Rearing honey bee colonies in laboratory for control experiments against Varroa mite in Egypt

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ABSTRACT— Rearing honey bee colonies in the laboratory in Dept. of Zool. , Fac. of Agric. ,Cairo Univ. by using of Apiguard , formic acid and sulfur to control varroa mite. Rate of efficacy %, number of dead mites on white card board, number of varroa mite on 100 honey bee worker, number of varroa on 20 brood cells were recorded and codified to compare among three treatments. These treatments were repeated weekly for 8 times through the experiment. Formic acid and sulfur preparations were saturated in cardboard strips and one strip was put in each colony. The acaricide Apiguard was used of 50 gel upper Frames of each colony. It was found that the efficiency of formic acid was the superior treatment followed by Apiguard then sulfur, being 92.4, 86.3, 76.9% efficiency, respectively.

A lot of advantages through rearing honey bee colonies in laboratory are promising to rear honey bee colonies in desert agriculture farming systems, especially in production of strawberry in mass production for exportation , in addition to gardens, roof garden, and in the open field to increase honey and crop pollination. This method protects bees from environmental changes, especially in cold winter as well as hot summer months from direct harmful. Also, it protects bees from the hazard of pesticides. Drafting, where they can close the chamber dissolved without closing the bee colonies and thus prevent the increase in the accumulation of water vapor and carbon dioxide ratio inside bee colonies as well as prevent global climate changes.

Key words: Honey bee, rearing, Varroa mite, control, indoor apiaries, Apiguard , formic acid , micronized sulfur .

1 INTRODUCTION

Honeybees are the most important insects that have benefited mankind for medicinal and nutritional purposes for thousand of years. Honeybees are of great economic importance to agriculture not only for honey production, but also for crop pollination. The ectoparasitic mite varroa destructor (Anderson and Trueman, 2000) is considered a severe pest for honey bees causing serious losses to the beekeepers (De Jong *et al*., 1982 and Baker and Peng, 1995). The most beneficial of all honey bees insect species (*Apis* spp.) are perhaps due to produce honey, royal jelly, pollen , propolis as well as for crop pollination . Honey bees products are well known possess great value for their use in the pharmaceuticals , food production and other industrial products (Wakhal *et al*.,1999).

Recent declines in many of these pollinators have been blamed on land-use changes, diseases, chemicals and climate change (Mullin, 2010). *Apis mallifera*, are the most exploited of all pollinators Produce such as watermelons, berries, nuts (mainly almonds), and many other fruits and vegetables need proper pollination in order to bear fruit and ensure larger yields.

Varroa destructor (Anderson and Trueman, 2000) is a common devastative parasite of honeybees, seriously affecting the industry. It also indirectly affects crop production by reducing numbers of bees required for crop pollination. The control of Varroa mite is especially difficult as the majority of mites live inside the sealed brood for reproduction and are well protected from different methods of control (Hoppe *et al*., 1989 and Tiwari *et al.,* 2014). Also varroa feed on the haemolymph of developing and adult bees. This infection results in transmission of secondary diseases such as virus diseases (Ball and Allen, 1988; Yang and Cox-Foster, 2007; Dainat *et al*., 2011). Acaricides are effective against varroa mite, but their application within the hives usually contaminates bees wax and honey. In addition, the Varroa mite can develop resistance to these chemicals (Ward *et al*., 2008). There is an urgent need, for alternative, sustainable forms of varroa management. In addition , *V. destructor* is considered to be the major pest of honey bees since it spread to *A. mellifera*. varroa mites shifted from their natural host, the Eastern honey bee *Apis cerana,* to the Western honey bee *A. mellifera,* about 70 years ago, after *A. mellifera* was introduced into the native range of *A. cerana* (Rosenkranz *et al*., 2010). Since then, commercial transportation of colonies and natural spread have been resulted in a global distribution of *V. destructor*, which has had dramatic consequences for both managed and wild populations of *A. mellifera*.

