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Controlling *Varroa destructor* Infesting Honey Bee *Apis mellifera* using Essential Oils as Diet Supplements and as Impregnated Paper

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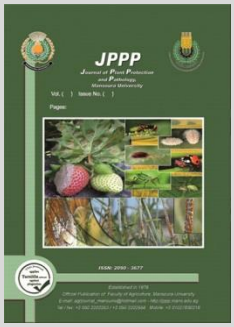

ABSTRACT

The results showed that all the evaluated supplementary diets were found to be accepted by the bees. Colonies fed on the supplementary diets mixed with different essential oils were found to have more individual bees, increased values of sealed brood area, more number of frames covered with bees and encouraged queens to lay more eggs than the controlled colonies. Diet [A] which has been mixed with mint oil proved to be better to be offered to bees during winter followed by diets [C], [B] and [D]. Feeding on diet [A] gave the highest means of sealed brood area (1348.0±13.93 inch2), number of eggs/queen/day (1195.64±12.35 eggs) and number of frames covered with bee (9.0±0.00 frames/hive) in winter season of 2019/ 2020. The fallen mites increased in November when the colonies fed on diet (A) & (B) recording high mean numbers of 176.0 & 201.0 mites (effectiveness= 80.11 & 80.33%) (2019 season) and 201.0 & 161.0 mites (effectiveness= 87.06 & 83.85%) (2020 season), respectively. Thyme oil impregnated papers was proved to be more effective during February of both seasons recording effectiveness of 77.84 and 83.95%, followed by mint oil (75.16 and 81.37%, respectively), while mustard oil impregnated papers was proved to be the least effective. Residues of the evaluated essential oils in honey were absent and that because these compounds quickly decreased due to their volatility, thus there cannot be any food safety risk for the consumer.

Keywords: Supplementary materials, Bees biological activity, *Varroa destructor*, Detection of essential oils

INTRODUCTION

Honey bee *Apis mellifera* is considered as one of the most important and beneficial whose damage has serious negative economic implication for both beekeeping industry and agriculture (Melathopoulos *et al*., 2000). In the dearth period because of less floral rewards, supplemental feeding is necessary for maintenance of the bee population (Dastouri and Sis, 2007).

The mite *Varroa destructor* has become a serious pest of *A. mellifera* all over the world (Rashid *et al*., 2014) since it causes serious weight loss, malformation and shortened life span of the bees. It also serves as a vector of diseases that lead to 100% bee mortality (Kanga and James, 2002). It feeds on haemolymph of larvae, pupae and adults during all honey bees life (Anderson and Trueman, 2000). It also decreases brood, colony ability to pollinate plant (De Jong *et al*., 1984). The extensive use 0f synthetic varroacides may increase varroa resistance and have their own negative effect on the quality of honey, wax and pollen grains (Calderone and Spivak, 1995; Tihelka, 2018). Recently, researches have been concentrated on the usage of some essential oils as well as volatile components to control varroa mite. Essential oils or any of their components have been tested to control the mite *V .destructor*. Their toxicity, repellent or attractive effect towards the mite and their influence on the mite's reproduction have been evaluated (Ruffinende *et al.*, 2007; Islam *et al.*, 2016; Rahimi *et al.*, 2017; Light *et al.*, 2020). Essential oils produced by plants are a rich source of metabolites that can have toxic or behaviour-modifying effects on arthropods. Some essential oils have shown promise in management of the mite *Varroa destructor* Anderson and Trueman.

The present investigation was carried out to evaluate certain essential oils (mint, camphor, thyme and garlic) as supplementary feeding agents that can be added to prepared diets to enhance the productivity of bees during the dearth period. Meanwhile, another group of certain essential oils (mint, camphor, thyme, garlic and mustard) were chosen and selected to evaluate their activity for controlling *V. destructor* through an impregnated papers technique. The evaluated essential oils were evaluated in the produced honey.

MATERIALS AND METHODS

This investigation has been carried out at a commercial apiary in Rosetta region, 40 km East of Alexandria city. Hybrids of Carniolan local strain queens (age of less than one year [9 months]) were used in the present experiments. The colonies of *Apis mellifera* were divided into 5 groups (4 treatments and 1 for control) (5 colonies for each group).(at completely randomized design (C R)

1. supplementary diet mixed with certain essential oils oils in winter seasons of 2018/2019 and2019/ 2020

A mixed supplementary diet consists of 75 g of skim soya bean powder, chickpeas and yellow corn, mixed

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with 300 g of sugar, 75 g of dry yeast, 15 g of cinnamon, 10 g of turmeric, 10 ml of lemon juice, 10 ml of apple cider vinegar and 135 g of honey. This mixture dough was repeated for four times. The first dough was mixed with 5 ml of peppermint oil (Diet A), the second was mixed with 5 ml of camphor oil (Diet B), the third was mixed with 3 ml of thyme oil (Diet C) and the fourth was mixed with 5 ml of garlic oil (Diet D). These four dough were offered to four groups of colonies, while the fifth group representing control (Diet E) was offered with sugar solution (2 sugar: 1water) (w/v). Each colony was supplied with a part of this mixed supplementary dough diet three times; 250 g each time in perforated polyethylene bag over the middle comb. The new amount of 250 g was offered to bees when the old one is finished. Then, a cardboard sheet greased with petroleum jelly was put in the bottom of the cell so that the varroa individuals that fall could be counted after applying the dough mixed with essential oils.

