

Full Length Research Paper

Morphophysiology of peppermint irrigated with salt water and bovine biofertilizer

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Peppermint is a medicinal plant grown worldwide, but it has not been extensively studied, especially the use of saline water for its cultivation. In this sense, the objective of this study is to evaluate the effect of saline waters on peppermint cultivation under the application of bovine biofertilizer. The experiment was carried out from September 2015 to December 2015 in a greenhouse of the Center of Human and Agrarian Sciences of the State University of Paraíba (UEPB) in the municipality of Catolé do Rocha-PB, Brazil. The experimental design was completely randomized, in a factorial scheme of 5 × 2, with 8 replicates. The treatments consisted of electric conductivity combined with irrigation water (ECw) of 1.0, 2.0, 3.0, 4.0, and 5.0 dS m⁻¹ in the presence and absence of bovine biofertilizer. The increase in electrical conductivity of the irrigation water levels from 1 dS m⁻¹ reduced the growth, development and production of peppermint biomass. Peppermint plants that received bovine biofertilizer had superior results in growth and biomass production. The application of bovine biofertilizer attenuates the effects of salty peppermint. The growth and production of peppermint biomass increased when the plants are irrigated with low salinity water (1 dS m⁻¹) using bovine biofertilizer.

Key words: *Mentha piperita* L., electrical conductivity, mitigating the salt stress.

INTRODUCTION

Peppermint (*Mentha piperita* L.) belongs to the Lamiaceae family. Originating in Europe and North Africa, it is widely cultivated in Brazil. It is a hybrid coming from the crossing of *Mentha aquatica* L. and *Mentha viridis* L. Botanical synonyms: *Mentha citrata* Ehrh. It is also

known as peppermint, mint, mint-kitchens, and sandalwood. It is an annual or perennial herb, aromatic, about 50 cm high, erect, and with branches that are dark green to purple. It has a quadrangular structure. The leaves have opposite arrangement, elliptic-acuminate,

jagged and pubescent. It has lilac flowers which are gathered in axillary glomeruli (Grandi, 2014).

The impact of salinity is considered a limiting factor in agricultural production in the arid and semi-arid regions of the world. In order to reduce the negative impacts on agriculture, alternatives are sought for the reutilization of unused areas, such as agricultural varieties tolerant to these conditions, as well as the prospection of substances capable of reversing the damage caused by salinity during cultivation (Kaiser et al., 2016).

To attenuate the effects of saline stress, organic input has been recommended as the bovine biofertilizer. Several studies have demonstrated the positive effects of organic inputs (El-Dardiry, 2007; Miranda et al., 2011). Among them is bovine biofertilizer; it improves the soil in terms of aeration (Aidyn et al., 2012) and it has complex substances used to mitigate the depressive effects of water salinity on plants.

In addition, bovine biofertilizer has a positive action; its composition has many beneficial substances. Among them are the humic substances which promote the reduction of the osmotic potential of soil solution, and stimulate the absorption of water and nutrients by plants in saline environments. The application of biofertilizer in the soil can induce increased osmotic adjustment in the plants by the accumulation of organic solutes, promoting the absorption of water and nutrients in adverse saline environments (Baldotto and Baldotto, 2014).

Due to the lack of studies on peppermint, especially in the semi-arid region, where it has low rainfall indexes, the use of low quality water in irrigation becomes relevant in this sense, to evaluate the effect of saline waters in the cultivation of mint using bovine biofertilizer.

MATERIALS AND METHODS

The experiment was carried out from September 2015 to December 2015 in a greenhouse at the Center for Human and Agrarian Sciences of the State University of Paraíba (UEPB) in the municipality of Catolé do Rocha-PB, (6° 20'38 "S ; 37° 44'48 "W). The climate of the municipality, according to the Koppen classification, is of the type BSW', that is to say, warm and dry type of steppe, with average monthly temperature superior to 18°C, throughout the year and 275 m of altitude.

Mint seedlings grown in 128-cell expanded polystyrene trays containing commercial Plantmax Hortaliças HT[®] substrate were obtained. The seedlings were grown in a greenhouse, with 60% shading, for 15 days, until it reaches about 10 cm in height. After acclimatization, they were transplanted to polyethylene pots with capacity of 8 dm³ in September 2015.

The experimental design was completely randomized, in a factorial scheme of 5 × 2, with 8 replications. The treatments consisted of electric conductivity combined with irrigation water

(ECw) of 1.0, 2.0, 3.0, 4.0 and 5.0 dS m⁻¹ in the presence and absence of biofertilizer bovine. The experimental units consisted of three plants.

To fill the vessels, a flavic Neosol was used with a sandy loam clay texture. Samples were collected in 0 to 20 cm layer in a native area located on UEPB campus. A subsample was withdrawn and analyzed chemically, with the following characteristics (Table 1).

Bovine biofertilizer was obtained by anaerobic fermentation, that is, in a hermetically sealed environment. To release the methane gas at the top of each biodigester one end of a thin hose was coupled and the other end was immersed in a vessel with water. For the preparation of the biofertilizer, 70 kg of bovine manure from lactating cows and 120 L of water were used. 5 kg of sugar and 5 L of milk were added to accelerate the metabolism of the bacteria.

