EVALUATION OF SOME PLANT OILS FOR CONTROLOF VARROA MITE (*Vharroa jacobsoni* Oudemans IN HONEY BEE (*Apis mellifera* Linnaeus, Hy*menoptera* Apidae) COLONIES

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ABSTRACT

The study consisted of two experiments which carried out during 2014 growing season. One conducted in the laboratories of the college of Agricultural Sciences, university of Sulaimani, and the other one was in Bazian apiary at 34 Nº and 38 Km west of Sulaimani, Iraqi Kurdistan region. Six plant oils in different concentrations were used for control of varroa and studying their effect on honey bees in laboratory and apiary.

The results showed that the highest number of fallen varroa"1.747"occurred at 100% thyme, while the lowest was "1.000" at 100% mint. The number of dead bee workers under laboratory condition. Were "2.307"when using thyme at 100%, but the lowest was 1.000 at 100% rosemary oil under laboratory condition.

In the field experiment, betony oil(50%) recorded the highest number of fallen varroa"43.33" after four weeks of application while the lowest were "1.833"was for eucalyptus(50%) after two weeks.

Results showed that, thyme oil with 50% concentration had the best results among other plant oils according to the results in both laboratory and apiary.

Key words: Varroa mites, plant oils, honey bees.

1.INTRODUCTION

Apiculture beings as a hobby then expanded into small business. It can provide marketable honey and use as a source of pollination for cultivated crops [5]. Ratti[2]] showed that western honey bees *Apis mellifera* are very important for sustaining of honey bees for crop pollination is over $ 2 billion annually. Honey bees have been estimated to account for at least 80% of all pollinators. In addition to pollination, bees play an important, age-old role as producer of honey and wax, which in turn find various nutritional and industrial uses, honey bees are, in economic terms, the third most important domestic animal after cattle and pigs, and before poultry [20].

Honey bees (*Apis mellifera*) are subjected to infestation by a number of natural

enemies, parasites and diseases. Within the parasites there are several types of mites

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causing devastation to honey bees (Varroa mite, Troilaelaps mite and tracheal mite). Varroa mites (Varroasis) have been reported as the biggest problem causing damage

for beekeepers throughout the world [21]. Accurate estimates of the effect of varroa on the apiculture industry are hard to find, but it is safe to assume that the mites have killed hundreds of thousands of colonies worldwide, resulting in billions of dollars of economic loss. Varroa mites have caused beekeeper production costs to rise, thus lowering the profit margin in beekeeping [9].

Hunt [21] , reported that Varroa mites were first discovered in Wisconsin, U.S.in 1987 ,after wards they have been found in every U.S. state except Hawaii.

In Iraq it was first detected in the mid-1980s [4].

Villalobos [24] mentioned that the Varroa mites feed on the haemolymph of developing larvae and adult bees. In 1990, Varroa mite was reported in all Arab countries [11]. These parasites not only ectoparasitically feed on bees, but also vertically transmit a number of deadly viruses to the honey bees [20].

Several attempts were used for controlling this pest worldwide including chemicals which causing toxicity of the honey. One of the safer ways for control is by using of plant oil. Several natural substances were used as they are biodegradable, safe for human and relatively inexpensive for the control of varroa [19]. Plant oils can be extracted from plant material by several methods, the steam distillation, expression, and so on. Among all methods, for example, steam distillation method has been widely used, especially for commercial scale production [23].

Plant oils are the production of plants used to repel predator insects or render the plant in some way, toxic to the insects if they are ingested. Plant oils have antimicrobial, antifungal, insecticidal, and miticidal effects on various pathogens and pests under both laboratory and field conditions. They are used for treating honey bee infested with parasitic mites varroa and acarine (tracheal mites), and their effects are usually between 50 and 95%.On the other hand, they cause contamination of wax and honey but with minimal levels. The most common oils for this purpose are thymol, eucalyptol and wintergreen, which can be applied alone or in concert [8].