Supplementary feeding was provided in parallel with feeding on sugar syrup (250 ml) every week for the all five groups to stimulate the activity of honey bees and this amount was doubled as the density of bees increased.

2. The paper impregnated with essential oils technique

In this experiment, a paper was impregnated with certain evaluated essential oils (peppermint, eucalyptus, thyme, garlic or mustard oil). These papers were used during the period started from the first of February 2020 or 2021 till the first of March, 2020 or 2021 where thirty Lingstroth colonies were selected. The colonies (30) with bees were divided into six groups of 5 colonies each (5 colonies / treatment) (treatments A-E) and the control (F). Two strips of oleander paper (7X15 cm) impregnated in the evaluated essential oil are hanged between the brood frames within the hive every week for four sequential weeks. A sheet of white paper greased with Vaseline is placed in the bottom of a hive so that varroa individuals fall on it can be easily collected and counted for the all running treatments and can be also compared to the control hives. Effectiveness percentages of the tested diets for controlling *Varroa* in each hive were determined according to a modified formula adopted by Gracia *et al.* (2017) as follows:

$$\text{Effectiveness in controlling Varroa mites \%} = \frac{\text{No. of Fallen mites in treatment} - \text{No. of Fallen mites in control}}{\text{No. of Fallen mites in treatment}}$$

3. Effect of mixed supplementary diet mixed with certain essential oils some biological activities

Sealed brood was measured by using the inch square frame method (Al-Tikrity *et al*., 1971 and Hassona, 2006). Also, the mean number of combs covered with bees was counted (frames/hives). Mean amount of food consumption (g), mean of converted food (g) and mean number of laying eggs/queen were also estimated.

4. Detection of essential oils in produced honey

The residual traces of the evaluated oils (mint, camphor, garlic, mustard and thyme) used in the impregnated papers technique were detected in five samples of produced citrus honey. GC-MS analysis was done to detect the residues of the evaluated essential oils at City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab, Alexandria, Egypt.

5. Statistical analysis

SAS program was used for data analysis by the aid of a personal computer. The analysis of variance (F. value) was done and both L.S.D and standard deviations were also determined. Data were analyzed using GIM procedure according to SAS (2004).

RESULTS AND DISCUSSION

1. The effect of mixed supplementary diet mixed with certain essential oils on *Varroa* during winter of two seasons of 2018/2019 and 2019/2020

The efficiency of the certain supplementary diets mixed with mint oil (5 ml) (diet A), camphor (5ml) (B), thyme oil (3 ml) (C) and garlic oil (5 ml) (D) was evaluated against Varroa individuals collected during winter of both seasons of 2019 and 2020 as compared with control (diet E). Data shown in Table 1 revealed that the fallen mites increased in November when the colonies fed on diet (A) & (B) recording high mean numbers of 176.0 & 201.0 mites (effectiveness= 80.11 & 80.33%) (1^{st} season) and 201.0 & 161.0 mites (effectiveness= 87.06 & 83.85%) (2^{nd} season), respectively. On the other hand, the mean numbers of the fallen mites decreased in December in all evaluated treatments during both seasons where the effectiveness of the evaluated essential oils was ranged between 69.52-80.37% (1^{st} season) and between 80.0-86.82%)(2^{nd} season), respectively. The mean numbers of mite as a result of using such essential oils mixed with supplementary diets were higher in January more than those detected in February, but the detected *Vorroa* individuals in both these two months were lower than those detected in November and December of both seasons. It is concluded that diet (A) (containing mint oil) showed the highest number of fallen mite individuals in November and December and that decreased the infestation and increased the strength of the colony. Rbee and Zedan (2018) showed that the highest reduction of the *Varroa* population parasite on adult bees (90.4, 85.6 and 81.9% reduction) resulted by their treatment with the mixture of Formic acid and Marjoram at the rates of 3, 2 and 1ml /colony followed by Spearmint mixture with Formic acid (77.0, 72.8 %) at 3ml/colony.

