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Chemical Composition, Yield and Contents of Essential Oil of *Lavandula hybrida* Reverchon Grown under Different Nitrogen Fertilizer, Plant Density and Location

OLCAY ARABACI*, EMINE BAYRAM†, HASAN BAYDAR‡, A. FERHAN SAVRAN**
TAHSIN KARADOGAN‡ and NAZAN OZAY†
Department of Field Crop, Faculty of Agriculture
University of Adnan Menderes 09100 Aydin, Turkey
Fax: (90)(256)7727233; Tel: (90)(256)772 7023; E-mail: oarabaci@adu.edu.tr

Effect of nitrogen fertilizer and plant density on the essential oil content, yield and oil composition of *Lavandula hybrida* Reverchon (lavandin) grown under different ecological conditions for initiating cultivation in Turkey were studied in this research during consecutive three years. Essential oil content and essential oil yield were affected by the experimental factors such as, location, fertilizer, plant density and their interaction. Essential oil content depending on fertilizer and plant density was between 4.900-5.617, 5.733-6.483 and 4.567-5.283% for experimental years, Aydin, Isparta and Canakkale locations, respectively. Under non-nitrogen fertilizer condition 80 × 20 cm plant density would be advisable for Aydin and Canakkale locations and under nitrogen fertilizer condition, 60 × 20 cm plant density would be appropriate for all locations. L × Y × F × Pd (Location × Year × Fertilizer × Plant density) interaction was found for essential oil yield. The major component of essential oil composition in *Lavandula hybrida* Reverchon was linalool, and this component was followed by linalyl acetate, camphor and 1,8 cineole.

Key Words: *Lavandula hybrida* Reverchon, Lavandin, Fertilizer, Plant density, Essential oil, Essential oil composition, Linalool.

INTRODUCTION

Lavender, a member of the *Lamiaceae* family, is an important aromatic and medicinal plant because of increasing economic value in the world. Essential oil obtained from its flowers is commonly used in perfumes, cosmetics, flavouring, pharmaceutical and detergent industries. Recently, antioxidant and medicinal properties of the plant were reported[1,2]. Also, essential oils of lavender have also received much attention as pest control agents because of their insecticide, repellent or antifeedant properties and fumigant toxicity of oil against eggs of stored product insect[3].

†Department of Field Crop, Faculty of Agriculture, University of Ege, 35100 Izmir, Turkey.
‡Department of Field Crop, Faculty of Agriculture, University of Suleyman Demirel, 32260 Isparta, Turkey.
**Faculty of Agriculture, University of Canakkale Onsekiz Mart, 17100 Canakkale, Turkey.

The genus *Lavandula* is divided into eight section and contains total number of wild species[4] to 39. Among the common and cultivated species are *Lavandula angustifolia* Mill. or *L. officinalis* Chaix or *L. vera* D.C. (true lavender), *L. dentate* L. (French lavender), *L. latiafolia* Medik. or *L. spica* (spike lavender), *L. stoechas* L. (Spanish or fringed lavender) and *L. hybrida* Reverchon or *L. intermedia* (lavandin, *L. angustifolia* × *L. latifolia*).

A hybrid of *L. officinalis* and *L. latiafolia* is *L. hybrida* or *L. intermedia*, as known lavandin. Lavandin is a sterile hybrid resulting from the natural crossing by pollination. It is indigenous to the Mediterranean area, growing in poor rocky soil, with 70-80 cm plant height, shrub form and perennial plant. Flowers rise on leafless stem and unbranded. Lavandin yields four times more oil per volume of plants than true lavender. Flowers preferred for sachets, potpourri and craftwork and essential oil by the perfume industry. Its essential oil today ranges among the world's 10 largest perfume oils from nature. Lavandin oil is used in large quantities for a fresh note in perfumes. It is well adapted for detergent products, needing no strong fixatives, but in soaps there is a necessity for good fixation[5]. The main chemical components of lavandin oil are linalool, linalyl acetate, camphor, cineole and terpineol.

Although the biosynthesis of secondary metabolites is controlled genetically, it is also affected strongly by environmental influences[6]. Few researches have been reported increased yield of essential oil in *Lavandula* plants. Tucker *et al.*[7] showed that lavandin, the yields of inflorescence and essential oil, were increased significantly by the addition of a 2.5 cm top dressing of white sand. El-Keltawi and Croteau[8] reported that *L. vera*, on foliar application of cytokinin, increased significantly the yield of essential oil. Many researchers reported that agricultural factors such as fertilizer, density, irrigation, planting time and harvesting time have a effect on quantitative and qualitative characters on a lot of medicinal plants[9-15].

