Honeybee Queen Rearing Methods and their Relation to Morphological and Physiological Characteristics and Queen Productivity

Al-Abbadi, Amal A. (*), H. A. Mesbah(**), Nagda A. Al-Sayed(**) and Nemat F. Mohanna(***)

*Corresponding Author, Dept. of Bio-Agricultural Technology, Faculty of Agricultural Technology, Al-Balqa Applied University, Al-Salt, Jordan.

** Faculty of Agriculture (Saba Basha), Alex., University, Alexandria, Egypt.

*** Dept. of Apiculture, ARC, Ministry of Agriculture, Alexandria, Egypt.

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ABSTRACT

To evaluate the following five different methods of queen rearing; Doolittle (Grafting), Hopkins, Miller, Natural and Smith ones. The main objectives of these practised rearing methods were to estimate the biometrical characters of mated queens and to study the different morphological and anatomical characters of raised virgin queens. Results showed that the highest mean number of reproduced workers (161443.3) was recorded for Hopkins rearing method and differed significantly from all the calculated means of other tested methods. The Natural rearing method showed the lowest mean number of 59840 workers. Concerning the results of honey yield, each of Hopkins, Miller, Smith and Doolittle methods were significantly different from the Natural one. The highest mean of fresh body weight of newly mated queens was found in Hopkins (255 mg) and/or Doolittle (250 mg) rearing methods. Regarding the morphological and anatomical characters the obtained results indicated that queen rearing method had a highly significant effect on the size of queen cell, fresh body weights of the derived virgin queens, abdomen length of virgin queen, ovarioles number and length of right ovary, length and width of hind wing, number of hooks of hind wing. Whereas, the highest mean percentage of protein content (71.02) was found for Miller method followed by Smith (61.79%), Natural (57.94%), Hopkins (56.31%), and the least was, however obtained in Doolittle (45.30%) methods. It could be concluded that Hopkins and Miller methods gave good results with regard to the colony productivity of brood and honey. These methods are simple, easy to practice and require no grafting or special tools, and, therefore are promising for the production of queens in narrow-scale purpose.

INTRODUCTION

In fact, honeybee queen is the mother of honeybee colony in which it is the most important individual. A colony is not productive unless the mother queen is a vigorous producer of eggs that are the main source of a

large population of workers. Wherever, the queen is more than that, she produces

Pheromones which maintain the cohesion of the colony. Moreover, the queen is the depository of all inherited characteristics of the species acquired through her progenitors and through the acquisition of sperm from drones at the time of mating (Laidlaw, 1992).

Naturally, bees rear queens under three conditions: swarming, which is the reproduction of colonies; queen supersedure, where a daughter queen replaces her mother queen because of aging, and queen replacement as a result of queen loss by accident (Mangum, 1997).

There are several natural rearing methods which were developed by many scientists and beekeepers to produce a good quality queens in a small scale (Townsend, 1880; Brooks, 1880; Alley, 1883; Hopkins, 1911; Miller, 1912; and Smith, 1912) – [C.F. (Abdellatif, 1994)]. On the other hand, the most popular artificial method of queen rearing is Doolittle method (1889), the so called grafting method, which aims to produce a large number of queens in a commercial scale.

Bilash (1963) verified some methods of queen rearing and quality of queens produced. Experiments showed that swarm queens were heavier and have more ovarioles than those reared artificially.

Gracy (1985) stated that Jay Smith used Doolittle method to rear queens. Later Smith became convinced that queens produced by Alley strip method were superior to grafted queens. In Smith's method the larva is not disturbed from it's bed of royal jelly and probably produces very good queens.

The Doolittle or grafting method is one most often employed by queen breeders. This method is somewhat labor intensive, but yields reasonably good queens in a commercial scale (Hayes, 1991).

The later auther reported that the Hopkins method of queen rearing allows the beekeepers to control to a large degree the quality and quantity of virgin queens. The least amount of manipulation is doing; no grafting, no specialized tools and no cell bars. He concluded also, if all conditions for Hopkins rearing method are favorable, the beekeeper will secure a maximum number of cells from 75 to 100 fine cells are not unusual.

Mangum (1997) reported that when naturally built queen cells are not available, queens could be reared using the Miller queen rearing method. He added that with a light nectar flow, the Miller method works easily, and as a bonus, the resulting queen cells tend to be larger.

There are several factors which may affect the production as well as characteristics of queens produced. Of these factors, rearing of queen from

fertilized eggs or newly hatched worker larvae may be considered the most important factor.

Vagt (1955) carried out some morphological investigations on emerging queens reared from different larval ages. He stated that queens could be reared from larvae ½, 1, 2, and 3 days old, but no queens could be reared from older larvae. Weaver (1957), Orösi-Pál (1960) and Jordan (1960) found that the weight and number of ovarioles of reared queens decreased as the age of grafted brood increased from egg to 3-day-old larvae. Cale (1963), Bilash (1963) and Alber (1965) reported that queens produced from grafted eggs were better than those obtained from grafted larvae. In a comparison between queens reared from newly hatched larvae and 2-day-old larvae, It was found that queens reared from newly hatched larvae were significantly heavier with larger spermatheca suggesting that they were of higher reproductive quality (Tarpy *et al.*, 2000).

Volosevich (1954) concluded that the quality of the queen could be determined by the number of ovarioles, length of ovary, diameter of spermatheca, length of 3rd and 4th tergites and length and breadth of fore wing. Cheng and Yuan (1985) mentioned that the productivity of queen depended upon her age, breed, weight at emergency, age of grafted larva and grafting methods, number of ovarioles, diameter of spermatheca, and number of spermatozoa in spermatheca.

