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Impact of essential oils of *Mentha spicata*, *M. pulegium*, *M. suaveolens* and *Artemisia herba* alba on *Apis mellifera* mortality

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ABSTRACT

The use of aromatic and medicinal plants is of great importance not only as a resource of medical care but also to control many pests including varroa, an ectoparasitic mite of honeybee. Based on this observation, we found it useful to conduct biotests to evaluate the Impact of Mentha spicata, *M. pulegium*, *M. suaveolens* and *Artemisia herba* alba essential oils on *Apis mellifera*. The bioassay results revealed that the tested oils exert a toxic effect on A. mellifera. Depending on concentrations and exposure times, mortalities recorded ranged from 0 to 100%. The LC50 values of tested essential oils, after 96 h of exposure, vary from 0.71 to 2.70 µl / l of air depending on the considered plant species. Moreover, the *M. pulegium* essential oil has been the most toxic comparing to others.

1. INTRODUCTION

The indiscriminate use of chemical pesticides has had harmful consequences, including a reduction in biodiversity and the destruction of a large number of useful organisms [1], such as decomposer organisms involved in building humus and biogeochemical cycles, as well as many pest predators [2- 4].

Faced with this situation, there is a whole range of systems and practices that allow a greater or lesser reduction in the use of pesticides in agriculture, and also significantly limit the potential extent of these chemical inputs in terms of undesirable effects. With this in mind, the use of plant extracts and essential oils with insecticidal activity offers some potential. Numerous studies have highlighted the negative effects of plant extracts and essential oils on phytophagous pests, but their possible effects on natural beneficials (enemies and pollinators) associated with protected crops have yet to be evaluated [5], [6]. In fact, the most interesting biological products used in plant protection are those which have a minimal impact on all the components of the agro-ecosystem except for the targeted pests [7].

With a view to finding alternative biodegradable products that are not harmful to auxiliary fauna and are compatible with integrated pest management, we set out to test the effect of certain aromatic and medicinal

plant (MAP) extracts on honeybees. The aim of this study was to extract essential oils from certain MAPs, namely: *Mentha viridis, M. pulegium, M. rotundifolia, Artemisia herba alba* and toxicological study of these extracts against the honey bee *Apis melifera*.

2. MATERIALS AND METHODS

2.1 Plants used

The biological tests were carried out using plant products from four plants: *M. pulegium, M. spicata, M. suaveolens* and *A. herba alba*. The plants used and their origins are shown in Table 1.

Table 1 Plants used in bioassays and their origins

Plants	Organs used	Source
M. pulegium	Leaves and flowers	Ben Smim region
M. spicata	Leaves and flowers	Rabat region
M. suaveolens (M. roduntifolia)	Leaves and flowers	Meknes region
A. herba alba	Leaves	Midelt region

2.2 Honey bee (Apis mellifera)

The hive being tested was obtained from a beekeeper in the Béni Mellal region. It is a colony of *Apis mellifera* in generally satisfactory condition, with no visible pathological symptoms. According to the beekeeper, it contains around 8,000 bees.

The hive was set up under natural conditions, in a location near the Plant Protection and Environment Department of the Meknes National School of Agriculture. Food was provided by sugar and water, as well as by the vegetation near the colony.

2.3 Drying plant matter

The leaves and flowers of M. spicata and M. suaveolens were placed, in thin layers, sandwiched between two absorbent papers, protected from light, in a dry, shaded and ventilated place for 7 and 5 days respectively. The other plants were collected in dry form.

The samples prepared in this way were then subjected to distillation after grinding.

2.4 Preparing essential oils

To extract the essential oils from the plants, the partially ground dry vegetative parts were subjected to hydrodistillation using a 'Clevenger' with a nominal capacity of 2 litres.

According to [8], hydrodistillation is carried out by dissolving the components of the existing essential oil in the plant cells, by means of the hot water (or steam) that enters the tissues during this operation. The constituents dissolved in the hot water then evaporate, and once they reach the surface of the plant tissue, the solution diffuses through the cell walls (hydrodiffusion).

The leaves and flowers of the plants used were placed with the water in a 2-litre ground flask at a rate of 100g/700ml of distilled water. The mixture was then brought to the boil (at 100°C). The vapours were condensed in a condenser and the essential oils were separated from the water by density difference.

Hydrodistillation was carried out until the essential oil reached a constant volume. The essential oils obtained were stored at 4°C in tubes protected from light until use.

