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Honeybee Queen Rearing: A Review of research (2000-2019) in Ethiopia

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In beekeeping, honeybee queen has significant effect on several production and productivity traits. Artificial queen rearing techniques are among the important approaches to help to produce of queens with desirable characters in beekeeping. The aim of the present review of literature is to assess progress of past research and identify gaps in artificial honeybee queen rearing. In Ethiopia, several studies have been conducted on various aspects of honeybee queen rearing. A number of queen rearing methods were evaluated and recommended for use. However, little or nothing is known on other important attributes of honeybee queen rearing like queen quality and fecundity.It is very much important for the future research works to focus 1st on how to be very sure that honeybee queen rearing is possible with all races of Ethiopian honeybee and 2nd test the produced queens on their quality and fecundity aspects.

Keywords: Honeybee, Queen rearing, Queen rearing method, honeybee larvae, honeybee pupae

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Introduction

In beekeeping, a honeybee queen has significant effect on several production and productivity traits such as disease resistance, prolificacy, and early population build-up, surplus honey storing tendency, and many other behavioral characters. Artificial queen rearing techniques are among the important approaches to help to produceof queens with desirable characters in beekeeping.

Queen rearing is one of the major objects of apiaries especiallyfor the commercial beekeepers, and it is themain factor to besuccessful in beekeeping (Morse, 1994). Rearing honeybeequeens occurs when the colony is in the process of swarming,supersedure or when the queen has been accidentally lost orkilled (Seeley, 1985). Although the rearing of queen bees canbe performed in the presence of the queen in a nurse colonyhowever higher effect can be achieved in queenfewercolonies (Morse, 1994; Crailsheim, *et al.,* 2013) and theabsence of emergency queen cells (Free *et al*., 1987). In allthese cases, adult workers rear new queens from worker larvaethat are less than 48 hrs old (Haydak, 1943). For successfulmanaging and rearing of queen bees, it is imperative to adopt beekeeping measures for colony development. Undertemperate conditions, the colony brood rearing cycle is characterized by complete cessation of brood rearing in thelate fall and reduction of colony size during the winter (Avitabile,1978). Limited brood rearing is initiated already during wintermonths and brood-rearing leading to colony expansion is ofteninitiated before nectar and pollen become available (Seeley,1978).

The highest number of queen cells is achieved by usingroyal jelly in July and August (Genc *et al.,* 2005). Queen beescan be reared fromthe end of March to September, but better quality of queens isobtained from the end of March until the end of April, (Koc and Ka-racaoglu, 2004). The acceptance and the ratio ofqueen emergence arehighest using royal jelly as the graftingsubstrate (El-Din, 1999).

In tropical or subtropical climates, where honey bees can rear brood continuously throughout the year, data on colonydevelopment is readily available. Compared to honey bees intemperate climates, colonies may respond more rapidly withincreased brood rearing when foraging conditions becomefavorable (Rinderer and Hellmich, 1991) Severalimprovements have been made in Doolittle’s grafting method.This method has been challenged as producing queens ofinferior quality to those produced from the egg. Several work of rearing honeybee queen artificially has been underway in the Ethiopian research system for the last couple of decades.Researchershave produced queens from eggs. However, no convenientand economical method has been developed. Consequently,the present work aimed to review past researches and gaps in artificial honeybee queen rearing in the Ethiopian research system.

Honeybee queen rearing methods and queen rearing success

The study indicated that colonies induced to different queen rearing techniques, in general, produced mature queen pupae (Tadele Alemu et al,2015). However, colonies assigned to overcrowding method did not respond well. Nuru and Dereje (1999) also argued that 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. 80-90 % of queen pupae were harvested fromsplitting, grafting, and natural cell cups techniques while the least 60-70 % was from Miller queen rearing technique (Fig. 1). The low response of Miller method could be attributed to some factors such as mechanical damage to the larvae during preparation of strips that led to higher rejection. Even though higher percentage of hatched queens was obtained from splitting, grafting, and natural cell cups, more virgin queens reached egg-laying stage for splitting technique only.

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 manyfacilities 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 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, the 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.

