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Evaluation of Some Natural Essential Oils and Formic Acid as A Mixture for Controlling Varroa Mite *Varroa destructor* (Anderson & Trueman) in Honey Bee Colonies

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ABSTRACT

Three different application methods were used for controlling varroa mites in honey bee colonies. A wood stick strips were soaked in a mixture of some essential oils and formic acid 65% and applied in honey bee colonies. Results showed that all experimental materials caused a significant reduction of varroa mite infestations when compared with control ones. The colonies get on a treated wood stick strips on the top of brood combs caused a highest reduction of varroa mites (91.3 & 84.7%) and (91.9 & 83.3%) on the brood & adult workers during October and November, respectively. the mean number of fallen varroa mites increased gradually after the first treatment compare to control in all treated colonies during October and November. During October, after the first treatment the highest mean number of fallen varroa mites were counted in case of held the wood stick strips between the brood comb (11.3). While, in November, after the first treatment a highest mean number of fallen varroa was recorded in colonies treated on the hive bottom board (11). According to the effect of tested materials on varroa mite injuries by grooming behaviour of bee workers, results revealed that there were some significant differences in the mean number of injuries varroa between either application methods or control. In October and November the highest deformed numbers of varroa were observed in Gnathosoma and legs of varroa mites during all experimental treatments.

Keywords: Essential Oils, Formic Acid, Mixture, Control, Varroa Destructor, Honey Bees.

1. Introduction

The ectoparasitic honey bee mite *Varroa destructor* Anderson & Trueman (Acari: Varroidae) is considering a destructive parasite of the Western honey bee *Apis mellifera* and is widely recognized to be one of the most reasons for colony collapse disorder (CCD) (Rosenkranz *et al.,* 2010a,b; Le Conte *et al.,* 2010). Varroa mites directly feeding on the fat body and hemolymph of bees (Ramsey *et al*. 2019), also it can transmit several virus diseases, including Deformed Wing Virus (DWV), which can be deathly to honey bee colonies (McMenamin and Genersch 2015; Martin and Brettell 2019). The combined effects of this parasite and virus have been consistently identified as the leading cause of overwintering honey bee hive mortality (Stahlmann-Brown *et al.,* 2022; McGruddy *et al.,* 2023). Although many organic acids have been increasingly used by beekeepers, the main control of Varroa has still largely depend on chemical acaricides worldwide (Rosenkranz et. al., 2010a, b; Tutun *et al*. 2018; Gregorc and Sampson 2019; Brodschneider *et al*. 2023). The acaricides such as tau-fuvalinate, fumethrin, coumaphos and amitraz have been used for decades in Varroa control (van der Steen and Vejsns, 2021).

Using acaricides inside beehives against varroa mites and repeated application during the year that led to contamination of honey and other hive products (Wallner, 1999; Martel *et al.,* 2007; Abou-Shaara, 2014; Pohorecka *et al.,* 2018 and Calatayud-Vernich *et al.,* 2018). Because of this repetitive treatment, the mite resistance to accaricides was development in varroa population (Elzen *et al.,* 1999; Lodesani

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et al., 1995; Baxter *et al.,* 1998; Mozes-Koch *et al.,* 2000; Spreafco *et al.,* 2001; Martin 2004; Pettis 2004; Rosenkranz *et al.,* 2010a, b; Jack and Ellis 2021; Mitton *et al.,* 2022).

Several components extracted from plants, including plant oils, have been shown to have biocidal activity (Masry *et al.,* 2020; Sabahi *et al.,* 2020). These compounds are considered to be more environmentally friendly acaricides and have been used as potential varroa mite control (Imdorf *et al.,* 1999; El- Hady *et al.,* 2015; Abu Bakar *et al.,* 2017; Bendifallah *et al.,* 2018; Kadhim *et al.,* 2022; Bava *et al.,* 2023). Several studies have conducted that EOs demonstrate effectiveness in varroa mite control while being safe to honey bees (Aglagane *et al.,* 2022 & Kebebe *et al.,* 2022). Formic acid and thymol are effective components in the control of varroa mites without any side effects (Soroker *et al.,* 2019).

The present investigation aimed to evaluate mixture of Thymol, Euculyptus, lemongrass, Mint oils and Formic acid by three different application methods for controlling varroa mite in honey bee colonies.

2. Materials and Methods

This work was carried out in the a private apiary yard at Qualiubia governorate, Egypt during the period from 1st October to 15th of December 2023.

2.1. Chemicals and Materials

The four tested essential oils lemongrass (*Cymbopogon citratus*), Thyme (Thymus vulgaris), Mint (*Mentha spp.*) and Eucalyptus(*Eucalyptus spp*.) were obtained from oil extraction unit, National Research Centre, Cairo, Egypt, whereas Formic acid 65% was bought from Al Gomhoria Pharm. Ind., Cairo, Egypt.

2.2. Preparing the mixture of essential oils and Formic acid

A combination of Thymol (30 mL.), Euculyptus (15 mL.), Lemongrass (15 mL.) and Ment (15 mL. essential oils was prepared and mixed with Formic acid 65% (300 mL.). The mixture was prepared by using 500 gm of Vaseline in a big Jar (2L) and left in a water bath for 15 minutes to become slightly soft. A previous amounts of each tested oil and formic acid 65% were added to Vaseline and by a glass stick were completely knead together to obtained a homogenate mixture. Four hundred of a wood stick strips (15 X 3 cm. and 1mm. thick) were soaked in the mixture for two hours and packing in plastic bag (ten strips for each) and were kept in the refrigerator until use. Through the experimental procedures another clean wood stripes were dipped in formic acid 65% alone for ten minute before colony treatments.

2.3. Experimental honey bee colonies

Twenty honey bee colonies of Carniolian hybrid *Apis mellifera* L. naturally infested by varroa mites were used for this study. The tested colonies were treated by a wood stick strips which saturated by oils and formic acid. Three application methods were used, on the bottom board, up of the brood combs and held wood strips between the brood combs. Four colonies were used for each application methods, Formic acid alone and the four untreated colonies left as control.

2.4. Determination of varroa infestation percentages

The percentages of varroa infestation in the experimental honey bee colonies were determined in worker brood cells and adult workers. For worker brood, 50 sealed worker cells were randomly selected and opened (pupae with pigment eyes), the pre-pupae or pupae inside it were carefully examined and the infested cells were counted. In adult workers, the infestation percentage was determined in approximately 100 living worker bees taking directly from the combs in brood nest (De Jong, 1988 and Branco *et al.,* 2006). The infestation percentage in worker brood cells and adult bee workers were determined once before the treatments and 2 times after treatments (after first treatment and end of treatments). The reduction percentages of varroa mite infestation were calculated from the collected data according to the equation of Henderson and Tilton (1955).