To calculate the average yield, the essential oils were weighed using a precision balance to determine their weight, and three repetitions were made for each plant material. The yield was determined in relation to the quantity of dry matter used initially, according to the relationship:

$$\text{Essential oil yield } (\boldsymbol{en}\ \%) = \frac{\text{Weight of the essential oil (en g)}}{\text{Weight of the dry matter use(en g)}} * 1000$$

2.5 Biotests

The method used for these trials consisted of placing five workers in each rearing box (plastic boxes with a capacity of one litre, part of the lid of which was replaced by a net with a mesh size of less than 2 mm). Each box contained four glass pillboxes filled with cotton wool, two of which were soaked in water and the other two in sugar syrup at a concentration of 500 g/l of distilled water. These boxes are then kept in a rearing room at a photoperiod of 12 h / 12 h. This set-up represents our experimental unit, which included five worker honey bees.

The boxes housing the bees were placed in large (fumigation) boxes with a nominal capacity of two litres. The large boxes contained pieces of filter paper onto which the essential oils of spearmint, round-leaved mint, spearmint or mugwort were injected using a propette. The concentrations used in the tests with these oils were 0 (control), 0.13, 0.25, 0.5 and 1 µl/l of air. The number of dead workers was counted daily for 4 days (according to [9]).

To test the effect of the essential oils of the plants studied, the trial was conducted using a completely randomised design (CRD), with 3 factors: treatment, concentration (four different concentrations as well as the control) and time (four levels). Each of the EO treatments was repeated four times.

2.6 Data analysis

To compare essential oil yields between the different plants used, a one-factor analysis of variance was carried out, after transforming the percentages into Arcsin (root (%)), followed by a Student-Newman-Keuls (SNK) test using SPSS version 16 software.

Gross mortalities were corrected using Abbott's formula. This correction gives the corrected mortality values as a percentage, based on the crude mortalities of the treated samples and those of the controls. It also makes it possible to exclude the bias due to the natural mortality observed under the experimental conditions. Abbott's formula is expressed as follows:

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

With:

T: percentage of deaths in the treated batch,

C: Percentage of deaths in the untreated batch (control).

The Logrank test was used to compare bee mortality caused by the different essential oils used. These are survival curves used according to [10].

Finally, the LC_{50} and LC_{99} after 24, 48, 72 and 96 hours of exposure were calculated using EPA Probit Analysis software (version 1.5).

3. Results

3.1 Essential oil yield of plants

Essential oils were extracted by hydrodistillation from four plants: spearmint (*Mentha pulegium*), white mugwort (*Artemisia herba alba*), round-leaved mint (*M. suaveolens*) and spearmint (*M. spicata*), chosen for their toxicological properties.

Statistical analysis of the variety of plant material on essential oil yield indicated a significant effect of the factor studied (F plant material = 209, 273; P plant material ≤ 0.05). The extraction yields obtained varied from one plant to another. These yields were of the order of 3.17 ± 0.15, 1.33 ± 0.15, 0.83 ± 0.15 and 0.73 ± 0.06% respectively for *M. pulegium, A. herba alba, M. suaveolens* and *M. spicata* (Table 2).

Table 2 Average yields of essential oils from the vegetative parts of the plants used

Plants	Average yields (%) ± standard deviation	Minimum	Maximum	Coefficient of variation (%)
***M. suaveolens* (=*M. roduntifolia*)**	0.83 [a] ± 0.15	0,70	1,00	18,33
A. herba alba	1.33 [b] ± 0.15	1,20	1,50	11,46
M. spicata	0.73 [a] ± 0.06	0,70	0,80	7,87
M. pulegium	3.17 [c] ± 0.15	3,00	3,30	4,82

* Means affected by the same lower case letter are not statistically different from each other (SNK test at 5% after transformation into Asin (root (%)).

3.2 The lethal effects of essential oils or lipids on A. mellifera

3.2.1 Effects of the essential oil of A. herba alba on the mortality of individuals of A. mellifer a

When *A. melifera* individuals were treated with *A. herba* alba essential oil, their responses varied according to the concentration tested (F= 6.093, P=0<0.05) and the duration of exposure (F= 17.929, P=0<0.05), with an interaction between the two factors (F= 2.754, P=0<0.005). The multiple comparison of mortality averages using Dunett's test and the SNK test showed that only the 0.5 and 1µl/l doses of air were significantly different from the control, giving 3 homogeneous groups. The 1er group consisted of the control (0 ±0,00 deaths), the 0.13 dose (5±10 deaths) and the 0.25 µl/l dose (10±12 deaths), the 2ème is made up of the 0.13 (5±10 deaths), 0.25 (10±12 deaths) and 0.5 µl/l doses (20±16 deaths), while the 0.5 (20±16 deaths) and 1 µl/l doses (30±12 deaths) form the last group (Table 3).