Biofertilizer treatments were applied 15 days after sowing (DAS), at 8 days interval; totalling 6 applications of 10% dosage of the vessel volume (0.8 dm³). Prior to application, bovine biofertilizer was diluted in water (5%), after which it was subjected to screen filtration to reduce the risks of obstruction of the watering system. The biofertilizer was analyzed (Table 1).

The water used for the irrigation was supplied from the local water supply and had electrical conductivity of 1.0 dS m⁻¹. Treatments with salinities were performed at 15 days after emergence. The plants were irrigated daily with each type of water, starting on the fifteenth day after sowing. Irrigation was performed manually by watering, providing a blade sufficient to raise the soil moisture at the field capacity level.

Different ECws were obtained by the addition of sodium chloride (NaCl) to the water from the local supply system, according to Rhoades et al. (2000). The quantity of salts (Q) was determined by the equation:

$$Q \text{ (mg/L}^{-1}\text{)} = \text{ECw} \times 640$$

where ECw (1.0 dS m⁻¹) is the desired value of electric conductivity from water.

The height of the plant, stem diameter and leaf area were evaluated at 30 and 90 DAS. In the measurement of plant height, a measuring tape graduated in cm was used, measuring the distance between the collar and the apex of the plant (Insertion of the youngest fully formed leaf). Measurements of stem diameter were performed with a digital caliper at 2 cm above the neck of the plant. The leaf area was obtained by measuring the width and length of the leaf.

From the mean monthly values of plant height, stem diameter and leaf area, their respective absolute growth rates (AGR) and relative growth rates (RGR) were calculated according to Benincasa (2003).

At 90 days after sowing, harvesting was performed in which the plants were separated into roots, stem and leaf; they were dried in an oven with forced ventilation at 65°C until constant weight was obtained. The fresh mass of the aerial part was determined by weighing in a precision scale of 0.0001 g. Later the parts of the plants (root, stem and leaves) were weighed in a precision balance of 0.0001 g. The total dry matter production data were used to calculate the percentages partitioned between vegetative organs and the rate of salinity tolerance. The data from saline treatments with the control (ECw = 1.0 dS m⁻¹) were compared, according to the methodology of Aquino et al. (2007).

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Table 1. Soil chemical attributes and bovine biofertilizer used in the experiment. Catolé do Rocha - PB, UEPB, 2015.

Characteristics	Soil	Bovine biofertilizer
CaCl ₂ pH	5.02	4.68
EC (dS m ⁻¹)	0.60	4,70
Ca ⁺² (cmol c dm ⁻³)	4.63	3.75
Mg ⁺² (cmol c dm ⁻³)	2.39	3.30
Na ⁺ (cmol c dm ⁻³)	0.30	1.14
K ⁺ (cmol c dm ⁻³)	0.76	0.71
P (mg dm ³)	0.70	14,45
Al ³⁺ (cmol c dm ⁻³)	0.00	0.00
H ⁺ + Al ³⁺ (cmol c dm ⁻³)	1.00	1.00
SB	7.78	7.76
Organic matter (g kg ⁻¹)	8.05	8.00

The data obtained were evaluated by analysis of variance using F test at 0.05 and 0.01 probability level. For significance level, linear and quadratic polynomial regression analysis was performed using the statistical software SISVAR 5.0. (Ferreira, 2011).

RESULTS AND DISCUSSION

There is interaction between the electrical conductivities of the irrigation water (ECw) and the bovine biofertilizer applied on the plant height (PH) at 30 and 60 DAS and for stem diameter (SD) at 90 and 120 DAS. The factors, electrical conductivity in irrigation water and the application of bovine biofertilizer exerted significant effects on all variables analyzed in the periods evaluated.

The plants irrigated with saline water (5.0 dS m⁻¹) showed a reduction in plant height in all periods. However, those treated with bovine biofertilizer demonstrated superiority in values, with 2 reductions, 22 cm at 30 DAS (Figure 1A) and 1.38 cm at 60 DAS (Figure 1B); with those without bovine biofertilizer had reductions of 1.19 cm at 30 DAS (Figure 1A) and 2.24 cm at 90 DAS (Figure 1B), with increased ECw.

At 90 and 120 DAS, plant height as a function of ECw decreased from 1.99 cm to 90 DAS (Figure 1C) and from 2.03 cm to 120 DAS (Figure 1C) for each unit increase of ECw. Bovine biofertilizer positively influenced plant height at 90 and 120 DAS (Figure 1D); the plants that received bovine fertilizer obtained values of 24.32 and 26.1 cm at 90 and 120 DAS, respectively. In addition, in the periods evaluated, bovine biofertilizer provided higher values, demonstrating the beneficial effect of the organic input on the height of the peppermint plant.

Bione et al. (2014) studied basil culture (*Ocimum basilicum* L.) and found that the electrical conductivity in the irrigation water reduced 0.0117 m plant height with

ECw unit 49 days after transplantation (DAT). In tomato, Guedes et al. (2015) observed that saline water negatively affected plants irrigated with water of 3.5 dS m⁻¹; there was a linear reduction from electrical conductivity.

Bovine biofertilizer has humic substances that improve soil structure, increase cell division and permeability of cell membranes; and consequently, provide greater absorption of water and nutrients in plants subjected to saline stress, leading to greater growth (Khaled and Fawy, 2011).