Varroa mites fallen on the hive bottom board, covered with sheet of white paper were collected and counted in all experimental colonies (treatments & control) at 24h.,48h., 72h. and 7 days after treatments.

2.5. Assessment of grooming behavior

To investigate grooming behavior in the in treated and untreated experimental colonies; the collected varroa mite on the bottom broad were carefully examined under dissecting microscope at 10X magnification (Boecking and Spivak, 1999; Flottum, 1997 and Ahmed *et al.,* 2017). The mean percentage of mite legs, Gnathosoma and dorsal shield injuries which caused by grooming behaviour of worker bees were observed and counted at 24 h., 48 h., 72 h. and 7 days post treatment.

2.6. Statistical analysis

Duncan's test ($P < 0.05$) was used to examine the collected data using one-way analysis of variance (ANOVA). Means ± SEM were used to express all data from biochemical and molecular genetics investigations. The Statistical Package for Social Sciences (SPSS 0.26 for Windows) was used to analyze the data.

3. Results and Discussion

Impact of the mixture of essential oils and Formic acid 65% for controlling varroa mites were illustrated in Table (1). Results showed that all experimental materials caused a significant reduction of varroa mite infestations when compared with control ones. The colonies get on a treated wood stick strips on the top of brood combs caused a highest reduction of varroa mites (91.3 & 84.7%) and (91.9 & 83.3%) on the brood & adult workers during October and November, respectively.

During October application methods between the brood combs results in a lowest reduction of varroa infestation on the brood (69.1%) and adult workers (82.4%). In November the lowest reduction of varroa infestation of worker broods was observed in colonies treated on the hive bottom board (82.8%), while it was (80.6%) for adult workers when held wood stick strips between the brood combs. Formic acid 65% reduced the varroa infestation to 78.3 & 72.4% in worker brood and 83.4 & 82.2 % for adult workers during October and November respectively.

Results of this study are consistent with previous studies which concluded that essential oils were effective in controlling varroa mites (Imdorf *et al.,* 1999; Abd El-Wahab *et al.,* 2012; Rashid *et al.,* 2012; Islam *et al.,* 2016; Abu Bakar *et al.,* 2019; Hýbl *et al.,* 2021). Results of this assessment were agreed with Faraj *et al.,* (2021) found a mixture of two or more of essential oils extracted from plant material could lead to a reduction of varroa mite infestations. Also Begna *et al.,* (2023) recorded that mixing some essential oils, a 1:1 mixture of carvacrol and thymol, decreased the number of *V. destructor*, rather than using single oil. Whittington *et al.,* 2000 and Dimetry *et al.,* 2005 observed that Neem oil spray caused 90-94% death of varroa mites but thymol oil spray reduced varroa to 79% in infested honey bee colonies .Fathy and Fouly 1995 found that 10 ppm of lemon grass oil caused 44.9% of varroa infestation reduction. Rbee and Zedan, 2018 showed that the highest reduction of the Varroa mite population on adult bee workers (90.4, 85.6 and 81.9 %) were resulted by treatment with Formic acid and Marjoram mixture at the rates of 3,2 and 1ml /colony followed by Spearmint mixture with Formic acid (77.0,72.8 %) at 3ml/colony. While, Formic acid treated alone at 3 ml/colony caused 77.5% and marjoram (71.9%). Also, the highest reduction percentage in brood infestation (75.9%) was obtained when the colonies treated with Formic acid followed by Marjoram, (58.6%).

Results in Table (2) revealed that the mean number of fallen varroa mites increased gradually after the first treatment compare to control in all treated colonies during October and November. During October, after the first treatment the highest mean number of fallen varroa mites were counted in case of held the wood stick strips between the brood comb (11.3) followed by Formic acid alone (10.1). It is clearly that the application method of held wood strips between the brood combs gave a highest mean of fallen varroa (16.5 and 14.5) followed by Formic acid (12.3 and 9.2) during the second and third treatment, respectively. In November, after the first treatment a highest mean number of fallen varroa was recorded in colonies treated on the hive bottom board (11) followed by that treated by the holding between the brood combs (10.5) and Formic acid (8.5). While the mean number was obtained in colonies get the wood stick strips on the top of brood combs (3.5) after the first treatment. Results in November also showed that the applied by holding between the brood combs had a highly number of fallen varroa (8 & 10) and (8.8 &8) after the second and third treatment, respectively. The lowest value was recorded in Formic acid alone (6 &6.3) after the second and third treatment, respectively.

Table 1: Impact of tested materials on the mean percentage of varroa mite infested brood and adult workers.

Method of application	During October						During November					
	Mean % of varroa infestation before treatment		Mean % of varroa infestation after fourth treatment		Reduction %	Reduction %	Mean % of varroa infestation before treatment		Mean % of varroa infestation after fourth treatment		Reduction %	Reduction %
	% on brood	% on adult	% on brood	% on adult	Brood	Adult	% on brood	% on adult	% on brood	% on adult	Brood	adult
On the hive bottom board	4.5± 1.8a	12±1.1a	1.5±0.5b	2.5±0.5b	81.1	84.1	7.5±1.7a	12.5±2a	2±0.0b	3±0.57b	82.8	82.9
On the brood comb	6.5±0.95a	12.5±a	1±0.75b	2.5±.57b	91.3	84.7	4±0.8a	8.5±1.7a	0.5±0.5b	2±0.81b	91.9	83.3
Held between the combs	5.5±0.95a	13±a	3±0.5b	3±0.75b	69.1	82.4	7±1.2a	11±2.3a	1±0.5b	3±0.57b	83.9	80.6
Formic acid 65%	6.5±1.25a	11.5±a	2.5±0.5b	2.5±0.5b	78.3	83.4	7.0±1.9a	6±1.2a	3±1.2b	1.5±0.5b	72.4	82.2
Control	6.5±0.95a	6.5±b	11.5±2a	8.5±0.9a	------	-----	9±2.08a	11±1.29a	14±2.1a	15.5±2a	-----	-----

Table 2: Impact of different application methods of tested material on the mean number of varroa fallen on the bottom board

