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Monthly Changes in the Natural Grooming Response in Workers of Three Honey Bee Subspecies Against the Bee Parasitic Mite *Varroa destructor*

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ABSTRACT

The seasonal variations in the number of damaged *Varroa* mites in the colonies of *Apis mellifera ligustica*, *A. mellifera carnica* and *A. mellifera syriaca* were studied using eight colonies for each honeybee subspecies. The results showed that the damaged mites in colonies of all three honey bee subspecies were noticed all over the year. Monthly changes in grooming behavior in the three subspecies showed the same general trend, the maximum percentages of the damaged mites were recorded during June correlated with high workers populated colonies and not correlated with the high *Varroa* infestation rates in the colonies. The occurrence of damaged mites in the colonies of the three subspecies in the summer and winter months provides evidence for active defense mechanisms against the parasitic mite *Varroa destructor* helping in reducing the initial mite mother population.

Keywords: Honeybees, *Varroa* mites, Defense behavior, Jordan.

INTRODUCTION

The defense mechanisms of the genus *Apis* to its parasitic mite *Varroa destructor* were well developed and performed with high variations to keep the population of the mite at the lowest level. There are several examples of such mechanisms; the limitation of the ontogenesis process in the *Varroa* mite on *Apis cerana* colonies to drone brood only (Koeniger et al., 1983; Rath, 1991), the differences in reproduction rate of the female mite in *Apis mellifera* colonies on both worker and drone brood (Martin, 1994; Al-Ghzawi et al., 2001a; Medina et al., 2002), brood capping period (Moritz, 1985), the removal behavior (Boecking and Drescher, 1992; Božič and Valentinčič, 1995; Fries et al., 1996; Rosenkranz et al., 1997; Bienefeld et al., 1999; Guzman-Novoa et al., 1999; Boecking and Spivak, 1999; Shannag et al., 2000) and the grooming behavior (Peng et al., 1987; Büchler et al., 1992; Moretto et al., 1991; Delfindo et al., 1992; Al-Ghzawi et al., 2001b; Zaitoun et al., 2001).

Grooming behavior of worker honey bees involves catching and killing *Varroa* mites by worker mandibles; depending on different factors such as bee subspecies, genetic factors, mite population, environmental conditions specially temperature and availability of nectar and pollen flow. The mite population seldom reached a destructive threshold in its original host *Apis cerana*, presumably due to mutual adaptation of eastern bee and *V. destructor* during co-evolution (Moritz, 1985). The removal of mite infested worker brood and

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grooming behavior are defense mechanisms of *A. cerana* toward *V. destructor* (Peng et al., 1987; Büchler et al., 1992; Rath, 1999). Many reports indicate the occurrence of *Varroa*-tolerance in European honey bees (Ritter et al., 1990; Böcking and Spivak, 1999). *A. mellifera* workers remove mite infested pupae from capped brood cells and groom *Varroa* mites but to a limited extent compared to *A. cerana* (Peng et al., 1987; Morse et al., 1991; Rosenkranz et al., 1997). The heritability material is known to affect the hygienic behavior of honey bees against *Varroa* mites. F1 colonies bred from colonies demonstrating high or low mite grooming ability showed the same ability as their parent colony (Moretto et al., 1993; Boecking and Drescher, 1998). Grooming behavior is known to be strongly influenced by environmental factors especially temperature (Marcangeli, 1997). Weak colonies or lack of incoming nectar showed to reduce the removal response to mite infested and dead brood cells (Spivak, 1996; Spivak and Gilliam, 1993). Moosbeckhofer (1997) observed low numbers of damaged mites in March with significant higher numbers in June of the same colonies and concluded that the proportion of damaged mites within a particular colony, even under the same environmental conditions, is not always consistent between collections.

