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## RESEARCH ARTICLE

### PERFORMANCE ASSESSMENT OF HONEYBEE (*APIS MELLIFERA BANDASII*) QUEENS REARED BY DIFFERENT QUEEN REARING TECHNIQUES

\*Tadele Alemu, Zewdu Ararso and Gemechis Legesse

Oromia Agricultural Research Institute, Holeta Bee Research Center, P. O. Box 22, Holeta, Ethiopia

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#### ABSTRACT

The experiment was conducted to assess the performance of honeybee (*Apis mellifera bandasii*) queens reared through different queen rearing techniques at Holeta Bee Research Center. A total of 20 local honeybee colonies with first super and uniform strength were selected and assigned randomly to splitting, Miller, overcrowding, grafting, and natural cell cup of queen rearing techniques. The success rate of the tested queen rearing techniques showed remarkable variations. But, those colonies assigned to overcrowding method did not totally respond. Brood area, brood solidity, swarming tendency and honey yield for colonies resulting from different queen rearing techniques were also evaluated. The result revealed that the highest hatchability was for the grafting and natural cell cup while the highest proportion reaching nuclei colony formation being for grafting. However, the highest rate of young queens starting egg laying was observed for those which were reared using splitting technique. The result also showed that, there was a significant ( $p < 0.05$ ) difference in the activity of brood rearing among the rearing techniques over the active seasons. Accordingly, Miller technique in the September-November active season exhibited significantly ( $P < 0.05$ ) higher brood rearing compared with the rest techniques except that of splitting. The solidity of brood of honeybee colonies reared by the four queen rearing techniques had no difference in the count of the empty cells in brood nests rather it was affected by the interaction of the rearing techniques and breeding seasons. Similarly, the number of queen cells constructed during brood rearing season showed no variation among colonies reared through different queen rearing techniques as well as between the two breeding season. Moreover, the variation in honey yield was not significant, it was comparatively better for the colonies obtained through splitting technique. Therefore, splitting can still be a useful technique as it doesn't demand additional equipment and facilities like that of the other queen rearing techniques.

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## INTRODUCTION

The honeybee queen is the key to success for both the colony and the beekeepers (Al-Fattah *et al.*, 2011) as it is the mother of the entire colony and the behavioral and general qualities of a particular queen are expressed in every one of her offspring. These traits can have profound effects on the behavior and health of the colonies. As a result the general colony performance like disease resistance, prolificacy and early population build up, surplus honey storing tendency and many behavioral characters are attributed to the nature of a queen (Morse, 1979). Buchler *et al.* (2013) indicated that use of standard and high quality queens is a prerequisite for any activities made on colony development as well as for economically successful beekeeping.

\*Corresponding author: Tadele Alemu,  
Oromia Agricultural Research Institute, Holeta Bee Research Center,  
P. O. Box 22, Holeta, Ethiopia.

It is generally believed that a queen of high quality should have high body weight, large number of ovarioles and large size of spermatheca (Carreck *et al.*, 2013) and high number of spermatozoa (Human *et al.*, 2013). This is very much dependent on the early growth pattern and physiological and anatomical developments that makes her pre-adult stages which is heavily reliant on the nourishment and total care she receives from nurse bees (Corona *et al.*, 1999; Evans and Wheeler, 1999; Barchuk *et al.*, 2007; Kamakura, 2011; Yamanaka and O'Connor, 2011; Cameron *et al.*, 2013; Crailshem *et al.*, 2013; Abbasi *et al.*, 2015). Several queen rearing techniques have been designed and developed for European evolved honeybee races. However, all these techniques are not equally suitable to all honeybee races all over the world (Morse, 1979; Disselkoe, 1988; Dodologlu *et al.*, 2004). Accordingly, few different queen rearing techniques have been tested for Ethiopian honeybee races and their response has been well documented (Nuru and Dereje, 1999; Zewdu *et al.*, 2013). But, the performance of the queens reared by these different

techniques has not been evaluated. Therefore, the aim of this study was to assess the performance of honeybee (*Apis mellifera bandasii*) queens reared by different queen rearing techniques.

