9%, when females were collected 15 d after the reproduction onset. Similarly, Tello and Vargas (2015) observed that, in collected females, when 50% of them began oviposition a CAC of 23.6% was achieved.

In this research, *D. coccus* collected in the post-reproductive stage could be classified as of first quality, according to the provisions of ITINTEC (1986); but according to Molero

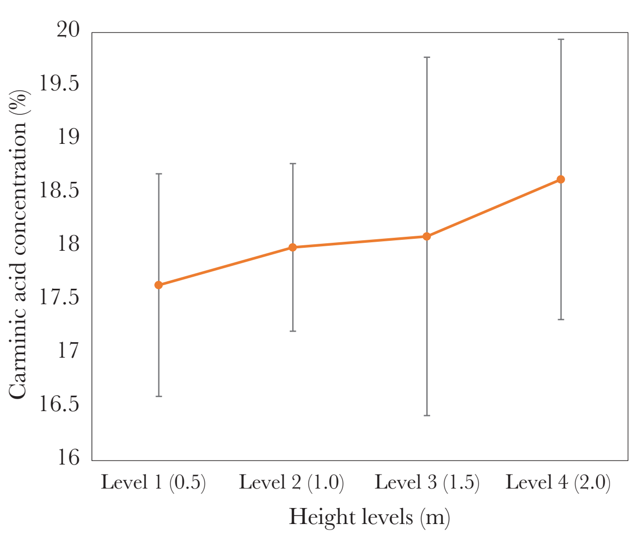

Figure 1. Carminic acid concentration (% ± standard error) in *D. coccus*, reared in a "Nopaloteca" with four height levels.

and Herrera (2013), they would be considered of medium quality (20 to 20.75 %). However, it is necessary to consider that, for those collected females in post oviposition, the biological cycle lasted 169 d after the infestation, 70 to 80% longer than a normal cycle. This implies the convenience of increasing the quality or sacrificing another crop cycle. Nevertheless, the reproduction process can be used to obtain new progeny, carry out another infestation for a successive generation, and generate a larger CAC value.

Dactylopius coccus fresh weight

The fresh weight (FW, g) of *D. coccus* was statistically influenced by the cultivars used as hosts for feeding (gl=1; Pr>F=0.001), by the height of the level within the structure (gl=3; Pr>F=0.001) and by the development phase in which they are collected (gl=1, Pr>F=0.001).

Concerning the cultivars used as food substrates, these showed a different behavior on the FW (g) of *D. coccus*. The statistically higher average values ($p \leq 0.05$) of FW per cladode (6.3 g) were attained obtained in the cv. Esmeralda, while the cladodes on cv. Villanueva produced 0.5 g less (5.8 g) (Figure 2).

The average yield of *D. coccus*, based on the FW (6.07 g) reached in both cultivars in this assessment could be considered high, according to the available literature. For example, Tovar *et al.* (2003) when evaluating three cultivars as a food substrate, recorded only 3.3 g per cladode in the cv. Villanueva. Also, these were superior to those registered by Coronado-Flores *et al.* (2015) using the same breeding system. In the case of the cv. Esmeralda, although no evidence was found to allow its comparison, it is possible to recommend it given its ease of handling, high productivity, and its performance in the FW, which is comparable to that reported in the literature.

The FW (g) of *D. coccus* per cladode depends on the height at which each level was located within the "Nopaloteca" ($p \leq 0.05$). The highest average FW value per cladode was reached at Level 2 (1.0 m), with 6.4 g while the lowest value was achieved in Level 1 (0.5 m)

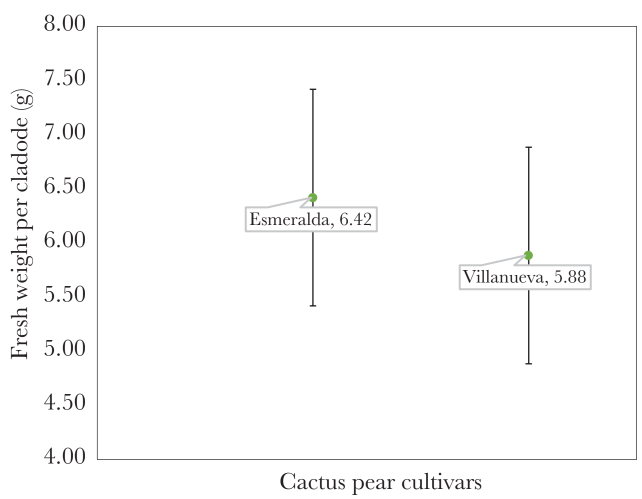

Figure 2. Fresh weight (g ± standard error) of *D. coccus* obtained in two *O. ficus-indica* (L.) Mill. cultivars used as food substrate in a "Nopaloteca".

