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AFFECT QUEEN STATUS ON BEE BREAD CONSUMPTION AND LONGEVITY OF HONEYBEE WORKERS (APIS MELLIFERA L.)

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ABSTRACT:

The investigation was carried out under laboratory conditions at Assiut Insect Research laboratory, Plant Protection Research Institute during the active season of 2012. The bee-bread conception and honeybee workers longevity were studied in reference to effects of the effects of certain queen statuses [egg-laying mated queen of one year old (M), supersedure queen (S), three-days old virgin queen (V), queen cell at the beginning of sealed (Q), without queen (W) and without queen / without bee bread (W/W)]. Means of bee bread consumption of newly emerged worker bees headed with tested queen statuses didn't differ significantly. The highest bee bread consumption through thirty- three days after emergence was recorded for the workers under Q status (48.513 mg). The lowest consumption was noticed for workers under V status (37.626 mg). Mortality rates of honeybee workers under different status of queen were calculated, indicated that the lifetime of honeybee workers under M, S, and Q statuses, was mostly similar, and recording 40.22; 39.12; and 39.1 days, respectively, while the workers under V status, their LT 50 extended to 41.55 days. For caged worker bees under W/W status, their longevity shortened LT 50 to 11.75 days in compared with the workers under W status where their LT 50 (21.50 days). It is clear that the different queen statuses play an important role on the bee bread consumption and also play an important role on influencing the longevity of honeybee workers.

Key words: Honeybee, *Apis mellifera* L., queen status, bee bread, consumption, mortality, longevity.

INTRODUCTION:

Pollen is the sole source of dietary protein as well as some lipids, vitamins, mineral amounts of carbohydrates (Herbert, 1992). Pollen is crucial for colonies as the sole source of protein for bread rearing. Honeybee pollen forager collects pollen from the anther of a plant, carries it on her corbiculae to the hive, and deposits the load of pollen in wax comb cells usually located near the brood (Winston, 1987). Pollen is further packed into the cell with the addition of a glandular secretions, thought to originate from both mandibular and hypopharyngeal glands, and is topped with a small cover of honey (Herbert, 1992 and Winston, 1987). The glandular additions step pollen grain germination over a two day period and begin the digestive processes. Nutritional behavior of honeybee workers varies drastically during their life.

Protein is consumed primarily by larvae through nurse bee food gland se-

cretions and therefore stored in cells near the brood rearing area in the center of the nest. The second greatest consumers of pollen are newly emerged adults followed by nurse bees. Protein consumption is necessary for complete development of muscles, and hypopharyngeal, mandibular, and wax glands (Herbert, 1992). Nurse bees consume and digest stored pollen and then convert it into proteinaceous secretions (Brouwers, 1982, Crailsheim, 1990 and Crailshien et al., 1992).

Beekeepers commonly provide a protein/ pollen supplements to colonies during period of low pollen availability to stimulate colony growth (Waller et al., 1981, Nabors, 2000, Safari et al., 2004 and Van der Steen, 2007).

In temperate climates, the lifespan distribution of worker bees is strongly bimodal (Fluri and Imdorf, 1989). During favorable conditions in summer, young workers conduct tasks inside the nest such as nursing, till 2- 3

weeks later to initiate foraging (seeley, 1982). The majority of bees die within 1-2 weeks of their first foraging flight (Visscher and Dukas, 1997) with a resulting adult lifespan of 3- 6 weeks for summer workers. However, when the favorable season ends, brood rearing and foraging ceases. Instead of dividing labor between nest tasks and foraging activities, the workers enter the diutinus winter bees stage (Amdam and Omholt, 2002), and can survive for 20 weeks or longer (Maurizio, 1950).

The extended lifespan of diutinus bees correlates with an increased amount of stored lipids and proteins in their hemolymph and fat body (Shehata et al., 1981 and Fluri et al., 1982). Honeybee, nutrition is vital for colony growth and maintenance of a robust immune system (Sagil and Breece, 2012).

In general, a larger adult population results in increased probabilities for colonies to reproduce and for swarms to survive; fitness traits (Cole, 1984; Lee and Winston, 1987; Pomeroy, 1979; and Seeley, 1985)

The objective of the current study was to determine the influence of the different queen statuses on bee bread consumption and longevity of honeybee *Apis mellifera* L. workers.

