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INFLUENCE OF CERTAIN TYPES OF BEE-STORED POLLEN ON OVARIAN DEVELOPMENT OF HONEY BEE WORKERS UNDER QUEENLESS CONDITIONS

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Abstract: The effect of seven types of bee-stored pollen (bee bread) from monofloral sources on ovarian development and egg-laying workers formation during queenless condition was studied in the apiary of Faculty of Agriculture, Assiut University. Pollens play an important and main role in the ovaries development of bee workers. All pollen types under experiment occurred well development in worker ovaries, in comparison with control (undevelopment). Broad bean pollen gave the highest effect on ovarian development, whereas coriander pollen gave the lowest effect. Workers fed on broad bean pollen gave the maximum value of ovarian development index (2.5), on the 15th day, followed by canola pollen, the index was 2.55, on the 18th day. Whereas, all workers fed on each of caper, maize, clover, fennel and coriander pollen gave the maximum value of index (2.45, 2.35, 2.35, 2.05 and 2.05, respectively), on the 21st day. Appearance of egg-laying workers was sooner in workers fed on broad bean and canola pollen than others, (latency period = 9 days), followed by caper pollen (12 days). While the latency period was 15 days for workers fed on maize, fennel and clover pollens. The slowest appearance of egg-laying worker was observed on the 18th day for workers fed on coriander pollen.

Key words: honey bee, laying worker, ovarian development, pollen, queenless.

Introduction

In the honey bee colony the queen has an influence on the behaviour and physiology of bee workers. Only queens can produce female offspring with the exception of the cape honey bee, in which workers also produce female offspring (Anderson, 1963). It is well known that presence of the queen in a bee colony inhibits the ovaries development of bee workers

(Velthuis, 1970). The ovaries of worker bees are normally rudimentary. However, ovaries of certain bee workers begin to develop, and become laying workers with removal or loss of the queen from the colony. The term "laying worker" is sometimes extended to include not only those workers which actually lay eggs, but also those possess ovaries either partially or fully developed. A 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 (Sakagami, 1959 and Khodairy, 1990). Speed of laying worker development 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 it (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 day-old) transfer to laying workers more and faster than other ages of workers (Khodairy, 2001). Nutrition and temperature (Lin and Winston, 1998), pollen consumption (Jay, 1975, Khodairy, 1990 and Bitondi and Simoes, 1996), 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, 1970) and mandibular gland secretions (queen-like pheromone) of queenless workers (Simon *et al*., 2001).

Also, vitellogenin levels (Bownes, 1986 and Velthuis *et al*., 1990), population size (Lin *et al*., 1999). In addition to certain of hormones; ecdysteroid levels (Robinson *et al*., 1991), juvenile hormone levels (Davey, 1996 and Pinto *et al*., 2000) and, dopamine and its metabolites; N-acetyl dopamine and norepinephrine (Sasaki and Nagao, 2001). Pollen is very important factor for honey bee colony; pollen is the only source of protein in the diet of the honey bee colonies and it also contains all of the lipids, vitamins and minerals necessary for normal growth and development of bee colony (Hydak, 1970 and Roulston and Cane, 2000).

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. So, the aim of the present study, is to investigate the effect of bee bread (pollen stored in comb cells) from seven monofloral sources on ovarian development, and egg-laying workers formation of queenless colonies.

Materials and Methods

The experiments were carried out in Faculty of Agriculture, Assiut University apiary during the season of 2006.

Bee-stored pollen (bee bread) extraction:

During the different times of 2005, bee bread (stored pollen in comb cells) was extracted from the bee colonies, as following protocol: Certain of bee colonies, housed in standard Langstroth hives, were placed in farms containing the following monofloral species: clover (*Trifolium alexandrinum*), broad bean (*Vicia faba*), canola (*Brassica napus*), maize (*Zea mays*), fennel (*Faeniculum vulgare*), coriander (*Coriandrum sativum*), caper (*Brassica sinapis*). All these plant sources were cultivated in Assiut Governorate. The main pollen sources for honey bee colonies in Assiut Governorate, were broad bean, maize, caper and clover (Hussein, 1982). At the end of the flowering seasons, the bee bread (pollen stored in comb cells) was extracted from the different bee colonies for each of monofloral farm. The extracted pollen was stored under freezing condition until used. For sure, identification of bee-stored pollens was done microscopically with comparison of pollen from the anthers of unopened flowers.