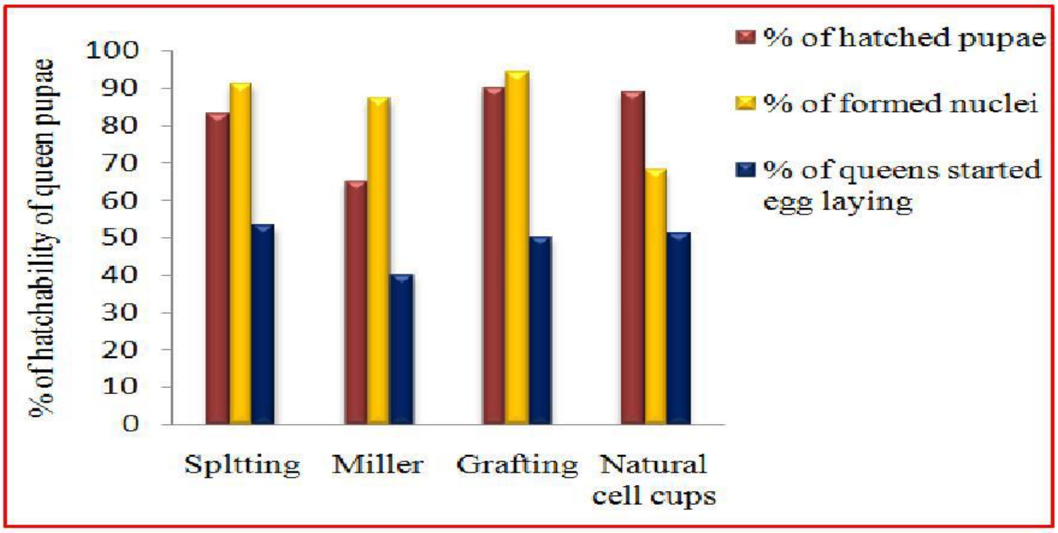

Figure 1: Percent of hatched pupae, formed nuclei, and queen started egg-laying for different queen rearing techniques (Tadele Alemu,2015)

The difference in honeybee queen rearing techniques has a profound effect on the success of honeybee queen rearing. Thesignificantdifference (*P < 0.0001*) was reported by Kibebaw Wakjira et al (**2019**) that sealing of queen cells when reared with Karl Jenter and Doolittle queen rearing techniques in Ethiopia. Analysis of the data (Fig 2) indicated that the percentsealing of cell cups from Karl Jenter was 42.75 while it was only 25.56 from grafted cell cups in Doolittle grafting out of the total 1600 larvae provided for each system. However, similar study reported no significant differences between the two techniques with regards to sealing of the queen cell cups based on accepted larvae (compare 54.24 and 53.93 for Karl Jenter and Doolittle grafting, respectively). The similarity in sealing level of queen cell cups from Karl Jenter and Doolittle grafting based on accepted larvae can be explained by several factors of which the amount of royal jelly produced by nurse bees to feed the larvae, number of available worker bees for nursing, nutritional quality of pollen and race of the bees may contribute and these required to be investigated.

In this study, sealing rate of the queen cells both on the basis of given and accepted larvae was generally lowercompared to previous reports. For example, Dhaliwal *et al.,* (2017) reported that sealing of cell cups ranged from 50.67% to 60.67% based on total cell cups given while it was ranged from 88.57 to 97.50% based on cell cups accepted for different queen rearing techniques (Emsen, B. 2006). Similarly, N. Adgaba *et al.,* (2018) reported that 71.84% of sealed larvae (from the total grafts) into pupae stage in wet grating for *A. mellifera jemenitica* in Saudi Arabia (Corona, M. et al, 1999).

Generally, indifferent queen rearing techniques, the rate of sealing queen cells is reported to be varied. But besides the types of techniques used, the population size of the colonies, the number of food resources available for the bees during the

breeding seasons, races of the bees were indicated as some of the factors to influence different parameters in queen rearing [15-17]. Therefore, the relatively low rate of sealed queen cells for both queen rearing techniques in this study could be one of or the combination of these factors and this suggests the importance of further investigations to determine the important factors that affect raising and sealing of queen cells in different queen rearing techniques under local conditions for different honeybee races.