MATERIALS AND METHODS:

The experiments were carried out in Assiut, Insect Research Laboratory, Plant Protection Research Institute during the active season of 2012.

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 queen right colony, then incubated at 32°C ±1 and 60% RH. , and the brood were observed until adults emergence. Experimental wooden cages of 15 × 15 × 5 cm, dimensions with a glass side and other was covered with black muslin. Every cage was provided with a vial of tap water and other vial of sugar solution 1:1 (w: v); bee bread and a pieces of wax foundation. The bee bread was changed in each cage every 3 days. Newly emerged

workers aged 0-12 hours were confined in the experimental cages (200 workers / cage as a replicate). The cages were continuously supplied with water, sucrose solution and bee bread. The cages were divided into six groups dependent on status of introduced queens as follows:

Group < 1 >, cages contained egg-laying mated queens: (M).

Group < 2 >, cages contained mated and egg-laying queens and replaced by honeybees

(supersedure queen) (S).

Group < 3 >, cages contained three-days-old virgin queens (V).

Group < 4 >, cages contained queen cells at beginning of sealed (Q).

Group < 5 >, cages without queens (queenless) as a control (W).

Group < 6 >, cages without queens / without bee bread (W/W).

Each group was four replicate. The cages were held in a dark incubator at $32^{\circ}\text{C} \pm 1$ and 60% RH.

Measurement of food consumption:

Each 3 days amount of food / cage, was compared to the number of live bees existing in each cage during the investigation. Food consumption was calculated daily, and represented as (Mg./ bee/

day). This procedure was repeated eleven times through three- days intervals. To evaluate the evaporation, four feeders of bee bread were put under the same conditions without bees. The decreasing in weight of the bee bred was calculated to correct the quantity of food consumption.

Measurement of workers longevity:

Dead bees in each cage were counted and removed every three- days intervals. The LT 50 (The time required to reach 50% mortality) was estimated. Values in day(s) of bee workers fed under different queen statuses were determined by a computerized probit analysis program.

Statistical analysis:

Data were analyzed using one-way ANOVA and presented as mean \pm S.E. (slandered error). Means were separated by Duncan's Multiple Range Test (DMRT). The median lethal exposure time (LT_{50} = time required to kill 50% tested bees) of each treatment was determined by a computerized probit analysis program and expressed in day (Finney, 1952). Figures and statistical analysis were done using Graph Pad Prism 5 TM software (San Diego, CA).

RESULTS AND DISCUSSION:

Determination of food consumption:

It was found that honey bee workers without queen (control) consumed 5.893 mg / day during the first three days after emergence and 3.615 mg / day from the 4th to the 6th day, then the amount decreased to 0.711 and 0.231 mg / day at the following periods till the end of the experiment at the 33rd days of worker age (Table 1 and figure 1).

It is obvious that, the honeybee workers consumed the amounts of bee bread in a similar trend at the several queen statuses. They started to consume a considerable amount at the first period after emergence (1 - 3 days),

then decreased gradually from the 4 - 6 days towards the progressing period till the end of the observed periods at 31 – 33 days for the most inspected queen statuses. The amount of bee bread consumption differed significantly over all the compared periods (Table 1).

The total bee bread consumed by the workers was calculated through the 33 days after emergence for each queen statuses. Also, the deviation in the total consumption of each queen status out from that of without queen status (control).

Table (1): Food consumption by honeybee workers headed by different queen status.

