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Effect of vermicompost on the nutraceutical quality of tender cladodes of *Opuntia ficus-indica*

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Abstract. The consumption of tender cladodes knows as "nopalitos" (*Opuntia ficus-indica* L. Miller.) has increased worldwide. That is because it is an important nutritional source in the human diet given its content of fiber, minerals, vitamins, phenolic compounds and secondary metabolites (antioxidants, phenols and flavonoids), which are essential for human health. The objective of this study was to evaluate the effect of doses of vermicompost (0, 5, 15 and 25 t ha^{-1}) on the yield and nutraceutical quality of tender cladodes of *O. ficus-indica*. The measured variables were tender cladodes production and nutraceutical quality. The results showed no significant effect on the content of total phenolic compounds, total flavonoids and antioxidant capacity. However, the application of vermicompost showed significant effects on the yield, in which higher estimates were achieved for the highest dose with up to 100% compared to the control. The data obtained indicate that the dose of 25 t ha^{-1} of vermicompost promotes and increase in yield without compromising the nutraceutical quality of "nopalitos".

Key words: Antioxidant activity, phytochemical compounds, organic fertilizer, semiarid, functional food.

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Introduction

The *Opuntia ficus-indica* (OFI) is considered a traditional food with centuries of tradition in Mexican culture. Its common name is "nopal" while the edible part is known as "nopalitos" (tender cladodes). Thus, nopalito is regarded as a vegetable. This has been documented in pre-Hispanic and later records (CONABIO, 2023). According to data from SIAP (2023), around 12,500 hectares were planted in Mexico in 2023. From those, just over 12,300 hectares were harvested, thus obtaining a national production of 872,300 t (SIAP, 2023). The main cactus producing countries are Brazil, Tunisia, Mexico, Morocco, Algeria and Italy, from largest to smallest area. However, Mexico is the nation that has the largest wild area, with more than 3,000,000 ha, followed by Brazil with 900,000 ha (Ciriminna *et al.,* 2019). Mexico complies with 50.92% of the nopalito imported by the United States of America, besides that, it is the fourth largest exporter of nopalito worldwide with revenues of $433.0 million dollars (mdd), only behind China, Belgium and Spain with revenues of $959.7, 532.0 and 468.5 million dollars respectively (Tridge, 2023).

The cactus is the fifth most important vegetable in Mexico with an annual per capita consumption of 6.3 kg, behind the potato 16 kg, green chili 14.3 kg, tomato 12.4 kg and onion 7.9 kg (Statista, 2023). Among vegetables, nopalito has a production of 872,300 t. The main producing entities or states in Mexico are Morelos, Mexico City, Mexico State, Jalisco and Puebla with an average yield of 70.5 t ha^{-1}, entities that together participate with 69.7% of the national production (SIAP, 2022). This crop is distributed throughout the American continent and can be grown at altitudes ranging from sea level to 400 masl. In Mexico, the genus *Opuntia* is found throughout the country (Ramirez-Serrano, 2021). The greatest species richness is in various regions of the northeast, northwest, and north-central Mexico (Scheinvar *et al.,* 2018).

In Mexico, organically grown nopalito is barely 0.064% with an average of almost 56 t ha^{-1}. Mexico City and Nuevo Leon State are the most important zones of organic nopalito with 4 ha each one (SIAP, 2022). The cultivation of nopalito is an area with great potential in Mexico. It is a cactus that is affected by a few factors. It withstands stress conditions, in addition to being a functional food consumed in different presentations, tender stalk as a vegetable in multiple food dishes (Román *et al.,* 2013); the fresh prickly pear fruit and in multiple processed presentations (Condori and Gutierrez, 2021); stalks as forage for livestock, mainly in times of drought (Inglese *et al*., 2018).

Mexico is the center of origin of cactus in a great diversity of genera and species (CONABIO, 2023). Of all, OFI is the most studied and in cultivated form it has demonstrated very outstanding general conditions. Various studies have proposed this ancient product as an alternative for the treatment and prevention of the diseases that most afflict the world's population (Lugo-Palacios *et al.,* 2022). OFI stands out as an alternative to improve diet due to its content of nutritional compounds, which gives it importance as a functional food. It is a vegetable that has great importance as an edible due to its high nutritional value. The compounds that stand out are fiber, minerals (calcium, magnesium and zinc), vitamins (A, B, C and K), chlorophyll, proteins, probiotics, among other nutraceutical compounds (Grajales *et al.,* 2023).

