



Strengthen of the Defense Behavior in Honey Bee Colonies (*Apis mellifera* L.) against *Varroa* mite (*Varroa destructor* Anderson & Trueman) Using Volatile Oils Under Arid Regions Conditions

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ABSTRACT

The efficacy of six essential oils compared with formic acid and Apistan stripes, against *Varroa* mite were evaluated. The infestation levels of these substances were evaluated in honeybee colonies during spring season, 2019 under arid regions environmental conditions. It is obvious that garlic oil was superior of all treatments in falling number of varroa mites after treatments with significant difference giving 46.66 ± 3.92 during the experimental period. Formic acid, clove oil, and apistan strips were the most effective of fallen *varroa* mites after garlic oil with insignificant differences among them represented 26.33 ± 0.33 , 21.33 ± 2.40 , and 20.66 ± 1.20 fallen mites/colony, respectively. On contrary, neem oil was the least in mean number of fallen *varroa* mites equalized with control colonies giving (0.33fallen mites/colony). For the reduction percentage on both brood and workers, the outstanding treatments were garlic oil, formic acid, and apistan strips represented 87.33, 87.33, and 82.56 %, respectively. On the other hand, the inferior reduction was observed for neem oil with mean reduction percentage 4.75 % for both brood and worker bees. This study indicates that using garlic oil, formic acid, and apistan strips mixture can be added to IPM programs for controlling *Varroa* mite.

INTRODUCTION

Bees play a critical role in agriculture and food development in the pollination of crops, which is vital for large global agricultural economies and the human food chain (Barry et al., 2018). Large scale losses of honey bee colonies in last years have perplexed beekeepers and bee researchers. These losses may be a result of natural causes or varroa infestation and viruses (Berthoud et al., 2010; Genersch et al., 2010; Dainat et al., 2012 and Francis et al., 2013). The ectoparasite mite *Varroa destructor* Andersen & Trueman live on the bodies of honey bees and feed on their haemolymph and fat body tissue (Ramsey et al., 2019). The most severe effects of varroa infestation on honey bees were including reduced lifespan (Kralj et al., 2007), decreased survivorship (Yang and Cox-Foster, 2007), and weight loss in

drones (Duay et al., 2002). Varroa mites are also efficient vectors for viral diseases transmission (Ball, 1983; Bowen-Walker et al., 1999; Chen, 2005 and Di Prisco, 2011). Grooming and hygienic behavior are defensive mechanisms that honey bees can limit varroa population growth (Peng et al., 1987; Rath, 1999 and Boecking and Spivak, 1999). The ability to groom depends on environmental factors as high temperature and low humidity (Currie and Tahmasbi, 2008 and Stanimirovic et al., 2010). Besides environmental factors, the bees' genetic make-up affects the ability to groom (Baçk and Wilde, 2015). Using chemicals to control Varroa mites has caused severe problems such as bee toxicity or contamination and increased probability of disease-resistance (Milani, 1995; Delaplane, 1997 and Watkins, 1997). For these reasons, recent researchers approached to control this mite by means which relatively safe to human and bees. Products with botanical origin have shown a wide range of biological activities including toxicity, repellence, antifeedant, and growth regulatory properties (Ciccina et al., 2000; Banchio et al., 2003, 2005; Ferrero et al., 2006; Jbilou et al., 2006 and Aivazi and Vijayan, 2009). Components of the essential oils were tested in previous studies and recommended that the product is a well-known effective compound against the varroa infection which can be used in place of chemical treatments such as coumaphos and amitraz (Gregorc and Planinc, 2004).

The aim of this work was to evaluate the miticidal effect of cheap, available, and mostly safe plant oils on *Varroa destructor* and the impact of these treatments on bees and colony defense.

MATERIALS AND METHODS

Study Area and Honey Bee Colonies:

Field experiments were conducted at Al-Ahsa province, Eastern Region, Kingdom of Saudi Arabia as arid regions during spring season 2019. Al-Ahsa lies at latitude 25° 25' 46" N, longitude 49° 37' 19" E, altitude of 121 m above sea level and about 400 km. east Riyadh city, Saudi Arabia. Twenty seven honey bee colonies (*Apis mellifera yemenitica*) headed by newly queen and nearly equal strength in brood, bees and food were used. Each colony contains seven combs covered with bees. The colonies have been divided in nine groups (each of three colonies) and mean values were taken. Temperature degree ranged between 25 to 45 and relative humidity from 10 to 15 % during the study period. Control colonies have no treatments (three replicates). Managements for all the honey bee colonies were the same.

