

The Stimulating Effect of Modified Hives (Langstroth) on Honey Bees (*Apis mellifera jemenitica* Hymenoptera, Apidae) to Propolis Production and its Relationship to Population Dynamics of Colonies and some Environmental Factors in Al-Baha province, Saudi Arabia

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Abstract

To evaluate the productivity of the bee hive from propolis and determine the plant species that visit it to produce propolis. The study was conducted in Al-Baha province in Saudi Arabia by using four modified hives. Hives with wall roughness modification were the best to stimulate honeybees to collect more resins and lipophilic materials ($P < 0.05$), while the best production was observed in July and August ($P < 0.05$), with temperatures ranging from 36.47 ± 2.66 - 37.21 ± 2.99 °C. Three plant species were visited by the honeybee *A. m. jemenitica* to collect resins and lipophilic materials. These plant species included *J. procera*, *A. tortilis*, and *F. palmata*. The propolis products had different colors according to the plant source. The results showed a significant correlation between the propolis yields and the strength of colonies. The correlation between propolis yield and temperature in 2014 was negative ($r = -0.19557$).

Keywords: Ficus palamata, Al-Baha propolis, Apis mellifera jemnetica

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1.1 Introduction

Propolis is one of the primary honeybee products, exhibiting a wide range of biological properties due to its complex chemical composition, diverse plant sources, and geographical origin (18). Propolis is known as an immune-stimulant; therefore, due to its antibacterial and antiviral effects, it is utilized as a preventative measure against colds (2). Additionally, it is used as a natural remedy for various skin ailments, offering soothing and healing properties (6). Resins and lipophilic materials are secreted from various parts of trees, such as buds, stems, and leaves. To produce propolis, honeybees forage on plants that secrete resins and lipophilic materials around their nest or hives, and they bring them into their hives for other workers to use against infections, strengthen wax combs, seal cracks, smooth interior walls, and reduce the entrance of hives (18). To enhance social immunity, honeybees deposit antimicrobial plant resins in their nest (19, 20). Physiological and behavioral defenses in eusocial insects, such as honeybees, contribute to social immunity; for example, painting the walls of the nest with plant resin to reduce pathogenic microbes and promote the colony's fitness. Resin collection increases when the colony is exposed to stress diseases (18). The antimicrobial analysis of plant resins from different plant species showed various inhibitory activities against the bacterial pathogen *Paenibacillus larvae* (20). Therefore, due to the therapeutic properties of propolis, beekeepers produce commercial quantities in some countries. Normally, few honeybee workers perform the resin collection task for propolis production based on the needs of their colony, which usually increase if the colony is infected. The colonies infected with chalk brood have increased propolis foragers to collect more resin, thereby reducing infection intensities (18). Many factors influencing a honeybee colony to produce large quantities of propolis are very critical (15). Therefore, propolis production may vary from 300 to 1,450 g/hive/year depending

on mechanical traps, rainfall, altitude, season, available sources, plant diversity, plant health status, and the tendency of colonies to produce propolis (5, 10, 4, 16). For example, the quantity of propolis per hive ranged from 154g to 430g throughout one year in apiaries, when using an intelligent propolis collector (17). Moreover, the propolis productivity of four subspecies of *A. mellifera* were estimated as follows: *A. m. caucasica*, *A. m. carnica*, *A. m. ligustica*, and *A. m. anatoliaca*, by using different traps, where they produced different quantities in averages of 27.34, 26.93, 26.12, and 39.67g/per/hive, respectively, during October to November 2004, where the propolis harvesting was performed every 15 days (1). Despite the importance of propolis, no study has investigated propolis produced by the native bees *A. m. jemenitica*. Therefore, this study aimed to evaluate the productivity of the bee hive by using four modified hives and plant species visited to produce propolis. Also, to investigate the effect of population dynamics of colonies and environmental factors on propolis production in Al-Baha province.

1.2 Materials and methods

Colonies of *A. m. jemenitica* were assigned in Wadi Feeg, between Al-Baha city and Buljurshi governorate. Four different modified Langstroth hives (48.58cm length X 37.30 cm width X 24 cm height) were used in this experiment. Also, normal Langstroth hives without any modification were used as a control.

1.2.1 Modified hives

Ventilation:

The first modification was to make 1 cm² rectangular holes on all sides of the top part of the hive, which makes more space between the hive and the outer cover and allows more air and light to enter the hive (Fig. 1).



Figure 1. Modified hives with 1 cm² rectangular holes on two sides of the top part of the hive

Light exposure:

The second modification was that one side of the wooden wall had been removed and replaced by a glass wall (4mm) in size to allow light to enter the hive. The inner side of this glass wall was covered with a plastic net to allow honeybees to deposit propolis to minimize the incoming light. (Fig. 2).



