

## EFFECT OF THREE VARIETIES OF BASIL AND THE NITROGEN SOURCE IN ESSENTIAL OIL CONTENTS

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### ABSTRACT

The study was conducted during the 2018 growing season in a field belong to Horticulture and Forestry Division/ Iraqi Ministry of Agriculture / Al-Najaf Agricultural Directorate. Three varieties of basil were tested as the first factor including *Ocimum basilicum* var. basilicum (sweet basil), *O. basilicum* var. purple (red basil) and *O. basilicum* var. citriodorum. (lemon basil). The second factor was the effect of three sources of nitrogen fertilizers including chemical using Urea at the level of 400 mg. L<sup>-1</sup>, Organic fertilizer using a liquid organic nitrogen at 4 ml. L<sup>-1</sup> and Bio- fertilizer using *Azotobacter chroococcum* at 5 gm. bacteria .m<sup>-2</sup> was applied to the soil while the control treatment was the spraying with distilled water. Randomized Complete Block Design (R.C.B.D.) was used as a factorial experiment in a split-plot system with three replicates for each treatment. The L.S.D. test was adopted to compare mean with a probability of 0.05 level (P <0.05).The Results showed superiority of red basil cultivar increased the active constituents content of Linalool to 64.96 mg.gm<sup>-1</sup> Myrcene to 29.87 mg.gm<sup>-1</sup>. While the Nitrogen source with Bio-fertilizer was superior increased Camphor ,Linalool, Limoine and Myrcene to 7.82,77.92,18.45and 35.86 mg.gm<sup>-1</sup> respectively, compared with control treatment.

Key Words: Basil varieties, Urea, Organic Fertilizer, Bio-fertilizer, Essential oil.

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\*The research is taken from the thesis of the first researcher

### INTRODUCTION

*Ocimum pp.L.* is a one of the important leafy vegetable plants which belong to Lamiaceae family, that's includes many aromatic plants that are spread all over the world, especially in the countries of East Asia, the Mediterranean basin and the African continent (14). It is also one of the most important sources of volatile essential oils, which are characterized by the strong aromatic smell because they contain terpenes, which collect inside specialized glandular trichomes (7). Myrcene and Linalool Therefore, fresh and dry green basil leaves are used, as well as their content of volatile oils and its various types in the fields of medicine, preventive and complementary therapies and pharmaceutical preparations due to their biological activity

and the lack of their side effects compared to the pain medications chemically (4). Dry basil leaves are also used as strong spices or flavors in preparing food. Wierdak (18) find the Plants belonging to the *Ocimum* genus are a rich source of essential oils that have expressed many biological activities and their use in therapeutic areas widely used, there is a large variety of chemical patterns varying in type and concentration within the same cultivar basil. Some studies showed a significant heterogeneity in morphological and chemical properties of basil varieties and indicated the importance of the *O.basilicum* var. citriodorum. (lemon basil) for the accumulation of the essential oil content in its leaves by 1.09% and high (20.5%) geranial, neral (15.8%), (10.7%) E-caryophyllene, (10.1%) bisabolene and (9.8%) linalool and

nerol 7.8%. So, the aim of this study is to know the effect of the source of nitrogen on content of some active constituents for three cultivars of basil plant under the environmental conditions of the Iraqi Middle Euphrates region.

## MATERIALS AND METHODS

A field experiment, arranged in a randomized complete blocks design with three replications, was conducted in the

Experimental field of Horticulture and Forestry Division of the Directorate of Agriculture Najaf province, during the growing season of 2018, Soil analysis tests were carried out before planting and fertilization by taking ten samples, to be analyzed in the analysis laboratory of the Department of Soil and Water Resources in the Faculty of Agriculture / University of Kufa, as shown in Table (1).

