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Impact of Outcross Mating on Brood Rearing Activity and Honey Production of Local Honey Bees in Libya

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ABSTRACT

This research aims to perform outcrossing mating on 60 beehives with virgin queens, followed by an investigation into the impact of hybrid vigor on both spring brood rearing activity and honey production. This outcrossing experiment was conducted with two beekeepers hailing from Tukra and Jirdinah in Lybia. The 60 beehives were divided into four groups to facilitate the investigation, with measurements taken on spring building and honey production. It was observed that bee colonies with outcrossing mating had significant increase in brood rearing activity compared to colonies that did not receive the outcrossing treatment. The results showed that colonies with outcrossing mating increased in hybrid vigor of 32.78% in spring brood rearing activity and 23.61% in honey production. The outcomes of this investigation have bestowed upon male honey bees a heightened level of power during the mating periods, allowing for the manipulation of mating through the alteration of the distance between colonies. This research ultimately provides profound insights into the complex mechanisms of honey bee mating and carries significant implications for beekeeping. It furthers our understanding of male honey bees' vital role in reproducing honey bees. It highlights the importance of manipulating the distance between colonies to optimize the mating process. This study also underscores the need for beekeepers to prioritize the selection of honey bees with desirable traits, as this will produce high quality offspring with improved characteristics.

Keywords: Outcrossing; hybrid vigor; spring brood; rearing activity; honey production.

1. INTRODUCTION

Approximately 125,000 honey bee colonies were present in Libya across various breeds. To enhance the quality of honey bees in the country, approximately 50,000 units consisting of colonies, package bees, and queens of *Apis mellifera* ligustica, along with roughly 3600 colonies of *Apis mellifera* carinnca were imported within the timeframe of 1970 to 1990, while a commercial hybrid line named Queen's wadi, which was a mixture of *Apis mellifera*. carnica and *Apis mellifera* lamarckii, was also made available in the Libyan market. The morphological analysis conducted on Libyan honey bees collected from coastal and desert regions revealed significant differences between the adjacent *A. m i*ntermissa bee population of Tunisia and Algeria and those of *A. m*. Lamarckii from Egypt. On the contrary, the Libyan honey bees exhibited a close association with *Apis mellifera* sahariensis. Moreover, the examination of wing variation angles indicated resemblances to *Apis mellifera* ligustica, as previously documented by Shaibi *et al.* (2009). Interestingly, the investigation also identified four novel haplotypes of oriental evolution lineage within the indigenous bee population of

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coastal regions in Libya. Conversely, the European *M* lineage was infrequent (Shaibi et al., 2009). It is noteworthy that numerous efforts were undertaken to import *A. m.* ligustica and *A.m.* carnica during the 1970s and 1990s, as recorded by Al Mahjoob *et al.* (1999). The objectives of any selective breeding program are to genetically enhance one or more traits that hold importance to the economy. The genetic enhancement of honey bees necessitated numerous technological and scientific breakthroughs. These breakthroughs encompassed fundamental investigations into honey bee morphology, physiology, and behavior and the creation of movable frames that regulate colony growth (Uzunov *et al.*, 2017).

Page and Laidlaw (1982) postulated that the presence of pepper pot brood in colonies featuring large sterile drones is indicative of a decline in brood population; this decline is a consequence of mating between drones and queens of the same lineage, resulting in an average loss of 25% of the brood. Delaplaine *et al.* (2013) observed a significance of the honey bee queen's ability to foster brood growth. This attribute is vital in ensuring the colony's membership increases, thus indicating the queen's fertility and activity. While the availability of nectar determines the worker population of each honey bee colony, the oviposition capacity plays a crucial role in generating populous hives, as noted by Moeller in 1958. Ruttner (1988) explained the genetic makeup of eggs fertilized by sperms carrying lethal alleles. If a sperm carrying a different lethal allele, such as (b, a), fertilizes the egg, the resulting queen or worker will have the composition a/b. On the other hand, if the egg is fertilized by a sperm carrying a similar allele (a), the individual will have a homozygous combination (a/a) that leads to death before maturity, usually in the egg stage. Kumar and Mall (2018) concluded that an inviable brood takes up the much-needed brood nest area in rapidly expanding colonies. The result of brood mortality caused by homozygous sex alleles is a reduction in individual and production, resulting in a slower pace of colony expansion, fewer colony divisions during a season in multivoltine populations, lower production of reproductive males, lower seasonal food productivity, and a decreased ability to survive unfavorable seasons. Salem (2009) expounded upon the notion that the efficacy of colonies and their honey production is contingent upon the efficiency of brood production. It is an established verity that the efficacy of honey production escalates proportionally with the population magnitude of the colony. Consequently, augmenting the population magnitude stands as the primary catalyst in augmenting productivity. One of the pivotal determinants of productivity is the quantum of production per laborious bee. This determinant amplifies as the population surges but dwindles post-attainment of a certain threshold. Consequently, preserving the laborious bees at the acme level of efficiency in the apiary engenders a contribution to the overall production quantum. Ergo, optimizing the population magnitude of colonies is an imperative measure to guarantee maximal productivity.