Table 1. Effect of supplementary materials mixed with essential oils on varroa during both winter seasons of 2018/2019 and 2019/2020

Month	Diet	Mean No. of collected fallen *varroa*/hive			
		2018/2019	Effectiveness (%)	2019/2020	Effectiveness (%)
November	A*	176.00±12.08[a]	80.11	201.00±7.14[a]	87.06
	B	178.00±8.60[a]	80.33	161.00±7.14[b]	83.85
	C	156.00±16.91[b]	77.56	140.00±12.14[c]	81.43
	D	136.00±6.96[c]	74.27	141.00±7.81[c]	81.56
	E	35.00±6.12[d]	-	26.00±2.92[d]	-
LSD		9.35		7.73	
December	A	163.00±6.63[a]	80.37	167.00±5.39[a]	86.82
	B	142.00±7.18[b]	77.46	149.00±10.30[b]	85.23
	C	140.00±12.25[b]	77.14	110.00±6.52[c]	80.00
	D	105.00±5.00[c]	69.52	117.00±5.39[c]	81.19
	E	32.00±3.56[d]	-	22.00±2.55[d]	-
LSD		9.29		7.93	
January	A	70.00±11.40[c]	48.29	73.40±12.77[b]	63.01
	B	82.00±8.60[b]	55.85	81.00±7.31[ab]	66.67
	C	98.00±8.60[a]	63.06	78.00±6.04[ab]	65.38
	D	80.00±3.54[b]	54.75	82.00±4.06[a]	67.07
	E	36.20±2.58[d]	-	27.40±2.29[c]	-
LSD		9.25		8.09	
February	A	39.00±6.00[c]	29.23	44.00±6.96[c]	56.82
	B	69.00±6.78[a]	60.00	57.00±10.68[b]	66.67
	C	58.00±8.00[b]	52.41	51.00±8.28[bc]	62.75
	D	67.20±5.32[a]	58.93	72.00±3.39[a]	73.61
	E	27.60±3.33[d]	-	19.00±1.18[d]	-
LSD		9.05		8.13	

***Soya bean + chickpeas + yellow corn mixed with mint oil (Diet A), mixed with camphor oil (Diet B), mixed with thyme oil (Diet C), mixed with garlic oil (Diet D) and diet(E) = control (sugar solution [2sugar:1water]).**

**** N.S = Not significant**

***** Means followed with the same letter(s) are not significantly different**

Moreover, the efficacy of peppermint for controlling varroa reached 58.5%, while it was 70.4% when the acaricide Mavric® was used (Nowar *et al.*, 2018). The fume of mint and thyme oil **might confuse mites and block their spiracles. It was noticed that mint oil does not mimic or interfere with normal colony pheromones which control activation inside the hive.**

2. The effect of mixed supplementary diet mixed with certain essential oils on some biological activities of bees during the winter season

A. Season of 2018/ 2019

As shown in Table 2 in November, the results revealed that both colonies fed on diets A and C (soya bean + chick peas + yellow corn mixed with either mint oil [A] or thyme oil [C]) consumed high amounts of food (121.0±3.32 and 125.0±5.00 g, respectively which led to high means of sealed brood area of 122.0±2.55 and 120.0±3.54 inch2. The mean number of egg laying/queen was 111.7±2.42 and 109.83±4.19 eggs/queen/day, respectively. Meanwhile, the mean number of covered frames with bees in the all running treatments was the same (3.6±0.24 frame/hive). Nevertheless, the statistical analysis showed that there were no significant differences between all the running treatments. In December, the mean amounts of food consumption were raised up to 172.0±5.83 and 186.0±5.10 g with the mean conversion rates of 0.72±0.02 and 0.82±0.01g for diets [C] and [D] (soya bean + chick peas + yellow corn mixed with garlic oil). The continuous feeding on diet [A] increased the sealed brood area (308.0±8.46 inch2) and also the mean daily eggs /queen (274.8± 8.91 eggs) as compared with control colonies (221.0±8.43 inch2 and 195.84±7.53 eggs). Concerning all the studied parameters, there were significant differences between the four evaluated diets and control. In January, it was also noticed that the continuous feeding on diet [A] showed the highesd means of sealed brood area of 884.0±6.78 inch2 and daily eggs /queen (783.6±6. 1 eggs) and this was reflected on the mean number of frames covered with bees (5.6±0.24 frames/hive). Although, bees consumed more amounts of diets [C] and [D], the means of sealed brood area and daily eggs /queen were the least as compared with the other two evaluated diets. On the other hand, all the parameters measured for diets [C] and [D] were still more than those of the control (sugar solution).

In February, the colonies fed on diet [A] were found to consume high amount that reached 280.0±9.08 g showing a higher food conversion rate of 0.25±0.01 g, producing a wider sealed brood area of 1142.0±14.63 inch2, laying the highest number of eggs by the queen (1141.2± 17.09 eggs/queen/day) and giving the highest number of frames covered with bees (7.4±0.24).

