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Original article

The responses of *Apis mellifera jemenitica* to different artificial queen rearing techniques

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ABSTRACT

In the current study, we investigated if any variations exist in acceptance rate of grafted larvae and quality of queens reared in different queen cell cup sizes, between wet and dry grafting and between queen right and queen less conditions of *A. m. jemenitica* colonies. The acceptance rate of grafted larvae in different queen cell cup sizes (7.0 mm, 7.5 mm, 8.0 mm, 8.5 mm) were varying from 69 to 71% and the variations were not significant among the different queen cups sizes but averagely lower than the acceptances recorded for other races. Out of the 172 dry grafted larvae, only 56.4% of them were accepted while in wet grafting out of 174 grafted larvae 77.01% were accepted. Regarding the rate of sealing, 48.84% and 71.84% of them sealed for dry and wet grafts, respectively. The observed variation in the rate of acceptance and sealing were significant ($N = 346$, $df = 1$, $P < 0.0001$) between the two techniques. However, there was no significant difference in fresh weight of emerged queens between the two grafting methods. Out of the 324 grafted larvae given to queen right and queen less starter colonies each; 106 (32.72%) and 252 (73.68%) were accepted in queen right and queen less starter colonies, respectively and the variation was highly significant at $P < 0.0001$. The total number of sealed pupae were 82 (25.31%) and 216 (63.16%) for queen right and queen less colonies, respectively and the variations was significant at $P < 0.0001$. From the study it can be concluded that *A. m. jemenitica* colonies can rear significantly more queens under wet grafting and in queen less colonies conditions than dry grafting and queen right conditions

1. Introduction

Artificial honey bee queen rearing is one of the indispensable parts of beekeeping to regularly re-queen colonies, to minimize swarming tendency, to enhance brood and honey production, to increase colony number and to improve their genetic characteristics (Morse, 1979, 1994; Crane, 1990; Laidlaw and Page, 1997). Honey bee queen is an important part of a colony because it is one of the most important factors that determine the productivity's of a colony (Laidlaw, 1979; Morse, 1979; Ruttner, 1983). Moreover, many desirable traits of a colony like gentleness and disease resistance are governed by the nature of the queen (Morse, 1979; Ratnieks and Nowogrodzki, 1988) which generally indicates the importance of artificial queen rearing.

Indeed, over many decades' different queen rearing techniques have been developed to rear many queens from a single colony (Johansson and Johansson, 1973; Morse, 1979; Harry and Laidlaw, 1981; Ruttner, 1983). However, the studies were limited to certain races and mostly under temperate climatic regions.

The responses of colonies towards different queen rearing techniques are greatly varying from ecology to ecology and race to race of honey bees. The presence of response variations towards different queen rearing techniques due to the differences in environmental, behavioral and biological factors have been well reported (Morse, 1994; Nuru and Dereje, 1999; Nuru, 2012; Crailsheim et al., 2013). Moreover, climatic conditions like temperature, relative humidity and pollen source plants are known as important factors in determining the acceptance and quality of artificially

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reared queens (Zhadanova, 1967; Koç and Karacaoğlu, 2004; Cengiz et al., 2009). In this regard, the great influences of environmental factors and the presence of response variations among different queen rearing techniques were well reported (Wen-Cheng and Chong-Yuan, 1985; Morse, 1994; Dodologlu et al., 2004; Cengiz et al., 2009; Crailsheim et al., 2013).

In addition, Wilkinson and Brown (2002) reported the presence of significant variations in length of queen cells reared by queen less and queen right colonies and they suggested the importance of further investigations to determine if there is variation among races of *Apis mellifera* in their ability or inclination to rear queens in queen right and queen less colony conditions. Moreover, Büchler et al. (2013) reported that, the acceptance rate of grafted larvae is highly affected by the presence or absence of queens and methods of rearing.

Moreover, Skowronek and Skubida, (1988) showed that grafting was more successful in queen cups diameter of 7.8–9.0 mm than in larger cups (10–12 mm) for *Apis mellifera*. The presence of acceptance variations towards different artificial queen cups cells dimensions has been reported for *Apis cerana* (Abrol et al., 2005). This may indicate the importance of determining if any variations exist in acceptance rate of larvae grafted in different queen cup cells sizes.

