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INFLUENCE OF DIFFERENT STATES OF QUEENS ON OVARIAN DEVELOPMENT OF HONEY BEE WORKERS

Mohammed M. Khodairy
Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt.

Abstract: The present study was carried out at the apiary of Faculty of Agriculture, Assiut University during active season of 2007. The effect of different states of bee queens on the development of worker ovaries were studied. These states of queens were: mated queens of 1-year-old, 1-month-old, supersedure, and virgin queens of 10, 7 and 3 day- old. All queens states under experiment occurred highly significant inhibition in the ovaries development of bee workers, in compared to queenless control bees. Bee workers headed with mated queens 1-year-old gave the minimum value of ovarian development index (1.02), inducing 95% ovarian inhibition as compared to queenless control workers. Whereas the minimum percentage of inhibition was recorded by workers headed with virgin queen 3-days old (37.2%), resulted 1.27 ovarian index. Stage I (slightly developed) of ovaries development of bee workers was appeared clearly on the 6th day for all treatments, then increased to reach maximum value between 12th and 18th day. The appearance of stage II (well developed) of ovaries development, which named egg-laying workers was recorded only in queenless control bees, on the 12th day, that reached maximum percentage on the 18th day (13.3%).

Key words: Honey bee, laying worker, ovarian development, queen state, queenless.

Introduction

The honey bee queen plays an important role in the behaviour and physiology of bee workers. it is well known that the presence of the queen in a bee colony inhibits the ovaries development of bee workers (Velthuis, 1970). The ovaries of worker bees are normally rudimenttary. In colonies with both of a laying queen and brood, only about one bee in 10.000 has fully-formed eggs in their ovaries (Ratnieks, 1993). This functional sterility is mediated by pheromonal signals produced by brood (Arnold *et al*., 1994) and bee queen (Hoover *et al*., 2003). When the bee colony is broodless or queenless, many adult workers activate their ovaries and become egg-laying workers (Ratnieks, 1993). One week or two after a honey bee colony has lost its queen, about half of its bee worker ovaries become active and the workers lay eggs (Khodairy, 1990). Speed of laying workers developm-ent varies greatly among subspecies of the western honey bee. Workers of the African subspecies develop ovaries and oviposit more quickly than workers of the European subspecies (Ruttner and Hesse, 1981). Ovary development in the

worker caste of bee colonies with queens has been observed in the preswarming period or in colonies with abnormal queens (Sakagami *et al*., 1963). Ovarian development increases slightly after swarming but not before (Kropácová and Haslbachová, 1970). Laying workers appear usually presumed to bee restricted to queenless colonies, which do not contain young brood to rear new queen (Winston, 1987). In general, queen inhibits ovaries development of workers (Velthuis, 1970) by queen pheromones (Butler and Fairey, 1963).

Development of laying workers is influenced by several environmental and innate factors, such as, seasonal variation; worker ovary development was lowest in spring, highest in mid-summer, and intermediate in fall (Hoover *et al*., 2006), the age of workers; the young ages (3, 6, 9 and 12 days old) transfer to laying workers more and faster than other ages of workers (Khodairy, 2001). Nutrition and temperature (Lin and Winston, 1998), pollen consumption (Khodairy, 1990; Bitondi and Simoes, 1996 and Khodairy and Moustafa, 2006), subspecies, races and hybrids (Ruttner and Hesse, 1981 and Khodairy, 2002), queen status and its pheromones (Butler and Fairey, 1963 and Free, 1977), amount of brood (Jay, 1975) and mandibular gland secretions (queen-like pheromone) of queenless workers (Simon *et al*., 2001).

The honey bee queen and their pheromones are important in sustaining cohesion and stability of the honey bee colony. By means of chemical substances, the queen inhibits oogenesis in workers and prevents the rearing of a new queen (Winston, 1987 and Pankiw, 2004). Appearance of egg-laying workers in the honey bee colonies is considered one of the important problems confronting the beekeepers, especially after colonies have been dequeened because it is very difficult to introduce a queen into queenless colony with egg-laying workers.