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Chamorro [6] showed that the natural products, such as essential oils, represent an alternative to synthetic acaricides for controlling *Varroa destructor* (Anderson and Trueman).

In the present study six plant oils (rosemary, thyme, eucalyptus, betony, lemon shell and mint) with three various concentrations were used for control varroa mites.

2. MATERIAL AND METHODS

2.1. Plant oils extraction

The extraction was carried out in Food Technology Department, Faculty of Agricultural Sciences**.**

Each of the above mentioned weights was placed in conical flask of the Clevenger and immersed with water. The system then has been turned on and the temperature was regulated at 100Cº (boiling degree) at which, the solutions were maintained for about 20 minutes. Later, it was lowered to 60Cº at which the oils began to extract that and took about three hours. The accumulated oils were placed in vials using pipette for the purpose. The amounts of oils obtained were (6.3, 6.6, 6.2, 1.8, 2.8, and 1.6) ml for eucalyptus, thyme, betony, mint, rosemary leaves, and lemon rind respectively.

Table (1): Plants used for oils extraction.

Plants	Scietifice Name	Weight	Oils (%)
Eucalyptus	*Eucalyptus deglupta* B.	185 gm	3.5 ml
Lemon rind	*Citrus limon* L.	145 gm	1.10 ml
Mint	*Mentha spicata* L.	80 gm	2.25 ml
Rosemary	*Rosemarinus offincinalis* L.	125 gm	2.24 ml
Betony	*Stachys hydrophila* L.	180 gm	3.42 ml
Thyme	*Thymus vulgaris* L.	150 gm	4.4 ml

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Figure (1): Clavenger apparatus for extracting plant oils.

2.2. Laboratory experiment

The plant oils extracted in the lab. as mentioned previously were used in the laboratory of the Department of Horticulture, Faculty of Agricultural Sciences, Sulaimani University in order to get familiar with their effects in controlling varroa as well as bee workers. Such procedure was as follows:

An experiment of six treatments with three levels of the plant oils in addition to the control was used in completely randomized design. Twenty infested bees were kept in the middle size plastic container and they were used as experimental unit for applying six various plant oils with three concentrations (50, 75, 100) % for each in addition to the control and each treatment was replicated thrice. Therefore, the total numbers of treatments were 57 and each one conducted in the plastic bags.

The method of [16] with some modification was applied. A sticky board with a mesh was placed in the inner side of the inverted container for the purpose of varroa collection. 0.2 ml of each of the plant oils (Eucalyptus, Betony, Thyme, Mint, Lemon rind and Rosemary) was

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taken and diluted with olive oil to (50, 75)% in addition to the pure (100 %). Little pieces of cotton were impregnated with such oils and placed inside the containers. Oils with lowest number of dead workers and highest number of fallen varroa had been selected as best oils and this process was continued for three consequential days. On this basis (50 %) of each thyme, betony and eucalyptus oils were selected and applied for the field experiment.

2.3. Field experiment:

Thyme, Betony and Eucalyptus oils effect on varroa and worker bees.

The study was carried out on August 19, 2014 until September 30 of the same year at Bazian Apiary. The selected oils (thyme, betony and eucalyptus) each with 50% were used as follows: 2 ml of such oils impregnated in a little piece of cotton were placed in each bee hive in addition to untreated hive as control. A sticky board was fixed in each hive for the purpose of varroa and workers collection. There were five combs per hive with 1 year old queen. Numbers of varroa and workers were counted for the first two weeks but no data had been taken during the third week while they were also taken for the last fourth and fifth weeks.

Figure (2): Effect of oils on honey bee colonies in the field

4.2. Sensory evaluation

In order to evaluate sensory characteristics of honey (taste, odor, color and consistency) treated with three types of plant oils (thyme, betony and eucalyptus) in addition to the control were used in laboratory and introduced to thirty persons (18 females and 12 males aged 24 to 65 years) familiar with honey bees. The scores given were (40%, 20%, 20% and 20%) for taste, odor, color and consistency

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respectively. Means were compared according to Duncan's multiple range taste at 0.05 level and completely randomized design with three replicates was applied for this purpose.

