Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(4): 683-692

Received: 19-05-2018
Accepted: 20-06-2018

Shakib Vaziritabar
Department of Animal Science, Islamic Azad University Varamin, Pishva Branch, Tehran, Iran

Sayed Mehdi Esmaeilzade
The Professional Instructor of Culturing Honey Bee, Number 2, Zibadasht, Karaj, Iran

Correspondence
Shakib Vaziritabar
Department of Animal Science, Islamic Azad University Varamin, Pishva Branch, Tehran, Iran

Preliminary attempts to rear larvae of the Iranian honeybee (*Apis mellifera meda*) colony and effect of different factors on graft acceptance in honeybee colonies in Karaj apiary

Shakib Vaziritabar and Sayed Mehdi Esmaeilzade

Abstract
The aim of this study was to determine effect of different factors on the acceptance rates of grafted larvae by using the prepared queen cell cups in Karaj apiary and if there is difference of larvae acceptance after grafting one day old larvae in artificial wax queen cells that were for one hour prior to grafting in starter colonies for polishing and artificial wax queen cells that weren't in contact with bees. A brood frame rotation schedule maintains the colony as a queen rearer for further batches of queen cells. Three factors *viz.* bee strength/crowdiness, queenliness of the colony and priming status of queen cell cups were evaluated on graft acceptance. The combined effect of the different levels of bee strength, queenliness and priming status of cell cups was non-significant on the mean number of accepted grafts (8.20-44.17 i.e. 7.85-50.18%). Quality of bee queens depends primarily on the age of the brood used for queen rearing.

Keywords: *A. mellifera meda*, Iranian honey bee, graft acceptance, queen cell cups, Karaj

1. Introduction
The number of accepted larvae depends on different factors, as described in detail by Ruttner [53]. The most important factors are: quality, strength and developmental stage of the nurse colonies, age of the workers and age of the grafted larvae, presence or absence of a queen in the rearing colony and the duration of the queenless stage, presence of open brood in the cell-starting colonies, number of grafted cells, rearing sequence and method of rearing. Larva age used in grafting has important effects on quality of queen bee in its rearing [63]. Environmental conditions are of major importance for final queen rearing success. Essential factors are: regulation of humidity and temperature by the rearing colony or in the incubator, and vitality of queen cells and the feed supply (nectar flow, supplemental feeding) of the nurse colony. A regular replacement of queens in bee colonies is a prerequisite for a successful beekeeping venture. Rearing efficiency is to a large extent dependent upon ambient conditions but it is largely influenced by the choice and the preparation of the nurse colony and by the rearing conditions. It is necessary to start with a nurse colony that is healthy and strong and has brood of different age [7, 33]. Rearing can be done in the presence of the queen but rearing performance is better in queenless colonies [66, 67] and in the absence of emergency queen cells Free *et al.* [31]. Cell cups in which the larvae are grafted can be made of plastic but bee wax cups perform better [11, 12]. The diameter of 9 mm is regarded as optimal, Weiss [70]. A moot point is the necessity to introduce empty cups in advance to precondition the colony. Queen rearing is one of the major objects of apiaries especially for the commercial beekeepers, and it is a main factor for success in beekeeping [47]. Rearing honeybee queens occurs when the colony is in the process of swarming, supersedure or when the queen has been accidentally lost or killed [59]. Although the rearing of queen bees can be performed in the presence of the queen in a nurse colony however a higher effectiveness can be achieved in queenless colonies Morse *et al.* [46]; Crailsheim *et al.* [15] and in the absence of emergency queen cells Free *et al.* [31]. In all these cases, adult workers rear new queens from worker larvae that are less than 48 hrs old [37]. For successful managing and rearing of queen bees, it is imperative to adapt beekeeping measures for colony development. Limited brood rearing is initiated already during winter months and brood rearing leading to colony expansion is often initiated before nectar and pollen become available [60].

Queen bees can be reared from the end of March to September, but better quality of queens is obtained from the end of March until the end of April, [41]. The acceptance and the ratio of queen emergence is highest using royal jelly as the grafting substrate [24]. At present, grafting is the most convenient and economical method of queen rearing. In tropical or subtropical climates, where honey bees are able to rear brood continuously throughout the year, data on colony development is readily available. Compared to honey bees in temperate climates, colonies may respond more rapidly with increased brood rearing when foraging conditions become favorable [54]. The quality of the queen bee determines the benefits received from honey bee colony. Various environmental factors affect the quality of the queen bees. These factors are: the age of the grafted larvae, origin of larvae, the number of young worker bees, food presence of starter and finisher colonies, and mated queen bees with enough drone bees [47]. One of the methods of queen rearing is to rear queen bees in queen right colonies. Acceptance of larvae is also affected by the number of grafted larvae. Smaller numbers are preferable [50] and they should not exceed 50 [38]. Furthermore the site of grafting can also be of some importance but no significant relationships have been demonstrated [29, 67]. The method consists of raising combs of brood above a queen excluder in a strong colony, and grafting 12-18 h old larvae into queen cell cups next to the brood in the upper chamber [71]. Larvae at any age up to the end of the third day have the potential of being reproductive queens. The quality of the queens decreases as the age of the grafted larvae increases. It is known that the honeybee queen is the key to success for both the colony and the beekeeper. The beekeepers have exploited the biology of queen rearing so as to offer queens for breeding or for commercial purposes. Honey bees (*Apis mellifera*) have needed to rear new queens for millions of years in order to survive as a species. They have evolved to rear queens in response to various conditions, such as accidental loss of the queen, or congestion of the nest cavity. Ever since Langstroth developed his moveable-frame hive, beekeepers have been devising ingenious methods of inducing their bees to rear queens 'on demand', whether it be a few queens for hobby beekeepers, or thousands of queens for commercial queen breeding and production programs. Beekeepers commonly transfer (graft) very young worker larvae into artificial queen cell cups, and introduce these into a queen less colony for acceptance and initial feeding. This starter colony is purposefully made queen less to take advantage of the natural response to this. If a queen is lost or killed, a sudden reduction in the level of queen pheromones in the hive usually triggers the worker bees to build emergency queen cells to rear a replacement queen; Huber [36]; Butler [9]; Butler [12]; Butler [11, 13]; Free [30]. Bees also naturally rear queens while in a queen right state. A new queen may be reared in a supersedure cell to replace a substandard or failing queen, and it is not unusual to later find the two queens, mother and daughter, laying in the same colony. Populous colonies preparing to swarm, will rear numerous *swarm* cells while the mother queen is still present in the colony. The factors that induce supersedure or swarming are complex, but queen pheromones again are believed to be a factor – poor distribution and low levels of pheromones per worker probably being important triggers (Butler [9, 10, 12]; Free [31] and Winston [69]. According to Doolittle [21] successfully reared queens in queen right colonies. The body weight of the queen is one of the first evaluations which breeders can make with emerged queen. Best evaluation of the queen weight is made inside first few hours after hatching because decline in the queen body weight is most rapid in first 36 hours [41]. Some of the basic factors for acceptance of grafted larvae by queen cell building colony are: strength of colony, food storage and number of grafted queen-cells, age of the worker bees, age of the grafted larvae, presence of queen and presence of open brood in rearing colonies [25, 55, 56]. Beside genetic origin of the queen, high quality queen cell is one of the most important steps in rearing biologically superior queens. The key in queen rearing is to take a young (12-24 hours old) larva from a worker cell and place ("graft") it into a queen cell cup suspended vertically in a hive. The larva is fed on a special royal jelly diet by the nurse bees. After 10-11 days, the queen cells, which are ready to emerge, can be transferred to queen less hives or mating nuclei ("nucs") [68]. The success and quality of queen production depends on strong, well fed and healthy nurse colonies and on suitable equipment and colony management. The development of modern queen rearing techniques started in the 19th Century. Doolittle emphasized the importance of simulating a swarming or supersedure situation in the cell building colonies and a constant, rich food supply for the production of high quality queens. The native honey bee in Iran is *Apis mellifera meda*. Engel [27]; calls this bee-The Median Honey Bee. It is most common in Iran and Iraq but does range into southeastern Turkey and northern Syria. Not much is known about this bee. We conducted this experiment to determine the effects of different factors on the acceptance rates of grafted larvae by using the prepared queen cell cups. The aim of the study was to analyze the effect of different factors on factors on the acceptance rates of grafted larvae by using the prepared queen cell cups in Karaj apiary and if there is difference of larvae acceptance after grafting one day old larvae in artificial wax queen cells that were for one hour prior to grafting in starter colonies for polishing and artificial wax queen cells that weren't in contact with bees. The present study were conducted to observe the impact of bee strength of colony/crowdiness, queenliness (queenless v/s queen-right) and priming status of the queen cell cups on the absolute number of accepted grafts and per cent graft acceptance during 2017-2018.

