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Máster Universitario en Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario

Effect of salinity and methyl jasmonate on the production and quality of sea fennel (Crithmum maritimum L.)

Autora: Hafise Varol

Dirección: Juan A. Fernández Hernández Codirección: María del Carmen Martínez

Ballesta

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HAFİSE VAROL

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ABSTRACT

In this project, *C. maritimum*, a facultative halophyte plant, was grown in floating systems and two experiments were carried out. Experiment 1 investigated the effect of salinity on growth and development. Therefore, biomass, leaf area, root growth parameters, mineral (anion and cation), post-harvest quality (% weight loss; L, a, b values; HUE, Chromaticity values), sensory and microbial quality analyzes were performed. In the Experiment 2, the effect of MeJa on salinity stress and the phytochemical content was investigated. To that, biomass and root growth parameters, mineral analysis (anion and cation), total phenolic component, flavonoid and antioxidant capacity analyzes were carried out. According to the results of the first experiment, *C. maritimum* baby leaf can be grown in floating hydroponic systems. NaCl increased root length and surface, while reduced the aerial parts of *C. maritimum*. Salinity hindered to intake of minerals of K⁺, Ca⁺, Mg⁺, NO₃⁻ and SO₄²⁻ from the roots. And also, NaCl treatments caused to accumulate Na⁺ and Cl⁻ ions in cell of *C. maritimum* plants. Salinity enhanced shelf life of *C. maritimum* by decreasing microorganisms. In the second experiment, MeJa addition reduced the adverse effect of salinity on biomass and recovered Ca⁺ and K⁺ ions. NaCl and MeJa treatments decreased

phenolic content. Also MeJa improved the effect of salinity on chlorophyll a, chlorophyll b and carotenoids. Maximum flavnoid content was obtained with MeJa treatment. MeJa addition did not affect antioxidant capacity in plants exposed to salinity but caused edible parts to grow and recovery minerals.

1. INTRODUCTION

1.1 The Importance of Baby Leaf as a Ready-To-Eat Food

Baby leaf vegetables are usually 12 cm in size that are harvested within 35-40 days 12 and used in the ready to eat vegetable industry. Baby leafy vegetables have soft leaves and their oxidation is minimal compared to mature vegetables, what is a benefit for the fresh-cut market³. Thus, basil (Ocimum basilicum), tatsoi (Brassica rapa subsp. narinosa), endive (Cichorium intibus), red, and green lettuce (Lactuca sativa L.), rucola (Eruca sativa (syn. E. vesicaria subsp. sativa (Miller) and Diplotaxis tenuifolia) spinach (Spinacia oleracea) are normally used in baby leaf production ^{4 5 6}. Vegetables reduce the risk of diabetes, cancer, and obesity thanks to the phytochemicals they contain ⁷. Due to the fact that baby leaf vegetables are richer in phytochemicals than mature vegetables, their nutritional value, shelf life, texture, and quality characteristics, their production and consumption have increased globally ⁸. When the amount of phenolic components is compared with mature vegetables, it was observed that the amount of baby leaves is quite high 9. In addition, some baby leaves have high antioxidant capacity due to which they have antiradical activity 9. Furthermore, baby leafy vegetables are important sources of minerals. Thus, green lettuce, swiss chard, watercress, lambs lettuce, wild rocket, organic wild rocket, spinach and parsley baby leaves contain K, Na, Ca, Mg, P, Fe, Mn, Zn, Cu minerals and show similar contents to mature vegetables ¹⁰. The quantity of vitamins and minerals in wild plants is generally very high respect to cultivated vegetables. Therefore, wild plants are very noteworthy for diet 11 and it is thought that they can be used in baby greens production due to their high nutritional values. As example, Sanguisorba minor Scop., Sinapis arvensis L., Taraxacum officinale Weber ex F. H. Wigg. wild plants are suitable to production of baby leafy vegetables. However, wild plants consumption can be considered a risk factor due to their high nitrate content, although their amount can be controlled when the plants are grown under controlled conditions (water, nutrient solution, substrates, etc.) ¹².

1.2 Baby Leaf Growing Media

According to research hydroponic systems are known as agricultural systems containing soil-free water and fertilizers ¹³. Rock wool, coir, perlite, vermiculite, gravel/quartz, sand, and expanded clay materials are used as plant support materials in soilless culture systems ¹⁴. Wick system, drip system, ebb and flow system, (deep) water culture system, nutrient film technique system, aeroponic systems, and floating systems, are used as hydroponic production methods ¹⁵. Soil or soilless growing environments, light, water and nutrients are required for growing baby leaf vegetables. For this reason, baby leaf vegetables are grown using aquaponic and hydroponic systems because of they provide the necessary intermediate conditions 16. Controlling environmental conditions such as nutrient solution affects the flavor and quality characteristics of plants ¹⁷. Greenhouse conditions are the most suitable and controlled conditions in which baby leaves can be grown. Perlite, coconut fiber and expanded vermiculite are common substrates for growing baby leaf vegetables 18 19. Therefore, the growing environment was thought to be important for baby leafy vegetables ²⁰. Thus, when growing lettuce grend rapid, lettuce new rapid, and lettuce delicato lettuce varieties, plants yielded the best results in terms of the plant height, number of leaves, and root wet weight when sandy soil and peat soil were used.

1.3 Floating System

In floating hydroponic systems, plants are grown in plastic or high-density polystyrene trays by filling suitable nutrient solution into the tanks. The amount of nutrient solution in the tanks varies between 150-250 liters/m². It is known that the use of floating hydroponic system enables producing cleaner foods, saving water. In addition, controlling nutrient solutions, can reduce nitrate content in vegetable production ²¹. In addition, the fact that it does not require electricity in some facilities is another advantage of floating systems ²². In addition, floating systems have the lowest cost among hydroponic systems ²³. It is known that floating culture systems are frequently used in agricultural researches, especially in the production of herbs and leafy vegetables, whereas their commercial use is less extended ²⁴ ²⁵. Like in other hydroponic systems, plant growing in a floating system may suffer hypoxia because the roots gradually consume the oxygen dissolved in the nutrient solution. By using pumps, pipes, and diffusers placed in the system, the plant roots are provided to receive oxygen ²⁶. In a study on spinach plants in floating systems, aerated and unventilated nutrient solutions were compared and was observed that aeration improves yield and reduces nitrate accumulation in spinach plants ²⁷. In another study with tomato seedling, different dissolved

oxygen concentrations in the nutrient solution affected some properties of plants such as fresh and dry weight, leaf area and, stem diameter ²⁸. Chemical reactions of ions in the solution can cause certain elements to precipitate and alter their bioavailability of plants. In order to increase the bioavailability of plants, the pH range appropriate for the grown plant should be determined and nutrient solutions must contain components in appropriate concentrations ²⁹. In commercial applications, it is known that strong acid or base solutions are added to change the pH of nutrient solutions. In hydroponic systems, the recommended pH range is 5.5-7.2 for the plants to benefit optimally nutrients uptake in the nutrient solution ³⁰. In addition, nutrient solutions with electrical conductivity between 1-3 dSm⁻¹ are normally used in hydroponic systems.



Picture 1: Sea fennel (C. maritimum) cultivars grown in floating system.

1.4 Seeds and Sowing of Baby Leaf

In baby leaf cultivation, ambient conditions, growing method, seed variety are important issues. Processes such as soaking, NaClO, GA₃, KNO₃, cold and heat can be applied to seeds to improve germination³¹. Plant density can affect yield and amount of dry matter. Lettuce (*Lactuca sativa* L. var. *longifolia*): 'Ronda' and 'Amadeus' varieties were grown in a floating system at plant densities of 316 and 620 plants / m^2 and higher fresh leaf yield was obtained from high-density plants. However, higher dry matter content and lower root weight were observed in low-density plants 32 .