The rate of hatching (out of the total given larvae) into the virgin queen stage in Karl Jenter and Doolittle graftingsystems were about 23 and 23.8%, indicating no significant difference between the two techniques (Fig. 2). However, the variations in queen emergence rates based on accepted larvae and sealed queen cells were found to be very significant ($P<0.001$). Accordingly, the emergence of queen bees based on accepted larvae and sealed queen cells were 29.22 and 55.71%, respectively for Karl Jenter while the corresponding rate of emergence for Doolittle grafting was46.47 and 86.68%, respectively indicating significant differences between the two methods. Dhaliwal *et al.,* (2017) reported that the rate of emerged queens based on accepted cells for different rearing techniques was significantly different [15]. According to the report by Dhaliwal *et al.,* (2017), the emergence of queen bees in the Cupkit apparatus and plastic cell cups were 83.28 and83.34%, respectively, while the respective rates for KarlJenter apparatus and wax cell cups were 52.20 and 54.73% inthat order [15]. In another report by Cengiz, Emsen, and Dodologlu (2009), 100.00% rate of queen bee emergence were recorded in queenright and queen less colonies for thegrafted larvae raised with the Doolittle method [18]. Similarly,ÖNK *et al.,* (2016) reported 100% queen bee emergencerate of accepted larvae for Caucasian race of A. mellifera honeybees [19]. the study indicated that sealing of queen cells and the emergence rate of queen bees on the basis of accepted larvaewas significantly ($P<0.001$) higher for Doolittle grafting compared to Karl Jenter system. However, the larval acceptance rate was significantly ($p<0.001$) higher for KarlJenter system compared to Doolittle grafting method (Fig 2). On the other hand, the queen emergence rate for the two techniques based based on given larvae was similar, indicating that accepted larvae were less successfully sealed and converted into virgin queen stage in Karl Jenter than Doolittle grafting. Therefore, both systems can be practiced for rearing queen bees as the number of queen bees obtained is similar. Though the beesresponded to the two methods similarly, the percentemergence was low for both methods. This could be due toenvironmental factors such as humidity and temperaturewhich may negatively affect the rearing colony and/or thefeed supply of the nurse colony. So, this should be the subjectof future investigation to identify important factors that affectdifferent queen rearing parameters, which result in low rateof queen emergence. If percent emergence of queen bees based on given larvae improved, at least Karl Jentersystem can be used to yield higher number of queens. If so,the method can be an excellent option for those who facedifficulties in identifying appropriate larval age and lack skillin grafting can opt for commercial queen bee rearing.However, there might be a difference in quality of the queens obtained from these two methods. Therefore, further study should be conducted to evaluate the performance of queens reared using the two techniques.

shortage. The most and foremost important thing that we have observed from this experiment is that colony management played a significant role.

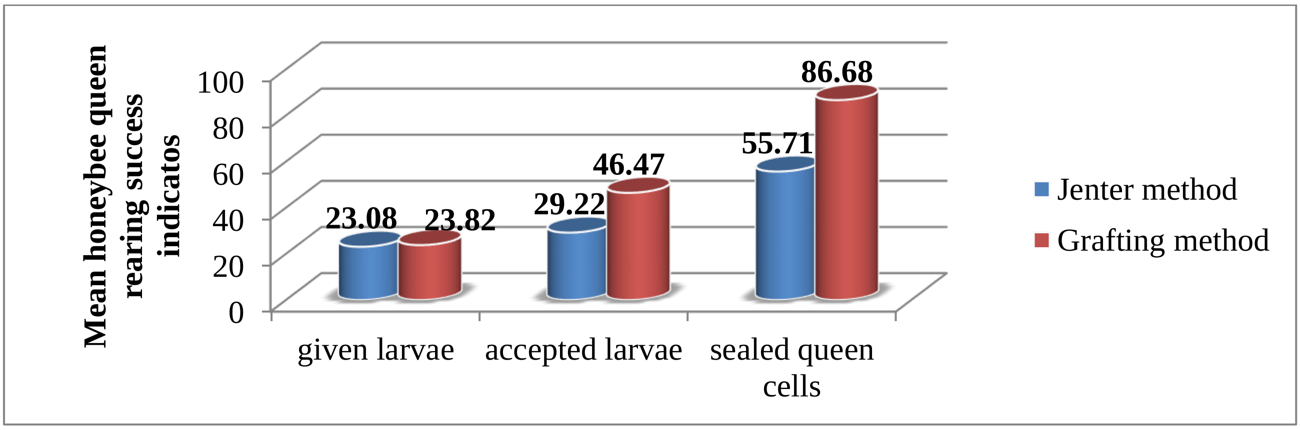

Figure 2: Mean comparison of different queen rearing success indicators of queen rearing practice using Karl jenter and Doolittle Grafting queen rearing methods(Kibebaw Wakjira et al.2019)

According to Abebe Jenberie Wubie (2014),*local honeybee colonies in the Waglasta have been found to emerge an average of 8.6 queen cells with a maximum and minimum of 15 and 5 queen cells respectively. The author has argued the possibility of queen rearing with local honeybee colonies.*

Splitting Honeybee as the best honeybee queen rearing technique for the local beekeepers

Splitting of honeybee coloniesis the process of rearrangement of hive resources and insertion of queen excluder for split colonies. Those combs with eggs, appropriate larvae, pollen, and honey are situated in the super above the queen excluder. The next day, splitting of the colonies is done carefully with much proportion of worker bees in the queenless super box by forcing them to usesmoke through the entrance. Subsequently, the queen's right split is moved 1 km away from the original place, whereas the queenless splits remain in their original places.

