Uttar Pradesh Journal of Zoology

Volume 45, Issue 19, Page 32-40, 2024; Article no.UPJOZ.4114
ISSN: 0256-971X (P)

Assessing Queen Cell Cups Priming from Combs of Different Honey Bee Species for Mass Rearing of *Apis mellifera* L. Queen Bee

Padiyar Suraj Ganpat [a], Ramanuj Vishwakarma [a*] and Tarak Nath Goswami [a]

[a] Department of Entomology, Bihar Agricultural University, Sabour, Bhagalpur-813210, Bihar, India.

Authors' contributions

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

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i194496

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://prh.mbimph.com/review-history/4114

Original Research Article

Received: 24/07/2024
Accepted: 28/09/2024
Published: 28/09/2024

ABSTRACT

Experiments were carried out at the Beekeeping-cum-Honey Production Unit, Department of Entomology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India, during 2020-21. The study was aimed to evaluate queen cell cups prepared from combs of different bee species to find out a suitable technique for royal jelly production through mass queen bee rearing of *Apis mellifera* species. The worker bees of *Apis mellifera* (AM) exhibited a low preference for working with combs produced by *Apis dorsata* (AD) and *Apis cerana* (AC) for queen cell construction amongst evaluated combs. In contrast, when queen cell cups were made using AM combs, the worker bees attempted to build 100% of the cells. The time required by AM worker bees to complete queen cell

BAU Communication No.: 1040/210831;
**Corresponding author: Email: entoramanuj@gmail.com;*

Cite as: *Ganpat, Padiyar Suraj, Ramanuj Vishwakarma, and Tarak Nath Goswami. 2024. "Assessing Queen Cell Cups Priming from Combs of Different Honey Bee Species for Mass Rearing of Apis Mellifera L. Queen Bee". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (19):32-40. https://doi.org/10.56557/upjoz/2024/v45i194496.*

construction on AM combs ranged from 7 to 15 days, however, they took 1.20 and 0.98 times longer to complete cells made with AD and AC combs, respectively. Similarly, queen bees showed reluctance to lay eggs in cells built on AD and AC combs. In queen cells constructed on AM combs, the queen bee began laying eggs on the second day after release into the nucleus hive and continued for five days, with an increasing trend in egg-laying activity. Among the different batches of eggs laid, the third batch was the most successful in respect of the larval growth and development. Additionally, larval growth showed a consistent upward trend during the first three days of observation for each laying batch. *A. mellifera* combs have been found to be more favourable for queen cell construction by worker bees, showing a strong preference for the queen's egg-laying performance, as well as optimal larval growth and development. Based on these findings, it is recommended that beekeepers may adopt this method to promote mass queen bee rearing and enhance royal jelly production.

Keywords: Apis mellifera; combs; honey bee; graft acceptance; queen cell cups.

1. INTRODUCTION

Beekeeping is only an enterprise which does not only improves the economic conditions of the beekeepers but also restricts the migration of rural youth to urban areas, thus helping holistic development of rural society by different means *viz.*management of hives, migration of honey bee colonies from one field to another, harnessing honey and other bee-hive products such as pollen, royal jelly, beeswax, propolis, and bee venom [1].

Now the time has come, the technique of producing other valuable hive product like royal jelly, etc. should be given top priority in beekeeping besides honey production. Royal jelly is a secretion product of the cephalic glands of nurse bees and served as the most important part of honeybee larvae diet, exclusive food of the queen honeybee (*Apis mellifera*) and plays a major role in caste determination. Due to its complex composition (water, proteins, lipids, carbohydrates, amino acids, mineral salts, vitamins, enzymes, hormones, oligo-elements, natural antibiotics), it has a multitude of pharmacological activities: antioxidant, neurotrophic, hipoglicemiant, hipocholesterole-miant and hepatoprotective, hypotensive and blood pressure regulatory, antitumor, antibiotic, anti-inflammatory, immunomodulatory and anti-allergic, general tonic, antiaging, etc. [2], the production of royal jelly in our country and state is still scanty. China is the world largest royal jelly producing country of which total production of royal jelly reaches to 3,500 tons that account for over 85% of the world total production [3 and 4]. Being the 8th leading country in the world in honey production, India has very few agencies/ organizations involved in royal jelly production. India is also import value-added products of royal jelly from China and other countries. Likewise the honey production business in the beekeeping sector, production, and marketing of royal jelly need to be encouraged for its commercialization and bestow the new opportunity to the beekeepers and unemployed rural people. Similarly, Bihar becomes one of the leading states of our country in honey production since last many years. But, as far as our knowledge no attempts have been made in the state by any of the agencies/ beekeepers/ organization for the promotion of production of royal jelly. The main provable reasons for that may be the unavailability of modern and scientific facilities in the state, lack of technical knowledge of the production of precious products produced by bees, lack of suitable market and appropriate prices of the hive products. Keeping in view of the above facts, the present experiment was aimed to evaluate queen cell cups prepared from combs of different bee species to develop a suitable technique for royal jelly production through mass qeen bee rearing of *Apis mellifera* species.

