The potential of using solarized cattle manure fertilizer and planting densities to cultivate the cactus *Opuntia ficus-indica* L.

Enrique Salazar-Sosa[1], Héctor Idilio Trejo-Escareño[2†], Ignacio Orona-Castillo[2], Enrique Salazar-Melendez[2], Luis Guillermo Hernández-Montiel[3], Bernardo Murillo-Amador[3*]

[1] Instituto Tecnológico de Torreón. Torreón, Coahuila, México.

[2] Facultad de Agricultura y Zootecnia. Universidad Juárez del Estado de Durango. Gómez Palacio, Durango, México.

[3] Programa de Agricultura en Zonas Áridas. Centro de Investigaciones Biológicas del Noroeste, S.C. La Paz, Baja California Sur, México

* Corresponding author: bmurillo04@cibnor.mx

† Héctor Idilio Trejo-Escareño, our colleague passed away on November 04, 2017. He will be sorely missed by us. This paper will be published in his memory.

Received: May 19, 2017; Accepted: March 28, 2018

ABSTRACT

Opuntia has been long cultivated for forage, as well as for human consumption. The objective was to evaluate yield, thickness, length and width of cladodes of *Opuntia* while in the soil, pH, electrical conductivity, organic matter and nitrates were evaluated. The experiment was conducted during three years 2008, 2009 and 2010 through a completely randomized block design with factorial arrangement with three replications. The factors were doses of organic fertilizer (0, 20, 40, 60 t ha^{-1}) and one inorganic fertilizer as control 100-100-0 kg ha^{-1} of N, P and K, respectively and planting densities (4,435, 8,871, 8,887 and 13,323 plants ha^{-1}) with different topological arrangements. The results showed greatest yield at 60 t ha^{-1} in 2009 and 2010. In 2008, yield was greater at 60 t ha^{-1} but was not significantly greater than 40 t ha^{-1}, however was greatest than control. All plant variables were greatest at 60 t ha^{-1}, although in some cases not significantly different from other dosages. For all 3 years, pH, electrical conductivity, organic matter and nitrate content at 60 t ha^{-1} and 13,323 plants ha^{-1} were unacceptable ranges and were not significantly worse than inorganic fertilizer, dosages or planting densities. In conclusion, organic fertilizer is an option to avoid use of non-organic fertilizers in order to improve *Opuntia* production as well as maintaining soil quality.

Keywords: cladode yield, cladode length, electrical conductivity, nitrates, organic matter, pH.

INTRODUCTION

Many current practices of industrialized agricultural production systems accelerate desertification by destroying natural vegetation and soil quality, thus representing a serious risk to native plants, wildlife and even human sustainability. In Northern Mexico, due to its arid climate the region is even more susceptible to agricultural damage. Previous studies in semiarid areas of Mexico have shown that the administration of nutritional soil supplements improve production yields of plants used as forage, thus playing a role in increasing productivity of livestock. However, the costs of commercial soil supplements are typically beyond the reach of the local farmers due to their low income. Hence, sustainable alternatives have been sought out such as using local natural resources to produce forage with the correct nutritional characteristics for the livestock (Baraza *et al.,* 2008).

In arid and semiarid areas, forages used by goats and sheep are characterized by large seasonal changes in production, annual fluctuations and seasonal variations in forage quality (Azocar, 2003). Mexico is one of the world's centres of species diversity (richness) for *Opuntia* cacti (Illoldi-Rangel *et al*., 2012). Among the cacti, *O. ficus-indica* Mill. is one of the most important and its tasty fruit and cladodes serve as forage and as a green vegetable (Moßhammer *et al*., 2005). Its use has increased steadily since the early 17^{th} Century with the introduction of livestock in semiarid areas and the subsequent decline of grasslands. Farmers use the *Opuntia* cladodes to feed their livestock after burning the spines, especially during droughts (Anaya-Pérez, 2003). Currently, *Opuntia* is being overexploited (Anaya-Pérez, 2003). It is been found that the productive and reproductive performance of cattle, sheep, and goats are higher when producers supplement the diet with cactus during the dry season (Flores, 1997). In addition, *Opuntia* species are considered to be a good and economic alternative for feeding goats, sheep and cattle when forage availability is low due to low rainfall or drought (Cordeiro Dos Santos and Gonzaga De Albuquerque, 2003; Felker, 2003). As a native plant of Mexico *Opuntia* is used for forage production in the Comarca Lagunera region located between the two states of Coahuila and Durango, Mexico (Moßhammer *et al*., 2005).

The Comarca Lagunera area produces manure in large quantities due to bovine exploitation of cattle for milk and meat under a system of intensive production. In this region there are around 600,000 heads of livestock for milk and meat exploitation (SIAP, 2015). In general, in this system the manure is collected and accumulates in stacks and afterwards it is incorporate into agricultural soils. However, the process of adding manure to soil should be carried out with appropriate organic materials. Otherwise, it can become a hot spot source of contamination leading to water pollution of aquifers and thus possibly posing a serious human health risk (Fortis-Hernández *et al*., 2007). It can also cause serious contamination of foods by pathogenic microorganisms present in the soil (Den Aantrekker *et al*., 2003).

