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# Determine the effect of some environmental factors on the values of carbohydrates, proteins, total phenols and total alkaloids in Eucalyptus *camaldulensis* Kirkuk-Iraq.

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## Abstract

This study was conducted to evaluate the effect of seasonal variations in the values of primary metabolites (carbohydrates and proteins) and secondary metabolites (total phenols and total alkaloids) in Eucalyptus *camaldulensis* grown in the front garden of Kirkuk University. Plant samples were collected from three sites (east, middle, and west of the garden) in three seasons (winter, spring, and summer) from 31/1/2022 to 5/7/2022. Variation recorded in primary and secondary metabolite compounds values. Carbohydrates' highest values 17.78% were recorded in the spring in the third site while the lowest values7.23% were recorded in the first site in the winter. Proteins' highest values 19.64% were recorded in the first site in the summer while the lowest values 4.25% were recorded in the spring. Total phenols higher values78.99 ppm and total alkaloids 2.84 ppm was recorded in the second site in the spring and the lowest value was 44.88 ppm, and 1.58 ppm, respectively, in winter in the first site. Phenolic compounds were identified: Rutin, Chlorogenic Acid, Quercetin, Gallic, and Salicylic. It had an effective role in protecting the eucalyptus plant individuals from environmental factors stress. Eucalyptus camaldulensis individuals tend to use primary and secondary metabolite compounds to protect their cells in stress conditions in the winter and summer seasons.

Key words: *Eucalyptus camaldulensis*, alkaloids, phenols.

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## Introduction

Plants accumulate a wide range of molecules classified into two categories: primary metabolites and secondary metabolites, which are called specialized metabolites because they differ from primary metabolites in that they are not directly responsible for the growth and development of plants under standard growth conditions [1]. Secondary metabolites are chemical compounds that are built during the metabolism of carbohydrates, fats, and amino acids, and are transformed into the acetate or Shikimic acid pathway. They are also built inside the plant during its growth period. These compounds include alkaloids, phenols, flavonoids, and terpenes [2] [3].

These metabolic compounds are released to the environment, especially the rhizosphere, by processes such as filtration, volatile emissions, and exudation of roots and plant residues [4]. These compounds are produced from various parts of the plant, mainly leaves, roots, stems, flowers, seeds, fruits, rhizomes, and even pollen grains [5]. The production of these secondary metabolic compounds is the most successful adaptation of plants during the development of different physiological mechanisms that enable plants to face stress conditions and biotic and abiotic threats. Thus, the production of these secondary metabolic compounds provides plants, unlike other organisms, with the ability to survive in different seasonal conditions without hibernation [6] [7] [8]. Carbohydrates are essential materials for the supporting tissues of the plant, represented by the cell wall (exoskeleton) that provides support and protection for the plant. The proportion of carbohydrates is estimated at two-thirds of the proportion of substances produced from metabolic processes [9].

As for proteins, they are distinguished by the large size of their molecules, as they consist of a large number of amino acids, and are characterized by the fact that they have specialized functional activity, as they are involved in the synthesis of enzymes and hormones, and they are the main engine for most of the vital activities and metabolic processes of the cell [10].

Eucalyptus has other names such as camphor, and frankincense. It is considered an evergreen perennial tree and is characterized by its rapid growth [11].

Eucalyptus trees are among the local trees in Iraq. The tree belongs to the exponential family Mytraceae and reaches a height of several meters [12]. Eucalyptus leaves are the main source for the release of secondary metabolites [13].

Our study aims to estimate the concentrations of some primary and secondary metabolite compounds of the *Eucalyptus camaldulensis* plant in three seasons within the study sites and to determine the effect of nutrient availability on their concentrations or ratios.

## Materials and methods of work:

### 1. study area

The study area is located at the University of Kirkuk near the main entrance of the university on the side of the university parking lot. It is a garden with an area of more than 1,500 square meters planted with *Eucalyptus camaldulensis* trees for more than three years. The distance between one tree and another is 1-1.5 meters (Plate 1 shows the study site). Three sites were chosen to collect samples of eucalyptus leaves from the east, middle, and west of the garden, with three replications for each season.