The aim of the present investigation, is to study the ovarian development of bee workers headed with different states of queens.

Materials and Methods

The experiments were carried out in Faculty of Agriculture, Assiut University apiary during the active season of 2007.

Preparation of bee cages and bioassay protocol:

The first hybrid of Carniolan honey bee, *Apis mellifera* L. workers were used in the present study. Sealed brood combs, containing hatching brood, were taken from queenright colony, then incubated at 32°C±1 and 60% RH., and the brood were observed until adults emergence. Five thousands and six hundreds workers, less than 12-hours old, were placed inside twenty eight wooden cages (12x12 x5 cm), two hundreds per cage. The cages were provided with a vial of tap water and a vial of sucrose solution (50% aqueous

sugar), bee bread and a piece of wax comb attached to the cage top. The cages were continuously supplied with water, sucrose solution and bee bread. The cages were divided into seven groups dependent on status of introduced queens as follows:

-group 1, cages contained mated and egg-laying queens 1-year-old.

-group 2, cages contained mated and egg-laying queens 1-month-old.

-group 3, cages contained mated and egg-laying queens, and replaced by honey bees (Supersedure queen).

-group 4, cages contained virgin queens 3 day-old.

-group 5, cages contained virgin queens 7day-old.

-group 6, cages contained virgin queens 10 day-old.

-group 7, cages without queens (control).

The cages were held in a dark incubator at 32°C±1 and 60% RH.

Determination of ovarian development:

To study the effect of the different status of queens on ovarian development in queenless condition, twenty bee workers were removed from each cage every three days. This procedure was repeated seven times at three- day intervals. Each worker was dissected under stereo-microscope (40 times magnification force) to determine the ovaries development by using the classification of development stages as given by Sakagami and Akahira (1958). According to this method, the degree of development classified as O, undeveloped (rudimentary); I, slightly developed (commencement of swelling and constriction) and II, well developed (distinct ova).

Also, the ovarian development index was calculated according to Jay and Jay (1976), as an indication of ovarian development for all stages determined in the bee worker samples. The mean of various scores when multiplied by the number of bees whose ovaries fall with each ovary development category; 0, undeveloped (score = 1); I, slightly developed (score = 2); II, well developed (score = 3). The index value 1.0 means that all workers with undeveloped ovaries, whereas the value 3.0 means that all workers with well developed ovaries.

The inhibitory effect of different status of queens on ovarian development of bee workers was calculated using the following suggested equation:

$$\text{Inhibition of ovarian development (\%)} = \frac{\text{IOI of control} - \text{IOI of treatment}}{\text{IOI of control (without queen)}} \times 100$$

IOI, means increase in ovarian development index more than score one (undeveloped ovary).

Statistical analysis:

The statistical analysis were conducted using the SAS general linear models procedure. Differences among means were determined by L.S.D. Significant differences at P<0.05 (SAS Institute, 1990).

Results and Discussion

In general, there were signify-cant differences in the ovarian development index of bee workers between the workers unheaded with queen (queenless) and the workers headed with all different states of queens. The general mean of ovarian develop-ment index was 1.02, 1.08, 1.13, 1.18, 1.22, 1.27 and 1.43 for worker bees headed with mated queens for each of 1-year-old, 1-month-old and super-sedure, virgin queens for each of 3-days old, 7-days old and 10-days old, and queenelss (con-trol), respecttively. Bee workers headed with mated queen 1-year-old gave the minimum value of ovarian index (1.02), inducing 95% ovarian inhib-ition as compared to queenless control bees. Followed by workers headed with mated queens 1-month-old resulted 1.08 ovarian index, inducing 81.4% ovarian inhibition (Table 1).

Table(1):Ovarian development index and inhibitory effect in honey bee workers headed with different states of bee queens, at a period of 21 days.

