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Article

Halophilic rhizobacteria maintain the forage quality of *Moringa oleifera* grown on a saline substrate

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Abstract:

In order to obtain an increase in the forage production of high-quality *Moringa oleifera* Lam., the use of cow dung manure can be combined with the inoculation of biofertilizers based on plant growth-promoting rhizobacteria (PGPR). This production was assessed in a greenhouse in Torreón, Coahuila, Mexico. Cow dung manure was utilized as part of the substrate (50% compost, 40% sand, and 10% perlite). Three inoculations into the tree were scheduled (at 40, 74 and 152 d after planting) with four PGPR strains; the treatments were: T1: *Bacillus paralicheniformis,* T2: *Acinetobacter guillouiae,* T3: *Aeromonas caviae,* T4: *Pseudomonas lini* and Control: Bacteria-free; strains from Poza Salada, Valley of Sobaco, Coahuila, Mexico. Three harvests were collected in the 2016-2017 summer-fall-winter cycle. Agronomic and bromatological variables were assessed in order to determine the production and the quality of the tree leaves. The *Pseudomonas lini* and *Bacillus paralicheniformis* strains provided a positive response in the development of forage *M. oleifera* during the summer-fall period, increasing the height in the first weeks of development and providing thicker, firmer diameters. The yield and the bromatological variables exhibited no differences between treatments; however, a good-quality forage was produced. In average, the leaves exhibited 13.56 % ashes, 70.15 % total digestible nutrients, 93.16 % *in vitro* digestibility of dry matter, 19.72 % neutral detergent fiber, 25.35 % acidic detergent fiber, and 24.15 % crude protein.

Key words: Biofertilizers, Biomass, Compost, Digestibility, Fertilizers, Inoculation, Protein, Ruminants.

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Introduction

The diet of ruminants, particularly of milk-producing cattle, must provide high levels of energy and protein[(1)]. Conventional feed concentrates are usually expensive[(2)]. The *Moringa oleifera* Lam. tree is a species with a high nutritional value and a good production of biomass, reaching an annual production of 25 $t\ ha^{-1}$ under dry tropical forest conditions[(3)]. In addition, the feeding costs are relatively low, ten times lower when using *M. oleifera* than when using balanced feeds[(4)]. Rations for milk-producing cattle formulated with *M. oleifera* forage provide a high protein value ranging between 15 and 30 % of NDF, with digestibility levels

of 52 to 85 %[5]. The supply of a fresh *M. oleifera* diet may give an unpleasant taste and smell to the milk; however, if the diet contains *M. oleifera* silage, the milk will exhibit good organoleptic characteristics[6]; therefore, *M. oleifera* is an option for supplementing the diet of the milk-producing cattle.

On the other hand, the intensification of the production of the milk industry increases the generation of dung, which entails a risk of contamination[7]. The excreta of the milk-producing cattle have less environmental impact when they are used as organic fertilizers[8]. However, they must be used carefully, as the five soluble salts accumulated (Na, K, Ca, Mg and S) may generate adverse effects[9]. The increase in salinity influences the quality of the forage, including organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF)[10]. More than half the Mexican territory is arid and semiarid, and its natural diversity, including its soil, is under threat[11]. The scenarios predicted for the future according to the climate change show the growing risk of salinization in various latitudes, which would require a special effort for maintaining the production of crops under saline stress[12].

The use of plant growth-promoting rhizobacteria (PFPR) based biofertilizers is an option for reducing the contamination of the soil, which is also partly caused by nitrogenous fertilizers[13]. The PGPRs have beneficial effects on the plants through direct and indirect mechanisms, such as nitrogen fixation, synthesis of phytohormones, phosphorus solubilization, secretion of siderophores, increased permeability of the roots, and induced systemic resistance, among others[14,15]. In fact, the inoculation of various strains of PGPR allows the development of plants in drought-stricken places, on soils that are contaminated with heavy metals, and even on saline soils[16]. The hypothesis assumes that, if crops are produced on heavy soils or on substrates with a high electrical conductivity, it is possible to inoculate halophilic PGPR in order to obtain a good yield and increase the quality, in this case, of *M. oleifera* forage. The purpose of this research was to assess the quality of the production of *M. oleiferea* inoculated with halophilic PGPR as forage, using compost and compost tea ―both from cow dung ― to irrigate the substrate.

Material and methods

This research was carried out at the "Antonio Narro" Autonomous Agricultural University, Lagoon Unit (Universidad Autónoma Agraria Antonio Narro Unidad Laguna, UAAAN-UL), located in Torreón, Coahuila, Mexico, at an altitude of 1,120 m asl, during the 2016-2017 summer-fall-winter cycle. The maximum and minimum temperatures were registered during the experiment.

A random blocks experimental design was utilized, with five treatments with four different PGPR (Table 1) and five repeats per treatment. The PGPR were provided by the Faculty of Biological Sciences of the Juárez University of the State of Durango, having been isolated from the rhizosphere of *Distichlis spicata* halophilic grass from Poza Salada in the Valley of Sobaco, in the municipality of Cuatrociénagas, Coahuila, Mexico[17].

