Asian Journal of Advances in Agricultural Research

5(4): 1-20, 2018; Article no.AJAAR.40051
ISSN: 2456-8864

Inducing Salinity Tolerance of Rosemary (*Rosmarinus officinalis* L.) Plants by Chitosan or Zeolite Application

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Authors' contributions

This work was carried out in collaboration between all authors. Author MNH designed the study and review the final manuscript; author SF participate in designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript; author SAA managed the analyses of the study and managed the literature searches; author NBIAA conducted the experimental schem. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJAAR/2018/40051
Editor(s):
(1) Muhammad Azam, Professor, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan.
Reviewers:
(1) Sofia Mendes Moreira Correia, University of Trás-os-Montes e Alto Douro, Portugal.
(2) Zeynel Dalkiliç, Turkey.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/23588

Original Research Article

Received 4th January 2018
Accepted 8th March 2018
Published 12th March 2017

ABSTRACT

Aims: Rosemary (*Rosemarinus officinalis* L.) is the foremost economically medicinal plants worldwide, its production like other crops affected negatively by environmental stresses. The adverse effects of salinity on crop production are more drastic. Application of chitosan or zeolite might considerably restore the plant productivity under the environmental stress. The present investigation aimed to evaluate the role of chitosan or zeolite on counteracting the deleterious impact of salinity on rosemary growth, oil percentage and its chemical compositions
Study Design: A factorial experiment was done in a randomized complete block design system with five replications.
Place and Duration of Study: The pot experiments were done at the Agric. Botany Experimental Farm and Laboratory, Fac. of Agric., Mansoura Univ., Egypt during 2015/2016 and 2016/2017 seasons.
Methodology: The factorial combinations of three zeolite concentrations (0, 4, 8 g/kg) as a soil

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additive and three chitosan foliar applications (0, 250 and 500 mg/l) and three salinity levels (0, 50, 100 mM NaCl) were considered.
Results: Shoot and root length, branches number, shoot and root dry weight were declined as a result of salinity. Similarly, the concentrations of photosynthetic pigments, minerals, and essential oil yield were decreased, whereas Na+ and Cl- increased in each season.
Application of chitosan or zeolite counteracted the depressing effects of salinity on plant growth, photosynthetic pigments, oil %, and minerals. They declined sodium and chloride concentration in each shoot and root compared to untreated plants.
Conclusion: It could conclude that zeolite at 8 g/kg soil or chitosan at 250 mg/l, showed a uniform impact in alleviating of rosemary growth inhibition and its productivity under salinity stress condition.

Keywords: Chitosan; chlorophyll; rosemary; salinity; zeolite.

1. INTRODUCTION

Medicinal plants are usually used in the manufacture of synthetic drugs based on its chemical structures [1]. Rosemary (*Rosmarinus officinalis* L.), a member of the Lamiaceae family contains flavonoids and essential oils. Rosemary essential oil is known to contain anti-microbial compounds and are therefore used in the manufacture of herbal shampoos to strengthen the hair and in the flavoring and conservation of food product [2,3]. In Egypt, rosemary was cultivated for a long time and had been used in folk medicine, as a spice and a natural preservative.

Salinity has drawn intensive attention worldwide like, Egypt. The deleterious effects of salinity may be attributable to osmotic and ionic effects as well as oxidative stress [4]. The free radicals induced by salinity disrupt normal metabolism through lipids peroxidation, protein denaturation and nucleic acids [5]. Several authors studied the impact of salinity on numerous plant growth traits and obtained negative responses [6,7], that found increasing salinity levels decreased markedly all studied growth characteristics.

Application of soil amendments or foliar application so as to enhance plant productivity is one amongst the foremost important approaches to overcome salinity stress. Because of its low cost and great versatility, zeolite plays a crucial role in agriculture. It can be used to improve the soils, boost the consequences impacts of fertilizers-alike, and as a component of substratum for the development of different crops [8,9]. Zeolite application results in improving water retention capability [10] and permitting some ions and blocking others [11]. In this concern, zeolite application is that the slow release of absorbed nutrients that stop fast leaching, therefore facilitating an adequate supply of nutrients to the crops [12]. Other studies showed that zeolite served as the stabilization agent in soybean growth, hindrance of salinization [13] and induced water availability in strawberry fields [14]. Recently, Hazrati et al. [10] proved that application of zeolite at 4 and 8 g/kg soil increased leaf numbers per plant, fresh weight of leaves, and water use efficiency.

Chitosan (β-1,4-linked glucosamine, CHI) has attracted tremendous consideration as potentially vital biological resources and environmentally friendly with numerous usage in agriculture [15]. CHI has been used in agricultural systems to induce crop productivity [16], to protect plants against oxidative stress [17] and to improve plant growth [18,19].

Therefore, the main objective of this study was to evaluate the role of zeolite or chitosan on counteracting the deleterious impact of salinity on growth characters and essential oil content as well as some biochemical characteristics of rosemary plant.

2. MATERIALS AND METHODS

Two pot experiments were done at the Agric. Botany Dept. Experimental Farm and Laboratory, Fac. of Agriculture, Mansoura University throughout the 2015/2016 and 2016/2017 years.

2.1 Plant Material

Uniform terminal cuttings of *Rosmarinus officinalis* L., 10-12 cm length, were planted within the nursery under shaded conditions for rooting. Once three months, on 15th October in both seasons, the rooted cuttings were separately transplanted in plastic pots 25 cm in inner diameter containing clay loamy soil. The physio-chemical characteristics of the used soil were estimated consistent with Chapman and Pratt [20] and presented in Table 1.

Table 1. Physiochemical characters of the experimental soil in 2015/2016 and 2016/2017 years

Soil properties	1st season	2nd season	Soil properties		1st season (meq/L)	2nd season (meq/L)
Clay %	42.1	41.2	Cations	Calcium	1.2	1.3
Silt%	25.6	24.5		Magnesium	1.0	1.1
Fine Sand%	24.3	23.4		Sodium	2.3	2.4
Coarse Sand%	8.0	7.9		Potassium	0.4	0.3
Hygroscopic Water%	5.1	5.2	Anions	Carbonate	----	----
SSP%	59	60		Bicarbonate	1.3	1.4
EC dSm-!	0.53	0.56		Chloride	2.4	2.7
pH (1:1.5, soil: water)	7.46	7.44		Sulphate	1.2	1.1

2.2 Experimental Design, Treatments and Growth Conditions

A factorial experiment was done in a randomized complete block design system with five replications. The factorial combinations of three zeolite concentrations (0, 4, 8 g/kg) as a soil additive and three chitosan foliar applications (0, 250 and 500 mg/l) and three salinity levels (0, 50, 100 mM NaCl) were considered.

The pots were held under natural environmental conditions and every pot were irrigated with tap water for 30 days, throughout that, the success of transplants took place, then the rosemary plants were subjected to salinity. Salinization was performed by adding NaCl solution to adjust the salt concentration within the soil at different examined. The water content of the pots was maintained at 75% field capacity until the end of the experiment. Zeolite concentration was mixed with the surface soil, whereas; chitosan was sprayed thrice until dropping after covering the soil surface. Initial chitosan treatments occurred one week after salt treatments with 21-day intervals.