## MATERIALS AND METHODS

The study was carried out at Holeta Bee Research Center from May 2011 to June 2013. For this study, 20 local honeybee colonies with uniform strength in Langstroth hive having one super box were used. These colonies were randomly allocated to splitting, Miller, overcrowding, grafting and natural cell cup queen rearing techniques.

### Queen rearing techniques

#### *Splitting*

Rearrangement of resources and insertion of queen excluder for split colonies was made one day in advance to splitting. Those combs with eggs, appropriate larvae, pollen and honey were situated in the super above the queen excluder. The next day, splitting of the colonies was done carefully with much proportion of worker bees in the queenless super box by forcing them using smoke through the entrance. Subsequently, the queen right splits were moved 1 km away from the original place, whereas the queenless splits were left in their original places.

#### *Miller*

In a Miller method new foundation sheets were given to selected mother colonies before a week to get newly laid eggs and young larvae. Dequeening and eliminating of uncapped brood for colonies assigned to Miller technique as starter were made one day in advance to inserting the eggs and young larvae from the mother colonies. Accordingly, three to four brood combs with eggs and appropriate larvae were chosen and taken from the colonies selected for mother colonies. Those selected brood combs were cut in a zigzag manner in order to expose the appropriate larvae at the edge of the combs. Then, the cut combs were immediately given to the dequeened colonies.

#### *Overcrowding*

Colonies assigned to this technique were overcrowded by reducing the supers as well as by preventing supering when they actually required additional space. Inspection was made nine days after removing super to observe the construction of queen cells.

#### *Grafting*

To rear queens using grafting method three colonies were made as starter colonies by dequeening them a day before grafting. After twenty four hours, the combs with young larvae were chosen and taken from the colonies selected for mother colonies. Twenty larvae were grafted in to 20 plastic cell cups and fixed on cell bars facing the cells upside down and given back to the starter colonies.

#### *Natural cell cup*

Like that of grafting, to rear queens by natural cell cup three queenless starter colonies were made a day before preparing the strips. Strips of cells containing twenty young larvae were prepared in the laboratory by destroying two cells and leaving every third cells intact on the strips. Then, the strip was fixed by tying on cell bar using frame wire facing the cells upside down just like that of grafting and given to starter colonies for rearing. Observations were made on the third day to count the number of larvae accepted for each technique.

#### **Finisher colony preparation**

Three queen right colonies with super box and queens restricted to brood chamber with queen excluder were prepared as finisher colonies a day before harvesting matured pupae from colonies of each technique. On the ninth day, number of queen cells constructed from each technique was recorded and sealed queen cells were cut and taken to the queen cages. Then, the pupae in the cages were given back to the finisher colonies for incubation until hatching. Data on number of harvested pupae, hatched out virgin queens, established nuclei colonies and queen started egg laying for each technique was recorded.

#### **Nuclei colony preparation**

Nuclei colonies were formed for the hatched out virgin queens from all techniques. Accordingly, one frame of sealed brood, pollen and nectar comb were given for each prepared nuclei boxes. Moreover, a good balance of young and flying workers bees were poured in to the formed nuclei boxes until the frames were well covered with bees. Two additional frames of foundation sheets were given to the formed nuclei boxes to keep the brood nest temperature. One of the newly emerged queens in cages were placed inside each of the prepared nuclei colonies for 24 hours so that the worker bees could recognize her before finally released.

#### **Brood area, brood pattern and swarming tendency evaluation**

The total brood area was measured every month of first (September to November) and second (April to June) active seasons to determine the brood population using 5cm x 5cm gridded wooden frame by putting it over each side of the brood combs. The total brood unit areas were calculated from the area occupied by the brood. Besides, brood pattern was also measured by inserting cardboard that delimits 100 cells over a section of sealed brood. The percentage of brood pattern was calculated by subtracting empty cells from that of restricted 100 cells (Delaplane *et al.*, 2013). Swarming tendency of the colonies was evaluated by counting the number of queen cells constructed from all colonies under the study. The counted cells were removed instantly to avoid double counting on subsequent observations.