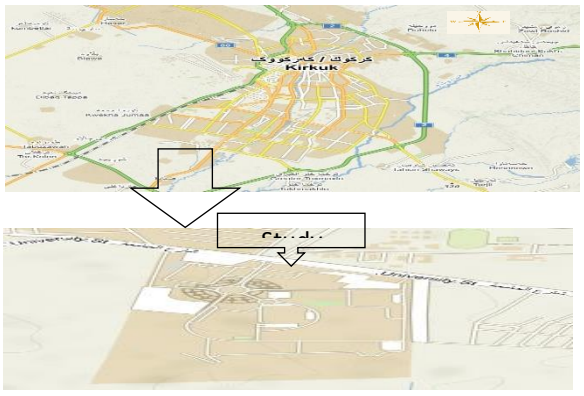


figure 1 study area

**2. Plant physiological characteristics**

. Estimation of the total content of carbohydrates: the phenolic sulfuric acid method was used according to the method of [14]. The spectrophotometer was used at a wavelength of 490 nm.

Estimation of the total content of proteins: [15] was used, which was modified by [16]. The spectrophotometer was used at a wavelength of 750 nm.

Estimation of the concentration of phenolic and alkaloid compounds: According to the method presented by [17], the examination was carried out using a high-performance liquid chromatography device (HPLC).

**Results and discussion:**

**Proteins**

Proteins recorded the following percentages, which are shown in figure 1. The lowest

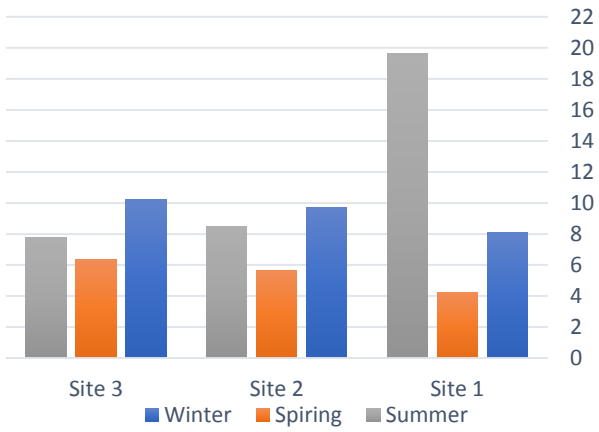


Figure 1 Proteins Percentages recorded at the sites

percentage of proteins was 4.25% in the spring season and the highest percentage reached 19.64% in the summer season for the first site.

Statistical analysis did not record significant differences ($p \leq 0.05$) between the averages relative to the sites and the study seasons (table 1), but the season's average values recorded a variation. It was observed that the protein values were high in the summer 11.96% and decreased in the spring to 5.41%. The decrease in the proportions of proteins can be attributed to its association with the high concentrations of secondary metabolite compounds in the spring, where the secondary metabolite

Table 1 Proteins values recorded at the sites

| Seasons | Site 1 | Site 2 | Site 3 | Mean |
|---|---|---|---|---|
| Winter | 8.08% | 9.74% | 10.20% | 9.34 a |
| Spring | 4.25% | 5.65% | 6.35% | 5.41 a |
| Summer | 19.64% | 8.49% | 7.75% | 11.96 a |
| Mean | 10.65a | 7.96a | 8.1a | |

compounds inhibit the activity of enzymes and factors that help in building protein or inhibition of ascorbic acid responsible for preserving proteins, or it may be the reason for inhibiting the fusion of amino acids which leads to protein reduction [18]. These secondary metabolic compounds can affect the processes of building and transporting amino acids and thus reduce the rate of protein building [19]. These compounds also reduce the absorption of ions and inhibition the growth process in plant cells by inhibiting protein synthesis [20].

Protein's low values in *Eucalyptus camaldulensis* leaves recorded with the high concentrations of secondary metabolic compounds may refer to secondary metabolic compounds causing a significant decrease in the protein content of the cowpea plant [21]. It was noted that there was a variation in the protein content at different sites, where the highest average was recorded at 10.56% for the first site, while the lowest average was recorded at 7.96% for the second site. The effect of secondary metabolite compounds may vary on the protein content in the plant, as these compounds can lead to an increase or decrease in the protein content [22].