Essential Oils and Chemicals:

Sex botanical volatile oils were selected to control varroa mites. The oils were: basil; *Ocimum basilicum*, neem; *Azadirachta indica*, thymus; *Thymus vulgaris*, clove; *Syzygium aromaticum*, garlic; *Allium sativum* and sidr; *Ziziphus spin-christi*. Also, formic acid 60% and Chinese Apistan stripes (10 % fluvalinate) were used. Cotton balls were prepared and saturated by 1 mL of oil (1 mL oil/ one ball/each colony/3 replicates) and hanged between the brood combs in each colony. A Cardboard (7x15x0.3 cm) was saturated with 10 mL of 60% formic acid and put onto the brood combs. One stripe of Apistan was hanged between two brood combs in treated colonies.

Evaluating the efficiency of the Tested Materials and Techniques:

The Varroa infestation (%) on bees and on brood was recorded before and after treatments according to the formula given by Henderson and Tilton (1955) as the following equation:

$$\text{The reduction rate of infestation} = 100 \times 1 - \{(Ta \times Cb) / (Tb \times Ca)\}$$

Where: Tb is % infestation of mite before treatment, Ta is % infestation of mite after treatment, Cb is % infestation of mite before treatment for the control and Ca is % infestation of mite after treatment for the control.

Mites Fall and Dead Bees:

The counts of dropped mites were recorded after 1, 2, 3, 7 & 14 days of the first application using paper sheets (51.5 x 36.5 cm) coated with Vaseline placed on the hive bottom board. Dead bees were daily counted to determine the effect of treatments on worker bees.

Determination Varroa Mites on Adult Bees and Brood:

About 100 bees were collected, if possible, from combs with open brood, and dipped in water to which detergent (washing-up liquid) has been added. The bees were collected in a wire net and removed after shaking for 3-5 minutes. Mites would have fallen off them and found at the bottom of the container (Ritter, 1981). The bees and *Varroa* mites were counted. Samples of 25 sealed worker brood cells in the middle of worker comb were determined. Their cells were scratched, the larvae were removed and the counts of *Varroa* mites in each cell were recorded.

Statistical Analysis:

The obtained data were tabulated to analysis of variance program (ANOVA) (Gomez and Gomez, 1984) followed by Multiple Range Test to compare means (Duncan, 1955).

RESULTS AND DISCUSSION**Fallen Varroa Mite:**

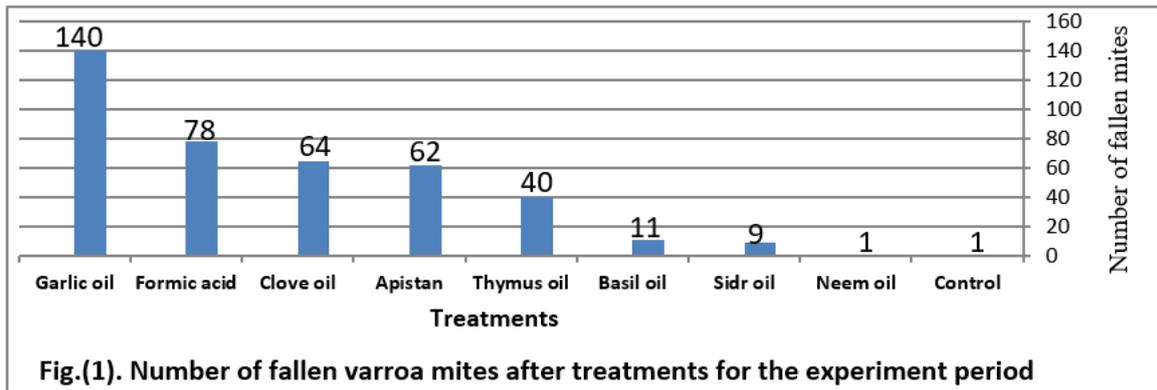
Data illustrated in Table (1) and Fig (1) showed the effect of volatile oils and chemicals on the number of fallen varroa mites during the study periods. It is obvious that garlic oil was superior of all treatments in falling number of varroa mites after treatments with significant difference giving 46.66 ± 3.92 during all the treatment period. Formic acid, clove oil, and apistan were the most effective of fallen varroa after garlic oil with insignificant differences among them represented 26.33 ± 0.33 , 21.33 ± 2.40 , and 20.66 ± 1.20 fallen mites/colony, respectively. On contrary, neem oil was the least in the mean number of fallen varroa mites equalized with control colonies giving (0.33 fallen mites/colony). This result is in agreement with Gonzalez-Gomez et al. (2006) they stated that there was no acute toxic effect of any of the neem extracts for varroa mites or bees.

