EFFECT OF QUEEN CELL CUP TYPES ON GRAFT ACCEPTANCE AND QUEEN BEE EMERGENCE IN *Apis mellifera* L.

Anisha Poudel[1], Min Raj Pokhrel[2], Govinda Prasad Pokhrel[3] and Bhawana Aryal[1]

[1]Faculty of Agriculture, Agriculture and Forestry University, Chitwan, Nepal
[2]Department of Entomology, Agriculture and Forestry University, Rampur, Chitwan, Nepal
[3]Agriculture Development Office, Dhading, Nepal
Correspondence: pokhrel.afu@gmail.com

ABSTRACT

Quality queen production is crucial for the multiplication of colonies, hive, and apiary management. A study was conducted at the Beekeeping Development Center, Bhandara, Chitwan, Nepal from April to June 2023 to assess the effectiveness queen cell cup types on grafted larvae acceptance by the workers for rearing and quality of the queen emerged. The experiment comprised four queen cell cup types as the treatments—made from bee wax (T_1), paraffin (T_2), blend of bee wax and paraffin (T_3), and plastic cups (T_4)—replicated across five queenless colonies considering randomized complete block design. Two-day-old larvae were grafted onto queen cell cups fitted on grafting frames and larval acceptance was checked after 72 hours. The quality of queen emerged across the type of cup cells was assessed considering queen quality attributes. Results indicated that mean larval acceptance was significantly higher in bee wax cells throughout the three months while acceptance was lowest in paraffin cell. The acceptance across wax-paraffin mix and plastic cup cell was moderate. The number of queen bees emerged from bee wax cups was significantly higher and displayed greater body weight and length as compared to other cell cup types. This study reveals bee wax cell cups are more suitable for worker larvae grafting for queen production in *Apis mellifera.*

Key words : Honey bees, queen production, grafting queen cell cups,

INTRODUCTION

Queen bee rearing is vital for apiaries to sustain productivity, with modern techniques serving as powerful tools for enhancing the economic, behavioral, and adaptive traits of honey bees (Dhaliwal, et al., 2017). The queen bee's attractiveness to workers decreases with age (De Hazan et al., 1989). Beekeepers can selectively raise queens as needed by employing different queen-rearing techniques, often involving the transfer of young worker larvae, through grafting, into artificial queen cell cups, which are then introduced to a queenless colony for acceptance and initial care (Vung et al., 2018). The acceptance and provisioning of artificial queen cells by workers are often influenced by queen cell cup material, its composition as the cups are constructed from various types of wax or plastic (Ebadi & Gary, 1980). Additionally, the characteristics of queens are influenced by various factors such as genetics, method of upbringing, the environment, the season in which they are raised, nutrition, the age of larvae, and the number of larvae selected for grafting (Ozbakir, 2021).

The type of materials used in making queen cells and precise timing of larvae grafting are crucial for beekeepers aiming to produce quality queens. As a result, it is critical to determine the acceptance of grafted larvae across types of queen cell cup materials (Sharma et al., 2020). More than 50,000 households in Nepal are involved in beekeeping, holding 125,00 beehives and producing about 1100 tons of honey per year (Devkota, 2020). However, there exists a significant limitation in the commercial production of queens as most beekeepers tend to prioritize honey production over the breeding of superior-quality queens. So, this research aims to select suitable types of cell cup materials suitable for larval growth, graft acceptance, and queen emergence. Also, the study focuses on selecting the best time to rear quality queens, and to recommend the best material for effective queen rearing.

MATERIALS AND METHODS

The research was carried out for three months from April to June 2023 at the Beekeeping Development Center, Chitwan, Nepal.

Experimental design

The experiment was designed using an RCBD that comprised four treatments and five replicates. Four types of queen cell cups made of bee wax (T_1), paraffin (T_2), 50% bee wax + 50% paraffin (T_3) and plastic (T_4) were tested for the grafting of honey bee (*Apis mellifera*) two-day-old larvae.

Cell builder and breeder colonies

Mite and disease-free hives consisting of six movable frames with a large number of nurse bees, sealed and unsealed worker broods were selected as builder colonies. The builder colonies were dequeened for 24 hours, and a grafting frame installed with a total of 20 queen cell cups was placed on each hive. Two-day old larvae sourced from the breeder colony, were used for grafting in the cell builder colonies. The cell builder colonies were thoroughly examined, and any emergency cells destroyed. The Doolittle technique of grafting was used for queen rearing in these queenless cell builder colonies (Doolittle et al., 1889).

