

## Comparing Effect of Plant-derived Oils on *Varroa destructor* Infesting Honeybee, *Apis mellifera*

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### ABSTRACT

In this research, we evaluated the application of different plant-derived oils (Thymol, Spearmint, Tea tree oil and mixture of spearmint and lemongrass) as control agents against the parasitic mite, *Varroa destructor* in the field. Four treatments of each tested material were applied to each colony during the treatment period. Data showed that all four tested compounds were effective in controlling *Varroa* oil and spearmint was the most effective substance as it had an efficiency of 85.9%, followed by spearmint & lemongrass and tea tree oils with an efficiency of 84.3% and 80.2%, respectively. Meanwhile, Thymol treatment had a lower efficacy, 78.1% compared to the other treatments and the natural mortality in control colonies was 12.3%.

**Key words:** *Apis mellifera*, *Varroa destructor*, Plant-derived oils, Infestation, Efficacy.

### INTRODUCTION

Honeybees are important insects economically and ecologically. The ectoparasite mite, *Varroa destructor*, is considered as one of serious and worldwide pest of honeybees, causing weight loss, malformation, and a shortened life-span. It also serves as a vector of diseases that may lead to 100% bee mortality within few months (Kanga and James, 2002). Synthetic acaricides, Coumaphos, Apistan, Amitraz, Folbex, Folbex-Forte and Apitol have shown to provide effective control against *V. destructor* (Henderson, 1988; Martin, 1994; Gregorc and Poklucar, 2003). However, the *Varroa* mites have evolved a resistance to many of these compounds (Floris *et al.*, 2004). Besides, these acaricides have significant drawbacks including contamination of honey; wax, and pollens (Calderone and Spivak, 1995).

Consequently, natural components might offer promising new avenues for the beekeeping industry. Many studies were carried out using some extracts of natural essential oil of various plants such as Rosemary, lemongrass, Camphor, Clove, Ginger roots, Fennel and Eucalyptus (Gregorc and Poklucar, 2003; Batish *et al.*, 2008), to minimize the pesticide residues in the colonies and the acaricide resistance (Wallner *et al.*, 1999). These products are generally inexpensive and had fewer health hazards to both man and honeybees (Melathopoulos *et al.*, 2000; Koumad and Berkani 2016). Therefore, further work is needed to assess the effects of different natural compounds on mites, bees and colonies.

This study was conducted to evaluate the use of different plant-derived oils, Spearmint (*Mentha spicata*), Lemongrass (*Cymbopogon citratus*), Thymol (*Thymus vulgaris*) & Tea tree oil (*Melaleuca alternifolia*), as alternative control methods that are safe, and environment friendly, against the parasitic mite, *V. destructor*.

### MATERIALS AND METHODS

This study was carried out in an apiary at Giza governorate in March and April 2015. Fifteen colonies of the honeybee, *A. mellifera* were identified as being infested with *V. destructor*. Each colony consisted of 10 full-depth combs of worker bees and a queen. The colonies were divided into five equal groups. The first and second groups were treated with one of the following substances, Thymol & Tea tree oil, respectively, while the third and fourth groups were treated with Spearmint oil & a mixture of Spearmint and Lemongrass, respectively, the fifth group served as control.

One application of the tested materials was applied weekly for four successive weeks (treatment period), using a white sticky paper (3x2 inches) saturated with 2 ml of each tested material & held between frames of each hive. Control colonies received the carrier only without any test material.

To monitor and capture *Varroa* populations before and after treatments for calculating the mite mortality, a corrugated plastic bottom boards were inserted to all hives's bottom prior to each treatment under the wire/wood frame, where falling mites were trapped and the wire kept bees from removing it (Sammataro *et al.*, 1998, Gregorc *et al.*, 2018). These boards were removed, replaced by new ones daily and the trapped mites were counted. The hive entrances kept open during the experiment and applications were carried out after sunset, when all honeybees had returned to the hives. The number of *Varroa* individuals that not killed by the treatments was also obtained by counting the *Varroa* drop-down after using formic acid (85%) for two consecutive weeks (treatment evaluation period).

The number of mites collected was recorded, the treatment efficiencies were estimated by comparing the number of *Varroa* that were killed before, and

then again after each application.

The efficiency of each application was determined according to the formula of Colin (1990)

$$X_n (\%) = \frac{A_n \times 100}{Z - Z_{n-1}}$$

$A_n$ : number of mites collected throughout the days following each application;  $Z$ : total number of collected mites [mites collected after 4 applications + remaining mites collected after treatment evaluation period]; and  $Z_{n-1}$ : number of collected mites before each application.