Plants were fertilized with 2.5 g ammonium sulfate (20.6% N), 3.59 g calcium super-phosphate (15.5% P_2O_5) and 1.25 g potassium sulfate (45% K_2O) per pot after 3 weeks from transplanting. All plants received traditional agricultural practices, consistent with the recommendation of ARC, Egypt, whenever they were required.

2.3 Sampling and Measurements

After 21 days from the last chitosan foliar spray (1st April), Rosemary plants were harvested and following parameters were measured.

2.3.1 Growth parameter

Plant height, number of branches per plant and both fresh and dry weights of shoots and roots were recorded.

2.3.2 Biochemical attributes

Photosynthetic pigments were extracted from the 1st mature leaf and determined spectrophotometrically as delineate by Lichtenthaler and Wellburn [21].

Ground dried materials for the shoot and root systems were used for mineral element determination. The dried materials were wet digested with $HClO_3/H_2SO_4$ mixture (1:1 v: v) as reported by Chapman and Pratt [20]. Total nitrogen was determined colorimetrically by Nessler's methods [22]; potassium by flame photometrically [23], and phosphorous according to the method of Cooper [24]. Moreover, sodium and chloride were extracted from dried plant materials consistent with Chaudhary et al. [25]. Sodium within the aqueous extracts was measured with a flame photometer, whereas, chloride was determined by titration with 0.001 N $AgNO_3$ using potassium chloride as an indicator.

2.3.3 Essential oil percentage and yield

The essential oil within the air-dried herb of each treatment was extracts determined by the water-distillation technique consistent with Egyptian Pharmacopoeia [26]. Essential oil yield (ml/plant) was estimated in proportion to the herb air-dry weight.

2.4 Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) technique by computer software MSTATC and significant treatment means were compared using least significance difference

(LSD) test at 0.05 probability level according to Gomez and Gomez [27].

3. RESULTS AND DISCUSSION

3.1 Growth Parameters and Essential Oil Yield

Tables 2 shows that plant growth represented as shoot and root length, and shoot and root dry weights of Rosemary were significantly declined due to salinity, and therefore the greatest reduction was assessed under high salinity. Essential oil % in rosemary shoot were enhanced by increasing salinity levels, this increase was accompanied by decreasing the essential oil yield due to decreasing shoot mass.

All plant growth and essential oil yield were significantly enhanced by the application of either zeolite or chitosan. The highest values were obtained by soil additive of 8 g/kg soil zeolite within each season comparing to control treatment.

As regards to the interactions, the data given within the same table disclosed that using chitosan or zeolite under low salinity level significantly enhanced all growth parameters and essential oil yield. The highest values were obtained by adding 8 g zeolite/kg soil. On the opposite, application of either zeolite or chitosan under severe salt stress counteracted the injuries of salinity on the growth parameter that enhanced growth parameter as compared with untreated plants under high salinity levels.

The responses of the Rosemary plant to a high level of salinity were reflected by decreases in shoot and root length, as well as shoot and root dry weight. The repressing effects of salinity on these parameters add more support to the previous finding [6,7,28,29]. The injurious impacts of salinity on plant growth are due to the inhibition of photosynthesis, the induction of growth inhibitor, and reduction of leaf area [30], leaf protein [28], and reduced ability to provide and utilize assimilates/photosynthates [30]. Alternatively, Romero and Maranon [31] added that the specific ion effects of salinity, caused toxicity and nutritional imbalance, leading to decline in potassium, calcium and phosphorous absorption. The deleterious impact of severe salinity concentration on growth may be ascribed to that salinity has been shown to reduce the synthesis of nucleic acid and protein in several plants that could result in a disturbance in metabolic activities, cell division and elongation [32].

The positive impacts of chitosan and/or zeolite on plant growth noticed in the present investigation may be attributed to its effect on inducing antioxidant system [28,33]. Also, chitosan or zeolite used enhanced potassium percentage (Table 4), that may increase the chloroplast number per cell, the cell number per leaf and leaf area [32], maintaining photosynthetic activity and metabolic transport [18,34]. Additionally, chitosan increased nitrate reductase, glutamine synthetase, and protease in the functional leaves that improved plant growth and development [35]. Khan et al. [36] found that foliar application of CHI enhanced net photosynthetic rate (PN), this increase was related with a rise in stomatal conductance (gs) and transpiration rate (E), without any effects on intercellular CO_2 concentration (Ci) that induced photoassimilate production.

It has been reported that increasing essential oil percentage within the plant may be a mechanism to adapt the plant to stress conditions [19,37]. In rosemary, salt stress stimulated essential oil accumulation owing to the higher density of oil glands. By exerting stress, the essential oil percentage is increased, but the shoot dry weight decreased, resulted in reducing essential oil [19, 38]. These results are in agreement with those reported earlier on other aromatic plant species such as *Pimpinella anisum* L. [39], *Satureja hortensis* L. [40]; *Mentha spicata* L. and *Rosmarinus officinalis* L. [41] and *Petroselinum crispum* [42].

Chemical compounds (secondary metabolites) in essential oil of plants are directly affected by environmental factors, especially osmotic stress [43]. Osmotic stress reduces the absorption and transfer of nutrients in plants, resulting in consequences including quantitative and qualitative changes in secondary metabolites like essential oils [42]. Various studies have shown that CO_2 and glucose are known as proper precursors for the synthesis of plant essential oil and which there's a direct correlation between photosynthetic products and essential oil production [44]. Consequently, physiological drought-induced damages like oxidative stress (ROS increase), photosynthetic pigment decomposition, alteration of membrane structure and proteins, deactivation of enzymes, stomatal closure, and dropped CO_2 under the stomata result in decreased photosynthesis (reduction of

glucose as essential oil precursor) and reduced cellular growth and development, ultimately resulting in qualitative and quantitative falls within the essential oil content of plants [42]. Among the chemical compounds of thyme essential oil, only three compounds (thymol, carvacrol, and trans-caryophyllene) rose significantly with increasing osmotic stress levels, that is keeping with alternative studies [38], probably indicating a type of plant adaptation to stress conditions. Zaghloul et al. [9] indicate that zeolite application enhanced essential oil percentage and yield.

3.2 Photosynthetic Pigments

Table 3 shows that chlorophyll a, chlorophyll b and total chlorophylls markedly reduced with increasing salinity levels in both seasons. The greatest decline was obtained under high salinity. Carotenoid concentration was parallel to chlorophylls except in the first season that low salinity nonsignificantly enhanced. The ratio between chlorophyll a and chlorophyll b was reduced in the first season and enhanced in the second season as compared with control plants.

Generally, under control, application of either chitosan or zeolite in most cases significantly increased photosynthetic pigments concentration as compared with untreated control plants. The highest values of chl a, b, total chlorophylls, and carotenoids (0.919, 0.494& 0.513, 0.274& 1.433, 0.769& 0.164, 0.061 mg/g FW) were obtained due to application of zeolite at 4 g/kg soil. However, application of chitosan or zeolite reduced chlorophyll a:b ratio in the 2nd season, whereas increased it in the 1st season.

The interaction treatments indicated that application of either chitosan or zeolite mitigated the injuries of salinity on photosynthetic pigment concentration. The highest values of chlorophyll a in the 1st season, total chlorophyll in both seasons were obtained by application of zeolite under moderate salinity. The highest chlorophyll a and carotenoids in the 2nd season were obtained by zeolite under normal condition (Table 3). The lowest chlorophyll a to chlorophyll b ratio was observed under the treatment of water under high salinity in the 1st season and low salinity level in the 2nd season.

