Journal of
Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2025-s1-003

Research Article

Open Access

Assessment of Priming Media and Queen Cup Materials in Grafting Techniques for Enhancing Queen Rearing Success

Mohammed Ahmed Alshogari[1,2], AM Raut[1*], Amine Assouguem[3,4] and SK Gharde[1]

[1]*Department of Entomology, School of Agriculture, Lovely Professional University, Punjab, India*

[2]*Department of Plant Protection, Faculty of Agriculture, Food and Environment, Sana'a University, Sana'a, Yemen*

[3]*Ethnopharmacology and Pharmacognosy Team, Faculty of Science and Technology, Moulay Ismail University, Meknes, Morocco*

[4]*Faculty of Science and Technology, Sidi Mohamed Ben Abdellah University, Morocco*

***Correspondence to:**
AM Raut
Department of Entomology,
School of Agriculture,
Lovely Professional University,
Punjab, India.
E-mail: ankushento@gmail.com

Received: January 21, 2025
Accepted: April 29, 2025
Published: May 05, 2025

Citation: Alshogari MA, Raut AM, Assouguem A, Gharde SK. 2025. Assessment of Priming Media and Queen Cup Materials in Grafting Techniques for Enhancing Queen Rearing Success. *J Food Chem Nanotechnol* 11(S1): S15-S20.

Copyright: © 2025 Alshogari et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC-BY) (https://creativecommons.org/licenses/by/4.0/) which permits commercial use, including reproduction, adaptation, and distribution of the article provided the original author and source are credited.

Published by United Scientific Group

Abstract

Artificial queen rearing is a revolutionary step for the beekeeping industry where beekeepers quickly multiply the bee population. A successful queen rearing program depends on various factors, including the age of larvae, the priming media, queen cup materials and bee flora. Therefore, this study aimed to evaluate the priming media and queen cup materials (bee wax, paraffin wax, and plastic) on successful queen rearing. The priming media like apple juice, commercial royal jelly enriched with honey, sugar syrup, honey solution, and a mixed solution were treated with 5 µl in each cup materials. The Doolittle grafting method was used to transfer young larvae (less than 24 h old) into queen cups. The result shows that the individual cup materials like bee wax and plastic cup treated with different priming media show significant impact on acceptance of grafted larvae and emergence of virgin queen. The plastic cup was treated with a mixture priming media most increased (90%) acceptance and the virgin queen emergence rate, followed by the bee wax queen cups treated with apple juice (50%). However, there was no impact of priming media treated with paraffin cups on larvae acceptance or queen emergence. Hence, it is concluded that the bee wax and plastic cups used for the artificial queen rearing where individual or mixed priming media performed better in emergence of queen.

Keywords

Apis mellifera, Grafting method, Queen rearing, Priming media, Cup materials

Introduction

Honeybee is an integral part of sustainable agriculture and directly impacting one-third of the world food crops [1]. Since the dawn of human civilization, beekeeping has not only contributed to the sustainability of the ecosystem but also provided a source of livelihood [2]. South and Southeast Asia regions are the habitat of diverse groups of bee species, each exhibiting distinct biological and ecological traits that influence their respective honey production capacities [3]. Honey production is greatly influenced by bee flora, environmental conditions and colony strength which highly rely on the queen age, health and its fertility [4, 5].

Queen rearing is a fundamental apicultural practice which is essential for enhancing genetic diversity, disease resistance, and overall productivity of honeybee colonies [6]. The selection and rearing of high-quality queen bees are paramount for maintaining the vigor, disease resistance, and overall productivity of honeybee colonies. By optimizing the genetic quality and health of bees through meticulous

queen rearing practices, beekeepers can ensure the sustainability and success of their apiaries [7, 8]. Therefore, a successful beekeeping program heavily relies on advanced queen rearing techniques, which directly influence egg production and colony expansion. Additionally, the timely replacement of aging queens and the introduction of new queens prior to the honey flow period are crucial for maximizing productivity and preventing colony loss [9]. Modern techniques like Doolittle grafting larvae have enhanced colony expansion and facilitated the propagation of desirable genetic traits within apiaries. Introducing a new mated queen minimizes the gap in oviposition and bee production, thus enhancing colony productivity [10-12]. While natural supersedure takes 25 - 30 days for a new queen to become mated and start laying eggs, introducing a young, fertilized queen reduces this gap to less than five days and lowers the risk of swarming compared to using older queens [13].

