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Developing Pollinating Insects Bees Honey *Apis cerana* by applying the Doolittle Method (Queen Rearing)

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Abstract

Insects hold significant importance in human life, exhibiting harmful and beneficial effects. Harmful insects, such as plant pests and disease vectors, threaten humans and animals, while beneficial insects contribute positively to human welfare and environmental health. They serve as agents of decomposition, recycle organic materials, and play a crucial role in the pollination of plants. Among these, bees are notable pollinators. Honeybees, in particular, are environmentally friendly insects that substantially benefit humans due to their role in plant pollination. This research was conducted using a Randomized Group Design (RAK) outlined by Steel and Torrie (1993), which involved seven treatments—six consisting of artificial feed and one control group. The findings indicate that the development of Apis cerana honey beekeeping, utilizing the Doolittle method, effectively increased the number of queen cells in royal jelly, with the most successful treatment being E, which had a ratio of 2:1 (200 grams of water to 100 grams of cane sugar). However, statistical analysis revealed no significant differences between this treatment and the others.

Keywords: Honeybee, Insect, Pollination, Pollinator, Sugar Cane

1. Introduction

Insects significantly impact human life, exhibiting both harmful and beneficial effects. Harmful insects, such as plant pests and disease vectors, threaten humans and animals, while beneficial insects contribute positively to human welfare and environmental health. These beneficial insects act as agents of life, aiding in the decomposition of organic materials and facilitating the pollination of plants. Among these, bees are notable pollinators. Honeybees, in particular, are environmentally friendly organisms that substantially benefit humans due to their role in plant pollination. The life cycle of bees progresses from eggs to larvae, pupae, and adult bees within the hive. Furthermore, bees (Apis sp.) produce several products that enhance human well-being (Joice et al., 2023).

Worker bees supply nourishment to the colony, catering to both larvae and adult bees. Bees also play a crucial role in boosting agricultural yields through pollination activities. Numerous crops, including mangoes, rambutans, lychees, watermelons, corn, and coconuts, rely on honeybees for pollination, increasing production two-fold. Honeybees collect nectar and pollen without causing harm to the plants (Nurdin, 2019).

Two species of cultivated honey bees in Indonesia are *Apis cerana* and Apis melifera. The *Apis cerana* species is still relatively understudied due to its reputation for being aggressive and wild. Bees require cultivating specific plants that serve as sources of nectar and pollen. However, not all plants are suitable for supporting the feeding needs of bees (Nurdin). 2019).

The feed experience of bees involves the collection of nectar and pollen. There are numerous sources of nectar and pollen available for the honey bee species *Apis cerana*, including flamboyant, sugar palm, watermelon, lychee, grapes, coffee, wood white, sweet orange, big orange, sunflower, apple, papaya, soybean, and cucumber. The primary nectar sources are the red calliandra flowers, mango, rambutan, cotton, beans, chili, langsat, water apple, and tamarind. According to Aisyah et al. (2023), corn, carrots, and guava are identified as the origins of pollen.

Pollen is a significant protein source that enhances the egg-laying capacity of queen bees and extends their lifespan. Consequently, pollen is vital for bee colonies in producing royal jelly. Nectar, a sugary liquid rich in water,

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provides bees with essential carbohydrates, hydration, vitamins, and minerals. This sweet substance can be secreted from flowers or leaf stalks, functioning not only as an energy source for bees but also as a raw material for the production of wax used to seal bee pupal cells (Sudarjat et al., 2020)

During periods of non-flowering, such as the dry or prolonged rainy season, it is imperative to provide alternative plant feed sources. Cane and palm sugar are commonly used as substitute food sources instead of nectar. The nutritional breakdown of cane and palm sugar is presented in Table 1. The high sucrose content in cane sugar and palm sugar makes them suitable for supplementation in the artificial feed of *Apis cerana* bees, intending to enhance royal jelly production.

An abundance of nectar and pollen will generate several *Apis cerana* bee products, including honey, wax, pollen, propolis, bee venom, and royal jelly. These products have potential applications in the pharmaceutical and food industries. Beverages and beauty products (Joice et al. . 2023).