The basic sources of bee colonies for beekeeping beginner farmers are those wild bees (feral bees) found in areas where human rich is limited due to different reasons. Therefore,beekeepers are expanding their apiaries and number of bee colonies simply by following the bees' natural reproduction pattern. At present, due to various factors, the honey bee colony population is in a state of continuous decline. Absconding is also becoming amajor problem in beekeeping development and is known to be a peculiar characteristic of the present-day beekeeping. As the result, it is becoming more difficult to obtain adequate swarms every year to start and expand an apiary (Nuru and Dereje, 1999). In most cases, farmers are also complaining that they are facing serious shortage of honeybee colonies.

Moreover, there is a high demand for honeybee colonies mainly due to the involvement of individuals and different NGOs in beekeeping development. Consequently, the price of honeybee colonies is becoming too costly for beekeeping farmers to expand their bee farms and engage in beekeeping, even though it can be used as an income source for the others (Nuru and Dereje, 1999; Abebe, 2008).

In the area, where reproductive swarming tendency is low, one of the major problems of apiculture is obtaining swarms either to start or to increase the existing stock. Thus, development, verification, and demonstration of simple ways of colony multiplication skills would be very important (Nuru and Dereje, 1999) and of these, splitting queen-rearing method have been rated as better in its simplicity for local conditions.

However, honeybees have their breeding strategies which are very dependent on the potential of the queen and the general activeness of the worker bees in a colony (Laidlaw *et al.,* 1962; Dan, 2006). These days, man has highly involved in facilitating and managing the breeding strategies of the colony (Snelgrove, 1981). Of course, there are different preconditions for successful queen breeding such as the presence of enough flowering plants, availability of freshwater, presence of plenty of young bees brood and store at exactly the right time, observed good behavior of the colony, the productivity of the colony, less tendency towards swarming, good record of gentleness, status of worker bees in nectar and pollen collection, availability of drones, availability of day-old eggs, availability of necessary beekeeping accessories and equipment (Jay, 1923; Laidlaw *et al.,* 1962; Snelgrove, 1981; Dan, 2006).

Even though the commercial lifespan of honeybee queen is two years, beekeepers in the area are not replacing their queens at this stage; rather they merely leave the colony to replace the queen after she ends her actual life span through the natural queen rearing process (Jim Cameron, *et al*, 1984; Graham, 1992). Nevertheless, having an old queen could result in the decline of the colony's productivity since the old queen couldn't administrate the colony efficiently (Jay, 1923; Snelgrove, 1981).

Thus, mitigating the satisfaction of the increasing demand of honeybee population by different newly emerging stakeholders through increasing the declining honeybee population using artificial queen rearing techniques is very critical at this moment (Jim Cameron, *et al*, 1984). Several studies were conducted to verify and demonstrate the reliability of splitting queen rearing technique about honeybee colonies. It was possible to understand that, even in areas where honeybee feed shortage is paramount importance and prevalence of longer dearth period and absconding is a peculiar characteristic, colony multiplication could be done and used as a means of alleviating serious colony

Table 2: Total queen cells constructed, colonies adapted, absconded and honey produced

Name of Participant	Date of Splitting	Location	AEZ	Total queen cells developed	Total colonies absconded		Honey harvested before splitting	Honey harvested after splitting		
					Mother	Daughter		Mother colony	Daughter colony	Total
Endeshaw Endalew	11/1/2001	A	Midland	15	0	1	8	12	0	12
Mulugeta Asmare	12/1/2001			7	0	0	7	4	8	12
Getu Molla	11/1/2001			7	0	1	9	12	0	12
Molla Berie	11/1/2001			5	1	1	10	0	0	0
Tefera Ageze	11/1/2001			13	0	0	12	18	19	37
Sub Total				47	1	3	46	46	27	73
Average per Colony				9.4	0.2	0.6	9.2	9.2	5.4	14.6
Kinfe Gebeyaw	26/12/2000	B	Lowland	8	1	0	9	0	16	16
Hante Gebeyaw	26/12/2000			5	1	1	8	0	0	0
Tekle Dessie	26/12/2000			7	0	1	10	16	0	16
Gebremichael Gebreselasie	26/12/2000			9	0	0	12	15	4	19
Worash Gebeyaw	26/12/2000			10	0	0	12	20	18	38
Sub Total				39	2	2	51	51	38	89
Average per Colony				7.8	0.4	0.4	10.2	10.2	7.6	17.8
Grand Total				86	3	5	97	97	65	162
Overall Average per Colony				8.6	0.3	0.5	9.7	9.7	6.5	16.2

