The Impact of Honey Bee Pollination on the Amount of Essential Oils

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Summary

The objective of this research was to study the impact of honey bee (*Apis mellifera* L.) pollination as an agricultural method for increasing the essential oil quantity. The quantity of essential oil in pollinated and unpollinated *Melissa officinalis* L., *Mentha piperita* L., *Origanum heracleoticum* L., *Salvia officinalis* L. and *Thymus serpyllum* L. plants was studied and significant differences were observed. Blossoms and stems from twenty pollinated and twenty unpollinated plants were used during mass blooming in a modified Clevenger apparatus for two-hour distillation under 100 °C. The statistical analysis proved that there was a larger quantity of essential oil in plants pollinated by honey bees than in unpollinated plants. The increase of essential oil from *M. officinalis* was 42.32%; from *M. piperita* was 24.96%; from *O. heracleoticum* was 18.91%; from *S. officinalis* was 23.90% and from *T. serpyllum* was 35.52%. The most visited plants per m^2, during their blooming belonged to *T. serpyllum* and the least visited plants during their blooming belonged to *M. officinalis*. According to pollination intensity *S. officinalis* and *M. officinalis* flowers were the least visited, while *M. piperita* flowers were the most visited.

Key words

agriculture and agroecology, honey bees, essential oil, pollination, aromatic plant

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Introduction

Most of angiosperm plants need a pollination to complete reproduction (Ollerton et al., 2011). The production and quality of around 70% of crops are directly affected by pollination (Klein et al., 2007). There is a variation in the pollination because not all pollinators are equally effective during pollination (Krauss et al., 2017). Insufficient quantity and quality of pollen reaching the stigmas is a pollination deficit (Vaissière et al., 2011). Insufficient quantity and quality of pollen could decrease the reproductivity of plants as well as the output (Castro et al., 2021). There are numbers of ways to measure the pollinators' effectiveness (Ne'Eman et al., 2010). The methods that partition effectiveness into qualitative and quantitative components are most used (Willcox et al., 2017). The frequency of pollinators visiting is called quantitative component (Vázquez et al., 2015). The result of pollinators visiting is a kind of qualitative component (Dellinger et al., 2019).

Worldwide agriculture consists of increasingly pollinator dependent plants (Aizen et al., 2009). One third of the food supply depends on insect pollination (Jivan and Bura, 2013; Said et al., 2015). The pollination is a factor of yield stability in pollinator dependent crops (Garibaldi et al., 2011; Deguines et al., 2014). The higher yields at pollination service have been confirmed (Garibaldi et al., 2013). A honey bee (*Apis mellifera* L.) is able to find and collect pollen, dew and nectar with high productiveness. This makes the honey bee the most useful insect in pollination of crops, achieving high yields in agriculture (Bura et al., 2005). The closer the honey bee hives are to the blooming plants, the greater the pollination effects and production are (Tabară, 2005). The increase in agricultural yield performed by the pollinating honey bee workers, is near 15 times higher than the production of honey bee products (Bura et al., 2003). The larger number of pollinators of rapseed are representatives of Hymenoptera 83%, especially honey bees (*A. mellifera*), *Diptera* 12%, *Lepidoptera* 3% and *Coleoptera* 2%. The plants without pollination have significantly lower yield parameters such as weight of 1000 seeds, oil content and seed germination than the pollinated plants (Amro, 2021). The honey bee is considered to be the most successful pollinator of canola (Rosa et al., 2010). Kiwifruit farmers often use honey bee colonies for pollination (Tacconi et al., 2016). In Citrus crops, 95% fruit forming is possible after pollination with honey bees (Satpute et al., 2021). Without pollination fruit yield in pears would be reduced by 50% and that of apples by 71–92% (Hünicken et al., 2021).

Essential oil takes a small but important part of a plant's composition and for this reason aromatic and medicinal plants are used in several industries. Essential oils play a major role in traditional medicine in many countries and are used because of their pharmacological activities. Essential oils have antifungal, insecticidal, and antibacterial properties (Ayvaz et al., 2010). They could be applied also in food preservation (Bassolé and Rodolfo-Juliani, 2012). The antimicrobial activity of essential oils is dependent on their chemical constituents (Sotomayor et al., 2004).