Royal jelly is a substance produced through the activities associated with cultivating honey bees. It is intricately linked to their diet, which comprises pollen and nectar—essential raw materials for synthesizing royal jelly. The quantity of royal jelly generated is directly proportional to the availability of nectar and pollen. To ensure a sufficient yield of royal jelly, it is imperative to understand the fundamental techniques involved in forming queen cells, which pertain to the propagation of queen bees. This result encompasses both natural and artificial methods of queen rearing. Without the establishment of queen cells, royal jelly production is not feasible. Furthermore, larvae destined to become queen bees must consume royal jelly; otherwise, they will not develop into queens. If a queen bee within a colony perishes without a suitable replacement, the colony faces the risk of extinction (Grouth, 2020).

The effectiveness of the Doolittle method in producing royal jelly has been investigated in the Apis mellifera species, with successful outcomes. However, the same method failed to yield royal jelly in the local *Apis cerana* bees from North Sulawesi. As a result, this study decided to utilize the *Apis cerana* species for further investigation. Due to a lack of comprehensive research on the subject, the breeding and capturing of *Apis cerana* bees is insufficiently documented in both technique and theoretical literature. This phenomenon occurs due to the widespread perception of honey bees as hazardous insects, which impedes the advancement of research in honey bee breeding, particularly in the case of *Apis cerana* bees, known for their untamed and aggressive nature in contrast to imported bees (Apis mellifera) (Joice et al.). . 2015). This research aimed to evaluate the optimal feed derived from cane sugar and palm sugar for the queen-rearing technique to enhance queen cells' development and the royal jelly secretion in *Apis cerana*.

2. Material and Methods

This study was conducted from October 2024 to December 2024 in the Village Kembes, District Tombulu, Regency Minahasa, North Sulawesi 1°27′22.3″N 124°49′38.1″E. Materials used in the study are the sugar cane solution, sugar sugar palm, and water. The bees used Apis, the cerana kept in a box colony.

The primary tool utilized in this study comprises a total of 35 boxes, which includes over 28 colony boxes, 6 empty boxes, and 1 box designated for breeder colonies. Each box is dimensioned at 36.0 cm in length, 18.0 cm in width, and 26.0 cm in height. Additional equipment includes a brush cleaner, a container for artificial bee feed, protective clothing, hand sheaths, socks, face masks, and 24 Doolittle frames, each measuring 34 cm in length and 16 cm in width, with a square shape. Furthermore, there are 240 queen cell bowls, each with a base size of 6 mm and a height of 10 mm, arranged with 10 bowls per Doolittle frame. The supporting tools encompass grafting sticks, knives, tweezers, brushes, queen dividers, larval containers, scales, temperature measuring devices, magnifying glasses, and labels.

This study used a Randomized Group Design (RAK), as Steel and Torrie (1993) outlined. The experimental design incorporated seven treatments: six artificial feed formulations and one control group. The treatments are as follows: Control (no addition of sugar and water), 200 grams of cane sugar combined with 200 grams of water, 100 grams of cane sugar with 200 grams of water, 66 grams of cane sugar with 200 grams of water, 200 grams of palm sugar with 200 grams of water, 100 grams of palm sugar with 200 grams of water, and 66 grams of palm sugar with 200 grams of water. 28 colony boxes were utilized across the seven feed compositions, each treatment being replicated four times. The design Model for this research is a Completely Randomized Design model with the following equation:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

i = 1, 2, …, 7
j = 1, 2, …, 7

With the understanding that:

Y_{ij} = Observation value of queen cell formation or royal jelly production in the i-th treatment in the j-th replication.
μ = Average queen cell formation or royal jelly production
α_i = Effect of the ith artificial feed treatment
ϵ_{ij} = Effect of experimental error from the i-th artificial feed treatment on the j-th replication.

2.1. Superior Queen Rearing method is Emergency Cell

Feed artificial, given one Sunday before observation, is sugar sugarcane. The sugar palm is weighed and dissolved in boiling water based on size composition/treatment. After that, it is cooled. All feed is artificially poured into a container placed on the top frame inside the comb box of

bees. Giving feed done every day at 7.00 WITA on colony bee

The bee queen is separated from its colony And placed in the empty box for three days, followed by One frame full comb with a cell nest And part bee worker to look after the bee queen. Then, observe how many cells the queen formed from five combs nest because if the cell queen formed in the cell, there is royal jelly. Afterward, the bee queen maintenance bee returned to box his colony for four days before implementing observation on Sunday second. Observation Sunday to two until Sunday to four procedure his work the same like observation First parts a to d.