Figure 2. Modified hive with a glass wall (4mm) in size.

Wall roughness:

The third modification was a plastic net on the inner walls of the hive was placed to increase the roughness of the surface of the hive walls (Fig.3). This modified hive was used by (3).



Figure 3. Modified hive with net plastic on the inner wall

Light alteration period:

The fourth modification was to include lamps that emit 300-650 nm waves continuously on the inner side of the hive. These lamps were covered by proper glass plates, and the glass plates were covered with a plastic net (Fig. 4).



Figure 4. Modified hive with lamps that emit 300-650 nm waves on the inner side of the hive.

1.2.2 Population dynamics:

To measure the strength of each honeybee colony and the number of workers, the area of closed and open brood was estimated according to (9). The standard frame was divided into 32 squares, and the area of each square was 5×5 cm. Then, each square, honeybee workers, closed brood cells, and open brood cells were calculated. This method was repeated monthly during the study period. The method was applied to all colonies of the experiment.

1.2.3 Experiment Location:

The location was in Wadi Feeg, where *J. procera*, *A. tortilis*, and *F. palmata* are the dominant sources for propolis production. Wadi Feeg is located between Al-Baha city and Baljurshi governorate (coordinates = 19°45'0" N - 41°30'0"E; Fig.6). The experiments were performed using honeybee colonies of *A. m. jemenitica*, where five replications of each modification were conducted. The experiment was a randomized complete block design (R.C.B.D) in which the colonies were assigned randomly to the different treatment groups. The colonies were kept under the same environmental conditions with regular management practices. For each treatment, the propolis was collected monthly throughout six months, and the amount of crude propolis was weighed and recorded. The data were statistically analyzed to assess significant differences among treatment groups and months.



Figure 5. Map showing the site of Apiary in Al-Baha province, Saudi Arabia.

1.2.3.1 Meteorological data collection:

Meteorological data were collected using a small weather station, including thermometers and hygrometers. Temperature and relative humidity were recorded

using a thermos-hygrograph (a German device). In this study, temperature and relative humidity were recorded daily throughout the six months of this research project.

1.2.3.2 Statistical analysis:

Five replicates were used for each treatment, and each hive was considered as one replicate. Means and standard deviation were calculated using the SAS program version 9.2. Data were processed by a general linear model (GLM). The least significant difference (LSD) between different treatments was determined. Correlation coefficients between propolis yield and population dynamics of colonies were calculated, and their levels of significance were determined at $P < 0.05$.

1.3 Results and Discussion:

1.3.1 Population dynamics:

The results showed that the population dynamics of honeybee *A. m. jemenitica* colonies were affected by limited factors within the six months (April-September 2014). Throughout the measurement of colony strength, the number of workers of each colony showed differences from April to September 2014. The best peak of workers was significant in May ($P < 0.05$), and the lowest peak was observed in September. The best peak of closed brood was also in May and June ($P < 0.05$), while the least peak was in September. Also, the highest peak of open brood was in May and June, while the lowest peak was in September (Table 1). The results showed a significant correlation between the yield of propolis and the strength of honeybee colonies with wall roughness, light alternation period, and light exposure modified hives. Although there was a correlation between the yield of propolis and the strength of colonies, the highest amount of propolis was obtained in July and August. While the results showed propolis yield produced in modified hives (ventilation), and control hives had a negative correlation

with the strength of colonies ($r = -0.41$, $r = -0.74$), respectively. This may indicate there are other factors to induce honeybee workers to collect plant exudates, such as specific volatile compounds, disease resistance traits, hygienic behavior, and resistance to parasites. This is consistent with (7), who stated that colonies with high hygienic behavior (uncapping brood sealed) produced more propolis than others. Also, the line traits of the queen contribute to propolis production, as found by (11); the hygiene and defense behavior of two generations of Africanized *Apis mellifera* play a role in honey and propolis production. This confirms the importance of selecting a honeybee queen to collect more propolis. Moreover, propolis production is related to the site of the apiary (14).

Table 1. Population dynamic of honey bee *Apis mellifera jemenitica* colonies (Means±SD) from April to September 2014. Means with the same letter (a, b, c, d) are not significantly different at $P \leq 0.05$.

