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Gas Exchange and Growth of Medicinal Plant Subjected to Salinity and Application of Biofertilizers

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Abstract

The objective of this study was to evaluate the use of biofertilizers and saline waters on gas exchange and growth of medicinal plant *Plectrantus amboinicus*. The experiment was conducted in the period February to May 2013 in a greenhouse. The experimental design was completely randomized in a 2 × 4 factorial arrangement, with two levels of salinity of irrigation water (ECw: 0.7 and 3.1 dS m^{-1}) and four levels of bovine liquid biofertilizer applied to the soil, corresponding to 0%, 10%, 20% and 30% of the soil volume, with five replications. The experiment lasted 60 days, counted from the beginning of the treatments. The stomatal conductance (gs), photosynthesis (A), transpiration (E), intrinsic water use efficiency (WUEi) were performed at the end of the experiment, and the height, number of leaves and stem diameter at the beginning and at the end. Generally plants subjected to salinity of irrigation water of 3.1 dS m^{-1} had the lowest values of gas exchange. Moreover, the application of biofertilizers and the interaction between this and salinity did not affect any growth variable studied except the stem length in the final phase which was influenced by salinity at 5% probability by F test. The average values of this variable were 57.22 cm and 69.65 cm when applied water ECw: 0.7 to 3.1 dS m^{-1}, respectively. The application of biofertilizers can reduce the effect of salinity on the final plant height of *Plectrantus amboinicus*, especially when the plants were fertilized with a dose of 20% of biofertilizers.

Keywords

Medicinal Plants, Salt Stress, Fertilizers

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1. Introduction

High concentration of salts is an important characteristic of the soils of semi-arid and arid regions of the world and is a continuing threat to crop production [1]-[3]. It has been estimated that high soil salinity brought about by mismanaged irrigation systems undermines the yield productivity of at least one-third of the world's irrigation lands [4]. Recent trends and future projections suggest that the need to produce more food and fiber for the expanding population will lead to an increase in the use of salt-prone water and land resources for crop-production systems, and this will be met by using salt-tolerant crops or halophytes [5] [6]. Biosaline agriculture is now becoming a reliable strategy for using saline environment [7].

As [8] there are several effects caused by salt stress in plants: the osmotic effect, the toxic effect of mineral elements (chlorides, boron, sodium) and the indirect effects, which occur when high concentrations of sodium or other cations in solution interfere with the physical condition of the soil or the availability of certain elements, affecting the growth and development of plants indirectly. The excess salts from irrigation water cause a reduction in photosynthetic rates, stomatal conductance and transpiration of plants. In addition to the gas exchange variables [9] found declines in chlorophyll levels, which result from imbalances in the physiological and biochemical activities promoted by the content of salts, above the limit tolerated by crops. However, saline soils can be utilized by growing salt tolerant crops. For example, *A. majus* is a potential medicinal crop, it could be grown on salt-affected lands if it possesses high degree of salt tolerance [10].

One of the alternatives that can minimize the deleterious effects of salinity and other adverse effects of the environment is the use of organic substances. The organic material functions as an organic soil amendment, since it can reduce the exchangeable sodium percentage (ESP) due to the release of CO_2 and the production of organic acids during their decomposition, increase water retention and acts as a source of calcium and magnesium rather than the sodium [11]. An example of this was observed by [12], to detect the mitigating action of bovine biofertilizer in plants of paluma guava (*Psidium guajava*) subjected to salinity of irrigation water. However, little is known about the use of biofertilizers on medicinal plants.

Of the potential medicinal plants being cultivated these days, *Plectranthus amboinicus* (Lour) Spreng, belonging to family Lamiacea, is a traditional medicinal herb which is also known as Karpuravalli, Omavalli in Tamil, Patta ajavayin, Patharcur in Hindi, Country borage in English [13]. This species is a large succulent aromatic perennial herb which together with *Plectranthus barbatus* have the widest geographical range of family Lamiacea occurring beyond Africa and Asia continents into the Americas [13]. The species *Plectranthus amboinicus* has a variety of properties. The leaves have been used in malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, anthelmintic, colic and convulsions [13], are often rubbed into the hair and body after bathing [14], are mixed with sugar and used as an intoxicant [15], used as insect repellants [16], in food stuffings [17], for flavouring and marinating beef and chicken [18]-[22], to mask odor of strong smells associated with goat, fish and shellfish [14]. Also this plant is offered to the spirits when a house is being built [14].

