

Protective effect of purple lettuce (*Lactuca sativa* L.) aqueous extract on physiological quality of lettuce seedlings subject to salt stress

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 lot of anthocyanins, which give 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 inhibits 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.

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 other horizon 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 thesequence of events related to germination [8]. Furthermore, as observed by Rodrigues et al., [9], the use of

increasing levels of salts in irrigation water influence 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 Akladios [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.

MATERIAL 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

seed 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⁻¹ water) 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 determine the SL and RL lengths, these being placed in paper bags to dry in an oven at $70 \pm 2^\circ\text{C}$ 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 confirmed 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 these seedlings emerged at 12 days after sowing, considering a seedling emerged, which had the cotyledon totally free and normal. The results were expressed as percentage of seedling emergence; c) **Leaf area (LA)**: it was performed using a non-destructive method in two stages,

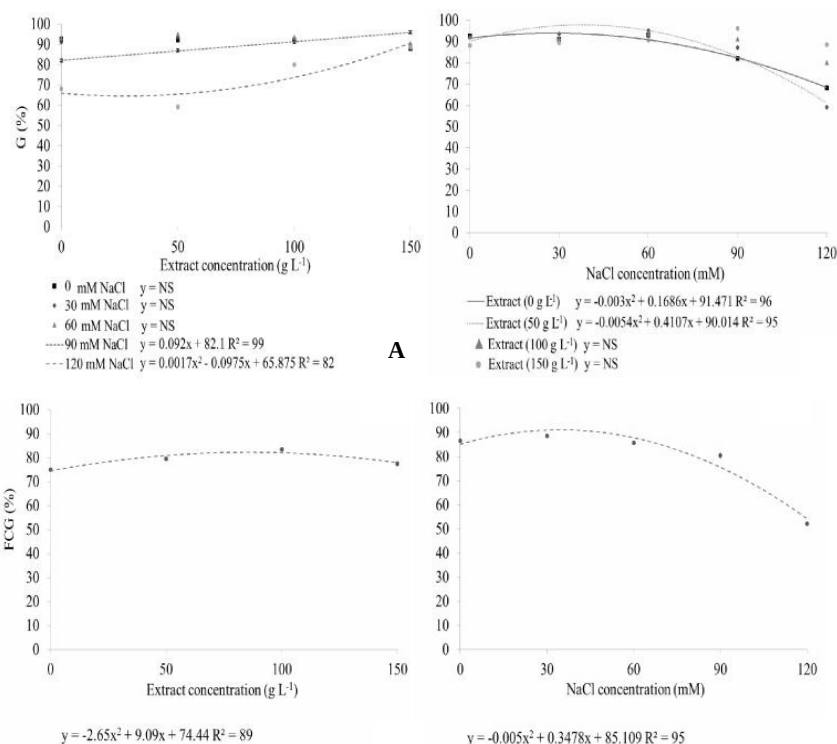
at 14 and 24 days after sowing, using the product of the main vein's length and the maximum width of the sheet, multiplied by the correction factor 0.75; proposed by Pereira et al., [30] to lettuce. The results were expressed as cm^2 seedling⁻¹; d) Shoot length (SL): it was measured 24 days after sowing, 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): after the measurement of shoot length, the same seedlings were weighed at analytical balance to obtain 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].

RESULTS

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Experiment I



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The data from the first experiment showed that germination responds to interaction

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between the two factors studied, while the other tests showed responses to isolated factors. There

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was an increase in germination percentage with increasing purple lettuce extract

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concentration combined with salinity at concentrations of 90 and 120 mM of NaCl (Figure

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1A). However, in the absence and lower concentration of purple lettuce extract, salt led to

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reduce germination (Figure 1B). The first counting of germination showed no interaction

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between factors; however, there was an increase in the number of germinated seedlings with the applicati

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on of lettuce extract, with a minimum point at 1.72 g L⁻¹ (Figure 1C). As for the

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salt factor, the maximum point was reached at 34.78 mM NaCl, followed by reduction with

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increased salinity treatment (Figure 1D).