The international food safety laws establishes that manure needs to be treated prior to its application through composting, pasteurization, steam drying or ultraviolet radiation, so it doesn't exceed quantity of heavy metals, faecal coliform bacteria and helminths eggs. A method that has been used with success in the disinfection of agricultural soils involves heating through plastic covers that have the capacity to capture solar radiation and to increase the temperature considerably; this method is known worldwide as solarisation and this method has been used with success to eliminate immature states and adults of arthropods, plant pathogens (fungi, bacteria and nematodes), seeds and overgrowth propagules (Katan, 1996). This technique offers a very promising alternative for the control of plant diseases caused by soil microorganisms without the need to use chemical products (Jiménez, 1995). In this sense, solarized cattle manure can be used for fertilization purposes to reduce farmer's efforts to resolve soil fertility problems, as well as improve water retention capacity, which helps to stimulate plant development in order to achieve a greater production, among other benefits. In addition, the high cost of chemical fertilization encourages the use of cattle manure by farmers which not only improves physical and chemical soil characteristics but also increases forage production (Salazar-Sosa *et al*., 2010b).

The use of different doses of organic matter such as cattle manure as a fertilizer in improving yield and other traits of *Opuntia* spp have been reported previously (Murillo-Amador *et al*., 2000; Rodriguez *et al*., 2001; Gutiérrez-Ornelas *et al*., 2007; Zúñiga-Tarango *et al*., 2009; Martínez-López *et al*., 2009). In addition, the use of different plant densities in *Opuntia* spp have also been studied and measured and its effects on production, yield, physiological traits and so on have been reported (García de Cortázar and Nobel, 1990; García de Cortázar and Nobel, 1991; Murillo-Amador, 1996; Dubeux *et al.,* 2006). In this study, we hypothesized that there is an optimum dosage of organic fertilizer and an optimum planting densities that will lead to higher cladode yield and their components of *O. ficus-indica* Lisa cultivar, as well as improving soil characteristics. The results were compared to an inorganic fertilizer (control) that is used by farmers in the study region. To our knowledge, this is the first report of investigation in *O. ficus-indica* using sterilized solarized cattle manure as an organic fertilizer in a sustainable production system over an extended period (3 years).

MATERIALS AND METHODS

Ethics statement

The research conducted herein did not involve measurements with humans or animals. The study site is not considered a protected area. For locations/activities, no specific permissions were required and the field studies did not involve endangered or protected species. However, to carry out research activities on the lands administered by University Juarez of Durango State (UJED). The plant material used for this study was cultivated under experimental field conditions at UJED. *Opuntia ficus-indica* is not considered an endangered species and sampling therefore had negligible effects on broader ecosystem functioning.

Study area

The work was carry out in the region known as “Comarca Lagunera”, which is located in the central part of northern México, in Coahuila and Durango States. The climate of this region is classified as of steppe (BS) and desert (BW); known as an arid climate with rains in summer and relatively mild winters. The annual average precipitation is 258 mm with an annual average evaporation of about 2,396 mm. Yearly average temperatures is 22 ºC with ranges from 40 °C as a maximum and 4 °C as a minimum. The relative humidity in the region varies according to year season, with 31% spring, 47% in summer, 58% in autumn and 40% in winter (García, 2004).

Experimental site

Field research was carried out in the Experimental Station of the Faculty of Agriculture and Animal Science (FAZ) of the University Juarez of Durango State, located at 25° 47' 02.39" LN and 103° 20' 42.6" LW at 1,112 masl in the Ejido Venecia, municipality of Gómez Palacio, Durango, Mexico.

Experimental conditions

Soils.

For soil analysis, two individual samples were taken, and then mixed to form a composite soil sample (0-30 cm) per plot. Laboratory results showed that native soils are alluvial, aridosol type. The type of soil in which the experiment was conducted is clay type, which according to Matus *et al*. (2000) interferes with the mineralization of nitrogen. The influence of the soil type (clay) on nutrients should be explained for the given growth conditions.

Organic fertilizer characteristics.

The fertilizer used was solarized cattle manure with a pH of 7.6, 0.63 dS m^{-1} of electrical conductivity (EC), 5.47% of organic matter (OM), 1.12% of total nitrogen, 0.11% of ammonium (NH_4), 0.35% of phosphorus, 3.38% of calcium, 0.71% of magnesium, 3.27% of potassium, 0.97 ppm of sodium, 560 ppm of molybdenum, 12,300 ppm of iron, 198 ppm of zinc, 45 ppm of copper and 410 ppm of boron. The humidity of the cattle manure at the time of application was 8%. The organic fertilizer and the inorganic fertilizer (N, P, K) were applied manually and incorporated directly into the soil in a circumference around the plant, opening a groove 30 cm of deep using a hoe. After, the groove was covered. The application was for only one time in January 2008.

Plant material.

One-year-old uniform and healthy rooted cladodes of the cultivar "Lisa" were dehydrated during 20 days under shade and were planted directly in the ground for rooting, where they remained for 30 days. Once cladodes took root, then uniform and healthy cladodes were selected and planted in the field. The plants were transplanted in March of 2003 and before the first harvest in December of 2008, the plants grew during five year, time enough to complete "three levels" of cladodes growth as it is described by Janczur *et al*. (2014).

***Cultural practices*.**

The plants were adequately irrigated and protected from pests and diseases throughout the growing period according the standard cultivation of organic agriculture of the region. The land was prepared by two crisscross ploughings and two harrowing; then it was levelled and furrowed for transplanting.

Variables measured in harvested cladodes.