Woyke (1976) posited that the scattering of the brood could be attributed to three possible factors. The first is a high level of inbreeding, which leads to the homozygosity of the sex locus. Secondly, the queen may have irregularly deposited the eggs. Lastly, for unknown reasons, the brood may not have been nurtured to the adult stage. These factors could significantly influence the reproductive success of a bee colony and thus warrant further investigation. One of the primary objectives in apiculture is to augment honey production proportionately to the population magnitude (Harbo, 1986) and enhance the average lifespan of worker bees. Besides the factors mentioned above, a critical component of colony management is the regulation of queen mating to ensure optimal brood production. This system not only aids in elevating productivity but also serves as a valuable tool for beekeepers (Woyke, 1984).

Delaplane *et al.* (2013) demonstrated the method of determining brood strength by placing a grid of 100 hexagonal wax cells over a section of sealed brood and calculating the percentage of empty cells to estimate brood strength. This process is repeated on different sections of the brood to obtain a mean of at least ten observations. The population of adult bees within the colony primarily affects the productivity of colonies. Colonies with larger populations tend to perform better than those with smaller populations, as bees' strength in a colony strongly correlates with honey production. Several essential factors influence colony strength, such as egg laying rate, viability rate, brood care, development time, and lifespan (Gregorc & Lokar, 2010).

Woyke (1984) study yielded conclusions on the variables significantly influencing honey production. Franks *et al*. (2002) noted that the production of honey is influenced by various factors, including the number of workers within the colony, which is closely associated with the queen's fertility and her capacity to lay eggs, as well as the strength, activity, longevity, tolerance to adverse environmental conditions, and disease resistance of the workers. Spivak *et al*. (1998) concluded that genetic factors and environmental conditions influence the quantitative trait of honey production. Multiple gene pairs control this trait, which requires time and effort. Additionally, the productivity of honey bees is influenced by their crossing.

The complex interplay between genetics, environment, and honey bee crossing underscores the need for careful management of honey production. Weather and other environmental factors can severely influence honey production, which makes it necessary to control these factors to ensure optimal honey production. In conclusion, honey production is a multifaceted process that requires a comprehensive understanding of genetics, environment, and honey bee crossing to ensure the best outcomes. Moreover, future research on honey production should continue to explore these factors and their complex interrelationships.

2. MATERIALS AND METHODS

The out-crossing experiment was conducted in cooperation with two beekeepers from Tukra and Jirdinah (Fig. 1). The distance between Tokra and Gardena is about 100 Km. Tokra region is located in the province of Barqa (Cyrenaica), east of Benghazi, about 65 km, between longitude 20.59 and latitude 32.53. It is a coastal region bordered by the Mediterranean Sea to the north and surrounded by green mountains to the south and east. Tukra has a semi-arid climate. It is warm to hot all year round. The average annual temperature for Tukra is from 10 °C to 33°C degrees, and there is about 248 mm of rain in a year. It is dry for 250 days a year with an average humidity of 42% (The Benina International Airport meteorological station). Gardena region is located in the province of Burqa (Cyrenaica), southwest of Benghazi, and between longitude 20.20 and latitude 31.79. It belongs to the Benghazi Governorate and is considered one of the ancient regions in Libya.