Days following treatment	Ovarian development index at different queen states (mean ± SD)						
	Mated queens 1-year-old	Mated queens 1-month-old	Super-sedure queens	Virgin queen 10day-old	Virgin queen 7day-old	Virgin queen 3day- old	Control
3	1.01 ±0.03	1.03 ±0.04	1.05 ±0.06	1.17 ±0.04	1.17 ±0.04	1.10 ±0.04	1.10 ±0.04
6	1.02 ±0.03	1.05 ±0.06	1.10 ±0.04	1.18 ±0.04	1.22 ±0.04	1.24 ±0.04	1.18 ±0.04
9	1.03 ±0.04	1.10 ±0.06	1.11 ±0.04	1.25 ±0.04	1.28 ±0.06	1.29 ±0.03	1.30 ±0.06
12	1.02 ±0.03	1.10 ±0.05	1.17 ±0.04	1.17 ±0.04	1.29 ±0.03	1.32 ±0.03	1.47 ±0.06
15	1.02 ±0.03	1.12 ±0.08	1.20 ±0.06	1.20 ±0.00	1.24 ±0.04	1.30 ±0.04	1.60 ±0.08
18	1.02 ±0.03	1.13 ±0.01	1.17 ±0.07	1.15 ±0.04	1.18 ±0.04	1.37 ±0.04	1.67 ±0.17
21	1.02 ±0.03	1.05 ±0.06	1.13 ±0.06	1.17 ±0.06	1.17 ±0.04	1.30 ±0.09	1.70 ±0.09
Grand mean ±SD	1.02E ±0.004	1.08DE ±0.04	1.13CDE ±0.05	1.18BCD ±0.04	1.22BC ±0.05	1.27B ±0.09	1.43A ±0.24
Ovarian inhibition (%)	95.0	81.4	69.8	58.1	48.8	37.2	--

Means have the same letter(s) do not differ significantly at 0.05 level of probability.

Whereas the minimum percentages of inhibition were recorded by workers headed with virgin queens for each of 10-days old (58.1%), 7-days old (48.8%) and 3-days old (37.2%) as compared to queenless control bees. Stage I (slightly developed) of ovaries development of bee workers was appeared clearly on the 6th day for all the treatments, then increased to reach maximum value between the 12th and the 18th day for all bee workers headed with queens, and the 18th and the 21st day for queenless bees. The general mean percentages of category I were 2.0, 7.7, 13.0, 18.0, 22.1, 26.8 and 34.14% for worker bees headed with mated queens for each of 1-year-old, 1-month-old and supersedure, virgin queens for 10day-old, 7day-old and 3day-old, and queenless control, respectively. The appearance of stage II of ovaries development (well develo-ped) recorded only on the 12th day for queenless control bees, then increased to reach maximum value on the 18th day (13.00%).

Appearance of egg-laying worker bees recorded only in queenless control bees on the 12th day, then increased to reach maximal percentage on the 18th day (13.3%) (Fig. 1, A,B,C,D and Fig. 2, A,B,C).

Honey bee worker ovaries are undeveloped in the presence of a laying queen, and worker reproduction is rare in most honey bee populations under queenright condition (Visscher, 1989 and Oldroyd *et al*., 1994). In the absence of a queen, some workers ovaries develop, and they begin to lay drone eggs. The most critical influences on worker ovaries development are the presence of a queens and/or their brood (Jay, 1968).

The honey bee queens produce pheromones which play important roles in maintaining the coherence of honey bee colonies, such as attracting a retinue of workers around her (Pankiw *et al*., 1995), inhibit queen cell production (Pettis *et al*., 1995), inhibit worker ovaries activation (Butler and Fairey, 1963 and Hoover *et al*., 2003). Pheromone secretions of honey bee queen are partially responsible for the inhibition of worker ovaries development (Slessor *et al*., 1988).