The improvement of queen quality is significantly influenced by environmental conditions and management practices [14-16]. In addition to this, selection of young larvae less than one day old significantly resulted in quality queen bee [17]. The priming of cells before grafting larvae serve to ease the grafting process, reduce dehydration and damage to the larvae, increase acceptance rates, and enhance the overall quality of the queen bee [18]. Therefore, it is advisable to rear a queen bee during a period of moderately warm weather, abundant drones, rich nectar and pollen, and ideal mating rates to ensure optimal productivity [19, 20].The success of queen rearing hinges on the acceptance of grafted larvae and the emergence of the queen, which are influenced by the priming media and queen cell cup materials used [21-23]. The priming media in queen cups is important for queen larval development, with different grafting techniques, such as wet and dry grafting affecting queen emergence and acceptance rates [9, 24, 25].

Globally, beekeepers have tested various priming media like distilled water, royal jelly, sugar solution, honey solution, coconut water, apple nectar, and cola for queen rearing, but research on their impact on larvae acceptance remains limited [2, 11, 19, 21, 26]. Previous research resulted that the queen cups made with old brood comb beeswax, fresh beeswax, and wax blends significantly impact larval acceptance, queen emergence rates, and queen morphometric traits [2, 17, 19, 22]. The selection of eggs from mother colonies has a significant impact on the physiological traits of the resulting queens. Specifically, queens that develop from eggs sourced from one-day-old larvae tend to have higher weight, more ovarioles, and longer queen cells compared to those that develop from two or three-day-old larvae in the queen rearing [8]. However, the choice of queen cell cup materials not only affects larval acceptance, and queen emergence but also the overall development and quality of the queen bee. Studies have shown that materials like royal jelly and bee wax can increase larvae acceptance rates and enhance the morphological traits and health of queen bees compared to artificial substitutes [2, 19, 21, 27]. Hence, considering the context, the current study aimed to address this gap, numerous studies on individual aspects of queen rearing, limited research has been conducted on the combined effects of priming media and queen cup materials on larval acceptance and queen emergence, which this study aims to address.

Materials and Methods

Study area

The research was carried out at Apiculture Area, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India during the Spring season. The geographical coordinates of the experimental site are N 31° 13' 4" – E 75° 46' 10". The mother colony and cell building colonies were located on the same site. Approximately two kilometers from the apiary location, numerous crops grow to provide nectar and pollen sources. The orange, guava, and ber orchard are around 500 - 600 m away from the apiary.

Preparation and selection of breeder (mother) colonies

Breeder colonies with strong worker populations, high brood raising capacity, excellent honey production, and gentleness were maintained through regular supplementation of sugar syrup, pollen, and sealed worker B in different colonies within the apiary [28]. Young larvae, aged between 24 and 48 h, were selected from these colonies for grafting into queen cell builder colonies [27].

Preparation of queen cell builder colonies

To facilitate the creation of queen cell builder colonies, three Langstroth beehives were used, and each brood chamber measured 41 × 51 × 26 cm. The process began with the removal of the queens from their respective colonies, followed by equalizing the colonies to ensure that each contained four frames of emerging capped (B), two frames with honey and pollen (HP), three grafting frames (GF), and one feeder (F). Each colony's adult bee population covered six frames on both sides, representing a strong colony as described [29]. This frame arrangement was based on previous works [30, 31], with a slight modification to optimize frame position. The frame sequence for all queen cell builder colonies was organized as follows: F, HP, B, GF, B, GF, B, GF, B, HP throughout the experiment. Additionally, each colony provided supplementary feeding, which included 0.5 L of a 50% sugar solution (1 part sugar 1 part water) and 50 g of a protein supplement. The protein supplement, composed of 70% low-fat soybean flour, 20% pollen, and 10% honey, was formulated to provide essential amino acids and nutrients required for larval development and increased nurse bee activity, thereby enhancing queen cell acceptance and emergence.