Worldwide, there is a trend to adopt better habits; this includes healthy eating and preserving the environment (Meléndez *et al.,* 2020). Nutraceutical compounds are of utmost importance for human health, the OFI highlights the content of total phenols, total flavonoids and antioxidant activity. The antioxidant capacity delays the oxidation of cells. Cells that present oxidative stress develop pathologies and alterations that favor degenerative diseases (Mejia-Reyes *et al.,* 2022). Recent studies worldwide have emphasized research on these compounds in fruits and vegetables (Sarker *et al.,* 2020). Phenolic compounds influence the quality of food and are found in most products of plant origin; properties such as color, flavor and aroma are attributed to them (Creus, 2004). The consumption of foods that contain these compounds has a positive impact on the prevention of diseases related to the cardiovascular system and some types of cancer (Pineda-Lozano *et al.,* 2021; Santacruz-Terán and Mantuano-Morán, 2021). The flavonoids are one of the two groups of phenolic compounds; they are divided into anthocyanins, aurones, chalcones, flavonoids, flavones, flavones and isoflavones (Gracia-Nava, 2009). The flavonoids in general are used, like phenolic compounds, for the prevention of diseases of the cardiovascular system, breast cancer and Alzheimer's, and they also delay the aging of the immune system, nervous and reproductive system (Liu *et al.,* 2021; Montané -Ojeda *et al.,* 2021).

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On the other hand, manure is a pollution problem in La Comarca Lagunera, Mexico (Acevedo-Peralta, 2017). This problem can be solved by giving a transformation process to this product to produce mainly compost and vermicompost. The organic fertilizers are an alternative for fertilizing Opuntia to produce tender cladodes and other crops (Lugo-Palacios *et al.,* 2022). The manure, vermicompost and other organic fertilizers have proven to be capable of providing sufficient nutrients for adequate development and production of various crops (Abou El-Goud, 2020). The objective of this study was to evaluate the effect of doses of vermicompost (0, 5, 15 and 25 t ha-1) on the yield and nutraceutical quality of tender cladodes of *O. ficus-indica*.

Materials and Methods

Study area

The experiment was carried out in the experimental field of the Facultad of Agricultura and Zootecnia, located at kilometer 28 of the Gómez Palacio-Tlahualilo highway in the Ejido Venecia, municipality of Gómez Palacio, Durang, Mexico. The study area is located at 25°47'1.14 "N and 103°21'1.92" W at 1096 meters above sea level. The average annual precipitation is 230 mm (Thornthwaite, 1948). The experiment was done during the agricultural cycle of 2023 in a cactus orchard with a topographic arrangement of 1.5 m × 0. 8m with 8,333 plants ha^{-1} an age of 17 years, using the smooth forage variety, and minimal tillage soil management (Figure 1).

Treatments

The treatments evaluated were three doses of fertilization with vermicompost (0 -control- 5, 15 and 25 t ha^{-1}). The application of the treatments was carried out in February 2023 incorporating the vermicompost into the soil. A completely randomized experimental design was used, with five replications per treatment with four cactus plants and 20 plants per treatment.

Figure 1. Aerial view of the experimental area.

Plant density

The previously established cladodes were selected in December, with spacings of 1.5 m × 0.8 m, with 8,333 plants ha^{-1}, The total area of the experiment was 180 m^2, corresponding to 18 m × 10 m, length and width, respectively.

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Measured variables

The harvest of tender cladodes was scheduled 90 days after the application of the treatments, obtaining fresh nopalitos and the cut was carried out on April 28, 2023. The green tender cladodes yield was determined by collecting data at the time after cutting 3 nopalitos per plant which were weighed on a precision scale, thus obtaining the total weight.

The sample preparation required 1 g of plant material weighed, placed in a screw-cap test tube, and covered with 10 mL of absolute ethanol. The tubes were placed on a circular rotary shaker (Scientific ®) at 20 °C for 12 h and at 20 rpm. Subsequently, the tubes were centrifuged at 3000 rpm for 5 min and the supernatants were extracted for the corresponding analysis described below.

The determination of total antioxidant capacity was measured. The ABTS+ trial was carried out according to the methodology proposed by Aubad *et al.* (2007). The radical is generated by an oxidation reaction of ABTS (Sigma Aldrich, USA.) with potassium persulfate. In the evaluation, 20 µL of sample and 980 µL of the ABTS+ solution in phosphate buffer at a pH of 7.4 are used. After 30 minutes, the absorbance is read at 743 nm by spectrophotometry. The calibration curve was performed using Trolox as a standard and the results were expressed as total antioxidant capacity in micromol equivalents of Trolox per 100 grams of sample (µm TEAC•100 g^{-1} DW).