As mentioned earlier in the different scientific works about the appearance of a positive bee-*Varroa* interaction, the question is "Did domestic and feral colonies which live many years adapt to live under the pressure of *Varroa* mites or they defend the mites actively during the summer as well as winter time?". The present study was carried out to investigate the changes in the active response of three subspecies; *A. mellifera ligustica*, *A. mellifera carnica* and *A. mellifera syriaca* against *Varroa*-mite during the summer and winter months of the year under semiarid Mediterranean conditions. Basic knowledge on the continuity of the defense mechanisms all over the year is required to reduce the initial mite mother population and to use it in a successful mite pest management program.

MATERIALS AND METHODS

Three honeybee subspecies; *Apis mellifera ligustica*, *Apis mellifera carnica* and *Apis mellifera syriaca* were used. For each honeybee subspecies, eight untreated colonies established in 10-framed Langstroth hives were used. All colonies were equalized to have similar amounts of stored food and brood combs; each containing an average of 4.5 brood combs at the beginning of the experiment. During the investigation, the colonies were subjected to different apiculture practices such as shading, feeding, supering and swarm control, as needed. In November 2004, the colonies were moved to a private apiary located in the northern Jordan Valley, North Shuna (32°37" N, 35°36" E) and kept there until the middle of April, which synchronized with the cessation of citrus blooming. Thereafter, honeybee hives were relocated for the rest of the year in the upland at Irbid (32°30" N, 35°59" E), Jordan. The study started in January and continued to the end of December 2005.

The monthly changes in the response of *A. mellifera* worker bees to *Varroa*-infestation have been determined by collecting fallen mites on the bottom-board traps from naturally infested colonies. Two mm mesh screen wire was inserted under the brood nest on the bottom board of the hive. The edges of each insert were smeared with vaseline to prevent the dropping but still living mites from escaping as well as to avoid attacking these mites by ants and other predators. Each insert was removed and replaced immediately by a new one every two days; 180 single collections were examined for all colonies all over the year without using smoke, in order to avoid any disturbance of the hive. On each sampling

date, collected mites were immediately examined carefully under a stereomicroscope at x 40 magnification for injuries and abnormalities. To estimate the infestation rate of *Varroa* mites in the hive, 200 worker bees were randomly selected from each experimental colony at one month intervals. Bee samples were kept in a plastic bag, transferred to the laboratory and exterminated after freezing. Then, samples were inspected individually under a dissecting microscope for any infestation by *Varroa*, and the percentage of infested bees was recorded. During the entire experimental period, populations of worker bees were determined at 21 days intervals using the procedures described by Gerig (1983). In that method, an empty frame divided into twelve sections, each of which is able to contain 200 bees was superimposed on tested comb. A visual estimation for the percentage of bee capacity on each section of the comb was conducted. After that, the number of sections occupied with bees was multiplied by 200 to find the total number of bees on each comb side. Data were subjected to analysis of variance (ANOVA), and means were compared using Fisher's least significant differences (Steel and Torrie, 1980).

RESULTS

Monthly Changes in Groomed Mites

Figure (1) shows the monthly change in the percentage of damaged mites dropped on the inserts throughout the year. The monthly percentage of groomed mites per colony was calculated to be 19.75% (range 6-38 %) for *A. mellifera syriaca,* 10.75% (range 4-25 %) for *A. mellifera ligustica* and 11.5% (range 3-26 %) for *A. mellifera carnica*. In June, the maximum percentages of damaged mites were 25%, 26% and 38% for *A. mellifera ligustica, A. mellifera carnica* and *A. mellifera syriaca,* respectively. The percentage of damaged mites decreased in amount gradually during the summer months followed by a low rate throughout the winter months, dropping to the minimum for the year in December which accounted to be 4%, 3% and 6% damaged mites for *A. mellifera ligustica, A. mellifera carnica* and *A. mellifera syriaca*, respectively. Significant differences ($P \leq 0.05$) were detected between the grooming rates of *A. mellifera ligustica and A. mellifera carnica* as new introduced subspecies and the native bees' *A. mellifera syriaca* during the period extended from March to September, but no significant differences ($P \leq 0.05$) were detected between the grooming rates of the three subspecies during the rest of the year. *A. mellifera ligustica* and *A. mellifera carnica* workers showed similar trends in grooming rates towards *Varroa* mites with no significant differences all over the study period.

