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The effect of foliar application of chitosan on yield and essential oil of savory (*Saturejaisophylla* L.) under salt stress

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ABSTRACT

Background & Aim: *Satureja Isophylla* L. is a medicinal herb which belongs to the family *Lamiacease*. Salinity affects the growth, the quality and quantity of essential oils of medicinal plants. Chitosan is also considered as a biological elicitor which plays a role in improving production of secondary metabolites of medicinal herbs. The current project was conducted to evaluate the effect of different concentrations of chitosan on growth indices and the quality and quantity of essential oil under salt stress (salinity).

Experimental: In this study, savory was treated by chitosan at three levels (0, 0.2 and 0.4 g/l) and NaCl with three concentrations (0, 50, and 100mM). After a two-week treatment, the dry weight of the root and the stem, the herb height, and the number of leaves and lateral branches were measured. In addition, the composition of the herb's essential oil was analyzed and identified by GC/MS.

Results: The results showed that salt stress decreased the dry weight of the root and stem, the herb height, and its number of leaves and lateral stems. Chitosan treatment could amend this reduction. Sodium chloride mutual treatment with the concentration of 50 mM as well as that of chitosan with the concentration of 0.2 g/l had a significant effect on the increase of the yield and quality of the essential oil. The results of the GC/MS analysis showed that 14 main compositions were detected in the essential oil, in which the major amount belonged to P-Cymene (3.13%), Y-Terpinene (28.97%) and Caryacrol (59.64%). The findings indicated that the utmost amount of Carvacrol was obtained by the increment of salt stress at the salt concentration of 100 mM and with chitosan concentration of 0.4 g/l; moreover, the greatest amount of P-Cymene and Y-Terpinene was obtained by the increment of salt stress with salt concentration of 50 mM and chitosan concentration of 0.4 g/l.

Recommended applications/industries: Chitosan is recommended to be exploited in industry as it has moderating effects against salt stress in addition of the increment influence on the certain secondary metabolites of savory.

1. Introduction

Savory is an annual herb in the family Lamiaceae (Azami- Ghadikolaei and Jafari., 2012) that has thin branches willing to burgundy and green and leaves mixed with the red cross, square stem,

irregularpink to blue and white bisexual flowers whit capsules containing fruit has dark brown seeds (Mahdavi *et al.*, 2011). Distance of leaves is exclusively as a nervure andcan be seen as tiny dots containing aromatic tuber in their level and frequency.

Researchers claimed that major compounds of savory species are comprised of phenolic monoterpenes such as thymol and carvacrol and often exist with γ-terpinene, linalool, and p-cymene; furthermore, this group of phenolic compounds has antioxidant and antimicrobial properties (Kamkar *et al*., 2013). Plants produce a large and diverse group of organic compounds called secondary metabolites. Multiple properties of secondary metabolites, as drugs, herbicides, biological, flavoring agents, colors, poisons, hallucinogenic substances and perfumes, considered them for using in biotechnology (Esma'ilzadeh Behabadi and Sharifi, 2013).This accumulation of metabolites in plants often occurs when the plant is exposed to various stresses (Larcher *et al*., 2001). After drought, salinity is the most important and most common environmental stress throughout the world, including Iran. Salt stress is regarded as a limiting factor in performance of the plants. Now many compounds are used in order to reduce the damaging effects of stress on plants. The use of biological elicitorin many plants is one of the ways to reduce the harmful effects of abiotic stresses (Goriketal., 2008). Biological elicitors include polysaccharides, proteins, glycoproteins or parts of the cell wall of fungi, plants (cellulose and pectin) and micro-organisms (chitin and glucan) is (Esma'ilzadeh Behabadi and Sharifi, 2013). Chitosan is one of the incentives including incentives that stimulate plant defense mechanisms in response to stress (Kowalski *et al*., 2006). It can also greatly affect the reduction of salinity and increased plant growth (Esma'ilzadeh Behabadi and Sharifi, 2013).Chitosan works as a bio-stimulant that came through the induction of plant defense systems and alters and enhances the production of secondary metabolites in medicinal plants (Cheng *et al*., 2006). In Artemisia (Artemisia annua) the use of chitosan increases the Artemisiain plant (Kaya., 2008). It also increases the production of Silymarin with the use of chitosan has been reported in cell suspension culture of Silybummarianum (Sanches-Sampedro*et al*., 2005). To the best of our knowledge there is no report on the mutual effect of chitosan and salt stress on the savory's growth and performance as well as the content of its essential oil. Therefore, the current project was conducted to evaluate the effect of different concentrations of chitosan on growth indices and the quality and quantity of essential oil under salt stress (salinity).

