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Impact of Water Stress and Temperature on Metabolites and Essential Oil of *Rosmarinus officinalis* (Phytochemical Screening, Extraction, and Gas Chromatography)

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ABSTRACT

Currently, climate change is disrupting life on Earth by causing imbalances in the biosphere. This work aimed to evaluate the impact of climate change on the content of primary and secondary metabolites and the yield of essential oil of *Rosmarinus officinalis*. Thus, the results of the conducted experiment show that the content of primary metabolites decreased with increasing temperature and decreasing precipitation along the experiment(proteins from 7.61% to 7.14%, carbohydrates from 6.92% to 5.64%, fats from 1.48% to 1.29% and dietary fiber from 4.96% to 4.22% and mineral composition: Ca from 7.67% to 5.98%, Mg from 8.61% to 7.01%, Fe from 7.53% to 7.21% and Mn from 6.85% to 3.97%), and the content of secondary metabolites increased in the second year when increasing the temperature by 5 °C and water stress by 50% (coumarin from 6.59% to 10.99%, saponins from 7.15% to 8.46%, tannin from 3.92% to 5.95%, alkaloids from 6.69% to 15.62% and flavonoid from 8.02% to 15.75%),but in the fourth year when the temperature continued to increase and water stress was 75% the content of secondary metabolites decreased (coumarin from 10.99% to 8.27%, saponins from 8.46% to 7.87%, tannin from 5.95% to 4.85%, alkaloids from 15.62% to 10.68% and flavonoid from 15.75% to 11.36%)and the same results were obtained for the yield of essential oil which increased in the second year and decreased in the fourth year. This GC analysis of the three essential oil samples shows that the majority of compounds in the three essential oils studied are cineole (S1:45.98%, S2:55.36%, S3:43.08%) followed by camphor (S1:17.44%, S2:21.44%, S3:21.56%) and Alpha-pinene (S1:9.30%, S2:8.34%, S3: 9.17%) and other compounds in low percentage.

Keywords: climate change, primary metabolites, secondary metabolites, essential oil yield, *Rosmarinus officinalis.*

INTRODUCTION

Climate change means a lasting change in weather parameters [Cisse, 2022]. These global climate changes caused by anthropogenic activities since the industrial revolution cause imbalances in the exploitation of natural resources [Ubertos, 2022] as well as changes in the global temperature and precipitation of the Earth that are alarming results of climate change [Konte, 2021], these changes are mainly due to the intensification of the emission of greenhouse gases [Thibaudon, 2022]. Recent studies show that the emission rate of greenhouse gases currently expects a temperature increase of 5.3 °C in 2100 if no intervention occurs. Climate change is currently considered the main factor threatening global biodiversity [Kitoko, 2022]. In addition to global warming

which has already reached 1.1 °C in the last decade compared to the pre-industrial era, the influences on ecosystems are both more extensive and higher than estimated in previous reports. This will lead to the majority of regions, regressions in the animal and plant kingdom, and even extinctions on a planetary scale [Éric Brun et Lisa Bostvironnois, 2022]. Climate change is a time bomb that has several consequences which reverberate on plant biodiversity and the chemical compositions of plants [Picon-Cochard, 2013]. Currently, the world population by its greed and its unawareness contributes to the accentuation of the consequences of climate change which will reflect on plant biodiversity and chemical compositions of plants. Thus, the meteorological parameters can directly affect the production of biomass, but also indirectly on the chemical compositions of plants. Changes in chemical compositions were analyzed in Rosmarinus officinalis planted for three years under different climatic factors.

The main objective of this paper was to better understand the response of plants to climate change, in terms of primary and secondary metabolites and activities, and to give recommendations for adaptation to the effects of climate change.

STUDY AREA

Taounate is located in the pre-Rifa and Rifa area in the north of the Kingdom, it is bordered by the province of Elhoceima and Chefchaouen in the north, the Wilaya of Fez in the south, the province of Taza in the east and the province of Sidi Kasem and Ouazane in the west. The climate of the province is of the Mediterranean type, characterized by the alternation of two seasons, one wet and cold, the other hot and dry. During the summer season, temperatures can exceed 45 °C. The average rainfall of 790 mm per year, with a maximum that can sometimes reach 1800 mm in JbelOutka. With a warm Mediterranean climate with dry summer, annual rainfall of 640 mm, and is located at an altitude of 600 m.

METHODOLOGY

The data on the therapeutic use of the three plants were obtained using a questionnaire form with residents of the Taounate region and this plant was grown in the field to predict the expected effects of climate change on the chemical compositionsof these plants', views of their importance in our daily life and their various uses.

Origin of plant material

Samples of three planted plants were taken from the Taounate region.

Transplantation of samples

Preparation of the planting substrate

The land well in the two fields was well plowed a few weeks before the transplant to avoid the grass, to mix the different edaphic factors, and to avoid the problem of asphyxiation of the roots at the time of watering and the weeds were also eliminated weekly after transplanting to prevent nutrient competition with the needed plants.

