

**EFFECT OF GENOTYPE, WEIGHT OF HONEYBEE VIRGIN
QUEENS AND BROOD STATUS ON SOME PARAMETERS
USED FOR JUDGING THE QUEENS FROM THEIR
INTRODUCTION UNTIL EGG-LAYING**

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ABSTRACT

Four Parameters were chosen in this study on which the effect of both weight and genotypes of honeybee queens as well as brood status were studied. One of these parameters is related to the pre-mating period, being the acceptance rate of the introduced queens, and the others are related to post-mating period, being mating success, pre-oviposition period and sperm counts. The genotype of the queens had a significant effect on both pre-oviposition and the number of spermatozoa in the spermatheca, whereas this effect was not significant on acceptance rate and mating success. On the other hand, the weight of the queens showed a significant effect on both acceptance and pre-oviposition period, but did not show a significant effect on mating success and sperm count. A trend response was established to test the linear dependence of both acceptance rate and pre-oviposition period on the weight, and the result verified this relationship, being positive with the former and negative with the latter. The comparison between different genotypes under study showed the superiority of the F1-Italian hybrid queens to the remaining genotypes.

The effect of sealed brood was superior to the effect of the other brood status for all parameters under study. On the contrary, the treatments containing unsealed brood showed an inhibitory effect on most of the parameters under study, when both compared with the treatment containing normal brood.

Key words: *acceptance rate, Apis mellifera, capped brood, genotypes, honeybee of mating success, pre-oviposition period, sperm counts, uncapped brood, virgin queens, weight.*

1. INTRODUCTION

Mating of virgin queens is the last step in producing young laying queens. The new emerged queen has to attain the reproductive capability of a colony, and in order to obtain a full-laying queens, they have to be cared for, either during the breeding period or after their emergence. During the breeding period, the breeder can play an important role to establish a suitable situation for achieving this purpose, whether by choosing the right larvae-age for grafting or by establishing the suitable situation in the breeding colony (Laidlaw, 1979). During the post emergence period, the breeders have to treat the emerging queens in a proper manner from the emergence until their mating. There are many parameters that could be used to judge and select the queens after their emergence. Some of these parameters are to be considered during the pre-mating and others are related to post-mating period (Huang and Zhi, 1985). In order to obtain the optimal values of these parameters, it is very important, for the breeder, to be aware of the different factors which influence them. Some of these factors are related to the queen itself (Szabo, 1973) and others are related to the situation of the breeding colony (Pilipenko, 1976) or the external environment, in which this colony is existed (El-Sarrag and Nagi, 1988). The genetic-relationship between the introduced queens and bees in the receiver colony is one of the factors, which need to be shed the light on.

Fert (1997) reported, that rejection behaviour of the colony towards queens is much more correlated with the introduction of a queen of a different race from that of the colony. The weight of the queens at emergence is considered to be a useful index for beekeeper in selecting queens (Szabo, 1973), since it is related to

to other economical characters, such as the amount of brood that the queen can produce (Avetisyan, 1961).

In this study, two factors related to the virgin queens, being the weight and the genetical origin of the introduced virgin queens, and one factor related to the brood, being brood status, were studied to clarify the effect of them on some parameters used for judging honeybee queens.

2. MATERIALS AND METHODS

This experiment was carried out during the summer of 2002 in the Faculty of Agriculture, Minia University, while the queens were prepared in the Beekeeping Section of the Plant Protection Institute.

Searching for the best genotype, weight of honeybee queens and brood status, 112 mating nuclei, with 5 frames in each, were prepared for receiving 112 virgin queens of different genotypes. The queens were reared artificially by Dolittle method (Laidlaw, 1979) in cell-building colonies by grafting larvae 24 hours old. 9 days later the queen cells were removed from the cell-building colonies and placed under hemi-spherical cages in queenless colonies until their introducing in the mating nuclei.