3. RESULTS AND DISCUSSION

3.1- Laboratory experiment

Data in table 2 indicate that the three concentrations of thyme oils were superior significantly to the other sources of the oils with respect to the fallen varroa. 100% betony was superior significantly to the eucalyptus, mint, rosemary, lemon rind and the control except 75% and 50% betony with no significant differences at 0.05level.With regard to the dead workers, the maximum numbers were recorded for %100 of both thyme and mint oils which were superior significant to the rest of plant oil concentrations except 75% thyme, 100% betony, 100% eucalyptus and 75% mint.

Table (2): Effect of different concentrations of every type of plant oils on the number of fallen varroa and dead workers.

Treatments	Concentrations (%)	No. of fallen varroa	No.of dead workers
Thyme	100	1.747 a	2.307 a
	75	1.650 a	1.637 abc
	50	1.617 a	1.127 c
Betony	100	1.390 b	1.960 ab
	75	1.213bc	1.323 bc
	50	1.180bc	1.080 c
Eucalyptus	100	1.177 c	1.763 abc
	75	1.177 c	1.297 bc
	50	1.133 c	1.137 c
Mint	100	1.000 c	2.113 a
	75	1.133 c	1.587 abc
	50	1.130 c	1.280 bc

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Rosemary	100	1.043 c	1.000 c
	75	1.043 c	1.133 c
	50	1.000 c	1.123 c
Lemon shell	100	1.043 c	1.043 c
	75	1.080 c	1.043 c
	50	1.043 c	1.123 c
Control	**0**	1.087 c	1.127 c

Numbers within a column carrying the same letters are not significantly different according to Duncan's multiple range test at 0.05 level

The result agreed with [19] who indicated that the mean number of mites collected from the colonies which received four applications of thyme was significantly higher than those received camphor and basil oil and all the treatments were superior to the control. The result also agreed with [13]; and [14] who all evaluated essential oils and their components as control agents for varroa and indicated that many of those compounds was biologically toxic to varroa without injuring bees.

The result also agreed with [6] who found mites mortality was much higher when they were exposed to *Tagetesminuta* essential oils than when they were not. However, the result disagreed with [10] who showed that caraway, eucalyptus, lemon, rosemary and rose essential oils were more effective than chenopodium, clove, cinnamon, fennel, garlic, geranium, geraniol, matrecary, peppermint and thyme. Increasing the time of exposure led to an increase in the toxicity of these essential oils against Varroa mite for instance after 48 hours of exposure they were more effective than 24 hours. Also [22]showed that the number of dead mites as a result of spraying with 3, 4 and 5 % solution of active ingredients of both *Heterothecalatifola* and *Tagetesminuta* essential oils did not differ significantly at 5% level but they are superior to the control.

Al- Zarog and El-Bassiouny[13], found that the treatment of thyme, mixture of (fenugreek and santonica), fenugreek only and santonica alone, were decreased the adult honey bee infestation with varroa

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mite, the results showed that the thyme was superior on other treatments.

The result disagrees with [17] showed that the overall mean mortality for the number of mites for different concentrations i.e. 5%, 10% and 15% of tobacco essential oil extract were 4.15, 2.95 and 2.35, respectively. This clearly showed that 5% is the most effective compared with 10% and 15% concentrations.

The result agrees with [22] who concluded that the values of mite mortality sprayed with 3, 4 and 5% concentrations of both *Tagetesminuta* and *Heterothecalatifola* did not differ significantly at 5% level. Also found that at the high concentration (5%) *Tagetesminuta* essential oil did not show bee toxicity and significant differences in adult bee toxicity between treated and control groups were not observed ($P \leq 0.05$).

