An-Najah National University

Faculty of Graduate Studies

The Influence of Salinity on the Chemical, Physiological and Morphological Changes of *Rosemary*

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Dedication

To my husband for his support, love, and encouragement

To my kids for their patience for being far from me, as I was busy all the time

To my parents for helping, taking care and praying for me

To my family who supported me and shared my worries

To my colleagues at NARC for their continuous support

To all who prayed for me

To all whom I loved and knew

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إقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل العنوان:

The Influence of Salinity on the Chemical, Physiological and Morphological Changes of *Rosemary*

أقر أن ما شملته هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي لدي أي مؤسسة علمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: اسم الطالب: دنيا غسان الفاكر

Signature: التوقيع:

Date: التاريخ: 7/4/2021

List of Contents

List of Tables

List of Figures

The Influence of Salinity on the Chemical, Physiological and Morphological Changes of *Rosemary*

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Abstract

Salinity is a major factor that inhibits plant growth. This study was aimed to investigate the effects of salinity on plant growth, relative leaf water content, physiological - morphological and chemical composition of the extracted rosemary essential oil and the antioxidants under saline variable concentrations. A factorial arranged hydroponics experiment with three salinity levels of irrigation water (0.45,2,4,6,8 and 10 ms/m NaCl)were applied. Salinity was foundsignificantly decrease growth of rosemary (arrange between 1-5% of length and weight), but it showed positive effect on the concentration of chemical constituents as leaf and root Na^+ and Cl^- ions concentration was increased by salinity (Na^+ concentrations ranged from 7 to 8 ml/l and the chloride ion concentration was measured a range from 88 to 90 mg/l). Essential oil extract had higher concentration under saline stress(Essential oil extract had higher concentration under saline stress Ec10 (5g/L) about $5*10^{11}$unitofarea concentration).Characterization and evaluation of chemical composition with GC-MS for the plants under salinity stress compared with those fed with fresh water (essential oil and polyphenol content) ingredientswas achieved. A number of chemical constituents such as, total alkaloids, polyphenols, flavonoids, chlorophyll and carotenoid determinations of leaves were carried out using UV-Vis

instrument. The major constituents of the rosemary essential oil reported in literature being α- pinene, 1,8-cineol and camphor; associated with variable amounts of camphene, limonene, borneol, verbenone. The commonly known phytochemical compounds include acridone alkaloids, volatile substances, terpenoids, flavonoids and furoquinolines (a group of alkaloids with simple structure). Morphologicalchanges (leaf size, stem length, weight of green and dry matter) was also monitored for rosemary growth under saline environment in terms of the tolerance of the plants to the various salinity,the optimal growth under different salinity was shown at Ec4(3g/L) equivalent to 6 ms/ m, with average height 63 cm comparing with 56.5 cm height tap water irrigated plant, as so as similar results for all other morphological characters.

CHAPTER ONE
INTRODUCTION

1. Introduction

In recent years observed amazing interest of the scientific community for aromatic plant because could be noticed. Theyare an inexhaustible source of raw material for preparing some vital phytochemicals, extracting polyphenols, and superb source of essential oil. However, the growth of such herbal plants might be affectedby many variables. The impact and brutality of the environmental conditions plays an important and main role in shaping the crop production. Salinity stress is one of these main issues regulating agricultural production (Abdo,2020).

Salinization of soils or waters is one of the world’s greatest serious environmental difficulties in agriculture (Said-Al Ahl& Omer ,2011). In fact, almost 40% of the world’s land surface is affected by salinity-related problems (Monda & Kaur, 2017). A saline soil is commonly defined as one in which the electrical conductivity (EC) of the saturation extract (EC) in the root zone exceeds 4 dsm-1 roughly (40 mM NaCl) at 25 °C(Monda & Kaur, 2017).

Irrigation with salt water could denoteacceptance to improve the value of the raw materials of these evolving crops(Sany et al., 2020).On the other hand, waterquality continues to decline in arid and semi-arid area, so the use of saline water in agriculture now seems inevitable (Sarmoum et al., 2019).

The therapeutic plants have become vital in the global context today as it offers solutions to the major concerns of human mankind and used in many ways for treatments,especially with their numerous properties such as Phytol and antioxidants(Ayoob et al., 2018)(Said & Omer, 2011).

Herbal remedy is considered an essential part of the Palestinian believes and plays a fundamental and central role in the current public health care (Jaradat, 2005).A high percentage of Palestinian herb exports to Israel are re-exported under an Israeli brand name to western and other important target markets (D’Agnanno, 2015).Human being have used herbs as both as a food source and as medicine for at least several thousand years Ancient Arabic medicine was influenced by medicinal practices, (Abu-Rabia, 2005).Therapeutic plants are widely used in non-industrialized societies, mostly because they are readily available and cheaper than syntheticmedicines.

The yearlyworldwide export rate of the thousands of categories of plants with suspected medicinal possessionswas estimated to be US$2.2 billion in 2012. In 2017, the prospectiveworldwide market for botanical extracts and medicines was projected at several hundred billion dollars.

Aromatic and medicinal plants are still a key part of substitute and traditional medicine in the emergingcountries,several herbal therapies are presentlybroadly used in medicine (Abu-Rabia, 2005). The use of medicinal herbs as anti-inflammatory, antifungal, and palliative drugs is common in Algeria. In most cases, the active molecules of the herbs are unknown. Studying the biological and pharmacological properties of medicinal (Ullah et al., 2019). It is necessary to determine the environmental factors under which medicinal and aromatic plants give higher yields and better quality (Said-AL-Ahl & Omer, 2011).

Salt tolerant plant can be used to produce economically important materials such as antioxidant and essential oils(Kiarostami et al., 2010).The salinity tolerance depends on the interaction between salinity and other environmental factors (Sany et al., 2020).Medicinal and aromatic plants are cultivated for diverse plant parts and their active ingredients are used in many ways especially for drugs (Said-AL-Ahl & Omer, 2011). The progressive decline of water resources is leading towards the unavoidable use of saline water for irrigation resolutions with the risk of salt buildup in the root zone and consequential damage to crop production and soil fertility. Thus, the objective of the study is to design this irrigation experiment with saline water at different concentrations and applying it to rosemary plants shown in **Figure 1.1** below

Figure 1.1: Rosemary plant(Boutekedjiret et al., 2004)

1.2 Rosemary (*Rosmarinusofficinalis L.):*

Rosmarinusofficinalis L. is a woody aromatic shrub belonging to the *Labiatae*(*Lamiaceae*) family. It grows about one meter tall. The young branches are pubescent and turn woody uponmaturing. The leaves are simple, opposite, sessile, lineal, curvaceous, and perennial and their edges roll up. The upper and lower surfaces of the leaves are covered by secretory hairs that give the plant a pleasant camphoraceous aroma. The flowers are small, bilabiate, pale blue or lilac with violet spots, arranged in dense axillary or terminal clusters. They appear by the end of the spring or beginning of the summer. The fruit is a bright brown tetra-achene. Rosemary grows in the dry warm regions of southern Europe, especially the Mediterranean area. The wild forms grow on every kind of substrate up to 2.800 meters'altitude. The major rosemary producers are Spain, Tunisia, Morocco and, to a lesser degree, Portugal, Turkey, India and former Yugoslavia.

Rosmarinusofficinalis, is a plant native to the Mediterranean region Rosemary is a draught tolerant plant is a woody and aromatic herb (Boutekedjiret et al., 2004). That can live for 15-20 years. It is an evergreen shrub with an average height of 4 feet (1, 2 m). Rosemary has flowers of white, purple or blue color and fragrant, leathery leaves, which look like pine needle. Rosemary is a bee plant and attracts bees, as they enjoy rosemary pollen. It is propagated by seeds or cuttings during spring and prefers warm and moderately dry climates. The stems are collected during the summer and are often left to dry. The leaves can be used as flavor boosters in cooking, but they are mostly distilled for their essential oil. Rosemary essential oil is widely used as ingredient in hair growth shampoos and various lotions, soaps and other cosmetic products. It is also used widely in the pharmaceutical industry, as rosemary oil has been reported to ease colitis, asthma and eczema, Rosemary needs at least 6-8 hours of daily sun exposure and prefers warm and moderately dry climates. The plant originated from Mediterranean countries, where average temperatures of 68-86°F (20-30°C) during spring-early summer are common. Soil temperatures above 65°F (18°C) favor growth and regeneration after harvest. However, the plant is resilient and can definitely tolerate lower temperatures as well,we can start rosemary plants from seeds or cuttings.

The average rosemary yield of fresh plant material is 20 tons per hectare, or 8 tons (17636 lbs.) per acre (keep in mind that 1 hectare = 2, 47 acres 454///= 10.000 square meters, 1 ton = 1000 kg = 2205 lbs.). The average //relation “dried: fresh” material is normally 1:3. Consequently, the average rosemary yield of dried material is 6, 7 tons per hectare or 2,7 tons (5952 lbs.).However,this involves the whole plant (stems and leaves), the average yield of dried leaf is 2,5 tons per hectare, or 1 ton (2205 lbs.), the average concentration of essential oil is close to 0,3% of total fresh plant mass harvested. Consequently, you can expect an average yield of 60 kg (132 lbs.) essential oil per hectare, or 24 kg (53 lbs.) per acre.

Thepreviously discussed average yields are annual. The rosemary is a perennial plant and -if the conditions are optimum- a healthy and well cared plant can live up to 20 years. If you grow rosemary for essential oil, knowing when exactly to harvest is very important for the quantity and quality of essential oil (Sarmoum et al., 2019). It requires extensive research and constant “trial and error” effort. First, you have to decide if you plan to produce essential oil from leaves, flowers, stems or all of the above (distill the whole plant). Stems and leaves -when distilled- can normally produce essential oil of higher quantity but of lower quality, compared to the flowers rosemary oil is colorless or pale yellow liquid and volatile (Ullah et al., 2019). Rosemary is the most effective herb with a wide range of uses (Sasikumar, 2012).It is commerciallyavailable for use as an antioxidant (Sasikumar, 2012). The herb rosemary hasbeen used as a food seasoning and as drug since ancient times(Kiarostami, 2010) and recently antioxidant properties are well documented(Santos et al., 2012)it is considered one of the most significant sources for the extraction of phenolic compounds with robust antioxidant activity due to their phenolic hydroxyl groups, besides of its other beneficial effects like antimicrobial, anti-inflammatory and anti-carcinogenic activities(Mreno et al., 2012). Rosemary contributes to development of wide range of human diseases, cancer, cardiovascular diseases, arthrosclerosis, hypertension, ischemia, diabetes, neurodegenerative diseases, rheumatoid arthritis and aging(Butu et al., 2013).Rosemary is credited as a carminative (falvanoids) anti-depressant, anti-spasmodic (volatile oil), rubefacient (phenolics) antimicrobial (diterpenes), emmenagogue (oleanolic acid), anti-inflammatory (carnosol), carcinogen blocker and liver detoxifier(carnosol and whole plant extract), anti-rheumatoid (ointment of rosemary oil)(Sasikumar, 2012). Antioxidants are compounds capable of delaying retarding or thwartingoxidation(Butu et al., 2013).Numerous studies have indicated that the consumption of antioxidant compounds protect cells against impairment of reactive oxygen species (Santos et al., 2012)Rosemary extracts have three groups of compounds: phenolic diterpenes, flavonoids and phenolic acids (Butu et al., 2013).

The study was focused on the use of native plants as a source of raw materials to start the experiment, and rosemary was best candidate plantbecause it is one of the most widely grown plant in Palestine. It grows on calcaneus soil, semi-arid area known for low rain fall. *Rosmarinusofficinalis* L. (Lamiaceae) is a small evergreen plant which grows wild in most Mediterranean countries (Sany et al., 2020).The main producers are Italy, Dalmatia, Spain, Greece, Turkey, Egypt, France, Portugal and North Africa (Svoboda & Deans, 1992). Essential oils of *R. officinalis*, known as rosemary oils, are obtained by steam distillation of the fresh leaves and twigs. The rosemary oil is used as a spicefor food stuffs, such as meat salami and sauces (Lee et al., 1999), but due to its chemical active constituent's properties, it is used as an antioxidant (for food preserving), antibacterial and antifungal agents against some spoilage organisms (Abdo, 2020). The oil is also used in traditional medicine as tonic pulmonary antiseptic, choleretic and colagoguicagents (Said AL-Ahl & Omer, 2011). The chemical constituent isseparated into:

1) **Flavonoids**: heterosides of luteolol, diosmetol and methoxylated flavones in C-6 and/or C-7; phenolic acids (2-3%), especially caffeic derivatives: caffeic acid, chlorogenic acid and rosmarinic acid.

2) **Terpenoids:** Rosemary also contains tricyclic diterpenes: carnosic acid and carnosol (mainly), rosmanol (in concentrations higher that 4%) and triterpenes: ursolic acid and oleanolic acid (2-4%).

3) **Essential oils:** The leaves of rosemary contain between 1.0-2.5% essential oil. Such composition may markedly vary according to the chemotype and the development stage at which the plant has been

harvested. The main constituents are α-pinene (25%), 1, 8-cineole (variable concentration 12-50%), camphor (10-25%), camphene (5-10%), borneol (1-6%), bornyl acetate (1-5%) and α-terpineol (12-24%).

1.3 Essential oils

Essential oils are volatile and liquid aroma compounds from natural sources, usually plants,and are formed in the chloroplast of the leaves, they also may be found in different parts of the plant(Ayoob et al., 2018).Most essential oils are composed of terpene and their oxygenated derivatives. Its extract obtained by steam distillation or solvent extraction of different parts of the aromatic plants including the buds, flowers, leaves, roots and stems(Santos et al., 2012)Rosemary oil is rich in components which help improve the long term memory and concentration, recently it used in medicine as painkiller in case of migraine or digestion problems(Elhassan & Osman, 2014) Major and active constituents of rosemary essential oils were 1,8-Cineole (52.8 %),camphor(11.9 %), α-pinene(10.2 %), β-pinene, camphene (3.0 %), borneol(7.5 %) (7, 21) as shown in **Figure 2.**

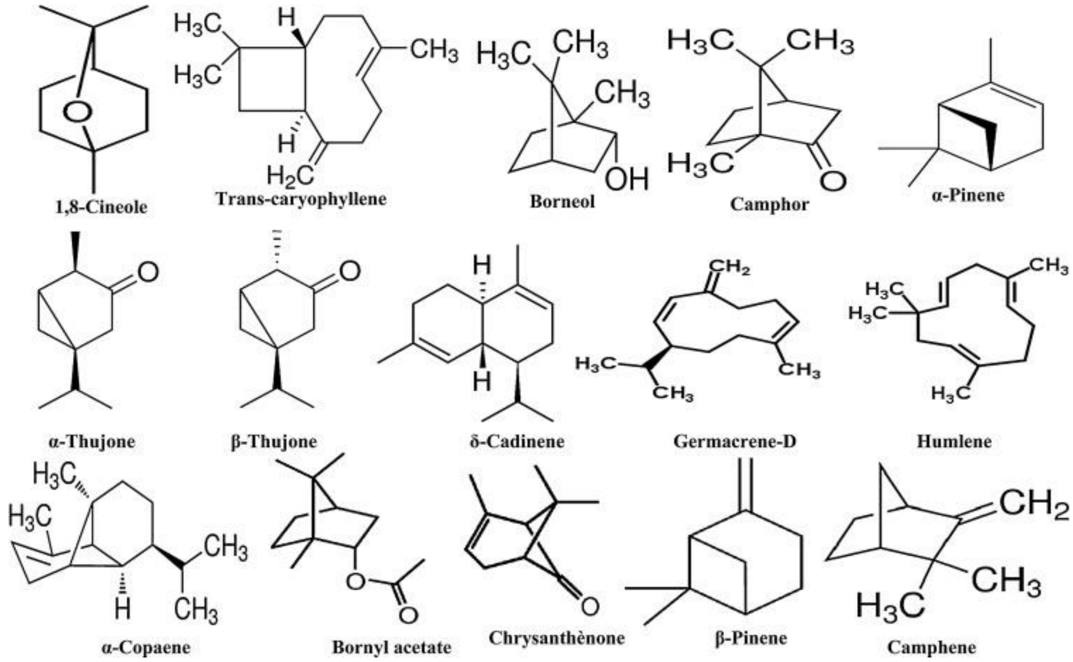


Figure 1.2: Major and active constituents of rosemary essential oils (Hamdi et al., 2011).

