Evaluation of the Development of Roselle *(Hibiscus Sabdariffa* L.) in Two Soil Types with Interaction of Bacterial Cells and Vermicompost

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Abstract— *In this study, we tested the growth of two varieties of Roselle (Hibiscus sabdariffa) cultivated in two types of soils and the effects of the inoculation of seeds with cells of bacterial strains and vermicompost. (H. sabdariffa) are a member of the Malvaceae family and an important plant because its calyxes are used to produce beverages, prepare food, in the textile industry, cosmetology, perfumery, medicine, etc.*

This research was carried out at the Postgraduate College, Montecillo Campus, Mexico. The experimental design was completely randomized blocks with a factorial arrangement (4x3x2): four levels of organic matter factor, three levels of bacterial strains factor, and two levels of soil factor with three repetitions. The variables tested were: germination percentage (GP), plant height (PH), stem diameter (SD), the number of leaves (NL), foliar area (FA cm^2), radical volume (RV cm^3), dry aerial part biomass weight (DAPBW), dry root biomass weight (DRBW), dry calyx biomass weight (DCBW) and chlorophyll content (CC). The effects of soil and bacterial strains showed significant differences ($p \leq 0.05$) on the agronomic characteristics of (H. sabdariffa) plants. The organic matter factor only presented significant differences ($p \leq 0.05$) in the germination percentage and growth variables of the vegetative phase.

To increase crop production, the use of wastewater and microorganisms is proposed. Vermicompost exploded the percentage of germination, height, the number of leaves and stem diameter during the first weeks of growth, presenting important results in Creole variety (farmers' local variety). By inoculating seeds with cells of bacterial strains, A9m and A7 helped significantly to stimulate plant growth, finding a better response in A9m strain. The effects of soil factor showed significant differences ($p \leq 0.05$) in soil irrigated with residual water over agronomic characteristics satisfying the nutritional requirements of the crop.

Keywords— ***Germination percentage, Malvaceae, Soil microorganisms, Organic matter, Wastewater.***

I. INTRODUCTION

Roselle or Roselle rose *(Hibiscus sabdariffa* L.) is a plant that has medicinal properties backed by scientific studies; however, it is also used to dye food, soft drinks, syrups, among other products. It is of great importance to the State of Guerrero, Mexico, with a first-place nationally, in harvest area and calyx production (SIAP, 2016).

In this water-scarce world, wastewater has been used for people to grow crops because it is free, it contains a lot of plant nutrients that also are free but, all these benefits do not consider the cost of the health care of the goods consumers. The Mezquital Valley in central Mexico is an example of this issue. In sum, crops will continue to be irrigated with wastewater due to growing water shortages, but this situation is a question of management and socioeconomic costs.

Plant Growth-Promoting Bacteria (PGPB) has been used primarily in agriculture to promote seed germination, vigor, plant growth, and improve crop yield (Kloepper, *et al.,* 1989), and for the control of plant pathogen microorganisms (Beneduzi, *et al.,* 2012). Plant growth-promoting bacteria, in addition to their agricultural applications, have been used successfully to mitigate the damage of plant growth caused by wastewater discharges into crop soils, so that crops thrive and they produce satisfactorily because bacteria have the ability to improve wastewater bioremediation processes. Some other rhizobacteria promote crop plants and the plants used in bioremediation processes to allow rapid extraction of hazardous materials from soils (Bashan *et al.,* 2008; McGrath *et al.,* 1993).

Another cost-effective solution is the use of vermicompost. Studies show that organic fertilizers protect and develop the life of microorganisms by improving soil structure and allowing the recycling of organic matter (Ruíz, 2011). The vermicompost stores mainly nitrogen, phosphorus, potassium, sulfur and prolonged-release micronutrients (García, 2011; Varela and Martínez, 2013), used as a growth medium in plant species under greenhouse conditions (Gardezi *et al.*, 2008), increasing microbial load and plant growth hormones, highlighting a sustainable agriculture that prevents degradation of soils with inorganic fertilizers.

There is little information on the use of biofertilizers and organic fertilizers in the cultivation of Roselle, and the accumulation in edible parts of the plant by toxic elements present in the soil, therefore, the information that is required to get in this investigation consists of determining the practical, economic, and safe culture medium for the cultivation of *H. sabdariffa* testing two types of soil interacting with bacterial strains and vermicompost. This will give farmers options to use organic fertilization, reduce pollution, and gain new ecosystem-friendly technologies.