Climatic conditions of transplantation

The transplantation of the samples was carried out under the following conditions:

For the open part, the cuttings are transplanted under the current conditions of temperature and precipitation. In the closed part, the temperature and the irrigation were controlled. CO_2 is not a limiting factor because the Taounate region is far from urban pollution. For the closed part, the climatic conditions have been accentuated (increase in temperature and reduction in irrigation)

The three samples were transplanted under the following conditions:

- Sample 1 – normal seasonal average temperature and precipitation;
- Sample 2 – the temperature was increased by 5 °C and water stress of 50% in a closed room;

Table 1. Climatic conditions for transplantation

The mean seasonal temperature in C0	The mean seasonal temperature in C0				Average seasonal precipitation in mm			
	Pertemps	Tee	Autumn	Hiver	Pertemps	Tee	Autumn	Hiver
Plante 1	16.25	34	21.25	6.75	42	21.75	70.25	55.25
Plante 2	21.25	39	26.25	11.75	34.5	10.87	35.125	27.625
Plante 3	26.25	44	31.25	16.75	21	7.25	23.41	18.41

- Sample 3 – the temperature was increased by 10 °C and water stress of 75% in one room close.

Phytochemical screening

It is a technique to determine the bioactive compounds contained in a plant organ. Phytochemical groups are numerous, including alkaloids, saponins, steroids, coumarins, sterols, terpenes, cardiotonic glycosides, polyphenols (flavonoids, anthocyanins, tannins).

Preparation of the extract

The crude extracts were obtained by extraction with solvents of different polarities, such as ether, methanol, and chloroform.

Preliminary phytochemical analyses

Alkaloids

The alkaloids have been characterized using Wagner's, Dragendorff's, and Mayer's reagents. Six (6) mL of the methanolic extract were evaporated to dryness. The residue is taken up in 6 mL of 60% alcohol. The addition of 2 drops of Dragendorff's reagent to the alcoholic solution causes a precipitate or an orange coloration. Adding 2 drops of Wagner's reagent to the alcoholic solution causes a reddish-brown colored precipitate and indicates a positive reaction. The addition of 2 drops of Mayer's reagent to the alcoholic solution causes a white precipitate.

Flavonoids

The flavonoids were sought by the cyanidin reaction. 1 mL of the methanolic extract was evaporated to dryness and the residue was taken up in 2.5 mL of hydrochloric alcohol diluted 2 times. By adding 2 to 3 shavings of magnesium, there is a release of heat that an orange-pink or purplish color. The addition of 3 drops of isoamyl alcohol intensified this coloration which confirms the presence of flavonoids.

Tannins

The search for catechin tannins was carried out using Stastny's reagent. First, 1 mL of the methanolic extract was evaporated to dryness. After adding 3 mL of Stastny's reagent to the residue, the mixture was kept in a water bath at 80 °C for 30 min. The observation of a precipitate in large flakes characterizes the presence of catechin tannins. For gallic tannins, the previous solution was filtered. The filtrate was collected and saturated with sodium acetate. The addition of 3 drops of FeCl3 causes the appearance of an intense blue-black color, a sign of the presence of gallic tannins.

Coumarins

The search for coumarins was carried out by evaporating 1 mL of the ethereal extract to dryness, the residue was taken up in 2 mL of hot water. Then, 0.5 mL of 25% NH_4OH was added and observation was carried out under the UV lamp at 366 nm, intense fluorescence indicates the presence of coumarins.

Saponins

In a test tube, place 10 mL of the aqueous extract. After stirring for 15 seconds, the tube was allowed to settle. A persistent foam height greater than 1 cm indicated the presence of saponins.

Mineral composition

The leaves were dried in an oven at 70 °C for 24 hours to eliminate free water in the plant and more accurately assess the ash yield. Then, a 100 g sample was taken, then put in an oven at 450 °C for two hours (calcination). The ashes thus obtained were weighed and then recovered in a solution containing 65% nitric acid and 35% hydrochloric acid. After filtration, dilutions for the assay were prepared. The dosage involved some heavy metals such as aluminum, cadmium, and cobalt, in addition to other minerals involved in metabolisms (even at low doses), such as iron, copper, zinc, sodium, calcium, potassium, and magnesium. The assay was carried out only on the filtrate. The elements are determined by spectrophotometry (UV) at specific wavelengths for each element.

Organic composition

For organic analysis, the sample was dried in the open air and the shade. This method allows better conservation of all the plant's constituent elements, including the volatile compounds.

Protein and amino acid composition

The protein assay was carried out according to the modified method of Lowry (1959) (Tan et al. 1984). This is a sensitive method the principle of which is based on the reaction of the Folin-Ciocalteau reagent with the phenolic groups of aromatic amino acids and the formation of a blue color that absorbs at 750 nm. The standard range is produced with BSA (bovine serum albumin) at 0.1 mg/ml.

The free amino acid composition was determined from the methanolic and aqueous extracts. Samples of these extracts as well as reference amino acids were deposited on chromatographic plates (CCM) under the following conditions:

- stationary phase –silica gel, thickness 0.2 mm on the plastic plate;
- mobile phase – n-butanol / acetone / acetic acid/water (7 / 7 / 2 / 4);
- developer – ninhydrin;
- reference amino acids –Alanine, Arginine, Asparagine, Aspartate, Valine, Cysteine, Histidine, Glutamine, Glutamate, Isoleucine, Leucine, Lysine, Metthionine, Proline, Phenylalanine, Threonine, Tryptophan.

Quantitative analyses

Colorimetric methods based on the use of a visible UV spectrophotometer were used to assess the number of phenolic compounds in plant matter.

