Effect of Fertilization and Irrigation on Essential Oils Extracted from Laurel (*Laurus nobilis* L.) Leaves

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Summary

This study focuses on the effects of fertilization and irrigation on the percentage of essential oil extracted from laurel (*Laurus nobilis* L.) leaves. Trees in the Aleppo Ecopark were treated with three different NPK fertilizers and three different irrigation water rates for two consecutive years, while the control group was left untreated. Phosphorus and potassium fertilizers were applied at the end of January, nitrogen fertilizer at the beginning of March. Additional irrigation was applied three times a year in summer (May, July and September). The leaves of each treatment were collected, dried and the essential oil was extracted with a clavinger. In addition, the extracted oil for each treatment was analyzed by gas chromatograph equipped with mass spectrometer. Results have shown that the fertilization and irrigation processes led to an increase in the essential oil percentage that was extracted from bay leaves and affected its quality, giving the highest percentage of oil, reaching 0.93% when treated with 1.5 kg of urea, 0.75 kg of triple superphosphate, 0.75 potassium sulphate and irrigation with 100 liters of water per tree. Fertilization and irrigation also influenced the proportions of essential oil components. Fertilization with 2 kg urea, 1 kg triple superphosphate, 1 kg potassium sulphate and irrigation with 150 litres of water for each tree gave the highest concentration of 1-8 cineole compounds, which reached 50.23%.

Key words

essential oil, fertilization, irrigation, laurel, *Laurus nobilis* L.

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Introduction

Laurel (*Laurus nobilis* L.), an important aromatic medicinal plant, belongs to the laurel family (Lauraceae), order Laurales, suborder Magnoliidae, class Magnoliopsida (Cronquist, 1981). The Mediterranean region is the original home of the laurel, and it is also widely cultivated in Europe, California, the United States of America and some Arab countries (Sharma et al., 2012).

The leaves contain 0.6-2.6% aromatic essential oil, consisting mainly of cineol, linalool, eugenol, geraniol and tannins (Attard and Pacioni, 2011).

The fruits contain: laurel acid, pinene, philanderine philandering, volatile essential oil (3%), eugenol, tannins, mucilages, resinous substances, linalool, cineol and geraniol. The fruit also contains 24-55% fatty substances for texture. Oil is obtained by steaming and pressing the fruits thus producing the precious product (Marzouki et al., 2009).

Chemical analysis of laurel leaves by gas chromatography (GC) yielded 276 chemical compounds, of which 50 were identified (Hokwerda et al., 1982). The main compound in most studies was 1.8 cineole with varying concentrations. In Turkey, it ranged from 59.9% to 46.6% (Ozcan et al., 2010), while the concentration in Croatia decreased to 45.5% (Politeo et al., 2007), in Morocco to 39.81% (Cherrat, 2014) and the lowest cineole concentration in Portugal was 27.2% (Ramos et al., 2012). Besides cineole, there are several other important compounds, such as sabinene, α-pinene, linalool, α-terpinyl acetate, β-pinene, methyl eugenol, 2-carene, trans-ocimene, cis-ocimene, trans-caryophyllene and many other minor compounds.

The cultivation and production of aromatic medicinal plants is one of the most important sectors of plant production in the world and an important source of natural products used in the pharmaceutical or food industry as well as in cosmetics, perfumes, soaps and detergents. These plants contain many active ingredients and essential oils. Improving plant growth and producing oils of better quantity and quality is one of the important issues to be studied through agricultural practices that affect the morphological, physiological and productive properties of the plant (Alwan, 2001).

Agricultural treatments such as fertilization, irrigation and timing of sampling have a great influence on the percentage and content of essential oil of the plant.

The composition of the essential oil is influenced by the availability of nutrients in the soil, with important and less important mineral elements being involved in the basic building of organic compounds in almost all phases of plant life processes; they also influence the composition of the chemical content of the essential oil. As for the compounds that are not included in the composition of organic compounds, they influence the necessary activation of enzymes in the life processes.

Nitrogen is the main influencing factor in the formation of essential oil in many aromatic plants. Increasing the nitrogen content in the soil leads to an increase in the amount of essential oil and its content of methylchavicol and asarone and decreases the content of linalool. In other plant species, increasing the potassium content in the soil leads to an increase in the content of essential oil and the essential oil components cineole, linalool, eugenol and cadinene (NurzyĚska-Wierdak,2013).