II. MATERIALS AND METHODS

2.1 Experimental site

The experiment was carried out under greenhouse conditions at the Postgraduate College, Montecillo Campus, State of Mexico, in the spring and summer of 2016. At 19° 27´ N and 98° 54´ W with an altitude of 2245 meters above sea level. With a mild climate, and an average annual temperature of 16.4° C and a rainfall of 762.7 mm per year, National Meteorological (Service, Servicio Meteorológico Nacional) (SMN, 2010). The greenhouse comprises symmetrical triangular flat roofs. The roof is glass with a 70% luminosity, and metal structure. Ventilation is through the front and side windows (Fig 1).

FIGURE 1. Greenhouse in the Postgraduate College, Montecillo Campus.

2.2 Plant material and substrate

Two varieties of Roselle, commercial (H1038) and Creole (farmers' local variety) from the state of Guerrero were used. We used two types of soils: one irrigated with sewage water that had been watered for decades with water from the Emiliano Zapata dam between the boundaries of the states of Guerrero and Morelos, which houses urban wastewater from surrounding towns. The other soil was irrigated with clean water from a well. Soil samples were obtained at 0-5 cm, 5-10 cm, 10-40 cm deep, the characteristics and method are shown in Table 1.

TABLE 1
ANALYSIS OF TWO TYPES OF SOIL IRRIGATED WITH CLEAN AND RESIDUAL WATER

	Soil sample						
	Irrigated with clean water			Irrigated with the residual water			
	0-5 cm	5-10 cm	10-40 cm	0-5 cm	5-10 cm	10-40 cm	Method
pH	7.21	7.35	7.36	7.36	7.39	7.45	Potentiometric
EC dSm^{-1}	0.46	0.55	0.4	0.71	0.46	0.43	
OM %	0.31	0.27	0.4	0.67	0.34	1.88	Walkley and Black
N $mgKg^{-1}$	13	9.3	8.4	13	16.7	9.9	Extracted with potassium chloride
CEC Cmol+/Kg	53	52	48	58	57	54	Olsen
P $mgKg^{-1}$	7.39	6.55	3.72	8.1	18.18	5.9	Flame Emission Spectrophotometry
K $mgKg^{-1}$	204	384	382	470	398	226	Atomic absorption spectrophotometry
Ca $mgKg^{-1}$	4085	4111	2810	4222	4376	4140	
Mg $mgKg^{-1}$	494	464	387	926	822	846	
Fe $mgKg^{-1}$	2.02	1.3	1.82	2.74	1.87	1.38	
Cu $mgKg^{-1}$	0.77	0.74	0.71	1.59	1.7	1.51	
Zn $mgKg^{-1}$	0.82	0.47	0.38	0.74	0.58	0.52	
Pb mgKg-1	ND	0.009	ND	ND	ND	0.011	
Cr mgKg-1	ND	ND	ND	ND	0.020	ND	
Cd mgKg-1	ND	0.004	ND	ND	0.007	ND	

pH: hydrogen potential, EC: electrical conductivity, OM: organic matter, CEC: cation exchange capacity, ND not detected.

Roselle varieties used were commercial H1038 (Hybrid variety) and Creole (Farmers' local variety), planted in 2.5 kg polythene bags filled with two types of soil.

Seeds inoculation with cells of microbial strains A9m and A7 (provided by the Molecular Genetics Laboratory, Faculty of Genetics, Postgraduate College, Montecillo Campus) was done thirty minutes before sowing by adding 200 µl of bacterial suspension per batch of 100 seeds.

Vermicompost was used as organic matter, made with 60 kg of bovine manure, 25 kg of melon residues, and 15 kg of wheat. The mixture interacted with earthworms for four months. The Vermicompost in doses of 0, 24.05g, 48.08g, 72.05g, equivalent to 0, 25, 50, 75 t ha-1 of organic matter, was added to four 2.5kg polyethylene bags containing soil. Irrigation was every third day, applying the same amount of water with a container during the development of the investigation.

2.3 Variables tested

The variables tested were: germination percentage (GP), plant height (PH), stem diameter (SD), the number of leaves (NL), foliar area (FA, cm^2), radical volume (RV cm^3), dry aerial part biomass weight (DAPBW), dry root biomass weight (DRBW), dry calyx biomass weight (DCBW), and chlorophyll content (CC).