Based on the foregoing, the aim of the present study was to evaluate the use of biofertilizers and salt on gas exchange and development of medicinal plants of the *Plectranthus amboinicus*.

2. Materials and Methods

2.1. The Experiment Site

The experiment was conducted in a greenhouse owned by the Center for Teaching and Research in Urban Agriculture (NEPAU), Federal University of Ceará-UFC, located in Fortaleza-CE, latitude 3˚44' S, longitude 38˚33' W, in altitude of 20 m during the period from February to May 2013. The climate is Aw (rainy tropical) according to Köeppen classification. The average values of temperature and relative humidity inside the greenhouse were 30.02˚C and 79.5%, respectively.

2.2. Plant Material

For this study we used the species (*Plectranthus amboinicus* (Lour) Spreng). The seedlings of this species were produced by vegetative propagation (cuttings) and placed in polyethylene bags containing a mixture of substrate as aloof + earthworm humus, in a 2:1 ratio, where they will remain for a period of 30 days. Subsequently, seedlings were selected for uniformity, height and diameter of the pile, and transplanted to plastic pots with a capacity of 7.5 L, containing the same substrate used for the training of seedling. The characteristics of the substrate were pH in H_2O (1:2.5) = 6.7; Ca^{2+} = 5.1 $cmol_c/dm^3$; Mg^{2+} = 3.6 $cmol_c/dm^3$; Na = 1.39 $cmol_c/dm^3$; K^+ = 2.15 $cmol_c/dm^3$; SB = 12.25 $cmol_c/dm^3$; H + Al = 1.65 $cmol_c/dm^3$; Al^{3+} = 0.00; V% = 88.13; P = 493.84 mg/dm^3; C = 1.43%; M.O. = 2.46%.

Before application of treatments the seedlings were kept for 10 days in greenhouse with 50% brightness, in order to restore the stress suffered at transplanting and started to issue new roots and leaves. The experiment lasted 60 days, counted from the beginning of the application of the treatments, the plants were kept in a greenhouse with 50% brightness.

2.3. Experimental Design and Treatments

The experiment was conducted according to a completely randomized design in a 2 × 4 factorial arrangement, with two levels of salinity of irrigation water ECw: 0.7 and 3.1 dS m^{-1} and four levels of biofertilizers applied liquid bovine to the ground, corresponding to (0, 10, 20 and 30%) of the volume of the soil, with five replications.

Water sources used were from two wells, one with electrical conductivity of 0.7 dS m^{-1} and the other with ECw of 3.1 dS m^{-1}. Irrigation was performed every two days and the amount of water applied was estimated with the objective of soil to reach field capacity, adding a leaching fraction of 0.15 for the water percole the bottom of the vessels in order to avoid excessive accumulation of salts. The application of water was manual, localized way to prevent direct effects on the leaves.

The biofertilizer was prepared by anaerobic fermentation of manure in black plastic pots containing fresh water at a ratio of 50% (volume/volume = v/v) for a period of thirty to sixty days. The results of three chemical analyzes of biofertilizers performed throughout the experiment are shown in **Table 1**.

2.4. Variables Evaluated

At the end of the experiment were performed on fully expanded leaf measurements of stomatal conductance (gs), net photosynthetic rate (A) and transpiration rate (E), using an infrared gas analyzer (IRGA) model Li-6400XT (Licor, USA). The measurements were conducted between 08:00 and 12:00h under natural conditions of air temperature, and CO_2 concentration, and photosynthetic active radiation of 1600 μmol m^{-2} s^{-1}. From the data obtained was estimated intrinsic water use efficiency (A/gs). During the measurements of gas exchange is also estimated the IRC, using laptop SPAD 502 (Minolta).

Height was determined with the aid of a graduated ruler, the number of leaves was determined by manual counting and stem diameter using a caliper.

2.5. Statistical Analyzes

The results were statistically analyzed using the "ASSISTAT 7.6 BETA" program. The data of analyzed variables were subjected to analysis of variance and subsequently as significant by the F test, submitted to Tukey's

Table 1. Chemical attributes of biofertilizer used in the experiment.