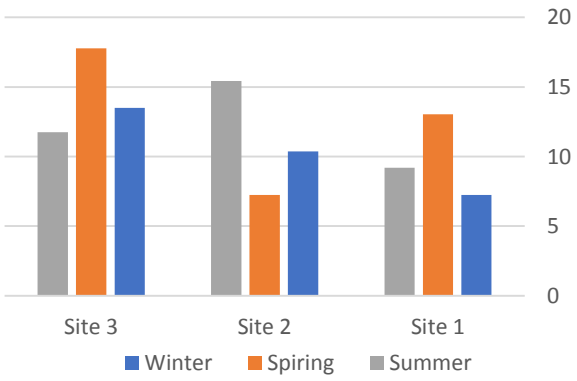


Fig 2 Carbohydrate percentages recorded at the sites

In addition, the study area is considered a traffic point for many cars, and the reduction of the protein content in the plant in the winter and spring seasons may be a result of the exposure of the plant to pollution as a result of the heavy traffic in the study area, which led to the adoption of the plant's behavior. Defensively during the period of exposure to pollution and because the heavy metals that are emitted from vehicle exhausts are washed away by rainwater in January and April and are deposited in the soil, thus posing a threat to the plant [23]. The protein content of *Oleander sp*. leaves has recorded low rates as a result of plant exposure to pollutants, including heavy metals emitted from vehicle exhausts in traffic areas [24].

## Carbohydrates.

The lowest percentage of carbohydrates was 7.23% recorded in the first site in the winter season, and the highest value was 17.78% for

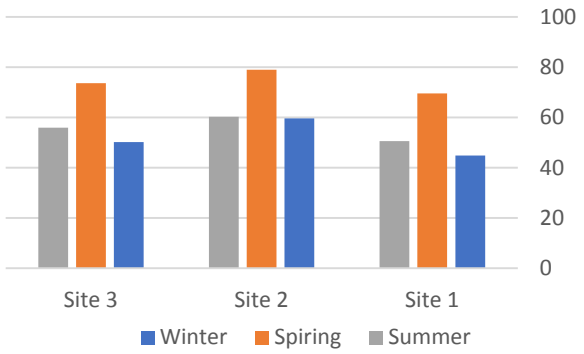


Figure 3 Total phenols values (ppm) recorded at the sites

the third site in the spring season (figure 2). Statistical analysis did not record significant differences between carbohydrate values ($p \leq 0.05$). The highest average was recorded in the spring, reaching 12.68% (table 2).

Table 2 Carbohydrate values recorded at the sites

| Seasons | Site 1 | Site 2 | Site 3 | Mean |
|---|---|---|---|---|
| Winter | 7.23% | 10.37% | 13.49% | 10.36 a |
| Spring | 13.03% | 7.23% | 17.78% | 12.68 a |
| Summer | 9.20% | 15.43% | 11.74% | 12.12 a |
| Mean | 9.82a | 11.01a | 14.33a | |

The plant consists of carbohydrates increase with secondary metabolite compounds, as plants always seek to contain and convert large nutrients into more stable compounds such as primary metabolite compounds (carbohydrates and proteins) and secondary metabolite compounds, where the transport of elements into the cell is a stabilizing factor in cell structure and plant tissues [25]. Therefore, researchers have given great attention to secondary metabolite compounds because of their significant impact on chlorophyll content and increase the absorption of light energy and photosynthesis, which increases plant carbohydrate content [26]. The addition of *Graecum sp.* residues containing secondary metabolite compounds to the shoot system of fenugreek caused a clear stimulation in the carbohydrate content of two wheat cultivars compared to the control [27]. Studying the effect of hot and cold-water extracts of the soft roots of *Chenopodium album* L. caused an increase in the dry weight and carbohydrate content of the leaves of the wheat plant [28].

## Total phenols

Total phenols values are shown in figure 3. The lowest value of total phenols was 44.88 ppm recorded in the first site in winter and the highest value was 78.65 ppm recorded in the second site in spring.

Statistical analysis did not record significant differences ($p \leq 0.05$) between the averages relative to the study seasons but noted that there is a discrepancy in the values of total phenols recorded between the averages. The highest average 74.07 ppm was recorded in the spring season, while the winter and summer seasons converged. 55.57 ppm, and 51.54 ppm, respectively (table 3). Seasonal variations have significant effects on the availability of secondary metabolites in plants. The relationship between the secondary metabolites and seasonal variation is dominated by environmental factors that affect metabolic concentrations and responses in plants [29].

The study seasons during which plant samples were collected are distinguished by different environmental factors such as soil moisture, evaporation rate, light intensity, photosynthesis efficiency, and plant water potential.

Table 3 Total phenols values (ppm) at the sites

| Seasons | Site 1 | Site 2 | Site 3 | Mean |
|---|---|---|---|---|
| Winter | 44.88 | 59.58 | 50.16 | 51.54 a |
| Spring | 69.58 | 78.99 | 73.65 | 74.07 a |
| Summer | 50.58 | 60.25 | 55.89 | 55.57 a |
| Mean | 55.01 a | 66.27 b | 59.9 a | |

The variety in the concentrations of polyphenols in *Tulbaghia violacea*, *Hypoxis hemerocallidea*, *Merwilla plumbea*, and *Drimia robusta* was referred to as changes in environmental factors [30]. *Grewia Flava* leaves collected in South Africa contained high concentrations of phenols in the spring growing season documented the effects of environmental factors [31].

