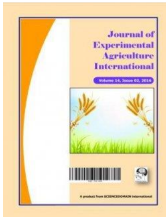

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Protective Effect of Purple Lettuce (*Lactuca sativa* L.) Aqueous Extract on Physiological Quality of Lettuce Seedlings Subject to Salt Stress

Cristiane Deuner[1*], Carolina Terra Borges[2], César Iván Suárez Castellanos[3], Sidnei Deuner[4], Francisco Amaral Villela[3] and Géri Eduardo Meneghello[3]

[1]Federal Institute of Rio Grande do Sul (IFSul), Campus Visconde da Graça, Pelotas, RS, Brazil.
[2]Sementes Lannes, São Gabriel, RS, Brazil.
[3]Postgraduate Program in Science and Seed Technology, Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas (FAEM-UFPel), Pelotas, RS, Brazil.
[4]Department of Botany, Institute of Biology, Federal University of Pelotas (UFPel), Pelotas, RS, Brazil.

Authors' contributions

This work was carried out in collaboration with all authors. Author CD designed and installed the study, conducted the study analyzes, statistical analysis, wrote the protocol and wrote the manuscript. Authors CTB and CISC installed the experiment, performed the analyzes of the study, aided in statistical analysis. Author SD assisted in the projection of the study, carried out analyzes of the study, assisted in the bibliographic researches and revision of the manuscript. Authors FAV and GEM helped in the projection of the study, assisted in the bibliographic research and revision of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The salinity is an environmental stress that can affect germination of seeds. The lettuce has carotenoids, vitamin C, high level of phenolic compounds and high antioxidant activity, which can help reduce the stress caused by salt. The purple varieties still have a lots of anthocyanins, which

**Corresponding author: E-mail: cdeuner@yahoo.com.br;*

gives them greater antioxidant power. The objective of this study was to evaluate the possible protective effect of purple lettuce leaf extract on the physiological quality during seed germination and early growth of lettuce seedlings subjected to salinity. Therefore, two experiments were conducted, one in the laboratory and the other in the greenhouse. In both experiments, lettuce seeds, cv. Regina, were subjected to irrigation with purple lettuce extracts, variety Batavia cacimba, and salt solutions during the early stages of seedling development, evaluating parameters related to germination and biomass production. The use of aqueous extract of purple lettuce promotes the germination, the first counting of germination, the shoot length and the dry mass of seedlings under laboratory conditions. The aqueous extract of purple lettuce, though not inhibit the effect of salt, it assists the initial development of lettuce seedlings. The salt affects the development of lettuce seedlings in the laboratory and in the greenhouse.

Keywords: Lactuca sativa L.; salinity; germination; growth.

1. INTRODUCTION

Salt stress involves changes in several physiological and metabolic processes, depending on the severity and duration of stress, it can inhibit the production of crops [1,2]. According to Ribeiro et al. [3], salinity can be defined as a situation of excess of soluble salts, exchangeable sodium or both, on the superficial layers or others horizons of soil, affecting plant development. High levels of soluble salts, especially sodium chloride (NaCl), reduce water potential in the substrate, leading to less capacity for water uptake by the seed, which may reduce the percentage of germination or even inhibit the process altogether, due to osmotic and toxic effects [4]. Furthermore, the production of reactive oxygen species (ROS) is enhanced during salt stress in plants [5]. This may lead to oxidative damage to various cellular components such as proteins, lipids and DNA, disrupting vital cellular functions [6].

Lopes and Macedo [7] evaluated the germination of Chinese cabbage seeds under the influence of water content and salt stress. The authors concluded that salinity affects the physiological manifestation of the germination and vigor of seeds. The decrease in the percentage of seeds germinated may be linked to the difficulty of water absorption due to very negative water potential, particularly at the start of imbibition. This influence on water uptake may impair the sequence of events related to germination [8]. Furthermore, as observed by Rodrigues et al., [9], the use of increasing levels of salts in irrigation water influences the development of lettuce (*Lactuca sativa* L.) cultivars, decreasing linearly the fresh weight, shoot dry mass, leaf number, and root dry mass. Some authors [10,11] claim that salt stress causes a reduction in plant growth due to nutritional imbalances caused by the effects of excess salt on the absorption and transport of nutrients. Farias et al. [12] indicate that salinity reduces the activity of ions in solutions and alters the absorption, transport, distribution and assimilation of nutrients in the plant.

However, plants respond differently to salt stress, adapting various defense mechanisms with changes in morphology and growth patterns as well as physiological and biochemical responses [13]. One mechanism that plants have developed to grow under stress conditions is the production of a wide variety of secondary metabolites [14], which assist in the detoxification of ROS.

Many studies have investigated the antioxidant properties of fruits, vegetables and medicinal plants [15,16,17]. Some substances such as β-carotene, vitamins C and E and phenolic compounds are related to the antioxidant capacity of various vegetables [18]. Furthermore, endogenous protectors such as osmoprotectors, plant hormones and antioxidants have been effective in reducing the damage induced by salt plants [19,20,21,22].