Method of application	Time	October				November			
		T1	T2	T3	T4	T1	T2	T3	T4
On the hive bottom board	24h.before treatment	**6.5**	---	---	---	---	---	---	---
	24 h.	10.25	14.25	23.5	17.75	23.5	17.75	16.25	11
	48 h.	9.75	10	14.75	11.5	16.75	11	12.25	1.5
	72 h.	7.75	5.75	5.5	3.75	3.25	4.5	2.25	0.0
	7 days	3	2.5	2.25	1.0	0.5	0.75	0.25	0.0
	Mean	**7.6**	**8.12**	**11.5**	**8.5**	**11**	**8.5**	**7.75**	**6.25**
On the brood comb	24h.before treatment	**3.5**	---	---	---	---	---	---	---
	24 h.	8.25	11.75	10.25	13	6.25	20.75	19.25	9.75
	48 h.	6.25	8.5	7	8.25	3.25	10.25	11.5	0.75
	72 h.	6	4.75	3.5	4.25	1.25	4	1.25	0.25
	7 days	2.25	2.25	1.25	0.5	0.0	0.5	0.0	0.0
	Mean	**5.6**	**6.8**	**5.5**	**6.5**	**3.5**	**8.8**	**8**	**2.7**
Held between the brood combs	24h.before treatment	**7**	---	---	---	---	---	---	---
	24 h.	15.75	27.25	27.25	23	22	18.25	25.75	11.75
	48 h.	14	20.5	18.5	15.25	15.5	10.5	12.5	1.25
	72 h.	11.75	12.25	9.5	8.25	4	2.75	1.75	0.75
	7 days	3.75	6.25	2.75	2.25	0.75	0.5	0.0	0.0
	Mean	**11.3**	**16.5**	**14.5**	**12.2**	**10.5**	**8**	**10**	**4.6**
Formic acid 65%	24h.before treatment	**4.5**	---	---	---	---	---	---	---
	24 h.	16.75	24	19	18	17	16	16.25	8.75
	48 h.	13.25	14.75	12.75	10.25	13.25	6.75	8	0.75
	72 h.	8.25	8.25	3.75	3.75	3.75	1.25	1	0.0
	7 days	2	2.25	1.25	0.5	0.25	0.0	0.0	0.0
	Mean	**10.1**	**12.3**	**9.2**	**8.1**	**8.5**	**6**	**6.3**	**4.75**
Control	24h.before treatment	**2.5**	---	---	---	---	---	---	---
	24 h.	6.25	7.5	4.75	4.5	4.75	8	6.75	4.25
	48 h.	4.75	5.75	2.75	1.5	2.75	2	1	0.25
	72 h.	4.25	2.75	1.25	0.75	2	0.25	0.5	0.5
	7 days	2.25	1.25	0.0	0.0	0.25	0.0	0.25	0.25
	Mean	**4.4**	**4.3**	**2.2**	**2.25**	**2.4**	**2.6**	**2.1**	**1.3**

T1: First treatment T2: Second treatment T3: Third treatment T4: Fourth treatment

Concerning to the grooming behavior of honey bee colonies against varroa mites in treated or untreated colonies, the investigated results were recorded in Tables 3,4,5,6. Data revealed that there were some significant differences in the mean number of injuries varroa between either application methods or control.

In October and November the highest deformed numbers of varroa were observed in Gnathosoma and legs of varroa mites during all experimental treatments. Application methods of wood strips impregnated with mixture of essential oils and Formic acid (65%) that put on the brood combs or held between the brood combs causes the highest mean number of deformed varroa through the different treatments. Whereas, application on the brood combs recorded 18.1% deformed varroa after the first treatment during November as well as 40.8 & 57.2% after the second and fourth treatment during October and November, respectively. Application method by held between the brood combs resulted in 45.1% after first treatment of October and 34.6 & 25.9% deformed varroa after third and fourth treatment of October. Also the same application method causes the highest deformed percentage 33 &48.7% of varroa mites after second and third treatments during November, respectively.

Table 3: Impact of oils and Formic acid treatment on the injuries varroa mites during first treatment.

Type of treatment	Time	First treatment of October								First reatment of November							
		Mean no. of fallen varroa	Mean no. of Normal varroa		Mean no. of injuries varroa		Types of varroa injuries %			Mean no. of fallen varroa	Mean no. of Normal varroa		Mean no. of injuries varroa		Types of varroa injuries %		
			Mean	%	Mean	%	Mouth	Leg	Body		Mean	%	Mean	%	Mouth	Leg	Body
On the hive bottom board	**24 h.**	10.25	6.75	65.8	3.5	34.1	50	28.5	21.4	23.5	19.75	84.1	3.75	15.9	46	40	13
	48 h.	9.75	7	71.7	2.75	28.2	45.5	36.3	18.2	16.75	14.25	85.1	2.5	14.9	40	40	20
	72 h.	7.75	4.25	54.8	3.5	45.1	50	35.7	14.3	3.25	3	92.3	0.25	7.7	100	0	0
	7 days	3	1.5	50	1.5	50	50	33.3	16.7	0.5	0.5	100	0.0	0.0	0.0	0.0	0.0
	Mean	**7.6**	**4.8**	**60.5**	**2.81ab**	**39.3**	**48.8**	**33.4**	**17.6**	**11**	**9.3**	**90.3**	**1.6a**	**9.6**	**62**	**26.6**	**11**
On the brood comb	**24 h.**	8.25	5.5	66.6	2.75	33.3	63.6	27.2	9.1	7.75	6.25	80.6	1.5	19.3	33.3	50	16.6
	48 h.	6.25	4	64	2.25	36	44.4	33.3	22.2	5	3.25	65	1.75	35	42.8	57.1	0.0
	72 h.	6	4.25	70.8	1.75	29.1	57.1	28.6	14.3	1.25	1.25	100	0.0	0.0	0.0	0.0	0.0
	7 days	2.25	1	44.4	1.25	55.5	60	20	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**5.6**	**3.6**	**61.4**	**2.0b**	**38.4**	**56.7**	**27.2**	**16.4**	**4.6**	**3.5**	**81.6**	**1.6a**	**18.1**	**38.1**	**53.5**	**8.3**
Held between the brood combs	**24 h.**	15.75	9.5	60.3	6.25	39.6	40	28	32	22	15.5	70.4	6.5	29.5	38.46	34.6	26.9
	48 h.	14	6.75	48.2	7.25	51.7	41.4	31.1	27.6	15.5	14.5	93.5	1	6.4	75	25	0.0
	72 h.	11.75	6.75	57.4	5	42.5	45	25	30	4	3.75	93.7	0.25	6.2	100	0.0	0.0
	7 days	3.75	2	53.3	1.75	46.6	42.9	42.9	14.2	0.75	0.75	100	0.0	0.0	0.0	0.0	0.0
	Mean	**11.3**	**6.25**	**54.8**	**5.1a**	**45.1**	**42.3**	**31.7**	**25.9**	**10.5**	**8.6**	**89.4**	**2.6a**	**10.5**	**71.1**	**29.8**	**26.9**
Formic acid 65%	**24 h.**	16.75	11.25	67.1	5.5	32.8	22.7	36.3	40.9	17	15.25	89.7	1.75	10.2	42.8	42.8	14.2
	48 h.	13.25	8	60.3	5.25	39.6	38.1	33.3	28.5	13.25	11.5	86.7	1.75	13.2	42.8	42.8	14.3
	72 h.	8.25	5.25	63.6	3	36.3	41.7	33.3	25	3.75	3.5	93.3	0.25	6.6	100	0.0	0.0
	7 days	2	1.5	75	0.5	25	50	50	0.0	0.25	0.25	100	0.0	0.0	0.0	0.0	0.0
	Mean	**10.1**	**6.5**	**66.5**	**3.6ab**	**33.4**	**38.1**	**38.2**	**31.4**	**8.5**	**7.6**	**92.4**	**1.25a**	**7.5**	**61.8**	**42.8**	**14.2**
Control	**24 h.**	6.25	4	64	2.25	36	33.3	55.5	11.1	4.75	4.75	100	0.0	0.0	0.0	0.0	0.0
	48 h.	4.75	3.75	78.9	1	21.1	25	50	25	2.75	2.75	100	0.0	0.0	0.0	0.0	0.0
	72 h.	4.25	3.25	76.5	1	23.5	50	25	25	2	2	100	0.0	0.0	0.0	0.0	0.0
	7 days	2.25	1.75	77.8	0.5	22.2	50	50	0.0	0.25	0.25	100	0.0	0.0	0.0	0.0	0.0
	Mean	**4.4**	**3.18**	**74.3**	**1.2b**	**25.7**	**39.5**	**45.1**	**15.3**	**2.4**	**2.4**	**100**	**0.0a**	**0.0**	**0.0**	**0.0**	**0.0**