1.5 Irrigation and Fertilization of Baby Leaf

Fertilization should be carried out in accordance with the correct rate, correct source, correct placement and correct timing factors, because irrigation and fertilization has a great importance in terms of yield and quality in vegetable growing ³³. While less irrigation causes the yield and quality of the plants to decrease, excessive irrigation causes the plants to decrease their resistance against diseases ³⁴ ³⁵. Since baby leafy vegetables are consumed

raw, they should be microbially safe³⁶ ³⁷. Therefore, the quality and characteristics of the irrigation water used, and the conditions of the European Commission, World Health Organization and Codex Alimentarius Commission should be taken into consideration by the producers³⁶. The water source should not contain pathogens, and floating systems should be used to prevent the contact of plants with water ³⁶ ³⁷ ¹⁹. Water and macro and micro elements are used in nutrient solutions ³⁷. The concentration of the nutrient solution is very important for the yield of baby leaf vegetables³⁷. In lettuce cultivation, the increased in the concentration of the nutrient solution in the floating system increased the fresh yield and leaf mineral content of the plant ³⁷ ¹². Nitrogen is one of the main fertilizers used in vegetable production, but an excessive use by producers in order to increase yield causes a decrease in vegetable quality, exceeding the limits set by the European Commission, and provoking water and environmental pollution. Therefore, excessive nitrogen use negatively affects the quality of the baby rocket. In order to overcome this issue, researchers are focusing their attention on the use of alternative means, such as plant biostimulant application. Thus, in a recent study, it was determined that plant-based biostimulants instead of nitrogen had a positive effect on baby leaf quality, maintaining nitrate content under the legal European Comission limits ³⁸.

1.6 Pest and Diseases of Baby Leaf

As the ready-to-eat baby leaf industry has gained great importance in recent years, it is necessary to fight against harmful insects and diseases during the production phase. Although baby leaf vegetables are produced in a short time, it is known that insecticides are generally ineffective ³⁹. It is recommended to implement an Integrated Pest Menagement strategy to prevent pests in baby leafy vegetables ^{40 41}. According to a study, diamondback moth, cabbage center grub, aphids, thrips, leaf miner, rutherglen bug, cabbage white butterfly are known as the main pests in baby leaf vegetables, while *Helicoverpa spp.*, jassisds, flea beetle, shore flies, fungus gnats, green mirid, cabbage cluster moth, mites, carabid beetle are known as minor pests ⁴¹. In addition, there are beneficial insects for baby leafy vegetables such as wasps, spiders, lacewings, hover fly, lady beetles. Pirate bugs, soldier beetle, red and blue beetle, assassin bug, damsel bug and big eyed bug are known as mirator beneficial insects ³⁹. It is known that bacterial, fungal, viral diseases and physiological disorders are seen in leafy vegetables. Leaf spot diseases are seen respectively on spinach and baby leaf lettuce due to bacterias of *Stemphylium botryosum* and

Xanthomonas campestris pv. *vitians* ⁴² ⁴³. Varnish spot diseases are seen butterhead lettuce (*Lactuca sativa* L. var. *capitata*) due to bacterias *Pseudomonas cichorii* ⁴⁴. Downy mildew disease is seen spinach (*Spinacia oleracea* L.) due to fungal (*Peronospora effusa*) ⁴⁵. Russet spotting disease is seen in iceberg lettuce (*Lactuca sativa* L.) due to physiological disorder ⁴⁶. Beet western yellows virus (BWYV) and turnip mosaic virus (TuMV) causes diseases in the Brassicaceae family. In Lettuce (Asteraceae) lettuce mosaic virus (LMV), mirafiori lettuce virus (MiLV); lettuce big-vein virus (LBVV) - (Lettuce big-vein disease), lettuce necrotic yellows virus (LNYV), turnip mosaic virus (TuMV) viruses cause diseases ⁴⁷.

1.7 Post-Harvest Handling of Baby Leaf

Processes such as cutting, washing, rinsing and packaging are applied to baby leafy vegetables postharvest and these affect the quality of baby leafy vegetables. The properties of bioactive compounds, taste, color, odor, and physical appearance in baby leafy vegetables change during the post-harvest process ⁴⁸. When harvesting baby leaf vegetables, they should not exceed the 'baby stage' ⁴⁹. Leaf length (cm) and petiole length (cm) are maturity parameters for the harvest phase of baby leafy vegetables for the fresh-cut industry. For example, for red and green batavia, red chard, rocket, spinach baby leaf vegetables, the minimum, optimum, and maximum leaf lengths are 5, 10, and 12 cm, respectively 1. Methods and genetic factors applied in the pre-harvest, harvest and post-harvest stages affect the quality of fresh-cut vegetables ⁵⁰. The shelf life time of young leafy vegetables varies according to the type of vegetable ⁵¹. Baby leafy vegetables generally have a shelf life of 7 to 10 days after packaging ⁵². Storage temperature is known as the critical point for the nutritional value of baby leafy vegetables. It has been determined that baby leaf vegetables can be stored at temperatures between 0-7 C, 75-85% RH, controlled atmosphere (0.5 kPa O₂ + 10 kPa CO₂) conditions, using polypropylene (PP) film, polyethylene terephthalate (PET) boxes, and MAP technology ⁵³ ⁴⁸.

1.8 Halophytes

Human activities, climate change and increasing population cause reduction in arable lands due to the salinization and declining of fresh water availability ⁵⁴. Therefore, new crops tolerant and /or resistant crops to salinity and drought could be used and new strategies should be applied to increase food production ⁵⁵. Halophytes are salt-tolerant species that could be cultivated in salty soil or using sea water due to using mechanisms inorganic ions

like Na and Cl⁻. There are three basic mechanisms to tolerate salt as salt excluding, salt excreting and salt accumulating. Roots of halophyte plants act as an ultrafiltration in only salt excluding mechanism. *Ceriops candolleana*, *Bruguiera gymnorrhiza* halophyte plants possess this mechanism. In salt excreting mechanism, foliar glands regulate the salt concentration. *Avicennia officinalis* and *A. alba* halophytes are known to have this mechanism. Finally, salt accumulates in cells and tissues in the salt accumulating mechanism. *Sonneratia apetala*, *S. acida* and *S. alba* halophyte plants have this mechanism ⁵⁶. Some halophytes have been used as human food and animal feed. This is advantageous against food shortage in the future ⁵⁷.

1.8.1 Crithmum maritimum

Sea fennel (Crithmum maritimum L.) is the only species of the genus Crithmum. In German it is named Meerfenchel or Seefenchel; in French Fenouil Marin or passepierre; in Italian finocchio Marino or critama; in Turkish Kaya korugu or Deniz rezenesi ⁵⁸ ⁵⁹. Among halophytes, sea fennel is a perennial, edible and medicinal halophyte species very common in some Mediterranian countries such as Spain, Greece, and Tunisia 60 61 62. These species can be used as dried, salad or pickled ⁶³. The species *Crithmum maritimum* L. belongs to the genus Crithmum, the family Apiaceae, the order Apiales, the class Magnoliopsida, the division Magnoliophyta, the super-division Spermatophyta, the Subkingdom Tracheobionta, and the kingdom Plantae ⁶⁴. C. maritimum L. has succulent leaves, thick and gnarled roots. Their flowers are yellowish or greenish-white colour ⁵⁸. Their fruits are composed of a porous outer coat. It is thought that at the maturation stage, this structure may have a crucial role in seed dispersion and germination ⁶⁴. C. maritimum is an underutilized crop for commercial cultivation until now 65. During the last decade, the floating culture system has become very popular for aromatic and medicinal vegetable cultivation due to rapid plant growth, crop quality, high yield, easy harvest, optimization of water and fertilizer use ⁶⁶, as far as it could be used for *C. maritimum* cultivation.

1.8.2 Bioactive Compounds in C. maritimum

It is known that this specie has been used as food, spice and, medicinal herb ⁵⁸. *C. maritimum* L. has important economic and medicinal potentials. This edible aromatic plant has also a powerful scent. It is known that their organs contains bioactive substances that could be used as aromatic, antimicrobial, medicine and insecticide ⁶⁷ ⁶⁸ ⁶⁹. Recent studies have shown that

C. maritimum is rich in terms of flavonoids, carotenoids, vitamin C and medicinal components ⁷⁰ ⁷¹ ⁷². It has been determined that limonene (22.3%), y-terpinene (22.9%) and thymol methyl ether (25.5%) are the main components of *C. maritimum* oil ⁷³. In leaves of *C. maritimum*, a phenolic compound never described in a halophyte before: chlorogenic acid (CGA) has been also determined⁶⁸. According to quantitative analyses of the content of tannins and total polyphenols of *C. maritimum*, the content of tannins ranged from 0.10 to 2.65% and the content of total polyphenols varied from 4.72 to 9.48% ⁷⁴. Also, *C. maritimum* contains chemical components such as saponins, monounsaturated fatty acids (oleic, linoleic acid), saturated fatty acids (palmitic and stearic), linolenic acid, essential oil and falcarindiol. Their antimicrobial, antioxidant and antibiotic activities have been determined by biological tests. It has diuretic, anti-scorbutic, digestive and purgative properties ⁷⁵. It was determined that *C. maritimum* contains some important phenolic substances including p-hydroxybenzoic and ferulic acids, epicatechin, pyrocatechol and 4-hydroxybenzaldehyde⁶². Generally, it has been concluded that, this plant should be commercialized in order to be used in the food, cosmetics and pharmaceutical industries ⁷⁶.