Abbas and Akladious [23] studied the application of carrot root extract in the induction of salinity tolerance in cowpea seedlings. Seed imbibition of carrot extract resulted in increased plant growth, yield, and tolerance to salt stress. The authors observed that the results of the beneficial effects of carrot root extract against abiotic stress may have been caused by its bioactive content and nutritional components, since the root contains anthocyanins, amino acids, proline, sugar, carotenoids, flavones, proteins and fibers.

Lettuce (*Lactuca sativa* L.) is considered the most important leafy vegetable in Brazil. The plant is consumed worldwide and widely cultivated in many countries [24]. Just like carrots, it has high nutritional value, containing carotenoids, vitamin C and high content of phenolic compound [25]. The red or purple varieties have large amount of anthocyanins, which gives them greater antioxidant activity [26].

The objective of this study was to evaluate a possible protective effect of purple lettuce leaf extract on physiological quality, during seed germination and the early growth, of lettuce seedlings, cv. Regina, subjected to salt stress.

2. MATERIALS AND METHODS

The study was conducted at the Didactic Laboratory of Seed Analysis (Departamento de Fitotecnia da Faculdade de Agronomia Eliseu Maciel) and Plant Physiology Laboratory (Departamento de Botânica, Instituto de Biologia) of the Federal University of Pelotas (Universidade Federal de Pelotas, UFPel), Pelotas-Rio Grande do Sul (RS), Brazil. The plant material used was seeds of lettuce, cv. Regina, and aqueous extract of purple lettuce leaves, variety Batavia cacimba, being conducted two experiments, as follow:

2.1 Experiment I

To evaluate the physiological quality during germination of lettuce seeds, cv. Regina, under controlled conditions in growth chamber (BOD), four purple lettuce extract concentrations (0, 50, 100 and 150 g lettuce leaves L water^{-1}) combined with five concentrations of sodium chloride (NaCl) solutions (0, 30, 60, 90 and 120 mM) were tested, using distilled water as control (to zero concentration of two factors combined). To obtain the plant extracts, purple lettuce plants in good health were purchased at a local fruit bowl on the day of use. Initially, the leaves were thoroughly washed in distilled water, and then performed a slight drying with a paper towel. After the leaves were weighed as each treatment and ground in a blender containing distilled water for 2 minutes. Performed grinding, the extracts were filtered on filter paper and transferred to the refrigerator maintained at 10°C, where they remained undisturbed for 24 hours, and then be used in conducting the tests. The combination of factors was performed by adding the corresponding amount of NaCl to purple lettuce extracts, before making the tests.

The physiological quality was evaluated as follows: a) Germination (G): the analysis was performed with four replications of 50 seeds, in transparent plastic boxes, on two sheets of blotter paper moistened with 2.5 times the weight of dry paper with each treatment solution. The boxes were placed in a germinator at constant temperature of 20°C and photoperiod of 12 hours. The counts were made in the fourth and seventh day, according to the Rules for Seed Analysis – RAS [27], computing the percentage of normal seedlings for each repetition. b) First counting of germination (FCG): it was performed together with the germination test. The counting was performed the fourth day after sowing and the evaluations were made according to RAS [27], computing the percentage of normal seedlings for each repetition. c) Shoot and root length (SL and RL, respectively): it was performed with eight replicates of 15 seedlings for each treatment. The shoot and the main root length were measured at seventh day after sowing with the aid of a graduated ruler (mm). d) Total dry mass (TDM): it was performed with the same seedlings used to determining SL and RL the lengths, these being placed in paper bags to dry in an oven at 70 ± 2°C until constant weight, determining the dry mass in an analytical balance and the results were expressed in mg seedling^{-1}.

2.2 Experiment II

To evaluate the initial growth of lettuce seedlings, cv. Regina, in the greenhouse, three purple lettuce extract concentrations were tested (0, 75 and 125 g of lettuce leaves L water^{--1}) in combination with five NaCl concentrations (0, 30, 60, 90 and 120 mM) constituting 15 treatments. Distilled water (zero-level combination for both factors) was used as the control. Factor combinations were performed as in Experiment 1.

Lettuce seeds, cv. Regina, were sown in polystyrene trays, using washed and autoclaved sand as substrate. Initially, three seeds were sown per cell, and thinning carried out after emergence, leaving only one seedling per cell. Each treatment was conformed by four replications of 18 cells, totaling 72 cells or seedlings per treatment. The solutions of each treatment were applied immediately after seeding and subsequently at 4, 9, 15 and 22 days after sowing, by volume of 15 mL per cell in each application. Hoagland nutrient solution [28] was added by volume of 10 mL in a concentration of 50% of the recommended dose (half-strength) at seven and 11 days after seeding, and 6 mL in a concentration of 100% of the recommended dose (full-strength) was administered at 16 and 20 days after sowing.