In October F= 3.40* and in November F= 0.79^{NS}, varroa injury means in a column with dissimilar letters differ significantly at 0.05 level of probability.

Table 4: Impact of oils and Formic acid treatment on the injuries varroa mites during second treatment.

Treatment	Time	During October								During November							
		Mean no. of fallen varroa	Mean no. of Normal varroa		Mean no. of injuries varroa		Types of varroa injuries %			Mean no. of fallen varroa	Mean no. of Normal varroa		Mean no. of injuries varroa		Types of varroa injuries %		
			Mean	%	Mean	%	Mouth	Leg	Body		Mean	%	Mean	%	Mouth	Leg	Body
On the hive bottom board	**24 h.**	14.25	9.75	68.4	4.5	31.5	33.3	38.9	27.8	17.75	13.75	77.4	4	22.5	37.5	50	12.5
	48 h.	10	6.25	62.5	3.75	37.5	46.7	33.3	20	11	10	90.9	1	9.1	50	50	0.0
	72 h.	5.75	3.75	65.2	2	34.7	50	37.5	12.5	4.5	2.75	6.1	1.75	38.8	42.9	42.9	14.3
	7 days	2.5	2.25	90	0.25	10	100	0.0	0.0	0.75	0.75	100	0.0	0.0	0.0	0.0	0.0
	Mean	**8.12**	**5.5**	**71.5**	**2.6ab**	**28.4**	**57.5**	**36.5**	**20.1**	**8.5**	**6.8**	**49.2**	**2.2a**	**23.4**	**43.5**	**47.6**	**8.93**
On the brood comb	**24 h.**	11.75	6.5	55.3	5.25	44.6	38.1	28.6	33.3	20.75	18.25	87.9	2.5	12.1	50	50	0.0
	48 h.	8.5	5	58.8	3.5	41.1	42.9	28.6	28.6	10.25	8.75	85.3	1.5	14.6	33.3	66.6	0.0
	72 h.	4.75	3	63.1	1.75	36.8	42.9	57.1	0.0	4	3	75	1	25	75	25	0.0
	7 days	2.25	2.25	100	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.5	100	100	0.0	0.0
	Mean	**6.8**	**4.2**	**69.3**	**3.5ab**	**40.8**	**41.3**	**38.1**	**30.9**	**8.8**	**7.5**	**85.2**	**1.4a**	**15.9**	**64.5**	**35.4**	**0.0**
Held between the brood combs	**24 h.**	27.25	18.25	66.9	9	33.1	38.9	30.5	30.5	18.25	14.25	78.1	4	21.9	43.7	43.7	12.5
	48 h.	20.5	14.75	71.9	5.75	28.1	47.8	26.1	26.1	10.5	8	67.1	2.5	23.8	40	40	20
	72 h.	12.25	7.75	63.2	4.5	36.7	44.4	50	5.6	2.75	1.75	63.6	1	36.3	50	50	0.0
	7 days	6.25	4.25	68	2	32	37.5	50	12.5	0.5	0.25	50	0.25	50	100	0.0	0.0
	Mean	**16.5**	**11.25**	**67.5**	**5.3a**	**32.4**	**42.1**	**39.1**	**18.6**	**8**	**6.1**	**64.7**	**1.9a**	**33**	**58.4**	**33.4**	**8.2**
Formic acid 65%	**24 h.**	24	17.25	71.8	6.75	28.1	37.1	40.7	22.2	16	13.75	85.6	2.25	14.1	44.4	44.4	11.1
	48 h.	14.75	10.5	71.2	4.25	28.8	41.2	35.3	23.5	6.75	5	74.1	1.75	25.9	42.8	42.8	14.3
	72 h.	8.25	6.25	75.7	2	24.2	62.5	25	12.5	1.25	1.25	100	0.0	0.0	0.0	0.0	0.0
	7 days	2.25	2.25	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**12.3**	**9.1**	**79.6**	**4.3a**	**20.2**	**46.9**	**29.5**	**19.4**	**6**	**5**	**86.5**	**1.3a**	**13.3**	**43.6**	**43.6**	**12.7**
Control	**24 h.**	7.5	5.75	76.6	1.75	23.3	57.1	28.6	14.3	8	6.75	84.3	1.25	15.6	60	40	0.0
	48 h.	5.75	5.25	91.3	0.5	8.7	100	0.0	0.0	2	1.5	75	0.5	25	100	0.0	0.0
	72 h.	2.75	2	72.7	.75	27.2	66.6	33.3	0.0	0.25	0.25	100	0.0	0.0	0.0	0.0	0.0
	7 days	1.25	1.25	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**4.3**	**3.6**	**85.2**	**0.75b**	**14.8**	**55.9**	**15.5**	**3.6**	**2.6**	**2.1**	**86.4**	**0.4a**	**13.5**	**80**	**20**	**0.0**

In October F= 2.48 ^{NS} and in November F= 0.86 ^{NS},varroa injury means in a column with dissimilar letters differ significantly at 0.05 level of probability.