1.8.3 Mineral Content of *C. maritimum*

Due to the increasing interest of people in the "natural life style", consumption of wild plants as vegetables is increasing, and the mineral content of wild plants is being investigated by scientists. The mineral content of *C. maritimum* per 100 g fresh leaves is as follows: N (360 \pm 88 mg), P (19 \pm 3.1mg), Na (290 \pm 26 mg), K (310 \pm 34 mg), Ca (97 \pm 6 mg), Mg (82 \pm 6 mg), Fe (2.3 \pm 0.3 mg), Cu (0.12 \pm 0.01mg), Zn (0.46 \pm 0.05 mg), Mn (0.71 \pm 0.06 mg), K / Na (1 \pm 0.1wt.), Ca / P (5.10 \pm 0.54 wt.), K / (Ca + Mg) (0.68 \pm 0.07 meq) ⁷⁷. As a result of mineral analysis of *C. maritimum* plants collected from the Thigzert region of Algeria, amounts of minerals were identified such as K, 5.2 g/kg dw, Mg 3.4 g / kg dw, Ca 23.3 g / kg dw ⁷⁸. When the mineral content of C. maritimum halophyte plants obtained from Spain, was analyzed, it was found high Na+ content ⁷⁷. In addition, it is also thought that different agricultural practices can improve the mineral content of plants ⁷⁹.

1.8.4 Effect of Salt Stress in C. maritimum

Important most of arable land in the world are negatively affected by salinity stress. Salinity causes changes in the biochemical and physiological activity of seeds, reducing the germination percentage of seeds ⁸⁰. The fact that *C. maritimum* seeds have a spongy structure

reduces the effect of stress by providing the ion balance of the environment with the seeds. That is, the spongy coating protects the seeds of *C. maritimum* from damage caused by Na ⁺ and Cl⁻ accumulation ⁶⁴. Besides, ascorbic acid (40 or 60 mM) and ethanol (% 96) treatments were caused an increase in the germination percentage of *C. maritimum* seeds ⁸¹. Regarding *C. maritimum* root and shoot growth biomass, root length, and the number of leaves were observed to be maximum at medium salinity levels (50 mM) and minimum at high salinity levels (200 mM) ⁷⁰. While the amount of sodium and chlorine increased in *C. maritimum* shoots in proportion to the increase in salinity, the amount of calcium, magnesium, and potassium decreased ⁷⁰. In addition the amount of antioxidant enzymes in the roots were decreased with increasing salinity, while in the shoots antioxidants were only enhanced at the concentration of 50 mM ⁷⁰. When the salt levels increased the osmotic balance of the plants was disturbed due to the accumulation of Na ⁺ and Cl⁻ in the cells of the plant and the growth was reduced ⁸².

1.8.5 Effect of MeJa in Salt Stress

It has been reported methyl jasmonate and jasmonic acid protected plants against pathogens, insects, injuries, and in addition, they play a role in germination, ripening of fruits and development of roots 83 84. The jasmonates (jasmonic acid and MeJa) are synthesized from linolenic acid by the octadecanoic path in plants, cause signaling in plants and play a role in gen activation and regulation ⁸⁵ ⁸⁴. In relation to the possible role of MeJa on salinity stress, it has been demonstrated that MeJa reduced salinity stress in strawberry plants (cv. Camarosa), increasing root and shoot dry weight of plants, through an enhanced antioxidant enzyme activity 86. It is also known that salinity stress increases the activity of antioxidant enzymes in plants, as well as stimulates lipoxygenase enzyme. Lipoxygenase enzyme is involved in the synthesis of jasmonic acid as a result of its stimulation ^{87 88 89}. Also, in *Cakile* maritime and Thellungiella salsuginea halophytes, salinity caused oxidative stress and thus increase jasmonic acid accumulation in leaves and roots 90. In order to determine the effect of methyl jasmonate on high salt stress, when different MeJa concentrations (0, 0.01, 0.03, 0.05, 0.1) were applied, to Limonium bicolor, it was found that treatment with 300 mM NaCl led to dramatic inhibition of seedling growth, but it was significantly alleviated by the application of 0.03 mM MeJa ⁹¹.

1.8.6 Effect of Meja as Plant Elicitor of Phytochemicals

It has been described that MeJa increased the bioactive compounds composition when is applied to plants due to its elicitor properties 92 93. It was due in the halophyte ice plant (Mesembryanthemum crystallinum) to a stimulating effect on the WI12 gene expression that produces cell wall protein⁹⁴. In Halophyte *Capparis spinosa* species, MeJa increased the rutin (important type of flavonoid compound) biosynthesis of by gene expression 95 96. When different concentrations of methyl jasmonate were applied to increase the phytochemical content of broccoli and radish shoots, low MeJa concentrations increased the glucosinolate content, and high concentrations reduce it ⁹⁷. In another study conducted to increase the nutritional value of broccoli shoots, methyl jasmonate at 10 and 25 µM concentrations increased the vitamin C amount, total glucosinolates, caffeoyl-quinic acid derivatives, flavonoids, sinapic and ferulic acid derivatives and total phenolics of plants. However, in terms of phytochemical content 25 µM MeJA concentration and 10mM MeJA concentration gave similar results. This is evidence that low elicitor concentrations will give better results than higher concentrations to increase the phytochemical content of plants ⁹⁸. MeJa (100 μM) treatment in light and dark environment in "Zi Ying" mustard (Brassice junca var. Tumida Tsen et Lee) plants significantly increased the anthocyanin accumulation of the plants⁹⁹. In general, studies show that MeJa application increases the phytochemical content of plants.

2. OBJECTIVES

The main objective of the project was to study the cultivation of Sea fennel (*C. maritimum*) in floating system under greenhouse conditions determining the salinity and methyl jasmonate effects on plant growth and postharvest quality. For this, partial objectives were:

- To determine the effect of salinity on growth, harvest quality and shelf life during storage as fresh-cut product.
- To determine the effect of methyl jasmonate on growth, functional elements and shelf life during storage as a fresh-cut product of plants grown under salinity.

3. MATERIAL and METHODS

3.1 Cultivation and Experiment Design

The experiments were conducted during the autumn and spring seasons of 2019 and 2020 at the Technical University of Cartagena, Spain (UPCT; lat. 37° 41' N; long. 0° 57' W). Plants

were grown in an unheated 145 m² greenhouse covered with thermal polyethylene. Aeration was provided and each level of treatment was carried out in a stainless steel flotation bed with dimensions 1.35 × 1.25 × 0.2 m covered with PVC liner. The tap water in the beds was replaced with a nutrient solution (pH of 5.8 to 5.6 and EC around 2.8 dS/m), containing the following elements in mol/L: NO3⁻, 7200; NH4⁺, 4800; H2PO4⁻, 2000; K⁺, 6000; Mg²⁺, 1500; Ca²⁺, 2000. A commercial mixture of microelements at a concentration of 0.02 g L⁻¹ (Nutromix, Biagro S.L., Valencia, Spain) and Fe chelate at a concentration of 0.02 g L⁻¹ (Sequestrene, Syngenta AG, Basel, Switzerland) were added to the solution. The EC and temperature of the nutrient solution were monitored during the growing season using Campbell CS547 sensors (Campbell Scientific Inc., Logan, UT, USA). At harvest, a total of 10 plants per tray were taken and shoot and roots separated for measurements and keep into N₂ liquid for subsequent lyophilization.

3.2 Experiment 1: Effect of Salinity on Postharvest

The plants were grown for 1 month in normal conditions (control) and then the salinity treatment was applied for 1 month. Seeds were sown in 60 x 40 cm styrofloat trays and 4 plants were left per fissure (400 plants/ha). The trays of 60 × 41 cm have pyramidal-trunk 172 mm long fissures 20 mm apart and grouped in three for a total of 42 fissures per tray; fissures measure 10 mm on the top and 2.5 mm on the bottom, leading to a volume of 32.4 cm³ per fissure. After sowing the trays were then transferred to flotation beds floating on fresh tap water. Nutrient solution was replaced weekly and pH measured every three days and adjusted to 6.5. The plants were harvested when they were 2 months old. Two treatments were considered, control plants (Con) and plants treated with 150 mM NaCl (NaCl) for 1 month. Each treatment had three replications. Growth and yield parameters (root parameters), mineral ion concentrations (anions and cations) and postharvest quality (% weightloss, chromaticity, HUE, CO₂, O₂, sensorial quality, microorganisms) were determined.