2. Materials and Methods

This research was conducted at the apiary of the Faculty of Agriculture, Islamic Azad University (I.A.U) in Karaj, Iran, during the spring and summer seasons of 2017 and 2018. The location of the apiary is characterized by moderately high nectar flow and by a high number of bee colonies. The different levels of various treatments, evaluated for royal jelly production during the experiment have been given below:

2.1 Bee strength and crowdiness in cell builder colonies

1. 10 bee-frames on 10 combs.
2. 15 bee frames on 15 combs.
3. 20 bee-frames on 20 combs.
4. 10 bee frames on 10 combs.
5. 15 bee-frames on 15 combs.
6. 20 bee frames on 20 combs.
7. bee frames on 10 combs.
8. 15 bee frames on 15 combs.
9. 20 bee frames on 20 combs.

2.2 Queenliness of cell builder colonies

1 Queenless.
2 Queen-right

2.3 Priming status of queen cell cups

2.3.1 Primed

2.3.2 Not primed

Various practices are used to simulate natural conditions to rear queens. The most efficient and widely used methods worldwide involve the Doolittle grafting technique which enables large scale production levels (Doolitle 1888) [20]. Grafting is the physical transfer of larvae from worker cells of a selected breeder colony into artificially made queen cell cups. The cups are attached to wooden bars on grafting frames, which are introduced into cell builder's strongly populated colonies in which the queen has been purposely removed. His method of queen rearing in queen-right colonies with the old queen isolated by a queen excluder [21] is still applied. Modified Doolittle method as standardized by Singh [64] was basically followed for the study. The above experiment was conducted using 135 grafts in the artificial wax cell cups without any artificial feeding to the experimental colonies. Larvae aged up to 24 hrs were used for rearing. Their age was estimated by size. The larvae were grafted from comb cell to bee wax queen cups 9 mm in diameter using a conventional Chinese grafting needle.

Nine starter colonies for experiment were formed four days prior grafting. Each starter colony was prepared with shaken bees from 40 frames of brood (where most of the nurse bees are expected to be found) [58] and 8 frames of honey and pollen. One frame of young brood was present in starter colony when there were no queen cells, but before inserting grafted cells brood frame was removed so it is ensured that most of nursing bees will be on queen cells. Every three treatments each starter received 135 grafted cells and totally 6 repeated series were performed. After the second series of grafting, starters were refreshed with young bees. Artificial wax queen cells were used for grafting one day old larvae to get high quality queens [64]. Two groups of 4 starter colonies were formed. In the first group, one hour prior to grafting, queen cells were added for polishing (group P). After removal of the queen cell from colony for grafting, it was clearly evident that bees added some new wax on edges of the queen cell. Chinese grafting tool was used for grafting, so there was no need to prime queen cells with royal jelly since some of it is grafted together with the larvae. After grafting, polished queen cells were added back to group P starters, while control group (group C) received grafted larvae in queen cells that were not in contact with bees before grafting. Upon receiving grafted material, each starter was fed with 1 liter of sugar syrup (60%). After 24 hours, checking for accepted larvae was made. Afterwards, the queens were weighed with electric balance to the nearest 0.01 mg and dissected for measuring diameter of spermatheca as described in [23]. At an interval of 24 h the consumed sugar syrup was replenished to its old level by pouring measured quantity of the syrup. The added quantity was recorded daily. In a colony having no space left in the brood chamber, super was added to accommodate the feeding mug.

2.4 Colony Set-up

Eight to 24 hours before the first grafting, each rearer is arranged so most of the sealed brood is above a queen excluder, and the queen and most of the unsealed brood are below the queen excluder, as shown in (Fig. 1). If the queen is not found, combs are shaken free of bees before they are placed above the queen excluder. At the same time the graft frame containing 12 - 24 empty artificial wax queen cups is added to the top brood box to allow the bees to polish the cell cups and add a small rim of beeswax to each. This also ensures that the cups are warmed to brood-nest temperature. It is not known how much each of these factors contribute to good graft acceptance, but this preparatory period for the cups takes virtually no extra effort on the beekeeper's part. A comb of pollen is put in the top box close to the graft bar, and a comb of young larvae, preferably also with some pollen stores, is also placed adjacent to the graft bar. This young brood attracts nurse bees to the graft area. Also extra boxes are added on top if required.