Well-developed and mature cladodes were sampled one time per year during the three evaluation years (December 2008, December 2009 and December 2010) and the following variables were measured: cladode thickness (CT, in mm) using a digital calliper (General No. 143, General Tools® Manufacturing Co., Inc., New York, USA), cladode width (CW, in cm), cladode length (CL, in cm) and cladode yield (CY, in t ha^{-1}).

Variables measured in soil.

As previously explained, a composite sample of the surface soil (0-30 cm) was taken, immediately cooled after sampling and then transported in a portable icebox to the laboratory. The variables measured in soils were carried out during three years, twice in 2008 (January and December) and once in 2009 and 2010 (December in both years). The pH in the soil extract was measured using a potentiometer (pH Orpt series 700 of Oakton® Instruments, USA); electrical conductivity in the soil extract was measured with EC Test 11 of Oakton® Instruments, USA; organic matter was determined using the Walkley-Black method and nitrate (NO_3) content was measured using a colorimetric method (Page *et al*., 1982). The NO_3 was analysed with a Spectrometer (Spectronic® Unicam Mercers Row Cambridge CB5 8HY, UK)

Experimental design

The experiment was conducted as a completely randomized block design with factorial arrangement with three replications, containing three rooted cladodes per replication for each treatment for a total of 180 rooted cladodes. The first factor were different doses of organic fertilizer (0, 20, 40, 60 t ha^{-1}) and one plot with inorganic fertilizer applied at dosages of 100-100-0 kg ha^{-1} of nitrogen, phosphorus and potassium, respectively, which is applied by farmers used in this experiment as a control of and will be referred to as control subsequently in the text. The second factor were different planting densities (4,435, 8,871, 8,887 and 13,323 plants ha^{-1}) with different topological arrangements (Fig. 1). Yield and some yield components of *O. ficus-indica* and soil characteristics of the experimental site were measured during three continuos years (2008, 2009 and 2010).

Statistical analysis

Data were analysed using univariate analysis of variance (ANOVA). Data were compared using ANOVA according to a factorial design with two ways of classification, those being organic fertilizer doses (0, 20, 40, 60 t ha^{-1}) with a plot of inorganic mineral fertilizer (100-100-

0 kg ha^{-1} of N, P and K, respectively) and planting densities (4,435, 8,871, 8,887 and 13,323 plants ha^{-1}) during three evaluation years (2008, 2009 and 2010). In the ANOVA analysis the interaction was not included because of there not exist synergism or interference among factors under study, according to Sokal and Rohlf (1998). The difference among the means was determined by the Fisher LSD multiple range test (p=0.05). In all cases, mean values were considered significantly different when $p \leq 0.05$. All analyses were done with SAS® software program for Windows® (SAS Institute, 1996).

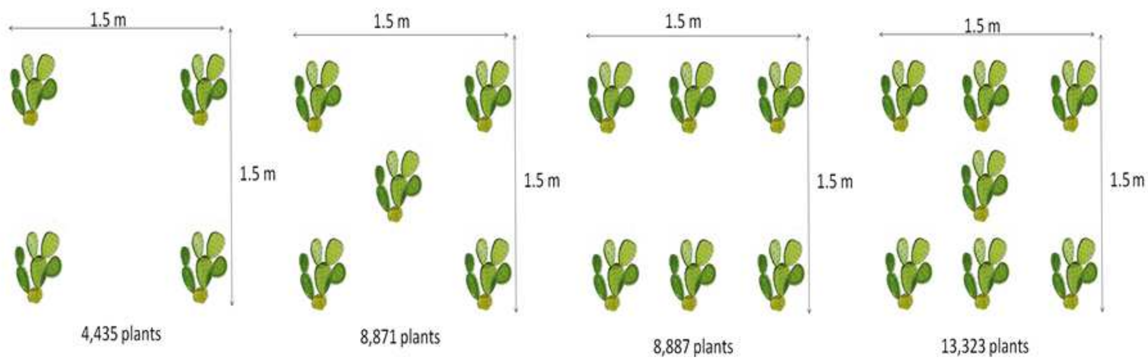

Figure 1. Topological distribution for each plant density of *Opuntia ficus-indica* in the field. Once cladodes took root, uniform and healthy cladodes were selected and planted in the field. The plants were transplanted in March of 2003 and before the first harvest in December of 2008, the plants growth during five year, time enough to complete "three levels" of cladodes growth as it is described by Janczur *et al*. (2014).

RESULTS

Cladode yield

Cladode yield (CY) exhibited significant differences between doses in 2008 and 2009 and plant densities in 2008 and 2010. The largest CY during the three years was with 60 t ha^{-1} while lowest CY was at control and 0 t ha^{-1} in 2008, 2009 and 2010, respectively(Table 1). Cladode yield was higher with 13,324 plants ha^{-1} during the three years while the lowest occurred with 4,435 plants ha^{-1} (Table 2).

Cladode thickness

Cladode thickness (CT) did show significant differences among doses in 2010 and plant densities in 2008 and 2010. Highest CT was with 60 t ha^{-1} during the three years, while the lowest CT was with 0 t ha^{-1} in 2008 and 2009, while in 2010 at the control (Table 1). Cladode thickness was highest at 8,871 plants ha^{-1} (2008 and 2010) and 13,323 plants ha^{-1} (2009)

while thinnest cladodes were harvested in 8,887 plants ha^{-1} (2008 and 2010) and 4,435 plants ha^{-1} (2009) (Table 2).