3.3 Experiment 2 : Effect of MeJa in Salt-Stressed Plants

In this experiment, polystyrene trays containing 54 cells were used. Every cell contained 2 plants. Thus, the plant density was around 400 plants/ha. Five treatments were considered; control plants (Con1), control plants spraying with Tween 20 (1ml L⁻¹) + 0,2% EtOH (Con 2), addition of 150 mM NaCl to the nutrient solution (NaCl), spraying with 0.5 mM methyl

jasmonate (MeJa) and 150 mM NaCl + 0.5 mM MeJa (NaCl + MeJa). MeJa was diluted in Tween 20 1 ml por L+ 0.2 EtOH in order to facilitate MeJa leaf penetration . NaCl was applied to the nutrient solution whereas an amount of 100 mL of MeJa was sprayed to the leaves three times (every 10 days). Nutrient solution was replaced weekly and pH measured every three days and adjusted to 6.5. A total of 2 replicates per treatment were carried out. Yield and quality analysis, fresh and dry weights of shoots, root parameters and stem length, shoot colour, clorophyll and carotenoids, content of ascorbic acid, phenolics compunds and antioxidant capacity as DPPH were determined.

3.4 Fresh and Dry Weights of Shoots and Roots

The fresh weight of roots and shoots was measured on a scale (Model: RADWAG PS 4500/C2) that had the level of accuracy of 0.0001 g. The dry weight of shoots and roots were determined by drying in an oven at 60°C until constant weight.

3.5 Root Parameters

Root length (cm), project area (cm²), surface area (cm²), average diameter (mm) and root volume (cm³) parameters were measured using a Winrhizo LA 1600 root counter (Regent Inc., Quebec, Canada) ¹⁰⁰.

3.6 Colour

Colour measurements were performed using a colorimeter. Color measurements was measured on six plants per replicate using a colorimeter model (Minolta CR-10; Konica-Minolta Sensing Inc., Osaka, Japan) equipped with the a^* (redness) b^* (yellowness) colour scale, according to the procedure described by Montesano et al. ⁷¹ ¹⁰¹. HUE angle (H*) as H*= tan-1 (b^*/a^*) and chromacity (C*) as C* = ($a^*2 + b^*2$) were also calculated ⁷¹.

3.7 Sensory and Visual Quality Analysis

The sensory quality was evaluated in a tasting room after 7 d of cold storage by a test panel consisting of 11 people. Visual quality factors (overall visual quality and global quality) were scored on a 9-point hedonic scale (l=extremely poor, 3=poor, 5= acceptable and limit of usability, 7= good, and 9= excellent). Disorders (browning visual dehydration, off-odors, off-color, and off-flavors) were scored according to the following scale of damage incidence and severity: 1= none, 2=slight, 3=moderate (limit of usability), 4=severe, 5=extreme ¹⁰².

3.8 Contents of Mineral

0.2~g DW of ground shoot tissue was mixed with 50 ml distilled water in an orbital shaker (Stuart SSL1, Stone, UK) for 45 min at $110~\rm rpm$ at $50~\rm ^{\circ}$ C. While Metrosep A SUPP 5 column (Metrohm AG, Zofingen, Switzerland) was used for flow anions at $0.7~\rm ml~min^{-1}$ flow rate, Metrosep C 2-250 column was used at $1.0~\rm flow~min^{-1}$ flow rate for cations to apply ion chromatography method 103 .

3.9 Phenolic Compounds

To determine phenolics analysis, the methodology of Tarazona-Díaz et al. 104 was performed 104 . Samples of 0.5 gram of FW. sample was centrifuged at 1,200 x g for 15 min (Heraeus Fresco 21; Thermo Scientific, Osterode, Germany) at 4 °C using 3.0 ml 100% (v / v). The FolinCiocalteu colorimetric method was chosed to determine the phenolic content. The procedure of Singleton and Rossi 105 was used for this purpose. A (0.1 ml) aliquot of the extract supernatant, (0.15 ml) of FolinCiocalteu reagent, and (1.0 ml 4 g l $^{-1}$) NaOH / (20 g l $^{-1}$) Na₂CO₃ were mixed. Absorbance was measured at 750 nm by spectrophotometric (SmartSpecTM Plus; BioRad Laboratories, Inc., Hercules, CA, USA) method. Results (CAE) were expressed in kg $^{-1}$ FW chlorogenic acid 103 .

3.10 Chlorophylls and Carotenoids

For chorophyll determination, 50 gr of lyophilised sample in 1 ml de methanol was used. For the analysis of chlorophyll and carotenoids, firstly the samples were extracted using 1 ml of methanol and 100 mg of sample (samples were triturated with liquid N_2). After mixing with vortex, extract was incubated overnight at 4 °C. Then, 16 g of extract was centrifuged at 4 °C for 5 minutes and methanol extract was obtained. Chlorophyll a and b and total carotenoids were measured by the method determined by Lichtenthaler and Buschmann 106 . Measurement was made with ethanol extracts. Spectrophotometric measurements were carried out by measuring the absorbances at 665, 652, and 470 nm and mathematical expressions corresponding to methanol as the solvent was applied 106 .

3.11 Flavonoids

Method of Zhishen was used to determination of total flavonoid content 107 . In order to determine the total flavonoid content, $100 \,\mu\text{L}$ of the hydro-methanolic extract were dissolved

using 400 microliters of pure water. 30 μ L of 5% sodium nitrite and 30 μ L of 10% aluminum chloride and 200 μ L of 1M sodium hydroxide was added to the solution, at intervals of 5 minutes. The volume of the mixture was completed to 1 ml by adding pure water. The absorbance of samples as measured at 510 nm. The results were expressed in mg of rutin equivalents per kilogram of fresh weight (mg Rutin kg-1 FW)⁷⁸.

3.12 Antioxidant Capacity

The antioxidant capacity was evaluated in terms of free radical-scavenging capacity ¹⁰⁸ with the modifications described by Perez-Tortosa et al. ¹⁰⁸.

3.13 Shelf Life During Storage Analysis

Shelf life and storage of C. maritimum conditions were tested. For that, plants were transported immediately after harvesting to a disinfected cold room at 10 °C, where all shoots free from defects were taken. They were disinfected washing for 2 min with a solution containing 100 ppm NaOCl and 0.2 g. 1.-1 citric acid. Then, the shoots were rinsed with tap water to eliminate chlorine residues. Excess surface water was removed by using a handheld salad spinner for the 30s. Then, 20 g of shoots were placed in 0.04-mm thick polyethylene bags, and the control group was stored at 5°C in dark condition for 7 days. Microbial growth was assessed after 7 days of storage. Samples of 10 g fresh weight (FW) from each treatment was blended with 90 ml of sterile tryptone phosphate water at pH 7.0 for 1 min in a sterile bag by using a stomacher as reported in Niñirola et. al., ¹⁰⁹. Serial dilutions were prepared in 9 ml tryptone phosphate water. From each dilution, 1 ml aliquots were aseptically pipetted for microbial population counting. Plate count agar (PCA) for both mesophilic aerobic microorganisms, incubated at 26 o c for 3 d, and psychrophilic microorganisms, incubated at 40 C for 10 d, was used. Microbial counts will be reported as long as 10 colony-forming units (CFU) per gram of FW. Changes in O2 and CO2 levels were determined by gas chromotography.

3.14 Statistics

Statistical analyzes were made with IBM SPSS Statistics 20 program. Analysis of variance was done using ANOVA. The differences were set at the 5% level. Results are expressed as mean and standard deviation.

4. RESULTS

4.1 Experiment 1: Effect of Salinity on Growth Postharvest Parameters of *C. maritimum*

4.1.1 Biomass, Leaf Area and Root Growth Parameters

Biomass, determining as fresh weight per plant did not show significant differences between under salinity and non-salinity growth conditions. Similarly, leaf area remained unchanged after NaCl addition with regard control plants, showing the ability of *C. maritimum* to tolerate saline environmental conditions (Table 1).

Table 1. Effect of 150 mM NaCl on fresh and dry weight of whole plant and leaf area (n=20).

Sample	Weight (gr/plant)	Leaf area (cm²/plant)
Control	$2.1 \pm 0.25a$	$3.43 \pm 0.38 \ a$
NaCl	$2.45 \pm 0.27a$	$2.54 \pm 0.17a$

Although numerical values were higher for control plants, no significant differences were found in the root growth parameters, between 0 and 150 mM NaCl-treated plants (Table 2).

Table 2. Effect of 150 mM NaCl on root growth parameters (length, root area, root diameter and root volume) (n=20).