Table 1. Mean number of fallen varroa mites after daily sequence from certain treatments

Days Treatment	Number of varroa mites (fall/colony)					Total
	1 day	2 day	3 day	7 day	14 day	
Basil oil	1.33d \pm 0.33	0.66cd \pm 0.33	0.33d \pm 0.33	0.66cd \pm 0.66	0.66bcd \pm 0.33	3.6d \pm 1.45
Neem oil	0.00d	0.33cd \pm 0.33	0.0d	0.0d	0.0d	0.33d \pm 0.33
Sidr oil	1.0d \pm 0.57	0.66cd \pm 0.33	0.66d \pm 0.33	0.0d	0.66bcd \pm 0.33	3d \pm 0.0
Thymus oil	3.66d \pm 0.88	2.66bc \pm 0.88	3.0c \pm 1.15	2.66b \pm 0.66	1.33bc \pm 0.33	13.33c \pm 2.18
Clove oil	8.66c \pm 1.2	3.66b \pm 0.88	7b \pm 0.57	0.33d \pm 0.33	1.66b \pm 0.33	21.33b \pm 2.40
Garlic oil	14.66b \pm 1.76	12.33a \pm 1.45	12.0a \pm 1.52	6.0a \pm 0.57	1.66b \pm 0.33	46.66a \pm 3.92
Apistan	11.0bc \pm 1.52	2.66bc \pm 0.88	0.66d \pm 0.33	1.66bc \pm 0.33	4.66a \pm 0.33	20.66b \pm 1.20
Formic acid	19.66a \pm 2.90	3.33b \pm 0.88	1.33cd \pm 0.33	0.33d \pm 0.33	1.66b \pm 0.66	26.33b \pm 0.33
Control	0.00d	0.0d	0.0d	0.0d	0.33cd \pm 0.33	0.33d \pm 0.33
LSD at 5%	4.029	2.334	2.087	1.232	1.094	6.924
F	28.14	23.25	34.25	22.73	13.86	43.925
P	.0000	.0000	.0000	.0000	.0000	.0000

Mean \pm Standard Error of three values

Values followed by the same letter (s) in a column are not significantly different according to Duncan test at level 0.05



It clears that, the effect of formic acid on fallen mites starting strongly and significantly during the first day of treatment giving (19.66 ± 2.90) then sharply declined till the last day of treatments (14 days) represented 1.66 ± 0.66 . This is due to the environmental conditions existing in the apiary region as temperature degree exceeds $45\text{ }^{\circ}\text{C}$ and relative humidity was 10% RH. For the previous reasons, the evaporation rate of formic acid was high on the first day of application. Our results are in agreement with Ritter and De Jong (1984) they found that in tropical climates the parasite seems to be less virulent. In addition, Moretto and de Mello (1999) supported these findings mentioned that in sub-tropical climates the infestation rate is lower than in temperature climates. It is clear that garlic oil has a stable and continuous effect on mites fallen during the first three days of treatment represented 14.66 ± 1.76 , 12.33 ± 1.45 , and 12.0 ± 1.52 fallen mites/colony, respectively. The same trend was observed for both clove and thymus oils but with minor effect. El-Zemity *et al.* (2006) and Damiani *et al.* (2009) stated that an increase in the exposure time led to an increase in the toxicity of the essential oils against varroa mites and all essential oils they tested caused mite mortality without severe harmful effects on adult bees.