Preparation of extracts

First, 2 g of plant material were dissolved in 2×20 ml of a 50% (v/v) methanol solution. The mixture was stirred for 6 hours, then filtered. The extract collected was centrifuged (1536 × g) for 20 minutes, and the filtrate obtained was kept cool (+4 °C) until it was analyzed. For the exudates, on average 60 mg of the extracts were dissolved in 2 ml of a hydro-methanolic solution (50/50 v/v ml), the mixture was shaken intermittently for 30 minutes,and then filtered. The filtrate obtained was used for quantitative assays (UV-visible spectroscopy).

Dosage of flavonoids

The reagents used are colorless solutions of sodium nitrite (NaNO2, 5%) and aluminum chloride (AlCl3, 10%). The principle of the method is based on the oxidation of flavonoids by these reagents, which leads to the formation of a brownish complex that absorbs at 510 nm. The comparison of the D.O. observed with that obtained by a catechin standard of known concentration makes it possible to evaluate the total content of flavonoids. The total flavonoids were evaluated by colorimetry, in a 10 ml flask 250 µl of the extract and 1 ml of distilled water were successively introduced. At the initial time (0 minutes) 75 µl of a $NaNO_2$ (5%) solution was added, and after 5 minutes 75 µl of $AlCl_3$ (10%) was added. At 6 minutes, 500 µl of NaOH (1N) was added and 2.5 ml of distilled water was successively added to the mixture. The absorbance of the mixture obtained was directly measured with a UV-visible spectrophotometer at 510 nm [Bouterfas et al. 2013].

Dosage of tannins

The dosage of hydrolyzable tannins was carried out according to the protocol of Mole and Waterman. First, 0.2 g of the crushed leaves were macerated for 18 h in 10 ml of 80% methanol, the mixture was filtered using Whatman paper. Then, 1 ml of the filtrate was added to 3.5 ml of a solution prepared from ferric trichloride ($FeCl_3$) 0.01 M in hydrochloric acid (HCl) 0.001 M. After 15 seconds, the absorbance of the mixture was read at 660 nm. The hydrolyzable tannins were expressed by the following formula (1):

$$TH\ (\%) = (A \times M \times V)/E\ mole \times P \qquad (1)$$

where: TH – hydrolyzable tannins; A – absorbance; $E\ mole$ – 2169 of gallic acid (constant expressed in moles); M – mass = 300; V – volume of the extract used; P – sample weight.

The method of Swain and Hillis quantification of condensed tannins was applied. First, 0.2 g of the crushed leaves were macerated for 18 h in 10 ml of 80% methanol. The mixture was filtered using Whatman paper. Then, 1 ml of filtrate was added to 2 ml of a solution prepared from 1% vanillin in 70% sulfuric acid. The entire mixture was placed in a water bath for 15 min at 20 °C away from light. The absorbance of the mixture was read at 500 nm. The condensed tannins are expressed by the formula (2):

$$TC\ (\%) = (5.2 \times 10^{-2} \times A \times V)/P \qquad (2)$$

where: TC – condensed tannins; 5.2×10^{-2} – constant expressed in equivalent of cyanidins; A – absorbance; V – volume of the extract used; P – sample weight.

Dosage of alkaloids

The assay was done by the spectrophotometric method described by Sreevidya N. and Mehrotra S. A quantity of 5 mL of extract solution was taken and the pH was maintained between 2 and 2.5 with dilute HCl. Then, 2 mL of Dragendorff's reagent was added thereto and the precipitate formed was centrifuged. The complete precipitation of the centrifugate was checked by adding Dragendorff's reagent to it. The centrifuged mixture was decanted completely. The precipitate was washed with alcohol. The filtrate was discarded and the residue was then treated with 2 mL of disodium sulfate solution. The brownish-black precipitate formed was then centrifuged. Completion of precipitation was checked by adding 2 drops of disodium sulfate. The residue was dissolved in 2 mL of concentrated nitric acid, with warming if necessary. This solution was diluted to 10mL with distilled water. Then, 1mL of this diluted solution was taken and 5 mL of thiourea solution was added thereto. Absorbance was measured at 435 nm. The standard curve was produced from a stock solution of atropine at 10 mg/L, ranging from 0 to 1 mg/mL. The absorbances were read with a spectrophotometer at 435 nm against the white tube prepared under the same conditions by replacing the extract with distilled water. The alkaloid contents of the leaves were evaluated from the linear regression line and expressed in gram equivalent of atropine per 100 g of powder or 100 mL of liquid medium.

Coumarins

The different plant samples (10 g) were crushed or transformed into powder before being extracted at room temperature with 100 mL of methanol for 24 hours under mechanical stirring. The methanolic extracts thus obtained after filtration were directly used for the characterization of coumarins. To determine the coumarin content, 1 gram of finely ground fresh sample of each species was extracted with 10 mL of 80% methanol for 10 minutes and filtered. The filtrate thus obtained was diluted to one hundredth (1/100).