1.4 Alkaloids

Alkaloids are foundmainly in plants and especially common in certain families of flowering plants (Lee et al., 1999). Thechemicalarrangements of alkaloids are extremely variable, it contains at least one nitrogen atom in an amine-type structure .Alkaloids are colorless, nonvolatile, crystalline solids as illustrated in **Figure 3**.

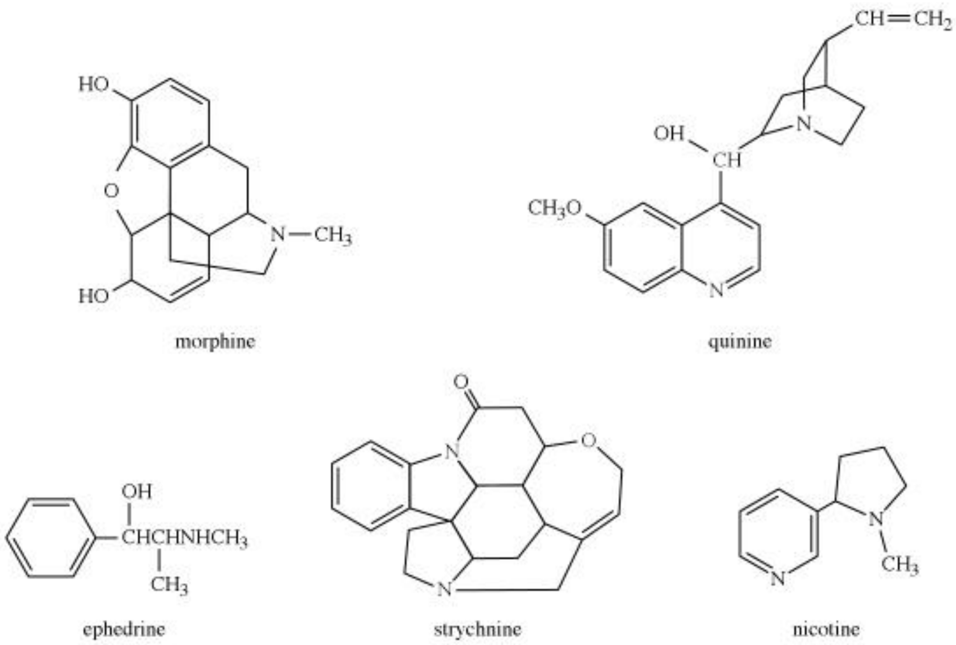


Figure 1.3: The chemical structures of alkaloids in Rosemary (Lee et al., 1999).

1.5 Polyphenols

Polyphenols are micronutrients that we get through certain plant-based foods. They are packed with antioxidants and potential health benefits. It has thought that polyphenols can improve or help treat digestion issues, weight management difficulties, diabetes, neurodegenerative disease, and cardiovascular diseases. (Nieto et al., 2018)

Rosemary and its polyphenolicditerpenes (carnosic acid and carnosol) are known to possess anti-oxidant activity that may be beneficial for cancer control. (Hendel et al., 2016)

Intake of antioxidants has been linked with reduced levels of oxidative damage to lymphocytic DNA, which mean polyphenols may protect cell constituents against oxidative diseases. (Pendey & Rizvi, 2009) Some of the (poly) phenolic compounds present in the rosemary extract in Figure 4.

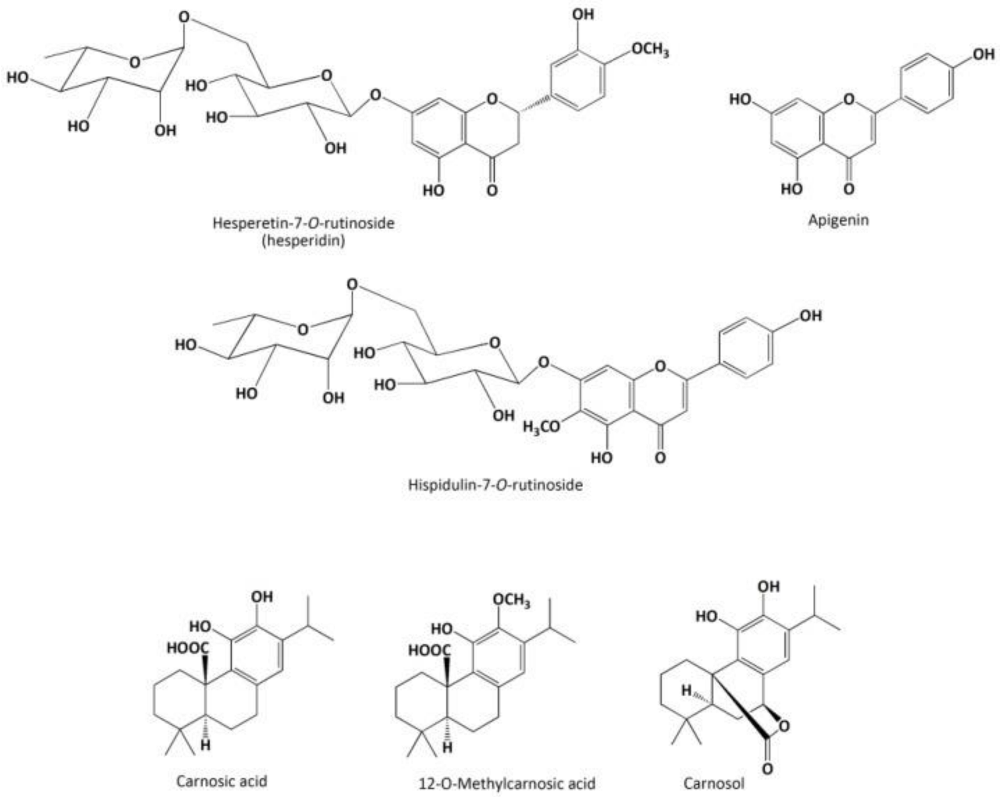


Figure 1.4: some of the most common polyphenols in rosemary plants (Mena et al., 2016).

CHAPTER TWO
MATERIALS AND METHODS

2. Methodology

2.1 Chemicals

Polar and non-polar solvent such aschloroform, ethanol, acetonitrile, bromocresolgreen, hydrochloric acid , sodium hydroxide,Follin-Ciocalteu ,sodium carbonate , and hexane.

All the chemicals were of analytical reagent grade except where stated otherwise, solutions were prepared using double distilled water.

2.2 Experiment description

The activity of the project was carried out at the National Agricultural research center (NARC). The selected medicinal plants were Rosemary *(Rosmarinusofficinalis L. (Lamiaceae*) (48 plants in pots) divided into six rows (8 pots for each raw). The first row was the control that was irrigated with tap water three times per week, meanwhile others were irrig ated with different saline concentrations. During the past months, planting the Rosemary plants were actively conducted and irrigated with fresh wate runtil plants becamerelated to the effect offer she water for about two months. Rosemary's then weres tarted to beirrigated with saline water with [0g/L;1g/L; 2g/L; 3g/L; 4g/L; 5g/L]of sodium chloride for three month, this is equivalent to 0.45, 2, 4, 6, 8, 10, ms/m respictively.

The chemical analysis for polyphenol, essential oils, alkaloids pigments, and chlorophyll were measured. Ash content were measured after the air drying of the plants. First harvest (first stage) were made after one months of irrigated with saline water and the plants shoot length and weight were measured. In addition to that the weights of the roots were recorded.

Figure 2.1: rosemary plants after two months of irrigation with saline water

Stage 1: irrigation

1) 48 rosemary plants were purchased from a local farmer and were planted in pots.

2) Sequential irrigation was accomplished with fresh water at first.

3) Monitoring the progress of plant growth and response.

4) One row that consist of eight plants will be irrigated with fresh water only throughout the experiments.

5) The2nd, 3rd, 4th, 5th and 6th rows that consist of 10 plants each was irrigated water with an EC2, EC4, EC6, EC8, and EC10.

Figure 2.2: rosemary plants after one month of irrigation with saline water

Table 2.1: the amount of NaCl applied for each plant.(added to water that irrigated with)

Row number	NaClquantity for eachplant(mg)	NaCl in ppm for each plant	mM of NaCl for each plant	Salinity millisiemenes per meter(ms/m)
1	0(blank)	0	0	0.45
2	1000	1000	17	2
3	2000	2000	34	4
4	3000	3000	51.3	6
5	4000	4000	68.4	8
6	5000	5000	84.5	10

2.3 Plant sample collection

Fresh plant leaves of rosemary (Rosmarinusofficinalis L. Lamiaceae) were collected from NARC Jenin. The leaves were dried in a cool dark place at room temperature (25o C) for 4 days. The average moisture content for the dry plant material was measured. The dried samples were ground prior to extraction of essential oil and alkaloids. UV-Vis of Carotenoid and chlorophyll will be performed from fresh sample.Qualitative estimation (Test for alkaloids) was carried out by Dragendroff's method (Sarmoum et al., 2019).

Figure 2.3: Rosemary plants shoot and root after harvesting

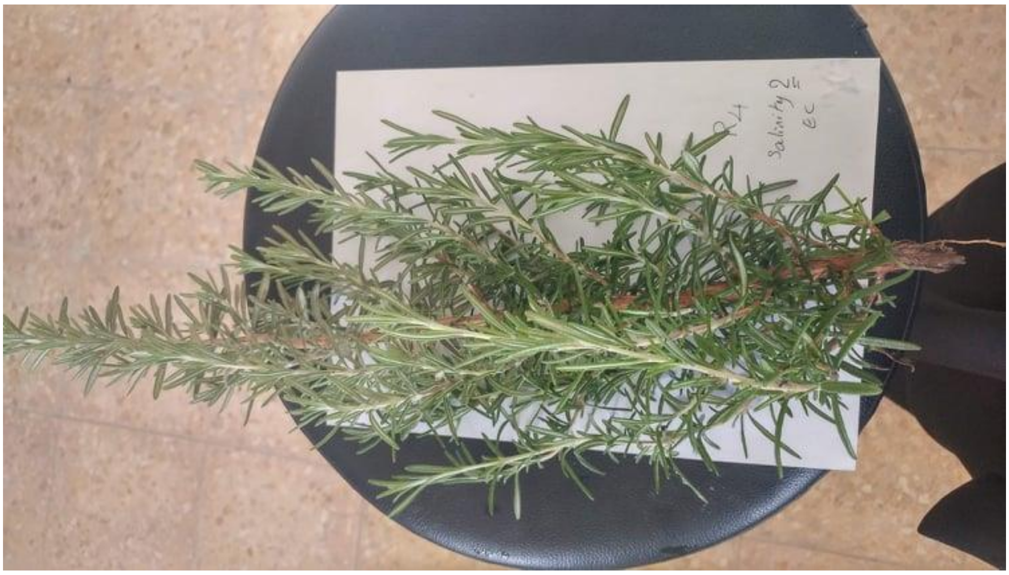


Figure 2.4: rosemary plant shoot for the row irrigated with saline water (EC: 2ms/m)

2.4 Equipment

Identification of components

For the identification of the components of the oil composition, gas chromatography–mass spectrometry (GC–MS)(Thermo Finnigan) will be used to analyzeorganic molecules, as well as using UV-Vis instrument(Agilent Technologies). Soxletapparatus, distillation apparatus rotatory evaporator, heatmantel,water path,separatory funnels, volumetric flask ,kejlhal apparatus(Vapodest). , ankom fiber analyzer , muffle furnace, grinding machine , automatic titrator , glass cell ,flame photometer (Jenway) , flasks and beakers .

2.5 Methodology

2.5.1 Essential oil extraction:

To extract essential oil both steam and hydro-distillation was used (Boutekedjiret et al., 2003). The prepared air-dried plant material of rosemary was powdered and 100 g were submitted to hydro distillation for 3-4 hours with 1000ml distilled water using a Clevenger-type apparatus, Theessential oil were extracted by hexane (20ml) for three times in separatoryfunnel and the collected hexane were evaporated undernitrogen to 10ml and injected to GC-MS for analysis.

Figure 2.5: Distillation appartus for essential oil extraction

Figure 2.6: Separation apparatus (Separatory funnel)

2.5.2 GC-MS analysis

GC-MS analysis was performed on Gas Chromatography-Mass Spectrometer-Quadrupole, with (30m x .25mm i.d, film thickness .25µm) capillary column at flow rate 1.47ml/min, helium was used as the carrier gas with velocity 47.6 cm/sec. Injection temperature was 250°cand the mode of injection was split, detector temperature was 200°c, oventemperature programmed at 60^0C for 4min, then raised to 240°C at rate 6^0C/min. Hold time 2min, then raised to 280^0C at rate 10^0C/min. The interface temperature was 260°c, quadrupole mass spectrometer scanning range was 40-350m/z. (Abo-alrub, 2017). Determination of the oil components was carried out by comparing their mass spectra with those from NIST library database. And 1µl of the oil inhexane, was used for injection (Hailemariam, 2016).

Figure 2.7: Thermo- Finnegan GC-MS instrument

2.5.3 Determination of the total phenolic content

About 2g of the powdered plant material were extracted using 20 ml of ethanol water (80:20) as solvent at 40c° for 4 days in dark place. The content of total polyphenols was quantified in the ethanol extract by the Follin-Ciocalteuspectrophotometric method using Gallic acid as standard. A 1000µl of the extract was mixed with 2.5 ml of Follin-Ciocalteureagent (10x dilution), after 5 min reaction ,2.5ml of Na_2CO_3 solution (7.5%w/v) was added and allowed to stand for 2h. The absorbance was measured at 765nm in a spectrophotometer, the concentration of total phenolic compounds was expressed as mg Gallic acid equivalents (GAE) per g dry extract by using an equation obtained from standard Gallic acid graph.

