

Effects of organic amendments and humic substances on tender cladode production of *Opuntia ficus-indica* (L.) Mill.

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Abstract. Humic and fulvic acids are widely recognized as biostimulants capable of improving nutrient availability and plant growth. This study evaluated the effects of organic amendments and humic substances on the production of tender cladodes of *Opuntia ficus-indica* (L.) Mill. The experiment was conducted under a randomized complete block design with four replicates and 15 treatment combinations derived from three substrate conditions (unamended soil, bovine manure, and poultry manure) and five humic/fulvic acid treatments (no acid application, humic acid at 2.5 and 5.0 g L⁻¹, and fulvic acid at 2.5 and 5.0 g L⁻¹). Organic amendments were applied once at planting at a rate equivalent to 50 t ha⁻¹, whereas humic and fulvic acids were applied periodically through irrigation. Tender cladodes were harvested at 20 ± 1 cm in length, and cumulative production and number of tender cladodes were recorded throughout the production cycle. Poultry manure significantly increased both cumulative tender cladode production and the number of tender cladodes compared with the unamended soil treatment. Fulvic acid application also resulted in greater production and a higher number of tender cladodes than humic acid and the no-acid treatment. The combination of poultry manure and fulvic acid produced the highest number of tender cladodes. These results indicate that poultry manure and fulvic acid constitute effective management alternatives for improving tender cladode production of *Opuntia ficus-indica* under calcareous soil conditions.

Keywords: bovine manure, poultry manure, leonardite, humic substances, *Opuntia ficus-indica*.

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Introduction

Few species match the cactus pear (*Opuntia ficus-indica* (L.) Mill.) in practical versatility. Long before the colonial period, this plant was a staple in the diets of American populations, a role that remains unchanged to this day. Its importance extends significantly to the livestock sector, especially during extreme droughts. Its value lies in its high-water content, which often exceeds 94%, and its remarkable nutritional advantages (Guzmán-Loayza and Chávez, 2007; Nefzaoui *et al.*, 2014). The cactus pear provides fiber and minerals such as calcium, magnesium, potassium, and iron, as well as important natural antioxidants such as flavonoids.

For those using it as a vegetable, harvest timing is critical; peak nutritional value occurs within a narrow window between days 15 and 25. In addition to its nutritional

value, *O. ficus-indica* is recognized for its remarkable tolerance to environmental stress. It thrives across a wide range of conditions, from sea level to 3,000 meters (Maiuolo *et al.*, 2024). The species maintains growth and survival across a broad temperature range. Whether facing a 60 °C peak or a sudden -8 °C plunge, the species remains viable (Morales *et al.*, 2025). This level of resilience has become increasingly important under current agricultural and environmental conditions. This is largely because intensive farming practices -as noted by Cox *et al.* (2011)- continually degrade the soil's integrity.

This decline is a major hurdle, particularly in Mexico. According to PUEIS-UNAM, visible damage has already taken hold in 64 % of the country (Ortiz-García *et al.*, 2022). It is no longer just a theoretical concern; it is a direct threat to the agricultural landscape. In this context, the prickly pear cactus takes on renewed purpose, not only as a consumer product but also as a tool for rehabilitating unproductive land (Meaza *et al.*, 2024).

An important strategy for improving soil quality is the integration of cactus pear cultivation with organic amendments to restore the soil system over time. Today, choosing inputs like poultry manure, biochar, or vermicompost is no longer about cutting costs. It has become a major environmental priority, primarily to prevent ongoing contamination of aquifers. Unlike synthetic options, these organic materials mineralize much more slowly.

This timing is critical because it lets the plant draw in nutrients right as it grows, while the soil's physical structure gets a chance to strengthen (Pikula, 2024). Similar effects have been reported for humic and fulvic acids derived from leonardite, which have become an important tool for improving plant nutrition. Their primary function is to enhance cation exchange capacity (CEC), acting as a safeguard. This keeps vital elements like calcium and potassium from simply washing away through leaching. Instead of losing those nutrients, these acids help lock them into stable complexes. This ensures, as López-Salazar *et al.* (2018) have pointed out, that they remain in forms available for root uptake.

Beyond nutrition, these compounds help stabilize pH through a buffering effect and boost soil microbiology (Nardi *et al.*, 2016). Whether sprayed onto leaves or applied directly to the soil, recent studies confirm that these applications increase both yield and chlorophyll levels (Kareem *et al.*, 2024; Shahrajabian and Sun 2024).

