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Behavioural Resistance of Libyan Honeybee against Varroa mite: Preliminary Study

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Abstract: ***This study was designed to evaluate the behavioural resistance of Libyan honeybee. A freeze-killed brood assay was used to explore the hygienic behaviour, while grooming behavior was assessed using anti-varroa bottom board. All colonies (100%) were found to show hygienic behaviour, with some other colonies having a high level of removal of sealed dead larvae and pupae. Higher infestation rate of varroa was detected in colonies performed lower hygienic behavior. There was a positive correlation between grooming behavior and number of falling varroa on bottom board of bee hives. For the first time since Varroa invaded Libya, our results provide evidence that untreated local honeybee colonies can survive the mite, which may be the basis for integrated Varroa management.***

Keywords: Libyan honeybee, hygienic behavior, grooming behavior, *Varroa destructor*

1. Introduction

Honeybee colonies (*Apis mellifera* L.) are susceptible to several diseases caused by different pathogens and parasites. One of the most destructive is varroa mite (*V. destructor*), by feeding on hemolymph of adult and developing bees, spreading disease, and reducing their lifespan [1] [2]. In 1976, *V. destructor* was reported to be present in Libya [3]. Mites were introduced with infested bee packages imported from Bulgaria to Algaba lElakder resulted in the establishment of varroa, and then spread rapidly throughout the country [4]. Although varroa can be treated using many miticides, there have been recent reports of resistance to many products in different areas of the USA, Italy, Mexico and UK [5-8]. It is therefore important to investigate other methods of treating or managing varroa in honeybee colonies.

Researchers have shown that honeybees has a specific behavioural response enhances colony resistance to certain diseases, particularly American Foulbrood and varroa mites performing tasks such as cleaning and foraging [9-11]. The behavioural resistance, including grooming and hygienic behavior may influence colony defence, which would directly contribute in slowing varroa buildup population. Hygienic behaviour is the dominant natural defence against *V. destructor* mites infesting brood cells, during its reproductive phase [12]. Whereas grooming behavior is mainly effective defence against phoretic phase of *V. destructor* mites infesting adult bees.

Hygienic behaviour involves the identification and uncapping of cells containing diseased and parasitized larvae and pupae, and their removal. In grooming, worker bees detect and remove phoretic mites from themselves (auto-grooming) or from nest mates (allo-grooming) [13]. As a behavioural trait of the bees, it might contribute to overall resistance against *V. destructor*. The purpose of this study was determined if Libyan honeybees have any heritable defense mechanism against *V. Destructor* which be readily incorporated into breeding programs.

2. Materials and Methods

The study was conducted at Spring 2014 in an apiary consisted of 21 colonies of *A. mellifera* located in the Research Station, University of Tripoli. These colonies occupied hives with 2 Dadant chambers, colonies naturally infested with bee parasites and known as 4-years miticide free. The presence of numerous flowers during the study assured continuity of foraging, no robbing among the colonies was observed.

2.1 Behavioral resistance of honeybee colonies

2.1.1Evaluation of bee hygienic behavior of the experimental colonies

Colonies were tested for hygienic behavior using freeze-killed brood assay [14]. A thin wooden piece, 5×6 cm in diameter, was placed on frame of sealed brood, this comb (containing approximately 100 cells) was cut with sharp knife, then placed into jar and frozen at approximately –20°C for 24 hours, the selected frame was marked to indicate its location within the brood nest later. The comb was allowed to defrost before being replaced into its original hive. After 24 hours and 7 days, the frame was photographed by a digital camera and returned to the hive. All photographed frames were downloaded on a computer, and the percentage of cells from which larvae have been uncapped and removed was determined.

2.1.2Evaluation of bee grooming behavior of the experimental colonies

Ten colonies were equipped with screened bottom boards operated with the drawer (closed bottom) underneath covered with paper smeared with a thin layer of Vaseline oil to capture the fallen parasites [15]. The anti-varroa screened bottom board promotes the natural grooming behavior of the honeybee. Number of fallen mites after three days of Vaseline sheet installation was recorded (i.e. mite per day). Calculate the total colony infestation rate from the weekly mite fall by multiplying the daily mite drop by 250–500 or 20-40 when brood is absent or present, respectively [16].

2.2 Colony infestation rate by varroa

2.2.1Infestation rate of adult worker bees

A sample of Approximately 300 bees per colony was taken directly from the combs of the hives into a jar, and the alcohol wash technique was used to assess infestation rate of mites [17]. In the lab, containers were placed for 30 min on the shaker to dislodge parasites. Content was pour over sieve to separate the parasites from the bees. Finally, parasites were examined under a microscope at 40X magnification to differentiate mite from bee louse (*B. coeca*). Total number of mites and number of bees in each sample was recorded then percentage (i.e. number of mites per 100 bees) was calculated.