The objective of this research was to determine the effect of nitrogen fertilizer and plant density on the essential oil content, yield and oil composition of *L. hybrida* Reverchon (lavandin) grown under different ecological conditions for initiating cultivation of this plant in Turkey.

EXPERIMENTAL

This research was carried out during three consecutive years, 2000, 2001 and 2002 in three ecologically different locations, Aydin (latitude 37º44' N, longitude 27º44' E, altitude 65 m), Isparta (latitude 37º45' N, longitude 30º33' E, altitude 997 m) and Canakkale (latitude 40º08' N, longitude 26º24' E, altitude 75 m) Province in Turkey. Soil characteristics of field experiment area and climatografic date on long-term of locations were presented in Table-1. *Lavandula hybrida* Reverchon, rooted stem cutting was used as a material in this study.

TABLE-1
STRUCTURE AND CHEMICAL COMPOSITION OF SOIL AND CLIMATE DATA ON LONG-TERM IN AYDIN, ISPARTA AND CANAKKALE

	Location		
	Aydin	Isparta	Canakkale
pH H_2O	7.91	8.20	7.88
$CaCO_3$ (%)	5.7	5.7	0.35
K^+ (kg ha^{-1})	77	104	202
Organic matter (%)	2.01	1.30	2.18
Mean temperature (^oC)	17.5	12.1	15.5
Highest monthly temp. (June) mean (^oC)	28.1	23.1	37.5
Lowest monthly temp. (Jan.) mean (^oC)	8.0	1.7	6.6
Annual total rainfall (mm)	658	581	439

This experiment was conducted on the base of experiment in randomized complete block design. The treatments consisted of two levels of nitrogen fertilizer (0, 10 kg ha^{-1}) and four levels of plant density (20 × 20, 40 × 20, 60 × 20 and 80 × 20 cm) were arranged in a split-plot design with three replications. Nitrogen fertilizer rates treatments were in the main plots and plant density levels in the subplots.

Rooted cutting was transplanted into experimental field on 11, 6 and 28 April 2000, Aydin, Isparta and Canakkale locations, respectively. Plots consisted of 6 rows of 4 m length in 20 × 20 cm row apart, and the other plots consisted of 3 rows of 4 m length. Irrigation and other field practices had been done as needed. The plants on the four central rows in 20 × 20 cm plots and central row in the other plots were harvested. Lavandin plant was not harvested in year 2000, to encourage root grown in the first year. Other years, harvests were taken during at the full flowering stage, 25-30 cm below floral stalks with spikes. Samples of each plot, corollas was removed from the spikes, were air-dried in the shade at room temperature for 10 d and stored until analysis inside paper bags in the dark.

The essential oil was extracted from drug flores by hydro-distillation for 3 h under continuous steam using Clevenger apparatus according to standard procedure described in European Pharmacopoeia[16] for determining the essential oil content (%) and the essential oil content was measured as percentage (v/w).

To determine essential oil composition, the essential oil samples were analyzed in using a Carlo Erba Fractovap series 2350 gas chromatograph (GC) equipped with a flame ionization detector at the central laboratory of the Ege University. A glass column (3 m long, 3.18 mm internal diameter), packed with 3% OV-1 50 chromosorb 80/100-mesh, was used. Carrier gas was N_2 at a flow rate of 25 mL/min. Each GC runs lasted for 20 min.

Temperatures of injector, column and detector were 250, 110 and 250ºC, respectively.

Peaks taken from GC were identified using the retention times obtained for reference standards of 1,8-cineole, linalool, camphor, borneol, α-terpineole, linalyl acetate and bornyl acetate. Relative content (%) of individual constituents of the oil was calculated proportionally on the basis of the peak area corresponding to each component.

Statistical analyses corresponding to analysis of variance of a split-split plot design as locations in main plots and nitrogen fertilizer in subplots and plant density in minor plots were performed and the results were presented as mean values. The significance of difference between any two treatmental means was determined using least significant difference (LSD) values at 5 and 1 % probability levels ($p < 0.05$ and $p < 0.01$).

RESULTS AND DISCUSSION

The results of variance analysis for consecutive two years are showed in Table-2. Essential oil content and essential oil yield were affected by the experimental factors: location, fertilizer, plant density and their interaction (Table-2).