with only 5.7 g on average per cladode. Likewise, the levels located at the extremes at 0.5 and 2 m in height obtained the lowest average FW values with 5.7 and 5.9 g per cladode, indicating that the intermediate levels provided better characteristics for the development of *D. coccus* (Figure 3).

Contrary to the behavior shown in the CAC, the highest average FW values were reached in the middle part of the "Nopaloteca" with values higher than 6 g per cladode, but the CAC in the females from the first three levels were lower at 18%, which shows a divergence between performance and CAC. The observed trend indicates that there are probable complex interactions between TPROM, HRPROM and ILTOTAL that affect each level, with the growth and development of *D. coccus*, suggesting that it has differential requirements for its optimal development, survival, reproduction, and CAC, such and as stated (Méndez-Gallegos *et al.*, 1993; Méndez-Gallegos *et al.*, 2014; Tello and Vargas, 2015; Zhang, 2017).

In this regard, Campos-Figueroa and Llanderal-Cázares (2003) found no difference in FW between the three levels evaluated, although they attained higher mean FW values than in this research (7.8 g per cladode). Similarly, Aldama-Aguilera and Llanderal-Cázares (2003) attained 8.6 g per cladode; in both studies the cv. Atlixco was used. They were also lower than those reported by Hernández *et al.* (2013) who obtained 9.3 g per cladode, under the same system in Peru, using another cultivar and a different protection structure; however, the values reached in this investigation were higher than those registered by Tovar *et al.* (2005) but using a rearing system in microtunnels.

In relation to the development phase in which the adult females of *D. coccus* are collected, it was observed that the detached females after concluding the reproductive process registered the highest average FW values with 6.3 g, statistically higher ($p \leq 0.05$) than the FW of those females collected before the beginning of reproduction (5.8 g), although the difference was only 0.5 g. Contrary to expected values, the average FW values of *D.*

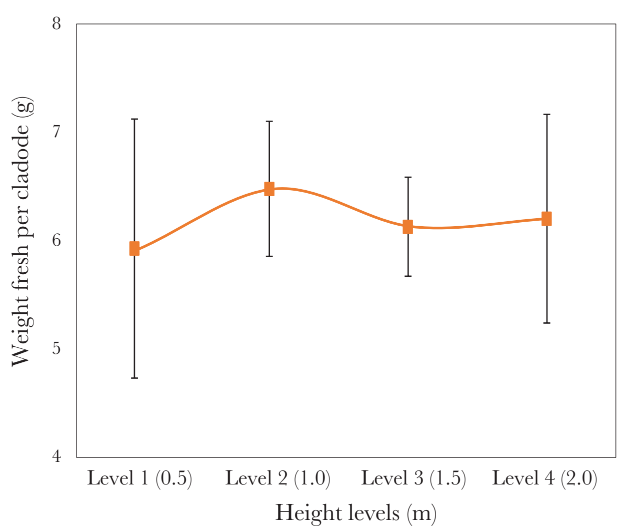

Figure 3. Average fresh weight values (g ± standard error) of *D. coccus*, reared at four levels of height (m) in a "Nopaloteca", under confinement conditions.

coccus in the post-oviposition phase were higher, despite having undergone a reproduction process. This could be associated with the fact that the females in this phase did not die, as there was no lethal factor, but rather continued to increase their volume and reproduce for a longer period of time.

Correlation analysis

When studying the association between the evaluated parameters and the climatic variables studied, a positive correlation was observed between total illumination (ILTOTAL), maximum temperature (TMAX), and average temperature (TPROM) with CAC (AC), suggesting that there is a direct relationship of these variables with the CAC. Likewise, the minimum temperature (TMIN) has a positive effect on the CAC up to a certain limit and then decreases. The FW of the adult females of *D. coccus* was positively correlated with the average temperature (TPROM), minimum temperature (TMIN), and with the total illumination (ILTOTAL) also within certain limits (Figure 4).

