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Positive effect of shade and vermicompost application in the growth of pitahaya (*Hylocereus ocamponis* and *Hylocereus undatus*)

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Abstract. Pitahaya (*Hylocereus* spp.) is a cactus of horticultural importance for its edible fruits. There is little information about the response of these plants to shading and fertilization conditions, which can limit pitahaya growth. The objective of this study was to evaluate the effect of shade (no shade, 35, and 50 % shade) and the application of vermicompost (0 and 3 kg) on plant growth, biochemical response and stem nutrient content in two species of pitahaya (*Hylocereus ocamponis* and *H. undatus*) grown in a greenhouse. The pitahaya cuttings were placed in 10 L pots with 12 kg of a mixture of mountain and leaf soil. The plants were maintained in a glass greenhouse under 12 different treatments, generated by the combination of three factors, species, shade, and fertilization based on vermicompost. The results showed that the total chlorophyll concentration of the stems was 23 % higher in plants grown under 35 % shade than in no shade. *Hylocereus ocamponis* had the highest growth and dry weight accumulation under 35 % shade, whereas *Hylocereus undatus* showed greater adaptability to cultivation under full sunlight and with 35 % shade. *Hylocereus ocamponis* was found to be more sensitive to high levels radiation. The nitrogen, Ca, Mg and Zn contents were 11, 21, 11 and 38 % lower in pitahaya stems grown under 50 % shade than in no shade. In general, vermicompost improved the contents of nutrients in the plants. The cultivation of two pitahaya species with 50 % shade is not recommended, given the lower accumulation of dry weight, growth and because of favors the etiolation.

***Keywords:** chlorophylls, growth, nutrient concentration, phenolic, photoinhibition.*

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Introduction

The pitahayas (dragon fruit) are hemiepiphytic plants of the family Cactaceae, comprising 18 species of the genus *Hylocereus* that grow in temperate, tropical, subtropical, semiarid, and arid regions of the world (García-Rubio *et al*., 2015; Mercado-Silva, 2018). The photosynthetic Crassulacean Acid Metabolism (CAM) allows them to fix CO_2 at night when the evapotranspiration rate is lower, which increases their water use efficiency (Gilman and Edwards, 2020).

Therefore, these species can be cultivated in regions with low available soil moisture, high temperatures and high solar radiation. These characteristics have favored the introduction of pitahaya in regions with various edaphoclimatic profiles. For this reason, it is currently cultivated in several countries. The pitahaya fruits are considered exotic given their shape, flavor, skin color, and, in some species, pulp color, and have a high concentration of the antioxidant pigments betalains (García-Rubio *et al*., 2015; Ibrahim *et al*., 2018; Subandi *et al*., 2018; Hernández-Ramos *et al*., 2020). The pithaya fruits is a source of proteins, fiber, phenolic compounds, flavonoids, phytosterols, triterpenes (Hernández-Ramos *et al*., 2023).

The pitahayas are native of Americas; Mexico has been considered the place of origin of several Cactaceae because it has the greatest diversity of native species (Novoa *et al*., 2015). Furthermore, Mexico has approximately 50 % of the species of the genus *Hylocereus* identified to date (Ramírez-Rodríguez et al., 2020). This plant is found in the wild, in backyard cultivation, and in commercial orchards of 11 of the 14 biogeographic provinces of Mexico. The most widely distributed species are *H. undatus*, *H. purpusii*, *H. ocamponis*, and *H. escuintlensis* (García-Rubio *et al*., 2015). In Mexico, more than 50 % of the territory corresponds to arid and semiarid zones; there, pitahaya production has prospered as a profitable activity and a viable alternative to recover abandoned lands. However, these regions commonly present slightly alkaline soils with high $CaCO_3$ content, which may impede P, N, Fe, B, Mn, Cu, and Zn availability for the plant (Bertrand *et al*., 2003). In addition, pitahaya is commonly grown in open fields without shade in these regions; thus, it is exposed to high solar radiation and high temperatures.

The radiation intensity and insufficient nutrition can limit pitahaya growth and productivity. The intense radiation affects flowering and photosynthesis because it influences the degree of CO_2 assimilation, malic acid accumulation, and gas exchange, thus affecting the growth and productivity of pitahaya plants (Raveh *et al*., 1998; Tomaz de Oliveira *et al*., 2021b). In addition, there is little information about the nutritional management of pitahaya. Consequently, fertilization is based on empirical recommendations or those reported in other countries and considering the doses applied to other Cactaceae. The latter could explain the low yield of pitahaya production in Mexico, 6.35 t ha^{-1} in open field in 2023 (SIAP, 2024), compared to that reported by Mizrahi (2020) and Nguyen (2020) for pitahaya production in Israel (35 t ha^{-1}) and Vietnam (30 to 50 t ha^{-1}) under conditions of shade and fertigation. In addition, it is important to highlight that the production systems of pitahaya vary with production areas, from agroforestry systems or open-pit production in Latin America, to greenhouse production in the USA and in various countries in Europe, where the use of shading nets inside of the greenhouses is common (Trindade *et al*., 2023). In Mexico, official statistics did not report the pitahaya cultivation under shade net or greenhouse conditions (SIAP, 2024). However, the pitahaya grown under shade nets is common in the Mixteca Poblana Region, Mexico, and there is a company that pitahaya cultivation in greenhouse conditions in Zempoala, Hidalgo, Mexico (L. Hernández-Ramos, personal communication, September 26, 2024).

The high levels of solar radiation, temperature and vapor pressure deficit could be reduced under greenhouse and shade net conditions. Therefore, it is important to study the response of pitahaya species to shading in greenhouse and fertilization conditions. The objective of this study was to evaluate the effect of shade (no shade, 35, and 50 % shade) and application of vermicompost (0 and 3 kg) on plant growth, biochemical response and stem nutrient concentration in two species of pitahaya (*Hylocereus ocamponis* and *H. undatus*) grown in a greenhouse.

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Material and Methods

Experimental site

This study was conducted under transparent glass greenhouse conditions at the Universidad Autónoma Chapingo located at 19° 29' 35.7" LN and 98° 53' 7.9" LW, an altitude of 2,256 m, in the period of July 2018 to August 2019. The pitahaya plants were grown in three subplots with different shadings, for which a black high-density polyethylene monofilament shade mesh (35 and 50 %) or no shade mesh was used. The temperature (°C), relative humidity (%), and radiation intensity (luxes) were recorded in each subplot using a HOBO® U12-013 data logger (Burlington, Vermont, USA). The value of radiation intensity (Lux) was multiplied by the conversion factor (Thimijan and Royal 1982) to get photosynthetic photon flux densities (PPFD, µmol m^{-2} s^{-1}).

Plant material and substrates

The plant cuttings of 38 ± 1 cm in length were obtained from the two pitahaya species from Molcaxac, Puebla, Mexico, red pitahaya "solferina" (*Hylocereus ocamponis*), endemic specie of the México, and white pitahaya (*H. undatus*), non-genetically modified genotypes. The plant cuttings were planted in a 10 L black polyethylene bag with approximately 12 kg of a mixture of mountain soil and leaf soil (1:1 ratio, v/v). The rooted cuttings (stems) with a thin and abundant root system over 30 cm long were obtained after two months. All plants were manually watered weekly with clean tap water with a pH of 7.1 and electrical conductivity of 0.42 dS m^{-1}.

The soil used (mixture of mountain soil and leaf soil) had a loamy texture (33.50, 28.00, and 38.50 % of sand, silt and clay, respectively), moderately acid pH (6.20), high organic matter content (7.60 %), and low electrical conductivity (0.30 dS m^{-1}). The nutrient content (mg kg^{-1}) were, N-NO_3 = 11.20 (intermediate), P = 6.90 (low), K = 91.20 (very low), Ca = 796 (moderately low), Fe = 45.10 (high), Mg = 102 (moderately low), Cu = 0. 62 (moderately low), Zn = 1.20 (moderately low), Mn = 2.50 (low), B=0.30 (very low), Na = 31.60 (moderately low), Al = 7.40 (very low), and S = 29.90 (very high). The soil analysis results were interpreted according to the classification of Castellanos *et al*. (2000).