Rutin, Chlorogenic Acid, Quercetin, Gallic, and Salicylic. identified in *Eucalyptus camaldulensis* leaves (table 4). There was recorded a variation in the concentrations.

Table 4 Phenols values (ppm) at the sites

| Season | Sites | Rutin | Chlorogenic | Salicylic | Galic | Qurcetine |
|---|---|---|---|---|---|---|
| Winter | 1 | 18.59 | 5.15 | 18.59 | 10.58 | 13.65 |
| | 2 | 23.65 | 7.58 | 20.56 | 14.59 | 17.49 |
| | 3 | 20.11 | 5.98 | 13.44 | 12.49 | 15.99 |
| Spring | 1 | 20.15 | 6.58 | 20.56 | 13.25 | 15.49 |
| | 2 | 25.49 | 8.99 | 22.59 | 16.49 | 19.58 |
| | 3 | 23.69 | 6.25 | 21.56 | 14.22 | 17.46 |
| Summer | 1 | 17.22 | 6.00 | 14.58 | 11.25 | 14.25 |
| | 2 | 20.25 | 7.23 | 18.55 | 13.25 | 16.25 |
| | 3 | 18.56 | 6.55 | 15.59 | 12.59 | 15.22 |

These compounds were less divergent than what was recorded by [32], where diagnosed Chlorogenic acid Catechin, P-coumaric acid, Benzoic acid, Cinnamic acid, Genisticacid, Kaempferol, Quercetin, Rutin, Ferulic acid and Epigallocatechin. may be severely affected plants with more environmental conditions in study area and seasons.

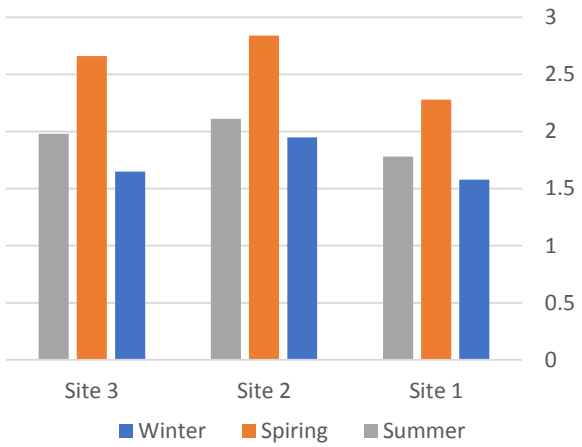


Figure 4 Total alkaloids values (ppm) at the sites

**Total alkaloids**

Total alkaloid values are shown in figure 4. Low variance in concentrations ranged between the highest value of 2.84 ppm in the second site in spring and the lowest value of 1.58 ppm in the first site in winter.

The statistical analysis documented significantly different in the averages of the sites ($p \leq 0.05$). The second site recorded the highest average of 2.3 ppm, while the first and third sites were 2.09 ppm and 1.88 ppm, respectively (table 5).

Table 5 total alkaloids values (ppm) at the sites

| Seasons | Site 1 | Site 2 | Site 3 | Mean |
|---|---|---|---|---|
| Winter | 1.58 | 1.95 | 1.65 | 1.72 a |
| Spring | 2.28 | 2.84 | 2.66 | 2.59 a |
| Summer | 1.78 | 2.11 | 1.98 | 1.95 a |
| Mean | 1.88 a | 2.3 b | 2.09 a | |

Results noted a discrepancy between the values with a non-significant difference, as the highest average of 2.59 was recorded for the spring season compared to the winter and summer seasons. Total alkaloid values decreased in the winter and summer seasons, reaching an average of 1.95 and 1.72, respectively. The secondary metabolite compounds are affected by environmental factors compatible with the stages of growth, as most of the medicinal and aromatic plants

change their content of secondary metabolite compounds with the change of climatic conditions, such as *Digitalis lanata* L. and *Adonis outumnalis* L., whose content of chemical compounds increases with increasing humidity and low temperatures [33] and our results are consistent with [34], which showed that *Fago pyrum esculentum* L. needs moderate climatic conditions to increase its content of secondary metabolites.