The reduction in photosynthetic pigment concentration by salinity was confirmed by earlier reports [6,7]. Salinity stress, enhances the activity of chlorophyll degrading enzyme chlorophyllase and interferes with the *de-novo* synthesis of chlorophyll-binding proteins [45]. Moreover, salinity can lead to the destruction of the fine structure of chloroplasts, instability of pigment-protein complex [46], which leads to oxidation of chlorophyll and decreased its concentration [47] and alteration in the content and composition of carotenoids. Accumulation of sodium and chloride (Table 5), could affect chlorophyll biosynthesis due to its effect on the activity of Fe-containing enzymes, cytochrome oxidase. Yeo and Flowers [48] showed that there's a negative relationship between total chlorophyll and sodium content where sodium and chloride ions penetrate the chloroplasts [49]. The hormonal imbalance under salt stress could have an effect negatively through decreasing the biosynthesis of cytokinin in salinized plant root and translocated to the shoot [46] and induced ABA accumulation leading to inducing chlorophyll breakdown [50] or inhibiting chlorophyll synthesis [51]. In addition, salt stress accelerates leaf senescence through inhibitory chlorophyll synthesis [52].

The superiority of either chitosan or zeolite in photosynthetic pigments was in harmony with [19,53] for chitosan, and [54] for zeolite; that indicated application of chitosan considerable enhanced photosynthetic pigment concentration and accelerate carotenoid biosynthesis that protects chlorophyll from oxidation. The influence of CHI on alleviating the water stress impact on the photosynthetic pigments might be due to the fact that CHI increased the endogenous level of cytokinins that stimulates chlorophyll biosynthesis. Chibu and Shiayama [55] proved these stimulative effects to more availability of amino compounds released from CHI. Data in the present investigation indicate that foliar application of CHI enhanced considerably either nitrogen and potassium content in the plant shoot (Table 4) that can be taking part in a very important role in increasing the number of chloroplast per cell, the cell size and number per unit area as well as enhanced the synthesis of chlorophyll [56]. Moreover, Si form application could increase the chlorophyll concentration and chloroplast ultrastructure under stress conditions [57]. These results are in accordance with El-Saedy et al. [58] who show that application of Si enhanced total chlorophyll contents in grapevine leaves.

Data in the same table show that Chla:b ratio increased due to salinity, proved that chl b was degraded at a higher rate than chl a (Table 3). This could be explained by the fact that the first step in the chl b degradation involves its

conversion to chl a [59]. The increase in the ratio of Chla:b has been connected with lower levels of light-harvesting chlorophyll proteins; LHCPs [60]. The decline in LHCPs content is an adaptive defense mechanism of plant organs that permits them to endure the environmental stresses [61].

Photosynthesis is limited to salinity that could result in photoinhibitory injury. Photoinhibitory injury could be avoided by photorespiration, through scavenging systems that remove ROS with carotenoids [62]. Carotenoids are synthesized and accumulate in chloroplast wherever they play a crucial role in the light harvesting complex assembly and performance. Salinity induced a specific change in the level of carotenoids that reduced carotenoid concentration (Table 3) due to degradation of β-carotene and formation of zeaxanthin, that defend the plant against photoinhibition [63], and no longer available for protection against additional damage, resulting in long-term chlorosis [64]. Thus, it looks that the decrease in carotenoids levels under saline stress could resultin chlorophyll degradation [65].

3.3 Minerals Percentage

Data in Tables 4 and 5 indicate that salinity stress caused disturbance in the electrolyte balance, leading to the deficiency of some nutrients and the excess of certain unwanted ions. Data presented in Table5 prove that sodium percentage and chloride concentration were increased with salinity. This increase was accompanied by a corresponding decreased in nitrogen, phosphorous, potassium and K^+/Na^+ ratio in both shoot and root system. The great reduction occurred under high salinity levels compared with control plants.

Application of either zeolite or chitosan, in special, 8 g/kg zeolite, significantly increased in most cases the K^+/Na^+ ratio, nitrogen, phosphorous and potassium percentage, but declined the percentage of sodium and chloride concentration in both shoot and root systems during the two experimental seasons. Additionally, they counteracted the injury's effects of salinity on minerals either in the shoot and root systems. The highest values of macronutrients in most cases obtained by application of 8g/kg soil zeolite under low salinity as compared with untreated control plants. Alternatively, the lowest sodium and chloride obtained because of the applying of zeolite at 8 g/ kg soil under normal condition.

On the other hand, an increase in chloride accumulation accompanied by a decline in shoot nitrate concentrations of plants was observed due to the competition between chloride and nitrate that decreases the nitrate [66]. Silberbush and Ben-Asher [67] found that despite drastic reductions in leaf nitrate concentrations under salinity. These conclusions are supported [68]. Saline conditions will influence the various steps of nitrogen metabolism, reduction and protein synthesis [69]; ammonium uptake [70]. Frota and Tucker [71] expressed that the decline in nitrogen under salinity could also be due to a reduction in water absorbed and also the decrease in root permeability. Accordingly, Papadopoulos and Rending [72], proved that the impact of salinity on leaf nitrogen and total nitrogen uptake was chiefly through the suppressing impact of salinity on root growth and water uptake.

The influence of salinity on phosphorous content in crop plants is variable and depends upon the cultivars. In our work, the results in Tables 4 and 5 indicate that increasing the salinity level promoted a reduction of phosphorous concentration in plant tissue. This negative relationship between phosphorous and salinity level could also be due to the postulation of Greenway et al. [73] who attributed the reduction in phosphorous and its uptake by tomato plants under saline conditions to a decline in the root absorption potential and to a decrease in the translocation of phosphorous upward through the root as a result of the increase in the osmotic pressure of the root medium. Champagnol [74] found that unlikely chloride and phosphate ions are competitive in terms of plant uptake. However, Papadopoulos and Rendig [72] concluded that chloride might have suppressed phosphorous accumulation in tomato. In other cases, reduction in plant phosphorous content might result from reduced activity of phosphorous in the soil solution due to the high ionic strength of the media [75]. The decline in P concentration with increased salinity levels might have occurred attributable to decreased P transport under high salt concentrations [76]. This reduction could be due to precipitate phosphorous ions with calcium in salt-stressed soil and become unavailable to plants. Furthermore, the reduction in P accumulation under salinity may be explained by the fact that Na ion increased the soil pH that successively declined the availability of P to the plants.