From tables 2 can be observe that the thyme, Betony, eucalyptus at 50% concentration are the best treatments regarding to the highest number of fallen varroa and the lowest number of dead worker bees, compared with the other treatments.

Data in table 3indicates that 50% thyme plant oil is superior significantly to the other types of the oils with regard to the number of fallen varroa, on the other hand, there were no significant differences among all the oils with respect to the number of dead workers. 75% thyme oil was superior significantly to the other types of the oils with regard to the number of fallen varroa, on the other hand, there were no significant differences among all the oils with respect to the number of dead workers.

Thyme 100 % was superior significantly to the other types of plant oils followed by the betony, (eucalyptus, mint, rosemary, lemon rind and the control) showed no significant differences among them while regarding for the fallen varroa, 100% thyme and mint oils were dominant to all other types of the oils except eucalyptus with regard to dead workers.

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Table (3): The effect of type of plant oils with different concentrations of oils on each of fallen varroa and dead workers.

Treatments	No. of fallen varroa			No. of dead workers		
	Concentration (%)			Concentration (%)		
	50	75	100	50	75	100
Thyme	1.617 a	1.650 a	1.747 a	1.127 a	1.637 a	2.307 a
Betony	1.180 b	1.213 b	1.390 b	1.080 a	1.960 a	1.323 b
Eucalyptus	1.133 b	1.177 b	1.177 c	1.137 a	1.297 a	1.763 ab
Mint	1.130 b	1.133 b	1.000 c	1.280 a	1.587 a	1.043 b
Rosemary	1.000 b	1.043 b	1.043 c	1.123 a	1.043 a	1.043 b
Lemon rind	1.043 b	1.080 b	1.043 c	1.123 a	1.043 a	1.043 b
Control	1.087 b	1.087 b	1.087 c	1.127 a	1.127 a	1.127 b

Numbers within a column carrying the same letters are not different significantly according to Duncan's multiple range test at 0.05 level.

4.2 Field experiments

Using 50% plant oils on the fallen varroa and dead workers in the field after two, four and six weeks.

Table 4 indicates that 50% thyme was superior significantly to the 50% betony and 50% eucalyptus oils in addition to the control on the number of fallen varroa after two, four and six weeks. Regarding the dead workers, no significant differences were found among them but thyme was superior to the control after two weeks, while after four weeks no significant differences were

noticed among all the four treatments . On the other hand, 50% thyme gave best result which was not significantly different with 50% betony after six weeks.

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Table (4):Effect of plant oils on the number of fallen varroa and dead workers in the fields after two, four and six weeks.

Treatments	two weeks		four weeks		six weeks	
	No. of fallen varroa	No. of dead workers	No. of fallen varroa	No. of dead workers	No. of fallen varroa	No. of dead workers
Thyme	9.000 a	1.833 a	43.333 a	1.167 a	21.500 a	0.500 a
Betony	3.500 b	1.333 ab	14.500 b	1.167 a	11.333 b	0.333 ab
Eucalyptus	1.833 b	0.667 ab	7.000 b	1.167 a	8.667 b	0.000 b
Control	1.167 b	0.500 b	5.333 b	1.167 a	7.167 b	0.333 ab

Numbers within a column carrying the same letters are not different significantly according to Duncan's multiple range test at 0.05 level.

The result agreed with [1] who mentioned that the highest total number of dead varroa mites were counted in the tested colonies after the first and second week of treatment particularly in 100% of all tested volatile oils in comparison with control colonies. Treatment with Citronella oil (100%) caused the highest number of dead varroa fallen on the sheet in comparison with the other tested volatile oils and control. Increasing the mean number of fallen varroa mites on the sheet in tested honey bee colonies treated with Citronella oil (100%) may be due to the activity of the defense behaviour mechanisms of honey bee workers by these plant volatile oils against varroa mites. The same authors stated that some defence behaviour mechanisms against varroa mites were detected in some races and hybrids of honey bees. These mechanisms resulted in increasing the number of fallen varroa mites on the bottom board of bee hive.