Bee Mortality:

Table 2 illustrated the mean number of dead worker bees after treating the honey bee colonies with different treatments. The daily dead bees were counted during the study period. It is clear that there were insignificant differences between honey bee colonies with different treatments in the daily dead worker bees treated with certain oils and chemicals. In addition, all the treatments were safe for worker bees at the applied dose. Data reveals that colonies treated with sidr oil gave the highest number of dead bees (4.33 ± 1.85) followed by colonies treated with basil, thymus oil and formic acid represented 2.66 ± 2.18 , 2.66 ± 1.66 , and 2.0 ± 0.57 , respectively. Additionally, the highest number of dead bees was observed mainly during the first day of application for all treatments. It is clear that the number of dead bees in all treatments is not in big concern. So, it could be concluded that all the treatments can be used safely for bees.

Reduction Percentages of Varroa Mites:

Data in table (3) showed the mean reduction percentages of varroa mites on brood and adult honey bees treated with certain chemicals and nonchemical substances.

It could be mentioned that most reduction in varroa on worker brood was achieved by basil, clove, and garlic oils with percentages 91, 89.33, and 87 %, respectively. On contrary, the least reduction percentage was recorded with neem oil represented 9.5 % on brood. Generally, all the treatments have reduction percentages of varroa on worker brood except control colonies. The highest reduction on adult workers was recorded by using formic acid, apistan, and garlic oil with percentages 100, 93.13, and 87.66 %, consistently. The lowest reduction percentage of varroa mites for worker bees was showed using basil oil (24.16 %). It is clear that any of neem, sidr, and clove oil has a reduction percentage of varroa on worker

bees. For the reduction percentage on both brood and workers, the outstanding treatments were garlic oil, formic acid, and apistan strips represented 87.33, 87.33 and 82.56 %, respectively. On the other hand, the inferior reduction was observed for neem oil with reduction percentage (4.75 %) for both brood and worker bees.

Table 2. Mean number of dead bees after daily sequence from certain treatments.

Days Treatment	Number of dead bees (bee/colony)					Total
	1 day	2 day	3 day	7 day	14 day	
Basil oil	0.66cd±0.33	0.0b	0.0a	0.33a±0.33	1.66a±0.33	2.66ab±2.18
Neem oil	0.0d	0.0b	0.0a	0.0a	0.0a	0.0b
Sidr oil	3a±0.57	0.33a±0.33	0.0a	1.0a±1	0.0a	4.33a±1.85
Thymus oil	1c±1	0.0b	0.66a±0.66	0.0a	1.0a	2.66ab±1.66
Clove oil	0.0d	0.0b	0.0a	0.0a	0.0a	0.0b
Garlic oil	0.0d	0.0b	0.0a	0.0a	0.0a	0.0b
Apistan	0.0d	0.0b	1.0a±1.0	0.0a	0.0a	1.0ab±1.0
Formic acid	2b±0.57	0.0b	0.0a	0.0a	0.0a	2.0ab±0.57
Control	0.0d	0.0b	0.0a	0.0a	0.0a	0.0b
LSD at 5%	0.873	0.330	1.19	1.04	1.924	3.478
F	13.75	1.46	0.88	0.925	0.88	1.84
P	.0000	0.469 ns	0.547ns	0.519ns	0.544ns	0.134ns

Table 3. Mean reduction percentages of varroa mites on brood and adult bees with different treatments.

Treatment	% infestation in brood cells (25 cells)		Reduction %	% infestation on adult bees (100 workers)		Reduction %	Mean %
	before	after		before	after		
Basil oil	5.0	0.66	91.0	2.66	1.00	24.16	57.58
Neem oil	4.0	3.33	9.50	2.00	2.00	-	4.75
Sidr oil	3.66	0.66	66.66	2.33	1.33	-	33.33
Thymus oil	5.0	0.66	77.7	3.33	0.66	48.26	62.98
Clove oil	5.66	0.66	89.33	2.66	0.33	-	44.66
Garlic oil	7.33	1.0	87.0	3.66	0.33	87.66	87.33
Apistan	9.33	1.66	72.0	4.00	0.33	93.13	82.56
Formic acid	8.66	1.33	69.0	4.00	0.0	100	87.33
Control	7.66	7.66	-	5.33	4.66	-	-