Grafting with different approaches such as wet or dry grafting were tested by different researchers (Ratnieks and Nowogrodzki, 1988; El-Din, 1999; Büchler et al., 2013) and reported the high rate of acceptance and queen emergence using royal jelly as grafting substrate. Moreover, significantly higher morphological values were also recorded for queens reared from wet than dry grafting method (Kamel et al., 2013). However, Wilkinson and Brown (2002); Cushman (2013) reported a good acceptance rate from dry grafting. Under Saudi Arabia conditions where temperature is high and the relative humidity is very low, wet grafting may contribute for better acceptance of larvae which required to be investigated.

Grafting methods with queen less or queen right colonies were also tested and different performances were recorded for different queen quality parameters (Laidlaw, 1979; Laidlaw and Page, 1997; Emsen et al., 2003; Cengiz et al., 2009, Ahmad and Dar, 2013; Büchler et al., 2013). Generally, in different approaches the rate of acceptance and quality of the reared queens reported to be varied. Besides the types of techniques used, the status of colonies being populous (two or three story) with young worker bees covering the brood with sufficient food resources reported to influence the number and quality of the reared queens (Morse, 1979; Laidlaw, 1979; Wilkinson and Brown, 2002; Büchler et al., 2013). However, under local conditions the *A. m. jemenitica* colony size (strength) is generally low and averagely occupy a nest volume of 12.28 ± 5.98 L (Nuru et al., 2016) and mostly kept in less than 10 frame hives without super which is one of the challenge to directly adopt queen rearing techniques developed for other races; which requires all possible modifications for better acceptances.

In the current study, we compared the acceptance, sealed and emerged rate of grafted larvae and quality of queens reared by *A. m. jemenitica* in different queen cell cups sizes; between dry and wet grafting and also under queen right and queen less colony conditions under typical dry environmental conditions.

2. Materials and methods

2.1. Experimental colonies

The colonies considered in this study were at least phenotypically the endogenous honey bee race (*Apis mellifera jemenitica*), which are widely used by beekeepers throughout the study area.

2.2. The effect of different queen cell cup sizes on the acceptance rate of grafted larvae

Since *A. m. jemenitica* is reported as the smallest honey bee race in the *Apis mellifera* species, it was important to determine the suitable size of artificial queen cell cups diameter for the race. To determine the suitable diameter of the artificial queen cups of the race, first, the natural queen cups diameter of the race was determined through measuring the diameters of naturally built queen cells. For this purpose, 60 fully built natural queen cells were obtained from six different colonies. The natural queen cells were carefully cut with sharp blade at height of 6–8 mm from the base then the diameters of the cups at rim were measured and recorded. The natural diameter of the queen cups of the race was varying from 6.34 mm to 8.91 mm with mean of 7.72 mm ($N = 60$). Then, the data were categorized into three groups (small, medium and large sizes) with nearly equal frequencies. According to the categorized data, the average diameters of small, medium and large queen cell cups size groups were 7.0 mm, 7.5 mm and 8.0 mm, respectively. The average natural queen cell cups diameter of each category was taken as bases for production of artificial queen cell cups.

Then wooden dipping sticks with three different sizes that match with each queen cell cups diameter category were carefully prepared and used to produce artificial queen cell cups of each category. Along with the three cup diameter categories, the standard queen cell cup size (with diameter of 8.5 mm), which is widely used for commercial queen rearing purpose was also included as control and comparisons. Sufficient numbers of queen cell cups of each category were produced using pure beeswax. So, four different queen cell cup diameter categories (7.0 mm, 7.5 mm, 8.0 mm and 8.5 mm) were used to test if any variations exist in acceptance rate of grafted larvae among the different queen cell cup sizes. The testing was made on 12 colonies (4 colonies per batch) in three batches. For each colony 36 queen cups (9 cups from each size category) were given. The 36 cups (12 cups/bar) were fixed on three wood bars that hanged on standard Langstroth frames. The four types of cups were arranged on bars alternatively with equal probability of being in different positions of the bars and frame (Fig. 1).

A suitable age of larvae for grafting was determined by caging queens from selected colonies with full drawn-out worker's brood combs using queen isolator. First, the full drawn-out worker's brood combs were inserted in the middle of brood chambers to be polished by worker honey bees. On the next day, the queens were confined for 24 h with combs polished by worker bees then the queens were released. Grafting was done at fourth day of queen confining when sufficient 24 h old larvae were available. The prepared queen cup cells were also inserted in to de-queened colonies to be polished by worker bees 14–16 h prior to grafting.

Before introducing the grafted larvae to starter colonies, all combs with open broods (eggs and young larvae) were removed to avoid rearing of emergency queens from their own broods. For each queen cell cup size category, an average of 101.5 larvae and a total of 406 larvae were grafted.

