



PROTECTIVE ROLE OF 2-BORNEOL IN THE EMERGENCE OF *Allium cepa* SEEDS EXPOSED TO NaCl

PAPEL PROTETOR DO 2-BORNEOL NA EMERGÊNCIA DE SEMENTES DE *Allium cepa* EXPOSTAS AO NaCl

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Abstract

It has been proposed that borneol acts as a compound responsible for inducing tolerance to salt stress in the *Allium cepa* model organism. This study investigated the effect of borneol variation (100, 300 and 500 µg/mL) on the phytotoxic potential in seed emergence, growth of seedlings, roots and leaves in *A. cepa* under field conditions, normal and induced by NaCl (150 mM). Changes in and lipid peroxidation (LPO) levels was evaluated as markers of oxidative stress, and the antioxidant activity of borneol was evaluated by DPPH test. The exposure of *A. cepa* to 150 mM of NaCl significantly decreased the emergence capacity of the seeds. Nevertheless, borneol at concentrations of 100 and 300 µg/mL significantly reduced the salt stress and improved the emergence rate and the number of roots. Borneol significantly reduced lipid peroxidation (LPO) caused by NaCl, but, did not have any effect on the total iron content. Borneol exhibited DPPH radical scavenging activity similar to that of ascorbic acid used as standard antioxidant. The nutrients with the highest accumulation in the soil were P > S > Ca > N > K > Mg. Moreover, the pH was ideal for the cultivation of the species. The results of this study suggest that borneol can be used as a potential tolerance regulator to improve resistance to salt stress in *Allium cepa*.

Keywords: Oxidative stress, salinity, phytotoxicity, borneol, *A. cepa*.

Resumo

Foi proposto que o borneol atua como um composto responsável por induzir tolerância ao estresse salino no organismo modelo *Allium cepa*. Este estudo investigou o efeito da variação do borneol (100, 300 e 500 µg / mL) sobre o potencial fitotóxico na emergência de sementes, crescimento de mudas, raízes e folhas de *A. cepa* em condições de campo normais e induzidas por NaCl (150 mM). As mudanças nos níveis de peroxidação lipídica (LPO) foram avaliadas como marcadores de estresse oxidativo, e a atividade antioxidante do borneol foi avaliada pelo teste DPPH. A exposição de *A. cepa* a 150 mM de NaCl diminuiu significativamente a capacidade de emergência das sementes. No entanto, o borneol nas concentrações de 100 e 300 µg/mL reduziu significativamente o estresse salino e melhorou a taxa de emergência e o número de raízes. O borneol reduziu significativamente a peroxidação lipídica (LPO) causada pelo NaCl, mas não afetou o teor de ferro total. O borneol exibiu atividade sequestrante de radical DPPH semelhante à do ácido ascórbico usado como antioxidante padrão. Os nutrientes com maior acúmulo no solo foram P > S > Ca > N > K > Mg. Além disso, o pH era ideal para o cultivo da espécie. Os resultados deste estudo sugerem que o borneol pode ser usado como um potencial regulador de tolerância para melhorar a resistência ao estresse salino em *Allium cepa*.

Palavras-chave: Estresse oxidativo, salinidade, fitotoxicidade, borneol, *A. cepa*.



INTRODUCTION

Among abiotic stresses, salinity is a serious environmental problem, affecting productivity in agriculture worldwide [1]. About 20% of the world's cultivated land, including one-fifth of irrigated agricultural areas, face salinity threats [2-3]. Salt stress affects seed emergence [4], causing a reduction in germination rate at the beginning of seedling development [5]. In addition to affecting the emergency, salt stress is very harmful and limits plant growth [6-7], and act by decreasing the osmotic potential, as well as causing ion toxicity due to the accumulation of Na⁺ and Cl⁻ [3]. Moreover, it causes several disturbances in plants [8], causing morphological changes such as decrease in leaf quantity, root length, plant size and fruit production, as well as reduced production of secondary metabolites [9], since the stages of seed germination and development of initial seedlings are generally more sensitive to salt stress [10].

Notably, problems with soil salinity are more severe, particularly in semi-arid regions due to limited precipitation and increased temperature associated with climate change [11-14]. Some compounds may influence salt stress, such as the inhibition of seed emergence by NaCl, which can occur through the inhibition of ethylene [15]. Other compounds can affect emergence of seeds, such as monoterpenes, which are the main constituents of essential oils, known for numerous biological activities [16]. They inhibit seed emergence and suppress initial root growth [17-20]. However, some studies indicate that monoterpenes can act as attractive pollinators and as natural protectors [21-23].

The monoterpene 2-Borneol occurs naturally in more than 260 plants and is commonly found as a major component of the essential oils of some aromatic plants, including the species of the families Dipterocarpaceae, Lamiaceae, Valerianaceae, Asteraceae, and also citrus peels oils, spices such as nutmeg, ginger and thyme [18,24-25]. Besides that, it is a fragrance ingredient used in decorative cosmetics, fine fragrances, shampoos, soaps and other personal care products, as well as non-cosmetic products such as cleaning products and household detergents [26], and also for medicinal purposes [27]. However, little is known about 2-borneol, and studies related to its phytotoxicity and biochemical effects are scarce. Even though, the literature reports that 2-borneol, presents high phytotoxicity, when combined with other monoterpenes [28], the exact mode of inhibitory action of monoterpenes remains unknown [20].

Phytotoxicity studies use model organisms, including the species *Allium cepa*, characterized as a low-cost, easy-to-handle sensitive experimental model in in vitro tests [29], and has advantages over other short-lived models [30]. In addition, *A. cepa* has been used to assess DNA damage (genotoxicity) [31], such as chromosomal aberrations and disorders in the mitotic cycle, as well as genetic toxicology of nanoparticles [32-35], and pesticide impacts [36]. Given these advantages, this model has been widely used to assess the impacts caused by xenobiotics, characterizing an important tool for environmental monitoring studies, whose satisfactory results have been reported in different studies [30-37].



The influences of some factors are essential to understand the ecophysiological and biochemical aspects related to seed germination, including temperature [38-39]. In order to understand the effects of temperature on seed emergence, changes in percentage, germination speed over time can be evaluated [40]. Soil type, fertility and physical condition contribute to the success or failure of the establishment of the crop [41].

The type of soil determines whether it is well drained, has the potential to form crusting and its water retention capacity. Heavy clay soils are more prone to the formation of crusts, which causes stress on seeds and seedlings of young plants when they emerge from the soil [42]. In addition, the emergence of seedlings depends on the energy contained in the endosperm or cotyledons, the depth at which the seed is sown [43], and the water available in the soil [44]. A rapid and uniform emergence of seeds, followed immediate seedling emergence are highly desirable characteristic in seedling formation [45].

Under conditions of oxidative stress, triggered by salinity and other factors such as dry and low or high temperature, plants produce Reactive Oxygen Species (ROS), harmful to their growth, due to their negative effects on the subcellular components and plant metabolism, leading to oxidative destruction of cells [46,47-48]. This is because reactive species cause deterioration of membrane lipids, leading to increased leakage of solutes [49].

Considering the above-mentioned information, the development of methods to induce salt tolerance and mitigate the effects of stress on emergence in plants, to facilitate the establishment of the crop in saline soils is relevant. However, the phytotoxic role of borneol in plants is not well established and considerable attention should be emphasized. The objective of this study was to analyze the phytotoxic effect of 2-borneol on emergence of seedling, roots and leaves growth in *A. cepa* under normal conditions and NaCl-induced conditions, as well as to examine changes in iron levels, lipid peroxidation (MDA concentration), in addition to the antioxidant potential of borneol by the DPPH assay.

MATERIAL AND METHODS

Reagents

The monoterpenoid 2-borneol ($C_{10}H_{18}O$) was purchased from Chengdu Biopurify Phytochemicals Ltd. (China). It has as a synonymous name: 2-hydroxybornane; Bingpian catalog number: BP0049, CAS N °: 507-70-0, molecular formula: $C_{10}H_{18}O$ and molecular weight: 154.253 M. It comes from the defensive secretion of the flies *Pristiphora erichsonii* and *Pristiphora wesmaeli*, with purity degree: 95% ~ 99%. The other reagents were obtained from Sigma Aldrich, Brazil.



Soil Physicochemical Analysis

Soil samples from the studied area were collected between 20 cm and 40 cm deep in the soil, and then sent to the laboratory, where the pH, phosphorus, potassium, sodium, calcium, magnesium, aluminum, hydrogen, aluminum concentration and the cation exchange capacity (CTC) were analysed, following the methodology proposed by [50].

Sowing site

The seeds of *A. cepa* L. used for germination were purchased from a local country house. NaCl (sodium chloride), distilled water and acetic acid (CH_3COOH) were also acquired. The seeding of *A. cepa* seeds was carried out under "Horta Dois Irmãos" field conditions in the city of Altaneira, in the state of Ceara, in the Microregion of Caririçu, South Ceara State, Brazil.