Preparation of priming media (substrates)

To evaluate the efficacy of five different priming media, excluding the control (dry grafting without priming media), in promoting queen acceptance and emergence for rearing of queen. The apple juice (B Natural®) was purchased from a local market. The royal jelly enriched with honey (Bro™ Bee Raw Organic) in its undiluted form was used in this study. The priming media consisted of apple juice (S_1), commercial royal jelly enriched with honey (S_2), sugar syrup (1:1 with distilled water) (S_3), honey solution (1:1 with distilled water) (S_4), mixtures solution (apple juice, commercial royal jelly enriched with honey, sugar syrup, honey solution) (1:1:1:1) (S_5), and control (dry grafting without priming media) (S_6). Worker larvae less than 24 h old were grafted in ten queen cups per priming media.

Queen cell cup materials

The three queen cup materials like plastic, bee wax, and paraffin wax were evaluated against the grafted larvae for the development of queen bee. All the cup sizes were kept 9 mm diameter and 10 mm heights. The silicon mold was used for preparation of bee wax and paraffin wax cups (Supplement file) while plastic cups were purchased from the market. Young worker larvae (< 24 h old) were grafted into three frames with 60 queen cell cups for each material and introduced to the cell builder colonies.

Grafting method

The larvae aged less than 24 h old were collected from the mother colonies. The larvae along with royal jelly picked with the help of plastic grafting tools. In each cell builder colony, three grafting frames were kept for each queen cup along with different priming media. The grafting frame had two removable wooden cell bars assembled by cell bar holder, cell cup holder, and cell cup. Each wooden cell bar had ten queen cups fixed. Each cell builder colony had 60 queen cups treated with six priming media including the control that lack of any priming media (Figure 1). Before a day of grafting process, all grafting frames excluding priming media and larvae were kept into the cell builder colony for polishing [16]. After polishing grafting frames, approximately 5 µl of a specific priming media was applied to the base of each queen cup by using the micropipette.

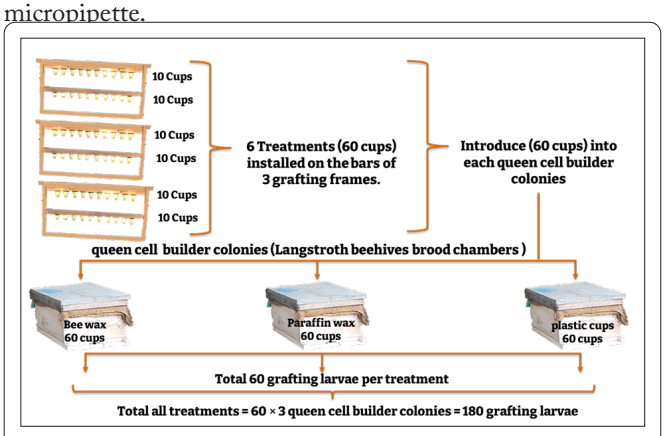


Figure 1: Systematic representation of artificial queen rearing by G methods.

Data Collection

The acceptance or rejection of larvae was observed 72 h after grafting, whereas the number of emerging queens was recorded at eleven to twelve days after grafting. The acceptance and queen emergence percentage from the grafted larvae were calculated by formulas [2].

$$Percentage\ of\ acceptance = \frac{Number\ of\ larvae\ accepted}{Total\ number\ of\ larvae\ grafted} \times 100$$

$$Percentage\ of\ emergence = \frac{Number\ of\ queens\ emerged}{Total\ number\ of\ larvae\ grafted} \times 100$$

Statistical analysis

The data were analyzed using a two-way analysis of variance to determine the effects of cup materials and different priming media. The significance level for all tests was set at 0.05, and Duncan's new multiple range tests were used to rank the groups. The effects of the priming media on the acceptance and emergence rate were performed by ANOVA using SPSS software (version 22).