Data on the rate of acceptance, number of sealed larvae and number of queens emerged and weight of newly emerged queens were recorded and compared among the cell cups sizes groups. Moreover, after the queens have emerged, data on the length of queen cells and the diameter of the tips of queen cells were recorded.

2.3. The effect of wet and dry grafting on the acceptance, sealed and emerging rates and weight of newly emerged queens

To test the effect of wet and dry grafting on the acceptance, sealed and emerging rate of grafted larvae, and weight of newly

Fig. 1. Arrangements of different size queen cups on wood bars and frame.

emerged queens, a total of 12 starter colonies in three batches (four colonies/batch) were used. For each colony between 24 and 36 grafts with equal proportion, a total of 346 queen cell cups were used. The cups were inserted in to de-queened colonies 14–16 h before grafting for polishing purpose. For wet grafting half of the queen cups were primed with 4 μl dilute fresh royal jelly of 1:1 (distilled water to royal jelly) ratio while the remaining half left for dry grafting. The fresh royal jelly was obtained from the same apiary in the same season through de-queening of some colonies. The royal jelly was kept in the refrigerator until it was used. The arrangement of the queen cups for dry and wet grafting was alternative, one after the other to be equally distributed in different position of wood bars and frames with equal chances. Then predetermined one day old larvae were grafted. The starter colonies were inspected on 3rd and 5th day of grafting and data on number of accepted and sealed grafts were taken. Then sealed pupae were caged and incubated in strong colonies two days earlier than the expected date of queen emergence. Finally, the numbers of emerged queens were recorded and their fresh weights were taken using sensitive electronics balance (Kern ABS, Kern & Sohn GmbH, Germany) with high precision (0.0001). Then the data were compared between dry and wet grafting.

2.4. Responses of A. m. jemenitica to rear queens under queen right and queen less colony conditions

To assess the possibilities of rearing queens in queen right colonies and to compare their responses with that of queen less colonies, a total of 18 colonies in 10 frame hives with a single super having relatively uniform strength were used. The test was done in three batches by assigning six colonies per batch. Half of the colonies in each batch were made queen less and the remaining half were left as queen right. The queens under queen right treatment colonies were confined at the base of the hives using queen excluder while the colonies in the queen less group were de-queened 14–16 h before the introduction of grafted larvae and in both groups, eggs and open broods were removed. For each colony, a day old 36 grafted larvae were given. Then the rate of acceptance, sealed pupae and emerged queens and fresh weight of the queens at emergence were recorded and compared.

2.5. Statistical analysis

The data was analyzed for the presence of significant variations in performances of colonies among the different treatment groups using ANOVA procedures. Moreover, pair-wise correlation and descriptive analysis were also conducted. Computations were made by JMP-5 statistical software (SAS, 2002) at 95% level of significance.

3. Results and discussion

3.1. Effect of different queen cell cup sizes on the acceptance rate of grafted larvae

The diameter of the natural queen cell cups of the *A. m. jemenitica* varied from 6.34 to 8.91 mm with mean of 7.72 mm ($N = 60$), which is relatively smaller than the queen cell cups of the European races which has been reported to be 8–9 mm diameter at rim (Coloss, 2017).

The general acceptance rate of grafted larvae in different queen cell cup sizes varied from 69 to 71%, which is relatively lower than the records of Guler and Alpay (2005) who reported an average of 75.83 ± 1.41% acceptance for different *Apis mellifera* genotypes. Moreover, Koç and Karacaoğlu (2004) reported 79.1–95.8% acceptance rate under Turkey conditions. In addition, the mean acceptance rate is lower than Wilkinson and Brown (2002) findings, who compiled 14 years of grafting data on colonies those have given 6666 grafted larvae, and they reported an average of 81% acceptance rate with range of 25–100% success variations indicating the presence of significant differences among colonies.

The rate of sealed larvae (from the total grafts) into pupae stage in different queen cell cup sizes was also varied from 56% to 64% and rate of emerging of queen was varying form 39–44%. However, the variations in acceptance, sealed and queen emergence rates that reared in different queen cell cup sizes were not statistically significantly different. The current result disagrees with the findings of Skowronek and Skubida (1988) who reported; the more acceptances of smaller queen cups with diameter of 7.8–9.0 mm than the larger queen cups with 10–12 mm diameters for *Apis mellifera*.