Table 2. Effect of Salinity, either zeolite or chitosan, as well as their interactions on growth characters and essential oil yield of Rosemary plants

Treatments	Shoot Length (cm)		Root Length (cm)		Shoot dry weight (g)		Root dry weight (g)		Essential oil %		Essential oil yield (g/plant)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Salinity												
C	41.33± 7.03a	34.26± 8.46a	16.46± 3.76a	21.86± 4.26a	17.66± 4.14a	7.21± 1.19b	5.59± 1.94a	1.41± 0.42a	0.583± 0.19c	0.578± 0.18c	10.435± 1.30a	4.176± 1.57b
MS	39.40± 9.94a	31.60± 10.3b	15.80± 6.89a	19.73± 1.01b	15.29± 1.14b	9.01± 1.89a	5.69± 1.03b	1.39± 0.13a	0.669 0.24±b	0.661± 0.22b	10.708± 1.07a	6.386± 1.61a
SS	35.26± 8.19b	26.06± 6.06c	12.80± 3.79b	15.06± 6.61c	10.45± 1.26c	5.16± 1.32c	3.98± 1.24c	0.87± 0.16b	0.748± 0.20a	0.736± 0.17a	8.095± 1.25b	3.919± 0.14b
LSD 5%	2.71	1.80	1.53	2.11	2.27	0.57	0.56	0.13	0.01	0.01	1.54	0.39
Antitranspirants												
W	33.66± 9.21c	26.22± 4.69c	13.00± 3.83c	16.11± 5.68b	10.84± 2.19	4.967± 1.38d	3.60± 0.18d	0.89± 0.29d	0.512± 0.16e	0.517± 0.17e	5.281± 1.41d	2.419± 0.22e
Ze1	41.55± 6.93a	33.88± 5.47a	15.77± 3.72ab	20.55± 5.00a	16.81± 2.61	7.62± 1.35b	5.52± 0.22b	1.33± 0.70b	0.713± 0.14b	0.706± 0.15b	11.758± 2.37b	5.316± 1.18b
Ze2	41.11± 10.01ab	36.00± 5.94a	17.66± 3.61a	22.44± 5.94a	18.54± 2.82	10.41± 1.99a	6.59± 0.23a	1.78± 0.12a	0.813± 0.19a	0.783± 0.19a	15.022± 2.42a	8.323± 0.98a
Chi1	39.33± 6.00ab	29.66± 5.16b	14.55± 3.40bc	17.55± 5.55b	13.65± 2.95	6.63± 1.66c	5.07± 0.20bc	1.11± 0.50c	0.676± 0.14c	0.676± 0.11c	9.090± 1.44c	4.454± 0.82c
Chi2	37.66± 7.81b	27.44± 4.17bc	14.11± 3.52bc	17.77± 5.81b	12.49± 2.55	6.02± 1.29c	4.65± 0.23c	1.00± 0.61cd	0.620± 0.11d	0.611± 0.10d	7.579± 1.39c	3.623± 0.41d
LSD 5%	3.506	2.336	1.977	2.726	2.931	0.741	0.726	0.176	0.015	0.022	1.997	0.504
Interaction												
CW	38.33± 5.77ab	30.00± 5.29b	16.00± 5.29bcd	21.00± 8.71ab	15.48± 0.44b-e	7.05± 1.58cd	4.82± 1.15def	1.28± 0.42cde	0.417± 0.04i	0.420± 0.05h	6.452± 0.80c-f	2.953± 0.43d
CZe1	43.33± 5.77a	36.66± 6.86a	16.33± 5.03bcd	22.33± 1.15ab	20.00± 1.99ab	7.29± 1.03cd	5.74± 2.43bcd	1.42± 0.41bcd	0.650± 0.02e	0.637± 0.06de	12.993± 0.90b	4.640± 0.61c
CZe2	43.33± 11.54a	38.66± 2.30a	17.33± 3.05abc	23.66± 3.05a	19.23± 1.82ab	7.38± 0.86c	6.62± 1.31ab	1.61± 0.62bc	0.683± 0.01d	0.663± 0.04d	13.140± 1.03b	4.894± 0.50c
CChi1	41.66± 5.77a	35.33± 5.03a	16.33± 2.30bcd	21.00± 0.00ab	16.96± 5.10a-d	7.28± 1.34cd	5.58± 1.65bcd	1.40± 0.19bcd	0.607± 0.01f	0.617± 0.04e	10.273± 2.91bc	4.495± 1.06c

Treatments	Shoot Length (cm)		Root Length (cm)		Shoot dry weight (g)		Root dry weight (g)		Essential oil %		Essential oil yield (g/plant)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
CChi2	40.00± 0.00ab	30.66± 1.15b	16.33± 5.03bcd	21.33± 3.05ab	16.67± 1.24a-d	7.07± 1.82cd	5.17± 1.90cde	1.36± 0.33bcd	0.560± 0.06g	0.553± 0.07f	9.321± 0.52bcd	3.899± 0.84c
MSW	31.33± 2.30c	25.00± 3.46c	12.66± 1.15def	14.33± 1.15c	9.98± 1.48efg	5.09± 1.49ef	3.84± 0.96fgh	0.87± 0.12g	0.513± 0.01h	0.510± 0.02g	5.107± 1.30ef	2.600± 0.85d
MSZe1	43.33± 5.77a	38.66± 4.16a	17.66± 6.11ab	23.66± 4.16a	18.61± 1.36abc	9.56± 2.51b	6.34± 1.57abc	1.63± 0.47b	0.683± 0.02d	0.677± 0.03d	12.750± 2.06b	6.482± 1.93b
MSZe2	43.33± 11.54a	39.66± 4.16a	20.66± 4.16a	26.00± 2.00a	21.88± 3.95a	17.00± 1.13a	7.52± 1.96a	2.51± 0.38a	0.873± 0.01a	0.840± 0.08ab	19.112± 3.58a	14.263± 0.52a
MSChi1	39.66± 1.15ab	27.33± 1.15bc	14.00± 2.00b-f	17.00± 8.58bc	13.39± 2.90s-f	6.78± 1.37cd	5.58± 1.99bcd	1.03± 0.33efg	0.660± 0.02a	0.667± 0.02d	8.814± 1.60cde	4.526± 0.98c
MSChi2	39.33± 9.54ab	27.33± 2.61bc	14.00± 3.46b-f	17.66± 4.16bc	12.57± 2.14d-g	6.62± 2.32cd	5.53± 0.21bcd	0.94± 0.29fg	0.617± 0.01de	0.613± 0.01e	7.765± 1.30c-f	4.057± 1.35c
SSW	31.33± 9.26c	23.66± 2.30c	10.33± 3.05f	13.00± 2.00c	7.06± 2.98g	2.75± 0.62g	2.51± 0.80b	0.53± 0.14h	0.607± 0.01f	0.620± 0.02e	4.287± 1.86f	1.705± 0.35e
SSZe1	38.00± 3.46abc	26.33± 2.23bc	13.33± 3.05c-f	15.66± 4.16c	11.81± 2.49cde	6.00± 1.98cde	4.48± 0.61d-g	0.95± 0.36fg	0.807± 0.02b	0.803± 0.01b	9.539± 2.23bcd	4.826± 1.65c
SSZe2	36.66± 9.54abc	29.66± 3.05b	15.00± 3.29b-e	17.66± 4.16bc	14.51± 0.58b-e	6.857± 1.35cd	5.62± 1.21bcd	1.24± 0.47def	0.883± 0.01a	0.847± 0.07a	12.824± 0.66b	5.812± 1.43b
SSChi1	36.66± 5.77abc	26.33± 4.16bc	13.33± 2.03c-f	14.66± 4.57c	10.62± 1.69efg	5.84± 0.94de	4.04± 0.46efg	0.91± 0.31fg	0.760± 0.07c	0.743± 0.01c	8.188± 1.04cde	4.341± 0.72b
SSChi2	33.66± 3.05bc	24.33± 3.05c	12.00± 2.00ef	14.33± 1.71c	8.23± 1.15fg	4.37± 1.41f	3.25± 1.17gh	0.71± 0.14gh	0.683± 0.02d	0.667± 0.04d	5.650± 1.02def	2.911± 0.91d
LSD5%	ns	4.046	ns	ns	ns	1.284	ns	0.305	0.026	0.299	ns	0.873