2.2.2 Infestation rate of sealed brood

For sampling parasitic mites in worker brood cells, two frames with recently sealed brood were chosen from each colony. Then one- hundred sealed brood cells were randomly selected. Each cell was uncapped, the pre-pupa or pupa inside it was carefully examined and any detected female mites were counted. The walls of the cells and removed caps were also examined as the mite frequently hides there [17]. Total number of inspected cells and number of adult mites was recorded and percentage of infestation of sealed brood was calculated.

2.3 Statistical Analysis

Data for mean varroa infestation, and hygienic behaviour were compared between treatments using mixed model analysis of variance (ANOVA) SPSS® for Windows™ Version 14. Prior to analysis, each variable was visually tested for normality using P-P plot and Levene's test was used to test the assumption of equality of error variance [18]. If significant differences between treatments were detected, their means were separated using Duncan multiple rang test. In all cases, significance was accepted at the 0.05 level.

3. Results and Discussion

3.1 Hygienic behaviour of honeybee colonies

It has been stated that most colonies will remove dead brood from combs. However, it is the length of time taken to do this that determines whether the colony is deemed hygienic or not [18]. The results of the freeze-killed brood assay are presented in Table 1 showed that experimental colonies performed different levels of hygienic behaviour after 24h of comb replacement. Five colonies (cleaned >95% of dead brood) performed high hygienic, 6 colonies (cleaned 95-65% of dead brood) performed medium hygienic, and 6 colonies (<65% of dead brood) performed high hygienic behaviour. However, all colonies removed dead brood before the 7th day of comb replacement and considered to be hygienic. On the other hand, we observe a difference between the removal of larvae on both sides of the comb in some colonies.

Table 1: Infestation rate of *V. destructor* on sealed brood of colonies with different levels of hygienic behaviour

Hygienic level	Number	Mean no. of*V. destructor* / 100 sealed brood cell
High	5	9.3 ± 2.9^{a}
Medium	6	13.7 ± 3.8^{a}
Low	6	30.1 ± 3.5^{b}

Colonies classified by freeze-killed brood assay into threes hygienic levels. Values are Mean±SE. Means followed by the same letter are not different at the $p \leq 0.05$ level. Means were separated using Duncan's test.

On this study, there was a difference between the experimental colonies in varroa infestation rate on brood (Table .1), and clearly it was significantly higher ($p<0.005$) in colonies with low hygienic level. Most colonies will remove freeze-killed brood eventually if it is left inside a colony. Indeed, this was supported by the results of this study, where many colonies had removed some of the dead brood. Colonies selected for hygienic behavior had lower mite levels than non-hygienic ones [19]. The removal of parasitized brood is thought to break reproductive cycle of varroa due to the removal before instars development in brood cell [20]. It seems that hygienic behavior of Libyan honeybees may directly contributed in slowing varroa buildup population during its reproductive phase when infesting brood cells.

Colonies, strains, races and species of bees are highly variable as regards their hygienic behavior [21]. Workers of Africanized bee colonies are more efficient at removing brood infested with varroa than are European bees, left under the same conditions [22] [23]. It has been reported that EHB in Mexico were able to remove only 8.0% of infested brood while AHB removed up to 32.5% showing a possible mechanism that could contribute to the tolerance of AHB toward *V. destructor* [24]. Thus, selective removal behavior as a type of hygienic behavior can be effective in the control of varroa.

There have been reported that 10-15% of European bees colonies exhibit hygienic behavior [20]. In Tunisia, *A. m. intermissa* bees removed on average 15.5% of the pupae in naturally infested cells [25] and *A. m. carnica* colonies removed 16.6% [26]. Another report confirmed Varroa tolerance in France in *A. m. intermissa* imported from Tunisia [27]. The mean percentage removal of brood experimentally infested with one living mite per cell by 76 colonies not preselected for hygienic behavior was 23.5% (tested three times during 1997) [28]. Only 9.2 % of these colonies removed more than 50% of the infested brood. While *A. m. ligustica* colonies that had been pre-selected for hygienic behavior in the US (28 colonies total, 1994-1997) removed an average of 52.1 % of the experimentally infested pupae, compared to 17.4% in colonies selected for non-hygienic behavior (19 colonies) [19] [29]. However, our study showed that 31% of the experimental colonies exhibit hygienic behavior.