For the other evaluated diets in February, the average of food consumption, sealed brood area, eggs laid by the queen and the number of frames covered with bees were 233.0-281g, 1120.0-1155 inch2, 917.4-1134.0 eggs and 6.0-6.4 frames, respectively.

By the end of February, the feeding on the evaluated supplemented diets (A, B, C and D) was stopped and at the beginning of March, the colonies were prepared to feed naturally during the citrus nectar season. During the period of March, it was found that the sealed brood area started to be increased (1346.0, 1300.0, 1256.0 and1204.0 inch2 regarding the all running treatments (A, B, C and D). Meanwhile, the mean number of eggs laid by the queen also increased showing the highest number of 1193.6 eggs for those colonies started in March to feed on diet [A] and also giving a high number of frames covered of bees (9.0 frames). It could be concluded that diet [A] (soya bean +

chick peas + yellow corn mixed with mint oil) would be the best to be offered to bee colonies during the dearth period. Diet [A] was found to increase the biological activity (sealed brood area and number of frames covered with bees) and encouraging the queens to lay more eggs.

Table 2. The effect of mixed supplementry materials diet mixed with certain essential oils on some biological activities of bees during the winter season of 2018/2019.

Month	Treatment	Mean area of sealed worker brood (inch^2)	Mean No. of daily egg/queen	Mean No. of frames covered with bees	Mean amount of food consumption (g)	Mean of food Conversion$^{\#}$ (g)
	Diet (A)*	122.00±2.55	111.70±2.42	3.60±0.24	121.00±3.32^{a}	0.99±0.03^{c}
	Diet (B)	105.00±3.54	96.08±3.20	3.40±0.24	113.00±5.39^{a}	1.08±0.03^{ab}
November	Diet (C)	120.00±3.54	109.83±4.19	3.60±0.24	125.00±5.00^{a}	1.04±0.04^{bc}
	Diet (D)	107.00±4.36	100.04±4.78	3.60±0.24	119.00±5.57^{a}	1.12±0.07^{a}
	Control (E)	105.00±4.47	98.20±5.05	3.60±0.24	0.00±0.00^{b}	0.00±0.00^{d}
	LSD	N.S**	N.S	N.S	19.34	0.06
	Diet (A)	308.00±8.46^{a}	274.80±8.91	4.00±0.00^{a}	163.00±6.63^{b}	0.53±0.02^{c}
	Diet (B)	233.00±4.36^{b}	206.58±3.85	3.40±0.24^{b}	162.00±3.74^{b}	0.70±0.02^{b}
December	Diet (C)	239.00±5.57^{b}	212.88±5.54	4.00±0.00^{a}	172.00±5.83^{ab}	0.72±0.02^{b}
	Diet (D)	228.00±8.15^{b}	201.98±7.26	3.80±0.20^{ab}	186.00±5.10^{a}	0.82±0.01^{a}
	Control (E)	221.00±8.43^{b}	195.84±7.53	4.00±0.00^{a}	0.00±0.00^{c}	0.00±0.00^{d}
	LSD	41.72	N.S	0.51	18.43	0.06
	Diet (A)	884.00±6.78^{a}	783.60±6.10^{a}	5.60±0.24^{a}	188.00±5.83^{c}	0.21±0.01^{b}
	Diet (B)	656.00±12.88^{b}	581.86±11.40^{b}	4.60±0.24^{c}	194.00±5.10^{bc}	0.30±0.01^{a}
January	Diet (C)	654.00±13.64^{b}	580.08±7.20^{b}	5.20±0.20^{b}	206.00±6.00^{ab}	0.32±0.01^{a}
	Diet (D)	622.00±8.60^{c}	551.68±7.67^{c}	5.20±0.20^{b}	210.00±7.07^{a}	0.34±0.01^{a}
	Control (E)	600.00±8.37^{c}	532.06±7.43^{d}	5.00±0.00^{b}	0.00±0.00^{d}	0.00±0.00^{c}
	LSD	30.62	18.06	0.32	15.49	0.06
	Diet (A)	1142.00±14.63^{ab}	1141.20±17.09	7.40±0.24^{a}	280.00±9.08^{a}	0.25±0.01^{a}
	Diet (B)	1120.00±8.37^{b}	1099.80±8.19	6.00±0.00^{b}	281.00±12.08^{a}	0.25±0.01^{a}
February	Diet (C)	1148.00±18.28^{ab}	917.40±200.22	6.40±0.24^{b}	247.00±4.90^{b}	0.22±0.00^{a}
	Diet (D)	1155.00±17.32^{a}	1134.26±17.06	6.40±0.24^{b}	233.00±12.00^{b}	0.20±0.01^{a}
	Control (E)	841.00±11.87^{c}	825.80±11.64	6.20±0.20^{b}	0.00±0.00^{c}	0.00±0.00^{b}
	LSD	33.65	N.S	0.57	16.43	0.06
	Diet (A)	1346.00±16.31^{a}	1193.60±14.51^{a}	9.00±0.00^{a}	0.00±0.00	0.00±0.00
	Diet (B)	1256.00±15.36^{c}	1113.86±13.54^{c}	9.00±0.00^{a}	0.00±0.00	0.00±0.00
March	Diet (C)	1300.00±12.25^{b}	1153.06±10.90^{b}	8.80±0.20^{a}	0.00±0.00	0.00±0.00
	Diet (D)	1204.00±17.20^{d}	1063.98±13.47^{d}	8.60±0.24^{a}	0.00±0.00	0.00±0.00
	Control (E)	1204.00±17.20^{d}	1067.98±15.25^{d}	8.00±0.00^{b}	0.00±0.00	0.00±0.00
	LSD	40.66	40.21	0.49	-	-