Results

Acceptance rate of queen larvae *A. mellifera* L.

The acceptance rate of grafted larvae in plastic and bee wax cups with mixture priming medium (apple juice, commercial royal jelly enriched with honey, sugar syrup and honey solution in a 1:1:1:1 ratio) was highly significant. However, as a priming media, apple juice, sugar syrup, commercial royal jelly enriched with honey, and mixture solution were reported to have a 50% larvae acceptance rate in bee wax cups, while apple juice and mixture solution functioned equally well in plastic cups (Figure 2).

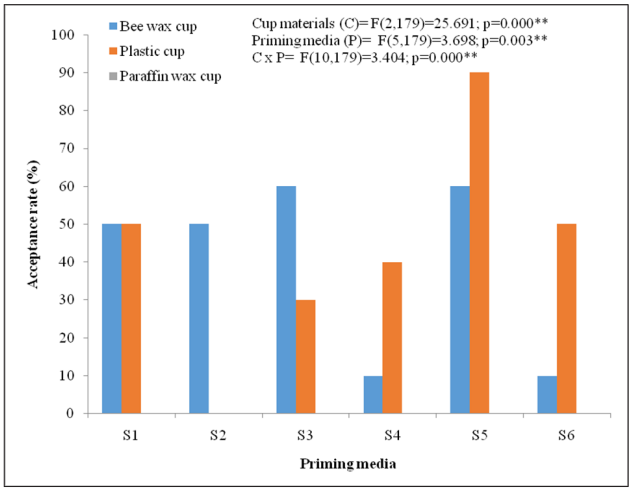


Figure 2: Effect of queen cup materials and priming media on acceptance rate of queen larvae *A. mellifera* L. (S_1 = apple juice @ 5 µl, S_2 = commercial royal jelly enriched with honey @ 5 µl, S_3 = sugar syrup @ 5 µl, S_4 = honey solution @ 5 µl, S_5 = mixtures solution (apple juice, commercial royal jelly enriched with honey, sugar syrup, honey solution) (1:1:1:1) @ 5 µl, and S_6 = control).

Emergence rate of queen *A. mellifera* L.

The statistically highly significant ($F_{(5,9)}$ = 3.628; p = 0.000) queen emergence rate (43.33%) was obtained in mixture media as primer while there was no significant difference in bee wax and plastic queen cup materials (Figure 3). All the treatments like priming media except honey solution @ 5 µl in bee wax queen cups and commercial royal jelly enriches with honey @ 5 µl in plastic queen cups were found significantly (p < 0.01) emerged the queen after grafting. None of the queen emerged by using various priming media ejected in paraffin wax queen cups.

Discussion

Pollinators, including bees, are under threat due to excessive pesticide use, habitat loss, and rising temperatures. These factors disrupt their vital functions and exacerbate global food security concerns [32-35]. However, addressing these challenges requires transitioning to ecological farming systems and raising awareness about sustainable pollination and beekeeping practices including bee health [36]. A major pollinators like