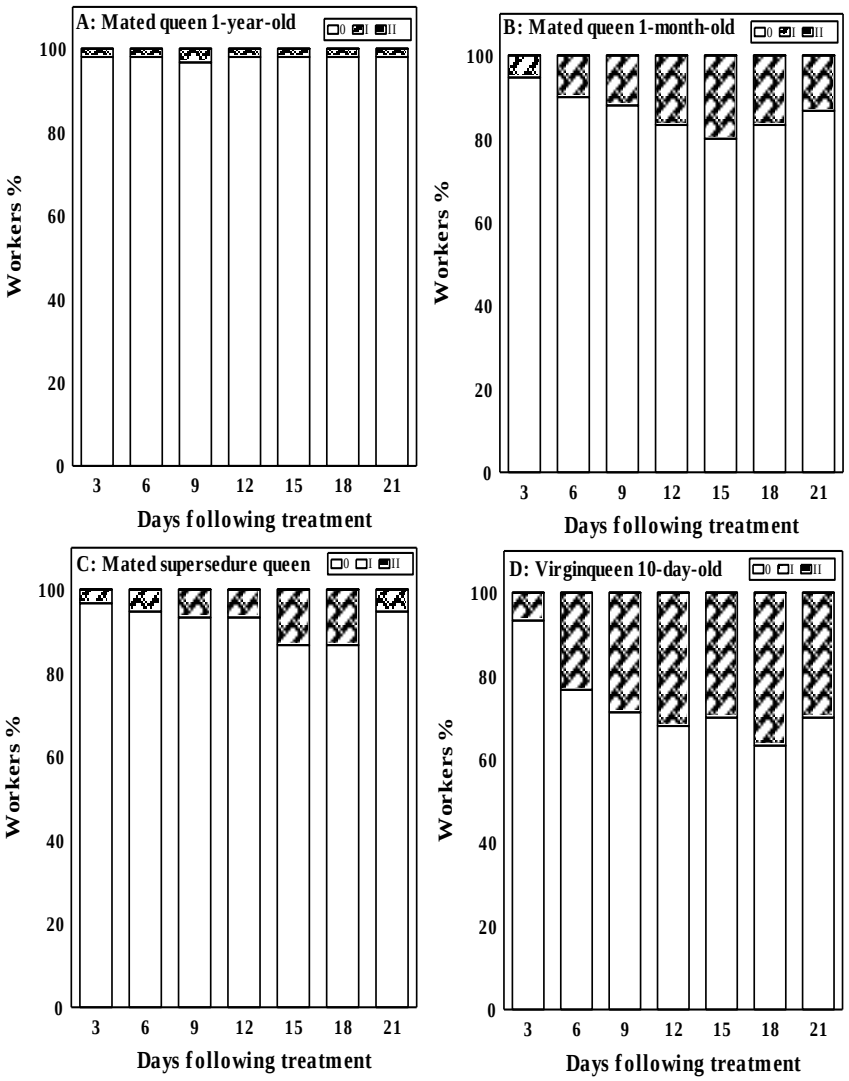

Fig. 1. Ovarian development of bee workers under laboratory condition headed with different status of queens, at a period of 21 days.

Degree of ovary development:
0, undeveloped I, slightly developed II, well developed