2.1. Effect of genotypes and weight of honeybee queens on their acceptance rate, mating success, preoviposition period and sperm counts

Out of 112 orphan mating nuclei used in this study, 72 were chosen randomly for receiving the same number of virgin queens. The queens tested in this experiment were grouped according to their genotypes and weight. The tested genotypes were chosen to represent the most common honeybees prevailing in Egypt, as follows:

Carniolan queens (*Apis mellifera carnica*)

Italian queens (*A. m. ligustica*)

Egyptian queens (*A. m. lamarckii*)

First-hybrid Carniolan queens (F1-Carniolan)

First-hybrid Italian queens (F1-Italian)

Before introducing the queens, they were weighted and classified accordingly to three weight-classes as follows:

In the first class, the weight ranged between 140 – 160 mg.

In the second class, the weight ranged between 161 –180 mg.

In the third class, the weight ranged between 181 – 200 mg.

The queens were introduced into their transferring cages in the nuclei, and two days later, the cages were opened manually to free the queens, and allowed to mate naturally.

2.1.1. The effect of both genotype and weight of virgin queens on their acceptance rate

Having introduced the different queen groups to their nuclei, the acceptance rate was estimated after two days from their introduction time. The loss rate of the introduced virgin queens was analyzed using contingency-table analysis using statistical package system developed by Holm(1997). Differences at 5% level of probability were considered to be significant.

2.1.2. Effect of both genotype and weight of virgin queens on their mating success

Having released the virgin queens in their nuclei, they were checked regularly every two days for eggs and the mating rate of the tested queens have been determined. The queens were considered to be successfully mated when the first area of capped worker brood had appeared.

Mating success was treated statistically by using contingency-table analysis, and the 0.05 level was used as a criterion for the presence of significant differences.

2.1.3. Effect of both genotype and weight of honeybee queens on the pre-oviposition period of the naturally mated queens

Having seen the first egg-area of the mated queen, the onset of oviposition was determined in each nuclei. For this purpose, the nuclei were checked in two periods during the day: At 9 a.m and at 6 p.m. The pre-oviposition period is the time between the queen-introduction and the onset of the oviposition, which was tested statistically using two way analysis of variance, followed by LSD test for multiple comparisons between the means.

2.1.4. Effect of both genotype and weight of naturally mated queens on the number of spermatozoa in the spermatheca

The successfully mated queens were killed and dissected in all treatments after oviposition onset and the appearance of the first worker sealed brood; then the number of spermatozoa in the spermatheca was counted using the spectrophotometer (Harbo, 1975). The differences between treatments were analyzed by two way analysis of variance, followed by LSD test for multiple comparisons between the means.

2.2. Effect of brood status on acceptance rate, mating success, preoviposition period and sperm counts

Fourty mating nuclei were prepared for reciving 40 first-hybrid of Carniolan Queens. The nuclei were so managed that they will contain different brood status in the different treatments as follows:

10 nuclei with only capped brood

10 nuclei with unsealed brood

10 broodless nuclei

10 nuclei with capped and uncapped brood (normal brood)

The four parameters were measured and analyzed as mentioned in the first experiment. The means of acceptance rates and mating success were treated statistically using contingency-table analysis, whereas pre-oviposition periods and the number of spermatozoa in the spermatheca were analyzed using one way analysis of variance, followed by LSD test for multiple comparisons between the means.

3.RESULTS

3.1. Effect of both queen genotype and weight

3.1.1. Acceptance rate

As shown in Table (1) and Figure(1), the acceptance rates ranged between 66% and 100% for queens with light and heavy weight values, respectively. The effect of weight on this parameter was significant.

Also, there was a linear tendency of the acceptance rate to increase with the increasing of weight for all genotypes under study; this linear relationship was statistically significant.

Another notation could be seen from Table (1), when the values of both light-weighted (between 140 and 160mg) and heavy-weighted queens (between 181 and 210 mg) were compared. The values of the acceptance rates in the light-weighted queens ranged from 50% and 80%, whereas similar values of this parameter were obtained in the heavy-weighted ones.

Comparing the different genotypes with each other, the first Italian queen hybrid had the highest acceptance rate (93%), while the Egyptian queens had the lowest value (75%). When the hybrid queens were grouped and compared with the pure ones, it was clear that the average values of acceptance rates of the hybrid (90%) were superior to those of the pure ones (80%). These differences, however, were not significant, and therefore, the genotype of virgin queens has no effect on the acceptance rate of the introduced virgin queens.