Preparation of queen cell cups

One of the commercial plastic queen cell cups and other three queen cup cells instantly made from fresh *Apis mellifera* brood wax and paraffin and a blend of 50% bee wax and 50% paraffin were used as the treatments for grafting of larvae. The cell ups were built by using a cylinder-shaped wood mold that was then placed into the melted wax. Once the wax had adhered to the mold, it was lifted, left to cool scraped off to get artificial queen cells (Minarti et al., 2022). Similarly, all the queen cup cell types were prepared separately the day before grafting. Thus, a total of 100 cup cells each type consisting of 25 were used across five grafting frames. The grafting frame was designed to hold 20 queen cell cups five each of four types and were randomly allotted accommodating 10 cells each on two bars of the grafting frame (Fig. 1). Two-day old worker larvae sourced from the breeder colony were grafted onto the bottom of each of the queen cell cups and secured to the rearing frame using a grafting needle.

Fig. 1. Queen cell cups in grafting frame

Larval acceptance records

For each treatment, the numbers of accepted larvae out of total grafted were recorded after 72 hours of grafting.

The larval acceptance rate = $\frac{\text{Total accepted larvae}}{\text{total grafted larvae}}$

After 6 days of grafting the accepted cells were further observed to check whether the queen cells covered with wax and sealed by workers indicating pupal stage. After 10 days of grafting, the queen pupal cells were transferred to nucleus hives and allowing queen emergence. The emerged queens from each type of queen cell cups were weighed and measured using an electronic weighing machine and measuring scale, respectively.

Fig. 2. A grafting frame showing accepted larvae

Statistical analysis

The resulting data was tabulated in Microsoft Excel and further analyzed by R-STUDIO. Duncan's Multiple Range Test (DMRT) was employed to find out the significant differences between the mean values at a 5% level of significance. The significance was determined using the format of the ANOVA table. The coefficient of variance, grand mean, and standard error of mean were calculated using R-STUDIO.

RESULTS

Effects of queen cell cup types on larval graft acceptance and queen emergence

The mean larval graft acceptance and queen bee emergence across queen cell cup types was different (Table 1). The mean larval acceptance was highest in honey bee wax cell cups as compared to plastic cups, and a blend of bee wax and paraffin cell cups while, the least larval acceptance was observed in paraffin cup cells. Similarly, the number of queen emergence was highest from wax comb cells followed by plastic cups and wax-paraffin blend cell cells and the least number of queens emerged from paraffin made queen cell cups.

Table 1. Larval graft acceptance and queen bee emergence across the types of queen cell cup and rearing months in Beekeeping Development Center, Bhandara Chitwan 2023

	Larval Acceptance (Number)			Queen Emergence (Number)		
Treatments \ Months	April	May	June	April	May	June
Bee wax	4.6^{a}	4.8^{a}	4.8^{a}	4.4^{a}	4.6^{a}	4.2^{a}
Paraffin	2.4^{b}	2.6^{c}	2.4^{c}	1.8^{c}	2.4^{b}	2.2^{c}
Bee wax + Paraffin	3^{b}	3.8^{b}	2.8^{c}	3^{b}	3.4^{b}	2.4^{c}
Plastic cup	3^{b}	3.6^{b}	3.8^{b}	2.8^{b}	3^{b}	3.2^{b}
Grand mean	3.25	3.7	3.45	3	3.35	3
F test	**	***	***	***	***	***
SEM (±)	0.313	0.234	0.177	0.267	0.324	0.227
LSD (0.05)	0.96	0.72	0.54	0.82	0.99	0.7
CV (%)	21.57	14.17	11.53	19.95	21.62	16.94

CV: Coefficient of Variance; *, ** and *** represent significance at 5%, 1% and 0.1% level of significance, respectively; SEM: Standard error of mean; LSD (0.05): Least Significant Difference at 5% level of significance; same letters in the superscript indicate the similar effect according to DMRT at 0.05 level of significance.