Figure1. 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 solution effect.

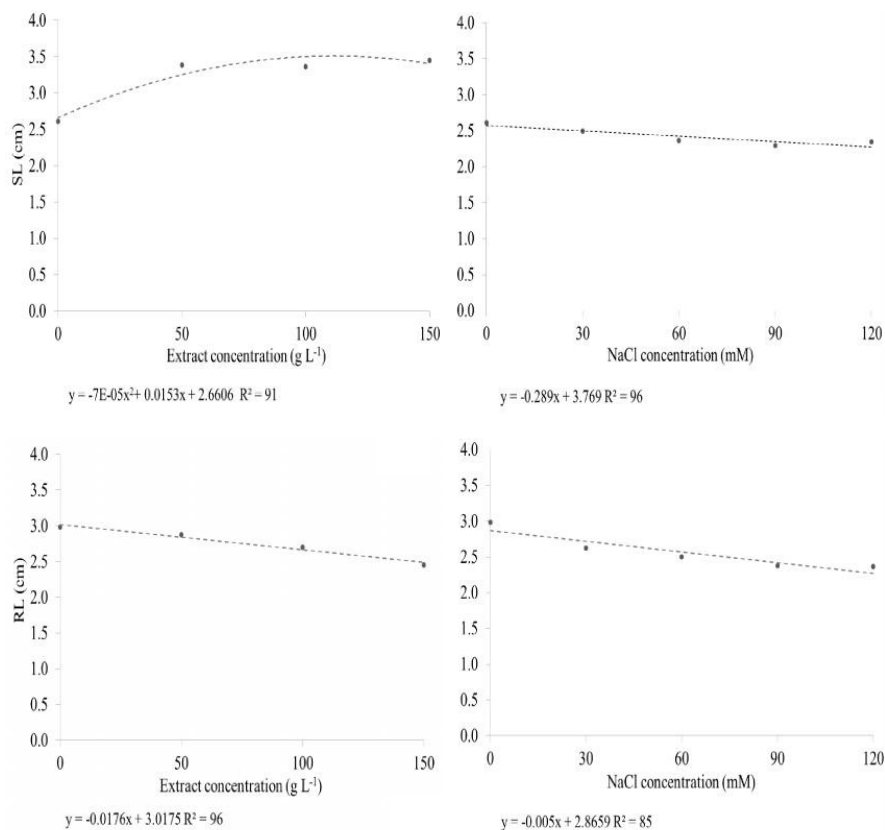


Figure2. Shoot length (SL) and root length (RL) of lettuce (*Lactuca sativa* L.) seeds, cv. Regina, cultured in the presence of purple lettuce aqueous extract and NaCl solutions. The left column (A,C) – purple lettuce extract effect; right column (B,D) – salt solution effect.

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

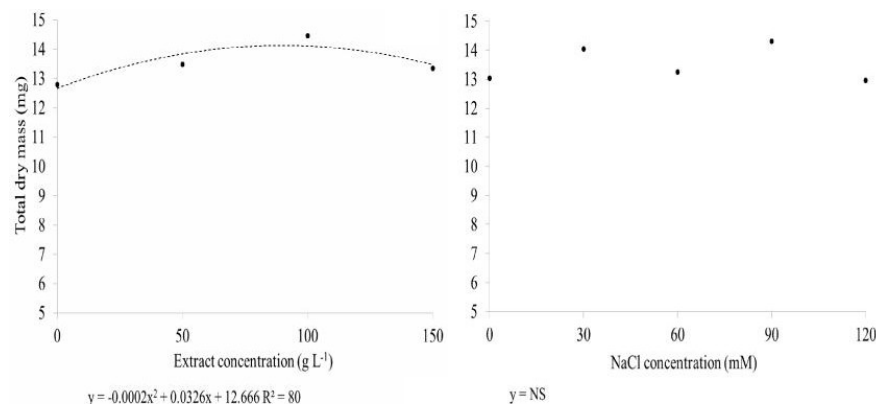


Figure 3. Total dry mass of lettuce (*Lactuca sativa* L.) seeds, cv. Regina, cultured in the presence of purple lettuce aqueous extract and NaCl. The left column (A) - extract effect; right column (B) - salt effect.