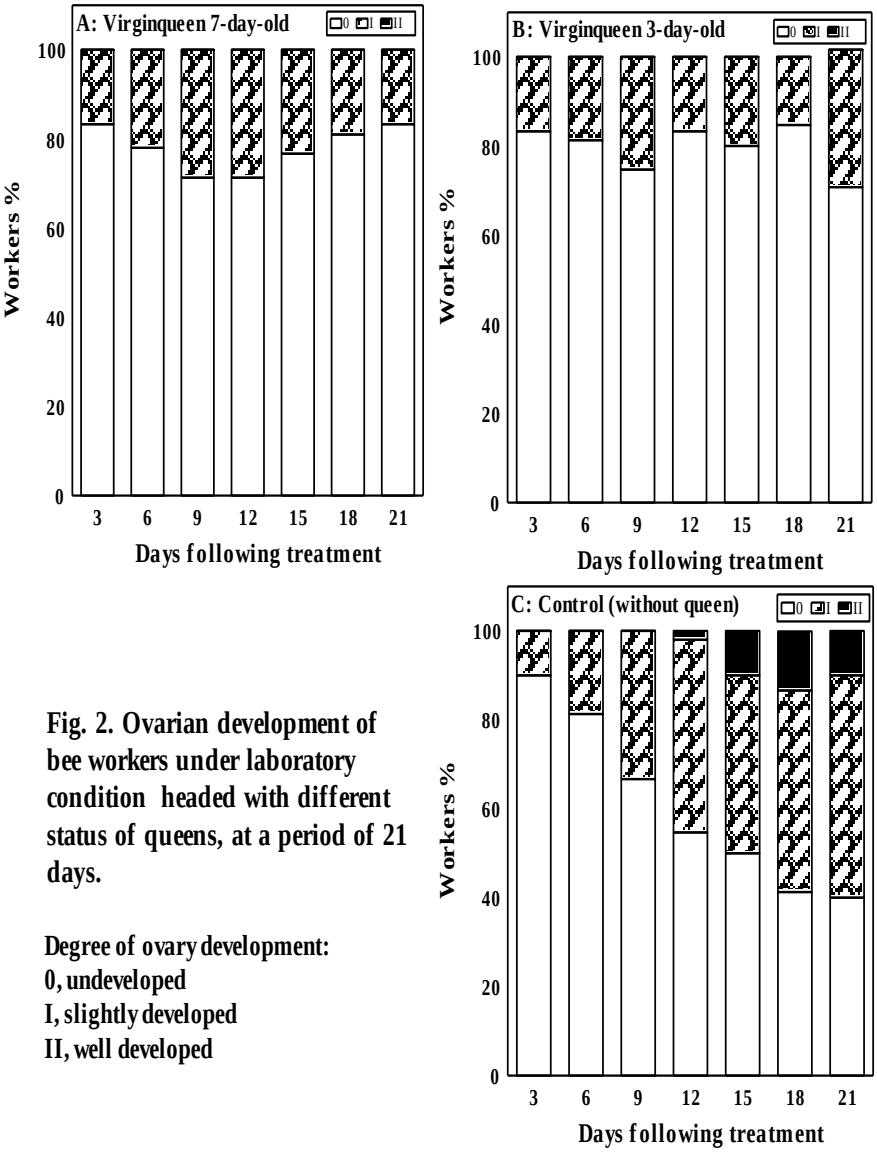

Fig. 2. Ovarian development of bee workers under laboratory condition headed with different status of queens, at a period of 21 days.

Degree of ovary development:
0, undeveloped
I, slightly developed
II, well developed

Queen mandibular pheromones, particularly 9-oxo-2-decenoic acid, is responsible for the ovary-regulating pheromonal capability of honey bee queens, although other factors are required for complete inhibition (Pettis *et al*., 1995 and Hoover, 2005). The queen mandibular pheromone is composed of five constituent compounds: 9-oxo-2-decenoic acid (9-ODA), both enantiomers of 9-hydroxy-2-decenoic acid (9-HAD), methyl p-hydroxybenzoate (HOB), and 4-hydroxy-3-methoxyphenylethanol (HVA) (Slessor *et al*., 1988). The quantity in a single mated and laying queen is produced in 24 hours was 200 µg (9-ODA), 80 µg (9-HDA), 20 µg (HOB) and 2 µg (HVA) (Naumann *et al*., 1991 and Pankiw *et al*., 1996). The studies of Lin *et al*. (1999) and Hoover (2005) clearly demonstrated that the five-components queen mandibular inhibit ovarian development in worker bees, especially, the 9-ODA pheromone that was the major and more inhibitory effect on worker ovaries.