2. MATERIALS AND METHODS

Experiments were carried out at Beekeeping -cum- Honey Production Unit and Department of Entomology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India located at 25^{0}15$'$ North Latitude, 86^{0}57$'$ South Longitude and altitude of 46 meters above the MSL during 2020-21 to evaluate queen cell cups prepared from combs of different bee species to develop a suitable technique for royal jelly production using *Apis mellifera* bees.

For the experiment, raw wax from different species of honey bees was collected from locally available colonies. The collected wax was melted

using a melting apparatus for 30 minutes, and then used to prepare queen cell cups with a queen cell cup-forming stick. The prepared cups were grafted onto the underside of each movable bar using raw wax. The frames, along with the queen cell cups, were placed inside a nucleus hive, which was supplemented with one or two established frames containing high-quality brood, worker bees, honey, and pollen to facilitate further queen cell construction and preparation for egg-laying [5].

Regular inspections were carried out daily to monitor the development of queen cells in the grafted cups, with progress recorded systematically. Once the queen cell construction was complete, a healthy and productive queen bee was introduced into the nucleus hive for a few days to lay eggs in the cells. Egg-laying activity was carefully observed at regular intervals to assess the queen's performance and preference, and larval developmental progress was also recorded.

This method was followed with slight modifications based on the recommendations of Vaziritabar and Esmaelizade [6], Wu et al. [7], Ruttner [8], Ebadi and Gary [9] and Dhaliwal et al. [10]. The collected data were analyzed using simple means and ranges.

Image 1. *Apis dorsata* comb

Image 2. *Apis cerana* combs

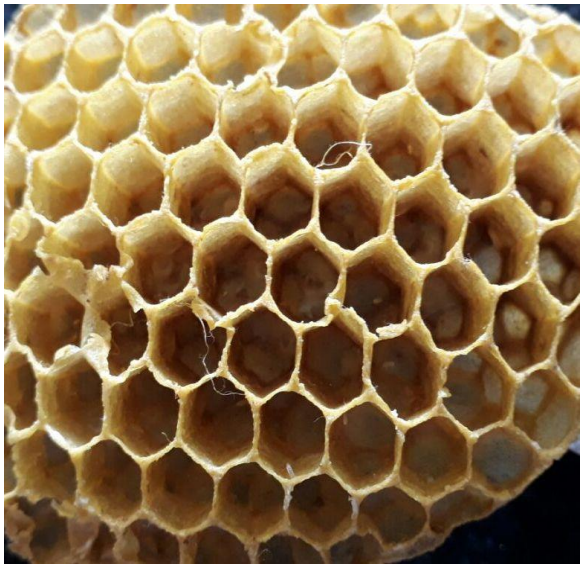

Image 3. *Apis mellifera* comb

Image 4. Prepared queen cell cups

3. RESULTS AND DISCUSSION

3.1 Building of the Queen Cells Made with Combs of Different Species of Bees

During the investigation, queen cell cups were prepared using raw wax collected from colonies of the rock honey bee (*Apis dorsata* - AD), the Italian honey bee (*Apis mellifera* - AM), and the Indian honey bee (*Apis cerana* - AC) to assess the adaptability of AM worker bees. The results, including the number of days required by AM worker bees to construct queen cells using combs from different bee species, are presented in Fig. 1. Of the 30 queen cell cups made from AD combs, AM worker bees attempted to build only six cells (20%). The time required for building these cells ranged from 0 to 27 days, indicating a very low preference of AM worker bees for queen cell construction using AD combs.