Modification: Ventilation			
Month	Worker /cm ²	Closed brood /cm ²	Open brood/cm ²
April	21401.6± 2518.7 bc	10958.8± 3506.7 b	6794.2±1737.1 b
May	31257.6± 4236.0 a	16628.0± 4760.9 ba	14499.2± 2556.7 a
June	28208.8± 6540.4 ba	17540.6± 8698.6 a	10961.6±6508.5 a
July	13734.4± 5399.8 d	1997.2±1428.2 c	3046.4± 1317.1 b
August	22288.8± 7501.6 bc	3726.4± 3690.3 c	3291.2± 1462.8 b
September	15569.6± 8018.9 cd	1523.2± 914.3 c	3372.8± 1344.2 b

Continue table 1:

Modification: Light exposure			
Month	Worker/cm ²	Closed brood /cm ²	Open brood /cm ²
April	20838.4± 5489.3 bac	9654.0± 1290.8 b	6436.0±860.5 cb
May	29363.2± 7782.0 a	16856.4±4191.3 a	14851.2±4045.1 a
June	26446.8± 7704.7 ba	12915.0± 7061.5 ba	8024.0±5538.5 b

July	16102.4± 7924.8 c	1904.0± 1246.4 c	2584.0± 2042.2 cd
August	18752.0± 6426.0 bc	4352.0± 2815.2 c	3291.2± 1472.3 cd
September	16413.2± 5054.9 c	1305.6± 742.4 c	2393.6± 1579.0 d

Continue table 1:

Modification: Wall roughness			
Month	Worker/cm ²	Closed brood /cm ²	Open brood /cm ²
April	23654.4± 4712.0 b	12304.8±3134.7 b	21157.6± 30246.3 a
May	33152.0± 5800.3 a	16903.0± 1805.9 a	17027.2±3459.8 ba
June	25811.2±4098.2 b	16777.2± 5938.8 a	6473.6±3751.2 ba
July	16102.4± 2594.0 c	2339.2±728.5 c	2529.6±997.5 b
August	16043.2± 4405.7 c	2992.0± 1429.6 c	2584.0± 372.4 b
September	8806.0± 4166.4 d	1360.0± 480.8 c	2910.4±754.7 b

Continue table 1:

Modification: Light alteration period			
Month	Worker/cm ²	Closed brood /cm ²	Open brood /cm ²
April	18585.6± 3210.7 b	10692.0± 2305.1 b	6762.2±1474.6 b
May	26521.6± 5399.8 a	20049.6±3354.2 a	16074.4±3404.9 a
June	30705.6± 4767.7 a	19702.2± 12417.5 a	14734.0± 8709.5 a
July	15155.2± 6174.9 cb	2611.2± 1068.2 c	2420.8± 753.5 b
August	14444.8± 4442.2 cb	3944.0± 2293.9 cb	3073.6± 1060.4 b
September	10966.8±2867.1 c	1115.2± 765.7 c	2964.8±1110.7 b

Continue table 1:

Control			
Month	Worker/cm ²	Closed brood /cm ²	Open brood /cm ²
April	19712.0± 5632.0 bc	9208.8± 4785.7 ba	6076.8± 3310.8 b
May	27942.4± 8603.3 a	14193.2± 6302.6 a	11430.4±6625.6 a
June	22022.4± 5571.5 ba	11808.0± 12141.8 a	6147.4± 6230.3 ba
July	12328.4± 3888.9 dc	3454.4± 3046.5 bc	1831.6± 1502.9 b
August	11320.2± 6731.1 d	2040.0± 1508.3 bc	1692.2± 1318.4 b
September	7503.6± 3944.8 d	870.4±697.4 c	1441.6±1365.4 b

1.3.2 Propolis production:

The results showed that the modified hive of wall roughness was the best to stimulate honeybee workers to collect more resin to produce propolis, as shown in Figures 3 and 6. This result is consistent with the results of (3) who used the same method of wall roughness to illustrate the effect of propolis on the immune system and survival of that colony. Also, wall roughness was better in surviving during the period

of study, which extended from April to September 2014-2015. The highest amount of propolis produced by honeybee workers was found in July and August 2014 (Figure 7). Also, the second modification to stimulate honeybees to collect more resins and lipophilic materials was ventilation (Fig.1). The results showed the propolis production was very low (3g/hive/month), which may be due to many reasons including low plant diversity of the study area. This results consistent with what found (8 and 17) where indicated that propolis production is influenced by the diversity of plants in the area of the apiary. Moreover, the quality of the produced propolis is subjected to different factors such as plant species, geographic area, time of collection, and bee species. On the other hand, the genetic trait of some *A. mellifera* controls propolis production; of 100 colonies of *A. mellifera* of Africanized honeybees subjected to evaluate their propolis production, only 25 colonies produced propolis with an average of 85.45 g, and the remaining colonies didn't produce propolis (12). This result supports the results of this study, where the amount of propolis yield produced by our hives was 45.6 g/hive/year. Moreover, the aggressiveness of honeybees is another factor to induces bee hives to increase collecting resin from plants to produce propolis. This consistent with (13) mentioned that colonies that have superior hygienic behavior produce more propolis.