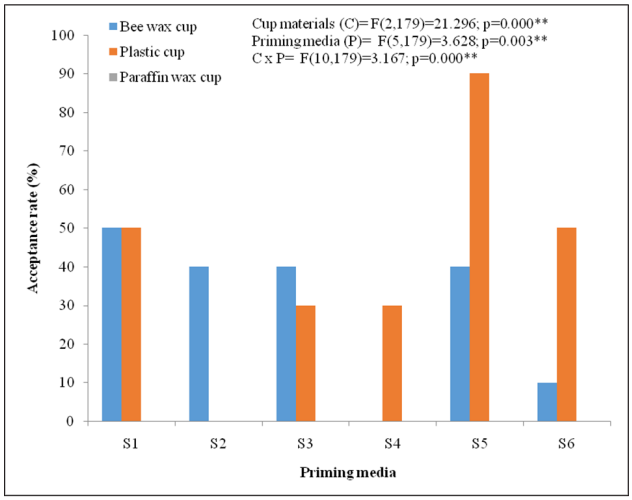


Figure 3: Effect of queen cup materials and priming media on queen emergence after successful G (S_1 = apple juice @ 5 µl, S_2 = commercial royal jelly enriched with honey @ 5 µl, S_3 = sugar syrup @ 5 µl, S_4 = honey solution @ 5 µl, S_5 = mixtures solution (apple juice, commercial royal jelly enriched with honey, sugar syrup, honey solution) (1:1:1:1) @ 5 µl, and S_6 = control).

honeybees play a critical role in the pollination of crops and thereby conserving and restoring local biodiversity [37-40].

The colony's performance totally depends on the quality of queen and population of workers. However, the queen bee is a mastermind of the colony and plays an indispensable role in the colony's survival and organized the various duties as per the colony requirement [6].Therefore, high quality queen in colonies performed significantly in increased brood production, reduced swarming behavior, high yield of bee by-products and less prone to pest and disease [5, 7].The inferior quality of queen leads to loss of the colony [22] and that case replacement or redevelopment of queen is the only way to alive the colony for the beekeeper. Similarly, bee breeders produce high quality queen from pest-disease free colony to maintain the genetic diversity [41-44]. Many factors like strength of the cell builder colony, number of nurse bees, abiotic factors especially temperature and humidity, natural flora, artificial diets, queen cup materials and size, age of larvae, priming media and its pH, surface texture, and arrangement of the grafted frame were responsible for the quality queen output. Moreover, our findings revealed that the highest larval acceptance and emergence rate was significantly achieved in apple juice and mixtures solution used as the priming media in bee wax cup and plastic cup materials. Therefore, apple juice as a sole priming media will enhance the larval acceptance and finally emergence of queen because of the acidic nature of the priming media [11]. Similarly, the sole priming media like apple nectar, royal jelly, honey solution, and sugar syrup performed significantly better than the mixed or diluted priming media [2, 11, 19, 21, 23, 45]. In addition to this, queen cup materials are also very important factors for artificial queen rearing. However, bee wax queen cups had a significantly higher rate of acceptance and emergence of queens observed in our research. Worker bees accepted bee wax cups due to their similar properties to natural bee wax, which is made from honeycomb. A similar result observed that the maximum emergence of queen obtained from bee wax cups [2, 19, 23, 45]. The present research finding is not line with [17] who obtained maximum emergence rate of queen in plastic cups by using the Doolittle method.

The modern techniques of queen rearing like Doolittle methods, cup kit, Karl Jenter are the powerful tools for developing quality queen with adaptive traits [18, 31]. As a success of bee breeding program based on materials and tools used for queen rearing, colony trails and performance of matted queen which improve the economic value of beekeepers for longer time [16]. Bee wax and plastic cups size (9 mm diameter and 10 mm height) were used and found significant impact on acceptance and queen emergence in present research. This result lined with [46] where the success of grafting was not affected by different grafting queen bee cup sizes and maximum virgin queen emergence in queen less colonies. This is because of pheromone triggers workers to develop for emergency queens to ensure colony survival. The position cell bar in grated frame is also very important factor for successful queen breeding and in our research two grafted cell bars were observed in acceptance rate. The acceptance rate varied between placement of grafted bar where slightly higher efficiency was found in middle bar as compared to upper and lower bar in grafted frame [47]. Generally, artificial rearing of queen bees is affected by many factors like cup materials, priming media, agro-ecological zone, mother colony and age of larvae for grafting exploring the success for queen rearing. In this regard, further studies are needed to determine the effect of various priming media, diets, and seasons on the morphometric parameters of the emerging queen and its reproduction capability along with honey production.

Conclusion

The result concluded that the sole priming media along with the addition of natural royal jelly in the queen cups during the grafting will encourage the acceptance and emergence of quality queens. However, the potential application of the research outcome will explore the colony multiplication, minimizing the swarming behavior, improve the genetic characteristics of mother colony, and reviewing the lost colony. Future research needs to focus on morphometric and genetic parameters of quality queen production by using different priming media and suitable queen cup materials in various agro-climatic zones.