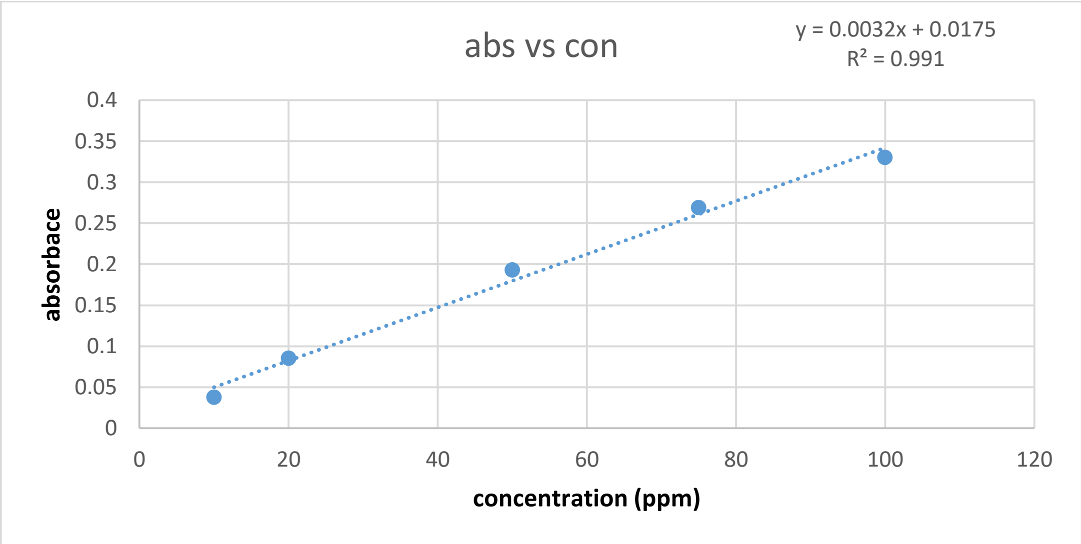


Figure 2.8: Calibration curve for polyphenol using Gallic acid standard

2.5.4 Chlorophyll measurements:

Acetonic extract of rosemary leaves were centrifuged at 300rpm for 10 min.Theextraction solution were prepared (80 ml acetone: 20ml distilled water) then added to 2.5g of the rosemary leaves, shaked well and centrifuged .The supernatant was collected and measured at three wavelength on a Cary 60 Agilent spectrophotometer UV-VIS, on 470,663 and 646nm wavelength .The concentration of chlorophyll was calculated according to the equation (Dumbravă et al., 2012)

Chl a = 12.21. (A663) – 2.81. (A646),

Chl b = 20.13. (A646) – 5.03. (A663),

Chltotal = 17.32. (A646) + 7.18. (A663),

Where:

Chl a – chlorophyll a, in mg/l

Chl b – chlorophyll b, in mg/l

Chltotal – total chlorophylls content, in mg/l

A663 –sample absorbance at 663 nm,

A646 – sample absorbance at 646 nm.

For determination of carotenes and Xanthophyll’s

= [(1000. A470) – (3, 27.Chl a) – (1, 04.Chl b)] / 229, Where: Chl a – chlorophyll a, in mg/l

Chl b – chlorophyll b, in mg/l,

A470 –sample absorbance at 470 nm.

2.5.5 Alkaloid extraction

Determination of total alkaloids extraction: The air dry plant materials (10g) were ground and then extracted with ethanol for 24 h in a (soxhlet) apparatus. The extract was filtered and ethanol was evaporated at a temperature of 45°C on a rotary evaporator to dryness under vacuum. A part of residue was dissolved in 2 N HCl before filtration. One ml the filtrate was transferred to a separatory funnel and washed (3 times) with 10 ml chloroform the pH was neutralized with 0.1 N NaOH(32). Then 5 ml bromocresol green solution and 18 ml of phosphate buffer were added to the solution. After shaking, the complex formed was extracted with 1, 2, 3, and 4 ml of chloroform with vigorous shaking. The extract was collected in 10ml volumetric flask and get to the volume with chloroform.

Preparation of solutions:

Bromocresol green solution:

69.8 mg bromocresol green was heating with 3 ml of2N NaOH and 5 ml distilled water until it dissolved then diluted to 1000 ml distilled water.(Biju et al., 2014)

Phosphate buffer solution (PH 4.7):

71.6 g Na2HPO4 was weight and dissolved in 1 L distilled water after adjusting

To PH. 4.7 with 0.2 M citric acid which was prepared by weighing 42.02 g citric acid and dissolving in 1 L distilled water,(Biju et al., 2014)

Caffeine standard solution:

Accurately1mg pure atropine was dissolved in 10 ml distilled water, aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of caffeine standard solution was transferred to different reparatory funnels. 5 ml PH 4.7 phosphate buffers and 5 ml bromocresolgreen solutionwere added the mixture was shacked with 1,2,3 and 4 ml of chloroform and the extract was collected in 10ml volumetric flask then completed to the volume with chloroform(Shams et al., 2008) . The absorbance of the yellow mixture was measured at 470 nm on Agilent Spectrophotometer (carry 60) against of blank prepared as above without adding atropine. The calibration curve was prepared.

Figure 2.9: Soxhlet extractor for alkaloid extraction

Figure 2.10: Rotatory evaporator to evaporate ethanol

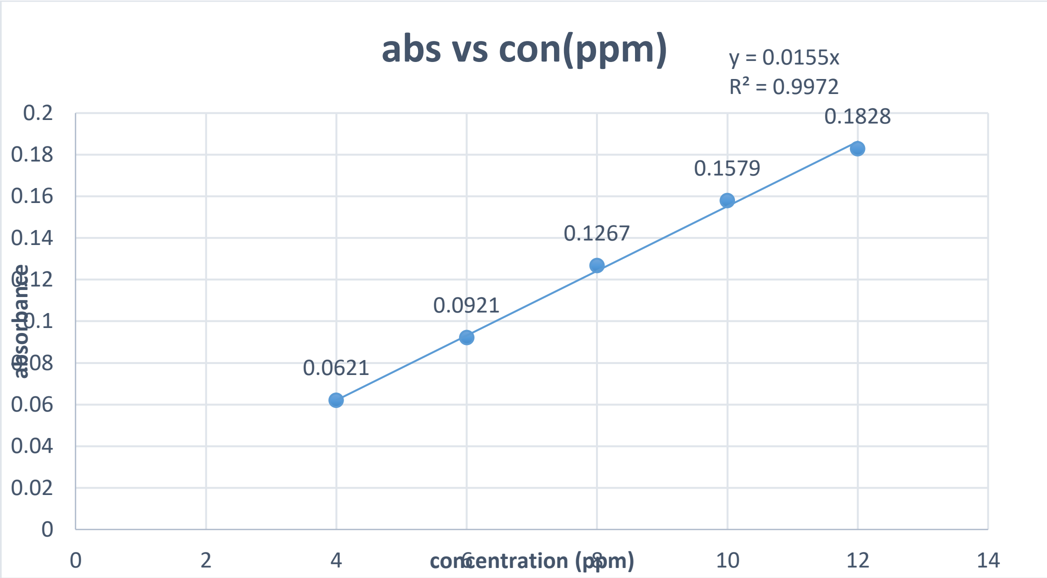


Figure 2.11: Calibration curve for alkaloid

2.5.6 Determination of sodium and potassium:

2g of air dry rosemary leaves were converted into Ash in muffle furnace at 550 ^{0}C overnight and digested with concentrated nitric acid (20ml) then the volume become to 50ml with DW and measured with Jenway PFP7 flame photometer.

2.5.7 Determination of total nitrogen:

for determination of total nitrogen 0.2gm of grinded air dry leaves of rose marry were ignited with concentrated sulfuric acid with tablet catalyst in block digester and cooled, then steam distillation were done and the distilled were collected in 70ml of boric acid (20g boric acid in 1L distilled water and 0.2g of methyl red indicator, 0.1gm of bromocresol green indicator in 100ml ethanol). The boric acid with distillate were titrated with 0.1N HCl to the end point vTN %= N (HCl)*V (HCl)*1.4/weight of sample.

CHAPTER THREE
RESULTS AND DISSCUTIONS

3. Results and Discussion

The results and data from the experimental setup is shown respectively in the following sections. The physiological characterization of the plantsare given in Table. 3.1, which reflects the length in centimeters of the plants during stage 1 of their growth. In this stage, the plants height ranged between 55 to 70 centimeters with an average range of 56 to 63 centimeters. The plant roots and plant shoots of are shown in Table 3.2

3.1 Physiological results:

The effect of salinity on the rosemary plants length, root, green matter in the stages one, two and three are shown in the obtained data given in tables 3.1 - 3.8".

Table 3.1:Plants length in cm at the end of stage one for plants irrigated with different concentrations of salinity with EC in the range 0-10 ms/m, where tap water is used as control and the average is taken for two plants.

Ec (ms)	plant 1	plant 2	Average
Tapwater	55	58	56.5
2	61.5	55.5	58.5
4	56	70	63
6	61	64	62.5
8	66	57	61.5
10	56	56	56

Table 3.2: Plant root and plant shoot in grams at the end of stage 1 for plants irrigated with different concentrations of salinity with EC in the range 0-10 ms/m, where tap water is used as control and the average is taken for two plants.

Ec (ms)	plant1 (root)	plant2 (root)	Plant1 (shoot)	Plant2 (shoot)	Average shoot	Average root
tw	24.43	17.7	43.13	37.68	40.40	21.07
2	18.57	17.03	31.69	36.11	33.90	17.8
4	15.66	14.05	33.34	30.52	31.93	14.86
6	21.39	21.54	39.51	37.21	38.36	21.46
8	10.04	10.05	32.45	33.32	32.83	10.04
10	11.72	10.38	34.99	30.09	32.54	11.05

Table 3.3: Plants length in cm at the end of stage 2 for plants irrigated with different concentrations of salinity with EC in the range 0-10 ms/m, where tap water is used as control and the average is taken for two plants.

Ec (ms)	plant 1	plant 2	Plant 3	Average
Tw	66	64	65	65
2	60	63	64	62.3
4	64	69	70	67.7
6	65	60	66	63.7
8	64	64	63	64.0
10	64	60	67	63.7

Table 3.4:Plants weight in grams at the end of stage 2 for plants irrigated with different concentrations of salinity with EC in the range 0-10 ms/m, where tap water is used as control and the average is taken for two plants.

Ec (ms)	plant1 (root)	plant2 (root)	plant1 (shoot)	plant 2 (shoot)	av shoot	av root
Tw	28.24	18.23	103.70	92.60	98.15	23.235
2	22.69	40	80.7	76.325	78.5125	31.345
4	33.83	18.73	58.06	45.45	51.755	26.28
6	16.42	14.79	64.75	61.25	63	15.605
8	15.54	18.87	63.59	68.28	65.935	17.205
10	19.90	14.76	55.65	71.89	63.77	17.33

Table3.5: Plants weight in grams at the end of stage 2 for plants irrigated with different concentrations of salinity with EC in the range 0-10 ms/m, where tap water is used as control and the average is taken for two plants.

Ec (ms)	plant1+2 (root)	plant1 (shoot)	plant2 (shoot)	Av shoot	av root
Tw	44.77	53.36	60.21	56.785	22.385
2	58.33	52.78	52.84	52.81	29.165
4	53.04	47.02	40.08	43.55	26.52
6	42.88	48.59	65.35	56.97	21.44
8	60.01	33.66	48.11	40.885	30.005
10	47.62	43.59	64.10	53.845	23.8

Table 3.6: Plants length in cm at the end of stage 3 for plants irrigated with different concentrations of salinity with EC in the range 0-10 ms/m, where tap water is used as control and the average is taken for two plants.

Ec (ms)	plant 1	plant 2	plant 3	Average
Tw	63	43	60	55.3
2	57	62	63	60.7
4	63	68	64	65.0
6	63	64	62	63.0
8	63	60	63	62.0
10	58	58	62	59.3

Table3.7: Plants weight in grams at the end of stage 3 for plants irrigated with different concentrations of salinity with EC in the range 0-10 ms/m, where tap water is used as control and the average is taken for two plants.

Ec (ms)	plant 1	plant 2	plant 3	average wt
Tw	81.5	80	83	81.5
2	90	86.5	96.5	91.0
4	114	100	95	103.0
6	100	106	92	99.3
8	87	90	80	85.7
10	66	76	67	69.7

Table 3.8: Plants weight in grams at the end of stage 3 for plants irrigated with different concentrations of salinity with EC in the range 0-10 ms/m, where tap water is used as control and the average is taken for two plants.

Ec (ms)	plant 1 shoot	plant 2 shoot	plant root wt (gm)	AVERAGE
tw	53.4	60.2	44.8	56.8
2	52.8	52.8	58	52.8
4	47	40.1	68.1	43.55
6	48.6	65.4	42.9	57
8	53.7	54.4	60	54.05
10	43.6	64.1	47.6	53.85

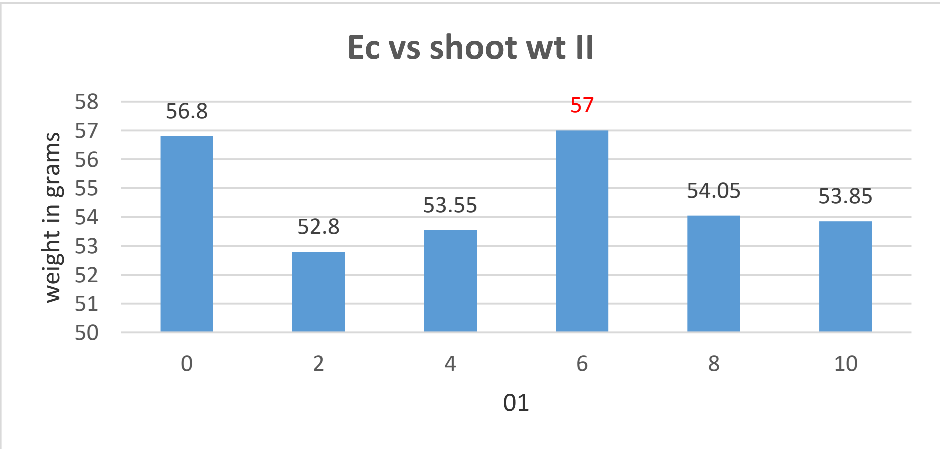


Figure 3.1: the effect of salinity on shoot weight of stage II

Figure 3.1shows the effect of salinity on shoot weight of stage II, which indicates that there is no big difference in weight of the shoot and at EC6 the biggest weight.

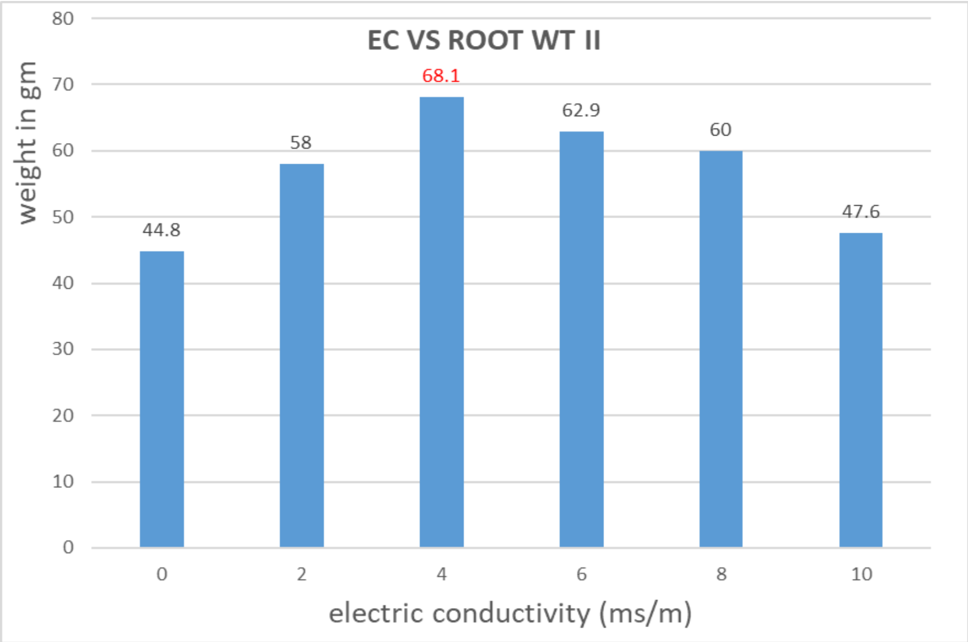


Figure 3.2: The effect of salinity on root weight in grams at the end of stage 2 where EC was in the range 0.0 - 10 ms/m and water tap was used as control (blank).