2. Materials and Methods

2.1 Seed selection and planting

The seeds of *Satureja Isophylla* L. were provided by Pakan Bazr Company, Isfahan, Iran. They were identified in Islamic Azad University – Falavarjan Branch Herbarium and were coded as 114/010/001. 27 pots with 25cm diameter were selected and filled with appropriate sandy soil, and the seeds were sown in the soil equally. Planting Environment was in greenhouse conditions and the temperature was 22±4centigradedegree.

2.2. Measurement of growth parameters (initial)

Before the treatment and when the herb height reached as far as 8-10 cm, 4 plants were randomly selected from each pot. Then, the number of leaves, the stem length and the number of lateral branches were investigated.

2.3. The treatment plants with a solution of sodium chloride and chitosan

After initial measurement of growth parameters, treatment was done with sodium chloride solution with a concentration of salt (0, 50 and 100 mM) over a week with four replications. On the weekend, chitosan treatment was done by spraying the solution with concentration of 0, 0.2 and 0.4 g/l to the aerial parts. Two weeks after the last chitosan treatment, the plants were harvested and prepared for the process of extraction and investigation of the essential oil components.

2.4. Measurement of growth parameters (secondary)

Before the second harvest of the plants, those plants which had been randomly selected were examined in terms of growth parameters such as root length, number of leaves and number of lateral buds. Moreover, to measure the dry weight of the root and the stem, the plants were dried and weighed separately.

2.5 Analysis and identification of essential oils components

30g of dried shoots were grinding and extraction was done in device Clevenger (Glass Factory Model Tears Tehran). Extraction time for each sample was about 5.3 hours and the volume of essential oils was individually measured and recorded.

After sample preparation, the essential oil was injected into the GC / MS to determine the type of constituents. Gas chromatography was 7890 Agilent equipped with HP-5column (30 m × 0.25 mm, 0.25 µm film thicknesses). Oven temperature was set up at 60 °C initially, and then raised at rate of 4°C/min to 300 °C. Injection chamber temperature was set at 290 °C, and helium was used as carrier gas at a flow rate of 8.0 ml/min. The gas chromatograph was coupled to an Agilent 5975 C (Agilent Technologies, Palo Alto, CA, USA) mass selective detector (MSD) and quadrupole EI mass analyzer (Agilent Technologies, Palo Alto, CA, USA). Operating parameters for the EI-MS were: ionization voltage, 70 eV and ion source temperature, 220 ºC. Identification of spectrum was done by calculation of retention indices and comparison with those of authentic standards and mass spectra of standard composition (Wiley and NIST) .Tests were performed with four replications and were analyzed using SPSS version 19. Mean comparison was evaluated with Duncan test and Graphs were plotted using Excel software.

3. Results and discussion

The effects of various chitosan concentrations on certain growth parameters of the savory as well as that effect on its essential oil which was under salt stress have been explored. The ANOVA, as the statistical procedure, was run on the data related to the savory's shoot dry weight under salt stress and various chitosan concentrations. The results indicated that the shoot dry weight reduced with increase in salinity. In addition, increasing chitosan at different concentrations, compared to the control group, led to decrease in the dry weight. Further, a significant difference was observed compared to absence of chitosan in treatments with different concentrations of chitosan and sodium chloride, in concentrations of 0.2 and 0.4 g/l chitosan and 100 mm sodium chloride, chitosan control (Figure 1). According to Figure 2, it can be seen that the root dry weight decreased with increasing in salinity. Root dry weight increased in different concentrations of chitosan, but the difference in concentration of 0.4 g/l chitosan was statistically significant compared to the control group. In treatments with different concentrations of chitosan and sodium chloride, it was observed that the root dry weight increased at concentrations of 50 and 100 mM of Sodium chloride. However, this increase showed a statistical significance, compared to the control group, at chitosan concentration of 0.2 g/l.