Table 1: Characteristics of plant growth-promoting rhizobacteria (PGPR) used in each treatment (T)

	Strain ID	Bacterium Genus/Species	Production of IAA (µg)	PS	DPS (mm)	TS (%)
T1	LBEndo1	*Bacillus paralicheniformis*	23.444±2.531	+	4.589±0.221	15
T2	NFbEndo 2M2	*Acinetobacter guillouiae*	+	+	+	<5
T3	KBEndo3	*Aeromonas caviae*	+	+	+	<5
T4	KBEcto4	*Pseudomonas lini*	36.730±0.011	+	4.112±0.042	15
Co	Bacteria-free					

IAA= Indole-acetic-acid; PS= production of siderophores; DPS= degree of phosphate solubilization; TS= tolerance to salinity; Co= control.
Source: Palacio-Rodríguez *et al*[17].

Direct planting of *M. oleifera* L. in black polyethylene bags with an 18 L capacity, on July 10, 2016. The utilized substrate was a mixture of compost (50 %), sand (40 %) and perlite (10 %). The compost was acquired at the Ampuero ranch; the solarization method was applied to it before use[18]. One seed was placed in each bag at a depth of 5 cm. Before planting, the substrate was washed with one liter of water per kilogram of substrate, in order to reduce its salinity. The plant pots were arranged in four rows, with a topological herringbone arrangement, with a separation of 0.25 x 0.25 m between stems and with a density of 16 trees m^{-2}.

The bacteria were inoculated 40 d after the planting, placing 3 mL at a concentration of $1x10^8$ ufc mL^{-1} of PGPR at the stem base; other inoculations were carried out 8 d after the first and second cuttings.

The variables evaluated were: yield, bromatological variables and leaf/stem ratio. The sampling was carried out when the tree reached an average height of 1.50 m and before the beginning of the flowering, leaving a remaining forage at a height of 0.25 m.

Irrigation was applied with 1 L compost tea, every other day. This tea was obtained by submerging 5 kg of cow dung compost in a net within 200 L of water. The water was placed on the previous days in order to allow the chlorine that it might have contained to evaporate. In each preparation, 90 g of unrefined brown sugar were added, and aerators were placed within the 200 L container during 12 h. After this time, the net that contained the compost was removed, and the compost tea was ready to be used. Table 2 shows the chemical composition of the compost and of the compost tea thus obtained.

Table 2: Chemical composition of macro- and micronutrients of the compost and compost tea utilized for the substrate and for irrigation of *M. oleifera*

			----------Macronutrients----------						
Component	**pH**	**CE**	**N**	**P**	**K**	**Ca**	**Mg**	**Organic carbon**	**OM**
		mS/cm	----------%----------						
Compost	8.35	12.77	0.11	0.45	2.95	18.8	0.94	17.75	30.60
Compost tea	7.52	3.27	0.25	0.15	0.28	1.33	0.12	-----	0.52
			----------Micronutrients----------						
Component	pH	CE	Na	Fe	Cu	Zn	Mn	Bo	
		mS/cm	%	----------ppm----------					
Compost	8.35	12.77	0.43	5100.00	62.00	200.00	390.00	1.00	
Compost tea	7.52	3.27	0.18	3.21	0.86	2.96	3.44	-----	

The only infestation which occurred during the experiment was with *Tetranychus urticae*, which was controlled by means of applications of eBioluzión Plus vO® (Febea bio), a broad-spectrum organic insecticide.

The agronomic variables —height of the tree, stem diameter, number of stalks, number of leaves, leaf size, root weight, fresh and dry weight of the forage, yield, leaf/stem ratio— were evaluated once a week.

The height of the tree was measured with a grade rod placed at the basal part of the soil and measuring the height up to the apex of the apical branch. The stem diameter was measured with a caliper, 3 cm above the stem base. The leaf size was measured from the primary rachis to the apical foliole, using a measuring tape. A digital scale was used to weigh the root, for which purpose the stem was cut from the base, and all the substrate was removed in order to maintain the largest amount of root. The forage was weighed fresh in a digital scale at the moment of cutting with pruning shears, leaves and stems together and separately (leaves without the rachis and stalks including the rachis of the leaves). In order to estimate the dry weight, the forage samples were taken to the laboratory; each sample was placed in a paper bag with its respective label and dried in a forced-air oven at 60 °C during 24 h until obtaining a constant weight. The dry weight was estimated using an analytical scale and was subsequently utilized as a basis for estimating the dry matter yield, by adding the dry weight of the leaves and that of the stalks and stem. The leaf/stem ratio was estimated by dividing the dry weight of the leaves (DWL) by the dry weight of the stems (DWS) with the equation L/S = DWL/DWS.

The bromatological variables were measured only at the last cutting and included: fresh matter (FM), dry matter (DM), ashes, fat, neutral detergent fiber (NDF), acidic detergent fiber (ADF), crude fiber (CF), crude protein (CP), nitrogen-free extract (NFE), non-fibrous carbohydrates (NFC), in vitro dry matter digestibility (IVDMD), net energy lactation (NE_L), and total digestible nutrients (TDN).