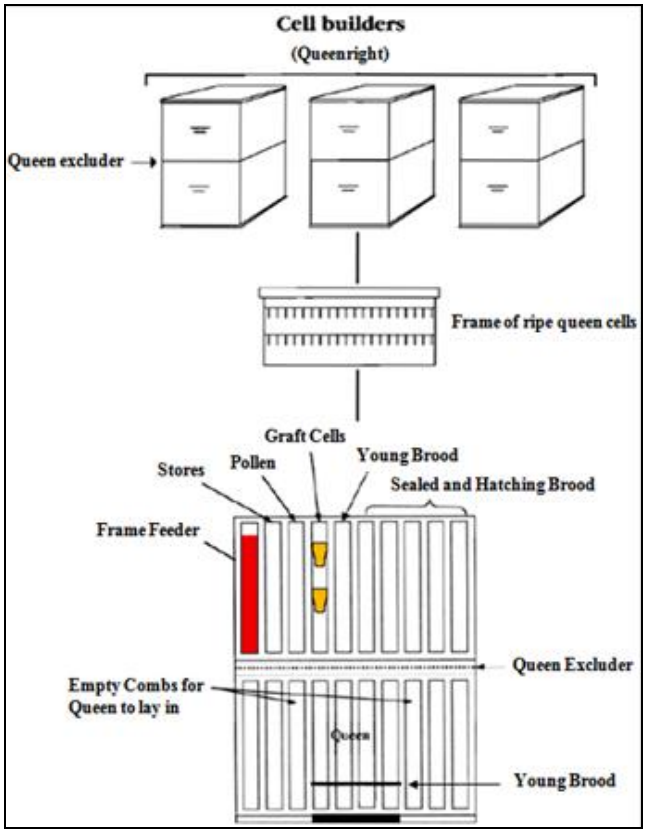

Fig 1: The set-up of the queen-right colony. (Photo by Shakib Vaziritabar).

The builder cum finisher colony were adjusted as H, P, SB, EB, YL, GL, P, OL, EB, H Where (H = honey frame, P = pollen frame, SB = sealed brood, EB = emerging brood, YL = young larvae, GL = grafted larvae, OL = old larvae) to attract nurse bees for initiating and nursing the grafted larvae [25] (Fig. 7C).

The larvae grafted in the cups were inspected on the 3rd day of grafting. These were considered 'accepted' when the larvae remained in the cup and fed by the bees. When the cup was found sealed, it was considered as 'Finished cell'. The depth (rim to inner bottom of the cell) and outer width of the queen cell were measured with Vernier caliper after the queen has emerged out of it. To record the weight of the queen, it was taken out of colony after one day of its emergence. Then it was placed in a vial of known weight and weighed.

2.5 The graft frame

The graft frame consists of a normal brood frame with wax, modified to accept two horizontal wooden cell bars. These cell bars are temporarily removed from the frame for ease of grafting. The cell bars have about two inches (5cm) free space beneath them to provide room for the bees to build the queen

cells. A part-depth saw cut along the length of the underside of each cell bar allows wax queen cups with base pegs to be pushed in bee wax cell cups (Fig. 2).

2.6 Equipment for queen rearing

Most systems of queen rearing use standard beekeeping equipment but employ some specialized equipment during the process. Most of the specialized equipment is inexpensive or can be constructed by the beekeeper.

2.6.1 Capping

In order to investigate parameters of adult honey bees, capping of the rearing plates with perforated bees wax and turning the plates to a vertical position on day eleven has been proven as a useful add-on (Riessberger-Gallé *et al*. [52] and Brodschneider *et al*. [6]. Wooden cell cups used as queen cell cup were attached to a bar with melted wax. The cups are pressed into the soft barely melted wax and spaced about 2.5 cm from center to center, fifteen cups were placed on each bar. The forming sticks were dipped into barely melted wax three or four times. The queen cells cups were kept in feeder colony until the cups were completely closed (Fig. 2).

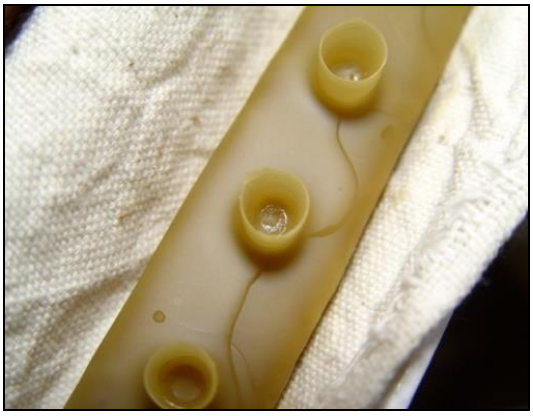

Fig 2: Prepare cell bars ahead of time and wooden cell cups used as queen cell cup were attached to a bar with melted wax. The cups are pressed into the soft barely melted wax and spaced about 2.5 cm from center to center, fifteen cups were placed on each bar.

2.6.2 Cell cups

Larvae are placed in artificial queen cell cups (grafted). The cups are placed on bars which, in turn, are placed in frames (Fig. 3). Queen cell cups should measure 8-9 mm in diameter at the rim.

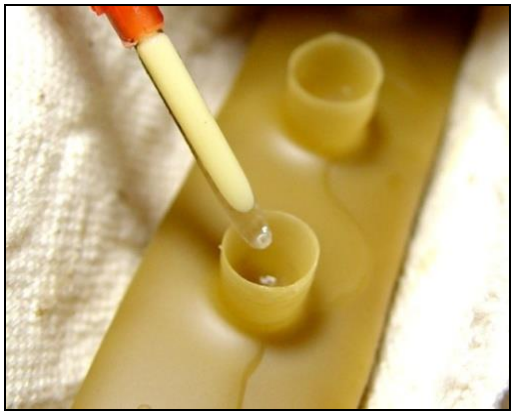

Fig 3: Young larvae of less than 24 hatches were transferred into queen cell cups and place into cell builder as quickly as possible. It is possible to graft without royal jelly or any liquid (dry grafting). (Photo by Shakib Vaziritabar, during 2017-2018).

Cell cups can be produced from beeswax as described by Ruttner [53] and Laidlaw [43]. Cells should always be rinsed, after removal from the dipping sticks ("cell mandrel"), to eliminate traces of soap. Cups made in advance should be kept free of dust by storing in a sealed box. Most queen producers attach their homemade beeswax cell cups directly to a cell bar with hot wax. Queen producers dip the base of the cell cups in molten beeswax (beeswax melts at 62.3 - 65.2 °C) and firmly push the cup base onto the cell bar as the wax cools.

2.6.3 Preparing Supplies/Chinese Grafting needle

A "Chinese" grafting needle is a handy and inexpensive grafting tool that looks like a ball point pen. It consists of a spring loaded bamboo plunger that slides along a thin tongue of flexible plastic. The flexible tongue slips easily under a larva and then a press on the plunger will deposit the larva and any royal jelly that was picked up in the cell to be grafted. A non-slip grip in the middle section gives excellent control. Modern versions of this tool have injection molded plastic parts, which may help with cleanliness. Chinese grafting needle should be modified when used for transferring honey bee larvae from combs to artificial queen cell cups (Fig. 4A). In these instances, the flexible tip of the tools should be trimmed to approximately 2.5 mm in width to allow for the larvae to be picked up from the base of the cell unhindered.

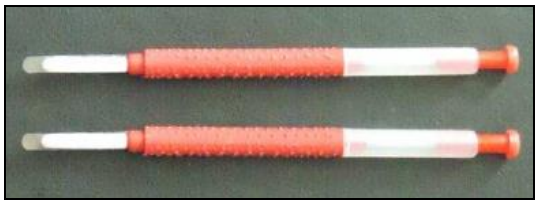

Fig 4A: A "Chinese" grafting needles used for transferring honey bee larvae from combs to artificial queen cell cups. The flexible tongue slips easily under a larva and then a press on the plunger will deposit the larva and any royal jelly that was picked up in the cell to be grafted.