Monthly Changes in the Infestation Rates of Adult Worker Bees

Results indicated that all honeybee colonies showed a single peak of *Varroa* mites within the year (Figure 2). However, the infestation rate of *Varroa* mites declined substantially at the beginning of each year and dropped to a minimum of 4.2%, 3.4% and 3.8% in March in all colonies of *A. mellifera syriaca, A. mellifera ligustica* and *A. mellifera carnica*, respectively. Infestation rate of worker bees by *Varroa* mites augmented from May up very rapidly until it reached the season's maximum of 26.5 %, 33.2% and 31% in September for *A. mellifera syriaca, A. mellifera ligustica* and *A. mellifera carnica*, respectively. The increased percentage of groomed mites did not correlate with the increased number of *Varroa* mites in the hives of the three bee subspecies ($r = 0.337$, – 0.002 and 0.343 for *A. mellifera ligustica*, *A. mellifera carnica* and *A. mellifera syriaca,* respectively).

Monthly Changes in the Number of Worker Bees

Under the migratory conditions, the colonies of *A. mellifera syriaca, A. mellifera ligustica* and *A. mellifera*

carnica showed a double cycle of worker population within the year as shown in Figure (3). In January and February, the colonies of the three subspecies showed a steep rise in the population of adult bees reaching the first peak in March. *A. mellifera ligustica* produced the higher population (28200 bees) with significant differences at ($P \leq 0.05$) from the other two subspecies (20000, 18400 bees) for *A. mellifera carnica* and *A. mellifera syriaca* respectively. After honey harvesting in April, the colonies showed a slight reduction in bee populations. The colonies rebuilt up their worker population with significant differences ($P \leq 0.05$) at the second peak in June (24600, 18000 and 21400 bees for *A. mellifera ligustica*, *A. mellifera carnica* and *A. mellifera syriaca*, respectively). The population of adult bees decreased after honey harvesting in July dropping to the minimum for the year in December. The increased number of damaged mites by the three honey bee subspecies positively correlated with the increased number of worker bees in the hives ($r = 0.554$, 0.687 and 0.705 for *A. mellifera ligustica*, *A. mellifera carnica* and *A. mellifera syriaca*, respectively).

DISCUSSION

Inspection of all dropped mites in each hive under a stereomicroscope revealed that the proportion of injured mites varied among the months within each honeybee subspecies. Significant fluctuations appeared in the percentage of damaged mites that had dropped on the inserts in eight experimental colonies of each tested honeybee subspecies. The maximum percentage of damaged mites was found in June, while the lowest percentage was recorded in December. Results demonstrated that *A. mellifera syriaca* is more efficient in damaging *Varroa* mites compared to the other two subspecies during the study period. Grooming behavior towards *Varroa* mites is known as a defense mechanism of the Asian bees (Peng et al., 1987) as well as in the western honeybees (Moosbeckhofer, 1992; Ruttner and Hänel, 1992; Bienefeld et al., 1999) with clear differences in its effectiveness. Büchler et al., (1992) and Fries et al., (1996) reported that *A. cerana* is more effective in both removing mites and causing mite damage compared to *A. mellifera* colonies. Moretto et al., (1993) found that Africanized workers were seven times more efficient than Italians in eliminating mites from their bodies. The results showed that as the environmental factors are changed by time of the year, this will affect the active reaction of the worker bees against the *Varroa* mites. These results agree with the findings of Marcangeli (1997), who reported that grooming behavior is known to be strongly influenced by environmental factors. Also, these results agree with a report by Moosbeckhofer (1997), who observed in Austria low numbers of damaged mites during the mild spring time in March (11%) and significantly higher numbers of damaged mites in June (42%) during the summer time.