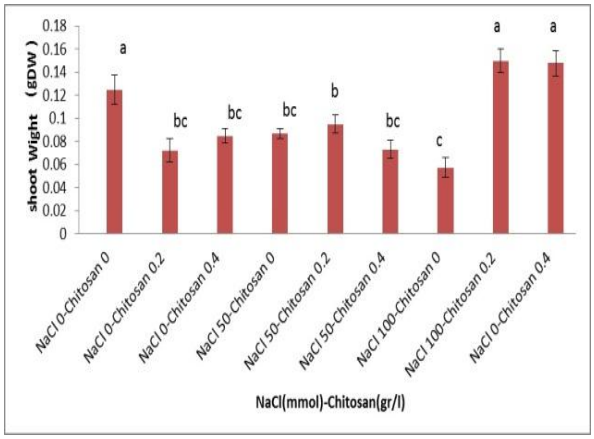

Fig. 1. Effect of salinity and chitosan on the shoots dry weight in the savory. Dissimilar letters indicate significant differences as per the Duncan test ($p \leq 0.05$).

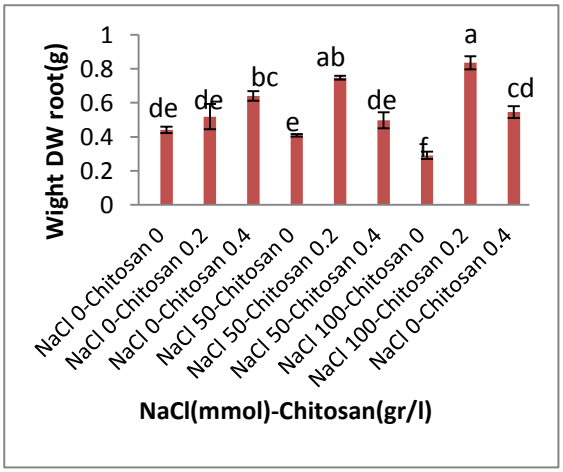

Fig. 2. Effect of salinity and chitosan on the roots dry weight in the savory. Dissimilar letters indicate significant differences as per the Duncan test ($p \leq 0.05$).

The other reports indicated that slow growth, reduction of dry matter production and also reduction of the ultimate performance is due to salt stress in most plants, such as wheat, barley, beans and cotton (Parida and Das, 2005). Increase in salt concentration reduced wet and dry weight of root of *Nigella Sativa* (Husseini *et al*., 2006).

Also the reduction of the salt-affected shoot dry weight in Seville (Rostami Hir *et al.*, 2004), subterranean clover (Galshi, and Soltan, 2002) and fennel (Safarnejad *et al.*, 2003) have been reported. Plant dry weight reduced because of the toxicity of ionic and osmotic adjustment and the reduction of nutrient uptake and photosynthesis disruption during salinity stress. Applying different chitosan concentrations in bean led to increase in the dry weight of stems and roots (Singla and Gary, 2005). A test was run to evaluate the pea seedling growth under different levels of chitosan salinity.The results demonstrated that with increasing salinity levels, dry weight of shoot and roots significantly decreased (Mahdavi *et al.*, 2013). Chitosan increased the chlorophyll and carotenoids of plant by activating the expression of genes in the biosynthesis of photosynthetic pigments (Naderi and Khajeh., 2015). Therefore, as a result of increasing the photosynthesis of the plant, chitosan caused to rise the dry weight of the plant (Heng *et al.*, 2012).

Figure 3 shows that the plant height reduced through increasing in salinity. Likewise, the plant height increased in treatments at different chitosan concentrations and the height reached its maximum level at concentration of 0.2 g/l. Also, in a treatment with different concentrations of chitosan and sodium chloride, a significant difference was observed in all concentrations, compared to the control group.