#### **Honey yield evaluation**

In the study area honey was harvested during both first and second active seasons. The major honey harvesting month was

in June followed by minor harvesting in November. To obtain honey yield data, frames with sealed honey combs were taken out of the hives and each frame with sealed honey was weighed before the extraction with centrifugal honey extractor. Then, the empty frames after extracting the honey were weighed. Finally, net honey yield data was obtained by deducting weight after extraction from the weight before the extraction.

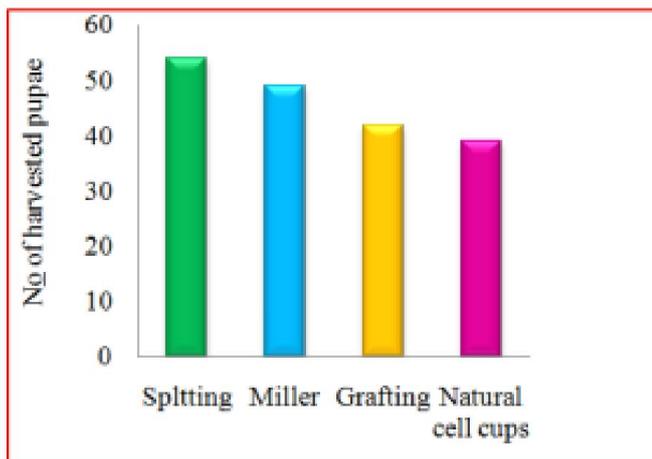
### Data Management and Statistical Analysis

Data were entered into computer using Microsoft excel and data analysis was carried out using the descriptive statistical package. Data were analysed by General Liner Model (GLM) SPSS version 20. LSMEANS at 5% significant level was used for comparison of means.

## RESULTS AND DISCUSSION

### Response of colonies to different queen rearing techniques

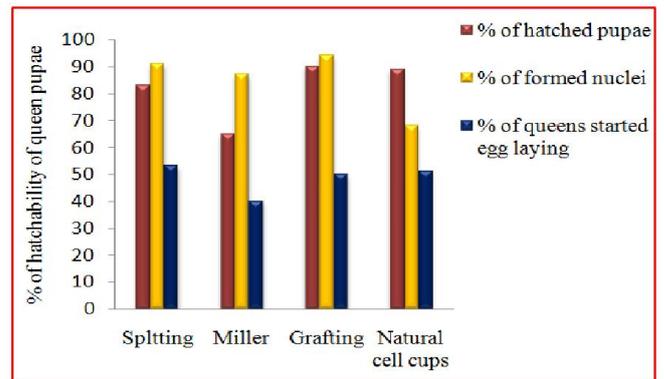
The result indicated that colonies induced to different queen rearing techniques in general produced mature queen pupae. However, those colonies assigned to overcrowding method did not respond during the study period. This result is in consent with report by Nuru and Dereje (1999) that indicated raising queens using overcrowding technique may not always give good responses. Weiss (1983) also indicated that weather, nectar and pollen flow conditions influence the reproductive instinctive behavior of the honeybees. The highest number (54) of queen pupae were harvested from splitting technique while the least (39) is from natural cell cup (Fig. 1). The low response of natural cell cup could be attributed to some factors such as mechanical damage to the larvae during preparation of strips and the age variation of the larvae that were offered to starter colonies that led to higher rejection.



**Figure 1. Production of mature queen pupae from colonies under different queen rearing techniques**

Like that of the number of queen pupae, there was a variation in the percentage of the hatchability of the pupae among the techniques. The efficiency of hatching queens was 90%, 89%, 83%, and 65% for grafting, natural cell cups, splitting and Miller, respectively (Fig. 2). This indicates that percent hatchability of virgin queens was higher for grafting and

natural cell cups. Even though higher percentage of hatched queens was obtained from grafting and natural cell cups, more (53%) virgin queens reached egg laying stage for splitting technique and the least (40%) was observed for Miller technique.