Figure 3.2 shows the effect of salinity on root weight of stage II. It is observed that at EC4, the plant had the biggest root system byweight,and the weight become smaller as the EC was above or below EC4.

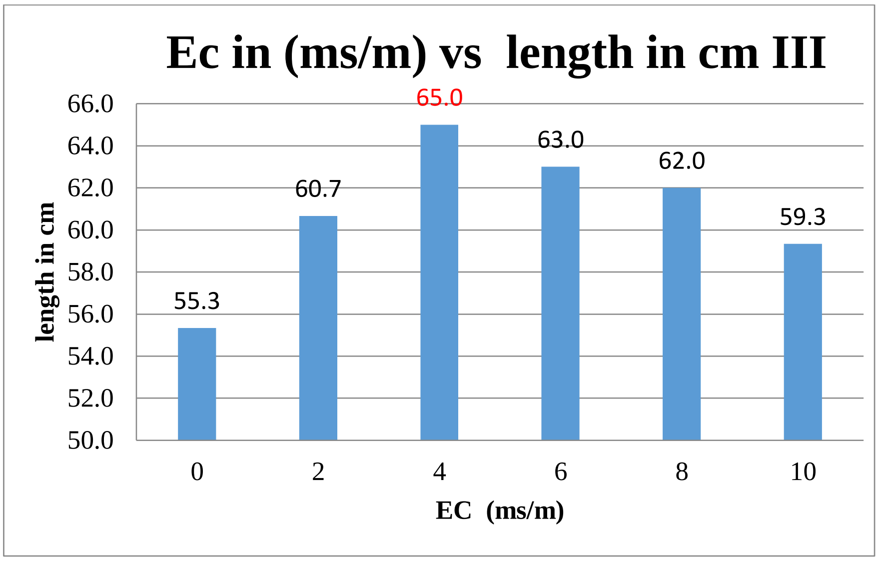


Figure 3.3: The effect of salinity on plant height of stage III where EC was in the range 0.0 - 10 ms/m and water tap was used as control (blank).

Figure 3.3 shows the effect of salinity on plant length of stage III. On the y-axis we have the length of the plants in centimeters, and on the x-axis we have the EC in ms/m.Within this chart, it is noted that at EC =4, the plants had highest length and there was no critical differences between other salinity levels.

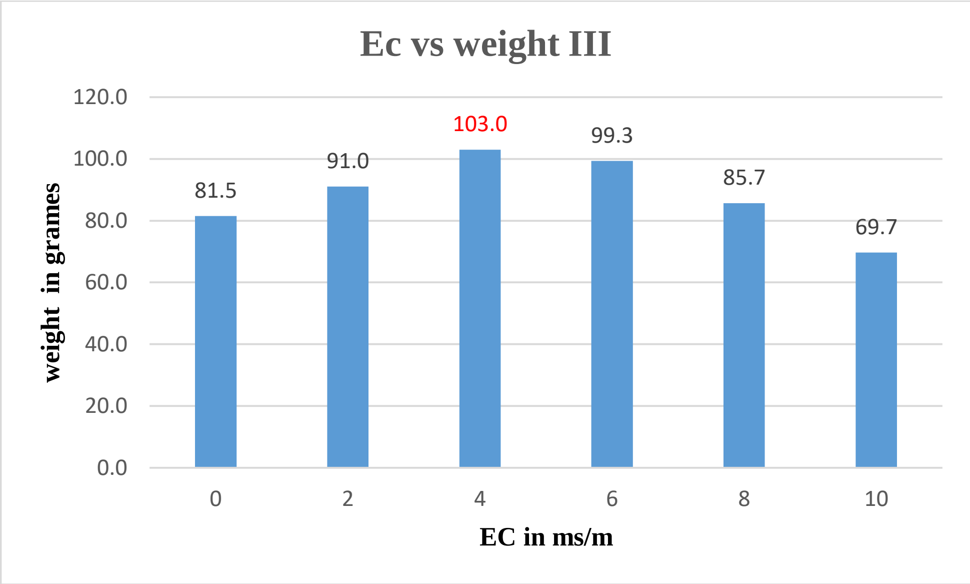


Figure 3.4: The effect of salinity on plant weight in grams at the end of stage 3 where EC was in the range 0.0 - 10 ms/m and water tap was used as control (blank).

Figure 3.4 shows the effect of salinity on plant weight of stage III. It shows that at EC4 plant weight was the biggest and there is no critical different between other salinity levels.

3.2 Chemical results:

Table 3.9 shows the chemical data and analyses for the soil. The pH ranges from 7.34-7.5, indicative of neutral soil. The EC ranged from 0.44-.51 ms/m, while K^+ and Na^+ concentrations ranged from 7 to 8 mg/l. The total nitrogen ranged from 0.32-0.41 mg/l. The chlorine ion concentration was measured a range from 88 to 90 mg/l. Finally the phosphorous measurements were from 15.2 to 17.3 mg/l.

Table3.9: Analysis of soil used in pots for growing of rosemary

Sample	TEST	pH	EC mg/l	K mg/l	Na mg/l	TN mg/l	Cl mg/l	P mg/l
1		7.4	0.51	8	8	0.36	90	17.3
2		7.34	0.50	8	7	0.32	90	16
3		7.5	0.44	7	7	0.41	88	15.2

Chlorophyll Results:

Chlorophyll was determined in the extract by measuring the absorbance at three wavelengths (470, 663, and 446 nm)using Cray 60 Agilent Spectrophotometer and by employing the equations in section 2.5.4.

Table 3.10: Chlorophyll and cartinoid in mg/l by measuring the absorbance in the extract at three wavelength at the end of stage one

Sample	A470	A663	A646	Chlor A	Chlorb	Chlo total	carotinoids
TW	3.95	3.46	2.94	33.99	41.78	75.8	16.57
EC2	3.88	3.34	2.88	32.69	41.17	82.6	16.29
EC4	3.88	3.45	3.34	32.74	49.88	73.9	16.25
EC6	3.93	3.44	2.87	33.94	40.47	74.4	16.49
EC8	3.92	3.38	2.74	33.57	38.15	71.7	16.47
EC10	3.88	3.37	2.59	33.87	35.19	69.1	16.30

Table3.11:Chlorophyll and cartinoid in mg/l by measuring the absorbance in the extract at three wavelength at the end of stage 2

sample	A470	A663	A646	ChlorA	Chlorb	chlo total	carotinoids
TW	1.72	1.50	0.75	16.21	7.55	23.76	7.25
EC2	2.70	2.10	1.20	22.27	13.59	35.86	11.41
EC4	1.65	1.35	0.75	14.38	8.31	22.68	6.96
EC6	2.10	1.70	0.92	18.17	9.97	20.14	5.87
EC8	1.27	0.95	0.66	9.74	8.51	18.25	5.37
EC10	1.10	0.92	0.49	9.86	5.24	15.09	4.64

Table 3.12:Chlorophyll and cartinoid in mg/l by measuring the absorbance in the extract at three wavelength at the end of stage 3

sample	A470	A663	A646	CLORA	CLORPB	total clor	carotinoids
TW	2.00	0.49	0.30	5.13	3.50	8.63	8.63
EC2	1.68	0.26	0.23	2.46	3.44	5.90	7.27
EC4	2.00	0.19	0.21	1.77	3.31	5.07	8.68
EC6	2.67	0.29	0.34	2.63	5.29	7.93	11.58
EC8	2.65	0.32	0.35	2.96	5.48	8.44	11.50
EC10	2.38	0.24	0.27	2.23	4.11	6.35	10.33

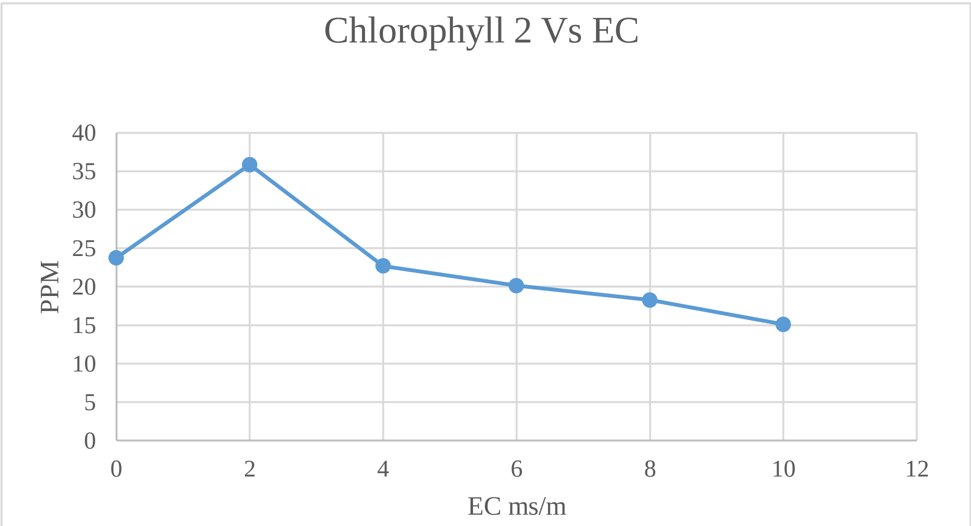


Figure 3.5: The effect of salinity on the concentration of total chlorophyll in

Figure 3.5 shows the effect of salinity on plant total chlorophyll concentration in stage II. it shows that at EC2 the highest concentration and there is no critical different between other salinity levels.

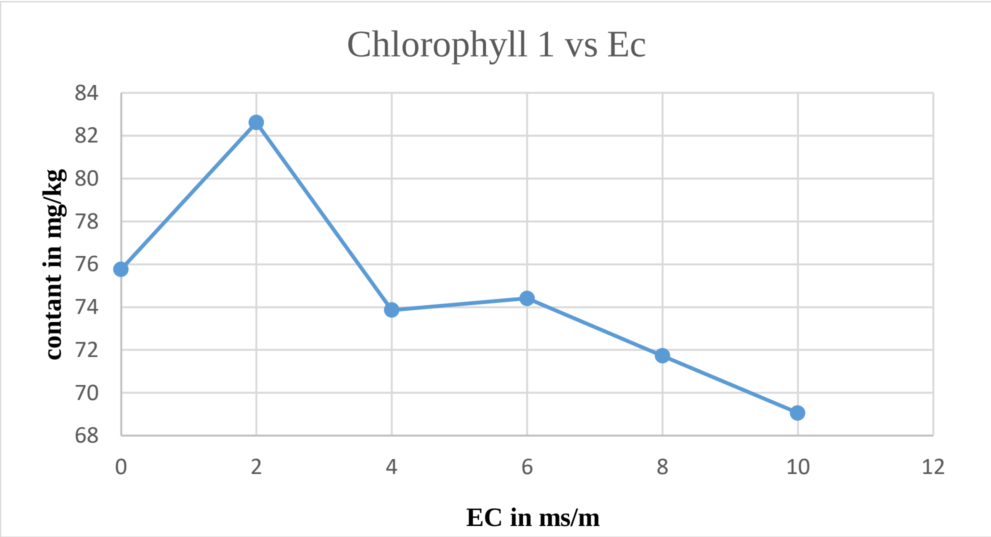


Figure 3.6: The effect of salinity on the concentration of total chlorophyll stage I

Figure 3.6 shows the effect of salinity on plant total chlorophyll concentration in stage I. It alsoshows that at EC2 the highest concentration and there is no critical different between other salinity levels.

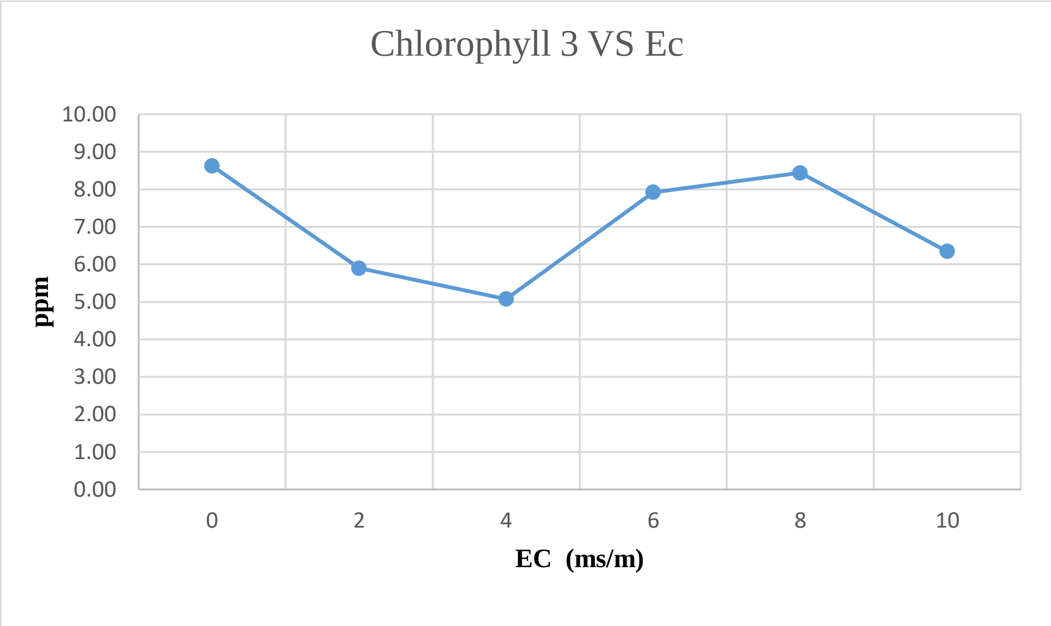


Figure 3.7: The effect of salinity on the concentration of total chlorophyll stage III

Figure 3.7 shows the effect of salinity on plant total chlorophyll concentration in stage III. Ital. so shows the highest concentration of chlorophyll was at EC8 and there is no critical different between all salinity levels.

Carotene results

Figure 3.8, through Figure 3.10 shows the effect of salinity on concentration of carotene from stage I, II and III growth period. It appears that the effect of salinity on plant carotene concentration was negligible at the different stages of growths.