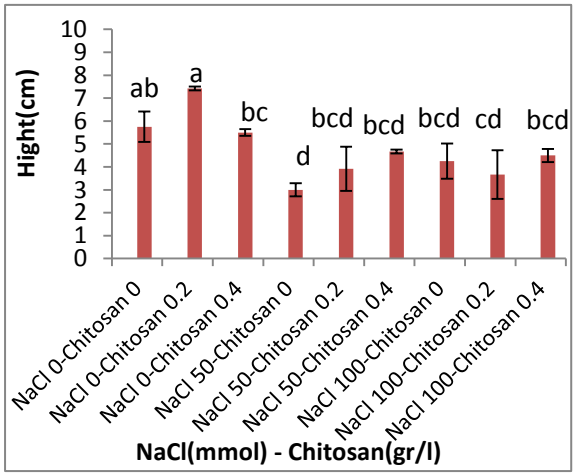

Fig. 3. Effect of salinity and chitosan on the height of *Satureja Isophylla* L. Dissimilar letters indicate significant differences as per the Duncan test (p≤0.05).

Figure 4 shows that the number of leaves reduced through increasing in salinity; while the number of leaves increased at chitosan concentration of 0.2 g/l and no significant difference was observed in chitosan treatment with 0.4 g/l, compared to the control group. The number of leaves reached its maximum level when there was a treatment containing different chitosan and sodium chloride concentrations (sodium chloride concentration 0 and 50 mM as well as chitosan concentration 0.2 g/l). Furthermore, the number of leaves reduced at chitosan concentration 0.2 g/l and sodium chloride concentration 100 mM.

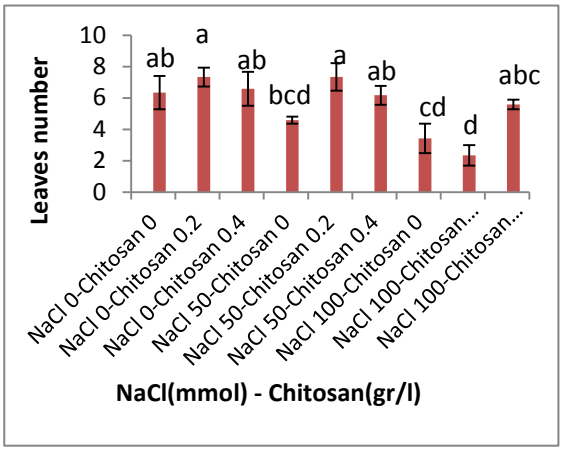

Fig. 4. Effect of salinity and chitosan on the number of leaves *Satureja Isophylla* L. Dissimilar letters indicate significant differences as per the Duncan test (p≤0.05)

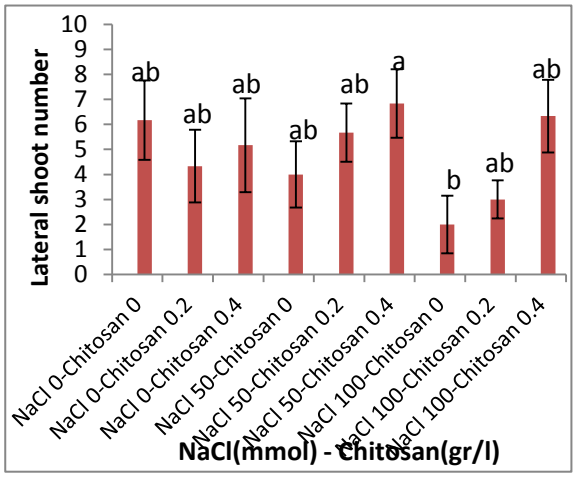

Fig. 5. Effect of salinity and chitosan on the number of lateral shoots of *Satureja Isophylla* L. Dissimilar letters indicates significant differences as per the Duncan test (p≤0.05).

As shown in Figure 5, the number of lateral shoots of *Satureja Isophylla* L. reduced through the increase in salinity and this difference was statistically significant at sodium chloride concentration 100 mM, compared to the control group. There were no significant differences in

chitosan treatment and also in treatment with various chitosan concentrations and sodium chloride except in chitosan with 0.4 g/l concentration and sodium chlorid with 50 mM concentration in which the number of branches has reached to its maximum level (Figure 4).