The samples were dried at 60 °C during 24 h, until a constant weight was obtained, and subsequently crushed through a 1 mm sieve before analysis. The ashes were analyzed using the AOAC procedure[19]. The fat was drawn using the Goldfisch method. The NDF and the ADF were obtained using the Van Soest method[20]. The CF was determined with the Weende method. CP was determined with the Kjedahl method. The IVDMD was obtained using a Daisy incubator (Ankom Technology). The NFE, NFC, NFE and TDN were calculated using the following formulas: NFE (%) = 100 – (DM + CP + CF + Fat + Ashes), NFC (%) = 100 – (CP + NDF + Fat + Ashes), NE_L=1.044 - (0.0124*ADF) and TDN = 31.4 + (53.1* NE_L).

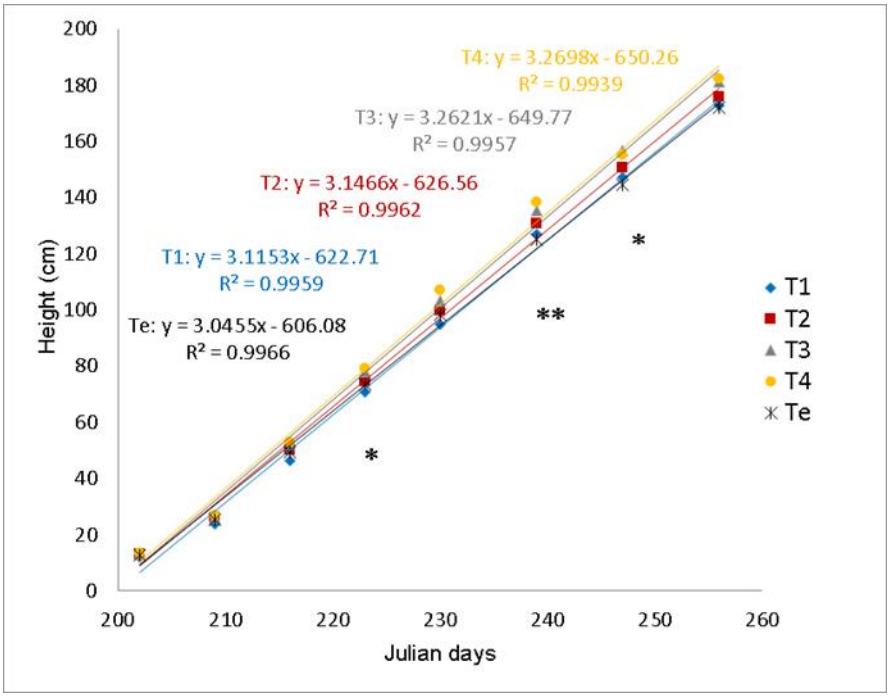
The variables were subjected to a variance analysis using the SAS statistical software for Windows, version 9.0. In those cases in which differences were found between means, the least significant difference (LSD) test was applied, with a significance of α= 0.05. The Microsoft Excel 2010 software was utilized to determine the regression equation for the variable height.

Results

Growth

The growth of the *M. oleifera* tree from the time of the planting to the first harvest exhibited a significant difference between the treatments on the Julian days 216 to 247. In the last week before the first harvest, the growth was not affected by the applied treatments and exhibited no significant differences; this may be due to the onset of the flowering (Figure 1). This growth occurred in the summer, which shows a linear tendency. The tree height increased in average 3.16 cm per day, attaining a mean height of 1.76 m at d 66. During this period, the tree exhibited the greatest growth in the experiment. The temperatures shown in this period, ranging between 20-22 °C and 38-42 °C, favored growth.

Figure 1: Growth of *Moringa oleifera* from the time of the planting to the first harvest (66 days) 2016



T1: *Bacillus paralicheniformis*, T2: *Acinetobacter guillouiae*, T3: *Aeromonas caviae*, T4: *Pseudomonas lini*, Co: Bacteria-free. (*, **: Indicates a significant and a highly significant difference, respectively, between treatments for the respective date).

In the second harvest of the tree, a significant difference in growth was obtained from Julian day 277 to Julian day 299, except for the five weeks that preceded the harvest. This second harvest took place in the fall; the growth also exhibits a linear tendency. However, it shows a reduction of 52 % with regard to the summer growth. The average increase is 1.51 cm per day. This decrease, along with the lowering of the temperature, ranging between and 15-16 °C and 36-40 °C, is ascribed to the change of season. The mean height of 1.50 m for cutting was reached 77 d after the first harvest. The tree required 11 d more to attain the average height before harvesting.

After the second harvest, which coincided with the beginning of the winter, the tree did not exhibit any growth during the first month, due to the low temperatures, which ranged between 9.5-10.5 °C and 32.5-35.5 °C. Thus, the third harvest showed no significant difference between treatments. The data of the growth resemble a second-degree polynomial regression due its slowness. The average height for the cutting was reached after 117 d. The tree required 40 d more than the second harvest, and 51 d more than the first harvest, to attain the average height for cutting. The first flowerings (2 %) occurred during this last harvest. The pest that befell during the development of the experiment was *Tetranychus urticae*.