The determination of total phenolic compounds was performed using a modification of the Folin-Ciocalteu method (Singleton *et al.,* 1999); 50 µL of reconstituted extract was mixed with 3 mL of distilled water in a test tube. After, 250 µL of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis MO, USA) was added and vortexed for 10 s. Afterward 3 min of reaction, 750 µL of sodium carbonate (20% w/v) were added and stirred for 10 s, followed by 950 µL of distilled water, vortexed again and allowed to react for 2 h at room temperature and in a dark place. The absorbance of the solution was obtained reading at 765 nm in a Genesys (USA) 10 UV spectrophotometer. The phenolic content was calculated using a calibration curve using gallic acid as a standard, and the results were reported in micromoles of gallic acid equivalent per gram of sample (µm AGE•g^{-1} DW).

The quantification of flavonoid content was determined using the method developed by Zhishen *et al.* (1999), applying the following modifications, initially 250 mL of the extract of each sample was placed with 1000 mL of deionized water, 75 µL of $NaNO_2$ and allowed to react for 5 min. Next, 150 µL of 10% AlCl3 solution and 500 µL of 1 M NaOH were added. The mixture was centrifuged at 3500 rpm for 5 min. The solution obtained was observed in a spectrophotometer to measure the absorbance at 510 nm by spectrophotometry (Genesys 10 UV spectrophotometer®). Total flavonoid content was expressed in µm quercetin equivalent per g dry weight sample (µm QE•g^{-1} DW). Analyzes were performed in triplicate.

Statistical analysis

The data were analyzed according to the completely random model:

$$\text{Yij} = \mu + \text{Fi} + \varepsilon\text{ij} \qquad (1)$$

Where: Yij: observed value; µ = general constant; Fi: effect of the fertilization strategy (i = control without fertilization, fertilization with VC with doses of 5, 15 and 25 t ha^{-1} respectively); εij: random error, associated with each observed value. After this procedure, the comparison of means was applied through least significant difference (LSD) with α=0.05. All statistical procedures were performed using SAS® (Statistical Analysis System) software using a one-way ANOVA (p≤ 0.05).

Results and discussion

The analysis of yield, total antioxidant capacity, total phenolic compounds, and total flavonoids was carried out with respect to the application of VC (Table 1). The nutraceutical variables did not show significant differences between the VC doses. In contrast, the yield showed significant differences between VC doses; the higher dose displayed the higher green tender cladodes yield (Table 1).

Table 1. Yield and nutraceutical variables of *Opuntia ficus-indica* from different doses of vermicompost.

Treatments (VC doses t ha^{-1})	Variables			
	Yield (t ha^{-1})	**Total antioxidant capacity (TEAC•100 g $^{-1}$ DW)**	**Total phenolic compounds (µm GAE•g^{-1} DW)**	**Flavonoids (µm QE•g^{-1} DW)**
0	13.74±6.29 b	20367±5616 a	53.8±37.7 a	148.8±32.4 a
5	18.92±11.35 b	25348±890 a	96.9±35.5 a	141.0±17.4 a
15	26.20±12.94 ab	20074±8916 a	67.7±46.2 a	136.24±10.55 a
25	38.65±10.33 a	17900±11280 a	60.5±39.8 a	132.6±44.5 a
P Value	0.0101	0.4969	0.3675	0.8346

The values represent the mean ± the standard deviation (n= 5). *Values in columns with different letters differ significantly (LSD p ≤ 0.05). DW = Dry Weight.

In the doses of 25 t ha^{-1} the tender cladodes yield was 38.65 t ha^{-1} FW. The coefficient of variation was 43%, r^2 = 0.49 and RMSE (Root-Mean-Square Error) = 10.51. The green tender cladodes increased as VC doses increased.

As stated previously, the total antioxidant capacity did not show significant differences between VC doses (Table 1). The coefficient variation was 36%, r^2 = 0.134 and RMSE (Root-Mean-Square Error) = 7730.77. Although of null significance, values show numerical increment in relation to VC doses. The total phenolic compounds did not show significant statistical difference between the treatments (Table 1). The coefficient of variation increased (57%), r^2 = 0.174 and RMSE = 40.02. The results show that VC doses did not influence synthesis of this compound. In the case of total flavonoids also did not show significant differences. The coefficient of variation was 21%, which is acceptable, r^2 = 0.050 and RMSE = 29.33.

The results shown in this study are similar those reported by Moreno-Reséndez *et al.* (2020) who reported no significant differences in the nutraceutical variables in the Chicomostoc, Chapingo and Narro varieties of *Opuntia ficus indica.* However, an impact on performance has been found, with higher doses of organic fertilizers, the biomass production is greater (Zúñiga-Tarango *et al.,* 2009). Likewise, planting density is also usually an important factor in yield (Ruiz-Espinoza *et al.,* 2008).

