International Journal of Environment and Climate Change

Volume 13, Issue 11, Page 1668-1678, 2023; Article no.IJECC.107484
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Comparative Quality Traits of *Apis mellifera* L. Queens Raised through Standard Queen Rearing Methods in the Spring Breeding Season

Rajan Kamboj [a++*], Surender K. Sharma [b#], Mandeep Rathee [c†] and Naveen Rao [d‡]

[a] Department of Entomology, CCS Haryana Agricultural University, Hisar, Haryana, India.
[b] Bee Research Station, Nagrota Bagwan, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India.
[c] Krishi Vigyan Kendra, Kaithal, CCS Haryana Agricultural University, Hisar, Haryana, India.
[d] Regional Research Station, Faridkot, PAU, Ludhiana, Punjab, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2023/v13i113322

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/107484

Original Research Article

Received: 09/08/2023
Accepted: 15/10/2023
Published: 26/10/2023

ABSTRACT

The present investigations were undertaken to assess the quality traits of *Apis mellifera* L. queens raised through standard queen rearing methods namely Doolittle, Miller, Smith and Swarming instinct during the spring breeding season, 2016-17 at the experimental apiary located at CSK

[++] *Ph.D. Scholar;*
[#] *Principal Scientist;*
[†] *Training Assistant;*
[‡] *Senior Research Fellow;*
**Corresponding author: E-mail: kamboj.rajan6@gmail.com, rajankamboj@hau.ac.in;*

HPKV, Bee Research Station, Nagrota Bagwan, Himachal Pradesh. A fixed number (n = 24) and age (≤ 24 hours) of either larvae (Doolittle) or eggs (Miller and Smith) were used to rear the queen cells. The significantly highest acceptance was recorded for Doolittle method (13.50 ± 1.44; 56.25%) followed by Miller method (11.25 ± 1.31; 46.88%) while the acceptance was least for the Smith method (10.25 ± 1.31; 42.71%). The maximum number of sealed queen cells/colony (9.00 ± 1.22) and neonate queens/colony (6.00 ± 0.91) was also witnessed in the Doolittle method followed by the Miller method (8.00 ± 1.08 sealed queen cells/colony; 4.25 ± 0.85 queens/colony) and Smith method (7.75 ± 0.85 queen cells/colony; 2.75 ± 0.62 queens/colony). The Doolittle method also produced the largest queen cells (25.86 ± 0.89 mm × 12.11 ± 0.23 mm) followed by the Miller method (25.00 ± 0.33 mm × 11.93 ± 0.44 mm), whereas the smallest queen cells (21.12 ± 0.24 mm × 10.23 ± 0.75 mm) were witnessed in the colonies with swarming instinct. The newly emerged queens from Doolittle, Miller, swarming instinct and Smith method had the mean body weights of 201.75 ± 10.06, 191.00 ± 8.82, 186.75 ± 6.54 and 184.00 ± 7.73 mg, respectively. The queens raised using the Doolittle method, initiated egg laying 3-4 days earlier (18.75 ± 0.48 days) compared to other methods (22.00 ± 0.41 days in Miller; 22.25 ± 0.48 days in Smith and 22.50 ± 0.87 days in swarming instinct method). Overall, the Doolittle method produced the highest quality queens and Miller method was the next best alternate. It is thus advocated to use the Doolittle method for mass rearing of high-quality queens on a commercial scale.

Keywords: Honey bee; Apis mellifera; queen rearing; doolittle method; miller method; queen cell cups; grafting.

1. INTRODUCTION

Honey bees and other pollinators have an immense economic and ecological impact on the cultivated and wild ecosystems [1]. The global economic value of insect pollination was estimated at 9.5 per cent amounting to USD 169.6 billion or ₹ 13,93,785.5 crores [2]. The direct contribution of insect pollination to Indian agriculture is estimated at staggering ₹ 1,12,615.7 crores (USD 22.52 billion) annually, representing 8.7 per cent, besides an increase in the quality traits, seed production, breeding efficiency, etc. [3]. In India, the annual economic value of pollination from the honey bees, pegged at ₹ 385.7 crores (USD 0.047 billion) at the base value of year 2014 [4]. The honey bees are considered key-stone species among the animal pollinators attributed to their pollination potential in 75 per cent of the world's cultivated crops. The honey bees support humanity via increased production, food security and maintaining the diversity in the global ecosystems. Globally, farmers manage only 11 species out of the 20,000 to 30,000 bee species known worldwide. The Italian honey bee, *Apis mellifera* L. (Apidae: Hymenoptera) is arguably the most recognizable and economically important commercial species with 91 million managed colonies at global level [5]. In India, *A. mellifera* was first successfully introduced through interspecific queen introduction technique at the Bee Research Station, Nagrota Bagwan, Himachal Pradesh in 1962 [6], 1965 in Punjab [7], and 1982 in Haryana [3]. Since then, beekeeping has been adopted as a source of livelihood by more than 912,882 registered beekeepers in India with 17,25,492 colonies. Of these, 906 and 366 registered beekeepers from Haryana and Himachal Pradesh possess 1,87,302 and 56,182 colonies, respectively [8]. The need for exploiting this avenue is realistic and income generating via pollination services, apitherapy and different products *viz*., honey, pollen, propolis, royal jelly, bee venom, beeswax, quality queens and drones, live colonies, etc.