The characters evaluated were: a) Emergence speed index (ESI): seeds were sown manually, approximately 0.5 cm deep, in polystyrene trays of cells containing sterilized sand. The observations were performed daily, counting the number of emerged seedlings per day, until that number remained constant. The speed index was calculated according to Vieira and Carvalho [29]; b) Seedling emergence (E): it was carried on with the same seedlings used in the ESI, counting the seedlings emerged at 12 days after sowing, considering as seedling emerged, which had the cotyledons totally free and normal. The results were expressed as percentage of seedling emergence; c) Leaf area (LA): it was performed using non-destructive method in two stages, at 14 and 24 days after sowing, using the product of the main vein´s length and the maximum width of the sheet, multiplied by the correction factor 0.75; proposed by Pereira et al. [30] to lettuce. The results were expressed as cm^2 $seedling^{-1}$; d) Shoot length (SL): it was measured 24 days after sowing, using 10 seedlings per replication for each treatment with the aid of a graduated ruler. The results were expressed in cm $seedling^{-1}$; e) Production of dry and fresh shoot mass (DSM and FSM, respectively)*:* after the measurement of shoot length, the same seedlings were weighed at analytical balance to btain the fresh mass and then placed in an oven at 70°C until constant weight, then weighed to obtain the dry mass. The results were expressed in g $seedling^{-1}$; f) Root volume (RV)*:* it was determined by placing the roots in a graduated cylinder containing a known volume of water. The root system volume corresponds to the volume of water displaced inside the cylinder after immersion of roots. By difference between final and initial volume of water, it was obtained the root volume (cm^3). g) Root dry mass (RDM): after measuring the volume of roots, they were placed in an oven at 70°C until constant weight, and then weighed at analytical balance to obtain the dry mass. The results were expressed in g $seedling^{-1}$.

The Experiment I was carried on completely randomized factorial design 4x5 (lettuce extract concentration x NaCl concentration) with four replications. The effects of factors were analyzed by polynomial regression at statistical program WinStat 2.0 [31]. The Experiment II was carried on completely randomized factorial design (3x5) (lettuce extract concentrations x NaCl concentrations) with four replications. Data were submitted to analysis of variance and subsequently the means compared by Least Significant Difference (LSD) with p=0.05 and polynomial regression at statistical program WinStat 2.0 [31].

3. RESULTS

3.1 Experiment I

The data from the first experiment showed that germination responds to interaction between the two factors studied, while other tests showed responses to isolated factors. There was an increase in germination percentage with increasing purple lettuce extract concentration combined with salinity at concentrations of 90 and 120 mM of NaCl (Fig. 1A). However, in the absence and lower concentration of purple lettuce extract, salt led to reduce germination (Fig. 1B). The first counting of germination showed no interaction between factors; however, there was an increase in the number of germinated seedlings with the application of lettuce extract, with a minimum point at 1.72 g L^{-1} (Fig. 1C). As for the salt factor, the maximum point was reached at 34.78 mM NaCl, followed by reduction with increased saline treatment (Fig. 1D).

Shoot length increased with the application of purple lettuce extract (Fig. 2A), reaching a maximum at 109.29 g L^{-1}, however, linearly reduced with increasing salt concentrations (Fig. 2B). But the root length was reduced for both factors evaluated (Fig. 2C, 2D).

The total dry mass of the seedlings differed significantly only for the treatments with lettuce extract application, reaching a maximum point at a concentration of 81.5 g L^{-1} (Fig. 3A, 3B).

3.2 Experiment II

For the seeds sowed in the greenhouse, there was interaction among the factors to the following variables: germination speed index, leaf area, shoot length, fresh and dry shoot mass, root volume and root dry mass. The studied factors did not influence seedling emergence (Fig. 4A).

The emergence speed index was significantly higher in the treatment with 125 g L^{-1} purple lettuce extract, in relation to the others, in the absence of salt as well as at concentration of 120 mM NaCl. Purple lettuce extract concentrations of 0 and 75 g L^{-1} showed a decreasing quadratic tendency, with maximum value at 29 and 1 mM of NaCl solutions, respectively (Fig. 4B).

As for leaf area (Fig. 5A, 5B), there was a significant increase with the application of lettuce extract, at both 14 (Fig. 5A) and 24 days (Fig. 5B) after sowing (DAS). Leaf area was approximately 20 cm^2 greater after treatment with 125 g L^{-1} of extract compared to control at 24 DAS (Fig. 5B). Also, comparing the lettuce extract concentrations within each salt concentration, the response variable was also positive, with significant increases, reaching values of approximately 9 cm^2 of difference between the concentrations 0 and 125 g L^{-1} of purple lettuce extract, on concentration of 120 mM salt (Fig. 5B).

Fig. 6 represents the stage of seedling development at 24 DAS.

Data relating to seedling length and fresh and dry mass of the shoot (Fig. 7) exhibited the same trend observed for leaf area. They were significant increases for the application of lettuce extract, both alone and in joint application to NaCl. There was no difference among the salt concentrations in the absence of extract, in the three variables evaluated.

Root volume and dry mass (Fig. 8) revealed a significant difference among treatment: even with increasing salt concentration, the addition of lettuce extract induced a significant increase in these variables. Both variables showed cubic tendency in relation to NaCl concentrations, with an increase up to a concentration of 30 mM salt (Fig. 8).