Elements	1st Application	2nd Application	3rd Application	units
N	0.7	1.1	1.0	$g \cdot kg^{-1}$
P	0.1	0.3	0.3	$g \cdot kg^{-1}$
P_2O_5	0.2	0.7	0.7	$g \cdot kg^{-1}$
K	1.5	1.7	1.5	$g \cdot kg^{-1}$
K_2O	1.8	2.1	1.8	$g \cdot kg^{-1}$
Ca	2.0	1.6	1.5	$g \cdot kg^{-1}$
Mg	0,5	0.8	0.6	$g \cdot kg^{-1}$
Fe	28.1	69.8	69.1	$mg \cdot kg^{-1}$
Cu	0.6	0.3	0.9	$mg \cdot kg^{-1}$
Zn	6.7	17.9	20.4	$mg \cdot kg^{-1}$
Mn	5.3	12.1	14.0	$mg \cdot kg^{-1}$

$p < 0.05$. In regression analysis, the equations that best fit the data were selected based on the significance of the regression coefficients at 1% and 5% probability by F test and greater coefficient of determination.

3. Results

3.1. Gas Exchange

In the analysis of variance shown in **Table 2**, it can be observed that with the exception of chlorophyll content all gas exchange variables were influenced by salinity. The biofertilizer and biofertilizer/salinity interaction did not affect the chlorophyll content, and intrinsic water use efficiency (WUEi), while stomatal conductance (gs), photosynthesis (A) and transpiration (E), were influenced by these two factors, the significance level of 1% probability by F test.

Observe in **Figure 1** that generally plants subjected to salinity of irrigation water from 3.1 dS m^{-1} had the lowest values of gas exchange. However, it appears that plants irrigated with irrigation conductivity of 0.7 dS m^{-1} water suffered an abrupt decrease when applied 30% of biofertilizer, which did not occur in plants under higher salinity.

3.2. Growth

In the analysis of variance presented in **Table 3**, it can be observed that no variable was influenced by salinity, biofertilizer or the interaction between these two factors, with exception of the height at the end of the experiment which was influenced by salinity at a significance level of 1% probability by F test. This shows the impossibility of detecting the benefit of the use of biofertilizers in alleviating the effect of salinity on plants of the *Plectranthus amboinicus*, at least at the levels used in the present study.

It is observed in **Figure 2** that the plants *Plectrantus amboinicus* collected at the end of the experiment, independent of the electrical conductivity of the irrigation water, had lower height values when doses were fertilized with 30% of biofertilizers. The highest value for this variable was observed in plants grown with 3.1 ECw water and fertilized with 20% of biofertilizers.

4. Discussion

Similar to that observed in this study, other authors have observed that salinity reduces gs, *A* and *E* in most plant species [23]-[25]. Salt-induced reduction of *A* can be caused by stomatal limitation with stomatal closure [26] [27],

Table 2. Values summarized the analysis of variance for stomatal conductance (gs), rates of net photosynthesis (A), transpiration (E), chlorophyll, intrinsic water use efficiency (WUEi) in plants of *Plectrantus amboinicus* under different levels of biofertilizers and water salinity irrigation.

Sources of variation	DL	Medium Square				
		gs	*A*	E	Chlorophyll	WUEi
Salinity (A)	1	0.11^{**}	129.70^{**}	55.13^{**}	0.11^{ns}	4532.85^{**}
Biofertilizer (B)	3	0.01^{**}	34.76^{**}	8.27^{**}	2.20^{ns}	46.17^{ns}
Int. (A × B)	3	0.02^{**}	45.90^{**}	13.44^{**}	10.75^{ns}	207.30^{ns}
Treatments	7	0.03^{**}	53.10^{**}	17.18^{**}	5.56^{ns}	756.18^{**}
Residue	32	0.0027	4.64	1.48	16.32	210.16
CV%	-	46.40	37.20	36.11	13.88	25.58
Salinity 0.7 dS m^{-1}	-	0.16a	7.59a	4.55a	29.16a	46.03b
Salinity 3.1 dS m^{-1}	-	0.06b	3.99b	2.20b	29.05a	67.32a
Biofertilizer 0%	-	0.13a	7.04a	3.76a	29.27a	59.35a
Biofertilizer 10%	-	0.14a	6.96a	4.06a	29.57a	56.64a
Biofertilizer 20%	-	0.12a	6.07a	3.65a	28.46a	56.62a
Biofertilizer 30%	-	0.05b	3.07b	2.04b	29.13a	54.09a

**, * and ns—significant at 1% and 5% of probability and not significant by F test, respectively. CV—coefficient of variation in percent. DF—Degrees of liberty. Means followed by the same lower case letter in columns do not differ by Tukey test ($p < 0.01$ and $p < 0.05$).