Soil pH

The soil pH in each pot was measured the two weeks (initial pH) and ten months (final pH) after the first application of vermicompost, with a soil potentiometer (pH Tester, Hanna GroLine HI981030, Woonsocket, USA).

Vermicompost application

The vermicompost used was from sheep manure obtained by pitahaya farmers from Molcaxac, Puebla, Mexico, who regularly use it for their crops. The characteristics of the vermicompost were as follows, C/N ratio = 13.90, pH = 8.40, electrical conductivity = 3.10 dS m^{-1}, organic matter = 57.60 %, ash = 42.40 %, moisture = 49.20 %, total N = 2.40 %, P = 0.30 %, K = 0.70 %, Ca = 7.70 %, Mg = 0.70 %, Na = 0.10 %, S = 0.40 %, Fe = 3362 mg kg^{-1}, Cu = 24.40 mg kg^{-1}, Mn = 182 mg kg^{-1}, Zn = 103 mg kg^{-1}, B = 93.20 mg kg^{-1} and organic C = 33.40 %.

The results of the soil and vermicompost analyses were used as a reference to estimate the amount of compost per pot. The following fertilization levels were established, 1) pots with pH 6.20 soil and no vermicompost (control), and 2) pots with pH 6.20 soil and application of 3 kg of vermicompost. The 3 kg of vermicompost was applied fractionally to the surface, 1 kg in October 2018, 1 kg in January 2019, and the rest in May 2019.

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Treatments, design, and experimental unit

The pitahaya plants were set up in a trellis staking system with stainless steel posts and wires. The plants were sown at 0.50 m among each plant and the rows were 1.20 m apart. The experiment was established under a split-split plot experimental design with shade levels, pitahaya species and fertilization as the main plots, subplot, and sub-subplots, respectively, with 12 treatments and four replications (Table 1). The experimental unit consisted of a pot with one pitahaya plant (stem).

Table 1. The treatments applied under a split-split plot experimental design, considering three factors, shade levels, *Hylocereus* species, and fertilization based on vermicompost application.

Shade levels	Species	Fertilization Vermicompost application and initial soil pH	Treatments
No shade	*H. ocamponis*	0 kg - pH 6.20	T_1
No shade	*H. ocamponis*	3 kg - pH 6.20	T_2
No shade	*H. undatus*	0 kg - pH 6.20	T_3
No shade	*H. undatus*	3 kg - pH 6.20	T_4
35 %	*H. ocamponis*	0 kg - pH 6.20	T_5
35 %	*H. ocamponis*	3 kg - pH 6.20	T_6
35 %	*H. undatus*	0 kg - pH 6.20	T_7
35 %	*H. undatus*	3 kg - pH 6.20	T_8
50 %	*H. ocamponis*	0 kg - pH 6.20	T_9
50 %	*H. ocamponis*	3 kg - pH 6.20	T_{10}
50 %	*H. undatus*	0 kg - pH 6.20	T_{11}
50 %	*H. undatus*	3 kg - pH 6.20	T_{12}

Response variables

Twelve months after potting the plants and applying the treatments, the plants were pruned to remove all the main sprouts and stems (aerial parts), leaving only the original cutting. The following variables were measured on the pruned material (stems and sprouts).

Dry weight accumulation. The material removed by pruning (stem and sprouts) was dried in a forced air oven (Binder®, Tuttlingen, Alemania) at 65 ºC until constant weight. Then, the dry weight (g) was determined by using an electronic balance scale (Scout Pro SP2001 Ohaus®, USA).

Stem thickness, length and growth rate. The thickness (cm), total length (cm) and growth rate (cm day^{-1} $plant^{-1}$) of the main stem were registered for each treatment.

Biochemical variables. Chlorophyll a (Cl_a), chlorophyll b (Cl_b), total chlorophyll (Cl_t) and carotenoids (Cart), concentration were measured by taking 500 mg of the macerated stem (fresh samples) that were placed in a vial with a lid, 10 mL of acetone (80 % v/v) were added and stored under dark conditions for 24 h at 6°C. The extract was measured at three wavelengths (470, 647 and 663 nm) in a spectrophotometric (Genesys 10s, Thermo Scientific, USA). The absorbance values (A) were used to calculate total concentrations as described by Lichtenthaler (1987). The equations used were, $Cl_a = 12.25\ A_{663} - 2.79\ A_{647}$; $Cl_b = 21.50\ A_{647} - 5.10\ A_{663}$; $Cl_t = 7.15\ A_{663} + 18.71\ A_{647}$; $Cart = (1000\ A_{470} - 1.82\ Cl_a - 85.02\ Cl_b)/198$. The results were expressed in mg per 100 g of fresh weight ($mg \cdot 100\ g^{-1}$ fresh weight).

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The content of total soluble sugars (TSS) was determined by the anthrone method. The anthrone reagent consisted of 0.40 g of anthrone (Merck) dissolved in 100 mL of concentrated sulfuric. The total soluble sugar was estimated from samples extracted with 80% hot ethanol and determined according to Witham *et al*. (1971). The concentration was calculated using a standard curve of glucose (Sigma-Aldrich, >99.5%). The results were expressed in percentage considering fresh weight (%, f. w.).

The starch content was quantified according to the method described by Vázquez-Martínez *et al*. (2015), with the use of diastase (Merck, Germany). The titratable acidity was evaluated in fresh samples according to the methods established by the Association of Official Analytical Chemists (AOAC, 2005). The results were expressed in percentage considering fresh weight (%, f.w.).

Quantification of total soluble phenolic compounds. The quantification of these metabolites was carried out according to the Folin-Ciocalteu method described by Singleton and Rossi (1965). The absorbance reading was performed at a wavelength of 760 nm. Concentrations were calculated using a standard curve of gallic acid (Sigma-Aldrich). Results were expressed as mg equivalent of gallic acid per 100 g of fresh weight (mg EAG 100 g^{-1} f. w.).

Nutrient analysis. The concentration of total P, K, Ca, Mg, Fe, and Zn were determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) using the equipment model 725-ES (Agilent®, Santa Clara, California, USA), prior to acid digestion (mixture of H_2SO_4: $HClO_4$ (relation 2:1 mL) and 2 mL of H_2O_2 to 30 %) of multiple elements in Digester® (Tecator Kjeltec FOSS, model DT 220, Hoeganaes, Sweden), as referred to by Alcántar-González and Sandoval-Villa (1999). The N content was measured by the micro Kjeldahl method (AOAC, 2005).

Statistical analysis

All data were expressed as the mean ± standard error. A mixed-model analysis of variance (mixed-model ANOVA) was performed considering the split-split plot experimental design, where treatments were considered as a variation factor of fixed effects and replication as a variation factor of random effects. The mean values within the treatments were compared with Tukey's test ($P \leq 0.05$) by using the software SAS 9.2 (SAS Institute, 2008). The student's t-test was applied to identify differences between the initial and final soil pH. The effects of significant factors on the evaluated parameters were adjusted by polynomial regression analysis.

Results and Discussion

Effect of vermicomposting on soil pH

Compared to the control (potted plants with pH 6.20 soil and no vermicompost), the pots with pH 6.20 soil increased their final soil pH in up to 1.70 units after applying 3 kg of vermicompost (Figure 1). This effect was caused by the vermicompost alkaline pH (8.4). Uz and Tavali (2014) stated that, despite the belief that any amendment with organic matter reduces soil pH, several organic fertilizers, such as vermicompost and manures, can be alkaline in nature. Therefore, these fertilizers increase soil pH, and their application at excessive levels could generate salinity problems (Uz and Tavali, 2014). In addition, soil pH impacts nutrient availability and microbial activity, as well as plant growth. The changes in soil pH due to vermicompost application can reduce the availability of P, N and micronutrients to plants (Bertrand *et al.*, 2003).