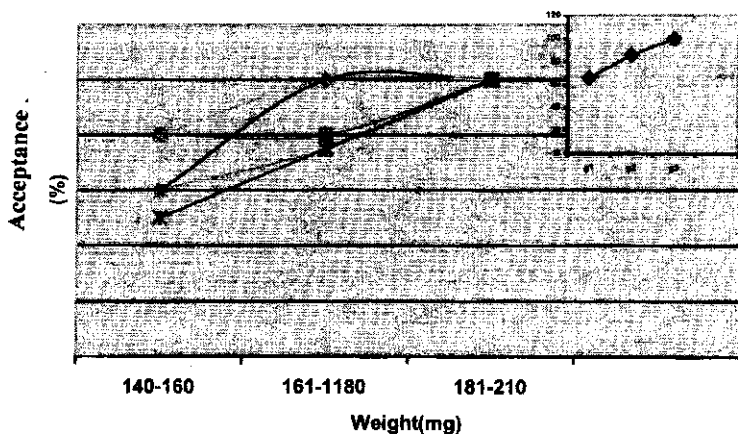


Fig. (1): Acceptance rates of the introduced queens of five genotypes under study (the upper right graph shows the linear relationship over all genotypes, $P < 0.05$).



Table (1) : Effect of genotypes and different weight categories of honeybee queens on their acceptance rate.

| Weight | 140-160mg | | 161-180mg | | 181-210mg | | Means* |
|------------------|-----------|----------------|-----------|----------------|-----------|----------------|----------|
| | No | Acceptance (%) | No | Acceptance (%) | No | Acceptance (%) | |
| Genotypes | | | | | | | |
| Carniolan | 5 | 60 | 4 | 100 | 6 | 100 | 86.67% a |
| F1-Carniolan | 5 | 80 | 5 | 80 | 5 | 100 | 86.67% a |
| Italian | 5 | 60 | 4 | 75 | 6 | 100 | 78.33% a |
| F1-Italian | 5 | 80 | 4 | 100 | 7 | 100 | 93.33% a |
| Egyptian | 4 | 50 | 4 | 75 | 3 | 100 | 75% a |
| Means* | | 66%a | | 86%ab | | 100%bc | |

* Any two means are significantly different if they are followed by letters that are all different ($P < 0.05$).

3.1.2. Mating success

As shown in Table (2), the average values of this parameter ranged between 71% and 90% in heavy- and light-weighted queens, respectively. These differences, however, were not significant (Fig. 2). Successful mating apparently did not depend on the weight of the virgin queens regardless of the genetical background of the queens.

Concerning the comparison between the different genotypes under study, and on the level of all weight classes, the first Italian hybrid showed the highest values of this parameter (100%), whereas the pure Italian queens had the lowest one (67%).

In the three weight classes, both of pure and hybrid Italian queens showed the same values of this parameter (67% and 100%, respectively).

Among the different genotypes, the hybrid ones (F1-hybrid Italian and Carniolan queens) were superior (92%) to those of the pure ones (71%), and this difference was statistically significant.

Accordingly, the genetical origin of the introduced virgin queens has a significant effect on their mating success, but in comparison with the acceptance rate, the weight of the introduced virgin queens did not show significant effect on this parameter.

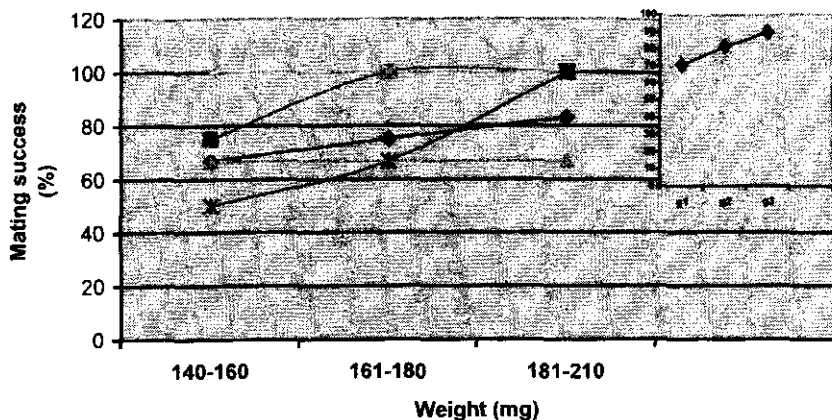


Fig. (2): Percentages of mating success of the five genotypes of honeybee queens under study (the upper right graph shows the linear relationship over all genotypes, $P > 0.05$)