Mean of consumption (mg. dry matter / bee / day) ± SE	Queen statuses					
	One year old mated queen (M)	Supersedure queen (S)	Three days old virgin queen (V)	Queen cell at beginning of sealed (Q)	Without queen (W)	Mean
1-3 days	4.871 AB ± 0.508	3.976 BC ± 0.122	2.976 C ± 0.328	4.273 BC ± 0.307	5.890 A ± 0.472	4.398 A ± 0.483
4-6 days	3.246 B ± 0.546	4.539 AB ± 0.121	4.652 A ± 0.254	4.749 A ± 0.135	3.613 AB ± 0.737	4.160 A ± 0.306
7-9 days	1.540 AB ± 0.486	1.265 AB ± 0.164	1.666 AB ± 0.130	2.196 A ± 0.311	0.711 B ± 0.070	1.476 B ± 0.244
10-12 days	0.963 AB ± 0.377	0.990 AB ± 0.110	0.653 B ± 0.147	1.984 A ± 0.715	0.639 B ± 0.144	1.046 BC ± 0.246
13-15 days	0.810 A ± 0.223	0.771 A ± 0.167	0.426 A ± 0.084	0.792 A ± 0.265	0.444 A ± 0.101	0.649 CD ± 0.087
16-18 days	0.692 A ± 0.234	0.433 A ± 0.136	0.556 A ± 0.192	0.636 A ± 0.118	0.340 A ± 0.087	0.422 CD ± 0.049
19-21 days	0.749 A ± 0.080	0.520 A ± 0.163	0.460 A ± 0.137	0.413 A ± 0.062	0.240 A ± 0.046	0.531 CD ± 0.065
22-24 days	0.291 A ± 0.108	0.394 A ± 0.138	0.520 A ± 0.042	0.326 A ± 0.047	0.316 A ± 0.064	0.369 D ± 0.041
25-27 days	0.245 A ± 0.048	0.398 A ± 0.145	0.241 A ± 0.048	0.378 A ± 0.055	0.240 A ± 0.071	0.300 D ± 0.036
28-30 days	0.297 AB ± 0.060	0.228 AB ± 0.031	0.314 A ± 0.074	0.157 B ± 0.020	0.246 AB ± 0.042	0.248 D ± 0.028
31-33 days	0.127 B ± 0.012	0.091 B ± 0.016	0.078 B ± 0.012	0.267 A ± 0.037	0.231 A ± 0.075	0.159 D ± 0.038
Mean	1.233 A ± 0.452	1.237 A ± 0.463	1.140 A ± 0.432	1.470 A ± 0.499	1.174 A ± 0.557	
Total (mg / bee / 33) day	40.683 B ± 5.310	40.815 B ± 4.071	37.626 B ± 4.583	48.513 A ± 7.328	38.739 B ± 4.925	
Deviation from control (%)	+ 5.018	+ 5.359	- 2.879	- 25.230	0.000	

Means followed by the same letter at the same column are not significant differences at 5 % level of probability.

I: Mated queen 1-year old; II: Mated supersedure queens; III: Virgin queens 3-day old; IV: Queen cells; V: Queenless; VI: Queenless / without bee bread

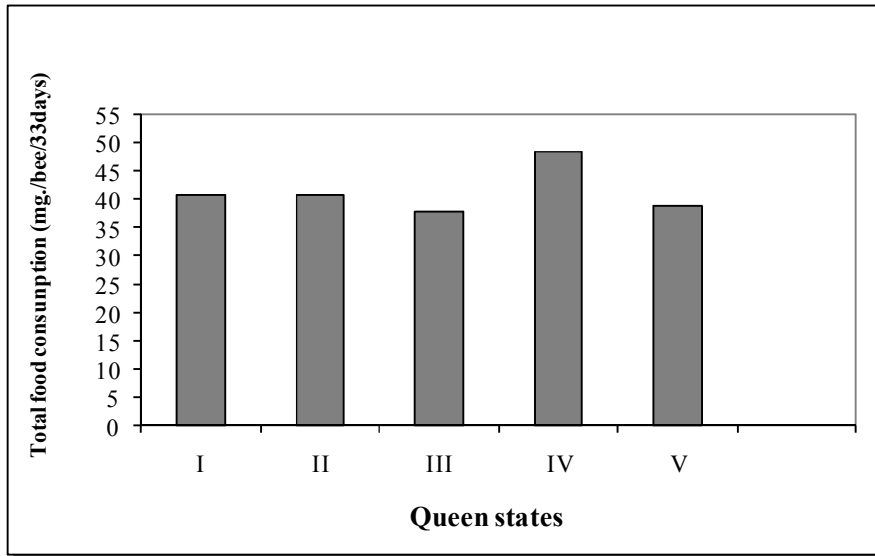


Fig. 1: Total bee bread consumed by honeybee workers headed with different status of queens.