C, control; MS, Mild Salinity; SS, Severe salinity; W, water; Ze1, Zeolite 4g/Kg; Ze2, Zeolite 8g/Kg; Chi1, Chitosan 250 mg/l; Chi2, Chitosan 500 mg/l .Values are givin as mean ±SD. Means in columns with different letters are significantly different at P<0.05 by Duncan's Multiple Range Test

Table 3. Effect of salinity, either zeolite or chitosan, as well as their interactions on photosynthetic pigments of Rosemary plants

Treatments	Chlorophyll a		Chlorophyll b		Total chlorophyll		Total carotenoids		Chlorophyll a:b ratio	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Salinity										
C	0.972± 0.04a	0.428± 0.02a	0.508± 0.01a	0.250± 0.08a	1.481± 0.06a	0.679± 0.03a	0.162± 0.01a	0.058± 0.09a	1.913± 0.49a	1.700± 0.74a
MS	0.902± 0.06a	0.360± 0.03b	0.467± 0.02b	0.212± 0.02b	1.370± 0.08b	0.573± 0.05b	0.167± 0.01a	0.045± 0.05a	1.910± 0.35a	1.723± 0.59a
SS	0.603± 0.03b	0.284± 0.01c	0.371± 0.02c	0.168± 0.09c	0.974± 0.05c	0.452± 0.03bc	0.111± 0.01b	0.053± 0.02a	1.632± 0.55b	1.943± 0.36a
LSD 5%	0.083	0.059	0.040	0.032	0.108	0.085	0.044	Ns	0.173	Ns
Antitranspirants										
W	0.797± 0.14b	0.294± 0.01c	0.449± 0.09b	0.175± 0.01c	1.247± 0.43b	0.470± 0.03c	0.158± 0.01a	0.043± 0.00a	1.761± 0.51a	2.063± 0.03a
Ze1	0.919± 0.18a	0.494± 0.04b	0.513± 0.13a	0.274± 0.02b	1.433± 0.11a	0.769± 0.06b	0.144± 0.01a	0.061± 0.01a	1.762± 0.81a	1.817± 0.08a
Ze2	0.812± 0.14ab	0.315± 0.01bc	0.426± 0.12b	0.197± 0.01bc	1.238± 0.70b	0.513± 0.02bc	0.154± 0.01a	0.043± 0.00a	1.894± 0.39a	1.617± 0.02a
Chi1	0.795± 0.17b	0.347± 0.02a	0.425± 0.13b	0.210± 0.01b	1.221± 0.12b	0.558± 0.03bc	0.141± 0.01a	0.057± 0.01a	1.803± 0.48a	1.697± 0.06a
Chi2	0.806± 0.14b	0.644± 0.01b	0.429± 0.17b	0.395± 0.01a	1.236± 0.57b	1.038± 0.29a	0.136± 0.01a	0.098± 0.02a	1.873± 0.44a	1.714± 0.05a
LSD 5%	Ns	0.077	0.051	0.042	0.139	0.110	Ns	Ns	Ns	Ns
Interaction										
CW	0.806± 0.01de	0.412± 0.03ab	0.431± 0.03d-g	0.260± 0.01ab	1.238± 0.04de	0.673± 0.01ab	0.175± 0.05a-d	0.046± 0.00b	1.868± 0.12a-d	1.584± 0.23b
CZe1	0.816± 0.18de	0.636± 0.17ab	0.513± 0.18cd	0.292± 0.09ab	1.329± 0.03d	0.929± 0.08ab	0.113± 0.00bcd	0.124± 0.00ab	1.644± 0.86a-d	2.235± 0.17ab
CZe2	0.927± 0.17cd	0.308± 0.09b	0.442± 0.19c-g	0.193± 0.08ab	1.370± 0.16cd	0.501± 0.01b	0.155± 0.03a-d	0.027± 0.00b	2.041± 0.49a	1.607± 0.15b
CChi1	1.235± 0.09b	0.416± 0.02ab	0.614± 0.05ab	0.259± 0.07ab	1.849± 0.08b	0.676± 0.02ab	0.159± 0.06a-d	0.049± 0.01b	2.013± 0.26ab	1.585± 0.35b

Treatments	Chlorophyll a		Chlorophyll b		Total chlorophyll		Total carotenoids		Chlorophyll a:b ratio	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
CChi2	1.077± 0.04bc	0.369± 0.01ab	0.539± 0.00bc	0.246± 0.04ab	1.617± 0.03bc	0.616± 0.01ab	0.210± 0.01a-d	0.044± 0.01b	2.000± 0.09ab	1.488± 0.29b
MSW	0.990± 0.12cd	0.238± 0.01b	0.508± 0.03cde	0.166± 0.07b	1.499± 0.10cd	0.405± 0.02b	0.228± 0.04abc	0.028± 0.00b	1.949± 0.37abc	1.429± 0.08b
MSZe1	1.440± 0.01a	0.611± 0.01ab	0.684± 0.02a	0.386± 0.07ab	2.124± 0.03a	0.997± 0.02ab	0.234± 0.06a	0.023± 0.00b	2.102± 0.05a	1.578± 0.06b
MSZe2	0.616± 0.11ef	0.313± 0.03b	0.328± 0.04h	0.177± 0.07b	0.943± 0.14f	0.490± 0.04b	0.162± 0.01a-d	0.046± 0.01b	1.882± 0.35a-d	1.768± 0.01b
MSChi1	0.810± 0.09de	0.245± 0.01b	0.447± 0.05c-f	0.124± 0.00b	1.258± 0.04de	0.370± 0.01b	0.105± 0.05cd	0.055± 0.01b	1.821± 0.40a-d	1.971± 0.09ab
MSChi2	0.656± 0.05ef	0.392± 0.10ab	0.366± 0.04fgh	0.210± 0.02ab	1.025± 0.06ef	0.602± 0.01ab	0.107± 0.06cd	0.073± 0.01ab	1.796± 0.29a-d	1.868± 0.07b
SSW	0.595± 0.04f	0.232± 0.07b	0.408± 0.06e-h	0.099± 0.01b	1.004± 0.03ef	0.331± 0.02b	0.073± 0.01d	0.055± 0.01b	1.465± 0.33d	3.175± 0.05a
SSZe1	0.502± 0.11fg	0.236± 0.01b	0.342± 0.05gh	0.144± 0.02b	0.844± 0.05f	0.380± 0.01b	0.084± 0.01d	0.037± 0.00b	1.539± 0.42cd	1.638± 0.03b
SSZe2	0.893± 0.11cd	0.326± 0.01b	0.507± 0.03cde	0.222± 0.01ab	1.400± 0.14cd	0.548± 0.02ab	0.145± 0.01a-d	0.055± 0.01b	1.758± 0.11a-d	1.477± 0.01b
SSChi1	0.340± 0.14g	0.381± 0.02ab	0.214± 0.05i	0.247± 0.01ab	0.555± 0.19g	0.628± 0.03ab	0.160± 0.01a-d	0.066± 0.00ab	1.574± 0.32bcd	1.536± 0.09b
SSChi2	0.684± 0.11ef	0.245± 0.03b	0.381± 0.09fgh	0.726± 0.02a	1.066± 0.02ef	1.897± 0.05a	0.092± 0.01d	0.178± 0.00a	1.824± 0.33a-d	1.890± 0.03b
LSD5%	0.018	0.077	0.09	0.072	0.241	0.191	0.098	0.042	ns	ns