**Table 1: Physical and chemical properties of the experimental soil**

Physical analysis		Chemical analysis			
Sand	200 gm.kg <sup>-1</sup>	pH	7.3	Mg <sup>++</sup> (m.equ.L <sup>-1</sup> )	2.71
Silt	350 gm.kg <sup>-1</sup>	EC mmohs/cm	1.98	Na <sup>+</sup> (m.equ.L <sup>-1</sup> )	9.24
Clay	450 gm.kg <sup>-1</sup>	Total N%	0.72	HCO <sub>3</sub> <sup>-</sup> (m.equ.L <sup>-1</sup> )	0.32
Soil texture	Clay loam	Total P%	0.32	Cl <sup>-</sup> (m.equ.L <sup>-1</sup> )	4.39
Organic matter (O.M)	4.9 gm.kg <sup>-1</sup>	Total K%	0.75	SO <sub>4</sub> <sup>-</sup> (m.equ.L <sup>-1</sup> )	19.72
field capacity %	28.32 %	Ca <sup>++</sup> (m.equ.L <sup>-1</sup> )	3.75	Fe (mg.L <sup>-1</sup> )	0.40

The experiment included two factors, which included three varieties of basil, *Ocimum basilicum* var. *basilicum* (sweet basil), *O. basilicum* var. purple (red basil) and *O. basilicum* var. *citriodorum*. (lemon basil), Its symbol (V1, V2 and V3) respectively and the second factor, it was using different sources of nitrogen fertilization, as it included both the nitrogenous fertilizer urea CO (NH<sub>2</sub>)<sub>2</sub> containing 46%N which sprayed on the vegetative at a concentration of 400 mg. L<sup>-1</sup> (5), Symbolize it (N1). The second was the liquid organic nitrogen (plus Optimus) prepared with nano-technology, which contains organic nitrogen at a concentration of 4% and the product was produced by the company Agri. Sciences, as it was sprayed on the vegetative growth at the level of 4 ml. liters<sup>-1</sup> according to the recommendation of the producing company has code (N2) and third source is addition of the soil applied *Azotobacter chroococcum*, with a concentration of CFU106 ml<sup>-1</sup> at 5 gm. bacteria. m<sup>-2</sup>, The symbol has N3 (8), the control treatment (without addition) that is sprayed with distilled water only and has a

symbol of N0. All necessary cultural practices and plant protection were followed uniformly for all the plot during the entire period of experimentation fifteen plants were randomly selected from each plot for extraction essential oil from basil leaves. Randomized Complete Block Design (R.C.B.D.) was used as a factorial experiment (3×4) in a split-plot design with three replicates for each treatment. The comparison was done using L.S.D. test to compare means with a probability of 0.05 level (P < 0.05).

### Essential oil extraction

According method Kmiecik *et al.* (9) essential oils were extracted from air dried plant material (leaves) by hydro-distillation, using a modified Clevenger type apparatus and lasted 3 h. The distilled essential oils had been dehydrated with 25 ml C<sub>4</sub>H<sub>10</sub>O and stored at 4 °C in dark airtight bottles until further.

### Measuring some active constituents in volatile oil

Some of the active compounds from the oil extracted were measured by the GC

chromatography device to estimate the concentration of each of the following compounds: Camphor, Linalool,  $\alpha$ -pinene, limonine and myrcene (12).

## RESULTS AND DISCUSSION

### 1- Camphor

It is noticed from Table (2) the superiority of red basil cultivar plants in increasing the active compound content of camphor, as it achieved a concentration of  $6.43 \text{ mg.gm}^{-1}$  compared to the plants of the class of lemon basil and sweet basil cultivar which reached  $5.42$  and  $4.71 \text{ mg.gm}^{-1}$  respectively.

The treatment of fertilization with bio-fertilizer and spraying with liquid organic nitrogen achieved significant increase in the concentration of the active compound camphor amounted to  $7.82$  and  $6.91 \text{ mg.gm}^{-1}$ , While the interaction between the plant variety and the type of nitrogen source showed the superiority of the red basil plants treated with the bio- fertilizer *Azotobacter chroococcun* (V2N3) significantly, it reached  $8.96 \text{ mg.gm}^{-1}$  compared with the intervention treatment of the lemon basil cultivar without spraying with nitrogen V3N0 as it reached  $2.13 \text{ mg.gm}^{-1}$ .