Agronomic variables

Of the assessed agronomic variables, the stem diameter exhibits a significant difference between treatments in the second and third harvest (Table 3). The treatments that had the largest stem diameter were T1: *Bacillus paralicheniformis* and T4: *Pseudomonas lini*, which are statistically equal. After the first harvest, growth begins again; the main stem, which is 0.25 cm high, has side shoots or secondary stems. The number of secondary stems varies; in the experiment, up to 8 side shoots were registered, but only 1 to 4 developed satisfactorily. The same happened after the third harvest.

Table 3: Means for the variables stem diameter (SD), number of stems (NoS), number of leaves (NoL), leaf size (LS) and root weight (RW), in the evaluation of *M. oleifera*

	First harvest									
Treatment	**SD (cm)**		**NoS**		**NoL**		**LS (cm)**		**RW (g)**	
T1	1.413	a	1.00	a	14.56	a	41.75	a		
T2	1.363	a	1.00	a	14.88	a	41.72	a		
T3	1.381	a	1.00	a	14.31	a	41.66	a		
T4	1.450	a	1.00	a	14.13	a	43.69	a		
Co	1.394	a	1.00	a	14.19	a	42.59	a		
	Second harvest									
T1	2.110	a	1.85	a	11.90	a	40.95	a		
T2	1.915	b	1.65	a	11.70	a	42.45	a		
T3	1.915	b	1.65	a	11.35	a	37.25	b		
T4	2.015	ab	1.60	a	11.65	a	42.75	a		
Co	1.930	b	1.60	a	11.50	a	43.00	a		
	Third harvest									
T1	2.440	a	2.85	a	14.60	a	34.05	a	220.35	b
T2	2.230	bc	3.35	a	14.65	a	33.20	a	178.90	bc
T3	2.195	c	2.80	a	16.40	a	33.85	a	159.10	c
T4	2.355	ab	2.75	a	15.00	a	36.50	a	341.50	a
Co	2.190	c	2.70	a	11.65	b	36.35	a	156.10	c

T1= *Bacillus paralicheniformis*, T2= *Acinetobacter guillouiae*, T3= *Aeromonas caviae*, T4= *Pseudomonas lini*, Co= Bacteria-free.

[ab] Different letters indicate a significant difference between treatments ($P<0.05$).

The root of *M. oleifera* is bulbous. The weight of the root collected in the third harvest exhibits a significant difference between treatments, being the largest ―341.54 g― in T4, *Pseudomonas lini*.

In the third harvest, the number of leaves is statistically equal between the treatments, but higher than in the control. Treatment T3: *Aeromonas caviae* and T4: *Pseudomonas lini* have the largest number of leaves —16.4 and 15 leaves, respectively.

The size of the leaves in the trees is not affected by the treatments applied to any of the three harvests. The leaf size decreases with successive cuttings. The lengths of treatment T4, *Pseudomonas lini*, are 43.69, 42.75 and 36.50 for the first, second and third harvest, respectively.

The average yield of fresh matter was 9.44 t ha^{-1}, and that of leaf dry matter was 4.86 t. The average yield of stem fresh matter was 25.08 t, and that of stem dry matter, 7.08 t. The yield was not affected by the applied treatments (Table 4).

Table 4: Means for the yield variable in t ha^{-1} in the evaluation of *M. oleifera* inoculated with four PGPR for the first, second and third harvest

	First harvest		**Second harvest**		**Third harvest**		**Total**	
	LY	**SY**	**LY**	**SY**	**LY**	**SY**	**LY**	**SY**
Fresh matter								
T1	2.68	9.06	3.47	9.08	3.64	8.20	9.79	26.34
T2	2.58	9.10	3.30	7.95	3.66	7.14	9.54	24.19
T3	2.59	9.07	2.80	6.26	3.51	6.37	8.90	21.70
T4	2.52	10.04	3.19	8.73	3.83	8.53	9.54	27.30
Co	2.61	9.10	3.50	8.85	3.31	7.92	9.42	25.87
Dry matter								
T1	1.40	2.49	2.04	2.79	1.43	2.01	4.87	7.29
T2	1.36	2.46	2.04	2.60	1.41	1.82	4.81	6.88
T3	1.39	2.50	1.95	2.40	1.41	1.72	4.75	6.62
T4	1.41	2.64	2.06	2.75	1.49	2.03	4.96	7.42
Co	1.47	2.54	2.07	2.74	1.39	1.90	4.93	7.18

T1= *Bacillus paralicheniformis*, T2= *Acinetobacter guillouiae*, T3= *Aeromonas caviae*, T4= *Pseudomonas lini*, Co= bacteria-free; LY= leaf yield; SY= stem yield.
(P>0.05).

The leaf/stem ratio exhibits an increase with successive harvests. The average leaf/stem ratio of the first harvest was 0.556; in the second harvest it was 0.768, and in the third, 0.754. The average leaf percentage was 35.72, 43.40 and 42.96 % for the first, second and third harvest, respectively. Table 5 shows the leaf/steam ratio and the leaf percentage exhibited by the tree; these variables were not affected by the treatments.

