

**INTERACTION BETWEEN WATER SALINITY AND MIXED
BIOFERTILIZER AT ACCUMULATION OF BIOMASS AND WATER
USE EFFICIENCY IN COWPEA PLANTS**

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ABSTRACT

The biofertilizer has been used to reduce the effects of salinity on plants. In this context the present work had as objective to evaluate the accumulation of phytomass of plants of cowpea (*Vigna unguiculata*) grown under different levels of salinity and biofertilizers. The experiment was conducted in a greenhouse at the experimental farm of the University of International Integration Lusophone Afro-Brazilian, with duration of 70 days. The experimental design utilized was completely randomized in 3 x 4 factorial arrangements, with three water salinity levels of irrigation and four levels of biofertilizer, with five repetitions. Were evaluated the efficiency of water use, dry mass of the aerial part, of the root, and the reason between them. It was observed that the application of biofertilizer significantly influenced the accumulation of phytomass in the evaluated variables. The variables dry mass of the aerial part, from root, and the ratio between them, presented better results when the plants were biofertilized with doses higher than 1600 mL, in all salinity levels of the irrigation water. The application of doses between 1600 and 2400 mL of biofertilizer is efficient for the phytomass accumulation of cowpea for all irrigation water salinity levels. Then, the biofertilizer presents itself as a sustainable alternative, with great potential for use in agriculture.

Keywords: Irrigation, Organic fertilization, Salinity, Sustainability, *Vigna unguiculata*

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is the most cultivated annual legume in Brazilian semi-arid areas. It belongs to the family Fabaceae, the plant is a rustic species of easy handling and well adapted to the edaphoclimatic conditions of the Northeast region [1]. Its cultivation presents itself as an activity of great importance for agricultural development in Brazil, both on the economic aspect as in nutritional status, because it constitutes one of the main elements of the diet of populations, being widely cultivated in small properties and family agriculture [2]. In addition to their use in human food, can be used in animal feed, such as grass, and yet as green manure and soil cover [3].

It is grown, basically, in regime of subsistence, in the North and Northeast regions, mainly because of its adaptation to the edaphoclimatic conditions. The culture has great importance in the diet of populations that live in these regions, because it provides a food of high nutritional value and therefore, one of the major components of the diet. Among the cultivars used most often in the Northeast, stands the "Setentão", which, in addition to the excellent quality of seed with respect to color and size, presents good productive capacity [1, 4].

Despite being a culture moderately tolerant to saline stress, the effects of salinity is set as one of the main factors that contribute in morphophysiological alterations that reduces their productivity, because these affect the development of the plant by means of nutritional imbalance, water deficit and the toxicity caused by ions [1, 5]. These effects also affect the net CO₂ assimilation, inhibit the leaf expansion and accelerate the senescence of mature leaves, reducing, consequently, the area intended for photosynthesis, the biomass and total production of assimilates [6, 7].

Several alternatives are being studied to minimize the effects of the use of saline water for irrigation and, concomitantly, encouraging the acquisition of nutrients by plants in terms of salinity, in order to increase their productivity. Some studies have demonstrated that the use of biofertilizers in saline environments can partially mitigate the effects of salinity on growth of plants [8, 9, 10].

In cowpea, [11] observed that the increase in the salinity of the waters detracted from its initial growth, but with less intensity in the substrate where it was applied the biofertilizer. [12] and [13] also found beneficial effects of biofertilizer in saline environment on plants of cherry tomatoes and maize, respectively.

The dung is a solution widely adopted for the supply of nutrients such as nitrogen, phosphorus and potassium, and its use in liquid biofertilizers incorporated to the soil, enjoys the bean registering an increase in their productivity [14]. Considering the above, the objective of this

work was to evaluate the phytomass accumulation of plants of cowpea cultivated under different salinity levels and doses of fertilizer.

