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Study of the age of grafted larvae on some morphological characteristics of queen honey bees (*Apis mellifera*)

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ABSTRACT: Queen bees are castes in the colony tasked with producing eggs to survive a bee colony. The Queen is the only one tail in the colony and can produce eggs to be a prospective queen bee, worker and stud. The eggs of would-be queen bees and would-be worker bees are fertilized fertile eggs. The fertilization process occurs shortly before the egg is laid inside the base of the hive so that when hatching, the larvae of the would-bees Queen and prospective worker bees are the same. Honeybee cultivation, in general, still relies on conventional means, especially in the development of queen populations as well as bee populations within colonies. Beekeepers still rely heavily on the rejuvenation of queen bees through natural means by relying on colonies to not optimal the time and results obtained. The short-term goal is to get quality queen bees with the best morphol-ogy mass and quickly through grafting worker larvae. The long-term goal is to assist farmers in the procurement of quality queen bees in a mass and fast way as needed with optimal results. Method : (1). Make a queen by grafting worker larvae then developed inside a particular colony until becoming an individual to get the queen bee. (2) Devel-op grafting queen bees to get the best production.

Keywords: Performance; Production; Colonies; Bees; Different larvae

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INTRODUCTION

Queen bees are one of the castes in each colony. The Queen has a larger body size a length and weighs 2.8 times the worker's weight to be identified easily. Each honeybee colony is only a queen tasked with laying eggs for the survival of the colony. Queen is formed from fertilized larvae (fertile)so are the larvae of prospective worker bees. The Queen grows inside a special cell tube with a different shape and a larger size. This shape and size can make it easier for worker bees to care for future queens by feeding them royal jelly.

The Queen can live to 5 (five) years, but productivity will decrease from the age of 2 (two) years. Naturally, a new queen of the new Queen will be formed in the colony if the old Queen dies, is lost or unproductive. This phenomenon will encourage worker bees to create new but commercially unprofitable queens because it takes a long time. According to Mahbobi (2012), age affects significantly about characters of queen bees.

The supplement of feed significant effect on the morphological characteristic. Futhrrmore Okuyan (2018). The physical parameters of queen bees investigated from their research were queen weight, length of some body part, the width of thorax, head and wing.

Some need to be research into the making of queens from the larvae of prospective worker bees and then observe the morphology of the resulting Queen covering the Queen's weight, the length of the Queen and the length of the abdomen. The abdominal length was not investigated in the previous research. It became good information that explains this research because we find out about abdominal length. It is crucial to know how to get the best queen bees grafted from larvae of different ages by that explanation. This research aims to for the first to make a queen bee of several different larvae ages. The second, studying morphological profiles include queen weight, queen length and abdomen length.

MATERIALS AND METHODS

This study uses experimental methods with Complete Randomized Design '*Rancang Acak Lengkap"* (RAL)consists of 3 treatments, each with five replays. Each replay consists of 5 future queens. According to Mahbobi (2012), the research method about grafting was that the Queen was confined to three empty combs. After that, provide larvae for grafting in which the age of the larvae was known. It was two bars consist of 24 artificial queen cell cups. Every eight larvae divided into three age groups, 1 day old, 2 days old, and 3 days old, were grafted into the queen cell cups cover by a drop of water with royal jelly.

Research Treatment is:

Treatment 1(T1): grafting of larvae aged 1 day

Treatment 2 (T2): grafting of larvae aged 2 days

Treatment 3 (T3): grafting of larvae aged 3 days

Research Procedures

Determine Source colonies of larvae

Colonies source bee larvae selected about the colony's fertility based on the nest area (eggs, larvae and pupae). Fertile colonies will produce higher amounts of puppies (Gary and Martson, 1971).