In contrast, for queen cell cups made from AM combs, the worker bees attempted all 30 cells (100%) for queen cell construction. The time required to build the cells ranged from 7 to 15 days, showing that AM combs were highly preferred by AM worker bees for rapid queen cell formation, followed by AD and AC combs.

Similarly, in the case of queen cell cups made from AC combs, worker bees attempted only six cells (20%), with the time required ranging from 0 to 23 days. No attempts were made to build in the remaining 24 cups. This reflects the minimal preference of AM worker bees for constructing queen cells from AC combs.

In conclusion, AM worker bees exhibited a clear preference for queen cell construction using AM combs, completing the task in significantly less time compared to AD and AC combs. They required 1.20 times more time for AD combs and 0.98 times more time for AC combs, demonstrating that AM combs are the most suitable for queen cell building.

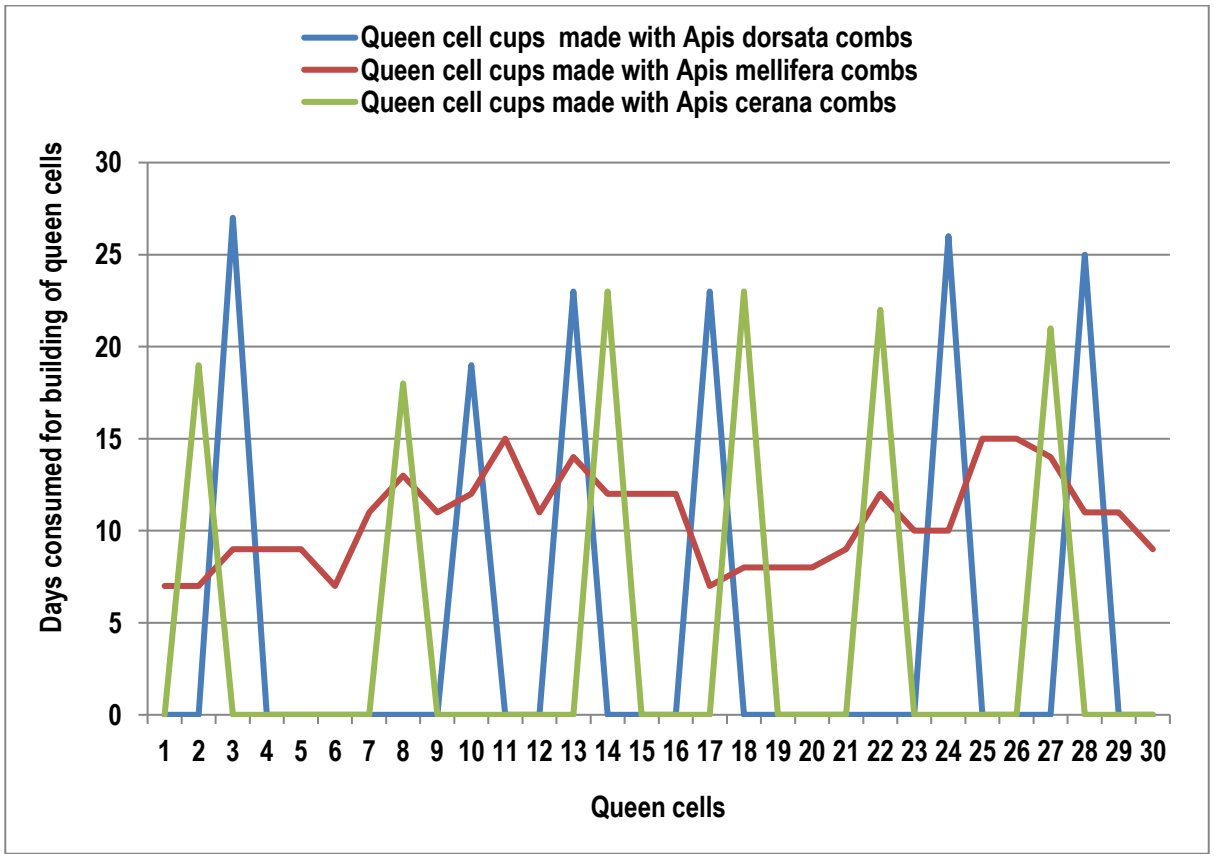

Fig. 1. Days consumed by the *A. mellifera* worker honey bees to build the queen cells made with combs of different species of bees