Acknowledgements

The entire authors are thankful to Republic of Yemen Government for financial support for the study. Also, we would like to thank Lovely Professional University for providing research and laboratory facilities.

Conflict of Interest

None.

References

1. Khalifa SA, Elshafiey EH, Shetaia AA, El-Wahed AAA, Algethami AF, et al. 2021. Overview of bee pollination and its economic value for crop production. *Insects* 12(8): 688. https://doi.org/10.3390/insects12080688

2. Sharma A, Rana K, Sharma HK, Sharma A. 2020. Evaluation of priming media and queen cup material on larval graft acceptance and queen emergence in *Apis mellifera* L. *J Entomol Zool Studies* 8(4): 1089-1097. https://doi.org/10.22271/j.ento.2020.v8.i4q.7268
3. Anandaraj K. 2017. Studies on the role of selective breeding and queen rearing techniques in the production of tolerant strain in *Apis cerana indica*. Annamalai University.
4. Mahbobi A, Farshineh-Adl M, Woyke J, Abbasi S. 2012. 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). *J Apic Sci* 56(1): 93-98. https://doi.org/10.2478/v10289-012-0010-1
5. Ozbakir GO. 2021. Effects of rearing method on some morphological and reproductive organ characteristics of queen honeybees (*Apis mellifera* L.). *Med Weter* 77(2): 89-94. http://dx.doi.org/10.21521/mw.6496
6. Mattiello S, Rizzi R, Cattaneo M, Martino PA, Mortarino M. 2022. Effect of queen cell size on morphometric characteristics of queen honeybees (*Apis mellifera ligustica*). *Italian J Animl Sci* 21(1): 532-538. https://doi.org/10.1080/1828051X.2022.2043790
7. Hatjina F, Bieńkowska M, Charistos L, Chlebo R, Costa C, et al. 2014. A review of methods used in some European countries for assessing the quality of honeybee queens through their physical characters and the performance of their colonies. *J Apic Res* 53(3): 337-363. https://doi.org/10.3896/IBRA.1.53.3.02
8. Yu L, Shi X, He X, Zeng Z, Yan W, et al. 2022. High-quality queens produce high-quality offspring queens. *Insects* 13(5): 486. https://doi.org/10.3390/insects13050486
9. Büchler R, Andonov S, Bienefeld K, Costa C, Hatjina F, et al. 2013. Standard methods for rearing and selection of *Apis mellifera* queens. *J Apic Res* 52(1): 1-30. https://doi.org/10.3896/IBRA.1.52.1.07
10. Guzman-Novoa E. 2007. Elemental Genetics and Breeding for the Honeybee. University of Guelph.
11. Contreras-Martinez CA, Contreras-Escareño F, Macias-Macias JO, Tapia-Gonzalez JM, Petukhova T, et al. 2017. Effect of different substrates on the acceptance of grafted larvae in commercial honeybee (*Apis Mellifera*) queen rearing. *J Apic Res* 61(2): 245-251. http://dx.doi.org/10.1515/jas-2017-0019
12. Smilga-Spalvina A, Spalvins K, Veidenbergs I. 2024. Field study: factors influencing virgin queen bee acceptance rate in *Apis mellifera* colonies. *Czech J Anim Sci* 69(4): 155-164. https://doi.org/10.17221/22/2024-CJAS
13. Winston ML. 1991. The biology of the honeybee. Harvard University Press, England.
14. Nuru A. 2012. Suitable Honeybee Colony Multiplication Technique using Intermediate Top-Bar Hives. In Proceedings of the 3rd International Conference on Apitrade Africa.
15. Crailsheim K, Brodschneider R, Aupinel P, Behrens D, Genersch E, et al. 2013. Standard methods for artificial rearing of *Apis mellifera* larvae. *J Apic Res* 52(1): 1-16. https://doi.org/10.3896/IBRA.1.52.1.05
16. Büchler R, Andonov S, Bernstein R, Bienefeld K, Costa C, et al. 2024. Standard methods for rearing and selection of *Apis mellifera* queens 2.0. *J Apic Res* 64(2): 1-57. https://doi.org/10.1080/00218839.2023.2295180
17. Dhaliwal NK, Singh J, Chhuneja PK. 2017. Comparative evaluation of Doolittle, Cupkit and Karl Jenter techniques for rearing *Apis mellifera* Linnaeus queen bees during breeding season. *J Appl Nat Sci* 9(3): 1658-1661. https://doi.org/10.31018/jans.v9i3.1417
18. Sharma A. 2019. Studies on Queen Rearing in *Apis mellifera* L. under Mid–Hill Conditions of Himachal Pradesh. (Doctoral dissertation)