Abdel Rahman and Rateb[2] indicated that the colonies treated with several concentrations (10, 25, 50, 75 and 100)% of lemon juice, the maximum mean number of dead mites was observed after

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the first treatment then, gradually decreased from the first spray to the fifth spray.

Ismaeil stated[14] that the infestation on adult worker bees decreased from 16.00 to 7.53%, 14.67% to 4.60%, 14.07% to 6.27% and 13.50% to 6.40% (reductions were 66.6%, 77.7%, 68.4% and 66.3%) in colonies treated with basil, geranium, eugenol and mixture, respectively. The higher reduction% of mites that occurred on bees compared to brood may be due to the direct exposure of mites to the released volatiles used. Therefore; the result agrees with the present study accordance to the effect of different essential oils on the control of honey bee mites.

Qayyoum[18] showed that the infestation on adult worker bees decreased from 11.41%, 9.43 and 10.84% (reductions were 61.4%, 68.7% and 65.8%) in colonies treated with neem oil, mixture (neem oil, garlic oil and tobacco oil) and tobacco oil, respectively. The higher reduction% of mites that occurred on bees compared to brood may be due to the direct exposure of mites to the released botanical used.

On the other hand [18] showed that the average numbers of dead worker bees were 3.12, 2.2, 9.3 and 0.91/ colony for neem oil, mixture (neem oil, garlic oil and tobacco oil), tobacco oil treatment and control respectively. The average number of dead bees was significantly high in case of mixture (neem oil, garlic oil and tobacco oil).

4.3. Sensory evaluations of honeys:

Figure (3) indicates the taste evaluation for honey treated with three types of oils (thyme, eucalyptus and betony) in addition to the control. No significant differences were found among the treatments.

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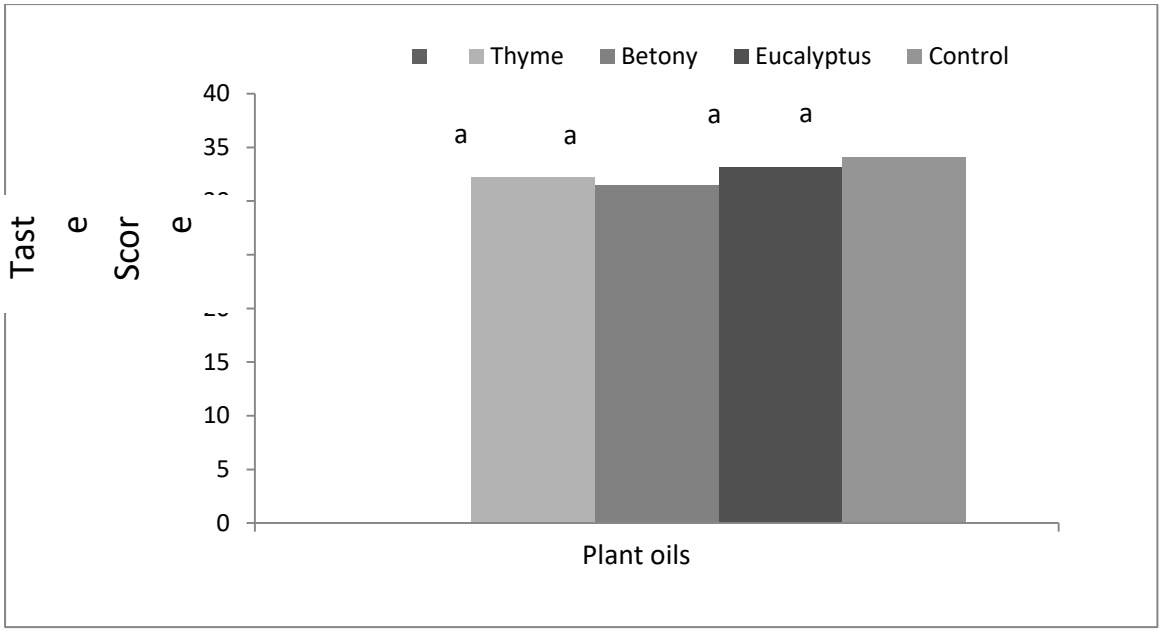

Figure (3): Taste score of honey treated with three types of oils compared with the control.