Based on this background, it was hypothesized that the use of organic amendments and the application of humic and fulvic acids would increase the number and production of tender cladodes. Therefore, the objective of this study was to evaluate the effects of bovine manure and poultry manure, applied alone or in combination with humic substances, on the number and production of tender cladodes of *Opuntia ficus-indica*.

Material and Methods

Study site location

The study was conducted at the experimental field of the Faculty of Agronomy, Universidad Autónoma de Nuevo León (UANL), in General Escobedo, Nuevo León, Mexico (25°47'06" N, 100°17'13" W; 510 m a.s.l.). The area is characterized by a semi-arid climate, with a mean annual temperature of 20 °C and annual precipitation ranging from 400 to 600 mm.

Experimental design and crop management

The experiment was established using a randomized complete block design with four replicates. Treatments consisted of 15 combinations derived from three substrate conditions (unamended soil, soil amended with bovine manure, and soil amended with poultry manure) and five humic/fulvic acid treatments: no acid application, humic acid at 2.5 g L⁻¹, humic acid at 5.0 g L⁻¹, fulvic acid at 2.5 g L⁻¹, and fulvic acid at 5.0 g L⁻¹ (Table 1). Because the zero-dose level corresponded to the absence of humic/fulvic acid application, dose was analyzed within the humic and fulvic acid treatments rather than as a fully crossed independent factor. Organic amendments, consisting of bovine manure and poultry manure, were applied once at planting at a rate equivalent to 50 t ha⁻¹ (301.2 g per plant) and incorporated into the substrate before planting the mother cladodes. The poultry manure had been previously subjected to heat treatment to reduce potential pathogen contamination and ensure sanitary quality.

Table 1. Treatment combinations used in the experiment.

Treatment	Substrate (S)	Acid treatment	Dose (D, g L ⁻¹)
1	Bovine manure (BM)	No acid	0
2	Bovine manure (BM)	Humic acid (H)	2.5
3	Bovine manure (BM)	Humic acid (H)	5.0
4	Bovine manure (BM)	Fulvic acid (F)	2.5
5	Bovine manure (BM)	Fulvic acid (F)	5.0
6	Poultry manure (PM)	No acid	0
7	Poultry manure (PM)	Humic acid (H)	2.5
8	Poultry manure (PM)	Humic acid (H)	5.0
9	Poultry manure (PM)	Fulvic acid (F)	2.5
10	Poultry manure (PM)	Fulvic acid (F)	5.0
11	Control (unamended soil)	No acid	0
12	Control (unamended soil)	Humic acid (H)	2.5
13	Control (unamended soil)	Humic acid (H)	5.0
14	Control (unamended soil)	Fulvic acid (F)	2.5
15	Control (unamended soil)	Fulvic acid (F)	5.0

Application of humic and fulvic acid treatments

Humic and fulvic acids were applied to the soil as aqueous solutions at concentrations of 2.5 and 5.0 g L⁻¹. Irrigation was performed every 20 days throughout the experiment. Applications of humic and fulvic acids were made every second irrigation event, resulting in treatment applications at 40-day intervals. A total of 3.5 L of solution was applied per pot at each application, for a total of 17 applications during the crop cycle. The application rates were selected according to the manufacturer's instructions.

Soil analysis

Table 2 presents the physical and chemical properties of the soil used in the experiment prior to the application of organic amendments and humic/fulvic acid treatments. The soil was characterized by a high calcium carbonate content (39.1%), alkaline pH (8.2), and low organic matter content (1.9%), indicating typical calcareous soil conditions.

Table 2. Initial physicochemical characteristics of the experimental soil.

Parameter	Value	Parameter	Value	Parameter	Value
Sand (%)	27	ESP	2.57	Fe (ppm)	8.57
Clay (%)	34	CaCO ₃ (total, %)	39.08	Zn (ppm)	3.55
Silt (%)	39	CEC (meq 100 g ⁻¹)	26.81	Mn (ppm)	12.85
Base saturation (%)	49.92	Organic matter (%)	1.89	S (ppm)	41.74
Field capacity (%)	35.3	NO ₃ ⁻ -N (ppm)	3.30	B (ppm)	1.06
Permanent wilting point (%)	21	P (ppm)	50.30	Na (ppm)	48.65
Bulk density (g cm ⁻³)	1.4	K (ppm)	318.95	Humic mat. (%)	0.05
pH	8.21	Ca (ppm)	4957.39	Humic acid (%)	0.007
EC (dS m ⁻¹)	1.13	Mg (ppm)	224.04	Fulvic acid (%)	0.041

ESP=Exchangeable Sodium Percentage. CEC= Cation Exchange Capacity.