**Figure 2: Percent of hatched pupae, formed nuclei and queen started egg laying for different queen rearing techniques**

Splitting is found to be a useful queen rearing technique due to not only the more percent of virgin queens reared through this method reached egg laying stage but also does not require much facilities as that of grafting (Nuru and Dereje, 1999). It only requires rearrangement of resources and insertion of queen excluder one day in advance of splitting. Specifically, in the Miller method new foundation sheet should be given a week before to selected mother colonies to get newly laid eggs and young larvae. Starter colony formation for grafting and natural cell cup techniques should take place 24 hrs before larva is given to formed starter colonies since colonies do not recognize the given larvae at that moment and even they removed out the larvae from artificial cell cups (Nuru and Dereje, 1999). Also, in grafting process of transferring one day old larvae from the worker cell to the artificial cell cups requires materials like grafting tool, cell cups, cell bar, cold light or magnifying glass and royal jelly (Ratnieks and Nowogrodzki, 1988; Johnstone, 2008; Knoxfield, 2008; Buchler *et al.*, 2013). Similarly, preparation of strips of cells containing young larvae has to be conducted in the laboratory in the case of natural cell cup technique (Zewdu *et al.*, 2013). However, splitting almost doesn't demand additional equipment. Therefore, with its higher fertile queen production rate, it is a better rearing technique especially for resource poor and less skilled local beekeepers.

### Brood area, brood pattern and swarming tendency evaluation

The Two-Way General Linear Model (GLM) computed on brood area and brood solidness data from the four treatments showed that there was an interaction between treatments (rearing techniques) and seasons at p values equals to 0.004 and 0.0142 for brood area and brood solidness, respectively. Nevertheless, neither the main effects (the treatments and the seasons) nor their interaction was significant for the number of queen cell constructed. General Linear Model (GLM) analysis of the brood area, brood solidness and queen cells constructed of resulting colonies from four rearing techniques during spring (September to November) and autumn (April to June) is depicted in Table 1.

**Table 1. Average brood area, empty cells and number of queen cells performances in two breeding seasons of colonies reared by different queen rearing techniques**

Breeding seasons	Treatment	Brood area	Empty cell per 100 cells	No of queen cell constructed
Spring	Splitting	7083.33±757.92 <sup>ab</sup>	9.00±1.71 <sup>ab</sup>	0.17±0.10 <sup>a</sup>
	Miller	8371.43±701.70 <sup>a</sup>	7.93±1.59 <sup>b</sup>	3.00±0.92 <sup>a</sup>
(September to November)	Grafting	5330.14±701.70 <sup>bc</sup>	6.36±1.59 <sup>b</sup>	0.86±0.92 <sup>a</sup>
	Natural cell cup	3407.14±701.70 <sup>cd</sup>	12.57±1.59 <sup>a</sup>	1.29±0.92 <sup>a</sup>
Autumn	Splitting	3233.25±656.38 <sup>d</sup>	1.62±1.48 <sup>c</sup>	0.63±0.86 <sup>a</sup>
	Miller	2525.00±587.08 <sup>d</sup>	1.90±1.32 <sup>c</sup>	0.70±0.80 <sup>a</sup>
(April to June)	Grafting	1889.14±701.70 <sup>d</sup>	5.79±1.59 <sup>bc</sup>	0.71±0.92 <sup>a</sup>
	Natural cell cup	2697.86±701.70 <sup>d</sup>	1.33±1.59 <sup>c</sup>	1.00±0.92 <sup>a</sup>

Values followed by different letters within a column are significantly different at  $\alpha < 0.05$

