

## EVALUATION OF SOME ESSENTIAL OILS FOR CONTROLLING VARROA MITES AND THEIR EFFECTS ON BROOD REARING ACTIVITY IN HONEY BEE COLONIES.

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### ABSTRACT

This study was carried out to assess the efficacy of controlling essential oils i.e. Thymol, Cinnamon and Anise oils with different concentrations compared to 65% Formic acid in controlling *Varroa destructor* mites infesting colonies of *Apis mellifera* during the winter and summer of 2013 season. Randomized complete block design was applied at this study. The apiary was divided into 7 groups (3 colonies / group). Three application of the oils. Average of *Varroa* infestation level before treatment ranged between 21–29 in winter and from 37-47 in summer. The treatments were significantly effective when applied in summer than the winter season and when used at 30% concentration than those obtained at 15% concentration compared to the control treatment. Average efficacies percentage ranged between 80.95% (Cinnamon oil 30%) to 86.99 (Thymol 30%) in winter and from 67.46 % (Thymol 30%) to 82.16% (Cinnamon 30%). Thymol oil was the best in winter, however, Cinnamon was the best in summer. Anise oil 30% was intermediate 80.00 and 77.11% in winter and summer treatments respectively. In concern with the effect of the tested oils on brood rearing activity, Anise and Cinnamon oils at 30% concentration were the best (74.54 and 74.35 % . respectively) during January – February. However, in significant differences were found between the tested oils at 30% concentration during August–September. Thymol was the best at 15 % concentration ( 55.38%). However, Cinnamon oil 30% was the best (64.54%) followed by Thymol (56.52%) .

**Keywords :** *Varroa destructor* , *Apis mellifera* , essential oils.

### INTRODUCTION

*Varroa destructor* mite has become a serious pest of *Apis mellifera* all over the world ( Baker and Peng, 1995; Rashid *et al.*, 2014). It cause serious losses in apiculture of *A. mellifera*. It feeds on haemolymph of larvae, pupa and adult bees during all life ( Anderson and Trueman, 2000). It also, decrease brood, colony ability to pollinate plants ( De Jong *et al.*, 1984). *V. destructor* associated with the virus diseases, which six bee viruses were detected in bee infested by *varroa* ( Shimanukiet *al.*, 1994; and Correa-Marqueset *al.*, 2003). Using of synthetics varroacides may due to increase of *varroa* resistance to these products ( Thomson *et al.*, 2002). Recently, beekeepers have used some essential oils as well as volatile compounds to control *varroa* mites ( El-Shemy, 1997 ; Allamet *et al.*, 2004; Sammataro *et al.*, 2004 and Ruffinengeet *al.*, 2007). Studies have shown that higher concentrations of formic acid can kill mites under capping (Calderon *et al.* 2000, Amrine *et al.* 2006). The present study aim to evaluate three essential oils i. e. Thymol, Cinnamon and Anise with two concentrations (15 and 30%) in

controlling applications compared to formic acid to controlling *varroa* mites in both capped brood and on adult bees in addition, the effects of these treatments on brood rearing.

## MATERIAS AND METHODS

Present study was confirmed in an apiary selected at Ayash El-Mehalla ElKobra District, Gharbia Governorate in 2013 season. All colonies had brood nests composed of one standard deep and one shallow hive body. Each brood box contained 5 frames in winter season and 8 frames in summer season, had approximately equal numbers of bees, and had a laying queen.

### Evaluated materials

Essential oils in two concentrations (15 and 30%) were used in this study to management *varroa* mites comparing to Formic acid 65% given in Table (1). Colonies were divided into 7 groups, three colonies for each concentration as well as 3 colonies as a control (without treatment). Three applications of each treatment were applied from 16 January to – February and from 15 August to – September, 2013. A completely randomized design was applied in this treatment.

**Table.1. Essential oils and their concentrations.**

Serial No.	Name of acaricides	Concentrations %
1	Thymol	15% & 30%
2	Anise oil	15% & 30%
3	Cinnamon oil	15% & 30%
4	Formic acid	65%

### Application methods:

- 1- The mixture of Essential oil Thymol or Anise and Cinnamon was prepared in two concentrations, 15 and 30% by mixing with olive oil (15ml: 85ml and 30ml:70 ml, respectively). Talcum powder was added to each mixture at rate 1 oil: 2 powder) to make solid mix (3x3x1 cm) weighted 30 gm. Each mixture was placed on the top of combs, changed every 10 days.
- 2- 120 ml of 65% Formic acid was kept in a corked plastic bottle with cotton strip to regulate 4 mL evaporation / daily of Formic acid. The bottle was adjacent to an empty space above the brood.