Cladode width

Cladode width (CW) did not show significant differences among doses and plant densities during any of the years; however, during the three years cladodes were wider in those plants with 60 followed by 40 t ha^{-1} while smallest CW were found in the control (Table 1). The cladodes were widest at 8,871 plants ha^{-1} (2008 and 2010) and 13,323 plants ha^{-1} in 2009 whereas smallest CW were at 4,435 in 2008 and 2009 and 13,323 plants ha^{-1} in 2010 (Table 2).

Cladode length

Cladode length (CL) revealed significant differences among doses during the three years and plant densities in 2009 only. The longest cladodes were harvested at 60 t ha^{-1} during the three years; whereas the shortest cladodes were collected at 0 t ha^{-1} and control during 2008, 2010 and 2009, respectively (Table 1). Cladodes were longest during 2009 as follow: 8,877 > 13,323 > 8,871 plants ha^{-1}(Table 2).

Soil characteristics

pH. The pH showed significant differences among doses and plant densities in all evaluated years. In January (2008) the highest pH was at the control while the lowest was at 20 t ha^{-1}. In December (2008 and 2010) the highest pH was at 0 t ha^{-1} whereas in 2009 was at 40 t ha^{-1}. The lowest pH in 2008 (January), 2009 and 2010 was at 20 t ha^{-1}, while in 2008 (December) was lowest in 40 t ha^{-1} (Table 3). In 2008 (January), 2009 and 2010, the pH was highest in soil sampled in 8,871 plants ha^{-1}, and in 2008 (December) was highest at 13,323 plants ha^{-1}, while lowest pH was observed at 4,435 plants ha^{-1} in 2008 (January) and 2009 (Table 4).

Electrical conductivity (EC).

Electrical conductivity showed significant differences among doses and plant densities in 2008 (January and December) and 2010. Electrical conductivity showed highest values at 60 t ha^{-1} in 2008 (December), 2009 and 2010, while in January of 2008 was highest at 0 t ha^{-1}, showing that as doses of organic fertilizer increase, the values of EC increased, except during January (2008) where was lowest at 0 t ha^{-1}. The lowest EC was obtained at 0 t ha^{-1} in 2008 (December), 2009, 2010 but in 2008 (January) the lowest was in the control (Table 3). In 2008 (January), the highest EC was found in 4,435 plants ha^{-1}, while in 2008 (December) EC was highest in 8,887 plants ha^{-1}, but in 2009 and 2010 was highest in 8,871 plants ha^{-1} (Table 4).

Table 1. Effects of doses of organic and one inorganic fertilizer (control) in cladode yield, thickness, length and width of *Opuntia ficus-indica* Lisa cultivar.

Doses ($t\ ha^{-1}$)	**Cladode yield ($t\ ha^{-1}$)**			**Cladode thickness (mm)**		
	2008 **	2009 **	2010 ns	2008 ns	2009 ns	2010 **
0	15.33±3.50 b	20.86±1.45 c	44.53±9.22 a	18.88±0.53 a	12.58±0.56 a	22.31±0.86 bc
20	17.26±1.62 b	22.38±2.56 c	46.82±7.18 a	19.26±0.74 a	14.58±0.68 a	23.18±1.03 abc
40	18.71±3.84 ab	22.96±3.73c	62.93±6.69 a	20.15±0.86 a	16.13±2.39 a	23.93±1.07 ab
60	29.19±8.96 a	58.14±8.24 a	66.33±8.92 a	20.41±1.44 a	17.99±1.23 a	24.70±1.00 a
100-100-0*	11.77±1.82 b	40.37±4.47 b	64.10±9.21 a	19.54±0.58 a	17.47±1.06 a	21.61±0.91 c
	Cladode length (cm)			**Cladode width (cm)**		
	2008 **	2009 **	2010 **	2008 ns	2009 ns	2010 ns
0	24.78±0.55 c	25.66±0.51 bc	23.91±1.02 c	14.28±0.20 a	14.49±0.35 a	14.17±0.39 a
20	25.67±0.40 bc	26.04±0.42 abc	24.36±0.68 bc	14.33±0.28 a	14.68±0.17 a	14.49±0.22 a
40	26.42±0.51 ab	26.99±0.46 ab	26.81±0.91 ab	14.75±0.18 a	14.78±0.28 a	14.74±0.38 a
60	27.47±0.61 a	27.57±0.89 a	27.37±1.22 a	14.85±0.19 a	14.97±0.28 a	14.82±0.43 a
100-100-0*	25.06±0.51 bc	25.04±0.77 c	25.08±0.63 abc	14.20±0.19 a	13.91±0.36 a	13.77±0.33 a

* 100-100-0 is the control, an inorganic fertilizer at NPK dosages of 100-100-0 kg ha^{-1}, respectively. *= Significant at $p \leq 0.05$; **= Significant at $p \leq 0.01$; ns= not significant. Mean ± SE followed by the same letter in each column are not significantly different (Fisher LSD p=0.05).

Table 2. Effects of plant densities in cladode yield, thickness, length and width of *Opuntia ficus-indica* Lisa cultivar.