The *A. mellifera* queen alone has uncountable significant effects on the colony and is one of the most important factors in the overall health, survival, growth and productivity of a colony and apiary as a whole [9]. In recent past, among the abiotic factors, queen failure in often associated with honey bee colony losses worldwide [10]. In addition to the environmental and management practices, different queen-related parameters, *viz*. genetic make-up, age and rearing conditions of larva destined to be queen, size of queen cell, weight of neonate queen, number of mating flights, mating success, quality of drones she mated with, size of spermatheca and ovaries, quantity of sperms stored, egg laying capacity of the newly mated queen, queen longevity, production of queen substance, innate immunity to pathogens, mites and insecticides, hygienic behaviour, etc., have a direct bearing on the performance of honey bee colony [10-18]. The colonies headed by high-quality queens are

characterized by lower swarming and stinging tendency, higher hygienic behaviour, resistance of pathogen and mites, higher colony strength, greater brood area and food reserves, brood solidness, a greater number of divisions, rapid multiplication and increased honey or pollen yields in the honey flow season [19,15]. Hence, high-quality queens are desirable for running a healthier and profitable apiary. In addition, the worst winters in Himachal Pradesh and scorching summer months in Haryana are characterized by a dearth or shortage of bee friendly flora [20,21]. Under these stressful environmental conditions, the hive reserves decline continuously, egg laying by the queens is minimal, brood area shrinks, strength lowers down and ultimately the colony ceases to death [22].

To ensure better growth of the colonies throughout the year and maximize the monetary outputs, the beekeepers must rear and provide high-quality queens prior to the onset of honey flow season *i.e.*, winter and spring season in Haryana and Himachal Pradesh, respectively. Therefore, mass rearing of high-quality *A. mellifera* queens is an essential part of beekeeping and a practical means of providing new queens to queen less colonies and replacement of the old and unreproductive queens [23], as the colony performance depends upon the status of the queen [24]. Considering above mentioned facts and importance of queen bees, Doolittle, Miller, Smith and swarming instinct methods were deployed to mass rear and study the quality traits of *A. mellifera* queens.

2. MATERIALS AND METHODS

2.1 Experimental Site

The present investigation was carried out at the apiary of Chaudhary Sarwan Kumar Himachal Pradesh Agricultural University Palampur, Bee Research Station; Nagrota Bagwan (H.P.), India during the spring breeding season of the calendar year 2016-2017. The experimental apiary was located at an altitude of 907.31 m above mean sea level (amsl) and between 32º07′ North latitude and 76º24′ East longitude.

2.2 Materials and Equipment Used

Langstroth hives, hive stands, gloves, bee veil, hive tool, smoker, commercial cane sugar, bee-collected pollen, half-frame feeders, buckets, stirring rod, distilled water, beakers, muslin cloth, queen cell protector, queen cage, queen gate, queen excluder, grafting needle, beeswax, match stick, stainless steel pot, queen cell cup forming wooden stick, queen cell cups, queen rearing frame, wooden cell bars, camel hair brush, bee brush, comb foundation sheets, measuring grid frame, empty frames, Petri plates, spatula, spoons, markers, battery operated headlight, magnifying lens, knife, saw, nails, hot water bath, Vernier caliper, electronic weighing balance, etc. were the major materials and equipment used during the research work.

2.3 Pre-requisites for Mass Queen Rearing

2.3.1 Mother colony

Two prolific and healthy *A. mellifera* mother colonies (10 bee frame each) with high quality queens were maintained at the experimental apiary. The colonies were regularly fed with freshly prepared sugar syrup (50:50 sugar: water solution @ 500 ml/week/colony) and pollen substitute (@ 100 gm/week/colony). Sealed healthy worker brood was provisioned from other colonies as and when required [25]. These colonies were used to transfer larvae for grafting in Doolittle method. The Miller and Smith method combs were placed in the mother colonies for egg laying by the queen during the 2016-17 spring season (1st February to 15th March).

2.3.2 Cell builder colony

Four healthy and strong (nine bee frame) colonies, for individual queen rearing method were established as cell builder colonies. A sufficient number of nurse bees were maintained by providing the extra young bees and sealed brood frames to the colony. Four days before the larval or egg grafting, these colonies were made queen less [19]. In Doolittle method, 4 queen less colonies, each provided with a larval (n=24) grafted queen rearing frame per colony served as a replication. For Miller as well as Smith method, single comb with eggs (n=24) was placed in individual cell builder colony, replicated four times. Likewise, a set of four colonies with swarming instinct was investigated for the quality traits of naturally emerged queens. Thus, a total of 16 cell builder colonies were used for mass rearing of queens. The comb arrangement of cell builder colony as per Mckinley [26] and Gatoria et al. [25] is described underneath:

H S S E Y C P E S H

Where,

H - Honey comb
S - Sealed brood comb

E - Emerging brood comb
Y - Young larvae brood comb
C - Queen rearing frame with grafted larvae/ Comb strips with eggs
P - Pollen comb