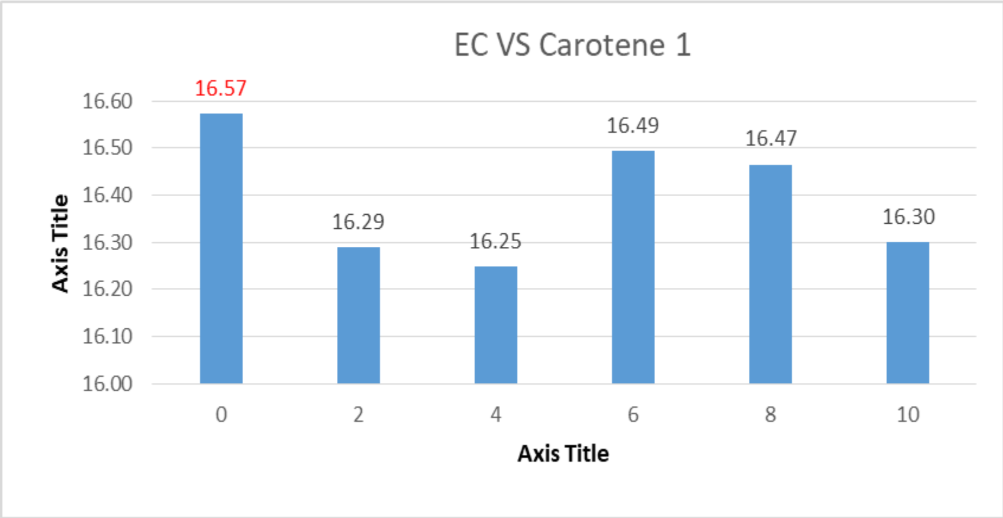


Figure 3.8: The effect of salinity on the concentration of carotenein ppm stage I

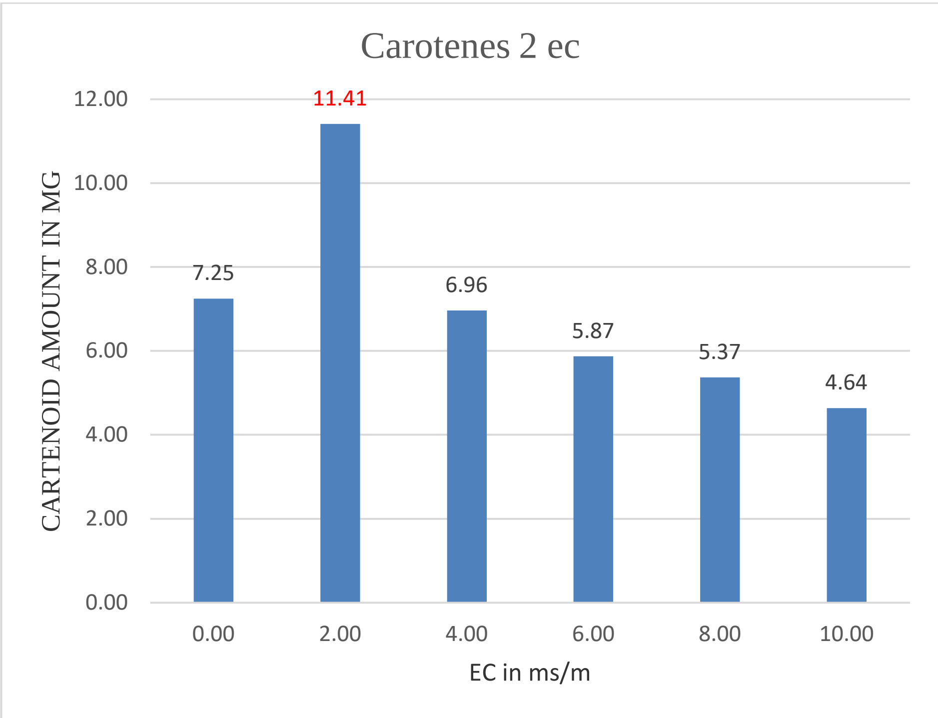


Figure 3.9: The effect of salinity on the concentration of carotene in ppm at the end of stage II

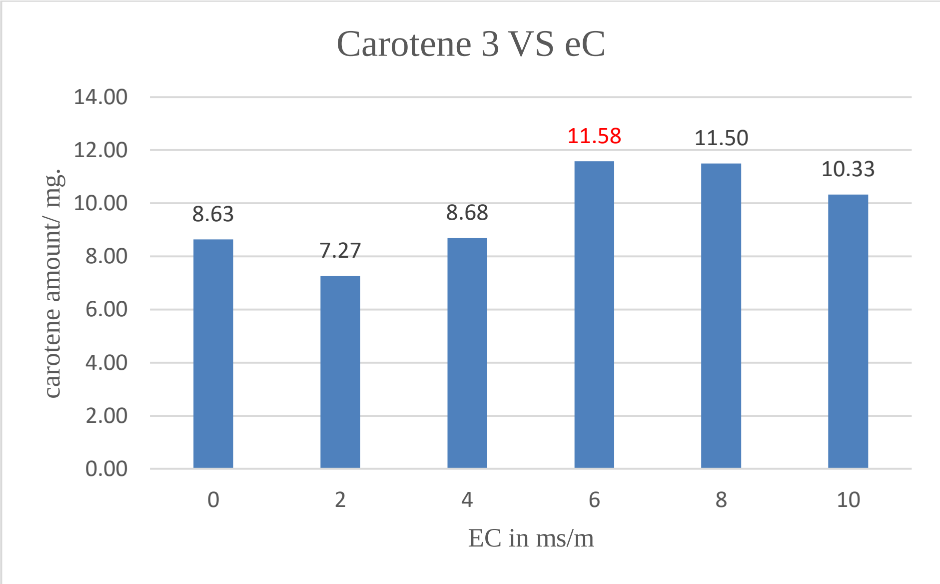


Figure 3.10: The effect of salinity on the concentration of carotene in ppm at the end of stage III

Alkaloids Results:

Table 3.13 through 3.15 show the data for the alkaloids in the sample from stages I, II, and III respectively. The Figures in 3.11 through 3.13 shows the effect of salinity on plant alkaloid concentration. Apparently, one can observe as salinity increase the concentration of alkaloid increase.

Table 3.13: Total alkaloid in mg/kg at the end of stage1

EC	r1	r2	r3	average	Std
tw	0.4	0.4	0.3	0.37	0.047
EC2	1.3	1.3	1.4	1.33	0.047
EC4	1.8	1.6	1.8	1.73	0.094
EC6	2.1	2.3	2.1	2.17	0.094
EC8	2.8	2.6	2.5	2.63	0.125
EC10	3.6	3.4	3.4	3.47	0.094

Table3.14:Total alkaloid in mg/kg at the end of stage2

EC	r1	r2	r3	average	std
tw	0.3	0.4	0.35	0.35	0.041
EC2	0.9	0.7	0.5	0.70	0.163
EC4	1.3	1.5	1.6	1.47	0.125
EC6	2.3	2.5	2	2.27	0.205
EC8	3.4	2.9	3.1	3.13	0.205
EC10	5.7	5.3	5.1	5.37	0.249

Table3.15: Total alkaloid in mg/kg at the end of stage 3

Ec	R1	R2	R3	AV	std
TW	2.1	2.2	2.1	2.13	0.047
Ec2	2.7	2.8	2.7	2.73	0.047
Ec4	3.5	3.4	3.4	3.43	0.047
EC 6	6	5.9	6	5.97	0.047
EC8	6.7	6.8	6.8	6.77	0.047
EC10	7.4	7.3	7.2	7.30	0.082

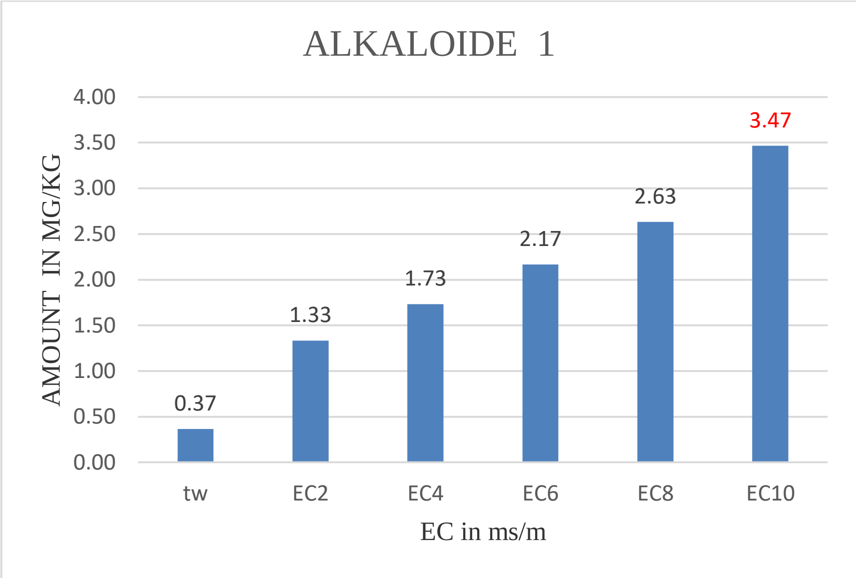


Figure 3.11: The effect of salinity on the concentration of alkaloid in mg/kg at the end of stage I

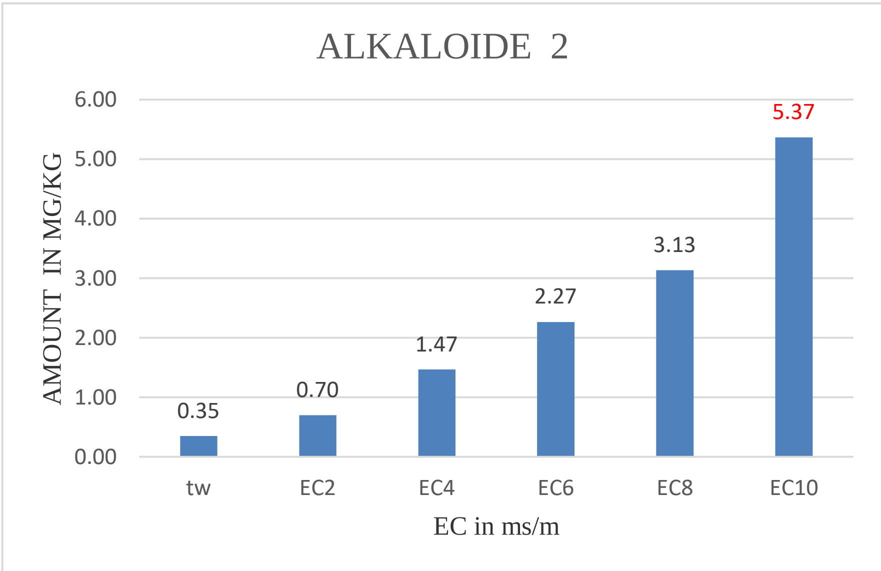


Figure 3.12: The effect of salinity on the concentration of alkaloid in mg/kg at the end of stage II

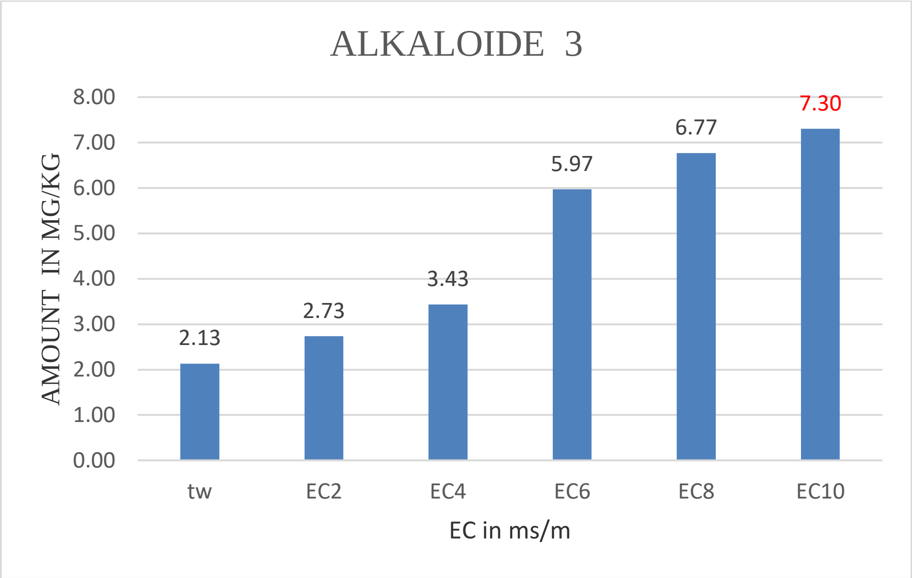


Figure 3.13: The effect of salinity on the concentration of alkaloid in mg/kg at the end of stage II

Polyphenols Results:

Table 3.16 through 3.18show the data for the polyphenol content in the sample from stages I, II, and III respectively. The Figures in 3.15 through 3.16 shows the effect of salinity on polyphenol concentration in rosemary sample during stages I , II, and III. These figures show that the concentration of polyphenols ranges from 50 to 70 ppm.

Table 3.16: Polyphenols in mg/kg at the end of stage1

Ec	R1	R2	R3	AV(mg/g)	Std
TW	55.4	52.3	54.8	54.17	1.34
Ec2	61.2	63	70	64.73	3.80
Ec4	59.8	55.1	59	57.97	2.05
EC 6	59.8	57.3	65.1	60.73	3.25
EC8	67.2	63.5	66	65.57	1.54
EC10	57.4	52	50	53.13	3.13

Table 3.17: Polyphenols in mg/kg at the end of stage2

Ec	R1	R2	R3	AV(mg/g)	Std
TW	62.9	60.2	65.1	62.73	2.00
Ec2	68.2	74.1	69	70.43	2.61
Ec4	55.8	57.5	60	57.77	1.72
EC 6	62.5	59.6	61.2	61.10	1.19
EC8	57.3	58.6	57.6	57.83	0.56
EC10	58	55.5	57.6	57.03	1.10

Table 3.18: Polyphenols in mg/kg at the end of stage3

Ec	R1	R2	R3	AV(mg/g)	Std
TW	70	71.5	71.3	70.93	0.66
Ec2	42	43.1	41.5	42.20	0.67
Ec4	38	38.9	39.2	38.70	0.51
EC 6	31	33.2	32.9	32.37	0.97
EC8	32.2	33.1	31.5	32.27	0.65
EC10	34.2	35	34.9	34.70	0.36

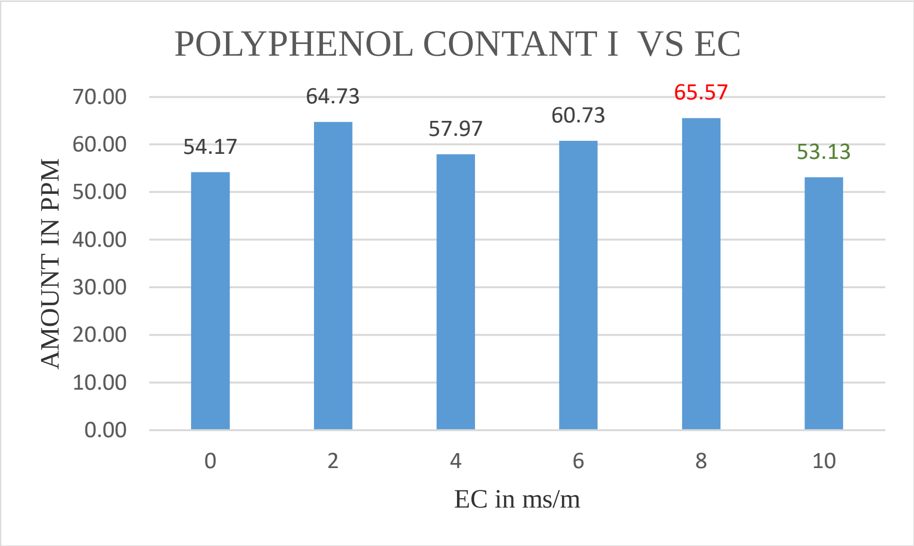


Figure 3.14: The effect of salinity levels on the concentration of polyphenol content as Gallic acid of stage I

