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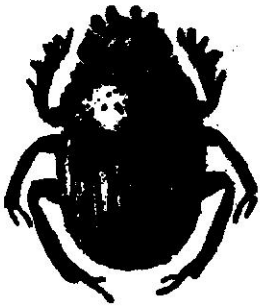

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CONTROL OF VARROA MITE IN HONEYBEE COLONIES USING BOTANICAL SMOKE AND ESSENTIAL OILS

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INTRODUCTION

Honey bees and beekeeping are important components of agriculture and considered the most active plant pollinators, provides 80% of all insect pollination for cultivated crops (Pimentel *et. al.*, 1997). An estimated one third of the human diet is directly or indirectly dependent on bee pollination (Delaplane and Mayer, 2000).

Varroa destructor Anderson & Truman, (Acari, Varroidae) is a haemolymph - sucking specialized group of mites, closely associated with bees in the genus Apis, which reproduce only within the sealed brood of honeybees (Martin, 2001). Infestation of *Apis mellifera* (Hymenoptera, Apidae) colonies, by the kin species *Varroa Jacobsoni* was first found in Philippines (De Jong *et al.*, 1982). During the mid-twentieth century Varroa mites have spread throughout most of the world (Martin, 2001). Varroa was registered in Egypt in 1983 according to (Wienands and Madel, 1988). The reproductive cycle of the Varroa mite is closlely associated with the cycle of honeybee development. As the mites develop and reproduce in workers and drone brood cells (Rosenkranz *et al.*, 2010) and spend part of their life cycle on nurse bees. Numerous of treatments which existed for controlling Varroa mite depended mainly on the application of chemical miticides (Abd Al-Fattah *et al.*,1991). However, the wide spread use, and over use of these chemicals resulted in the rapid development of chemical resistance in numerous mite population (Lodsawi *et al.*, 1995; Mathieu and Faucal, 2000 and Rodrı´guez-Dehaibes *et al.*, 2005). The rapid development of mites resistant to multiple classes of chemical pesticides emphasizes the need to develop alternative mite control tactics using natural plant derived acaricides with desirable qualities such as low cost, low toxicity to mammals, negligible environmental effect, and generally accepted by the public (Abou-El-Enain *et al.*, 2007). Therefore this work was carried out to evaluate the efficacy of some botanical sources in controlling Varroa mite.

MATERIAL AND METHODS

This work was conducted in the apiary of Agriculture Experimental Station, Faculty of Agriculture, Cairo University during autumn 2011 and spring 2012 by using 15 honeybee colonies headed by mated first hybrid carniolan queens.

A-Botanical smokes

Two materials of botanical smokes sources were tested, the first was mature red sumac seed (*Rhus coriaria*) homogenized and the second was the fresh leaves of eucalyptus plants, (*Cinnamomus camphora*). They used by handful in lighted smoker throughout 20 slow puffs of the smoker delivered for each brood chamber (Zawislak, 2008). Smoke treatments were administered using bee smoker and after the treatments occurred, the cover of the hive was immediately placed and the entrances of hives were not sealed, smokes were allowed to dissipate at its natural rate. This method might be used by individual beekeeper as a practical treatment. During autumn season 2011, smoke treatment was applied once every three days for three times while during spring 2012, the treatment was conducted for continuous 9 days.

B- Essential oils

Two main sources of essential oils were used in this study, the first was Mint oil (*Mentha piperita*) and the second was Eucalyptus oil (*Cinnamomus camphora*). Each treatment was prepared by mixing 50 g of pure commercial Vaseline with 5 cm^3 of concentrated commercial oils and placed in petri dishes under the brood combs (Espinosa- Montano & Guzman- Novoa, 2007). During autumn 2011, and spring, 2012 the study was conducted for 9 days respectively and the treatment of oil mix was renewed every 3 days placed under the brood combs of each colony the (bottom board).