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. Six thousands and four hundreds workers, less than 12-hour-old, were placed inside thirty-two wooden cages (12x12x5 cm), two hundreds per cage. The cages were provided with a vial of tap water and a vial of sucrose solution (50% aqueous sugar), and a piece of bee comb attached to the cage top. The cages were continuously supplied with water and sugar solution. The cages were provided with enough and equal amount of bee bread from the seven sources under experiment. Four cages (replicates) were used for each treatment (seven types of stored pollen) and control (without pollen). The cages were held in a dark incubator at 32°C$\pm$1 and 60% RH.

Determination of ovarian development:

To study the effect of the different types of pollen on ovarian development in queenless condition, ten 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.

Statistical analysis:

The statistical analysis was conducted using F-test. Differences among means were determined by Duncan's Multiple Range test (SAS Institute, 1990).

Results and Discussions

In general, there was a significant difference in the ovarian development index of bee workers between the workers fed on sugar solution (control) only and the workers fed on single pollen from seven plant sources under experiment. The general mean of index was 2.07, 1.89, 1.68, 1.62, 1.59, 1.55 and 1.46 for bees fed on broad bean, canola, caper, maize, clover, fennel and coriander pollen, respectively, whereas, the index value was 1.04 for control (no pollen).

Bee workers fed on broad bean pollen gave the maximum value of index (2.50) on the 15th day, whereas it was 2.55 on the 18th day for bees fed on canola pollen. Workers fed on each of caper, maize, clover, fennel and coriander pollen gave the maximum value of index (2.45, 2.35, 2.35, 2.05 and 2.05, respectively) on the 21st day (Table 1).

Stage I (slightly developed) of ovaries development of workers was recorded on the 3rd day for all treatments. Whereas appearance of stage II (well developed) was different for the treatments. After six days the percentage of workers at category II, was 55.0 and 20.0% for bees fed on broad bean or canola, pollen, but it was not recorded in the others. After 12 days it was 5.0% for bees fed on caper pollen, whereas was 20, 20, and 5.0% on the 15th for bees fed on maize, fennel and clover pollen. On the 18th day, it was 35.0% for bees fed on coriander pollen. The general mean percentages of category II were 36.79, 27.86, 25, 17.14, 11.43, 10.71 and 8.57% for bees fed on broad bean, canola, caper, maize, coriander, fennel and clover pollen, respectively (Figs, 1 and 2). Appearance of egg-laying workers was sooner in bees fed on broad bean and canola pollen than other treatments (latency period = 9 days), followed by the bees fed on caper pollen (12 days), while the latency period was 15 days for each of bees fed on maize, fennel and clover pollen. The slowest appearance of egg-laying workers was observed on the 18th day for bees fed on coriander pollen, (Table 2). In most treatments, appearance of egg-laying workers increased, then decreased, after that it increased again. This observation may be due to two reasons, the first; queen-like pheromone is produced by the laying workers that help to inhibit ovaries development and play a major role in the development of reproductive organs among workers (Simon *et al*., 2001). The second reason, is that duration of laying worker phase is much influenced by colony condition e.g., sometimes these laying workers are very severely attacked and may be killed, then they decrease and within a few days laying workers increase again (Velthuis, 1985).

It is clear, from the present results under queenless condition, that the tendency of honey bee workers to produce laying workers relatively depend on the type of pollen. It is known, that the quality of plant pollens differ, especially in protein component.

Pollen is very important for bee colonies, it is the only source of protein in the diet of bees, and it also contains all of the lipids, carbohydrates, vitamins and minerals necessary for normal growth and development of bee colonies (Hydak, 1970 and Roulston and Cane, 2000).