In the first year, the plants were not harvested to encourage more root development. In 2001 and 2002, plants were harvested at three locations.

Locations, fertilizer and plant densities significantly affected the essential oil content in both years. Significant interactions were detected between experimental factors. Y × F × Pd and L × F × Pd interactions were found to be significant for essential oil content. The effect of years on the experimental factors was not to take into consideration. However L × F × Pd interaction for essential oil content characters was evaluated (Table-2).

Essential oil content ranged from 4.567 to 6.100 under non-fertilizer condition and from 4.650 to 6.483 % under fertilizer condition (Table-3). Under non-nitrogen fertilizer condition, the highest essential oil contents were obtained from 40 × 20 and 80 × 20 cm plant densities at Aydin and Canakkale locations, respectively. However plant density did not affect the essential oil content in Isparta location. The highest essential oil content under nitrogen fertilizer condition was produced in 20 × 20 cm and 60 × 20 cm plant densities in Aydin and all plant densities except 20 × 20 cm in Isparta. However plant density did not change essential oil content in Canakkale under nitrogen fertilizer condition (Table-3). When all data were evaluated together, it was concluded that under non-nitrogen fertilizer condition 80 × 20 cm plant density would be advisable for Aydin and Canakkale locations and under nitrogen fertilizer condition, 60 × 20 plant density would be appropriate for all locations. These results indicate that appropriate plant densities to receive the highest essential oil content depend on locations and fertilizing conditions.

TABLE-2
ANALYSIS OF VARIANCE OF *LAVANDULA HYBRIDA* REVERCHON GROWN IN YEAR 2001 AND 2002

Variation sources	S.D.	Mean squares	
		Essential oil content (%)	Essential oil yield (L ha^{-1})
Location (L)	2	19.560**	12259.406**
Replication (R) × L	6	1.119**	100.231
Year (Y)	1	7.563**	1550.332**
L × Y	2	53.216**	3942.785**
R × Y × L	6	0.261	187.082
Fertilizer (F)	1	0.840*	1005.012**
L × F	2	0.124	199.889
Y × F	1	1.480**	0.524
L × Y × F	2	0.012	397.859*
Error-1	12	0.093	79.621
Plant density (Pd)	3	0.226	700.596**
L × Pd	6	0.626**	404.649**
Y × Pd	3	0.207	131.618
L × Y × Pd	6	0.170	251.359**
F × Pd	3	0.442**	176.195*
L × F × Pd	6	0.416**	126.364*
Y × F × Pd	3	0.326*	69.643
L × Y × F × Pd	6	0.204	153.937*
Error	72	0.105	50.986

*Significant at $p < 0.05$, ** Significant at $p < 0.01$.

Marotti *et al.*[17] reported that essential oil content was ranged 1.04-1.71 %. Baydar *et al.*[18] determined that the essential oil content was 3.4 % in the first year and 6.7 % in the second year in Isparta ecological conditions. Renaud *et al.*[19] indicated that essential oil contents among lavandin cultivars were varied 7.1-9.9 %. Our results were higher than reported by Marotti *et al.*[17] and Baydar *et al.*[18] and lower than as reported Renaud *et al.*[19].

TABLE-3
ESSENTIAL OIL CONTENT OF *LAVANDULA HYBRIDA* REVERCHON GROWN IN YEAR 2001 AND 2002

Fertilizer	Plant density (cm)	Aydin	Isparta	Canakkale
0 kg ha^{-1}	20 × 20	4.900 b	5.733 a	4.650 b
	40 × 20	5.483 a	6.017 a	4.567 b
	60 × 20	4.983 b	5.983 a	4.733 b
	80 × 20	5.200 ab	6.100 a	5.283 a
10 kg ha^{-1}	20 × 20	5.617 a	5.667 b	5.067 a
	40 × 20	5.333 ab	6.483 a	4.650 a
	60 × 20	5.417 a	6.217 a	4.883 a
	80 × 20	4.900 b	6.433 a	4.800a
$LSD_{0.05}$	L × F × Pd = 0.496			