2.4 Methods of Mass Queen Rearing

2.4.1 Doolittle method

2.4.1.1 Preparation of artificial queen cell cups

A 10 cm long wooden dipping stick (A.I. Root Company, USA) with a 9.5 mm diameter at a point 12 mm from the tip tapering to 8 mm at the tip as described by Laidlaw and Eckert [27] was employed to build the queen cell cups. The wax cell cups were prepared with clean beeswax, which was molten by keeping stainless steel pot filled to half its capacity with beeswax into a hot water bath. For preparing the queen cell cups, the wooden stick was first dipped in cold water for 5-10 minutes and subsequently dipped 3 or 4 times in molten wax. Then the wooden cell forming stick was immersed immediately into cold water so that the wax cup becomes hard enough and gets detached easily. A total of 400 queen cell cups of 8 mm across mouth and 10 mm depth were prepared. Before grafting the larvae, the queen rearing frames were constructed form normal wooden frames using wooden cell bars and beeswax.

2.4.1.2 Construction of the queen rearing frame

For constructing the queen rearing frame from a normal bee frame, one wooden bar was inserted across the sidebars of main frame at a distance of 5.6 cm from the top bar. Another such bar was inserted 7.5 cm below the first wooden bar. Below each of these bars, a wooden bar was nailed to hold the artificial cell cups. The exposed base of secondary bars was then affixed with a 1 cm thick beeswax strip base to retain the artificially prepared wax cell cups.

2.4.1.3 Grafting of larvae in wax cell cups

The prepared wax cell cups were then carefully mounted onto the waxed wooden bars. The wax cell cups were affixed at a distance of 2 cm on the wax strip cell cup base fastened on the secondary wooden bar of queen rearing frames. The upper and middle wooden bar were affixed with 12 wax cell cups each at a distance of 2 cm. The queen rearing frame was then placed in the cell builder colony and left for about 30 minutes so that the worker bees could clean and polish the cups. The queen rearing frame was then removed from the cell builder colony and smeared with royal jelly collected using a sterilized camel hair brush. One day old (≤ 24 hours) larvae were selected from the mother colony and transferred into the primed wax cell cups using a grafting needle [28,29].

2.4.2 Miller method

For raising queen cells as per the Miller method, the wax foundation sheets were cut into V-shape, 2/3rd from the lower side. Such foundation sheets were then kept in the mother colonies for the raising the combs. These raised combs were taken out immediately after the queen has laid the eggs. The edges of the combs were then left with only 24 eggs. The rest of the eggs were removed carefully. These frames with eggs (≤ 24 hours) were then marked and given to the cell builder colonies for raising the queen cells. The frames were then placed in the center of a strong queen-right colony [30].

2.4.3 Smith method

The methodology of raising combs with eggs was similar as described for Miller method, except, the shape of wax foundation sheet being rectangular. The horizontal wooden bars of the queen rearing frame were attached with beeswax to the strips of cells containing eggs (≤ 24 hours), and then the bars were put into frames parallel to the top bar. Each frame was left with 24 eggs to raise queen cells by removing the alternate 2 eggs in a horizontal line and kept in the center of the cell builder colony [31,29].

2.4.4 Swarming instinct method

Four queen-right cell builder colonies were congested in the brood chamber and supplemented with sugar syrup, bee-collected pollen and sealed brood frames. Consequently, an increase in the population inside the colony led to overcrowding and insufficient availability of queen substance to the workers. Thus, swarming instinct aroused in these colonies and workers started constructing queen cells at the edges of the frames. Queen was allowed to lay eggs, but as soon as the open queen cells with royal jelly were observed, the colonies were made queen less. The developing queen cells were observed till queen emergence [27,29].

2.4.5 Observations recorded

2.4.5.1 Acceptance of eggs and larval grafts

In case of Doolittle method, after two days of grafting, the number of grafted larvae accepted

by the nurse bees for raising the queen cells were recorded and converted to percentage acceptance. Similarly, when the queen cells became apparent in Smith and Miller method, per cent acceptance was worked out. In colonies where artificially swarming was induced, the number of raised queen cells was recorded.

2.4.5.2 Number of sealed queen cells

The total number of queen cells completely sealed by the workers was counted for each colony and converted to per cent. The queen cell protectors were then installed to protect the sealed queen cells from any damage.

2.4.5.3 Size of queen cells

The diameter of central, top and bottom parts of the fully developed queen cells was measured with the help of a Vernier caliper. The depth (rim to inner bottom part) of these queen cells was measured after the emergence of the queens for each colony.

2.4.5.4 Queen emergence

The number of the queens emerging from the sealed queen cells confined to queen cell protectors were counted for individual colony and converted to per cent emergence.

2.4.5.5 Weight of neonate queens

Immediately after the emergence, the neonate queens were carefully shifted to pre-weighed plastic queen cages. Before capturing the queens for weighing, the plastic cages were marked and numbered accordingly. The queens were then weighed by placing the carrier cages on a digital electronic balance. Each such queen was weighed and released into the nucleus colonies to estimate the pre-oviposition period and other colony growth parameters.