In the present study, the mated queens 1-year old gave the highest inhibitory effect (95%) on the worker ovarian development, followed by the mated queens 1-month-old (81.4%). Whereas, the lowest effect (37.2%) resulted by the virgin queens 3-day-old. It is clear that the inhibitory effect of queen on worker ovarian development increase by increasing queen age. This may be attributed to queen pheromones, especially 9-oxo-2-decenoic acid (9-ODA) increase by increasing age and depend on status of honey bee queens. This finding is confirmed by Pankiw *et al*. (1994), who showed that the virgin queens produce significantly less 9-ODA pheromone than mated queens. Also, the present results are confirmed by Apšegaite and Skirkevičius (1999), who showed that the physiological state and age of bee queens are of significance to the pheromone they produce, the content of 9-ODA in the extract of a newly emerged bee queen is the lowest, while its content in the extract of a 2-year-old intensively egg-laying queen is the highest. During the first eight days of adult life, the content of 9-ODA increases very rapidly (up to 6.5 times). During the period in which a bee queen is mated and begins to lay eggs, the rate of the increase in the content of this acid decreases nearly twice. The content of 9-ODA in the extract of recently mated and egg-laying bee queens is not stabilized. The development of honey bee queen are related with great physiological changes in their organs. These charges are determined by maturation, mating, egg-laying and aging. The physiological changes influence also their pheromones, it has been noted by many authors that maturation of bee queens influences also the content of 9-oxo-2-decenoic acid (9-ODA),

the most abundant component of their pheromones (Butler and Paton, 1962). The content of 9-ODA in virgin queens to be lower than in mated queens (Boch *et al*., 1975). Slesser *et al*. (1990) and Apšegaite (2003) showed that the largest amounts of 9-ODA are released by mated egg-laying queens 2-year-old and more than 21-day-old mated queens.

In the present study, the inhibitory effect of supersedure mated queens were less than both of 1-year-old and 1-month-old mated queens. This may be due to the decrease in their pheromones and confirmed by many author e.g., Pankiew *et al*. (1996) they showed that the drone-laying queens possess lower amounts of 9-ODA than the mated queens. These variations in the qualitative and quantitative composition of the queen pheromones greatly depend on the physiological state of the individual (Slessor *et al*., 1990; Pankiw *et al*., 1996). It has been revealed that 1-year-old mated queens are more attractive to worker bees than virgin or newly mated queens (Winston, 1987; Free *et al*., 1992). There is revealed large variation in the amounts of 9-ODA carried by different queens (Slessor *et al*., 1990; Pankiw *et al*. 1996). Mated queen honey bees produce about 12-400 µg of 9-ODA per day (Naumann *et al*., 1991). Queen honey bees must produce sufficient amounts of 9-ODA in order to take up a dominant position in a colony (Saioviici, 1983). The amounts of 9-ODA in the mandibular glands of mated queens increase with time spent in isolation (Naumann *et al*., 1991). As well known, queen do not secrete pheromones responsible for attracting worker bees on the day of emergence, bees can not successfully distinguish their extracts. Whereas the worker bees highly significantly differentiated extracts of 7-days old virgin queens of different races, as well as the extracts of mated and unmated queens. Successful discrimination of extracts of virgin queens depend on their ages (Levchenko *et al*., 1995).

There are other pheromones produced by queen also may be involved in the regulation of worker ovary development. A second queen source of inhibitory pheromones (Winston and Slessor, 1998), tergal gland secretions may regulate worker ovary development (Wossler and Crewe, 1999). Tergal pheromone inhibits ovarian development when tested in small groups of caged workers.