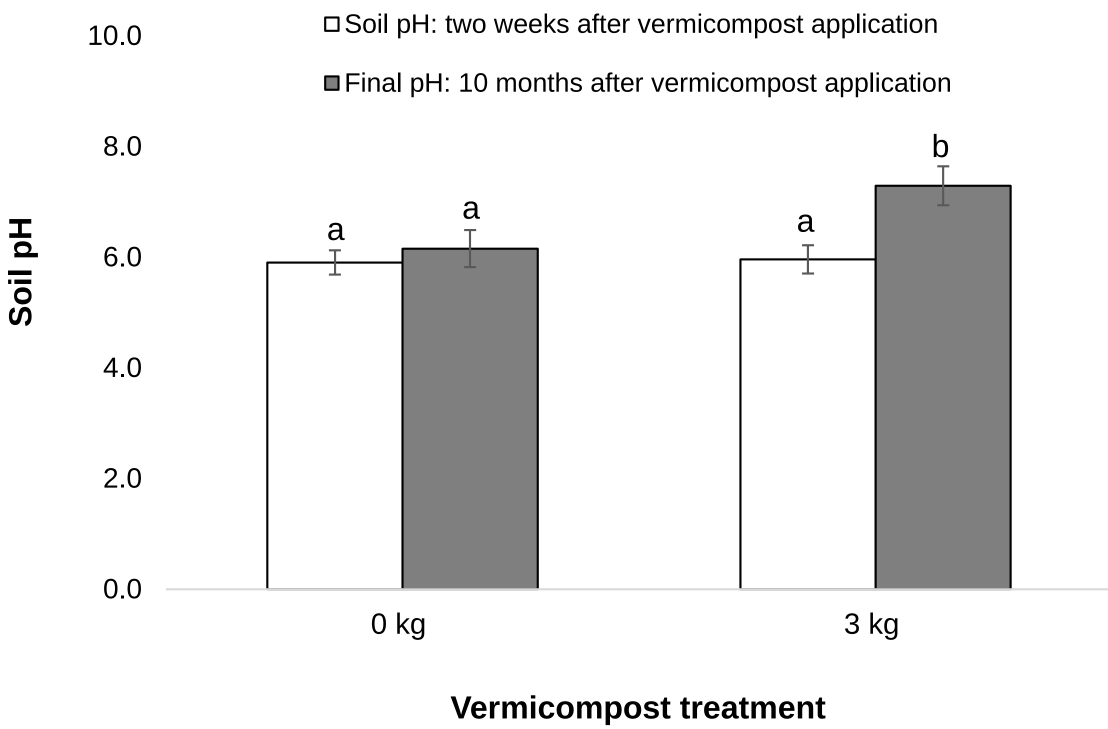

Figure 1. The changes in the soil pH of soil in plant pots with pitahaya (*Hylocereus* spp.), after sub-treatments of fertilization were applied. The values represent means of 24 independent replicates (n= 24) ± standard error. Different letters in the same sub-treatment indicate significant difference by paired sample T-Student test (P ≤ 0.05).

Effects of shading on environmental factors

The average daily values of temperature, relative humidity and photosynthetic photon flux densities (PPFD) under three shade levels (0, 35 y 50 %) are shown in Figure 2. The highest temperature was observed between 12 and 15 hours, associated with an increase of PPDF. In the shade mesh at 50 %, solar radiation (PPFD) and air temperature were reduced by more than 78.74 and 18.18 %, respectively, and relative humidity increased by 43.47 % relative to under full sunlight (no shade, control). According to Ahemd *et al*. (2016), shading improves the distribution of the environmental parameters in greenhouses and to maintain greenhouse air temperature and relative humidity at appropriate levels for plant growth. Also, the selection of the appropriate shade level increase water use efficiency due to decreasing plant transpiration.

Stem thickness, total length, dry weight and growth rate

The stem thickness and total length were affected by the shadow factor (Table 2). The pitahayas grown without shade had 27.0 % (up to 1.5 cm more than the plant grown under shade) greater stem thickness (Figure 3A) than plants with 35 and 50 % shade, because more radiation can influence the cell dimensions of the parenchymal tissue. Herman *et al*. (2001) reported greater dimensions and more parenchyma cell layers in some Opuntia species exposed to intense light.

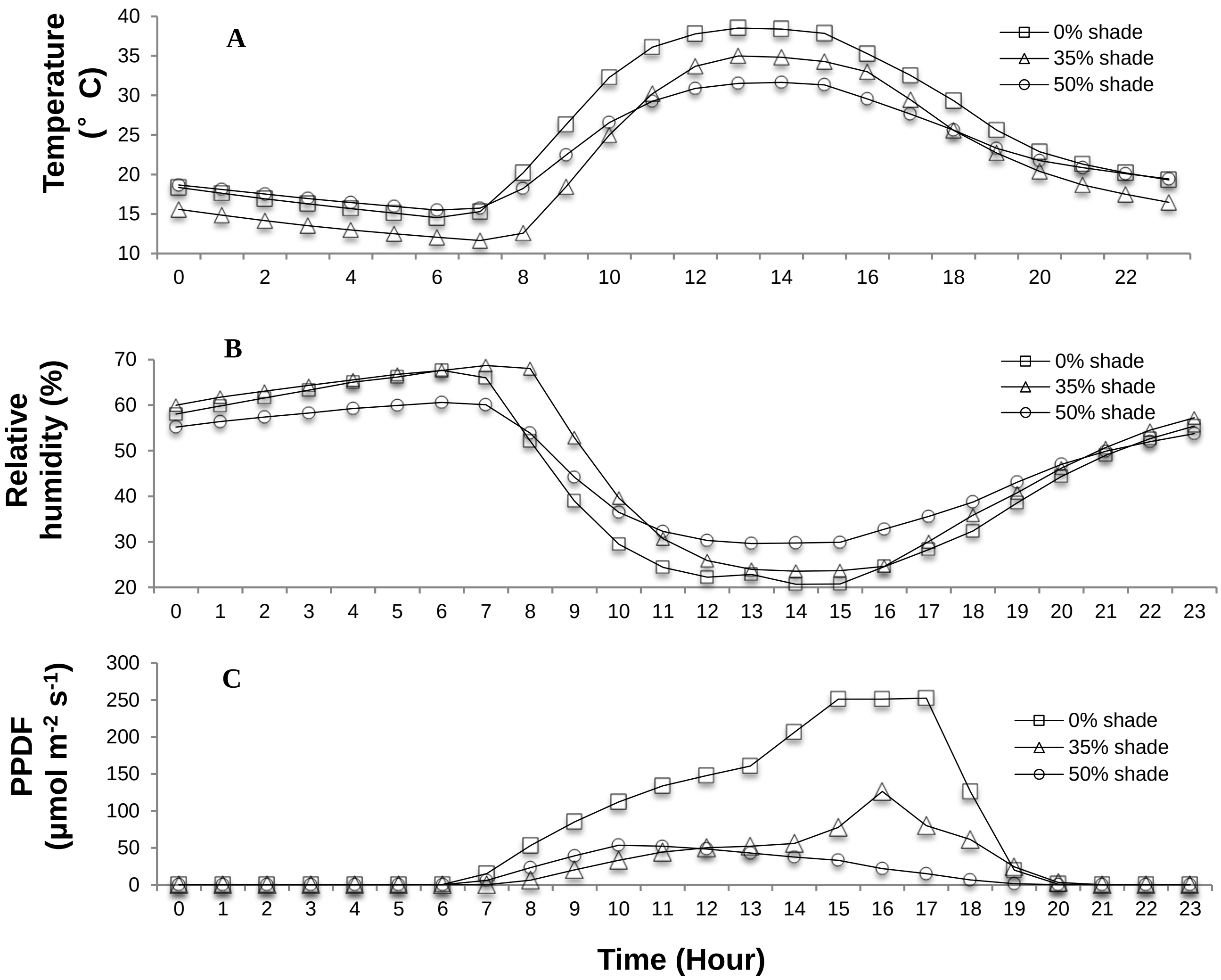

Figure 2. The average daily values of temperature (A), relative humidity (B) and photosynthetic photon flux densities (PPFD) (C) where the pitahaya plants of *H. ocamponis* and *H. undatus* were grown under three shade levels (0, 35 y 50 %) in greenhouse. The data are the average of 11,500 readings made during the period from July 2018 to August 2019.