Table 3 Average cumulative mortality (± standard deviation) of Apis melifera individuals (N=5 per concentration) due to essential oils of Artemisia herba alba leaves.

Hours after treatment	Concentrations (µl/l of air)				
	Indicator	**0,13**	**0,25**	**0,5**	**1**
12h	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00
24h	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00
36h	0 ± 00	0 ± 00	0 ± 00	5 ± 10	10 ± 12
48h	0 ± 00	5 ± 10	10 ± 12	20 ± 16	30 ± 12

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Figure 1 shows that the higher the concentration of essential oil, the higher the percentage of mortality. Furthermore, for each concentration tested, the mortality of honey bee individuals increased with the duration of exposure. In fact, the mortality of individuals was not only recorded after 36 hours of exposure. The highest mortality was recorded after 48h of exposure by the concentration 1 µl/l of air with 30 ± 12 deaths, followed by that marked by the concentration 0.5 µl/l of air with 20 ± 16 deaths, and in last place, we find the concentrations 0, 25 and 0.13 µl/l with respectively 10 ± 12 and 5 ± 10 deaths, whereas at the level of the control no deaths were recorded.

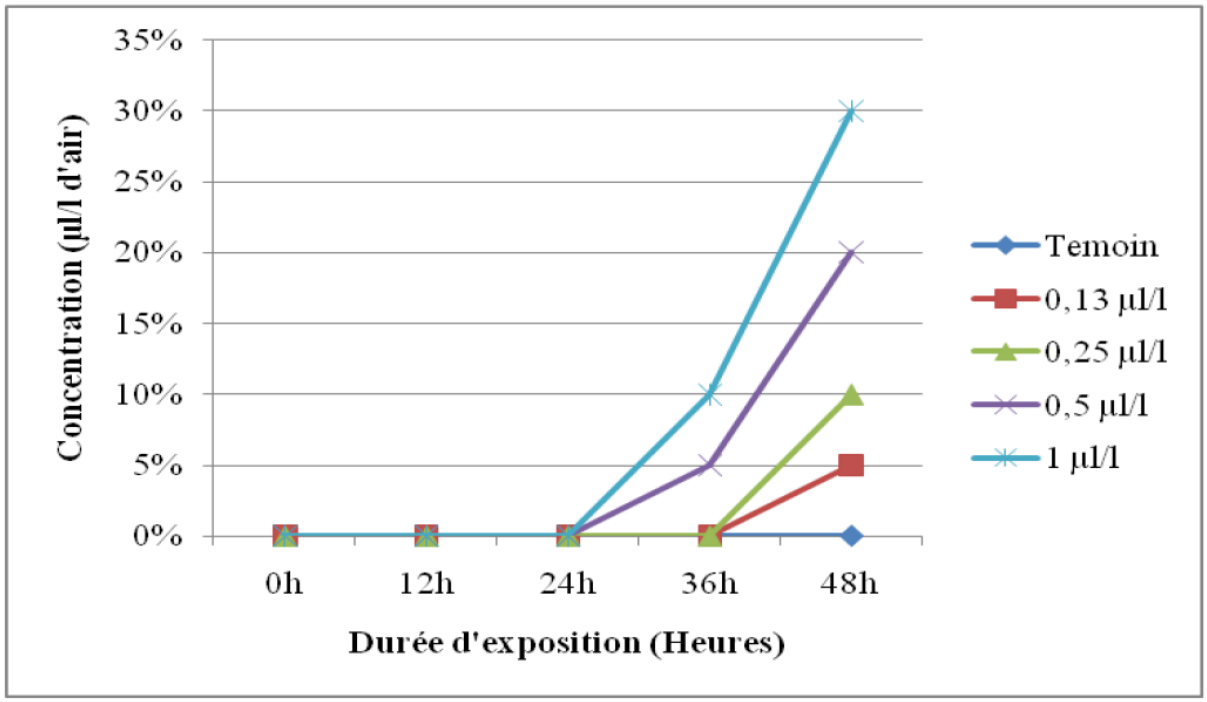

Figure 1 Mortality of *A. melifera* due to essential oils from the leaves of *A. herba* alba as a function of concentration and duration of exposure of individuals.

