

The relationship between *Varroa destructor* infestation and virgin queen's acceptance, mating success and onset of oviposition in honeybee colonies

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ABSTRACT

This study was conducted to evaluate the role of level infestation of the *Varroa destructor* mite on virgin queen's acceptance, mating success and pre-oviposition period when their introduced into Carniolan honeybee, *Apis mellifera* colonies. According to level infestation of mite, the colonies were divided into three groups, each contained 15 colonies, low ($5\% \pm 0.3$); moderate ($12\% \pm 0.7$) and high ($20\% \pm 0.9$).

The results showed that the varroa level significant influence virgin queen's acceptance, mating success and somewhat influence their onset of oviposition. The results mentioned that, low infestation level was more accepted and reached mating success 100% than both moderate and high levels (mating success were 91.67% and 80.0%, respectively). Pre-oviposition periods were (8.9 ± 1.05 ; 11.1 ± 0.89 and 11.6 ± 1.45 days), recorded at low, moderate and high mite infestation levels, respectively. Beekeepers can get an economic benefit from introducing queen bees and improving introduction acceptance and mating success by control varroa mite before queen introduction.

Key words: honey bee, *Apis mellifera*, queen's acceptance, mating, oviposition, *varroa destructor*.

INTRODUCTION

Varroa destructor (Anderson and Trueman, 2000) formerly named *Varroa jacobsoni* Oudemans is potentially the main parasite of *Apis mellifera* L. The varroa mite is an external parasite of the honey bee that feeds on the hemolymph of immature and adult bees (Harbo and Harris, 2001). Parasitism causes weight loss, wing deformities and sometimes loss of appendages of the emerging bee (De Jong *et al.*, 1982). High infestation of varroa in colonies that lack innate mite suppression characteristics, ultimately cause the collapse of untreated colony in a few years. Colony collapse is due not only to mite infestation, but also to secondary viral, bacterial and fungal infections (Hung *et al.*, 1996). This parasite has spread rapidly and now infests most of the world's *Apis mellifera* causing much concern to beekeepers (Rinderer *et al.*, 2001 and Sanford, 2001). Periodic requeening is considered an important management practice in commercial beekeeping to develop strong colonies and to obtain a maximum production during nectar flows (Guzman-Nova *et al.*, 1998). The queen quality in turn depends on genetic, environmental factors and good management. Beekeepers replace queens in colonies to assure that colonies will have vigorous queens that are less likely to fail at a critical time in the annual colony cycle (Furgala and McCutcheon, 1992) or to improve the genetic stock. Requeening is also required as colonies led by young queens are more productive than

those led by old queens (Kostarelou- Demiandou *et al.*, 1995) Progeny of old queens could also have undesirable characteristics, e.g., high susceptibility to diseases and strong aggressive behaviour (Free, 1987). Beekeepers also introduce queens to queenless colonies they have made by dividing existing colonies to increase the number of colonies they own.

Many factors had been considered to influence the acceptance of introduced virgin queens in honey bee colonies such as the weight of the virgin queens at emergence (Szabo, 1977), the age of the virgin queen at introduction time (Abdel-Rahman *et al.*, 2008), the attractiveness and behavior of the virgin queens towards the workers (Yadava and Smith, 1971), the age of the workers in the receptory colonies (Szabo and Townsend, 1974), the duration of queenlessness of the receptory colonies and the caging period of the virgin queens before being released (Mantilla and Gonçalves, 1987).

Little or no data are available on the effect of varroa mite infestation on virgin queen's acceptance, mating success and initiation of oviposition in honey bee colonies.

The aim of the present work was carried out to determine the influence of different population levels of varroa mites on queen introduction, mating success and onset of oviposition in Carniolan honey bee.

MATERIALS AND METHODS

Field experiments were carried out at Sehel Seleim location, Assiut Governorate, Upper Egypt, during February and March, 2010.

Queens were reared by dry grafting method according to Doolittle, 1889, using about 1 day old worker larvae from one Carniolan queen breeder. Rates of mite infestation for adult bees were determined in the established colonies from samples of (200-400) living worker bees taking directly from the combs icing sugar shake method (Mark and Cliff, 2001). The infestation rate was calculated by the following formula (Alloui *et al.*, 2002).

$$T_x = \frac{N_v \times 100}{N_a}$$

Where, T_x : infestation rate; N_v : number of varroa; N_a : Number of honeybees.

Table 1: Infestation rate of varroa mite in the three groups before the virgin queens introduction into experimental colonies.