The Chinese grafting needle should be modified differently when used transferred into wax cups (Fig. 4B). The tools should be modified by removing their springs and plungers. The plunger and spring are easily removable by pulling firmly on the plunger, and sliding off the plunger and spring from the tool.

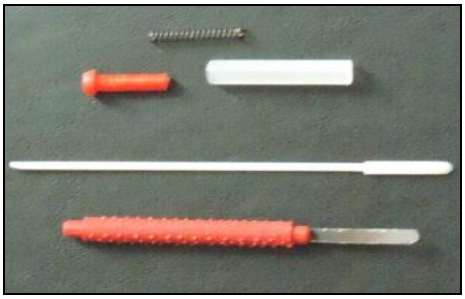

Fig 4B: A "Chinese" grafting needle buildings.

2.7 Grafting procedure

Respect of the following conditions when transferring the larva from its original cell to the artificial queen cell (Fig. 5) ensures quality queen production:

1. Grafting the larvae from the worker comb to the queen cells should be done rapidly and with suitable

environmental conditions (24-26°C and RH > 50%).

2. The best place to perform the grafting is in a honey house or a laboratory room, as larvae are sensitive to high temperatures, direct sun light (UV) and low humidity. Grafting in a room is comfortable for the operator and protects against robbing bees. The location of the grafting room should be just a few steps from the breeder colonies and the nurse colonies that receive the grafted cells.
3. Cold lighting must be used to avoid generating too much heat which may damage the larvae.
4. The cells and the brood comb should be kept out of the bright sunlight as much as possible. When the weather is hot and dry, a damp cloth may be spread over the cells to prevent them from drying out. A damp cloth also protects the larvae from light and dust.
5. With experience and speed, three bars (60 cups) can be completed in 8-10 minutes or less. As soon as one bar is finished, it should be covered with the damp cloth. The grafted cells should be placed into the starters as soon as possible.
6. In general, grafting is easier from dark wax combs rather than from light wax combs because of the better contrast with the small white larvae. The use of a cool light or an illuminated grafting magnifier will help one see the larvae better. Grafting should be done preferably in a room or in indirect light to ensure the larvae do not dry out or become damaged by UV radiation from direct sunlight (Fig. 5).

Fig 5: A larva taken from dark comb is transferred into wax cups by using Chinese grafting needle. (Photo by Shakib Vaziritabar, grafting is in a laboratory room, during 2017-2018).

Grafting is either done with a flexible 'spatula-like' tool, such as the Chinese grafting tool, or a solid metal tool, such as a dentist's excavator. The Chinese grafting tool has the advantage of transferring a bed of royal jelly along with the larvae, but good acceptance rates have been obtained from dry grafting with a metal tool or a fine wetted paintbrush. Each larva is picked up by approaching from the outer convex curve of its 'c' shape.

2.8 Cell builder colony preparation

Fig 6: The comb arrangement in cell builder colony and location of different provisions and grafted larvae *A. mellifera* meda (Iranian honey bee) cell builder cum finisher colony (H= honey frame, P= pollen frame, SB= sealed brood, GC= grafted cells, EC= empty comb).

In the case of queen-right 15 and 20 bee-frame strength colonies, the queen cells raising frame(s) was/ were given in brood /lower chamber and the existing queen bee was restricted in the upper chamber with the use of horizontal queen excluder in between the two chambers.

2.9 Priming of queen cell cups

Dry grafting (without priming the cell cups with royal jelly) or wet grafting (after priming queen cell cups with speck of royal jelly) of larvae were evaluated.

2.10 Statistical analysis

Statistical analysis was made in Statistical 12 software. Data were statistically analyzed using Factorial Completely Randomized Design for determining the significance of differences of various levels of the treatment means and the means of combinations (interactions) among the various levels of different treatments.

3. Results and Discussion

The grafted larvae were introduced into either a queen-less or a queen-right cell raising colony. For grafting, larvae less than 24 hold were transferred to dry wax cell cups melted completely closed. Their heights were measured and they were placed into incubator (37.0±0.05 C and 59-64% RH) until queen emerged. When grafting for a frame was completed, it was immediately put into a cell builder colony (Fig. 7A). Shortly after emergence queens were weighed and pre-oviposition period, diameter of spermatheca and number of spermatozoa was examined. The queens were marked, numbered and were placed in mating colonies (nuclei) in cages (Fig. 7B). Queens were permitted to be naturally mated after 24 hours. To obtain large numbers of larvae of the right age for grafting, empty brood combs can be added to a breeder colony, or a breeder queen caged overnight on an empty comb (i.e. using a queen excluder cage), four days prior to grafting (Fig. 7C).

Fig 7: The set-up of the queen-right colony and location of different provisions and grafted larvae in *A. mellifera meda* cell builder cum finisher colony. (Photos by Shakib Vaziritabar, University of Varamin-Pishva in Iran, during 2017-2018).

3.1 Checking Acceptance

Between one and three days after grafting, the graft frame is checked to assess cell acceptance. It is always handled gently without shaking or jarring, but can be turned upside down to check the contents of the cells. Normally the bees have further extended the walls of accepted cells with beeswax (see Fig. 8), and each accepted larva is floating on a deep bed of royal jelly (see Fig. 9).

Fig 8: A frame of newly accepted queen cell grafts. (Photo by Shakib Vaziritabar, in Karaj's apiary, during 2017-2018).

Fig 9: Newly accepted queen cell grafts. Larva is very well fed, floating on a deep bed of milky-white royal jelly visible through the prepared queen cell cups. (Photo by Jeff Harris and Audrey Sheridan, Mississippi State University).

We have sometimes found that the first one or two batches of grafts of the year placed in a rearer have a poor acceptance, but then the batches to follow have a high acceptance rate. Occasionally, however, a colony keeps giving poor graft acceptance rates, or destroys cells it has started. Possible reasons include the presence of a second queen located in the upper brood box, or a damaged queen excluder allowing the queen to move through. In total 3.710 larvae was grafted. The percentage of accepted queen cells in group P and C was 75.9% and 64.2% respectively which is significant difference ($t=-2.25$, $p<0.05$) (Table 1.). The results of acceptance rate are similar to Ebadi and Gary [28] and Gancer *et al*. [35]; who have 76.6% and 73.4% respectively.

Table 1: Acceptance of dry grafted larvae during 2017-2018.

Group	Larval acceptance			Min-Max	Average
	accepted	rejected	total		
Control	1465	390	1855	56-72.5	64.2% (±12.8)
Polishing	1650	205	1855	68-79.8	75.9% (±8.4)

Note: (Source: Field Survey, 2017-2018).