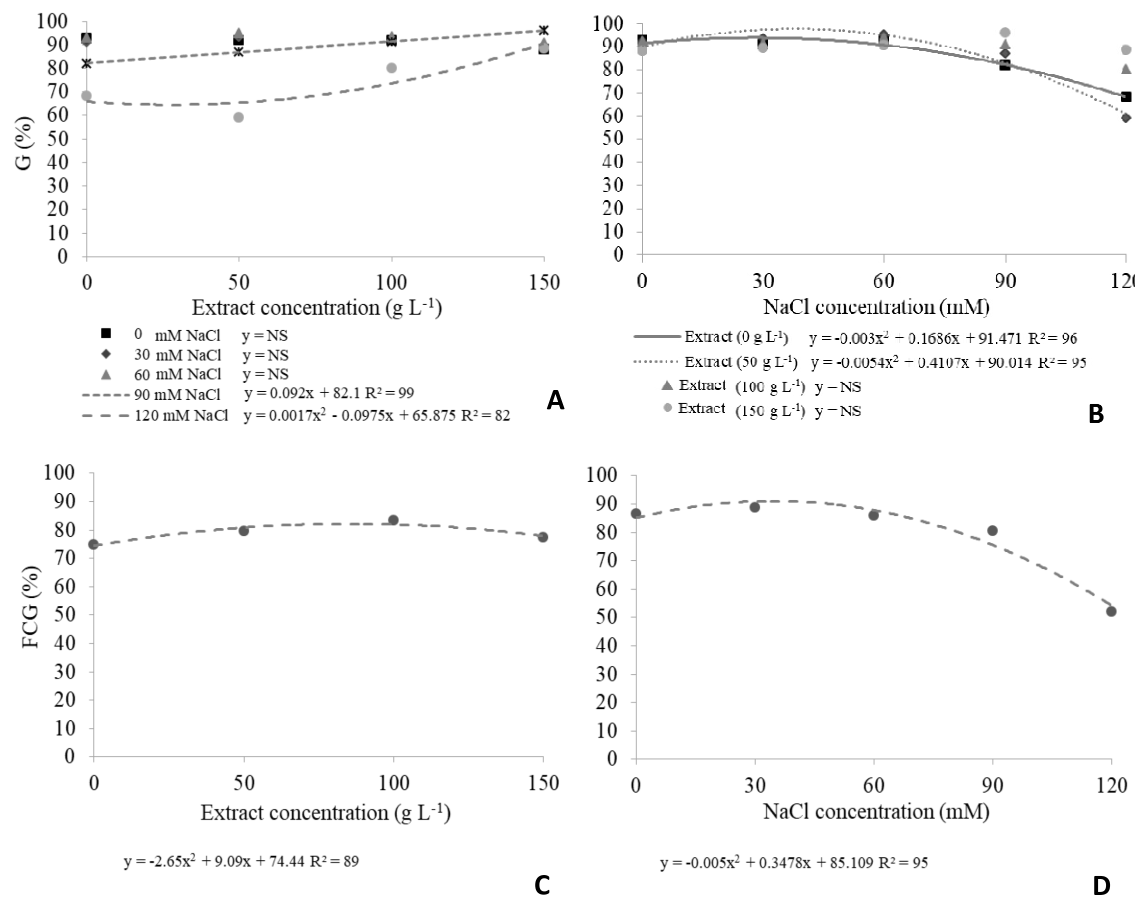

Fig. 1. Germination (G) and first count of germination (FCG) of lettuce (*Lactuca sativa* L.) seeds, cv. Regina, cultured in the presence of purple lettuce aqueous extract and NaCl solutions. The left column (A, C) – lettuce extract effect; right column (B, D) – salt solutions effect