The absence of significant variations in acceptance rate of different queen cell cup sizes could be due to the fact that the bees can modify the rim of the cups according to their sizes (Fig. 2). On other hand, the bees may naturally tolerate wide range of cell cup sizes as it can be witnessed from the relatively wide range of natural queen cell cups diameters (6–9 mm) as observed in this study for *A. m jemenitica*.

The average length of sealed queen cells varied from 18.11 ± 2.60–18.44 ± 1.91 mm ($N = 146$) and there were no

Fig. 2. Showing how the *A. m jemenitica* modify (narrowing) the rim of the queen cell cups according to their size.

significant differences in the lengths of sealed queen cells, among the different queen cell cups diameter. The average length of matured queen cells recorded in this study (18.11 ± 2.60–18.44 ± 1.91 mm) was much shorter than the sizes recorded for European races 26.70–30.82 mm (Wilkinson and Brown, 2012); 24.80 ± 0.3 mm (Dodologlu et al., 2004) and 21.7 ± 0.27–25.1 ± 0.25 mm (Koç and Karacaoğlu, 2004) which could be due to the relatively smaller size of the *A. m. jemenitica*.

Moreover, the diameter of the tip of queen cells (after the queens have emerged) was averagely varied from 5.28 ± 0.61–5.46 ± 0.57 (N = 146) and the variation was not significantly different among different queen cell cup diameters. This result indicates that, even if the queen cell cups diameters were initially varying, there was no significant variation in the diameter of queen cells tips after queen emerged. The absence of significant variations in rate of acceptance, sealing, number of queens emerged, length of queen cells and diameter of queen cell tips could be due to the adjustments of the sizes of cells by worker bees to match the body size of the queens irrespective of the initial queen cell cups sizes.

The relatively low rate of acceptance of grafted larvae in this study could be due to their strength in which in most cases the colonies were in base hives with 10 frames only. In this regard, low rate of acceptance (33%) of grafted larvae as a result of small colony size (only 10 frames with 3 brood combs) was reported for hybrid of *Apis mellifera scutellata* and *Apis mellifera capensis* in South Africa (Wilkinson & Brown, 2002). Moreover, relatively dry weather conditions, limited nectar and pollen sources and short flowering duration may have contributed for the low acceptance rate. In this regard, the negative influence of higher temperatures and insufficient nectar and pollen resources on the acceptance rate of the grafted larvae and weight of queens at emergence is well reported (Zhadanova, 1967; Abdellatif et al., 1970). Factors such as: quality, strength and developmental stage of the nurse colonies, age of the workers, age of the grafted larvae, presence or absence of queen in the rearing colony and duration of the queen-less stage, presence of open brood in the cell-starting colonies, number of grafted cells, rearing sequence and method of rearing reported to affect the acceptance rate of grafted larvae (Ruttner, 1983).

3.2. The effect of wet and dry grafting on the acceptance rate and weight of newly emerged queens

Out of the 172 dry grafted larvae, only 56.4% of them were accepted while in wet grafting out of 174 grafted larvae 77.01% of them were accepted (Table 1). The variation in the acceptance rate was significantly different ($N = 346$, $df = 1$, $P < 0.0001$) between the two methods. Regarding the rate of sealing, in dry grafting out of 172 grafted larvae, only 48.84% of them sealed, while in wet grafting out of 174 grafted larvae 71.84% them were sealed. The observed variation in the rate of sealing of grafted larvae was significant ($N = 346$, $df = 1$, $P < 0.0001$) between the two techniques. The significantly higher acceptance and sealing rate of wet grafting over dry grafting could be due to the advantages of wet grafting in preventing the grafted larvae from desiccation due to the low humidity conditions of the area. Similarly, Ratnieks and Nowogrodzki (1988), El-Din (1999) and Büchler et al. (2013) reported the high rate of acceptance of grafted larvae using royal jelly as grafting substrate.

However, once the larvae were sealed; the rates of emerged queens were generally low in both techniques which were only 46.00% and 44.79% of total grafted larvae for dry and wet grafting, respectively. The variations in the rate of emerged queens were not significantly different between dry and wet grafting. The sharp declining of the rate of emerged queens was associated with the sudden occurrence of severe cold weather during testing period that affected the proper incubation of the sealed queen pupae. Moreover, the length of queen cells and diameter of the tips of queen cells were not significantly varied between wet and dry grafting. In addition, the average weight of newly emerged queens were 0.14 ± 0.03 g (N = 60) and 0.14 g ± 0.02 (N = 70) for dry and wet grafting, respectively. However, there was no significant difference in fresh weight of emerged queens between dry and wet grafting methods. However, Genc et al. (2005) reported better quality queen bees using royal jelly into queen cell cups than dry grafting.