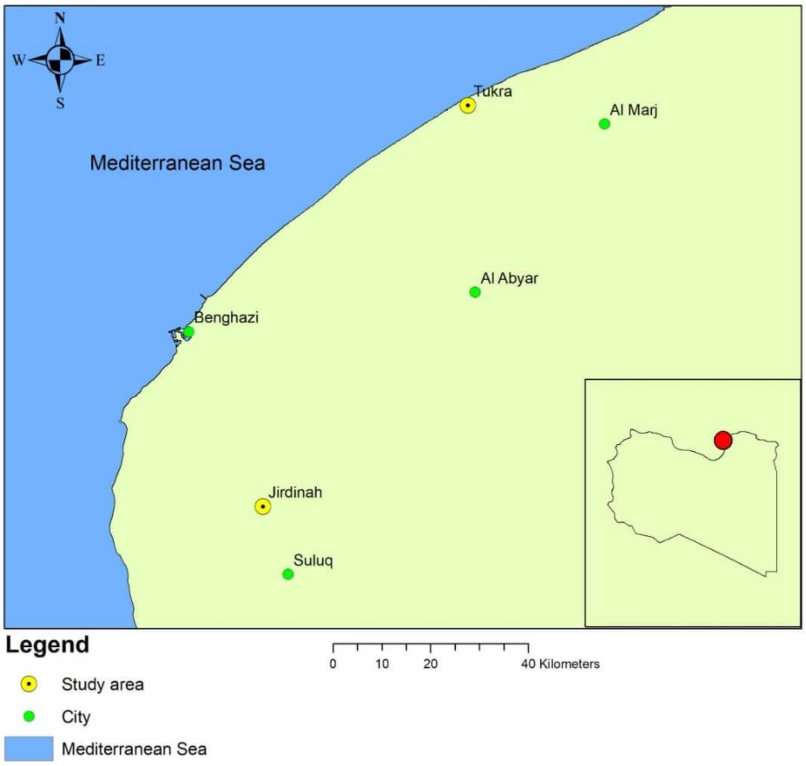


Fig. (1): The location of the outcrossing experiments (Tukra and Jirdinah).

Mating crossings were conducted between queens from Tukra and Jirdinah by using 15 hives for each treatment (Table 1).

Table (1): Mating crossing used in the present study.

	Mating Crossing
1	Queen (Tukra) + drones (Jirdinah)
2	Queen (Jirdinah) + drones (Tokra)
3	Randomly mating (Tukra) (Control)
4	Randomly mating (Jirdinah) (Control)

15 hives were used for each mating crossing.

Jirdiniah has a semi-arid climate, it is warm to hot all year round, and the average annual temperature for Jirdinah is 39 ºC degrees, with about 148 mm of rain in a year. It is dry for 273 days a year with an average humidity of 53 % (The meteorological station of Benina International Airport). To assess the spring brood rearing activity, a novel frame was incorporated into the hive, bearing distinct markings, and left to sit for twelve days. The queen laid her eggs inside the wax cells. At the same time, on the ninth day, the bee workers sealed the wax cells and the larvae inside it into the prepupal stage. The frame was inspected and photographed on the twelfth day, providing valuable insights into the brood development process. The spring brood rearing activity was determined by the geometric shape, which is subsequently placed on the brood comb, followed by the calculation of the number of sealed brood cells. For a single season, honey production levels were assessed in sixty hives where bees had foraged on Thymus capital. The method used to determine honey production involved calculating the weight of filled honey comb containing honey in each hive and subtracting the weight of the empty comb. This was done according to the procedure outlined by Arathi and Spivak (2001).

3. RESULTS

The investigation focused on analyzing the effects of outcrossing mating of bee queens on the spring brood rearing activity. The study revealed notable variations in spring brood rearing for different mattings ($P<0.5$). Specifically, the average spring building value was 0.81 when queens were mated with drones from Gardena apiary and 0.76 when queens were mated with drones from Tukra apiary. This is comparable to the control group, where queens and drones were from the same apiary and brood rearing activity values (0.61 & 0.56) for Tukra and Jirdinah, respectively.

Additionally, the percentage of hybrid vigor for spring brood rearing was 32.78 % and 24.59 % when queens were mated outside, or when queens from another apiary were mated inside the apiary, respectively.

The findings of this study showed that there exists a notable variation in honey yield among different mating types ($P<0.5$). A higher production was observed in instances where queens were mated with drones from Gardena apiary (4.92 kg) in comparison to those mated with drones in Tukra apiary (3.64 kg) and controls (3.98 and 3.26 kg, respectively). Additionally, the hybrid vigor was 23.61 % for queens mated with drones outside the apiary, while it was -8.86% for those mated with drones inside the apiary.

Analysis of variance for spring building and honey production was conducted using SPSS one way ANOVA, and LSD as mean separation test.

Statistical model:

$Yij = \mu + Ti + eij$

Over all meanμ = Yij: trait studied

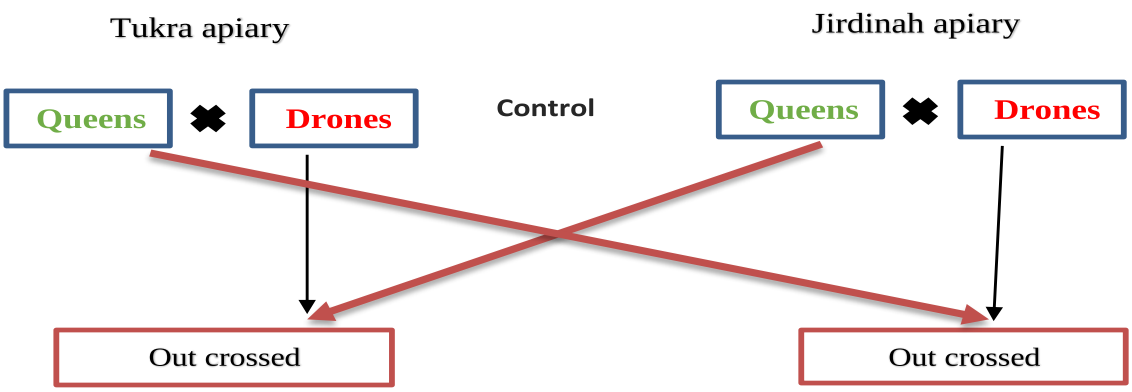
Ti = effect of crossing group

eij = random error

4. DISCUSSION

Numerous studies, including those by Woyke (1955) and Wray *et al.* (2011), have established that crossing plays a significant role in the spring brood rearing activity, with queens mating with up to twenty males to obtain the most significant proportion of different genes. However, in our study area, we have noted that 67.2 % of beekeepers have fewer than ten beehives at the beginning of breeding, contradicting Milner (1996) assertion that the number of beehives should be more than 60. Furthermore, beekeepers in our study area tend to breed queens from the same apiary and depend on artificial swarming, increasing homologous genes and significantly affecting the spring brood rearing.

Table (2) demonstrates a significant difference between queens mated by drones from outside the apiary, queens from outside mated with drones from inside the *apiary*, and those left to mate with males of the



Traits:
1- Spring building
2- Honey production

Fig: (2): Plan of mating.

Table (2): Means and standard deviation of spring sealed brood rearing and honey production for different types of crosses.

Mating outcrossing	Means and standard deviation	
	Spring brood rearing	Honey production (kg)
Queen from Tukra apiary and drones from Jirdinah apiary	0.81 ± 0.061 a	4.92± 1.29 a
Queen from Jirdinah apiary and drones from Tukra apiary	0.76 ± 0.012 b	3.64 ± 1.10 b
Randomly mating at Tukra apiary (Control)	0.61 ± 0.17 b	3.98 ± 1.33 a
Randomly mating at Jirdinah apiary (Control)	0.56 ± 0.018	3.26 ± 1.2 b

15 hives were used for each mating crossing

and the mating process occurs randomly. It is It It is worth noting that the distance between apiaries is less than three kilometers, which enhances proximity breeding. Additionally, 64% of beekeepers used artificial swarming for reproduction, a common practice. Beekeepers rely heavily on seasonal foraging; most check their hives weekly. However, Varroa, Nosema, and foulbrood are the significant problems facing honey bee production. Unfortunately, honey production is low, ranging from 50 to 300 kg annually. Nevertheless, local honey is characterized by higher hygienic behavior, with 89 % of beekeepers indicating this trait.
Outcross mating is a common beekeeping practice, leading to hybrid vigor in spring brood rearing activity and honey production. Our study showed that outcrossing contributed significantly to the honey production process, with a 23.61 % increase. Additionally, it is worth noting that the published version of the manuscript.

Competing interests

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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تأثير التزاوج التهجيني على نشاط تربية الحضنة وإنتاج العسل لنحل العسل المحلي في ليبيا

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يهدف هذا البحث إلى دراسة تأثير قوة الهجين على كل من نشاط تربية حضنة الربيع وإنتاج العسل بإجراء عملية تزاوج على 60 خلية نحل مع ملكات عذراء. أجريت تجربة التهجين هذه مع اثنين من مربي النحل من توكرة وجردينهم في ليبيا. تم تقسيم خلايا النحل الستين إلى أربع مجموعات لتسهيل عملية البحث، وأخذ قياسات بناء الربيع وإنتاج العسل. وقد لوحظ أن طوائف النحل ذات التزاوج التهجيني شهدت زيادة كبيرة في نشاط تربية الحضنة مقارنة بالطوائف التي لم تتلقى معاملة التهجين. أظهرت النتائج أن الطوائف ذات التزاوج الخارجي زادت في قوة الهجين بنسبة 32.78% في نشاط تربية حضنة الربيع و23.61% في إنتاج العسل. لقد منحت نتائج هذا البحث ذكور نحل العسل مستوى مرتفعًا من القوة خلال فترات التزاوج, مما يسمح بممارسة التزاوج من خلال تغيير المسافة بين المستعمرات. يوفر هذا البحث في النهاية رؤى عميقة حول الآليات المعقدة لتزاوج نحل العسل وإجراءات مهمة على تربية النحل. أيضاً يعزز فهمنا للدور الحيوي لذكور نحل العسل في تكاثر نحل العسل وكذلك يسلط الضوء على أهمية تنظيم المسافة بين المستعمرات لتحسين عملية التزاوج. تؤكد هذه الدراسة أيضًا على حاجة النحالين إلى إعطاء الأولوية لاختيار نحل العسل ذو السمات المرغوبة، حيث سيؤدي ذلك إلى إنتاج ذرية عالية الجودة ذات خصائص محسنة.

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