# Honey Bee (*Apis mellifera* L.) Pollination as an Ecological Method to Increase the Quality of Lavender Essential Oil

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**Summary**

The essential oil of lavender is important worldwide. The aim of the study was to investigate the effects of pollination by honey bees (*Apis mellifera* L.) as an ecological method to increase the quality of the essential oil of lavender (*Lavandula angustifolia* Mill.). Fifteen components were determined in the essential oil samples tested and included in this study. Three groups of organic compounds were found: Terpenes, Terpenoids and Ketones. The percentages of the major chemical components in the essential oil of bee-pollinated lavender plants are: linalool - 30.7%, linalyl acetate - 30.3%, lavandulyl acetate - 5.2% and terpinen 4-ol - 6.5%, whereas these results from unpollinated lavender plants are as follows: linalool - 25.5%, linalyl acetate - 27.6%, lavandulyl acetate - 4.4% and terpinen 4-ol - 5.5%. Importantly, statistically significant differences were found when comparing the results of these essential oil components. It was found that the essential oil of the pollinated plants contained more than 5% linalool and almost 3% more linalyl acetate. These two ingredients were the most important components of lavender essential oil. The experimental study showed a higher content in the essential oil of the pollinated lavender plants for nine of the fifteen components examined.

**Key words**

honey bees, pollination, lavender, essential oil, ecology

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## Introduction

Lavender (*L. angustifolia* Mill.) is a plant species native to the Mediterranean region, but it is also cultivated in countries such as Bulgaria, Moldova, Russia, Ukraine, Tajikistan and Georgia (Muntean, 1990; Terziev et al., 2006). In countries such as France, Italy, Spain, Greece and North Africa, it grows at an altitude of 1800 m above sea level (Coiciu and Racz, 1962). Lavender is a plant that prefers aerated soils with a light mechanical composition and southern exposure (Atanasova and Nedkov, 2004). Lavender is mainly grown for the production of high quality essential oil and extracts used in the cosmetic, perfume and pharmaceutical industries (Yankulov, 2000; Lubbe and Verpoorte, 2011; Woronuk et al., 2011; Buljat et al., 2019). Lavender has been attributed with antibacterial and antioxidant effects (Lee and Shibamoto, 2002; Romeo et al, 2008; Woronuk et al, 2011; Jinau, 2013; Carrasco et al, 2016; Giovannini et al, 2016; Sharifi-Rad et al, 2017). The main essential oil components of *L. angustifolia* are: linalool (25-38%) and linalyl acetate (25-45%) (Lis-Balchin, 2002).

The selection results of lavender showed that the chemical composition of the essential oil in it varies over a wide range. The Bulgarian variety "Yubileyna" contains a high proportion (8-10%) of terpinen-4-ol, - one of the most active ingredients with pesticidal effects (Stanev, 2008). During distillation, the chemical profile of the essential oil can also be influenced (Schmidt, 2010). The extraction solvents and extraction times play an important role in the quality of the extracts (Mierina et al., 2018). Pollinated lavender plants produce a greater amount of essential oil than unpollinated ones (Radev, 2020). The most commonly used method for extracting lavender essential oil is steam distillation (Détár et al., 2020; Dong, 2020).

Pollination plays a major role in the reproduction of most floral species by allowing taxa to share genetic material from other species. There is not much information how each insect species chooses which flowers to visit (Cseke et al., 2007). The flowers of taxa attract pollinators by producing different chemical components. The main essential oils in lavender the linalool and linalyl acetate probably play such key role. The ingredients give each plant taxon a unique aroma (Galen, 1985). According to Knudsen et al. (2004) flowers of entomophilous pollinated floral species produce higher quantities of compounds. The floral species are visited by great number of insects, but just a few impact pollination (Kandori, 2002). Most taxa flowers are pollinated by insects because they feed nectar and pollen (Proctor et al., 1996). The bees are the best insect pollinators. They are attracted by plants according flower morphology, colour and aroma (Endress, 1994). Honey bees visit a wide range of floral species (Paldi et al., 2003; Laloi and Pham-Delegue, 2004).

The aim of the study was to investigate the effects of pollination by honeybees (*A. mellifera*) on increasing the amount of key constituents such as linalool and linalyl acetate in the essential oil of lavender (*L. angustifolia*).