2. MATERIAL AND METHODS

2.1 Description of the experimental area

The experiment was conducted in a greenhouse, during the period from July to September 2016 in an area of an experimental farm of the University of International Integration of the Lusophony Afro-Brasileira (UNILAB), located on Piroás, municipality of Redenção, in the Maciço of Baturité - CE, Brazil, at a latitude of 04°14'53"S, longitude 38°45'10"W and an average altitude ranging from 240 to 340, the local climate is classified as Aw', i.e., tropical rainy season.

2.2 Experimental treatments, and vegetative material used

The experimental design was completely randomized, in a 3 x 4 factorial arrangement, with three levels of salinity of irrigation water (E_{cw}: 0.5; 2.5 and 4.5 dS m⁻¹) and four levels of Biofertilizer mixed (cattle manure, chicken, wood ash), in liquidation, applied to the soil, corresponding to (0, 5, 10 and 15%) of the volume of the soil, which corresponds to 0, 800, 1600 and 2400 mL, respectively, with five replications. The experiment lasted 70 days, counted from the start of the application of treatments, being the plants maintained in a greenhouse with 50% of brightness.

We used the seeds of cowpea (cultivar Setentão), being the same provided by Seed Technology Laboratory of the Federal University of Ceará (UFC) and sown in plastic pots with capacity for 16 L, containing soil of the region (typic eutrophic red-yellow) and with a layer of gravel at the base number 1, in order to facilitate the drainage of irrigation water. We used 3 seeds per pot at a depth of approximately 3 cm and after a week made the manual thinning, leaving only one plant of cowpea in each vessel. We used a total of 60 plants, these being identified randomly, in accordance with the experimental and after 3 weeks of planting was done the staking of same with the aid of a string, stakes and steel wire.

2.3 Soil conditions and the mixed biofertilizer

To analyze the conditions of the soil used in the experiment, before the completion of the same, soil samples were collected (composed and representative of the different treatments), these being sent for chemical analysis in the Laboratory of Chemistry and Soil Fertility at the UFC, presented in Table 1.

Table 1: Chemical attributes of the soil layer of 0 to 0.20 m depth, where the plants of cowpea were grown.

C	N	C/N	MO	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	H ⁺ +Al ³⁺	Al ³⁺	S	T	P
(g Kg ⁻¹)				(cmolc Kg ⁻¹)						(mg m ³)		
11.6	1.2	10	20.1	4.2	1.9	0.21	0.26	1.65	0.15	6.6	8.2	42.33

Source: Prepared by the author, 2016.

The aerobic biofertilizer used was prepared in case of polyethylene, with capacity of 500 L, using goat manure (100 L), wood ashes (10 L) and water (210 L). Were performed 5 applications of fertilizer mixed liquid, these being provided once per week, in accordance with the doses calculated for the treatments 0, 5, 10 and 15% of the volume of the soil (0, 800, 1600 and 2400 mL, respectively), from 7 days after germination (DAG) and with the objective of achieving the nutritional needs of culture.

The application of the biofertilizer was performed manually and located to avoid that this enters in contact with the leaves of the plants. A sample of each of the five applications of fertilizer supplied to the plants were sent to the Laboratory of Soil and Water from the UFC for chemical analysis. The results of this analysis are available in Table 2.

Table 2: The chemical composition of the fertilizer applied in plants of cowpea, Redenção - CE, 2016.

Nº The application	(mg L ⁻¹)										
	N	P	P ₂ O ₅	K	K ₂ O	Ca	Mg	Fe	Cu	Zn	Mn
1	129.2	51.38	104.75	3028.85	3695.19	255.65	719.12	4.76	0.01	4.21	5.09
2	186.7	50.95	116.67	3038.46	3926.92	282.47	892.69	2.05	0.01	3.47	7.47
3	108.2	54.72	86.6	2575.76	3142.42	195.17	614.94	2.4	0.01	4.41	4.39
4	107	53.06	121.51	2783.51	3395.88	320.25	728.3	4.54	0.01	6.67	8.28
5	130.27	50.03	104.90	3106.64	3790.10	263.38	738.69	3.44	0.01	4.69	6.31

Source: Prepared by the author, 2016.