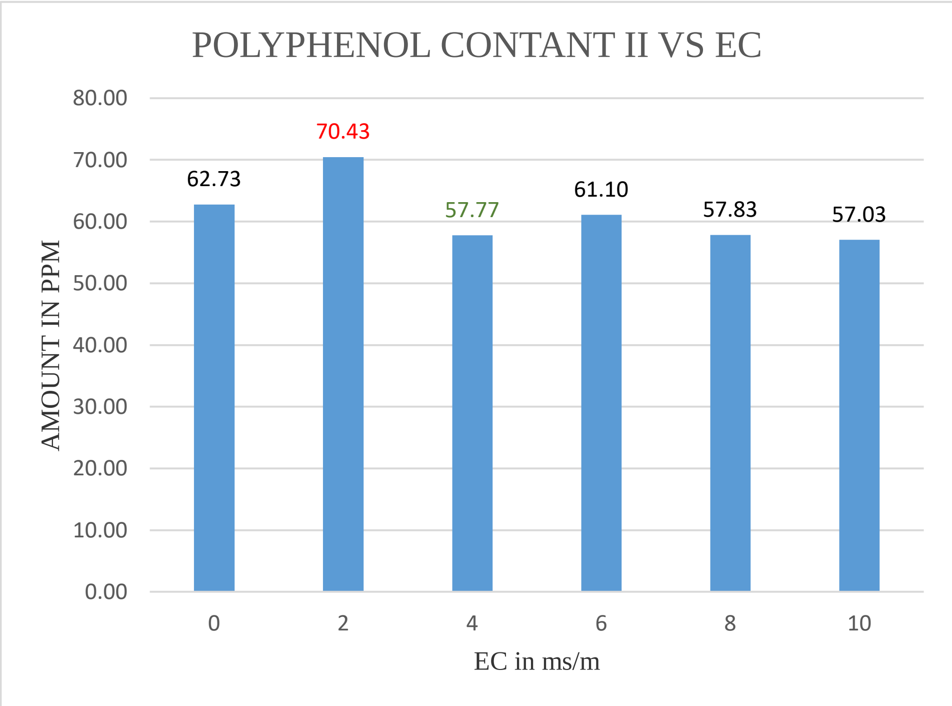


Figure 3.15: The effect of salinity levels on the concentration of polyphenol content as Gallic acid of stage II

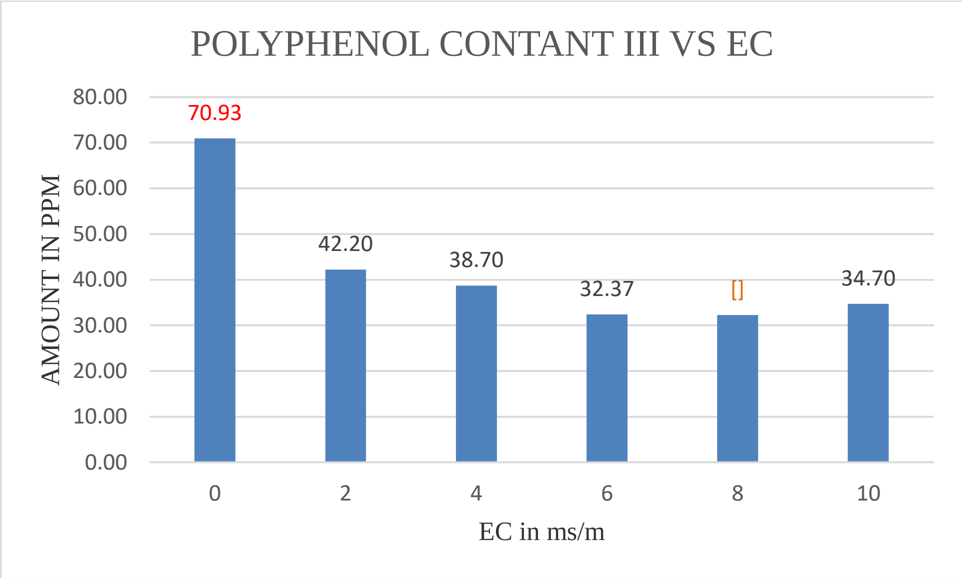


Figure 3.16 : the effect of salinity levelson the concentration of polyphenol content as Gallic acid of stage III.

Ash content

The ash content test and data are summarized in table 3.19, Table 3.20 and table 3.21 for stages I, II, and III respectively. The data almost the same from EC2 to EC 6, but at EC8 it was variable, then back to normal at EC10 for stage I growth.

Table 3.19:% ash content in the three stages

Sample	% Ash		
	Stage 1	Stage 2	Stage 3
TW	8.82	6.86	9.42
EC2	10.01	7.17	12.53
EC4	9.88	6.64	11.09
EC6	9.10	7.74	11.09
EC8	4.51	9.05	12.29
EC10	10.40	9.55	13.55

Sodium (Na^+) and Potassium(K^+)

Table3.20: Na & K in mg/l r in three stages

Stage 1

Ec	Na	K	Na/k	K/NA
TW	900	1000	0.9	1.11
Ec2	1000	700	1.4	0.70
Ec4	3200	700	4.6	0.22
EC 6	1000	800	1.3	0.80
EC8	750	800	0.9	1.07
EC10	500	900	0.6	1.80

Stage 2

EC	Na	k	Na/K	K/Na
tw	500	450	1.1	0.90
EC2	1250	250	5.0	0.20
EC4	1000	300	3.3	0.30
EC6	500	350	1.4	0.70
EC8	250	350	0.7	1.40
EC10	200	400	0.5	2.00

Stage 3

EC	Na	K	Na/K	K/Na
TW	750	400	1.9	0.53
EC2	1000	350	2.9	0.35
EC4	2500	350	7.1	0.14
EC6	2250	300	7.5	0.13
EC8	500	300	1.7	0.60
EC10	500	300	1.7	0.60

Essential oil Results Stage 2:

Essential extracts acquired from the GC-MS instrument are shown that GC Trace with correspond ding MS spectrum of water that no detection of the essential oil “cineol” in tap water so this GCMS serves as blank against which essential oil from rosemary was compared with all this results appears clearly at the appendix.

It is noteworthy to indicate that as EC rise, the essential oil Cineol increased as shown in the area under the peak for each chromatogram trace.

GC-MS spectrums for the essential oil in the extract for sample irrigated with various concentration of saline water are given in Appendix A, while the Retention Time (R) for some officials essential oil composition is given in Appendix B.

Table 3.21summarizes the area under the peak foressential oil cineol from each EC. There is a direct relationship between EC and Cineol concentration. Probably, this can be explained by the dehydration of plant and the cineolconcentration rising as a direct effect of that.

Table3.21: unit area

EC	**Unit Area**
TW	ND
2	1588966495
4	2257848623
6	2289601811
8	41274198366
10	54839009428

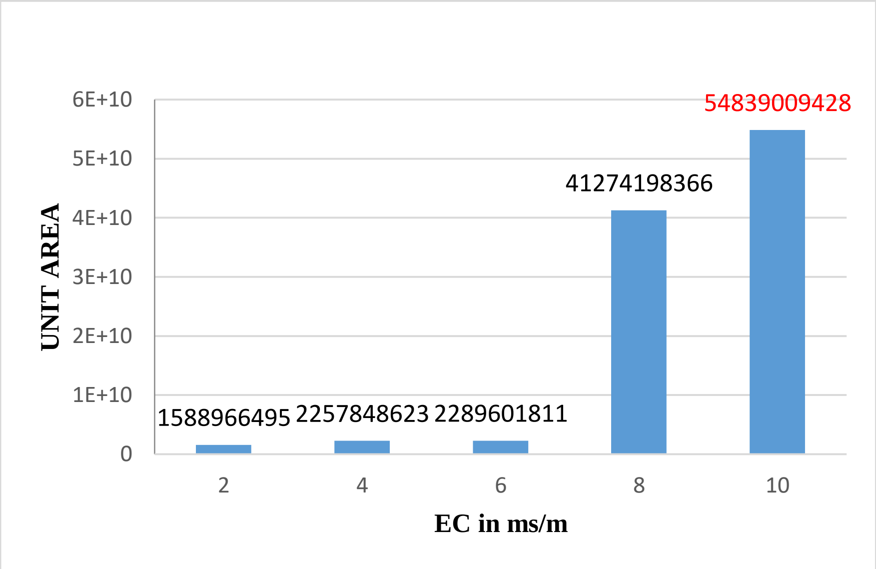


Figure 3.17: The effect of salinity on the concentration of cineol

3.3 Discussion

According to many old studiessalinity reduces the yield of essential oils in plants belonging to the Lamiaceae family, possibly because of the limited supply of calcium (Ca) from the roots to the branches and alterations in the ratio of Ca to abscisic acid in the leaves, but recent studies include this search revealed thesignificantly affects of the chemical compound including essential oils of rosemary compounds whichcorrespond Langroudi M. (Langroudi et al., 2013), this result similar to the obtained results in this study whereas **e**ssential oil content at different level of salinity was measuredas the salinity increases the concentration of the essential oil increases, so that at EC 10 the cineol has the largest area this due to the stress of salinity enforce the plant to produce more oil. Also at low level salinity (tap water) the cineol was not detected.

About 22 organic components were detect in the rosemary extracts as was shown in table 3.23Salinity did not influence the concentration of carotene or polyphenols at each stage of growth similar results reported toSadia et al. (Sadia et al., 2016).

Aswell as for alkaloids; salinity increase the concentration of alkaloid increase, this result also become similar to that reported bySadia et al. (Sadia et al., 2016) which explain that the alkaloids only highly increased at 50 mMNaCl salinity, also increased to some extent at 100 mM salinity but decreased at 150 mM salinity as compared to control one.

3.4 Conclusion

Medicinal and aromatic plants are cultivated for different plant parts and their active constituents are used in many ways, especially for drugs, The general objective of the future studies is better understanding of the response of medicinal and aromatic plants to salinity stress by evaluation of the relative tolerance of different medicinal and aromatic plants and their sensitivity at different plant stages; and how different environmental conditions affect salt-stressed medicinal and aromatic plants; morphological and physiological traits that participate to salinity tolerance in medicinal and aromatic plants, as well as Rosemary presents effective mechanism for tolerating salinity we can exploit it in the manufacture of synthetic drugs based on the chemical structures of the natural products because of its beneficial constituents. In the present work:

1. Essential oil content at different level of salinity was measured. As the salinity increase the concentration of the essential oil increase, so that at EC 10 the cineol has the largest area this due to the stress of salinity enforce the plant to produce more oil. Also at low level salinity (tap water) the cineol was not detected.
2. About 22 organic components were detect in the rosemary extracts as was shown in table 3.23.
3. Salinity did not influence the concentration of carotene or polyphenols at each stage of growth.

References

- Abdo Q. FAO,(2020). **water productivity baseline: concepts and definitions.**
- Abo-alrub M. (2017). **Extraction, Characterization and Application of Essential oil from Native Palestinian Plants, faculty of Graduate Studies.** An Najah National University, Nablus- Palestine
- Abu-Alnaeem MF, Yusoff I, Ng TF, Alias Y, Raksmey M. **Assessment of groundwater salinity and quality in Gaza coastal aquifer, Gaza Strip, Palestine: An integrated statistical, geostatistical and hydrogeochemical approaches study.** Sci Total Environ. 2018 Feb 15;615:972-989. doi: 10.1016/j.scitotenv.2017.09.320. Epub 2017 Oct 17. PMID: 29751448.
- Abu-Rabia A. (2005). **Herbs as a Food and Medicine Source in Palestine.** Asian Pacific J Cancer Prev, 6, 404-407.
- AL-Massry K., Farouk A., Abouzeid M., (2013). **Free Radical Scavenging Activity and Lipoxygenase Inhibition of Rosemary (RosmarinusOfficinalis L) Volatile Oil. National Research Center, Flavor and Aromatic Chem.** Dep., Tahrirst. ,Dokki, Giza , Egypt. P 536-54, Vol.11.
- Ayoob R., Rahman M. and Rehman SH. (2018).**Essential Oil Composition of Romaine so fficinalis L**.EC Microbiology. 14.2 :29-3
- Baker B.P. and Grant J.A. (2016). **Rosemary & Rosemary Oil Profile Active Ingredient Eligible for Minimum Risk Pesticide Use**

New York State Integrated Pest Management, Cornell University, Geneva NY.

- Biju j., SulaimanC.T. ,Satheesh G., and Reddy V. R. (2014). **spectrophotometric estimation of total alkaloids in selected justiciaspecies,** Int. J. Pharm Pharm. Sci., Vol 6, Issue 5, 647-648.

- Boutekedjiret C.,Bentahar F.,Belabbes R. and Bessiere J.M.(2003). **Extraction of rosemary essential oil by steam distillation and hydro distillation**.
- Boutekedjiret C., Belabbes R., Bentahar F., Bessiere J.M., (2004). ***Isolation of Rosemary Oils by Different Processes***, **Journal of Essential Oil Research 16(3):195-199.**

- Brian B. P., Grant J. (2018).**Rosemary& Rosemary Oil Profile**. New York State IPM Program.

- Butu M., Andrei N., Rodino S. (2013).**Evaluation Of Total Polyphenol Content Of The Extracts Obtained From Selected Romanian Plant Species.StudiaUniversitatis "VasileGoldiş", SeriaŞtiinţeleVieţii Vol.** 23, issue 4, 2013, pp. 467-471
- D'Agnanno A., (2015). **Herbs and medicinal plants in Palestine, Published on December 3,** Jerusalem (Palestine).

- DumbravăD.G. , Moldovan C., Raba D.I., Popa M.V.(2012).***Vitamin C, chlorophylls, carotenoids and xanthophylls content in some basil (Ocimumbasilicum L.) and rosemary (Rosmarinusofficinalis L.) leaves extracts,*** **Journal of Agroalimentary Processes and Technologies**, 18 (3), 253-258

- ElhassanI., and Osman N. (2014). **New Chemo type Rosmarinusofficinalis L. (Rosemary) "R. officinalis ct. bornyl acetate". Pharmaceutical Industries Department, Industrial Research and Consultancy Centre,** P.O. Box 268, Khartoum, Sudan, Vol 2(4).
- Hailemariam S. (2016). **Extraction and Characterization of Essential Oil from Rosemary Leaves,** Addis Ababa, Ethiopia.
- Santos R., Shetty K.,Cecchini A., Miglioranza L.,(2012). **Phenolic compounds and total antioxidant activity determination in rosemary and oregano extracts and its use in cheese spread**. CiênciasAgrárias, Londrina, v. 33, n. 2, p. 655-666

- Hendel N.; Larous L. Belbey L., (2016). ***Antioxidant activity of rosemary (Rosmarinusofficinalis L.) and its in vitro inhibitory effect on Penicilliumdigitatum.*** **International Food Research Journal;** Selangor Vol. 23, Iss. (4) : 1725-1732.