C- Measuring Varroa mite drop

Sticky board traps were used to collect Varroa mites that fell from bees inside the hive. The trap that consists of adhesive contact paper covered with Vaseline was applied at the bottom of the hive with sticky side up. After treatment, each trap was carefully removed and account of all mites by visual inspection. The numbers of mites captured from treated colony were compared to those from control ones.

Evaluation of tested materials

The efficacy of tested materials and techniques were evaluated according to the Equation of Henderson & Tilton (1955) as follows:,

$$\% \text{ reduction of infestation} = 100 \text{ X } 1\text{- (Ta X Cb)/ (Tb/Ca)}$$

Where T = % infestation of treated mites.
C = % infestation of untreated mites.
A = after, b = before treatment.

The percentage of varroa infestation on sealed brood counted on 100 worker sealed brood cells. The selected cells were scratched and all adult stages of Varroa female mite in each cell were counted. The collected data were statistically analyzed. Treatment means were compared at 5% probability levels by LSD test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

A-Botanical smokes

Autumn 2011

Over the entire test, the daily mean number of mites caught on a sticky board traps for the smoke application of sumac seeds ranged from 0.5 to 10 with an average of 4.2 mites /col. The smoke with eucalyptus leaves gave a mean number of fallen Varroa ranged from 0 to 6.75 mites with an average of 2.28 mites/col. Using sumac seed smoke reach its peak after 24 hours of the second application 10 mites/ col. For Eucalyptus leaves smoke, the most effective peak occurred after 72 hours of the first and second treatments, (6.75 mite /col. for both), respectively (Table 1).

TABLE (I)

Mean no. of fallen varroa mite after three smoking application of sumac seeds and eucalyptus leaves during autumn, 2011 (Sep. 28 to Oct. 15) and spring 2012 (Mar. 14 to Apr. 6)

Fallen mites after / Smoke with	First treatment			Second treatment			Third treatment			Mean ±SD
	24h.	48h.	72h.	24h.	48h.	72h	24h.	48h.	72h.	
Autumn 2011										
Sumac seeds	3	0.75	5.75	10	3.5	4.5	8	2	0.5	4.2±3.0
Eucalyptus leaves	0.25	1	6.75	3	1.75	6.75	1	0	0	2.3±2.6
Control	2.5	3.5	2	3.5	0.5	1	0.5	0	1	1.6±1.2
Spring 2012										
Sumac seeds	5.3	5.7	3.2	5.5	2.7	2.5	2.5	3	3.3	3.7±1.3
Eucalyptus leaves	6.2	3	3	5	2.6	4.6	4.6	4	2.8	4.0±1.2
Control	3.5	4.5	3	4.5	1.5	1.5	1	0	1.5	2.3±1.5

Spring 2012

The daily mean number of fallen mites caught after the application of sumac seeds smokes ranged from 2.5 to 5.7 with an average of 3.7 mites/ col., while for Eucalyptus leaves smoke treatment, the results ranged from 2.6 to 6.2mites/col. with an average of 4 mites/trap. Using sumac seed smoke, peak appeared after 48 hours of the first application 5.7 mites/ col. For Eucalyptus leaves smoke the most effective peak occurred after 24 hours of the first treatment 6.2 mites /col. Generally the effect of sumac seeds and eucalyptus leaves smokes was more effective during autumn than spring (Table 1).

B- Essential oils:

Autumn 2011

Over the entire test, the daily mean number of mites caught on a sticky board traps for the application of mint oil was ranged from 1.7 to 10.8 mites with an average of 7.6 mites /col. Using eucalyptus oil gave a mean number of fallen Varroa ranged from 1.5 to 19.5 mites with an average of 11.7mites/col. The mint oil treatment reaches its peak after 48, 24 and 72hours of the first and second applications (10.8 mites/ col.). For Eucalyptus oil treatment the most effective peak occurred after 24 hours of the second application, (19.5 mites /col.) (Table 2).