The present work aimed to study the effect of different presumed methods of queen rearing on the biometrical characters of resulted mated queens, besides the morphometrical and physiological characteristics of produced virgin queens

MATERIALS AND METHODS

The present study was carried out at the apiary of the Research unit of Apiculture, El-Sabahia Research station, Agric. Res. Centre, Alexandria, Egypt. Bees used were Carniolan-Egyptian first hybrid which is well known and usually used in the Egyptian apiaries. The experiments were performed during the queen-rearing period in Egypt along two successive years of 2003 and 2004.

Five different rearing methods of honeybee queens were practised to evaluate their efficiency on queen production and the produced queens. These methods are Doolittle (grafting) method as an artificial rearing one, Hopkins, Miller, Natural and Smith rearing methods. The resulted virgin queens of each rearing method were directly introduced to the mating nuclei.

Rearing of Honeybee Queens

Queen Rearing Methods

In any queen rearing method, one must choose a rearing colony that serves as a profitable source of new queens. The desired rearing colony should have the characteristics that the beekeepers want to reproduce in other multiplied colonies. Honey production, wintering ability, gentleness, early colony build up and resistance to diseases are the most required and wanted characters by the beekeepers. On the other hand, the queen cell building colony which is a queenless nursing colony should have abundant bees of different ages, two combs of sealed brood and two combs of honey and pollen. During queen cell construction, the nursing colony must be daily fed with diluted sugar syrup (1:1 sugar: water). Just before the queen rearing frame is placed in the nursing colony, brood combs should be examined and any queen cell must be destroyed. At the time of queens rearing, attention is made for the presence of drones of good stock.

Doolittle Method (Grafting)

Doolittle or grafting method is performed by grafting one-day-old larvae into the given queen cups to the queenless building colony.

In the carried on experimental work, the rearing colony was provided with new well-constructed worker comb (from which the worker bees had just emerged), which is preferred by the queen to deposit her eggs. This comb was placed in the centre of the brood nest of the colony. As soon as this comb had been contained 24-hour-old larvae, it was taken out from the queen rearing colony. The selected larvae for transferring were those lying on abundant royal jelly in their worker cells.

Twelve cups were fixed on each of three horizontal wooden bars using melted wax. The bars were fitted into a special frame, then they were transferred to the cell building colony at several times for few hours before grafting in order to let the bees clean and prepare the queen cell cups.

At the grafting performance the smooth tip of the grafting tool was slipped under the larva, lifting it out with small portion of its surrounding jelly. Then larva was deposited on a drop of freshly diluted royal jelly obtained from the natural queen cells.

After 10 days post grafting the mature queen cells were carefully separated in a well-aerated shadowy place using a sharp knife.

Hopkins Method

A frame of brood comb was removed from the centre of brood nest of a rearing colony and replaced by a new well-constructed and ideal comb for queen cells building purpose. A modification was done by us using 3-day-old egg for rearing purpose instead of the newly hatched larvae. The fit and best side of the comb containing the three-day-old egg was chosen for

queen cells building. Herein, the surface of the comb was prepared by destroying two of worker cells and leaving the third one within each row using the pencil tip. Then, this prepared surface was horizontally laid flatwise with cells facing down over the combs of brood nest of the queenless colony. To provide more support for the rearing comb, the upper frame bar appendages were carefully cut. Wooden pieces of 2cm thickness were put under each side of the rearing comb as a necessary mean to hold it apart from the combs of brood nest to leave a space that is enough for drawing large queen cells. The comb was also covered with a piece of cloth to provide a suitable temperature to stimulate queen rearing and queen cells building. Upper story was added to provide a good environment and enough protection for the produced queen cells. After 11 days from rearing date, the frame with all adhering mature queen cells was removed from the building colony, placed on its bottom bar and cut around each queen cell with a serrated sharp knife.

Miller Method

A newly constructed comb was placed in the brood nest of the queen rearing colony. Sequently, the queen rearing comb was checked every couple of days until the revealance of deposited eggs. When the eggs in the comb reached 3-day-old* it was removed after brushing off the bees, covered with a moistened towel to prevent the desiccation of eggs then transferred to illuminated chamber, and by the aid of a sharp knife the queen rearing frame was trimmed into three triangular shaped strips. The triangularly cut comb gave the bees a space enough to build larger queen cells. It is worthy to mention here that in Miller method three triangular shaped strips of foundation wax are fixed to the frame of rearing comb and is placed in rearing colony for worker bees to construct it and for queen to fill it with eggs. It is usually noticed that the worker bees construct the foundation wax, complete its building and finally, the triangular shape of rearing comb is lost. So in our experimental work the rearing comb was trimmed after the construction and filling of eggs.

Along the edges of both the sides of trimmed comb, a pencil tip was inserted into some of these cells to destroy the developing eggs. Two adjacent eggs were destroyed and the third was left, the same action was repeated along all the edges of the comb. The existing drone cells along the edges of the comb were carefully destroyed and terminated. The queen rearing frame was placed in a strong nursing colony which was queenless for at least one day. On the eleventh day of rearing period (as the queens

* As a modification these eggs were used instead of the newly hatched or young (1 – 3 days old) larvae for rearing purpose.

were reared from eggs), the mature queen cells were separated using a serrated sharp knife. This process was easy because the cells were constructed and built along the edges of the triangularly trimmed comb.

Natural Rearing Method

The naturally built queen cells in the queenless colonies were used. Larger queen cells with proper shape were chosen, while the small and undesired ones were destroyed.

The mature queen cells were separated from the comb using sharp serrated knife. A plug of the comb underlying the queen cell was cutout, to protect the developing queens.

Smith Method

This method is a modification of that adopted by Alley, 1883 and is used for the production of high number of queens.

In this initiated method by Smith in 1912 – [C.F. Abdellatif, 1994)] the rearing colony is adapted to accommodate 9 combs and is divided into two compartments using a queen excluder which is higher above the top of the frames by about ¾ inch. The small compartment contains the queen and implies three combs of the standard langstroth size, while the large one contains six combs. Each compartment has its own inner cover in addition to a common outer cover.