Table 5: Table of the leaf/stem ratio and leaf percentage in the evaluation of *M. oleifera* inoculated with four PGPR in each of the three harvests

	Leaf/stem ratio	Leaf percentage
First cutting		
T1	0.561	35.9
T2	0.552	35.5
T3	0.555	35.7
T4	0.534	34.8
Co	0.580	36.7
Second cutting		
T1	0.731	42.2
T2	0.787	44.0
T3	0.813	44.8
T4	0.751	42.9
Co	0.757	43.1
Third cutting		
T1	0.709	41.5
T2	0.775	43.7
T3	0.822	45.1
T4	0.734	42.3
Co	0.731	42.2

T1= *Bacillus paralicheniformis*, T2= *Acinetobacter guillouiae*, T3= *Aeromonas caviae*, T4= *Pseudomonas lini*, Co= bacteria-free.
(P>0.05).

Bromatological variables

Although the chemical composition of the tree was determined only in the last harvest, the values obtained are very good. In average, 13.5 % of leaf ashes, 70.15 % of TDN, 93.6 % of IVDMD, 19.72 % of NDF, 25.35 % of ADF, and 24.15 % of CP. The averages obtained for the stems were 11.21 % of ashes, 45.32 % of TDN, 61.83 % of IVDMD, 59.07 % of NDF, 58.01 % of ADF and 7.23 % of CP. Table 6 lists the bromatological variables analyzed for each treatment.

Table 6: Means by treatment of the bromatological analyzes expressed as percentages

Treatment	T1	T2	T3	T4	Co
Leaf					
FM	74.01	74.87	73.22	73.62	73.23
DM	25.99	25.13	26.78	26.38	26.77
Ashes	13.55	14.55	13.78	12.89	13.07
Fat	4.49	4.70	4.77	4.92	4.29
NDF	19.26	20.24	20.09	19.33	19.70
ADF	24.77	24.06	25.53	26.39	26.01
CF	9.70	9.33	9.61	9.12	9.05
CP	23.79	24.45	23.46	23.57	25.49
NFE	22.48	21.84	21.61	23.11	21.33
NFC	38.91	36.06	37.91	39.28	37.45
IVDMD	94.89	91.70	93.49	93.98	91.76
NE_L	0.737	0.746	0.727	0.717	0.722
TDN	70.53	70.99	70.02	69.46	69.71
Stem					
FM	81.78	82.76	81.71	82.82	82.72
DM	18.22	17.24	18.29	17.18	17.28
Ashes	11.21	11.47	11.07	10.68	11.63
Fat	2.02	1.99	1.95	1.74	1.61
NDF	58.62	56.77	58.27	60.94	60.75
ADF	60.71	57.99	67.94	64.66	63.95
CF	37.62	38.67	38.06	39.18	41.36
CP	7.49	7.00	7.28	6.84	7.55
NFE	23.44	23.64	23.37	24.38	20.57
NFC	20.66	22.79	21.44	19.82	18.46
IVDMD	60.38	65.38	61.90	60.86	60.65
NE_L	0.291	0.325	0.202	0.242	0.251
TDN	46.87	48.66	42.10	44.26	44.73

T1= *Bacillus paralicheniformis*, T2= *Acinetobacter guillouiae*, T3= *Aeromonas caviae*, T4= *Pseudomonas lini*, Co= bacteria-free.

Fresh matter (FM), Dry matter (DM), neutral detergent fiber (NDF), acidic detergent fiber (ADF), Crude fiber (CF), Crude protein (CP), Nitrogen-free extract (NFE), non-fibrous carbohydrates (NFC), in vitro dry matter digestibility (IVDMD), net energy lactation (NE_L), total digestible nutrients (TDN).

(P>0.05).

Discussion

Growth

At the germination state, *M. oleifera* reaches a growth rate of 100 % when using direct planting in the bags, in a substrate with an electrical conductivity (EC) of 12.77 mS/cm. This agrees with the findings of Noreem *et al.*[21] in the sense that *M. oleifera* seeds germinate only at salinity levels of 5 and 10 dS/m and at EC levels ≤ 15 and 20 dS/m. The average growth of *M. oleifera* trees obtained with the 4 treatments and by the control of the experiment for the first, second and third harvests is 176.75 cm, 140.39 cm and 120.50 cm, respectively. The time intervals for each harvest were 66, 77 and 117 d, respectively. In a comparative study under similar conditions to those of this experiment, *Moringa oleifera* and *Leucaena leucocephala* obtained 95 % germination between the time of germination and the initial growth phase; at 13 weeks, the seedlings reached a height of 53.2 cm at d 91, using in the substrate 60 % alkaline loamy silt, 10 % sand, and 20 % composted cow dung[22]. The halophilic PGPR inoculated into the *M. oleifera* trees allowed a satisfactory growth. T4 exhibited a significant height between treatments of 138.31 cm at d 47, which amounts to a 61.68 % increase, compared to that obtained by the abovementioned authors and in half the time.