It was observed that increased ECw promoted a reduction in stem diameter at 30 DAS in the order of 0.36 mm and at 60 DAS of 0.37 (Figure 2A). However, at 60 DAS, bovine biofertilizer positively influenced the diameter of the peppermint stem, presenting a value of 2.06 mm, while without bovine fertilizer application, a lower value (1.95 mm) was observed (Figure 2B). At 90 and 120 DAS, the peppermint plants reduced with increased ECw. With increased ECw, there was a decrease in the order of 0.28 mm in the presence of biofertilizer and 0.39 mm without bovine fertilizer at 90 DAS (Figure 2C); there was reduction of 0.26 mm with bovine biofertilizer and 0.39 mm without bovine fertilizer (Figure 2D).

In the culture of eggplant and castor bean, Lima et al. (2015, 2008) observed that increased saline in the irrigation water promoted a reduction in stem diameter. Studying tomato, Medeiros et al. (2014) found that the interaction of salinity × biofertilizers has significant effect and that the plants that received bovine biofertilizer had superior results with maximum diameter value at maximum salinity estimated to be 2.64 irrigation water dS m⁻¹.

Reduction in the diameter of the stem occurred due to the toxic effect of the ions Na⁺ and Cl⁻ which cause

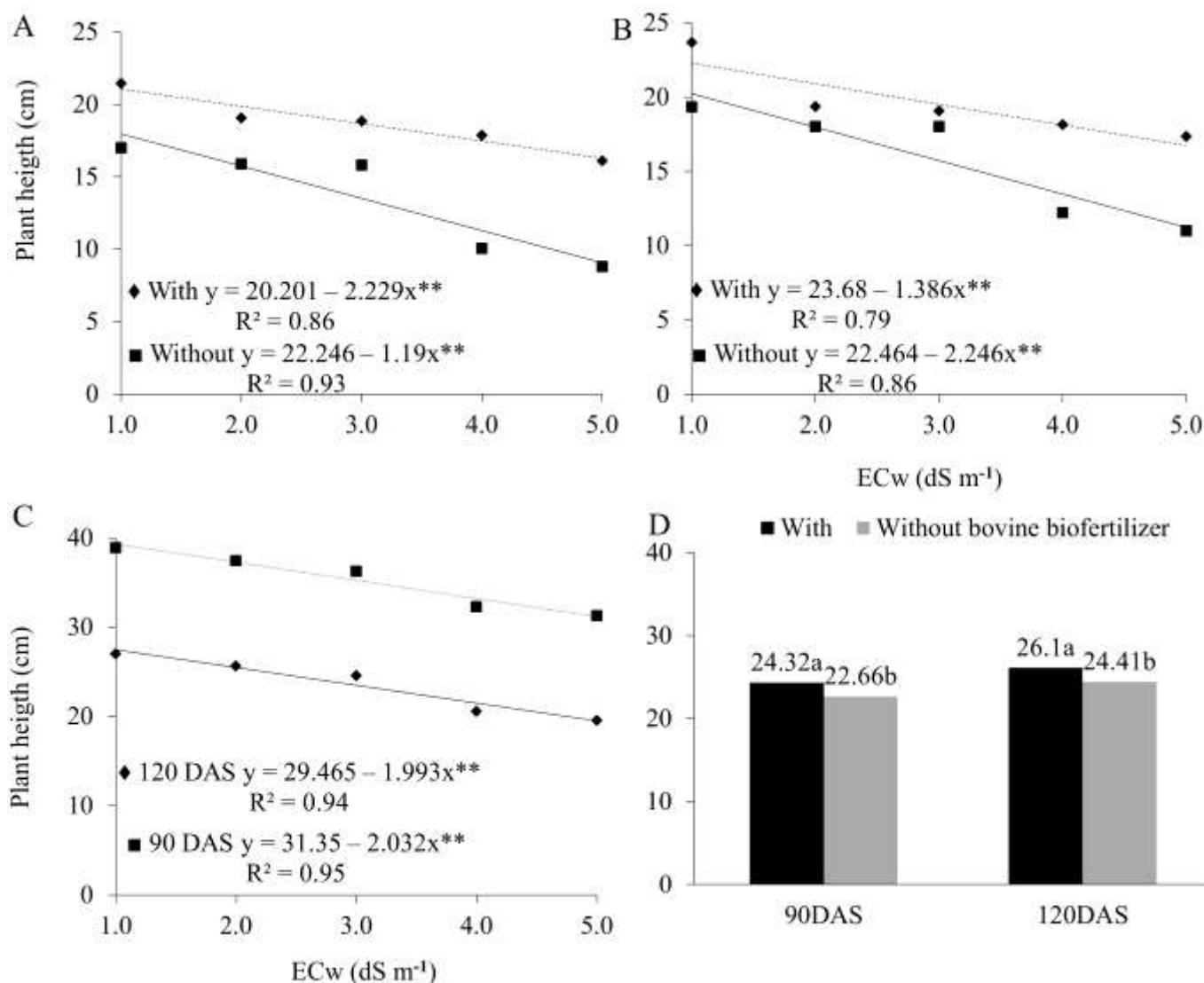


Figure 1. Effect of electrical conductivity in the irrigation water over the height of the ground peppermint plant with and without biofertilizer to 30 (A), 60 (B), and 90 to 120 DAS (C and D).

decreased absorption of water and nutrients and imbalance in the cationic sheet and plant metabolism. This leads to loss in growth and production (Nivas et al., 2011). However, with the application of bovine biofertilizer, plants irrigated with saline water were superior, because of the organic raw material and nitrogen supply. Thus, it is believed that bovine biofertilizer reduces the effect of high concentrations of saline waters and favors the development of plants (Sá et al., 2015).