2.4 Irrigation

The water sources used were prepared, in tanks with a capacity of 200 L, being used NaCl, CaCl₂.2H₂O and MgCl₂.6H₂O in proportions of 7:2:1. The concentration of salts was calculated by the following equation [C_s (mmolL⁻¹) = EC x 10], in which: C_s = concentration of salts; CE = pre-established electrical conductivity [15].

The irrigation was performed every two days and the amount of water applied was estimated with the goal of reaching the soil field capacity, adding a fraction of leaching of 0.15 for water percolation at bottom of vessels, in order to avoid the excessive accumulation of salts. The application of the water was also performed manually and located to avoid that this enters in contact with the leaves of the plants.

2.5 Variables analyzed

At the end of the experiment, 70 days after planting (DAP), the plants were divided into parts (leaf, stem and root), allocated in paper bags are duly identified and placed for drying in an oven with forced air circulation at 75°C until constant mass, for obtaining of the dry mass of aerial part, root and reason dry mass of aerial part and root dry mass.

The 70 DAP, were also performed measurements of gas exchange in Leaf fully developed, in the zone between 9h00min and 12h00min, under-radiation saturante and under ambient conditions of temperature and CO₂ concentration, using an infrared gas analyzer IRGA (LCI System, ADC, Hoddesdom). With these data, it was possible to obtain water use efficiency (EUA), dividing the values of photosynthesis by transpiration and efficiency of transpiration (ET), dividing the values of transpiration by photosynthesis.

2.6 Statistical analyzes

Qualitative data of the analyzed variables were submitted to analysis of variance and later, when significant by F test, submitted to the test of Tukey Test with $P < 0.05$. For the data of quantitative nature conducted a regression analysis, the equations that best fit to the data, were selected based on the significance of the regression coefficients to 1% and 5% probability by the F test and with greater determination coefficient, or higher R². For the statistical analysis we used the computational program "Assistat 7.6 BETA".

3. RESULTS AND DISCUSSION

In the analysis of variance presented in Table 3, we can observe that the salinity factor only the variable reason of the dry mass of aerial part and root dry mass (MSPA/MSR) showed no significant effect. As for the biofertilizer factor, as well as the interaction between the factors

salinity and biofertilizer, it was observed that all variables had suffered significant influence at the level of 1% probability by F.

Table 3: Summary of the analysis of variance for shoot dry mass (MSPA), root (MSR), efficiency of water use (EUA) and efficiency of transpiration the reason dry mass of aerial part and root dry mass (MSPA/MSR) of plants cowpea, subjected to three levels of salinity (Ecw: 0.5, 2.5 and 4.5 dS m⁻¹) and four doses of fertilizer (0, 800, 1600 and 2400 mL), Redenção – CE, 2016.

Sources of Variation	Mean Square				
	MSPA (g)	MSR (g)	MSPA/MSR (g/g)	EUA (μmol m ⁻² s ⁻¹)	ET (μmol m ⁻² s ⁻¹)
Salinity (S)	74.42**	1.28*	0.20 ns	1.88 ns	0.09 ns
Biofertilizer (B)	362.94**	3.12**	2.32**	0.92 ns	0.02 ns
Int. S x B	89.24**	2.01**	0.81**	0.06*	0.10 ns
Residue	14.37	0.22	0.15	2.00	0.84
Overall Average	53.06	9.28	5.68	2.67	0.46
CV (%)	6.77	6.03	6.83	22.90	16.23

** Significant by F test at 0.01 probability; * Significant by F test at 0.05 probability; ns - not significant.

Source: Prepared by the author, 2016.