Figure 4 indicates the odour evaluation for honey treated with three types of essential oils (thyme, eucalyptus and betony) in addition to the control. No significant differences were found among the treatments.

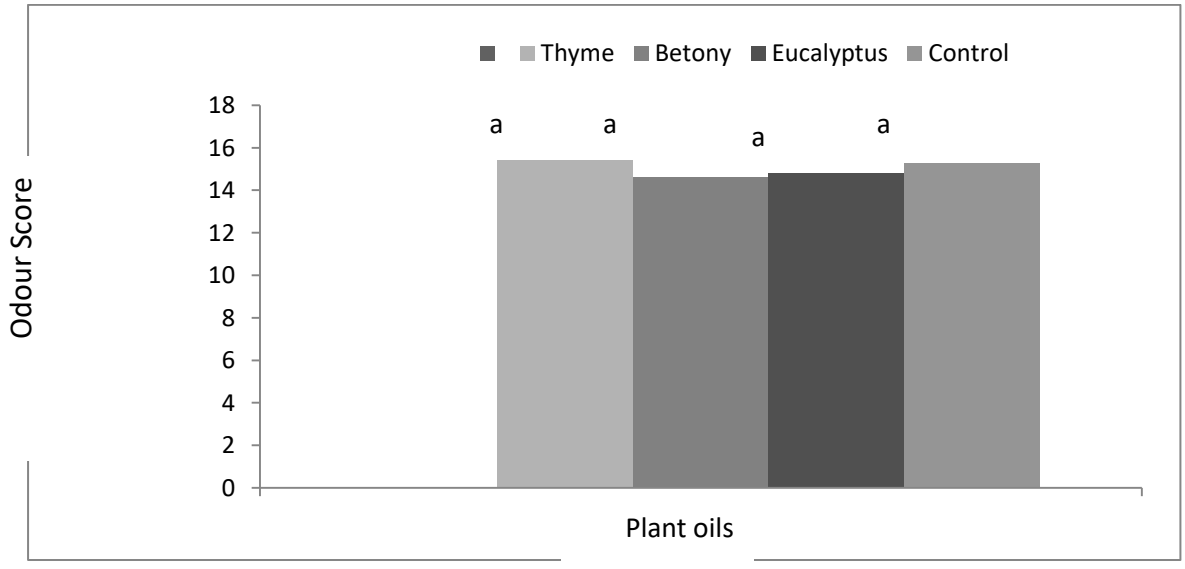

Figure (4): Odour score of honey treated with three types of oils compared with the control.

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Figure 5mentioned the colour evaluation for honey treated with three types of essential oils (thyme, betony and eucalyptus) in addition to the control. No significant differences were found among the treatments.