3.2.2 Effects of Mentha pulegium essential oils on the mortality of A. melifera individuals

Mortality of *A. melifera* individuals due to the application of *M. pulegium* essential oils varied with the concentration applied (F= 19.828, P=0<0.05) and the duration of exposure (F= 8.585, P=0<0.05), with no interaction between the two factors (F= 0.716, P=0.730>0.005). The multiple comparison of mortality means using Dunett's test and the SNK test showed that all the doses tested were significantly different from the control, with the exception of the 0.13 µl/l dose of air. Thus, 3 distinct homogeneous groups were obtained, with the 1er group consisting solely of the control (0 ±0.00 deaths), the 2ème group consisting of the 0.13 dose (15±10 deaths) and the 0.25 µl/l dose (20±16 deaths), while the 0.5 µl/l (35±19) and 1 µl/l air doses (55±19 deaths) make up the last group (Table 4).

Table 4 Average cumulative mortality (± standard deviation) of *Apis melifera* individuals (N=5 per concentration) due to essential oils from *Mentha pulegium* leaves.

Hours after treatment	**Concentrations (µl/l of air)**				
	Indicator	**0,13**	**0,25**	**0,5**	**1**
12h	0 ± 00	0 ± 00	5 ± 10	10 ± 12	20 ± 16

24h	0 ± 00	5 ± 10	10 ± 12	20 ± 16	30 ± 12
36h	0 ± 00	10 ± 12	15 ± 10	25 ± 10	35 ± 10
48h	0 ± 00	15 ± 10	20 ± 16	35 ± 19	55 ± 19

Figure 2 below shows that the essential oil of M. pulegium causes significantly higher mortality in A. melifera than in the control batch.

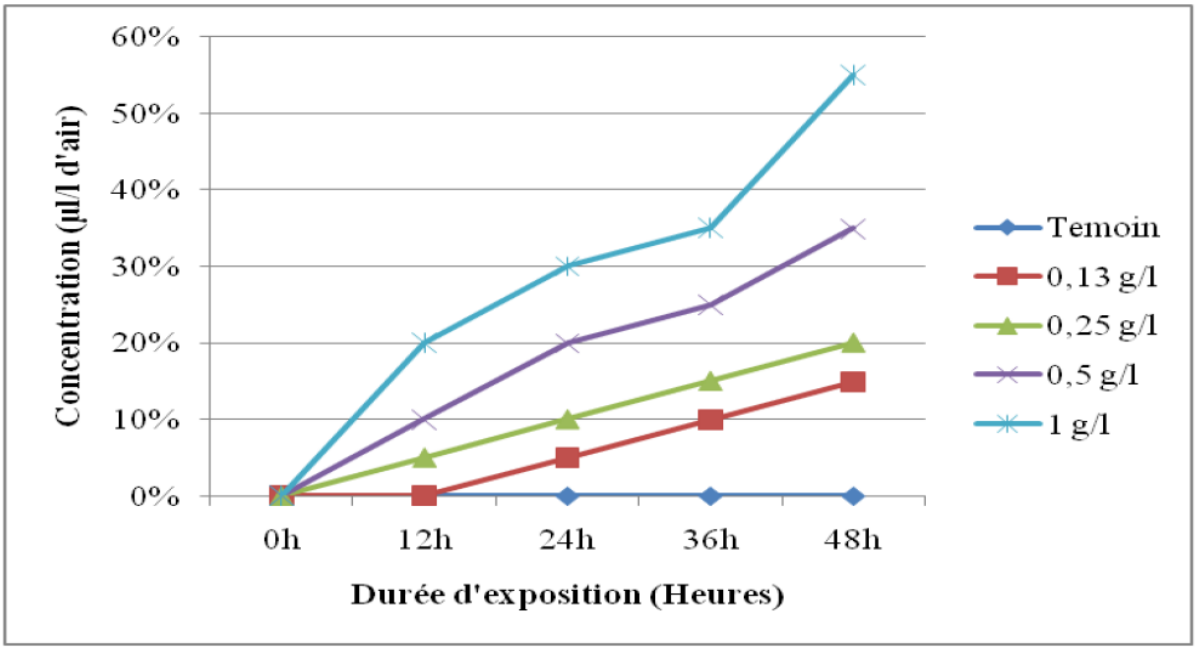

Figure 2 Mortality of *A. melifera* due to essential oils from the leaves of *M. pulegium* as a function of concentration and duration of exposure of individuals.

The same graph also shows that the mortality rate of *A. melifera* individuals increases as the concentration increases and the duration of exposure increases.

Between 12h and 48h of exposure, it increased from approximately 0± 00 to 15± 10, from 5± 10 to 20± 16, from 20± 16 to 35± 19 and from 20± 16 to 55± 19 % of individuals treated respectively with 0.13, 0.25, 0.5 and 1 µl/ l of air.