The coumarin content was determined by spectrophotometry. To do this, the coumarin calibration curve was drawn according to the method described by Malik et al. (2012) using coumarin stock solution (100 µg/mL) at the maximum wavelength of 290 nm. The stock solution was obtained by dissolving 100 mg of coumarin (2H-1-benzopyran-2-one) in 10 mL of 80% methanol. The test solution with a concentration of 10 µg/mL gave solutions of different concentrations after a series of dilutions in 80% methanol. Three hundred microliters of this test solution were used to make the balayage in the area of 200 and 400 nm. The maximum wavelength obtained was 290 nm. Next, the calibration curve was performed with coumarin stock solution (100 µg/mL) at the maximum wavelength. The concentrations of 50 µg/mL, 25 µg/mL, 12.50 µg/mL, 6.25 µg/mL, 3.125 µg/mL, 1.5625 µg/mL, d 0.78185 µg/mL obtained after dilution of the stock solution were used to draw the standard curve the equation line of which is: $y = 0.059x + 833$ and $R^2 = 0.996$. The coumarin contents were determined using 300 µL of fresh extracts for direct determination at a wavelength of 290 nm [Soulama 2013].

Determination of saponins

The powder was taken up in MeOH (2 mg/ml) and filtered before use as the analyte. Then, 250 µl of a solution of vanillin (8%, w/v) in absolute EtOH was added to 250 µl of the analyte. To this mixture, 2.5 ml of H_2SO_4 (72%, v/v) was added. The whole mixture was incubated in a water bath at 60 °C for 10 min, then cooled in an ice bath for 5 min. The absorbance was read at 475 nm and compared with diosgenin (10–40 µg/ml) under the same conditions as the analyte from the calibration curve. The content of steroidal saponins (Q_2) was expressed as diosgenin equivalent per 100 grams of dry matter (g ED /100 g MS) according to the equation (3):

$$Q_2 = (V_2 \times C_2)/ m_2 \quad (3)$$

where: V_2 – volume of the analyte (ml); C_2 – concentration of the extract (µg /ml); m_2 – mass of the dry matter [Baguia-Broune et al. 2018].

Essential oil

The plant material consists of leaves dried in the shade. Fractions of about 100 g of dried leaves were subjected to hydro distillation for 3 h using a Clevenger-type apparatus.

Gas chromatography

After the extraction of essential oils and to make a comparison of the chemical compositions of the three samples under different climatic

conditions, gas chromatography for the three samples was carried out.

The GC analysis was performed using a chromatograph equipped with a flame ionization detector (FID) and two capillary columns of different polarities type OV. 101 (25 m × 0.22 mm × 0.25 µm) and Carbowax 20 M (25 m × 0.22 mm × 0.25 µm). The carrier gas was helium with a flow rate of 0.8 ml/min and the oven programming temperature was between 50 and 200 °C with a gradient of 5 °C/min. The GC/MS coupling was performed on a DB1 fused silica capillary column (25 m × 0.23 mm × 0.25 µm) with helium as the carrier gas and the same temperature programming as the GC.

Table 2. Nutritional values

Primary metabolites	Presence		
	E1	E2	E3
Proteins	+++	+++	++
Carbohydrates	+++	++	+
Fats	++	+	+
Dietary fiber	+++	++	++

Table 3. Amino acids of the three samples

Primary metabolites	Presence		
	E1	E2	E3
Alanine	-	-	-
Arginine	-	-	-
Asparagine	-	-	-
Aspartate ou Acid Aspartique	+	+	+
Cystéine	+	+	+
Glutamate ou Acid Glutamique	-	-	-
Glutamine	-	-	-
Glycine	++	+	+
Histidine	+	+	+
Isoleucine	++	+	+
Leucine	+++	++	+
Lysine	++	+	+
Méthionine	-	-	-
Phénylalanine	+++	+	+
Proline	++	++	+
Pyrrolysine	-	-	-
Sélénocystéine	-	-	-
Sérine	++	+	+
Thréonine	-	-	-
Tryptophane	-	-	-
Tyrosine	+	-	-
Valine	++	+	+

RESULTS

Phytochemical screening

Primary metabolites

It was noted from Table 2 that proteins, carbohydrates, and dietary fibers are quite present in sample one followed by two and finally three but lipids are not very frequent.

Table 3 shows the presence of amino acids in the three samples studied and it was noted that the amino acids (Aspartate or Aspartic Acid, Cysteine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Phenylalanine, Proline, Serine, Valine) are quite frequent in sample one and low in sample three and two; on the other hand, the amino acids (Alanine, Arginine, Glutamate or Glutamic Acid, Glutamine, Methionine, Pyrrolysine, Threonine, Tyrosine, Tryptophan) are present in low concentration or absent.

It can be seen in Table 4 that generally the mineral compositions varied from one sample to another and most of the mineral compounds decrease the sample one to sample three and Ca, P, K, Mg,Fe is the most frequent compared to the others.

Secondary metabolites

The data in Table 5 shows the frequency of secondary metabolites of Rosmarinus officinalis and it is noted that the different secondary metabolites are quite high in sample two and low in sample three and sample one and also there is a variation of its metabolites depending on the type of extraction solvent. Thus, the best extraction solvent is ethanolic.

Table 4. Mineral compositions

Primary metabolites	Presence		
	E1	E2	E3
Ca	+++	++	++
P	+++	+++	++
K	+++	++	++
Na	+	+	+
Cl	+	+	+
S	++	++	+
Mg	+++	++	++
Fe	+++	+++	++
Mn	+++	+++	++
Zn	++	++	+
Pb	+	+	+
Se	+	+	+
Cu	+	+	+
Co	++	++	++

Table 5. Extracts

Compounds chemicals	Presence								
	E1			E2			E3		
	E	C	ET	E	C	ET	E	C	ET
Alkaloids	+	++	+++	++	+++	++++	+	++	+++
Coumarins	++	+	+++	+++	+++	++++	++	++	+++
Flavonoids	+	+	++	+	+++	+++	+	++	++
Tanins	+++	+	+++	++	++	+++	++	+	++
Saponins	+++	++	++	+++	+++	+++	++	++	++

Note: (+++) – strongly present; (++) – moderately present; (+) – weakly present; (-) – negative test.