Crude protein in an essential dietary component for the development and well being of bee colony. The pollen with crude protein levels less than 20% are considered to be below and can not satisfy colony requirements for optimum production (Kleinschmidt and Kondos, 1976).

Also, Kleinschmidt *et al*. (1974) found that the digestive capacity of bees limit protein intake and pollens containing less than 20% crude protein did not appear to satisfy nutritional requirements of bee colony.

Ovarian development, as a measuring factor, is considered to be a physiological parameter used to evaluate the quality of a pollen diet is the extent of ovary development in bee workers. Pollen that is protein-rich usually promotes ovaries and egg development in workers that are caged without queens (Maurizio, 1954 and Lin and Winston, 1998), and a lack of pollen protein can retard or prevent ovary development (Harris and Harbo, 1990).

In the present results, bees fed on faba bean, canola or caper pollen gave a high ovarian development, because these types of pollen have very high crude protein levels, when compared to other pollen sources. Canola pollen have crude protein of about 23 to 27% (Rayner and Langridge, 1985 and Stace, 1996) and faba bean pollen has 24% (Somerville, 1995). Bees fed on maize, fennel or coriander pollen gave a low ovarian development because these pollens may have low levels of crude protein. Stace (1996) found that the crude protein levels of maize pollen was very low (14-15%). Whereas bees fed on sugar solution and no pollen (control) gave undeveloped ovaries. Pollen protein promotes growth of the fat body (Maurizio, 1954), and haemolymph vitellogenin titre has been linked to the level of pollen in the diet of workers. In contrast, worker ovarian development provides a direct measure of the ability of bees to convert pollen proteins into vitellogenin (Bitondi and Simŏes, 1996).

Ability of workers for transferring to laying workers in queenless conditions depend on pollen sources. This could be due to the consumption of pollen to develop their organs and then the yolk precursor protein or vitellogenin level increases to reach maximum amount at nursing period.

Pollen can promote ovarian development of queenless workers. Honey bees fed on a mixture of pollen and honey developed ovaries in queenless condition, whereas bees fed on honey only had little ovarian development (Jay, 1975 and Khodairy, 1990). Moreover, restricted consumption of pollen by queenless bees retards worker Oogenesis because of the lack of protein for ovarian development (Harris and Harbo, 1990).