M. oleifera has a slow initial growth rate at the low temperatures of the fall-winter season[23]. Tropical climates seem to be the best for growing *M. oleifera*; yet, a limited but satisfactory growth can be attained in less than optimal climates, since the trees seem to tolerate a lower growth temperature through physiological adaptations[24]. The findings of the authors agree with those obtained through the present experiment. Evidently, the growth of the *M. oleifera* tree is affected by a reduction in the temperatures and by attack by *T. urticae*. However, the growth persisted.

Agronomic variables

The nutritious quality of *M. oleifera* is determined partly by the conditions in which it develops. Low temperatures delay its growth[22]. The planting density affects the development of the roots, the stem diameter, and the biomass. The higher the planting density, the thinner and more fragile the stem diameter[25]. In this experiment, the trees were planted in black polyethylene bags, with a density of 16 trees m^{-2}, where none of the alterations indicated by the abovementioned authors are to be expected. Using lower planting

densities favors harvesting at greater heights, as well as the development of thicker stems and a larger number of side shoots[26].

However, Figure 2 shows the lodging exhibited by the control treatment before the third harvest, due to the thin stems and to the salinity saturation resulting from constant irrigation with compost tea, which had an EC level of 3.27 mS/cm. It may be said that inoculation with halophilic PGPR provides greater firmness and thickness, and therefore greater resistance, to the stems. A comparative study of the germination and initial growth phases resulted in a 0.92 cm diameter at wk 7, which is far less than the stem thickness obtained in the present experiment. The number of leaves per branch obtained was 16, which is similar to that obtained in this experiment[21]. The thinness of the stems is caused by the high concentrations of Na+ in the solution, which inhibits the absorption of nutrients, causing a reduction in the K+ and Ca+ concentrations in the tissues of the stems and the root[27]. The root is an essential part for the development of plants. A well-developed root can draw more nutrients, as well as more Ca^{2+}, which provides firmness and structure to the cell wall. Halophilic PGPR allow the absorption of nutrients in saline substrates without causing nutritional disorders in successive harvests.

The experiment exhibits a reduction of the leaf size with successive harvests and with the changing seasons and temperatures. These changes cause the loss of basal leaves, which has an impact on the yield. Table 4 shows the yield of the *M. oleifera* tree, considering a density of 16 trees m^{-2}. Most other researches by other authors are open-air, while a few others are carried out in a greenhouse, but only at germination and seedling level. In an open-air research carried out in northeastern Mexico with a density of 11 and 33 trees m^{-2}, respectively, 14.4 and 14.5 t ha^{-1} of total dry matter were obtained in all three harvests[28]. In Nicaragua, the open-air biomass production was evaluated at various planting densities, reaching a DM production of 11.6 t ha^{-1} after one year, with a density of 100 000 trees per ha and eight harvests per year[3]. A study on the open-air establishment of *M. oleifera* with various planting densities resulted in 100.98 g of DM at a density of 98,764 trees per ha[24].

Figure 2: Lodging of *Moringa oleifera* due to thin stem diameters in treatment **Co**, bacteria-free, during the period between the 2016-2017 second and the third harvests (117 days)

M. oleifera leaves are the part that contains the largest amount of usable nutrients. The stems provide nutrients in lower amounts. The leaf/stem ratio presented in Table 5 shows the proportion of grams of leaf DM per gram of stem. It may be observed that the average proportion increased by 0.22 g of leaf DM in the second harvest. This may be ascribed to the number of stalks. In the second and third harvest, the proportion remained similar. In the study carried out in northeastern Mexico, the leaf/stem ratio was lower in the second harvest, and higher in the third[27]. The purpose of producing good forage with this type of trees is to obtain the largest amount of leaves and the lowest number of stems.

Bromatological variables

Forages are sensitive to salinity at various degrees. As salinity increases, its biomass is reduced[10]. The bromatological analysis to which *M. oleifera* trees were subjected (Table 6) shows that the nutritional content is good, i.e. despite having grown on a saline substrate, the amount of forage did not diminish. Although no difference is shown between the treatments, It is possible to speculate that, at the fourth cutting, the control treatment will reduce its quality and biomass due to salinity saturation. A study of the chemical composition of the

leaves of *M. oleifera* trees planted at an altitude of 1,100 masl estimated a content of ashes of 13.3 %; 29 % of CP, 8.5 % of crude fiber (CF), 42.7 % of nitrogen-free extract (NFE), 16.8 % of NDF, 12.1 % of ADF, and 34.5 % of non-fibrous carbohydrates (NFC)[29]. The data obtained by these authors are very similars to those obtained in this research at a similar altitude.

The bromatological characterization of *M. oleifera* leaves carried out at different stages of growth without irrigation or fertilization determined that, as the age of the side shoot increases, its nutritional quality decreases; the amount of NDF and acidic detergent lignin increases, CP, IVDMD and TDN decrease[30]. This situation did not occur in the present research. The various researches on *M. oleifera* have yielded different results as to the bromatological analyses, but the variations are due to the diverse conditions under which the tree is produced.