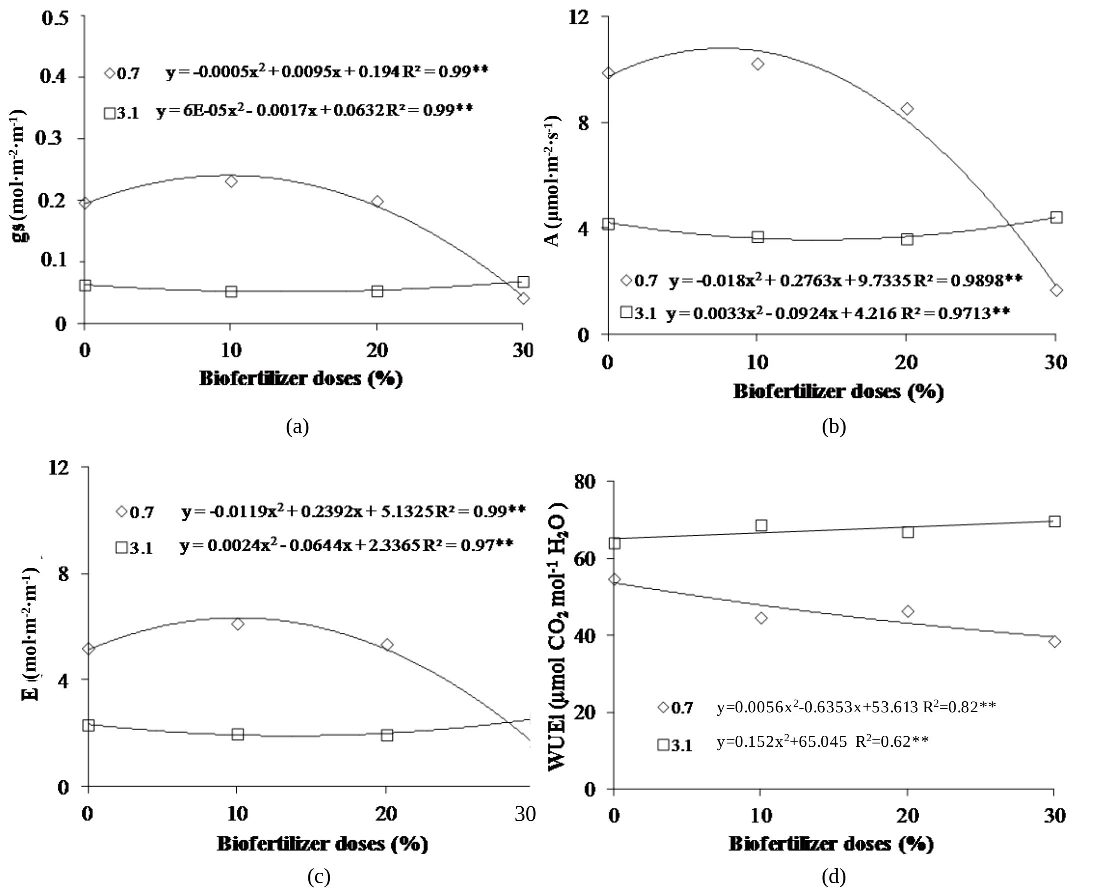

Figure 1. Stomatal conductance (gs), rates of net photosynthesis (A), transpiration rate (E), intrinsic water use efficiency (WUEi) in plants of *Plectrantus amboinicus* under two levels of irrigation water salinity (ECw: 0.7 and 3.1 dS m^{-1}) and four doses of biofertilizer.

Table 3. Values summarized analysis of variance for the height, number of leaves and stem diameter of plants of *Plectrantus amboinicus* under different levels of biofertilizers and salinity of irrigation water.