Over the osmotic stress resulting from salinity, production and transformation of hormones including cytokinin and auxin decreased which caused division and elongation of cells. Thus, during salinity plant height and number of branches will be reduced. The findings of the studies conducted on basil (Hassani, 2003) and plantajo (Ashraf and Orooj, 2006) were in line with the results of the current study concerning the reduction of the plant height under salt stress.

Chitosan as a biological stimulus may induce signs for the synthesis of plant hormones such as auxin and gibberellin and increase plant growth by some signaling pathways related to auxin biosynthesis and the dependent pathway of tryptophan (Uthairatanakij *et al*., 2007). Soaking seeds of pearl millet in chitosan, increased seed yield and plant height, compared to control (Sarathchandra and Jaj., 2004).

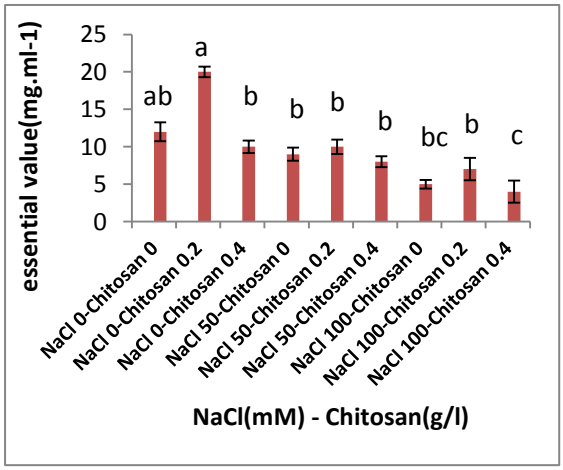

Fig. 6. The effect of salinity and chitosan on the essential volume of *Satureja Isophylla* L. essence. Dissimilar letters indicate a significant difference as per the Duncan test ($p \leq 0.05$).

It was indicated that chitosan had stimulatory effects on the growth of wheat seedlings (Wei *et al*., 2007), corn (Winter *et al*., 2001) and peanut (Winter *et al*., 2002). The positive effect of chitosan on the growth of various plants such as cabbage (Hirano, 1988), soybean sprouts (Lee *et al*., 2005) basil (Kim, 2005) has also been reported.

Considering the findings of the essence volume in savory which was under salt stress and different chitosan concentrations, Figure 6 indicated that the essential oil volume reduced due to increase in salinity. In a treatment containing different chitosan concentrations, it was observed that the amount of essential oil volume increased in chitosan concentration of 0.2 g/l and a significant difference, compared to the control group, was shown. In treatments with different concentrations of sodium chloride and chitosan significant difference are also observed in comparison with control. So that the minimum volume of essential oil of savory was observed in concentration of 0.4g/l and 100mM of chitosan and sodium chloride, respectively. In a similar study, the effect of salinity on mint and marjoram species was studied and it was found that salinity reduced 21 percent of oil yield (CroteauEl-Keltawi., 1987). According to other study on dill plant, salinity reduces the total oil concentration (Udagawa *et al*., 1995). Ozturkand *et al*. (2004) also stated that the increasing in salinity was associated with a reduction in the amount of essential oil in Lemon balm.

Other studies which were conducted to explore the effects of salinity on the amount and yield of oil essential in other medicinal herbs indicted that salt stress reduced the yield of dill essential oil (Riaze *et al*., 2007), the amount of fennel essential oil (Ashraf and Akhtar., 2004), and saddle essential oil (Ashraf and Orooj, 2006). Oil yield loss as a result of salinity may be due to the detrimental effect on growth and performance of plant (Dow *et al*., 1981).

The results of the composition of the essential oil of aerial parts in Table 1 indicated that 14 main compounds were detected in the essential oil, and the greatest amount of them was reported to be P-cymene (3.13%), γ-Terpinene (28.97%) and Carvacrol (59.64%). This findings showed that the greatest amount of carvacrol obtained by increasing salt stress in salt concentration 100 mM and chitosan concentration 0.4 g/l. Moreover, the greatest amount of P-cymene and γ-terpinen obtained by increasing salt stress in salt concentration of 50 mM and chitosan concentration of 0.4g/l.

Table 1. Effect of chitosan foliar application and salt treatment on concentrations (%) of essential oil constituents.