3.2 Egg Laying Preference of the *Apis mellifera* Queen Honey Bee

The egg-laying preferences of *Apis mellifera* queen bees in queen cells constructed from the combs of the rock honey bee (*Apis dorsata* - AD), the Italian honey bee (*A. mellifera* - AM), and the Indian honey bee (*A. cerana* - AC) were visually observed, and data were recorded over five days. The results regarding the egg-laying performance of *A. mellifera* queens in cells made from the combs of different bee species are presented in Table 1.

From the data in Table 1, it is clear that, similar to the behaviour of AM worker bees, the AM queens showed no preference for laying eggs in cells made from AD and AC combs. Throughout the five-day observation period after their release into nucleus hives, no attempts were made by the queens to lay eggs in any of the cells constructed from AD or AC combs.

In contrast, positive and encouraging results were observed when queen bees were provided with queen cells made from AM combs. The queen began laying eggs on the second day after being introduced into the nucleus hive, with an increasing trend in egg-laying activity over the five days. On the second day, the queen had laid eggs in only two cells (6.66% of the cells), but by the fifth day, 22 cells (73.33%) were occupied with eggs. This demonstrates a clear preference for AM comb cells for egg-laying by *Apis mellifera* queens.

3.3 Performance of the Larval Development of *Apis mellifera* in Mass Queen Rearing Frame

Visual observations on the growth and development of *Apis mellifera* larvae in mass queen-rearing frames were conducted over a period of three days after hatching, as shown in Table 2. Thirty cells were marked in each nucleus hive, and data on larval growth were recorded daily for three days post-hatching.

From Table 2, it is evident that the larval growth on the first day of observation in the first laying showed good condition in three larvae (60%) out of five, while two larvae were in poor condition. An increasing trend in larval growth was observed over the three days. By the third day, the overall health of two larvae was categorized as very good, one larva as good, and two larvae remained in poor condition.

In the second laying, on the first day of observation, four larvae (80%) exhibited poor growth, while only one larva showed good development. However, the overall performance of larval growth gradually improved, with four larvae (80%) displaying good to very good growth by the third day of observation.

In the third laying, only 10 out of the 30 marked cells were occupied by larvae. On the first day, four larvae showed good growth, while six were categorized as poor. A similar trend in larval growth was observed in the third laying as in the first and second layings. By the third day, nine larvae (90%) demonstrated good growth and appearance.

This pattern of gradual improvement in larval development over the observation period indicates consistent progress in larval health across the three layings, with the third day generally yielding the best outcomes.

The findings of Ruttner [8] align with those of the present study. He reported that the diameter of artificial queen cups plays a crucial role in their acceptance and the subsequent development of queens. He investigated the performance of various-sized mandrils used to prepare artificial queen cell cups, finding that a 9 mm diameter mandril was the most effective and widely accepted. Additionally, he noted that the type of wax whether virgin or oldhad no significant effect on the acceptance of queen cell construction. Natural queen cups on older combs are typically made from old wax. Plastic cups, introduced more recently, have also shown good results [11].

The present study's findings regarding the acceptance of queen cell cups prepared from waxes of different bee species are in agreement with Weiss's observations. The results of acceptance rates for egg-laying and larval development are also consistent with the findings of Vaziritabar and Esmaelizade [6], who reported that the first one or two batches of queen bee larvae grafts often have poor acceptance, while later batches show significantly higher acceptance rates. Occasionally, however, a colony may continue to exhibit low graft acceptance rates or even destroy cells it has begun to develop. In their study, the percentage of accepted queen cells in group polishing and control treatments was 75.9% and 64.2%, respectively.