Quantitative analyses

Primary metabolites

It can be seen from the results of Table 6 that the content of proteins, carbohydrates, and dietary fibers is high in sample one and low in samplestwo and three and the content of lipids is low in the three samples.

Table 6. Protein, carbohydrate, fat, and dietary fiber content

Primary metabolites	Content (%)		
	E1	E2	E3
Proteins	7.61	7.43	7.14
Carbohydrates	7.61	6.92	5.64
Fats	1.48	0.98	1.29
Dietary fiber	4.96	4.22	2.93

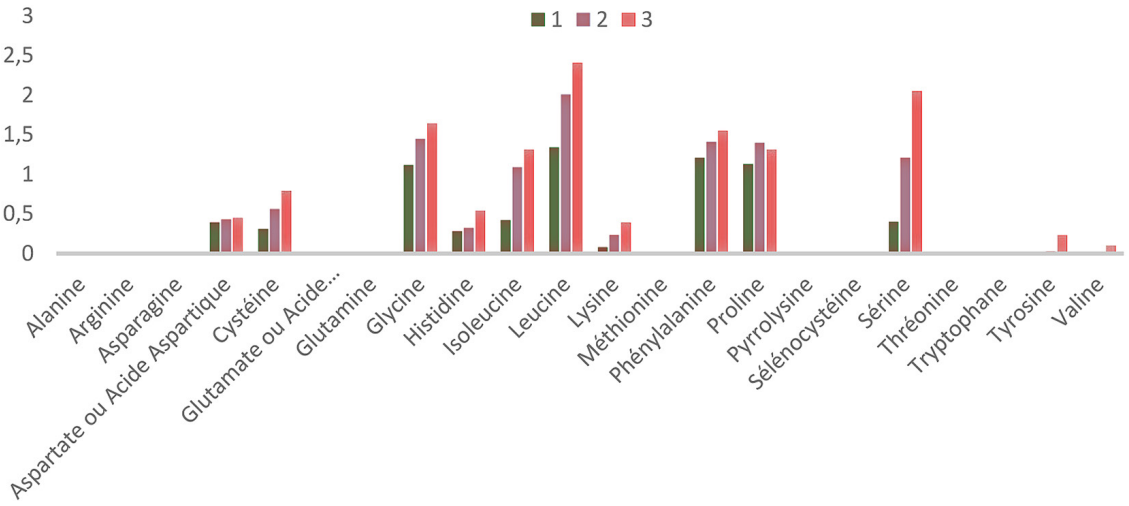

Figure 1. Amino acid content

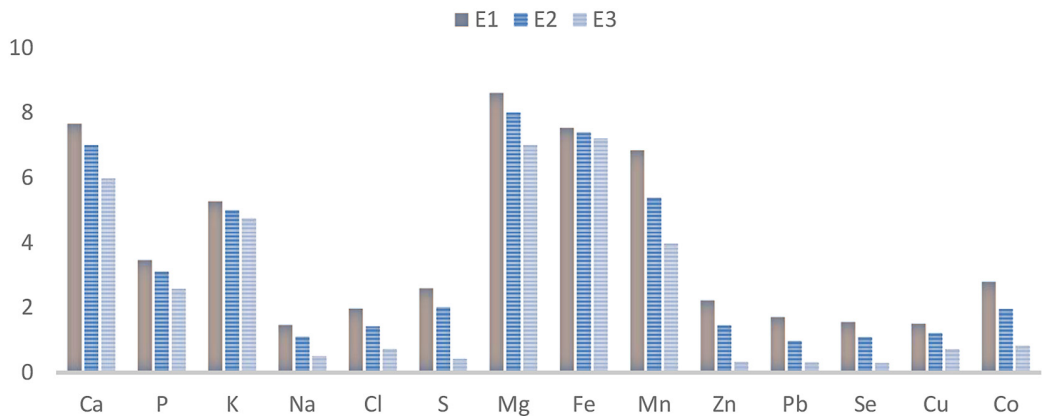

Figure 2. Content of mineral compositions in dry matter

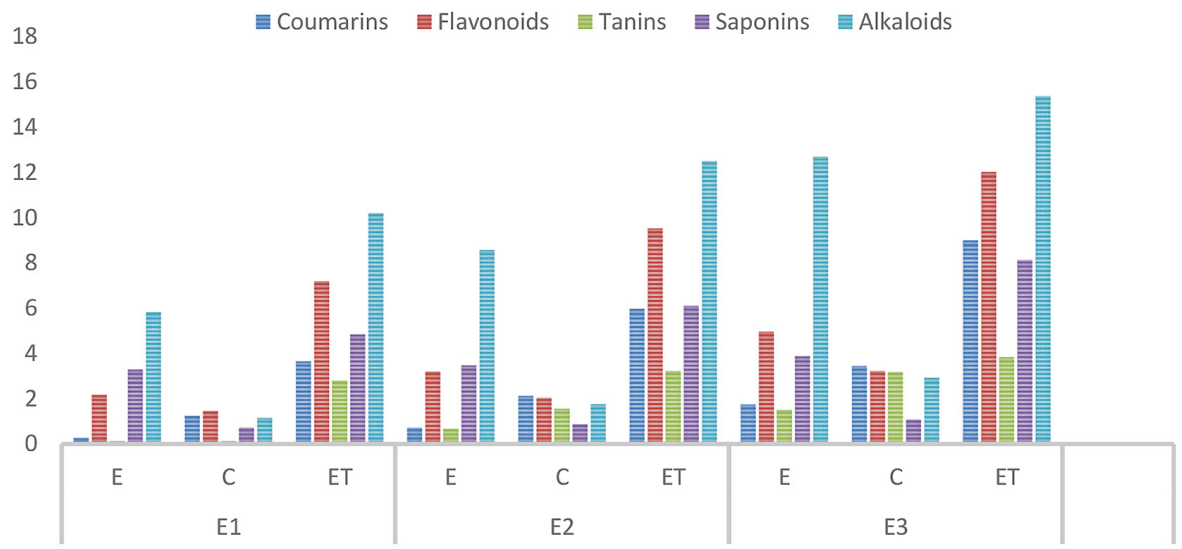

Figure 3. Content of extracts (E= Ethereal; C= Chloroformic ; ET= Ethanolic; E1 or 1=Sample 1 ; E2 or 2=Sample 2 ; E3 or 3=Sample 3)