A pilot test and series of field experiments were carried out two or three times under rainy conditions or with supplementary irrigation, in the dry season during the 2018-2019 period. For each experiment, plots were selected using NPK fertilizer ($50 \text{ kg}\cdot\text{ha}^{-1}$ N, $22 \text{ kg}\cdot\text{ha}^{-1}$ P, $83 \text{ kg}\cdot\text{ha}^{-1}$ K), according to [51].

The study was carried out through a randomized block design with three repeated plots per treatment, in which each plot had an area of 16 m^2 for the cultivation of *A. cepa* in a line by groove with a distance of 8 cm, space between the lines of 12 cm and space between the grooves of 30 cm.

Land preparation, planting and crop management followed the local practices and were applied uniformly to all treatments. Data on emergence, growth and related parameters were collected and analyzed.

Culture of *Allium cepa* and experimental treatments

The inhibition of root growth in *A. cepa* was observed to examine the toxicity of untreated and treated seedlings, according to previous experiments carried out by [52]. It was performed under field conditions. The seeds of *A. cepa* seeds ($n = 20$ seeds) were exposed to 150 mM of NaCl for 20 minutes and then treated with 100, 300 and 500 $\mu\text{g}/\text{mL}$ of the monoterpene borneol, while the control was treated with 3% acetic acid, NaCl (150mM) and Borneol. All the groups remained at room temperature for 24 h. Seedling production of *A. cepa* was carried out by direct seeding. For each treatment, in the greenhouse, 20 seeds were sown directly. At the end of the 45-day period, the mean effective concentration (EC_{50}) as well as (EC_{20}) was determined by linear regression. The number of roots and the relative inhibition of root growth was also calculated and expressed as a percentage.



Measurements of Root and shoot length

Root lengths were measured using a digital paquimeter to determine the growth rate of roots and aerial parts. The number of roots was also evaluated. Measurements were performed on $n = 8$ plants per treatment after 45 days.

Preparation of Sample for biochemical assays

After performing the experimental tests and treatments, the treated onions were cut, weighed and homogenized in cold 0.1 M phosphate buffer, pH 7.4 (ratio of 0.02 leaf: 40 μL buffer) and centrifuged at 10,000 rpm for 10 min. Each experiment was performed with three replications. The supernatant was separated from the leaf tissues and used to determine antioxidants and oxidative stress-related assays.

Determination of iron levels

The iron (II) ions content was determined by measuring the intensity of the orange complex formed with 1,10-phenanthroline and free Fe^{2+} in the supernatant of control and treated groups. The content of free Fe^{2+} was determined using a modified method from [53] and [54]. Briefly, 10 μL of 1,10-phenanthroline (0.25%) was added to the reaction mixture containing 110 μL of saline (0.9% NaCl), 60 μL of 0.1 M Tris-HCl (pH 7,4), and 20 μL of the supernatant and then incubated for 60 min at room temperature. Iron (II) sulfate was used to perform the standard curve. The absorbance was measured after incubation at 492 nm, using a microplate reader and the results were expressed in mol of Fe (II)/g of tissue.

Measurement of malondialdehyde (MDA) content

Thiobarbituric acid reactive substances (TBARS) were measured to determine the lipid peroxidation (LPO) of the products as a measure of oxidative stress. Three samples per group, in triplicate, were homogenized and centrifuged at 10,000 rpm for 10 min. The reaction mixture containing 100 μL of the supernatant, 100 μL of 10% trichloroacetic acid (TCA) and 100 μL of 0.75% of 2-thiobarbituric acid (TBA prepared in 0.1 M HCL) was incubated at 95 °C for 1 hour. After cooling, the mixtures were centrifuged at 10,000 rpm for 10 minutes, and the absorbance was measured at 405 nm, using 250 μL of the reaction mixture [55]. The MDA used for the standard curve was obtained by hydrolysis of 1,1,3,3-tetramethoxypropane (TMP). The results were expressed in MDA mol (Malondialdehyde)/g of tissue.



Measurement of the antioxidant potential of 2-borneol

The antioxidant capacity of 2-borneol was measured as reported by [56]. The sample of 50 μL of the borneol at different concentrations (10, 30, 50 e 100 $\mu\text{g}/\text{mL}$) were mixed with 100 μL of freshly prepared DPPH (1,1-diphenyl-2-picrylhydrazyl) solution (0.3 mM in ethanol). Thereafter, the plate was held in the dark at room temperature for 30 min. Reduction of the DPPH radical was measured by monitoring the absorption drop at 517 nm using a microplate reader (SpectraMax, Sunnyvale, CA, USA). The Ascorbic acid was used as a standard antioxidant.

Statistical analysis

One-way Analysis of variance (ANOVA) was performed between the different treatments. Significant differences between treatments were assessed by the Tukey post-test ($p < 0.05$) and the data were expressed as the mean \pm SEM of 3 repetitions. For the toxicity the LC_{50} and LC_{20} were calculated.

RESULTS

Physicalchemical Soil Analysis

The results presented in Table 1 showed sandy soil texture, with expressive values for soil granulometry, between thick and thin sand. For the other soil compositions (silt, clay and natural clay), lower values were obtained (Table 1). In decreasing order, the macronutrients with the highest accumulation in the soil were: $\text{P} > \text{S} > \text{Ca} > \text{N} > \text{K} > \text{Mg}$. The pH was ideal for the cultivation of the species. Regarding the assortment complex, the Exchange Capacity Unit, the Total Exchange Capacity (T), the Saturation by Bases (V) (which was 96%) and the Saturation per Aluminum (m) (which was 0%) were evaluated. There was a high capacity for total exchanges and a predominance of the phosphorus element in the study area (Table 1).

Table 1. Initial characteristics of the soil used for germination experiment in tray (continua)

Parameters	Values
Granulometric composition	
Thick sand (g/kg)	366
Thin sand (g/kg)	314
Silt (g/kg)	210
Clay (g/kg)	110
Natural clay (g/kg)	49
Flocculation degree (g/100g)	55
Global density (g/cm^3)	1.20
Particle density (g/cm^3)	2.47
Ph	7.4
C.E (dS/m)	2.29
Assorted complex	
Ca^{2+} (cmol _c /kg)	14.50
Mg^{2+} (cmol _c /kg)	2.90

Table 1. Initial characteristics of the soil used for germination experiment in tray. (conclusão)

Parameters	Values
Granulometric composition	
Na ⁺ (cmol _c /Kg)	0.91
K ⁺ (cmol _c /kg)	3.37
H ⁺ + Al ³⁺ (cmol _c /kg)	1.16
Al ³⁺ (cmol _c /kg)	0
S (cmol _c /kg)	21.7
T (cmol _c /kg)	22.8
V (%)	95
PST	4
C (g/kg)	29.40
N (g/kg)	3.37
C/N	9
MO (g/kg)	50.69
P assimilable (mg/kg)	496

Emergence of *Allium cepa* strain

The effect of 2-borneol monoterpene (Figure 1A) on the emergence of *A. cepa* is depicted in Figure 1B. As expected, treatment with NaCl (150 mM) caused a significant reduction in the germination of *A. cepa* when compared with the control group. However, co-treatment with borneol at concentration of 100 and 300 µg/mL attenuated the inhibition of the germination caused by NaCl when compared with NaCl alone, and this was reverted to control level on day 15 (Figure 1B).

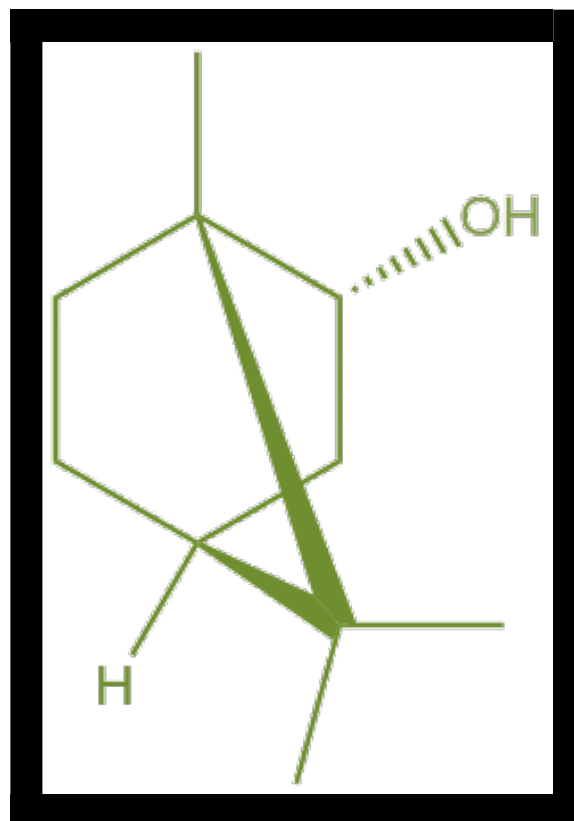


Figure 1A. Chemical structure of the compound 2-Borneol.