Table 1. Egg laying preference of *Apis mellifera* queen honey bee in queen cells made with combs of different species of bees

Marked Cell No.	Queen cell cups made with the combs of														
	Apis dorsata					*Apis mellifera*					*Apis cerana*				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
1	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
2	×	×	×	×	×	×	×	×	✓	✓	×	×	×	×	×
3	×	×	×	×	×	×	×	✓	✓	✓	×	×	×	×	×
4	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
5	×	×	×	×	×	×	×	✓	✓	✓	×	×	×	×	×
6	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
7	×	×	×	×	×	×	×	×	✓	✓	×	×	×	×	×
8	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
9	×	×	×	×	×	×	✓	✓	✓	✓	×	×	×	×	×
10	×	×	×	×	×	×	×	×	×	✓	×	×	×	×	×
11	×	×	×	×	×	×	×	×	×	✓	×	×	×	×	×
12	×	×	×	×	×	×	×	×	×	✓	×	×	×	×	×
13	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
14	×	×	×	×	×	×	×	×	×	✓	×	×	×	×	×
15	×	×	×	×	×	×	×	✓	✓	✓	×	×	×	×	×
16	×	×	×	×	×	×	×	×	×	✓	×	×	×	×	×
17	×	×	×	×	×	×	×	×	×	✓	×	×	×	×	×
18	×	×	×	×	×	×	×	×	✓	✓	×	×	×	×	×
19	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
20	×	×	×	×	×	×	✓	✓	✓	✓	×	×	×	×	×
21	×	×	×	×	×	×	×	×	×	✓	×	×	×	×	×
22	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
23	×	×	×	×	×	×	×	×	✓	✓	×	×	×	×	×
24	×	×	×	×	×	×	×	×	×	✓	×	×	×	×	×
25	×	×	×	×	×	×	×	×	×	✓	×	×	×	×	×
26	×	×	×	×	×	×	×	✓	✓	✓	×	×	×	×	×
27	×	×	×	×	×	×	×	×	×	✓	×	×	×	×	×
28	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
29	×	×	×	×	×	×	×	✓	✓	✓	×	×	×	×	×
30	×	×	×	×	×	×	×	×	✓	✓	×	×	×	×	×

✓ Egg laying; × Do not lay the egg

Table 2. Visual observations on larval development of *Apis mellifera* in mass queen rearing frame till three days after hatching

Marked Cell No.	Performance of the larval development after hatching								
	1st laying			2nd laying			3rd laying		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
1	-	-	-	-	-	-	-	-	-
2	-	-	-	G	G	G	-	-	-
3	G	VG	VG	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
5	G	G	G	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-
7	-	-	-	P	P	G	-	-	-
8	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	G	G	VG
11	-	-	-	-	-	-	P	G	G
12	-	-	-	-	-	-	P	G	G
13	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	G	G	G
15	P	P	P	-	-	-	-	-	-
16	-	-	-	-	-	-	P	P	G
17	-	-	-	-	-	-	P	G	G
18	-	-	-	P	G	VG	-	-	-
19	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	P	P	P
22	-	-	-	-	-	-	-	-	-
23	-	-	-	P	P	P	-	-	-
24	-	-	-	-	-	-	G	G	G
25	-	-	-	-	-	-	G	VG	VG
26	P	G	VG	-	-	-	-	-	-
27	-	-	-	-	-	-	P	G	G
28	-	-	-	-	-	-	-	-	-
29	G	P	P	-	-	-	-	-	-
30	-	-	-	P	G	G	-	-	-

VG- Very Good; G-Good; P-Poor

The acceptance rates observed in this study are also similar to those reported by Ebadi and Gary [9] and Khan et al. [12], who found that queen cups made from old brood comb beeswax had an acceptance rate of 86.6%, fresh beeswax had an acceptance rate of 76.6%, and cups made from capping wax, beeswax foundation, or a mix of paraffin and old beeswax had a 70% acceptance rate. Similarly, Gancer et al. [13], Sharma et al. [14] and Lashari et al. [15] reported acceptance rates of 76.6% and 73.4%, which are in line with the current findings.