For the leaf area, there are data that decrease the linear model in all periods (Figure 3A); this shows the extent to which increased ECw of 1.0 to 5.0 dS m⁻¹ led to reductions of 156.66, 157.06, 85.12 and 83.76 cm² for the

leaf area of peppermint at 30, 60, 90 and 120 DAS, respectively (Figure 3A). However, the plants that received bovine biofertilizer presented a larger leaf area and higher results in the periods evaluated: 580.55, 634.26, 852.55, and 898.51 cm² at 30, 60, 90 and 120 DAS, respectively against 502.2, 55.42, 788.7, and 827.95 cm² at 30, 60, 90 and 120 DAS, respectively (Figure 3B).

The decline in leaf area, emergence of new leaves and death and leaf fall occur due to the effects of saline stress, since the plant uses these strategies as a way to reduce water losses (Mahmoud and Mohamed, 2008). The emergence of new leaves and/or foliar senescence occur also because these organs are

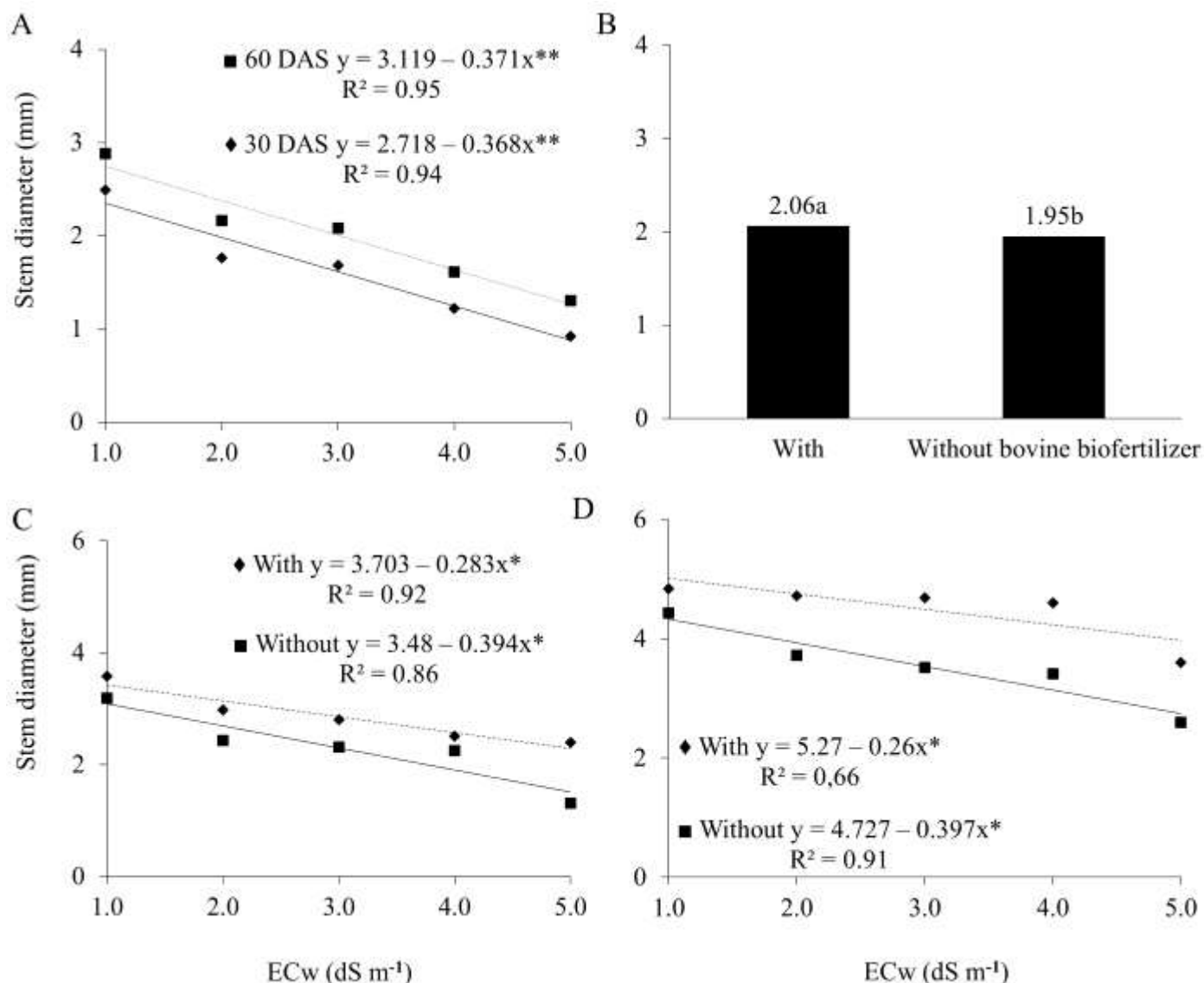


Figure 2. Effect of electrical conductivity in the irrigation water on the diameter of peppermint stem at 30 and 60 DAS (A) in the soil with and without biofertilizer to 60 (B), 90 (C) and 120 DAS (D).

sensitive to salinity and reduce in size and number in the presence of high concentrations of salts. The deleterious effect of salinity on leaf area was also observed by Medeiros et al. (2011), Vieira et al. (2016) and Lycoskoufis et al. (2012) in tomato.