Low infestation (Mean % \pm S.D.)	Moderate infestation (Mean % \pm S.D.)	High infestation (Mean % \pm S.D.)
5 \pm 0.4 ^c	12 \pm 0.7 ^b	20 \pm 0.9 ^a
n = 15		

^{a, b, c} Means at the same row differ significantly at $p < 0.05$.

Forty-five colonies of Carniolan (*Apis mellifera carnica* Pollmann) honey bees, were chosen for this work. They divided into three groups (each of fifteen colonies,) nearly equal strength contained sealed and unsealed brood of different stages, adult bees of different ages and stored food (honey and bee bread). We chose first group colonies with low infestations (5% \pm 0.3), second group colonies with moderate infestations (12% \pm 0.7) and third group with high infestations (20% \pm 0.9). The virgin queens were weighed, using an analytical scale to the nearest 0.001 mg. within 5-minutes of emergence and were marked with paint (Medina and Gonçalves, 2000).

The weights of virgin queen at emergence were ranged between 150-160 mg. for each.

Two days before introduction of virgin queens, the mated queens of colonies were removed. The dequeened colonies were inspected carefully and queen cells were destroyed. At 3-days-age, fifteen caged virgin queens for each group were introduced in dequeened colonies. Releasing of the caged virgin queens was done after two days of introduction. After 24-hours from releasing of queens, the colonies were examined in order to check the acceptance or rejection by workers. The colonies were checked in the morning (08:30 -10:30 am) when virgin queens were not performing orientation or mating flights and their observation were possible (Medina and Gonçalves, 2000). Queens that were not injured by workers after liberation and found alive were considered accepted. Queens found dead inside the cage, injured after liberation or queens which were not found were considered as rejected (Rhodes *et al.*, 2004). After queen release, all frames in the colonies were inspected daily for 25-days in order to observe the presence of eggs laid into the cells then percentages of queen mating success and onset of oviposition after queen releasing were recorded (El-Sarrag and Nagi, 1989).

Percentages of queen's acceptance and queen mating success were transformed using arcsin method, then, analysis of variance (ANOVA) was carried out using MSTAT-C software program (MSTAT-C, Michigan State University Version 2.10) and least significant difference (LSD) values were calculated when F-value were significant for infestation effects according to the method of waller and Duncan (Waller and Duncan, 1969).

RESULTS

To help beekeepers to increase colony production, improve queen quality, introduction acceptance and mating success, we studied influence of varroa mite infestation on queen's acceptance mating and initiation of oviposition in Carniolan honeybee.

Table 2: Number and percentage of the virgin queen's acceptance from the three groups.

	Low infestation	Moderate infestation	High infestation
Introduced	15	15	15
Accepted	15	12	10
Acceptance (%)	100 ^a	80 ^b	66.67 ^c

^{a, b, c} Means at the same row differ significantly at $p < 0.05$.

The number of virgin queens introduced and accepted between the three groups were significantly different ($p < 0.05$). The highest percentage (100%) was recorded at low infestation colonies. While, the lowest acceptance (66.67%) was found at high infestation group (Table 2).

Table 3: Number and percentage of the virgin queens mating success from the three groups of varroa mite infestation.

Groups	Low infestation	Moderate infestation	High infestation
Accepted	15	12	10
Mated	15	11	8
Mating success (%)	100 ^a	91.67 ^b	80.0 ^c

^{a, b, c} Means at the same row differ significantly at $p < 0.05$.

Data presented in Table 3 show queen mating success percentages. The results assure that, the maximum percentage (100%) of mating success was observed at low infestation group. Mating success percentage was (91.67%) found at moderate group, while, the minimum percentage (80%) was noticed at high infestation group. Results differed significantly between the three groups ($p < 0.05$).

Table 4: Pre-oviposition period (in days) of the queens from the three groups of varroa mite infestation.

	Low infestation	Moderate infestation	High infestation
N	15	11	8
Means \pm S.D.	8.9 \pm 1.05 ^b	11.1 \pm 0.89 ^a	11.6 \pm 1.45 ^a
Range	6 – 12	8 – 14	8 - 15

Means headed by the same letter at the same row don't differ significantly at $p < 0.05$.

The mean (\pm S.D.) onset of oviposition was significant between low infestation group on slide long and both high and moderate infestation groups on the other hand. These results indicating that rate of mite infestation had influenced on the time of oviposition. ($p < 0.05$) (Table 4). The low infestation level showed the shortest pre-oviposition period (8.9 \pm 1.05 days) with a range of 6-12 days. The pre-oviposition period (11.1 \pm 0.85 days) was recorded at moderate infestation group with a minimum of 6 days and a maximum of 14 days. The longest onset of oviposition (11.6 \pm 1.45 days) was noticed at high infestation colonies with a range of 8-15 days.