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The secondary metabolites found in *Opuntia ficus indica* make it a functional food. The stage of maturity is important according to the consumer's nutritional requirements (Hernández-Becerra *et al.,* 2022). The range of values obtained in this study for total antioxidant capacity, total phenolic compounds and total flavonoids was from 2135.71 to 26040.48 TEAC•100 g $^{-1}$ DW, 14.95 to 132.03 µm GAE•g^{-1} DW and 85.73 to 204.14 µm QE•g^{-1} DW, respectively. However, the results of the present study show the same trend as reported previously in the Comarca Lagunera (Moreno-Reséndez *et al.,* 2020).

The recorded values of 14.95 to 132.03 µm GAE•g^{-1} for total phenolic compounds exceeded values of 0.63 to 24.46 µm GAE•g^{-1} in varieties of *Opuntia ficus indica* such as Jade and Milpa Alta (Cruz-de la Cruz *et al.,* 2021). Also, the recorded values of 85.73 to 204.14 µm QE•g^{-1} for total flavonoids exceeded values of 0.33 to 9.24 µm GAE•g^{-1} in the variety *Opuntia gosseliniana* (Manzanarez-Tenorio *et al.,* 2022). The phenolic compounds and total flavonoids demonstrate a numerical increase in their values due to the incorporation of VC. However, the recorded values of 26040.48 TEAC•100 g $^{-1}$ for the total antioxidant capacity were lower than 30,000 to 620,000 µm TEAC•g^{-1} in the variety OFI cv. Milpa Alta (Figueroa-Pérez *et al.,* 2018).

The vermicompost could be very effective in increasing the size, yield and quality of vegetables as tomatoes; this occurs due to an increase in macro and micronutrients, moisture retention and improvement in the population of soil microorganisms (Ahirwar and Hussain, 2015). In eggplant, the incorporation of VC against chemical fertilization increased 190% of the yield (Abou El-Goud, 2020). In addition, in beans plants, significant differences were observed between VC and chemical fertilization, promoting a higher growth, yield and quality of the bean; the conditions provided by VC are better water drainage, soil aeration and root of the bean plant (Mahmoud and Gad, 2020).

The use of VC shows that it acts as a moderator and causes the production of metabolites in buckwheat. Metabolites such as phenolic and flavonoids related to greater antioxidant properties (Mohammadi *et al.,* 2023). However, water stress is also related to the activation of enzymes (SOD, APX and CAT) that increase antioxidant activity in *Thymus vulgaris* L. (Rahimi *et al.,* 2023). Because of that, it is probable that the best conditions provided to *Opuntia ficus indica* in this experiment did not activate defense mechanisms like other studies.

The *Opuntia ficus indica* after processing shows stability for total antioxidant capacity during storage, which ensures nutraceutical quality for human consumption (Liguori *et al.,* 2021). The information on the *Opuntia ficus indica* promotes cladodes as a functional food for the agri-food and pharmaceutical industries (Tahir *et al.,* 2019).

Conclusions

The green tender cladodes (nopalitos) yield showed significant differences between VC doses, increasing yield as VC doses increased; however, the total antioxidant capacity, total phenolic compounds and total flavonoids did not show significant differences between VC doses. The results reported in this study showed that VC promotes an increase in yield without affecting the nutraceutical quality of green tender cladodes.

ETHICS STATEMENT

Not applicable

CONSENT FOR PUBLICATION

Not applicable

COMPETING INTEREST

The authors declare that they have no competing interests.

AVAILABILITY OF SUPPORTING DATA

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTION

Conceptualization, R.E.L.-P. and A.D.L.-P; methodology, R.E.L.-P. and M.G.R-.A.; software, R.E.L.-P. and A.D.L.-P; validation, R.E.L.-P., A.D.L.-P, J.G.L.-O, R.Z.-V. and M.G.R.-A.; formal analysis, R.E.L.-P., A.D.L.-P, J.G.L.-O, R.Z.-V. and M.G.R.-A.; investigation, R.E.L.-P.; resources, R.E.L.-P., A.D.L.-P, J.G.L.-O, R.Z.-V. and M.G.R.-A.; data curation, R.E.L.-P., A.D.L.-P. and J.G.L.-O; writing—original draft preparation, R.E.L.-P.; writing—review and editing, R.E.L.-P., A.D.L.-P., J.G.L.-O, R.Z.-V. and M.G.R.-A.; visualization, R.E.L.-P.; supervision, J.G.L.-O.; project administration, R.E.L.-P.; funding acquisition, R.E.L.-P. and M.G.R.-A.

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