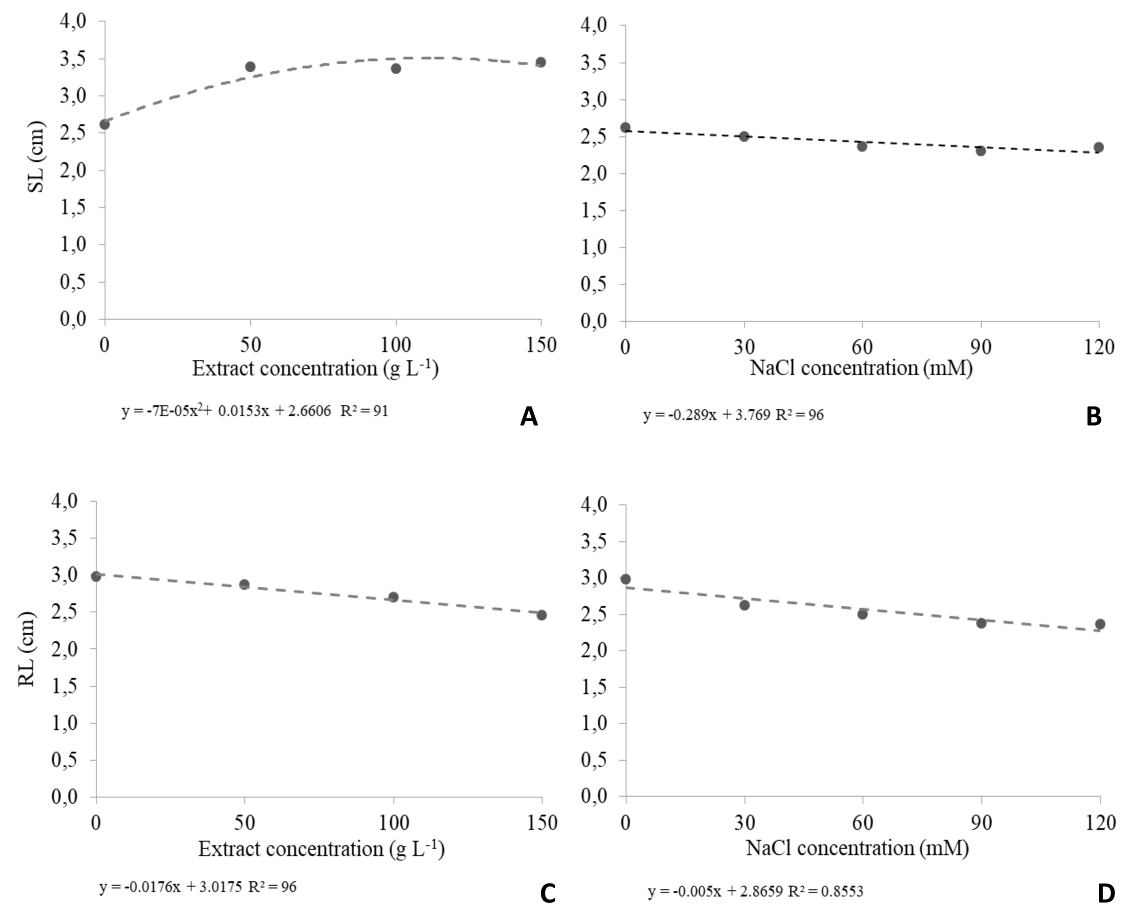

Fig. 2. Shoot length (SL) and root length (RL) of lettuce (*Lactuca sativa* L.) seeds, cv. Regina, cultured in the presence of purple lettuce aqueous extract and NaCl solutions. The left column (A, C) – purple lettuce extract effect; right column (B, D) – salt solutions effect

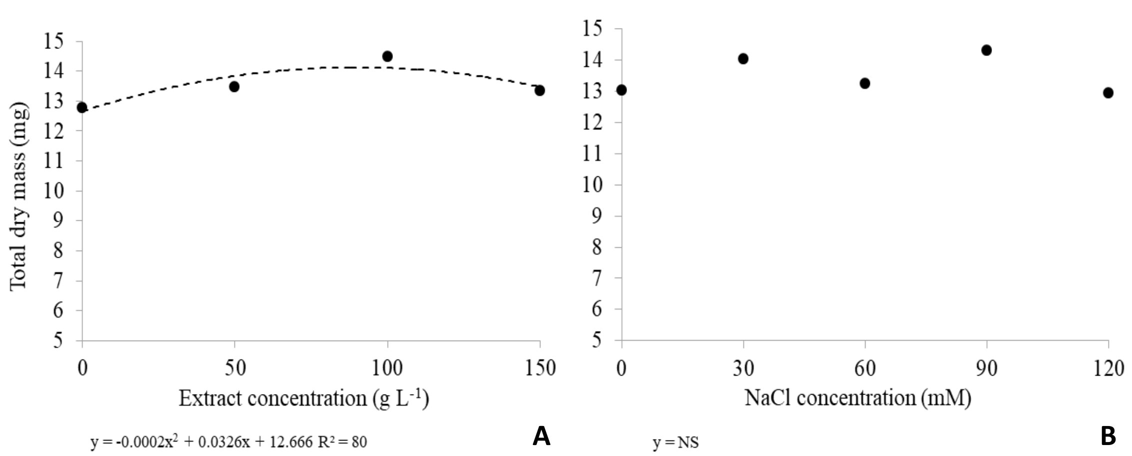

Fig. 3. Total dry mass of lettuce (*Lactuca sativa* L.) seeds, cv. Regina, cultured in the presence of purple lettuce aqueous extract and NaCl. The left column (A) - extract effect; right column (B) - salt effect

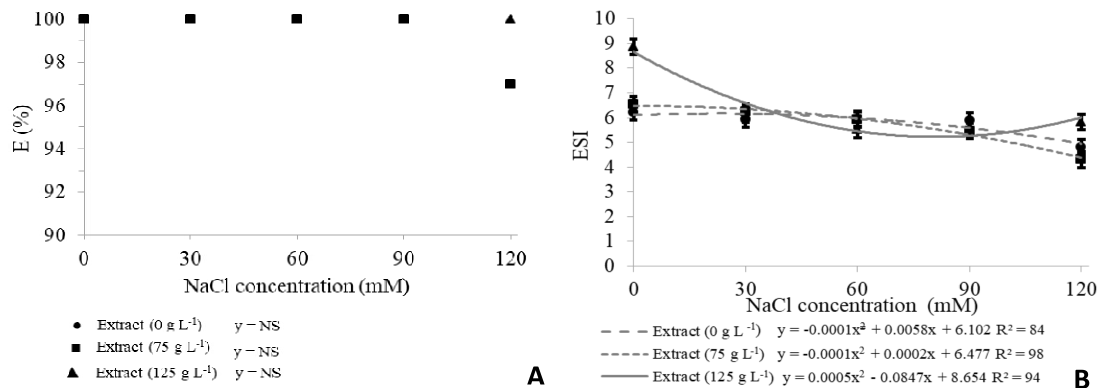

Fig. 4. Seedlings emergency (E) (left column (A)) and emergence speed index (ESI) (right column (B)) of lettuce (*Lactuca sativa* L.) seedlings, cv. Regina, cultured in the presence of aqueous extract of purple lettuce and NaCl solutions. The bars correspond to the amount of LSD (Least Significant Difference) among the factor levels of the purple lettuce extract (p =0.05); overlapping bars indicate that the treatments are statistically equal