C, control; Ms, mild salinity; Ss, severe salinity; W, water; Ze1, zeolite 4g/kg; Ze2, zeolite 8g/kg; Chi1, chitosan 250 mg/l; Chi2, chitosan 500 mg/l.Values are givin as mean ±sd. Means in columns with different letters are significantly different at p<0.05 by Duncan's Multiple Range Test

Table 4. Effect of salinity, either zeolite or chitosan, as well as their interactions on nitrogen, phosphorous and potassium percentage in both shoot and root of Rosemary plants

Treatments	Nitrogen in shoot		Nitrogen in root		Phosphorous in shoot		Phosphorous in root		Potassium in shoot		Potassium in Root	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Salinity												
C	2.340± 0.57a	2.656± 0.17a	2.852± 0.64a	2.265± 0.58b	0.697± 0.22a	1.000± 0.24a	0.516± 0.15a	0.663± 0.18a	2.587± 0.26a	2.515± 0.79a	2.322± 0.71a	2.878± 0.94a
MS	2.180± 0.11a	2.082± 0.20b	2.435± 0.50b	2.690± 0.10a	0.708± 0.27a	0.949± 0.21a	0.487± 0.19a	0.504± 0.10b	2.022± 0.67b	2.448± 0.78a	1.847± 0.70b	2.502± 0.81b
SS	1.637± 0.13b	1.160± 0.10c	1.674± 0.99c	2.353± 0.20b	0.457± 0.21b	0.526± 0.24b	0.292± 0.02b	0.285± 0.02c	1.233± 0.12c	1.542± 0.21b	0.955± 0.10c	1.663± 0.18c
LSD 5%	0.161	0.056	0.206	0.119	0.063	0.077	0.053	0.054	0.333	0.240	0.177	0.173
Antitranspirants												
W	1.259± 0.12c	1.284± 0.19e	1.757± 0.15d	1.547± 0.11d	0.431± 0.02d	0.544± 0.06e	0.286± 0.03c	0.322± 0.03d	1.178± 0.57d	1.469± 0.40d	1.043± 0.55c	1.552± 0.30e
Ze1	2.250± 0.86b	2.335± 0.14b	2.543± 0.10b	2.572± 0.55b	0.736± 0.03b	0.962± 0.05b	0.514± 0.03a	0.569± 0.03ab	2.627± 0.63a	2.478± 0.45b	2.134± 0.71a	2.702± 0.50b
Ze2	2.608± 0.41a	2.739± 0.15a	2.979± 0.11a	3.042± 0.77a	0.856± 0.04a	1.156± 0.07a	0.573± 0.03a	0.604± 0.02a	2.231± 0.17ab	2.888± 0.49a	2.351± 0.18a	3.113± 0.42a
Chi1	2.094± 0.70b	1.846± 0.12c	2.286± 0.12bc	2.680± 0.96b	0.605± 0.03c	0.813± 0.04c	0.417± 0.02b	0.510± 0.03b	1.985± 0.38bc	2.082± 0.80c	1.574± 0.12b	2.366± 0.15c
Chi2	2.051± 0.91b	1.627± 0.16d	2.039± 0.12c	2.339± 0.13c	0.477± 0.01d	0.649± 0.04d	0.369± 0.02b	0.415± 0.05c	1.716± 0.80c	1.925± 0.22c	1.439± 0.13b	2.037± 0.11d
LSD 5%	0.208	0.072	0.266	0.154	0.081	0.100	0.069	0.069	0.430	0.310	0.229	0.224
Interaction												
CW	1.971± 0.23efg	2.536± 0.04c	2.767± 0.20b-e	2.018± 0.25fg	0.506± 0.06cd	0.937± 0.01cd	0.454± 0.18c-f	0.534± 0.15b	2.201± 0.67bcd	2.246± 0.46cde	2.044± 0.53de	2.268± 0.20c
CZe1	2.274± 0.40b-e	2.794± 0.01b	2.895± 0.50bc	2.370± 0.08de	0.738± 0.03b	1.054± 0.01bc	0.533± 0.07cd	0.669± 0.16a	3.232± 0.95aa	2.717± 0.40bc	2.762± 0.47ab	2.851± 0.13b
CZe2	2.559± 0.47abc	2.644± 0.01c	3.209± 0.56ab	2.763± 0.09c	0.976± 0.02a	1.130± 0.23b	0.585± 0.19bc	0.671± 0.12a	2.358± 0.54bcd	2.739± 0.54bc	2.403± 0.78bcd	3.546± 0.60a

Treatments	Nitrogen in shoot		Nitrogen in root		Phosphorous in shoot		Phosphorous in root		Potassium in shoot		Potassium in Root	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
CChi1	2.417± 0.63a-d	2.648± 0.03c	2.933± 0.68bc	2.109± 0.04ef	0.778± 0.02b	1.032± 0.04bc	0.521± 0.05cd	0.711± 0.05a	2.537± 0.60a-d	2.470± 0.60cd	2.224± 0.63cd	2.986± 0.00b
CChi2	2.479± 0.39a-d	2.659± 0.02c	2.458± 0.22c-f	2.065± 0.16fg	0.485± 0.01cd	0.845± 0.26de	0.491± 0.16cde	0.732± 0.01a	2.604± 0.55abc	2.403± 0.80cd	2.179± 0.40cde	2.739± 0.57b
MSW	1.218± 0.55i	0.888± 0.14h	1.381± 0.19ij	1.826± 0.03fg	0.491± 0.04cd	0.499± 0.11gh	0.266± 0.00g	0.252± 0.06ef	0.722± 0.07f	1.439± 0.33fg	0.677± 0.11gh	1.529± 0.07e
MSZe1	2.661± 0.78ab	2.806± 0.45b	2.827± 0.70bcd	2.447± 0.18d	0.927± 0.02a	1.181± 0.26b	0.672± 0.16ab	0.692± 0.14a	2.806± 0.54ab	3.143± 0.53ab	2.605± 0.74abc	3.456± 0.13a
MSZe2	2.715± 0.36a	3.661± 0.11a	3.432± 0.15a	2.840± 0.07c	1.020± 0.01a	1.579± 0.26a	0.729± 0.01a	0.724± 0.13a	2.605± 0.15abc	3.658± 0.33a	2.873± 0.55a	3.591± 0.53a
MSChi1	2.161± 0.15c-f	1.592± 0.02e	2.181± 0.56fgh	3.181± 0.46b	0.548± 0.01cd	0.798± 0.24def	0.402± 0.05def	0.488± 0.02bc	2.134± 0.38bcd	1.798± 0.44ef	1.551± 0.43f	2.201± 0.33cd
MSChi2	2.145± 0.15def	1.464± 0.14f	2.357± 0.98d-g	3.157± 0.51b	0.556± 0.01c	0.687± 0.12ef	0.368± 0.04efg	0.363± 0.01cde	1.843± 0.12cde	2.201± 0.07cde	1.529± 0.33f	1.730± 0.60e
SSW	0.588± 0.17j	0.428± 0.01j	1.122± 0.26j	0.798± 0.14h	0.296± 0.05e	0.195± 0.02i	0.138± 0.01h	0.180± 0.01f	0.610± 0.07f	0.722± 0.53h	0.409± 0.07h	0.767± 0.13f
SSZe1	1.814± 0.25fgh	1.404± 0.20fg	1.906± 0.27gh	2.901± 0.46bc	0.543± 0.08cd	0.651± 0.17fg	0.339± 0.04fg	0.347± 0.02de	1.843± 0.67cde	1.574± 0.47fg	1.036± 0.13g	1.798± 0.24de
SSZe2	2.551± 0.46abc	1.912± 0.02d	2.296± 0.67efg	3.521± 0.56a	0.571± 0.05c	0.760± 0.14def	0.405± 0.02def	0.418± 0.00bcd	1.731± 0.74de	2.268± 0.40cde	1.776± 0.35ef	2.201± 0.20cd
SSChi1	1.704± 0.10gh	1.299± 0.15g	1.745± 0.87hi	2.749± 0.01c	0.489± 0.09cd	0.609± 0.12fg	0.329± 0.06fg	0.331± 0.01de	1.282± 0.27ef	1.977± 0.00def	0.946± 0.44g	1.910± 0.50cde
SSChi2	1.530± 0.61hi	0.758± 0.05j	1.302± 0.50ij	1.796± 0.60g	0.388± 0.04de	0.416± 0.03h	0.250± 0.09gh	0.148± 0.01f	0.700± 0.00f	1.170± 0.04gh	0.610± 0.07gh	1.641± 0.00e
LSD5%	0.360	0.125	0.462	0.267	0.141	0.173	0.119	0.121	ns	0.538	0.397	0.387