—◆— Carniolan —■— F1-Carniolan —▲— Italian —×— F1-Italian —*— Egyptian

Table (2) : Effect of genotypes and weight of the virgin queens on their mating success.

| Weight \ Genotypes | 140-160mg | | 161-180mg | | 181-210mg | | Means* |
|--------------------|-----------|--------------------|-----------|--------------------|-----------|--------------------|----------|
| | No | Mating success (%) | No | Mating success (%) | No | Mating success (%) | |
| Carniolan | 3 | 66.67 | 4 | 75 | 6 | 83.33 | 75% ab |
| F1-Carniolan | 4 | 75 | 4 | 100 | 5 | 100 | 91.67%ab |
| Italian | 3 | 66.67 | 3 | 66.67 | 6 | 66.67 | 66.67 a |
| F1-Italian | 4 | 100 | 4 | 100 | 7 | 100 | 100 b |
| Egyptian | 2 | 50 | 3 | 66.67 | 3 | 100 | 72.22 ab |
| Means* | | 71% a | | 81.66% a | | 90% a | |

* Any two means are significantly different if they are followed by letters that are all different ($P < 0.05$).

3.1.3. Pre-oviposition period

As the results indicated, the pre-oviposition period was affected significantly by the weight of the queens. The value of this parameter

in the heavy-weighted queens (181–210 mg) was lower than those of the light-weighted ones (140–160mg) and they were 7.38 and 10.63 days, respectively. In other words the light queens (140 mg and lighter) started laying eggs approximately 3 days later than the heavy queens (weighted 181 mg and heavier).

The linear relationship between the weight of the queens and time elapsed since their introduction until they start egg-laying was found to be significant (Figure 3).

Concerning the genotype, the results showed also significant effect especially between both Egyptian and first hybrid Italian queens, which viewed the shortest pre-oviposition period, on one side and Carniolan, F₁-Carniolan hybrid and Italian queens on the other side (Table 3).

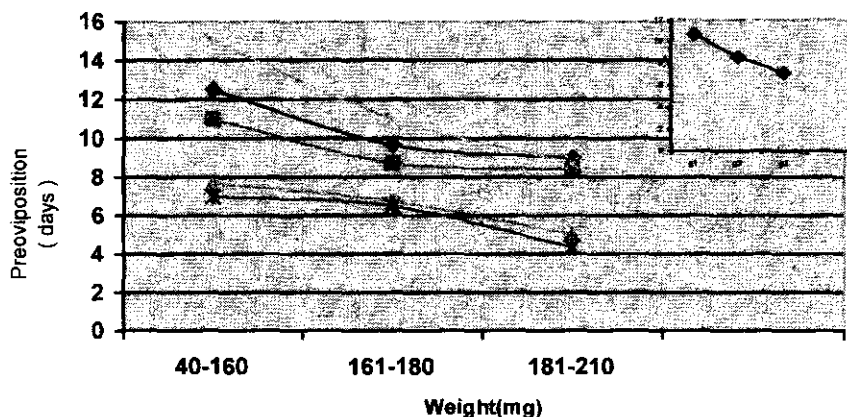


Fig. (3): The relationship between weight and preoviposition period of five genotypes of honeybee queens under study (the upper right graph shows the linear relationship over all genotypes, $P < 0.05$).