Densities	**Cladode yield (t ha^{-1})**			**Cladode thickness (mm)**		
(Plants ha^{-1})	2008 **	2009 ns	2010 **	2008 **	2009 ns	2010 **
4,435	10.32±2.06 b	28.04±2.56 a	37.18±4.19 c	18.78±0.42 bc	14.89±0.72 a	22.68±0.85 b
8,871	13.77±1.51 b	29.15±4.51 a	49.12±7.13 bc	21.20±0.31 a	16.85±0.64 a	25.55±0.61 a
8,887	14.06±1.71 b	35.12±7.94 a	64.32±9.16 ab	17.84±0.48 c	15.26±0.92 a	20.43±0.61 c
13,323	35.65±6.53 a	39.45±5.66 a	77.15±4.91 a	20.77±1.24 a	17.61±2.04 a	23.93±0.94 ab
	Cladode length (cm)			**Cladode width (cm)**		
	2008 ns	2009 *	2010 ns	2008 ns	2009 ns	2010 ns
4,435	25.37±0.72 a	25.20±0.41 b	25.55±1.21 a	14.22±0.21 a	14.16±0.22 a	14.28±0.34 a
8,871	26.73±0.49 a	26.50±0.89 a	26.95±0.63 a	14.69±0.21 a	14.49±0.35 a	14.89±0.31 a
8,887	25.89±0.31 a	26.81±0.43 a	24.98±0.63 a	14.49±0.17 a	14.75±0.26 a	14.24±031 a
13,323	25.54±0.44 a	26.53±0.49 a	24.55±0.82 a	14.52±0.20 a	14.87±0.23 a	14.18±0.33 a

*= Significant at $p\leq0.05$; **= Significant at $p\leq0.01$; ns= not significant. Mean ± SE followed by the same letter in each column are not significantly different (Fisher LSD p=0.05).

Organic matter content.

Soil organic matter content (OMC) showed significant differences among doses during all evaluated years, whereas for plant densities in 2008 (January and December) and 2010 only. The OMC increased in all years in 60 t ha^{-1}, while lowest OMC was in 0 t ha^{-1} or in the control (Table 3). The highest OMC was at 4,435 plants ha^{-1} during 2008 (January and December) and 2010, whereas in 2009 was highest at 13,323 plants ha^{-1} (Table 4).

Nitrates content.

Nitrates content (NC) showed significant differences among doses in 2008 (January and December) and 2010 and between plant densities in 2008 (January and December) and 2009. Highest NC was showed at 20 t ha^{-1} in 2008 (January) while in 2008 (December) and 2010 highest NC was at 60 t ha^{-1} increasing in 2008 (December) 2009 and 2010 as doses of organic fertilizer increased (Table 3). Except in 2008 (December) the highest NC was found in 4,435 plants ha^{-1} (Table 4).

DISCUSSION

In terms of organic fertilizer dosages and cladode yield, in this study the general trend found was that highest dosages increased cladode yield. Similar results have been found in other studies (Vázquez-Alvarado and Gallegos-Vázquez, 1995), where they found that highest dosages of organic fertilizer increased cladode yield. Also, Vázquez-Alvarado and Gallegos-Vázquez (1997a) studied different doses of cow manure (200, 400, and 600 t ha^{-1}) and found that the number of green cladodes “nopalitos” increased with dosages of 400 and 600 t ha^{-1}, hence increasing the overall cladode yield. In a second evaluation, Vázquez-Alvarado and Gallegos-Vázquez (1997b) found that the length and the weight of green cladodes “nopalitos” increase with very high dosages of 600 t ha^{-1} of cow manure (10X the optimum dosage of present study). Rodríguez *et al*. (2001) using doses of cow manure (0, 400, and 600 t ha^{-1}) found annual production of 10.86, 20.57, and 18.05 kg m^{-2}, respectively, being 400 t ha^{-1} the best dose. Similarly, Lopez *et al*. (1999) using three doses of cow manure (0, 690, and 940 t ha^{-1}) found that as doses increased, the nutrients concentration increased and the yield increased.

In this study, cladode yield increased in 2008, 2009 and 2010 with organic fertilizer at dosages of 60 t ha^{-1} and were higher in 2009 and 2010 than those reported by a recent study by Salazar-Sosa *et al*. (2010a) who reported a yield of 35.6 t ha^{-1} with organic fertilizer dosages of 60 t ha^{-1}; nevertheless the yields reported in this study, were lowest than those reported by Martínez-López *et al*. (2009) where they reported a yield of 74.3 t ha^{-1} of *Opuntia* green cladodes “nopalitos”; however it was with a higher organic fertilizer dosage (100 t ha^{-1}). Similarly, Zúñiga-Tarango *et al*. (2009) reported highest cladode yield of another *Opuntia* variety called "Jalpa" at 100 t ha^{-1} of organic fertilizer (solarized cattle manure). Reveles-Hernández *et al*. (2010) concluded that *Opuntia* cladode yield responds favourably to the application of fertilizer and high plant densities. Also, Murillo-Amador *et al*. (2005) reported

Table 3. Effects of doses of organic fertilizer and one inorganic fertilizer (control) in soil characteristics cultivated with *Opuntia ficus-indica* Lisa cultivar.