C, control; MS, mild salinity; SS, severe salinity; W, water; Ze1, zeolite 4g/kg; Ze2, zeolite 8g/kg; Chi1, chitosan 250 mg/l; Chi2, chitosan 500 mg/l.Values are givin as mean ±sd. Means in columns with different letters are significantly different at p<0.05 by Duncan's Multiple Range Test

Table 5. Effect of salinity, either zeolite or chitosan , as well as their interactions on sodium, chloride percentage and potassium sodium ratio in both shoot and root of Rosemary plants

Treatments	Sodium in shoot		Sodium in root		Potassium: Sodium in shoot		Potassium: Sodium in root		Chloride in Shoot		Chloride in Root	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Salinity												
C	2.025± 0.97c	1.994± 0.26c	1.842± 0.65c	1.865± 0.42c	1.358± 0.88a	1.430± 0.31a	1.318± 0.50a	1.584± 0.84a	73.55± 13.89c	77.62± 7.21c	36.92± 7.84c	62.48± 2.55b
MS	3.648± 0.51b	2.997± 0.33b	2.667± 0.34b	3.042± 0.80b	0.661± 0.15b	0.869± 0.13b	0.780± 0.10b	0.951± 0.18b	136.57± 10.5b	139.91± 11.3b	90.78± 6.95b	91.73± 2.99a
SS	4.352± 1.15a	3.523± 0.64a	3.198± 0.52a	3.590± 0.86a	0.326± 0.01c	0.491± 0.15c	0.343± 0.08c	0.530± 0.07c	181.62± 10.9a	176.17± 10.7a	99.87± 5.99a	92.30± 0.00a
LSD 5%	0.293	0.178	0.217	0.155	0.150	0.169	0.124	0.106	8.003	5.242	4.942	8.002
Antitranspirants												
W	4.502± 1.08a	3.841± 0.86a	3.403± 1.99a	3.886± 0.62a	0.384± 0.02c	0.449± 0.02d	0.411± 0.08d	0.512± 0.08e	195.32± 14.82a	198.16± 15.14a	95.131± 16.46a	77.78± 11.85c
Ze1	2.704± 1.03d	2.325± 0.34d	2.266± 1.06c	2.355± 0.10d	1.164± 0.37a	1.205± 0.19b	1.044± 0.11b	1.227± 0.91b	98.76± 15.69d	99.08± 14.77d	68.47± 18.52cd	70.05± 17.20d
Ze2	2.259± 1.35e	2.169± 0.46d	1.932± 0.90d	2.088± 0.88e	1.093± 0.09a	1.504± 0.45a	1.310± 0.10a	1.590± 0.11a	88.51± 16.82d	88.67± 14.99e	63.26± 17.14d	71.15± 13.49d
Chi1	3.262± 1.21c	2.667± 0.52c	2.533± 1.04bc	2.749± 0.16c	0.725± 0.09b	0.846± 0.06c	0.681± 0.07c	0.991± 0.10c	126.38± 11.79c	123.38± 15.11c	71.00± 19.41c	105.71± 11.93a
Chi2	3.982± 1.61b	3.187± 0.24b	2.712± 1.07b	3.083± 0.87b	0.558± 0.09bc	0.647± 0.05cd	0.623± 0.08c	0.788± 0.10d	144.05± 12.11b	146.89± 11.43b	81.41± 19.19b	86.14± 12.45b
LSD 5%	0.379	0.230	0.280	0.200	0.194	0.218	0.161	0.137	10.332	6.767	6.381	10.330
Interaction												
CW	2.476± 0.63d	2.712± 0.54ef	2.132± 0.33ef	2.177± 0.20gh	0.912± 0.48cde	0.832± 0.20cd	0.958± 0.19cd	1.042± 0.00c	99.40± 14.2f	98.54± 19.3g	51.59± 17.3e	51.59± 18.0d
CZe1	1.597± 0.60ef	1.441± 0.20h	1.664± 0.40fg	1.842± 0.07hi	2.001± 0.78a	1.897± 0.53a	1.669± 0.25ab	1.548± 0.13b	68.63± 12.8hi	71.94± 3.27ij	30.29± 6.55fg	25.56± 5.81e
CZe2	1.486± 0.13f	1.218± 0.13h	1.374± 0.30g	1.575± 0.07i	1.592± 0.48b	2.275± 0.52a	1.774± 0.35a	2.248± 0.28a	51.59± 12.8i	61.06± 4.91j	19.40± 10.7g	28.87± 5.91e