It was found that the highest food consumption was recorded for the workers which headed by queen cells at beginning of sealed (48.513 mg). While, the lowest consumption was noticed for the workers under three - days old virgin queen (37.626 mg). Queen cell status is different significantly from all the other compared statuses.

Table 1 and Figure 1 show the superiority of bee bread consumption

for the workers which headed by queen cells at beginning of sealed (48.513 mg) over any all other queen statuses. The percentages of deviation from control (without queen) + 5.018; +5.359; - 2.873; and +25.230 % were recorded in case of one-year old mated queens; supersedure queens; three- days old virgin queens and queen cells at beginning of sealed, respectively.

The amount of bee bread consumption differed significantly over all the compared periods. Workers started to

consume a considerable amount at 1- to 3-days old after emergence, then decreased gradually. Similar results were also obtained by (Haydak, 1970 and Jaycox and Parise 1981) who reported that mass consumption of pollen begins when workers are from 42 – 52 hours old and reaches a maximum around day-live of worker age and then decreased to low level by time.

Determination of worker's longevity:

The cumulative mortality percentages as well as the longevity of workers at each queen status were recorded. The half-life was estimated as LT 50 (number of days required for 50 % of the bee to die). The mortality percentages and LT 50 were illustrated in (Fig. 2, A-F). Data revealed that caged bees without queens / without bee bread shortened the longevity of honeybee workers (LT 50, 11.75 days) in compared with those without queen (LT 50, 21.50 days).

The results also indicated that, the lifetime of honeybee workers headed with mated queen- one year old; supersedure queen and queen cell was mostly similar, recording 40.22; 39.12; and 39.10 days, respectively, while those headed with virgin queen three- days old , their LT 50

extended to 41.55 days. These results indicated that protein played an important role in influencing the longevity of the honeybee workers. This conclusion is in agreement with those of Erickson and Herbert, 1980. Also, obtained results indicated that the absence of the queen, leads to reduction in the workers survival. This supports earlier conclusions that queenlessness may cause enough stress to kill certain workers (Milne, 1982; and Delaphane and Harbo, 1987), and that the level of queen pheromone in the colony affects worker survival (Jaycox, 1970). Queen mandibular pheromones (QMP) produces many nurse-like physiological changes, such as increased levels of lipids and vitellogenin RNA in the fat bodies of workers (Fischer & Grozinger 2008),