2.4.5.6 Pre-oviposition period

The neonate queens emerging from the sealed queen cells were immediately kept in plastic queen cages provided with honey to feed upon. After 24 hours, these carrier queen cages were placed in nucleus colonies to evaluate the pe-oviposition period of these queens. Young and unmated *A. mellifera* queens are known to perform mating flights after six days emergence in the drone congregation areas [32-34] and 1 to 4 days after the last mating queen starts egg laying [35]. Hence, status of the released queen bees, whether mated or not, was monitored continuously after 3rd day of their release in the nucleus colonies. For each released queen, the pre-oviposition period i.e., onset of egg laying was measured in terms of the time taken to lay the first egg (days). The data were tabulated and analyzed using CPCS1 analysis software.

3. RESULTS AND DISCUSSION

3.1 Acceptance of Larval Grafts or Eggs

The acceptance of larval grafts (in wax cell cups) and eggs (in comb strips) varied for queen rearing by the nurse bees significantly among the different queen rearing methods (Table 1). The mean strength of cell builder colonies of *A. mellifera* varied from 8.40 ± 40 to 9.44 ± 0.28 bee covered frames/colony in different queen rearing methods. Doolittle method resulted in significantly highest mean acceptance (13.50 ± 1.44 accepted queen cell cups/colony) followed by Miller method (11.25 ± 1.31 accepted eggs/colony) and Smith method (10.25 ± 1.31 accepted eggs/colony). Of the 24 larval grafts or 24 eggs exposed to the nurse bees, the respective percentage acceptance was 56.25, 46.88 and 42.71 for Doolittle, Miller and Smith Method.

In conformity, Gatoria et al. [19], Vaziritabar and Esmaeilzade [36] and Wakjira et al. [37] recorded 54.25, 50.80 and 50.81 per cent larval acceptance rates, respectively in 10-frame strength queen less cell builder *A. mellifera* colonies using the Doolittle method. Contrary to the present findings, Thakur et al. [38], Kumar et al. [39], Emson et al. [40] and Okuyan and Akyol [41] reported comparatively higher acceptances rates of 77.80, 84.46, 83.0 and 81.2 per cent, respectively, using 1-day-old larvae for rearing queens in the Doolittle method. Gencer et al. [42] reported 64.4 and 87.5 per cent acceptance of 1-day-old larval grafts with the Doolittle method in sugar syrup (1 lit./day/colony) and sugar syrup + 10 g bee-collected pollen fed colonies, respectively.

The grafted larvae raised with Doolittle method had remarkably higher acceptance in queen less (88.10%) as compared to queen right colonies (76.66%) [43]. The cell builder colonies fed with pollen supplement had higher acceptance (41.0%) of 1-day-old larval grafts in the Doolittle method compared to unfed colonies (31.0%) [44]. Adgaba et al. [45] reported comparatively higher acceptance of cell cups in the Doolittle method in queen less (73.68%) as compared to

queen right (32.72%) cell builder colonies with natural swarming instinct. While rearing *A. mellifera* queens with Doolittle method, Cengiz et al. [46] recorded higher acceptance rate (82.35%) of 1-day-old larval grafts in supplementary fed queen less cell builder colonies compared to unfed colonies (62.74%). Rearing *A. mellifera* queens with Doolittle method, Dhaliwal et al. [47] obtained 42.0 and 40.0 per cent acceptance of larval grafts in plastic cell cups and wax cell cups, respectively during autumn season. Acceptance was highest in the larval grafts of Cup kit apparatus (54.0%). However, better results were obtained by Dhaliwal et al. [48] during the spring season. Sharma et al. [49] recorded 81.25 and 87.50 per cent acceptance of larvae, grafted in cell cups made from fresh wax cappings and primed with royal jelly, using Doolittle method during autumn and spring season, respectively at Nauni, Solan, H.P.

3.2 Queen Cell Formation and Emergence of Neonate Queens

The formation of queen cells and queen emergence varied significantly among the different queen rearing methods (Table 2). The mean strength of cell builder colonies of *A. mellifera* varied from 7.81 ± 0.37 to 9.50 ± 0.35 bee covered frames/colony in different queen rearing methods. The Doolittle method resulted in the highest mean number of sealed queen cells (9.00 ± 1.22/colony), which was 37.50 per cent of the grafted larval cups (Table 2). Miller method was the second-best option that resulted in 8.00 ± 1.08 number of sealed queen cells/colony, *i.e.*, 33.33 per cent. Smith and Swarming instinct methods could develop only 7.75 ± 0.85 and 5.25 ± 0.48 numbers of sealed cells per colony. Quite higher conversion of accepted cell cups to sealed queen cells was observed by Adgaba et al. [45] in the Doolittle method in queen less (63.16%) as compared to queen right (25.31%) cell builder colonies that were compacted using a queen excluder to induce swarming instinct. Dhaliwal et al. [47] reported that 93.1 and 86.3 per cent of the accepted cells got completely sealed in Doolittle method when larval grafting was done in plastic cell cups and wax cell cups, respectively.