19. Rafique MK, Mahmood R, Qadir ZA, Bodlah I, Shaheen FA. 2019. Effects of rearing interlude and grafting technique on honeybee *Apis mellifera* L. queen under field conditions. *Pak J Zool 51*(6): 2369-2372. https://doi.org/10.17582/journal.pjz%2F2019.51.6.sc1
20. Guda AZG, Elshemy AAE, El-Enany YE. 2024. Effect of rearing season on queen and the reproduction of *Apis mellifera carnica* and *A. mellifera ligustica*. *Indian J Entomol* 86(1): 1-5. https://doi.org/10.55446/IJE.2023.885
21. Ma C, Ahmat B, Li J. 2022. Effect of queen cell numbers on royal jelly production and quality. *Curr Res Food Sci* 5: 1818-1825. https://doi.org/10.1016/j.crfs.2022.10.014
22. Lashari MA, Ghramh HA, Ahmed AM, Mahmood R, Rafique MK, et al. 2022. Aptness of diverse queen cup materials for larval graft acceptance and queen bee emergence in managed honey honeybee *(Apis mellifera)* colonies. *J King Saud Univ Sci* 34(4): 102043. https://doi.org/10.1016/j.jksus.2022.102043
23. Kamboj R, Sharma SK, Rathee M, Rao N. 2023. Comparative quality traits of *Apis mellifera* L. queens raised through standard queen rearing methods in the spring breeding season. *Int J Environ Clim Change* 13(11): 1668-1678. https://doi.org/10.9734/ijecc/2023/v13i113322
24. Kamel SM, Osman MAM, Mahmoud MF, Mohamed KM, Abd Allah SM. 2013. Morphometric study of newly emerged unmated queens of honey bee *Apis mellifera* L. in Ismailia Governorate, Egypt. *Arthropods* 2(2): 80-88.
25. Adgaba N, Al-Ghamdi A, Tadesse Y, Alsarhan R, Single A, et al. 2019. The responses of *Apis mellifera jemenitica* to different artificial queen rearing techniques. *Saudi J Biol Sci* 26(7): 1649-1654. https://doi.org/10.1016/j.sjbs.2018.08.028
26. Gene F, Emsen B, Dodologlu A. 2005. Effects of rearing period and grafting method on the queen bee rearing. *J Appl Anim Res* 27(1): 45-48. https://doi.org/10.1080/09712119.2005.9706535
27. Ustadi U, Ikhsan N, Junus M, Suyadi S. 2022. Improving the larvae acceptance rate and morphometric characteristics of queen *Apis cerana-javana* by grafting larvae at different ages. *Biodiversitas J Biol Diversity* 23(1): 1-5. https://doi.org/10.13057/biodiv/d230121
28. Gatoria GS, Singh B, Singh L. 2000. Effect of diameter of queen cell cups on mass rearing of *Apis mellifera* L. queen bees. *Indian Bee J* 62(1): 59-61.
29. Delaplane KS, Van Der Steen J, Guzman-Novoa E. 2013. Standard methods for estimating strength parameters of *Apis mellifera* colonies. *J Apic Res* 52(1): 1-12. http://dx.doi.org/10.3896/IBRA.1.52.1.03
30. McKinley WM. 1963. Grafting-how it is done. *Glean Bee Cult* 91: 404-409.
31. Gatoria RS, Chhuneja PK, Singh J, Singh B. 2004. Mass queen bee rearing in *Apis mellifera* L. *Indian Bee J* 66(1): 41-50.
32. Kremen C, Williams NM, Thorp RW. 2002. Crop pollination from native bees at risk from agricultural intensification. *Proc Nat Acad Sci 99*(26): 16812-16816. https://doi.org/10.1073/pnas.262413599
33. Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, et al. 2007. Importance of pollinators in changing landscapes for world crops. *Proc Biol Sci* 274(1608): 303-313. https://doi.org/10.1098/rspb.2006.3721
34. Garibaldi LA, Carvalheiro LG, Leonhardt SD, Aizen MA, Blaauw BR, et al. 2014. From research to action: enhancing crop yield through wild pollinators. *Front Ecol Environ* 12(8): 439-447. https://doi.org/10.1890/130330
35. Zhao H, Li G, Cui X, Wang H, Liu Z, et al. 2022. Review on effects of some insecticides on honey bee health. *Pestic Biochem Physiol* 188: 105219. https://doi.org/10.1016/j.pestbp.2022.105219
36. Pervez M, Manzoor F. 2023. Honey bee losses and pesticides threat: an Asian perspective. *J Apic Res* 62(1):64-75. https://doi.org/10.1080/00218839.2022.2103331
37. Spivak M, Mader E, Vaughan M, Euliss NH. 2011. The plight of the bees. *Environ Sci Technol* 45: 34–38. https://doi.org/10.1021/es101468w
38. Bond JK, Hitaj C, Smith D, Hunt K, Perez A, et al. 2021. Honey bees on the move: from pollination to honey production and back. Economic Research Report.