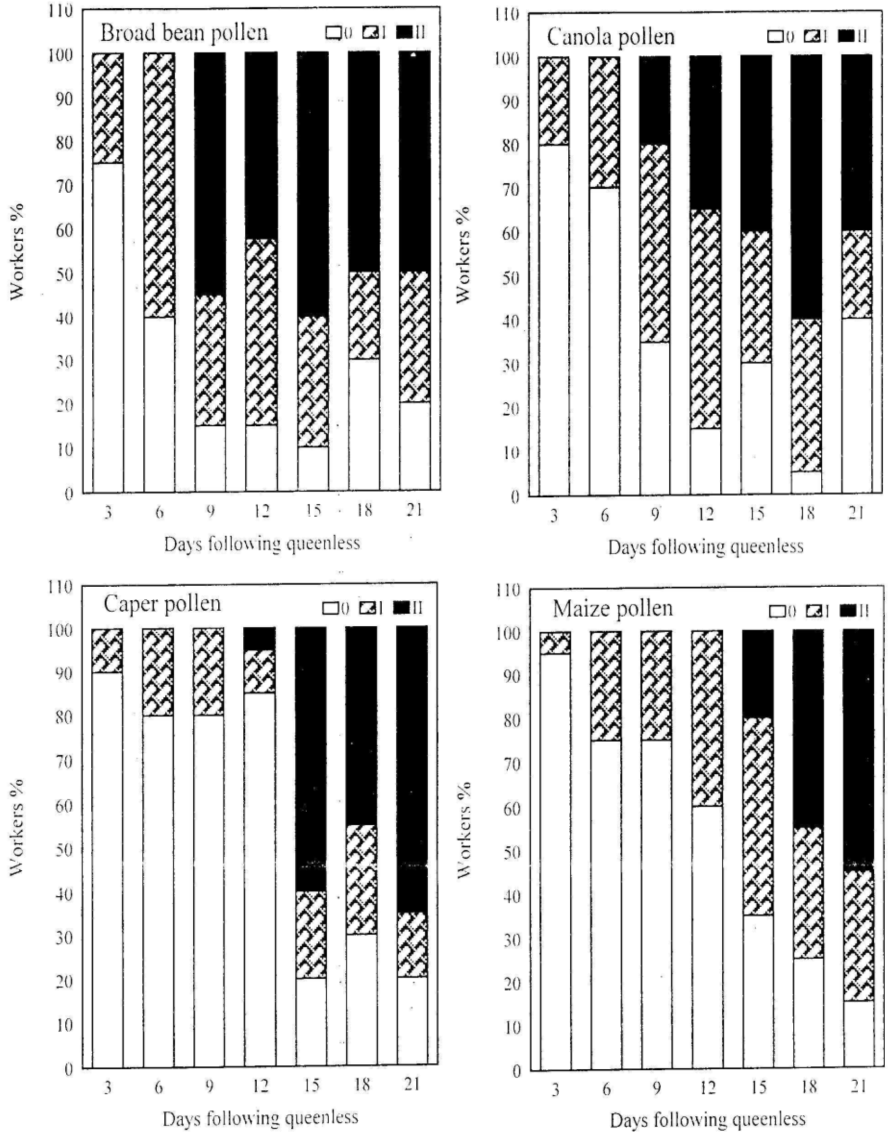

Fig.1. Ovarian development of bee workers in queenless condition which fed on broad bean, canola, caper and maize bee-stored pollen, at a period of 21 days.

Degree of ovary development:

0, undeveloped I. slightly developed II, well developed

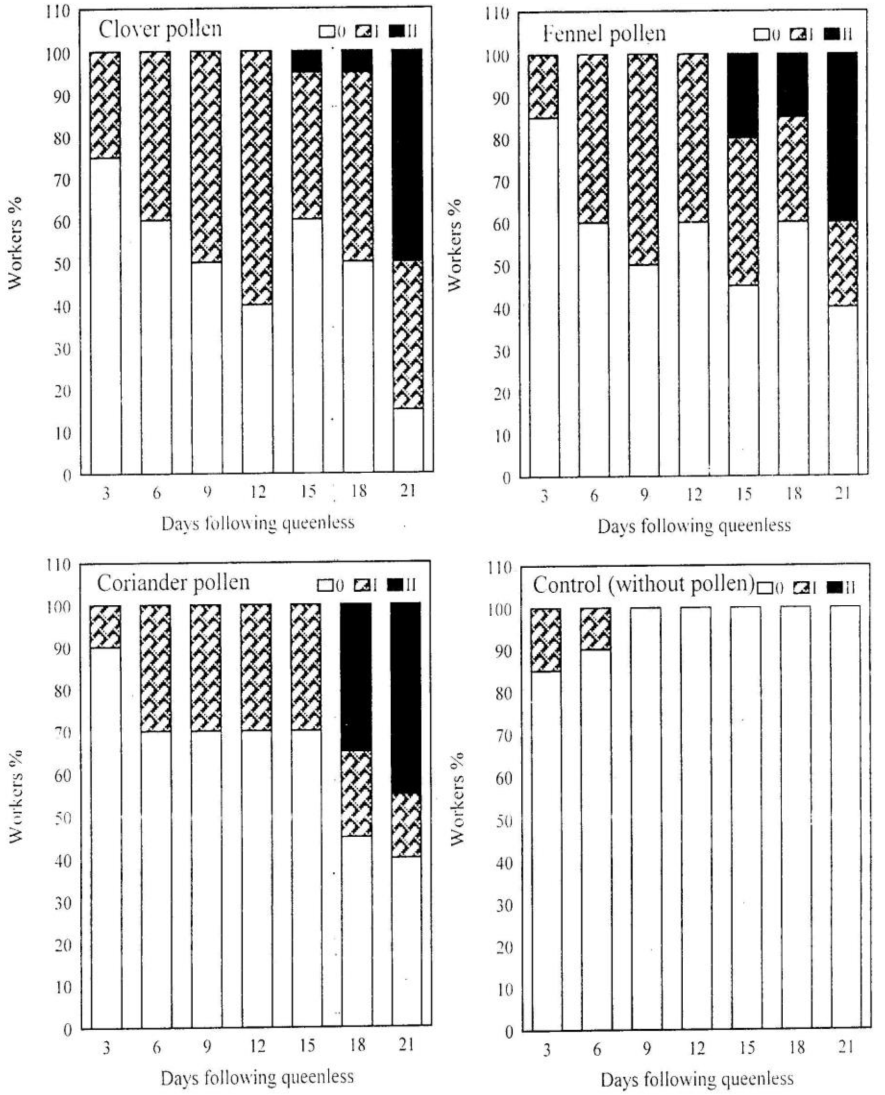

Fig.2. Ovarian development of bee workers in queenless condition which fed on clover, fennel, coriander and control (without pollen) bee-stored pollen, at a period of 21 days.

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

Table(1): Ovarian development index in queenless bee workers which fed on bee-stored pollen from seven monofloral sources, at a period of 21 days.

Days following queenless	Avg. of ovarian development index ± SD							
	Broad bean	Canola	Caper	Maize	Clover	Fennel	Coriander	Control
3	1.25 ±0.10	1.20 ±0.16	1.10 ±0.12	1.05 ±0.10	1.25 ±0.10	1.15 ±0.10	1.10 ±0.12	1.15 ±0.10
6	1.60 ±0.00	1.30 ±0.12	1.20 ±0.16	1.25 ±0.10	1.40 ±0.00	1.40 ±0.00	1.25 ±0.19	1.10 ±0.12
9	2.40 ±0.37	1.85 ±0.19	1.20 ±0.16	1.25 ±0.10	1.50 ±0.12	1.50 ±0.12	1.30 ±0.12	1.00 ±0.00
12	2.30 ±0.22	2.20 ±0.33	1.25 ±0.25	1.40 ±0.16	1.60 ±0.28	1.45 ±0.30	1.30 ±0.12	1.00 ±0.00
15	2.50 ±0.35	2.10 ±0.26	2.40 ±0.46	1.85 ±0.10	1.45 ±0.19	1.75 ±0.10	1.30 ±0.12	1.00 ±0.00
18	2.15 ±0.10	2.55 ±0.30	2.15 ±0.25	2.20 ±0.28	1.55 ±0.19	1.55 ±0.19	1.90 ±0.48	1.00 ±0.00
21	2.30 ±0.20	2.00 ±0.33	2.45 ±0.34	2.35 ±0.19	2.35 ±0.19	2.05 ±0.19	2.05 ±0.25	1.00 ±0.00
Grand mean ± SD	2.07d ±0.46	1.89cd ±0.49	1.68bcd ±0.62	1.62bc ±0.51	1.59bc ±0.36	1.55bc ±0.28	1.46b ±0.36	1.04a ±0.06

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

Table(2): Appearance time of egg-laying workers in queenless bee workers which fed on bee-stored pollen from seven monofloral sources, at a period of 21 days.

Days following queenless	Percentage of laying workers						
	Broad bean	Canola	Caper	Maize	Fennel	Clover	Coriander
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	55.0*	20.0*	0.0	0.0	0.0	0.0	0.0
12	42.5	35.0	5.0*	0.0	0.0	0.0	0.0
15	60.0	40.0	60.0	20.0*	20.0*	5.0*	0.0
18	50.0	60.0	45.0	45.0	15.0	5.0	35.0*
21	50.0	40.0	65.0	55.0	40.0	50.0	45.0
Mean	36.79	27.88	25.0	17.14	10.71	8.57	11.43
Latency period (days)	9	9	12	15	15	15	18

* Means of the first appearance of egg-laying workers.

