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Effects of essential oils extracted from Moroccan medicinal and aromatic plants on *Apis mellifera*

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A B S T R A C T

The use of essential oils from medicinal plants as natural alternatives to chemical pesticides has gained significant attention due to their environmental benefits. This study aims to evaluate the toxic effects of essential oils from *Mentha spicata*, *Mentha pulegium*, *Mentha suaveolens*, and *Artemisia herba-alba* on honeybee (*Apis mellifera*) mortality, focusing on their potential use as biopesticides. Essential oils were extracted through hydrodistillation, and bioassays were conducted at varying concentrations over a 96-hour period. Mortality rates were recorded, and LC50 values were calculated to determine toxicity levels. Results showed that all essential oils tested were toxic to honeybees, with mortality increasing with concentration and exposure time. *Mentha pulegium* exhibited the highest toxicity, with an LC50 of 0.71 µl/l after 96 hours, while *M. spicata* and *A. herba-alba* displayed lower levels of toxicity. The findings suggest that while these essential oils may be viable for pest management, their impact on beneficial pollinators like honeybees must be carefully managed. The study concludes that optimization of dosage and application methods is essential to minimize risks to pollinators while effectively controlling pests.

1. Introduction

Growing concerns over the environmental impact of chemical pesticides have led to an increased focus on natural alternatives for pest control. Essential oils from plants like Mentha spicata, Mentha pulegium, Mentha suaveolens, and Artemisia herba-alba are promising due to their insecticidal properties and biodegradability. These oils align with the goals of integrated pest management (IPM), which seeks sustainable solutions that minimize harm to ecosystems. While many studies confirm the effectiveness of these oils against agricultural pests [1-3], their effects on non-target organisms, especially honeybees (Apis mellifera), are not fully understood.

Research in this field shows conflicting results. Some studies emphasize the oils' low impact on pollinators when used at controlled doses [4,5], while others highlight their potential risks, particularly at higher concentrations [6,7]. This divergence underscores the necessity of further research to clarify the risks associated with essential oils and their compatibility with pollinator protection.

This study investigates the toxicity of essential oils on honeybee mortality through controlled bioassays, focusing on lethal concentration values (LC50). The goal is to determine safe usage levels of these oils, offering insights into their application in pest management while safeguarding pollinators.

2. Materials and Methods

2.1. Plant Material

The biological tests were conducted using extracts from four plant species: Mentha pulegium, Mentha spicata, Mentha suaveolens, and Artemisia herba-alba. Plant samples (leaves and flowers) were collected from different regions in Morocco (Rabat, Meknès, Midelt, and Ben Smim). The plant material was dried in a shaded, well-ventilated area for 5–7 days. Once dried, the plant material was ground into a fine powder to prepare it for essential oil extraction.

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2.2. Essential Oil Extraction

The essential oils were extracted using a Clevenger-type apparatus via hydrodistillation, following the method described by Benjilali [8]. A total of 100 g of ground dry plant material was placed in a 2-liter round-bottom flask with 700 ml of distilled water. The mixture was heated to 100°C, and the vapors were condensed and collected. Essential oils were separated from the water by density differences and stored at 4°C in dark tubes until further use. The extraction process was repeated three times for each plant to calculate the average yield. The yield of essential oils was calculated using the formula:

$$\textbf{Essential oil yield (\%)} = \frac{\text{Weight of the essential oil (en g)}}{\text{Weight of the dry matter use(en g)}} * 100$$

2.3. Test Organism: Apis mellifera

The honeybee (*Apis mellifera*) colony used for bioassays was obtained from a beekeeper in the Béni Mellal region. Approximately 8000 bees were maintained in a hive under natural conditions near the Plant Protection and Environment Department at the National School of Agriculture in Meknès. The bees were provided with sugar water and access to local vegetation.

2.4. Bioassays

Toxicity tests were conducted by exposing honeybee workers to different concentrations of essential oils. Plastic boxes (1 liter capacity) containing five bees each served as experimental units. Two containers filled with cotton were soaked with distilled water, while two others were soaked with sugar syrup (500 g/l of distilled water). These boxes were placed inside larger fumigation chambers (2-liter capacity). Filter paper soaked with the essential oils (*M. spicata*, *M. pulegium*, *M. suaveolens*, or *A. herba-alba*) at concentrations of 0.13, 0.25, 0.5, and 1 µl/l of air was introduced into the fumigation chambers using a micropipette. Control groups were exposed to air with no essential oils. Mortality was recorded daily for 96 hours.

2.5. Data Analysis

Mortality rates were corrected using Abbott's formula to account for natural mortality in control groups. The corrected mortality rate was calculated as:

Percentage of corrected mortality = (T-C) / (100-C) *100

where T is the mortality rate in treated groups, and C is the mortality rate in control groups. The lethal concentration (LC50 and LC99) values were determined using Probit Analysis software (EPA Probit Analysis Program, version 1.5).
Data were analyzed using SPSS version 16. Analysis of variance (ANOVA) was performed to compare essential oil yields, and post-hoc Student-Newman-Keuls (SNK) tests were used to determine significant differences.