With this, comparison between honey produced from mother colonies before splitting and produced honey from both mother and daughter colonies after splitting has revealed that honey production after splitting has exceeded the production before splitting with a 27 and 38 kg of honey yield increase in locations A and B respectively with a total percentage increase of 40.12 (Table 3). This could confirm that splitting is not only able to increase the number of colonies but also the amount of honey produced after splitting. With this, comparison between honey produced from mother colonies before splitting and produced honey from both mother and daughter colonies after splitting has revealed that honey production after splitting has exceeded the production before splitting with a 27 and 38 kg of honey yield increase in locations A and B respectively with a total percentage increase of 40.12 (Table 3). This could confirm that splitting is not only able to increase the number of colonies but also the amount of honey produced after splitting.

Season and queen rearing in Ethiopia

Tables 1 and 2 illustrates rates of different queen rearing parameters of *A. mellifera* bandasii colonies to Jenter and

Grafting queen rearing techniques under two seasons. The difference between seasons in terms of larva acceptance and sealed queen cell rates was statistically significant ($P<0.01$).Larva acceptance and sealed queen cell rates were found to be higher in September and October than April and May. Similarly, Gene, Emsen, and Dodologlu (2005) showed that rearing seasons were found to be significant in affecting acceptance of larvae. This difference in performance level in different seasons may arise from the fact that different floral resources may be preferred by honeybees to focus more on breeding offspring in one season and to focus on honey storage in another season. Another explanation for lower acceptance in April and May could also be lower swarming tendency of the bees during this season under local conditions. However, the other queen rearing parameters were not influenced by the seasons. This result is in line with a previous study report by Nuru and Dereje (1999) on the responses of local honeybees to different queen rearing method

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 a few honeybees forages in the second active season. During the first active season, a lot of potentially pollen and nectar-producing plant species such as *Trifollium species,Bidens species, Ceolasia argentea, Guizotia scabra, Vicia faba,Plantago lanceolatum* and, different grasses and weeds were blooming, whereas only *Eucalyptus globules* wereflowering 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 parameters 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 expressed as a 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 an unacceptable level of brood solidness. Similarly, a 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).

Rearing Seasons	**Accepted larvae (%)**	**Given larvae (%)**	**Accepted larvae (%)**	**sealed queen cells (%)**
Sept - Oct	84.13±4.39a	50.13±5.53a	56.11±10.08a	56.36±12.41a
April - May	72.25±5.86b	35.37±7.00b	52.37±9.35a	55.07±15.57a
Overall	78.19±7.94	42.75±9.82	54.24±9.78	55.71±13.90
Sept - Oct	58.00±7.05a	28.50±5.98a	55.52±10.21a	90.83±14.50a
April - May	43.62±5.76b	22.63±3.58b	52.33±8.70a	82.54±17.32a
Overall	50.81±9.66	25.56±5.70	53.93±9.47	86.68±16.31
LSD	27.31	17.06	N.S.	30.97

Conclusion

Honeybee queen does significantly influence several production and productivity traits such as disease resistance, prolificacy, and early population build-up, surplus honey storing tendency, and many other behavioral characters. Artificial queen rearing techniques are among the important approaches to help to produce of queens with desirable characters in beekeeping.

In Ethiopia several studies have been conducted with pupae production, percent hatchability of ripening pupae, percent of virgin queens started egg-laying, brood area, and brood percent of brood solidness as a center of attention for research. In this fashion splitting and Miller's method of honeybee queen rearing has been demonstrated to relatively produce a higher number of matured pupae production whereas the highest rate of young queens starting egg laying was observed for splitting. However, little or nothing is known on other important aspects of honeybee queen rearing like queen quality and fecundity. Therefore, it is very much important for future research works to focus 1st on how to be very sure that honeybee queen rearing is possible with all races of Ethiopian honeybee and 2nd test the produced queens on their quality and fecundity aspects.

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