Figure 1.Larvae Source Colony

Preparing equipment for grafting larvae

Prepared equipment that will be used in the process of grafting larvae, including:

a. Leverage tool to separate attachments between frames to know it is from the box.
b. Brush to get rid of bees when retrieving larval source hive frames.
c. Grafting tool for grafting larvae
d. Bowl of the future Queen to place larvae
e. Bar frames to place a bowl of the future Queen
f. Cell builder, which is honeybee colony without a queen to build a bowl hive containing grafted larva

Figure 2. Queen Bowl

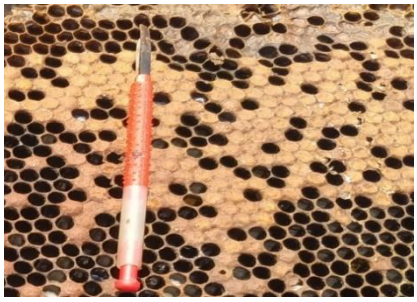

Figure 3.Grafting tool

Prepare Cell Builder

At the same time as the creation of colonies, the creation of cell builders whose stages include:

1. Choose 1 colony that has a queen.
2. Move multiple bee combs.
3. Prepare 1 empty box to put on top of the box that has a queen.
4. It seals between the bottom box and the top box so that the Queen cannot get into the top box.

5. Preparing queen cells and pasted on frames as many as 20 seeds/frames/super boxes.
6. Insert 1 frame into each box.
7. Insert a colony comb into a super box, given several empty combs.
8. After 1 day, observe the development of larvae.

Grafting Larvae Treatment

After the egg hatches, the 4th day can be grafting larvae. According to Kuntadi (2013) The grafting process includes the following stages:

9. On the 4th day, the larvae are taken from the colony, making a box using grafting tools and made larvae aged 1 day (P0).
10. Larvae are placed on the Queen cell that has been affixed to the frame.
11. The Queen cell frame is placed on super box 1.
12. On the 5th day, the larvae are taken from the colony, making a box using grafting tools and made larvae aged two days (P1).
13. Larvae are placed on the Queen cell that has been attached to the frame.
14. The Queen cell frame is placed on the super box 2.
15. On the 6th day, the larvae are taken from the colony, making a box using grafting tools and made into larvae aged three days (P2).
16. Larvae are placed on the Queen cell that has been attached to the frame.
17. Queen cell frame placed on super box 3.
18. Label each frame and stup according to the given treatment.

Figure 4. Larvae Grafting

Controlling Larvae

Some things done at this stage include:

19. Control every two days for ten days.
20. To see the development of larvae characterized by the increase of royal jelly in the Queen cell.
21. After the larvae are five days old, the Queen cell will be covered in beeswax.
22. Wait up to 10 days, and the larvae enter the pupae phase.
23. After ten days, cage installation aims to secure the prospective queen bee so as not to escape.
24. Carry out regular checks for three days after captivity to make sure all prospective queen bees have hatched.

On the 4th day after the captivity, the future queen bee remains in a cage and can be taken.

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Figure 5. Grafting Results

Variable Observation

- On the 14th day (two days before the future Queen leaves the bowl), each pupa is moved to a colony that does not have a queen by inserting the pupa into the Queen's lair (queen cage)
- The Queen's variables were observed a few days later, covering the length, width and number of abdominal segments.
- Variable observations are made using the Queen being dissified first, then measured the length and width of her abdomen using the callipers
- The abdominal segment is observed directly by calculating the number of segments of the abdomen.

Data Analysis

The data obtained are tabulated and calculated the default values and deviations if there is a difference, followed by the Duncan double distance test. Complete RandomIzed Design linear model (RAL)as follows:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}(1)$$

Description:

Y_{ij} = observations on the treatment to-i and replay to the-j

μ = mean population (general middle value)

τ_i = the effect of treatment on-i

ε_{ij} = attempt error on treatment to-i and replay to the-j

Where (i = 1, 2,.., t dan j =1,2,…r)

RESULT AND DISCUSSION

The research was conducted at a grazing site in Argosuko Village, Malang District. Bee colonies are located in areas with the primary source of feed from waru plants, coffee, Leucaena glauca, tomatoes, papaya and shrubs. In Indonesia, the ideal environment temperature for bees growth is 26^0C. The previous research was conducted in Iran (Mahbobi, 2012) and Turkey (Okuyan, 2018). It has different condition of the environment. In Iran, the ambient temperature about 20^0C and Turkey, according to the territory, some territory in the winter up to -13^0C.