Furthermore, attempts have been made to determine the optimal cutting time for *M. oleifera* in which the best quality forage may be obtained. The open-air and rain-fed production of *M. oleifera* and its chemical composition were assessed at various densities and cutting times, and a recommendation was made to harvest *M. oleifera* at intervals of 75 d in order to obtain a better quality forage and a larger DM yield, as the nutritional value of the *M. oleifera* forage in terms of CP and IVDMD remains constant at different intervals of the harvest. The first year, with 8 harvests, yielded 18.54 % of DM, 8.58 % of ashes, 32.12 % of NDF, 22.76 % of ADF, 22.63 % of CP, and 70.09 % of IVDMD[31].

M. oleifera has been utilized as an alternative protein supplement. Various bovine and caprine species have been fed with different percentages of *M. oleifera* in combination with diverse forages and concentrates[2,32-34]. This protein supplement may be administered fresh or as silage; the chemical composition does not present much variation. Fresh *M. oleifera* had 19.3 % DM, 24.10 CP, 45.3 % NDF, 29.9 % ADF, and 10.3 % ashes, while *M. oleifera* silage had 26.70 % of DM, 22.6 % of CP, 43.50 % of NDF, 29.10 % ADF, and 11.6 % ashes. The considerable difference between these two forms of supplement is the strong taste that fresh *M. oleifera* gives to milk, whereas *M. oleifera* silage does not change its organoleptic characteristics[6]. The results obtained in this research meet the parameters required in a forage for its inclusion in the formulation of a balanced diet (Table 6). The CP and the IVDMD are indicative values of a good forage (24.15 and 93.16 %, respectively), which were obtained in this research. As stated above, the higher the percentage of IVDMD, the lower the content of lignin. A high percentage of IVDMD indicates that the consumption of DM in animals will increase. The previous researches show that, in average, the forage contains the elements required to benefit the animals that ingest it. Even when produced under saline conditions and inoculated with the halophilic PGPRs necessary for its development, it is possible to obtain a high quality forage, as was the case in the present experiment.

Conclusions and implications

The results of this study show that growing *M. oleifera* under saline conditions and with inoculation with halophilic PGPRs does not lower its quality for use as forage and allows it to meet the characteristics required for its inclusion as a protein supplement in the nutrition of various animal species. The control treatment showed a systemic resistance to salinity; however, before the third cutting, it exhibited lodging of the stems. Further research using halophilic PGPRs inoculated into various forages grown on different soils with salinity issues is required to enable planting in places that have hitherto been considered uncultivable.

Literature cited:

1. Hoffmann EM, Muetzel S, Becker K. Effects of *Moringa oleifera* seed extract on rumen fermentation *in vitro*. Arch Anim Nutr 2003;57(1):65-81.

2. Mendieta-Araica B, Spörndly R, Reyes-Sánchez N, Spörndly E. Moringa (*Moringa oleifera*) leaf meal as a source of protein in locally produced concentrates for dairy cows fed low protein diets in tropical areas. Livest Sci 2011;137(1):10-17.

3. Mendieta-Araica B, Spörndly E, Reyes-Sánchez N, Salmerón-Miranda F, Halling M. Biomass production and chemical composition of *Moringa oleifera* under different planting densities and levels of nitrogen fertilization. Agrofor Syst 2013;87(1):81-92.

4. Pérez ÁR. *Moringa oleifera*: una alternativa forrajera para ovinos. Culiacán, Sin, Méx: Universidad Autónoma de Sinaloa; 2011.

5. García QII, Mora-Delgado J, Estrada AJ, Piñeros VR. ¿Cuál es el efecto de la *Moringa oleifera* sobre la Dinámica Ruminal? Revisión sistemática. Rev Inv Vet Perú 2017;28(1):43-55.

6. Mendieta-Araica B, Sporndly E, Reyes-Sanchez N, Sporndly R. Feeding *Moringa oleifera* fresh or ensiled to dairy cows - effects on milk yield and milk flavor. Trop Anim Health Prod 2011;43(5):1039-1047.

7. Sun J, Zeng B, Chen Z, Yan S, Huang W, Sun B *et al*. Characterization of faecal microbial communities of dairy cows fed diets containing ensiled *Moringa oleifera* fodder. Sci Rep 2017;7:1-9.

8. Galindo PFV, Hernández MF, Rangel PP, Valencia RT, Castruita MÁS, Vidal JAO. Caracterización físico-química de sustratos orgánicos para producción de pepino (*Cucumis sativus* L.) bajo sistema protegido. Rev Mex Cienc Agríc 2014;5(7):1219-1232.

9. Larney FJ, Olson AF, Miller JJ, Tovell BC. Soluble salts, copper, zinc, and solids constituents in surface runoff from cattle manure compost windrows. Can J Soil Sci 2014;94(4):515-527.

10. Robinson PH, Grattan SR, Getachew G, Grieve CM, Poss JA, Suarez DL *et al*. Biomass accumulation and potential nutritive value of some forages irrigated with saline-sodic drainage water. Anim Feed Sci Technol 2004;111(1):175-189.