Doses	**pH**				**Electrical conductivity** (dS m^{-1})			
(t ha^{-1})	2008† **	2008†† **	2009†† *	2010†† **	2008 **	2008 **	2009 ns	2010 **
0	7.96±0.05 bc	8.18±0.03 a	7.62±0.05 ab	8.27±0.10 a	4.52±0.61 a	2.51±0.19 e	1.25±0.05 a	1.15±0.20 d
20	7.75±0.06 d	8.14±0.03 b	7.35±0.35 b	7.45±0.07 d	3.29±0.30 b	2.67±0.30 d	1.48±0.11 a	2.31±0.21 b
40	7.93±0.03 c	8.08±0.03 c	7.87±0.03 a	7.47±0.09 d	2.88±0.30 c	3.13±0.18 b	1.68±0.04 a	2.40±0.17 ab
60	7.99±0.03 ab	8.10±0.02 c	7.74±0.04 a	7.70±0.13 c	2.81±0.32 d	3.29±0.19 a	1.70±0.25 a	2.74±0.18 a
100-100-0*	8.02±0.04 a	8.10±0.01 c	7.72±0.05 a	8.02±0.15 b	2.63±0.05 e	2.91±0.30 c	1.46±0.10 a	1.54±0.12 c
	Organic matter (%)				**Nitrates (mg kg^{-1})**			
	2008 **	2008 **	2009 **	2010 **	2008 **	2008 **	2009 ns	2010 **
0	1.45±0.02 b	1.24±0.04 c	1.86±0.10 b	1.73±0.08 b	7.78±0.75 b	2.78±0.35 e	5.41±0.78 a	6.25±2.23 b
20	1.51±0.03 b	1.33±0.03 b	2.02±0.09 b	2.54±0.22 a	11.98±3.29 a	3.94±0.72 c	6.43±0.68 a	6.47±2.41 b
40	1.53±0.06 b	1.39±0.03 a	2.28±0.11 a	2.68±0.24 a	5.33±0.63 e	4.42±0.73 b	6.89±1.14 a	12.83±2.88 b
60	1.64±0.05 a	1.40±0.03 a	2.29±0.07 a	2.71±0.24 a	6.22±0.95 d	7.41±2.63 a	7.10±0.65 a	23.08±4.46 a
100-100-0*	1.44±0.08 b	1.10±0.07 d	1.84±0.10 b	1.54±0.18 b	7.00±1.37 c	3.61±0.54 d	6.12±0.85 a	13.74±2.97 b

* 100-100-0 is the control, an inorganic fertilizer at NPK dosages of 100-100-0 kg ha^{-1}, respectively.. †= January sampling. ††= December sampling. *= Significant at p≤0.05; **= Significant at p≤0.01; ns= not significant. Mean ± SE followed by the same letter in each column are not significantly different (Fisher LSD p=0.05).

Table 4. Effects of plant densities in soil characteristics cultivated with *Opuntia ficus-indica* Lisa cultivar.

Densities	pH				Electrical conductivity (dS m^{-1})			
(Plants ha^{-1})	2008† **	2008†† **	2009†† **	2010†† **	2008 **	2008 **	2009 ns	2010 **
4,435	7.84±0.05 c	8.11±0.01 b	7.36±0.28 b	7.95±0.15 a	4.56±0.49 a	3.20±0.16 b	1.56±0.08 a	1.68±0.19 b
8,871	8.00±0.02 a	8.10±0.01 bc	7.80±0.03 a	7.96±0.13 a	3.60±0.17 b	3.17±0.11 c	1.70±0.21 a	2.44±0.29 a
8,887	7.89±0.04 b	8.07±0.02 c	7.68±0.05 a	7.58±0.06 b	2.51±0.09 c	3.31±0.21 a	1.43±0.08 a	2.16±0.14 a
13,323	7.99±0.04 a	8.20±0.02 a	7.79±0.06 a	7.63±0.14 b	2.24±0.16 d	1.94±0.16 d	1.37±0.04 a	1.83±0.19 b
	Organic matter (%)				**Nitrates (mg kg^{-1})**			
	2008 **	2008 **	2009 ns	2010 **	2008 **	2008 **	2009 **	2010 ns
4,435	1.59±0.03 a	1.39±0.02 a	1.93±0.04 a	2.78±0.17 a	13.55±2.39 a	4.24±0.48 b	7.90±0.62 a	19.85±5.27 a
8,871	1.57±0.06 a	1.22±0.07 d	2.09±0.07 a	2.33±0.24 b	7.43±0.57 b	2.64±0.42 d	4.73±0.60 b	14.97±5.15 a
8,887	1.42±0.05 b	1.32±0.03 b	2.03±0.13 a	1.96±0.22 b	4.86±0.52 c	7.51±2.09 a	6.41±0.57 ab	10.17±2.29 a
13,323	1.47±0.04 b	1.25±0.04 c	2.18±0.12 a	1.89±0.17 b	4.80±0.87 c	3.35±0.46 c	6.52±0.94 a	10.51±4.49 a

†= January sampling. ††= December sampling. *= Significant at $p\leq0.05$; **= Significant at $p\leq0.01$; ns= not significant. Mean ± SE followed by the same letter in each column are not significantly different (Fisher LSD $p=0.05$).

that *Opuntia* responds well to the application of nitrogen, while López-García *et al*. (2003) reported that in Coahuila, Mexico, *Opuntia* cladode yield varies from 5 to 15 t ha^{-1}.