In addition to supplying nutrients and organic matter to the plants, the application of bovine biofertilizer contributes to the tolerance of the plants to salinity, providing improvements in the germination, growth and biomass production (Lancet et al., 2006).

There is a significant effect of electrical conductivities on irrigation water (ECw) in all variables analyzed. For the bovine biofertilizer factor, only significant effect was

observed for the absolute growth rate in plant height (AGRph), absolute growth rate (AGRsd) and relative stem diameter (RGRsd), dry mass of the root (DMR), dry mass of the aerial part (DMAP) and total dry matter (TDM). There was a significant effect of the ECw × bovine biofertilizer interaction on the variables AGRph, RGRph, AGRsd, RGRsd and DMR.

It was observed that the interaction between ECw and bovine biofertilizer exerted significant effects on absolute (AGRph) and relative (RGRph) growth rates of plant height and absolute (AGRsd) and relative (RGRsd) stem diameter; with increased ECW, there were reductions of 0.0083 cm⁻¹ day in AGRph (Figure 4A) and 0.0062 cm

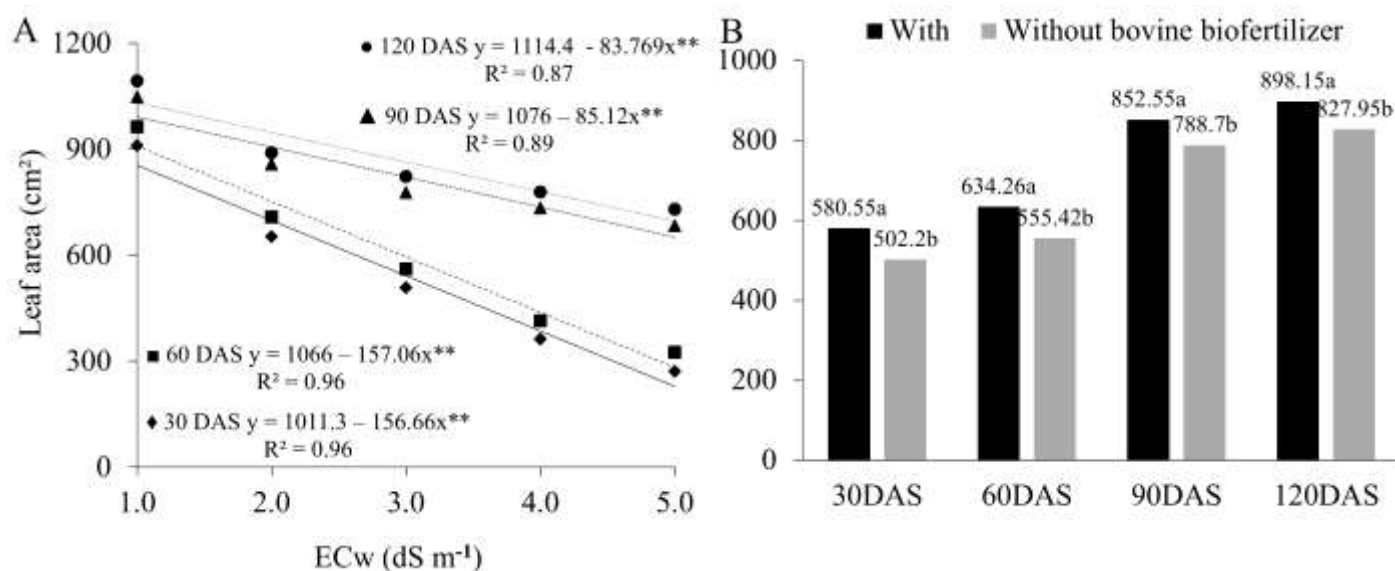


Figure 3. Effect of electrical conductivity in the irrigation water (A) on the leaf area peppermint soil with and without biofertilizer (B) 30, 60, 90 and 120 DAS.

cm⁻¹ day⁻¹ in RGRph (Figure 4B) in plants treated with biofertilizer against 0.0124 cm⁻¹ in day AGRph (Figure 4A) and 0.0106 cm⁻¹ day⁻¹ in RGRph (Figure 4B) in plants treated with bovine fertilizer.

In the absolute growth rate of leaf area (AGRlf), it is noticed that ECw interfered negatively, decreasing linearly with reductions of 0.0088 cm² day⁻¹ for each unit increase in ECw (Figure 4C). It can be seen in Figure 4D that the peppermint plants when subjected to ECw 5.0 dS m⁻¹ showed relative growth rate of leaf area (RGRlf) lower compared to plants irrigated with water of lower salinity (1.0 dS m⁻¹), presenting reductions 0.0037 cm² day⁻¹ per unit increase of ECw.