The experimental block design was handled completely randomly with 24 treatments (4x3x2) in a factorial arrangement with three repetitions. The factors and levels studied were: (1) soil with two levels, irrigated with wastewater and irrigated with clean water; (2) vermicompost, with four levels, 0, 24.05 g, 48.08 g, 72.05 g; (3) bacterial strains, with three levels, without strains, with strains A9m and A7. Analysis of variance analyzed the response variables with a level of significance of $p \leq 0.05$ and means separation tests with Tukey ($p \leq 0.05$).

III. RESULTS AND DISCUSSION

3.1 Physical and chemical properties of soil

The soil used had a clayey texture, with a moderately alkaline pH. The soil irrigated with clean water showed a pH of 7.36 in comparison with the soil irrigated with residual water that had a pH of 7.45 in sample 10-40 cm deep. The results of electrical conductivity (EC), organic matter (OM), cation exchange capacity (CEC), nitrogen (N), phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), zinc (Zn) and copper (Cu) were higher in soils irrigated with sewage water. The highest concentration of N was in upper layers (0-5 cm to 5-10 cm), with a slight increase in soils irrigated with wastewater because of the contribution of organic matter (Ramón-Zamora*et al*., 2009). Likewise, the content of available P presented average concentrations and content of K showed high levels, according to the norm NOM-021-RECNAT-2000. The levels of Cr, Cd, and lead (Pb) were below the limit of concentration to be considered contaminants.

3.2 Germination percentage

Table 2 shows the germination percentage associated with organic matter at different doses and seeds inoculated with cells of bacterial strains in two different soils. There were no significant differences in soil and bacterial strains factors tested ($p \leq 0.05$), however, there was a significant effect on levels of organic matter factor on the germination percentage. The 50% dose of the organic matter showed a greater positive effect on the germination percentage for H1038 variety, while a 75% dose of the organic matter showed a greater effective impact on Creole variety (Fig 2). This result shows that the addition of the organic matter of culture media consistently improves the germination percentage of seedlings (Domínguez *et al*., 2010; Atiyeh *et al*., 2002).

TABLE 2
GERMINATION PERCENTAGE OF ROSELLE VARIETIES H1038 AND CREOLE IN TWO TYPES OF SOIL ASSOCIATED WITH ORGANIC MATTER AND CELLS OF BACTERIAL STRAINS

Factors and levels	H1038	Creole
	Germination (%)	
Soil (S)		
S. irrigated with clean water	68.33 a	51.11 a
S. irrigated with wastewater	72.78 a	52.78 a
Organic matter		
0 g	60.00 c	39.89 c
24.05 g	66.67 bc	50.00 b
48.08 g	81.11 a	48.89 bc
72.05 g	74.44 ab	70.00 a
Bacterial strain		
Without strain	67.50 a	48.33 a
With A9m strain	75.83 a	52.50 a
With A7 strain	73.33 a	56.67 a

Means with the same letter in the same column are statistically equal (Tukey, p≤ 0.05).

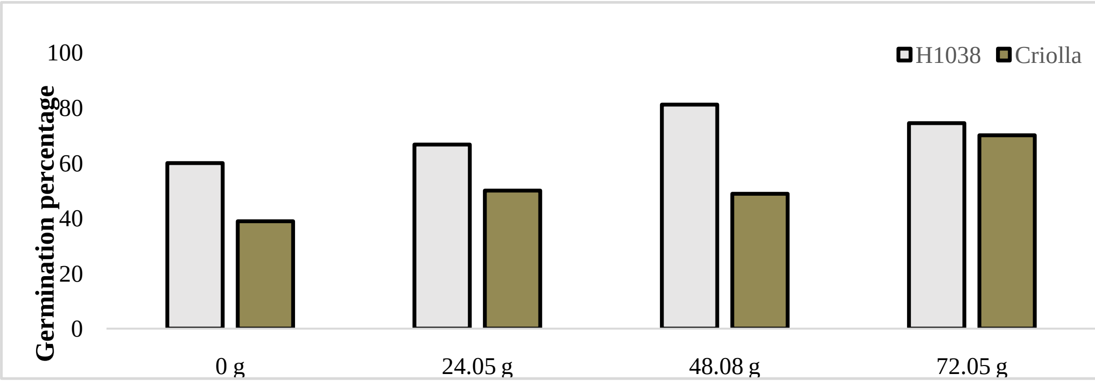

FIGURE 2:Effect of Organic Matter Added in Two Varieties of (H. Sabdariffa) on Germination Percentage

3.3 Agronomic characteristics

Table 3 presents the results of the agronomic characteristics of two varieties of *(H. sabdariffa)* associated with organic matter and bacterial strains in two types of soil.