For the variable MSPA and the interaction between the factors salinity and biofertilizers in the same, adjusted to a linear equation for growing plants irrigated with water of 0.5 dS m⁻¹ with 0.0049g unit increase in function of doses of fertilizer. For those irrigated with 2.5 dS m⁻¹ showed a quadratic behavior, where the plants when subjected to a dose of fertilizer for 1912.5 mL showed a maximum point of MSPA, with value of 63.43g, being approximately 25.29% higher than the lowest value (47.39 g), found in plants irrigated with water of 2.5 dS m⁻¹ and subjected to a dose of 0 mL of biofertilizer. Already for plants irrigated with 4.5 dS m⁻¹, adjusted a linear equation increasing with increasing unit of 0.0033g depending on the doses of fertilizer (Figure 1).

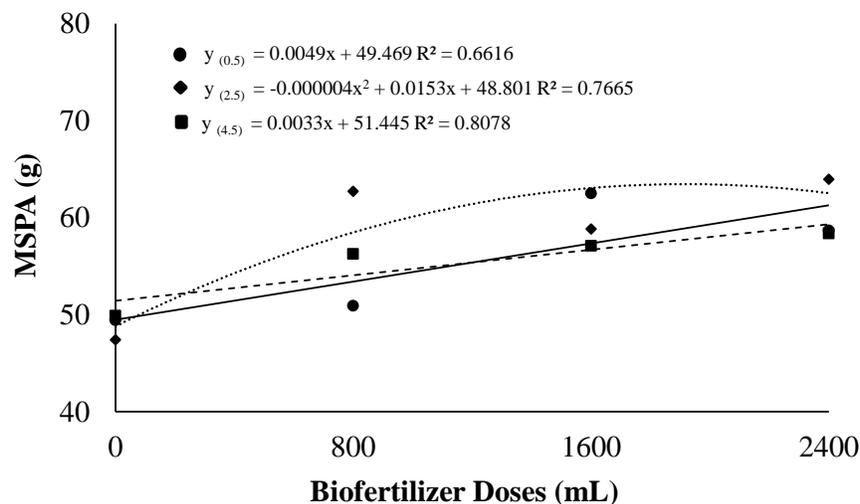


Figure 1: Dry mass of aerial part (MSPA) of plants cowpea, subjected to three levels of salinity (Ecw: 0.5, 2.5 and 4.5 dS m⁻¹) and four doses of fertilizer (0, 800, 1600 and 2400 mL), Redenção - CE, 2016.

Source: Prepared by the author, 2016.

Similar results were found by [16], which when working with beans (*Phaseolus vulgaris* L.) observed that the dry mass of aerial part was influenced significantly by the application of the biofertilizer. Then, the positive results for this variable when the plants were subjected to a dose of 2400 mL of biofertilizer, possibly due to the greater availability of nutrients and by improving soil physics cultivated.

For the variable dry mass of the root (MSR), plants irrigated with water of Ecw 0.5 and 2.5 dS m⁻¹ showed a linear increase of 0.0003 and 0.0014 g as a function of doses of fertilizer, respectively. When the plants were irrigated with water of 4.5 dS m⁻¹, they presented constancy in the value of 9.23 g (Figure 2).