In contrast to the monthly changes of worker bees inside the colonies, *Varroa* mites showed a single peak within the year. The infestation rates of this parasitic mite flux out of synchrony with the number of worker bees during the year. That is, when the bee numbers increase in early spring and reach their maximum during the late months of spring, the number of infested worker bees were at the lowest. The increased percentage of groomed mites negatively correlated with the increased number of *Varroa* mites in the hives of the three bee subspecies. These results are in agreement with the results of Moosbeckhofer (1992), who found a significant negative correlation between the proportion of damaged mites and the population size of the *Varroa* mites in the infested colonies. Guzmán-Novoa et al., (1999) found also that the number of injured mites significantly correlated with the mite population growth in the hives. On the other hand, these results were in

disagreement with the results of Boecking and Drescher (1998), who found that number of damaged mites found on the bottom board increased as the total number of living mites in the colonies increased.

All experimental colonies of the three subspecies showed a double cycle of worker population within the year. With the availability of nectar and pollen from wild plants and vegetables grown in the Jordan valley, the colonies of all three subspecies showed the first peak in March, while in the up-lands they rebuilt up their worker population showing the second peak in June. The population of adult bees in colonies of the three subspecies decreased after honey harvesting in July as a consequence of the lack of the main nectar and pollen flow dropping to the minimum for the year in December. These results are in agreement with the observations made by Avitabile (1978), Free (1986) and Liebig (1993), who stated that the seasonal regularity of fluctuations in the workers population cycle correlated to the recurrence of various nectar flows and pollen yields in any year. In Jordan, Al-Ghzawi et al., (2001c) and Zaitoun (2000) detected that workers population increased from the early stages of minor nectar and pollen flow throughout January until the second honey flow in June. High temperature, drought conditions and reduced numbers of wild flowers cause a steep decline in the worker numbers during August and September, which is in accordance with these results.

When the percentage of naturally groomed mites was matched up to the total number of worker bees, their percentages were in synchrony with bees almost all over the year. That is, when the bee numbers increased through out January to March and reached their height during the period between March and June; damaged *Varroa* mites were at their highest during May and June. Damage rate of *Varroa destructor* by worker bees augmented from June down until it reached the season's minimum in December synchronized with the minimum of worker population in the colonies of *A. mellifera ligustica*, *A. mellifera carnica* and *A. mellifera syriaca*. The present results demonstrate that the larger number of bees in summer time together with high temperatures and forage availability could be responsible for the increased damage rate at that time in all three honey bee subspecies. This synchronization of the increased number of damaged mites by three honey bee subspecies is significantly correlated with the increased number of worker bees in the hives. These results are in agreement with those obtained by Bienefeld et al. (1999), who reported the increased damaging rate of *Varroa* mites during the night as a result of the presence of all worker bees inside the hive at night.

In conclusion, all honeybee subspecies actively groomed the *Varroa* mites with significant different rates during the different months of the year. This fluctuation throughout the months of the year must be taken into consideration when studying grooming activity of any bee subspecies. This activity of defense behavior can be enhanced by the increasing of colony workers, population and suitable environmental conditions, but not affected by the number of mites inside the colonies, so beekeepers must maintain healthy large colonies to enhance this natural defense behavior. Furthermore, colonies with high grooming rates should be selected for further studies in breeding a mite-tolerant line.