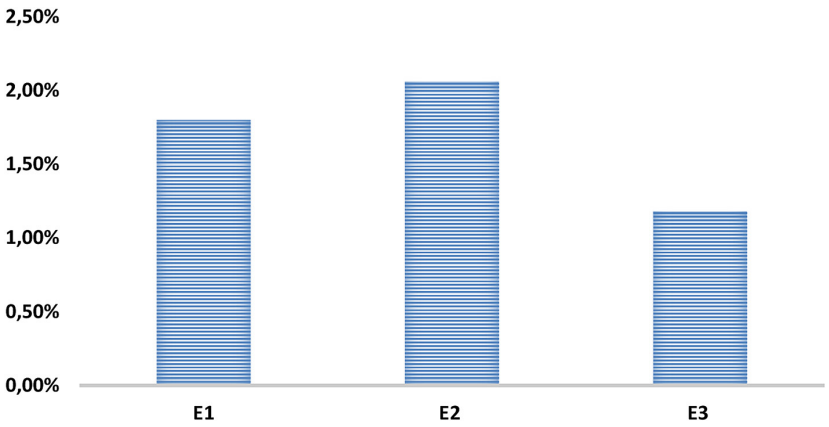

Figure 4. Essential oil yield of the three samples

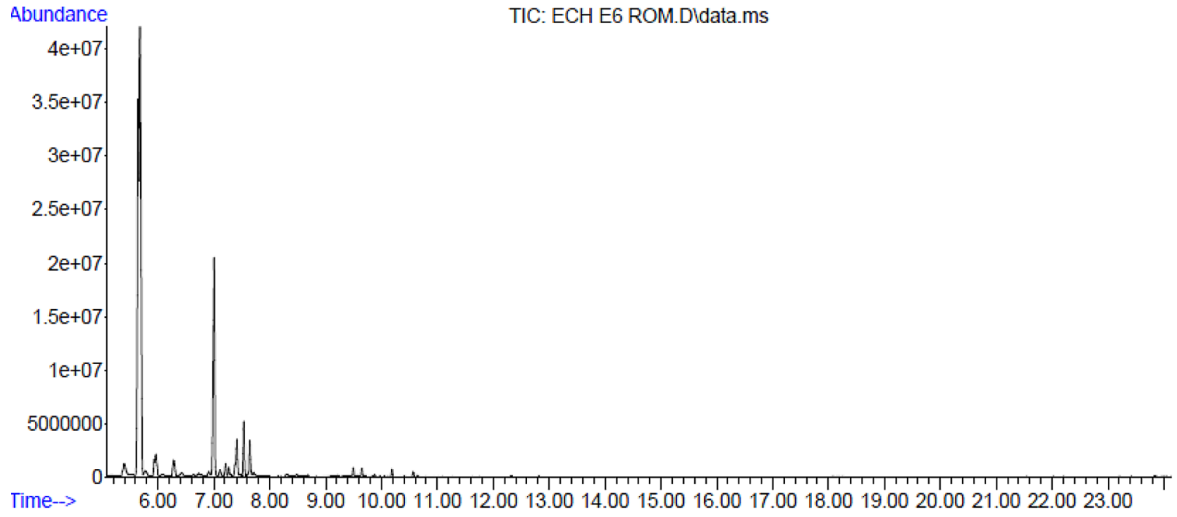

Figure 5. Gas chromatogram (GC) of essential oils extracted from sample one

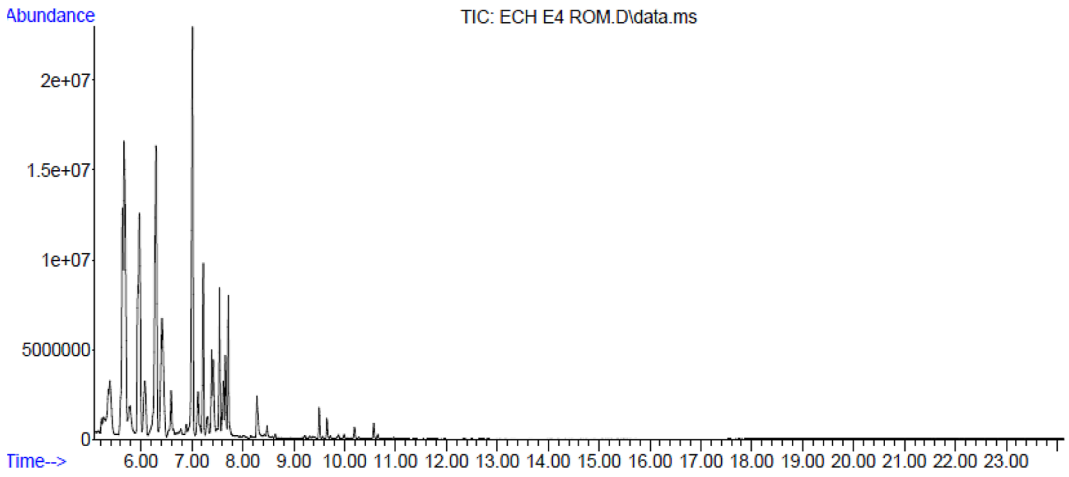

Figure 6. Gas chromatogram (GC) of essential oils extracted from sample two

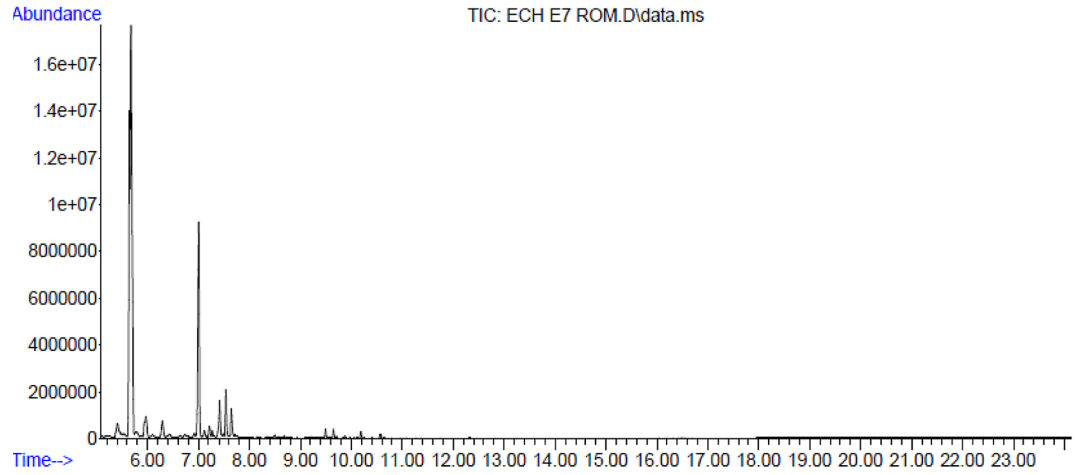

Figure 7. Gas chromatogram (GC) of essential oils extracted from sample three

Table 7. Chemical composition in percentage of essential oils of *Rosmarinus officinalis* of three samples under different climatic conditions

Compounds	Components	Relative percentage		
		S1	S2	S3
1	Alpha-pinene	9.30	8.34	9,17
2	Camphene	4.66	4.14	4,56
3	Beta-pinene	3.85	0.23	9,04
4	a-Terpinene	0.22	0.04	0,08
5	p-Cymene	2.55	0.71	2,07
6	Limonene	0.09	Tr	Tr
7	Cineole	45.89	55.36	43,08
8	Beta-myrcene	3.99	1.93	1,99
9	Linalool	0.35	0.12	0,41
10	Camphre	17.44	21.44	21,56
11	Bornéole	1.09	2.44	0,85
12	a-Terpineole	3.92	4.03	1,66
13	Verbenone	0.54	0.12	0,38
14	Acetate de Bornyle	5.99	1.05	5,42
15	B-Caryophyllene	0.03	0.03	0,09
16	a-Caryophyllene	0.07	Tr	0,08
Total		99.98	99.98	99.98

It is noted from Figure 1 that the amino acid content is highest in sample one and low in sample two followed by sample three. From Figure 2 the content of the mineral compositions in sample one and higher than the mineral compositions of samples two and three and Ca, P, K, Mg, and Fe are the most present compared to the others.

Secondary metabolites

It is observed from Figure 3 that the content of coumarins, flavonoids, tannins, saponins, and alkaloids is higher in sample two compared to the other samples and it is also noted that the content of secondary metabolites also varied depending on the extraction solvent.

Yield of essential oil

It can be seen from Figure 4 that the yield of essential oils is highest in sample two followed by sample one and finally sample three.

Gas chromatography

The chemical composition of the essential oils of *Rosmarinus officinalis* from the three samples is represented in Table 4. The results of gas chromatography analysis of the essential oils were shown in Figures 5, 6 and 7.

It can be seen from Figures 4, 5, 6 and Table 4 that the majority compounds in the three essential oils studied are cineole followed by camphor and other compounds in low percentage so for the percentage of cineole, the highest is sample two 55.36% followed by sample one 45.89% and finally sample three 43.08% and for camphor the highest percentage is sample two 21.44% followed by sample three 21.56% and finally sample one 17.44%.