The difference between detect, uncap and removal of dead larvae on both sides of the comb observed in our study could be explained by many factors. It is possible that during the freezing process one side of the comb section was damaged

more than the other. Thus, dead larvae were removed that would not normally have been detected by the bees, leading to a higher removal rate on the damaged side. It is also possible that the brood on one side was considerably older than brood on the other side, thus influencing the rate of removal; younger brood may be removed more quickly than older brood [14].

It has been suggested that the assay should be carried out at least twice, and the colony only deemed hygienic if greater than 95% of dead brood is removed on both occasions [20]. However, in the current report, just a snapshot of expression of hygienic behaviour was determined. If Libyan beekeepers would like to breed hygienic bees and wish to be certain that their colony is hygienic, a second test would be required.

3.2 Grooming behaviour of honeybee colonies

Grooming behavior was evaluated by assessing the natural fall of varroa mites through screen bottom board hives. In addition, infestation level of varroa mite on adult was assessed in the experimental colonies to investigate the efficacy of grooming in control of varroa mite.

There was apperant difference in the rate of falling mites (ranged from 2 – 12.5 mite / day/ colony) during spring (Fig.1). This would be opvious when multiplying the daily mites drop by 20-40 (when brood is present) to estimate the infestation rate of varroa (80- 500 mite/ colony) [16]. Similarly, there was a difference between colonies in varroa infestation rate (ranged from 1.8 – 10.9 mite / 100 adult bee). We recorded a higher infestation rate in colonies with high dropped mites.

In hygienic colonies, during the removal process, the female mite that has parasitized larvae may escape and re-enter a different brood cell or attach it to adult bees. In the latter case, grooming behavior, the other physiological resistance of bees would allow them to get remove varroa from their bodies, and force mites to fall on the bottom boards. Acorrelation (r=0.822, p= 0.0035) between the rate of falling mites and varroa infestation level (Fig.1) for expression of grooming behaviour was found in the current study. It seems that grooming behavior of Libyan honeybees may directly contribute in slowing varroa buildup population during its phoretic phase when infesting brood cells.

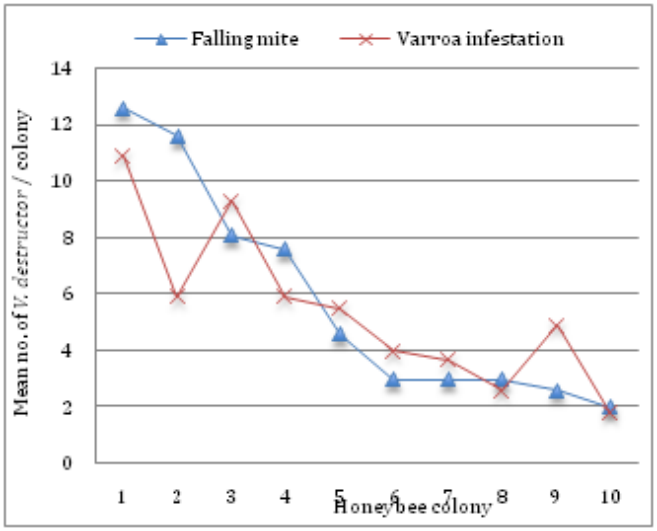

Figure1: Number of falling varroa and infestation rate of *V. destructor* on adult bees.

Number of falling varroa/ colony/ day by using anti-varroa bottom boards.

Number of varroa per 100 adult bees.

In Brazil, artificial infestation of bee colonies with adult varroa females has showed that Africanized bee workers were almost eight fold more efficient in getting rid of the mites on their bodies compared to pure Italian bee workers [22]. Of the total number of infested workers in the studied colonies, 31.0% removed the parasite due to their own action and the action of nest-mate bees [30]. The active defense of the Africanized bees is recorded to be similar to that of the natural host *A. cerana* [31]. The results of reports in Mexico [32] [33] were in agreement with those reported from Brazil. They suggested that grooming behavior may be an important mechanism conferring tolerance of honey bee colonies toward varroa mites in Mexico. There is also evidence for mite resistance in *A. mellifera* from far-eastern Russia (Primorsky) originating from honey bees imported in the mid 1800's; those honey bee colonies have a strong, genetically based resistance to the parasite [34].

In conclusion, Libyan honeybees have good hygienic behavior level and seem to be tolerant to varroa mite. This study has given an insight into the expression of hygienic behaviour in honeybee colonies in Libya. It may in turn lead to greater understanding of the bee resistance, and may provide different ways of preventing common diseases that affect honey bee colonies.

It is recommended that, at this stage, further trials with a larger scale study could give more information about how widespread hygienic behaviour is, and may lead to a greater understanding of why some parts of Libya have more disease than other areas. The simple assay used could give beekeepers the option to selectively breed for bees expressing hygienic behaviour.

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