2.2. Retrieval Production Royal jelly

The production of royal jelly is closely associated with the formation of queen cells, as the absence of queen cell formation results in the absence of royal jelly. The collection of royal jelly involves preparing the necessary equipment for harvesting, including a knife, tweezers, a royal jelly container, and a brush. And an insulated container of frozen water. Additionally, royal jelly is collected or harvested at 6:30 AM WITA. Next, in the preferred method (emergency cell), all combs that contain queen cells are removed and cleaned using a brush to remove any bees attached to the frame or comb. The queen cells from each treatment group are removed and transferred into separate plastic containers. Subsequently, the plastic material is placed inside a container designed to maintain a low temperature, such as an ice thermos. Subsequently, a knife should be employed to separate the queen cells constructed by the bees, followed by the cleansing of each cell to remove any propolis. Subsequently, tweezers were used to extract bee larvae, and a drawing brush was utilized to eliminate royal jelly from the queen cells, followed by filtration to remove any impurities. Finally, the resulting substance is transferred into the designated container. Moreover, the royal jelly is preserved in a freezer before transportation to the laboratory, where it is examined and quantified for its composition.

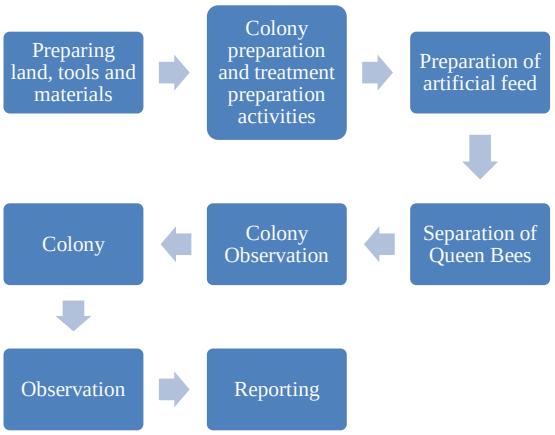

Figure 1. Research flow diagram

3. Results and Discussion

3.1. Formation Cell Queen with Doolittle Method With Bowl Cell from Factory

The findings regarding queen cell formation through the Doolittle method, utilizing queen cell cups sourced from the factory across seven different artificial feeding treatments for the *Apis cerana* colony, were insufficient for royal jelly analysis. This limitation arose due to the minimal number of queen cells produced, resulting in an inadequate quantity of royal jelly for analysis. Furthermore, research conducted by Grouth (2020) indicates that attempts at queen rearing using the Doolittle method with *Apis cerana* have yet to yield significant results. This result is attributed to the more aggressive and wild nature of *Apis cerana* compared to the imported Apis mellifera species. So, as the study by Rompas et al. (2015) said, queen-rearing or Doolittle methods yield 51 bowls cells (Figure 2).

Table 1. Data from observations of queen cell formation using the Doolittle method using cell bowl from factory

Treatment	Test				Total	Average
	I	II	III	IV		
A	0	2	4	3	9	2.25
B	1	0	2	4	7	1.75
C	0	2	3	2	7	1.75
D	1	1	2	4	8	2.00
E	4	5	7	5	21	5.25
F	2	0	3	3	8	2.00
G	0	1	3	1	5	1.25

Note: A. 200 grams of cane sugar + 200 grams of water, B. 100 grams of cane sugar + 200 grams of water, C. 66 grams of cane sugar + 200 grams of water, D. 200 grams of palm sugar + 200 grams of water, E. 100 grams of palm sugar + 200 grams of water, F. 66 grams of palm sugar + 200 grams of water, G. Control (no additional sugar and water).

Figure 2. Formation of Queen Cells by the Doolittle Method with cell cups from the factory

Table 2. Data from Observations of Total Queen Cell Formation Using the Doolittle Method with an artificial cell bowl from the *Apis cerana* bee comb

Treatment	Test				Total	Average
	I	II	III	IV		
A	5	15	7	8	34	2.25
B	17	16	18	12	62	1.75
C	20	23	14	12	54	1.75
D	19	16	2	4	61	2.00
E	13	23	25	14	75	5.25
F	20	11	8	15	54	2.00
G	8	12	8	9	37	1.25

Note: A. 200 grams of cane sugar + 200 grams of water, B. 100 grams of cane sugar + 200 grams of water, C. 66 grams of cane sugar + 200 grams of water, D. 200 grams of palm sugar + 200 grams of water, E. 100 grams of palm sugar + 200 grams of water, F. 66 grams of palm sugar + 200 grams of water, G. Control (no additional sugar and water).