DISCUSSION

Primary metabolites

Nowadays, climate change is a major problem that threatens life on Earth because of its negative effect on the biosphere, both on plant and animal biodiversity. Thus, the results obtained in the conducted experiments showed that for the sample planted under normal conditions, the content of simple compounds (carbohydrates, lipids, proteins, and dietary fibers) varied in a decreasing way from sample one to three, depending on the increase of temperature and the decrease of precipitation as in sample two and three. Thus, the same results were obtained for mineral compositions and amino acids which are high in sample one to decrease with increasing temperature and decreasing precipitation which is confirmed by [Alhaithloul et al. 2019] who found that water deficiency directly affects the growth, photosynthetic activity, and the slowing down of the passage of water to the roots which will result in the decrease of the entry of nutrients it is a reciprocal relationship as well as [Yang et al. 2022] and [Hessini et al. 2022] who found that the treatments of water deficit decreased the production of biomass and the treatment of water stress 50% led to a significant increase in the levels of total phenolic, benzoic and cinnamic acids compared to the well-watered leaves.

Secondary metabolites

The analysis of the results of the three samples of Rosmarinus officinalis under various climatic conditions shows that their sample grown under normal climatic conditions in the first year the content of secondary metabolites increases in the second year with the increase of temperature and decrease of precipitation, but when the climatic conditions continued to worsen in the fourth year the content of secondary metabolites decreased, as it was found [Podda et al. 2019] that the deficit irrigation can modify the content of secondary metabolites in an increase of temperature and precipitation. Climatic conditions continued to worsen in the fourth year; the content of secondary metabolites decreased, as it was found [Gao et al. 2020] that the deficit irrigation can modify the content of secondary metabolites increasing their content and also [Jan et al. 2021] found that the highest percentage of secondary metabolites is those of plants under unfavorable conditions and [Ahmed et al. 2019] found that the content of secondary metabolites is high in the case of stress.[Ashraf et al. 2018] showed that the plants that suffered a water deficit increased their content of bioactive compounds; in turn, [Sancho-Knapik et al. 2017], [Chávez-Arias et al. 2022], [Griesser et al. 2015] and [Baher et al. 2002] showed thatthe plants under water stress have a high content of phenolic compounds to defend themselves or adapt to the difficult climatic conditions

The yield of essential oil

The resultsobtained in thisstudy show thatthereisan increase in the content of essential oilsfromsample one plantedundernormal conditions to sampletwounderhigh climatic conditions and for samplethree in the fourthyearwhen the climatic change continued to accentuatetheir a decrease in the yield of essential oil.As it was found by [Sarmoum et al. 2019], the plants subjected to light stress contain the highest content of essential oils in comparison with the one that undergoes severe stress and [Keshavarz 2020], [Rahimi et al. 2022] showed that the non-irrigated rosemary represents the highest yield of essential oils compared to the other two samples irrigated by tap water and salt water and (Uma), [Xu et al. 2022] found that the yield of essential oils increased under conditions of water deficit and [Saber et al. 2021] found that the drought tended to increase the oil content and decrease the protein content.

Gas chromatography

The results obtained show that the climate change today is a time bomb that can give catastrophic results especially on plant biodiversity and chemical compositions of plants, so this study shows that the majority compounds in the three essential oils studied are cineole followed by camphor and other compounds in low percentage. Similarly, the highest percentage of cineole is sample two 55.36% planted under average climatic conditions followed by sample one 45.89% grown undernormal climatic conditions and finally sample three 43.08% which was planted undersevere climatic conditions and for camphor the highest percentage is sample two 21.44% followed by sample three 21.56% and finally sample one 17.44%. Thus, this result was confirmed by [Farhoudi 2013] who found that the majority compounds of rosemary under control are: 1,8-cineole, camphene, β-Pinene and their percentages increase under stress and drought conditions and also [Department of Watershed and Rangeland Management, University of Kashan, Iran. and Bidgoli 2018] who found that The important compound in essential oils is Camphor and their percentage increased with the level of drought stress.

CONCLUSIONS

Rosmarinus officinalis is a plant that occupies a primary place in the life of various populations and especially the rural population seen the difficult recourse to their medicine and seesmajor occupation in our daily life, it is necessary to predict their behaviors towards climatic changes. Thus, the results obtained showed that for the sample the content of carbohydrates, lipids, proteins, and dietary fibers is high and it decreases with the increase of temperature and the decrease of precipitation as in samples two and three, For secondary metabolites, it was obtained that the content of secondary metabolites increased in the second year (sample two) when the temperature increased and the precipitation decreased. However, in the fourth year, the content of secondary metabolites diluted when the temperature continued to increase and the precipitation decreased (sample three), whereas for essential oils, the results obtained show that in the first two years the content of secondary metabolites increased and the precipitation decreased. The results obtained show that in the first two years there was an increase in the content of essential oils from sample one planted under normal conditions to sample two under high climatic conditions and for sample three in the fourth year when the climatic conditions continued to increase there was a decrease in essential oil yield.This study showed the highest percentage of cineole is sample two 55.36% planted under average climatic conditions followed by sample one 45.89% grown under normal climatic conditions and finally sample three 43.08% which was planted under severe climatic conditions and for camphor the highest percentage is sample two 21.44% followed by sample three 21.56% and finally sample one 17.44%. Finally,it can be said that climate change currently has a detrimental effect on our life, requiring to take intoconsideration the measures of climate change to mitigate its adverse effects on humanity in general.

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