Root Parameters	Length (cm)	SurfArea (cm²)	AvgDiam (mm)	RootVolume (cm ³)
Control	546,73±53,86a	63,46±6,44a	0,38±0,01a	0,60±0,07a
NaCl	541,16±45,11a	54,51±4,03a	0,33±0,02a	0,46±0,05a

4.1.2 Mineral Ion Concentrations (Anions and Cations)

Exposure of *C. maritimum* plants to salinity caused a decrease in the concentration of NO_3^- , NO_2^- , Br^- , SO_4^{2-} anions compared to control plants, while an increase in the concentration of C1- PO_4^{3-} anions was observed. However, oxalate $(C_2O_4^{2-})$ concentration remained unaltered after salinity addition with respect to control (Table 3). It has been observed that concentrations of K $^+$, Ca $^+$, and Mg $^+$ decreased with saline treatment while concentrations of Na $^+$ and NH_4 $^+$ increased (Table 4).

Table 3. Effect of 150 mM NaCl Salinity on anions content (n=10).

ANIONS

	NO ₃ -	NO ₂ ·	Cl.	Br ⁻	PO ₄ ³⁻	SO ₄ ² -	C ₂ O ₄ ² -
Treatment	(mg/kg FW)	(mg/kg FW)	(mg/kg FW)	(mg/kg FW)	(mg/kg FW)	(mg/kg FW)	(mg/kg FW)
Control	1530,31±586,47a	743,69± 525,85a	1810,97±120,56b	152,00±2,82a	947,01±94,14a	1699,55±32,28a	88,19±33,59a
NaCl	1263,03±19,79b	203,83±177,23b	6718,18±1029,31a	125,98±3,46b	1154,39±142,73a	671,25±47,66b	88,70±28,87a

Table 4. Effect of 150 mM NaCl Salinity on cations content (n=10).

CATIONS

Treatment	Na ⁺ (mg/kg FW)	K+(mg/kg FW)	NH4 ⁺ (mg/kg FW)	Ca ²⁺ (mg/kg FW)	Mg ²⁺ (mg/kg FW)
Control	777,57±54,46a	3642,49±109,48a	50,92±20,89b	1108,08±20,91a	379,07±12,46a
NaCl	4639,88±703,02b	1070,45±345,86b	425,31±49,74a	532,05±143,54b	203,42±10,47b

4.1.3 Postharvest Quality

4.1.3.1 Weightloss Percentage of C. maritimum Plants

It was observed that the exposure of plants to salinity increased weightloss with regard to control plants. Percentage of weightloss at day 6 was higher than at day 12 for both treatments (Table 5).

Table 5. Effect of 150 mM NaCl on % weightloss (n=20).

%Weightloss

Treatment	Day 6	Day 12
CONTROL	1,45±1,49b	0,32±0,16c
NaCl	2,74±1,67a	1,24±0,38b
Significance (1)		
Treatment ***		-
Day of Storage ***		-
Treatment x Day	Storage *	-

⁽¹⁾ Significance of F-test: *** and ns, respectively, significant for $P \le 0.05$ and $P \le 0.001$.

4.1.3.2 L, a and b Values

Considering the change of colour parameters, it has been observed that the L value in control plants ranged from 35.79 to 37.96, a value ranged from -11.44 to 10.63 and the value of b ranged from 17.33 to 19.11. In plants exposed to salinity, L value varied from 38.12 to 40.12, a value from -11.13 to 11.04 and b value from 19.25 to 21.99. From the statistic analysis no

significative effect of day of storage was observed in colour parameters in any treatment. However, salinity increased L values at all storage days (Table 6). Intereaction between treatment and days of storage showed no significant differences.

Table 6. Effect of NaCl on L, a, and b values (n=10)

Colour	Treatment		Control		NaCl
	L	3	57,84±1,91ab	3	9,17±0,89ab
Day 0	a	-	11,44±0,95a	-	11,13±1,32a
_	b		18,51±3,13a		21,99±0,41a
	L		35,79±1,35b		40,12±1,16a
Day 6	a	10,63±1,16a		11,04±0,69a	
	b		17,33±3,71a		20,63±1,73a
	L	3	57,96±2,41ab	3	8,12±0,88ab
Day 12 —	a	-	11,86±1,52a		10,66±0,44a
	b		19,11±3,36a		19,25±1,45a
Significance (1)		L	A	В	
Treatment		*	Ns	Ns	
Day of Storage		ns	Ns	Ns	
Treatment x Day storage		ns	Ns	Ns	

⁽¹⁾ Significance of F-test: * and ns, respectively, significant for $P \le 0.05$

4.1.3.3 HUE Values

Regarding HUE values, no significance differences were observed between control and NaCl-treated plants at any day of storage. Similarly, no effect day was showed in each treatment (Table 7).

Table 7. Effect of NaCl on HUE values (n=10).

HUE °

Treatment	Day 0	Day 6	Day 12
Control	122,08±3,22a	121,89±1,74a	121,96±0,86a
NaCl	116,78±1,38a	118,18±0,29a	119,06±1,62a
Significance (1)			
Treatment		ns	
Day of Storage		Ns	
Treatment x Day	storage	Ns	

⁽¹⁾ Significance of F-test: ns, not significant for $P \le 0.05$.

4.1.3.4 Chromaticity Values

Chromaticity values remained unchanged with the time of storage in both treatments (Table 8). However, at day 0, chromacity was higher in NaCl-treated plants compared to control plants, whereas an increase in the days of storage (6 and 12d) reported no significant differences between the Chromacity values of control and NaCl-treated plants (Table 8).

Table 8. Effect of NaCl on chromaticity values (n=10)

Chromaticity			
Treatment	Day 0	Day 6	Day 12
Control	21,83±1,48a	22,47±0,78a	22,50±2,10a
NaCl	24,66±0,53a	23,40±1,07a	22,02±0,62a
Significance (1)			
Treatment	*	•	
Day of Storage	ľ	Ns	
Treatment x Day sto	orage r	ıs	

(1) Significance of F-test: * and ns, respectively, significant and not significant for $P \le 0.05$.

4.1.3.5 CO₂ and O₂ Values of C. maritimum Plants

The CO₂ and O₂ values did not show significant differences between control and NaCl-treated plants (Table 9). However, while CO₂ was enhanced as time of storage was increased in both treatments, O₂ was reduced during the period of storage.

Table 9. Effect of NaCl to CO₂ and O₂ values of *C. maritimum* plants (n=10)

Atmosphere		CO_2			O_2	
Treatment	Day 0	Day 6	Day 12	Day 0	Day 6	Day 12
NaCl	0,03d	4,8±0,4c	9,7±0,3a	21a	16,2±0,4b	11,3±0,3d
Control	0,03d	5,2±0,8c	8,1±0,6b	21a	15,8±0,8b	12,9±0,6c
Significance (1)	CO ₂	O ₂				
Treatment	ns	ns				
Day of Storage	***	***				
Treatment x Day storage	*	*				

⁽¹⁾ Significance of F-test: ***, * and ns, respectively, significant for $P \le 0{,}001, P \le 0{,}05$ and not significant for $P \le 0{,}05$.

4.1.3.6 Sensorial Quality of C. maritimum Plants

The sensory quality of *C. maritimum* plants were evaluated at days 0, 6, and 12 of storage. At day 0, the visual appearance of the plants was excellent, while the NaCl treated plants

had a good visual acceptability. Visual appearance quality was decreased for both treatments as the period of storage increased, being lowest at 6 day than at 12 day. In general, the visual appearance of the control plants was better than NaCl-treated plants (Table 10). No changes were observed in the color parameter of the plants at days 0, 6, and 12, for any treatment. The colour determined a good acceptability for all plants (Table 10). The texture (crisp) was "perfect" in both control and NaCl treated plants at day 0. However at day 6, control plants had a "good" texture and NaCl-treated plants has a "normal" texture. At day 12, both plants (0 and 150 mM NaCl) had similar and "normal" texture. Flavor (freshness) was determined as perfect for control plants at day 0 and good for NaCl-treated plants. At day 6 and 12, the flavor (freshness) was clasified good and normal, respectively, for both groups of plants. Salinity or storage time had no effect on the aroma of the plants and in all conditions, the aroma of *C. maritimum* plants was "excellent". Global acceptability, was better for control plants than NaCl-treated plants during the entire storage period, but this value decreased from day 0 to day 12.

Table 10. Effect of NaCl on sensorial quality (n=10).