Experiment II

For the seeds sowed in the greenhouse, there was an 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 (Figure 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 a 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 (Figure 4B).

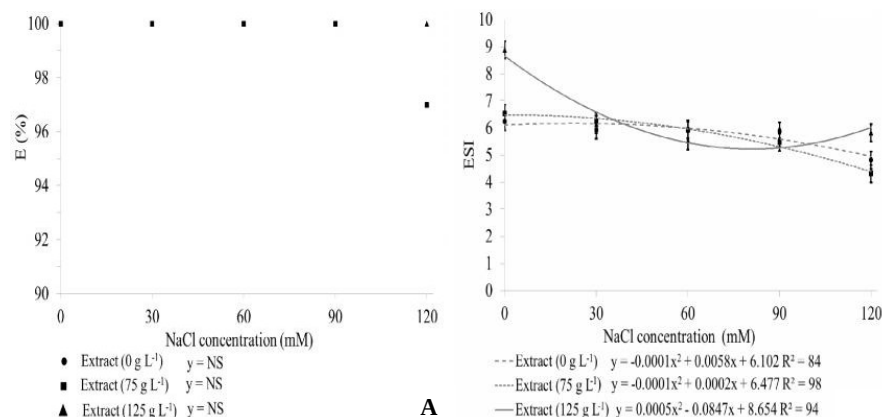
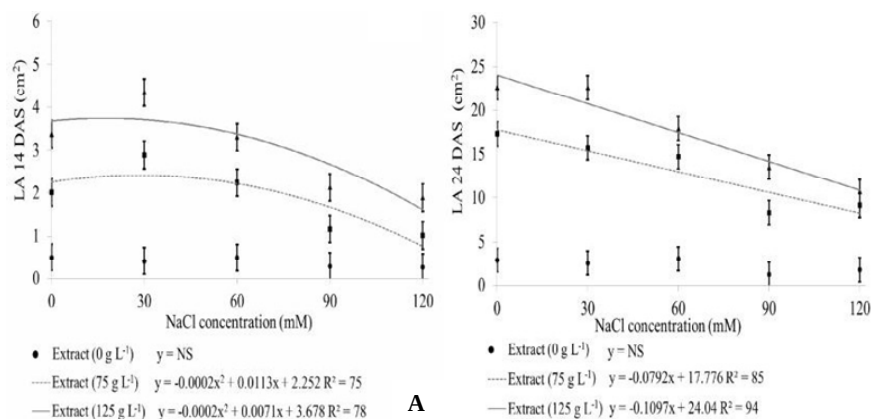


Figure 4. Seedling emergence (E) (left column (A)) and emergence speed index (ESI) (right column (B)) of lettuce (*Lactuca sativa* L.) seedlings, cv. Regina, cultured in the presence of aqueous extract of purple lettuce and NaCl solutions. The bars correspond to the amount of LSD (Least Significant Difference)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

As for leaf area (Figure 5A, 5B), there was a significant increase with the application of lettuce extract, at both 14 (Figure 5A) and 24 days (Figure 5B) after sowing (DAS). Leaf area was approximately 20 cm² greater after treatment with 125 g L⁻¹ of extract compared to control at 24 DAS (Figure 5B). Also, comparing the lettuce extract concentrations with in each salt concentration, the response variable was also positive, with significant increases, reaching values of approximately 9 cm² of difference between the concentrations 0 and 125 g L⁻¹ of purple lettuce extract, on concentration of 120 mM salt (Figure 5B).