Organic fertilizer generally increased thickness, length and width of cladodes as doses increased. The general trend is in agreement with other studies, such as reported by Murillo-Amador *et al*. (2000) who used different doses of organic fertilizer (0, 400, and 800 t ha^{-1} of cow manure) and reported that the number, fresh and dry weight, length and width, stem area, and average weight of green cladodes increased as levels of doses of cow manure increased. With respect to cladode length, width, and thickness, Reyes-Agüero *et al*. (2005) reported minimum thickness of 10 mm and maximum of 30 mm in *O. ficus-indica* and concluded that thickness varies depending on the species.

A study carried out by Martínez-González *et al*. (2001) reported cladode thickness of the variety Copena T5 with maximum values of 28 mm and minimum 20 mm, values that are in the range those found at the present study. The cladode yield was largest with a plant density of 13,323 plants ha^{-1} for the three years of evaluation, showing that with higher plant densities, the yield increased. This is evidence that plant density had not reached limiting values since yield increased even though solar radiation and the supply of water and nutrients were constant. Similar results were reported by Ruiz-Espinoza *et al*. (2008) using plant densities of 30,000, 60,000 and 90,000 plants ha^{-1}, showing that cladode yield increased as plant densities increased. Also, the results are similar for those reported by García-Velázquez and Grajeda-Gómez (1991) and Murillo-Amador (1996).

In the present study, cladode length and width showed significant differences among planting densities during 2009 only, these results are contrary to those reported by Ruiz-Espinoza *et al*. (2008). However, they harvested green cladodes (nopalitos) in a short time (14 or 18 days) that showed minimum and maximum 11.99 and 14.26, 4.17 and 7.54 cm of length and width, respectively. In the present study, the cladodes harvested were mature not green cladodes and only two irrigation periods per year were applied, that are in the range of those reported by Reyes-Agüero *et al*. (2005), where they obtained minimum values of 27 cm of cladode length and maximum of 63 cm in one genotype of *O. ficus-indica*.

In terms of the soil pH with respect to organic fertilizer doses, the range of the pH was 7.35 to 8.27 and with different plant densities pH was 7.36 to 8.20. The values of pH found in the present study are in the range known to be optimal for cactus species (Flores-Ortiz and Reveles-Hernández, 2010). However, De Kock (2003) revealed that *Opuntia* tolerates relatively high values of pH and can support up to a pH of 8.5. The range of soil electrical conductivity (EC) in the doses of organic fertilizer was 1.15 to 4.52 dS m^{-1} while in the plant densities was 1.37 to 4.56 dS m^{-1}. These values are in the optimal range for *Opuntia* according to De Kock (2003) who reported that for a better development of the *Opuntia* plants, the maximum soil EC must not exceed 5-6 dS m^{-1}. In the present study, the EC values do not increased as doses of organic fertilizer increased, contrary to that indicated by Castellanos *et al*. (2000) who specified that with the application of manure, the EC tends to increase. The results of our study suggest that soluble ions may have moved deeper in the soil depth or were uptake by plants.

The soil organic matter content (OMC) in the doses of organic fertilizer showed the range of 1.10 to 2.71% and in the plant densities was 1.22 to 2.78%. A clear trend was observed when OMC increased in all years in a dose of 60 t ha^{-1}, i.e. OMC content increased as doses of organic fertilizer increased. Also, the OMC increased in 2009 and 2010 compared to 2008 (January and December) due to biodegradation of manure that contains more than 5% of organic matter; however, no clear trend was observed of increasing or decreasing OMC as plant densities increased. The results of this study coincides with those reported by Zúñiga-Tarango *et al*. (2009) where they concluded that manure applications in *Opuntia* increase the mineral content of organic matter in the soil at the end of the experiment. Meanwhile, Salazar-Sosa *et al*. (2010a) reported higher values of organic matter in the soil when used solarized cattle manure as organic fertilizer. Also, Salazar-Sosa *et al*. (2009) concluded that with application of 60 t ha^{-1} of bovine manure, the content of organic matter increased on the soil, which coincides with Castellanos *et al*. (2000).

Soil nitrates content (NC) in 2008 (December), 2009 and 2010 show a trend that as doses of organic fertilizer increased, NC in the soil increased, showing the lowest values at 0 t ha^{-1} of organic fertilizer No clear trend was found between NC and plant densities. As is known, nitrogen is also a required element for the plants because it is a structural constituent of components of the cellular wall, as well as non-structural components such as amino acids, enzymes, chlorophyll, and nucleic acids. Inorganic nitrogen is absorbed by the plants in the form of NO_3^-, although in some circumstances ions of NH_4^+ can be assimilated. The abundance and mobility of the nitrate ion has a very important position in the normal metabolism of the plants, being the higher nutrient in most soils as a nitrogen form and frequently it is the main limiting factor in plant growth. Also, nitrogen limits strongly the productivity of the plants. Therefore, any improvement in the absorbed efficiency of NO_3^-, can produce higher protein content and high yields (Gallegos-Vázquez, 1999).

Valdez-Cepeda and Blanco-Macías (2002) indicates that NO_3 are a form of nitrogen available to plants, is soluble and moves with the water applied to the soil, is easily leached. However, the results of our study do not match with these authors, because of the total soil nitrates content by doses, was highest in 2008 (January), decreased in 2008 (December) but increased newly in 2009 and 2010. When the total NC was analysed by densities, the NC showed the same trend, i.e. showed highest values in 2008 (January), decreased in 2008 (December) but increased again in 2009 and 2010. These results shows that some nitrates were leached from January to December 2008, but other nitrates remained leaving it available to the plants, which is an advantage because of the nitrates is the main way that it is absorbed by plants, including *Opuntia*. In *O. engelmannii* (Nobel *et al*., 1987) and *O. ficus-indica* Esmeralda variety (Mondragón and Pimienta-Barrios, 1990) found a positive response to the application of nitrogen fertilizers, which is expressed in terms of number of green cladodes. It has been demonstrated clearly that the increase of nitrogen induces vegetative and floral bloom (Valdez-Cepeda *et al*., 2003) and higher production of cactus pear (Nerd and Mizrahi, 1992).