Sensorial Quality

	Day	7 0	Da	ay 6	Da	y 12
	CONTROL	NaCl	CONTROL	NaCl	CONTROL	NaCl
Acceptability						
Visual Appearance	5	4	4,5	4	4	3,5
Colour	4	4	4	4	4	4
Texture (Crisp)	5	5	4	3,5	3	3
Flavor (Freshness)	5	4	4	4	3	3
Aroma	5	5	5	5	5	5
Global Acceptability	5	4,5	4	3,5	3,5	3
Alterations						
Strange Smells	5	5	5	5	5	5
Mechanical Damage	4	4	4	4	3,5	3,5
Observations		Salty taste, on a scale of 1 to 10 as salty, 7.5		Follow the salty taste, the same		İgual a dequa

Acceptability:1=Very bad, 2=bad, 3=Normal, 4=Good, 5=Excelllent Alterations:1=Extreme presence,2= Important presence, 3=Acceptable, Commercialization Limit, 4= Slight Presence, 5= Absence

4.1.3.7 Firmness

Salinity in general decreased firmness in all days of storage excepting at day12 where no significant differences were found between control and salinity firmess values. The day of sotrage had no effect on the vegetable firmess in any of the treatments.

Table 11. Effect of NaCl on firmness of *C. maritimum* plants during storage (0, 6, 12 days).

Firmness						
Treatment	Day 0	Day 6	Day 12			
Control	576,79±63,84a	594,79±63,39a	617,23±58,57a			
NaCl	412,17±43,34b	435,30±46,12b	526,37±63,27ab			
Significance (1)						
Treatment	*		-			
Day of Storage	ns		-			
Treatment x Day storage	ns		-			

(1)Significance of F-test: * and ns, respectively significant and not significant for $P \le 0.05$.

4.1.3.8 Microbial Quality

Microbiological analyzes were carried out determining psychrophiles, yeast and moulds, and enterobacteria at 0,6, and 12 days storage for control and NaCl-treated plants (Table 12). At all storage days, the number of psychrophiles, molds and yeasts, enterobacteria were higher in the control plants than in the plants exposed to NaCl. Therefore, salinity reduced the number of microorganisms with the exception of mesophiles which were similar for both treatments. The effect of storage day was similar in control and NaCl-treated plants where the levels of microrganisms were increased.

Table 12. Effect of salinity for the number of psychrophiles, mesophyll, mold, and yeast, enterobacter microorganisms of *C. maritimum* plants during the 0, 6 and 12 days storage period (n=10).

		Day 0	Day 6	Day 12
Psycrophile	Control	5,00±1,12b	6,06±0,18ab	6,40±0,00a
-	NaCl	3,48±0,13c	5,92±0,22ab	6,37±0,05a
Mesophile	Control	4,22±0,15c	5,67±0,31b	6,31±0,01a
•	NaCl	3,88±0,27c	5,55±0,16b	6,30±0,00a
Mold and Yeast	Control	3,28±0,55bc	3,95±0,27ab	4,30±0,26a
·	NaCl	2,91±0,13c	3,71±0,20abc	3,40±0,17bc
Enterobacteria	Control	3,69±0,47c	5,62±0,39ab	6,14±0,17ab
·	NaCl	0,00±0,00d	5,42±0,30b	6,29±0,16a
Significance (1)	Psycrophile	Mesophile	Mold and Yeast	Enterobacteria
Treatment	*	ns	**	***
Day of Storage	***	***	**	***
Treatment x Day storage	*	ns	ns	***

4.2 Experiment 2: Effect of MeJa in Salt-Stressed C. maritimum Plants

4.2.1 Biomass and Root Growth Parameters

Leaf biomass, as edible and comercial part of the plant, was evaluated determining fresh (FW) and dry (DW) weights after treatments addition (Table 14). The higher FW was observed in Con1 plants without significant differences regarding Con2. NaCl reduced the FW of *C.maritumum* plants while MeJa partially recovered the FW in NaCl+MeJa-treated plants compared the only NaCl addition, reaching values similar to Con2. The only MeJa addition caused a reduction in FW regarding Con1, but not with respet Con2. In a similar way, DW was higher in Con1 plants and it was signifivative reduced by NaCl with regard the rest of treatments. MeJa addition enhanced DW compared with the only NaCl treatment and NaCl+MeJa-treated plants showed similar DW values than Con2 and MeJa plants. Finally, the colour parameter did not show significant changes in any of the treatments.

Table 13. Fresh weight (FW), dry weight (DW), and of the aerial part of *C. maritimum* plants grown under hydroponic conditions with the following treatments; Con1, Con2, MeJa, NaCl-MeJa, NaCl (n=20).

	FW	\mathbf{DW}	Chlorophyll Index (SPAD)
Control 1	11,51 ±0,77a	1,33 ±0,09a	65,86 ±0,85a
Control 2	$8,92 \pm 0,81 ab$	1,03 ±0,09b	65,08 ±0,92a
MeJa	7,21 ±0,43b	0,83 ±0,05b	$66,49 \pm 0,57a$
NaCl	5,25 ±0,49c	$0,60 \pm 0,06c$	65,72 ±0,64b
NaCl+ MeJa	7,94 ±0,55b	0,91 ±0,06b	65,82 ±0,61a

Root length and root area were higher in NaCl-treated plants compare with the rest of treatments, which did not show significant differences in these parameters (Table 13). Root diameter remained unmodified after the treatment applications. However, root volume was significantly higher in NaCl-treated plants in relation to Con1- and MeJa-treated plants.

Table 14. Root growth parameters (Length, surface area, average diameter, root volume) of *C. maritimum* plants grown under hydroponic conditions with the following treatments; Con1, Con2, MeJa, NaCl-MeJa, NaCl (n=20).

Treatment	Length (cm)	SurfArea (cm ²)	AvgDiam (mm)	RootVolume (cm ³)
Control1	222,48 ±38,19b	35,46 ±5,66b	0,54 ±0,04a	0,49 ±0,08b
Control2	$207,84 \pm 29,75b$	38,99 ±5,98b	0,60 ±0,04a	$0,62 \pm 0,12ab$
MeJa	207,90 ±25,61b	33,14 ±4,64b	0,50 ±0,03a	0,46 ±0,086b
NaCl-MeJa	219,14 ±37,8b	44,25 ±6,69ab	0,65 ±0,05a	0,75 ±0,12ab
NaCl	347,57 ±49,33a	65,05 ±8,59a	0,63 ±0,05a	$1,01 \pm 0,13a$

4.2.2 Mineral Ion Concentrations (Anions and Cations)

Cations (Mg⁺², Ca⁺², NH4⁺ and Na⁺) and anions (F⁻, Cl⁻, NO₂, Br⁻, NO₃⁻, PO₄⁻,C₂O₄²⁻) were determined in the leaves of *C. maritimum* (Table 15). Regarding cations, Mg²⁺ content was higher in NaCl + MeJa plants compare with the only NaCl-treated plants but no significant differences in Mg²⁺ concentration were found in Con1, Con2, MeJa and NaCl+MeJa plants. Ca²⁺ levels were similar in all treatments with the exception of NaCl treated plants where Ca content was decreased. K⁺ content was similar in Con1, Con2 and MeJa plants and it deceased in NaCl and NaCl+MeJa treated plants. The two MeJa treatments increased the NH4⁺ content regarding Con1, Con2 and MeJa treatments, which did not show significant differences between them in the NH4⁺ concentrations. Finally, Na⁺ was higher in NaCl treatments compared with Con1, Con2 and MeJa plants.

Table 15. Cations (Mg⁺², Ca⁺², K⁺, NH4⁺, and Na⁺) (mg Kg⁻¹ FW) of *C.maritimum* leaf tissues under the different treatments (Con1, Con2, MeJa, NaCl, NaCl+MeJa), (n=10).

Cationes	\mathbf{Mg}^{+2}	Ca^{+2}	\mathbf{K}^{+}	NH4 ⁺	\mathbf{Na}^{+}
Control1	$309,00 \pm 6,09ab$	997,72 ±10,036a	2411,01 ±48,78ab	277,59 ±23,13a	977,27 ±70,43b
Control2	299,25 ±20,21ab	901,05 ±53,26a	2295,72 ±125,96ab	251,19 ±14,84a	$1086,10\pm67,04b$
NaCl	255,03 ±53,10b	692,83 ±36,78b	1628,10 ±113,72c	384,90 ±15,00ab	2698,07 ±16,54a
MeJa	283,91 ±12,15ab	926,53 ±28,21a	2901,40 ±121,81a	449,18 ±104,60b	1308,48 ±51,43b
NaCl+ MeJa	$342,78 \pm 10,98a$	1044,16 ±88,79a	2006,77±134,36b	447,61 ±48,50b	2903,18 ±44,96a

Regarding anions, F⁻ and NO₃⁻ content did not show significant differences between treatments in the leaves (Table 16). Cl⁻ was higher in the NaCl and NaCl+ MeJa treated plants compared with the rest of treatments. NO₂⁻ was only decreased in NaCl⁻ treated plants and Br⁻ was increased in both MeJa treatments with regard the rest of treaments. PO₄³- was enhanced in MeJa and NaCl+MeJa-treated plants but the increment was higher in

NaCl+MeJa plants. Finally $C_2O_4^{2-}$ was similar in both control treatments but it was increased in NaCl treated plants.