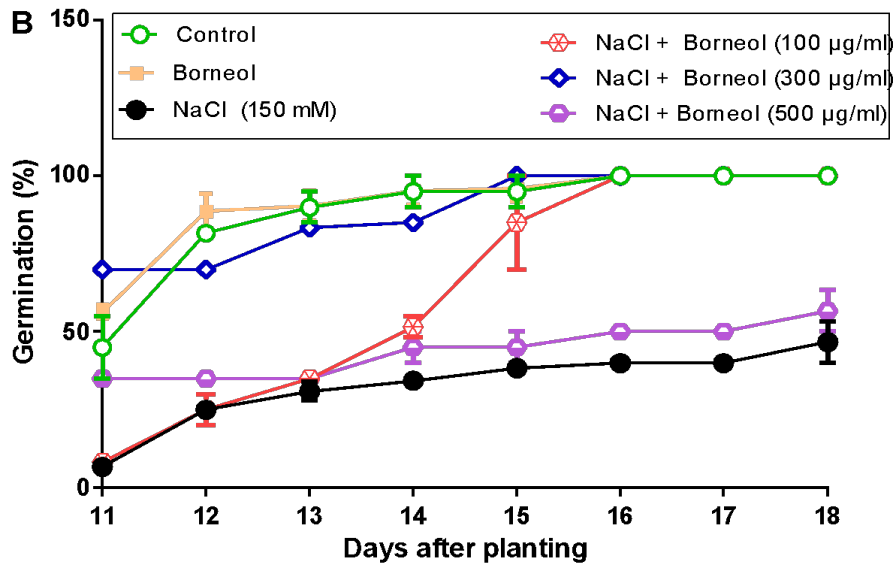


Figure 1B. Germination curve from the eleventh day (11th) after planting. *Indicates difference when compared to the control, while “#” indicates the difference when compared to NaCl ($p < 0.05$).

The different concentrations for the NaCl + Borneol group in the emergence of *A. cepa* did not show any significant changes when compared to the negative control. However, at the end of the 45-day period, when compared to treatment with NaCl (150 mM) alone, there was a significant emergency improvement after treatment with Borneol ($p < 0.05$, Figure 1C).

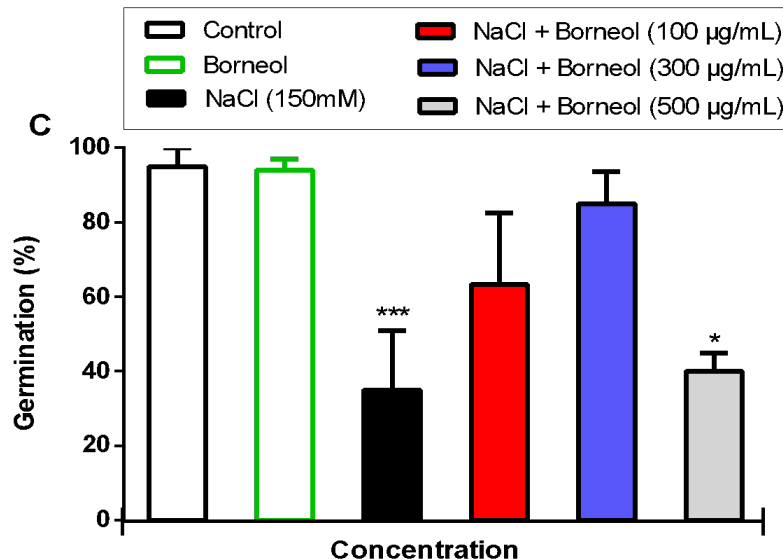


Figure 1C. Borneol effect on seed emergence in the presence of NaCl. Germination rates were coded 45 days after planting under various treatments: control; treatment with 150 mM of NaCl, 100, 300 and 500 µg/mL. Data were expressed using the mean \pm SEM for 3 repetitions. * Indicates significant difference in relation to the control.

NaCl (150 mM) exhibited LC_{20} and LC_{50} of 12.00 and 19.06 mM respectively, indicating bigger toxicity in relation to treatments in association with borneol, with higher values of CL_{20} and

CL₅₀, a fact that demonstrates the protective effect of borneol (Table 2).

Table 2. LC20 and LC50 data regarding emergency inhibition.

Treatment	CL ²⁰	CL ⁵⁰
NaCl (150 mM)	12.00	19.06
NaCl (150 mM) + borneol (µg/mL)	18.65	30.77

Figure 2A shows that NaCl (150 mM) significantly the leaf length of *A. cepa* when compared with the control ($p < 0.05$), and this effect was reverted with co-treatment with borneol all the concentration tested when compared to NaCl (150 mM) alone ($p < 0.05$, Fig. 2 A).

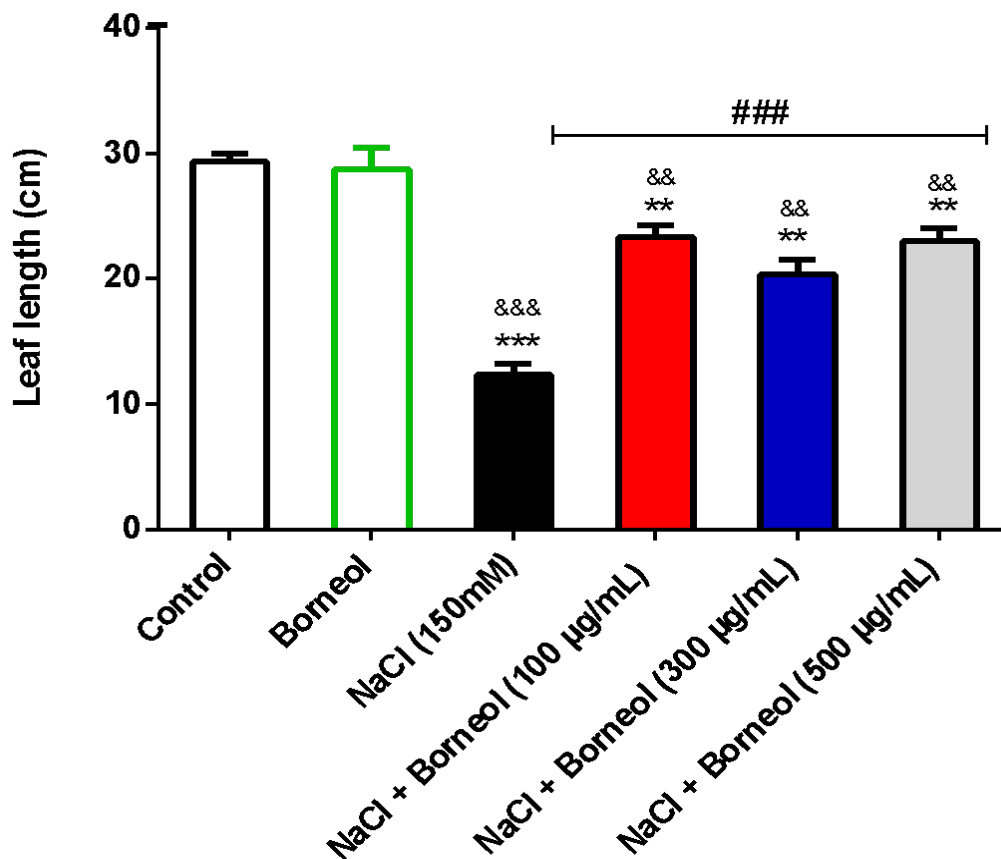


Figure 2A. Effects of different concentrations of NaCl + Borneol on leaf length in *A. cepa* L. Vertical bars denote SEM, n = 8. * Indicates a significant difference in relation to the control; & Indicates a significant difference in relation to the Borneol; # Indicates a significant difference in relation to NaCl ($p < 0.05$).

When analysing the effect of different concentrations of NaCl alone and in association with Borneol, it was observed a decrease in the overall morphology of the leaves in the NaCl group when compared to the other groups (Figure 2B).



Figure 2B. Morphological performance of *A. cepa* L., cultivated in field conditions. The plants showed differences in general morphology, observed mainly through the length of the aerial part and the root. (C) = control.

Similarly to what observed with the leaf length, NaCl (150 mM) also caused a significant reduction of root length (Figure 3A) and the number of roots (Figure 3B) when compared with their respective control ($p < 0.05$). However, when combining NaCl (150 mM) with different concentrations of borneol, the reduced root length and number of roots were significantly prevented in comparison with NaCl-treated *A. cepa* (Figure 3A and 3B). Noteworthy is the fact that borneol at 500 µg/mL in association with NaCl seemed to be toxic to the roots when compared the control (Figure 3B).