The largest brood area was recorded in queens reared through Miller followed by splitting during the spring. But brood area difference between the two techniques was non significant ( $p < 0.05$ ) rather the results were significant compared to the same techniques in autumn as well as to the rest two techniques in spring. On the other hand, the least brood area was obtained from colonies reared through grafting technique. Though this was with the smallest brood area, it was not statistically significant ( $p < 0.05$ ) from the colonies obtained through splitting, Miller, and natural cell cup in autumn, and natural cell cup in the spring. Therefore, these results suggest that queens reared by Miller and splitting techniques during spring displayed better brood rearing activity. The current result is also in line with the previous findings of Nuru and Dereje (1999). This is due to the availability of adequate pollen and nectar producing honey plants in the first active season while there are only few honeybee forages in the second active season. During first active season, a lot of potentially pollen and nectar producing plant species such as *Trifolium species*, *Bidens species*, *Ceolasia argentea*, *Guizotia scabra*, *Vicia faba*, *Plantago lanceolatum* and, different grasses and weeds were blooming, whereas only *Eucalyptus globules* was flowering in the study area during the second active season.

The quantity of brood area reflects the rate of population growth that can be used to anticipate the size of adult honeybee population in the future (Harbo, 1993). Emsen (2006) also indicated that estimating of the colony population development is the most important parameter to be considered in any activities of honeybee colonies that can be evaluated through total brood area measurement. In addition to brood area, brood pattern is also one of the important parameter used to determine the strength and well beings of honeybee colonies which depends on the quality of honeybee queens. The solidity of brood of honeybee colonies reared by the four queen rearing techniques had no difference in the count of the empty cells in brood nests rather it was affected by the interaction of the rearing techniques and breeding seasons. Accordingly, the highest counts of empty cells were obtained from natural cell cups followed by splitting techniques during spring breeding season. This record was 7.93 and 6.36 for Miller and grafting in spring, respectively. All the techniques in the autumn breeding season fall in a very good brood pattern (Laidlaw, 1979). Fewer than 11% brood solidness that expressed as percent of empty

worker cells in a brood patch of 100 cells is considered as very good brood pattern (Laidlaw, 1979). According to Delaplane et al. (2013) the acceptable level of empty cells is typically less than 10%. Therefore, this result indicated that only the natural cell cup technique failed in unacceptable level of brood solidness. Similarly, number of queen cells constructed during brood rearing season showed no variations among colonies reared using the four queen rearing techniques as well as between the two breeding seasons (Table 1).

### Honey yield evaluation

Honey yield from the colonies reared using different queen rearing techniques showed that there was no significant yield variation among the techniques (Table 2). However, it was comparatively better for the colonies obtained through splitting technique. In a given apiary honeybee colonies might produce more honey than others within uniform environmental conditions and type of flora. This production differences can be due to variations in the strain of bee and the quality of the queen in the individual colonies (Knoxfield, 2008).

**Table 2. Average honey yield in kilograms per colony reared by the different queen rearing techniques per season**

Queen rearing techniques	Honey yield (Kg)
Splitting	5.1±1.0 <sup>a</sup>
Miller	3.8±1.8 <sup>a</sup>
Grafting	4.8±0.3 <sup>a</sup>
Natural cell cup	3.2±0.6 <sup>a</sup>

Because colonies headed by high quality queens can build adequate foragers that enable the colonies to store surplus honey. Hence, a high egg laying queen is required to sustain a large number of worker bees. The larger the population of worker bees, the higher is the number and proportion of foragers that serve as taskforce to bring in nectar and store it as honey.

### Conclusions and Recommendations

The colonies from queens reared through the four rearing techniques had performed differently in terms of matured pupae production, percent hatchability of ripen pupae, percent of virgin queens started egg laying, brood area and brood percent of brood solidness. Our results demonstrated that splitting and Miller displayed relatively higher number of matured pupae production whereas the highest rate of young queens starting egg laying was observed for splitting. Similarly, better brood rearing activity was recorded for queens from Miller and splitting during the first active season (September to November) and the percent of their brood solidness was within the acceptable range. The number of queen cells constructed during brood rearing season showed no variations among colonies reared through different queen rearing techniques as well as between the two breeding seasons. Moreover, the variation in honey yield was not significant, it was comparatively better for the colonies obtained through splitting technique. Therefore, for these facts rearing queens using splitting during the spring could be forwarded as a better technique for resource poor and less skilled local beekeepers.

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