On the other hand, research shows that the quantity and quality of essential oils are influenced by the amount and type of irrigation water. Irrigation at 55% of field capacity resulted in a significant increase in the amount of essential oil extracted from *Agastache foeniculum* compared to the irrigation treatment at 100% of field capacity, which gave the lowest value. Among the experimental treatments, the effect of the amount of irrigation water on the components of this oil from methyl chavicol and limonene was low (Omidbaigi and Mahmoodi, 2013).

Since the effect of irrigation is in contrast to the effect of fertilization on the amount of oil extracted from laurel leaves (as shown in previous studies), the research aims to study the combined effect of fertilization and irrigation together, as they are the two most commonly used agricultural practices by farmers.

Materials and Methods

Plant Materials

18-year-old laurel trees planted in the Aleppo Ecological Park were fertilized and watered for two years, then their leaves were collected to extract and analyze the oil.

Soil Properties

A composite sample of five individual samples was taken from the study area at a depth of 30 cm, each sample weighing 1 kg. It was cleaned, pneumatically dried, ground and sieved with a 2 mm sieve. This soil was analyzed according to the accepted methods (Ryan et al. 1998) to determine its physical and chemical properties in the laboratories of the Soil Department of the Faculty of Agricultural Engineering of Aleppo University.

Fertilization and Irrigation

The trees in the Ecological Park were divided so that each treatment included 6 trees (3 female, 3 male). Light pruning was carried out (removal of dry, diseased, broken, weak branches and debris) and the following fertilization and irrigation were applied in two consecutive years.

A0: First treatment: (control): Trees left untreated.

A1: Second treatment: NPK fertilized at a rate of 1.5 kg urea 46%, 0.75 kg triple superphosphate 46% and 0.75 kg potassium sulphate 50%. Irrigation was 100 liters per tree.

A2: Third treatment: NPK fertilized at the rate of 2 kg urea 46%, 1 kg triple super phosphate 46%, 1 kg potassium sulphate 50%, irrigation 150 liters per tree.

A3: Fourth treatment: NPK fertilized at a rate of 2.5 kg urea 46%, 1.25 kg triple super phosphate 46%, 1.25 kg potassium sulphate 50% with irrigation of 200 liters per tree.

The dates of the treatments were:

In the first year, phosphate and potassium fertilizers were added on January 28, 2019 and nitrogen fertilizers on March 1, 2019. Supplementary irrigation was applied three times a year, on May 17, 2019, July 12, 2019, and September13, 2019.

In the second year, phosphate and potassium fertilizers were added on July 02, 2020 and nitrogen fertilizers were added on March 02, 2020. Al-Rai was applied on May 15, 2020, July 16, 2020, September 15, 2020.

Extraction of Essential Oil

The leaf samples were dried in the shade until their weight was stable. The essential oil was extracted from the leaves by placing 50 g of dried and crushed bay leaves in 600 ml of distilled water in a clavinger distiller for 3 hours.

The extracted essential oil samples were treated with anhydrous sodium sulphate to get rid of moisture and the essential oil was stored in the refrigerator at 5 °C until the tests were carried out (Erturk, 2006).

Determination of the Chemical Composition

Determination of the chemical composition of the essential oils was carried out using a GC (gas chromatography) instrument (Agilent model 7890A, Agilent Technologies, USA), available in the Organic Physics Laboratory in the Faculty of Science, Department of Chemistry, University of Aleppo. The instrument was equipped with a MS (mass spectrometer) (Agilent model 5975C, Agilent Technologies, USA).

GC process: the oil was dissolved in hexane, an amount of 2 µl was taken and manually injected into the instrument using a non-polar capillary column 5dp, the carrier gas was helium, the flow was 0.6 mL min^{-1}, the fractionation ratio was 1:50, the injector temperature was 300 °C.

The thermal program started at 60 °C, then the temperature was increased at a rate of 3 °C min^{-1} to 165 °C and this temperature was held for 5 minutes, then it was increased at a rate of 20 °C min^{-1} to 300 °C and held for one minute.