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

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214 **Figure 5.** Leaf area (LA) of lettuce (*Lactuca sativa*
215 *L.*) seedlings, cv. Regina, cultured in the presence of aqueous

216 extract of purple lettuce and NaCl solutions. Left column (A) - 14 DAS - days after sowing;

217 right column (B) - 21 DAS - days after sowing. The bars correspond to the amount of LSD (Least

218 Significant Difference) among the factor levels of the purple lettuce extract ($p=0.05$); overlapping bars indicate

219 that the treatments are statistically equal.

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225 **Figure 6.** Development of lettuce (*Lactuca sativa*
226 *L.*) seedlings (24 days after sowing). 1: control (water); 2: 75 g

227 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

228 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

mMsalt;13:125gL⁻¹ ofextract+60mMsalt;14:125gL⁻¹ ofextract+90mMsalt;15:
125gL⁻¹ ofextract+120mMsalt.

Data relating to seedling length and fresh and dry mass of the shoot (Figure 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.

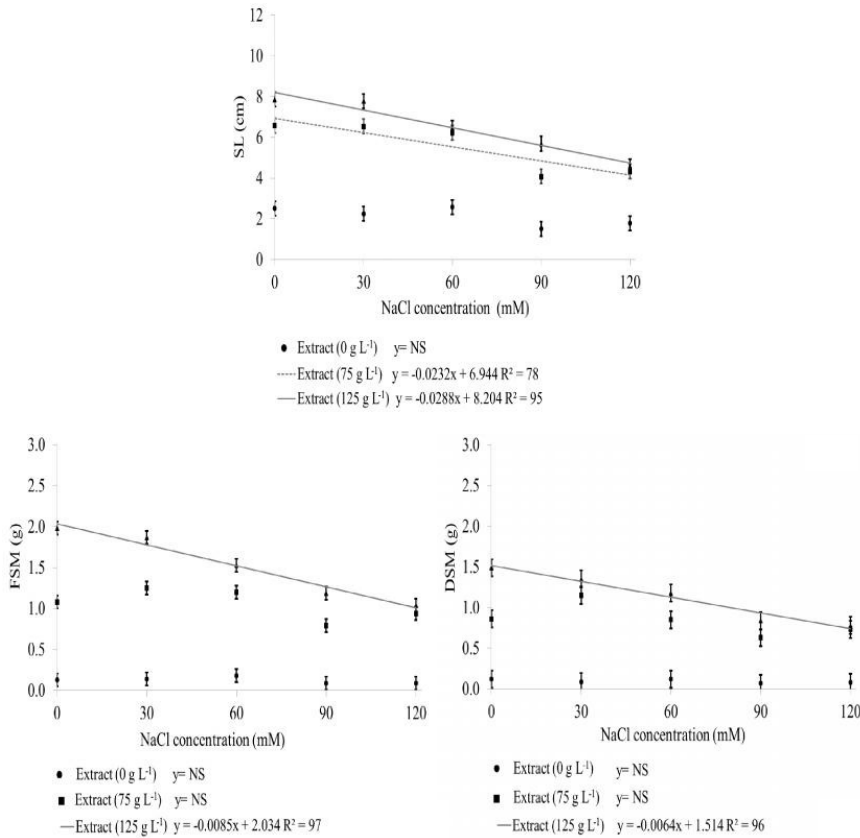


Figure 7. Shoot length (SL), fresh and dry shoot mass (FSM; DSM, respectively) of lettuce (*Lactuca sativa* L.) seedlings, cv.

Regina, cultured in the presence of purple lettuce aqueous extract and NaCl solutions. The bars
correspond to the amount of LSD (Least Significant Difference) among the factor levels of the purple lettuce
extract ($p=0.05$); overlapping bars indicate that the treatments are statistically equal.

Root volume and dry mass (Figure 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 (Figure 8).