Under Egyptian conditions, during the last few decades many complaints have been received from honey bees beekeeping due to the harmful effect of varroa mite on adult honey bees and broods. Recent studies have been confirmed these complains , where its substantial contribution to honey bee losses across the Northern hemisphere (El- Shemy, 1997; Allam, 1999 ; Abd El-Halim, *et al.,* 2006 ;Abdel Rahman and Rateb, 2008 and Allam and Zakaria, 2009). In addition , *V. destructor* can reproduce on both male and female brood of *A. mellifera*, thus attaining a longer reproductive season and larger mite populations. With larger numbers of mites in a colony, a greater proportion of bees and larvae are affected. Without treatment, a colony of *A. mellifera* infested with *V. destructor* dies within one to three years (Korpela *et al*., 1992 and Fries *et al*., 2006).

This work aims to evaluate the efficiency of Apiguard , formic acid and micronized sulpur to manage Varroa mite on honey bees rear in bee house in the laboratory as a new culture control against varroa and prevent climate changes. Also, assessment

the effect of Apiguard , formic acid and micronized sulpur on the colony strength. Moreover, studying the effect of rearing honey bees in the laboratory on the colony strength.

2 MATERIALS AND METHODS

Bee house measurements :

Bee house was built in laboratory in Dept. of Agric. Zool. in basements of Plant Protec. building Fac. of Agric. , Cairo Univ. Roll polestar and plastic wire were used to close a portion from the laboratory to make bee house in 360 cm width, 432 cm length and 360 cm height. In this bee house there are no tubes for exiting bees ,where exiting from two windows (115 cm width and 95 cm length) on height of 190 cm from the ground of the laboratory.

Colonies in bee house:

Twelve colonies were put in the bee house. Colonies nearly similar in their strength and headed by hybrid Carniolan queens (*A. mellifera carnica).*

Desgin of control experment :

The experiment was carried out during Septamber10 to October30, 2014 to evaluate the efficiency of Apiguard (thymol), formic acid and micronized sulfur on the controlling varroa mites infected bee colonies in a bee house. Apiguard was added to the colony by peeled back the lid of the tray and placed , gel side up on top of the brood frames. Enough space for the bees to get into the tray was taken into consideration then the hive closed . Strips of carton paper (8 x 25 cm) saturated by formic acid were put between each two frames .Micronized sulfur was sprayed at 0.25 % concentration on all component of the colony . These treatments were replicated for 8 times with one week interval. white cardboard (coated with vaseline) located under the colony combs to stick of the fallen varroa. The number of the fallen varroa was counted each week .

Assessment of the efficacy levels:

The infestation levels in all experiments colonies were determined before and after each application. The following data were recorded:

- Number of dead mites fallen down on a white card board (coated with Vaseline) located under the colony.
- Number of mites in random sample of approximately 100 live bees.
- Number of mites in 20 cells of each worker and drone brood (if available) or in 20 workers cells if drone brood was not available.
- The area of sealed worker brood (colony strength).

The efficacy of the treatments was calculated by using the following formula (Allam *et al.*, 2003 and Marinelli *et al.*, 2004) .

$$\text{Rate of efficacy \%} = \frac{\text{No. of dead mites*}}{\text{Total No. of mites **}} \times 100$$

* Dropped mites due to treatment +natural mortality

** Dropped mites + No. of mites on 100 live bees + No. of mites in 20 brood cells.

Estimation of colony strength:

The effect of the tested materials, *i.e.* Apiguard, formic acid and micronized sulfur on the strength of the colonies was assessed by counting the covered frames by adult worker bees. In this, respect , the strength of each colony was measured along the period of the experiment(Septamber10 to October 30,2014) and recorded . Also, the effect of rearing honey bee in bee house on colony strength was estimated in comparison with rearing in out door during Septamber, 2014 to August, 2015.

Statistical analysis:

Data were statistically analyzed and treatments were determined according to Duncan's multiple range test (Duncan, 1955).

3 RESULTS AND DISCUSSION

Data listed in Table (1) show that the number of dead mites on white cardboard was ranged between 25.7-96, 41-101, 8.7-53 with the average of 65.5, 73.4, 24.6 mites/colony/week in case of using Apiguard , formic acid and micronized sulfur, respectively.

the number of alive varroa mite on broods was ranged between 3.7-11.7, 1-7.7, 2.7-8 with the average of.5, 3.3, 5.2 mites/20 brood cells/colony/week in case of using Apiguard , formic acid and micronized sulfur, respectively.