A sheet of unwired foundation wax with dimensions of 9 ½ X 5 ½ inches was fixed on the center of each lateral frame of the smaller compartment of the colony, while the remaining part of each frame was occupied with a thin wooden board. The third middle frame was provided with a sheet of unwired foundation wax with the same previous dimensions. Firstly, the fixed foundation wax to these frames was constructed in the larger compartment of the colony. Thereafter, a sealed brood comb was put in the smaller compartment between both the lateral combs. Later on, the sealed brood comb was replaced by the frame which implies the small sheet of well-constructed foundation wax. Within 24 hours, this small sheet of wax was filled with eggs; the frame was removed and transferred to the larger compartment of colony until eggs hatching. Ordinarily, this process was sequently repeated to get more eggs for queen rearing purpose. Four days later, the wax area that contained the newly hatched larvae was separated using a warm sharp knife, cut into strips containing two rows of cells with the newly hatched larvae in each strip. Then these strips were attached to wooden bars of the frame using melted wax. Also, within each of attached strips, every third cell that containing a larva was left intact and the in-between two cells were crushed. The prepared frame with two or three wooden bars with attached strips was placed in a queenless building colony. The queen cells were matured after 10 days of rearing time. Then

these cells were separated from the wooden bar of the frame using a sharp knife and were partioned into separate cells.

Queen Cells Care

In all above mentioned rearing methods, extreme caution was done to avoid damaging of the growing pupae inside the removed queen cells. Thereafter, each of the removed queen cells from the queen building colony was fixed well and caged under a half ball cage –5cm in diameter made of screening galvanized hardware cloth (1mm hole size). They were caged on a dark old comb with some honey in a strong queenless colony supplied with large population of young workers, worker brood, honey and pollen.

All the newly emerged queens of each of experimented rearing methods mentioned before were weighed. Some of these queens were introduced to mating nuclei and the others were used for the morphometrical and physiological measurements.

Queen introduction

Queen introduction is the procedure that has been used to provide a queen to a colony of honeybee (McCutcheon, 2001).

Four mating Langstroth nuclei were prepared for each queen rearing method. Each weighed and caged virgin queen was introduced into the mating nucleus, which consisted of one brood comb of different ages and a comb of honey and pollen, with enough number of bees covering the three combs.

After three days from the queen introduction, the cage that left was taken away and the virgin queen was free. The mating nuclei were re-observed 10 days later to detect and inspect the sequency of eggs oviposition as indicator to the mating completion of the virgin queens. The mated queens were weighed and returned immediately to their nuclei. The pre-oviposition period was determined as a period lasted from the beginning of virgin queen release until the time at which the mated queen started egg laying.

Biometrical Measurements

The productivity and biological characters of the mated queens resulted from the five tested rearing methods were studied. Colony productivity included both the parameters of worker brood production and honey yield. On the other hand, the measured biological characters were the fresh body weight of mated queen, the pre-oviposition and oviposition periods and the fresh weight of laid eggs.

Workers Brood Production

For each rearing method the viability and fertility of queens were investigated. Estimation of the brood rearing activity of all the derived queens of the different tested rearing methods was done during the years

of 2003 and 2004. A typical langstroth frame with dimensions of 17 X 8 inches was divided into square inches by means of wire (Al-Tikrity *et al.*, 1971) and used to evaluate the quantity of sealed brood. The frame was laid against each side of brood comb and the area occupied by sealed brood was measured.

The counts of worker brood were done at 12 days intervals. The obtained total number of square inches for each colony per month was multiplied by 27.5; which has been considered as the number of worker cells per square inch.

Honey Yield

The honey yield was evaluated at the end of two successive seasons during the year of 2004. The first season was on June when the clover (*Trifolium alexandrium*) flow was still occurring. The second one was at the end of September where the Brazil pepper tree (*Schinus terebinthifolius*) was mostly the only source of pollen and nectar during this period (Mohanna, 1989).

The honey production was determined for each colony of the different performed queen rearing methods by weighing the honey combs before and after honey extraction.

Queen Egg Weight

An analytical balance was used to weigh the individual glass coverslip. A group of twenty eggs was weighed and the mean weight of one egg was calculated (Mackasmiel and Fell, 2000). Four replicates, each represented by 20 eggs, were done for each of tested queen rearing method.

Morphometrical measurements

Some of the newly emerged virgin queens of the different performed rearing methods were weighed to obtain their fresh and/or dry body weight; others were preserved in 70% ethyl alcohol for morphometrical measurements. Each queen rearing method was represented by twelve replicates.

Queen Cell Size

The inner size of queen cell was determined by estimating the volume (in cubic millimeters) of water amount which has been injected in the queen cell using a 1ml plastic syringe.

Fresh and Dry Body Weight of the Newly Emerged Virgin Queens

The fresh body weight of the newly emerged virgin queen was determined by weighing the emerged queens individually within 24 hours post emergence. These weighed virgin queens were preserved in small vials in a deep freezer until determining their dry body weight. Dry body

weight was estimated after keeping the sampled queens for 24 hours in an oven at 105°C (Yakoub, 2002).

Length and Width of Queen Abdomen:

The length and width of the virgin queen abdomen were measured in millimeter using a thick millimetrical ruler paper.

Number and Length of Virgin Queen Ovarioles and Diameter of Spermatheca:

After the extirpation of the wings and legs of the preserved virgin queens it was placed upon its abdomen in the dissecting plate, the abdominal cuticle was carefully cut along both sides using a fine scissors. A cut was also done on the midline of the dorsal tergites that were carefully removed using a minute dissecting needle. Then, the internal organs were cleared, became easy to recognize and deal with. Later on, the dissected virgin queen was immersed in 70% ethyl alcohol for easiest removal and disattachment of the interior parts in a proper way. The alimentary canal was carefully freed from the investing tissues and excluded from the abdomen. Using a minute dissecting needle, the attached tracheae to the ovaries were carefully extirpated. The ovaries became completely loose and could be isolated from the queen's abdomen. The right ovary was separated from the left one using a scissor. Each ovary was immersed in xylene for 8-10 minutes for partial dissolving the investing tissues. Each ovary was put on a lens paper to get rid off xylene residue, then washed with tap water. A drop of Puri's medium (Puri, 1931) was added to each ovary to get further loosing of the ovarioles.