- Isaac J. and Sabbah W. **The Intensifying Water Crisis in Palestine Applied Research Institute – Jerusalem (ARIJ).**

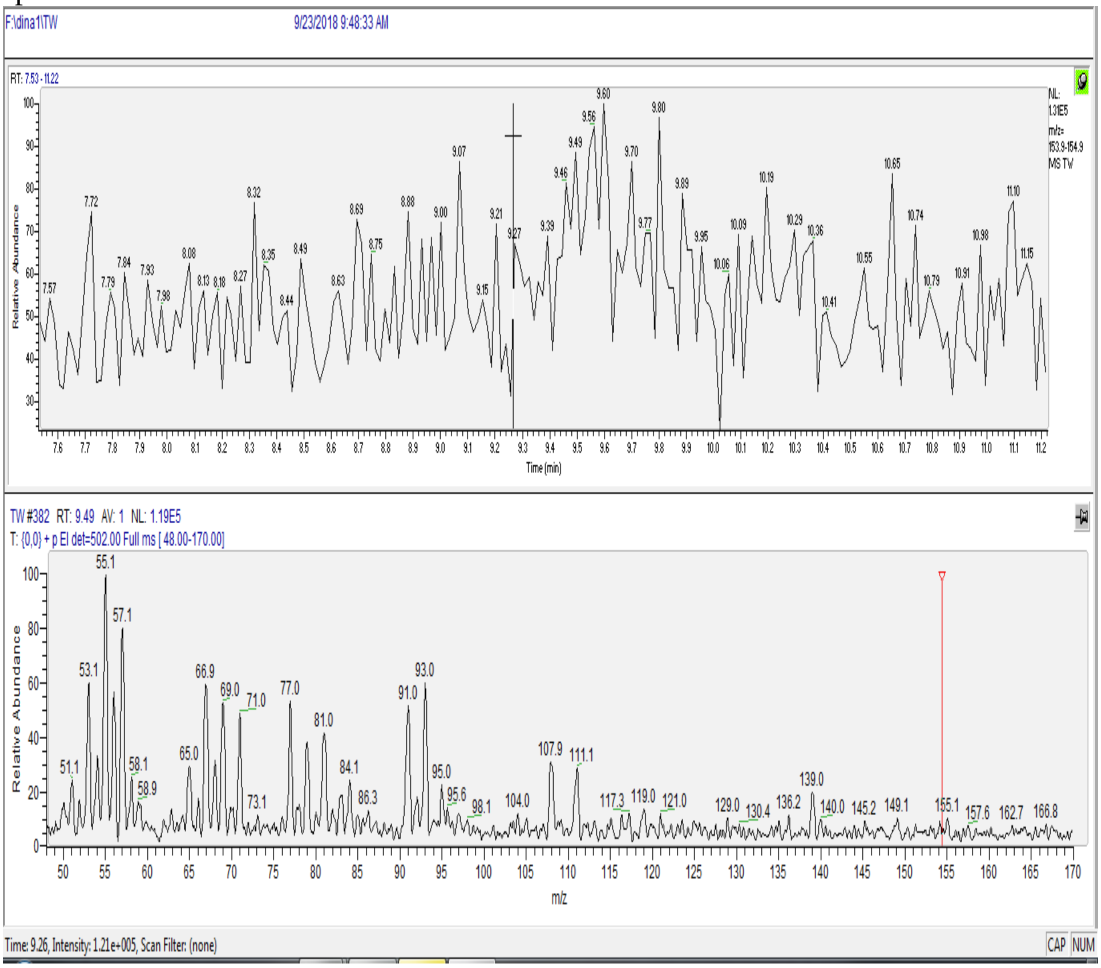
- Jaradat, N.,(2005). **Medical Plants Utilized in Palestinian Folk Medicine for Treatment of Diabetes Mellitus and Cardiac diseases J.** Al-Aqsa Unv., 9.
- Kiarostami KH., Mohseni R., Saboora A.,(2010). ***Biochemical changes of Rosmarinusofficinalis under salt stress.*** **journal of Stress Physiology & Biochemistry**, Vol. 6 No. 3, pp. 114-122 ISSN 1997-0838 Original Text Copyright
- Langroudi M.E., Sedaghathoor Sh. and Bidarigh S.,(2013). **Effect of Different Salinity Levels on the Composition of Rosemary (Rosmarinusofficinalis) Essential Oils.** American-Eurasian J. Agric. & Environ. Sci., 13 (1): 68-71.
- Lee, J., Kassis, S., Kumar, S., Badger, A. and Adams, J., 1999. p38 **Mitogen-Activated Protein Kinase Inhibitors— Mechanisms and Therapeutic Potentials.** *Pharmacology & Therapeutics*, 82(2-3), pp.389-397
- Mena P. , Cirlini M.,Tassotti M., Herrlinger K., Dall'Asta CH. (2016). **Phytochemical Profiling of Flavonoids, Phenolic Acids, Terpenoids, and Volatile Fraction of a Rosemary (Rosmarinus Of ficinalis L.) Extract.**19;21(11):1576.,10.3390/molecules
- Mondal, H. and Kaur, H., 2017. ***Effect of Salt Stress on Medicinal Plants and its Amelioration by Plant Growth Promoting Microbes*. International Journal of Bio-resource and Stress Management,** 8(3), pp.477-487.

- Moreno S., Sana A., Gaya M., Barni M., Castro O. and Baren C.(2012). **Rosemary Compounds as Nutraceutical Health Products.FFyB-UBA, Junín 956 2º piso (1113) CABA Argentina**
- Nieto G.,Ros G., and Castillo J. (2018). **Antioxidant and Antimicrobial Properties of Rosemary (Rosmarinusofficinalis, L.):** A Review, Department of Food Technology and Human Nutrition, Veterinary Faculty, University of Murcia, Espinardo, 30071 Murcia, Spain; gnieto@um.es (G.N.); gros@um.es (G.R.), 4 September 2018
- Pandey **K.** and Rizvi S. (2009). **Plant polyphenols as dietary antioxidants in human health and disease**, 2(5): 270–278.
- Qurie M., DaghraS.,Khamis M., KananA., BarghouthiZ., AlimariA., Nussiebah S. and KaramanR. (2019). ***Rosemary (Rosmarinusofficinalis) plants irrigation with secondary treated effluents using Epuvalisation technology.*** **Net Journal of Agricultural Science.**7(2):69-77
- Sadia B., Irfan Sh., and Bazai Z. A. (2016).**Chemical Composition Of Rosemary (RosmarinusOfficinalis L.) Leaves Under Salt Stress.** Pure and Applied Biology. 5 (2), pp355-360.
- Said-Al Ahl HAH., Omer EA.,(2011). **Medicinal and aromatic plants production under salt stress.Department of Cultivation and Production of Medicinal and Aromatic Plants, National Research Center Dokki**. Vol. 57 No. 1

- Santos R., Shetty K.,Cecchini A., Miglioranza L.,(2012). **Phenolic compounds and total antioxidant activity determination in rosemary and oregano extracts and its use in cheese spread.** CiênciasAgrárias, Londrina, v. 33, n. 2, p. 655-666.
- SanyH.,Alharbi B.,AlmutairiKH., Said-Al AhlH.,Mauro R. and Astatkie T. (2020). **Effect Department of Botany (Plant Physiology Section), Faculty of Agriculture**, Cairo University, Giza, Egypt. Vol. 20 Supplement 1, pp. 2471-2477
- Sarmoum R., HaidS., Biche M., Djazouli Z., Zebib B. and MerahO. (2019). **EffectofSalinityandWaterStressontheEssentialOil Components of Rosemary (Rosmarinusofficinalis L.).**Agronomy 2019, 9, 214; doi:10.3390/agronomy9040214
- Sasikumar B.(2012). **Handbook of Herbs and Spices, Indian Institute of Species Research, India**. Second edition, vol. 1.
- Shamsa F., Monsef H., Ghamooshi R. and Verdian-riziM. (2008). ***Spectrophotometric determination of total alkaloids in some Iranian medicinal, plants,*** Journal of Applied Horticulture 32(1):17-20 .
- Ullah M., Rasheed M. and Mahmood I. (2019). **Niazboo (OcimumBasilicum) As Medicinal Plant Establishes Against Salinity and Sodicity.Adv Biotech & Micro**. 12(5): 555846
- Yubin JI, MiaoYu, Bing W. and Yao Z. (2014).***The extraction, separation and purification of alkaloids in the natural medicine,*** **Journal of Chemical and Pharmaceutical Research**, 6(1):338-345

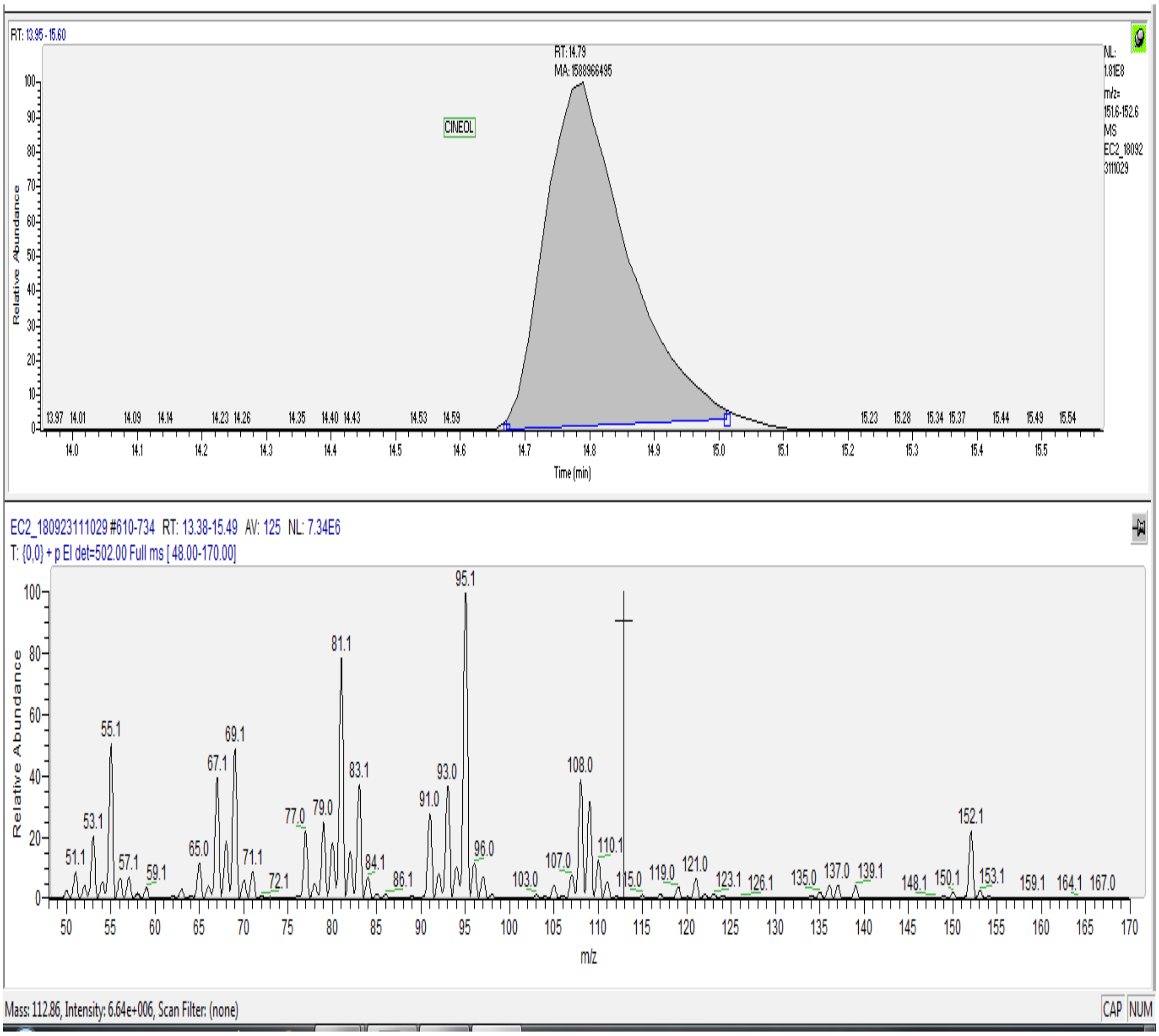
Appendix

Tap water



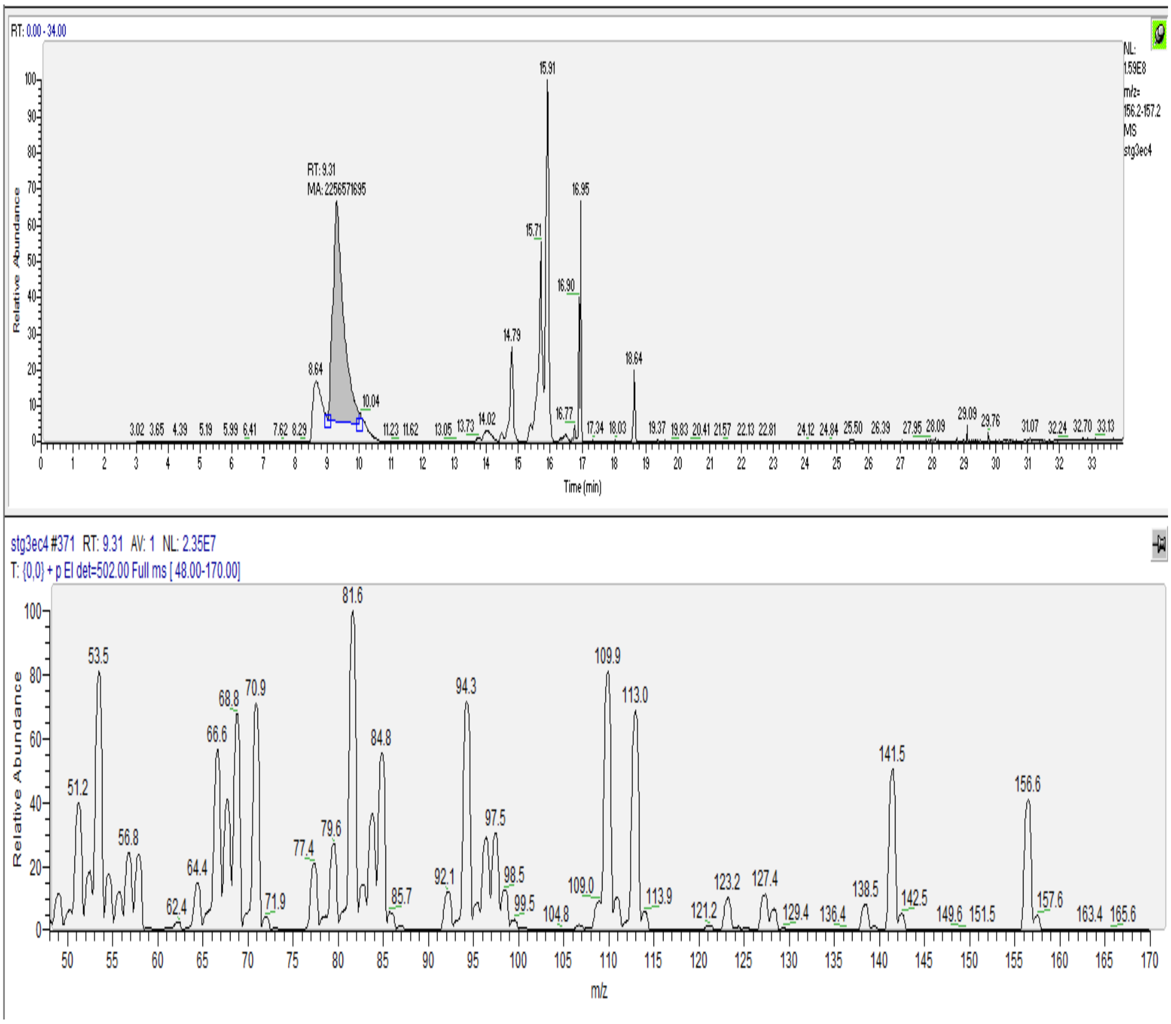
GC Trace with correspondingMS spectrum of water showing no detection the essential oil “cineol”intap water.