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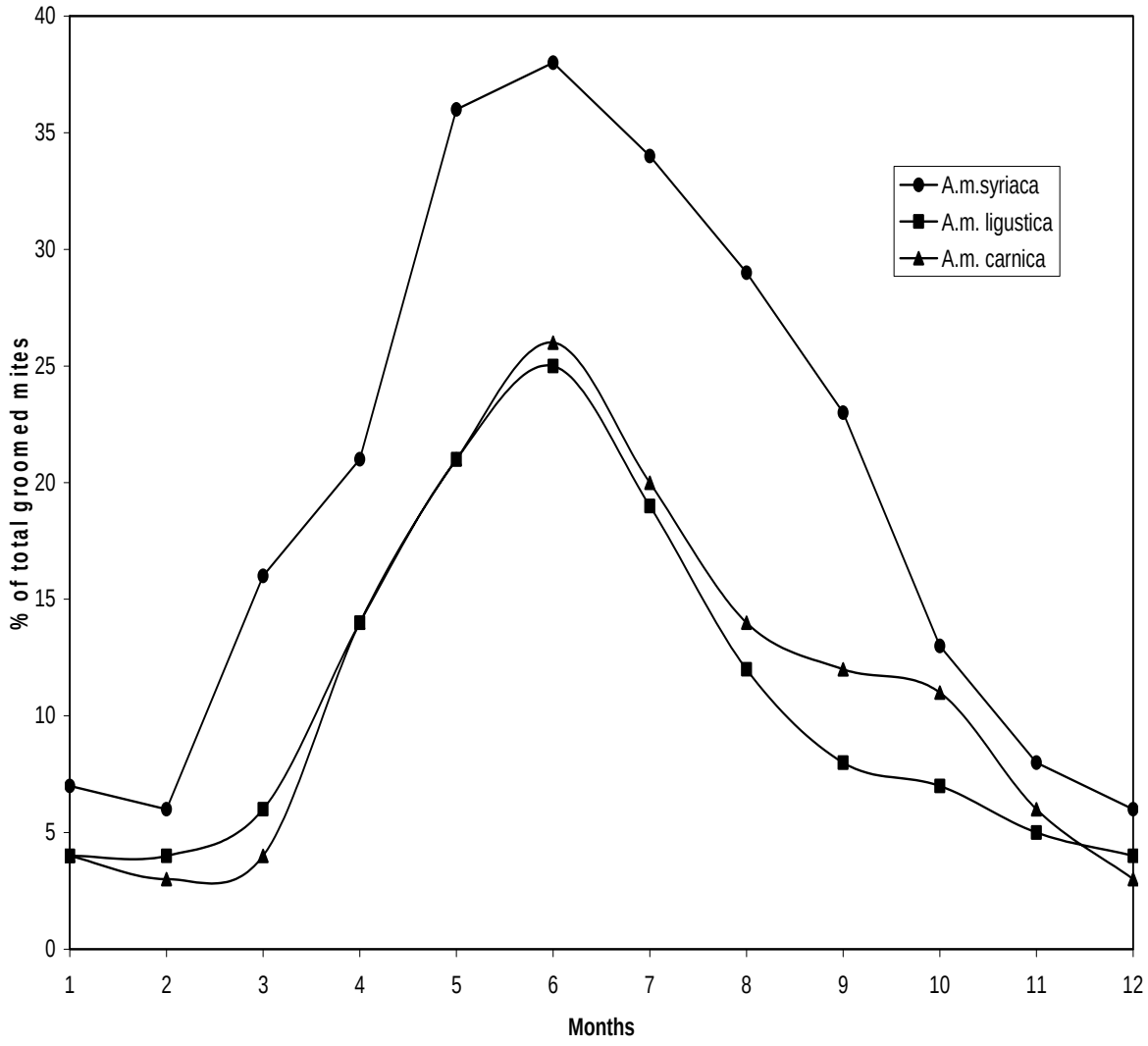

Figure (1): Monthly changes in the percentage of total damage mites of *Apis mellifera syriaca, A. mellifera ligustica* and *A. mellifera carnica* colonies throughout the study period.