RI	compound	NaCl 0 Chitosan 0	NaCl 0 Chitosan 0.2	NaCl 0 Chitosan 0.4	NaCl 50 Chitosan 0	NaCl 50 Chitosan 0.2	NaCl 50 Chitosan 0.4	NaCl 100 Chitosan 0	NaCl 100 Chitosan 0.2	NaCl 100 Chitosan 0.4
5.11	α-Thujene	0.81	0.69	0	0.25	0	0.65	0.38	0.39	0.32
5.27	α-Pipene	0.45	0.38	0	0.13	0	0.4	0.23	0.24	0.21
6.27	β-Pinene	0.22	0.18	0	0	0	0	0	0	0.15
6.54	β-Myrcene	1.09	0.98	0	0.41	0	0.94	0.69	0.66	0.67
6.94	α-Phellandrene	0.28	0.25	0	0.11	0	0	0.17	0.18	0.17
7.26	α-Terpinene	3.56	3.19	0	1.4	2.17	3.14	2.29	2.37	2.21
7.48	*P*-Cymene	3.13	2.56	0	1.28	2.8	3.64	2.66	2.34	2.3
7.6	delta-3-Carene	0.36	0.44	0	0.16	0	0.36	0.25	0.32	0.23
8.5	γ-Terpinene	28.97	25.93	19.99	13.01	25.03	29.03	21.29	21.17	19.54
12.14	Terpinene-4-ol	0.23	0.25	0	0	0	0	0	0	0.22
14.27	Thymol	0	0	0	0.12	0	0	0	0	0.19
16.33	Carvacrol	59.64	63.85	80.01	82.36	69.94	61.36	71.59	71.96	72.02
19.9	β-Caryophyllene	0	0.22	0	0	0	0	0	0	0.16
22.59	β-Bisabolene	0.6	0.91	0	0.29	0	0.47	0.44	0.37	0.69
		99.34	99.83	100	99.52	99.94	99.99	99.99	100	99.08

Salinity affected the essence contents of medicinal herbs by changing their metabolism. Although the main compositions of chamomile oil increased under salt stress, certain main compositions of essential oil of marjoram and fennel such as carvacrol, p-cymen and y-terpinen decreased under salt stress (Razmjoo *et al*., 2008). Effect of Salinity on the reduction of thymol in thyme has also been reported (Babaee *et al*., 2010).

Chitosan can alter the function of genes by modifying the activity of enzymes that activated certain biosynthetic pathways in plants (Zhang *et al*., 2006). Chitosan is effective in stimulating the production of secondary metabolites, such as alkaloids, flavonoids and paranoid (Namdeo., 2007; Ionkova., 2007).

Increasing in the amount of phenolic compounds and flavonoids in the Pune plant treated with chitosan has been reported (Heng *et al*., 2012). Vasconsuelo *et al*. (2004) reported that Application of chitosan in Rubia led to increase in the production of anthraquinone).

It was also reported that the use of chitosan in cell suspension cultures resulting from cultivation of *Citrus grandis* caused an increase in linalool and limonene in this plant (Putalum *et al*., 2006).

The results of the present study revealed that the salt stress decreased the growth and essential oil yield in *Satureja Isophylla* L. Application of chitosan as a biological stimulus can adjust such stress via inducing the herb's defense routes. Moreover, chitosan treatment caused changes in the amount of metabolites of *Satureja Isophylla* L. and such changes can be affected by chitosan concentration.

4. Conclusion

The results of the present study revealed that the salt stress decreased the growth and essential oil yield in *Satureja Isophylla* L. Application of chitosan as a biological stimulus can adjust such stress via inducing the herb's defense routes. Moreover, chitosan treatment caused changes in the amount of metabolites of *Satureja Isophylla* L. and such changes can be affected by chitosan concentration.

5. References

Ashraf, M.,Akhtar, N. 2004. Influence of salt stress ongrowth ion accumulation and seed oil content in sweetfennel. BiologiaPlantarum, 48: 461-464.

Ashraf, M.,Orooj, A. 2006. Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plantajowan (*Trachysprmumammi*

L.) Sprague). Journal of Arid Environmental, 64: 209-220.