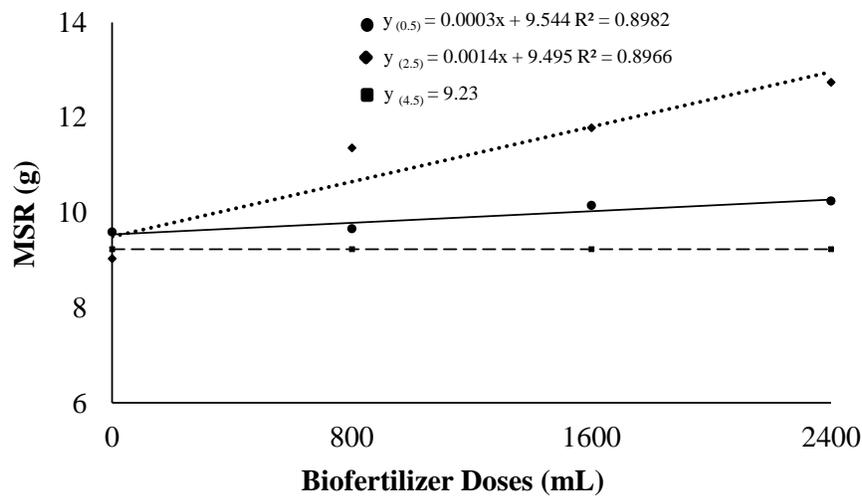


Figure 2: Dry mass of the root (MSR) Plants of cowpea, subjected to three levels of salinity (ECw: 0.5, 2.5 and 4.5 dS m⁻¹) and four doses of fertilizer (0, 800, 1600 and 2400 mL), Redenção - CE, 2016.

Source: Prepared by the author.

The growth, the increase of biomass, productivity and the nutritional status of the bean plant are affected by a large number of factors, highlighting the fertilization carried out [17]. Therefore, the linear increase observed in the MSR when plants were irrigated with waters of ECw 0.5 and 2.5 dS m⁻¹, possibly, were influenced by the organic fertilization performed with the biofertilizer. These results are similar to those found by [18], who working with *Vigna unguiculata*, observed a significant increase in dry mass of stem and higher averages in the dry mass of the root in accordance with the increase in the doses of fertilizer.

As regards the variable reason dry mass of aerial part and root dry mass (MSPA/MSR) and the interaction between the factors salinity and biofertilizer in the same, adjusted to a linear equation for growing plants irrigated with water of 0.5 dS m⁻¹ with unit increase of 0.003g depending on the doses of biofertilizer. For those irrigated with 2.5 dS m⁻¹, it was observed that the plants when subjected to a dose of fertilizer for the 2400 mL showed a maximum point of reason, with value of 6.85g/g. Already for plants irrigated with water of 4.5 m⁻¹, there was a linear increase as a function of doses applied, and this is possibly due to the beneficial effect on biomass accumulation of the aerial part and the improvement of water retention in the soil (Figure 3).

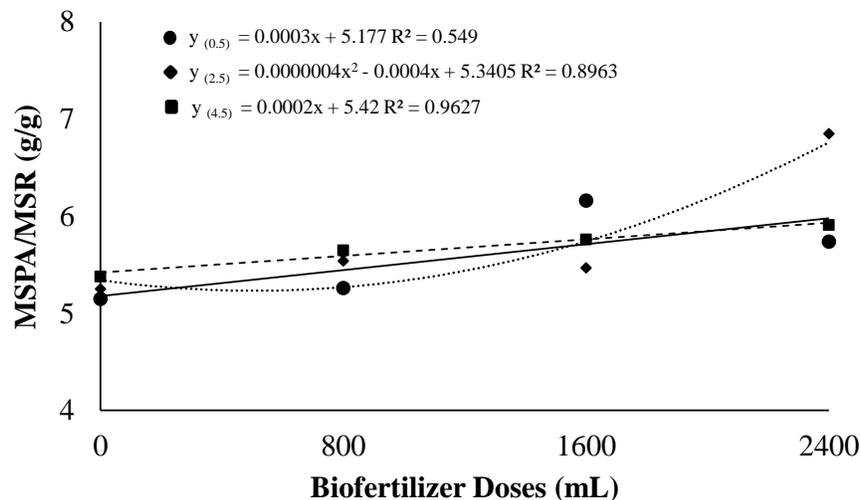


Figure 3: The ratio of the dry mass of aerial part and root dry mass (MSPA/MSR) Plants of cowpea, subjected to three levels of salinity (E_{cw}: 0.5, 2.5 and 4.5 dS m⁻¹) and four doses of fertilizer (0, 800, 1600 and 2400 mL), Redenção - CE, 2016.

Source: Prepared by the author, 2016.