Figure 3. Formation of Queen Cells by the Doolittle Method made from *Apis cerana* combs from the factory.

The results above show that the provision of cane sugar and palm sugar solutions as artificial feed to the *Apis cerana* bee colony with the highest average was treatment E, which was 18.75 queen cells/comb/colony, ranging from 12-25 cells/comb/colony, and the lowest average results were in treatment A, which was 8.50 cells/comb/colony with a range of 4-14 cells/comb/colony. The results of the normality test of the queen cell formation data for each treatment carried out using the Shapiro-Wilk test are presented in Table 3.

The normality test results of the queen cell data showed that the data in the seven treatments tended to be normally distributed.

The homogeneity test of the queen cell formation variance in all treatments was carried out using Levene's Test, and the results are presented in Table 4 below. The test results showed that there was a non-homogeneous variance. The results of the homogeneity of variance test stated that not all varieties of queen cell formation in all treatments were the same, so the hypothesis of queen cell formation was tested using the Kruskal - Wallis ANOVA. The tests and their results are presented in Table 5.

Table 3. Testing the Normality of Queen Cell Data

Treatment	Statistics	Sig.(p)	Decision
A	0.98	0.899	Spread Normal
B	0.89	0.414	Spread Normal
C	0.86	0.264	Spread Normal
D	0.93	0.647	Spread Normal
E	0.83	0.177	Spread Normal
F	0.96	0.804	Spread Normal
G	0.79	0.086	Spread Normal

Note. Normal if sig. ≥ 0.05
Abnormal if sig.< 0.05

Table 4. Homogeneity Test for Queen Cell Formation Varieties

Levene Statistics	df1	df2	Sig.	Decision
5.256	6	21	0.002	Variety is not homogeneous

Note: If sig. ≥ 0.05: homogeneous
If sig. < 0.05: not homogeneous

Table 5. Anova Queen Cell Formation

Treatment	*Median*	*Mean Rank.*	χ^2	Sig. (p)
A	8.00	7.38	8.962	0.176
B	16.50	18.38		
C	12.50	13.50		
D	15.00	17.75		
E	19.00	20.88		
F	13.00	14.88		
G	8.50	8.75		

The findings in Table 5 indicate no statistically significant difference among the median values of queen cells across the seven treatments (p = 0.176, greater than α = 0.05). Nevertheless, it is noteworthy that treatment E resulted in the highest median of queen cell formation when compared to the other treatments.

According to Grouth (2020), an adequate artificial feed for bee colonies, particularly in the absence of natural feed, consists of a syrup or sugar solution with a ratio of 1:2. The natural formation of queen cells through the emergency cell method is evidenced in treatments B (cane sugar) and E (palm sugar), where the application of artificial feed comprising 100 grams of sugar mixed with 200 grams of water yielded the highest number of queen cells, despite the lack of statistical significance.

From an economic perspective, treatments B and E are particularly advantageous, as the sugar volume utilized—100 grams of sugar combined with 200 grams of water—produced a greater quantity of queen cells compared to treatments A and D, which employed a higher sugar volume of 200 grams mixed with 200 grams of water.

3.2. Royal Jelly production using the artificial Doolittle bowl method from *Apis cerana* Combs

The results of observations of royal jelly production using this method from the seven artificial feed treatments given to the *Apis cerana* bee colony and the results of descriptive analysis, data normality testing, homogeneity testing, and variance analysis are presented successively below (Tables 10, 6, 7 and 8).

Table 6. Descriptive Statistical Values of Royal Jelly Production using the Doolittle method (gr/cell/comb)

Treatment	Average	Standard Deposit	Minimum	*Median*	Maximum
A	2.225	0.250	2,090	2.105	2,600
B	2,545	0.286	2.120	2,665	2,730
C	2.393	0.355	2,070	2.395	2,710
D	2,650	0.060	2,590	2,640	2,730
E	3.268	0.573	2,580	3,370	3,750
F	2,520	0.283	2.110	2.605	2,760
G	2.287	0.199	2.100	2.260	2,530

Note: A. 200 grams of cane sugar + 200 grams of water, B. 100 grams of cane sugar + 200 grams of water, C. 66 grams of cane sugar + 200 grams of water, D. 200 grams of palm sugar + 200 grams of water, E. 100 grams of palm sugar + 200 grams of water, F. 66 grams of palm sugar + 200 grams of water, G. Control (no additional sugar and water).