Babaee, K.,AminiDehaghi, M.,ModaresSanavi, SAM.,Jabbari R.2010.Water deficit effect onmorphology, prolin content and thymol percentage of thyme (*Thymus vulgaris* L.). Iranian Journal of Medicinaland Aromatic Plants, 26: 239-251.

Cheng, X., Zhou, U.,Cui ,X. 2006. Improvement of phenylethanoid glycosides biosynthesis in (*Cistanchedeserticola*)cell suspension cultures by chitosan elicitor. Biological Journal, 121: 253–260.

Conrath, U., Domard, A., Kauss, H. 1989. Chitosan-elicited synthesis of callose and of coumarin derivatives in parsley cell suspension cultures. Plant Cell Report, 8: 152-155.

Dow, A.I., Cline, T.A. , Horning, E.V. 1981. Salt Tolerance Studies on Irrigated Mint

Bulletin of Agriculture Research Center, Washington State University, Pullman (1981), p. 11

El-Keltawi, NE., Croteau R. 1987. Influence of foliar applied cytokinins on growth and essential oil content of several members of the lamiaceae. Phytochemistry 26(4): 891-895.

Esma'ilzadeh Behabadi, S., Sharifi, M. 2013. Increase secondary metabolite production plants using biological Alysytvrhay. Journal of Cells and Tissues,20:119-128.

Galshi, S.,Soltani, A. 2002. Evaluation growth, nitrogen fixation and salinity tolerance of five varieties of *Subterranean clover*. Journal of Agricultural Sciences and Natural Resources Gorgan, 18:71- 83.

Hassani, A. 2003. Water and sodium chloride salinity stress on morphological and physiological properties of basil. PhD. thesis. College of Agriculture, Tarbiat Modarres.

Hirano, S. 1988. The activation of plant cells and their self defence function against pathogens in connection with chitosan. Nippon NogeikagakuKaishi.(in Japanese with English summary). 62:293-295

Heng, YC., Xavier, F., Lars, P., Christensen, GK.2012. ChitosanOligosaccharides Promote the Content ofPolyphenols in Greek Oregano *(Origanumvulgare ssp. hirtum*). Journal.Agricalture. Food Chemistry,60:136−143.

Ionkova, I.2007. Biotechnological Approaches for the Production of Lignans. Pharmacognosy Reviews, 1: 427-438.

Kamkar, A.,Toriean, F .,AkhondzadehBasti, A.,Misaghi, A.,Shariatifar, N. 2013. Study the chemical composition of essential oils Savory (*Saturejahensis* L.) and compared the antioxidant activity of the extract and alcohol. Journal of Veterinary Research, 20:183-190.

Kaya, Z.2008. Effect of varing nitrogen doses on yield, yield components and artemisinin content of (*Artemisia annua*L.).Ind Crop Prod, 27:60-64.

Kim H. 2005. Characterization of bioactive compounds in essential oils, fermented anchovysauce, and edible plants, and, induction of phytochemicals from edible plants using methyl jasmonate (MeJA) and chitosan. PhD Thesis Clemson University. USA. ‹pp: 178

Kowalski, B., Jimenez, F., Herrera, l.,Agramontepenalver, D. 2006. Application of soluble chitosan in vitro and in the greenhouse To increase yield and seed quality of potato minitobers. Potato Research,49:167-176.

Lee, Y., Kim, Y., Kim, S. 2005. Changes in the respiration, growth and vitamin c contentof soybean sprouts in response to chitosan of different molecular weights. Horticalture Science, 40:1333-1335.

Lee, Y., Kang, C., Lee, Y. 1999. Effects of chitosan on production and root control of soybean sprouts. Korean Journal of Crop Science, 44:368-372.

Larcher, W. 2001. Physioligical plant ecology. Springer-VerlagVerlag Berlin Heidelberg New York. Germany.

Mahdavi, B., ModaresSanvi, A., Aghaalikhani, M., Sharifi, M. 2011. The effect of different densities chitosan on seed germination and antioxidant sunflower under drought stress.Journal of Plant , 3:352- 365.