## Materials and methods

### Sampling

The lavender plants of the variety "Hemus" on the area of 0.2 ha used in this study were grown in the area of Belozem, Bulgaria. The region is part of the Plovdiv administrative district (N 42.201860/E 25.049330). For pollination, we placed 40 hives with *A. mellifera* bees near the sampling area. Flowers and stems from 20 pollinated and 20 non-pollinated plants were used for distillation. The non-pollinated plants were covered with a metal grid with 5 mm holes before flowering.

Two hours of hydrodistillation in a modified Clevenger apparatus yielded the essential oil (Georgiev and Stoyanova, 1997). Identification of the main components of the essential oil was carried out using an Agilent 7820 gas chromatograph under the following working conditions: a column oven with dimensions 28.0×30.5×16.5 cm. Temperature programming: from 8 °C to 425 °C, maximum operating temperature up to 400 °C. Temperature setpoint resolution of 1 °C, max. temperature ramp rate 75 °C $min^{-1}$, max. run time 999.99 min, temperature programming ramps 5, ambient rejection < 0.01 °C per 1 °C, oven temperature ramp ≤ 2%, repeatability of programme temperature ≤ 1% and carrier gas velocity max. 100 mL $min^{-1}$. The accuracy of the results in the determination of the peaks was checked using standardised reference materials (BDS ISO 3515, ISO 1124-1, ISO 11024-2). All variants were carried out in three repetitions and the data were statistically evaluated.

### Statistical analysis

The data were statistically analysed with the statistical software Statistica V 7.0. The T-test for independent variables was used. The samples were treated as independent at a significance level of $P \leq 0.05$.

## Results

The chemical composition of the essential oils of pollinated and non-pollinated lavender plants was determined. Fifteen components were determined in the essential oil samples examined and included in this study (Table 1). The major components of lavender essential oil were: linalool, linalyl acetate, lavandulyl acetate and terpinen 4-ol.

Three groups of organic compounds were found - terpenes, terpenoids and ketones. The terpenes were the most numerous - 53.3% of the total, the terpenoids detected were 33.3% and the ketones detected were only 13.3%. The essential oil of the pollinated plants was found to contain more than 5% linalool and almost 3% more linalyl acetate. These two ingredients were the most important components of lavender essential oil (Table 1). Importantly, when comparing the results of the major essential oil constituents of linalool, linalyl acetate, lavandyl acetate and terpinen-4-ol, statistically significant differences were found ($P$ = 0.000000, $P \leq 0.05$) (Table 1).

The results were also statistically significant for the essential oil from pollinated plants for the following compounds: 3-octanone, camphor, borneol, lavandulool and α-terpineole ($P$ = 0.003602; $P$ = 0.000314; $P$ = 0.000204; $P$ = 0.000007 and $P$ = 0.000106, $P \leq$ 0.05) (Table 1).

The results for limonene+1.8 cineole were similar for both essential oils from pollinated and unpollinated lavender plants ($P$ = 0.287864, $P \leq 0.05$).