Table 16. Anions (F-, Cl⁻, NO₂-, Br⁻, NO₃-, PO₄³-, C₂O₄²-) (mg Kg⁻¹ FW) of *C.maritimum* leaf tissues under the different treatments (Con1, Con2, MeJa, NaCl, NaCl+MeJa), (n=10).

Aniones	F -	Cl ⁻	NO_2	Br ⁻	NO ₃ -	PO ₄ ³ -	$C_2O_4^{2-}$
Control1	$20,\!88 \pm \!0,\!97a$	2285,63 ±162,74b	$963,25 \pm 8,94a$	$109,62 \pm 0,90b$	296,95 ±23,32a	944,38 ±25,93c	116,46 ±4,42b
Control2	25,83 ±4,56a	2515,93 ±104,79b	845,95 ±36,81a	105,87 ±0,86b	229,12 ±8,13a	911,26 ±25,64c	108,28 ±1,11b
NaCl	21,71 ±0,87a	4385,71 ±31,93a	453,87 ±90,22b	109,38 ±0,67b	255,84 ±9,10a	940,02 ±14,36c	134,28 ±1,89a
MeJa	25,83 ±1,25a	2840,38 ±4.00b	970,44 ±12,22a	120,20 ±1,40a	$276,49 \pm 1,66a$	1082,62 ±23,03b	128,90 ±7,94ab
NaCl+ Meja	24,81 ±0,75a	4986,10 ±39,67a	954,57 ±15,37a	132,47 ±0,81a	292,59 ±0,74a	1278,91 ±20,64a	126,25 ±6,58ab

4.2.3 Total Phenolic Compounds, Flavonoids and Antioxidant Capacity

Total phenolic compounds were determined in the leaves of *C.maritimum* plants (Table 17). The content of total phenolic compounds was smilar in Con1, Con2 and MeJa plants but it was incrased in NaCl- and NaCl+MeJa- treated plants.

Table 17. Total phenolic compounds (GAE mg Kg-¹ FW), Flavonoids (mg Rutin kg-1 FW), antioxidant capacity (mg DPPH Kg-1 FW) of *C.maritimum* leaf tissues under the different treatments (Con1, Con2, MeJa, NaCl, NaCl+MeJa), (n=10).

Treatment	Total Phenolic Compounds (GAE mg Kg ⁻¹ FW)	Flovanoids (mg Rutin kg-1 FW)	Antioxidant Capacity (mg DPPH Kg-1 FW)
Con 1	891,79±15,79a	1965,41 ± 47,17 b	113,64 ± 7,17 a
Con 2	883,09±8,11a	$1968,\!39\ \pm 6,\!30\ b$	110,85 ± 5,47 a
NaCl	833,53±9,42b	$2167,24 \pm 22,09a$	$109,92 \pm 5,84$ a
MeJa	901,50±9,71a	$2186,94 \pm 5,97$ a	108,81 ± 1,77 a
MeJa+NaCl	844,40±7,08b	$2273,53 \pm 0,60$ a	$117,78 \pm 1,09$ a

Flavonoids content was increased in NaCl, MeJa and NaCl+MeJa treates plants with regards both controls.

Finally, no significant differences were found in total antioxidant capacity between treatments (Table 17).

4.2.4 Chlorophylls and Carotenoids

Clorophyll a was higher in MeJa-treated plants regarding the rest of treatments, while a reduction in clorophyll b was induced by MeJa in both MeJa and NaCl+MeJa treatments. Finally, carotenoids were increased in MeJa treated plants.

Table 18. Chlorophyll a (mg/dry weight), chlorophyll b (mg/dry weight) and carotenoids (mg/dry weight) of *C.maritimum* leaf tissues under the different treatments (Con1, Con2, MeJa, NaCl, NaCl+MeJa), (n=10).

Treatment	Ca (mg/ dry weight)	Cb(mg/ dry weight)	C(X+C) (mg/ dry weight)
Con1	12,68 ±0,05 a	7,51 ±0,40 a	2,43 ±0,09 b
Con2	15,41±0,23 a	7,92 ±0,03 a	2,62 ±0,03 b
NaCl	16,13 ±0,45 a	8,85 ±0,29 a	2,86 ±0,21 b
MeJa	17,77 ±0,05 b	4,98 ±0,03 b	4,25 ±0,00 a
NaCl + MeJa	14,22 ±0,01 a	4,24 ±0,08 b	3,40 ±0,01 ab

5. DISCUSSION

5.1 Experiment 1: Effect of Salinity on Postharvest Quality of C. maritimum

It is known that floating systems are an excellent medium for producing ready to eat vegetables with high quality, efficiency, economy, and bioavailability 110. Similarly, the growth and development of C. maritimum in floating systems was adequated and no nutritional deficiencies symptoms were observed in the plants. It has been reported that C. maritimum plants can tolerate extreme salinities ranged from 0 to 512 mM NaCl ¹¹¹. These authors showed that the percentage of survival remained unchanged until 171 mM NaCl in an Algerian C. maritimum population and significant reductions of shoot height were observed at \geq 341 mM NaCl. Similarly, in our *C. maritimum* plants from South Spain, no effect on the biomass and root growth parameters were observed when plants were grown in hydroponics at 150 mM NaCl. This fact corroborates the idea that C.maritimum is a facultative halophyte, since plants did not require salinity to reach a maximal growth. However, Ben Hamed et al. (2004) showed a reduction in the biomass, the leaf number and total leaf area in a Tunisian C. maritimum population after 5 weeks of 150 mM NaCl treatment. These results pointing out the importance of the genotype in the salinity tolerance acclimatation. Mineral analysis showed important restrictions of salinity in nutrients such as K, Ca, Mg, NO₃- and SO₄²-acquisition, which could be due in part to the inhibition of ion transport by salinity rather than a reduction of root intrinsic growth and performance, as can be deduced of root growth parameters. In any case, mineral decreases did not limit the overall plant growth under salt stress and the observed increased in the leaf Na and Cl concentrations could be ameliorated by an ion compartimentation into the leaf vacuoles ¹¹². C.maritimum cells have high concentrations of Na + and Cl content. Na+ ions were accumulated by compartmentation in Na + vacuoles 113. Therefore, an osmotic effect of NaCl on plant nutrition together a reduced root to leaf translocation may explain leaf minerals redutions of some ions in our plants. The percetange of weightloss with storage time was higher in salineplants compare to control. However, this fact was not related with firmess, it was lower than control, but remained constant during storage days for salt-treated plants. Percentage of weightloss was in all cases lower than 3% and fresh horticultural products lose their fresh apparence with weightloss percentages higher than 3% ¹¹⁴. Similar results were found in Salicornia ramosissima and Sarcorconia perennis, two halophytes with low % weightloss percentages during storage time from 7 to 21 days ¹¹⁵. The present results showed that C.maritimum can be stored until 12 days without senescence effects. The acceptability of sensorial parameters of the *C.maritimum* saline-treated plants was slightly lower than control plants. C.maritimum has been used as a new spice with high sensorial evaluation of their attributes ⁶¹. The interest on wild species as new healthy vegetables and as links with the nature and traditional varieties, makes C.maritimum a valuable specie since a gastronomic point of view. It is known that salt stress may modify quality factors improving bioactive composition in halophytes 116 117 118. However, there is a lack of information about how a pre-harvest condition, such as salinity, may act on the maintenance of baby-leaf products shelf-life, especially in halophytes. In our experiment, the postharvest CO₂ and O₂ production was similar in salt-stressed fresh cut products compared to control. This result indicated that salinity did not accelerate respiratory metabolism neither intensifiy senescence processes. In cauliflower florets, salt stress (4.0 dS m⁻¹) increased both CO₂ and O₂ content from 0 to 7 days of storage, but after 7 days, control florets showed an acceleration in respiratory metabolism compared with the florets from saline treated plants. This fact could be due to the delay caused by salinity in the complex metabolic network that acts on postharvest biological decay ¹¹⁹. This was not the case of *C.maritimum* plants, a facultative halophyte, where 150 mM NaCl had similar effect than control plants on CO₂ and O₂ atmosphere. Microbial contamination is an important factor diminishing baby leaf quality ¹²⁰. In fact, several pre and post harvest strategies has been studied in order to cope with microbial contamination and maintain safety fresh leafly vegetables ¹²⁰. In general a lower

microbial level was observed in our plants under salinity as the storage period was increased. It has been reported that plants grown in soils contained a higher level of microorganisms than plants grown in hydroponics ¹²¹. Also, water irrigation during production was evaluated as a major potential risk for microbial baby leaf contamination ¹²² ¹²³. In this sense, the use of halophyte plants as *C.maritimum* growing in floating systems under salinity could represent an advantage.