3.2.3 Effects of Mentha Routundifolia essential oil on the mortality of A. melifera individuals

The analysis of variance of the effect of the factors duration of exposure and treatment concentration in the case of the use of essential oil of round-leaved mint itself showed an individual effect of the two factors on honey bee mortality with an interaction between the said factors (F= 3.137, P=0.002<0.05). The multiple comparison of mortality means using Dunett's test and the SNK test showed that, with the exception of the concentration 0.13µl/l of air, all the doses were significantly different from the control. Thus, three homogeneous groups were obtained: the 1er group was composed of the control and the 0.13 µl/ l dose (5± 10% deaths), while the 2ème group was formed by the 0.25 (15± 10% deaths) and 0.5 µl/ l doses (25± 10% deaths), while the 1 µl/ l air dose (40± 10% deaths) formed a separate group (Table 5).

Table 5 Average cumulative mortality (± standard deviation) of *Apis melifera* individuals (N=5 per concentration) due to essential oils from *Mentha Routundifolia* leaves.

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Hours after treatment	Concentrations (µl/l of air)				
	Indicator	**0,13**	**0,25**	**0,5**	**1**
12h	0 ± 00	0 ± 00	0 ± 00	0 ± 00	5 ± 10
24h	0 ± 00	0 ± 00	5 ± 10	10 ± 12	20 ± 00
36h	0 ± 00	0 ± 00	10 ± 12	15 ± 10	30 ± 12
48h	0 ± 00	5 ± 10	15 ± 10	25 ± 10	40 ± 10

Figure 3 shows that the higher the dose of essential oil, the higher the percentage of deaths. This finding was also shown by EL Kassimi (2009) after applying the essential oil of *M. rotundifolia* at different doses to *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Furthermore, for each dose tested, the mortality of *A. melifera* individuals increased with the duration of exposure, from 0±00 to 5±10, from 0±00 to 15±10, from 0±00 to 25±10 and from 5±10 to 40±10% of individuals treated respectively with 0.13, 0.25, 0.5 and 1 µl/ l of air.

These results show that low doses do not cause significant mortality in honeybees, unlike high doses, where mortality was estimated at 40% after 2 days' exposure.

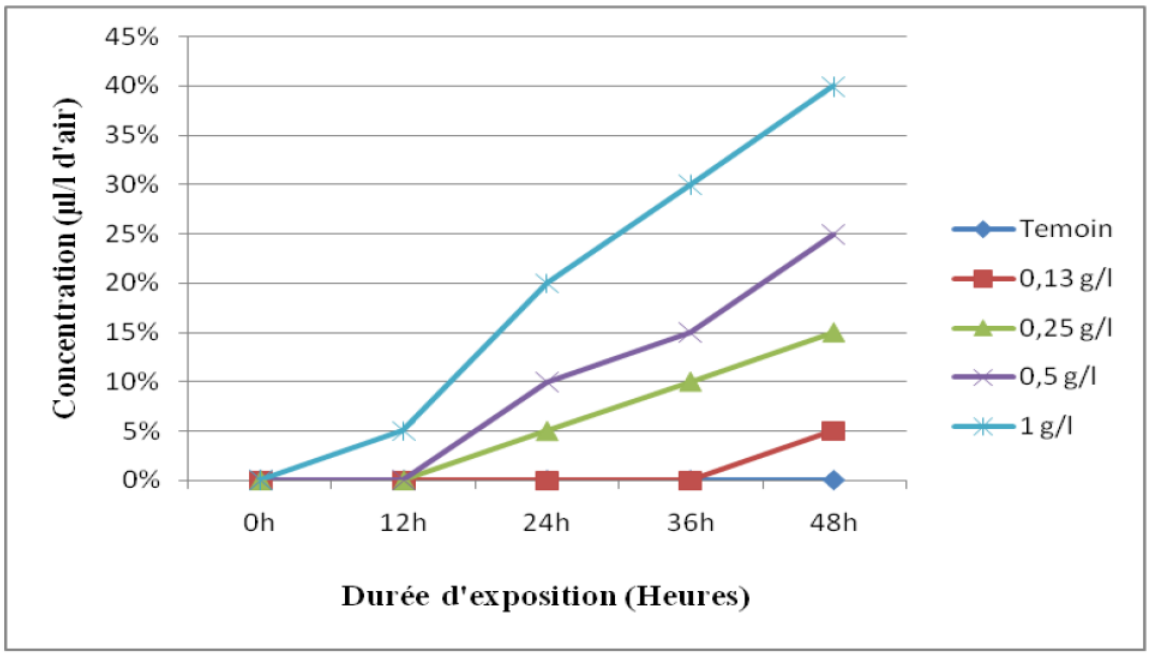

Figure 3 Trends in mortality of *A. melifera* due to essential oils from the leaves of *M. Routundifolia* as a function of concentration and duration of exposure of individuals.

