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Hygienic Behavior of Honeybee (*Apis mellifera* L.) and Efficiency of Volatile Oils against *Varroa destructor*

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ABSTRACT

The study was carried out at the apiary of Department of Plant Protection Faculty of Agriculture Al-Azhar University, Cairo, Egypt, to determine the effects of diurnal and nocturnal periods on hygienic behavior of Carniolan F_1 and Italian F_1 hybrids colonies under Nasr City environmental conditions, and to evaluate the using of plant oils against *Varroa destructor* mite. Hygienic behavior in honeybee *Apis mellifera,* is measured by determining the rate of the bees uncap and remove dead sealed brood. Significant differences between hygienic behavior during diurnal and nocturnal activities, as well as between hybrids were found. The average of hygienic percentages at diurnal of Italian F_1 hybrid colonies were 63.33, 82.67, and 85.33 % and for Carniolan F_1 hybrid colonies were 45.33, 59.67, and 77.33 % after 12, 18 and 24 hrs, respectively. The average of hygienic percentages at nocturnal of Italian F_1 hybrid colonies were 93.00, 98.00, and 100.00 % and for Carniolan F_1 hybrid colonies were 67.00, 83.67, and 95.00 % after 12, 18 and 24 hrs, respectively. The highest mean numbers of fallen mites/colony (39.67 mites) were obtained from colonies treated with olive oil, followed by 35.00 mites from colonies treated with Mitac, then 27.00 mites from colonies treated with mustard oil. It could be advisable to use plant oils to control *Varroa* mite infesting honeybee colonies to avoid the appearance of mite resistant strains and products pollution.

INTRODUCTION

A standard field assay was used to select colonies for hygienic behavior by freeze-killing approximately 200 cells of wax-capped pupae and recoding the time, it took for the bees in the colony to detect, uncap and remove the dead brood (Spivak and Downey, 1998).

The efficiency of hygienic behavior was evaluated in hygienic and non-hygienic races of bees using two types of combs (new and old), as well as at different periods of the day (night and day) (Pereira *et al.,* 2013).

Behavior studies of social insects, such as ants and honeybees, have revealed an elaborate system of division of labor (Gordon, 1996). Bees have also been observed removing sick or dead brood, which has been named hygienic behavior. Hygienic behavior of a bee is defined as the ability to detected and uncap cells with dead or diseased brood and remove it from the nest (Goncalves *et al.*, 1970). Hygienic behavior is consider a major mechanism of resistance against parasites and pathogens (Spivak and Downey, 1998).

Honeybees that have the genetic predisposition to perform hygienic behavior are characterized by their ability to detect, uncap and remove diseased and mite-parasitized brood from the nest, limiting disease transmission and reducing the reproductive success of the parasitic mite *varroa destructor* (Spivak and Reuter, 2001).

Alternative control strategies including the use of natural products such as organic acids, plant extracts and essential oils against *V. destructor* have been developed. In the last decade, several studies throughout the world have confirmed the use of formic acid (Thomas, 1997), oxalic acid (Nanetti *et al.,* 2003), thymol and eucalyptol (Melathopoulos and Gates, 2003) in *Varroa* control.

A worker bee can groom all the mites stuck to its body by its legs and jaws; otherwise, it does some special movements to attract other bees attention to make clean up the parasites from its body (Peng, *et al.,* 1987). The death of the colonies depends up on the mite density (Garedew *et al*., 2004), which can be controlled by the application of several rather effective acaricides (Ritter, 1990). However, the use of these agents has important disadvantages as their residues contaminate bee products like honey, wax, propolis and royal jelly (Greef *et al*., 1994). The combatting *Varroa* mites by plant oils alone or in an IBM program showed significantly effective against *Varroa* in treated colonies compared to untreated ones (Ismail *et al.,* 2006).

The present study aimed to investigate the hygienic behavior of two races of honeybees during diurnal and nocturnal periods, and evaluate using of plant oils against *V. destructor* mite.