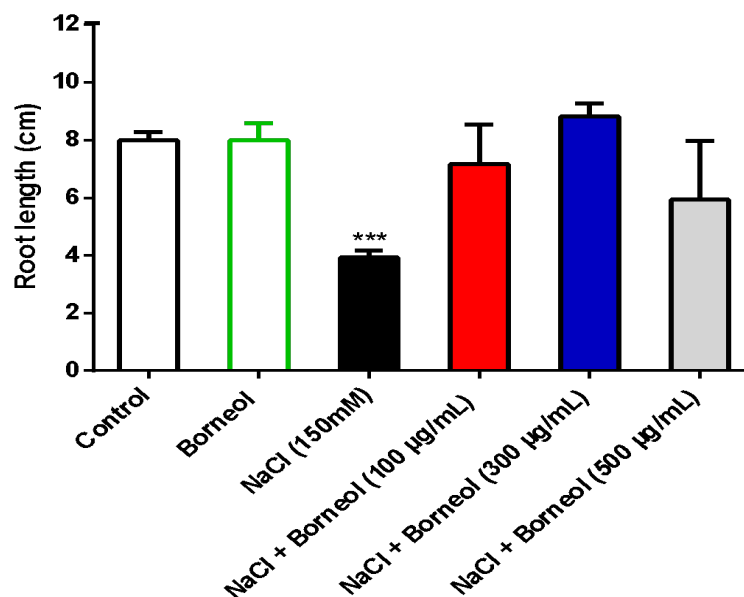


Figure 3A. Effects of different concentrations of NaCl + borneol on root length in *A. cepa* L..

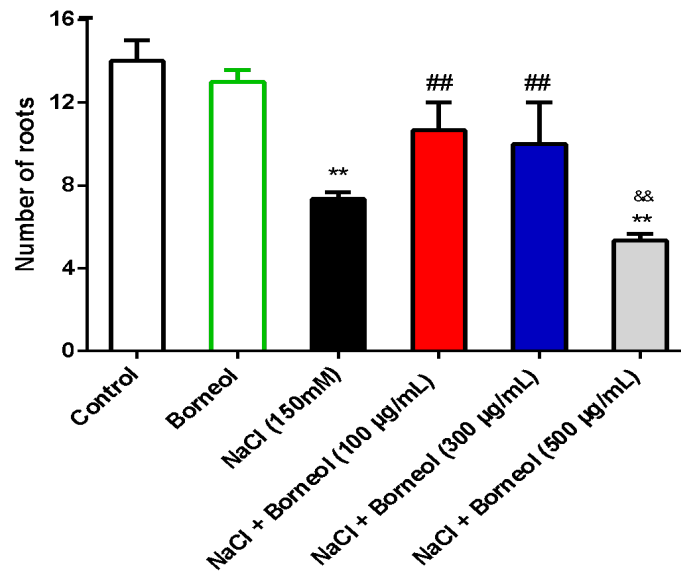


Figure 3B. Effects of different concentrations of NaCl + Borneol on the number of roots in *A. cepa*, 45 days after emergence. Bars. * indicates significant difference in relation to control; & Indicates a significant difference in relation to the Borneol; # indicates significant difference in relation to NaCl ($p < 0.05$).

Biochemical assays

Malondialdehyde (MDA), one of the secondary products of lipid peroxidation (LPO), is used as an index of lipid damage. For this reason, the effect of NaCl + borneol on MDA levels after exposure was analyzed in this study. The concentration of thiobarbituric acid reactive substances (TBARS) was observed for samples treated with different concentrations of borneol (100, 300 and 500 $\mu\text{g/mL}$) (Figure 4). NaCl (150 mM) significantly increased the MDA content of the leaves of *A. cepa* when compared to the control ($p < 0.05$). Interestingly, borneol, at all the concentrations tested significantly reduced MDA levels to almost control value (Figure 4, $p < 0.05$).

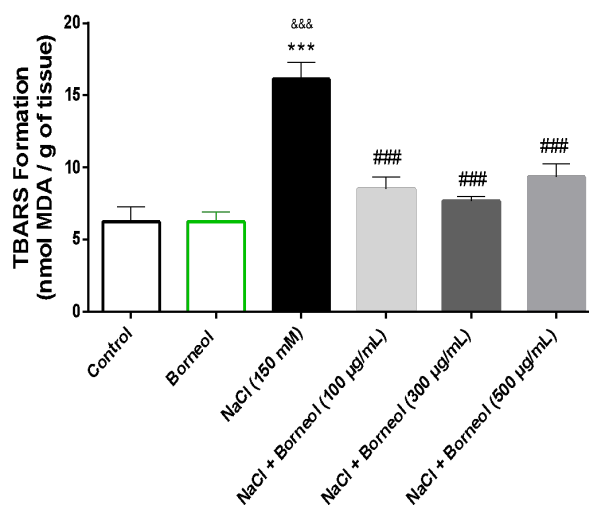


Figure 4. Content of malondialdehyde (MDA) in the tissue sample of leaves of *A. cepa* L. * Expresses significant difference in relation to the control; & indicates a significant difference in relation to the Borneol; # expresses significant difference in relation to NaCl (150 mM).

In order to investigate possible oxidative damage, after exposure of *A. cepa* to different concentrations of borneol, the levels of free Fe²⁺ ions were measured in the tissue of *A. cepa* leaves. As shown in Figure 5, the exposure of *A. cepa* did not cause a significant change in the total iron content compared to the control and NaCl-treated groups.

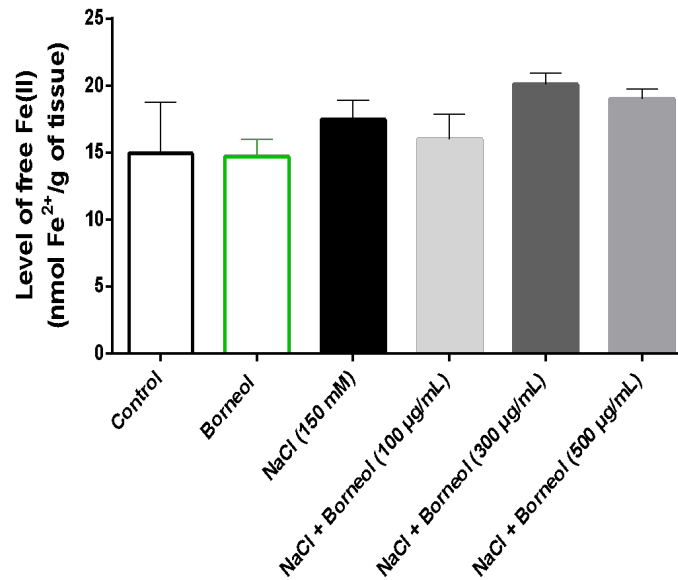


Figure 5. Total iron levels in *A. cepa* treated with Borneol monoterpene at different concentrations of 100, 300 and 500 µg/mL.

Evaluation of the antioxidant assay (DPPH)

The potential of borneol in reducing DPPH is shown in Figure 6. In general, the results show that 2-borneol exhibited slightly less antioxidant activity (IC₅₀ of 56.4 µg/mL), in comparison to the standard antioxidant drug, vitamin C (IC₅₀ 42.5 µg/mL).

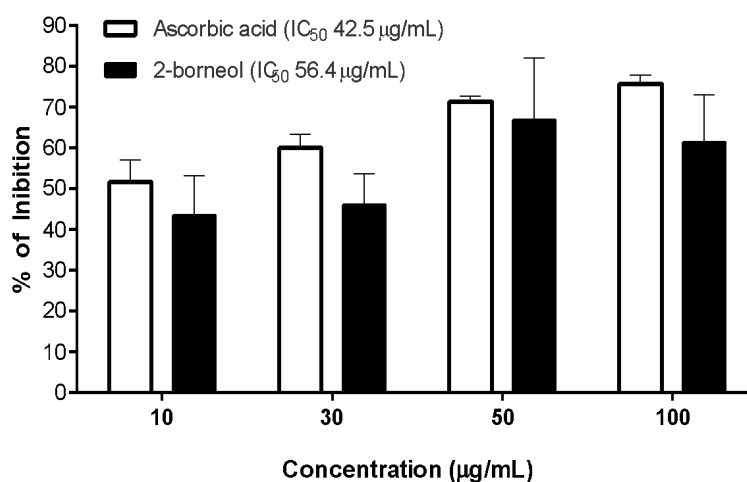


Figure 6. Percentage of DPPH radical's inhibition by 2-borneol and vitamin C. Data are expressed as average ± SEM, of n = 3 independent experiments.



DISCUSSION

Allium cepa is relatively sensitive to soil acidity, developing better in soils with a pH from 6.0 to 6.5 [57-58], which is in agreement with this study in which the pH is 7.4 (neutral) and ideal for growing onion. In acidic soils, liming is a fundamental process to promote the neutralization of exchangeable aluminum (which is a toxic element in plants) and increase the availability of phosphorus, calcium, magnesium and molybdenum [59]. Moreover, the use of organic fertilizers, as a traditional practice, has attracted considerable attention to the supply of various nutrients to crops, such as manure.

Manure improves the soil permeability to air and water, as well as the absorption of nutrients. This type of fertilizer also provides nutrients, increases the moisture retention capacity and cation exchange capacity (CEC) of the soil, besides moderating the soil pH [59-60]. The *A. cepa* crop may have a better development in medium texture soils, that are fertile and with adequate rates of organic matter, as well as a good retention. These soils are the more indicates because it has the good physical conditions and higher productive efficiency, but they must be free physical impediments (compacted layers, stones, gravels and crusting) [61].

