EFFECT OF MONTHLY CHANGES (MAY – SEPTEMBER) ON LARVAE ACCEPTANCE RATE AND ROYAL JELLY PRODUCTION IN APIS MELLIFERA COLONIES IN BUMTHANG

PRASAD BHUJEL[1*], LHABA DORJI[1], KABIRAJ GURUNG[1], JAMBAY DORJEE[1], AND VIJAY RAIKA[2]

[1]National Livestock Research Centre, Department of Livestock, Ministry of Agriculture and Livestock
[2]National Highland Development Centre, Wangdue

*Author for correspondence: pbhujel@moal.gov.bt

ABSTRACT: *This study investigates the influence of colony status (queenright vs. queenless) and seasonal variations on royal jelly production and larvae acceptance rates in Apis mellifera colonies. The study was conducted at the National Livestock Research Centre (NLRC) apiary in Bumthang, Bhutan, from May 2023 to September 2024. Eight colonies, each with equal adult bee populations and brood percentages, were selected for the study. The colonies were maintained under controlled conditions, with sugar syrup supplementation during the rainy season to ensure uninterrupted nectar flow. Larvae grafting was carried out between May and September each year. Royal jelly produced was harvested and weighed at regular intervals. The results indicated significant seasonal effects ($p < 0.05$) on both royal jelly production and larvae acceptance rates. Highest production and acceptance were recorded in June, with queenright colonies yielding average royal jelly of 48.0g and 67.9% acceptance rate, while queenless colonies produced an average of 47.0g and had 66.5% acceptance rate. By September, royal jelly production and larvae acceptance rates had declined. Queenright colonies had average production of 16.3g and a 23.0% acceptance rate, while queenless colonies had average production of 16.9g and a 23.9% acceptance rate. The study suggests that royal jelly production is influenced by nectar availability, with peak production occurring when nectar flow is abundant in June. Based on the findings, it is recommended to use faster-developing colonies to improve royal jelly production. A similar study is recommended in different geographical locations (warmer southern districts) to further explore regional variations in royal jelly production.*

Keywords: *Apis mellifera*; Bumthang; Larvae acceptance rate; Queenless; Queenright; Royal jelly

1. INTRODUCTION

Royal jelly is premium hive products from *Apis mellifera* managed beekeeping. It is produced by the young bees of age between six to 12 days old from their hypopharyngeal and mandibular glands. The primary constituents of royal jelly are water, proteins, carbohydrate, lipids and 10- hydroxy-2-decenoic acid, ash, pH and acidity (Balkanska et al. 2013, Buttstedt et al. 2014 and Maghsoudlou et al. 2019).

China is the largest producer of Royal jelly globally producing about 4000 metric tons (MT) annually which contributes about 85% of its production worldwide. Countries like South Korea, Taiwan and Thailand produce the Royal jelly for commercial market and Japan is the largest importer of Royal Jelly (Cao et al. 2016).

Several factors influence the quality and quantity of royal jelly production, including the colony's health of colony, the standard of tools and equipment used, and the length of the production period (Chen et al. 2015; Lashari et al. 2022). The skills of beekeepers in managing hive conditions feeding practices, and the number of queen cups provided for queen rearing also play a crucial role (Sahinler and Kaftanoglu 2005). Additionally, the materials used for grafting larvae into queen cups, seasonal variations, hive arrangement techniques,

and the specific race of honeybee affect overall production outcome (Balkanska et al. 2013; López et al. 2022).

On average, the acceptance rates were 82.2% in queenless colonies and 72.1% in queenright colonies during the six months grafting period from April to September (Şahinler & Kaftanoğlu, 2005). The study by Van and Littlejohn (2015), found that the average yields of royal jelly from queenless and queenright colonies were 7.1g and 7.4g irrespective of hive design. A similar study conducted in South Korea recorded an average production of 11.54g and 18. 16g per graft, respectively from 36.6 and 49.7 accepted queen cells during the period of nectar availability and non-availability from the natural forage source (Lee et al. 2017). The production of royal jelly decreases from Spring and become minimum in Autumn season due to the decline in fresh nectar and pollen in the foraging areas (Sahinler and Kaftanoglu 2005). The common practice to harvest the royal jelly is three days (72 hours) after grafting the 24 hours young larvae (Chen et al. 2015).

Apis mellifera was introduced in Bhutan during the 1980's and kept in Bumthang and Chukha Dzongkhags for honey production (Verma 1990). From these two Dzongkhags, *Apis mellifera* is successfully managed in Bumthang but did not thrive in Chhukha because of predators, mainly hornet and unfavorable monsoon weather condition (Verma 1990). Besides honey, beekeepers can produce royal jelly, bee pollen and propolis among others, but are not produced by Bhutanese beekeepers on a commercial scale. Realizing the commercial value, royal jelly is included in the 13th Five Year Plan (FYP) document of the Ministry of Agriculture and Livestock (MoAL), under ongoing research and trials. It is expected to be classified as one of the high value, low volume livestock products in the 14th FYP. Therefore, the trial was conducted to study the effect of honey production months on acceptance rates and royal jelly yield from *Apis mellifera* in Bumthang to generate baseline information on royal jelly production.