The Doolittle method recorded the highest mean number of emerged neonate queens (6.00 ± 0.91/colony) with 67.36 per cent emergence. From the sealed queen cells in the Miller method, 4.25 ± 0.85 queens (55.01%) emerged successfully, whereas under Smith method merely 2.75 ± 0.62 queens (34.26%) emerged per colony. The lowest number of queens per colony emerged from the sealed queen cells in the swarming instinct method (2.25 ± 0.62). Dodologlu et al. [50] reported 100.0 per cent queen emergence from the sealed queen cells in Doolittle method, depicting a high precision of queen rearing. Similarly, Wakjira et al. [37] recorded that 25.56 per cent of the accepted *A. m. bandasii* queen cells got completely sealed, whereas neonate queens emerged from only 23.10 per cent of the sealed queen cells under the Doolittle method. Adgaba et al. [45] using Doolittle method recorded higher percentage of queen emergence in queen less (54.39%) as compared to queen right (20.68%) cell builder colonies with natural swarming instinct. Dhaliwal et al. [47] reported maximum queen emergence in Doolittle method when larval grafting was done in plastic cell cups (84.7%) followed by Cup kit apparatus (75.6%) and larval grafting wax cell cups (74.8%). Deploying Doolittle method of queen rearing, Sharma et al. [49] recorded 75.00 and 81.25 per cent queen emergence from the sealed queen cells during autumn and spring season, respectively at Nauni, Solan, HP.

Table 1. Acceptance of eggs or larval grafts in different methods of *A. mellifera* queen rearing

Queen rearing method	Colony Status	Mean colony strength (No. of bee covered frames/colony)	Eggs (No./colony)	Grafted larvae (No./colony)	Mean acceptance (No./colony) (Mean ± SE)	Per cent acceptance (%)
Miller	Queen less	*8.81 ± 0.37	24	-	11.25 ± 1.31	46.88
Smith	Queen less	8.40 ± 0.40	24	-	10.25 ± 1.31	42.71
Doolittle	Queen less	9.18 ± 0.34	-	24	13.50 ± 1.44	56.25
Swarming	Queen right	9.44 ± 0.28	-	-	-	-
C.D. (p=0.05)		1.0	-	-	-	-

**Figures are the mean values of four replications; 1 colony = 1 replication*

Table 2. Queen cell formation and queen emergence in different methods of *A. mellifera* queen rearing

Queen rearing method	Colony Status	Mean colony strength (No. of bee covered frames/colony)	Sealed queen cells No./colony	%age	Number of neonate queens emerged/colony (Mean ± SE)	Per cent emergence (%)
Miller	Queen less	7.81 ± 0.37	8.00 ± 1.08	33.33	4.25 ± 0.85	55.01
Smith	Queen less	8.40 ± 0.40	7.75 ± 0.85	32.29	2.75 ± 0.62	34.26
Doolittle	Queen less	9.18 ± 0.34	9.00 ± 1.22	37.50	6.00 ± 0.91	67.36
Swarming	Queen right	9.50 ± 0.35	5.25 ± 0.48	-	2.25 ± 0.62	41.25
C.D. (p=0.05)		1.0	NS	-	-	2.4

Figures are the mean values of four replications; 1 colony = 1 replication

Table 3. Dimensions of the finished queen cells in different methods of *A. mellifera* queen rearing

Queen rearing method	Colony status	Diameter of queen cell (mm)			Depth of queen cell (mm)
		Top	Mid	Bottom	
Miller	Queen-less	13.58 ± 0.71	11.93 ± 0.44	7.48 ± 0.53	25.00 ± 0.33
Smith	Queen-less	13.31 ± 0.26	11.32 ± 0.23	6.95 ± 0.21	23.07 ± 0.40
Doolittle	Queen-less	14.26 ± 0.61	12.11 ± 0.23	7.67 ± 0.30	25.86 ± 0.89
Swarming	Queen-right	10.97 ± 0.43	10.23 ± 0.75	7.35 ± 0.01	21.12 ± 0.24
C.D. (p=0.05)		1.4	1.3	NS	1.9

3.3 Size of Queen Cells

Both the diameter and depth of the finished queen cells varied significantly among the different queen rearing methods (Table 3). The top diameter (distal end of the queen cell) and mid diameter (center of the queen cell) of Doolittle-raised queen cells had the highest mean values (14.26 ± 0.61 and 12.11 ± 0.23 mm). The mean top and mid diameter of the queen cells raised by other methods were in the descending order of the Miller method (13.58 ± 0.71 and 11.93 ± 0.44 mm), Smith method (13.31 ± 0.26 and 11.32 ± 0.23 mm) and swarming instinct method (10.97 ± 0.43 and 10.23 ± 0.75 mm), respectively.

Similarly, the mean bottom diameter of queen cells was maximum in the Doolittle method (7.67 ± 0.30 mm); however, it was insignificant among the methods used. The minimum bottom diameter of the queen cell was recorded by the swarming instinct method (7.35 ± 0.01 mm). The mean size of the queen cells (depth x mid diameter) was found to be the largest for the Doolittle method (25.86 0.89 mm x 12.11 0.23 mm) followed by Miller method (25.00 ± 0.33 mm × 11.93 ± 0.44 mm) and Smith method (23.07 ± 0.40 mm × 11.32 ± 0.23 mm), while the least size of finished queen cells (21.12 ± 0.24 mm × 10.23 ± 0.75 mm) was measured for the queen cells reared from the swarming instinct method. Gencer et al. [42] and Emsen et al. [40] reported sealed queen cells of 25.20±0.04 and 25.60±0.04 mm length under Doolittle method of queen rearing. In conformity, Dodologlu et al. [50] reported higher mean length of the sealed queen cells in Doolittle method (24.80 mm) as compared to the naturally sealed queen cell (19.47 mm). Cengiz et al. [46] reported significantly higher mean length of queen cells in queen less (30.71 ± 0.14 mm) compared to the queen right colonies (25.13 ± 0.18 mm). Ahmad and Dar [43] reported a greater depth (31.00 mm) of queen cell in queen less colonies compared to queen right colonies (26.60 mm). The length of sealed queen cell was significantly greater in artificially fed queen less cell builder colonies (29.05 mm) compared to the unfed colonies (27.03 mm) [46].