Ec2



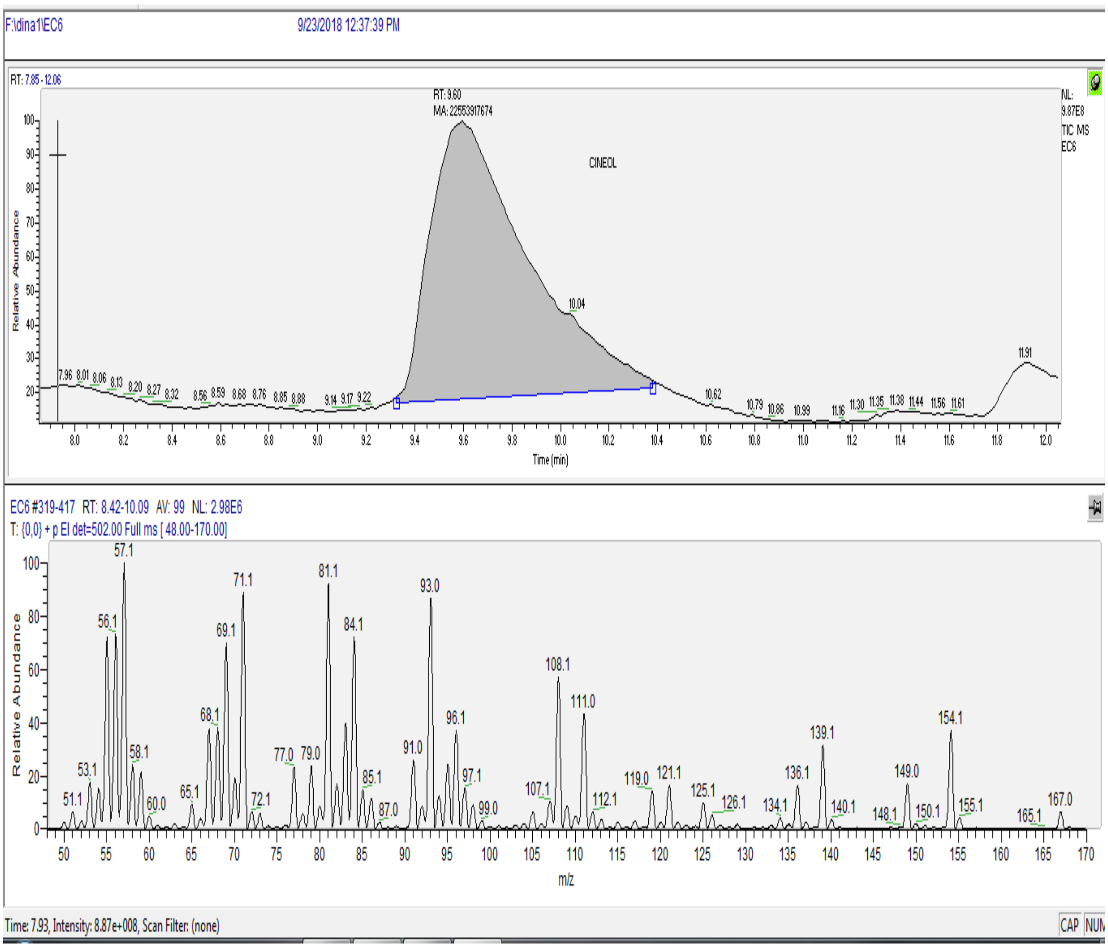
This GCMS demonstrates presence of essential oil cineol in rosemary extracts at salinity 2ms/m

Ec4



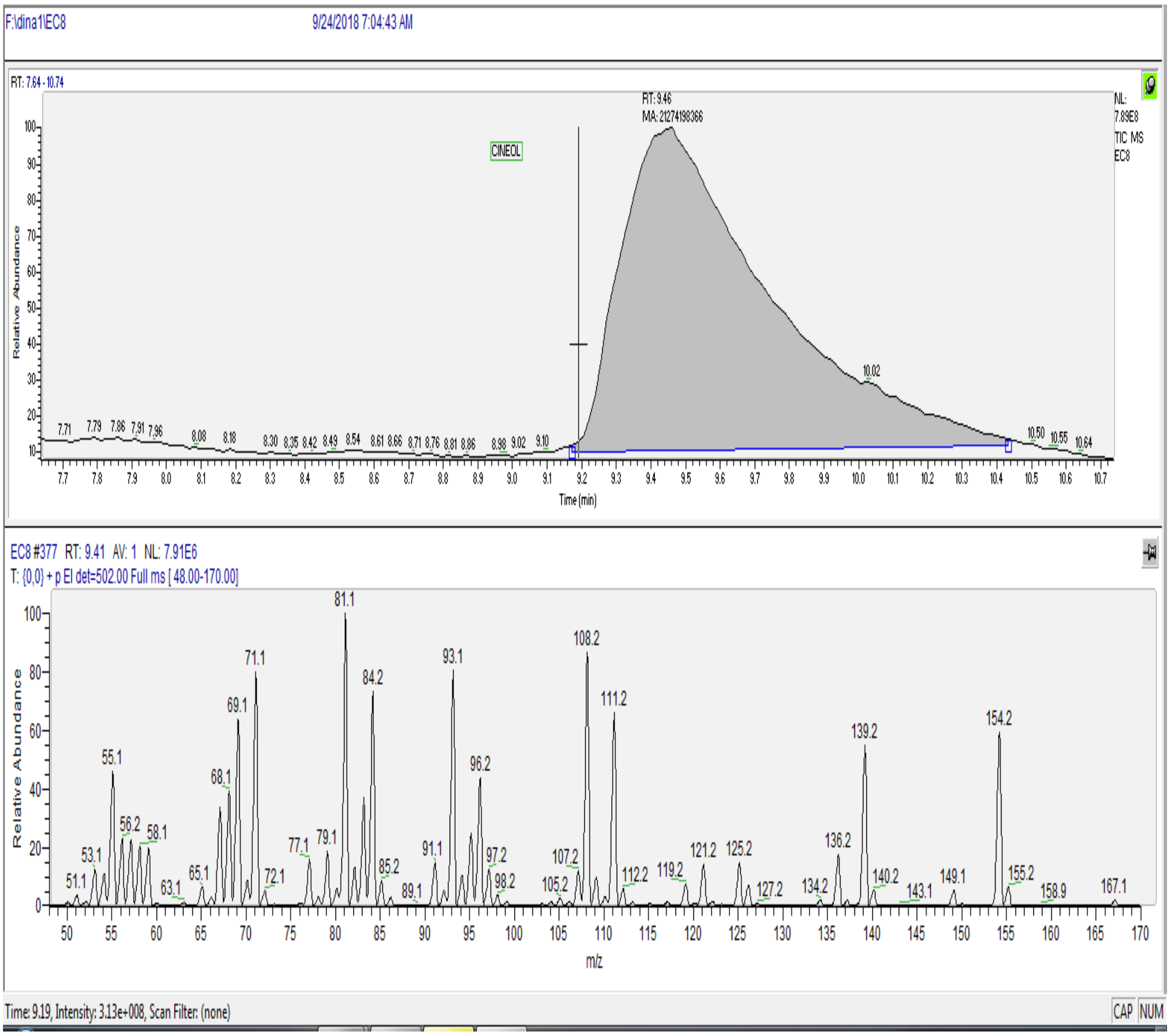
This GCMS demonstrates presence of essential oil cineol in rosemary extracts at EC4

Ec6



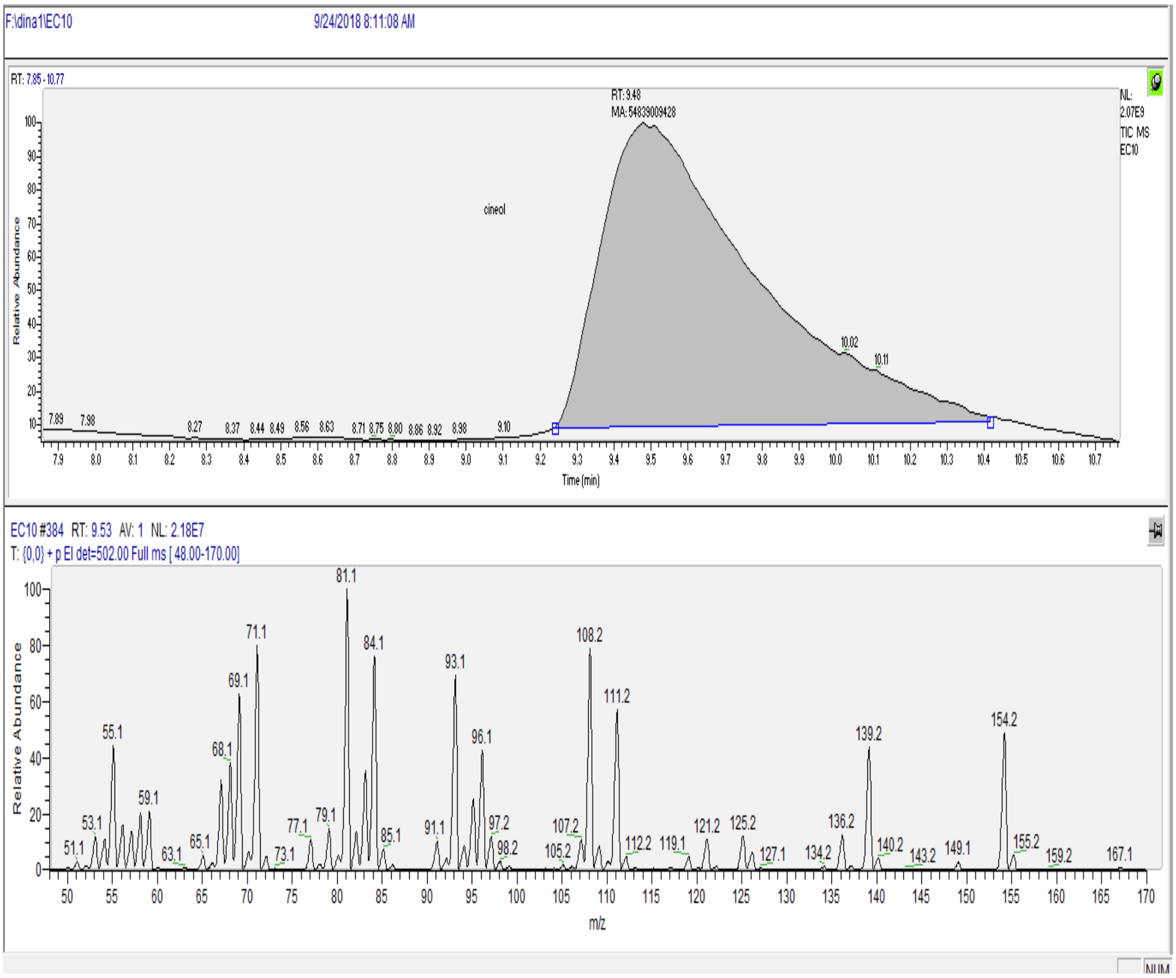
This GCMS demonstrates presence of essential oil cineol in rosemary extracts at EC6

Ec8



This GCMS demonstrates presence of essential oil cineol in rosemary extracts at EC8

Ec10



This GCMS demonstrates presence of essential oil cineol in rosemary extracts at EC10

Some of R. (Retention Time) officials essential oil composition

No	R.Time	Name of constituent	Area%
1	6.162	α-pinene	7.67%
2	7.009	Camphene	4.19%
3	8.109	β—pinene	1.06%
4	9.856	D-limonene	0.41%
5	10.594	Eucalyptol	12.84%
6	15.447	Borneol	3.61%
7	15.631	Camphor	6.41%
8	18.188	Bornyl acetate	20.27%
9	18.677	Copaene	0.30%
10	20.239	Caryophyllene	13.61%
11	21.265	α-caryophyllene	2.53%
12	21.817	β—cubebene	0.78%
13	22.442	α-muurolene	0.52%
14	22.893	Germacrene D	0.82%
15	23.070	γ-cadinene	1.87%
16	23.682	Calamenene	0.27%
17	24.801	Caryophyllenyl alcohol	0.18%
18	25.416	Caryophyllene oxide	2.02%
19	26.002	Cubenol	0.28%
20	26.117	12-oxabicyclo[9-1-0]dodeca-3,7-diene	0.30%
21	26.453	Tau-cadinol	0.50%
22	26.732	Unidentified	0.57%
23	27.152	Longipinene epoxide	1.67%
24	30.127	Hexadecanoicacid,methyl ester	4.71%
25	31.331	Farnesyl acetate	0.67%
26	33.344	9-octadecenoic acid (z)-,methyl ester	7.88%
27	33.632	9,12-octadecadienoic acid (z,z)-, methyl ester	4.05%

جامعة النجاح الوطنية

كلية الدراسات العليا

تأثير الملوحة على التركيب الكيميائي والصفات الشكلية والوظيفية لنبات اكليل الجبل

إعداد

دينا غسان العكر

إشراف

د. رائد الكوني

د. عروه حوشيه

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم البيئية، بكلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2021

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الملخص

تعتبر الملوحة عاملاً رئيسياً يثبط نمو النبات، وحيث أن تملح التربة أو المياه من أكبر الصعوبات البيئية الخطيرة في الزراعة في العالم، هدفت هذه الدراسة إلى معرفة تأثير الملوحة على نمو النبات، والمحتوى النسبي لمياه الأوراق، والتركيب الفسيولوجي –الشكلي والكيميائي للزيت العطري من مستخلص إكليل الجبل ومضادات الأكسدة تحت تراكيز متغيرة من المحلول الملحي.

تم تطبيق تجربة الزراعة المائية مرتبة على ستة مستويات ملوحة لمياه الري (ملم كلوريد الصوديوم). تم الكشف عن ان الملوحة لم تقلل بشكل كبير من نمو إكليل الجبل (1–5٪ من الطول والوزن)، وحيث أظهرت تأثيرًا إيجابيًا على تركيز المكونات الكيميائية حيث زاد تركيز الأوراق والجذر من الصوديوم والكلورايد (تراوحت تراكيز Na + من 7 إلى 8 وتم قياس تركيز أيون الكلور من 88 إلى 90).

كان لمستخلص الزيت العطري تركيز أعلى تحت ضغط الملح (كان لمستخلص الزيت العطري تركيز أعلى تحت إجهاد ملحي 5) Ec10 جم / لتر) حوالي 5*1011 وحدة من تركيز المنطقة). تم توصيف وتقييم التركيب الكيميائي باستخدام جهاز GC–MS للنباتات تحت ضغط الملوحة مقارنة بتلك التي يتم تغذيتها بمكونات المياه العذبة (محتوى الزيت العطري).

تم إجراء عدد من الفحوصات الكيميائية مثل Alkaloids Polyphenols، chlorophyll and cartenoids للأوراق باستخدام جهاز .Uv–Vis المكونات الرئيسية للزيت العطري لإكليل الجبل التي ذكرت في الدراسة اشتملت –α: بينين، 1،8–سينول وكافور. تشمل المركبات الكيميائية

النباتية المعروفة بمضادات الاكسدة على (Alkaloids, polyphenols, cartenoids, chlorophylls). كما تم رصد التغيرات الشكلية (حجم الورقة، طول الساق، وزن المادة الخضراء والجافة) لنمو إكليل الجبل في البيئة المالحة من حيث تحمل النباتات للملوحة المختلفة، وظهر النمو الأمثل تحت الملوحة المختلفة في ((L/.(3g) Ec4 بمتوسط ارتفاع 63 سم مقارنة بالنباتات المروية بمياه الصنبور بارتفاع 56.5 سم، وكذلك كانت النتائج مماثلة لجميع الصفات الشكلية الأخرى.