Table 5: Impact of oils and Formic acid treatment on the injuries varroa mites during third treatment.

Treatment	Time	During October								During November							
		Mean no. of fallen varroa	Mean no. of Normal varroa		Mean no. of injuries varroa		Types of varroa injuries %			Mean no. of fallen varroa	Mean no. of Normal varroa		Mean no. of injuries varroa		Types of varroa injuries %		
			Mean	%	Mean	%	Mouth	Leg	Body		Mean	%	Mean	%	Mouth	Leg	Body
On the hive bottom board	**24 h.**	23.5	17.25	73.4	6.25	26.6	40	32	28	16.25	11.75	72.3	4.5	27.7	50	44.4	5.5
	48 h.	14.75	10.25	69.5	4.5	30.5	50	27.8	22.2	12.25	10.25	83.6	2	16.3	50	37.5	12.5
	72 h.	5.5	3.25	59.1	2.25	40.9	55.6	33.3	11.1	2.25	1.75	77.7	0.5	22.2	50	50	0.0
	7 days	2.25	1.75	77.7	0.5	22.2	0.0	100	0.0	0.25	0.25	100	0.0	0.0	0.0	0.0	0.0
	Mean	**11.5**	**8.2**	**69.9**	**3.4ab**	**30.1**	**36.4**	**48.2**	**15.3**	**7.75**	**6**	**83.4**	**1.75a**	**16.5**	**50**	**43.9**	**6**
On the brood comb	**24 h.**	10.25	6.75	65.8	3.5	34.1	57.1	35.7	7.1	19.25	17.75	92.2	1.5	7.8	50	50	0.0
	48 h.	7	4.75	67.8	2.25	32.1	22.2	66.6	11.1	11.5	10.25	89.1	1.25	10.8	40	60	0.0
	72 h.	3.5	2.75	78.5	0.75	21.4	66.6	33.3	0.0	1.25	0.0	0.0	1.25	100	60	40	0.0
	7 days	1.25	1.25	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**5.5**	**3.8**	**78.1**	**1.6ab**	**21.9**	**48.6**	**45.2**	**6.6**	**8**	**7**	**60.4**	**1.0a**	**39.5**	**50**	**50**	**0.0**
Held between the brood combs	**24 h.**	27.25	16.5	60.5	10.75	39.4	37.2	30.2	32.6	25.75	18.75	72.8	7	27.1	25	53.5	21.4
	48 h.	18.5	12.5	67.5	6	32.4	45.8	37.5	16.6	12.5	8.25	66	4.25	34	47.1	35.3	17.6
	72 h.	9.5	5.75	60.5	3.75	39.4	40	46.6	13.3	1.75	0.25	14.2	1.5	85	50	33.3	16.6
	7 days	2.75	2	72.7	0.75	27.2	66.6	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**14.5**	**36.7**	**65.3**	**5.3a**	**34.6**	**47.4**	**36.9**	**15.6**	**10**	**6.81**	**51**	**3.2a**	**48.7**	**40.7**	**40.7**	**18.5**
Formic acid 65%	**24 h.**	19	15	78.9	4	21.1	50	18.7	31.2	16.25	13.5	83.1	2.75	16.9	36.3	54.5	9.1
	48 h.	12.75	7.75	60.7	5	39.2	45	35	20	8	6.75	84.3	1.25	15.6	80	20	0.0
	72 h.	3.75	3.75	100	0.0	0.0	0.0	0.0	0.0	1	0.25	25	0.75	75	33.3	66.6	0.0
	7 days	1.25	1.25	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**9.2**	**6.9**	**84.9**	**2.25ab**	**15.1**	**47.5**	**26.8**	**25.6**	**6.3**	**5.1**	**64.1**	**1.2a**	**35.8**	**49.8**	**47.1**	**3.1**
Control	**24 h.**	4.75	4	84.2	0.75	15.7	66.6	33.3	0.0	6.75	5.75	85.2	1	14.8	50	50	0.0
	48 h.	2.75	2.25	81.8	0.5	18.1	50	50	0.0	1	1	100	0.0	0.0	0.0	0.0	0.0
	72 h.	1.25	1.25	100	0.0	0.0	0.0	0.0	0.0	0.5	0.25	50	0.25	50	100	0.0	0.0
	7 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.25	0.25	100	0.0	0.0	0.0	0.0	0.0
	Mean	**2.2**	**1.9**	**88.7**	**0.3b**	**11.3**	**58.3**	**41.7**	**0.0**	**2.1**	**1.8**	**83.8**	**0.3a**	**16.2**	**75**	**25**	**0.0**

In October F= 2.21 ^{NS} and in November F= 1.48 ^{NS}, varroa injury means in a column with dissimilar letters differ significantly at 0.05 level of probability.

Table 6: Impact of oils and Formic acid treatment on the injuries varroa mites during fourth treatment.