TABLE-4
ESSENTIAL OIL YIELD OF *LAVANDULA HYBRIDA* REVERCHON GROWN IN YEAR 2001 AND 2002

Fertilizer	Plant density	Aydin		Isparta		Canakkale	
		2001	2002	2001	2002	2001	2002
0 kg ha^{-1}	20 × 20	33.347 ab	31.840 b	36.407 a	37.490 a	20.497 a	14.359 a
	40 × 20	44.090 a	57.807 a	35.720 a	28.030 ab	5.413 b	16.095 a
	60 × 20	24.757 b	61.264 a	25.340 ab	19.433 b	6.217 b	10.338 a
	80 × 20	23.643 b	56.598 a	18.287 b	18.023 b	11.750 ab	11.491 a
10 kg ha^{-1}	20 × 20	33.860 a	69.369 a	52.577 a	49.977 a	25.060 a	10.629 a
	40 × 20	29.567 a	60.634 ab	56.290 a	27.377 b	6.973 b	17.183 a
	60 × 20	28.840 a	53.703 b	31.407 b	23.943 b	8.707 b	13.038 a
	80 × 20	29.030 a	70.712 a	32.307 b	19.723 b	12.807 b	11.331 a

$LSD_{0.05}$ L × Y × F × Pd = 11.635

The effect of experimental factors on essential oil yield was significant. L × Y × F × Pd interaction was found for essential oil yield (Table-2). Under non-nitrogen fertilizer condition, the highest essential oil yield was obtained from 40 × 20 cm in 2001 and all plant densities except 20 × 20 cm in 2002 at Aydin location. In both years, the highest essential oil yield was detected in 20 × 20 cm at Isparta location. The highest essential oil yield was obtained from 20 × 20 cm plant density growing condition in year 2001, but plant density did not affect the essential oil yield in year 2002 at Canakkale location. Under nitrogen fertilizer condition, the essential oil yield did not varied at all plant density in year 2001 and the highest essential oil yield was obtained from 80 × 20 cm and 20 × 20 cm plant density in year 2002 at Aydin location. In year 2001, the highest yield was obtained from 40 × 20 cm and 20 × 20 cm plant densities, in year 2002 and 20 × 20 cm plant density in Isparta location. For Canakkale location, the highest essential oil yield was determined from 20 × 20 cm plant density in year 2001 and plant density did not affect the essential oil yield in year 2002.

It was concluded that under non-nitrogen fertilizing conditions 40 × 20 cm plant density would be suggested for Aydin and 20 × 20 cm would be appropriate plant density for Isparta and Canakkale for high essential oil yield. For nitrogen fertilizing condition 20 × 20 cm would be recommended for all locations.

Marotti *et al.*[17] found that essential oil yield of three varieties of *Lavandula hybrida* Reverchon was varied from 15.1 to 17.4 L ha^{-1}. Baydar *et al.*[18] reported that essential oil yield in Isparta location was between 1.93-9.89 kg ha^{-1}. Lis-Balchin[5] reported that the yield of lavandin was up to 120 kg/ha. Our results were higher than previous studies[17,18]. Especially, the results obtained from Isparta location were higher than Baydar's *et al.*[17] in the same location.

The major component of essential oil composition in *Lavandula hybrida* Reverchon was linalool and this component was followed by linalyl acetate, camphor and 1,8 cineole (Figs. 1 and 2). In 2001, linalool ratio was 40.70-48.28, 40.60-46.92 and 41.16-45.94 % in Aydin, Isparta and Canakkale location, respectively. Linaly acetate, second component, was 25.82-33.97, 22.24-25.32 and 31.77-36.23 % in Aydin, Isparta and Canakkale location, respectively (Fig. 1). Nitrogen fertilizer in Aydin location did not significantly affect the major components of essential oil composition and non-nitrogen fertilizer increased both linalool and linalyl acetate content. Nitrogen fertilizer increased linalool content in Isparta and Canakkale locations; nevertheless same condition was decreased linalyl acetate. The highest value of linalool in both growth conditions was arrived in 40 × 20 cm plant density.