MATERIALS AND METHODS

The experiments were performed at the apiary of the Department of Plant Protection Faculty of Agriculture Al-Azhar University, Cairo, Egypt. Six Italian hybrid (F_1) and 6 Carniolan hybrid (F_1) honeybee colonies in the same strength (brood, adult bees and stored food) headed by young sister open mated queens were subjected to this experiment. Three colonies of each hybrid for diurnal and 3 colonies for nocturnal periods. Hygienic behavior was determined by using pin-killing 100 caped worker brood cells and introduced to experimental colonies at the day for the diurnal and at the night for the nocturnal colonies. The diurnal colonies started at 0600 hrs, and the removed dead brood were counted after 6.00, 12.00 and 24.00 hrs. The nocturnal colonies started at 1800 hrs and the removed dead brood were recorded after 12.00, 18.00 and 24.00 hrs.

Eighteen Carniolan F_1 hybrid honeybee colonies in the same strength (brood, adult bees and stored food) headed by young sister open mated queens were divided into 6 groups of 3 replicates for each group. All colonies. The first group was treated by Mitac, while the 2^{nd}, 3^{rd}, 4^{th} and 5^{th} groups were treated by Olive oil, Mustard oil, Cinnamon oil and Mentha oil, respectively, whereas the 6^{th} group was untreated (control). A cardboard (4×4×0.4 cm) impregnated with 2.5 ml of each oil and Mitac were placed on top of the combs. A white paper sheet (51.5×36.5 cm) coated with thin vaseline layer was put on the bottom board under the brood combs of each colony to capture the fallen *varroa* mites and checked daily for the presence of dead mites which were counted and recorded (Ritter *et al.,* 1989; Milani, 1990). This procedure was repeated 1, 2 and 3 days after application.

Statistical analysis:

Data collected were statistically analyzed and the treatment means were compared at 5% probability levels by LSD test (SPSS software ver. 20 for windows 7 following the methods of (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Data presented in Table (1) and Figure (1) showed that, there were significant differences between hygienic behavior during diurnal and nocturnal periods, and between Italian F_1 and Carniolan F_1 hybrids. The average of hygienic percentages at diurnal of Italian F_1 hybrid were 63.33, 82.67 % and for Carniolan F_1 hybrid colonies were 85.33 and 45.33, 59.67 or 77.33 % after 12, 18 or 24 hrs, respectively. The average of hygienic percentages at nocturnal of Italian F_1 hybrid were 93.00, 98.00 or 100.00 % and for Carniolan F_1 hybrid colonies were 67.00, 83.67, 95.00 % after 12, 18 or 24 hrs, respectively. The superiority of nocturnal hygienic may be due to the crowdedness of bees in hives during the night as a result of stopped the outside activities.

Data presented in Table (2) showed significant differences between plant oils in controlling *Varroa* mite. The highest mean numbers of fallen mites/colony (39.67 mites) were obtained from colonies treated with olive oil, followed by 35.00 mites from colonies treated with Mitac, then 27.00 mites from colonies treated with mustard oil. The differences between Mitac and each of olive and mustard oil were non-significant, while the differences among Mitac, olive oil, and each of cinnamon oil, Mentha oil and control, were significant. On the other hand the differences between menthe oil and control were non-significant. Controlling *Varroa* mites using plant oils alone or in an IBM program showed significantly effective against *Varroa* in treated colonies compared to untreated ones (Ismail*et al.,* 2006).

Data illustrated graphically in Fig. (2) showed that the highest numbers of fallen *Varroa* mites (18.33 mites) were obtained after 24 hrs of treated with olive oil, followed by 16.35 mites after 24 hrs of treated with Mitac, then 11.67 mites after 24 hrs of treated with mustard oil.