The total length of stem was higher in plants grown without shade and with 35 % shade (Figure 3B). However, pitahaya stem growth and dry weight accumulation were affected by the species × shade interaction (Figures 3C and 3D), indicating different adaptability of *Hylocereus* species to different levels of shade. Similarly, Raveh *et al.* (1998) found that pitahaya (*H. polyrhizus*) is sensitive to high solar radiation, but the percentage of shade suitable for its cultivation will depend on the degree of sensitivity, which varies among species and genotypes. In the present study, the cultivation of *H. ocamponis* with 35 % shade mesh resulted in greater length and dry weight accumulation, until the 27.20 % greater than plants without shade and 48.30 % greater than plants in 50 % shade, which is related to a higher photosynthetic rate. In contrast, cultivating *H. undatus* without shade achieved greater length and dry weight accumulation, until the 17.60 % greater than that observed in plants grown in 35 % shade and 50.70 % greater than plants in 50 % shade. The plant growth depends on photosynthesis and the accumulation of reserves (starch) due to the greater demand for photoassimilates for growth and development. Therefore, the higher productivity of a crop will be related to a higher photosynthetic rate (Behling *et al*., 2015). Based on this information, the low growth of pitahaya at 50 % shade mesh could be due to a low photosynthetic rate. Concerning pitahaya plants in 50 % shade, the lower accumulation of dry weight, total length and stem thickness should be attributed to insufficient light that impairs growth and favors etiolation in pitahaya, resulting in plants with thin stems and low accumulated biomass (Tomaz de Oliveira *et al*., 2021a). Regarding the cultivation of *H. ocamponis* without shade, this treatment caused low stem growth and dry weight accumulation, and it was statistically like the values found in plants grown with 50 % shade. Therefore, it can be stated that *H. ocamponis* was found to be more sensitive to high levels radiation and better adapted to cultivation with 35 % shade than *H. undatus*.

The dry weight was the only variable affected by fertilization without interaction with another factor (Table 2). Those plants grown on vermicompost applied to soils had greater dry weight accumulation (44.71 g plant^{-1}), with a value of about 25.34 % more than those plants without application (35.67 g plant^{-1}). According to Lahbouki *et al*. (2023), vermicompost is a carbon-rich organic matter and its application serves as a biostimulant by improving the growth, physiological and biochemical responses of cactus under field drought conditions.

Table 2. The F-values correspond to the analysis of variance for the split-split plot experimental design with three levels of shading, two pitahaya species and two fertilization conditions.

Variables	F-values						
	E	S	F	E × S	E × F	S × F	E × S × F
Dry weight	1.19^{NS}	20.41**	7.46*	6.77*	3.04^{NS}	1.03^{NS}	0.81^{NS}
Stem thickness	2.82^{NS}	20.08**	4.38^{NS}	0.20^{NS}	0.09^{NS}	4.36^{NS}	0.44^{NS}
Stem length	1.56^{NS}	7.40**	1.65^{NS}	0.79^{NS}	0.29^{NS}	1.89^{NS}	1.11^{NS}
Growth rate	0.29^{NS}	8.42**	0.52^{NS}	4.29*	0.53^{NS}	0.27^{NS}	0.97^{NS}
Chlorophyll a	1.96^{NS}	14.58**	4.04^{NS}	0.63^{NS}	0.46^{NS}	0.19^{NS}	0.69^{NS}
Chlorophyll b	0.78^{NS}	4.36*	1.17^{NS}	2.55^{NS}	1.77^{NS}	2.29^{NS}	1.99^{NS}
Total chlorophyll	0.71^{NS}	12.02**	3.52^{NS}	0.05^{NS}	0.36^{NS}	0.24^{NS}	0.51^{NS}
Total carotenoids	4.32^{NS}	6.70*	0.35^{NS}	2.15^{NS}	1.79^{NS}	2.24^{NS}	0.83^{NS}
Soluble sugar	0.40^{NS}	6.51*	0.80^{NS}	0.43^{NS}	0.13^{NS}	2.76^{NS}	0.35^{NS}
Total starch	3.10^{NS}	97.03**	1.01^{NS}	2.60^{NS}	1.95^{NS}	1.59^{NS}	1.15^{NS}
Titratable acidity	1.18^{NS}	294.90**	2.43^{NS}	2.74^{NS}	0.16^{NS}	3.59^{NS}	0.09^{NS}
Total phenols	0.14^{NS}	8.64**	0.27^{NS}	0.14^{NS}	0.39^{NS}	3.18^{NS}	0.68^{NS}
Nitrogen (N)	0.42^{NS}	9.79**	0.04^{NS}	0.81^{NS}	2.55^{NS}	0.83^{NS}	1.10^{NS}
Phosphorus (P)	1.88^{NS}	1.80^{NS}	1.81^{NS}	5.56*	1.79^{NS}	10.71**	1.70^{NS}
Potassium (K)	0.41^{NS}	1.56^{NS}	4.66*	0.76^{NS}	0.16^{NS}	2.33^{NS}	0.86^{NS}
Calcium (Ca)	2.11^{NS}	53.35**	0.16^{NS}	2.24^{NS}	0.27^{NS}	2.14^{NS}	2.14^{NS}
Magnesium (Mg)	1.61^{NS}	7.19*	10.06*	1.68^{NS}	1.15^{NS}	1.45^{NS}	1.75^{NS}
Iron (Fe)	35.97**	0.44^{NS}	43.27**	50.30**	3.27^{NS}	16.11**	1.48^{NS}
Zinc (Zn)	13.83**	15.68**	2.14^{NS}	0.33^{NS}	1.54^{NS}	0.29^{NS}	0.04^{NS}

E= species; S= shade; F= fertilization; E × S, E × F, S × F and E × S × F interactions between variation factors; *= indicates that at least one level within the variation factor produced a significant effect in relation to the rest ($P < 0.05$); **= indicates that at least one level within the variation factor produced a significant effect in relation to the rest ($P < 0.01$), ns= indicates a non-significant effect ($P \geq 0.05$) between levels within the variation factor.