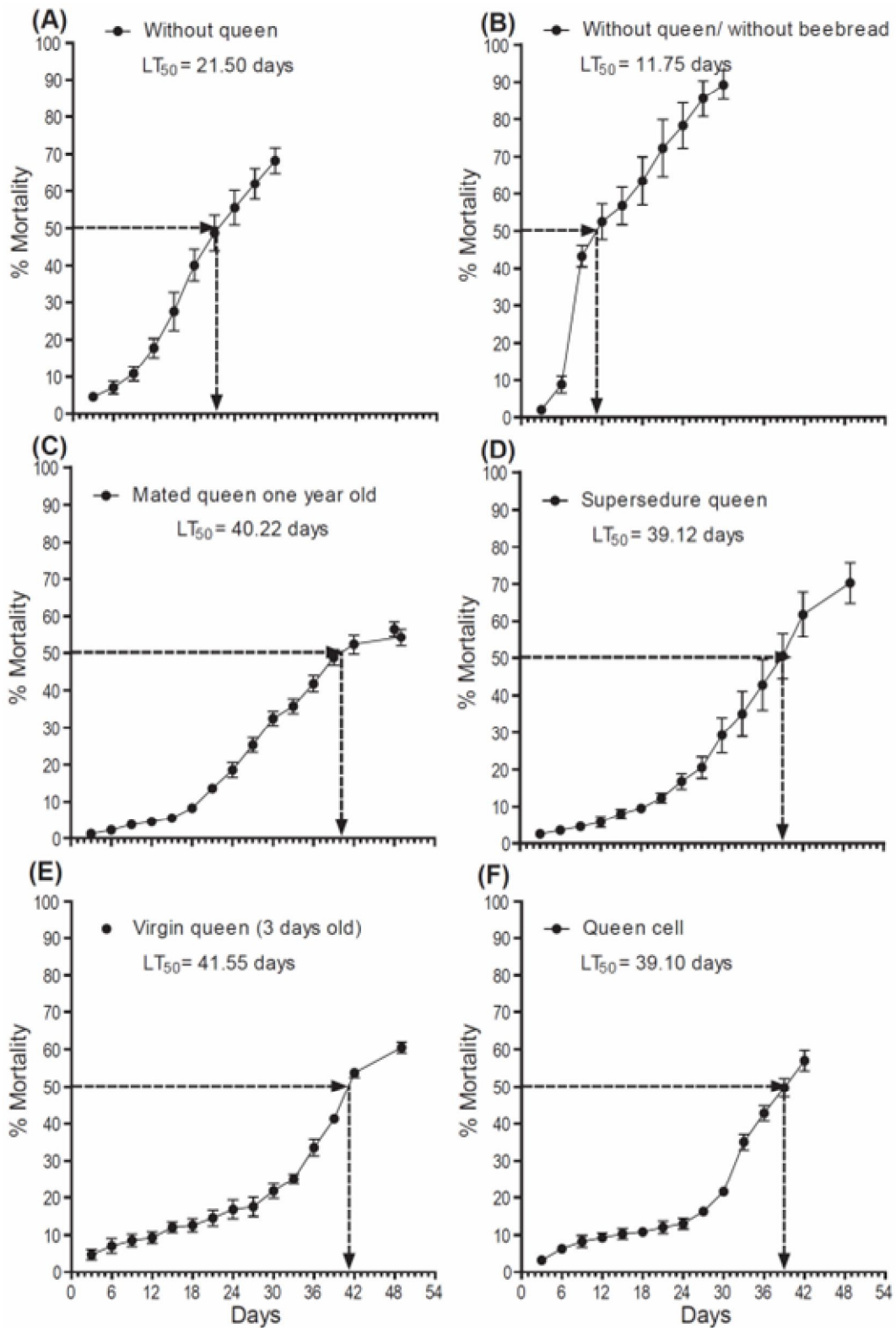


Fig. 2: Cumulative mortality percentage of honeybee workers headed with different queen statuses.

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تأثير حالة الملكة علي معدل استهلاك خبز النحل وطول العمر في شغالات نحل العسل

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تم إجراء هذا البحث تحت الظروف العملية في معمل بحوث الحشرات بأسيوط التابع لمعهد بحوث وقاية النباتات وذلك خلال موسم النشاط من عام ٢٠١٢م وفي هذا البحث تم دراسة تأثيرات بعض حالات الملكة (ملكة ملقحة عمر سنة، ملكة عذارى عمر ثلاثة أيام، ملكة إحلال ملقحة، بيت ملكي، غياب الملكة و غياب الملكة بدون وجود خبز نحل) علي كل من استهلاك خبز النحل وطول العمر لشغالات نحل العسل. لم يوجد أي اختلاف معنوي بين متوسطات إستهلاك شغالات النحل حديثة الخروج في حالات الملكة المختبرة. سُجل اعلي استهلاك لخبز النحل خلال ٣٣ يوما بعد الفحص وذلك للشغالات التي علي راسها بيت ملكي (٤٨,٥١٣ مجم). ولوحظ أقل استهلاك في حالات الملكة العذراء. وتم حساب معدلات الموت للشغالات تحت حالات الملكة المختلفة. وأشارت النتائج الي تشابه في طول عمر الشغالات وذلك في حالات الملكة الملقحة وملكة الاحلال والبيت الملكي حيث سجلت قيم الفترة اللازمة للموت ٥% من الافراد (LT 50) ٢٢,٤٠، ١٢,٣٩، ١٠,٣٩ يوما علي التوالي، بينما تلك التي علي رأسها ملكة عذراء امتدت الفترة اللازمة لموت ٥٠% من الافراد (LT 50) الي ١١,٥٥ يوماً. أما الشغالات الموجودة في أقفاص بدون ملكات وبدون خبز نحل فقد قَصُرَ طول العمر الي ١١,٧٥ يوماً وذلك مقارنة بتلك التي بدون ملكة الي ٢١,٥٠ يوماً. أظهرت الدراسة انه يوجد دورا واضحا لحالة الملكة علي استهلاك خبز النحل وكذلك أيضا تأثير تلك الحالات والتغذية البروتينية علي طول عمر شغالات نحل العسل.