Varroa mite is controlled chemically (acaricides), but this technique results in the contamination of hive products with their residues (Peter, 1999 and Sammataro *et al*., 2004). In addition, these chemicals lead to occurrence of developed *Varroa* resistant strains (El-Zen *et al*., 1999). It is better to use natural materials in controlling *Varroa* mite and avoid using pesticides in colonies of honeybees (Manosur *et al*., 2007).

It can be concluded that, based on the obtained results, it is advisable to use olive oil or mustard oil to control *Varroa* mite infesting honeybee colonies to avoid the appearance of mite resistant strains and products pollution.

Table 1. Effect of diurnal and nocturnal periods on hygienic behavior percentages of Italian F_1 and Carniolan F_1 hybrids under Nasr city conditions, Cairo, Egypt.

Treatments Time (hrs.)	Italian F_1 hybrid						Carniolan F_1 hybrid					
	Diurnal			Nocturnal			Diurnal			Nocturnal		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
12	59	62	69	90	96	93	55	39	42	77	64	60
18	78	82	88	100	99	95	75	50	54	87	84	80
24	80	85	91	100	100	100	80	75	77	100	85	100
Mean		77.11 b			97.00 a			60.78 B			81.89 A	
Mean				87.06 a						71.33 b		

Data followed by the same litters are not significantly different at 5 % by Duncan, 1955.

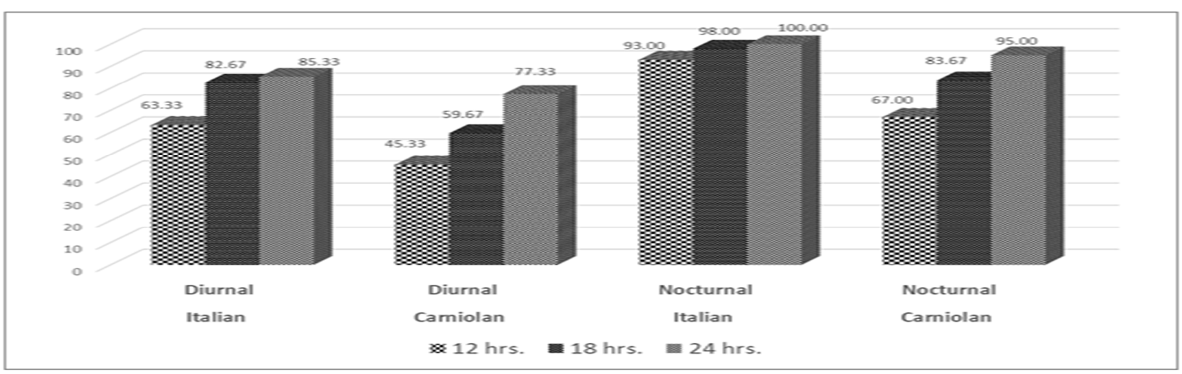

Fig 1. Effect of diurnal and nocturnal periods on hygienic behavior percentages of Italian F_1 and Carniolan F_1 hybrids under Nasr city conditions, Cairo, Egypt.

Table 2. Effect of Mitac and plant oils against *Varroa* mite on honeybee under Nasr city conditions, Cairo, Egypt.

Treatments	Mitac			Olive oil			Mustard oil			Cinnamon oil			Mentha oil			Control		
Time (hrs.)	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
24	17	15	17	17	19	19	11	13	11	09	10	08	05	04	04	01	01	02
48	13	11	12	11	13	12	08	10	11	07	08	09	03	02	03	01	02	01
72	07	06	07	08	11	09	05	06	06	04	05	03	02	03	02	02	01	01
Total	37	32	36	36	43	40	24	29	28	20	23	20	10	9	9	4	4	4
Mean		35.00 ab			39.67 a			27.00 bc			21.00 c			9.33 d			4.00 d	

Data followed by the same litters are not significantly different at 5 % by Duncan, 1955.