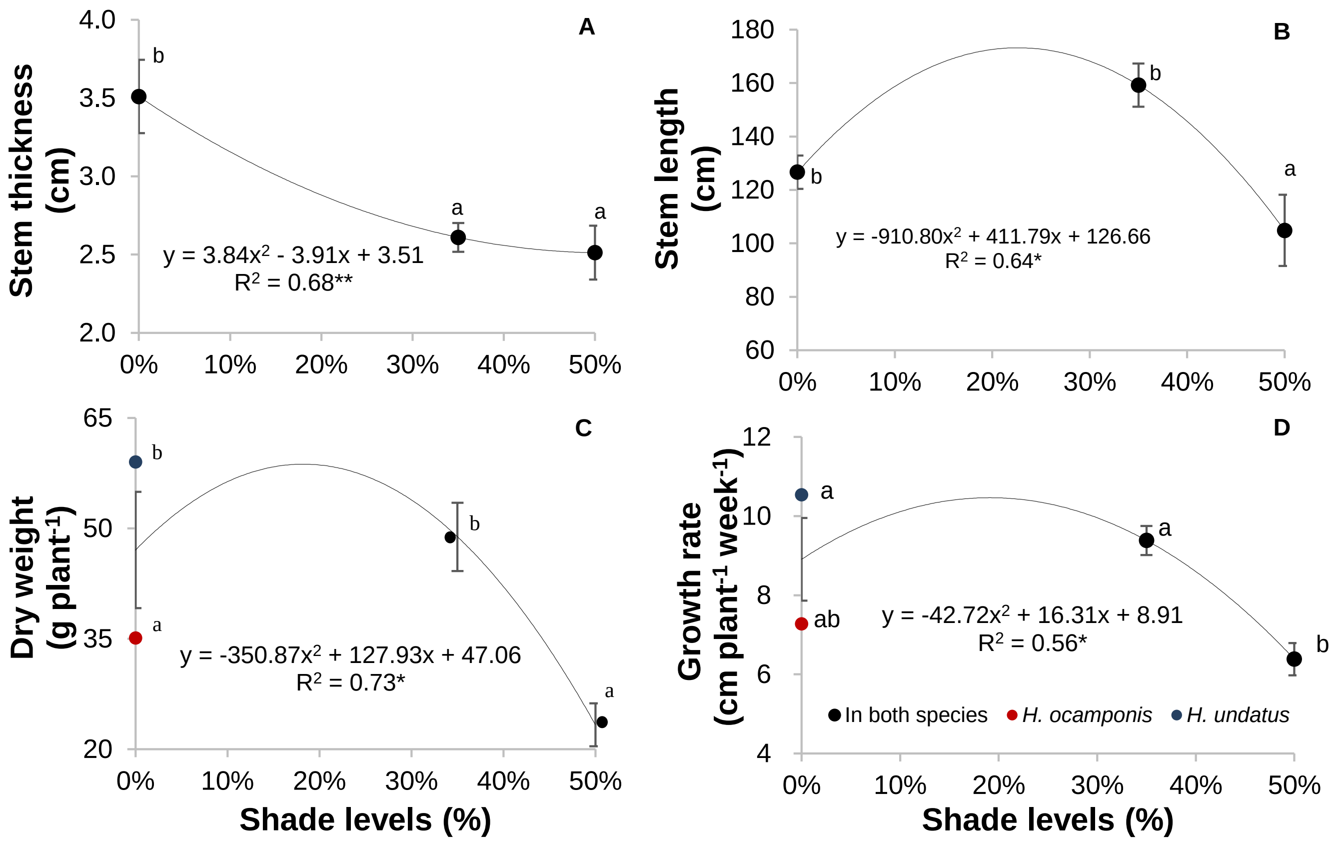

Figure 3. The effect of shading on the stem thickness (A), stem length (B), and effect of the species × shade interaction on dry weight accumulation (C) and growth rate (D) of pitahaya. The data are expressed as mean ± standard error. The values at each shade level represent the means of four independent replicates (n= 4). Significant at * P <0.05; ** P <0.01. Different letters indicate significant difference by Tukey test (P ≤ 0.05).

Biochemical variables

The biochemical variables evaluated were only affected by the shadow factor (Table 2). The concentration of chlorophylls was 9.98 % higher in *H. undatus* compared to *H. ocamponis*. The total chlorophyll and carotenoids concentration in pitahaya stems were affected by the shade (Figures 4A and 4D). The concentration of these pigments was different between plants grown without shade and with 35 % shade. The total chlorophyll and carotenoids concentration in plants grown without shade were 27.9 and 25.10 % lower than that found in pitahayas grown under 35 % shade, respectively. The decrease in total chlorophyll and carotenoids concentration in pitahayas grown without shade suggests photoinhibition, which can destroy photosynthetic pigments (photooxidation) in cases of extreme solar radiation. This result agrees with Raveh *et al*. (1998) and Tomaz de Oliveira *et al*. (2021a), who reported lower chlorophyll concentrations in *H. costaricensis* and *H. polyrhizus* grown without shade, respectively. It also corresponds with the reports of Andrade *et al*. (2006), who indicated that *H. undatus* can suffer photoinhibition when grown at a photosynthetic photon flux density (PFFD) greater than 20 mol m^{-2} day^{-1} (equivalent to 231.50 µmol m^{-2} s^{-1}). This value is close to that found in the present research in plants grown without shade (maximum PFFD of 252.50 µmol m^{-2} s^{-1}). In addition, the low concentration of photosynthetic pigments in plants grown with 50 % shade suggests that the sunlight stimulus is insufficient for chlorophyll synthesis, since this synthesis requires the presence of protochlorophyllide oxidoreductase, a light-dependent enzyme involved in the conversion of protochlorophyllide to chlorophyll (Gabruk and Mysliwa-Kurdziel, 2015).