3. Results and discussion

3.1. Essential Oil Yields

The extraction of essential oils from the four plant species (*Mentha spicata*, *Mentha pulegium*, *Mentha suaveolens*, and *Artemisia herba-alba*) revealed significant variations in yield. The highest yield was observed with *Mentha pulegium*, which produced 3.17 ± 0.15% essential oil, while the lowest was with *Mentha spicata* (0.73 ± 0.06%). These results are consistent with previous studies that reported *M. pulegium* as a rich source of essential oils, likely due to its high content of volatile compounds such as pulegone [8]. The variation in essential oil yield among plant species can be attributed to factors such as the specific biochemical makeup of each species, the plant part used, and the environmental conditions during growth and collection [6].

3.2. Toxicity to Apis mellifera

The bioassays performed on *Apis mellifera* revealed that all tested essential oils had toxic effects, with mortality rates increasing with both concentration and exposure time (Table7). *Mentha pulegium* showed the highest toxicity, with an LC50 of 0.71 µl/l after 96 hours of exposure, followed by *Mentha suaveolens* (LC50 = 1.44 µl/l), and *Artemisia herba-alba* (LC50 = 2.49 µl/l). *Mentha spicata* had the lowest toxicity (LC50 = 2.77 µl/l).

The high toxicity of *M. pulegium* can be attributed to its chemical composition, particularly its high concentration of pulegone, which is known for its potent insecticidal activity [3,9]. This compound's efficacy as an insecticide has been documented in other studies, where it demonstrated lethal effects on a variety of insect species, including *Culex pipiens*, *Sitophilus oryzae*, *Aphis fabae*, *Acyrthosiphon pisum*, *Macrosiphoniella sanborni*, *Myzus persicae*, *Planococcus citri*, *Aonidiella aurantii*, and *Chrysomphalus aonidum* [2,10,11,12]. The non-target organisms like *Coccinella septempunctata* and *Adalia bipunctata* were also highly susceptible to the essential oil vapours of this plant [3,10].

In a comparable way, the high mortality rates observed in the groups treated with *Mentha suaveolens* essential oil can likely be attributed to the specific chemical composition of the oil, particularly the predominance of piperitenone oxide. This compound is recognized for its strong insecticidal activity, which has been documented in various studies [13,14,15,16]. The synergistic effects of piperitenone with other minor components could also contribute to the potent insecticidal properties observed.

The essential oil of *Artemisia herba-alba* has, in turn, demonstrated significant insecticidal activity against a wide variety of insect pests. Its efficacy is largely attributed to its rich composition of bioactive compounds such as thujone, and camphor. Several studies have highlighted its potential as a natural alternative for controlling pest species like *Bemisia tabaci*, *Aphis gossypii*, *Thrips tabaci* and *Cydia pomonella* [17,18, 19]. Finaly, *Mentha viridis*, commonly known as spearmint, is a well-recognized aromatic plant with essential oil that exhibits notable insecticidal properties. These effects can be attributed to its rich chemical composition, which includes high concentrations of bioactive compounds such as carvone, limonene, and α-pinene [20,21, 22, 23].

3.3. Tables

Table 1
Toxicity of the essential oils of the plants tested on *Apis melifera* workers.

Biopesticides	Duration (h)	LC_{50} (µl/l of air)	LC_{99} (µl/l of air)
M. spicata	24	13,38	1638,51
	48	2.771	326,732
	72	2,49	756,11
	96	1,266	30,781
M. pulegium	24	2,49	169,27
	48	0,865	55,741
	72	0,70	27,47
	96	0,712	47,557
M. suavelens	24	2,98*	70,56
	48	1,439	54,338
	72	1,39	106,1
	96	1,255	86,07
A. herba alba	24	0	0
	48	2,490	169,275
	72	2,2	139,47
	96	2,202	139,471

4. Conclusion

The results of this study demonstrate that essential oils from Mentha species and *Artemisia herba-alba* possess strong insecticidal activity, particularly against honeybee (Apis mellifera) workers, with varying degrees of toxicity depending on the concentration and exposure duration. Among the oils tested, Mentha pulegium showed the highest toxicity, followed by Mentha suaveolens, Artemisia herba-alba, and Mentha spicata. These findings suggest that while these essential oils could be useful for pest control in agricultural settings, their application must be carefully managed to avoid harm to beneficial pollinators like honeybees. Future studies should focus on optimizing the dosage and application methods to minimize ecological risks while maximizing the pest control efficacy of these natural biopesticides.

This study contributes valuable insights to the development of eco-friendly pest management strategies, but further research is needed to better understand the long-term impact on non-target species and ecosystem health.

Conflicts of interest statement

If no conflict of interest is declared, the following statement will be published in the article: “The authors declare no competing financial interest”.

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