### Assessment of bee infestation

For bees, pre-treatment count of mites was taken before application. About 100 adult bees/ colony were used to calculate *varroa* infestation using alcohol wash technique (De Jong *et al.*, 1982), Post-treatment Assessment count of mites was taken after the end of each treatment application. For brood, the mite infestation was evaluated by opening cells of sealing brood before treatment and mite infestation was assessed in inch<sup>2</sup>, three replicates / colony (Burgett and Burikam, 1985).

### Efficacy of oils.

Effectiveness % of the tested treatments were determined according to the formulae adopted by Liorente J-Martines(1989) as follow:

**Effectiveness in controlling Varroa mites %=**

$$\frac{\text{Final of fallen mites in treatment} - \text{Final of fallen mites in control}}{\text{Final of fallen mites in treatment}} \times 100$$

Whoever, on brood rearing activity, an area of 5x5 cm in the middle of a worker comb (sealed brood) was selected after treatment to determine the effect of oils on brood rearing activity.

**Effectiveness in brood rearing activity %=**

$$\frac{\text{Final of increasing in treatment} - \text{Final of increasing in control}}{\text{Final of increasing in treatment}} \times 100$$

**Statistical analysis.**

Randomized complete block design were applied to this experiment Mean mite mortality (fallen mites) and brood rearing activity were compared by analysis of variance technique to test the significance of data using the least significance test (LSD) at 5% probability level (Montgomery, 2001).Duncan analysis was used to compare between mean of fallen mites.

## **RESULTS AND DISCUSSION**

Efficacy of some essential oils at different concentrations as well as Formic acid 65% was evaluated for controlling of *varroa* mites during the winter and summer seasons of 2013. Each treatment was applied three times from the first of January to the half of February and from the half of August to the half of September. Pre-treatment count of fallen mites was taken before treatment. The pre-count of the beginning of fallen mites was higher in summer than the winter season. It ranged from 21 to 29 infestation in winter and from 39 to 47 in summer.

Data in Table (2) revealed that from January to February, the fallen mites increased and the first application was the highest one. Thymol 30% showed the highest fallen mites (225 mites) at 1<sup>st</sup> application, followed by 94 mites at the 2<sup>nd</sup> application then 50 mites at the 3<sup>rd</sup> application.. In case of Cinnamon 30% recorded the second rank, it showed 103, 102 and 47 fallen mites, respectively. Anise oil 30% came in the 3<sup>rd</sup> rank, the fallen mites were 155 mites at the 1<sup>st</sup> application, 48 mites at the 2<sup>nd</sup> application then 38 mites at the 3<sup>rd</sup> application. Consequently, it could be ranking the essential oils according to the total fallen mites in descending order as follow, Thymol 30% (369 mites), Cinnamon oil 30% (252 mites) then Anise oil 30% ( 241 mites). In concentration 15 5 of the essential oils, Anise oil came in the first rank (205 mites) followed by thymol (175 mites) then cinnamon oil (142 mites). Formic acid 65% showed the highest total fallen mites (573 mites) compared to the tested essential oils.

**Table.2. Effectiveness of essential oils for controlling of *Varroa* mites during January – February, 2013.**

Treatment	Pre-treatment count	1 <sup>st</sup> Application	2 <sup>nd</sup> Application	3 <sup>rd</sup> Application	Total fallen mites	Efficacy %
	No. of mites	2/1 – 14/1	15/1-26/1	28/1 – 10 / 2		
Thymol 15%	23	82e	59d	34d	175	72.57
Anise oil 15%	29	89de	78c	38d	205	76.58
Cinnamon oil15 %	24	61	37e	44c	142	66.19
Thymol 30%	21	225a	94b	50b	369	86.99
Anise oil 30%	25	155b	48de	38d	241	80.00
Cinnamon oil 30 %	24	103cd	102b	47bc	252	80.95
Formic acid 65%	22	108c	223a	242a	573	91.62
Control	21	16f	15f	17e	48	
L.S.D at 5%		14.75	12.87	5.41		