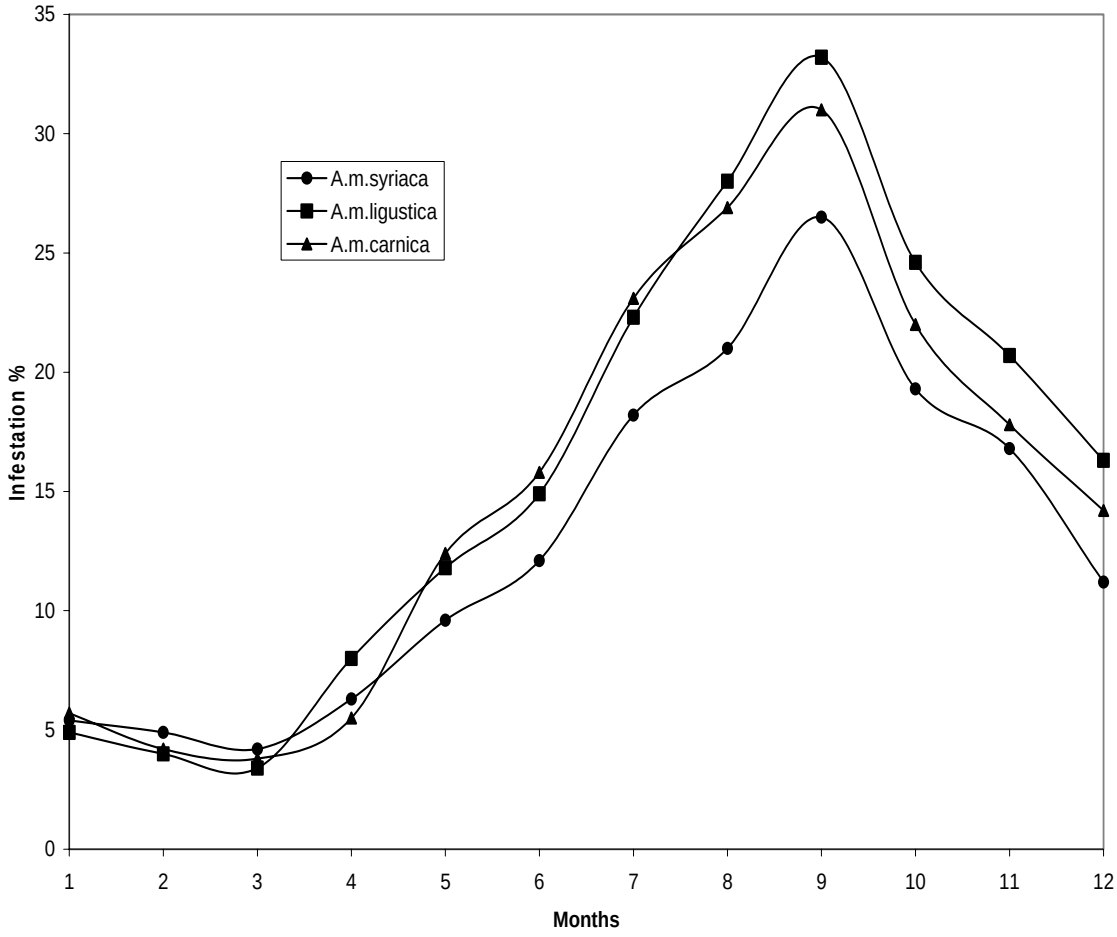

Figure (2): Monthly changes in the infestation rates of *Apis mellifera syriaca, A. mellifera ligustica* and *A. mellifera carnica* bees with *Varroa* mites throughout the study period.