Table (3): Effect of genotypes and different weight categories of honeybee queens on their pre-oviposition period.

| Weight Genotypes | 140-160mgs | | | 161-180mgs | | | 181-210mgs | | | Means* |
|---------------------|------------|---------|--------|------------|--------|-------|------------|--------|-------|----------|
| | No | Mean | Range | No | Mean | Range | No | Mean | Range | |
| Carniolan | 2 | 12.5 | 12-13 | 3 | 9.67 | 8-11 | 5 | 9 | 7-12 | 10.38 ab |
| F1-Carniolan | 3 | 11 | 10-12 | 4 | 8.75 | 6-10 | 5 | 8.4 | 7-11 | 9.38 a |
| Italian | 2 | 15 | 14-16 | 2 | 11 | 10-12 | 4 | 8.75 | 8-10 | 11.58 b |
| F1-Italian | 4 | 7.69 | 6-9.38 | 4 | 6.75 | 4-9 | 7 | 6.42 | 4-10 | 6.47 d |
| Egyptian | 1 | 7 | ----- | 2 | 6.5 | 6-7 | 3 | 4.33 | 3-6 | 5.94 d |
| Means* | | 10.63 a | | | 8.53 b | | | 7.09 b | | |

*Any two means are significantly different if they are followed by letters that are all different ($P < 0.05$)

Table (4): Effect of genotypes and different weight categories of honeybee queens on the number of spermatozoa in the spermatheca.

| Weight Genotypes | 140-160mgs | | | 161-180mgs | | | 181-210mgs | | | Means* |
|---------------------|------------|--------|-----------|------------|-------|-----------|------------|-------|-----------|---------|
| | No | Mean | Range | No | Mean | Range | No | Mean | Range | |
| Carniolan | 2 | 2.7 | 2.38-3.02 | 3 | 3.46 | 2.48-5.08 | 5 | 3.72 | 2.58-5.85 | 3.29 ab |
| F1-Carniolan | 3 | 3.57 | 2.62-5.38 | 4 | 3.32 | 2.45-5.21 | 5 | 3.75 | 2.38-4.81 | 3.55 ab |
| Italian | 2 | 2.68 | 2.62-2.74 | 2 | 2.64 | 2.6-2.68 | 4 | 3.49 | 2.5-4.63 | 2.94 a |
| F1-Italian | 4 | 3.53 | 2.62-5.96 | 4 | 3.47 | 2.66-4.78 | 7 | 5.04 | 3.37-5.98 | 4.01 b |
| Egyptian | 1 | 2.54 | ----- | 2 | 2.63 | 2.42-2.84 | 3 | 2.55 | 2.45-2.68 | 2.57 a |
| Means* | | 3.00 a | | | 3.1ab | | | 3.7 b | | |

* Any two means are significantly different if they are followed by letters that are all different ($P < 0.05$).

3.1.4.Sperm counts

As shown in Table (4), the average values of this parameter ranged between 3 millions in light-weighted queens and 3.71 in heavy-weighted queens. The correlation between weight and number of spermatozoa over all genotypes, however, was statistically not significant (Fig. 4).

With regard to the genotype effect, the results showed a significant effect on this parameter. When the comparison was carried out between all genotypes under study, the first Italian hybrid was superior (4 millions) to the other genotypes (3.29, 2.94, 2.57 and 3.54 millions, in Carniolan, Italian, Egyptian, and F1-hybrid Carniolan bees, respectively). The hybrid queens showed superiority when they were grouped and contrasted against the pure ones, being 2.93 in the former and 3.77 million in the later.

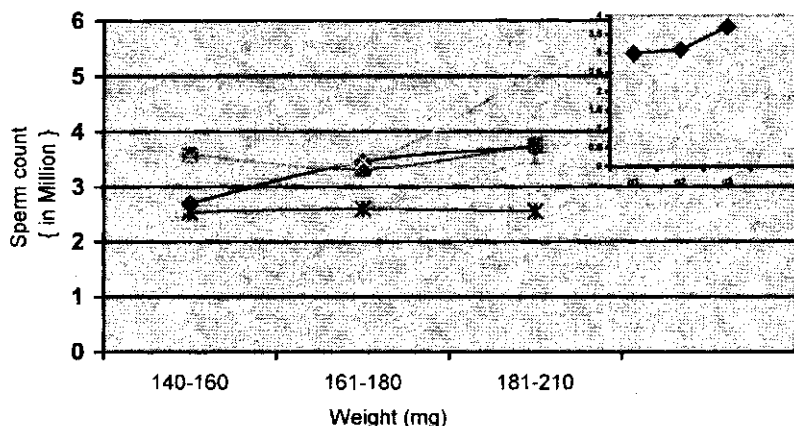
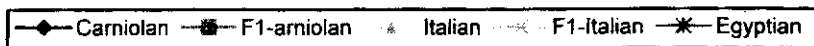


Fig. (4): Average values of sperms count in the spermatheca in the five genotypes of honeybee queens under study (the upper right graph shows the linear relationship over all genotypes, $P > 0.05$).