The evaluation of soil quality in relation to the indexes provides clear approaches and schemes in order to interpreting the values of the measured indicators. Total organic matter, carbon and pH are most frequently proposed evidence of soil quality, followed by available phosphorus and other evidence of water storage and apparent density (all mentioned as >50% of the indicator sets). Texture, available potassium and total nitrogen are also frequently used (>40%). Among the chemical indicators of the soil, the organic carbon content, the pH, P and K available, total N, electrical conductivity, cation exchange capacity and mineral nitrogen have been reported more frequently than all other indicators [62].

In general, the soil is considered saline when the amount of salts present in it is able to affect the development of plants. For most cultures, this occurs when the electrical conductivity of the saturation extract is equal to or greater than 4dSm^{-1} , when they have a pH below 8.5, and a exchangeable sodium percentage (ESP) below 15% [63-64]. In this study, the soil has a sodium nature, with pH 7.4, Na^+ (cmol_c/kg), 0.91, (ESP) 4.0, ECs lower than 2.29dSm^{-1} (Table 1).

Soils with saline concentrations equal or greater than 0.5% cause serious problems in the emergence of *A. cepa* seeds [61]. Although salinity has a negative effect on plants, the physical properties of the soil have a positive effect depending on the concentration and composition of the salts, because in high concentrations they promote the flocculation of the particles, which results in a good structure in the entire soil profile [64]. Nonetheless, the predominance of monovalent cations, especially sodium, promotes a considerable increase in the expansion of clay particles [65], causing the dispersion and formation of impermeable layers, hindering the movement of the air and water in the soil [66]. These changes occur when the percentage of exchangeable sodium is greater than 15%.



In general, soil salinity occurs with the accumulation of certain ions, mainly Na^+ and Cl^- [67]. The predominance of these ions in the growth medium, besides causing toxicity, causes changes in the plant's ability to absorb, transport and use the ions necessary for growth. Thus, Ca^{2+} deficiencies can be induced by excess of Na^+ or SO_4^{2-} , as well as K^+ deficiencies can be induced by excess of Na^+ or Ca^{2+} and also high concentrations of Mg^{2+} , which can inhibit the absorption of K^+ or Ca^{2+} [68]. Among these ions, Cl^- is the most toxic anion and Na^+ is the cation most harmful to the plant metabolism.

In this study, the treatment of *A. cepa* with NaCl showed considerable inhibition in the emergence of the seeds. This may be related to the toxic effect resulting from the excessive accumulation of Na^+ and Cl^- ions, responsible for inhibiting the reduction of cotyledon reserves and the growth of the embryonic axis, according to previous experiments carried out by [69]. This explains the low percentage of seed germination observed in this study for the NaCl-treated group. In the NaCl + borneol group (100 and 300 $\mu\text{g}/\text{mL}$) (Figure 1C), there was an increase in the emergence of *A. cepa* seeds, a fact that suggests a positive influence of borneol, in these tested concentrations, to mitigate the toxic effect of Na^+ and Cl^- ions. However, in the NaCl (150 mM) + borneol (500 $\mu\text{g}/\text{mL}$) group, exposure to high salt concentrations (salt stress) visibly resulted in a very low rate in the emergence of *A. cepa* seeds.

There are scientific reports that higher salt concentrations, that is, salt stress, causes a reduction in seedling growth, as observed, for example, in studies with sunflower seeds [69], in which the authors demonstrated the formation and accumulation of reactive species such as the superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) at the cellular level [70]. In fact, reactive species accumulation causes the oxidation of membrane lipids, proteins and nucleic acids and, consequently, leads to cell death [71-73]. In addition, it can cause toxic effects due to ions accumulation [70], especially during the germination process, causing various physiological and biochemical disturbances to plants [74].

Seed emergence is a determining phase for the life cycle, establishment and growth of *A. cepa* plants, a species known for showing resistance and productivity in saline soils [75-76]. According to [10], the germination stages of seeds and initial seedlings are more sensitive to salt stress than adult plants, but such reduction in germination power serves as an indicator of the degree of tolerance of the species to salinity [77]. In the case of *A. cepa*, it is important to emphasize that mechanisms responsible for the tolerance to salinity in onion seeds associated with the role of the borneol monoterpene are still not completely understood.

In this study, the analysis of root and leaf growth exposed to 150 mM NaCl revealed that the growth of *A. cepa* was strongly inhibited by salinity. The effect of different concentrations of borneol in association with NaCl on the leaf length of *A. cepa* L., as shown in Figure 2A, showed a significant reduction in the isolated NaCl (150 mM) group when compared with the control. However, the other groups evaluated (NaCl [150 mM] + 100 $\mu\text{g}/\text{mL}$, NaCl [150 mM] + 300 $\mu\text{g}/\text{mL}$ and NaCl [150 mM] + 500 $\mu\text{g}/\text{mL}$ of borneol), showed an evolution in leaf length of the *A. cepa*



when compared only to NaCl (150 mM), similar to the control (Figure 2A).

In a pattern approximated to that observed in the control, borneol (100 µg/mL and 300 µg/mL) in association with NaCl (150 mM), managed to improve the number of roots in *A. cepa* (Figure 3B). On the other hand, NaCl (150 mM) alone or when co-exposed with 500 µg/mL of borneol largely decreased the production of roots in *A. cepa*, indicating a probable negative effect of the excess of borneol. In agreement with this finding, [78] reported that the reduction in the size and accumulation of dry matter in the leaf and also in the root of *A. cepa* is associated with the osmotic, toxic and nutritional effects of excess salts on the seedling root.

In general, plants get the nutrients they need to grow from the soil. The absorption of nutrients is differentiated throughout the life cycle, intensifying in the flowering, formation and growth of the fruits or the reserve organ of the plant to be harvested [59]. Onion growth is slow, up to 115 days old. From there, the general development is intensified, starting the formation of the bulb and the translocation of photoassimilates, as well as other compounds to the bulb [79-80]. Otherwise, the onion's tolerance to salinity and to high concentrations of Na⁺ and Cl⁻ ions is high in seed emergence, but very low during seedling growth, increasing again in the stage of three to five leaves. Depending on the composition of the saline solution, ion toxicity or nutritional deficiencies may arise due to the predominance of a specific ion effect or competition between cation and anion [81]. The leaves change from rich green to blue-green with salt stress and the tips of the leaves express burn symptoms normally associated with salt stress [82].

According to [83], the inhibition of root growth is the expected response for plants submitted to salt stress, since they are the most vulnerable organs for being directly exposed to the effects of salinization [9]. High salt concentrations can cause a reduction in water potential in plant tissues, besides causing a limitation in root growth, since the rates of cell stretching and division depend exactly on the extensibility process of the cell wall [84], generating changes in various physiological and metabolic processes of plants, as well as changes in the root structure in response to gravity ("halotropism"), including reductions in elongation and root growth [85-89].

The variable root length of *A. cepa* determined after a period of 45 days of exposure to different concentrations of borneol in association with NaCl (150 mM) revealed little effect on root length (Figure 3A). Root growth is considered an important morphological adaptation under water and salt stress, because it allows the increase of the area of water and nutrients absorption, preventing mineral deficiency and dehydration [90-92]. The inhibition of root growth due to salt stress has been widely reported, especially in cultivated plants [93-97]. In the present study, root length reduction was used as an indicator of growth inhibition in *A. cepa* seedlings exposed to salinity. The root growth may be restricted both by water deficit induced by high solution osmolarity and by ionic toxicity, involving metabolic and physiological damage [9].

Martino et al. [98], demonstrated that monoterpene compounds can exhibit phytotoxic activity, can be used as potential bioherbicides and as lead structures for the development of new potentially safe and ecocompatible pesticides. However more studies are needed to investigate



costs, selectivity, safety and mode of action.

To address whether NaCl (150 mM) alone and in association with borneol caused oxidative stress, the malondialdehyde content of *A. cepa* leaves was evaluated. Among the lipid peroxidation (LPO) products formed that react with thiobarbituric acid (TBA), malondialdehyde stands out mainly, although other aldehydes and compounds such as sugar, amino acids and bilirubin can also react with TBA [99]. In this study, treatment with NaCl (150 mM) exhibits a significant increase in the content of malondialdehyde in relation to the negative control. On the other hand, treated groups with NaCl-treated with borneol significantly reduced the formation of TBARS, compared to NaCl (150 mM) group (Fig. 4). These findings indicate that borneol possibly provided greater tolerance to salt, through decreasing the formation of lipid peroxidation products.