Vitellogenin, reproductive protein, is normally controlled by genetic or hormonal mechanisms, resulting in a vitellogenin production restricted to the vitellogenic phase of Ooegenesis (Bownes, 1986). The egg-laying bees have the highest level of vitellogenin on their first days, a value decreases with increasing time (Engels, 1974). The vitellogenin is the major haemolymph protein of queens (Engels, 1987), also, it is produced by workers, but only during the nurse age (Rutz and Lüscher, 1974). The foragers of queenless *Apis cerana* colony had higher levels of protein in their haemolymph than those of a queenright colony (Naim *et al*., 1985).

The present results are confirmed by the following observations: the protein quality depends on pollen sources and vitellogenin synthesis depends on pollen consumption. Bitondi and Simǒes (1996) found that the vitellogenin is dependent on pollen consumption. Workers fed on a 50% pollen diet had higher vitellogenin levels than workers fed on a 15% pollen diet and a 0% pollen diet severely impaired the increase in vitellogenin titer. In a queenless condition some workers increase vitellogenin production and become egg layers (Velthuis *et al*., 1990).

Nurse bees functioning as pollen-digesting units affect the ovarian development of other workers and to a less extent vitellogenesis via food exchange (Lin *et al*., 1999). They suggest that the nurse bees could play an important role in mediating worker fertility via trophallaxis, possibly by differentiating worker dominance status, and generally only young workers become fertile when a queen is lost in a colony. Vitellogenin is a more sensitive parameter to measure bee fertility and might be a useful tool to further explore ovary development and egg laying in bee workers.

Also, certain hormones play an important and diverse role in regulating the feeding, reproduction and vitellogenesis. Juvenile hormones regulate vitellogenesis in the fat body, vitellogenin uptake by the ovaries (Tobe and Stay, 1985 and Davey, 1996) and oviposition behavior (Cayre *et al*., 1994). A low juvenile hormone titer at the beginning of adult life, combined with the intense pollen consumptions for the initiation and progress of vitellogenin synthesis with accumulation in haemolymph. Such bees apparently can react very quickly to queen loss and become laying workers (Pinto *et al*., 2000).

Low titers of juvenile hormone are detected in young bee workers which synthesis vitellogenin, whereas high titers are present in foragers that no longer produce vitellogenin (Robinson *et al*., 1991). Vitellogenin starts to accumulate by days 3-4 and remains at a high level until the end of the second week, then it decreases (Rutz *et al*., 1976, Fluri *et al*., 1982 and Engels *et al*., 1990). By the time, the juvenile hormone titer starts to increase (Huang *et al*., 1991).

On the other hand, the interactions between juvenile hormone and ecdysteroids play a central role in the regulation of a particular process such as vitellogenesis (Hagedorn, 1983). Worker bees have a low ecdysteroid titer relative to queens, and mated queens have a higher than virgin queens, suggesting that this hormone may be involved in reproduction (Kaatz, 1987). The ecdysteroid titers were low in both nurses and foragers, high in laying workers and laying queens (Robinson *et al*., 1991).

Also, dopamine and its metabolites; n-acetyldopamine and norepinephrine, change the reproductive states of honey bee workers. The brain levels of dopamine and its metabolites were positively correlated with ovary development and its level was higher in queenless than queenright workers (Sasaki and Nagao, 2001).

Process of transferring from normal workers to laying workers is very complicated when queen is lost. Ages of workers, 3, 6, 9 and 12-day-old, apparently can react and become egg layers. In young ages of bee workers, the physiological interaction occur between a low juvenile hormone, certain levels of ecdysteroids and dopamine and its metabolites, combined with intense pollen consumptions leading to progress of vitellogenin synthesis and accumulation in haemolymph. Such bees (3, 6, 9 and 12-day-old), apparently can react very quickly to queen loss compared with the other ages and become laying workers (Khodairy, 2001).