The number of alive varroa mite on adult bees was ranged between 2-5.7, 0.7-9, 0.0-5 with the average of 3.9, 2.7, 2.2 mites/100 adult bees/colony/week in case of using Apiguard , formic acid and micronized sulfur respectively.

The number of alive varroa mite on the broods was greatly higher in the control treatment in comparison with the three tested treatments ,*i.e.* Apiguard , formic acid and micronized sulphur, being 11.4, 6.4 , 3.3 and 5.2 , respectively. The same trend was observed in case of the alive varroa on bees, being 16.7, 6.9, 2.7 and 2.2, respectively.

High numbers in control in comparison with the three treatments reflected that the success of Apiguard , formic acid and micronized sulphur, in controlling varroa mite on adult worker bees and in brood cells.

There were significant differences among the eight treatments of Apiguard, formic acid, and sulfur in number of dead varroa mite. This result indicated the importance of the eight treatments, because the data were significantly differed from one treatment to another. Whereas data in control did not significantly differed as in three tested materials.

Number of alive varroa in worker brood were significantly differed due to the treatment with Apigaurd, formic acid, and sulfur every two weeks and in the control every week . These result recommend taking of the sample from worker brood every two weeks, especially in unfavorable weather conditions . On the other hand, number of alive varroa mite on adult bees was not significantly differed weekly but monthly.

The hemophagous honey bee mite *Varroa destructor* is still the greatest threat for apiculture. No other pathogen has had a comparable impact on both beekeeping and honey bee research during the long history of apiculture.

The obtained data revealed that Apiguard , formic acid and micronized sulfur resulted in increasing the number of dead varroa mites 8 weeks after treatment compared with control treatment. In addition, the number of dead mites was decreased, in most cases, gradually by increasing the period after the treatment. In addition, formic acid was the superior treatment for increasing the number of dead varroa mites 8 weeks after treatment followed by Apigurad then micronized sulsphur. The number of alive varroa mite on the broods was greatly higher in the control treatment in comparison with the three tested treatments. The same trend was observed in case of the alive Varroa mite on bees.

Many researchers used these materials in controlling varroa mites on honey bee colonies (El-Shaarawy, 1999 ;Mattila, and Otis ,1999 ; Trouiller, 2004 ; Amrine and Noel, 2007 and Rashid, *et al*., 2011 and 2012). However, Amrine and Noel (2007) reported that varroa mite has not yet shown resistance to formic acid, and researchers are not sure how the compound actually kills mites. although formic acid is effective (70- 80%), but it is not as effective as synthetic chemicals such as fluvalinate. He added that success depends on the amount used, the strength of the colony, and the ambient temperature . rates of dead mites are obtained when outside temperatures are high enough to achieve good evaporation.

Also, Hooper (2005)) used the spray application method to test two colloidal sulfur formulations in Field tests. Results were very good, with an average 52% mite drop compared to 26% for the control over a three-day treatment period. These data agree with our result however found that mean number of rate of efficiency % was 76.9% in compared to 41.7 % for the control over eight-weekly treatment period.

During the acute phase, it will not only want to control varroa mite in the hives, but will also want to reduce mite invasion. Choosing a method that offers control for an extended period of time is therefore important. Both formic acid and Apiguard provide very good mite control and also offer protection over six to eight weeks.

After applying a mite control, it is important to sample some of the colonies again to make sure mite populations have been reduced to low levels , where if mite numbers are still high, it is needed to re-apply a control, even if this means removing the honey from the hives. These data are in agreement with those obtained by Goodwin(2004) how reported that traditional "*Varroa* miticides", the mode of action for thymol causes the disruption of cell membranes and processes of mites in a general way instead of being highly specific to nerve channels. This means that pesticide resistance is less likely. Apiguard is a gel formulation of thymol designed to be easy to apply, while at the same time providing a more controlled release of vapours than other methods.. The product could be used in the autumn , which Goodwin(2004) also ,recommended that formic acid (systematically called methanolic acid ; organic acid) is the simplest carboxylic acid. It is an important intermediate in chemical synthesis and occurs naturally, most famously in the venom of bee and ant stings. formic acid (65%) Formic acid is a fumigant that kills Varroa mite by respiratory inhibition. Methods of application concentrate on ensuring high levels of formic acid vapour are present in the colony for various periods of time. The chemical is generally applied in the late summer and/or spring.