3.3. Response of the local bees to rear queens under queen right and queen less colony conditions

Out of the total 324 grafted larvae given to the queen right starter colonies, only 106 (32.72%) were accepted. While in the case of queen less starter colonies, out of 342 grafted larvae a total of 252 (73.68%) were accepted (Table 2). The variation in the acceptance rate of grafted larvae between queen right and queen-less colonies was significant at $P < 0.0001$. In queen right starter colonies from the total grafted larvae only 82 (25.31%) were sealed in to pupae; while in queen less starter colonies out of the total grafted larvae 216 (63.16%) were sealed in to pupae. The variation in the rate of sealed larvae between queen less and queen right starter colonies was significant at $P < 0.0001$.

The total number of queens emerged from queen right starter colonies were only 67 (20%) while in queen less starter colonies 186 (54.39%) queens were emerged. The variation between the two treatment groups was significant at $P < 0.0001$. Similarly, significantly higher rate of acceptance of grafted larvae was reported for queen less starter than queen right starter honey bee colonies (Cengiz et al., 2009; Ahmad and Dar, 2013). Moreover,

Table 1
Comparisons in rate of acceptance, sealing of grafted larvae and emergence of queen, cup sizes, and weight of queens at emergence between dry and wet grafting.

Response variables	Percentage		Test		
	Wet	Dry	DF	X^2-value	P-value
Accepted grafted larvae	77.01(N = 174)	56.4 (N = 172)	1	16.568	0.000
Sealed queen pupae	71.84	48.84	1	19.134	0.000
Emerged queens	44.79	46.00	1	0.047	0.829
	Wet	Dry	DF	t-value	P-value
Cup height	N = 68 (18.04 ± 1.70)	60 (18.40 ± 2.64)	1	0.924	0.358
Cup tip diameter	N = 68 (5.39 ± 0.54)	60 (5.32 ± 0.54)	1	−0.732	0.466
Weight of queens	N = 70 (0.14 ± 0.02)	60 (0.14 ± 0.03)	1	1.447	0.151

Table 2
Rate of acceptance, sealing of grafted larvae and emergence of queens sealed pupae reared in queen right and queen less starter colonies.

Parameters	QR	QL	Test		
Acceptance of grafts	N (%)	N (%)	DF	X^2-value	P-value
✓ Accepted	106 (32.72)	252 (73.68)			
✓ Rejected	218 (67.28)	90 (26.32)	1	112.33	0.000
Sealing of accepted larvae					
✓ Sealed	82 (25.31)	216 (63.16)			
✓ Unsealed	242 (74.69)	126 (36.84)	1	96.40	0.000
Emerging of sealed pupae					
✓ Emerged	67 (20.68)	186 (54.39)			
✓ Not emerged	25 7(79.32)	156 (45.61)	1	80.24	0.000
Emerging weight	70 (0.136 ± 0.01)	70 (0.141 ± 0.01)	1	2.302	0.023

N = number of grafts; DF: degree of freedom; QR: queen right; QL: queen less and numbers in parenthesis are percentages.

Morse (1994) and Crailsheim et al. (2013) reported the better effectiveness of rearing queens in queen less colonies than queen right colonies. In addition, the average weights of queens at emergence were 0.136 ± 0.01 g (N = 70) and (0.141 ± 0.01 g (N = 70) for queen right and queen less rearing colonies, respectively and the variations were significant at $P < 0.05$.

The relatively low acceptance, sealed and emerging rates of queen right colonies could be due to small colony size of the race. Maintaining small colony size of the race is well known and considered as adaptation to avoid risks in long dearth period of the region (Ruttner, 1988). The smaller the population is, the easier for dissemination of queen pheromone and recognition of the presence of the queen which might have affected the acceptance of grafted larvae. Raising of significant number of queens under queen right colonies conditions have been suggested for honey bee races (Ruttner, 1983; Laidlaw and Page, 1997; Wilkinson and Brown, 2012; Büchler et al., 2013) but such method might not be a good option to use for mass queen rearing in *A. m. jmenitica.* However, rearing queens in queen right colonies can be used to raise small number of queens for own consumption to increase colony stock size without having the chance to lose mother queens and colonies. From this study it can be concluded that in the dry climatic conditions of the study area where colony population size is limited *A. m. jemenitica* colonies can rear significantly more queens under wet grafting and in queen less colonies conditions than dry grafting and queen right colonies conditions.

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Further reading

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