***Soya bean + chickpeas + yellow corn mixed with mint oil (Diet A), mixed with camphor oil (Diet B), mixed with thyme oil (Diet C), mixed with garlic oil (Diet D) and diet(E) = control (sugar solution [2sugar:1 water]).**

**** N.S = Not significant**

 # Food conversion= amount of consumed food/ sealed worker brood

B. Season of 2019/ 2020

As shown in Table 3, the results of using supplementary diets mixed with essential oils for feeding honey bees during winter months (dearth period) of 2019/2020 season showed that the colonies fed on diet [A] in November consumed 134.0±6.2 g of the offered diet (with a conversion rate of 1.08±0.06 g) and this led to a high sealed brood area (125.0±5.0 inch2), 114.52±4.58 eggs/queen/day and 3.6±0.24 frames/hive.

Concerning the amount of consumed food and food conversion; the statistical analysis showed that there were significant differences between the means of these parameters due to the feeding on the all four evaluated diets and control. The colonies that have been fed on diets [C] and [D] consumed 131.0±6.78 and 120.0±3.54 g which led to sealed brood area of 126.0±6.78 and 116.0±5.1 inch2. Meanwhile, the daily laying eggs /queen reached 115.46±6.22 and 106.28±4.66 eggs, respectively and the numbers of the frames covered with bees were as the same (3.6±0.2.4 frames) in both treatments.

3. The effect of impregnated papers with certain essential oils on Varroa and some biological activities of bee during both seasons of 2020 and 2021

A. The effect on mean number of fallen mite individuals

The effect of impregnated papers with certain essential oils on *Varroa* during two seasons of 2020 and 2021 was investigated. The results shown in Table 4 illustrated that the effectiveness of the evaluated essential oils (mint, camphor, thyme, garlic and mustard oil) ranged between 62.5-77.84% (2020) and between 79.59 -83.95% (2021). Thyme oil impregnated papers was proved to be more effective during February of both seasons recording effectiveness of 77.84 and 83.95%, followed by mint oil (75.16 and 81.37%, respectively). On the other hand, mustard oil impregnated papers was proved to be the least effective treatment. The presented results are in agreement with those reported by Rahimi *et al.* (2017) who found that the use of the thyme extract influenced significantly the percentage of mortality of mites. Also, results of Ghafoor and Faraj (2017) showed that thyme oil (50% concentration) had the best results for controlling *Varroa* among other tested plant oils according to the results in both laboratory and apiary. El-Hady *et al.* (2015) found that the thyme oil was the best in winter for controlling *Varroa*.

Table 3. The effect of mixed supplementry materials diet mixed with certain essential oils on some biological activities of bees during the winter season of 2019/2020.