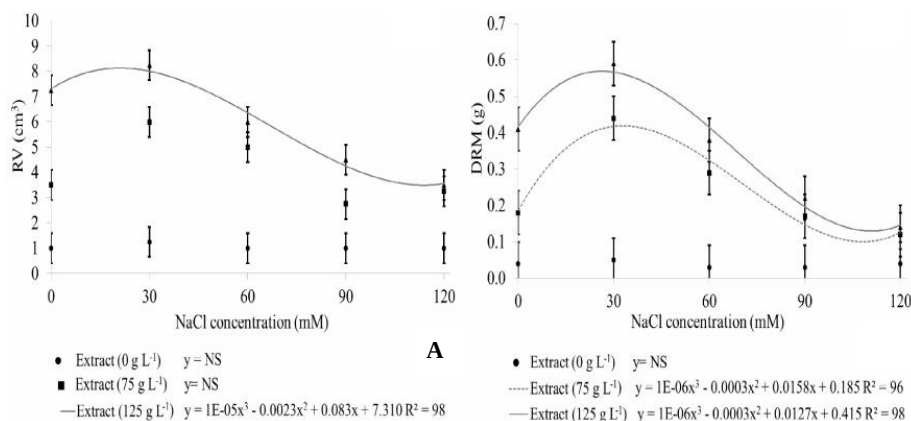


Figure 8. Root volume (RV) and root dry mass (RDM) of lettuce (*Lactuca sativa* L.) seedlings, cv. Regina, cultured in the presence of a faequeousextract of purple lettuce and NaCl solutions. The bars correspond to the amount of LSD (Least Significant Difference) among the factor level of the purple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

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 these 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 salinity stress, they observed a relatively high tolerance during germination, because a high level 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 high 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 Akladios [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 trait of length evaluated, both in control plants as those subjected to salinity.

The use of purple lettuce extract provided 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 cm² to 1.3 cm², 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 cm³ 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 as increase the potential for initial nutrition and water absorption in the plant [49]. It can also be observed that the salt showed a 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 CaCl₂ 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 by 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 stress 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 at 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.

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 increases the germination, first counting of germination, shoot length and dry mass of lettuce seedlings under laboratory conditions.

BIBLIOGRAPHIC REFERENCES

1. James RA, Blake C, Byrt CS, Munns R. Major genes for Na⁺ exclusion, Nax1 and Nax2 (wheat HKT1;4 and HKT1;5), decrease Na⁺ accumulation in bread wheat leaves under saline and waterlogged conditions. Journal of Experimental Botany. 2011;62:2939–2947.
2. Rozema J, Flowers T. Crops for a salinized world. Science. 2008;322:1478–1480.

- 374 3. RibeiroMR,BarrosMFC,FreireMBGS. Química dos solos salinos e sódicos. In: Melo,
375 V.F., Alleoni, L.R.F. (ed.). Química mineral do solo. Parte II – Aplicações.
376 Viçosa: Sociedade Brasileira de Ciência do Solo. 2009:449-484.
- 377 4. SeccoLB, QueirozSO, DantasBF, SouzaYA, SilvaPP. Germinação de sementes de
378 melão (*Cucumis melo* L.) em condições de estresse salino. Revista Verde. 2010;4:129–
379 135.
- 380 5. ApelK, HirtH. Reactive oxygen species: Metabolism, oxidative stress, and signal
381 transduction. Annual Review of Plant Biology. 2004;55:373–399.
- 382 6. GuptaB, HuangB. Mechanisms of salinity tolerance in plants: Physiological, biochemical,
383 and molecular characterization. International Journal of Genomics. 2014:1–18.
- 384 7. LopesJC, MacedoCMPde. Germinação de sementes de couve chinesa sob influência do
385 teor de água, substrato e estresse salino. Revista Brasileira de Sementes. 2008;30:079-
386 085.
- 387 8. MoterleLM, LopesPdeC, LuccaeBracciniAde, ScapimCA. Germinação de sementes e
388 crescimento de plântulas de cultivares de milho-pipoca submetidas a estresse hídrico e
389 salino. Revista Brasileira de Sementes. 2006;28:169-176.
- 390 9. RodriguesRR, BertossiAPA, GarciaGdeO, AlmeidaJRde, SilvaEAda. Salinidade no
391 desenvolvimento de cultivares de alface. Agrarian Academy. 2015;2:70-81.
- 392 10. CavalcanteLF, CordeiroJC, NascimentoJAM, CavalcanteIHL, DiasTJ. Fontes e níveis
393 da salinidade da água na formação de mudas de mamoeiro cv. Sunrise Solo. Semina:
394 Ciências Agrárias. 2010;31:1281-1290.
- 395 11. FerreiraRG, TávoraFJAT, HernandezFFF. Distribuição da matéria seca e composição
396 química das raízes, caule e folhas de goiabeira submetida a estresse salino. Pesquisa
397 Agropecuária Brasileira. 2001;36:79-88.