TABLE 3
AGRONOMIC CHARACTERISTICS OF (*H. SABDARIFFA*) ASSOCIATED WITH ORGANIC MATTER AND BACTERIAL STRAINS IN TWO TYPES OF SOIL

Factors and levels	Height cm		Stem diametermm		Number of leaves		Foliar area cm^2		Radical volume cm^3	
Soil (S)	H1038	Creole	H1038	Creole	H1038	Creole	H1038	Creole	H1038	Creole
S. irrigated with clean water	130.72 b	110.97 b	7.18 b	6.11 b	41.25 b	45.31 b	1202.28 b	758.55 b	5.11 b	4.65 b
S. irrigated with wastewater	147.71 a	129.41 a	8.36 a	7.08 a	49.83 a	59.47 a	1741.86 a	1070.95 a	7.45 a	5.55 a
Organic matter										
0 g	139.11^{NS}	119.17^{NS}	7.43^{NS}	6.61^{NS}	46.00^{NS}	53.83^{NS}	1365.17^{NS}	914.82^{NS}	5.67^{NS}	4.31 b
24.05 g	148.88	112.73	7.96	6.36	48.5	50.28	1671.39	818.78	6.59	4.68 ab
48.08 g	131.38	127.28	7.76	6.88	42.44	54.61	1329.89	1035.61	5.58	6.05 a
72.05 g	137.5	121.57	7.94	6.53	45.22	50.83	1521.83	889.79	7.27	5.37 ab
Bacterial strain										
Without strain	126.08 b	98.13 b	7.33 b	5.85 b	40.00 b	37.79 b	1100.58 b	576.22 b	5.99^{NS}	4.34 b
With A9mstrain	148.32 a	130.98 a	8.06 a	7.01 a	50.08 a	59.21 a	1728.17 a	1075.72 a	6.58	5.43 ab
With A7strain	143.25 a	131.46 a	7.93 ab	6.93 a	46.54 ab	60.17 a	1587.46 a	1092.30 a	6.27	5.53 a

Hybrid variety = H1038 and Creole variety = Farmers' local variety
NS = Not significant. Means with the same letter in the same column are statistically equal (Tukey, p≤ 0.05)

TABLE 3
CONTINUATION

Factors and levels	Aerial dry weight (g)		Root dry weight(g)		Calyx dry weight(g)		Chlorophyll content SPAD unit	
Soil (S)	H1038	Creole	H1038	Creole	H1038	Creole	H1038	Creole
S. irrigated with clean water	9.17 b	7.93 b	1.01 b	0.83 b	8.74 b	2.91 b	27.10 b	32.45 b
S. irrigated with wastewater	15.79 a	10.60 a	2.01 a	1.03 a	13.79 a	4.57 a	29.64 a	41.00 a
Organic matter								
0 g	11.40^{NS}	9.40^{NS}	1.40^{NS}	0.80 b	10.24^{NS}	5.02^{NS}	28.39^{NS}	36.24^{NS}
24.05 g	13.49	8.16	1.40	0.88 ab	12.84	3.26	27.24	35.46
48.08 g	13.49	10.29	1.55	1.07 a	10.48	3.69	28.46	38.87
72.05 g	11.53	9.21	1.70	0.98 ab	11.52	2.99	29.39	36.34
Bacterial strain								
Without strain	10.06 b	5.90 b	1.64^{NS}	0.82 b	6.06 b	1.54 b	27.70 a	30.35 b
With A9m strain	13.83 a	11.23 a	1.43	1.03 a	14.75 a	4.69 a	28.50 a	39.12 a
With A7 strain	13.55 a	10.66 a	1.47	0.95 ab	12.99 a	4.98 a	28.91 a	40.71 a

NS = Not significant. Means with the same letter in the same column are statistically equal (Tukey, p ≤ 0.05). g=gram, SPAD=Soil-Plant Analyses Development.

3.4 Soil analysis

The analysis shows that there were significant differences in all agronomic variables tested ($p \leq 0.05$) in the levels studied in the soil factors in both varieties (Table 3). This result reflected by a slight difference in the concentration of elements such as N, P, K, Mg in both soils, because soils irrigated with wastewater increase the content of nutrients and organic matter, favoring the development of crop and improvement of soil (Veliz*et al*., 2009). Plevich *et al*., (2012) observed similar results in alfalfa cultivation that presented an increase in nutritional value and production of plants, determining that incorporation of wastewater to soil exceeds the values, compared to soil irrigated with clean water.