The ovaries must not be left in Puri's medium for more than one minute to avoid the strong dissolving effect of the medium. In this concern, it was found better to repeat the addition and removal of the medium for 2-3 times, in each the medium was left for a period lasted less than one minute and tap water was used for washing the ovary and removing the residue of chemical materials. The ovaries became loose enough and ready for counting process. The terminal filament of the loosen ovary was cut, batches of the ovarioles were separated from each other. Each batch was divided into smaller batches which were separated from their bases to prevent any probable damage and to facilitate their easier counting.

A drop of water was put on the prepared ovary that mounted on a glass slide for measurement purpose. Counting the number of queen's ovarioles in both ovaries was achieved under a stereoscopic binocular microscope with a high magnification power.

After loosing and preparing of both ovaries, the existing spermatheca with surrounding tracheae was separated and the connecting tracheal net

was removed before measurement, then the sample was mounted on glass slide for measuring purpose.

Diameter of spermatheca and the length of ovarioles were determined using a stereoscopic binocular microscope with an eyepiece micrometer slide.

Other morphometrical measurements

The right wings were mounted on the glass slides for measuring the length and width of right fore and hind wings, cubital index and the number of hooks of right hind wing. These morphometrical measurements were also obtained using a stereoscopic binocular microscope with an eyepiece micrometer slide. Cubital index value was evaluated as the ration between both the veins a&b of the right fore wing.

Physiological Measurement:

Determination of Protein Content of Virgin Queen

The total protein content of the newly emerged virgin queens was determined using the described macro Kjeldahl method by the Association of Official Agricultural Chemists, AOAC (1964). Three replicates represented by three queens were used for each queen rearing method. The chemical analysis was carried out in Animal Production Department, Faculty of Agriculture, Al-Shatby, Alexandria Univ.

RESULTS AND DISCUSSION

Effect of Tested Queen Rearing Methods on the Rate of Produced Virgin Queens:

Table (1) shows the number of produced queen cells and emerging percentage in the five different used rearing methods. The highest percentage was in the Doolittle method (9615) and the lowest one was in the Hopkins method (5344). Several researchers used successfully the Doolitle method and it was preferred to other methods (Hayes, 1991 and Taber, 1993).

Effect of Tested Rearing Methods on the Biometrical Parameters of Raised Queens:

The productivity and biophysiological characters of the mated queens; after had been raised from the different tested rearing methods were studied.

Effect on the Rates of Reproduced Workers and Honey Yield:

The best performance of any derived queen is usually evaluated by measuring its brood rearing activity. The implies results in Table 2, declare the detected highly significant differences between the means numbers of reproduced workers in the five tested queen rearing methods in the year of 2003. The highest mean number of 161443.33 workers was recorded for

Hopkins rearing method and differed significantly from all the calculated means of other tested methods. In this concern, the Natural rearing method showed a lowest mean number of 59840 workers.

Meanwhile, results of year 2004 showed the insignificant differences between the mean numbers of reproduced workers in all of the tested rearing methods. The highest insignificant mean number of reproduced workers by Doolittle method comprised 263332, followed by Miller 18020.33, Hopkins 216296.67, Smith 146795 and finally the Natural one 51736.67.

From the above demonstrated data it could be revealed that the productivity of these produced queens to a more extent, depend upon the age of reared larvae. In each of Hopkins and Miller methods, the rearing of queens was carried out using 3-day-old egg and gave 16144333 workers which are significantly different from all other methods. Whereas in Doolittle method 24-hour-old larva was used for rearing purpose. Consequently, these three methods gave the highest means numbers of reproduced workers. The effect of the age of reared larva on queen productivity was supported by the findings of Cheng and Yuan (1985).

Also, in each of the tested rearing methods the mean number of reproduced workers was higher in the second year of 2004 than that in the first year of 2003, except the case of Natural rearing method which gave higher mean number of reproduced workers in the first year than that in the second one (Table 2).

In accordance to the aforementioned results of 2003 and 2004 seasons, it could be concluded that queens should be replaced every two years or whenever colonies show that they can benefit by the change. These obtained results support the findings of Szabo (1993) and Mangum (1996) who recommended requeening colonies after the second season of egg laying. The results were in disagreement with those of Morse (1978) and Ibrahim (2002) who stated that the queens should be replaced annually.

The exhibited results in Table 2 show significant differences between the calculated means numbers of laid eggs/queen/day in the tested rearing methods during the year of 2003. In this concept, Hopkins method gave the highest mean number of laid eggs /queen/day amounted to 672.68 eggs/day, followed by Smith, Miller, Doolittle and Natural ones which indicated mean values of 527.23, 510.21, 399.97 and 391.14 eggs/day, respectively. On the other hand, during the year of 2004 the obtained differences between the means numbers of eggs laid by queen/day for each rearing method were insignificant. In Doolittle method the mean number of laid eggs/queen/day was equal to 1066.47, followed by the

methods of Miller 846.73, Hopkins 832.50, Smith 629.11 and the least Natural one 303.0.

Concerning honey yield, Hopkins, Miller, Smith and Doolittle methods gave yield with no significant differences between them. Meanwhile, all method were significantly different from the Natural one (Table 2). The highest mean of honey yield in Kilograms per colony, comprising 10.78 kgs was found in case of Smith rearing method; followed by those detected in the rearing methods of Doolittle (9.35), Miller (7.23) and Hopkins (6.55 kgs/ colony) with no significant difference. The Natural rearing method gave the lowest mean of honey yield 3.13 Kg/colony, versus all the performed rearing methods which gave much more honey yield during the year of 2004.