Effects of queen cell cup types on emerged queen weight and length

The queen bees that emerged from bee wax cups were significantly heavier and longer, compared to others across all months. The mean weight of queen from bee wax was statistically greater (176.6 mg in April, 172.2 mg in May and 166.4 mg in June) than paraffin cell cups (157 mg in April, 157.8 mg in May and 153.8 mg in June). The queens emerged from bee wax cups during April were longer than in other months, while there was no significant difference in queen length from all grafts that emerged during May (Table 2).

Table 2. Effect of types of queen cell cups on queen bee weight (mg) and length (cm) across the different rearing period at Beekeeping Development Center, Bhandara Chitwan 2023

	Queen weight (mg)			Queen length (cm)		
Treatments \ **Months**	**April**	**May**	**June**	**April**	**May**	**June**
Bee wax	176.6[a]	172.2[a]	166.4[a]	1.94[a]	1.86	1.8[a]
Paraffin	157[c]	157.8[b]	153.8[b]	1.8[b]	1.7	1.68[b]
Bee wax + Paraffin	166.8[bc]	157.6[b]	158.2[ab]	1.84[b]	1.8	1.72[b]
Plastic cup	172.6[b]	159.2[b]	159.6[ab]	1.82[b]	1.82	1.72[b]
Grand mean	168.5	161.7	159.5	1.85	1.795	1.73
F test	**	**	*	*	NS	*
SEM (±)	3.233	2.355	2.721	0.029	0.044	0.024
LSD (0.05)	9.96	7.25	8.38	0.09	0.13	0.076
CV (%)	4.29	3.25	3.81	3.55	5.5	3.2

CV: Coefficient of Variance; *, ** and *** represent significance at 5%, 1% and 0.1% level of significance, respectively; SEM: Standard error of mean; LSD (0.05): Least Significant Difference at 5% level of significance; same letters in the superscript indicates the similar effect according to DMRT at 0.05 level of significance.

DISCUSSION

The findings demonstrated that the highest mean value of accepted larvae occurred with fresh bee-wax material and the larval acceptance rate with this substrate was much greater than that of larvae grafted to paraffin, wax-paraffin mix, and plastic cell cups. The number of queens emerged from bee wax cell cups was higher while both the acceptance and queen emergence was lowest on paraffin made cell cups. The acceptance of bee wax cups during the spring was higher (Lashari et al., 2022). Similarly, our findings indicated that larval acceptance was statistically significant in bee wax material during May (4.8) and June (4.8) followed by April (4.6). Likewise, Shafey et al. (2021) revealed that the greatest quantity of queen cell cups accepted occurred during May and June, while the fewest were accepted in November and December. Also, (Ahmad & Dar, 2013) found larval acceptance rates in queenright and queenless colonies were measured at 79% and 96.33%, respectively during June, surpassing rates observed in other months like May and July. Similarly, the maximum number of emerged queen bees were from bee wax cup during May (4.6), followed by April (4.4) and June (4.2) which was according to findings of (Shafey et al., 2021) where they found highest number of newly emerged queen recorded during May and June. Additionally, the study found that spring month exhibited the most favorable conditions for larval acceptance and queen emergence which was also supported by (Shafey et al., 2021).

The queens emerged from bee wax cups resulted in significantly heavy and long queens in all three rearing periods i.e. April, May and June 2023. While synthetic paraffin wax resulted in poor quality of queens having lighter weight and shorter length. The mean weight of the queen was significantly higher in bee wax during April (176.6 mg) followed by May (172.2 mg) and June (166.4 mg). In contrast, Dhaliwal, et al. (2017) reported that grafting in plastic cell cups can also be practiced for rearing good quality queen bees as the heaviest queen bees are obtained by this method. The study results with heavy newly emerged queens during April, May and June and similar trend of weight was found but it was during May, June and August (Shafey et al., 2021). Similarly, the mean length of queen was longer in bee wax during April (1.94 cm), followed by May (1.86 cm) and June (1.8 cm). While in the study of Shafey et al. (2021), the maximum length of newly emerged queen was recorded in May and June. Furthermore, Koç & Karacaoglu (2004) pointed out that honey bee queens can be successfully reared in the Aegean Region of Turkey from late March through September. However, they emphasized that the highest quality queens were obtained when rearing was done between late March and late April.