3.2.4 Effects of Mentha viridis essential oils on the mortality of A. melifera individuals

Analysis of variance of the effect of essential oil extracted from spearmint leaves on honey bee individuals showed that the two factors studied had a significant effect (F_{doses} =14.344; P_{doses} ≤0.05 and $F_{durée\ exposure}$= 16.708; $P_{durée\ exposure}$ ≤0.05) with an interaction between the two ($F_{doses\ *\ exposure\ duration}$=14.344; $P_{doses\ *\ exposure\ duration}$ ≤0.05). Thus, the percentage mortality of this species is influenced by the doses tested and this mortality varies with time. A multiple comparison of the mean mortalities using Dunett's test and the SNK

test showed that all the doses were significantly different from the control, except for the dose of 0.13 µl/l of air, which showed an efficacy similar to that of the control. Thus, this analysis made it possible to distinguish three distinct groups, one made up of the control (0±00% deaths) and the 0.13µl/l dose (5±10% deaths), the other by the 0.25 (15±10% deaths) and 0.5 µ/l doses (15±10% deaths), while the 3rd group was formed by the 0.5 (15±10% deaths) and 1 µl/l doses of air (30±12% deaths) (table 6).

Table 6 Average cumulative mortality (± standard deviation) of Apis melifera individuals (N=5 per concentration) due to essential oils of Mentha viridis seeds.

Hours after treatment	Concentrations (µl/l of air)				
	Indicator	**0,13**	**0,25**	**0,5**	**1**
12h	0 ± 00	0 ± 00	0 ± 00	0 ± 00	5 ± 10
24h	0 ± 00	0 ± 00	5 ± 10	5 ± 10	10 ± 12
36h	0 ± 00	0 ± 00	10 ± 12	15 ± 10	25 ± 10
48h	0 ± 00	5 ± 10	15 ± 10	20 ± 00	30 ± 12

Analysis of figure 4 shows that increasing the dose of spearmint essential oils applied to *A. melifera* individuals increased the mortality rate, but this increase was no longer significant until 36 or 48 h of exposure, which confirms the results of the analysis of variance. The mortality of individuals was not recorded until after 24 h of exposure to the doses of essential oils tested, except in the case of the 1µl/l dose of air, where mortality was marked 12 h after exposure, with a rate of 5 ± 10%. The highest mortality was recorded after 48 h of exposure for the 1µl/l dose with a rate of around 5± 10%, followed by the 0.5 µl/l dose which recorded a mortality rate of around 20± 00%, then the 0.25 µl/l dose with a mortality rate of 15± 10% and finally the low dose used in this test 0.13 µl/l with a mortality rate of around 5± 10%.