A variation of individual colony's foraging behavior may result in a significant difference in the honey yield obtained from colonies of similar strength. Although honey production is closely correlated to colony population size and also to the bee race with highly degree (Farrar, 1937, Geiger, 1969 and Sugden and Furgala, 1982).

Effect on the Biological Characters of Mated Queens:

The included results of statistical analysis of data in Table 3 show the deduced significant differences between the means of fresh body weight of newly mated queens in the performed rearing methods. The highest mean of fresh body weight of mated queen was found in Hopkins (255 mg) and Doolittle (250 mg) rearing methods, which were highly significantly different from the other tested ones. On the other hand, Smith rearing method showed the lowest mean value of 217.5 mg which was insignificantly different from the exactly equal values detected in Miller and Natural rearing ones (232.5 mg).

Also, the shown results in Table 3 declear the estimated rate of increase in the body weight of introduced virgin queens from emergence until the start of egg laying. There were insignificant differences among all of the tested rearing methods.

Concerning the pre-oviposition period, the presented results in Table 3 show significant differences among the different tested rearing methods. The highest mean value of pre-oviposition period was inspected in Hopkins method (12.0 days) which was insignificantly different from the exactly equal values inspected in Doolittle and Smith ones (10.66 days). The lowest mean value of 9.33 days was recorded for Miller rearing method. These obtained results are supported by the findings of Crane (1949) who stated that the pre-oviposition period ranged between 5 and 21 days. Laidlaw and Eckert (1950) who mentioned that the queen rearer must destroy all virgin queens, that have failed to mate within fourteen days.

Referring to the measured weight of queen egg, there were insignificant differences detected among the different rearing methods. That weight ranged from 0.104 mg in Smith method to 0.140 mg in Miller and Natural ones. This result was in agreement with those obtained by Tew (1997) and Mackasmiel & Fell (2000) who found that queen egg weight is ranged between 0.12 – 022 mg.

Results in Table 3 also elucidate the calculated mean values of oviposition period (an extended period from the date of queen mating until ceasing egg laying) in the various run methods. It was the highest in Hopkins method (15.66 months) but insignificantly differed from those in Doolittle (14.66 months) and Miller (14 months) methods. The lowest oviposition period of 8.33 months was recorded for the Natural method. It could be also noticed that the performed rearing methods under the control of beekeeper (i.e. Doolittle, Hopkins, Miller and Smith) gave highly fertile queens with longer oviposition period, in comparison to those derived in the Natural uncontrolled rearing method, in which large number of queens with undesired traits were produced.

It is worth mentioning that Hopkins and Doolittle followed by Miller rearing methods gave the best results concerning the weight of mated queen and oviposition period, besides the suitable egg weight and pre-oviposition period.

It could be also concluded that the tested rearing methods more or less affected the weight and reproductivity of the resulted mated queens, but had no significant effect on the weight of laid egg. In addition, it is clearly evident that as the pre-oviposition period lasted the proper range of time though it had no effect on the productivity of the resulted queens. For example, the well fecundate and long lasting queens with high brood and honey production in Hopkins method were characterized by long pre-oviposition period (12 days), but this period was lie within the acceptable range. The same trend of results was recorded for Miller method with short pre-oviposition period (9.33 days). On the other hand, Natural method with 10 days of pre-oviposition period showed a lowest oviposition period of 8.33 months and resulted in queens with low productivity of brood and honey.

Effect on the Morphometrical and Anatomical Characters of Virgin Queens:

Queen Cell Size:

As shown in Table 4 there are highly significant differences among the mean values of estimated queen cell size. The highest mean of queen cell size was found in case of Doolittle rearing method (0.85 mm^3), followed by Natural (0.82 mm^3) and Miller (0.78 mm^3) methods which significantly

differed from those recorded for Hopkins and Smith methods (0.72 and 0.71 mm^3, respectively). Miller's method was insignificantly different from Hopkins and Smith ones.

It could be concluded that the rearing method had an effect on queen cell size. A similar conclusion was stated by Salem (2002).

The highest mean value of queen cell size in Doolittle method may be attributed to the presence of a specific number of separated and far enough queen cells on each cell bar. In case of Miller method, the triangular shape of wax rearing comb certainly provides enough space for making properly large sized queen cell. This result was supported by the finding of Mangum (1997 a) who reported that the triangularly cut rearing comb in Miller method provided an extra space to build large queen cells.

Fresh Body Weight of Virgin Queen:

Results presented in Table 4 clearly show the highly significant differences among the estimated fresh body weight of the derived virgin queens from the tested rearing methods. From the table, it could be revealed that Miller method gave the heaviest weight value of 181.66 mg, versus Hopkins method which gave the lightest weight value of 153.33 mg. Remarkedly, each of Miller, Doolittle and Smith methods gave virgin queens with heavier fresh body weight than Hopkins and Natural ones. These heaviest weights may be a result of the comparatively lower number of produced queen cells which were separately far enough from each other. *Vice versa*, in Hopkins and Natural methods the large numbers of produced queen cells were closely attached to each other and caused the weight reduction of derived virgin queens. These results were in agreement with Salem (2002) where Miller method gave the highest fresh body weight with 158.53mg.

Dry Body Weight of Virgin Queen:

The exhibited results in Table 4 elucidate also the deduced significant differences among the calculated mean values of dry body weight of raised virgin queens in the tested rearing methods. The heavier dry weights of virgin queens in each of Hopkins (47.22 mg) and Doolittle (46.22 mg) methods were significantly different from these inspected in the other tested methods of Miller (39.95 mg), Natural (38.62 mg) and Smith (37.88 mg).