However, for the calyx number, there were no significant differences ($p \leq 0.05$) in any variety (Table 4). This is explained by the morphology of the plant, because the transition from flowering to fruiting is gradual, because the anthesis of the young flowers continues after the old flowers have already formed the capsules (Acosta, 1999), in addition, floral induction occurs when days are shorter (Arbex de Castro *et al*., 2004). However, an increase in elements such as nitrogen, phosphorus, and potassium have a positive response of the plant in variables such as weight of fresh and dry matter from the calyx (El-Sherif and Sarwat, 2007; Haruna *et al*., 2009) (Table 5).

TABLE 4
THE NUMBER OF CALYXES IN TWO VARIETIES OF THE ROSELLE CROP WITH DIFFERENT FACTORS AND LEVELS

Factors and levels	Number of Calyxes	
	number	
Soil (S)	H1038	Creole
S. irrigated with clean water	3.61^{NS}	13.28^{NS}
S. irrigated with wastewater	4.14	15.75
Organic Matter		
0 g	4.17^{NS}	15.06^{NS}
24.05 g	4.44	12.94
48.08 g	3.11	15.44
72.05 g	3.78	14.61
Bacterial strain		
Without strain	3.21^{NS}	7.67 b
With A9m strain	4.08	17.96 a
With A7 strain	4.33	17.92 a

NS = Not significant. Means with the same letter in the same column are statistically equal (Tukey, p ≤0.05).

TABLE 5
WEIGHT OF FRESH AND DRIED CALYX MATTER IN TWO VARIETIES OF THE ROSELLE CROP WITH DIFFERENT FACTORS AND LEVELS

Factors and levels	Calyx fresh matter weight (g)		Calyx dry matter weight (g)	
Soil (S)	H1038	Creole	H1038	Creole
S. irrigated with clean water	26.99 b	18.73 b	8.74 b	2.91 b
S. irrigated with wastewater	40.10 a	26.65a	13.79 a	4.57 a
Organic Matter				
0 g	29.62^{NS}	27.81^{NS}	10.24^{NS}	5.02^{NS}
24.05 g	39.15	20.17	12.84	3.26
48.08 g	31.01	24.95	10.48	3.69
72.05 g	34.41	17.84	11.52	2.99
Bacterial strain				
Without strain	19.73 b	9.30 b	6.06 b	1.54 b
With A9m strain	42.83 a	28.39 a	14.75 a	4.69 a
With A7 strain	38.08 a	30.38 a	12.99 a	4.98 a

NS = Not significant. Means with the same letter in the same column are statistically equal (Tukey, p ≤0.05).

3.5 Bacterial strains results

The results show significant differences ($p \leq 0.05$) in agronomic characteristics tested in Roselle plants from seeds inoculated with bacterial cells than in non-inoculated plants, except for the variables radical volume, dry root biomass weight and chlorophyll content in H1038 (Table 3). In Creole variety (farmers' local variety), the effect of strain A7 was numerically better in six agronomic characteristics tested, compared to three characteristics where strain A9m was superior. Higher values were found in six agronomic characteristics in plants generated from seeds inoculated with cells of strain A9m compared with those of strain A7 in variety H1038. Hassan, 2009 used (*Azospirillum lipoferum*, *Bacillus polymyxa*, and *Pseudomonas fluorescens*), getting that inoculation of bacteria along or combined with chemical fertilizers significantly improve the growth and increase characteristics of the calyx product of (*H. sabdariffa*) plants compared to control.

Regarding variables radical volume and dry root biomass weight in H1038 variety, there was no significant difference between inoculation and non-inoculation, probably because strains only acted in plant-microorganism interaction solubilizing minerals usable for the plant and not as phytostimulators that increase the number of radical hairs and lateral roots (Hernández *et al*., 2010). Likewise, the difference in chlorophyll content between both varieties marked by physiological maturity of the plant, when the basal leaves change color and dry, happening after flowering. The harvest period for H1038 variety is 120-180 days in contrast, Creole variety is of 160-180 days (Ariza *et al*., 2014), so there is a greater deficiency of nutrients in leaves of H1038 variety in all treatments (Fig 3).