Previous research established that honey bee pollinated lavandula plants produced a higher quantity of essential oil than unpollinated lavandula plants (Radev, 2020a). Honey bee pollination of agriculture crops is very important agro-technical measure for production increase. In the literature available to us there is not much information on the impact of pollination from honey bees to essential oil quantity. Some of the plants most used for production of essential oil are: *Melissa officinalis* L., *Mentha piperita* L., *Origanum heracleoticum* L., *Salvia officinalis* L. and *Thymus serpyllum* L.

The objective of this research was to study the impact of honey bee (*A. mellifera*) pollination as an agricultural method for increasing the essential oil quantity in *M. officinalis*, *M. piperita*, *O. heracleoticum*, *S. officinalis* and *T. serpyllum*.

Materials and methods

Sampling

M. officinalis (0.02 ha), *M. piperita* (0.02 ha), *O. heracleoticum* (0.02 ha), *S. officinalis* (0.02 ha) and *T. serpyllum* (0.02 ha) plants used in this study were cultivated in an apiary area (0.16 ha) in Belozem. The region of Belozem is located in Bulgaria (N 42.201860/E 25.049330). It is situated on 143 meters above sea level with a transitional-continental climate and saline soils. The experiment was conducted in 2020. For pollination we placed 40 bee hives with *A. mellifera* bees next to the sampling area. Twenty plants of each plant species were covered with a metal mesh with 5 mm holes before plants' flowering to protect them from pollinators, but sun light was allowed. The average daily attendance of honey bees was established every 20 minutes from 10 am till 17 pm for five days during the mass blooming of each plant species: 1) per square meter – by one m^2 of each end and one m^2 in the middle of the planted area; 2) visited flowers per minute in each m^2; 3) working day length was counted from sunrise to sunset.

Blossoms and stems from twenty pollinated and twenty unpollinated plants were used during mass blooming in the distillation. One thousand grams of each plant species were used. Under two-hour hydro distillation under 100 °C in a modified Clevenger apparatus the essential oil was obtained (Georgiev and Stoyanova, 1997).

All variants from pollinated and unpollinated plants were done in 3 replications and the data was processed statistically.

Statistical Analysis

The data was statistically processed by using statistical program Statistica V 7.0. It used T-test for independent variables at $P \leq 0.05$ level of significance. The samples were treated as independent, where: Mean 1 - average value for pollinated plants; Mean 2 - average value for unpollinated plants; t-value - coefficient of student; Df – degree of freedom; P – level of significance.

Results

The results presented in this study provide an additional insight into the impact of honey bee pollination on essential oil quantity in plants. The presented results are given for each plant species separately. Significant differences of essential oil quantity were observed between honey bee pollinated and unpollinated plants for the species described below.

M. officinalis

A comparison was made between honey bee pollinated and unpollinated *M. officinalis* plants, and a significant difference in the essential oil quantity was found: the mean of essential oil quantity of pollinated plants was 8.6 mL 1000 g^{-1}, whereas it was 4.96 mL 1000 g^{-1} in unpollinated plants ($P = 0.000001$, $P \leq 0.05$) (Table 1). The increase in the quantity of essential oil in the bee pollinated plants was found to be by 42.32% bigger.

M. piperita

A comparison was made between honey bee pollinated and unpollinated *M. piperita* plants, and a significant difference in the essential oil quantity was found: the mean of essential oil quantity of pollinated plants was 18.83 mL 1000 g^{-1}, whereas it was 14.13 mL 1000 g^{-1} in unpollinated plants ($P = 0.000000$, $P \leq 0.05$) (Table 1). The estimated increase in the essential oil quantity was 24.96% bigger.