Sources of variation	DL	Medium Square					
		Height		Number of leaves		Stem diameter	
		initial	end	initial	end	initial	end
Salinity (A)	1	2.50^{ns}	1543.80*	0.02^{ns}	90.00^{ns}	0.12^{ns}	1.52^{ns}
Biofertilizer (B)	3	11.84^{ns}	251.02^{ns}	1.49^{ns}	35.00^{ns}	0.48^{ns}	0.63^{ns}
Int. (A × B)	3	11.61^{ns}	166.83^{ns}	1.49^{ns}	490.20^{ns}	1.19^{ns}	1.20^{ns}
Treatments	7	10.41^{ns}	399.62^{ns}	1.28^{ns}	237.94^{ns}	0.73^{ns}	1.00^{ns}
Residue	32	14.51	212.03	2.07	245.00	0.80	0.82
CV%	-	20.24	22.95	22.60	19.49	13.61	11.62
Salinity 0.7 dS m^{-1}	-	18.57a	57.22b	6.40a	81.90a	6.64a	7.61a
Salinity 3.1 dS m^{-1}	-	19.07a	69.65a	6.35a	78.90a	6.53a	8.00a
Biofertilizer 0%	-	19.85a	60.85a	6.10a	82.20a	6.61a	7.44a
Biofertilizer 10%	-	17.55a	63.00a	6.00a	78.50a	6.61a	7.94a
Biofertilizer 20%	-	18.30a	70.60a	6.80a	81.80a	6.83a	7.87a
Biofertilizer 30%	-	19.60a	59.30a	6.60a	79.10a	6.29a	7.99a

**, * and ns—significant at 1% and 5% of probability and not significant by F test, respectively. CV—coefficient of variation in percent. DF—Degrees of liberty. Means followed by the same lower case letter in columns do not differ by Tukey test ($p < 0.01$ and $p < 0.05$).

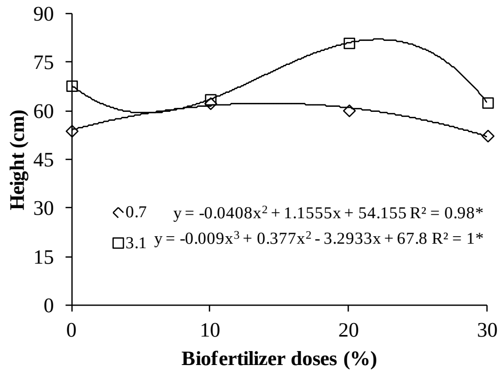

Figure 2. Plant height of *Plectrantus amboinicus* under two levels of salinity of irrigation water (ECw: 0.7 and 3.1 dS m^{-1}) and four doses of biofertilizer.

nonstomatal limitation or both limitations with stomatal closure at low tissue salt concentration, and a disturbance of photosynthetic activity at high tissue salt content [28] [29]. However, some researchers not find any significant effect of salt stress on gas exchange in herbal *A. majus* [10], *Hibiscus cannabinus* [30], *Hordeum vulgare* [31], *Trifolium repens* [32], *Triticum aestivum* [33] [34], and *Olea europea* [35].

According [2], inhibition of growth in plants grown under salinity may be due to osmotic effects of salts, reducing the availability of water in the soil solution and/or the excess of ions absorbed by the plant metabolism leading to toxic effects, and this phase that clearly separates species and genotypes that differ in tolerance or sensitivity to salinity. However, in the present work to fertilizing with biofertilizer at least up to 20%, seems to have alleviated the effect of salinity stress on plant height at the end of the experiment, whereas the values of this variable were higher when irrigated with saline water higher electrical conductivity.

Excess salts can alter the physiological and biochemical functions of plants, resulting in disturbances in water relations, changes in the absorption and use of essential plant nutrients, affecting their final growth and yield. However, according to [36], the intensity of salt stress is dependent on many factors, among them, the plant species, cultivar, developmental stage, saline medium composition, intensity and duration of stress, as well as the climatic conditions and irrigation management. This may explain in part the fact that salinity did not influence the studied plants.

Regarding the relationship beneficial for biofertilizer with salinity, some studies have shown that the use of biofertilizer in saline environments can partially mitigate the impact of salinity on the growth of plants [37]. The importance of the use of liquid biofertilizers, as simple or enriched microbial fermentation, is the quantitative elements in the diversity of chelated and made available for biological activity as an enzyme activator and plant metabolism nutrients.

5. Conclusions

The stomatal conductance, rates of net photosynthesis and transpiration showed lower values when the plants of *Plectrantus amboinicus* were irrigated with ECw: 3.1 dS m^{-1}. However, it can be seen that the interaction of biofertilizers to 30% with saline water benefited gas exchange of these plants.

The application of biofertilizers can reduce the effect of salinity on the final plant height of *Plectrantus amboinicus*, especially when the plants were fertilized with a dose of 20% of biofertilizers. This positive effect can be associated with better nutrition of the plant.

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