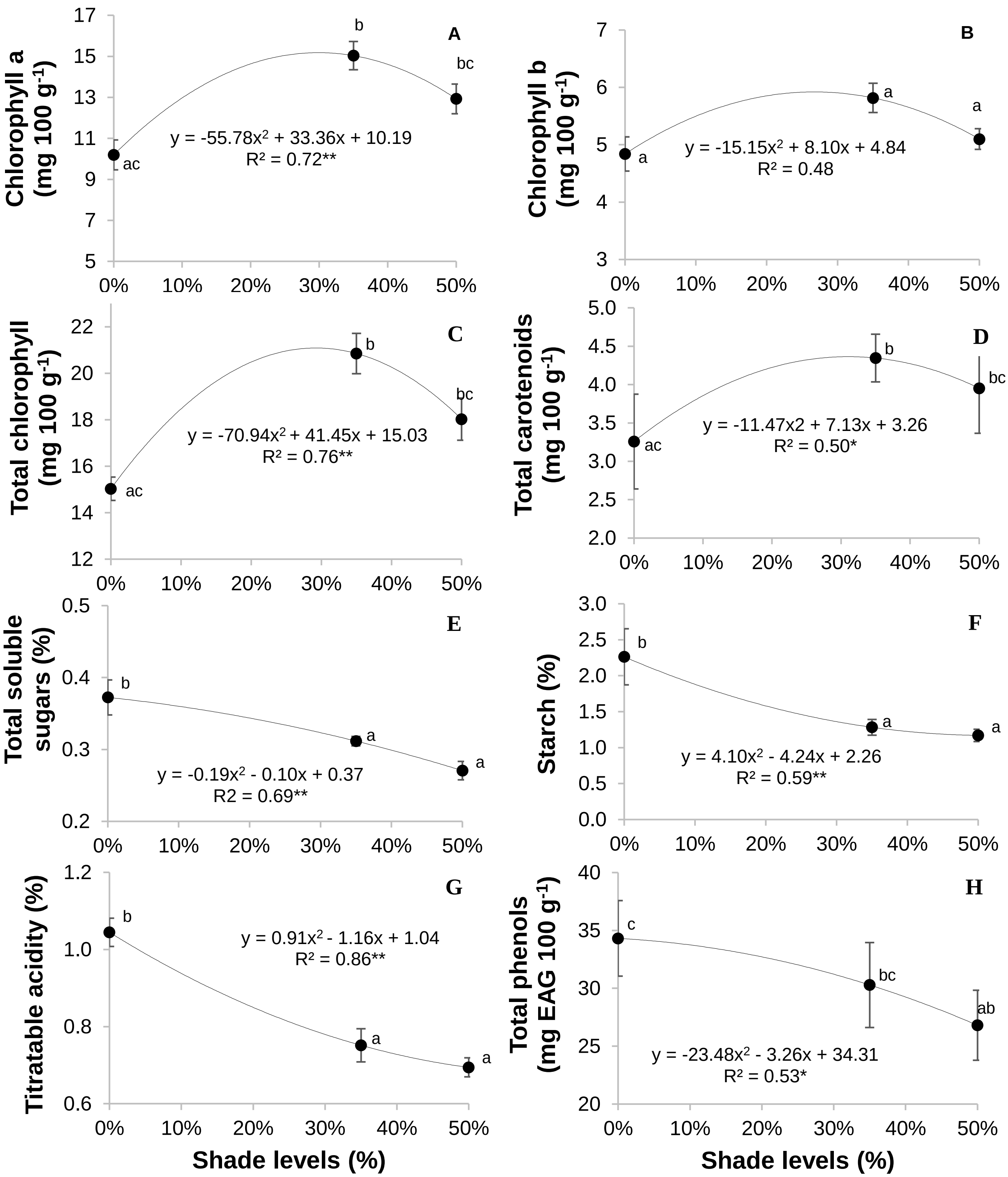

Figure 4. The effect of three shade levels on the chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), carotenoids (D), soluble sugars (E), starch (F), acidity concentrations (G) and total soluble phenols (H) of pitahaya stems (*Hylocereus* spp.). The data are expressed as mean ± standard error. The values at each shade level represent means of four independent replicates (n= 4). Significant at * $P < 0.05$; ** $P < 0.01$. Different letters indicate significant difference by Tukey test ($P \leq 0.05$).

The soluble sugars and starch are universally present in plants as an essential carbon and energy source. The concentrations of these compounds were affected only by the shadow factor (Figures 4E and 4F). The results of the present study showed that the plants grown without shade had the highest concentrations of total soluble sugars (including sucrose) and starch, even when they exhibited similar low chlorophyll concentrations as found in plants with 50 % shade. The above is probably due to light use efficiency in greater light use efficiency without shade, favoring the synthesis of photoassimilates and biomass production. The triose phosphates excess that are not used for sucrose synthesis are commonly used for starch synthesis, which acts as a reserve substance (Rosales *et al*., 2007).

The acidity (expressed as malic acid) in stems decreased linearly as shading levels increased throughout the experiment, showing highest values in plants without shade (Figure 4G). Several species possess an inherent capacity for the induction of CAM depending on environmental conditions (extreme temperature, radiation intensity, photoperiod, drought, plant nutrition, among others), the greatest accumulation of organic acids in vacuoles have been reported in plants under stress conditions (Qiu *et al*., 2023). The highest acidity concentration coincided with the highest concentration of total soluble phenols in plants grown without shade (Figure 4H), because light favors the synthesis of phenolic compounds as a mechanism of photo-oxidative stress protection (Csepregi and Hideg, 2017).

Nutrient analysis

A significant effect of shade (Table 2, $P \leq 0.05$) was only found in the N, Ca, Mg and Zn concentrations in the stem of *H. ocamponis* and *H. undatus*. The N, Ca, Mg and Zn concentrations (Figures 5A and 5D) decreased by 11, 21, 11 and 24 %, respectively, in plants with 50 % shade compared to those without shade and with 35 % shade. The light affects transpiration, which, in turn, can influence nutrient uptake and transport. The N (NO_3^-), Ca, Mg and Zn (as cations or cation complexes with organic acid anions) are transported through the xylem by transpiration-driven water flow a higher mass flow being associated with higher radiation intensity (Sago, 2016; White and Ding, 2023).

Furthermore, a lower photosynthesis rate due to plant shading could generate less available energy (ATP via photophosphorylation) for nutrient uptake (Pandey, 2015). These facts could explain the lower Ca, Mg, N and Zn concentrations in pitahayas grown at lower radiation intensity (50 % shade, Figure 5). Additionally, the upward translocation of nutrients in xylem sap occurs with the transpiration stream, physiological process that is influenced by environmental conditions such as temperature, light and relative humidity (White, 2012). In the present study the plant grown under 50 % shading was kept at lower temperatures and a higher relative humidity compared to other treatments (Figure 2). These environmental conditions could have caused lower transpiration and, consequently, low translocation of these macronutrients to the aerial parts of the plant, indicating low mobility of minerals from the roots (in the present study, mineral concentration was not determined in the original cutting (lower main stem and root by treatment).

On the other hand, Mg is an essential element for plant growth, central atom in the chlorophyll molecule, and a mineral essential as enzyme cofactors (Pandey, 2015). In the present study, there was no correlation relationship between chlorophyll concentrations and total magnesium content of the stems. The Mg concentration was lower in plant grown at 50 % shade (Figure 5C), possibly due to lower translocation of that element from roots to the aerial parts of the plant by lower transpiration because of higher environmental relative humidity compared to other treatments (Figure 2). The plants

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acquire Mg from the soil through diffusion processes and mass flow, this last process is a passive movement that is driven by the transpiration stream (Chen *et al*., 2018). Moreover, the higher concentration of Mg at higher solar radiation levels may be due to its thylakoid-protective functions, which participate in the dissipation of excessive light stress (Marschner and Cakmak, 1989).

Regarding the Zn concentration, this microelement is an important protective agent of the photosynthetic structure (leaves or stems) because high concentrations of this element are associated with a lower risk of damage by photooxidation when plants are exposed to high light intensity (Cakmak, 2000). Therefore, the higher concentration of Zn in plants grown without shade may be a protective mechanism against intense radiation. In addition, a significant effect of significant effect of the species factor was observed for the Zn concentration (Table 2). The *H. ocamponis* plants without shade showed a higher concentration of this micronutrient, 34 % higher than *H. undatus*. According to Cakmak (2000), Zn plays a fundamental role in several critical cell functions, such as gene expression and photosynthetic metabolism. The higher concentration of Zn in *H. ocamponis* grown without shade may be a protection mechanism against intense radiation, given that this species is more sensitive to high solar radiation.