O. heracleoticum

A comparison was made between honey bee pollinated and unpollinated *O. heracleoticum* plants, and a significant difference in the essential oil quantity was found: the mean of essential oil quantity of pollinated plants was 29.76 mL 1000 g^{-1}, whereas it was 24.13 mL 1000 g^{-1} in unpollinated plants ($P = 0.000000$, $P \leq 0.05$) (Table 1). The increase of the essential oil quantity was 18.91% bigger.

S. officinalis

A comparison between honey bee pollinated and unpollinated *S. officinalis* plants and a significant difference in the essential oil quantity was found: the mean of essential oil quantity of pollinated plants was 18.66 mL 1000 g^{-1}, whereas it was 14.2 mL 1000 g^{-1} in unpollinated plants ($P = 0.000000$, $P \leq 0.05$) (Table 1). The increase of the essential oil quantity was 23.90% bigger.

T. serpyllum

A comparison was made between honey bee pollinated and unpollinated *T. serpyllum* plants, and significant difference in the essential oil quantity was found: the mean of essential oil quantity of pollinated plants was 14.16 mL 1000 g^{-1}, whereas it was 9.13 mL 1000 g^{-1} in unpollinated plants ($P = 0.000000$, $P \leq 0.05$) (Table 1). The increase in the essential oil quantity was 35.52% bigger.

Most of visited plants per m^2, during their blooming belonged to *T. serpyllum* (Table 2), compared to other plants species ($P = 0.000000$, $P \leq 0.05$). Lesser visited plants during their blooming belonged to *M. officinalis* (Table 2) and $P = 0.000000$, $P \leq 0.05$, and they enjoyed the shortest working day compared to *M. piperita*, *S. officinalis* and *T. serpyllum* (Table 2) and $P = 0.005646$, $P \leq 0.05$, but similar to *O. heracleoticum* (Table 2) and $P = 0.204550$, $P \leq 0.05$. According to pollination intensity *S. officinalis* and *M. officinalis* flowers having close results were less visited compared to the other taxa (Table 2) and $P = 0.000000$, $P \leq 0.05$, while *M. piperita* flowers were the most visited (Table 2) and $P = 0.003443$, $P \leq 0.05$.

Table 1. Data for essential oil quantity for pollinated and unpollinated plants (mL 1000 g^{-1})

Plant species	Pollinated plants	Unpollinated plants	t-value (Df = 4)	P
	Mean ± SD	Mean ± SD		
M. officinalis	8.60±0.1	4.96±0.12	-52.36	0.000001
M. piperita	18.83±0.06	14.13±0.06	-95.94	0.000000
O. heracleoticum	29.76±0.06	24.13±0.06	-114.92	0.000000
S. officinalis	18.66±0.06	14.2±0.10	-127.00	0.000000
T. serpyllum	14.16±0.06	9.13±0.06	-102.67	0.000000

Note: Df - degrees of freedom; P – level of significance

Table 2. Daily attendance of honey bees every 20 minutes from 10 AM till 17 PM

Plant species	Plants (m^2)	Mean ± std	Flowers,per min.	Mean ± SD	Working day (h)	Mean ± SD
M. officinalis	12.9	12.9±0.9	15.1	15.1±1.1	12.9	12.9±0.7
M. piperita	21.1	21.1±0.7	26.2	26.2±1.5	14.1	14.1±0.4
O. heracleoticum	22.3	22.3±0.8	22.4	22.4±2	13.3	13.3±0.5
S. officinalis	20.1	20.1±1.1	12.4	12.4±1.9	13.9	13.9±0.4
T. serpyllum	25.1	25.1±1.3	21.4	21.4±3.2	14.6	14.6±0.5

Honey bees had the longest working day on *T. serpyllum* flowers compared to *M. officinalis*, *O. heracleoticum* and *S. officinalis* (Table 2) and $P = 0.013658$, $P \leq 0.05$, but similar working day on *M. piperita* flowers (Table 2) and $P = 0.0108864$, $P \leq 0.05$. Presented results could suggest that, the ecology of pollination and plant visiting are strongly dependant on plant species, flower morphology, flower numbers, provided nectar and pollen supplies and other factors.