From these results, it could be explained that the moisture content of the produced virgin queens by Miller, Smith and Natural methods (78.17 %, 78.04 % and 76.11%, respectively) were comparatively higher than that of Hopkins (69.20%) or/and Doolittle (73.12%) ones.

Moreover, the heavier dry body weights of reared virgin queens by each of Hopkins and/or Doolittle methods were noticeably coincided with the higher brood and honey productivity in regard to the resulted queen.

Length and Width of Queen Abdomen:

Results in Table 4 show that the mean of the longest abdomen length (13.3 mm) was revealed for the reared queens by Miller method; it was significantly different from all the calculated means in other tested rearing methods, except the Natural one (12.7 mm). Whereas, these shorter mean values of the abdomen length were 12.5, 12.5 and 12.1 mm for Doolittle, Smith and Hopkins methods, respectively.

Obviously, the abdomen length of virgin queen, to a less extent, was affected by some of the applied rearing methods. The mean value of longer abdominal length was in accordance with that value of larger queen cell size in case of Miller and Natural methods. Herein, the used artificial queen cell cups in Doolittle method must provide an extra length for the queen cell. But, as a result of applying this rearing method the larger queen cell size did not coincide with the longer abdomen length of resulted queen.

These obtained results regarding the abdominal length were in agreement with Salem (2002) who showed that the longest length of queen's abdomen was recorded for Miller method with 10.30 mm.

Regarding the effect of experimented rearing methods on the measured similar values of abdomen width, the obtained results showed the insignificant differences among the tested rearing methods (Table 4).

The reported findings are in agreement with Sharaf El-Din *et al.* (2000) who concluded that in Doolittle method the abdomen width of resulted virgin queen was in a range of 5.0 – 5.4 mm. Salem (2002) found also, that in case of Doolittle method the abdomen width of raised virgin queen was 4.73 mm, whereas it was 4.5 mm in case of Miller one.

Number and Length of Ovarioles:

The demonstrated statistical analysis of data in Table 4 show a highly significant difference between the mean number of counted right ovarioles in the resulted queens from Doolittle method (168.92) and the other methods. The mean numbers in the other tested methods, were insignificantly different in the mean values were 140.33 for Smith method, 136.33 for Miller, 134.66 for Natural and 130.5 for Hopkins one.

Meanwhile, Smith rearing method showed highly significantly different length of right ovary (3.9 mm), from those measured in each of the other tested methods, which ranged from 3.1 to 3.4 mm with no significant differences.

These above cited results clearly show that both the values of number and length of right ovary were affected by certain performed rearing methods. In this concern, Doolittle method gave the best result regarding the number of right ovarioles. This result contradicts those obtained by

Bilash (1963) who stated that swarm queens were heavier and had more ovarioles than those artificially reared.

In addition, results presented in Table 4 indicated that none of the performed rearing methods had significant effect on either the ovarioles number or the length of left ovaries. Despite, the made statistical analysis of data using T-test showed a significant difference between the mean of counted numbers of ovarioles in the left and right ovaries with mean values of 145.93 and 140.93 ovarioles, respectively. This result was in agreement with that arrived at by Moukayess (1979) where the number of ovarioles of the left ovary (173.9) was significantly higher than that of the right ovary (164.4).

Diameter of Spermatheca:

The carried out statistical analysis of data indicated that for all the tested rearing methods the estimated diameter of spermatheca of raised virgin queen was not significantly different (Table 4). This result surely decleared that the tested rearing method had no effect on the diameter of spermatheca.

Measurements of Right Fore and Hind wings:

Results concerning the effect of tested rearing methods on the measurements of fore right wing are included in Table 4 and elucidated that none of the performed rearing methods had a significant effect on the length and/or width of the right fore wings of emerged virgin queens.

On the other hand, the measurements of the length and width of right hind wings were significantly affected by these tested rearing methods.

Concerning the mean length of hind wing, it was revealed that Hopkins rearing method gave a longer one of 7.5 mm, which was highly significantly different from all the other tested methods of Miller (7.28 mm), Natural (7.22 mm), Doolittle (7.16 mm), and Smith 7.14 mm. The same trend of results was detected for the measured width of hind wing, whereas Hopkins method gave the wider hind wing (2.33 mm), followed by Miller (2.24 mm), versus Smith one which gave the narrower hind wing (2.12 mm). In this respect, it is worthy mentioning here that both of Hopkins and Miller methods were insignificantly different, in comparison to the other tested methods (Table 4).

Number of Hooks of the Right Hind Wing:

The implied statistical analysis of data in Table 4 show that all the tested rearing methods differed significantly from the Natural one. The highest mean value of counted hooks was recorded for Hopkins method (19), while the lowest one was obtained in case of Natural method (16.58). These obtained results are confirmed by the mentioned findings in the work of Salem (2002) who stated that the reared virgin queens by Miller method

had possessed 19 hooks the right hind wing. On the other hand, she showed that the reared virgin queens by Doolittle method had only 17.40 hooks the right hind wing.

Cubital Index of Right Wing:

Obviously, there was no revealance of any significant effect of each of the experimented rearing methods on the cubital index of right wing (Table 4).

Finally, in regard to all the assessed effects of different adopted rearing methods on the external and internal morphological characters of raised virgin queens, it could be concluded that most of the experimented rearing methods greatly affected the fresh and dry body weight of emerged virgin queen, queen cell size, abdominal length, number and length of right ovarioles and length, width and number of hooks of right hind wings.

Although, there is a voluminous work in the literature on the morphometrical characteristics of raised queens, unfortunately, few of these initiated research works, interested in correlating the resulted effects of tested rearing methods with the inspected characteristics of raised virgin queens.