Chemical composition of humic and fulvic acid products

Table 3 presents the chemical composition of the humic and fulvic acid products used in this study. Fulvic acid contained higher concentrations of N, P, K, Ca, Mg, Zn, and Mn than humic acid, whereas humic acid showed greater concentrations of Cu and Fe. Despite these differences, the amounts supplied through the applied doses and application frequencies were negligible relative to the crop nutrient requirements; therefore, neither product represented a significant source of mineral nutrition.

Table 3. Chemical composition and humic fractions of the humic and fulvic acid products used in the study.

Parameter	Humic acid	Fulvic acid
Mineral composition (%)		
Total Nitrogen	0.21	2.97
Total Phosphorus	0.10	0.17
Total Potassium	0.67	11.31
Calcium	0.60	1.52
Magnesium	0.35	1.38
Trace elements (ppm)		
Copper	83.24	31.24
Iron	9044.5	394.8
Zinc	92.97	377.27
Manganese	66.93	125.44
Humic fractions (%)		
Humic material	22.85	29.84
Humic acids	18.71	2.49
Fulvic acids	1.55	27.15

Response variables

Tender cladode production was recorded during 71 harvests conducted between March 10 and December 18, 2023. Cladodes were harvested when they reached a length of 20 ± 1 cm. Fresh weight was determined using a precision balance with a resolution of 0.001 g. Cumulative tender cladode production and the total number of tender cladodes were calculated from the production recorded throughout the harvesting period.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) according to the following model:

$$Y = \mu + S + HF + (S \times HF) + D(S \times HF) + \varepsilon \quad [1]$$

where Y is the observed response, μ is the overall mean, S is the substrate effect, HF is the humic/fulvic acid treatment effect, S \times HF is the interaction between substrate and humic/fulvic acid treatments, D(S \times HF) represents the dose effect evaluated within each substrate \times humic/fulvic acid combination, and ε is the experimental error.

The model evaluated the main effects of substrate and humic/fulvic acid treatments, their interaction, and the effect of dose (2.5 and 5.0 g L⁻¹) within each substrate \times humic/fulvic acid combination.

Prior to ANOVA, model assumptions were assessed by testing the normality of residuals and homogeneity of variances using the Shapiro-Wilk and Levene tests, respectively. When significant treatment effects were detected, means were compared using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$. All statistical analyses were performed using SPSS®.

Results and Discussion***Tender cladode production***

The analysis of variance for cumulative tender cladode production from March 10 to December 18, 2023, revealed significant effects of substrate and humic/fulvic acid treatments ($P < 0.05$), whereas the substrate \times humic/fulvic acid interaction and dose effects within the interaction were not significant (Table 4).

Table 4. Analysis of variance for cumulative tender cladode production (March–December 2023).

Source of variation	SS	df	MS	F-value	P-value
Substrate (S)	10209628.87	2	5,104,814.4	23.76	<0.001
Humic/Fulvic (H/F)	1502023.93	2	751,012.0	3.49	0.03
S \times H/F	701262.34	4	175,315.6	0.81	0.52
Doses within (S \times H/F)	1042748.82	6	173,791.5	0.80	0.56
Error	12028305.71	56	214,791.2		
Total	25930849.71	70			

The mean comparison of organic amendments showed that both bovine manure and poultry manure significantly increased tender cladode production compared with the control (unamended soil), whereas no significant differences were detected between the two organic amendments (Table 5). These results are consistent with previous reports indicating positive responses of cactus species to organic amendments. Silva *et al.* (2023) observed improvements in cladode quality following the application of a bovine manure-based biofertilizer, including increased concentrations of total sugars, phenolic compounds, and soluble solids. Such responses suggest that organic amendments may enhance nutrient availability and plant physiological performance, ultimately contributing to greater productivity.

Table 5. Effect of organic amendments on tender cladode production.

Organic amendment	Tender cladode production (kg)	95% confidence interval	
		Lower limit	Upper limit
Bovine manure	2387.37 ± 98.31 a*	2190.42	2584.31
Poultry manure	2637.83 ± 101.89 a	2433.72	2841.95
Control (unamended soil)	1738.67 ± 98.31 b	1541.72	1935.61

* Data are presented as mean ± SE. Values followed by different letters differ significantly according to Tukey's HSD test ($P < 0.05$).

Regarding the effects of humic and fulvic acids, the analysis of variance revealed significant differences among treatments (Table 4). As shown in the mean comparison (Table 6), application of fulvic acid resulted in a higher tender cladode production than that of both humic acid treatments and the control. This physiological response is associated with fulvic acids' ability to enhance cation exchange capacity and mobilize nutrients in the rhizosphere, thereby facilitating their uptake by plants.