The peppermint plants had reduced absolute growth rate of stem diameter (AGRsd) of 0.0023 mm day⁻¹ when the plants were treated with bovine biofertilizer and 0.0025 mm day⁻¹ without bovine biofertilizer (Figure 4E); while, the relative growth rate of stem diameter reduction was 0.002 mm⁻¹ day with biofertilizer bovine mm and 0.0021 mm⁻¹ day⁻¹ without bovine biofertilizer (Figure 4F).

The reduction in plant growth when subjected to salinity is caused by water deficit caused by excess soluble salts in the root zone. It causes a decline in turgescence, consequently resulting in a decrease in cell expansion, reducing the growth rate of plants (Khalid and Silva, 2010). This occurs due to the closure of the stomata and consequently less CO₂ assimilation limiting the photosynthetic processes (Debez et al, 2008; Taarit et al., 2010). It can also be caused by the energy expenditure that is required in the synthesis of organic solutes and in the processes of compartmentalization and

regulation of ion transport (Mendonça et al., 2007).

Plant growth rate is one of the most important parameters for evaluating the effects of saline stress as well as the capacity of the plant to overcome salinity, since plant growth processes are particularly sensitive to the effect of salts (Morais et al., 2011). Reductions in growth rates are mainly due to the deleterious effect of excess salts on plant metabolism (Santos et al., 2013).

ECw had a negative influence on shoot fresh mass (DMAP), dry shoot mass (DSM), dry mass of the root (DMR) and total (DMT) of the peppermint plants, according to the regression equations. The model to which the data fit better was linear, so that as the ECw increased, there were decreases of 2.62 and 3.25. The highest gains in DMAP, MSPA, DMR and DMT were observed in the plants treated with bovine biofertilizer, with values of 40.85, 0.89 and 4.20 g in DMAP, MSPA, DMR and DMT, respectively (Figure 5A); 11.05, 2.74 and 1528 g, respectively (Figure 5B).

When the plants are subjected to saline environments, some things occur, such as reduction in biomass production, which has already been observed by several authors (Freire et al., 2010; Gomes et al., 2011; Medeiros et al., 2011). Thus, to reduce energy costs, plants reduce leaf area, among other mechanisms to reduce water losses; as a consequence, there is less accumulation of biomass, since there is a proportional relationship between transpiration and plant production (DIAS et al., 2011).

In addition, saline stress has negative effects on the plant, such as changes in root growth and development; consequently, it interferes with the absorption of ionic