Treatment	Time	During October								During November							
		Mean no. of fallen varroa	Mean no. of Normal varroa		Mean no. of injuries varroa		Types of varroa injuries %			Mean no. of fallen varroa	Mean no. of Normal varroa		Mean no. of injuries varroa		Types of varroa injuries %		
			Mean	%	Mean	%	Mouth	Leg	Body		Mean	%	Mean	%	Mouth	Leg	Body
On the hive bottom board	**24 h.**	17.75	14.75	83.1	3	16.9	50	41.6	8.3	11	9.25	84.1	1.75	15.9	57.1	42.8	0.0
	48 h.	11.5	7	60.9	4.5	39.1	44.4	44.4	11.1	1.5	1	66.6	0.5	33.3	50	50	0.0
	72 h.	3.75	3.25	86.7	0.5	13.3	50	50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	7 days	1.0	1.0	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**8.5**	**6.5**	**82.7**	**2.0ab**	**17.3**	**48.16.**	**45.3**	**4.6**	**6.25**	**5.1**	**75.4**	**1.2a**	**24.6**	**53.5**	**46.4**	**0.0**
On the brood comb	**24 h.**	13	9.5	73.1	3.5	26.9	35.7	35.7	28.6	9.75	9.25	94.8	0.5	5.1	50	50	0.0
	48 h.	8.25	5.5	66.6	2.75	33.3	45.5	36.4	18.2	0.75	0.25	33.3	0.5	66.6	100	0.0	0.0
	72 h.	4.25	3.75	88.2	0.5	11.8	50	50	0.0	0.25	0.0	0.0	0.25	100	100	0.0	0.0
	7 days	0.5	0.5	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**6.5**	**4.81**	**81.9**	**1.6ab**	**18**	**43.7**	**40.7**	**15.6**	**2.7**	**2.4**	**42.7**	**1.6a**	**57.2**	**83.3**	**16.6**	**0.0**
Held between the brood combs	**24 h.**	23	14	60.8	9	39.1	41.7	33.3	0.25	11.75	8.25	70.2	3.5	29.8	28.5	50	21.4
	48 h.	15.25	10.75	70.5	4.5	29.5	38.9	27.7	33.3	1.25	0.25	20	1	80	75	25	0.0
	72 h.	8.25	6.25	75.7	2	24.2	37.5	62.5	0.0	0.75	0.5	66.6	0.25	33.3	100	0.0	0.0
	7 days	2.25	2	88.8	0.25	11.1	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**12.2**	**8.25**	**73.9**	**3.9a**	**25.9**	**54.5**	**30.8**	**16.7**	**4.6**	**3.0**	**52.3**	**1.6a**	**47.7**	**67.8**	**25**	**7.1**
Formic acid 65%	**24 h.**	18	15	83.3	3	16.6	33.3	41.6	25	8.75	7.75	88.5	1	11.4	50	50	0.0
	48 h.	10.25	7.75	75.6	2.5	24.4	50	30	20	0.75	0.25	33.3	0.5	66.6	50	50	0.0
	72 h.	3.75	3.25	86.7	0.5	13.3	50	50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	7 days	0.5	0.5	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**8.1**	**6.6**	**86.4**	**1.5ab**	**13.6**	**44.4**	**40.5**	**15**	**4.75**	**4**	**60.9**	**0.75a**	**39**	**50**	**50**	**0.0**
Control	**24 h.**	4.5	4.25	94.4	0.25	5.5	100	0.0	0.0	4.25	3.75	88.2	0.5	11.8	50	50	0.0
	48 h.	1.5	1.5	100	0.0	0.0	0.0	0.0	0.0	0.25	0.0	0.0	0.25	100	100	0.0	0.0
	72 h.	0.75	0.5	66.6	0.25	33.3	0.0	0.0	0.0	0.5	0.25	50	0.25	50	100	0.0	0.0
	7 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.25	0.25	100	0.0	0.0	0.0	0.0	0.0
	Mean	**2.25**	**2.1**	**87**	**0.33b**	**12.9**	**100**	**0.0**	**0.0**	**1.3**	**1.1**	**59.6**	**0.25a**	**40.4**	**83.3**	**16.6**	**0.0**

In October F= 1.56 ^{NS} and in November F= 0.81^{NS}, varroa injury means in a column with dissimilar letters differ significantly at 0.05 level of probability.

The obtained results are in agreement with the findings of Islam *et al.,* (2016) who recorded that the highest total number of dead Varroa mites fallen on the sheet was counted by Formic acid followed by lemon grass, thyme, mint and rosemary respectively. Abd El-Wahab *et al.,* (2012) summarized that a highest reduction percentage of varroa infestation on adult bee workers at the highest concentration (100%) of Formic acid, Thyme, Cinnamon, Lemongrass and Anise whereas more than 96% reduction in infestation percentage of worker brood. Increasing the mean number of varroa mite fallen on the hive bottom board in colonies treated with essential oils may be due to the activation of the defense behaviour mechanisms of adult bee workers by these plant oils against varroa mite. Zakaria and Allam (2007) observed that the mean number of cutting varroa bodies by bee workers was 18.6% with formic acid treatment, while in colonies treated with black cumin oil was 14.1% in comparison with control which recorded 4.3% of varroa mite fallen on the sheet. Salem *et al.,* (1998) found that the honey bee colonies which fed on Neem extract resulted the highest mean number of varroa mite fallen on the sheet. They suggested that these extracts caused changes in the haemolymph of honey bee workers and therefore increased the number of varroa mite fallen on the hive bottom board. Abd El-Wahab (2001) stated that some defense behaviour mechanisms against varroa mite were detected in some races and hybrids of honey bees. These mechanisms resulted in increasing the number of fallen varroa mite on the hive bottom board.

References

Abd El-Wahab, T.E., 2001. Physiological and morphological studies on the natural defense behaviour in honey bee colonies against varroa mites. Ph. D. Thesis, Fac. Agric., Cairo Univ., Egypt, 154 pp.

Abd El-Wahab, T.E., I.M.A. Ebadah, and E.W. Zidan, 2012. Control of varroa mite by essential oils and formic acid with their effects on grooming behaviour of honey bee colonies. J. Appl. Sci. Res. 2: 7674–7680.

Abou-Shaara, H.F., 2014. Continuous management of Varroa mite in honey bee, Apis mellifera, colonies. Acarina 22 (2): 149–156.

Abu Bakar, M., M.A. Aqueel, A.M. Raza, R. Mahmood, Z. Abdul Qadir, M. Arshad, and M. Sohail, 2019. Evaluation of essential oils for the management of parasitic bee mites, Varroa destructor (Acari: Varroidae) in vitro. Pak. J. Agric. Res. 32(4): 566– 571.

Abu Bakar, M., M.A. Aqueel, A.M. Raza, M.I. Ullah, M. Arshad, M. Sohail, and J. Molina-Ochoa, 2017. Evaluation of few essential oils for the management of parasitic bee mites, Varroa destructor (Acari: Varroidae) in Apis mellifera L. colonies. Pak. J. Zool. 45: 2005–2010.