Data in Table (3) revealed that the total fallen mites as a result to use the essential oils from August to September were more than those in from January to February, 2013. The total fallen mites due to the use of essential oils at 30% concentration were more than those at 15% concentration. On contrast of winter season, Cinnamon oil 30% showed the highest total fallen mites (684 mites) followed by Anise oil% (533 mites), It could be noticed that Thymol 15 % concentration was more effective (375mites) than the concentration 30% (447 mites). Also, Formic acid 655 gave the highest fallen mites (976 mites) compared to the tested essential oils and it was more effective in summer season than the winter season.

**Table.3. Effect of different essential oils for controlling the management of *Varroa* mites during August – September, 2013.**

Treatment	Pre-treatment count	1st Application	2nd Application	3 <sup>rd</sup> Application	Total fallen mites	Efficacy %
	No. of mites	17/8 – 26/8	27/8-6/9	7/9 – 15/9		
Thymol 15%	43	291a	107d	49d	375	67.46
Anise oil 15%	37	112c	110d	48d	270	54.81
Cinnamon oil15%	39	147b	106d	35d	288	57.63
Thymol 30%	47	157b	157c	61cd	447	72.70
Anise oil 30%	41	293a	151c	89c	533	77.11
Cinnamon Oil 30%	45	314a	257b	113b	684	82.16
Formic acid 65%	42	288a	320a	368a	976	87.50
Control	38	28e	33e	61c	122	
L.S.D at 5%		29.53	30.28	37.36		

The effectiveness of different essential oils for controlling *varroa* mites ranged between 66.19 to 86.99 % in winter (January – February) and 54.81 to 82.16% in summer (August to September). In the period from January – February, Thymol oil 30% was the best (86.99% followed by Cinnamon oil 30% (80.95%) then Anise oil 30% ( 80.00%). However, the same oils at 15% were less effective. Floris, *et al.*(2004) stated that both Thymol formulations reduced significantly the levels of mite infestations of

adult bees and sealed brood and its efficacy, expressed as percentage of mortality, was lower for both products (Apilife VAR  $74.8 \pm 13.1$  and  $81.3 \pm 15.5$ , Apiguard  $90.4 \pm 8.3$  and  $95.5 \pm 8.7$  for sealed brood and adult bees, respectively).

On the other hand, treatments from August to September showed that Cinnamon oil 30% was the best and occupied the first rank (82.16 % efficacy) in summer followed by Anise oil 30% (77.11%) then Thymol 30% (67.46%). Similar results with different oils were suggested by Whittington *et al* (2000) examined the botanical oils neem, thymol, and canola control of parasitic mites (*Varroa jacobsoni* and *Acarapis woodi*) in honey bee (*Apis mellifera*) colonies in British Columbia, Canada. Neem oil spray (5% solution) killed  $90 \pm 6\%$  of *varroa* mites, 3 times more than that died in the untreated group. Thymol-oil spray (4.8g Thymol/litre in 20% canola oil solution), thymol in vermiculite (3.6g/vermiculite block), and canola oil spray (20% solution) killed  $79 \pm 8\%$ ,  $68 \pm 6\%$ , and  $65 \pm 6\%$  of the *varroa* mites, respectively. Colonies treated with the Thymol-oil spray had a significantly lower tracheal mite infestation ( $1.3 \pm 7.5\%$ ) at the end of the treatment period than the untreated group ( $23.3 \pm 6.0\%$ ). All other treatments showed a slight but not statistically significant decrease in tracheal mite infestation levels. Also, Akyol and Yeninar (2008) reported that average efficacies of Thymovar and Bee Vital were found to be 96.91 and 88.66%, respectively. El-Zemity *et al.* (2006) who found that clove oil gave good results in controlling *varroa* mites. Fouly and Al-Dehghairi (2009) stated that clove showed 62% efficacy in controlling *varroa* mites. Hussein *et al.* (2001) tested the 6 plants oils to control *varroa* mites and rated clove oil, the best among the other oils.