- 398 12. FariasSGG,SantosDR,FreireALO,SilvaRB.Estressesalinonocrescimentoinicial
399 nutriçãomineral de Gliricídia(*Gliricidiasepium*(Jacq.) Kuntex Steud) emsolução
400 nutritiva.RevistaBrasileiradeCiênciadoSolo.2009;33:1499-1505.
- 401 13. BohnertHJ,NelsonDE,JensenRG.Adaptationstoenvironmentalstresses.PlantCell7,
402 1995:1099-1111.
- 403 14. RêgoJúniorNO,FernandezLG,CastroRDde,SilvaLC,GualbertoSA,PereiraMLA,
404 Silva MV da. Compostosbioativose atividadeantioxidante de extratosbrutos de
405 espéciesvegetaisdacaatinga.BrazilianJournalFoodTechnology.2011;14;50-57.
- 406 15. Oliveira M dos S, DorsGC, Souza-SoaresLA de, Badiale-FurlongE. Atividade
407 antioxidanteeantifúngicadeextratosvegetais.AlimentoseNutrição.2007;18:267-275.
- 408 16. ArbosKA, Freitas RJS de, StertzSC, Dornas MF. Atividadeantioxidantee teor de
409 fenólicostotaisemhortaliçasorgânicase convencionais. CiênciaeTecnologiade
410 Alimentos. 2010;30:501-506.
- 411 17. FormagioASN,MasettoTE,VieiraMdoC,ZárateNAH,DeMatosAIN,VolobuffCRF.
412 Potencial alelopático e antioxidante de extratosvegetais. Bioscience Journal.
413 2014;30:629-638.
- 414 18. McDonaldS,PrenzlerPD,AntolovichM,RobardsK.Phenolicscontentandantioxidant
415 activityofoliveextracts.FoodChemistry.2001;73:73-84.
- 416 19. IqbalN, MasoodA; Khan NA. Phytohormonesin salinity tolerance: ethylene and
417 gibberellins crosstalk. In: Khan, N.A., Nazar, R., Iqbal, N., Anjum, NA. (eds)
418 Phytohormonesandabioticstresstoleranceinplants.Springer.2012:77–98.
- 419 20. IoannidisNE,CruzJA,KotzabasisK,KrameDM.Evidencethatputrescinemodulates
420 thehigherplantphotosyntheticprotoncircuit.PLoSOne.7.2012;e29864.