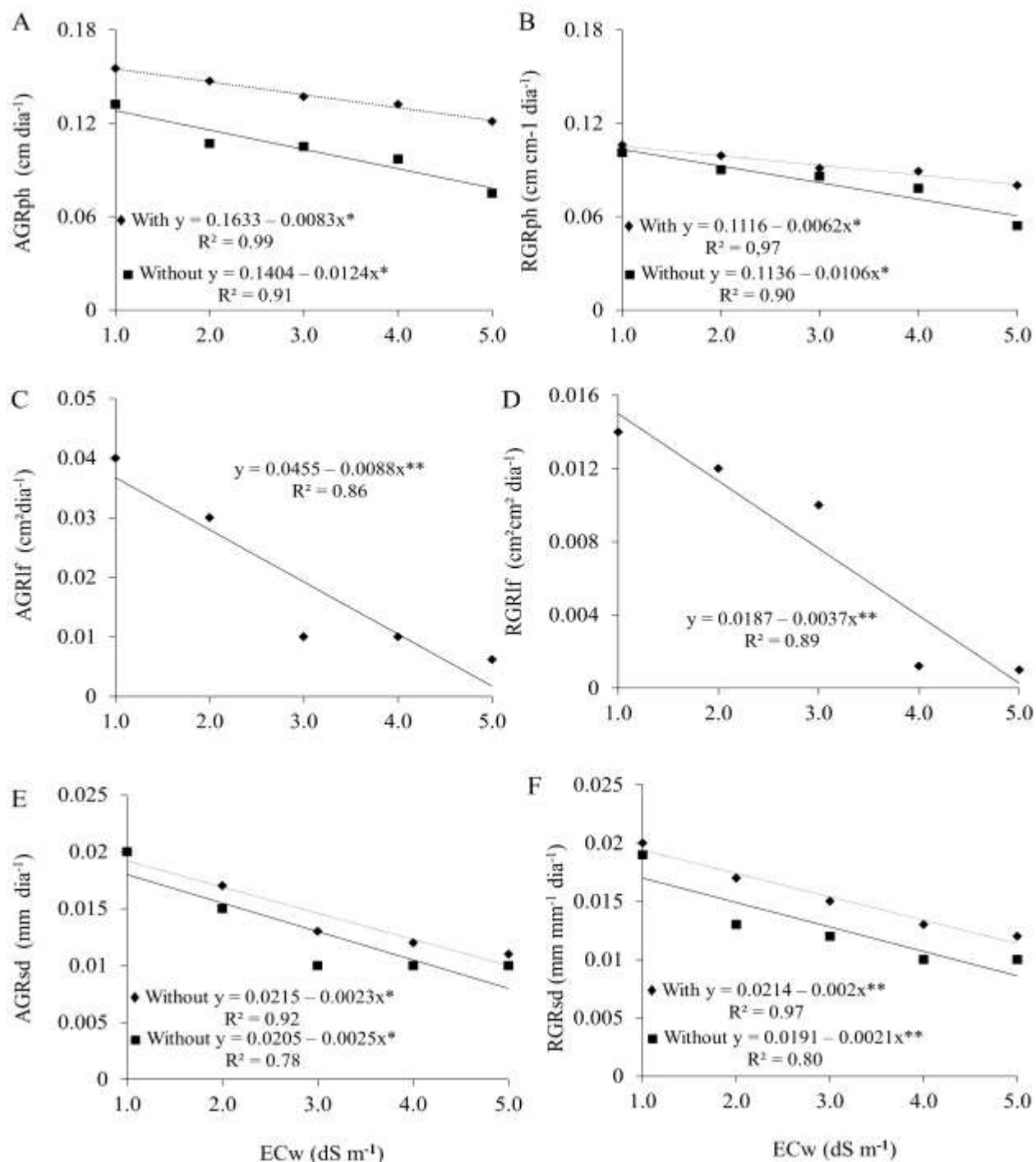


Figure 4. Effect of electrical conductivity in the irrigation water on the absolute growth rates and relative plant height (AGRph (A), RGRph (B)), leaf area (AGRlf (C), RGRlf (D)) and of stem diameter (AGRsd (E), RGRsd (F)) in the soil with and without peppermint biofertilizer.

water, hindering the development of crops; also, the well developed root system provides a greater area of absorption (Soares et al., 2011). However, it is not possible to determine the effect of the plant's nutrients on

the nutrient content of the plant.

Tabatabaei et al. (2007), studying peppermint (*M. piperita* L.) grown in hydroponics, found that increasing concentrations of electrical conductivity solutions

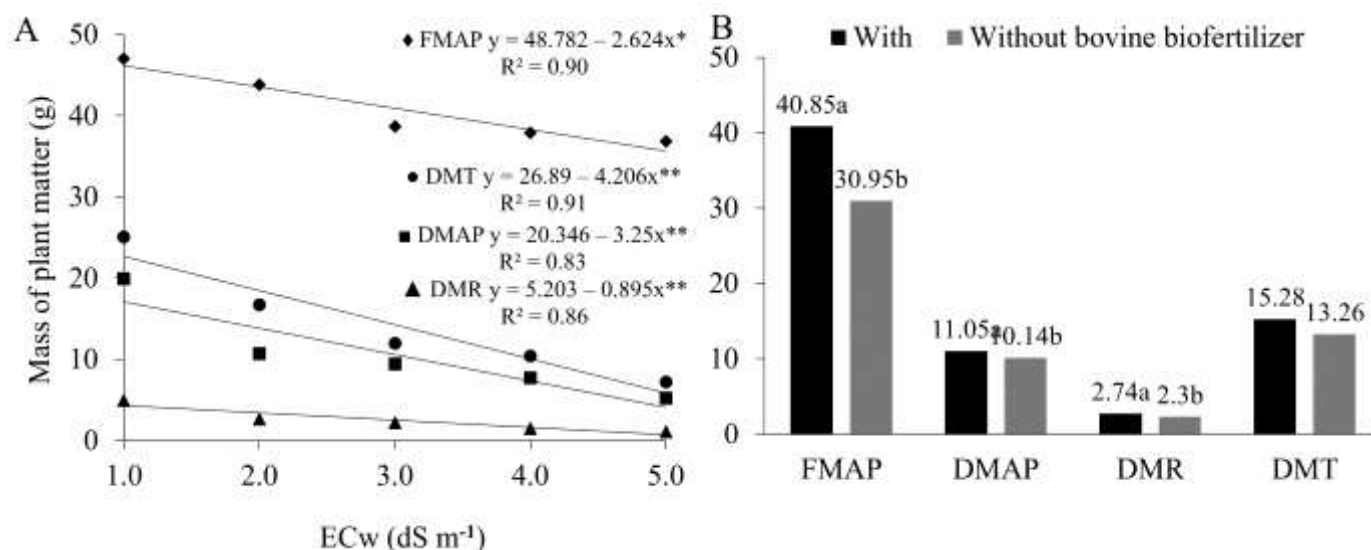


Figure 5. Effect of electrical conductivity in the irrigation water (A) on the mass of the plant matter on the ground with and without biofertilizer (B) peppermint.

adversely affected growth, essential oil content and production of biomass. The same authors found that the highest and lowest accumulation of fresh weight of the plants were obtained in 1.4 and 5.6 dS m⁻¹, respectively. Aziz et al. (2009) found that the mint biomass was reduced when plants were subjected to salinity of 2.56 dS m⁻¹, leading to a reduction of 30%. Tabatabaie and Nazari (2007) also observed that the concentration of NaCl adversely affect the growth of spearmint, wherein the higher biomass yields occurred at moderate levels of electrical conductivity (1.4 to 2.8 dS m⁻¹).

Heidari (2012), working with basil (*O. basilicum* L.) subjected to salinity in nutrient solution, observed a reduction of 17.8% in the mass production of fresh basil when the plants were irrigated with salinity waters over non-saline condition. The reduction in the biomass of the aerial part occurs due to the deviation of energy as a result of the increase of the salinity levels of the soil (Garcia et al., 2007).