4. CONCLUSIONS

On the basis of present investigation, it may be concluded that the queen cells constructed on *Apis mellifera* combs, the queen bee began laying eggs on the 2nd after release into the nucleus hive and continued for five days, with an increasing trend in egg-laying activity. Among the different batches of eggs laid, the third batch was the most successful. Additionally, larval growth showed a consistent upward trend during the first three days of observation for each laying batch.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as OpenAI © 2015–2024 have been used during editing of this manuscript.

ACKNOWLEDGEMENTS

Authors are grateful and thankful to the members of the Student's Advisory Committee, Chairman, Department of Entomology, Principal, Bihar Agricultural College, Sabour and Dean, Post Graduate Studies, Bihar Agricultural University, Sabour (India) for providing necessary facilities and supports.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar M, Vishwakarma R. Sociodemographic attributes of beekeepers and trends of Beekeeping in Bihar. Indian Journal of Entomology. 2021; 83(4):542-545.
   DOI: 10.5958/0974-8172.2021.00077.8
2. Pavel CI, Marghitas LA, Bobis O, Dezmeirean DS, Sapcaliu A, Radoi I, Madas MN. Biological activities of royal jelly - Review. Scientifical Papers, Anim. Sci. Biotechnol. 2011;44:108-118.
3. Cao LF, Zheng HQ, Pirk CW, Hu FL, Xu ZW. High royal jelly-producing honeybees (*Apis mellifera ligustica*) (Hymenoptera: Apidae) in China. J Econ Entomol. 2016; 109:510-514.
4. Zhang B, Liao C, Zeng Z. Research progress of the method for taking royal jelly. Journal of Bee. 2017;370:4-6 (in Chinese).
5. Fu-Liang Hu, Katarína Bíliková, Hervé Casabianca, Gaëlle Daniele, Foued Salmen Espindola, Mao Feng, Cui Guan, Bin Han, Tatiana Krištof Kraková, Jian-Ke Li, Li Li, Xing-An Li, Jozef Šimúth, Li-Ming Wu, Yu-Qi Wu, Xiao-Feng Xue, Yun-Bo Xue, Kikuji Yamaguchi, Zhi-Jiang Zeng, Huo-Qing Zheng, Jin-Hui Zhou. Standard methods for *Apis mellifera* royal jelly research. Journal of Apicultural Research. 2019; 58(2):1-68.
   DOI: 10.1080/00218839.2017.1286003
6. Vaziritabar S, Esmaelizade 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. Journal of Entomology and Zoology Studies. 2018;6(4):683-692.
7. Wu XB, Zhang F, Guan C, Pan QZ, Zhou LB, Yan WY, Zeng ZJ. A new method of royal jelly harvesting without grafting larvae. Entomological News, 2015;124: 277–281.
   DOI: 10.3157/021.124.0405
8. Ruttner. Breeding Techniques and selection for breeding of the honeybee. The British Isles Bee Breeders Association. 1988;151.
9. Ebadi R, Gary NE. Acceptance by honeybee colonies of larvae in artificial queen cells. Journal of Apicultural Research. 1980;19:127-132.
10. 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. Journal of Applied and Natural Science. 2017:9(3): 1658-1661.
11. Weiss K. The Influence of rearing conditions on queen development. In: Queen Rearing: Biological Basis and Technical Instruction, Ruttner, F. (Ed.). Apimondia Publishing House, Bucharest. 1983;83-148.
12. Khan KA, Ghramh HA, Ahmad Z, El-Niweiri MAA, Ahamed Mohammed ME. Queen cells acceptance rate and royal jelly production in worker honey bees of two Apis mellifera races. PLoS ONE. 2021;16(4):e0248593.pp1-13.
13. 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. 2003:1319-1322.
14. 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. Journal of Entomology and Zoology Studies. 2020:8(4):1089-1097.
    Available:https://doi.org/10.22271/j.ento.2020.v8.i4q.7268

15. Lashari MA, Ghramh HA, Ahmed AM, Mahmood R, Rafique MK, Ahmad S, AL-Shehri BM, MEA Mohammed, Khan KA. Aptness of diverse queen cup materials for larval graft acceptance and queen bee emergence in managed honey bee (*Apis mellifera*) colonies. Journal of King Saud University – Science. 2022; 34(4):102043.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/4114