3.4 Weight of Neonate Queens and Onset of Egg Laying by the Mated Queens

The mean weight of newly emerged queens varied non-significantly among the different queen rearing methods (Table 4). The neonate queens emerging from the sealed queen cells in Doolittle method were the heaviest with a mean weight of 201.75 ± 10.06 mg/queen and the weight of queens ranged from 181 to 224 mg. The *A. mellifera* queens with a weight ≥ 200 mg are considered of good quality [51]. The mean weight of neonate queens was 191.00 ± 8.82 mg,

Table 4. Weight of neonate queens and onset of egg laying by the mated queens in different methods of *A. mellifera* queen rearing

Queen rearing method	Weight of neonate queen (mg)		Pre-oviposition period (days)	
	Mean ± SE	Range	Mean ± SE	Range
Miller	191.75 ± 8.82	174 - 214	22.00 ± 0.41	21 - 23
Smith	184.00 ± 7.73	172 - 207	22.25 ± 0.48	21 - 23
Doolittle	201.75 ± 10.06	181 - 224	18.75 ± 0.48	18 - 20
Swarming	186.75 ± 6.54	171 - 202	22.50 ± 0.87	20 - 24
C.D. (p=0.05)	NS	-	1.8	-

186.75 ± 6.54 mg and 184.00 ± 7.73 mg/queen by Miller, swarming instinct and Smith method, respectively. The present findings are in accordance with Gatoria et al. [25], Dodologlu et al. [50] and Ahmad and Dar [43] who recorded 200.15, 206.13 and 202.19 mg weight of neonate queens developed from 1-day-old larval grafts in queen less colonies, respectively using Doolittle method. In conformity, Gregorc and Skerl [52] reported that mean weight of *Apis mellifera carnica* Pollman queens reared with Doolittle method ranged from 201.83 to 208.40 mg. Kumar and Mall [53] reported 187.58 and 205.25 mg weight of newly emerged and mated queens, respectively in the Doolittle method of queen rearing. Adgaba et al. [45] observed no significant difference in the mean weight of neonate queens emerging from queen less colonies (Doolittle method) (141.00 mg) and queen right (136.00 mg) cell builder colonies with natural swarming instinct. The emergence weight of queen was significantly greater in artificially fed queen less cell builder colonies (195.01 mg) compared to the queens reared from the unfed colonies (186.30 mg) [46]. Dhaliwal et al. [47] reported maximum weight of gynes in Doolittle method by grafting 1-day-old larvae in plastic cell cups (202.57 mg) followed by Karl Jenter apparatus (193.36 mg) and wax cell cups (187.77 mg), respectively.

The pre-oviposition period *i.e.*, the time taken by the neonate queens to lay the first egg after emergence varied significantly among the different queen rearing methods (Table 4). The queens emerging from the Dolittle method had the shortest pre-oviposition period (18.75 ± 0.48 days) and ranged from 18 to 20 days. Whereas, for the queens developed in other methods, the onset of egg laying was delayed with statistically similar number of days to lay the first egg (22.00 ± 0.41, 22.25 ± 0.48 and 22.5 ± 0.87 days under Smith, Miller and swarming instinct method, respectively). Moreover, the pre-oviposition period ranged from 20 to 24 days. In conformity, Dodologlu et al. [50] reported a pre-oviposition period of 10.9 days for the queens developed in Doolittle method and 11.1 days in naturally sealed queen cells. Arun [54] documented a pre-oviposition period of 29 to 30 days compared to 18-20 days in the Doolittle method of our findings, the differences might be due to climatic conditions and the season chosen for the study [55]. Ahmad and Dar [43] observed no significant difference in pre-oviposition period of queens emerging from queen less (12.54 days) and queen right colonies (12.59 days).

4. CONCLUSION

The Doolittle method is the most followed method for mass queen rearing of high-quality queens across the globe. In the present investigations as well, the queens reared from 1-day-old larvae with Doolittle method were of the highest quality with significantly greater acceptance of cell cups, a greater number of sealed queen cells, higher emergence of new queens characterized with greater weights and early onset of egg laying. Therefore, it is advocated to the beekeepers to learn this method, practice at their apiary and develop their own queens of superior quality.

RESEARCH CONTENT

The research content is original and has not been submitted or published elsewhere.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Neov B, Georgiev A, Shumkova R, Radoslavov G and Hristov P. Biotic and abiotic factors associated with colonies

mortalities of managed honey bee (Apis mellifera). Diversity. 2019;11:237. Available:https://doi.org/10.3390/d11120237.