It has been found that Apiguard , formic acid and micronized sulfur resulted in considerable increase in the colony strength compared with control treatment. In most cases, colony strength was gradually increased by lengthened the period of treatment . In addition, formic acid was the most efficient in this regard followed by Apigurad then micronized sulfur.

In general, keeping the colonies in the bee house is very useful for scabbing from the unfavorable environmental conditions such as frost, low and high temperature as well as scabbing from the attacking of wasp (*Vespa orientales*) beginning from mid of August of each year .This procedure made the colonies retains their strength through the most period of the year .

The obtained data revealed that colony strength was better under bee house conditions during autumn and winter months than that of out door conditions.. Meanwhile, it was better under out door conditions during spring and summer months , with exception of July and August of 2015 due to the high arise in the temperature and relative humidity.

The increase in colony strength under bee house conditions during autumn and winter months than that of out door conditions may be due to the warm weather under bee house conditions than that under out door conditions. On the other hand, colony strength was the best under out door conditions than that under bee house conditions during spring and summer months may be due to the lacking of sunshine under bee house conditions . However, it worth to mentioned that the lowering in colony strength under out door conditions through July and August of 2015 may be due to the high temperature and relative humidity than the mean of them in the previous years. Also, it was noticed that the queen put the egg in the near part of the frames from the windows of the bee house . This result give a reason to make a window in the bee house to interring sunshine to encouraging the queen to put the egg in hole frame.

The advantages or rearing honey bees in bee houses is to rear honey bee colonies in desert agriculture farming systems, especially in production of strawberry in mass production for exportation , in addition to gardens, roof garden, and in the open field to increase honey and crop pollination. This method protects bees from environmental changes, especially in cold winter as well as hot summer months from direct harmful. Also, it protects bees from the hazard of pesticides.

Table (1). Rate of efficacy % of Apiguard , formic acid and micronized sulphr controlling honey bee mite *V. destructor* during Septamber 11 to October 30,2014.

Periods (week)	Treatments											
	Apiguard			Formic acid			Micronized sulphr			Control		
	n.d.v.m*	n.v.v.b**	n.v.m.ab***	n.d.v.m	n.v.m.b	n.v.m.ab	n.d.v.m	n.v.m.b	n.v.m.ab	n.d.v.m	n.v.m.b	n.v.m.ab
1	96.0 ^{a}	11.7 ^{a}	5.7 ^{a}	101.0 ^{a}	7.7 ^{a}	9.0 ^{a}	46.7 ^{a}	8.0 ^{a}	5.0 ^{a}	30.0 ^{a}	12.0 $^{a\,b}$	19.0 ^{b}
2	88.7 ^{b}	9.7 $^{a\,b}$	5.7 ^{a}	97.0 ^{b}	5.0 ^{b}	5.0 ^{b}	53.0 ^{b}	7.0 ^{a}	3.7 ^{b}	27.7 ^{b}	13.7 ^{a}	21.0 ^{a}
3	84.0 ^{c}	7.7 ^{b}	4.7 ^{a}	90.7 ^{c}	4.0 ^{b}	2.0 ^{c}	29.0 ^{c}	6.0 ^{b}	2.7 ^{b}	25.0 ^{c}	9.7 ^{c}	12.7 ^{c}
4	77.7 ^{d}	5.7 ^{c}	4.7 ^{a}	87.7 ^{d}	2.7 ^{c}	0.7 ^{c}	23.7 ^{d}	6.0 ^{b}	3.0 ^{b}	22.0 ^{d}	1.0 ^{d}	18.7 ^{b}
5	64.0 ^{e}	4.7 $^{c\,d}$	3.0 ^{b}	73.0 ^{e}	2.0 $^{c\,d}$	1.7 ^{c}	14.7 ^{e}	5.7 ^{b}	1.3 $^{b\,c}$	17.0 ^{e}	13.0 ^{a}	21.0 ^{a}
6	47.0 ^{f}	3.7 ^{d}	3.0 ^{b}	51.3 ^{f}	2.3 ^{c}	1.0 ^{c}	11.0 ^{f}	3.7 ^{c}	0.7 ^{c}	15.0 ^{f}	8.0 ^{c}	14.7 ^{c}
7	40.7 ^{g}	4.7 ^{cd}	2.0 ^{b}	45.7 ^{g}	1.0 ^{d}	1.3 ^{c}	9.7 ^{f}	2.7 ^{c}	1.0 ^{c}	12.7 ^{g}	11.0 $^{a\,b}$	13.7 ^{c}
8	25.7 ^{h}	3.7 ^{d}	2.7 ^{b}	41.0 ^{h}	1.3 ^{d}	0.7 ^{c}	8.7 ^{g}	2.7 ^{c}	0.0 ^{c}	11.7 ^{g}	11.0 $^{a\,b}$	13.0 ^{c}
Average	65.5	6.5	3.9	73.4	3.3	2.7	24.6	5.2	2.2	20.1	11.4	16.7
R of E%	86.3			92.4			76.9			41.7		