The total duration of the analysis was set at 48.75 minutes.

Analysis by mass spectrometry was performed as follows: ion source temperature 230 °C, quadrupole temperature 150 °C, scan range 50-550, ion source energy 70 MeV.

The GC/MS computer was equipped with a library of mass spectra of natural organic compounds, including the essential oil constituents and a program to calculate the weight percentages of the constituents. Each GC/MS peak corresponded to a constituent of the essential oil, the mass spectra of all peaks (constituents) were recorded and compared by computer with the spectra stored in the library.

Statistical Analysis

The trial was designed as a randomised block design (RCBD), 6 trees were selected for each treatment (3 female and 3 male), so that the number of experimental trees was 24. Equal weights were mixed from the leaves of each treatment of trees to obtain a sample for each treatment, then the essential oil was extracted by clavinger with 3 replicates for each treatment. ANOVA Test was applied to identify significant differences between the remedies with LSD at $P < 0.01$ significance level.

Results and Discussion

Soil Properties

The soil texture was silty clay loam, moderately acidic, poor in organic matter (Table 1).

The content of soil was also poor of nutrient elements compared to the standard levels approved in the laboratories of the General Authority for Scientific Agricultural Research.

Table 1. Results of soil analysis

Texture	silty clay	**Ph (on 28 °C)**	6.7
EC (on 31 °C)	3 µMoh cm^{-1}	**Organic matter**	1.09 %
$Caco_3$	13.68 %	**N**	23.31 ppm
P	12 ppm	**K**	102.4 ppm
Zn	0.78 ppm	**Cu**	1.8 ppm
Fe	4.8 ppm	**Mn**	6.8 ppm

The Effect of Fertilization and Irrigation on the Percentage of Essential Oil Extracted from Laurel Leaves

The results (Table 2) show the superiority of the three fertilization and irrigation treatments over the control treatment (3 levels of fertilization and three levels of irrigation). The highest percentage of essential oil was found in the first fertiliser and irrigation treatment (1.5 kg urea, 0.75 kg triple superphosphate, 0.75 potassium sulphate and 100 liters of water for each tree), where the average percentage of essential oil for the leaves was 0.93% (volume/weight).

Table 2. Percentage of essential oil in the four treatments of leaves

	A_0	A_1	A_2	A_3
Percentage of essential oil	0.945 b	1.820 a	1.390 ab	1.528 a
LSD $P < 0.01$		0.726		

Note: **A_0** – control (trees left untreated); **A_1**- NPK fertilized at a rate of 1.5 kg urea 46%, 0.75 kg triple superphosphate 46% and 0.75 kg potassium sulphate 50%, Irrigation was 100 L per tree; **A_2** - NPK fertilized at the rate of 2 kg urea 46%, 1 kg triple super phosphate 46%, 1 kg potassium sulphate 50%, irrigation 150 L per tree; **A_3** - NPK fertilized at a rate of 2.5 kg urea 46%, 1.25 kg triple super phosphate 46%, 1.25 kg potassium sulphate 50% with irrigation of 200 liters per tree.

At the higher fertilization and irrigation levels, the differences between them and the first level were not significant.

These results are similar to those of Maatallah et al. (2013) on laurel and to the results of Omidbaigi and Mahmoodi (2013) on

Agastache foeniculum (Pursh) Kuntze that irrigation with a certain amount positively affects the percentage of essential oil extracted from the plant and that higher amounts of irrigation water negatively affect the percentage of essential oil.

These results are also similar to the results of Alizadeh et al. (2010) on *Satureja hortensis* L. and the results of Bufaloa et al. (2015) on *Ocimum basilicum* L., according to which mineral fertilization leads to an increase in the amount of essential oil extracted from the plant. This can be explained as follows: nitrogen and potassium have a positive effect on the proportion of the essential oil. In addition, there is the influence of other elements such as phosphorus (NurzyĚska-Wierdak, 2013).

Effect of Fertilization and Irrigation on the Compounds of the Essential Oil Extracted from Laurel Leaf

The number of chemical compounds of the essential oil from the GC-MS analysis was varied as the instrument yielded 31 compounds in the control treatment (Fig. 1), 41 compounds in the first stage of treatment with fertilization and irrigation (Fig. 2), 51 compounds in the second stage of treatment (Fig. 3) and 43 compounds in the third stage of treatment (Fig. 4).