It is clearly evident that control starter colonies had much wider range of grafted larvae acceptance (Fig.10) compared to polishing group. The results suggest that polishing of the queen cells before grafting is effective way to increase acceptance of grafted larvae.

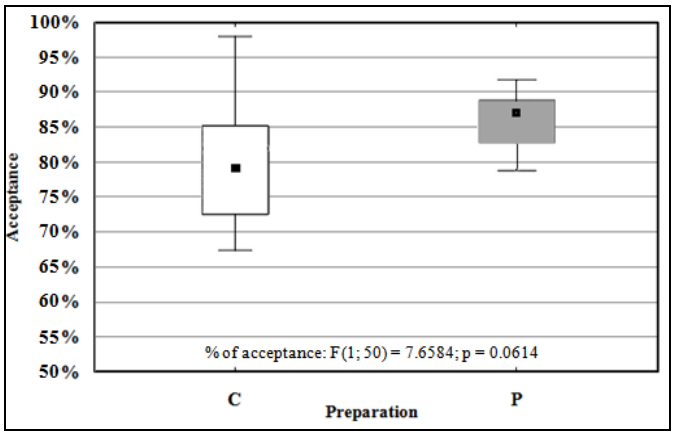

Fig 10: Acceptance of grafted larvae in C (Control) and P (polishing) groups.

Bee strength of 20/20 in the colonies resulted in the highest mean number of grafts accepted (26.18) cell cups and it was on par with all the other bee strengths except 8/10 bee-frame strength (12.10). Other bee strengths showed acceptance of 22.68 (10/10 bee-frames), 22.46 (16/20 bee frames), 19.68 (12/15 bee-frames) and 21.92 (15/15 bee frames) grafted cell cups (Table 2). The effect of queenliness on the number of the accepted grafts was significant. Queen-right colonies showed higher number of accepted grafts (26.18) than queenless colonies (16.50). The mean number of accepted cells in colonies provided primed cell cups was significantly higher (26.20) than those provided unprimed cell cups (14.30). The interaction between bee strength and queenliness of the cell builder colonies proved to be non-significant, with the mean number of accepted grafts ranging between 9.40-27.50. Bee strength of 16/20 frames coupled with priming of cell cups resulted into significantly higher graft acceptance (32.30) which was followed by 12/15 bee-frame strength under primed status of cell cups (30.12). The other combinations resulted in 10.27 (8/10 bee-frame colonies with primed cell

cups) to 28.53 (10/10 bee-frame colonies provided with primed cell cups) accepted grafts. The interaction among the different levels of three treatments proved to be non significant (8.20-44.17 grafts). Bee strength of 20/20 frames resulted in the highest per cent acceptance (28.40%) which was on par with 15/15 bee-frame strength (27.38%), 10/10 bee-frame strength (26.61 %), 16/20 bee-frame strength (26.53 %) and 12/15 bee-frame strength (24.57%) colonies. The least per cent graft acceptance was recorded in 8/10 bee-frame strength colonies (13.22%); (Table 3). The queenliness of cell-builder colonies showed significant effect on the percent acceptance of grafted cell cups. Between the two conditions (queenless and queen-right), it was higher in queen-right colonies (28.40%) than in queenless colonies (20.38%). Priming of cell cups proved to be better (27.69%) than no priming (18.31%) in this respect. Interaction among the Combinations of bee strength and queenliness of cell builder colonies was non-significant percent graft acceptance (8.14-27.50). The combined effect of the given levels of queenliness and priming status of cell cups was non-significant on the percent acceptance of larval grafts (15.01-36.23).

Table 2: Effect of bee strength and queenliness of cell builder (*A. mellifera* meda) colony on the number of grafts accepted under primed v/s. dry grafting condition during 2017-2018.

Bee strength (No. of combs/No. of bee frames)	Mean percent acceptance rates of grafted larvae by using queen cell cups *								
	Queen-right colony			Queen-less colony			Mean		Grand mean
	Primed cell cups	No priming	Mean	Primed cell cups	No priming	Mean	Primed cell cups	No priming	
8/10	16.92 (24.38)	15.09 (21.36)	15.70 (24.29)	9.05 (16.08)	12.58 (20.44)	9.56 (14.30)	11.48 (18.85)	12.26 (15.47)	13.22 (17.91)
10/10	50.18 (38.49)	20.29 (24.82)	27.57 (31.43)	29.78 (32.48)	20.44 (26.32)	26.24 (30.00)	33.83 (34.94)	18.20 (21.10)	26.61 (30.52)
12/15	48.69 (45.18)	13.49 (20.55)	30.66 (32.48)	18.30 (20.46)	7.85 (14.82)	16.28 (21.42)	30.52 (31.52)	15.69 (24.19)	24.57 (30.18)
15/15	34.92 (35.49)	24.18 (28.96)	30.81 (32.70)	36.98 (30.84)	21.32 (27.06)	22.50 (25.17)	40.92 (39.49)	22.02 (28.83)	2738 (31.11)
16/20	47.27 (43.15)	16.59 (21.67)	29.23 (32.13)	36.65 (30.57)	10.92 (18.49)	20.49 (23.59)	38.26 (38.25)	25.02 (30.55)	26.53 (30.41)
20/20	40.39 (39.76)	22.53 (27.63)	27.80 (31.66)	25.78 (28.80)	8.59 (14.07)	18.58 (23.39)	38.70 (38.55)	20.11 (25.85)	28.40 (31.46)
Mean	36.23 (37.69)	20.42 (25.60)	25.67 (28.19)	24.55 (27.99)	15.01 (21.05)	20.38 (26.78)	27.69 (30.24)	18.31 (21.76)	24.33 (28.45)

* Figures in parentheses are the means of $\sqrt{}$ percentage transformations.
LSD (p= 0.05) for: Bee strength (A) = (6.80), Queen-liness (B) = (4.52), Priming vs. no priming (C) = (4.52), A×B = (NS), B×C = (NS), C×A = (12.52). A×B×C = (NS).
Source: Field survey, 2017-2018.

Table 3: Effect of bee strength and queenliness of cell builder (*A. mellifera meda*) colony on graft acceptance under primed v/s dry grafting condition during 2017-2018.