These results corroborate those found by [19] that when working with cowpea (*Vigna unguiculata*), observed that the relationship MSPA/MSR has been increased by the application of earthworm humus incorporated into the soil. The best results are displayed when the plants were irrigated with 2.5 dS m⁻¹ and biofertilized with doses of 2400 mL, possibly due to the beneficial effects of organic compost to the soil, aiding the full development of bean.

As for the efficiency of water use, as regards the plants irrigated with 0.5 dS m⁻¹ was adjusted to a quadratic equation which presented maximum point of 3.43 $\mu\text{mol H}_2\text{O mol de CO}_2^{-1} \text{ m}^{-2} \text{ s}^{-1}$ when the plants were biofertilized with the dose of 1416.67 mL. The plants irrigated with 2.5 dS m⁻¹ showed a linear increase as a function of doses of fertilizer mixed, reaching the maximum point of 2.87 $\mu\text{mol H}_2\text{O mol de CO}_2^{-1} \text{ m}^{-2} \text{ s}^{-1}$ when biofertilized with a dose of 2400 mL, being above the minimum point (2.33 $\mu\text{mol H}_2\text{O mol de CO}_2^{-1} \text{ m}^{-2} \text{ s}^{-1}$) approximately 18.81%.

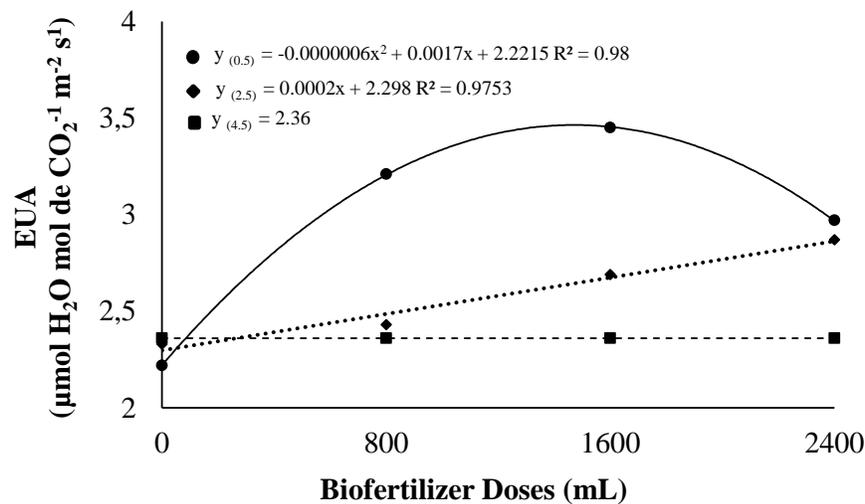


Figure 4: Water use efficiency in plants of cowpea, subjected to three levels of salinity (Ecw: 0.5, 2.5 and 4.5 dS m⁻¹) and four doses of fertilizer (0, 800, 1600 and 2400 mL), Redenção - CE, 2016.

Source: Prepared by the author, 2016.

In relation to the EUA, [20], to analyze different cowpea genotypes under water stress in the Mediterranean, verified values of intrinsic efficiency in water use between 0.8 and 1.63 $\mu\text{mol H}_2\text{O mol de CO}_2^{-1} \text{ m}^{-2} \text{ s}^{-1}$, confirming that the conditions of cultivation to which the genotypes were exposed in this work were adequate. When comparing the results found in the present study with those of [20], it is observed that, regardless of the salinity of the water and the dose of fertilizer, the plants grown in Redenção presented results well above, which may confirm the beneficial effect of the biofertilizer.

4. CONCLUSION

The application of doses between 1600 and 2400 mL of mixed biofertilizer proved to be efficient for the biomass accumulation of cowpea for both levels of salinity of irrigation water.

The plants showed a higher efficiency of water use when were biofertilized, independent of salinity of irrigation water.

The doses of mixed biofertilizer more efficient for the cultivation of cowpea in the climatic conditions of the Maciço of Baturité - Ceará were those of 2400 and 1600 mL, respectively. Then, the biofertilizer presents itself as a sustainable alternative, with great potential for use in agriculture.

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