Effect on the Protein Content of virgin Queens:

Honeybee protein content is differed by the season (Ivanov and Spasov, 1990) and by the quality and quantity of food (Standifer et al, 1970). A high level of haemolymph protein concentration was found to be correlated with an active neurosecretory system and developing ovaries (Hill, 1962). Haris and Harbo (1990) mentioned that since oogensis required protein, a lack of protein might have retarded ovary development. Saleem (2002) found that the nitrogen content varied with different queen rearing method. From Table 5 it could be shown the significant differences in the calculated mean percentages of protein content among the tested queen rearing methods. The highest mean percentage of 71.02 was found for Miller method followed by Smith (61.79%), Natural (57.94%), Hopkins (56.31%) and the least in Doolittle (45.30%) method.

Herein, the virgin queens in Miller method were raised from 3-day-old eggs and treated as queen larvae from the beginning of their life post hatching. In addition, a moderate number of large sized queen cell was produced. Queen's larvae obtained sufficient amount of food and received more royal jelly which greatly reflected on the protein content of their bodies. In smith method, queen was also confined in the smaller compartment part of rearing colony and forced to lay eggs in a small area of constructed wax. Then the newly laid eggs were re-placed in the larger compartment part; that enable them to receive high attention from the nurse bees. Whereas, the developing larvae were properly fed on sufficient

amount of royal jelly that may also affect the protein content of their bodies. On the other hand, in Natural rearing method worker bees usually select the larvae to rear queens from them. These larvae took great care and they were fed sufficiently.

In confirmation with the aforementioned results Salem (2002) made a comparison among different queen rearing methods, and concluded that Miller method showed the highest protein content of raised virgin queen.

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Table (1): Effect of Performed Rearing Methods on the Mean Number of Total Produced Queen Cells; Virgin Queens and Percentage of Emerged Queens.

Rearing method	Number of queen cells produced	Number of emerged virgin queens	Emergence percentage
Doolittle	20.80	20.00	96.15
Hopkins	26.20	14.00	53.44
Miller	21.25	14.25	67.05
Natural	20.30	12.00	59.11
Smith	19.50	13.17	67.54

Table (2): Effect of Different Performed Methods of Queen Rearing on the Rates of Reproduced Workers and Honey Yield/Colony.

Rearing Method	Mean Number of Reproduced Workers		Mean Number of eggs/queen/day		Honey yield Kg/colony 2004
	2003	2004	2003	2004	
Doolittle	76670.00 bc**	263332.00	399.97 b*	1066.47	9.35 a*
Hopkins	161443.33 a	216296.67	672.68 a	832.50	6.55 a
Miller	107143.00 b	218020.33	510.21 b	846.73	7.23 a
Natural	59840.00 c	51736.67	391.14 b	303.01	3.13 b
Smith	83240.00 bc	146795.00	527.23 ab	629.11	10.78 a
L.S.D.	46714.43	N.S.	5.36	N.S.	5.362

Table (3): Effect of Different Evaluated Rearing Methods on the Mean Weight and Reproductivity of Mated Queens.

Rearing Method	Weight of mated queen (mg)	Rate of weight increase (mg)	Pre-oviposition period (Day)	Weight of queen egg (mg)	Oviposition Period (Month)
Doolittle	250.0 a **	43.86	10.66 ab *	0.135	14.66 ab *
Hopkins	255.0 a	51.34	12.00 a	0.134	15.66 a
Miller	232.5 b	52.36	9.33 b	0.140	14.00 ab
Natural	232.5 b	48.10	10.00 b	0.140	8.33 c
Smith	217.5 b	32.70	10.66 ab	0.104	10.33 bc
L.S.D.	16.38	N.S.	1.63	N.S.	4.91

- Mean of four replicates were taken for each tested rearing method
- Values followed by the same letter(s) are not significantly different.
*Significant values, ** Highly significant values, N.S. Not significant values.

Table (4): Calculated Means of Measured Morphological Characters of Raised Virgin Queens from Different Adopted Rearing Methods.

Rearing method / Characters	Doolittle	Hopkins	Miller	Natural	Smith	L.S.D.
Queen cell size (mm^3)	0.85 a	0.72 b	0.78 ab	0.82 a	0.71 b	0.097
Virgin fresh body weight (mg)	174.16 ab	153.33 c	181.66 a	161.66 bc	172.50abc	19.820 **
Virgin dry body weight (mg)	46.82 a	47.22 a	39.65 b	38.62 b	37.88 b	6.730 *
Abdomen length (mm)	12.50 b	12.10 b	13.30 a	12.70 ab	12.50 b	0.780 *
Abdomen width (mm)	4.40	4.30	4.40	4.20	4.50	N.S.
Number of right ovarioles	168.92 a	130.50 b	136.33 b	134.66 b	140.33 b	20.510 **
Number of left ovarioles	157.17	132.00	148.08	144.42	147.42	N.S.
Length of right ovary (mm)	3.30 b	3.10 b	3.40 b	3.30 b	3.90 a	0.050 **
Length of left ovary (mm)	2.80	3.10	3.30	2.90	3.20	N.S.
Diameter of spermatheca (mm)	1.15	1.21	1.11	1.13	1.10	N.S.
Length of right fore wing (mm)	10.15	10.15	10.07	10.10	9.98	N.S.
Width of right fore wing (mm)	3.18	3.20	3.23	3.20	3.16	N.S.
Length of right hind wing (mm)	7.16 b	7.50 a	7.28 b	7.22 b	7.14 b	0.220 **
Width of right hind wing (mm)	2.14 b	2.33 a	2.24 ab	2.20 b	2.12 b	0.126 **
Number of hooks of right wing	18.18 a	19.00 a	18.80 a	16.58 b	18.80 a	1.760 *
Cubital index of right wing	2.76	2.62	3.00	2.81	3.14	N.S.

- Twelve replicates were taken for each tested rearing method.
- Values in the same line followed by the same letter(s) are not significantly different.
* Significant values, ** Highly significant values, N.S. Not significant values.

Table (5): Mean Percentage of Protein Content of Raised Virgin Queens, after using different rearing methods.