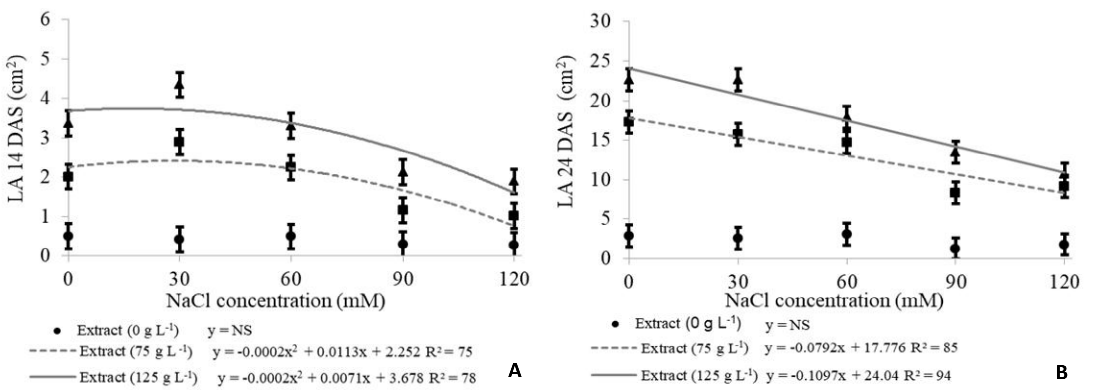

Fig. 5. Leaf area (LA) of lettuce (*Lactuca sativa* L.) seedlings, cv. Regina, cultured in the presence of aqueous extract of purple lettuce and NaCl solutions. Left column (A) - 14 DAS - days after sowing; right column (B) - 21 DAS - days after sowing. The bars correspond to the amount of LSD (Least Significant Difference) among the factor levels of the purple lettuce extract (p =0.05); overlapping bars indicate that the treatments are statistically equal

Fig. 6. Development of lettuce (*Lactuca sativa* L.) seedlings (24 days after sowing). 1: control (water); 2: 75 g L^{-1} of extract; 3: 125 g L^{-1} of extract; 4: 30 mM salt; 5: 60 mM salt; 6: 90 mM salt; 7: 120 mM salt; 8: 75 g L^{-1} of extract + 30 mM salt; 9: 75 g L^{-1} of extract + 60 mM salt; 10: 75 g L^{-1} of extract + 90 mM salt; 11: 75 g L^{-1} of extract + 120 mM salt; 12: 125 g L^{-1} of extract + 30 mM salt; 13: 125 g L^{-1} of extract + 60 mM salt; 14: 125 g L^{-1} of extract + 90 mM salt; 15: 125 g L^{-1} of extract + 120 mM salt