TABLE (II)

Mean no. of fallen varroa mite after three essential oil application of mint and eucalyptus during autumn, 2011 (Sep. 28 to Oct. 15) and spring 2012 (Mar. 14 to Apr. 6)

Fallen mites after / Smoke with	First treatment			Second treatment			third treatment			Mean ±SD
	24h.	48h.	72h.	24h.	48h.	72h.	24h.	48h.	72h.	
Autumn 2011										
Mint oil	10	10.8	9.3	10.8	10.3	10.8	2.3	1.7	3	7.6±3.8
Eucalyptus oil	10.75	8.25	14.25	19.5	24	14.25	6	6.5	1.5	11.7±6.7
Control	2.5	3.5	2	3.5	0.5	1	0.5	0	1	1.6±1.2
Spring 2012										
Mint oil	15	12.7	7.7	14.7	7.7	7	9	5.7	6.3	9.5±3.4
Eucalyptus oil	3.3.	7.7	4	6.3	4	5.3	5.3	3.7	3	4.7±1.5
Control	3.5	4.5	3	4.5	1.5	1.5	1	0	1.5	2.3±1.5

Spring 2012

The daily mean numbers of fallen mites caught after the application of mint oil ranged from 5.7 to 15 with an average of 9.5 mites / col., while for Eucalyptus oil, the results ranged from 3 to 7.7mite with an average 4.7 mites/col. Using mint oil, peak appeared after 24 hours of the first application, (15 mites/ col.), while the most effective peak of Eucalyptus oil occurred after 48 hours of the first treatment (7.7 mites /col.) (Table 2).

Reduction of infestation

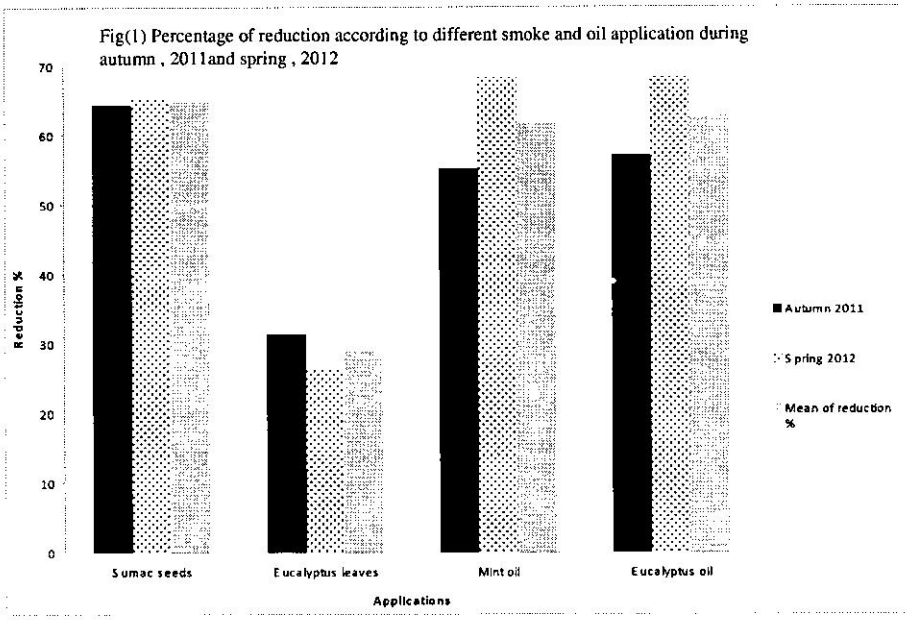
Using essential oils, especially eucalyptus oil (no. of fallen mites/col. 11.67 and 4.7 during autumn 2011 and spring 2012 respectively), were more effective than using botanical smokes.