- 421 21. Hossain MA, Munemasa S, Uraji M, Nakamura Y, Mori IC, Murata Y. Involvement of
422 endogenous abscisic acid in methyl jasmonate-induced stomatal closure in *Arabidopsis*.
423 *Plant Physiology*. 2011;156:430–438.
- 424 22. Ahmad P, Jaleel CA, Salem MA, Nabi G, Sharma S. Roles of Enzymatic and non-
425 enzymatic antioxidants in plants during abiotic stress. *Critical Reviews*
426 *in Biotechnology*. 2010;30:161–175.
- 427 23. Abbas SM, Akladios SA. Application of carrot root extract induced salinity tolerance in
428 cowpea (*Vigna sinensis* L.) seedlings. *Pakistan Journal Botany*. 2013;45:795–806.
- 429 24. Camili EC, Azevedo CCBV de, Bocuti ED, Silvério J de M, Barros K da C, Silva ARB
430 da, Seabra Júnior S. Cultivo consorciado de alface sob diferentes arranjos espaciais e
431 manejo do solo. *Revista Agrária*. 2013;6:110–120.
- 432 25. Collins A. Lettuce. In: *AC diet food and nutrition*. 2004. Available in:
433 <http://www.annecollins.com/dietnutrition/lettuce>. Accessed 15.03.2018
- 434 26. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds.
435 *Trends Plant Science*. 1997;2:152–159.
- 436 27. Brasil. Ministério da Agricultura e Reforma Agrária. Regras para análise de sementes.
437 Brasília: Mapa/ACS. 2009:399.
- 438 28. Hoagland DR, Arnon DI. The water-culture method for growing plants without soil.
439 *California Agricultural Experimental Station*. n.347. 1938
- 440 29. Vieira RD, Carvalho NM. Testes de vigor de sementes. *Jaboticabal: FUNEP*. 1994:164.
- 441 30. Pereira OCN, Bertonha A, Freitas PS, Gonçalves ACA, Rezende R, Silva FF. Produção
442 de alface em função de água e de nitrogênio. *Acta Scientiarum Agronomy*.
443 2003;25:381–386.
- 444 31. Machado Ade A, Conceição, AR. WinStat: sistema de análise estatística para Windows.
445 Versão 2.0. Pelotas: UFPel/NIA. 2003.

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- 446 32. Muscolo A, Sidari M, Panuccio MR, Santonoceto C, Orsini F, De Pascale S. Plant
447 responses in saline and semiarid environments: an overview. The European Journal of
448 Plant Science and Biotechnology. 2011;5:1-11.
- 449 33. Schleiff U, Muscolo A. Fresh look at plant salt tolerance as affected by dynamics at the
450 soil/root-interface using Leek and Rape as model crops. The European Journal of Plant
451 Science and Biotechnology. 2011;5:27-32.
- 452 34. Nass LL. Recursos genéticos vegetais. Brasília: Embrapa Recursos Genéticos Vegetais e
453 Biotecnologia. 2007:858.
- 454 35. Taiz L, Zeiger E. Plant physiology. 3. ed. Porto Alegre: Artmed. 2013:918.
- 455 36. Soares ANR, Gonçalves EP, Viana JS, Souto PC, Moura MF de. Physiological and
456 sanitary potential of peanut seed treated with cinnamon powder. African Journal of
457 Microbiology Research. 2015;9:1921-1927.
- 458 37. Parimelazhagan T, Francis K. Antifungal activity of *Clerodendrum viscosum* against
459 *Curvularia lunata* in rice seeds. Journal Mycology Plant Pathology. 1999;29:139-141.
- 460 38. Borella J, Martinazzo EG, Aumonde TZ, Amarante L, Moraes DM, Villela FA. Respostas
461 na germinação e no crescimento inicial de rabanetes sob aplicação de extrato aquoso de *Piper*
462 *mikanianum* (Kunth) Steudel. Acta Botanica Brasilica. 2012;26(2):404-409.
- 463 39. Almeida SP, Proença CEB, Sano SM, Ribeiro JF. Cerrado: espécies vegetais úteis.
464 Planaltina: EMBRAPA-CPAC. 1998:464.
- 465 40. Keshavarzi MHB. Studying the effects of different levels of salinity which caused by
466 NaCl on early growth and germination of *Lactuca Sativa* L. seedling. Journal of Stress
467 Physiology & Biochemistry. 2012;8:203-208.
- 468 41. Rabbani ARC, Nunes FBS, Carvalho SVA, Ferreira RA, Mann RS. Condicionamento
469 osmótico em sementes de girassol (*Helianthus annuus* L.). Revista Científica Udo
470 Agrícola. 2013;3:50-55.