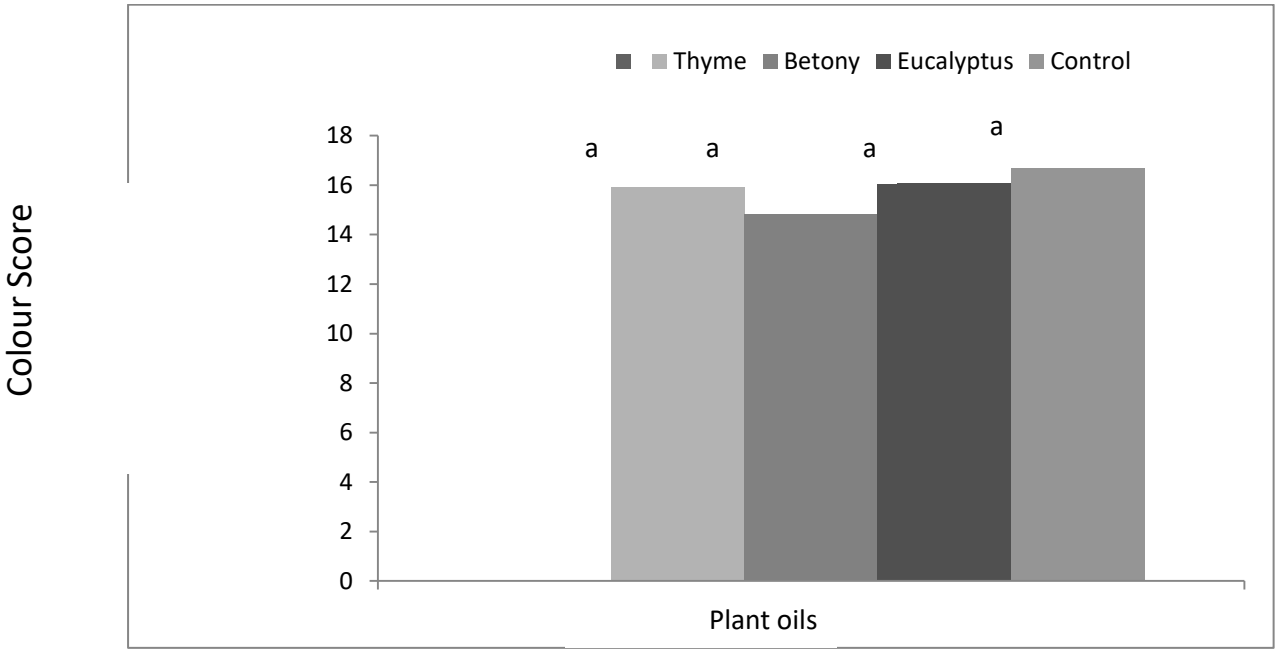

Figure (5): Colour scores of honey treated with three types of plant oils compared with the control.

Figure 6 indicates the consistency evaluation for honey treated with three types of essential oils (thyme, eucalyptus and betony) in addition to the control. No significant differences were found among the treatments.

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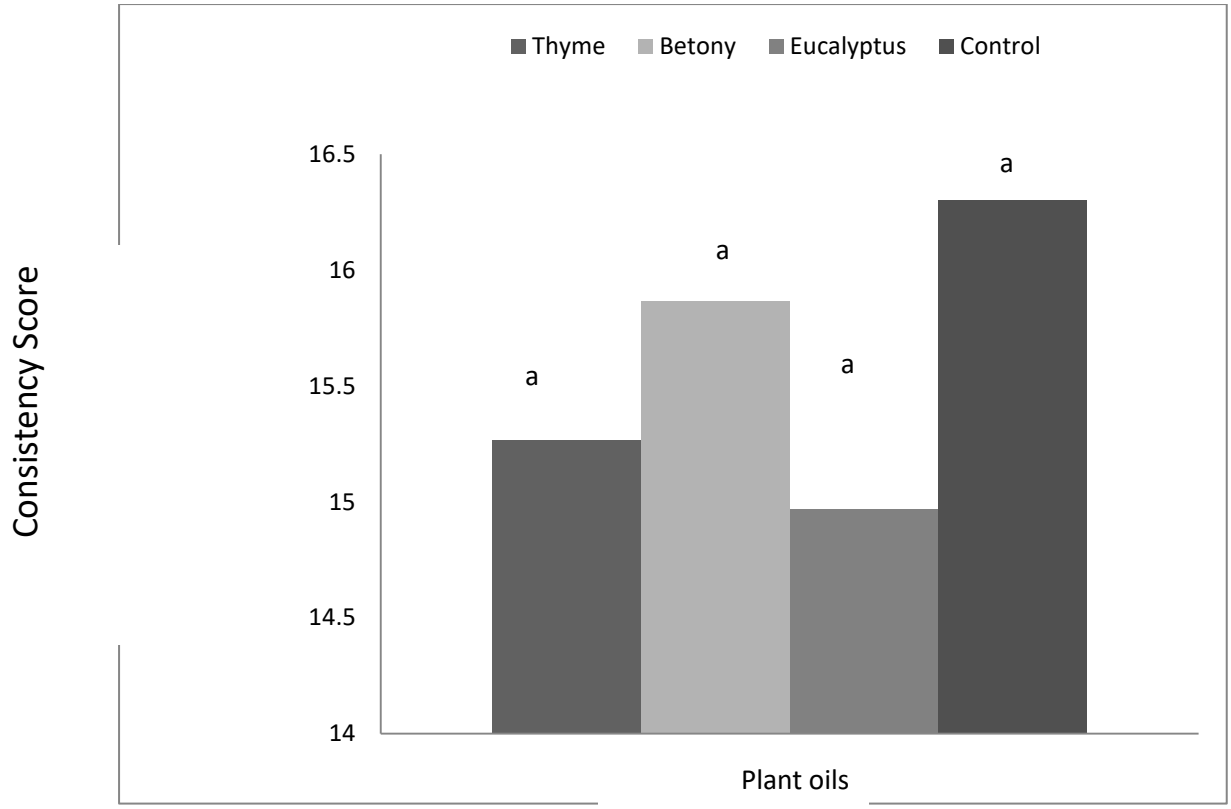