Also, Gathaara *et al*. (1989) reported that applying high concentrations of nitrogen, the vegetative growth is increased in *O. engelmannii*; however, the nitrogen requirements are lower than that corresponding to production of green cladodes “nopalitos” and cladodes for propagation and animal consumption (Valdez-Cepeda *et al*., 2003) Fertilization with inorganic products that contain nitrates or organic products such as cow manure increases the growth of agaves and cacti (Nobel, 1998). However, caution should be exercised regarding nitrogen fertilization because *Opuntia* species has the particularity of being able to absorb and to accumulate nitrates easily in its cladodes. Therefore, rooted cladodes can have toxic levels of nitrates for people and animals that consume those (Valdez-Cepeda *et al*., 2003). It is necessary to define the need for fertilization based on the chemical composition of the young harvested cladodes of different ages because fertilization changes according the production system.

In this study and in terms of soil, the increase of cladode yield in *Opuntia* with organic fertilizer dosages was favoured positively because of the soil type where the experiment was carried out was clay. Clay is a soil type that is known to retain much organic matter (Amato and Ladd, 1992; Hassink, 1994) since it tends to provide higher bio protection of organic matter degradation (Van Veen and Kuikman, 1990). This bio protection occurs when the organic matter is adsorbed on the surface of the particles of clay, or when it is embedded or coated by clay minerals (Golchin *et al*., 1994) or when located within the micro-aggregates away from the microorganisms (Elliott and Coleman, 1988).

One principal goal of agriculture is to produce crops that limit undesirable environmental effects and are eco-friendly, for this reason organic crop production has grown. However, it is desirable that crop yields be comparable or superior to conventional fertilization. In a review of organic crop yields, Kirchmann *et al*. (2008) reported yields of 25 to 50% lower than conventionally fertilized crops, and one main reason is lower nutrient availability, especially of nitrogen in manure fertilized crops (Kirchmann *et al*., 2016).

For conventional crops, yield of crops as wheat and rice is better with inorganic fertilization, presumably than with organic manure due to better nitrogen uptake. In contrast for our study, *Opuntia* had greater yield with organic fertilization than with conventional inorganic fertilization (100-100-0 of N, P and K, respectively). Why this is the case is unclear, however there are several possibilities. Even if it is assumed that nitrogen uptake is usually better with conventional fertilization, nonetheless this may have not played a factor if original soil is already rich in nitrogen which coincide to Falconnier *et al*. (2016) who reported that original soil type might influence yield. Another possibility is that *Opuntia* -a CAM plant-, and there are reports that nitrogen uptake is different compared to C_3 and C_4 plants (Bloom *et al*., 2012).

CONCLUSIONS

In the three years evaluated (2008, 2009 and 2010) it was found that significantly largest cladode yield occurred at the highest organic fertilizer dosage of 60 t ha^{-1} for a yield of 29.19±8.96, 58.14±8.24 and 66.33±8.92 t ha^{-1} in 2008, 2009 and 2010, respectively. In the three years evaluated it was found that thickness, length and width of cladodes were largest in 60 t ha^{-1} of organic fertilizer dosage, although in many cases not significantly different from other dosages. The results support the notion that higher organic fertilizer dosages provides nutritive components that allows for the growth and development of cladodes.

In terms of plant densities, in all three years it was determined that the yield was not affected negatively by the highest planting density of 13,323 plants ha^{-1}, in fact at this density the largest or not significantly different values where found for yield, thickness, length and width when compared to the lower planting densities. Results suggesting that higher planting densities are conceivable, since the inflection point where yield decreased due to plant overcrowding were not found.

It was found that soil organic matter and nitrate content increased in all years in the highest organic fertilizer dosage of 60 t ha^{-1}. In general, it was found that for all 3 years that the pH and electrical conductivity at the highest organic fertilizer dosage (60 t ha^{-1}) and highest planting density (13,323 plants ha^{-1}) showed inacceptable ranges and was not significantly worse than the chemical fertilizer, or the other lower dosages or planting densities.

Organic fertilizer based on solarized cattle manure provided largest yield compared to conventional inorganic fertilization, offering the possibility of reducing the use of chemical fertilizers whereas maintains soil quality; however, more studies will be required to unravel the better response of *Opuntia* with solarized cattle manure as an organic fertilization compared to conventional inorganic fertilization.

ACKNOWLEDGEMENTS

We would like to thank Diana Dorantes and Mike Matson for the help in reviewing the English of the manuscript. This research was supported by grants from Universidad Juárez del Estado de Durango and Centro de Investigaciones Biológicas del Noroeste, S.C. Corresponding author thanks to Consejo Nacional de Ciencia y Tecnología Mexico for the support granted to stay sabbatical abroad through the program “Support for sabbatical stays linked to the consolidation of research groups and/or strengthening of the national postgraduate program 2017-I”.

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