The findings presented above indicate that when cane sugar and palm sugar solutions were provided as synthetic sustenance to the *Apis cerana* bee colony, treatment E yielded the highest average production at 3,268 grams per cell per comb, with a range of 2,580-3,750 grams per cell per comb. Conversely, treatment A had the lowest average production at 2,225 grams per cell per comb, with a range of 2,090-2,600 grams per comb.

This study also revealed that the preferred feed for *Apis cerana*, when comparing cane sugar and palm sugar, is palm sugar in treatment E at a ratio of 2:1 (200 grams of water to 100 grams of palm sugar). Table 7 below presents the results of the Shapiro-Wilk normality test conducted on the data for royal jelly production within each treatment.

Table 7. Normality Test of Royal Jelly Production

Treatment	Statistics	Sig.(p)	Decision
A	0.672	0.005	Abnormal Spread
B	0.746	0.036	Abnormal Spread
C	0.764	0.052	Spread Normal
D	0.962	0.792	Spread Normal
E	0.866	0.282	Spread Normal
F	0.837	0.187	Spread Normal
G	0.922	0.546	Spread Normal

Note: A. 200 grams of cane sugar + 200 grams of water, B. 100 grams of cane sugar + 200 grams of water, C. 66 grams of cane sugar + 200 grams of water, D. 200 grams of palm sugar + 200 grams of water, E. 100 grams of palm sugar + 200 grams of water, F. 66 grams of palm sugar + 200 grams of water, G. Control (no additional sugar and water).
Note. Normal if sig. ≥ 0.05
Abnormal if sig.< 0.05

The normality test results of royal jelly production data showed that the data in the treatment tended to be normally distributed, and some were not normally distributed.

The homogeneity test of the variety of royal jelly production in all treatments was carried out using Levene's Test, and the results are presented in Table 8 below.

The variance test indicated that the variations in royal jelly production across different treatments were not uniform. Consequently, the hypothesis regarding royal jelly production was evaluated using the Kruskal-Wallis ANOVA. The findings from these tests are detailed in Table 9.

Table 8. Homogeneity Test of Royal Jelly Production Variety

Levene Statistics	df1	df2	Sig.	Decision
5,060	6	21	0.002	Variety is not homogeneous

Note: If sig. ≥ 0.05: not homogeneous
If sig.< 0.05: homogeneous

Table 9. Anova Royal Jelly Production

Treatment	*Median*	*Mean Rank.*	χ^2	*Sig* . (p)
A	2.105	6.75	11.816	0.066
B	2,665	17.38		
C	2.395	12.00		
D	2,640	18.13		
E	3,370	23.25		
F	2.605	15.38		
G	2.260	8.63		

Note: A. 200 grams of cane sugar + 200 grams of water, B. 100 grams of cane sugar + 200 grams of water, C. 66 grams of cane sugar + 200 grams of water, D. 200 grams of palm sugar + 200 grams of water, E. 100 grams of palm sugar + 200 grams of water, F. 66 grams of palm sugar + 200 grams of water, G. Control (no additional sugar and water).

The findings presented in Table 9 indicate no statistically significant difference in the median number of queen cells across the 7 treatments ($p = 0.066 > \alpha = 0.05$). As presented in Table 13, our data analysis indicates that the median royal jelly production in treatment E surpassed that of the other treatments, signifying a greater yield of royal jelly.

The analysis of variance results indicated no significant difference in the effect of artificial feed on royal jelly production between the several treatments administered to the bee colonies. It is hypothesized that bee colonies consume not only the artificial feed provided to them but also natural food sources in their natural environment.

Figure 4. Royal Jelly products for each treatment

4. Conclusion

The best method for multiplying queen cells in royal jelly from honey produced by *Apis cerana* bees through the use of the Doolittle technique showcases the development of beekeeping. Out of 21 bowls, the most effective ratio for the E treatment is 2:1 (200 grams of water combined with 100 grams of sugarcane sugar). Although statistics are essential in their way, they are not fundamentally different from other forms of treatment.

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