Figure (6): Consistency scores of honey treated with three types of oils compared with the control.

From the results of this experiment there were no significant differences among the tests.

The result agrees with [7] who found no significant differences among honey samples taken from different parts of Negiria with regard to taste, flavour and consistency.

The result disagreed with (Kaakeh and Gadelhak, 2005) who showed significant differences among 13 honey samples in terms of colour, taste and smell.

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تقيم كفاءة بعض زيوت المسخلصات النباتية في مكافحة حلم الفاروا (*Varroa jacobsoni*) في مستعمرات نحل العسل (*Apis mellifera*, Hymenoptera, Apidae)

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الخلاصة

شملت الدراسة تجربتين والتي اجريت خلال الموسم الزراعي سنة 2014 . احداها اجريت في مختبرات كلية العلوم الزراعية في جامعة السليمانية والاخرى في منحل بازيان عند 34 درجة شمالآ و 38 كم غرب السليمانية. استخدمت ست انواع من الزيوت النباتية . حيث تم استخدام كل نوع من الزيوت النباتية وبمختلف تراكيزها لمكافحة الفاروة ودراسة تأثيرها على النحل العسل في المختبر و المنحل. وبينت النتائج بأن اكبر عدد لفارواة المتساقطة)كانت "1.747" عند استخدام زيت الزعتر و بتركيز100% واقل عدد كانت "1.000" عند استخدام زيت النعناع و بتركيز 100%. واكبر عدد للشغالات الميتة كانت العسل في المختبر و المنحل. وبينت النتائج بأن اكبر عدد لفارواة المتساقطة)كانت "1.747" عند استخدام زيت الزعتر و بتركيز100% واقل عدد كانت "1.000" عند استخدام زيت النعناع و بتركيز 100%. واكبر عدد للشغالات الميتة كانت '2.307" عند استخدام زيت الزعتر و بتركيز 100% و اقلها كانت 1.000 في حالة استخدام زيت اكليل الجبل و بتركيز 100% تحت الظروف المختبرية. اما في التجربة الحقلية، فان زيت البنطج (50%) سجلت اكبر عدد للفاروا المتساقطة '43.33" بعد اربع اسابيع من المعاملة بينما اقل العدد كانت "1.833" و ذلك للزيت اليوكاليبتوس(50%). اظهرت النتائج بأن زيت الزعتر 50%اعطت افضل النتائج من بين بقية الزيوت الاخرى في كل من المختبر و المنحل

**The research is a part of M.Sc. thesis of second autho*

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