2. MATERIALS AND METHODS

2.1 Study area

The study was conducted by the National Livestock Research Centre (NLRC) in Bumthang from May to September in 2023 and 2024. The study site is located at Batpalathang in Bumthang Dzongkhag, at an elevation of 2650 meters above sea level (m.a.s.l) (Wangda et al. 2007). The region experiences a temperate climate, characterized by distinct seasonal variations. Summers are mild, while winters are cold with occasional snowfall, making the region suitable for temperate vegetation. The average annual temperature ranges from 10°C to 18°C, and annual rainfall varies between 700 to 1,000 mm, mostly during the monsoon season (June to September).

2.2 Sampling method

In total, eight *Apis mellifera Lingustica* colonies with equal number of adult bee population and brood percentage were randomly selected to carry out the trial. Each colony was placed with eight brood frames, keeping provision for one grafting frame and a frame feeder. A young queen mated in August 2022 at NLRC apiary was placed in the experimental colonies to maintain uniformity in queen age to control hive population, and all were placed in the same location throughout the trial duration.

2.3 Management of research hives

The selected hives were divided into two groups, each comprising of four hives – one group with queen and the other group without queen which was assigned a unique identification number engraved on plastic tags. The queenright colonies were set up in two brood boxes, with queen excluder placed atop of lower brood box to confine

the queen to the lower brood box. The queenless colonies were maintained in the single brood box. All the colonies were provided table sugar syrup as a feed supplement throughout the trial period, particularly during inclement weather conditions to ensure a constant nectar flow for brood growth and development in the hives. By end of September 2023, all the trial hives feed storages were maintained at 30kg for winter survival which lasts from November till March. Larvae grafting for trial was carried out from May to September in 2023 and 2024.

2.4 The royal jelly production

The young larvae of age 24 to 36 hours were grafted in food-grade pre-designed plastic queen cell cups. A drop of royal jelly solution made by mixing equal amount of distilled warm water and the fresh royal jelly was put in every queen cup of grafting frames. A single grafting frame has two grafting bars holding 68 predesigned food grade plastic queen cups in total. After grafting, the frames were placed in the middle of the brood box flanked with two brood frames consisting of open young brood to attract young bees to feed the grafted larvae. The inserted frames were taken out after 72 hours (3 days), and the bees were brushed off from the grafting bars. The number of accepted cells from the total grafted cells were manually counted and recorded immediately. With a warm stainless-steel kitchen knife, the wax portion from the plastic cell cups was removed and the larvae were carefully removed with steel tweezers. The amount of royal jelly from the random 10% of the accepted cups were individually extracted, weighed and recorded in the excel sheets for comparison. The royal jelly from all accepted cups were scooped out using a silicon royal jelly spoon and stored in the brown vials for study.

2.5 Data collection and analysis

The royal jelly production from individual experimental hives were collected, weighed and stored in the vials after each harvest with identification details including date of harvest and experimental group. The data obtained from the trial were entered in Microsoft excel and the dataset was imported into SPSS for analysis. The analysis of variance (ANOVA) was performed in SPSS to compare the means of different groups.

3 RESULTS AND DISCUSSIONS

3.1 Royal jelly production and acceptance rates

The impact of season on royal jelly production and larvae acceptance rate was significant ($p<0.05$). Table 1 summarizes the acceptance rates in queenright colonies from May to September. The average acceptance rates were 66.5%, 67.9%, 53.2%, 40.8% and 23.0%, respectively. The average royal jelly production in May, June, July, August and September in queenright colonies was 47.1g, 48.0g, 37.6g, 28.9g and 16.3g, respectively (Table 1; Figure. 1&2)

The average acceptance rate in queenless colonies in May, June, July, August and September were 64.9%, 66.5%, 51.1%, 45.3% and 23.9 % respectively. The average royal jelly production was 45.9g, 47.0g, 36.1g, 32.0g, 16.9g from May, June, July, August and September, respectively (Table 2: Figure. 1 and 2).