In order to reduce the effects of salinity, bovine biofertilizer was used, since it has some beneficial elements, mainly the diversity and availability of the essential nutrients used for biological activity (Alves et al., 2009). In addition, it has many beneficial substances: the humic substances which promote the reduction of the osmotic potential of soil solution, and stimulate the absorption of water and nutrients by the plants, in saline environments (Aydin et al., 2012).

In cherry tomato crop, Medeiros et al. (2011) observed that the application of bovine manure biofertilizers (with and without the addition of molasses, milk and agricultural gypsum) provided a positive effect on

biomass production even with increased salinity of irrigation water.

Behavior similar to the previous variables can be observed in the shoot root ratio and tolerance index, where the data conformed to the linear decreasing model, with a reduction of 0.065 in the shoot root ratio (Figure 6A) and 16.55% in the tolerance index (Figure 6B). In each unit increase of ECw, in higher salinity (5.0 dS m⁻¹), 0.52 value was obtained in the relation of root and shoot ratio plus 28.75% tolerance index; when the plants were irrigated with low salinity water (1.0 dS m⁻¹) a maximum value of 0.79 was obtained in the root and shoot ratio plus 100% of tolerance index.

Studying mint (*O. basilicum* L.), Bernstein et al. (2010) did not find a significant effect on the root/shoot ratio of the hydroponic basil, as a function of salinity. While Bione et al. (2014) observed that the ratio R/PA increased significantly with increasing salinity (8.94% per dS m⁻¹).

The tolerance of peppermint to salinity has been studied by several researchers, such as Khorasaninejad et al. (2010) who verified that the culture is considered moderately tolerant to salinity. However, the plants respond differently to salinity, that is, there is great variability depending on the species, genotype, the phenological stage of the same genotype and salt exposure period (Parida and Das, 2005; Munns and Tester, 2008).

Melo Filho et al. (2016) in peanut crop, Veras et al. (2016) in castor bean and Alves et al. (2016) in cotton observed that bovine biofertilizer does not mitigate the effect of salt stress; however, better results in growth rate, production and tolerance can be observed with the application of this input.

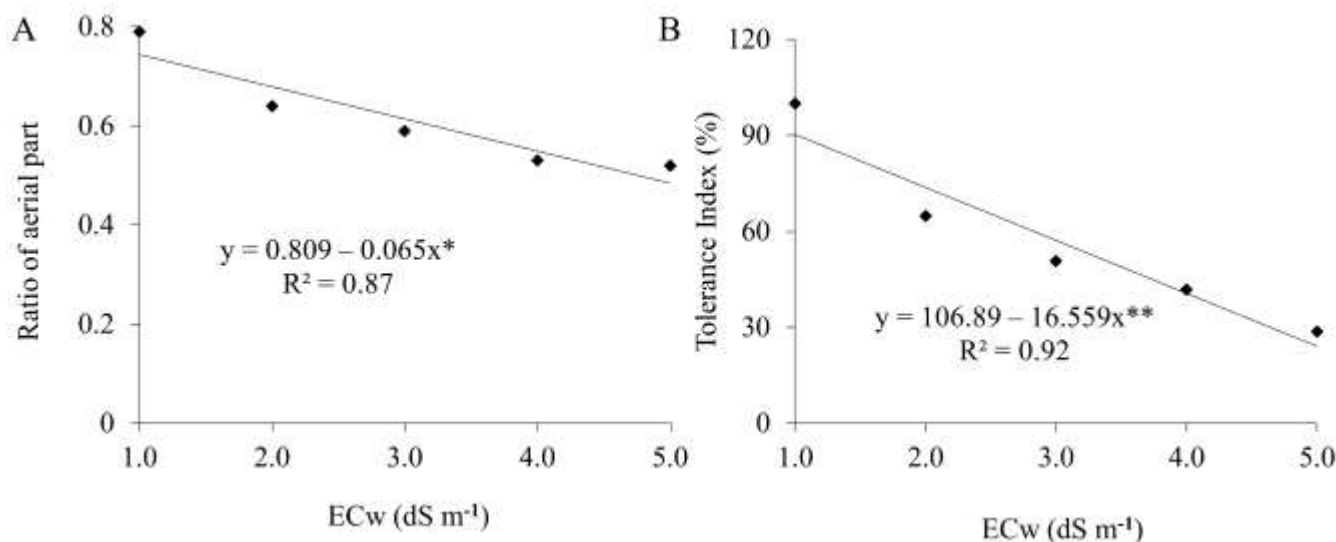


Figure 6. Effect of electrical conductivity in the irrigation water over the root shoot ratio (A) and tolerance index (B) peppermint.

Conclusion

The increase in electrical conductivity of the irrigation water levels from 1 dS m⁻¹ reduced the growth, development and production of peppermint biomass. Peppermint plants that received bovine biofertilizer showed superior results expressed in growth and biomass production. The application of bovine biofertilizer attenuates the effects of salty peppermint. The growth and production of peppermint biomass increase when the plants are irrigated with low salinity water (1 dS m⁻¹) in the presence of bovine biofertilizer.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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