39. Cappellari A, Bonaldi G, Mei M, Paniccia D, Cerretti P, et al. 2022. Functional traits of plants and pollinators explain resource overlap between honeybees and wild pollinators. *Oecologia1* 98(4): 1019-1029. https://doi.org/10.1007/s00442-022-05151-6

40. Papa G, Maier R, Durazzo A, Lucarini M, Karabagias IK, et al. 2022. The honey bee *Apis mellifera*: an insect at the interface between human and ecosystem health. *Biology* 11(2): 233. https://doi.org/10.3390/biology11020233

41. Meixner MD. 2010. A historical review of managed honeybee populations in Europe and the United States and the factors that may affect them. *J InvertebrPathol* 103: 80-95. https://doi.org/10.1016/j.jip.2009.06.011

42. Smith KM, Loh EH, Rostal MK, Zambrana-Torrelio CM, Mendiola L, et al. 2013. Pathogens, pests, and economics: drivers of honey bee colony declines and losses. *Eco Health* 10: 434-445. https://doi.org/10.1007/s10393-013-0870-2

43. Walsh E, Rangel J. 2016. Local honey bee queen production and quality. *Bee World* 93(2): 30-32. https://doi.org/10.1080/0005772X.2016.1211501

44. Walsh EM, Sweet S, Knap A, Ing N, Rangel J. 2020. Queen honey bee (*Apis mellifera*) pheromone and reproductive behavior are affected by pesticide exposure during development. *Behav Ecol Sociobiol* 74: 1-14. https://doi.org/10.1007/s00265-020-2810-9

45. Ebadi R, Gary NE. 1980. Acceptance by honeybee colonies of larvae in artificial queen cells. *J Apic Res* 19(2): 127-132. https://doi.org/10.1080/00218839.1980.11100011

46. Wahid F, Singh BKP, Singh SN. 2022. Different artificial methods for rearing queen of *Apis mellifera. J Entomol Zool Studies* 11(4): 38-42. https://doi.org/10.22271/j.ento.2023.v11.i4a.9214

47. Kruk C, Skowronek W. 2002. Effect of different factors on the efficiency of honey bee queen rearing. *J Apic Sci* 46(1): 41-50.