Figure 5. Research Sites

Environmental Conditions

Ambient air temperature and humidity are observed daily both inside and outside the colony box (Table 1). This condition significantly affects the daily life activity of bees. Based on Figure 1, the average temperature at the research site is suitable for honeybees' development. The availability of feed at research sites also affects the productivity and life of bees (Akyol *et.al*, 2008). The types of flowering plants containing pollen at the research site are quite diverse to be the natural feed of honeybees.

Table 1. Temperature and Humidity Data

	Temperature(°C)		Humidity (%)
	Out	In	
Average Up to	30,65	32,15	60,1
	0,58	0,89	0,85

The Length of the Queen's Abdomen from Grafting Larvae Of Different Lifespans

Observations of the Queen's abdomen's length developed from different larvae, and statistical analysis results are presented in Table 2.

Table 2 above shows that queens formed from larvae of different ages will produce significantly different abdominal lengths (P<0.05). The longer the lifespan of the larva, the less the length of the Queen's abdomen. The Queen formed from the larvae's lifespan of one day in real terms has a longer abdominal length than the Queen than the larvae lifespan of two and three days. The Queen of larvae lifespan of two days and three days statistically has an abdomen length that does not differ.

The difference in these observation results is caused by the feed that nurse bees give to larvae (Crenguţa *et.al*, 2011). The older the larvae, the more significant the portion of royal jelly as the main feed of larvae will be reduced and instead will begin to be given another feed in the form of bee bread (Graham, 1993).

The change in feeding will affect the development of larvae until they become new individuals (Arun, 2011)

Table 2. Observations results of Queen Bee Abdomen Length Research Results

Treatment	Average Abdomen Length (mm)
P_0	$9,21^{b}$±0,30
P_1	$8,98^{a}$±0,15
P_2	$8,88^{a}$±0,22

Description: different superscripts show real differences(P<0,05)

The Width of the Queen's Abdomen from Grafting The Age of Different Larvae

Observations of the Queen's abdomen's length developed from different larvae, and the results of statistical analysis are presented in Table 3. Table 3 above shows that statistically, the larva's age exerts an unreal influence (P>0.05) on the abdomen width of the Queen.

This means that the grafting queen of larvae of different ages produces the same abdominal width. This result is obtained because the bowl used for the manufacture of queens is made with the same diameter, so the Queen's development to the side depends on the bowl's size. The Queen formed of the same diameter will produce the same abdomen width.

Table 3. Queen Bee Abdomen Wide Observation Results Research Results

Treatment	Average Abdomen Width (mm)
P_0	$5,09\pm0,40^{ns}$
P_1	$4,97\pm0,23^{ns}$
P_2	$4,93\pm0,16^{ns}$

Description:ns = non significant

Number of Queen's Abdominal Segments from Grafting The Lifespan of Different Larvae

Observations of the number of abdominal segments of the Queen developed from different larvae, and statistical analysis results are presented in Table 4. Table 4 above shows that statistically, the larvae's age exerts an unreal influence (P>0.05) on the number of segments of the Queen's abdomen. This means that the grafting queen of larvae of different ages produces the same number of abdominal segments. This result is thought to have occurred because the Queen formed is a virgin queen who has not experienced a marriage in which the Queen's spermatheca pouch serves to store spermatozoa in an empty state.

According to Pavord (1975), the development of queen bees ranging from eggs to adults takes 15 days, and there are rings of 12 pieces, and the abdomen consists of 6 segments.

Table 4. Results of The Number of Queen Bee Abdomen Segments *Research Results*

Treatment	Average Number of Abdominal Segments
P_0	$5,80\pm0,42^{ns}$
P_1	$5,70\pm0,48^{ns}$
P_2	$5,70\pm0,48^{ns}$

Description:ns = non significant

CONCLUSIONS

Queen bees that develop from worker larvae aged 1 to 3 days can produce individuals with morphometrics (width and number of abdomen segments) relatively the same. However, the abdomen length of worker larvae age 1 day has a significant result.

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