All the oils were characterised by a high content of 1-8 cineole and a reasonable amount of limonene, eugenol, α-pinene, β-pinene, methyleugenol and L-4-terpinol. It is observed that fertilization and irrigation resulted in an increase in the concentration of 1-8

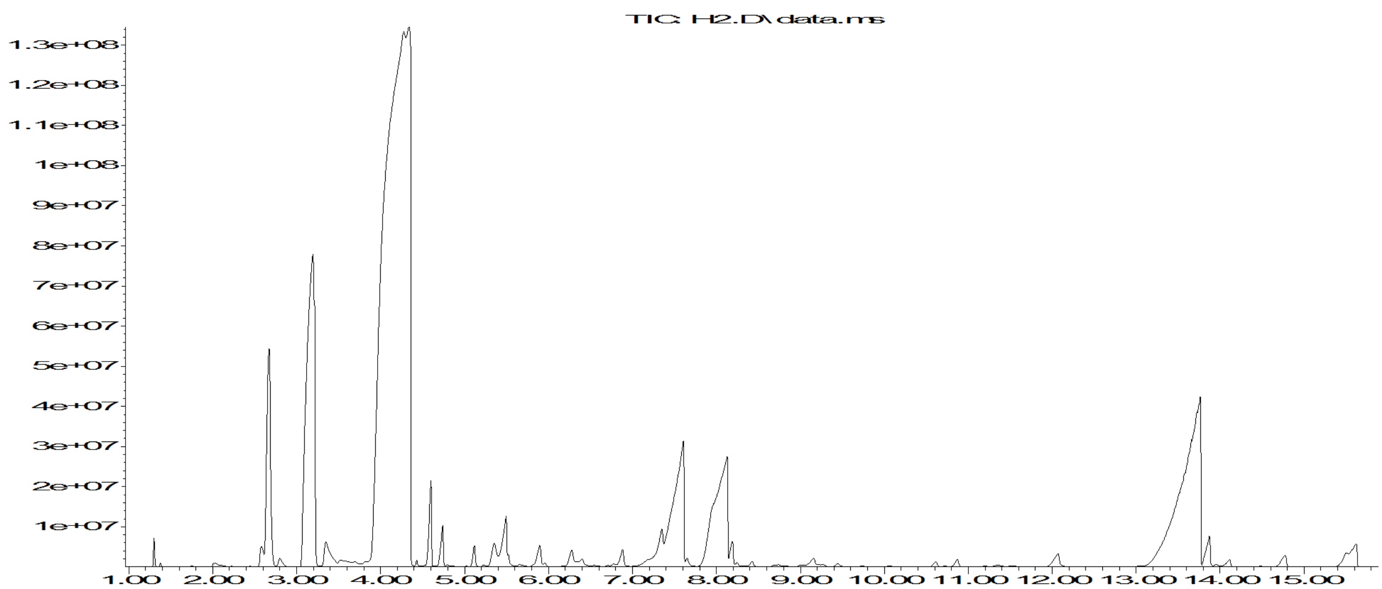

Figure 1. Chromatogram of the essential oil from laurel leaves in the control treatment