Rearing Method	Mean Percentage of Protein Content
Doolittle	45.30 c *
Hopkins	56.31 bc
Miller	71.02 a
Natural	57.94 abc
Smith	61.79 ab
L.S.D.	14.67

- Mean of three replicates were taken each represented by three virgin queens.
- Values with the same letter(s) were not significantly different.

* Significant values.

الملخص العربي

طرق تربية ملكات نحل العسل وعلاقاتها بالصفات المورفولوجية والفسيولوجية وانتاجية الملكات

*امل العبادى، **حسن على مصباح، **نجدة احمد السيد، ***نعمت مهنا

* كلية الزراعة التكنولوجية – جامعة البلقاء التطبيقية – الاردن

** كلية الزراعة – سابا باشا – جامعة الاسكندرية

*** قسم بحوث النحل – مركز البحوث الزراعية – الاسكندرية

أجريت هذه الدراسة في المنحل التجريبي لقسم بحوث النحل – محطة بحوث وقاية النبات– مركز البحوث الزراعية – الصباحيه – الإسكندرية وذلك باستخدام هجين النحل المصري الكرنيولي Carniolanتقييم خمسة طرق مختلفة لتربية الملكات وهي طريقة : دوليتل (التطعيم)، طريقة هوبكنز، طريقة ميلر، طريقة التربية الطبيعي وطريقة سميث، وذلك من خلال دراسة مقارنة للصفات البيولوجية والإنتاجية للملكات الملقحة الناتجة ومقارنة الصفات المورفولوجية والتشريحية والفسيولوجية المختلفة للعذارى الناتجة. وقد سجلت طريقة هوبكنز أكبر متوسط لعدد الشغالات الناتجة حيث بلغ العدد الناتج ١٦١٤٤٣,٣٣ شغالة بينما أعطت طريقة التربية الطبيعية أقل عدد للشغالات الناتجة بمتوسط قدره ٥٩٨٤٠. وكانت النتائج المسجلة في عام ٢٠٠٤ غير معنوية بالمقارنة بين طرق التربية المختلفة. كما وأظهرت الدراسة إن عدد الشغالات الناتجة في جميع طرق التربية كانت في السنة الثانية للإنتاج (٢٠٠٤) أعلى منها في السنة الأولى للإنتاج (٢٠٠٣) يستثنى من ذلك طريقة التربية الطبيعي حيث كان الناتج في عام ٢٠٠٣ أكبر منه في عام ٢٠٠٤. وعليه يوصي بتغيير الملكات كل سنتين أو عندما تظهر العلامات المميزة لضرورة إجراء هذا التغيير في الطائفة. تم تقدير إنتاج العسل في الخلايا التابعة لطرق التربية المختلفة خلال عام ٢٠٠٤ و أظهرت النتائج وجود فروق معنوية بين متوسطات كمية العسل المنتج في جميع طرق التربية بالمقارنة مع طريقة التربية الطبيعي؛ حيث تراوح متوسط إنتاج العسل للخلية الواحدة ما بين ١٠,٨٧ كغم عند استخدام طريقة سميث و ٣,١٣ كغم عند استخدام طريقة التربية الطبيعي. وفيما يتعلق بالصفات البيولوجية للملكات الناتجة فقد أظهرت النتائج وجود فروق معنوية بين طرق التربية المختلفة في متوسطات أوزان الملكات الملقحة، كما أوضحت النتائج وجود فروق معنوية بين طرق التربية المختلفة في فترة وضع البيض وكان أعلى متوسط لفترة وضع البيض ١٥,٦٦ شهرا عند

استخدام طريقة هوبكنز يليها في ذلك طريقة دوليتل (١٤,٦٦ شهرا) وطريقة ميلر (١٤ شـــهرا) ، بينمـــا سجلت طريقة التربية الطبيعي أقل مدة للفترة الإنتاجية للملكة بمتوسط قدره ٨,٣٣ شهرا. مما سبق يمكـــن القول بأن كل من طريقتي هوبكنز وميلر أعطت نتائج جيدة فيما يتعلق بإنتاجية الخلية من الحضنة والعسل ، بالإضافة إلى إن هاتين الطريقتين تعتبران من الطرق البسيطة والسهلة المراس و التعلم واللتان يمكـــن بواسطتهما إنتاج عدد مناسب من الملكات في كل مرة تربية. وفيما يتعلق بتـــأثير طـــرق التربيـــة علـــى الصفات الشكلية الظاهرية والتشريحية على الملكات العذارى فقد بينت النتائج وجود تأثير معنوي عـــالي لطرق التربية المختلفة على حجم البيت الملكي ومتوسط وزن الملكة العذراء وطول البطن وعدد الفـــروع المبيضية وطول أنابيب المبيض الأيمن وطول وعرض الجنـــاح الخلفـــي الأيمـــــن وعـــدد خطـــاطيف الجناح الأيمن الخلفي. الفروقات المحسوبة بين طرق التربية المستخدمة لم تكن معنوية فيما يتعلق بقيم كل من الصفات التالية: عرض البطن، عدد الفروع المبيضيـــة وطول أنابيب المبيض الأيسر، طول وعرض الجناح الأيمن الأمامي وكذلك دالة الجناح الأيمن. أظهرت الدراسة وجود فروق معنوية في نسبة المحتوى البروتيني للملكات العذارى الناتجة من طرق التربية المختلفة حيث كانت أعلى نسبة للمحتوى البروتينـــي (٧١,٠٢ ٪) في العذارى الناتجة عن استخدام طريقة ميلر للتربية مقارنة بنـــسب المحتـــوى البروتينـــي المنخفضة للملكات العذارى الناتجة باستخدام كل من طريقـــة سميث والتربية الطبيعي وهوبكنز ودوليتل (٦١,٧٩، ٥٧,٩٤، ٥٦,٣١ و ٤٥,٣٠ ٪ على التوالي).