Naderi, S., Khajeh, H., Ahmadi, H. 2015. Characteristics of the authors The Effect of Chitosan on Some Morphological Indices in *Lepidium sativum* L. The First Conference of Medicinal Herbs and Herbal Medicines , Tehran, Center for the Sustainable Development of Science and Technology Farzin.

Namdeo, A. 2007.Plant cell elicitation for production of secondary metabolites. Pharmacognosy Reviews, 1: 320-345.

Ozturk, A., Unlukara, A., Ipek, A. Gurbuz, B. 2004. Effects of Salt Stress and Water Deficit on

Plant Growth and Essential oil Content of Lemon Balm (Melissa officialis L.). Pakistan. Journal of. Botany. 36(4), 787-792.

Parida, A., Das, B. 2005.Salt tolerance and salinity effects on plants. Ecotoxicology and Environmental Safety, 60: 324-349.

Putalun, W.,Luealon, W., De-Eknamkul, W., Tanaka, H.,Shoyama ,Y.2007. Improvement of artemisinin production by chitosan in hairy root cultures of *Artemisia annua*L., Biotechnology,29: 1143- 1146.

Razmjoo, K., P. Heydarizadeh and M.R. Sabzalian. 2008. Effect of Salinity and Drought Stresses onGrowth Parameters and Essential oil Content of *Matricaria chamomila*. International Journal of Agricultural and Biology., 10, 451-475.

Rostami Hir, M.,Galshys, A.,Soltani ,A.,Zeinali, A. 2004. The effect of NaCl on the growth of soybean varieties in eleven biological determination. Journal of Agricultural Sciences and Natural Resources, Gorgan, 2: 127- 136.

Riaz, P., Surendra, S., Harting, H. 2007. Effect of irrigation intervals and splitted nitrogen on carvone content of *Anethum graveolense* L. grown in semi-arid region. Journal of Horticulture Environment Biotechnology, 41(4): 25-30.

Sarathchandra, R., Jaj, N .2004.A chitosan formulation Alexa induce downy mildew disease resistance and growth promotion in (*pearl millet*).Crop Protection, 23:881-888.

Safarnejad, A.,Salami, M.,Hamidi, H. 2007. Morphology Characterization of PP medicinal plants to salinity stress.Research and Development, 7: 123- 132.

Singla, R., Gary, N. 2005. In fluence of salinity on growth and yield attributes in chickpea cultivars.TurkishJornal of Agriculture and Forestry,29: 231-235.

Udagawa, Y. 1995. Some responses of dill (*Anethum graveolens*) and thyme (*Thymus vulgaris*), grown in hydroponic, to the concentration of nutrient solution. Acta Horticultyre. 396: 203–210.

Uthairatanakij, A.,Teixeirada, A., Obsuwan, K. 2007. Chitosan for improving orchid production and quality. Orchid Science and Biotechnology, 1:1-5.

Vasconsuelo, A.,Giulietti, AM., Boland, R. 2004. Signal transduction events mediating chitosan stimulation of anthraquinone synthesis in Rubiatinctorum. Plant Science, 53: 405-413.

Wanichpongpan, P.,Suriyachan, k.,Chandrkrachang, S. 2001. Effect of chitosan on the growth of gerbera flower plant. Gerberajamesonii, 4:198-201.

Wei, S.,Zang, X.,Xue, J., Xiang, G. 2007. Effect of chitosan on seeds germination and seedling physiological property of wheat. Periodicals. Core Journals Biology Journal, 2:1-24.

Winter, Y., House, P.,Xiujuan, W.,Zhimeng, Z.,Yourong, S. 2001. Effect of chitosan on physiological activities in germinating seed and seedling leaves of maize. Periodicals Hebetic Vocational and Technical Teachers College Journal,4:1-15.

Winter, Y., House, P.,Zhimeng, Z.,Xiujuan, W.,Xiaojun, H. 2002. Germinating seed of peanut effects of chitosan on some physiological activity in germinating seed of peanut.Core Journals Journal of peanut science, 1: 1-31.

Zhang, Y .,Mian, MR.,Bouton, JH. 2006. Recent Molecular and Genomic Studies on Stress Tolerance of Forage and Turf Grasses. Crop Science, 46: 497–51