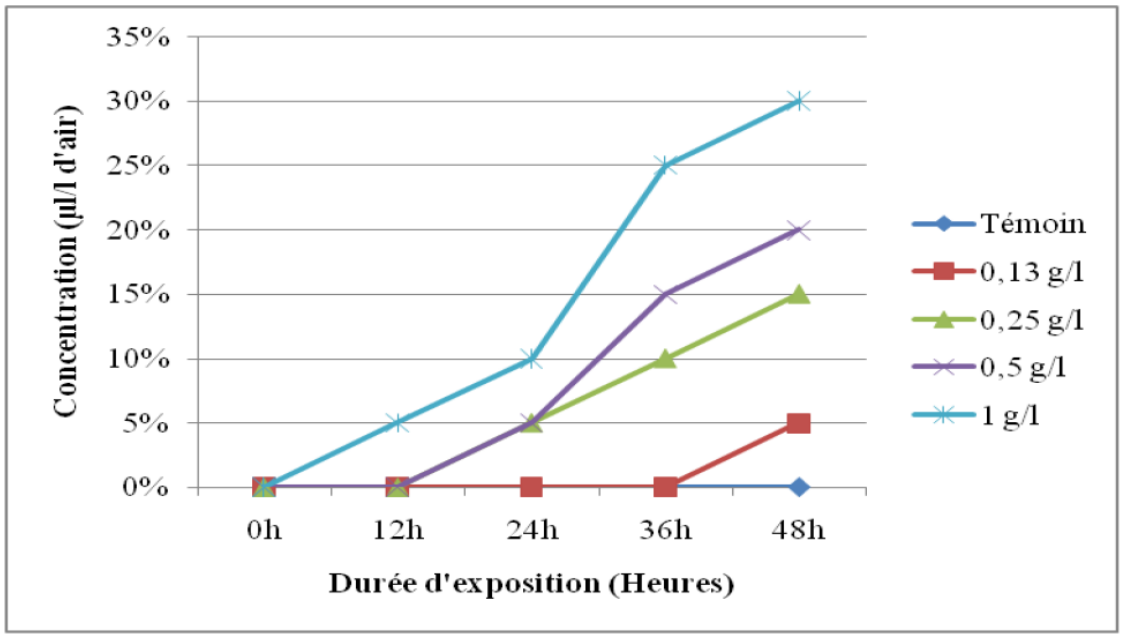

Figure 4 Mortality of *A. melifera* due to essential oils from the leaves of *M. viridis* as a function of concentration and duration of exposure of individuals.

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3.2.5 Calculation of lethal doses of the essential oils of the plants tested and toxicity of the essential oils tested on A. melifera

Toxicological tests using different concentrations of essential oils from the plants studied enabled us to calculate the lethal concentrations of these active ingredients.

Using the Probit Analysis Program version 1.5, we were able to identify the remarkable concentrations characteristic of these products (Table 7).

Table 7 Toxicity parameters of the essential oils of the plants tested on the mortality of Apis melifera workers.

Biopesticides	Duration (H)	LC_{50} (µl/l of air)	LC_{99} (µl/l of air)	Henry equation	χ^2 calculated < χ^2 tabulated (0.05; 3)= 3.84
M. spicata	24	13,38*	1638,51*	3,744 + 1,114 X	0,67
	48	2.771*	326,732*	4,503 + 1,123 X	0,275
	72	2,49	756,11	4,627 + 0,937 X	0,138
	96	1,266 [0,74 ; 8,87]	30,781*	4,827 + 1,678 X	2,376
M. pulegium	24	2,49*	169,27*	4,497 + 1,269 X	0,027
	48	0,865 [0,50 ; 7,43]	55,741*	5,080 + 1,286 X	0,108
	72	0,70 [0,44 ; 2,37]	27,47	5,223 + 1,461 X	0,042
	96	0,712 [0,42 ; 3,98]	47,557*	5,188 + 1,274 X	0,29
M. suavelens	24	2,98*	70,56*	4,196 + 1,693 X	0,388
	48	1,439 [0,77 ; 27,55]	54,338*	4,766 + 1,475 X	0,116
	72	1,39	106,1	4,822 + 1,236 X	0,264
	96	1,255 [0,65 ; 45,82]	86,07	4,875 + 1,267 X	0,033
A. herba alba	24	-	-	-	-
	48	2,490*	169,275*	4,497 + 1,269 X	0,027
	72	2,2	139,47	4,557 +1,291 X	0,391
	96	2,202*	139,471*	4,557 + 1,291 X	0,391

*: Confidence intervals are too wide and do not lend themselves to calculation

Analysis of the table (Table 7) summarising the lethal concentrations obtained following the application of the essential oils of the plants tested in this work showed that all these products are toxic to honey bee workers.

The results showed that the essential oil of *M. pulegium* was the most toxic. The low LC value$_{50}$ (0.865 µl/l of air) calculated after two days' exposure confirms the high degree of toxicity of this essential oil towards this insect. The same observation was made after 96 h of monitoring.

The essential oil of round-leaved mint showed an LC_{50} of the order of 1.439 µl/l of air after 48h of exposure. Finally, the essential oils of *M. spicata* and *A. herba* alba showed comparable toxicity with LC_{50} of 2.771 and 2.490 µl/l of air respectively after 48h.

Our results show that all the essential oils tested are toxic to *A. melifera* workers, but the order of toxicity varies from plant to plant. Spearmint proved to be the most toxic. This result can be explained by the chemical composition of this species, which is particularly rich in pulegone, a molecule known for its high insecticidal activity [12].

4. Discussion

4.1 Essential oil yield of the plants studied

It should be noted that the essential oil yield changes during the drying of the plant material prior to distillation. Initially, the yield increases sharply to reach a maximum and then decreases steadily [8]. The quality of the drying of the different samples in our study could also be at the origin of this variation in yield.

A multiple comparison of the mean yields of 3 replicates for each plant material using the Student-Newman-Keuls (SNK) test showed that the yields of *M. suaveolens* and *M. spicata* were not statistically different and formed a homogeneous group. On the other hand, the yields of *A. herba* alba and *M. pulegium* were significantly different and formed two separate groups. These results could be explained by the fact that the plants used in this trial were from different botanical families, the nature of the organs used for extraction and by the distinct constituents from one plant to another.