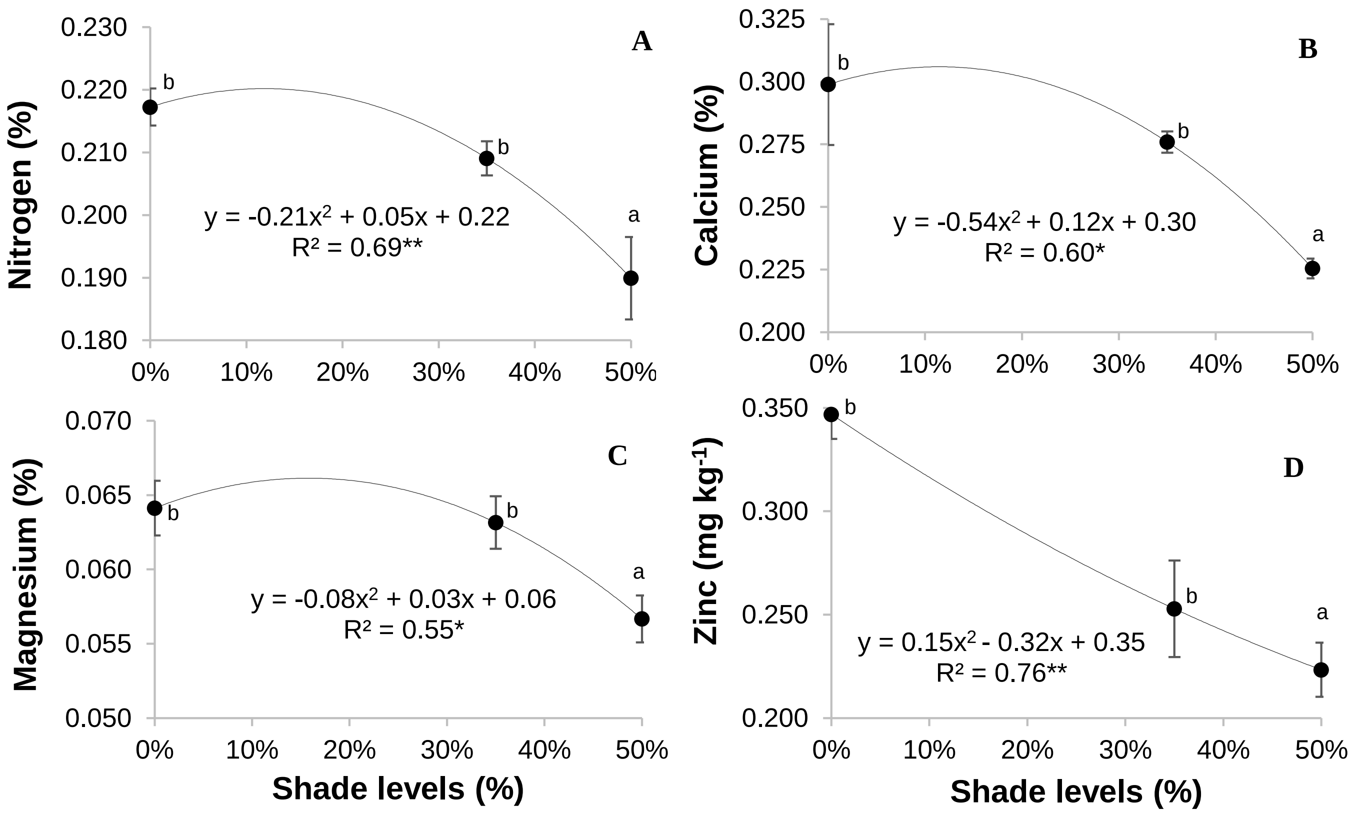

Figure 5. The effect of shading on the nitrogen (A), calcium (B), magnesium (C) and zinc (D) concentration of pitahaya stems (*Hylocereus* spp.). The data are expressed as mean ± standard error. The values at each shade level represent the means of four independent replicates (n= 4). Significant at * $P < 0.05$; ** $P < 0.01$. Different letters indicate significant difference by Tukey test ($P \leq 0.05$).

On the other hand, a different genotypic response was observed in P and Fe absorption among the plants with different shade exposure, since the species × shade interaction was significant (Table 2, P

≤ 0.05). The concentration of P (Table 3) was higher in *H. ocamponis* plants with 35 % shade, 24 % higher than in *H. undatus* plants. Schachtman *et al*. (1998) reported that the different absorption of this element between species could be associated with the presence of a gene encoding a P transporter and the fact that light can regulate root morphology and subsequent P absorption.

The Fe concentration was higher in *H. ocamponis* with 35 % shade, 10 and 11 % higher than in plants grown without shade and with 50 % shade, respectively. On the other hand, *H. undatus* grown in 50 % shade showed a similar Fe concentration to those grown without shade (P ≤ 0.05) but showed 12% more Fe than plants grown with 35 % shade (Table 3). Clark (1983) pointed out that each genotype develops different mechanisms to solubilize and absorb Fe efficiently, such as roots with a higher reduction capacity that produces H^+ ions or Fe^{3+} reducing agents (Krohling *et al.*, 2016).

Table 3. The effect of three shade levels and its interaction with other factors on potassium and iron concentrations of pitahaya stems.

Response variable	Significant Factor	Shadow level 0%	35%	50%
Effect of the shade × species interaction				
	Species:			
P (%)	*H. ocamponis*	0.051 ± 0.009^{ab}	0.053 ± 0.010^{a}	0.051 ± 0.004^{ab}
	H. undatus	0.042 ± 0.010^{c}	0.041 ± 0.004^{c}	0.046 ± 0.008^{bc}
Fe (mg kg^{-1})	*H. ocamponis*	64.860 ± 4.928^{ab}	67.770 ± 7.768^{a}	64.720 ± 2.617^{ab}
	H. undatus	54.230 ± 5.449^{cd}	52.240 ± 4.550^{d}	58.920 ± 2.618^{bc}
Effect of the applied shade × fertilization interaction				
	Fertilization:			
P (%)	0 kg of vermicompost	0.036 ± 0.010^{c}	0.035 ± 0.003^{c}	0.045 ± 0.002^{b}
	3 kg of vermicompost	0.057 ± 0.003^{ab}	0.059 ± 0.010^{a}	0.051 ± 0.002^{ab}
Fe (mg kg^{-1})	0 kg of vermicompost	46.530 ± 3.020^{c}	44.340 ± 2.389^{c}	58.040 ± 3.409^{b}
	3 kg of vermicompost	72.560 ± 2.150^{ab}	75.670 ± 4.738^{a}	65.600 ± 0.550^{ab}

The data are expressed as mean ± standard error. Different letters within each factor and mineral indicate significant difference by Tukey test (P ≤ 0.05).

A significant effect of the shade × fertilization interaction was found in P and Fe concentrations (Tables 2 and 3, P ≤ 0.05). The plants without vermicompost application shown a lower P concentration, 28.50 % less on average than those with vermicompost and soil pH of 6.20. The phosphorus is the macronutrient with the lowest availability in the soil. Since the soil used in this study had a low P level (6.90 mg kg^{-1}), vermicompost application contributed to this element's supply. The P is a vital nutrient for plant growth and productivity because it is involved in many cellular processes, such as the maintenance of membrane structures, synthesis of biomolecules, generation of high-energy molecules, cell division, enzyme activation/deactivation, and carbohydrate metabolism (Malhotra *et al*., 2018).

The lowest concentration of iron (Fe) was registered in crops without shade and with 35 % shade that did not receive vermicompost (Table 3); this concentration was 33 % lower than the other treatments. The Fe has several biochemical functions, such as chlorophyll synthesis, and N assimilation (Li *et al*., 2021). Thus, the plants that grew more (35 % shade and no shade) probably had diluted Fe in the plant due to the mechanisms mentioned. Furthermore, applying vermicompost to all shade levels yielded the Fe concentrations higher than that of the treatments without vermicompost, for one kilogram of vermicompost provided 3,362 mg of Fe.

A significant main effect of fertilization (vermicompost application in soils) was observed by Mg and K concentration ($P \leq 0.05$, Table 2) without interaction. The K and Mg concentrations were different between the plants cultivated with vermicompost (0.246 ± 0.032 % and 0.062 ± 0.004 %, respectively) and the no application treatment (0.200 ± 0.023 % and 0.057 ± 0.005 %, respectively), due to vermicompost was an important source of these nutrients.

Treatment clustering

The cluster analysis identified five main treatment groups (Figure 6) that showed similar effects on growth, chlorophyll concentration, and nutrient concentrations in the pitahaya stems.