Ritter (1984) mentioned that if the mite population is between ten and 100, then the examination of hive debris should allow for detection. Moreover, adult bees or brood examination are insufficient for population levels below 100 mites per colony (Fries et al., 1991 and Al-Ghamdi and Hoopingarner, 2004). As the mite fall (removal) was density-dependent. Namely, a negative correlation was found between mite fall percentages and mite infestation level. So, grooming is only beneficial at high levels of mite infestation (Kruitwagen et al., 2017). The results of our colony experiment were based on a relatively small mite infestation range. Contrasting, others contrasting results about the importance of grooming have been reported, some indicate grooming as an important resistance trait (Guzman-Novoa et al., 2012 and Rinderer et al., 2013) but others do not (Vandame et al., 2002 and Locke and Fries, 2011). Removal of infested brood and the inhibition of mite reproduction are more likely to explain other resistance strategies of bees (Kruitwagen et al., 2017). Kotwal and Abrol (2013) found varying results according to the season. In general, the performed studies on the use of these oils against varroa mite within colonies showed high degree of efficacy. On conclusion, varroa mite should be continuously (monthly) managed within honey bee colonies using mechanical methods or treatment with essential

oils. In severe cases, and especially during the fall and not during honey seasons, the use of chemical materials can be done with preference to formic or oxalic acid (Abou-Shaara, 2014).

Conclusion and Recommendation

The use of organic formic acid and/or essential oils (e.g. garlic, clove, and thymus) combined or through an IPM program to control *Varroa* mites is economically recommended. These materials are inexpensive, efficient, and relatively less toxic compared to very expensive and toxic acaricides randomly used in over-doses by beekeepers. It is agreed that there is no danger of contamination if the treatment is carried out properly, outside the period of production or storage of honey in the hive and when the temperature is under the 28 °C. It could be recommended to beekeepers to use garlic, clove, and thymus essential oils with 1 to 2 mL to control varroa mite infestation. They are not expensive, easy to apply, and most pose few health risks.

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ARABIC SUMMARY

تعزيز سلوك الدفاع في طوائف نحل العسل (*Apis mellifera* L.) ضد طفيل الفاروا (*Varroa destructor* Anderson & Trueman) باستخدام الزيوت الطيارة

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تم تقييم فعالية ستة زيوت عطرية مقارنة بحمض الفورميك وشرائط الأبيستان ضد طفيل الفاروا . تم تقييم مستويات الإصابة بهذه المواد في طوائف نحل العسل خلال فصل الربيع، ٢٠١٩ في ظل الظروف البيئية للمناطق القاحلة . أوضحت الدراسة أن زيت الثوم كان متفوقاً على جميع المعاملات في خفض أعداد طفيل الفاروا بعد المعاملة مع وجود فروق معنوية معطياً (٤٦,٦٦ ± ٣,٩٢) طوال فترة التجربة . كان حمض الفورميك، زيت القرنفل وشرائط الأبيستان الأكثر فعالية في تعداد الفاروا المتساقطة بعد زيت الثوم ولم تكن هناك فروق معنوية فيما بينها (٢٦,٣٣ ± ٠,٣٣، ٤٠,٤٠ ± ٢,٣٣ و ٢١,٢٠ ± ١,٢٠ طفيل/طائفة، على التوالي. على النقيض، كان زيت النيم هو الأقل في متوسط عدد طفيل الفاروا المتساقطة متساوياً مع الطوائف غير المعاملة معطياً (٠,٣٣) من الفاروا المتساقطة/ طائفة). بالنسبة لنسبة الخفض على كل من الحضنة والشغالات، كانت المعاملات المتفوقة هي زيت الثوم، حمض الفورميك وشرائط الأبيستان بنسبة ٨٧,٣٣ و ٨٧,٣٣ و ٨٢,٥٦٪ على التوالي . من ناحية أخرى، لوحظ الانخفاض الأدنى لزيت النيم مع متوسط إنخفاض بنسبة ٤,٧٥٪ لكل من الحضنة والنحل البالغ . تشير هذه الدراسة إلى أن استخدام زيت الثوم، حمض الفورميك وشرائط الأبيستان يمكن إضافتهم إلى برامج مكافحة المتكاملة للأفات للتحكم في طفيل الفاروا.