### 2- Linalool

show Table ( 2) the superiority of the plants of the red and lemon basil variety in increasing the content of the active compound Linalool, with an increase of  $64.96$  and  $63.90 \text{ mg.gm}^{-1}$ , which differed significantly About sweet basil cultivar which  $58.03 \text{ mg.gm}^{-1}$ .The treatment with the Bio-fertilizer was significant increasing the oil content of the active substance by  $77.92 \text{ mg.gm}^{-1}$  and with a significant compared with control treatment of  $37.66 \text{ mg.gm}^{-1}$ , While the interaction between the plant variety and the type of nitrogen source showed the superiority of the lemon basil plants treated with the Bio-fertilizer *Azotobacter chroococcun* V3N3 significantly, it reached  $88.45 \text{ mg.gm}^{-1}$  compared with the treatment of the sweet basil cultivar without spraying with nitrogen V1N0 as it reached  $34.08 \text{ mg.gm}^{-1}$

### 3- $\alpha$ – pinene

From data in the same table, we note the superiority the red basil cultivar in increasing the concentration of the active compound  $\alpha$ -pinene  $1.09 \text{ mg.gm}^{-1}$  camper by sweet and lemon basil reach  $0.98$  and  $0.93 \text{ mg.gm}^{-1}$ , respectively. The treatment of fertilization with the bio-fertilizer *Azotobacter chroococcun* and spraying with liquid organic nitrogen achieved significant increase in the concentration of the active compound  $\alpha$ -pinene amounted to  $1.30$  and  $1.20 \text{ mg.gm}^{-1}$ , While the interaction between the plant variety and the type of nitrogen source showed the superiority of the red basil plants treated with the bio-fertilizer *Azotobacter chroococcun* V2N3 and the interference of V2N2 significantly, it reached  $1.39$  and  $1.33 \text{ mg.gm}^{-1}$

### 4- Limonine

The results of the same table indicate significant difference for variety in Limonine content of red basil reached  $18.05 \text{ mg.gm}^{-1}$ , while the content of the plants of the class of lemon basil and sweet basil cultivar was  $17.60$  and  $16.56 \text{ mg.gm}^{-1}$ , respectively. The treatment of spraying with liquid organic nitrogen(N2) and Bio-fertilizer achieved significant superiority in increasing the concentration of the active compound by  $16.39$  and  $18.45 \text{ mg.gm}^{-1}$ , and with a significant difference from the urea spray treatment and control treatment  $8.63$  and  $6.83 \text{ mg.gm}^{-1}$ . Interference between the plant variety and the type of nitrogen source showed the superiority of the red basil plants treated with the bio-fertilizer *Azotobacter chroococcun* V2N3 and sprayed with liquid organic nitrogen(optmas) significantly, reaching  $23.58$  and  $23.09 \text{ mg.gm}^{-1}$  compared with the sweet basil cultivar without spraying with nitrogen V1N0 which arrived to  $9.59 \text{ mg.gm}^{-1}$

### 5- myrcene

The results of the table indicate the superiority of the plants of the red basil cultivar in increasing the oil content of compound myrcene, as it reached  $29.87 \text{ mg.gm}^{-1}$ .