11. Aguirre-Garrido JF, Montiel-Lugo D, Hernández-Rodríguez C, Torres-Cortes G, Millán V, Toro N *et al*. Bacterial community structure in the rhizosphere of three cactus species from semi-arid highlands in central Mexico. Antonie van Leeuwenhoek 2012;101(4):891-904.

12. Turral H, Burke J, Faurès JM. Climate change, water and food security. 36th ed. Roma, Italia: FAO; 2011.

13. Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Fact 2014;13(66):1-10.

14. Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J King Saud Univ Sci 2013;26(1):1-20.

15. Glick BR. Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012;2012:1-15

16. Parray JA, Jan S, Kamili AN, Qadri RA, Egamberdieva D, Ahmad P. Current perspectives on plant growth-promoting rhizobacteria. J Plant Growth Regul 2016;35(3):877-902.

17. Palacio-Rodríguez R, Coria-Arellano JL, López-Bucio J, Sánchez-Salas J, Muro-Pérez G, Castañeda-Gaytán G *et al*. Halophilic rhizobacteria from *Distichlis spicata* promote growth and improve salt tolerance in heterologous plant hosts. Symbiosis 2017;73(3):179-189.

18. Vázquez VC, Salazar SE, Fortis HM, Reyes OMI, Zúñiga TR, Antonio GJ. Uso de cubiertas plásticas para solarización de estiércol bovino. Rev Mex Cienc Agríc 2010;1(4):619-625.

19. AOAC. Official methods of analysis. 15th ed. Arlington, VA, USA: Association of Official Analytical Chemists. 1990.

20. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74(10):3583-3597.

21. Noreen F, Muhammad A, Muhammad S, Ghulam A, Mubshar H, Muhammad N *et al*. Germination, growth and ions uptake of moringa (*Moringa oleifera* L.) grown under saline condition. J Plant Nutr 2018;41(12):1-11.

22. Medina MG, García DE, Clavero T, Iglesias JM. Estudio comparativo de *Moringa oleifera* y *Leucaena leucocephala* durante la germinación y la etapa inicial de crecimiento. Zootecnia Trop 2007;25(2):83-93.

23. da Costa PF, Rabello dOPS, Borsoi A, Soares dVE, Taffarel LE, Tiago PJ *et al*. Initial growth of *Moringa oleifera* Lam. under different planting densities in autumn/winter in south Brazil. Afr J Agric Res 2015;10(5):394-398.

24. Muhl QE, Du Toit ES, Robbertse PJ. *Moringa oleifera* (Horseradish Tree) leaf adaptation to temperature regimes. Int J Agric Biol 2011;13(6):1021-1024.

25. Goss M. A study of the initial establishment of multi - purpose moringa (*Moringa oleifera* Lam) at various plant densities, their effect on biomass accumulation and leaf yield when grown as vegetable. Afr J Plant Sci 2012;6(3):125-129.

26. Padilla C, Fraga N, Scull I, Tuero R, Sarduy L. Efecto de la altura de corte en indicadores de la producción de forraje de *Moringa oleifera* vc. Plain. Rev Cubana de Cienc Agríc 2014;48(4):405-409.

27. Hu Y, Schmidhalter U. Drought and salinity: A comparison of their effects on mineral nutrition of plants. J Plant Nutr Soil Sci 2005;168:541-549.

28. Meza-Carranco Z, Bernal-Barragán H, Olivares-Sáenz E, Aranda-Ruiz J. Crecimiento y producción de biomasa de moringa (*Moringa oleifera* Lam.) bajo las condiciones climáticas del Noreste de México. TECNOCIENCIA Chih 2016;10(3):143-153.

29. Melesse A, Steingass H, Boguhn J, Rodehutscord M. In vitro fermentation characteristics and effective utilisable crude protein in leaves and green pods of *Moringa stenopetala* and *Moringa oleifera* cultivated at low and mid-altitudes. J Anim Physiol Anim Nutr 2013;97(3):537-546.

30. Méndez Y, Suárez FO, Verdecia DM, Herrera RS, Labrada JA, Murillo B *et al*. Caracterización bromatológica del follaje de *Moringa oleifera* en diferentes estadios de desarrollo. Cuban J Agric Sci 2018;53(3):1-10.

31. Reyes SN, Ledin S, Ledin I. Biomass production and chemical composition of *Moringa oleifera* under different management regimes in Nicaragua. Agrofor Syst 2006;66(3):231-242.

32. Moyo B, Masika PJ, Muchenje V. Effect of supplementing crossbred Xhosa lop-eared goat castrates with *Moringa oleifera* leaves on growth performance, carcass and non-carcass characteristics. Trop Anim Health Prod 2012;44(4):801-809.

33. Reyes SN, Spörndly E, Ledin I. Effect of feeding different levels of foliage of *Moringa oleifera* to creole dairy cows on intake, digestibility, milk production and composition. Livest Sci 2006;101(1):24-31.

34. Sultana N, Alimon AR, Huque KS, Sazili AQ, Yaakub H, Hossain J *et al*. The feeding value of moringa (*Moringa oleifera*) foliage as replacement to conventional concentrate diet in Bengal goats. Adv Anim Vet Sci 2015;3(3):164-173.