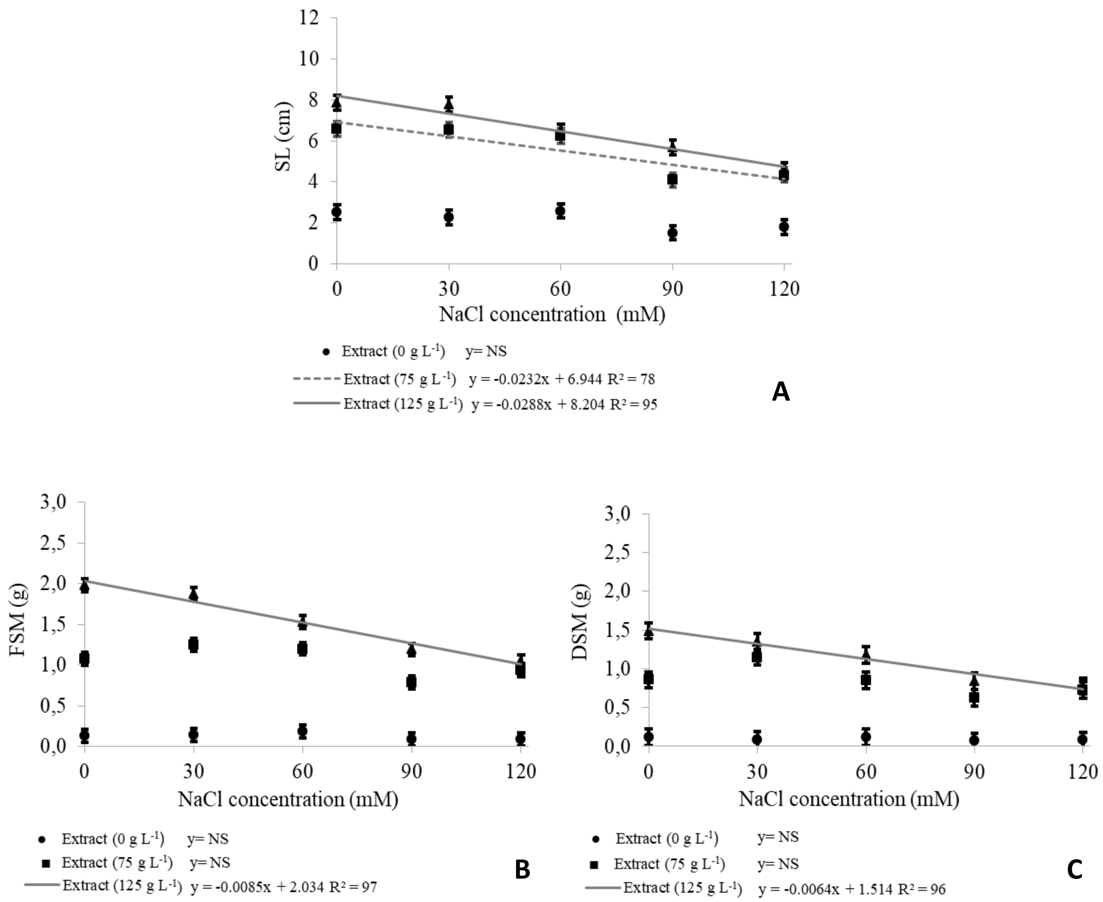

Fig. 7. Shoot length (SL), fresh and dry shoot mass (FSM; DSM, respectively) of lettuce (*Lactuca sativa* L.) seedlings, cv. Regina, cultured in the presence of purple lettuce aqueous extract and NaCl solutions. The bars correspond to the amount of LSD (Least Significant Difference) between the factor levels of the purple lettuce extract (p=0.05); overlapping bars indicate that the treatments are statistically equal

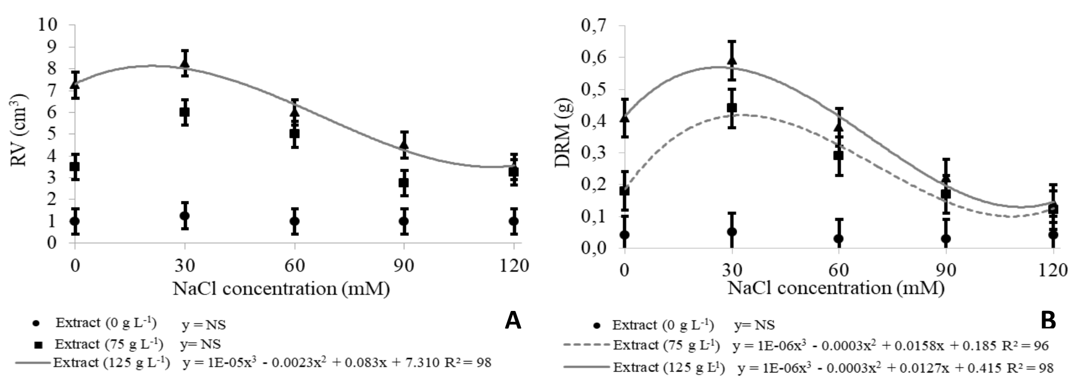

Fig. 8. Root volume (RV) and root dry mass (RDM) of lettuce *(Lactuca sativa* L.) seedlings, cv. Regina, cultured in the presence of aqueous extract of purple lettuce and NaCl solutions. The bars correspond to the amount of LSD (Least Significant Difference) between the factor levels of the purple lettuce extract (p=0.05); overlapping bars indicate that the treatments are statistically equal

4. DISCUSSION

Salinity affects plants in various ways, reducing growth, development and survival [32,33]. However, vegetables have two types of metabolites: primary and secondary. While primary metabolites are responsible for the survival of the plant, the secondary are intimately associated with defense strategies [34] in adverse conditions. According Taiz and Zeiger [35] secondary metabolites are distributed into terpenes, phenolic compounds and nitrogen-containing compounds. The lettuce contains high levels of phenolic compounds [25], in addition to anthocyanins present in red or purple varieties.

The present study demonstrated the positive effect of the application of purple lettuce extract on germination, first counting of germination, shoot length and dry mass of lettuce seedlings, cv. Regina, grown under controlled conditions. Some studies have reported the effect of aqueous extracts on seeds germination. Soares et al. [36] observed that the treatment of peanut seeds with cinnamon powder did not affect the germination and germination speed index of the seeds. Also, Parimelazhagan and Francis [37] found that the application of *Clerodendrum viscosum* leaf extract led to a higher percentage of germination and improved rice seedling development. In contrast, the percentage of germination and germination speed index of radish under action of *Piper mikanianum* aqueous extract were reduced in all treatments containing extract [38]. This suggests that the effect of a given type of extract depends on the sensitivity of the tested plant for the present compounds in the extract, because certain substances can inhibit the germination or growth [39].

Unlike lettuce extract, salinity affected negatively the germination and growth of lettuce seedlings. Similar results were observed in lettuce [40] and sunflower [41], where the increase of salinity in the substrate affected negatively the germination. However, Zapata et al. [42] studied nine varieties of lettuce under saline stress, they observed a relatively high tolerance during germination, because high levels of NaCl (150 mM) delayed germination, but the majority of seeds were able to germinate under these conditions. The salinity, depending on the crop, can act by inhibiting or delaying the germination of seeds, however, in the presence of compounds which act to enhance the germination and early seedling growth, the negative effect of salinity may be lower, as observed in this study by the application of purple lettuce extract.