Bee strength (No. of combs/No. of bee frames)	Mean number of accepted graft per (*A. mellifera.meda*) colony *								
	Queen-right colony			Queen-less colony			Mean		Grand mean
	Primed cell cups	No priming	Mean	Primed cell cups	No priming	Mean	Primed cell cups	No priming	
8/10	15.13 (3.98)	12.53 (3.58)	14.00 (3.62)	7.43 (2.70)	10.40 (3.28)	8.14 (2.31)	10.27 (2.38)	11.50 (2.66)	12.10 (2.90)
10/10	30.87 (5.65)	17.73 (4.20)	24.06 (5.00)	26.20 (5.09)	16.40 (4.54)	22.00 (4.26)	28.53 (5.34)	17.19 (4.70)	22.68 (4.84)
12/15	44.17 (10.72)	12.23 (3.35)	27.24 (5.21)	18.77 (4.78)	10.73 (3.53)	15.67 (3.58)	30.12 (5.28)	11.00 (3.01)	19.68 (3.83)
15/15	31.63 (5.67)	21.43 (4.47)	26.40 (5.24)	23.23 (4.68)	16.87 (4.82)	21.00 (3.26)	27.63 (5.19)	21.00 (5.37)	21.92 (4.64)
16/20	33.08 (5.05)	18.10 (4.42)	27.50 (5.83)	34.05 (5.29)	8.53 (2.84)	18.67 (3.99)	32.30 (5.92)	12.27 (4.00)	22.46 (4.62)
20/20	27.43 (5.68)	21.18 (4.30)	26.17 (5.02)	24.15 (4.96)	8.20 (2.19)	15.22 (3.37)	26.73 (5.18)	14.46 (3.70)	26.18 (5.39)
Mean	30.58 (5.42)	15.84 (4.15)	26.18 (5.39)	22.31 (4.52)	14.36 (3.75)	16.50 (3.42)	20.09 (4.05)	14.30 (3.65)	21.51 (4.38)

* Figures in parentheses are the means of $\sqrt{n+1}$ transformations.
LSD (p= 0.05) for: Bee strength (A) = (0.98), Queenliness (B) = (0.05), Priming vs. no priming (C) = (0.05), A×B = (NS), B×C = (NS), C×A = (1.64). A×B×C = (NS).
Source: Field survey, 2017-2018.

According to some authors the preconditioning positively influences the acceptance of grafted larvae Kither and Pickard [38]; Delaplane and Harbo; [24]. When grafted in cell cups the larvae normally are not placed on the bare bottom but on

diverse substrates that can serve as the food for the larvae or they just help maintain the right humidity. Small drop of royal jelly or of royal jelly diluted in plain water are the best substrates Ebadi and Gary [28]; Pickard and Kither [51]. The best quality of reared queens is secured by a double transfer that consists in the grafting of a larva to replace another one that has been removed from an already existing queen cell, Roberts [55] and Weiss [70]. According to Woyke [73, 74], each 1 day increase in the age of the grafted larvae, decreased the body weight, the size of spermatheca, and the number of ovarioles in the ovaries of the queen. Rawash *et al*. [56] found that virgin queens reared from larvae 1 day old were the heaviest and queens reared from 3 day old larvae were the lightest. Tarpy *et al*. [65] verified that low-quality queens are indeed produced from older worker larvae, as measured morphometrically and as measured by stored sperm counts. The location of a given queen larvae within the queen rearing colony and the number the colony received, were taken into consideration by many affect the quality of the resulting queens (Eckert and Shaw [26]; Rawash *et al*. [56]; Fell and Morse [29]; Spivak *et al*. [61]; Sharaf El-Din *et al*. [62]; Abd Al-Fattah *et al*. and [1, 2]. Larvae were grafted, and queen cells accepted, fed and finished in one colony with no queenless period required. The general principles of a queen right starter-finisher are described by Laidlaw [43], the method is commonly used to produce royal jelly or queens commercially. In honey bees (*Apis mellifera* L.), the quality of the queen undeniably affects the colony performance. Quality of the queen can be evaluated by characters of the queen live weight, weight and number of ovariole, size of the spermatheca, fecundity (number of eggs laid per day) and brood quality. All above mentioned characters are highly depending on the conditions when the queen is grown. Commercial rearing of queens requires huge production of high quality queens [5]. Doolittle [20] was first who grafted worker larvae to produce queens, and since then several research was made about factors affecting the success of grafting: age of grafted larvae [34, 50, 54], design and position of artificial queen cells [19, 35], priming queen cells with royal jelly before grafting [19, 25, 54], position of queen cell with transferred larvae in hive [36, 40, 55], feeding of queen rearing colony [33, 50]. Queens can be reared from worker larvae, in which the larvae are up to 3 days old, by grafting the larvae into artificial queen cell cups Weiss [70] and Dodologlu and Emsen, 1998 [22]. Queens can be reared from worker larvae, in which the larvae are up to 3 days old, by grafting the larvae into artificial queen cell cups, Weiss [72]; Dodologlu and Emsen 1998) [22]. Larvae at any age up to the end of the third day have the potential of being reproductive queens. The quality of the queens decreases as the age of the grafted larvae increases. According to Woyke [73, 74]; each 1 day increase in the age of the grafted larvae, decreased the body weight, the size of spermatheca, and the number of ovarioles in the ovaries of the queen. Rawash *et al*.[56]; found that virgin queens reared from larvae 1 day old were the heaviest and queens reared from 3 day old larvae were the lightest. Tarpy *et al*. [65]; verified that low-quality queens are indeed produced from older worker larvae, as measured morphometrically and as measured by stored sperm counts.

4. Conclusion

The present study concluded that the colonies showed that combined effect of the given levels of queenliness and priming status of cell cups was non-significant on the per cent acceptance of larval grafts (15.01-36.23). The combined effect of different levels of bee strength and priming status of cell cups was significant with maximum percent acceptance in 15/15 bee-frame strength colonies with primed cell cups (40.92%) which was on par with colonies with primed cell cups with bee-strength of 20/20 bee-frame (38.50%), 16/20 bee-frame (38.26%), 10/10 bee-frame (33.83%), 12/15 bee-frame (30.52%). This was followed by 16/20 bee-frame strength colonies with non-primed cell cups (25.02%) which were on par with other colonies with non-primed cell cups and bee strength of 15/15 bee-frame (22.02%), 20/20 bee-frame (20.11%), 10/10 bee-frame (18.20%), 12/15 bee-frame (15.69%), 8/10 bee-frame(12.26%) and least acceptance was observed in8/10 bee-frame strength colonies with primed cell cups (11.48%). Interaction among all the three combinations was non-significant in this respect (7.85- 50.18%). The above results showed the effect of bee strengths on graft acceptance for royal jelly production are in conformity with Aulakh *et al*. [3] who have also reported that graft acceptance by using 24 h old larvae in 15-20 bee frame strength *A. mellifera* meda colonies was significantly higher than 15 bee-frame strength colonies. Considering the effect of queenliness, queen-right condition proved to be better than queenless condition and our results are in conformity with Adam [4] who reported 80 percent acceptance of grafts using queen-right cell finisher colonies. The results of the effect of priming status of cell builder colonies are in conformity with those of Macicka [48] who reported that the mean acceptance of larvae grafted with cell cup priming was 75.9 percent in comparison with 64.2 percent without priming with royal jelly. The similar kinds of observations were recorded by Kitner and Pickard [38, 49]; Kumar Chhuneja and Kumar Gill [39] and Morton [45]. Positive correlation between weight of the queen with depth and width of finished queen cell, depicted that the size of the finished queen cells influenced emerging queen's weight to some extent and ultimately appeared to corroborate the influence of size of queen cell cups on queen weight observed By [36, 42]. Preparation of queen cells before grafting does not affect the queen weight and the spermatheca size.