3.1.5. Effect of the brood status on the four parameters under study

3.1.5.1. Acceptance and pre-oviposition period

The acceptance and oviposition time are the most sensitive parameters to both presence and kind of brood (Table 5). The highest acceptance rate obtained when mating nuclei were provided with only sealed brood (100%), whereas the lowest value was obtained in those without any brood. The presence of only sealed brood had the same effect on pre-oviposition period. While the shortest (5 days) value was obtained with adding of only sealed brood, the presence of unsealed brood delayed oviposition to 22 days, followed by those without any brood (17 days).

Table (5): Effect of brood status on the parameters under study.

| Brood status | Acceptance rate (%)* | Preoviposition period | Mating success (%) | Spermathecal content* |
|----------------|----------------------|-----------------------|--------------------|-----------------------|
| Sealed Brood | 100 a | 5.2 ba | 90 a | 3.11 a |
| Unsealed Brood | 70ab | 22.4 b | 71.42 a | 2.83 b |
| No Brood | 50 bc | 17.4 b | 80 a | 2.9 ab |
| Normal Brood | 70 ac | 8.42 c | 85.71 a | 3.08 ab |

*Any two means are significantly different if they are followed by letters that are all different ($P < 0.05$).

3.1.5.2. Mating success and spermathecal content

In comparison with the two previous parameters, mating success and spermathecal content were not affected greatly by the kind of brood existed in the nuclei. The mating success rates ranged from 71% (unsealed brood) and 90% (sealed brood), but without having significant differences.

Concerning sperm counts, the average values had the lowest range in comparison with all parameters under study (2.8 – 3.1 million). This parameter seems to be not affected by the kind of brood added to the nuclei. Both mating success and the number of spermatozoa are two parameters that have something to do with mating, carrying out outside the hive, and thus the brood had not a significant effect on them.

4. DISCUSSION AND CONCLUSION

The result of the first experiment showed the importance of the queen weight to both pre-oviposition period and acceptance rate. This could be explained by the fact that the heavy-weighted queens are more attractive to the bees (Rawash *et al.*, 1983 and Schaper, 1985). This attractiveness may encourage the acceptance of the queens easily, and the queen has take more attention from the bees than the light-weighted queens. This may be reflected by feeding the heavy-weighted queens more vigorously than the light ones, the process which may accelerate their maturation, their mating and consequently the onset of egg laying.

This confirms the results of Taranov (1976), who demonstrated that both pre-oviposition period and the acceptance rate of queens are closely related to their weight. Consequently, proper care for breeding a good quality queens assures high acceptance rate and a quicker egg-laying for naturally mated queens.

In contrary, the queen weight did not affect the number of spermatozoa and mating success. This result coincides with that obtained by Koeniger *et al.* (1995), who did not find any relationship between queen size and both mating success rate and spermathecal volume. The later being related positively with the number of spermatozoa it can contain (Kobel, 1968).

The pre-oviposition period is the only parameter being affected by both genotype and weight of queens. On the other hand, both the queen acceptance and mating success were not affected by the genetic-background of the tested queens. For studying the acceptance rate, the queens were introduced into hybrid colonies and therefore, the different relatedness degrees between genotypes of both queens and host colonies were generally ensured.

Although it was stated that queen honeybees provide genetic cues that can be used by workers to assess genetic relationship (Page and Erickson, 1986), no significant differences were observed between the acceptance rate of the queens used in this study. This result coincides with that obtained by Guzman *et al.* (1997), who found that requeening Africanized colonies with European queens did not affect negatively the queen acceptance rate. In contrary, Ebadi (1988) found differences in the acceptance rates between 5 different queen genotypes, but he did not view the genetic relationship between the introduced queens and the bees in the host colonies.