Four additional new compounds were identified from several glandular sources of queen (methyl-loleate, coniferyl alcohol, Hexadecan-1-ol and lindenic acid), that function with queen mandibular pheromones in attracting workers to around the queen. These compounds are in active alone, but greatly increase activity when combined with queen mandiublar pheromones. They also may be active in worker ovary regulation (Keeling *et*

al., 2003). Also, there are two esters produced by worker brood, ethyl palmitate and methyl linoleate both are inhibited ovary developpment when fed to workers (Mohammedi *et al*., 1998). It is clear, that the regulation of worker honey bee ovary development may seem overly complex, involving both queen and brood of honey bee (Hoover, 2005). Studies on the volume changes of the JH-producing corpora allata in workers allowed to suggest an effect of queen pheromone on the endocrine system of the receiver (Gast, 1967). Results of Khodairy and Tawfik (2003) indicated that the anti-juvenile hormone, precocene II induced decrease in corpora allata and inhibited ovary development of worker bees. Queen mandibular pheromones and its major component 9-oxo-2-decenoic acid inhibit the rate of biosynthesis of the juvenile hormone which is released by corpora allata (Kaatz *et al*., 1992).

It is clear, from the present study under laboratory condition, that the tendency of honey bee workers to produce laying workers mainly depend on the age of their headed queen, physiological state and other factors, determining quantitative and qualitative composition of the pheromones produced by bee queens. According to the present study and previous references, it can be concluded that the queen status play an important and main role in the transferring to laying workers, together with other important factors, such as seasonal variation, races, pollen consumption; vitellogenin levels (Velthuis *et al*., 1990) and certain hormones such as ecdysteroid levels (Robinson *et al*., 1991), juvenile hormone levels, (Davey, 1996 and Pinto *et al*., 2000), and dopamine and its metabolites, N-acetyldopamine and norepinephrine (Sasaki and Nagao, 2001).

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تأثير حالات مختلفة من الملكات على تطور المبايض لشغالات نحل العسل

محمد محمد خضيرى

قسم وقاية النبات – كلية الزراعة – جامعة أسيوط – أسيوط 71526 – مصر

أجريت هذه الدراسة بمنحل كلية الزراعة جامعة أسيوط خلال موسم النشاط لعام 2007. وذلك لغرض دراسة تأثير 6 حالات مختلفة من الملكات وهى : ملكات ملقحة عمر عام ، ملكات ملقحة عمر شهر ، ملكات ملقحة فى حالة إحلال ، ملكات عذارى عمر 3 يوم وعمر 7 يوم وعمر 10 أيام على درجة تطور المبايض فى شغالات نحل العسل وسرعة ظهور الشغالات الواضعة للبيض (الأمهات الكاذبة) . وقد أوضحت النتائج أن جميع حالات الملكات المستخدمة فى الدراسة أعطت تثبيطاً جيداً للمبايض بدرجة معنوية مقارنة بالكنترول (بدون ملكات) . وقد أعطت الملكات الملقحة عمر عام أقل قيمة لدليل تطور المبايض (1.02) ، مسجلة أعلى نسبة تأثير مثبط لمبايض الشغالات (95%) وذلك مقارنة بالكنترول . بينما سجلت أقل نسبة تثبيط للمبايض فى حالة استخدام الملكات العذارى عمر 3 يوم (37.2%)، مسجلة قيمة 1.27 لدليل تطور المبايض . وقد سجل أول ظهور للمرحلة I (المرحلة الثانية ذات التطور الخفيف) لتطور المبايض فى اليوم السادس من المعاملة وذلك لجميع الحالات تحت التجربة ، ثم يزداد ليصل إلى أعلى مستوى ما بين اليوم الثانى عشر والثامن عشر . بينما سجل ظهور المرحلة الثالثة والأخيرة II (تطور جيد) والتى تسمى الشغالات الواضعة للبيض فى معاملة الكنترول فقط (بدون ملكات) فى اليوم الثانى عشر ، ثم يزداد لتصل أعلى نسبة للشغالات الواضعة للبيض فى اليوم الثامن عشر (13.3%) . من الواضح أن الملكات تلعب دور هام وأساسى فى تثبيط تطور مبايض الشغالات ولكن يتم بصورة مختلفة على حسب حالة وعمر الملكة .