Month	Treatment	Mean area of sealed worker brood (inch^2)	Mean no. of daily egg/queen	Mean No. of frames covered with bees	Mean amount of food consumption (g)	Mean food Conversion$^{\#}$ (g)
November	Diet (A)*	125.00±5.00	114.52±4.58	3.60±0.24	134.00±6.20^{a}	1.08±0.06^{a}
	Diet (B)	117.00±5.39	104.98±6.01	3.40±0.24	110.00±5.70^{a}	0.95±0.06^{b}
	Diet (C)	126.00±6.78	115.46±6.22	3.60±0.24	131.00±6.78^{a}	1.05±0.07^{a}
	Diet (D)	116.00±5.10	106.28±4.66	3.60±0.24	120.00±3.54^{a}	1.05±0.08^{a}
	Control (E)	112.00±5.83	102.60±5.33	3.60±0.24	0.00±0.00^{b}	0.00±0.00^{c}
	LSD	N.S**	N.S**	N.S**	36.34	0.10
December	Diet (A)	322.00±8.60^{a}***	285.58±7.63^{a}	4.00±0.00^{a}	157.00±9.82^{a}	0.49±0.03^{b}
	Diet (B)	250.00±7.07^{b}	221.74±6.27^{b}	3.40±0.24^{b}	168.00±10.07^{a}	0.68±0.05^{a}
	Diet (C)	238.00±11.58^{b}	212.70±10.79^{b}	4.00±0.00^{a}	175.00±8.66^{a}	0.74±0.03^{a}
	Diet (D)	235.00±10.00^{b}	208.34±8.90^{b}	3.80±0.20^{ab}	173.00±10.20^{a}	0.74±0.04^{a}
	Control (E)	240.00±10.00^{b}	212.82±8.91^{b}	4.00±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{c}
	LSD	47.97	43.88	0.54	26.34	0.09
January	Diet (A)	844.00±16.00^{a}	748.62±14.20^{a}	5.60±0.24^{a}	188.00±5.15^{b}	0.22±0.01^{b}
	Diet (B)	674.00±9.27^{b}	597.78±8.22^{b}	4.60±0.24^{c}	174.00±14.35^{b}	0.26±0.02^{ab}
	Diet (C)	646.00±10.30^{b}	573.02±9.13^{bc}	5.20±0.20^{ab}	178.00±4.64^{b}	0.28±0.01^{ab}
	Diet (D)	640.00±9.49^{b}	567.66±8.36^{bc}	5.20±0.20^{ab}	218.00±6.04^{a}	0.34±0.01^{a}
	Control (E)	620.00±16.43^{b}	549.90±14.55^{c}	5.00±0.00^{bc}	0.00±0.00^{c}	0.00±0.00^{c}
	LSD	43.79	43.38	0.56	28.38	0.09
February	Diet (A)	1160.00±21.21^{a}	1099.94±20.13^{a}	7.40±0.24^{a}	270.00±19.36^{ab}	0.23±0.01^{a}
	Diet (B)	1128.00±10.68^{a}	1069.60±10.14^{a}	6.00±0.00^{b}	292.00±24.27^{a}	0.26±0.02^{a}
	Diet (C)	1138.00±10.68^{a}	1071.50±8.67^{a}	6.40±0.24^{b}	269.00±13.45^{ab}	0.24±0.01^{a}
	Diet (D)	1154.00±16.31^{a}	1094.26±15.47^{a}	6.40±0.24^{b}	239.00±16.16^{b}	0.21±0.02^{a}
	Control (E)	862.00±11.58^{b}	817.38±10.98^{b}	6.20±0.20^{b}	0.00±0.00^{c}	0.00±0.00^{b}
	LSD	39.99	42.86	0.49	29.39	0.09
March	Diet (A)	1348.00±13.93^{a}	1195.64±12.35^{a}	9.00±0.00^{a}	0.00±0.00	0.00±0.00
	Diet (B)	1248.00±14.63^{b}	1107.04±12.98^{bc}	9.00±0.00^{a}	0.00±0.00	0.00±0.00
	Diet (C)	1272.00±18.81^{b}	1128.30±16.65^{b}	8.80±0.20^{a}	0.00±0.00	0.00±0.00
	Diet (D)	1206.00±29.77^{c}	1069.80±26.41^{cd}	8.60±0.24^{a}	0.00±0.00	0.00±0.00
	Control (E)	1166.00±20.64^{d}	1028.90±18.80^{d}	8.00±0.00^{b}	0.00±0.00	0.00±0.00
	LSD	39.88	41.48	0.57	-	-

***Soya bean + chickpeas + yellow corn mixed with mint oil (Diet A), mixed with camphor oil (Diet B), mixed with thyme oil (Diet C), mixed with garlic oil (Diet D) and diet(E) = control (sugar solution [2sugar:1water]).**
**** N.S = Not significant**
***** Means followed with the same letter(s) are not significantly different**
 # Food conversion= amount of consumed food/ sealed worker brood

B. The effect on some biological activities of bee

The effect of impregnated papers with certain essential oils on some biological activity of honeybees was studied. Data illustrated in Fig. 1 showed that thyme and mint oil treatments increased the mean area of sealed brood during February 2020 and 2021 (1245.0 and 1230 & 1230.0 and 1212.0 inch^2, respectively). The increased sealed brood was reflected on the number of combs covered with bees (7.5 frames/colony for both oils in both seasons). The presented results are in agreement with those reported by Abd El-Wahab *et al.* (2016) who prepared a diet that contains certain main components (orange juice, mint oil (EO), turmeric and fenugreek powders and vitamins) (diet E). They found that colonies fed with diet E (containing mint) resulted in the highest number of combs covered with bees. Colonies fed on diet E consumed a higher amount /2 weeks interval, with no residues of patty, than the other tested diets during the experimental period.