Aglagane, A., E.M. Laghzaoui, B. Soulaimani, O. Er-Rguibi, A. Abbad, E.H.E. Mouden, and M. Aourir, 2022. Acaricidal activity of Mentha suaveolens subsp. timija, Chenopodium ambrosioides, and Laurus nobilis essential oils, and their synergistic combinations against the ectoparasitic bee mite, Varroa destructor (Acari: Varroidae). J. Apic. Res., 61: 9–18. [CrossRef]

Ahmad, S., M.A. Aziz, M. Ahmad, and I. Bodlah, 2017. Effect of queen age on hygienic and grooming behavior of Apis mellifera Ligustica against Varroa destructor (Anderson and Trueman). Asian J. Agri. & Biol. 5(3):113-118.

Bava, R., F. Castagna, E. Palma, M. Marrelli, F. Conforti, V. Musolino, C. Carresi, C. Lupia, C. Ceniti, B. Tilocca, P. Roncada, D. Britti and V. Musella, 2023. Essential oils for a sustainable control of honey bee varroosis. Vet. Sci., 10, 308.

Baxter, J., F. Eischen, J. Pettis, W. Wilson, and H. Shimanuki, 1998. Detection of fluvalinate- resistant Varroa mites in US honey bees. Am. Bee, J. 138: 291.

Begna, T., D. Ulziibayar, D. Bisrat, and C. Jung, 2023. Acaricidal toxicity of four essential oils, their predominant constituents, their mixtures against varroa mite, and their selectivity to honey bees (Apis cerana and A. mellifera). Insects, 14(9):735.

Bendifallah, L., R. Belguendouz, L. Hamoudi, and K. Arab, 2018. Biological activity of the Salvia officinalis L. (Lamiaceae) essential oil on Varroa destructor infested honey bees. Plants, 7(2): 44.

Boecking O., and M. Spivak, 1999. Behavioral defences of honey bees against Varroa jacobsoni Oud. Apidologie, 30: 141–158.

Branco, M.R., A.C. Neil and S.P. Robert, 2006. A comparative evaluation of sampling methods for Varroa destructor (Acari: Varroidae) population estimation. Apidologie, 37:452-461.

Brodschneider, R., J. Schlagbauer, I. Arakelyan, A. Ballis, J. Brus, V. Brusbardis *et al.,* 2023. Spatial clusters of Varroa destructor control strategies in Europe. J. Pest. Sci., 96(2):759–783.

Calatayud-Vernich, P., F. Calatayud, E. Simó, and Y. Picó, 2018. Pesticide residues in honey bees, pollen and beeswax: assessing beehive exposure. Environ. Pollut. 241: 106–114. https://doi.org/10.1016/j.envpol.2018.05.062

De Jong, D., 1988. Varroa jacobsoni does reproduce in worker cells of Apis cerana in South Korea., Apidologie, 19: 241-244.

Dimetry, N.Z., T.E. Abdel EL-Wahab and M.E. Zakaria, 2005. Effective control of varroa mite Varroa destructor Anderson & Trueman infesting honeybee colonies *Apis mellifera* L. by some natural products. Bull. Fac. Agric., Cairo Univ., 56: 295-308.

El-Hady, A.M., E.E. Nowar, and M.F. EL-Sheikh, 2015. Evaluation of some essential oils for controlling varroa mites and their effects on brood rearing activity in honey bee colonies. J. Plant. Prot. Path. 6(1): 235–243.

Elzen, P.J., J.R. Baxter, M. Spivak, W.T. Wilson, 1999. Amitraz resistance in varroa: new discovery in North America., Am. Bee. J., 139: 362.

Faraj, T.H.S., R.J. Rashed, and Z.S. Lazim, and D.M. Atta, 2021. Study the effect of some plants extracts to control Varroa destructor Anderson and Trueman mite (Acari: Varroidae), and activity of honey bees (*Apis mellifera* L.), (Hymenoptera: Apidae). Euph. J. Agri. Sci. 13(3): 60–69.

Fathy, H.M. and A.H. Fouly, 1995. The effect of some natural volatile oils to control the ectoparasitic mite varroa jacobsoni infesting honey bee in Egypt 1st Int. Conf. Pest. Control, Mansoura, Egypt, Sept., 311-319.

Flottum, K., 1997. 21st century Apiculture: A review of the East Lansings Symposium. Bee Culture, 125: 24-26.

Gregorc, A., and B. Sampson, 2019. Diagnosis of Varroa Mite (Varroa destructor) and sustainable control in honey bee (*Apis mellifera*) colonies a review. Diversity, 11(12):243

Henderson, C.F. and F.W. Tilton, 1955. Tests with acaricides against the Brown wheat mite. J. Econ. Ent., 48 (2): 157-161.

Hýbl, M., A. Bohatá, I. Rádsetoulalová, M. Kopecký, I. Hoštic˘ková, A. Vaníc˘ková, and P. Mráz, 2021. Evaluating the efficacy of 30 different essential oils against Varroa destructor and honey bee workers (*Apis mellifera*). Insects, 12(11):1045.

Imdorf, A., S. Bogdanov, R.I. Ochoa, and N.W. Calderone, 1999. Use of essential oils for the control of Varroa jacobsoni in honey bee colonies. Apidologie, 30(23): 209–228.

Islam, N., M. Amjad, E. Ul-Haq, E. Stephen, and F. Naz, 2016. Management of Varroa destructor by essential oils and formic acid in *Apis mellifera* Linn. Colonies. J. Entomo. Zool. Stud. 4(6): 97–104.

Jack, C.J., and J.D. Ellis, 2021. Integrated pest management control of Varroa destructor (Acari: Varroidae), the most damaging pest of (*Apis mellifera* L. (Hymenoptera: Apidae)) colonies. J. Insect. Sci., 21(5):6.

Kadhim, H.M., M.H. Hadi, and A.A. Hassoni, 2022. Study of the effectiveness of essential oils (anise, clove) solution in controlling varroa mites (Varroa destructor) on honey bees *Apis mellifera*. QJAS, 12(2): 130–136.

Kebebe, D., A. Gela, T. Damto, M. Gemeda, and G. Leggese, 2022. Evaluating the Effect of Plants Extracts against Varroa Mites (Varroa destructors) of honey bees (*Apis mellifera*). J. Chem. Mater. Res., 14: 26–30.

Le Conte, Y., M. Ellis, and W. Ritter, 2010. Varroa mites and honey bee health: can Varroa explain part of the colony losses? Apidologie, 41(3):353–363

Lodesani, M., M. Colombo, and M. Spreafico, 1995. Ineffectiveness of Apistan® treatment against the mite Varroa jacobsoni Oud in several districts of Lombardy (Italy). Apidologie, 26: 67-72.