Data obtained in the *Varroa* control groups were analyzed statistically using analysis of variance (ANOVA) and means were separated by a least significant differences test in SAS.

## RESULTS AND DISCUSSION

In the present study, several natural oils were used for the control of *Varroa destructor*, as they are biodegradable, safe for human and relatively inexpensive.

(Table, 1) indicated that the mean number of mites collected from the colonies that received four applications of the Spearmint (during the treatment period) was significantly higher (228.3 mites) than those that have received mixture of Spearmint & Lemongrass, Tea tree oil and Thymol (152.6, 125 and 115.6 mites, respectively).

Essential oils of Spearmint have proven to be the most effective against *Varroa destructor* with a mean

efficiency percentage of (85.91±3.5), followed by the mixture of Spearmint, Lemongrass and tea tree & Thymol oils with an efficiency of (84.35±2.1%, 80.21±3.2% and 78.17±3.4%) compared with the control group (12.3±1.07%) at 5% significant levels.

Our data showed that all essential oils were effective against *Varroa*. The averages efficacies of four essential oils were at similar levels, but Thyme essential oil had a lower effect compared with the others (Fig. 1). ANOVA showed significant differences between treatments with essential (F = 119.78, P <0.0001)

Several authors have evaluated essential oils and their components as control agents for *Varroa* (Sammataro *et al.*, 1998; Imdorf *et al.*, 1999; Ariana *et al.*, 2002; Ismail *et al.*, 2006), and indicated that many of these compounds are biologically toxic to *Varroa* without injuring bees, due to the activation of the defense behavior mechanisms of honeybee workers by these plant oils against *Varroa* mite (Islam *et al.*, 2016).

In this study, the Spearmint oil was the most effective against *Varroa*, which in agreement with Ariana *et al.*, 2002; Koumad and Berkani, 2016, who concluded that the Spearmint oil was effective against *Varroa* mite with a good effectiveness, greater than 80%, but ineffective at low dose.

Also, Spearmint & Lemongrass mixture gave high *Varroa* mortality; similarly, Islam *et al.*, 2016 recorded 80.12% of mite mortality after Lemongrass treatment.

Table (1). Effect of different plant-derived oils against *V. destructor* in *A. mellifera* colonies.

Group	Colony No.	Number of dead/fallen mites		Percentage of treatment efficiency
		Total number of collected mites after the treatment period	Total number of mites in each colony	
Thymol	1	121	161	75.15
	2	84	113	74.33
	3	142	167	85.02
Mean ±SE		115.6±16.9	147±17.07	78.17±3.4 <sup>a</sup>
Tea tree oil	1	98	113	86.72
	2	140	184	76.08
	3	137	176	77.84
Mean ±Se		125±13.5	157.6±22.4	80.21±3.2 <sup>a</sup>
Spearmint	1	251	283	88.69
	2	187	237	78.90
	3	247	274	90.14
Mean ±SE		228.33±20.6	264.6±14.07	85.91±3.5 <sup>a</sup>
Spearmint & Lemongrass	1	129	146	88.35
	2	173	214	80.84
	3	156	186	83.87
Mean ±SE		152.6±12.8	182±19.7	84.35±2.1 <sup>a</sup>
Control	1	19	139	13.6
	2	9	88	10.2
	3	28	212	13.2
Mean ±SE		18±9.5	146.33±35.8	12.36±1.07 <sup>b</sup>
F-value = 119.78		P < 0.0001		

On the other hand, Thymol revealed a lower efficiency (78.1%) in reducing *Varroa* mite when compared to other oils in the present study. But, Ariana *et al.*, 2002 and May-Itza *et al.*, 2007 found that Thymol and Thymol gel could eliminate ~90% of the mites in honeybee colonies.

Similarly, several authors evaluated this compound as control agent for *Varroa destructor* mite (Mattila *et al.*, 2000; Emsen and Dodologlu, 2009). However, significant bee mortality was reported when powdered Thymol was used against *V. destructor*, which was probably a consequence of the high surface area of Thymol or high ambient temperatures (Chiesa, 1991 and Gal *et al.* 1992). Therefore, numerous factors contribute to the overall efficacy of acaricides such as the concentration of compounds, delivery method, ambient temperature, and colony and apiary environment (Calderone and Spivak, 1995).

Generally, using essential oils seems to be promising as the best control method for *Varroa* mite (present study and previous research), it occurs in nature, safe to environment, relatively inexpensive, and support the need to overcome resistance issues with currently registered acaricides. Therefore, further work is needed to assess the effects of these compounds on mites, bees and colonies.

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