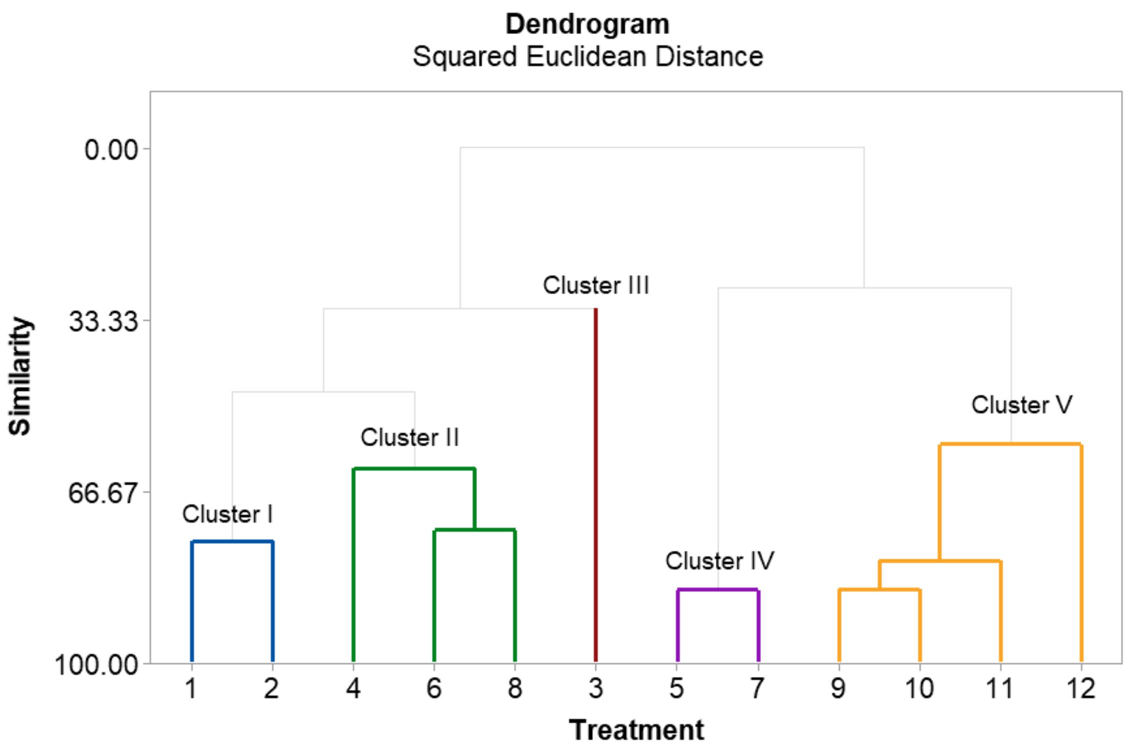

Figure 6. Similarity of treatments by hierarchical cluster analysis. **T_1:** *H. ocamponis*, no-shade and 0 kg of vermicompost; **T_2:** *H. ocamponis*, no-shade and 3 kg of vermicompost; **T_4**: *H. undatus*, no-shade and 3 kg of vermicompost; **T_6**: *H. ocamponis*, 35 % shade and 3 kg of vermicompost; **T_8**: *H. undatus*, 35 % shade and 3 kg of vermicompost; **T_3**: *H. undatus*, 0 % shade, and 0 kg of vermicompost; **T_5**: *H. ocamponis*, 35 % shade and 0 kg of vermicompost; **T_7**: *H. undatus*, 35 % shade and 0 kg of vermicompost; **T_9**: *H. ocamponis*, 50 % shade and 0 kg of vermicompost; **T_{10}**: *H. ocamponis*, 50 % shade and 3 kg of vermicompost; **T_{11}**: *H. undatus*, 50 % shade and 0 kg of vermicompost; **T_{12}**: *H. undatus*, 50 % shade and 3 kg of vermicompost.

The first cluster (I) consisted of two treatments with 76.43 % similarity, *H. ocamponis* grown without shade nor vermicompost (T_1) and *H. ocamponis* grown without shade and with vermicompost (T_2). The pitahayas of this group (Table 4) had a higher concentration of N, K and Zn, given that the higher radiation intensity probably favored absorption and mass-flow nutrient supply through the xylem associated with transpiration. However, these plants showed lower dry biomass accumulation and growth rate compared to plants of *H. ocamponis* grown with 35 % shade, probably associated with low concentrations of chlorophylls and carotenoids due to possible photoinhibition.

The second cluster (II) consisted of three treatments with 74 % similarity: *H. undatus* grown without shade and with vermicompost application and *H. undatus* and *H. ocamponis* with 35 % shade and with vermicompost application (T_4, T_6, and T_8). The cultivation with 35 % shade and vermicompost application allowed greater growth, stem length and dry weight accumulation for the two species of *Hylocereus*, confirming the greater adaptability of *H. undatus* at different radiation levels without affecting its productive potential.

The third cluster (III) consisted only of *H. undatus* grown in no shade and without application of vermicompost (T_3). These crop conditions allowed adequate growth, stem length and dry weight accumulation in plants (Table 4). However, the exposure of this species to full sunlight (cultivation without shade) and lack of fertilization caused an increase of phenolic compounds at concentrations of up to 37.76 mg EAG 100 g^{-1} f. w., which indicates stress in the plant. The plants accumulate phenolic compounds in their tissues, secondary metabolites that play a key role in the regulation of various abiotic stress factors, such as high solar radiation, low or high temperatures, pathogen infection, pest infestation, herbivore attack, and nutrient deficiency (Naikoo *et al*., 2019).

The fourth cluster (IV), with 85.92 % similarity, was composed of *H. ocamponis* and *H. undatus* grown with 35 % shade and without vermicompost (T_5 and T_7). This group was characterized by the highest values of total chlorophylls and carotenoids given that this level shade effectively protects the stems from sunburn and of the photooxidation. However, the lack of fertilization causes the lowest concentrations of P and K (Table 4) due to insufficient nutrition since the availability of these elements in the soil was low (6.90 mg of P kg^{-1}, a concentration considered low; 91.20 mg K kg^{-1}, a concentration considered very low in soils).

All treatments of *H. ocamponis* and *H. undatus* with 50 % shade (T_9, T_{10}, T_{11}, and T_{12}) integrated the fifth cluster (V), with 57.53 % similarity between observations. The plants in this cluster had lower dry weight, stem thickness, growth, and accumulation of N, Ca, Mg, and Zn (Table 4).

Conclusions

The cultivating of pitahaya with different levels of shade affected the growth, dry weight accumulation, stem thickness, nutrient (N, Ca, Mg and Zn), chlorophyll, carotenoids, starch, soluble sugars, acidity and phenols concentrations. *Hylocereus undatus* showed greater adaptability to the lack of shade (with vermicompost application) and 35 % shade, confirmed by higher values of growth, thickness, and dry weight accumulation. Conversely, *H. ocamponis* showed the greatest productive potential with 35 % shade and vermicompost application. However, *H. ocamponis* and *H. undatus* grown without shade showed greater phenolic accumulation. The cultivating of *H. ocamponis* and *H. undatus* with 50 % shade is not recommended, given the lower accumulation of dry weight, thickness, and growth, and the lower concentrations of Ca, Mg, and N.

Table 4. Mean values of growth, biochemical and nutritional variables obtained for each cluster.

Variables	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Dry weight (g plant^{-1})	35.11	58.35	52.43	42.99	23.31
Stem thickness (cm)	3.41	3.16	3.22	2.48	2.51
Stem length (cm)	127.88	150.36	111.13	162.76	104.85
Growth rate (cm plant^{-1} day^{-1})	7.28	10.16	9.45	9.34	6.38
Total chlorophylls (mg 100 g^{-1})	14.37	17.93	16.41	22.29	18.02
Total carotenoids (mg 100 g^{-1})	2.88	3.92	3.66	4.61	3.95
Total soluble sugars (%)	0.39	0.30	0.40	0.32	0.27
Total starch (%)	1.72	1.68	3.40	1.75	1.85
Acidity (%)	1.04	0.81	1.11	0.78	0.69
Total flavonoids	8.86	8.13	10.16	5.00	6.49
Total soluble phenolic (mg 100 g^{-1})	35.18	31.59	37.76	27.75	26.81
N (%)	0.21	0.21	0.18	0.21	0.19
P (%)	0.05	0.06	0.03	0.03	0.05
K (%)	0.26	0.24	0.19	0.18	0.24
Mg (%)	0.06	0.07	0.06	0.06	0.06
Ca (%)	0.17	0.17	0.19	0.16	0.13
Fe (mg kg^{-1})	64.86	73.12	40.44	44.34	61.82
Zn (mg kg^{-1})	0.36	0.22	0.26	0.28	0.22

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

All data generated or analyzed during this study are included in this published article.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

All authors contributed jointly to all aspects of the work reported in the manuscript. All authors have read and approved the final manuscript.

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