5. Acknowledgement

I thank Sayed Mehdi Esmaeilzade for helping to collect data in the field. We would like to thank Karaj beekeepers in the Alborz province of Iran for their kindness and for their permission to do research at their apiaries. We would like to thank anonymous and very competent reviewers, for their insightful comments on an earlier version of this manuscript.

6. References

1. Al-Fattah MAA, Haggag EI, Nora AM. Some factors affecting quality of artificially reared queens (*Apis mellifera*) within honey bee nursering colonies. Journal of Agriculture Science Mansoura University, 2007; 32:3151-3159.
2. Al-Fattah MAA, Mazeed AM, Al-Hady NA. Quality and quantity of honeybee queens as affected by the number and distribution of queen cells within queen rearing colonies. Journal of Apicultural Science. 2011; 55:31-43.
3. Aulakh RK, Gatoria GS, Chhuneja PK. Effect of colony strength and elevation of call bars in gyne cell rearing frame on the acceptance of grafted larvae up to the royal jelly collection stage in *Apis mellifera* Linn. Colonies. Indian Bee Journal. 2002; 64:71-72.
4. Adam B. Beekeeping at Buckfast Abbey. British Bee

Publications Ltd. Geddington, Northants. UK, 1975.
5. Büchler R, Andonov S, Bienefeld K, Costa C, Hatjina F, Kezic N *et al*. Standard methods for rearing and selection of *Apis mellifera* queens. In: The coloss Beebook, standard methods for *Apis mellifera* research. V Dietemann; JD Ellis; P. Neumann (Eds), IBRA, Treforest, UK. 2013; 1:08609-82742.
6. Brodschneider R, Riessberger-Galle U, Crailsheim K. Flight performance of artificially reared honey bees (*Apis mellifera*). Apidologie. 2009; 40:441-449.
7. Bilasz GD. O Wlijanii pczel kormilic na matok I ich potomstwo. Pchelovodstvo. 1962; 39:27-30.
8. Breed MD, Butler L, Stiller TM. Kin discrimination by worker honey bee in genetically mixed groups. Proceeding national Academy Science USA. 1985; 82:3058-3061.
9. Butler CG. The method and importance of the recognition by a colony of honey bees (*Apis mellifera*) of the presence of its queen. Transactions of the Royal Entomological Society of London. 1954; 105:11-29.
10. Butler CG. The significance of queen substance in swarming and supersedure in honey-bee (*Apis. mellifera* L.) colonies. Proceedings of the Royal Entomological Society of London. 1960; 35:129-132.
11. Butler CG. Pheromones of the honeybee (*Apis. mellifera* L.): the "inhibitory scent" of the queen. Proceedings of the Royal Entomological Society of London (B). 1968; 43:62-65.
12. Butler CG. Queen Substance. Bee World. 1959; 40:269-275.
13. Butler CG. The World of the Honey Bee. London, UK: Collins, 1974.
14. Chang SY. Effects of size and type of queen cup on the production of royal jelly and acceptance by nurse bees. Master Thesis, National Chung Hsing University Taichung, Taiwan, 1979.
15. Crailsheim K, Brodschneider R, Aupinel P, Behrens D, Genersch E, Jutta Vollmann J *et al*. Standard methods for artificial rearing of *Apis mellifera* larvae. Journal of Apicultural Research. 2013; 52:1-16.
16. Carreck NL, Andree M, Brent CS, Cox-Foster D, Dade HA, Ellis JD *et al*. Standard methods for *Apis mellifera* anatomy and dissection. Journal of Apicultural Research. 2013; 52:1-40.
17. Chuda-Mickiewicz B, Prabucki J. Temperatura gniazda rodziny wychowujacej matki pszczele. Pszczeln. Zesk. Nauk. 1995; 39:87-97.
18. De Grandi-Hoffman G, Spivak M, Martin JH. The role of thermoregulation by nest mates on the development time of honey bee (Hymenoptera: Apidae) queens. Annals of the Entomological Society of America. 1993; 86:165-172.
19. Doolittle GM. Scientific queen-rearing, Thomas G. Newman & Son, Chicago, Illinois, 1889, 978190-4846611.
20. Doolittle GM. Scientific Queen Rearing. Thomas G. Newman & Son, Chicago, Illinois, USA, 1888, 121-154.
21. Doolittle GM. Scientific queen-rearing as practically applied; being a method by which the best of queen-bees are reared in perfect accord with nature's ways. American Bee Journal. 1915; 28:126-139.
22. Dodologlu A, Emsen B. Relationship between larvae age and characteristics of queen honeybee (*Apis mellifera* L.) after single and double grafting. Journal of Veterinary and Animal Science. 1998; 21:379-385.
23. Dedej S, Hartfelder K, Aumeier P, Rosenkranz P, Engels W. Caste determination is a sequential process: effect of larval age at grafting on ovariole number, hind leg size and cephalic volatiles in the honey bee (*Apis mellifera carnica*). Journal of Apicultural Research. 1998; 37:183-190.
24. Delaplane KS, Harbo JR. A re-examination of double grafting. American Bee Journal, 1988; 128:439-440.
25. El-Din HAES. Biological and ecological studies on rearing honeybee (*Apis mellifera* L.) for commercial queen's production. Honeybee Science. 1999; 20:127-130.
26. Eckert JE, Shaw FR. Queen rearing and package bee production, Beekeeping, 1960, 293-296.
27. Engel MS. The taxonomy of recent and fossil honey bee (Hymenoptera: Apidae; *Apis*). Journal of Hymenoptera Research. 1999; 8:165-196.
28. Ebadi R, Gary NE. Acceptance by honeybee colonies of larvae in artificial queen cells. Journal of Apicultural Research. 1980; 19:127-132.
29. Fell RD, Morse RA. Emergency queen cell production in the honey bee colony, Insectes Sociaux. 1984; 31:221-237.
30. Free JB. Pheromones of Social Bees. London, UK: Chapman and Hall, 1987, 146-165.
31. Free JB, Ferguson AW, Simpins JR. Influence of immature queen honey bees (*Apis mellifera*) on queen rearing and foraging. Physiological Entomology. 1984; 9:387-394.
32. Gabka J, Ochnio M, Kaminski Z, Madras-Majewska B. Effect of age of eggs used for rearing honey bee queens on the number of received queen cells. Journal of Apicultural Science. 2011; 55:47-53.
33. Gatoria GS, Gupta JK, Thakur Singh J. Mass queen bee rearing and multiplication of honeybee colonies. AICRP on honeybees and pollinators ICAR, New Delhi and CCS-HAU, Hisar, 2011, 3-70.
34. Gatoria GS, Singh L. Effect of diameter of queen cell cups on mass rearing of *Apis mellifera* L. queen bees. Indian bee Journal. 2000; 62:59-61.
35. Gancer HV, Shah SQ, Firatli C. Effects of supplemental feeding of queen rearing colonies and larval age on the acceptance of grafted larvae and queen traits. Pakistan Journal of Biological Sciences. 2000; 3:1319-1322.
36. Huber F. Nouvelles observation sur les abeilles. 2nd edition translation 1926. Hamilton, Illinois, USA, 1814, 104-132.
37. Haydak MH. Larval food and development of castes in the honeybee. Journal of Economic Entomology. 1943; 36:778-783.
38. Kither GY, Pickard RS. Increasing the acceptance of transplanted honeybee worker larvae by queen-cell starter colonies with the use of partially drawn artificial queen cups. Journal of Apicultural Research. 1983; 22:175-183.
39. Kumar Chhuneja P, Kumar Gill A. Influence of different factors on graft acceptance in (*Apis mellifera* Linnaeus.) colonies during autumn in Punjab. Biological Forum-An International Journal. 2014; 6:148-151.
40. Konopacka Z. Technika wychowu matek pszczelich. Pszczelarstwo. 1971; 5:6-8.
41. Koc AU, Karacaoglu M. Effects of rearing on the quality of queen honeybees (*Apis mellifera* L.) raised under the conditions of Aegean Region. Mellifera. 2004; 4:34-37.