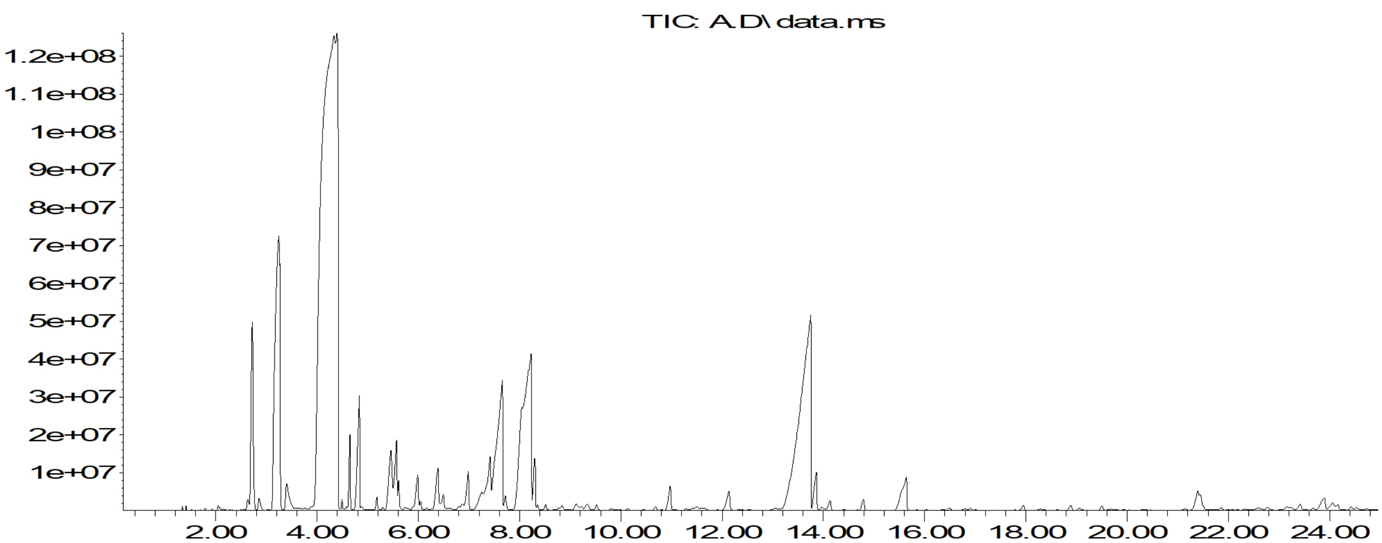

Figure 2. Chromatogram of the essential oil from laurel leaves in the A1 treatment (NPK fertilized at a rate of 1.5 kg urea 46%, 0.75 kg triple superphosphate 46% and 0.75 kg potassium sulphate 50%. Irrigation was 100 liters per tree.)

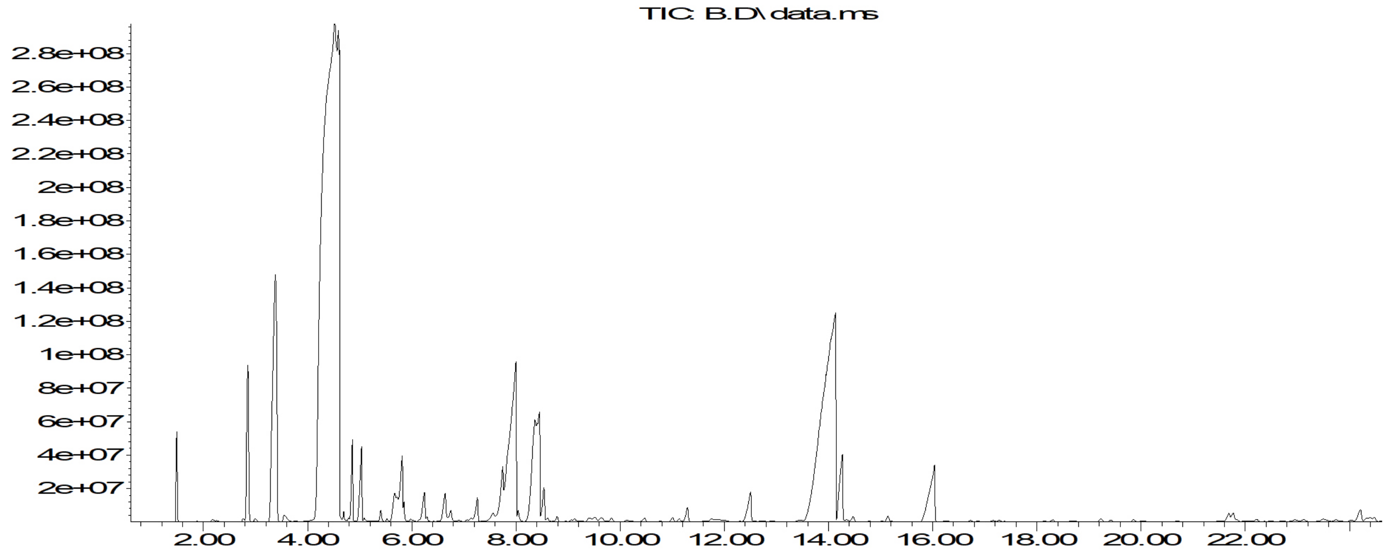

Figure 3. Chromatogram of the essential oil from laurel leaves in the third treatment