As highlighted earlier, the stress generated by reactive species leads to gradual peroxidation of cell membranes and inactivation of lipid and antioxidant enzymes [100-101]. Once these species are produced, they can cause damage to cellular components and tissues, especially proteins, lipids and nucleic acids, and can lead to cell death [73]. Moreover, reactive species are implicated in a variety of physiological processes in plants, from seed emergence to cell death [102-103]. More specifically, free radicals can inactivate enzymes and damage important cellular components, causing the degradation of phospholipids present in the cell membrane [104-105], polysaccharide degradation, enzyme denaturation and DNA breakdown [106], as well as the bleaching of chlorophyll pigments [73]. Therefore, the defence mechanisms against damage caused by free radicals are important in limiting the action of oxidative stress, depending on the duration of the stress and the plant's ability to survive these stresses. The plant can react with increased expression of enzyme genes with antioxidant functions and with the synthesis of reactive species-sequestering species [107].

Regarding the oxidative damage induced by salt stress, scientific evidence demonstrates that the antioxidant defense system plays an important role in plant protection. It was reported that salt stress causes reductions in the growth of cucumber root, which is associated with increased production of free radicals and damage to cell membranes, resulting in decreased activity of antioxidant enzymes [108]. In addition, it was found that treatment with NaCl caused suppression of seedling growth due to decreased antioxidant enzyme activity [109]. In this study, the level of free iron Fe^{2+} was measured in the treated onion leaves, in which there were no changes in the treated groups when compared to the control or with NaCl (150 mM) (Figure 5).

The *in vitro* antioxidant capacity of borneol was assessed by DPPH assay. Ascorbic acid exhibited the greatest elimination of DPPH free radicals, with IC_{50} 42.5 μ g/mL, while 2-borneol exhibited antioxidant activity (IC_{50} de 56.4 μ g/ mL), comparable to ascorbic acid (Figure 6). Taking these findings into consideration, it seems reasonable to suggest that the antioxidant capacity of borneol recovers seed emergence, leaf growth, and the number of roots in *A. cepa*, reducing the content of malondialdehyde under conditions of salt stress. This is supported by the finding that borneol present in fresh leaves of *Cinnamomum camphora* showed effective antioxidant activity



and reducing power [110].

The treatment of *A. cepa* with some compounds such as Na₂SeO₄, under field conditions, has shown a tendency to increase bulb yield and size, increase the bulb's total antioxidant capacity, and greater production of organic compounds [51].

The results described here demonstrated that the tolerance of *A. cepa* to NaCl improved significantly with the co-treatment with borneol. The incubation of *A. cepa* seeds in NaCl (150 mM) for 24 h resulted in a big increase in the concentration of Na⁺ ions in the plant tissue, which was mitigated by borneol. Therefore, the use of borneol allowed a flexible response to the emergence of onion seeds in conditions of salt stress, thus contributing to the survival of the crop in soils that present adversities in terms of salinity.

CONCLUSION

The exposure of *A. cepa* to NaCl at concentration of 150 mM decreased the seed's emergence capacity. NaCl + Borneol at concentrations of 100 and 300 µg/mL exhibited effective antioxidant when co-treated with NaCl. This was evidenced the reduction of salt stress caused by NaCl (150 mM), and the improvement of seed emergence rate, as well as the number of roots in *A. cepa*. In addition, borneol provided a greater tolerance of *A. cepa* to salt by decreasing lipid peroxidation but did not alter the free iron content. The nutrients with the highest accumulation in the soil were P > S > Ca > N > K > Mg. The pH was ideal for the cultivation of the species.

Conflicts of interest

The authors have nothing to declare.

Acknowledgement

The authors are grateful to the Cear Research Support Foundation (FUNCAP) for financial support of this research (concession BP3-0139-00018.02.00/18).

REFERENCES

- [1] KUSVURAN, A. The effects of salt stress on the germination and antioxidative enzyme activity of Hungarian vetch (*Vicia pannonica* Crantz.) varieties. Legume **Research-An Inter**. J. n. 38, p. 51-59, 2015.
- [2] HERNANDEZ, L.; ALEGRE, L.; MUNNE-BOSCH, S. Drought-induced changes in flavonoids and other low molecular weight antioxidants in *Cistus clusii* grown under Mediterranean field conditions, **Tree Physiol**. n. 24, p. 1303-1311, 2004.
- [3] ALSAEEDI, A. et al. Exogenous nanosilica improves germination and growth of cucumber by maintaining K⁺/Na⁺ ratio under elevated Na⁺ stress. **Plant Physiol. Biochem**. n. 125, p. 64-171, 2018.
- [4] MISRA, N.; DWIVEDI, U. N. Genotypic difference in salinity tolerance of green gram cultivars. **Plant Science**, v. 166, n. 5, p. 1135-1142, 2004.
- [5] ALMANSOURI, M.; KINET, J.-M.; LUTTS, Stanley. Effect of salt and osmotic stresses on germination in durum



- wheat (*Triticum durum* Desf.). **Plant and soil**, v. 231, n. 2, p. 243-254, 2001.
- [6] KHALIQ, A. et al. Salinity tolerance in wheat cultivars is related to enhanced activities of enzymatic antioxidants and reduced lipid peroxidation. **CLEAN-Soil, Air, Water**, v. 43, n. 8, p. 1248-1258, 2015.
- [7] BARNAWAL, D. et al. Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression. **Physiologia plantarum**, v. 161, n. 4, p. 502-514, 2017.
- [8] IVANOVA, K. et al. Influence of Salt Stress on Stomatal, Biochemical and Morphological Factors Limiting Photosynthetic Gas Exchange in *Paulownia Elongata*× *Fortunei*. *Comptes rendus de l'Académie bulgare des Sciences*. v. 68, n. 2, 2015.
- [9] MUNNS, R.; TESTER, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, v. 59, p. 651-681, 2008.
- [10] FOOLAD, M. R. Recent advances in genetics of salt tolerance in tomato. **Plant Cell, tissue and organ culture**, v. 76, n. 2, p. 101-119, 2004.
- [11] GUPTA, R. K.; ABROL, I. P. Salt-affected soils: their reclamation and management for crop production. In: **Advances in soil science**. Springer, New York, NY, 1990. p. 223-288.
- [12] MUNNS, R. Comparative physiology of salt and water stress. **Plant, cell & environment**, v. 25, n. 2, p. 239-250, 2002.
- [13] BARROS, M. de F. C. et al. Recuperação de solos afetados por sais pela aplicação de gesso de jazida e calcário no Nordeste do Brasil. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 8, n. 1, p. 59-64, 2004.
- [14] QADIR, M. et al. Phytoremediation of sodic and saline-sodic soils. **Advances in agronomy**, v. 96, p. 197-247, 2007.
- [15] SILVA, P. O. et al. Germination of salt-stressed seeds as related to the ethylene biosynthesis ability in three *Stylosanthes* species. **Journal of plant physiology**, v. 171, n. 1, p. 14-22, 2014.
- [16] WEIDENHAMER, J. D. et al. Just how insoluble are monoterpenes? **Journal of Chemical Ecology**, v. 19, n. 8, p. 1799-1807, 1993.
- [17] SINGH, H. P. et al. Comparative phytotoxicity of four monoterpenes against *Cassia occidentalis*. **Annals of Applied Biology**, v. 141, n. 2, p. 111-116, 2002.
- [18] SINGH, H. Pal et al. Phytotoxicity of the volatile monoterpene citronellal against some weeds. **Zeitschrift für Naturforschung C**, v. 61, n. 5-6, p. 334-340, 2006.
- [19] ZUNINO, M. P.; ZYGADLO, J. A. Effect of monoterpenes on lipid oxidation in maize. **Planta**, v. 219, n. 2, p. 303-309, 2004.
- [20] NISHIDA, N. et al. Allelopathic effects of volatile monoterpene produced by *Salvia leucophylla*: inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. **Journal of chemical ecology**, v. 31, n. 5, p. 1187-1203, 2005.
- [21] LANGENHEIM, J. H. Higher plant terpenoids: a phytocentric overview of their ecological roles. **Journal of chemical ecology**, v. 20, n. 6, p. 1223-1280, 1994.
- [22] ISMAN, M. B. Plant essential oils for pest and disease management. **Crop protection**, v. 19, n. 8-10, p. 603-608, 2000.
- [23] LEE, B.-H. et al. Fumigant toxicity of essential oils from the Myrtaceae family and 1, 8-cineole against 3 major stored-grain insects. **Journal of Stored Products Research**, v. 40, n. 5, p. 553-564, 2004.
- [24] KORDALI, S. et al. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. **Bioresource Technology**, v. 99, n. 18, p. 8788-8795, 2008.
- [25] HORVÁTHOVÁ, E. et al. Effects of borneol on the level of DNA damage induced in primary rat hepatocytes and testicular cells by hydrogen peroxide. **Food and Chemical Toxicology**, v. 47, n. 6, p. 1318-1323, 2009.
- [26] BHATIA, S. P.; LETIZIA, C. S.; API, A. M. Fragrance material review on borneol. **Food and chemical toxicology**, v. 46, n. 11, p. S77-S80, 2008.
- [27] WU, H.-Y. et al. The synergetic effect of edaravone and borneol in the rat model of ischemic stroke. **European Journal of Pharmacology**, v. 740, p. 522-531, 2014.
- [28] ASPLUND, R. Owen. Some quantitative aspects of the phytotoxicity of monoterpenes. **Weed Science**, v. 17, n. 4, p. 454-455, 1969.
- [29] IQBAL, M. et al. Bioassays based on higher plants as excellent dosimeters for ecotoxicity monitoring: a review **v Chemistry International**, v. 5, n. 1, p. 1-80, 2019.
- [30] LEME, D. M.; MARIN-MORALES, M. A. *Allium cepa* test in environmental monitoring: a review on its application. *Mutation Research/Reviews in Mutation Research*. v. 682, n. 1, p. 71-81, 2009.
- [31] PATNAIK, A. R.; ACHARY, V. M. M.; PANDA, B. B. Chromium (VI)-induced hormesis and genotoxicity are mediated through oxidative stress in root cells of *Allium cepa* L. **Plant growth regulation**, v. 71, n. 2, p. 157-170, 2013.