42. Kumar N, Chand S, Singh R. Effects of size of queen cup cells on queen production in *Apis mellifera* and *Apis cerana indica*. Indian Bee Journal, 2000; 62:63-65.
43. Laidlaw HHJR, Eckert JE. Queen rearing. University of California press Berkeley and Los Angeles, 1962, 8-165.
44. Mahbobi A, Farshineh-Adl M, Woyke J, Abbasi S. Effects of the Age of Grafted Larvae and the Effects of Supplemental Feeding on Some Morphological Characteristics of Iranian Queen Honey Bees (*Apis mellifera meda* Skorikov, 1929). Journal of Apicultural Science. 2012; 56:93-98.
45. Morton J. Methods of queen rearing and queen banking. Indian Bee Journal, 1992; 54:26-27.
46. Morse RA, Hooper T. The Illustrated *Encyclopedia* of Beekeeping (First Ed) Printed and bound by Buttler and Tanner Ltd. Forme; Somerset, U.K, 1985; 425.
47. Morse RA. Rearing Queen Honey Bees. Ithaca, New York, USA: Wicwas Press, 1979.
48. Macicka M. The effect of several factors on the acceptance of larvae and on queen weight. Pszczel. Zesz. Nauk. 1985; 29:73-80.
49. Pickard RS, Kither GY. Acceptance of transplanted worker larvae by queen-cell starter colonies. Journal of Apicultural Research. 1983; 22:169-174.
50. Pusca V. Influenta numarului de larve orescute asupra calitatii matcilor. Apicultural. 1970; 23:2-6.
51. Pickard RS, Kither GY. An evaluation of shaking and displacement methods for separating a sample of honeybees with large hypo-pharyngeal glands from the remainder of a colony. Journal of Apicultural Research. 1983; 22:101-106.
52. Riessberger Galle U, Vollmann J, Brodschneider R, Aupinel P, Crailsheim K. Improvement in the pupal development of artificially reared honey bee larvae. Apidologie. 2008; 39:595.
53. Ruttner F. Queen rearing: biological basis and technical instruction. Apimondia Publishing House, Bucharest, Romania, 1983, 09554-20830.
54. Rinderer TE, Hellmich RL. The process of Africanization, in: M. Spivak DJ. Fletcher MD. Breed (Ed.), The "African" honey bee. Westview Press, Boulder, Colorado, USA, 1991, 95-117.
55. Roberts WC. Save a step queen rearing. American Bee Journal, 1965; 105:446-447.
56. Rawash I, Gayarfh E, Helalyms E. Effect of larval age and number of cell cups on the quality of Cario-Egyption F1 hybrid of honeybee queens. Apiculture. 1983; 36:925-985.
57. Skowronek W, Bienkovska M, Kruk C. Changes in body weight of honeybee queens during their maturation. Journal of Apicultural Science. 2004; 48:61-68.
58. Sahinler N, Gul A, Sahin A. Vitamin E supplement in honey bee colonies to increase cell acceptance rate and royal jelly production. Journal of Apicultural Research. 2005; 44:58-60.
59. Seeley TD. Honey bee Ecology. A study of adaptation in social life. In: Krebs JR and Clutton-Brock (Eds). Monographs in Behavior and Ecology. Princeton University. Press, Princeton, NJ, 1985, 201.
60. Seeley TD. Life history strategy of the honey bee, *Apis mellifera*. Oecologia. 1978; 32:109-118.
61. Spivak M, Zeltzer A, De Grandi-Hoffman G, Martin J. Influence of temperature on the rate of development and color patterns of queen honey bees (*Apis mellifera* L.). Environmental Entomology. 1992; 21: 364-370.
62. Sharaf El-Din HA, El-Samni MA, Ibrahim RE. Biological and biometrical studies on honey bee queens *Apis mellifera* L. in Menoufia region, Zagazig. Journal of Agriculture Research. 2000; 27:704-713.
63. Snelgrove LE. Queen Rearing London, UK: Purnell and Sons Ltd, 1949.
64. Singh TP, 1997. Standardization of the techniques for royal jelly production by *Apis mellifera* L. colonies. M.Sc. Thesis Punjab Agricultural University, Ludhiana, India.
65. Tarpy DR, Keller JJ, Caren JR, Delaney DA. Experimentally induced variation in the physical reproductive potential and mating success in honey bee queens. Insectes Sociaux. 2011; 58:569-574.
66. Taranov GF. Weight of queens and their quality. Pchelovodstvo. 1973; 93:27-29.
67. Visscher K. Effect of location within the nest on acceptance of queen cells in honeybee colonies. Journal of Apicultural Research. 1986; 25:154-157.
68. Woodward D. Queen bee: biology, reading and breeding. Balclutha; New Zealand, 2007, 137.
69. Winston ML, Higo HA, Colley SJ, Pankiw T, Slessor KN. The Role of Queen Mandibular Pheromone and Colony Congestion in Honey Bee (*Apis mellifera* L.) Reproductive Swarming (Hymenoptera: Apidae). Journal of Insect Behavior. 1991; 4:649-660.
70. Weiss K. Neue Untersuchengen Zum doppelten Umlarven. Apidologie. 1974; 5:225-246.
71. Wilkinson D, Brown MA. Rearing queen honey bees in a queen-right colony. American Bee Journal. 2002; 142:270-274.
72. Weiss K. The influence of rearing conditions on queen development. In: F. Ruttner (Ed.) Queen rearing biological basis and technical instruction, 1983, 83-177.
73. Woyke J. Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. Journal of Apicultural Research. 1971; 10:45-55.
74. Woyke J. Rearing conditions and number of sperms reaching the queens spermatheca. Proceedings of 21th Apimondia congress, University. Maryland, USA, August, 1967, 14-17, 232-234.