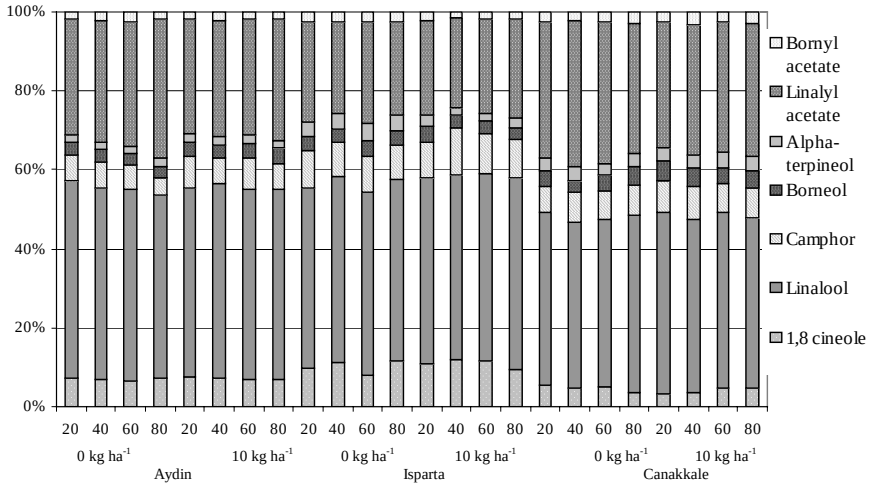

Fig. 1. Composition of essential oil content in year 2001

In year 2002, linalool content was 39.87-43.01, 36.33-43.30 and 37.66-42.22 % and linalyl acetate content was 32.46-35.83, 22.87-30.50 and 28.85-33.11% in Aydin, Isparta and Canakkale location for both components, respectively (Fig. 2). Under nitrogen fertilizer condition, linalool content increased and linalyl acetate content decreased in Aydin location, also under nitrogen fertilizer condition linalool and linalyl acetate increased in Isparta location and linalool content decreased and linalyl acetate content increased in Canakkale location. It showed that the components of essential oil depend on location and nitrogen application.

Previous researchers reported that linalool content was varied between 25-39.60% and linalyl acetate content was varied between 17.8-38%[17,19-22]. Our results obtained from all location were found higher than previous researchers.

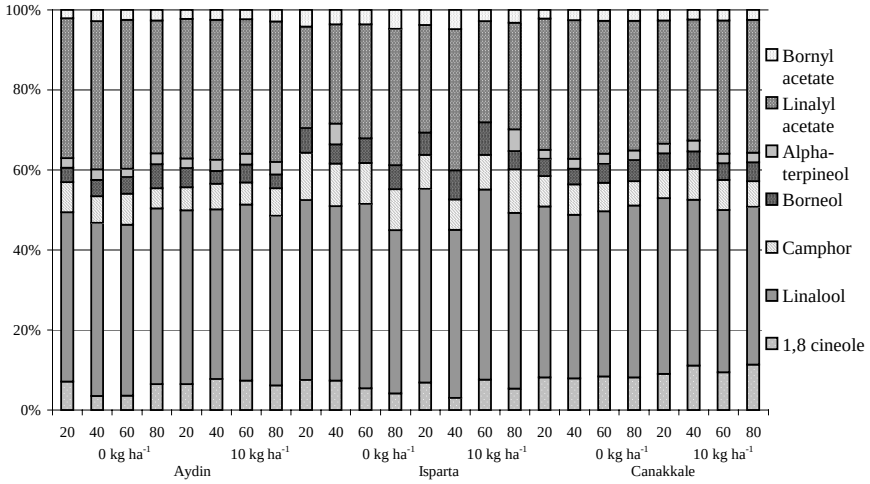

Fig. 2. Composition of essential oil content in year 2002

Tucker *et al.*[23] reported a very wide range of linalyl acetate from 7.46-56.60 % among the evaluated cultivars. Lawrence[24] also reported a wide range in linalool and linalyl acetate percentages depending on plant genotype and cultivated area.

The linalyl acetate content in our results showed that max 33.97% in one year and 35.83% in two years, was higher than the level of 25% set by International standards. This could be due to ecological differences, grow conditions (fertilizer, plant density *etc.*) and soil characterization *etc.*

Conclusions

In conclusion, fertilizer and plant density had significant effects on essential oil content and essential oil yield of *Lavandula hybrida* Reverchon (lavandin) under different ecological conditions. The results showed that to receive the highest essential oil content appropriate plant densities depend on locations and fertilizing conditions.

L × Y × F × Pd interaction was found to be significant for essential oil yield. For these results, non-nitrogen fertilizing conditions, 40 × 20 cm plant density for Aydin and 20 × 20 cm plant density for Isparta and Canakkale locations would be suggested for high essential oil yield. For nitrogen fertilizing condition 20 × 20 cm would be recommended for all locations. Ratio of the major component of essential oil composition, linalool, was varied between 36.33 and 48.28%. According to locations, non-nitrogen and nitrogen fertilizer grown conditions, ratio of essential oil components was varied.

It can be said that lavandin could be cultivated easily for all locations. Using suitable agronomical methods could increase essential oil content, yield and composition.

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