Treatments	Sodium in shoot		Sodium in root		Potassium: Sodium in shoot		Potassium: Sodium in root		Chloride in Shoot		Chloride in Root	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
CChi1	2.244± 0.46de	2.043± 0.33g	2.021± 0.35ef	1.820± 0.26hi	1.161± 0.66c	1.206± 0.09bc	1.110± 0.45c	1.646± 0.24b	73.84± 2.84gh	77.62± 10.7hi	33.13± 8.67f	132.5± 23.6a
CChi2	2.333± 0.49d	2.578± 0.53ef	2.021± 0.35ef	1.909± 0.07hi	1.140± 0.44c	0.940± 0.02bcd	1.082± 0.24c	1.437± 0.50b	74.31± 11.3gh	79.04± 14.2hi	50.17± 10.9e	73.84± 15.5c
MSW	5.476± 0.20a	4.094± 0.33b	3.849± 0.66ab	4.562± 0.93b	0.131± 0.01h	0.362± 0.02ef	0.179± 0.10g	0.338± 0.08fg	223.36± 9.97b	238.08± 25.4b	113.1± 11.4a	89.46± 4.91b
MSZe1	2.890± 0.58d	2.712± 0.33ef	2.444± 0.38de	2.310± 0.33g	0.972± 0.10cd	1.159± 0.01bc	1.069± 0.33c	1.499± 0.15b	88.50± 7.14fg	91.82± 11.4g	82.83± 16.3d	92.3± 0.00b
MSZe2	2.355± 0.61d	2.712± 0.33ef	2.065± 0.38ef	2.311± 0.60g	1.098± 0.26c	1.348± 0.03b	1.404± 0.47b	1.581± 0.44b	95.61± 15.9f	88.04± 10.2gh	82.83± 16.3d	92.30± 0.00b
MSChi1	2.935± 0.27d	2.311± 0.33fg	2.467± 0.33de	2.712± 0.20ef	0.734± 0.16def	0.784± 0.02cd	0.634± 0.25ef	0.814± 0.18cd	130.60± 11.3de	130.16± 6.55e	82.83± 16.3d	92.30± 0.00b
MSChi2	4.606± 1.11b	3.158± 0.26d	2.511± 0.61de	3.314± 0.33d	0.393± 0.17fgh	0.694± 0.17de	0.615± 0.19ef	0.520± 0.12ef	144.80± 2.84d	151.46± 9.97d	92.30± 0.00bcd	92.30± 0.00b
SSW	5.565± 0.48a	4.718± 0.33a	4.227± 0.48a	4.918± 0.07a	0.109± 0.00h	0.153± 0.00f	0.096± 0.01g	0.156± 0.02g	263.13± 7.14a	257.96± 5.91a	120.70± 0.00a	92.33± 0.00b
SSZe1	3.626± 1.79c	2.823± 0.13de	2.689± 0.57cd	2.912± 0.43e	0.518± 0.16fg	0.559± 0.01def	0.394± 0.07fg	0.635± 0.11de	132.03± 5.68de	133.48± 4.91e	92.30± 0.00bcd	92.30± 0.00b
SSZe2	2.935± 0.76d	2.578± 0.46ef	2.355± 0.13de	2.377± 0.60fg	0.588± 0.10efg	0.890± 0.24cd	0.753± 0.11de	0.939± 0.22c	125.36± 1.63e	116.91± 21.6f	87.56± 16.3cd	92.30± 0.00b
SSChi1	5.008± 1.06ab	3.648± 0.46c	3.113± 0.23c	3.715± 0.20c	0.279± 0.08gh	0.548± 0.29def	0.300± 0.11g	0.513± 0.18ef	174.6± 23.9c	162.35± 21.5d	97.03± 16.3bc	92.30± 0.00b
SSChi2	4.629± 0.54b	3.848± 0.11bc	3.603± 0.23b	4.027± 0.13c	0.140± 0.01h	0.307± 0.06ef	0.174± 0.07g	0.408± 0.01ef	212.96± 22.7b	210.16± 14.7c	101.76± 16.3b	92.30± 0.00b
LSD5%	0.657	0.399	0.485	0.347	ns	0.378	ns	0.238	17.897	11.721	Ns	17.895

C, control; MS, mild salinity; SS, severe salinity; W, water; Ze1, zeolite 4g/kg; Ze2, zeolite 8g/kg; Chi1, chitosan 250 mg/l; Chi2, chitosan 500 mg/l.Values are given as mean ±sd. Means in columns with different letters are significantly different at p<0.05 by Duncan's Multiple Range Test

The most abundant inorganic cation in vacuoles is potassium that plays a large part in maintaining cell turgor pressure and potassium-homeostasis between cytoplasm and the vacuole [77]. Excess of sodium and chloride creates a high ionic balance which will improve the selectivity of root membrane [78]. Sodium is often accumulated within the vacuoles wherever it will replace potassium both quantitatively and qualitatively [79]. Many glycophyte species can partially substitute sodium for potassium [80]. Several mechanisms could also be accountable for a reduction in potassium with increasing salinity, as well as the antagonism of sodium and potassium at the uptake site in roots [81]. In the present study, salinity considerably increased sodium concentration in shoot and root. This increase was accompanied by a reduction in potassium content in both shoot and root (Table 4), indicating a visible antagonism between potassium and sodium [82]. This antagonism may be due to the direct competition between potassium and sodium at a site of ion uptake within the plasmalemma [83]. Sodium enhanced the efflux of potassium into the growth medium, attributable to disturbed membrane permeability [69], possibly due to membrane integrity [52]. Moreover, sodium could also be interference with potassium uptake or transport [84] and inhibit transport of these ions into the roots [85]. Additionally, an excess of sodium content in root media leads to a passive accumulation of this ion as shown in Table 5 results in high sodium/potassium ratio [86].

The increase in sodium content chiefly within the vacuole provides an osmotic adjustment of salt-affected plants [87]. This accumulation may be due to the vital role of sodium in reducing the osmotic pressure that facilitates absorption of water required for plants to tolerate the harmful impact on growth caused by salinity. Many of the deleterious effects of sodium, however, appear to be associated with the structure change, including the lose its selective permeability [88].

The role of chitosan or zeolite on inducing ion accumulation is not totally understood, and there are few studies in this concern [9,53,89] under normal or stressed conditions. This increase may be due to enhanced nutrient uptake by improving membrane permeability (Table 3) and/or giving better-developed root system due to enhanced microbial activity in addition as increased root growth which might have facilitated more efficient nutrient absorption. In this concern, Mali and Aery [90] show that application of Si form, plants maintained plasma membrane fluidity, decreased the ratio of phospholipids to protein and enhanced plasma membrane H+-ATPase activity, thereby maintaining membrane selectivity to ion influx and enhance potassium uptake. The role of CHI on increasing ion contents may be due to its effects on stabilizing cellular membrane through increasing the antioxidant substances and saving cell membrane from oxidative stress therefore improved plant cell permeability resulting in increasing ion content. This observation was supported by the results of [17,91,92] who indicated that application of CHI significantly declined lipid peroxidation, due to stimulation of some antioxidants enzymes leading to decreasing membrane permeability and improved its functions. This increase could also be caused by the amino components in chitosan and or the higher ability of the plant to absorb nitrogen from the soil when chitosan was degraded. Additionally, application of zeolite increased nitrogen percentage, due to the better use efficiency of applied nitrogen fertilizers coupled with retarded nitrification process, enabling the slow availability of applied nitrogen, leads to reduced loss of nitrogen by volatilization. Zaghloul et al. [9] and Latifah et al. [89] indicate that application of zeolite increased NPK% in thyme plants.

The increase in P accumulation as results of zeolite and CHI application could also be resulted from the prevention of P fixation within the soil and the formation of humophospho complexes, which are easily assailable by the plants [93] and this explains the more of P percentage by rosemary plant in the present study.

4. CONCLUSION

It could be concluded that application of zeolite at 8g/kg soil as a soil additive or chitosan at 250 mg/l, to Rosemary plants under salinity stress stimulated its growth, essential oil percentage and yield increased photosynthetic pigments and ion percentage in the herb.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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