* = No. of dead varroa mite , ** = No. of alive varroa mite on worker brood and *** = No. of alive varroa mite on adult bees . Duncan multiple range significant at Alpha (0.05). Means with the same letter are not significantly different. a,b,c,d,e.f.g,h., values in the same column with different superscripts differed significantly.

Tabe (2). Effect of Apiguard , formic acid and micronized sulfur on colony strength due to controllinghoney bee mite *V.destructor* during Septamber, 10 to October, 30,2014.

Periods (week)	Colony strength due to treatment with			
	Apiguard	Formic acid	Micronized sulfur	Control
1	7.7 ^{a}	8.3 ^{a}	7.3 ^{a}	6.7 ^{a}
2	8.5 ^{a}	9.0 ^{a}	7.7 ^{a}	6.7 ^{a}
3	7.3 ^{a}	8.7 ^{a}	7.2 ^{a}	6.8 ^{a}
4	7.2 $^{a\,b}$	8.7 ^{a}	7.0 ^{a}	6.0 ^{a}
5	6.8 ^{b}	6.8 ^{b}	5.5 ^{b}	4.6 ^{b}
6	6.8 ^{b}	7.0 $^{b\,c}$	6.0 ^{b}	5.3 ^{b}
7	6.0 $^{b\,c}$	7.3 $^{b\,c}$	6.3 ^{b}	5.8 ^{b}
8	5.7 ^{c}	6.0 $^{b\,c}$	5.7 ^{b}	5.5 ^{b}
Average	6.9	7.7	6.6	5.9

Duncan multiple range significant at Alpha (0.05).
Means with the same letter are not significantly different.
a,b,c., values in the same column with different superscript it differed significantly**.**

Tabe (3). Effect of bee house on colony strength during Septamber,2014 to August,2015.

Periods (Month)	Colony strength	
	Out door	Bee house
September	5.7 ^{c}	7.5 ^{a}
October	4.3 ^{c}	6.1 $^{a\,b}$
November	3.7 ^{d}	5.0 $^{b\,c}$
December	3.3 ^{d}	4.4 ^{c}
January	3.1 ^{d}	4.8 ^{c}
February	4.6 ^{c}	5.3 $^{b\,c}$
March	6.4 ^{b}	6.4 ^{a}
April	7.8 ^{a}	7.0 ^{a}
May	8.5 ^{a}	7.1 ^{a}
June	8.7 ^{a}	7.2 ^{a}
July	6.5 ^{b}	7.0 ^{a}
August	4.2 $^{c\,d}$	7.2 ^{a}
Average	5.5	6.25

Duncan multiple range significant at Alpha (0.05).). Means with the same letter are not significantly different. a,b,c.d, values in the same column with different superscript it differed significantly**.**

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