These results affirmed that bees exhibit a preference for queen cell cups made from bee wax rather than made from alternative materials. The findings further supported that grafting during early spring resulted in a larger number of good-quality queen bees with heavier weights and longer lengths. The rationale behind the higher acceptance rate observed with natural bee wax can be hypothesized as stemming from the queen's inherent preference for the wax they produce within the hive environment.This inclination towards their own naturally generated wax, as opposed to synthetic alternatives, could be a result of the familiarity and compatibility of natural wax with the colony, ultimately leading to increased acceptance rates during queen rearing.

CONCLUSIONS

The queen cell cups crafted from honey bee wax showed the most promising results in terms of larval acceptance and quality queen emergence. The consistent highest rates of larval acceptance, queen emergence, and overall queen quality across multiple rearing periods spanning from April to June indicates the suitability of honey bee wax for making queen cells. Moreover, this study implies that the adoption of honey bee wax can significantly enhance the acceptability of grafted larvae for queen-rearing and multiplication.

ACKNOWLEDGMENT

We would like to thank the staff of the Beekeeping Development Center for their technical assistance in the study and like to acknowledge Prime Minister Modernization Agriculture Project (PMAMP) and Agriculture and Forestry University (AFU) for providing assistantship to the lead author during the time of research and report preparation.

REFERENCES

Ahmad, S. B., & Dar, S. A. (2013). *Mass rearing of queen bees , apis mellifera L. (Hym: Apidae) for bee colony development raised under the temperate conditions of Kashmir*, 8, 945-948.

De Hazan, M., Lensky, Y., & Cassier, P. (1989). Effects of queen honeybee (Apis mellifera L.) ageing on her attractiveness to workers. *Comparative Biochemistry and Physiology Part A: Physiology*, 93 (4), 777–783.

Devkota, K. (2020). Beekeeping: Sustainable Livelihoods and Agriculture Production in Nepal. *Modern Beekeeping-Bases for Sustainable Production*, 26, 1–11.

Dhaliwal, N. K., Singh, J., & Chhuneja, P. K. (2017). 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*, 9(3), 1658–1661.

Doolittle, G. M. (1889). Scientific Queen-rearing. York, PA : George W. York.

Ebadi, R., & Gary, N. E. (1980). Acceptance by Honeybee Colonies of Larvae in Artificial Queen Cells. *Journal of Apicultural Research*, 19 (2), 127–132. https://doi.org/10.1080/00218839.1980.11100011

Koç, A. U. & Karacaoglu, M. (2004). Effects of rearing season on the quality of queen honey bees (*Apis mellifera* L.) raised under the conditions of Aegean region. *Mellifera*, 4(7), 34–37.

Lashari, M. A., Ghramh, H. A., Ahmed, A. M., Mahmood, R., Rafique, M. K., Ahmad, S., AL-Shehri, B. M., Mohammed, M. E. A., & Khan, K. A. (2022). 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*, 34(4), 102043. https://doi.org/10.1016/ j.jksus.2022.102043

Minarti, S., Yusryansyah, A. R., & Masyithoh, D. (2022). The Effect of Queen Cell Size Difference in Morphometrics of Bees Queen Apis cerana. *Jurnal Ilmu-Ilmu Peternakan (Indonesian Journal of Animal Science*), 32 (3), 373–379.

Ozbakir, G. O. (2021). Effects of rearing method on some morphological and reproductive organ characteristics of queen honey bees (*Apis mellifera* L.). *Medycyna Weterynaryjna*, 77(2), 89–94. https://doi.org/dx.doi.org/10.21521/mw.6496

Shafey, A. S., Shebl, M. A., Mahmoud, M. F., & Kamel, S. M. (2021). Evaluation of Colony Parameters for Queen Rearing under Arid Ecosystem Conditions. *Journal of Applied Plant Protection*, 10(1), 69–76. https://doi.org/10.21608/japp.2021.234779

Sharma, A., Rana, K., Sharma, H. K., & Sharma, A. (2020). 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*, 8(4), 1089–1097.

Vung, N. N., Kim, I., Lee, M. Y., Kim, H. K., Kim, D. W., Woo, S. O., & Choi, Y. S. (2018). Effects of Larval Grafted Age for Artificial Queen-rearing on Queen Reproductive Potential and Growth of Apis cerana Colony. *Journal of Apiculture*, 33(4), 261–268. https://doi.org/10.17519/apiculture.2018.11.33.4.261