- [32] KUMARI, M. et al. Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of *Allium cepa*. **Journal of hazardous materials**, v. 190, n. 1-3, p. 613-621, 2011.
- [33] MODLITBOVÁ, P. et al. Short-term assessment of cadmium toxicity and uptake from different types of Cd-based Quantum Dots in the model plant *Allium cepa* L. **Ecotoxicology and environmental safety**, v. 153, p. 23-31, 2018.
- [34] FOUAD, A. S.; HAFEZ, R. M. The effects of silver ions and silver nanoparticles on cell division and expression of *cdc2* gene in *Allium cepa* root tips. **Biologia Plantarum**, v. 62, n. 1, p. 166-172, 2018.
- [35] RAJPUT, V. D. et al. Effects of copper nanoparticles (CuO NPs) on crop plants: a mini review. **BioNanoScience**, v. 8, n. 1, p. 36-42, 2018.
- [36] FATMA, F. et al. Phytotoxicity of pesticides mancozeb and chlorpyrifos: correlation with the antioxidative defence system in *Allium cepa*. **Physiology and molecular biology of plants**, v. 24, n. 1, p. 115-123, 2018.
- [37] LUO, Y. et al. Seed germination test for toxicity evaluation of compost: Its roles, problems and prospects. **Waste Management**, v. 71, p. 109-114, 2018.
- [38] BEWLEY J. D.; BLACK M. Seeds: Physiology of Development and Germination, 2nd (edn), **Plenum Press**, New York, London, USA. 1994.
- [39] DA SILVA, L. C. et al. Influence of temperature on the germination and root size of *Acmella oleracea* (L.) RK Jansen. **Revista Agro@mbiente On-line**, v. 14, 2020.
- [40] LABOURIAU, L. G. On the frequency of isothermal germination in seeds of *Dolichos biflorus* L. **Plant and Cell Physiology**, v. 19, n. 3, p. 507-512, 1978.
- [41] ORZOLEK, M. D. Establishment of vegetables in the field. **HortTechnology**, v. 1, n. 1, p. 78-81, 1991.
- [42] GRASSBAUGH, E. M.; BENNETT, M. A. Factors affecting vegetable stand establishment. **Scientia Agricola**, v. 55, n. SPE, p. 116-120, 1998.
- [43] HACKBART, V. C. dos S.; CORDAZZO, C. V. Ecologia das sementes e estabelecimento das plântulas de *Hydrocotyle bonariensis* Lam. 2003.
- [44] LEAL, C. C. P. et al. Water stress on germination and vigor of 'mofumbo' (*Combretum leprosum* Mart.) seeds at different temperatures. **Revista Ciência Agronômica**, v. 51, n. 1, 2020.
- [45] MARTINS, C. C.; NAKAGAWA, J.; BOVI, M. L. A. Efeito da posição da semente no substrato e no crescimento inicial das plântulas de palmito-vermelho (*Euterpe espirosantensis* Fernandes-Palmae). **Revista Brasileira de Sementes**, v. 21, n. 1, p. 164-173, 1999.
- [46] FOTOPOULOS, V. et al. Involvement of AsA/DHA and GSH/GSSG ratios in gene and protein expression and in the activation of defence mechanisms under abiotic stress conditions. In: Ascorbate-glutathione pathway and stress tolerance in plants. **Springer**, Dordrecht, 2010. p. 265-302.
- [47] GUNES, A. et al. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. **Journal of Plant Physiology**, v. 164, n. 6, p. 728-736, 2007.
- [48] DHANYALAKSHMI, K. H.; VIJAYALAKSHMI, C.; BOOMINATHAN, R. Evaluation of physiological and biochemical responses of rice (*Oryza sativa* L.) varieties to salt stress. **Indian Journal of Agricultural Research**, v. 47, n. 2, p. 91-99, 2013.
- [49] MISHRA, A.; CHOUDHURI, M. A. Effects of salicylic acid on heavy metal-induced membrane deterioration mediated by lipoxygenase in rice. **Biologia Plantarum**, v. 42, n. 3, p. 409-415, 1999.
- [50] TEIXEIRA, P. C.; DONAGEMMA, G. K.; FONTANA, A.; TEIXEIRA, W. G. (Ed.). Manual de métodos de análise de solo. 3. ed. **rev. e ampl. Brasília**, DF: Embrapa, pt. 1, cap. 4, p. 34-46, 2017.
- [51] PÖLDMA, P. et al. Selenium treatment under field conditions affects mineral nutrition, yield and antioxidant properties of bulb onion (*Allium cepa* L.). **Acta Sci. Pol., Hortorum Cultus**, v. 12, n. 6, p. 167-181, 2013.
- [52] FISKEŠJÖ, G. The *Allium* test as a standard in environmental monitoring. **Hereditas**, v. 102, n. 1, p. 99-112, 1985.
- [53] KAMDEM, J. P. et al. Antioxidant activity, genotoxicity and cytotoxicity evaluation of lemon balm (*Melissa officinalis* L.) ethanolic extract: Its potential role in neuroprotection. **Industrial Crops and Products**, v. 51, p. 26-34, 2013.
- [54] KLIMACZEWSKI, C. V. et al. Antioxidant activity of *Peumus boldus* extract and alkaloid boldine against damage induced by Fe (II)-citrate in rat liver mitochondria in vitro. **Industrial Crops and Products**, v. 54, p. 240-247, 2014.
- [55] BARBOSA FILHO, V. M. et al. Phytochemical constituents, antioxidant activity, cytotoxicity and osmotic fragility effects of Caju (*Anacardium microcarpum*). **Industrial Crops and Products**, v. 55, p. 280-288, 2014.
- [56] DUARTE, A. E. et al. Antimicrobial activity and Modulatory effect of essential oil from the leaf of *Rhaphiodon echinus* (Nees & Mart) **Schauer on some antimicrobial drugs. Molecules**, v. 21, n. 6, p. 743, 2016.
- [57] AFTAB, S. et al. Impact of potassium on the growth and yield contributing attributes of onion (*Allium cepa* L.). **Asian Research Journal of Agriculture**, p. 1-4, 2017.