DISCUSSION

The presence of the varroa mites in colonies has become ubiquitous in K.S.A. Varroa is becoming more difficult to control as mites develop resistance to widely used miticides (Sanford, 2001). The results showed that, the rate of mite infestation influence on the queen acceptance. There have been reports that rates of supersedure soon after introducing new queens appear to be higher than in past years especially for Italian honeybees (Guzman-Nova *et al.*, 1998; Petties *et al.*, 2004). Italian honeybees are known to be very susceptible to infestation by varroa mites. Supersedure and colony death were greater in colonies that were more highly infested (Cargel and Renderer, 2009). Supersedure was probably led to multiple queens' productions in the same colony. Under such condition worker bees from queenless colonies make a distinction between virgin queens produced naturally into the colonies (Medina and Gonçalves, 2000). The obtained results assure that, queen mating success influenced by the level of varroa infestation.

Many factors, such as traits related with the subspecies, climatic conditions during mating flights and availability of sexually mature drones in congregation areas could influence the orientation and mating flights of virgin queens and affect their mating success (Szabo *et al.*, 1987).

In the tropical climate of Brazil, Silva *et al.*, (1995) obtained a mating success of 36-93% varying with nectar flow status (flow or dearth), the age of virgin queens (1 to 6 days) and brood status of nuclei (broodless, with open brood, with capped brood). High mating success 93% was obtained during nectar flow in mating nuclei containing capped brood and where virgin queens were introduced at 3-4 days old. They also showed negative relationship between wind velocity and mating success.

In relation with the pre-oviposition period, it was found that rate infestation of varroa mite somewhat affect on initiation of oviposition.

Showronek *et al.*, 2002 recorded that, queen inseminated at the age of 6 days were found to have a very short latency period. By far the longest latency period was

found in queen insemination at the age of 13-days or more. Queen kept in mating nucleus starting egg laying significantly earlier than those kept in boxes (Chuda-Mickiewicz *et al.*, 2003).

Overall, the results presented here provide strong evidence that elevated levels of varroa in colonies decrease queen acceptance and mating success. Also, may be pre-oviposition period somewhat affecting as a result of mite infestation. For practical purpose to obtain a high acceptance and mating success, it might be productive for beekeepers to treat colonies they intend to re-queen or divide prior to queen introduction.

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ARABIC SUMMARY

العلاقة بين الإصابة بالفاروا و قبول الملكات العذارى ونجاح التلقيح وبداية وضع البيض في طوائف نحل العسل

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أجريت هذه الدراسة بغرض تقييم دور مستوى الإصابة بطفيل الفاروا على قبول الملكات العذارى ونجاح عملية التلقيح وكذلك على الفترة اللازمة لتبدا الملكة في وضع البيض وذلك عندما أدخلت في طوائف نحل العسل الكرنولي. تم تقسيم طوائف التجربة تبعا لمستوى الإصابة بالفاروا إلى ثلاث مجموعات تحتوي كل منها على 15 طائفة وهي: مجموعة منخفضة الإصابة (5% ± 0.3)، مجموعة متوسطة الإصابة (12% ± 0.7) والثالثة مرتفعة الإصابة (20% ± 0.9).

أظهرت النتائج أن مستوى الإصابة بالفاروا يؤثر وبصورة معنوية على قبول الملكات العذارى وعلى نجاح عملية التلقيح وتؤثر إلى حد ما على بداية وضع البيض. أشارت النتائج إلى أن الإصابة المنخفضة أظهرت وبوضوح أعلى نسبة من قبول الملكات ونجاح التلقيح بنسبة 100% وذلك عن كل من الإصابة المتوسطة والإصابة المرتفعة حيث كانت نسبة نجاح التلقيح بهما (91.67%، 80.0%) على الترتيب. سجلت فترات ما قبل وضع البيض (8.9 ± 1.05، 11.1 ± 0.89، 11.6 ± 1.45 يوما) وذلك في كل من مستويات الإصابة المنخفضة والمتوسطة والمرتفعة على الترتيب. وأنه يمكن للنحالين الحصول على فائدة اقتصادية من الملكات المدخلة وزيادة قبول الإدخال وكذا نجاح التلقيح وذلك بمكافحة طفيل الفاروا قبل إدخال الملكات.