Table 1: The acceptance rate (%) and royal jelly production per hive (g) in different months in queenright colonies

Months	Number of repetitions	Number of grafts	Number of larvae accepted	Acceptance rate (%)	Max-Min (%)	Royal jelly yield (g)
May	64	4352	2896	66.5 ± 6.5	88-28	47.1
June	64	4352	2955	67.9 ± 6.2	87-36	48.0
July	64	4352	2316	53.2 ± 7.4	85-15	37.6
August	64	4352	1776	40.8 ± 7.7	82-9	28.9
September	64	4352	1001	23.0 ± 3.3	39-2	16.3
Total/Average	64	21760	10522	50.2 ± 18.2	88-2	35.5

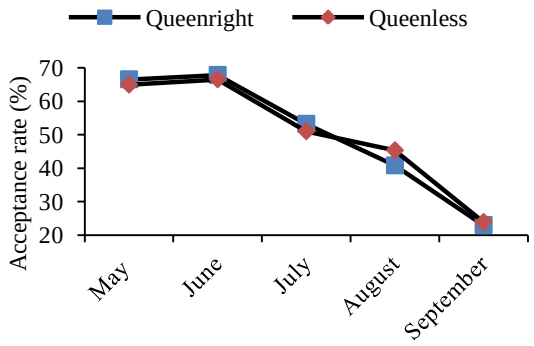

Figure 1: Monthly acceptance rate (%) in queenright and queenless colonies

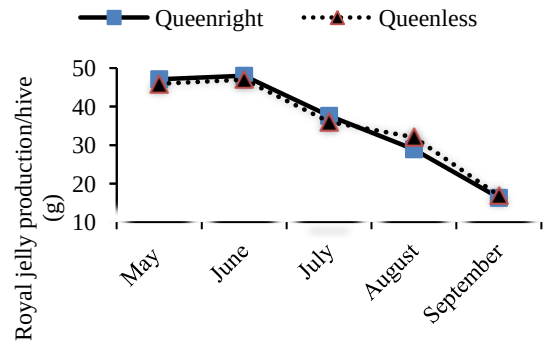

Figure 2: Monthly royal jelly production per hive (g) in queenless and queenright colony

Royal jelly production was highest in June and lowest in September in both queenright and queenless colonies. However, there was no significant difference in the average production of royal jelly between queenright and queenless colonies. Similarly, the acceptance rate was highest in June and lowest in September in both queenright and queenless colonies. However, the average acceptance rate was 1.3% higher in queenright colonies than in queenright colonies.

The average acceptance rate was 50.2% in queenright colonies and 50.3% in queenless colonies during the trial period. Şahinler and Kaftanoğlu (2005), during the six months grafting period from April to September, found average acceptance rates of 82.2% for queenless colonies and 72.1% for queenright colonies.

The royal jelly production in June was 1.9% more than in May, 21.7% more than in July, 39.9% more than in August, and 66.0% more than in September. The increase in royal jelly production from May to June, and continuous decline thereafter implies that royal jelly production decreases when there is a shortage of fresh nectar and pollen in the foraging areas of bees.

Table 2: The acceptance rate (%) and royal jelly production per hive (g) in different months in queenless colonies

Months	Number of repetitions	Number of grafts	Number of larvae accepted	Acceptance rate (%)	Max-Min (%)	Royal jelly yield (g)
May	64	4352	2823	64.9 ± 6.2	84-32	45.9
June	64	4352	2896	66.5 ± 4.1	84-35	47
July	64	4352	2222	51.1 ± 51.	85-18	36
August	64	4352	1971	45.3 ± 3.1	76-15	32
September	64	4352	1040	23.9 ± 0.9	56-10	16.9
Total/Average	64	21760	10952	50.3 ± 16.3	85-10	35.5

4. CONCLUSION

In conclusion, this study demonstrates that seasonal variation and colony status (queenright vs. queenless) significantly affect royal jelly production and larvae acceptance rates in *Apis mellifera* colonies. Royal jelly production peaked in June and declined by September, with queenright colonies showing slightly higher yields than queenless colonies. The findings align with previous studies that nectar availability drives royal jelly production, influencing colony productivity. June was the optimal month for royal jelly production, with a noticeable decline in both royal jelly production and acceptance rates as forage availability decreased. Effective hive management during the peak nectar flow periods can maximize royal jelly yields. Additionally, the results suggest that faster-developing colonies may further improve production. Further studies, particularly in warmer southern districts of Bhutan could provide valuable insights into regional factors influencing royal jelly production and support in the development of region-specific beekeeping strategies**.**

Acknowledgement

The authors would like to thank Department of Livestock, Ministry of Agriculture and Livestock (MoAL) for providing the fund support to carry out this study. Similarly, all the staff of the National Livestock Research Centre (NLRC), Bumthang who have contributed to this study are equally acknowledged. The study would not have been possible without their dedicated support. Lastly, the team would like to thank Livestock Research Technical Committee (LRTC) of NLRC for giving their valuable comments to improve this work and office administration for rendering the necessary field support to complete this study successfully.

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