**Table 1.** Composition (%) in essential oil from pollinated and unpollinated lavender plants (mean ± SD)

| Components | Pollinated plants | Unpollinated plants | t-value | df | P |
|---|---|---|---|---|---|
| | | | Terpenes | | |
| α-pinene | 0.30 ± 0.01 | 0.43 ± 0.01 | 15.92168 | 4 | 0.000091 |
| β-caryopyllene | 3.17 ± 0.01 | 4.2 ± 0.01 | 4.898979 | 4 | 0.008050 |
| borneol | 1.0 ± 0.02 | 0.73 ± 0.03 | 126.1487 | 4 | 0.000000 |
| camphene | 0.22 ± 0.01 | 0.26 ± 0.01 | -12.9704 | 4 | 0.000204 |
| lavandulool | 0.90 ± 0.01 | 0.65 ± 0.01 | -30.6186 | 4 | 0.000007 |
| limonene+1.8 cineole | 0.85 ± 0.01 | 0.84 ± 0.01 | -1.22474 | 4 | 0.287864 |
| linalool | 30.66 ± 0.02 | 25.55 ± 0.01 | -395.819 | 4 | 0.000000 |
| trans-β-ocimene | 0.30 ± 0.01 | 3.5 ± 0.01 | 391.9184 | 4 | 0.000000 |
| | | | Terpenoides | | |
| α-terpineol | 1.91 ± 0.02 | 1.64 ± 0.02 | -15.3093 | 4 | 0.000106 |
| camphor | 0.40 ± 0.01 | 0.25 ± 0.02 | -11.6190 | 4 | 0.000314 |
| lavandulyl acetate | 5.21 ± 0.02 | 4.43 ± 0.02 | -59.6439 | 4 | 0.000000 |
| linalyl acetate | 30.3 ± 0.01 | 27.68 ± 0.01 | -320.883 | 4 | 0.000000 |
| terpinen 4-ol | 6.51 ± 0.01 | 5.57 ± 0.01 | -115.126 | 4 | 0.000000 |
| | | | Ketones | | |
| 3-octanone | 0.40 ± 0.01 | 0.35 ± 0.01 | -6.12372 | 4 | 0.003602 |
| cis-β-ocimene | 1.84 ± 0.02 | 12.5 ± 0.02 | 652.7890 | 4 | 0.00000 |

Note: Mean 1 - average value for pollinated plants; Mean 2 - average value for unpollinated plants; t-value - coefficient of Student t-test; df – degree of freedom; P – level of significance

The constituents with higher levels in non-pollinated plants compared to pollinated ones were: α-pinene, camphene, β-caryopyllene, trans-β-ocimene and cis-β-ocimene ($P = 0.000091$; $P = 0.008059$; $P = 0.000000$; $P = 0.000000$ and $P = 0.000000$, $P \leq 0.05$ (Table 1).

Importantly, this study found higher essential oil content from pollinated lavender plants for nine out of fifteen ingredients. (Table 1) As expected, the non-pollinated plants looked fresh during 90% of the massive flowering, while the pollinated flowers had a shorter flowering period.

## Discussion

So far, no such research has been conducted in Bulgaria, so the data presented here represent new research. The main objective of the present study was to determine the impact of honeybee pollination on the quality of essential oil of lavender plants. This is a very important point in such studies because pollination affects the essential oil yield (Radev, 2020). In addition, the pollination effect must be taken into account, as pollinated plants flower earlier than unpollinated plants. Further research is needed to prove at which stage the essential oil in pollinated plants is of higher quality. This should be considered in future studies. A better understanding of the effects of ecological factors would give us more information about sustainable agriculture.

The essential oils of lavender have a spectrum of antimicrobial properties (Evandri, 2005), and the ingredients of the lavender plant are very important in several important industries such as pharmaceuticals, medicine and cosmetics.

In the present study, higher levels of terpinen-4-ol were found in pollinated lavender plants. According to the results of this study, pollination would help to improve the quality of lavender essential oil and use it as a potential biological pesticide. This biological pesticide could be used in organic agriculture and horticulture after it is tested and approved. According to Dikova et al. (2017), lavender plants with a higher content of terpinen-4-ol produce essential oils with an antiphytoviral effect. Lavender essential oil has an inhibitory effect against Tomato Spotted Wilt Virus.

The results presented in this study are within the range of the European Pharmacopoeia VII edition (2010), where the limits for the essential oil composition are given as follows: 20-45% linalool, 0.1-8% terpinen-4-ol, 25-47% linalyl acetate, max. 2.5% 1,8-cineole, max. 1% limonene, 0.1-8% lavandulyl acetate, max. 2% α-terpineol and max. 1.2% camphor.

Under the conditions of the experiment, significant differences were found between the pollinated and non-pollinated lavender plants with regard to the main components of the essential oil. This work has shown that the quality of lavender essential oil can be significantly improved by pollination by honey bees. This conclusion provides commercial lavender producers with a practical tool to increase the quality of the essential oil. The results of the study allow us to recommend pollination of lavender plants by honey bees to obtain better quality lavender essential oil. These initial results should encourage further studies in future research to confirm the need for pollination by honeybees.

## Conclusion

Pollination could help increase the quantity and quality of some essential oil components used in various industries, and of another component such as terpinen-4-ol, which could be used in crop protection.

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