**The effect of environmental factors on the values of primary and secondary metabolites.**

The variation in the values of primary metabolite compounds (carbohydrates and proteins) and secondary metabolite compounds (phenols and alkaloids) of eucalyptus trees is a clear indicator of the influence of environmental factors. Variations in weather factors for three seasons (winter, spring, and summer) affected the percentages of primary metabolites, although it was not significant ($p \leq 0.05$).

The percentages of carbohydrates in the spring season, site 3, were 17.78%, the highest percentage of their concentrations, and the lowest was 7.23%, recorded in the first site in the winter. The increase in the yield of carbohydrates was mostly due to the adaptation of weather factors to the optimum limits for photosynthesis. The gross primary productivity of carbohydrates was used in the formation of developing cells and tissues and was not used in the process of protection and self-defense against weather factors. The effect of tension weather factors on eucalyptus plants individuals was evident with the increase in summer temperatures, as the percentages of proteins increased, which were recorded in the first site, the highest percentage of 19.64% used by plants for protection in their living cells (Heat Shock Proteins), and their lowest percentages in spring were 4.25% due to using the bulk of its components in building new cells and tissues during the growing season [34].

Secondary metabolites products (total phenols and total alkaloids) recorded their highest values in the second site in the spring season 78.99 ppm, 2.84 ppm respectively consistent with the values of higher primary productivity in eucalyptus. The lowest values of total phenols 44.88 ppm and 1.58 ppm for total alkaloids were recorded in the winter season at the first site with low primary productivity of the plant consistent with the increase in stress of environmental factors and lack of suitable conditions for growth [32].

**Conclusion**

*Eucalyptus camaldulensis* individuals use the primary metabolites products in the construction of cells and tissues in optimum climate values during the spring season. The environmental factors of stress were recorded in the winter and summer seasons. *Eucalyptus camaldulensis* individuals tend to use primary and secondary metabolite compounds to protect their cells. Heat shock proteins (HSPs) cooperated with the diversity of the identified phenolic compounds, Rutin, Chlorogenic acid, Quercetin, Gallic and Salicylic used as defense compounds. They protect their cells through it.

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# تحديد تأثير بعض العوامل البيئية في قيم الكربوهيدرات والبروتينات والفينولات الكلية والقلويدات الكلية لنبات *Eucalyptus camaldulensis* كركوك–العراق.

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- تاريخ استلام البحث 16/1/2023 وتاريخ قبوله 28/2/2023
- البحث مستل من رسالة ماجستير للباحث الاول

**المستخلص**

أجريت هذه الدراسة لتقيم تأثير التغايرات الموسمية في قيم مركبات الأيض الأولي (الكربوهيدرات والبروتينات) ومركبات الايض الثانوي (الفينولات الكلية والقلويدات الكلية) لأشجار نبات اليوكالبتوس المزروعة في الحديقة الأمامية لجامعة كركوك. جمعت عينات النبات من ثلاث مواقع (الشرق، الوسط والغرب) في ثلاث فصول (الشتاء، الربيع والصيف) ابتداء من 31\1\2022 ولغاية 5\7\2022. سجلت النتائج تبايناً في تراكيز مركبات الأيض الأولي والثانوي. سجلت أعلى قيم الكربوهيدرات 17.78% في فصل الربيع في الموقع الثالث. أما أدنى القيم سجلت في الموقع الأول في فصل الشتاء وبلغت 7.23%. البروتينات سجلت أعلى نسبها 19.64% وأدناها 4.25% في الموقع الأول في فصل الصيف وفي فصل الربيع على التوالي. الفينولات الكلية والقلويدات الكلية سجلت أعلى قيمهما 78.99 جزء بالمليون و2.84 جزء بالمليون في موقع الثاني في فصل الربيع على التوالي. أدنى قيم الفينولات الكلية 44.88 جزء بالمليون، والقلويدات الكلية 1.58 جزء بالمليون سجل في فصل الشتاء في الموقع الأول. المركبات الفينولية المشخصة Chlorogenic Acid , Qurcetine , Gallic , Salicylic Rutin كانت لها دور فعال في حماية أفراد نبات اليوكالبتوس من شد العوامل البيئية. افراد نبات *Eucalyptus camaldulensis* استخدمت مركبات الايض الأولي والثانوي لحماية خلايا الانسجة النباتية من شد العوامل البيئية في فصلي الشتاء والصيف.

**الكلمات المفتاحية:** *Eucalyptus camaldulensis*، القلويدات، الفينولات.