Martel, A.-C., S. Zeggane, C. Aurières, P. Drajnudel, J.-P. Faucon, and M. Aubert, 2007. Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar or Asuntol 50. Apidologie, 38 (6): 534–544. https://doi.org/10.1051/ apido:2007038.

Martin, S.J., 2004. Acaricide (pyrethroid) resistance in Varroa destructor. Bee World, 85(4):67–69

Martin, S.J., and L.E. Brettell, 2019. Deformed wing virus in honeybees and other insects. Annu. Rev. Virol., 6:49–69.

Masry, S.H.D., T.E. Abd El-Wahab, and M. Rashad, 2020. Evaluating the impact of Jatropha oil extract against the varroa mite, Varroa destructor Anderson &Trueman (Arachnida: Acari: Varroidae), infesting honeybee colonies (*Apis mellifera* L.). Egypt. J. Biol. Pest. Control. 30(91): 2–7.

McGruddy R.A., M. Bulgarella, A. Felden, J.W. Baty, J. Haywood, P. Stahlmann-Brown, *et al.,* 2023. Are increasing honey bee colony losses attributed to *Varroa destructor* in New Zealand driven by miticide resistance? *bioRxiv. preprint. March.* 24: 2023. doi: 10.1101/2023.03.22.533871

McMenamin, A.J., and E. Genersch, 2015. Honey bee colony losses and associated viruses. Curr. Opin. Insect. Sci., 8:121–129.

Mitton, G.A., F. Meroi Arcerito, H. Cooley, G. Fernández de Landa, M.J. Eguaras, S.R. Rufnengo, and M.D. Maggi, 2022. More than sixty years living with Varroa destructor: a review of acaricide resistance. Int. J. Pest. Manag. https://doi.org/10.1080/09670874.2022.2094489

Mozes-Koch, R., Y. Slabezki, H. Efrat, H. Kalev, Y. Kamer, B.A. Yakobson, and A. Dag, 2000. First detection in Israel of fluvalinate resistance in the varroa mite using bioassay and biochemical methods. Exp. App. Acarol. 24: 35-43

Pettis, J.S., 2004. A scientifc note on Varroa destructor resistance to coumaphos in the United States. Apidologie, 35(1):91–92.

Pohorecka, K., T. Kiljanek, M. Antczak, P. Skubida, P. Semkiw, and A. Posyniak, 2018. Amitraz marker residues in honey from honeybee colonies treated with Apiwarol. J. Vet. Res. 62: 297–301. https://doi.org/10.2478/jvetres-2018-0043.

Ramsey, S.D., R. Ochoa, G. Bauchan, C. Gulbronson, J.D. Mowery, A. Cohen, D. Lim, J. Joklik, J.M. Cicero, J.D. Ellis, D. Hawthorne, and D. vanEngelsdorp, 2019. Varroa destructor feeds primarily on honey bee fat body tissue and not hemolymph. Proc. Natl. Acad. Sci. USA, 116(5):1792–1801

Rashid, M., E. Stephen, A. Mohsin, S. Raja, and G. Sarwar, 2012. Control of ectoparasitic mites in honeybee (*Apis mellifera* L.) colonies by using Thymol and Oxalic acid. Pak. J. Zool. 44 (4): 985–989.

Rbee, A.E.A. and O.A.A. Zedan, 2018. Essential oils and Formic acid as good elements in the integrated management of Parasite varroamite Varroa destructor (Anderson and Trueman) in honey bee colonies. Annals of Agric. Sci., Moshtohor, 56(3): 779 – 784.

Rosenkranz, P., P. Aumeier, and B. Ziegelmann, 2010a. Biology and control of Varroa destructor. J. Invertebr. Pathol., 103:96-S119

Rosenkranz, P., P. Aumeier, and B. Ziegelmann, 2010b. Biology and control of Varroa destructor. J. Invertebr, Pathol., 103:S96–S119

Sabahi, Q., N. Morfin, B. Emsen, H.A. Gashout, P.G. Kelly, S. Otto, A.R. Merrill, and E. Guzman-Novoa, 2020. Evaluation of dry and wet formulations of oxalic acid, thymol, and oregano oil for varroa mite (Acari: Varroidae) control in honey bee (Hymenoptera: Apidae) colonies. J. Econ. Entomol. 1–7.

Salem, M.S., M.E. Nour; S.A.S. ElMaasarawy and M.E. Zakaria, 1998. Testing medical plants extracts compounds on haemocytes and varroatosis in honeybees. J. Agric. Sci. Mansoura Univ., 23 (1): 447- 460.

Soroker, V., N.K. Singh, N. Eliash, and E. Plettner, 2019. Olfaction as a target for control of honey bee parasite mite Varroa destructor. In olfactory concepts of insect control-alternative to insecticides (1st Edition). Edited by J-F. Picimbon. Springer International Publishing, Heidelberg, Germany, 117-134.

Spreafco, M., F.R. Eördegh, I. Bernardinelli, and M Colombo, 2001. First detection of strains of Varroa destructor resistant to coumaphos. Results of laboratory tests and feld trials. Apidologie, 32(1):49–55

Stahlmann-Brown P., R.J. Hall, H. Pragert, and T. Robertson, 2022. Varroa appears to drive persistent increases in New Zealand colony losses. *Insects,* 13 (7): 589. doi: 10.3390/insects13070589

Tutun, H., N. Koç, and A. Kart, 2018. Plant essential oils used against some bee diseases. TURJAF, 6(1):34–45

Van der Steen, J., and F. Vejsnæs, 2021. Varroa control: A brief overview of available methods. Bee World, 98(2):50–56

Wallner, K., 1999. Varroacides and their residues in bee products. Apidologie 30 (2- 3): 235–248. https://doi.org/10.1051/apido:1999021

Whittington, R., M.L. Winston, A.P. Melathopoulos and H.A. Higo, 2000. Evaluation of the botanical oils Neem, Thymol and Canola sprayed to control Varroa jacobsoni Oud. (Acari: Varroidae) and Acarapis woodi (Acari: Tarsonemidae in colonies of honeybees (*Apis mellifera* L, Hymenoptera: Apidae). Amer. Bee J., 140: 567-572.

Zakaria, M.E. and S.F. Allam, 2007. Effect of some aromatic oils and chemical acaricides on the mechanical defense behavior of honeybees against varroa invasion and relationship with sensation responses. Journal of Applied Sciences Research, 3(7): 653-661.