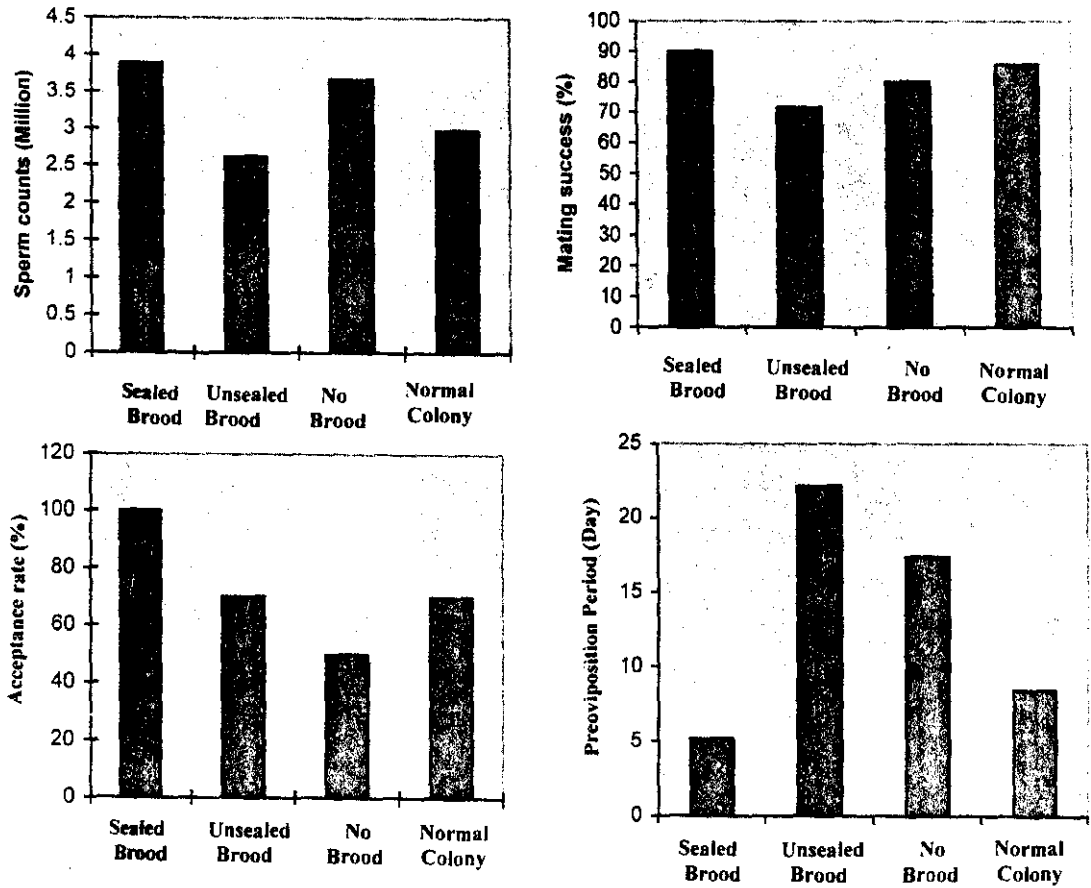


Fig. (5): Effect of brood status on the acceptance rate, preoviposition period, mating success and sperm counts.

Further studies are, therefore, needed using concrete genotypes of both queens and host colonies to test the behavior of the bees clearly.

With respect to mating success ratios, and although the general comparison between the 5 genotypes did not show significant differences, this difference has become obvious when the F_1 -hybrid queens were grouped and compared against the pure ones. The F_1 -hybrid queens seem to be more vigor and attractive to the drones and therefore a full-mating process could be ensured. Another interpretation could be drawn from the fact that, there are differences in the orientation ability of queen honeybee between the races (Adam, 1982). The low mating success values of the Italian queens (Table 3) could be, therefore, explained by high drifting value of this race in comparison to other honeybee races (Adam, 1982).