5.2 Experiment 2: Effect of MeJa in Salt-Stressed C. maritimum Plants

Since salinity has an important problem that prevents the growth and development of plants, scientific studies have been conducted on this subject ¹²⁴. Although halophytes have developed different strategies to cope with salt stress 125 70 126, in our second experiment salinity reduced leaf biomass. These results are in consonance with previous reports, where C.maritimum plants irrigated with NaCl, from 100 to 500 mM NaCl, reduced their shoot fresh and dry weights 82. Differences in the effect of NaCl on biomass regarding the first experiment could be due the fact that in the first experiment the total plant biomass (aerial part and root) was determined, whereas in the second experiment leaf biomass as edible part (aerial part) of the plant was considered. Therefore the sum of both shows no differences between Control and NaCl as the first experiment. In fact, root length, root area and root volume was significantly higher in NaCl-treated plants with regard the rest of treatments in the second experiment. In any case, MeJa addition recovered the adverse effect of salinity on biomass. It is known that MeJa alleviated the salt stress in glycophyte plants as rice (Oryza sativa) 127 and B. napus 128, but less is known about the addition of MeJa under salinity in halophyte plants. In Limonium bicolor, exogenous 0,03 mM jasmonic acid (JA) improved plants biomass after 300 mM NaCl addition, demonstrating that the most revelant factor involved in plant growth and salt tolerance by MeJa, was net photosynthesis ⁹¹. This could be the case of C.maritimum plants, but other mechanisms of MeJa for salt stress alleviation may operate and further research is needed. Considering the recent interest of this halophyte as traditional agri-food product, the increase of the edible part of the plants by MeJa under salinity resulted of great interest and visually the colour chlorophyll index (SPAD) plant acceptance was not modified by any of the treatments. It is well known that salinity may restrict the mineral uptake by the roots ¹²⁹. This was the case of several cations in our plants as Ca²⁺ and K⁺, demonstrating that limitation in the nutrient uptake may contribute to the general decrease in the leaf growth. Hamed et al. 82 found a gradual reduction of Ca2+ and K⁺ ions with increasing salinity, being the decrase higher in leaves and stems compare to the root. However, nutrient decrase did not induce any necrosis or chorotic lesions in the leaves of our sea fennel plants and nutrient limitation may be due to an osmotic effect rather than a reduction in the root growth. MeJa recovered Ca²⁺ and K⁺ ions levels in the leaves of the plants treated with salinity. It has been shown that MeJa may induce root hydraulic conductance in maize 130 and tomato plants 131 and therefore a higher water uptake may result in higher Ca²⁺ and K⁺ uptake and translocation. In carrots plants, MeJa induced changes improving mineral balance under salt stress through a reduction in the Na⁺ and Cl⁻ accumulation ¹³². However, in our plants, Na⁺ and Cl⁻ content were similar in the leaves of NaCl and MeJa+NaCl treated plants indicating the importance of the genotype on the effect of MeJa in saline ions uptake. As a facultative halophyte, C.maritimum may tolerate Na⁺ and Cl concentrations that could be compartimentalized in the vacuole 126. In fact, in a glycophyte as tomato, a higher Cl⁻ content induced the reduction of nitrate uptake ¹³³. In sea fenel plants the levels of NO₃ were similar in all treatments, indicating the lack of toxicity of Cl⁻ regarding NO₃⁻ translocation, but NaCl reduced NO₂⁻ content. MeJa addition restored the level of NO₂ as well as increased NH₄ concentrations regarding Con1 and Con2 plants. This effect of MeJa was describe previously for Vaccinium myrtillus L. plants, where MeJa up-regulated genes involved on nitrite transport and metabolism ¹³⁴. There is a lack of literature of the effect of MeJa on halophytes and to our best of knowledge any report concerning to the combined effect of MeJa to salinity on C.maritimum, but a similar upregulation by MeJa on the genes involved in nitrogen metabolism could operate. In any case, the level of nitrate ions are lower than other baby leaf crops as lettuce (from 700 to 1264 mg Kg-1FW), spinach (from 700 to 2013 mg Kg-1FW), kale (from 600 to 1181 mg Kg-1FW) and chard (from 900 to 1024 mg Kg-1FW) ¹³⁵. Similarly, low levels of nitrite were detected with regard lettuce and spinach (up to 197.5 mg kg⁻¹) ¹³⁶. Acceptable daily intake (ADI) for nitrate determined by the Scientific Committee on Food (SCF) was 0 to 3.7 mg/kg body weight/day, this means that if the average adult daily consumes approximately 400 g of various vegetables, the intake of nitrate is around 222.0 mg/day (FAO/WHO 2013). In this sense, the consume of 100 gr of C.maritimum baby leaf plants did not reach the ADI for nitrate and nitrite. A high content of phenolic compounds has been described in the aerial part of *C.maritimum* plants compared to other crops ¹³⁷. A range from 200 to 700 GAE mg Kg-1 FW of total phenolic compounds was detected in lettuce under diffent LEDs light

conditions ¹³⁸. Other vegetables as caraway (770 GAE mg Kg⁻¹ FW), Chives (567 GAE mg Kg⁻¹ FW), Cowpea (717 GAE mg Kg⁻¹ FW), Packchoi (820 GAE mg Kg⁻¹ FW) and Perilla leaf (687 GAE mg Kg-1 FW) showed lower levels of total phenolic compounds than C.maritimum plants grown in floating systems ¹³⁹. Thus, although both saline treatments, NaCl and MeJa + NaCl decreased the content of total phenolic compounds regarding Con 1 and Con2 the plant maintained its nutritional properties under all treatments. A scientific study on Sweet basil (Ocimum Basilicum L.) was indicatived of MeJa alleviating the effects of salinity stress on phenolic compound¹⁴⁰ . The effect of salinity stress on chlorophyll a, chlorophyll b and carotenoid values was no effect much with MeJa application. A similar situation has been observed in the scientific study on maize (Zea maize L.)¹⁴². In the present experiment, C.maritimum showed a good ability to tolerate elevated NaCl concentrations with chlorophyll concentration remaining unchanged with respect to controls. Although NaCl and MeJa increased the flavonoid content, the maximum level was reached with NaCl-MeJa application. A similar report on balackberries (*Rubus sp.*) supported this situation ⁸⁷. The results of our research have shown that the phytochemical content of vegetables can be enriched with MeJa 143 144.

6. CONCLUSIONS

In conclusion, hydroponic floating systems was an adequate cultivation system that allows *C.maritimum* plants to grow as a new baby leaf product. NaCl had no effect on *C. maritimum* whole-plant growth but decreased the aerial part of the plant while increase root length and surface, pointing out the facultavtive character of this halophyte and 150 mM NaCl a thereshold salnity concentration affecting plant growth.

In spite of the edible part reduction, NaCl did not affect acceptance of the product and enhanced the shelf life of *C. maritimum* baby by reducing the number of microorganisms during storage time.

MeJa was considered for the alleviation of NaCl effects on plant growth. Thus, MeJa addition increased the biomass of the edible parts regarding the only NaCl treatment. Also, while NaCl restricted minerals that K^+ , Ca^+ , Mg^+ , NO_3^- and SO_4^{2-} from the roots, MeJa addition recovered Ca^+ and K^+ ion content, but it did not restrict leaf Na^+ and Cl^-

accumulation. This recovering in ion balance increased the tolerance of plants to 150 mM NaCl salt stress.

Regarding nitrite and nitrate content, consumption of 100 gr of *C.maritimum* plants did not reach the ADI (0 to 3.7 mg/kg body weight/day), making them suitable vegetables as new foodstuff.

NaCl-treatments decreased total phenolic content regarding control, but *C.maritimum* increased their flavonoids content and carotenoids under combined NaCl and Meja treatments. In any case, the amount of phenolics was optimal for consume consideration of these plants.

Therefore, the addition of MeJa to NaCl-treated *C.maritimum* has to be considered in this vegetable production, since MeJa addition to saline plants exert a recovery of edible part growth, minerals and flavonoids content.

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