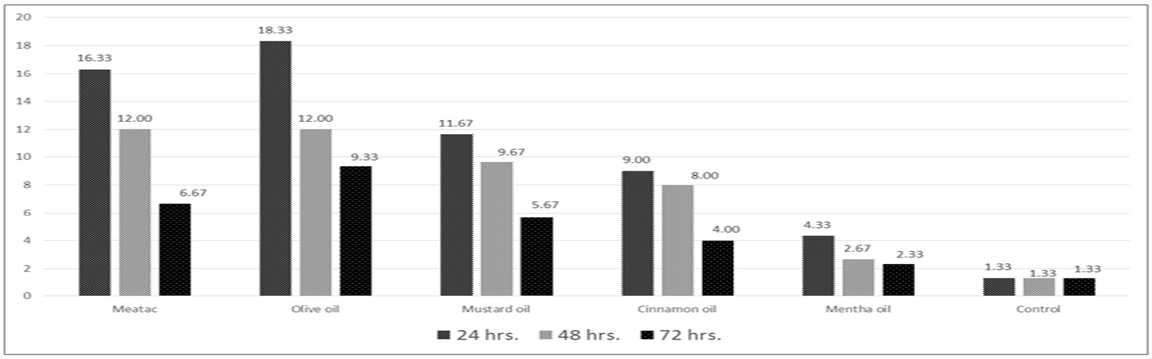

Fig 2. Effect of Mitac and plant oils against *Varroa* mite on honeybee at different perods under Nasr city conditions, Cairo, Egypt.

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دراسات على سلوك التنظيف ومكافحة الفاروا بإستخدام الزيوت النباتية على طوائف نحل العسل تحت ظروف مدينة نصر – القاهرة – مصر

جمعه فتح الله أبولبن
قسم وقاية النبات - كلية الزراعة - جامعة الأزهر - القاهرة.

أجريت هذه الدراسة في منحل كلية الزراعة – جامعة الأزهر بالقاهرة مدينة نصر خلال العام (٢٠١٧م) استخدمت فيه طوائف نحل كرينولى وإيطالي هجين أول . قسم هذا العمل الى تجربتين: التجربة الأولى كانت عبارة عن دراسة تأثير فترات الليل والنهار على سلوك التنظيف فى طوائف نحل العسل لكل من الهجين الأول الإيطالي والكرنيولى حيث إتضح من النتائج أن متوسط نسبة سلوك التنظيف أثناء فترات النهار لكل من الهجين الأول الإيطالي والكرنيولى كانت (٦٣.٣٣و ٨٢.٦٧و ٨٥.٣٣و – ٤٥.٣٣و ٥٩.٦٧و ٧٧.٣٣) بعد ١٢ – ١٨ – ٢٤ ساعة على التوالي بينما كان متوسط نسبة سلوك التنظيف اثناء فترات الليل لكل من الهجين الأول الإيطالي والكرنيولى كانت (٩٣.٠٠و٨٩.٠٠ و١٠٠.٠٠– ٦٧.٠٠و ٨٣.٦٧و ٩٥.٠٠) بعد ١٢ – ١٨ – ٢٤ ساعة على التوالي لكل من الهجين الأول الإيطالي والكرنيولى. أما التجربة الثانية فتم فيها دراسة تأثير الأنواع المختلفة من الزيوت النباتية والميتاك على أعداد الأفراد المتساقطة من الأكاروسات الكاملة لطفيل الفاروا فى طوائف نحل العسل مقارنة بالكنترول : حيث إتضح من النتائج ان متوسط عدد الأفراد المتساقطة كان (٣٥.٠٠و ٣٩.٦٧و ٢٧.٠٠و٢١.٠٠و٣٣.٩و ٤,٠٠) فرد/ طائفة لكل من الميتاك وزيت الزيتون الخردل والقرفة والنعناع ثم الكنترول على التوالى .كما أظهرت النتائج بأن الاستجابة لتأثير هذه المعاملات كان مرتفعاً بعد ٢٤ساعة ثم ٤٨ساعة وأقلهم بعد ٧٢ساعة وقد أثبت التحليل الإحصائى للنتائج وجود فروق معنوية.