Formatted: Portuguese (Brazil)

42. Zapata JP, Serrano M, Pretel MT, Amoroso AM. Changes in ethylene evolution and polyamine profiles of seedlings of nine cultivars of lettuce in response to salt stress during germination. *Plant Science*. 2003;164:557–563.
43. Oliveira F de A de, Carrilho M J S de O, Medeiros J F de, Maracá PB, Oliveira M K T de. Desempenho de cultivares de alface submetidas a diferentes níveis de salinidade da água de irrigação. *Revista Brasileira de Engenharia Agrícola e Ambiental*. 2011;15:771–777.
44. Morales MA, Olmos E, Torrecillas A, Alarcon JJ. Differences in water relations, leaf ion accumulation and excretion rates between cultivated and wild species of *Limonium* sp. grown in conditions of saline stress. *Flora* 2001;196:345–352.
45. Tester M, Davenport R. Na⁺ tolerance and Na⁺ transport in higher plants. *Annals of Botany*. 2003;91:503–527.
46. Mittova V, Tal M, Volokita M, Guy M. Salt stress induces up-regulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species but not in the cultivated species. *Physiology Plantarum*. 2002;115:393–400.
47. Sultana N, Keda T, Kashem MA. Effect of seawater on photosynthesis and dry matter accumulation in developing rice grains. *Photosynthetica*. 2002;40:115–119.
48. Crouch IJ, Van Staden J. Effect of seaweed concentrate on the establishment and yield of greenhouse tomato plants. *Journal of Applied Phycology*. 1992;4:291–296.
49. Mancuso S, Azzarello E, Mugnai S, Briand X. Marine bioactive substances (IPA extract) improve ion fluxes and water stress tolerance in potted *Vitis vinifera* plants. *Advances in Horticultural Science*. 2006;20:156–161.
50. Munns R, Tester M. Mechanisms of salinity tolerance. *Annuario Journal Plant Biology*. 2008;1:651–681.

- 494 51. DragoevaAP, KolevaVP, NanovaZD, GeorgievBP. AllelopathicEffects of Adonis
495 vernalisL.:RootGrowthInhibitionandCytogeneticAlterations.JournalofAgricultural
496 ChemistryandEnvironment.2015;4:48-55.
- 497 52. TasG, PapadandonakisN, SawasD. Responses oflettuce (*Lactuca sativa* L.
498 var.longifolia)growninaclosedhydroponicsystemtoNaCl⁺ orCaCl₂⁺ salinity.Journalof
499 AppliedBotanyandFoodQuality.2005;79:136-140.
- 500 53. PaulusD,DouradoNetoD,FrizzoneJA,SoaresTM.Produçãoeindicadoresfisiológicos
501 dealfacesobhidroponiacomáguausalina.HorticulturaBrasileira.2010;28:29-35.
- 502 54. PedóT,AisenbergGR,AumondeTZ,VillelaFA.Desempenhofisiológicodesementese
503 plântulasdegenótiposdeCucurbitaceaeSolanaceaeemambientesalino.Tecnologia&
504 CiênciaAgropecuária.2014;8(2):1-17.
- 505 55. UnlukaraA,CemekB,KaramanS,ErsahinS.Responseoflettuce(*Lactucasativa*var.
506 crispa)tosalinityofirrigationwater.NewZealandJournalCropHorticulturaeScience.
507 2008;36:265-273.
- 508 56. SoaresTM,SilvaEFF,DuarteSN,MeloRF,JorgeCA,Bonfim-SilvaEM.Produçãode
509 alfaceutilizandoáguassalinasemsistemahidropônico.Irriga.2007;12:235-248.
- 510 57. ZahirM,FarrukhH.EffectofNaCl salinityonthe germinationandseedlinggrowthof
511 sevenwheatgenotypes.PakistanJournalofBotany.2012;44:1845-1850.
- 512 58. Zia S, Khan MA. Comparative effect of NaCl and seawater on seed germination of
513 *Limoniumstocksii*.PakistanJournalofBotany.2002;34:345-351.
- 514 59. PuchooaD,RamburnRA.Studyontheuseofcarrotjuiceinthetissuecultureof*Daucus*
515 *carota*.AfricanJournalofBiotechnology.2004;3:248-252.