2. Gallai N, Salles JM, Settele J, Vaissiere BE. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ. 2009;68:810-821.
3. Chaudhary OP. Constraint analysis in beekeeping industry (pp. 40-55). In: Proceedings of the Workshop on Promotion of Honey bee keeping in Haryana, Panchkula, Haryana, India. 2014;256.
4. Chaudhary OP, Chand R. Economic benefits of animal pollination to Indian agriculture. Indian J. Agric. Sci. 2017;87 (9):1117-1138.
5. Phiri BJ, Fevre D, Hidano A. Uptrend in global managed honey bee colonies and production based on a six-decade viewpoint, 1961-2017. Sci. Rep. 2022;12:21298. Available:https://doi.org/10.1038/s41598-022-25290-3
6. Atwal AS, Goyal NP. Introduction of *Apis mellifera* in Punjab plains. Indian Bee Journal. 1973;35(1):1-9.
7. Atwal AS, Sharma OP. The introduction of Apis mellifera queens into Apis cerana indica colonies and the associated behaviours of the two species. Indian Bee Journal. 1968;30(2):41-56.
8. National Bee Board. State-wise details of the beekeepers registered with the Board up to March; 2023. Available:https://madhukranti.in/nbb/frm_appl_report_all.aspx?id=1 [Accessed on 2 April, 2023].
9. Ruttner F. Biogeography and Taxonomy of Honeybees. In: Springer Verlag, Berlin, Germany. 1988;284.
10. Amiri E, Strand MK, Ruepell O, Tarpy DR. Queen quality and the impact of honey bee diseases on queen health: Potential for interactions between two major threats to colony health. Insects. 2017;8:48.
11. Hoopingarner R, Farrar C. Genetic control of size in queen honey bees. J. Econ. Entomol. 1959;52:547-548.
12. Winston ML. The biology of the honey bee. In: Harvard University Press, Cambridge. 1987;180.
13. Oldroyd BP, Goodman RD, Allaway MA. On the relative importance of queens and workers to honey production. Apidologie. 1990;21:153-159.
14. Martin SJ, Medina LM. Africanized honey bees have unique tolerance to Varroa mites. Trends Parasitol. 2004;20:112-114.
15. Buchler R, Andonov S, Bienefeld K, Costa C, Hatjina F, Kezic N, Kryger P, Spivak M, Uzunov A and Wilde J. Standard methods for rearing and selection of Apis mellifera queens. J. Apic. Res. 2013;52(1):1-30.
16. Collins AM, Pettis JS. Correlation of queen size and spermathecal contents and effects of miticide exposure during development. Apidologie. 2013;44:351-356.
17. Koeniger G. Reproduction and mating behavior. In: Bee Genetics and Breeding, Academic Press (eds. Rinderer T E), Cambridge, USA. 1986;255-280.
18. Zhao X, Liu Y. Current knowledge on bee innate immunity based on genomics and transcriptomics. Int. J. Mol. Sci. 2022;23:14278. Available:https://doi.org/10.3390/ijms232214278.
19. Gatoria GS, Singh B, Singh L. Effect of diameter of queen cell cups on mass rearing of Apis mellifera L. queen bees. Indian Bee Journal. 2000;62(1):59-61.
20. Kumar J, Mall P. Effect of life stages and colony on live weight of newly emerged queen Apis mellifera ligustica. J. Exp. Zool. 2016;19(1):1379-1383.
21. Rathee M, Chaudhary OP and Dalal PK. Advertising the significance of diverse plant taxa to *Apis mellifera* L. foragers during dearth period. In: National Web Symposium on Recent Advances in Beneficial Insects: Natural Resins and Gums, organized by ICAR-IINRG, Ranchi, India. 2021;86.
22. Rathee M, Chaudhary OP, Yadav S, Dalal P. Estimating the influx of pollen into *A. mellifera* L. hives using Chaudhary's pollen trap during dearth period at Kaithal (Haryana). In: Proceedings of Virtual National Conference on Strategic Reorientation for Climate Smart Agriculture, PAU, Ludhiana, India. 2021;3:53-55.
23. Laidlaw HH, Eckert JE. Queen rearing. In: University of California Press, Berkeley, USA; 1962.
24. Tarpy DR, Hatch S, Fletcher DC. The influence of queen age and quality during queen replacement in honey bee colonies. Animal Behaviour. 2000;59(1):97-101.