(A) (B) (C)

FIGURE 3: Calyxes In Variety H1038 at 180 Days after Sowing (Das). (A: Clean Soil without Inoculation, B: Soil Irrigated with Residual Water and C: Inoculated with the Bacterial Strain

In relation to the number of calyxes and days to flowering, no significant differences were observed ($p \leq 0.05$). This is explained by the factors that can affect the performance and effectiveness of the inoculation, among the most prominent are the competition with the native microorganisms, physical and chemical characteristics of the soil, genotype and age of the plant to be inoculated, type of radical exudates and agricultural management (Castro *et al*., 2007).

Although there are huge information gaps in the evolution of calyxes during their fruiting (Ramírez *et al*., 2011), it is necessary to generate information on the agricultural characteristics of interest.

3.6 Organic matter factor

There was a significant effect of the levels of the organic matter factor in the germination percentage ($p \leq 0.05$). The dose with 48.08 g of the organic matter showed a greater positive effect on the germination percentage for the H1038 variety, while the dose with 72.05 g of the organic matter showed a greater effective impact on the Creole variety (Fig 2). This result shows that the addition of organic matter to the culture media consistently improves the germination percentage of seedlings (Domínguez *et al*., 2010; Atiyeh *et al*., 2002).

At the end of the experiment, 180 days after sowing (Fig 4-5), on the levels studied of organic matter factor, no significant differences were found in any of the agronomic characteristics ($p \leq 0.05$), except for variables of dry root biomass weight and radical volume in Creole variety, the dose of 48.08 g of organic matter showed greater effect. The in-depth distribution of organic matter and roots follow the same trend. These results differ with those obtained by Haruna *et al*., (2011) in the effect produced by *(H. sabdariffa)* in the field because of incorporating poultry manure as organic matter, and as reported by Anyinkeng and Mih, (2011).

FIGURE 4. Variety H1038 (Hybrid variety) at 180 days after sowing (DAS).

FIGURE 5. Creole variety (farmers' local variety) at 180 days after sowing (DAS).

However, significant differences were found in growth variables at 80 days after sowing ($p \leq 0.05$). For H1038 variety, it showed only a positive increase in stem diameter in doses of organic matter of 48.08 g and 72.05g (Table 6). In the Creole variety, the dose of 48.08g expressed a greater effect on the height, the number of leaves and the diameter of the plant stem. This may show that improved varieties, unlike Creole, have high development potential in the vegetative phase and greater tolerance to biological stress (SAGARPA, 2014, 2015; Hidalgo-Villatoro *et al*., 2009), even in poor soils.

TABLE 6
GROWTH VARIABLES OF TWO VARIETIES OF ROSELLE 80 DAYS AFTER PLANTING

Factor and levels	Height(cm)		Number of leaves		Stem diameter(mm)	
Organic matter	H1038	Creole	H1038	Creole	H1038	Creole
0 g	36.94^{NS}	34.39 b	14.44^{NS}	12.50 c	4.24 b	3.16 b
24.05 g	40.26	36.48 b	14.00	14.06 bc	4.52 ab	3.34 b
48.08 g	40.64	41.74 a	14.33	18.11 a	4.54 ab	3.96 a
72.05 g	40.74	42.79 a	16.78	17.94 ab	5.04 a	4.03 a

NS = Not significant. Means with the same letter in the same column are statistically equal (Tukey, $p \leq 0.05$).

Finally, the behavior of plants observed in the organic matter factor reflected by mineralization existing in soil, which can contain up to 45% of minerals (Labrador, 2001), influenced by climate and mineralogy of clays (Vogt *et al*., 1995; Geissen and Brümer, 1999) and by a vegetative phase of the genus (*Hibiscus*), in which amount of minerals and water absorbed is greater, during the first weeks of growth plant absorbs almost all nitrogen, phosphorus, and potassium(NPK) that will be necessary for the rest of the growing period, so a mixture of organic matter and soil will be necessary weeks before planting, to increase the use of nutrients provided by organic components.

IV. CONCLUSION

The four components: improved varieties, vermicompost, treated wastewater, and bacterial cells, play an important role in the quality, quantity, and health of plants for agricultural production with a suitable formula according to soil and water analyzes. The aggregation of organic matter shows positive effects on the percentage of the germination of both varieties and during the vegetative phase in Creole variety. Likewise, the vermicompost increased the emergency percentage of each variety studied and increased the radical volume. Plants with better agronomic characteristics were found in soils that were irrigated with sewage and inoculated with some bacterial strains in both varieties of Roselle. However, the use of microbial consortia that acts together in the crop system is suggested, having a more efficient and effective flow of nutrients in the plant's growth.

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