Table 6. Effect of humic and fulvic acid treatments on tender cladode production.

H/F treatments	Tender cladode production (kg)	95% confidence interval	
		Lower limit	Upper limit
No acid	2163.57 ± 94.6 b*	1974.06	2353.09
Fulvic acids (F)	2442.95 ± 94.6 a	2253.44	2632.46
Humic acids (H)	2111.82 ± 97.19 b	1917.12	2306.53

* Data are presented as mean ± SE. Values followed by different letters differ significantly according to Tukey's HSD test ($P < 0.05$).

Consistent with these findings, Zhang *et al.* (2021) reported that fulvic acids increase the availability of nutrients such as Ca, Fe, and Zn and enhance tomato yield by up to 35%, primarily through the promotion of a more vigorous root system that improves water and nutrient uptake. Similarly, Fang *et al.* (2020) found that fulvic acid applications improved photosynthetic performance and chlorophyll content, preserved chloroplast and mesophyll cell integrity, and increased the expression of genes associated with drought tolerance.

The positive effects of fulvic acids on nutrient acquisition have also been documented in other crops. Luna de Neto *et al.* (2025) reported increased accumulation of P and K in *Brassica oleracea* tissues, accompanied by improved growth and yield. Likewise, Ding *et al.* (2024) observed increases in plant height, chlorophyll content, biomass production, and root development in rice, with the most effective response obtained at 40 mg L⁻¹.

The present study was conducted in a calcareous soil with a pH of 8.2, conditions under which micronutrients such as Fe, Zn, and Cu tend to precipitate and become less available to plants. Therefore, compounds with chelating properties that enhance cation exchange capacity, such as fulvic acids, may play an important role in improving nutrient availability and uptake, which could explain the observed increase in tender cladode production.

Regarding the interaction between substrates and humic/fulvic acids, no significant effects were detected. Similarly, no significant differences in the effect of dose were observed within the interaction.

The analysis of variance for the number of tender cladodes recorded from March to December 2023 revealed significant effects of substrate, humic/fulvic acid treatment, and their interaction (Table 7).

Table 7. Analysis of variance for number of tender cladodes (March-December 2023).

Source of variation	SS	df	MS	F-value	P-value
Substrate (S)	1348.55	2	674.27	30.80	<0.001
Humic/Fulvic (H/F)	145.04	2	72.52	3.31	0.040
S × H/F	234.37	4	58.59	2.67	0.041
Dose within (S × H/F)	91.35	6	15.22	0.69	0.65
Error	1225.62	56	21.88		
Total	3088.31	70			

The mean comparison of organic amendment types showed that poultry manure produced more tender cladodes, followed by bovine manure, whereas the control produced the fewest tender cladodes (Table 8). This result supports the previously discussed benefits of poultry manure as an organic amendment and suggests it may be more effective than bovine manure for enhancing tender cladode production.

Table 8. Effect of organic amendment on number of tender cladodes.

Organic amendment	Number of tender cladodes	95% confidence interval	
		Lower limit	Upper limit
Bovine manure (BM)	21.77 ± 0.99 b	19.78	23.76
Poultry manure (PM)	26.60 ± 1.02 a	24.54	28.66
Control (unamended soil)	15.85 ± 0.99 c	13.86	17.83

* Data are presented as mean ± SE. Values followed by different letters differ significantly according to Tukey's HSD test ($P < 0.05$).

The superior performance of poultry manure may be related to its nutrient contribution and gradual nutrient release during the production cycle. In a 10-year study, Gaytán-Martínez *et al.* (2022) reported that poultry manure applied to vegetable nopal is an important source of macronutrients, including N, Ca, and Mg. However, the availability of P and K was reduced by 50-60%, likely due to soil properties that influence nutrient dynamics.

Humic and fulvic acid treatments also differed significantly (Table 7), and the mean comparison showed that fulvic acid application resulted in the highest number of tender cladodes (Table 9). Previous studies have reported similar positive effects of fulvic acids on plant growth and productivity. For example, Luna de Neto *et al.* (2025) observed increased phosphorus concentration in leaves, higher chlorophyll content, and greater potassium accumulation in cauliflower (*Brassica oleracea* var. botrytis) following fulvic acid application. Likewise, Mahdy *et al.* (2024) reported enhanced vegetative growth, greater pod production, and increased seed yield in pea plants treated with fulvic acids. In contrast, humic acid treatments did not differ significantly from the no-acid treatment (Table 9), a response consistent with the results obtained for tender cladode production.