25. Gatoria RS, Chhuneja PK, Singh J, Singh B. Mass queen bee rearing in *Apis mellifera* L. Indian Bee Journal. 2004;66(1&2):41-50.
26. McKinley WM. Grafting-how it is done. Gleanings in Bee Culture. 1963;91:404-409.
27. Laidlaw HH, Page RE. Queen Rearing and Bee Breeding. In: Wicwas Press, pp. 224, New York, USA; 1997.
28. Doolittle GM. Scientific queen rearing: As practically applied. An American Bee Journal Publication, Hamilton, USA. 1915;126.
29. Chhuneja PK. Traditional and commercial methods of mass queen bee rearing. In: Training course manual. Scientific Beekeeping and Integrated Pest Management, CCS Haryana Agricultural University, Hisar, Haryana, India. 2015;45-55.
30. Miller CC. Fifty Years Among the Bees. In: An A.I. Root Company Publication, Medina, Ohio, USA. 1911;322.
31. Smith J. Queen rearing simplified. In: An A.I. Root Company Publication, pp. 119 Medina, Ohio, USA; 1923.
32. Woyke J, Ruttner F. An anatomical study of the mating process in the honey bee. Bee World. 1958;39;3-18.
33. Zmarlicki C, Morse RA. Drone congregation areas. J. Apic. Res. 1963;2:64-66.
34. Woyke J, Jasinski Z, Prabucki J, Wilde J, Chuda-Mickiewicz B, Siuda M, Madras-Majewska B, Samborski J, Bratkowski J and Jojczyk A. Onset of oviposition by honey bee queens, mated either naturally or by various instrumental insemination methods, fits a lognormal distribution. J. Apic. Res. 2008;47(1):1-9.
35. Koeniger G, Koeniger N, Ellis J, Connor LH. Mating biology of honey bees (*Apis mellifera*), In: Wicwas Press, Kalamazoo, Michigan, USA. 2014;155.
36. Vaziritabar S, Esmaeilzade SM. 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. J. Entomol. Zool. 2018;6(4):683-692.
37. Wakjira K, Negera T, Dabela S, Alemu T. Comparing responses of local honeybees (*Apis mellifera* L.) to Karl Jenter and Doolittle grafting queen rearing methods. International Journal of Animal Science and Technology. 2019;3(3):35-40.
38. Thakur RK, Dogra GS, Gupta JK. Investigation on factors influencing queen rearing success in honeybees Apis mellifera L. Pest Management and Economic Zoology. 1998;6:61-63.
39. 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.
40. Emsen B, Dodologlu A, Genc F, Smith B H and Cobey S. Effect of larvae age and grafting method on the larvae accepted rate and height of sealed queen cell (*Apis mellifera* L.). J. Appl. Anim. Res. 2003;24:201-206.
41. Okuyan S, Akyol E. The effects of age and number of grafted larvae on some physical characteristics of queen bees and acceptance rate of queen bee cell. TURJAF. 2018;6(11):1556-1561.
42. Gencer 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. PJBS, Pakistan. 2000;3(8):1319-1322.
43. Ahmad SB, Dar SA. Mass rearing of queen bees *Apis mellifera* L. (Hymenoptera: Apidae) for bee colony development raised under the temperate conditions of Kashmir. Bioscan. 2013;8(3): 945-948.
44. Njeru LK, Raina SK, Kutima HL, Salifu D, Cham DT, Kimani JM, Mili EM. Effect of larval age and supplemental feeding on morphometrics and oviposition in honey bee *Apis mellifera* scutellat queens. J. Apic. Res. 2017;56:1-7.
45. Adgaba N, Al-Ghamdi A, Tadesse Y, Alsarhan R, Single A, Mohammed SE, Khan KA. The responses of *Apis mellifera jemenitica* to different artificial queen rearing techniques. Saudi J. Biol. Sci. 2019;26:1649-1654.
46. Cengiz M, Emsen B and Dodologlu A. Some characteristics of queen bees (*Apis mellifera* L.) rearing in queenright and queenless colonies. J Anim Vet Adv. 2009;8(6):1083-1085.
47. Dhaliwal NK, Singh J, Chhuneja PK. Comparative evaluation of Doolittle, Cupkit and Karl Jenter techniques for rearing *Apis mellifera* Linnaeus queen bees during breeding season. J. Appl. Nat. Sci. 2017;9 (3):1658-1661.

48. Dhaliwal NK, Singh J, Chhuneja PK. Comparative evaluation of mass queen bee rearing techniques for Apis mellifera (Hymenoptera: Apidae) in autumn season. J. Entomol. Zool. 2019;7(3):1062-1065.
49. Sharma A, Rana K, Sharma HK, Sharma A. Evaluation of priming media and queen cup material on larval graft acceptance and queen emergence in *Apis mellifera* L. J. Entomol. Zool. 2020;8(4):1089-1097.
50. Dodologlu A, Emsen B, Gene F. Comparison of some characteristics of queen honey bees (*Apis mellifera* L.) reared by using doolittle method and natural queen cells. J. Appl. Anim. Res. 2004;26(2):113-115.
51. Kahya Y, Gencer HV, Woyke J. Weight at emergence of honey bee (*Apis mellifera* caucasica) queens and its effect on live weights at the pre and post mating periods. J. Apic. Res. 2008;47(2):118-125.
52. Gregorc A, Skerl S. Characteristics of honey bee (*Apis mellifera* carnica Pollman 1879) queens reared in Slovenian commercial breeding stations. J Apic Sci. 2015;59(2):5-12.
53. Kumar R, Kundal N. Beekeeping status in Kangra district of Himachal Pradesh. J. Entomol. Zool. 2016;4(4):620-622.
54. Arun BC. Studies on the factor influencing the mass production of *Apis mellifera* L. queens. M.Sc. Thesis submitted to University of Agriculture Sciences, Bangalore, Karnataka, India, 2011;60.
55. Cengiz MM, Yazici K, Arslan S. The effect of the supplemental feeding of queen rearing colonies on the reproductive characteristics of queen bees (*Apis mellifera* L.) reared from egg and different old of larvae. Caucasian University J. Vet. 2019;25(6):849-855.

© 2023 Kamboj et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/107484