- [58] MOLLAVALI, M. et al. Nitrogen form and mycorrhizal inoculation amount and timing affect flavonol biosynthesis in onion (*Allium cepa* L.). **Mycorrhiza**, v. 28, n. 1, p. 59-70, 2018.
- [59] MENDES, A. M. S. et al. Nutrição mineral e adubação da cultura da cebola no Submédio do Vale do São Francisco. **Embrapa Semiárido-Circular Técnica** (INFOTECA-E), 2008.
- [60] MEKONNEN, D. A.; MIHRETU, F. G.; WOLDETSADIK, K. Farmyard manure and intra-row spacing on yield and yield components of Adama Red onion (*Allium cepa* L.) cultivar under irrigation in Gewane District, Afar Region, Ethiopia. **Journal of Horticulture and Forestry**, v. 9, n. 5, p. 40-48, 2017.
- [61] RESENDE, GM de; COSTA, N. D.; SOUZA, RJ de. Cultivo da cebola no Nordeste. Petrolina: Embrapa Semi-Árido, 2007. **Sistemas de Produção**, 3. 2012.
- [62] BÜNNEMANN, E. K. et al. Soil quality—A critical review. **Soil Biology and Biochemistry**, v. 120, p. 105-125, 2018.
- [63] DAKER, A. **Irrigação e drenagem: A água na agricultura**. Freitas Bastos, 1988.
- [64] RICHARDS, L. A. **Diagnosis and improvement of saline and alkali soils**. Handbook No. 60. US Department of Agriculture, Washington, DC, 1954.
- [65] FASSBENDER, H. W.; BORNEMISZA, Elemer. **Química de suelos con énfasis en suelos de América Latina**. lica, 1987.
- [66] ROLSTON, D. E.; BIGGAR, J. W.; NIELSEN, D. R. Effect of salt on soils. **California Agriculture** 38:11-13. 1984.
- [67] Saffaryazdi, A.; Lahouti, M.; Ganjeali, A. The effect of different concentrations of selenium on morphophysiological characteristics of spinach (*Spinacia oleracea* L.), **J. Hort. Sci.** 26, 292-300. 2012.
- [68] MARSCHNER, H. Marschner's mineral nutrition of higher plants. Academic press, 2011.
- [69] NUNES, R. T. C. et al. Desempenho germinativo de sementes de girassol submetidas ao estresse salino. *Cultura Agronômica: Revista de Ciências Agronômicas*, v. 25, n. 1, p. 79-92, 2016.
- [70] PINHEIRO, D. T. et al. Germination and antioxidant action in melon seeds exposed to salt stress. **Pesquisa Agropecuária Tropical**, v. 46, n. 3, p. 336-342, 2016.
- [71] SCANDALIOS, J. G. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. **Brazilian journal of medical and biological research**, v. 38, n. 7, p. 995-1014, 2005.
- [72] HALLIWELL, B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. **Plant physiology**, v. 141, n. 2, p. 312-322, 2006.
- [73] MILLER, G. A. D. et al. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. **Plant, cell & environment**, v. 33, n. 4, p. 453-467, 2010.
- [74] YACOUBI, R. et al. Proteomic analysis of the enhancement of seed vigour in osmoprimed alfalfa seeds germinated under salinity stress. **Seed Science Research**, v. 23, n. 2, p. 99-110, 2013.
- [75] ANSARI, N. A.; KHALEGHI, S.-S. Germination and emergence response of some onion cultivars of southern Iran to salinity stress. **Seed Science and Biotechnology**, v. 3, n. 1, p. 21-23, 2009.
- [76] BYBORDI, A.; SAADAT, S.; ZARGARIPOUR, P. The effect of zeolite, selenium and silicon on qualitative and quantitative traits of onion grown under salinity conditions. **Archives of Agronomy and Soil Science**, v. 64, n. 4, p. 520-530, 2018.
- [77] DE GÓIS, V. A.; TORRES, S. B.; PEREIRA, R. A. Germinação de sementes de maxixe submetidas a estresse salino. **Revista Caatinga**, v. 21, n. 4, 2008.
- [78] LACERDA, C. F. et al. Morpho-physiological responses of cowpea leaves to salt stress. **Brazilian Journal of Plant Physiology**, v. 18, n. 4, p. 455-465, 2006.
- [79] GAMIELY, S. et al. Onion plant growth, bulb quality, and water uptake following ammonium and nitrate nutrition. **HortScience**, v. 26, n. 8, p. 1061-1063, 1991.
- [80] BREWSTER, J. L. The classification, origins, distribution and economic importance of the major vegetable crops. Onions and Other Vegetable Alliums. Wallingford: **CAB International**, p. 1-18, 1994.
- [81] BERNSTEIN, Leon; FRANCOIS, L. E.; CLARK, R. A. Interactive effects of salinity and fertility on yields of grains and vegetables I. **Agronomy Journal**, v. 66, n. 3, p. 412-421, 1974.
- [82] SHANNON, M. C.; GRIEVE, C. M. Tolerance of vegetable crops to salinity. **Scientia horticulturae**, v. 78, n. 1-4, p. 5-38, 1998.
- [83] MAIA, J. M. et al. Salt-induced changes in antioxidative enzyme activities in root tissues do not account for the differential salt tolerance of two cowpea cultivars. **Brazilian Journal of Plant Physiology**, v. 22, n. 2, p. 113-122, 2010.
- [84] ASHRAF, M. P. J. C.; HARRIS, P. J. C. Potential biochemical indicators of salinity tolerance in plants. **Plant science**, v. 166, n. 1, p. 3-16, 2004.
- [85] HAJIBAGHERI, M. A. et al. Salinity resistance in Zea mays: fluxes of potassium, sodium and chloride, cytoplasmic concentrations and microsomal membrane lipids. **Plant, Cell & Environment**, v. 12, n. 7, p. 753-757, 1989.
- [86] POTTERS, G. et al. Stress-induced morphogenic responses: growing out of trouble?. **Trends in plant science**,



v. 12, n. 3, p. 98-105, 2007.

- [87] SUN, F. et al. Salt modulates gravity signaling pathway to regulate growth direction of primary roots in Arabidopsis. **Plant physiology**, v. 146, n. 1, p. 178-188, 2008.
- [88] BERNSTEIN, N.; ESHEL, A.; BEECKMAN, T. Effects of salinity on root growth. *Plant roots: the hidden half*, p. 1-784, 2013.
- [89] JULKOWSKA, M. M. et al. Capturing Arabidopsis root architecture dynamics with ROOT-FIT reveals diversity in responses to salinity. **Plant Physiology**, v. 166, n. 3, p. 1387-1402, 2014.
- [90] OKUSANYA, O. T. The effect of sea water and temperature on the germination behaviour of *Crithmum maritimum*. **Physiologia Plantarum**, v. 41, n. 4, p. 265-267, 1977.
- [91] CREELMAN, R. A. et al. Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings: analysis of growth, sugar accumulation, and gene expression. **Plant Physiology**, v. 92, n. 1, p. 205-214, 1990.
- [92] SAAB, I. N. et al. Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. **Plant physiology**, v. 93, n. 4, p. 1329-1336, 1990.
- [93] WAHID, A. et al. Short term incubation of sorghum caryopses in sodium chloride levels: changes in some pre-and post-germination physiological parameters. **Plant Science**, v. 139, n. 2, p. 223-232, 1998.
- [94] DASH, M.; PANDA, S. K. Salt stress induced changes in growth and enzyme activities in germinating *Phaseolus mungo* seeds. **Biologia plantarum**, v. 44, n. 4, p. 587-589, 2001.
- [95] PINHEIRO, H. A. et al. Leaf gas exchange, chloroplastic pigments and dry matter accumulation in castor bean (*Ricinus communis* L.) seedlings subjected to salt stress conditions. *Industrial Crops and Products*, v. 27, n. 3, p. 385-392, 2008.
- [96] FERREIRA-SILVA, S. L. et al. Changes in physiological indicators associated with salt tolerance in two contrasting cashew rootstocks. **Brazilian Journal of Plant Physiology**, v. 20, n. 1, p. 51-59, 2008.
- [97] DA SILVA, E. N. et al. Ion uptake and growth of pinhão-mansô under different salinity levels. **Revista Ciência Agronômica**, v. 40, n. 2, p. 240, 2009.
- [98] MARTINO, L. De et al. The antigerminative activity of twenty-seven monoterpenes. **Molecules**, v. 15, n. 9, p. 6630-6637, 2010.
- [99] MEAGHER, E. A.; FITZGERALD, G. A. Indices of lipid peroxidation in vivo: strengths and limitations. **Free Radical Biology and Medicine**, v. 28, n. 12, p. 1745-1750, 2000.
- [100] MITTLER, R. Oxidative stress, antioxidants and stress tolerance. **Trends in plant science**, v. 7, n. 9, p. 405-410, 2002.
- [101] TANOU, G. et al. Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. **The Plant Journal**, v. 60, n. 5, p. 795-804, 2009.
- [102] WANG, P. et al. Hydrogen peroxide-mediated activation of MAP kinase 6 modulates nitric oxide biosynthesis and signal transduction in Arabidopsis. **The Plant Cell**, v. 22, n. 9, p. 2981-2998, 2010.
- [103] FILIPPOU, P.; ANTONIOU, C.; FOTOPOULOS, V. Effect of drought and rewatering on the cellular status and antioxidant response of *Medicago truncatula* plants. **Plant Signaling & Behavior**, v. 6, n. 2, p. 270-277, 2011.
- [104] ABOGADALLAH, G. M. Insights into the significance of antioxidative defense under salt stress. **Plant signaling & behavior**, v. 5, n. 4, p. 369-374, 2010.
- [105] BARTOLI, C. G. et al. Interactions between hormone and redox signalling pathways in the control of growth and cross tolerance to stress. **Environmental and Experimental Botany**, v. 94, p. 73-88, 2013.
- [106] MATYSIK, J. et al. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. **Current Science**, p. 525-532, 2002.
- [107] MUNNÉ-BOSCH, S.; QUEVAL, G.; FOYER, C. H. The impact of global change factors on redox signaling underpinning stress tolerance. **Plant Physiology**, v. 161, n. 1, p. 5-19, 2013.
- [108] DUAN, J. et al. Exogenous spermidine affects polyamine metabolism in salinity-stressed *Cucumis sativus* roots and enhances short-term salinity tolerance. **Journal of plant physiology**, v. 165, n. 15, p. 1620-1635, 2008.
- [109] YAZICI, I. et al. Salinity tolerance of purslane (*Portulaca oleracea* L.) is achieved by enhanced antioxidative system, lower level of lipid peroxidation and proline accumulation. **Environmental and Experimental Botany**, v. 61, n. 1, p. 49-57, 2007.
- [110] SU, J. et al. Composition and biological activities of the essential oil extracted from a novel plant of *Cinnamomum camphora* Chvar. Borneol. **Journal of Medicinal Plants Research**, v. 6, n. 18, p. 3487-3494, 2012.