Table 4. The effect of different essential oils tested as impregnated papers technique on mean number of fallen Varroa during February of both years of study

Treatment	Year of study			
	2020		2021	
	Mean number of fallen Varroa	Effectiveness (%)	Mean number of fallen Varroa	Effectiveness (%)
A*	39.25±9.60	75.16	40.25±9.35^{a}**	81.37
B	27.50±6.61	64.54	40.25±6.30^{a}	81.37
C	44.00±10.09	77.84	46.75±13.33^{a}	83.95
D	26.00±6.47	62.50	39.50±7.57^{a}	81.01
E	30.50±7.03	68.03	36.75±7.08^{a}	79.59
F	9.75±0.85	-	7.50±0.64^{b}	-
LSD	-		24.65	

***Papers impregnated with mint oil (A), with camphor oil (B), with thyme oil (C), with garlic oil (D), with mustard oil (E) and control (F).**
**** Means followed with the same letter(s) are not significantly different**

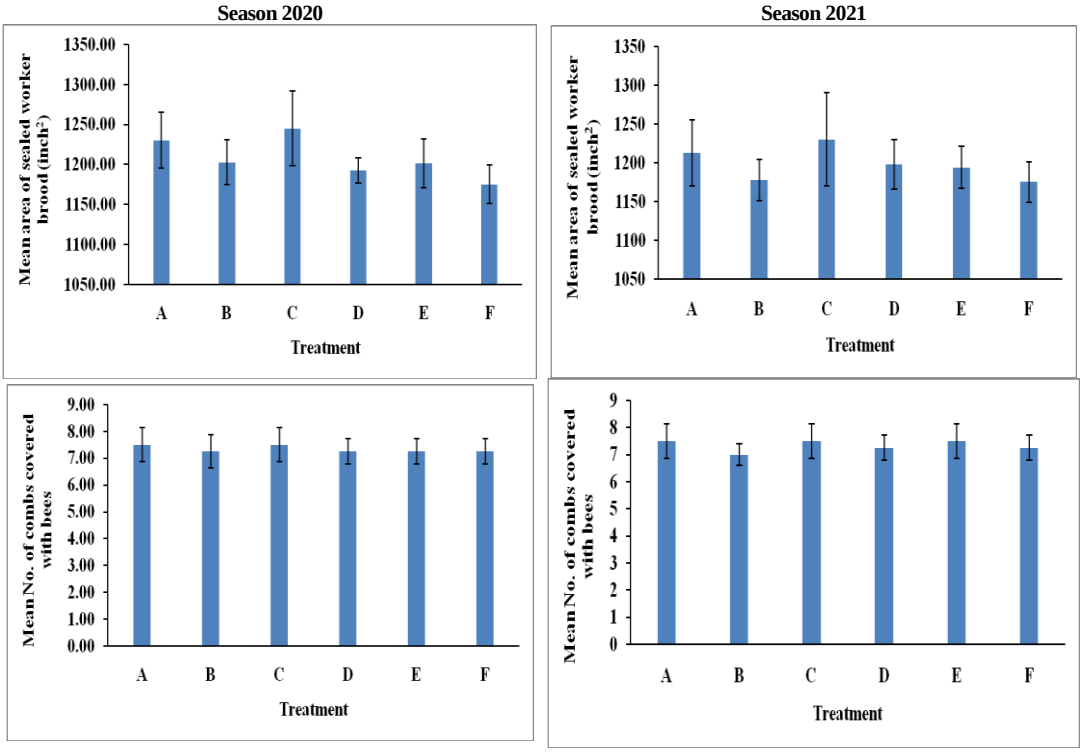

Fig.1. The effect of different essential oils tested as impregnated papers technique on some biological activities of bees (seasons 2020 and 2021).

*Honey produced from bees fed on soya bean + chickpeas + yellow corn mixed with mint oil (Diet A), mixed with camphor oil (Diet B), mixed with thyme oil (Diet C), mixed with garlic oil (Diet D) and diet (E) = mustard oil (honey produced from bees fed on sugar solution [2sugar:1 water]).

Residues of the evaluated essential oils in honey were absent (Fig.2) and that because these compounds quickly decrease due to their volatility, thus there cannot be any food safety risk for the consumer.

Bonvehí *et al.* (2016) reported that thyme oil was detected in 89% of the detected samples (mean, 0.78 µg/g) and menthol in 15% (mean, 0.27 µg/g), while camphor and eucalyptol were not found (< LOQ). There were no negative effects on sensorial quality of the honey. Thyme oil, as well as camphor and menthol and camphor, have a FAO GRAS status (Generally Recognized As Safe) in concentrations up to 50 mg/kg (50ppm). Thus, residues of these substances in honey are of no toxicological concern.