According to the present study and previous information, it can be concluded that the pollen quality play an important and main role in the transferring to laying workers, together with other important factors, such as seasonal variation (Hoover *et al*., 2006), races (Ruttner and Hesse, 1981 and Khodairy, 2002), pollen consumption (Jay, 1975, Khodairy, 1990 and Bitondi and Simoes, 1996); vitellogenin levels (Bownes, 1986, Velthuis *et al*., 1990 and Lin *et al*., 1999) 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).

It can be suggested and recommended that, after a colony dequeened, it is necessary to introduce a queen before appearance of egg-laying workers, within 9 days as a maximum, in both broad bean and canola seasons, within 12 days in caper season, within 15 days in each of maize, fennel and clover seasons, and within 18 days in coriander season, in Assiut region.

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تأثير بعض الأنواع من خبز النحل على تطور المبايض لشغالات نحل العسل تحت ظروف عدم وجود ملكة

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أجريت هذه الدراسة بمنحل كلية الزراعة بجامعة أسيوط فى موسم 2006 بغرض دراسة تأثير 7 أنواع من حبوب اللقاح (خبز النحل) وهى : الفول البلدى ، الكانولا ، الكبر ، الذرة الشامية ، البرسيم ، الشمر ، الكزبرة على درجة تطور المبايض فى شغالات نحل العسل وسرعة ظهور الشغالات الواضعة للبيض (الأمهات الكاذبة) . وقد لوحظ أن حبوب اللقاح تلعب دوراً رئيسياً وهاماً فى تطور المبايض . وقد أوضحت النتائج أن جميع أنواع حبوب اللقاح المستخدمة أعطت تطوراً جيداً لمبايض الشغالات بدرجات مختلفة مقارنة بالكنترول (بدون حبوب لقاح) الذى لم يعطى تطوراً للمبايض . وقد أعطت التغذية باستخدام حبوب لقاح الفول البلدى أعلى تأثيراً على تطور المبايض فى أقصر فترة بينما فى حالة التغذية باستخدام حبوب لقاح الكزبرة كانت أقل وأبطأ تأثيراً على تطور المبايض . وسجلت أعلى قيمة لدليل تطور المبايض (Ovarian development index) 2.5 عند اليوم الـ 15 فى حالة التغذية على حبوب لقاح الفول البلدى يليها حبوب لقاح الكانولا بقيمة 2.55 عند اليوم الـ 18. بينما فى باقى المعاملات التى تغذت على حبوب لقاح كل من الكبر والذرة الشامية والبرسيم والشمر كانت قيمة دليل التطور 2.45 ، 2.35 ، 2.35 ، 2.05 ، 2.05 على التوالى عند اليوم الـ 21 . وقد سجل أول ظهور للشغالات الواضعة للبيض فى حالة التغذية على كل من حبوب لقاح الفول البلدى والكانولا بعد 9 أيام من بداية التغذية ، يليها حبوب لقاح الكبر بعد 12 يوماً . بينما سجل أول ظهور للشغالات الواضعة للبيض بعد 15 يوماً فى حالة التغذية على حبوب لقاح كل من الذرة الشامية والشمر والبرسيم . وكان آخر ظهور للشغالات الواضعة للبيض بعد 18 يوماً فى حالة التغذية على حبوب لقاح الكزبرة . ومن خلال هذه النتائج يُنصح مربى النحل عند فقد أو تغيير ملكة الطائفة بسرعة إدخال الملكة البديلة فى فترة أقل من 9 أيام خلال موسم الفول البلدى والكانولا وفى فترة أقل من 12 يوماً فى موسم الكبر وفى فترة أقل من 15 يوماً خلال مواسم كل من الذرة الشامية والشمر والبرسيم وفى فترة أقل من 18 يوماً فى موسم الكزبرة وذلك قبل ظهور الشغالات الواضعة للبيض التى تحول دون قبول الطوائف للملكات ، وذلك فى منطقة أسيوط .