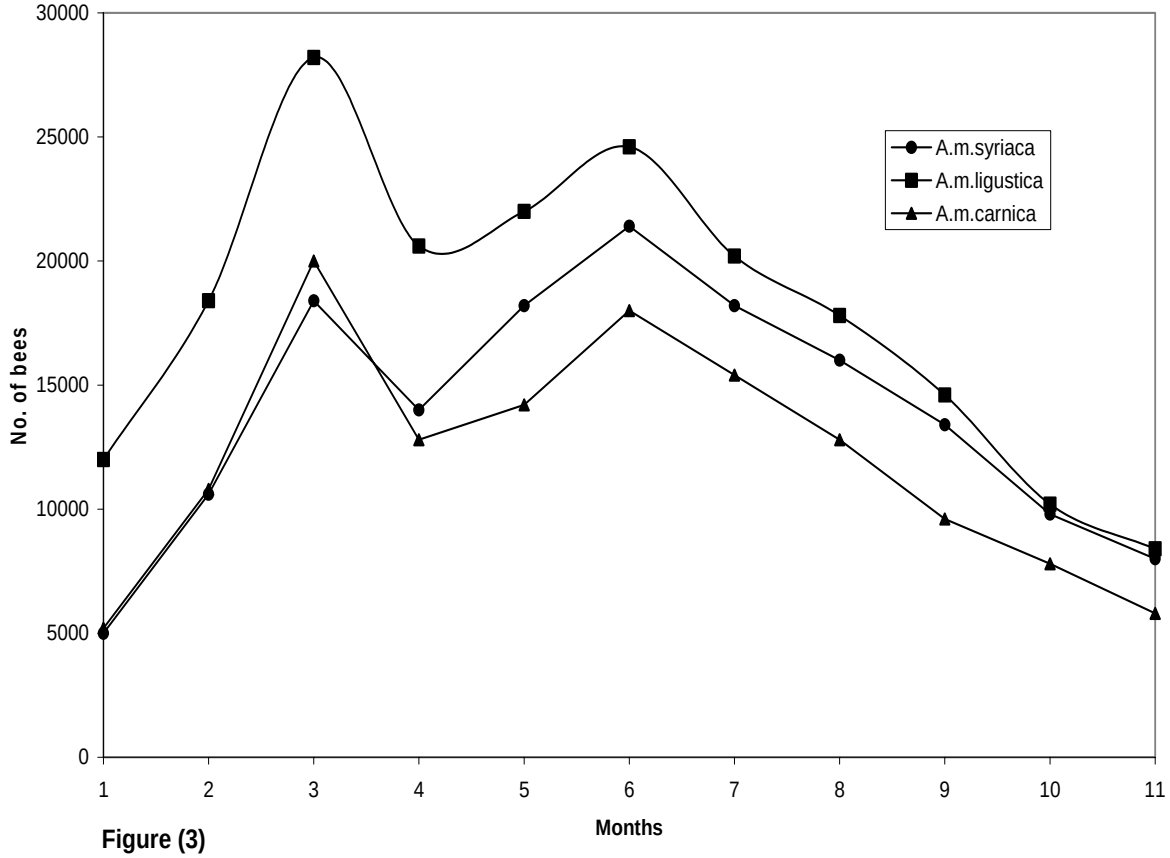

Figure (3): Monthly changes in the worker population in the colonies of *Apis mellifera syriaca, A. mellifera ligustica* and *A. mellifera carnica* throughout the study period.

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التغيرات الشهرية في سلوك التنظيف الذاتي الطبيعي لدى عاملات ثلاث سلالات من نحل العسل ضد حلم الفاروا

شاهرة زيتون* وعبدالمجيد الغزاوي**

ملخـص

تمت دراسة التغيرات الشهرية في مدى سلوك التنظيف الذاتي لثلاث سلالات من نحل العسل هي الايطالي (*Apis mellifera ligustica*) والكرنيولي (*A. mellifera carnica*) والبلدي السوري (*A. mellifera syriaca*) ضد حلم الفاروا. استخدمت في الدراسة ثماني خلايا لكل سلالة. اظهرت النتائج مقدرة عاملات هذه السلالات على الاستمرار في مهاجمة الحلم طوال اشهر السنة. سجل اعلى نشاط في مقاومة الحلم خلال شهري حزيران وتموز متطابقا مع وجود اعداد عاملات نحل مرتفعة داخل الخلايا وليس مع ازدياد نسبة اصابة العاملات بالحلم. ان وجود ظاهرة المقاومة الطبيعية لحلم الفاروا من قبل عاملات النحل يدل على وجود مقاومة نشطة لهذا الحلم تساعد في تقليل عدد امهات الحلم في الخلايا، وقد تستخدم في برامج التربية لانتاج سلالات من النحل اكثر مقاومة لهذه الافة.

الكلمات الدالة: نحل العسل، حلم الفاروا، سلوك الدفاع، الأردن.

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