Discussion

The results give the important contribution of honey bee pollination to essential oils yield of five crops. It was found that the absence of pollinators decreased essential oil yield. Regarding the role of honey bee workers (*A. mellifera*) as pollinator visitors, it was found it had a considerable effect on essential oil yield increase. Honey bees are considered as the most significant pollinator (Corbet et al. 1991). The results presented in this study showed an increase in the quantity essential oil from: 18.91% for *O. heracleoticum*, 23.90% for *S. officinalis*, 24.96% for *M. piperita*, 35.52% for *T. serpyllum* to 42.32% for *M. officinalis* species as a result of pollination. The content of *M. officinalis* essential oil ranged from 0.09 to 0.45% (Moradkhani et al., 2010). Essential oil content obtained from different countries of *S. officinalis* ranged as follows: in Tunisia - 1.02%, in France - 2.05%, in Romania - 2.30% in Hungary – 2.50% and in Portugal - 2.90% (Fellah et al., 2006). The result for *Thymus zygis* Loefl. ex L. is 1.1% (Amarti et al., 2011) and for *Thymus algeriensis* Boiss et Reut is 1.3% (Dob et al., 2006). Pollinators are significant agriculturally and ecologically. They are ecosystem service providers and increase the agricultural crops production (Ollerton, 2017). In protected systems 90% of the dominant pollinators are bees, such as honey bees (*Apis* spp.), bumble bees (*Bombus* spp.), stingless bees (Apidae: Meliponini) and solitary bees (*Amegilla* spp., *Megachile* spp., and *Osmia* spp.). Flies are just 9% of pollinators, while only 1% of taxa include *Lepidoptera* and *Coleoptera* representatives. It has been established that 96% of the studies point that pollinators improve the production of pollinated plants compared to unpollinated agriculture plants (Kendall et al., 2021). Pollination is an environmental phenomenon and ecological factor, an essential element of the existence of life for many biological species on Earth. The agricultural method of "honey bee pollination" is very easy to use and should be practiced, as it would be beneficial for both honey bees and pollinated plants in the following way: 1) honey bees will collect pollen, dew and nectar, and 2) the pollinated plants will produce not only more essential oil, but also more seeds for their reproduction.

Research works have proved that essential oil content depends on many factors, such as: flowering period, different geographic areas (Ayanoglu et al., 2005), harvesting period, origin of species, methodology of extracting (Karousou et al., 2005). Essential oil contents ranges between 0.01–0.25% (Bagdat and Cosge, 2006).

These differences could be due not only to the methods used, ecological or plant factors, but also due to the pollination factor. Pollinators are distributed unevenly in the environment. There may be many in one region and missing in another. In the present research all the studied plants were under same conditions, except the unpollinated plants which had no contact with pollinators. For future research studies in this topic the "pollinator factor" could be taken into consideration. Pollination could contribute to increasing the essential oil quality, and this opportunity could be used if possible. The use of pollination practice would provide better outcomes in terms of agroecology and sustainable agriculture. The use of pollination would increase the yields and the return on farmers' investments. This agricultural method has provided useful and valuable agricultural information.

There is a prerequisite for the application of honey bees (*A. mellifera*), for biological control against lavender pests (*Lavandula officinalis* L.) (Radev, 2020b). Honey bee crisis is threatening worldwide food supply. In spite of their importance for people, honey bees die at worrisome speed. In present years in the world, owing to pollution, pesticides and neglect there is registered an unseen percentage of honey bees disappearance. Taking this into account, an analysis of the influence of pesticides on honey bees should be done (Jivan and Bura, 2013). Honey bees' intoxication with pesticides could be acute and chronic. In acute intoxication, bees die from contact or ingestion of pesticides. In chronic intoxication, toxicity is intented in small and repeated doses, and may implicate the death of the whole honey bee family (Charrière and Imdorf, 1999). The overuse of herbicides must be restricted as well as other negative factors, which are the main reason for the decrease in the species diversity of honey bee flora (Radev, 2018).

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