The yield of essential oils from pennyroyal mint leaves and flowers was around 3.17%. This yield is comparable to that obtained by [13] (3.16%) and relatively higher than that obtained by [12], which was 2.33%. For Armoise blanche, the yield of essential oils obtained (1.33%) is slightly higher than that obtained by Amine et al. (2008), which was around 0.82%, and also higher than that obtained by [13] (1.04%). It should be noted that essential oil yields depend on a number of factors, including the species, the place and time of harvest, the phenological stage, cultivation practices and the extraction technique used [12].

For spearmint, the yield of essential oil obtained was 0.73%. This is lower than that reported by [14] (from 0.75% to 1.00%). Extractions of round-leaf mint showed a relatively low yield (0.83%). This is lower than that obtained by Daniel et al (2002) (1.02%) and slightly higher than that reported by [11] (0.73%). Generally speaking, the variation in essential oil yields obtained by hydrodistillation is closely linked to the phytochemical profiles of the plants (genetic, physiological, pedological and climatic) [15].

4.2 Effects of essential oils from the plants tested on honeybee mortality

The results of the effects of *A. herba* alba essential oil on the mortality of *A. mellifera* bees showed that the higher the concentration of essential oil, the higher the percentage of mortality. The mortality observed could be explained by the chemical composition of the essential oils of mugwort and in particular by the presence of α-thujone, known for its insecticidal activity [16]. In view of the results obtained, it can be said that the use of the essential oil of *A. herba* alba does not constitute a major threat to bees if it is used in low doses.

Mortality of *A. melifera* individuals due to the application of *M. pulegium* essential oils was significantly higher. The high mortality recorded in batches treated with *M. pulegium* essential oils could be due to the effect of its chemical composition and in particular pulegone and piperitenone oxide, known for their insecticidal activities, as major constituents (Koliopoulos et al. 2010).

In view of the results obtained, it can be said that the essential oil of *M. pulegium* proved to be more toxic to bees, given the mortality rate recorded after 48 hours with this oil, which exceeded 50%. This leads us to say that the oil of this species is not promising as a bioinsecticide and does not lend itself well to investigations in the field of biological control, unless it is used in a way that is compatible with the activity of bees, despite the fact that it has a negative impact against a number of different types of pest, namely : *Sitophilus oryzae*, *Rhizopertha dominica* [12] and others, because the most interesting biological products used in plant protection are those that have a minimal impact on all the components of the agro-ecosystem except for the targeted pests [17].

Similarly, the high mortality observed in batches treated with the essential oil of *M. rotundifolia* could be explained by the effect of its chemical composition, which is mainly represented by piperitenone oxide, known for its insecticidal activity (Koliopoulos, 2010).

Finally, the results of our study on the effect of *M. viridis* essential oil on honey bees showed that the oil tested does not pose a major threat when used in low doses. A study by Koliopoulos et al (2010) showed that piperitenone oxide, the most abundant compound in the essential oil of *M. viridis*, has strong larvicidal activity against *Culex pipiens* (Diptera: Culicidae) and was one of the most toxic molecules of the p-menthane type.

5. Conclusion

The effects of essential oils extracted from four different plants were tested on *A. melifera*.

The yields of essential oils extracted from the plants studied in the course of this work varied significantly according to the type of plant material and the vegetative part used. The leaves of *M. pulegium* gave the highest yield (3.17 ± 0.15), while the lowest essential oil content was found with *M. spicata* (0.73 ± 0.06).

The study of the toxicity of the essential oils of *M. viridis*, *M. pulegium*, *M. suaveolens* and *A. herba* alba to the workers of the Italian honey bee, *Apis mellifera*, showed that these biopesticides are generally toxic to this pollinator, but the order of toxicity varies according to the plant. The essential oil of *M. pulegium* proved to be the most toxic with an LC_{50} = 0.865 µl/l of air recorded after two days' exposure, followed by that of *M. suaveolens* with an LC_{50} of around 1.439 µl/l of air. Finally, the essential oils of *M. viridis* and *A. herba alba* showed comparable toxicity, with LC_{50} of 2,771 and 2,490 µl/l of air respectively.

Our study has shown that the use of essential oils extracted from the 4 plants in our study to combat harmful organisms, including the varroa mite, a mite that infests honey bees, could prove to be a very interesting avenue. However, the use of these EOs needs to be optimised in order to determine both the duration of exposure and the lethal concentration to avoid bee mortality.

6. References

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