CONCLUSIONS

The cultivars used as food substrate, the height of the level in the "Nopaloteca" and the collection phase influenced the yield and the CAC of *D. coccus*. From a productive point of

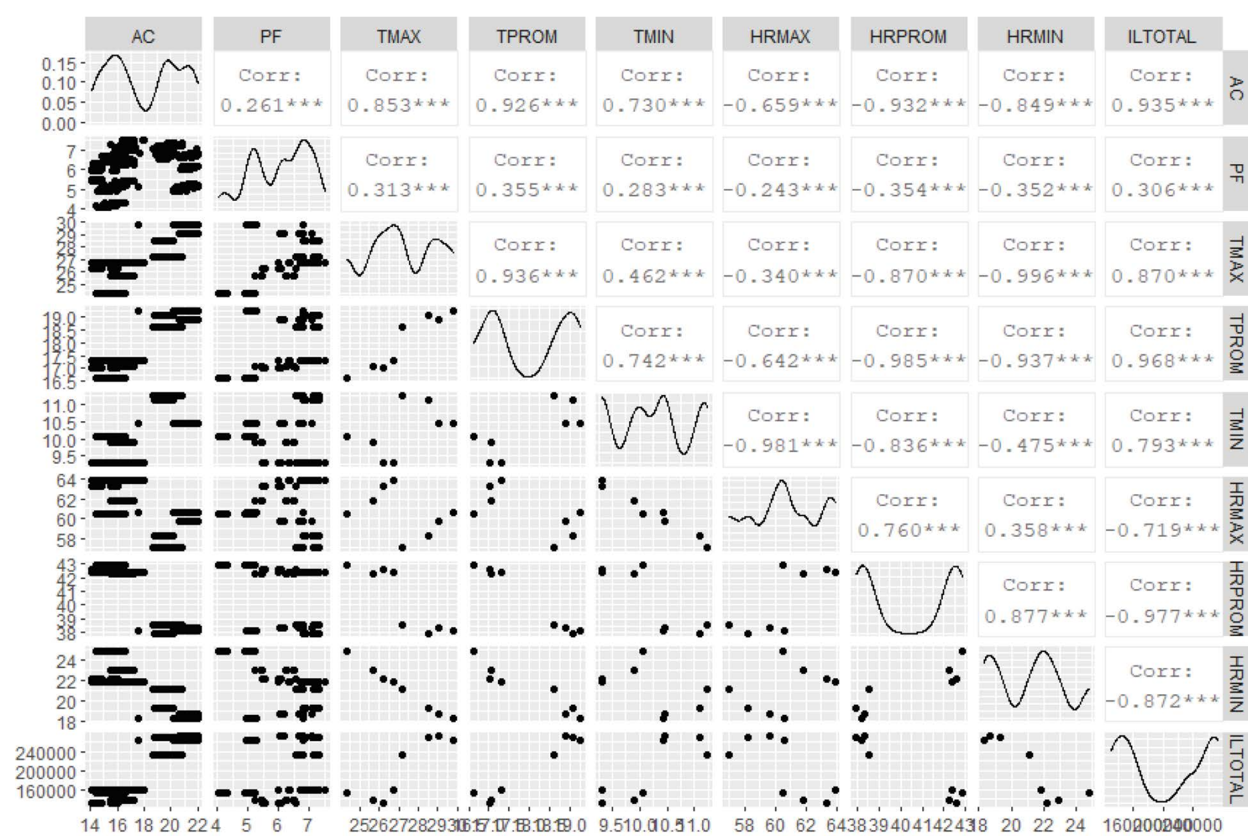

Figure 4. Correlation between fresh weight (FW) and carminic acid (CA) concentration of *D. coccus* and minimum relative humidity (HRM), average relative humidity (HRPROM), maximum relative humidity (HRMAX), minimum temperature (TMIN), average temperature (TPROM), maximum temperature (TMAX) and total illumination (ILTOTAL).

view, the cultivars used could be recommended in the commercial breeding of *D. coccus*, in the absence of other more suitable cultivars. The height of each level tested influences quality and performance, emphasizing that the higher the CAC, the lower the FW of the insect. The harvest, at the end of the reproductive phase, allowed to achieve a higher CAC value. The correlation analysis showed that there are specific limits between the physical factors evaluated and the growth and development of the insect. More detailed studies are needed to define these relationships and thus optimize performance and CAC under "Nopaloteca" conditions.

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