All parameters in Experiment II showed higher values with the use of purple lettuce extract, alone or combined with salt, and the best results were obtained with the application of 125 g L^{-1}. Abbas and Akladious [23] studied the effect of soaking of cowpea seeds in carrot root extracts and NaCl. The authors observed a significant effect of salinity in reducing the stem length, roots, number of leaves, fresh and dry mass of seedlings. Regarding the carrot extract, the authors found a stimulatory action on the growth of cowpea plants with the application of the extract, also they found that the concentration of 25 g 100 mL^{-1} was the best concentration to improving the traits of length evaluated, both in control plants as those subject to salinity.

The use of purple lettuce extract provide an increase in the leaf area of lettuce seedlings, when applied in isolation, as in the application with salt, demonstrating the protective effect of the extract. This result is highly positive from the agronomic standpoint, since the edible part of lettuce are the leaves, therefore, increases in leaf area are always desired.

Oliveira et al. [43] evaluated the performance of five lettuce cultivars under different levels of irrigation with water salinity (0.5, 1.5, 2.5, 3.5 and 4.5 dS m^{-1}). The authors observed that leaf area was significantly affected (p=0.01) by salinity in all cultivars. Leaf area ranged from 2.3 cm^2 to 1.3 cm^2, showing that the effect of salinity is influenced by genetic variation among cultivars. According to Morales et al. [44], not all parts of the plant are equally affected by salinity, and adaptation to salt stress varies between species and in the same genotype may vary between phenological stages. The decrease in plant leaf area in salinity conditions may be associated with one of the mechanisms of adaptation of plants to salt stress, reducing the transpiration surface [45].

Beyond the agronomic factor mentioned above, leaf area is important as a growth variable indicative of productivity, because the photosynthetic process depends on the interception of light energy and its conversion into chemical energy, which is a process that occurs directly on the leaf [35]. How the photosynthesis depends on the leaf area, crop yield will be higher how much faster the plant reaches the maximum leaf area index and the longer the leaf area remains active [43]. Also, according to Mittova et al. [46] and Sultana et al. [47], the reductions in leaf area and photosynthesis contribute, somehow, to adapt the crop to salinity. Reduced leaf area under salinity stress can be a survival mechanism for the conservation of water in an area of the plant characterized by lower transpiration.

The 125 g L^{-1} concentration of purple lettuce extract provided increases of more than 6 cm^3 in root volume and 0.5 g in root dry mass (relative to concentration 0 g L^{-1}). The root growth reinforcement by applying lettuce extract is important because it can reduce the shock of transplantation into horticultural crops [48], as well increase the potential for initial nutrition and water absorption in the plant [49]. It can also be observed that the salt showed cubic trend about volume and root dry mass, with an increase in these characters until the concentration of 30 mM, followed by reduction until the concentration of 90 mM. According to Munns and Tester [50], the inhibition of root system growth under saline conditions can be attributed to the reduction of photosynthesis, whereas the number and size of the leaves of glycophyte plants are reduced under saline conditions. Dragoeva et al. [51] studied the effect of aqueous extract of *Adonis vernalis* L. and observed that the extract reduced the length of *Triticum aestivum* roots. Tas et al. [52] studied the response of hydroponic lettuce to salinity produced by NaCl and $CaCl_2$ and found no significant effect of salinity on root length.

Paulus et al. [53] observed that increasing the salinity of the water reduced fresh and dry mass in two lettuce cultivars, and the reduction varied according to each cultivar. According to Pedo et al. [54], the smallest allocation of dry mass may result from the effect of high NaCl concentration on hydrolysis mechanisms and the mobilization of reserves for the seedling. Unlukara et al. [55], studying the response of curly lettuce at different levels of irrigation water salinity (0.75 to 7.0 dS m^{-1}), noted increase in the plant dry mass with increasing salinity. Soares et al. [56] found that the root system of lettuce was not affected by salinity. Still according to the authors, it is possible to use saline water for production of lettuce on hydroponics, because the tolerance to salts may be superior to that obtained in conventional soil-based cultivation.

Although abiotic stresses such as drought, salinity and extreme temperatures can reduce the yield of major crops and limit global agricultural production [57,58], various plant extracts can promote the growth of a particular tissue because they contain substances that trigger growth induction mechanisms in plants [59]. The results showed that the purple lettuce extract has a stimulatory action on the germination and early growth of lettuce seedlings, even under salt stress. Thus, future studies aimed the physiological and biochemical response mechanisms induced by purple lettuce extract must be explored, in order to confirm possible increases in production of different cultures.

5. CONCLUSION

Salt stress affects the development of lettuce seedlings, in the laboratory and in the greenhouse.

The purple lettuce extract does not inhibit the effect of salt but assists in the initial development of lettuce seedlings.

The use of purple lettuce extract increase the germination, first counting of germination, shoot length and dry mass of lettuce seedlings under laboratory conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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