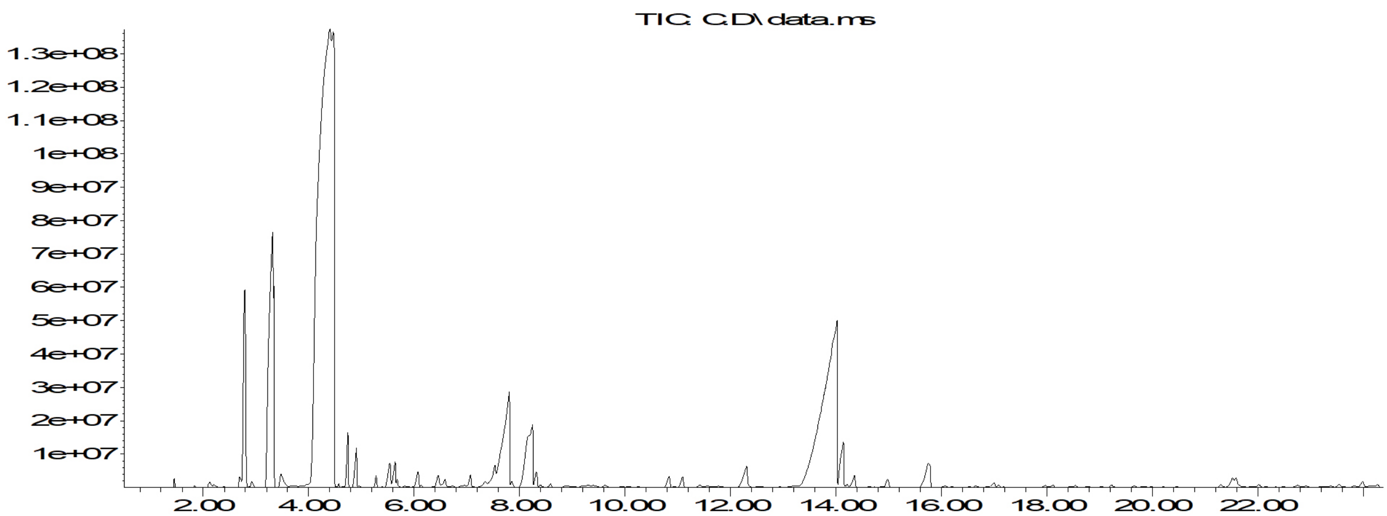

Figure 4. Chromatogram of the essential oil from laurel leaves in the fourth treatment

cineole, with the percentage increasing in the control treatment (50.23%) and in the third treatment (fertilization with 2 kg urea, 1 kg triple superphosphate, 1 kg potassium sulphate and irrigation with 150 litres of water per tree) to (54.22%) (Table 3). Fertilization and irrigation also increased the proportion of trichloromethane and limonene in the essential oil. For the compounds α-pinene, β-pinene, terpinene, eugenol, methyleugenol, mytenol, sabinol-trans, sabinol hydroxide, cis-p-mentha-2,8dien-1-ol, α-terpinol, 3-cyclohexene-1-methanol-α and α4-trimethyl-, fertilization and irrigation resulted in a decrease in their essential oil content (Table 3).

It is difficult to compare the results of oil yield (classes and compounds) because they depend on many factors, as described by Figueiredo et al. (2008). However, studies have shown that the influence of irrigation amount on essential oil content is small (Coban et al., 2018; Omidbaigi and Mahmoodi, 2013). Therefore, this may be the reason for the similarity of the results in this research to those of Alizadeh et al. (2010) showing that mineral fertilization leads to an increase in the percentage of essential oil and affects its quality, as the percentage of essential oil from *Satureja hortensis* L. is increased and its quality is affected by the use of mineral fertilisers of NBK, the elements Fe, Mn, Zn, B and Cu. The quantity of some oil components such as carvacrol, terpenes and terpenes was affected by the use of these fertilisers

These results are also similar to those of NurzyĚska-Wierdak (2013), according to which potassium fertilization slightly affects the essential oil components and increases the amount of 1-8 cineol and linalool but contradicts his study showing that the effect of fertilization increases the concentration of eugenol, whereas fertilization with higher potassium concentrations decreases the amount of eugenol. This may be due to the effect of nitrogen and phosphorus fertilization and the interaction between the three elements NPK.