As far as the timing of application, the number of fallen mites was higher after the 1<sup>st</sup> application than the 2<sup>nd</sup> and 3<sup>rd</sup> applications either in winter or summer treatments. These results are similar with other findings and treatments (Shoreit and Hussein, 1994) who found that the maximum fallen mites was happen after the first treatment. Abdel Rahman and Rateb (2008) suggested that the mean number of fallen mites were higher after 24 h., than after 48 h and after 72 which showed the lowest numbers. Also, Rashid *et al.* (2014) who stated that the mean number of fallen mites were higher after 24 h of treatment.

In case of Formic acid was more effective than the tested oils, which showed 91.62% in January to February and 87.50% in August to September. Also, the mean number of fallen *varroa* mites increased gradually with timing application. The number of fallen mites was lower in the first treatment than the second and third treatment either in winter or summer application. Similar results were obtained by Rashid *et al.* (2011) who stated that formic acid significantly killed higher number mites compared to thymol. Also, Calderon *et al.* (2014) found that formic acid 86% gave effectiveness in controlling *varroa* mites 96.9%, while Thymol released 94.7%.

#### **Effect of essential oils on brood rearing activity.**

Data in Table (4) showed that both of Cinnamon and Anise oils treatments increased brood rearing activities percentage than Thymol in the two concentrations 15 and 30% compared to formic acid and the control

treatments. No significant differences were found between Aniseoil, Cinnamon oil at 15 % and Thymol at 30% concentrations. Thymol oil 15 % recorded the lowest increase in brood rearing (200.99 inch<sup>2</sup>) and efficacy% 44.28%. No significant differences were found between the Anise and Cinnamon at 30% concentration (439.99 and 436.65 inch<sup>2</sup>). All the tested oils increased brood rearing compared to Formic acid( 297.99 inch<sup>2</sup>)and control treatment (111.99 inch<sup>2</sup>)

**Table.4. Effectiveness of essential oils on brood rearing activity during January – February, 2013.**

Treatment	1st	2 <sup>nd</sup>	3rd	Brood rearing (Inch <sup>2</sup> )	Efficacy %
	Application	Application	Application		
	2/1 – 14/1	15/1-26/1	28/1 – 10 / 2		
Thymol 15%	43	72.66	85.33	200.99d	44.28
Anise oil 15%	121.66	130.33	136.33	388.32b	71.16
Cinnamon oil 15 %	121	138	160	385.88b	70.97
Thymol 30%	103.33	132.66	155.33	391.32b	71.38
Anise oil 30%	128.33	144.66	167	439.99 a	74.54
Cinnamon oil 30 %	131.66	145.66	159.33	436.65a	74.35
Formic acid 65%	98.33	110	89.66	297.99c	62.41
Control	36.66	37	38.33	111.99e	
L.S.D at 5%				41.28	

Data in Table (5) showed contrast results during January – February, Out of concentration 15%, Thymol 15% ranked the first in increasing brood rearing activity(55.38%).However, Cinnamon oil 30% and Thymol 30 and15 % were the best 64.54, and 56.32 %, respectively. It could be noticed that Formic acid showed lower efficiency in summer (40.18%) than those in summer months (62.41%) compared the control treatment. No significant differences were found between the tested oils at 30% concentration.

**Table.5. Effectiveness of essential oils on brood rearing activity during August – September, 2013.**

Treatment	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	Brood rearing (Inch <sup>2</sup> )	Efficacy%
	Application	Application	Application		
	2/1 – 14/1	15/1-26/1	28/1 – 10 / 2		
Thymol 15%	148.33	181	203.33	532.66b	55.38
Anise oil 15%	81.66	100.66	117.33	299.65f	20.68
Cinnamon oil 15 %	110	116.33	132.33	358.66e	33.73
Thymol 30%	166.66	182.66	197.33	546.65b	56.52
Anise oil 30%	146.66	168	126	440.66c	46.06
Cinnamon oil 30 %	193.33	220	257	670.33a	64.54
Formic acid 65%	126	141	130.33	397.33d	40.18
Control	133.33	131	123.33	237.66d	
L.S.D at 5%				28.72	

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Table with 7 columns and 4 rows of data. The content is extremely faint and illegible, appearing to be a list of entries with various symbols and parentheses.