The reduction of infection for botanical smoke treatment ranged from 64.3% to 65.3% for sumac seeds smoke during autumn 2011, and spring 2012.Using Eucalyptus leaves smoke resulted 31.4% and 26.3 % reduction in mite population during the previous seasons, respectively, table(3). The percentages of reduction of infestation for essential oils treatments were 55.1% and 57.1% during autumn 2011 for mint and eucalyptus oil, while during spring 2012 the reduction percentages were 68.3% for both treatments respectively (Table 3, fig. 1).

TABLE (III)

Percentage of redaction according to different smoke and oil methods during autumn 2011 and spring 2012

Treatment	Season						Mean reduction %/season
	Autumn 2011			Spring 2012			
	Pretreatment Infestation level	Post treatment infestation level	Reduction %	Pretreatment infestation level	Post treatment infestation level	Reduction %	
Sumac seeds	8	2	64.3	6.2	1.5	65.3	64.8
Eucalyptus leaves	12.5	6	31.4	6.2	3.2	26.3	28.9
Mint oil	11.7	3.7	55.1	6.0	1.3	68.3	61.7
Eucalyptus oil	7.5	2.25	57.1	6.0	1.3	68.3	62.7
Control	5	3.5		5	3.5		



Botanical smokes caused non-significant decreases in mite population under the condition of this test. According to previously published studies, the specific smoke can cause greater than 90% immediate knockdown of Varroa mite (Adams, 1997). The previously published reports were mainly from studies conducted on small groups of infested bees held in cages outside the hive. In this artificial situation, mites may be more prone to releasing from bees due to repellant effect of honeybee alarm pheromone (Kraus, 1990) or some other self-preservation survival instinct within the mite. However removing all bees from a hive for a smoke treatment is not a practical method for Varroa control. When brood present, mites tend to spend a longer periods inside the pupal cells than outside. The duration of the bees capped time is 12-15 days depending on caste. Newly emerged female mite will spend an average of 4-6 days (20-25% of their life) in phoretic behavior on adult bees before entering a cell (Woyke, 1987 and Donze *et al.*, 1998) in early autumn, while the queen continues laying a large number of eggs in a heavily mite infested colony, it can be assumed that newly matured mites will be emerging constantly from pupal cells.

Mites in cells could not be affected by smokes (Zawislak, 2008), but only those phoretic on bees would be affected. The emerging mites may be numerous enough to replace those lost during smoke treatments. Ordinary and more frequent smoke treatment during colony inspection all over the year may be necessary to combat constantly emerging mites which could be a good strategy that can suppress

mite population below a damaging level and considerable benefit to the bees. Using sumac seed smoke caused a high rate of mite knockdown than eucalyptus leaves smoke where it potentially delays the buildup of phoretic mite numbers, which will in turn, limit the number of mites entering brood cell to reproduce.

A final consideration is that sumac seeds smoke could be used as a normal beekeeping practice during manipulating colonies.

Varroa infested colonies treated with essential oils showed noticeable reduction in population. Mint and eucalyptus oils have been found more effective during autumn and spring. The overall strategy of the experiment was to assess short term control of Varroa population and attempt to suppress Varroa mite population. Using oil mixed with Vaseline at the bottom brood chambers resulted in reduction of infestation level ranged from 56.12% to 68.3% during autumn and spring respectively. This may be due to the high temperature during autumn and low temperature during spring.

SUMMARY

The efficacy of using two different application methods (smoking and essential oils) on reduction of Varroa mite infestation level in honeybee colonies were studied at the apiary of Agriculture Experimental Station, Faculty of Agriculture, Cairo University throughout autumn, 2011 and spring 2012.

The obtained results showed that using essential oils were more effective than using botanical smokes as general conclusion, but the most effective application was using sumac seeds smokes which resulted 64.8% (mean reduction percent during autumn, 2011 and spring, 2012) and followed by Eucalyptus oil (62.7%), mint oil (61.7%) and finally the Eucalyptus leaves smoke that resulted the lowest percent of reduction (28.9%).

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