Table 3. Chemical composition of the laurel leaves essential oils

Compounds	A_0		A_1		A_2		A_3	
	RT	%	RT	%	RT	%	RT	%
Trichloromethane	1.304	0.136	2.632	0.317			1.487	0.675
α Pinene	2.683	3.14	2.714	3.51	2.775	3.443	2.875	1.91
β Pinene	3.2005	9.955	3.22	9.712	3.315	8.973	3.378	6.178
1,8-Cineole	4.3135	50.231	4.368	53.41	4.402	54.222	4.523	53.112
Terpinene	4.62	0.883	4.684	0.64	4.738	0.613	4.86	0.542
Sabinol-trans	4.938	1.56	4.835	0.78	4.896	0.572	5.038	0.482
Sabinene hydroxide	5.449	0.456	5.455	0.36	5.278	0.129	5.406	0.152
Linalool	5.507	1.24	5.57	1.41	6.014	1.52	5.8	2.033
Cis-p-Mentha-2,8dien-1-ol	5.897	0.51	5.988	0.48	6.068	0.213	5.987	0.067
Pinocarveol	6.278	0.456	6.388	0.66	6.455	0.491	6.235	0.542
2(10)-pinene-one,(±)-	6.88	0.247	7.987	0.39	6.582	0.267	6.747	0.189
αTerpinol	7.02	1.51	7.418	1.44	7.353	0.49	7.252	0.446
L-4-Terpinol	7.61	5.63	7.664	5.48	7.801	5.244	7.979	5.899
3-Cyclohexene-1-methanol-α,α4trimethyl-	8.127	3.23	8.23	2.022	8.213	2.346	8.414	1.893
Myrtenol	9.159	0.41	8.3	0.42	9.299	0.2	9.122	0.111
Bomylaceate	10.87	0.204	10.977	0.35			11.289	0.226
Laevo-β-Pinene	12.07	0.126	12.15	0.38			12.492	0.623
α-Terpinol aceate	12.67	0.32			14.004	1.117		
Limonene	13.7225	10.616	13.529	11.113	14.655	13.656	14.141	15.236
Eugenol	14.781	5.221	13.675	3.57	14.991	2.612	14.267	1.295
Methyl Eugenol	16.01	1.81	15.565	1.12	15.75	1.23	16.014	0.958
Ledenoxide	20.64	0.44	21.4	0.38	22.023	0.356	21.766	0.361
Caryophyllene oxid			21.848	0.22			23.199	0.109
Total		98.331		97.847		97.694		97.919

Note: A_0 – control (trees left untreated); A_1- NPK fertilized at a rate of 1.5 kg urea 46%, 0.75 kg triple superphosphate 46% and 0.75 kg potassium sulphate 50%, Irrigation was 100 L per tree; A_2 - NPK fertilized at the rate of 2 kg urea 46%, 1 kg triple super phosphate 46%, 1 kg potassium sulphate 50%, irrigation 150 L per tree; A_3 - NPK fertilized at a rate of 2.5 kg urea 46%, 1.25 kg triple super phosphate 46%, 1.25 kg potassium sulphate 50% with irrigation of 200 liters per tree.

Conclusions

Fertilization and irrigation influence the percentage of essential oil extracted from the bay leaves of *Laurus nobilis* L.. Fertilization with (1.5 kg urea, 0.75 kg triple superphosphate, 0.75 potassium sulphate) and irrigation with 100 liters of water for each tree gave the highest percentage, which reached 0.93%. Higher amounts of fertilization and irrigation did not produce significant benefits. Fertilization and irrigation affected the percentages of the components of the essential oil extracted from the leaves. Fertilization with 2 kg urea, 1 kg triple superphosphate, 1 kg potassium sulphate and irrigation with 150 liters of water for each tree gave the highest concentration of cineole 1-8, which was 50.23%.

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