Concerning the effect of different brood status, and as the results in Table (5) indicated there may be different effects of both capped and uncapped brood. Whereas the best results were obtained when only capped brood were added, these parameters showed inferior values when unsealed brood were existed in the nuclei. It could be concluded that the sealed brood has a stimulatory effect on all parameters, while the unsealed brood has an inhibitory effect, when both treatments were compared with the treatment without any brood. This could be attributed to the effect of the chemical substance (Pheromones), being emitted from the worker brood and releasing different behavior patterns and so different functions (Le-Conte *et al.* 1995 & Free, 1987). These difference functions could be explained by different pheromones' roles of both brood status, but yet no published data are available to support this explanation; Therefore, further studies are needed to explain chemically the different effects of both brood types in honeybee colonies. The inhibitory and stimulatory effects of the brood were stated to be found on many colony activities (Free, 1987).

The mating success was better in nuclei having brood than those without brood. Similar results were obtained by Hellmich *et al.* (1986), and El-Sarrag and Nagi, (1988). Among the different brood status, the presence of sealed brood resulted in the best mating and acceptance ratios. These results corroborate reports of superior values of these parameters when nuclei contain only capped brood (Silva *et al.*, 1995 & Johnsson and Johnsson, 1978).

Both the pre-oviposition period and the number of spermatozoa were found to be related positively with the presence of sealed brood. The presence of capped brood may affect the behavior of the bees, so that it may stimulate the bees to feed the queens vigorously. Offering food could well be associated with pheromones as reported by Free (1987). This behavior may accelerate the queen maturation and accordingly shorten their pre-oviposition period.

The important conclusions of this study could be summarized as follows:

- The high-weighted queens are more attractive to the bees than the light-weighted ones.
- The first hybrid queens are more attractive to the bees than the pure genotypes and have high values of all parameters used.
- Among hybrid queens, the F₁-hybrid Italian bees are superior to the F₁-Carniolan hybrid in all parameters used.
- The presence of sealed brood in mating nuclei is very important to obtain high-quality queens.

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تأثير كل من التركيب الوراثي وأوزان عذارى ملكات نحل العسل بالإضافة إلى نوع الحضنة على بعض المقاييس المستخدمة في الحكم على الملكات منذ إدخالها وحتى بداية وضع البيض

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ملخص

تم في هذا البحث اختبار تأثير كل من المصدر الوراثي ووزن الملكات العذارى لنحل العسل بالإضافة لتأثير نوع الحضنة على بعض المقاييس المستخدمة للحكم على جودة هذه الملكات. فبداية من تأثيرها على نسبة القبول عند الإدخال ومرورا بنسبة نجاح التلقيح وطول فترة ما قبل وضع البيض وحتى عدد الحيوانات المنوية داخل القابلة المنوية لوحظ تأثر هذه المقاييس بدرجات مختلفة . ففي حين لوحظ تأثر فترة ما قبل وضع البيض بكلا العاملين بصورة معنوية، فإن نسبة نجاح التلقيح لم تتأثر بهما بينما تأثرت نسبة القبول بأوزان العذارى المدخلة. تأثر عدد الحيوانات المنوية بصورة معنوية بالأصل الوراثي لهذه الملكات ، وعند دراسة شكل الإستجابة بين كل من الوزن والمقاييس المتأثرة به لوحظ وجود علاقة خطية حيث لوحظ وجود علاقة خطية موجبة بينه وبين نسبة القبول وسالبة مع فترة ما قبل وضع البيض، كما لوحظ عند مقارنة الأصول الوراثية ببعضها تفوق الهجين الأول الإيطالي كما لوحظ تفوق الهجن الأولى عن الأصول الوراثية النقية بالنسبة لجميع المقاييس تحت الدراسة . أما بالنسبة لتأثير نوع الحضنة الموجودة في نويات التلقيح المعدة لاستقبال الملكات العذارى فقد أظهرت النتائج تفوق تأثير الحضنة المغلقة عن بقية المعاملات في التجربة بالنسبة لجميع المقاييس المستخدمة في حين أظهرت الحضنة المفتوحة تأثيرا مثبطا علي هذه المقاييس وذلك عند مقارنتهما بتلك المعاملات المحتوية على نوعي الحضنة.

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