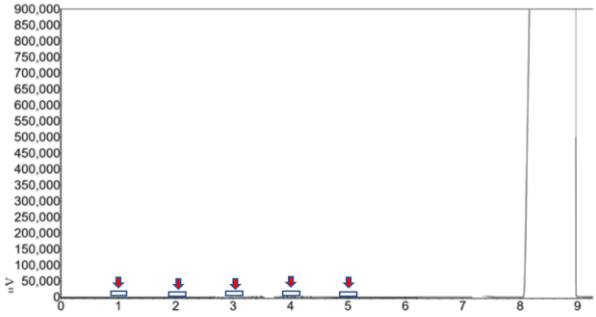

Fig. 2. Chromatogram of the detected essential oils that may be presented within the produced honey by these bees that fed on the different diets.

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مكافحة طفيل الفاروا الذي يصيب نحل العسل Apis mellifera باستخدام الزيوت الأساسية كمكملات غذائية وكورق مشبع

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أُجريت الدراسة الحالية لتقييم بعض الزيوت العطرية (النعناع ، الكافور ، الزعتر والثوم) التي تم خلط كل منها علي حدة مع المكملات الغذائية (75 جم من كل من دقيق فول الصويا خالي الدسم والحمص الشامي والذرة الصفراء تم خلطها مع 300 جم سكر ، 75 جم خميرة جافة ، 15 جرام قرفة ، 10 جم كركم ، 10 مل عصير ليمون ، 10 مل خل التفاح ، 135 جم عسل نحل) وذلك لتعزيز ورفع إنتاجية النحل خلال فترة عدم تواجد الرحيق وحبوب اللقاح (فترة القحط) بالمقارنة مع التغذية علي المحلول السكري (الكنترول) (بيئة E) (2سكر:1 ماء) (وزن/حجم) ، وفي نفس الوقت تم تقييم بعض الزيوت العطرية (النعناع ، الكافور ، الزعتر والثوم والخردل) لتقييم نشاطها لمكافحة أكاروس الفاروا وذلك من خلال تقنية تعليق الورقة المشبعة بهذه الزيوت داخل الخلية ، وعلاوة علي ذلك تم تقدير مدي تواجد هذه الزيوت في العسل المنتج. وقد أظهرت النتائج أن كل البيئات المجهزة والتي تم تقديمها للنحل و تقييمها كانت مقبولة بواسطة النحل وقد وجد أن النحل المتغذي علي تلك المكملات الغذائية التي تحتوي علي الزيوت العطرية أعطت المزيد من أعداد النحل وزادت قيم مساحة الحضنة وأعطت زيادة في أعداد البراويز المغطاة بالنحل وشجعت الملكات لوضع عدد اكبر من البيض بالمقارنة مع الكنترول. وقد أظهرت البيئة (A) التي تم فيها خلط العجينة الأساسية مع زيت النعناع أنها من أحسن المكملات الغذائية والتي تم تقديمها للنحل في فترة الشتاء تلاها البيئات (C) و (B) و (D). وقد أدت التغذية علي البيئة (A) إلي تحقيق أعلي متوسطات لكل من مساحة الحضنة (1348,0 ±13,0 بوصة مربعة) وعدد البيض الذي تضعه الملكة/يوم (1195,64 ±12,35 بيضة) وعدد البراويز المغطاة بالنحل (9,0 برواز/خلية) وذلك خلال شتاء موسم 2020/2019، وقد إزدادت أعداد الأكاروس (الفاروا) المتساقط داخل الخلية في نوفمبر عندما تمت التغذية علي البيئات (A) و (B) حيث سُجلت أعلي متوسط لعدد الأكاروس المتساقط (176,0 و 201,0 أكاروس) محققة بذلك نسبة كفاءة ضد الأكاروس وصلت إلي 80,11 و 80,33% في خلال موسم 2019 وكذلك أعلي متوسط (201,0 و 161,0 أكاروس) بنسب كفاءة 87,06 و 83,85 % في خلال موسم 2020 علي التوالي. وقد أظهرت النتائج أن الأوراق المشبعة بزيت الزعتر أنها أكثر كفاءة ضد الأكاروس خلال شهر فبراير من كلا موسمي الدراسة مسجلة نسب كفاءة وصلت إلي 77,84 و 83,95 % تلاه في ذلك زيت النعناع (75,16 و 81,37 % علي التوالي) بينما أثبت زيت الخردل أنه الأقل كفاءة ضمن الزيوت المختبرة. وبتقدير متبقيات الزيوت العطرية في العسل المنتج أظهرت النتائج غياب هذه الزيوت وقد يرجع هذا إلي أن هذه المركبات تتناقص سريعاً وذلك لتبخرها وعلي هذا لايمكن تواجد أي عامل خطورة علي أمان العسل المقدم كغذاء جراء إستعمال مثل هذه الزيوت الفعالة كمكمل غذائي ولمكافحة الفاروا.