Table 9. Effect of humic and fulvic acid treatments on number of tender cladodes.

H/F treatments	Number of tender cladodes	95% confidence interval	
		Lower limit	Upper limit
No acid	20.12 ± 0.95 b*	18.21	22.03
Fulvic acids (F)	23.20 ± 0.95 a	21.29	25.12
Humic acids (H)	20.25 ± 0.98 b	18.28	22.21

* Data are presented as mean ± SE. Values followed by different letters differ significantly according to Tukey's HSD test ($P < 0.05$).

A significant substrate × humic/fulvic acid interaction was detected for the number of tender cladodes (Table 7). Therefore, treatment means were compared for the different combinations of substrates and humic/fulvic acid treatments (Table 10). The interaction indicated that the lowest numbers of tender cladodes were obtained in the unamended soil treatments without acid application and with humic acid application. In contrast, the highest numbers of tender cladodes were recorded when poultry manure was combined with either fulvic acid or humic acid (Table 10).

Table 10. Effect of substrate × humic/fulvic acid interaction on number of tender cladodes.

H/F treatment	Organic amendment	Number of tender cladodes	95% CI (Lower)	95% CI (Upper)
No acid	Control (unamended soil)	12.75 ± 1.65 c	9.43	16.06
No acid	Bovine manure (BM)	23.87 ± 1.65 ab	20.56	27.18
No acid	Poultry manure (PM)	23.75 ± 1.65 ab	20.43	27.06
Fulvic acids (F)	Control (unamended soil)	19.12 ± 1.65 abc	15.81	22.43
Fulvic acids (F)	Bovine manure (BM)	23.75 ± 1.65 ab	20.43	27.06
Fulvic acids (F)	Poultry manure (PM)	26.75 ± 1.65 a	23.43	30.06
Humic acids (H)	Control (unamended soil)	14.12 ± 1.65 c	10.81	17.43
Humic acids (H)	Bovine manure (BM)	18.75 ± 1.65 abc	15.43	22.06
Humic acids (H)	Poultry manure (PM)	27.87 ± 1.78 a	24.29	31.45

* Data are presented as mean ± SE. Values followed by different letters differ significantly according to Tukey's HSD test ($P < 0.05$).

The positive response observed with organic amendments in the present study agrees with findings reported by Salazar-Sosa *et al.* (2018), who demonstrated that cattle manure fertilization improved cladode yield in *Opuntia ficus-indica* and contributed to maintaining soil quality. These results support the role of organic amendments as an effective strategy for enhancing productivity in cactus production systems while promoting more sustainable soil management practices.

Additional evidence supporting the beneficial effects of organic amendments was reported by Miranda e Silva *et al.* (2016), who found that increasing cattle manure application rates enhanced productivity in *Opuntia ficus-indica*, highlighting the importance of organic nutrient sources for sustained cladode production. Likewise, Santiago-Lorenzo *et al.* (2016) reported that poultry manure performed as well as or better than cattle manure for tender cladode production, which is consistent with the superior performance of poultry manure observed in the present study.

Conclusions

Organic amendments significantly increased both the number of tender cladodes and cumulative tender cladode production compared with the unamended soil treatment. Among the evaluated amendments, poultry manure produced the greatest response, followed by bovine manure. Fulvic acid application also increased the number of tender cladodes and cumulative tender cladode production, whereas humic acid did not differ significantly from the no-acid treatment. These results indicate that the combined use of poultry manure and fulvic acid represents a promising management strategy for improving tender cladode production under calcareous soil conditions.

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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Not applicable.

AUTHOR CONTRIBUTIONS

Conceptualization, G.N.M. and R.E.V.A; Methodology, G.N.M. and R.E.V.A; Software, G.N.M., R.E.V.A. and E.O.S; Validation, G.N.M., R.E.V.A., S.J.M.G., A.B.V., and E.O.S; Formal analysis, G.N.M., R.E.V.A., E.V.G.C., and E.O.S; Investigation, G.N.M., R.E.V.A. and E.O.S; Resources, G.N.M., S.J.M.G., A.B.V., and R.E.V.A; Data curation, G.N.M. and E.O.S; Writing—original draft preparation, G.N.M., R.E.V.A., S.J.M.G., and E.O.S; Writing—review and editing, G.N.M., R.E.V.A., E.O.S, E.V.G.C., S.J.M.G., A.B.V., and M.C.O.Z; Visualization, G.N.M; Supervision, G.N.M., R.E.V.A., S.J.M.G. and E.O.S; Project administration, G.N.M. and R.E.V.A. All authors have read and agreed to the published version.

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