**Table (2):The effect of cultivar and nitrogen source on leaves content of the active compound in essential oils**

Variety	Nitrogen Source	Characteristics				
		Camphor	Linalool	$\alpha$ -pinene	Limonine	Myrcene
Sweet basil (V1)	N0	2.59	34.08	0.51	9.59	13.62
	N1	4.85	53.60	0.97	15.94	26.15
	N2	6.52	70.43	1.16	19.75	31.73
	N3	7.73	74.02	1.29	20.98	35.65
Red basil (V2)	N0	3.12	40.95	0.64	10.93	16.45
	N1	5.37	67.92	1.03	13.10	28.02
	N2	8.30	79.66	1.33	23.09	36.38
	N3	8.96	71.31	1.39	23.58	38.66
Lemon basil (V3)	N0	2.13	39.95	0.52	8.95	12.92
	N1	4.11	61.73	0.88	12.85	23.63
	N2	5.93	65.49	1.10	20.62	29.34
	N3	6.68	88.45	1.22	22.02	33.29
L.S.D.0.05		0.32	1.12	1.06	3.06	1.43
Mean of Variety	Sweet basil (V1)	5.42	58.03	0.98	16.56	26.78
	Red basil (V2)	6.43	64.96	1.09	18.05	29.87
	Lemon basil (V3)	4.71	63.90	0.93	17.60	24.79
L.S.D.0.05		0.78	1.22	0.03	2.67	1.90
Mean of Nitrogen Source	N0	2.61	38.32	0.55	6.83	14.33
	N1	4.77	61.08	0.96	8.63	25.93
	N2	6.91	71.86	1.20	16.39	32.48
	N3	7.82	77.92	1.30	18.45	35.86
L.S.D.0.05		1.98	2.43	0.43	1.43	1.23

The fertilization treatment with the Bio-fertilizer *Azotobacter* succeeded by increasing the concentration of the myrcene compound significantly by 35.86 mg.gm<sup>-1</sup>, with a significant difference from the comparison treatment of 14.33 mg.gm<sup>-1</sup>, While the interaction between the variety and source of nitrogen source showed the superiority of the red basil plants treated with the Bio-fertilizer *Azotobacter chroococcun* (V2N3) significantly, it reached 38.66 mg.gm<sup>-1</sup> compared with the intervention treatment the lemon basil cultivar without spraying with nitrogen V3N0 as it reached 12.92 mg.gm<sup>-1</sup>.

The effect of the genetic factors related to the cultivars may have had a major role in the variability of the volatile oil and its active compounds (13), or the increasing concentration of active compounds Camphor, Linalool,  $\alpha$ -pinene, Limonine and Myrcene in *O. basilicum* var. purple (red basil), perhaps caused growth natural of red basil which character in Profuse growth (6 and 11), the reason for the increase in the active compounds of the volatile oil by the effect of the treatment of *Azotobacter chroococcun* is the increase in the amount of nitrogen installed by the bacteria that contribute in formation of the chlorophyll molecule and thus increase the efficiency of the photosynthesis process,

which led to an increase in total soluble carbohydrates (2). The increase in the production of carbohydrates as a result of the efficiency of the process of photosynthesis leads to an increase in the production of Terpenes compounds through the entry of carbohydrates in the process of glycolysis and the production of pyruvic acid and then mevalonic subsequently, which is the main key for the production of isoprene units C<sub>5</sub>H<sub>8</sub> which is the structural unit for the formation of turbine volatile oils (17), These results are in agreement with Salman (16) on the red basil and Abid Ameen (3) on the *Magnesium Fertilization*.

Also Perhaps the reason is due to the structural structure of organic fertilizers represented by the abundance of nutrients such as carbon and some micronutrients and their transporters of organic acids led to stimulating physiological processes, especially photosynthesis, which caused the increase and accumulation of nutrients manufactured in the plant, especially the total dissolved carbohydrates (1) which indicated that nitrogen fertilization increases the production of secondary metabolism compounds, which are an important source of formation of active compounds in the plant, these results are in agreement with Mahmood and Slman (10), Salama and Yousef (15).

## CONCLUSION

Under the experimental treatment of the present study it was revealed that the dominant constituent for linalool in three variety. While the component concentrated in *Ocimum basilicum* var. purple (red basil) whereas, At the same time, the chemical composition of *O. basilicum* var. *citriodorum*. (lemon basil ) hybrid plant differed substantially from the two basil varieties: it had middle value of compound content, represented by Camphor , linalool and limonene

with another prevalent constituent

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