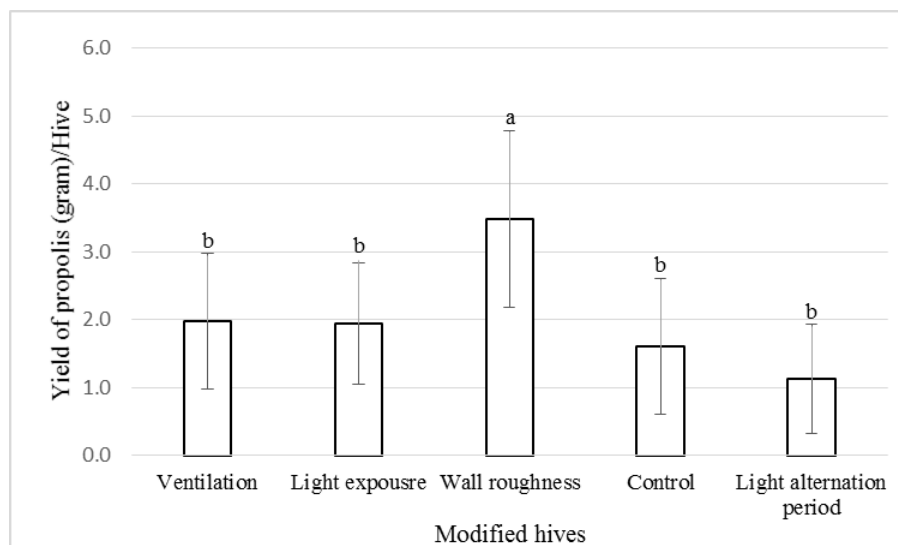


Figure 6. The mean \pm SD of five replicates of propolis yields produced by honeybees using different modified hives. Means with the same letters are not significantly different at $P \leq 0.05$ depending on LSD.

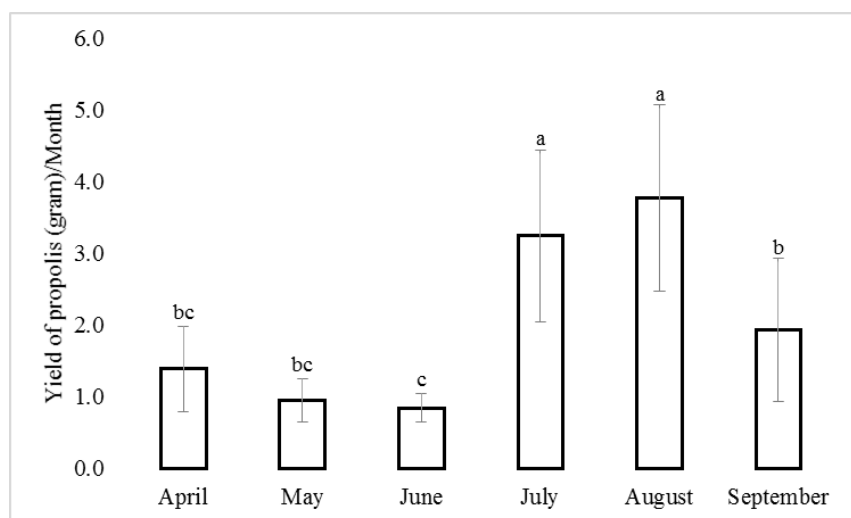


Figure 7. The mean \pm SD of five replicates of yields of propolis produced by honeybees (April-September 2014) in all different modified hives (Feeg village-Al-Baha province, Saudi Arabia). Means with the same letters (a, b, c) are not significantly different at $P \leq 0.05$ depending on LSD.

1.3.3 Ecology data:

Temperature and relative humidity were recorded using a thermos-hygrograph from Germany. The average temperature ranged from 34.5 to 37.33 C° and relative

humidity from 53.81 to 86.37 % in 2014 (Table 2). The results of the study show a negative correlation between the temperature and propolis yield in 2014. The same results found by (15 and 18). While light exposure, wall roughness, and light alternation period showed significant positive correlation between propolis yield and strength of colonies ($r = 0.94, 0.29, \text{ and } 0.21$), respectively. Although no correlation between temperature and relative humidity, honeybee *Apis mellifera jemenitica* produced more propolis from July to August, when the mean temperature ranged from 36.47 ± 2.66 to $37.21 \pm 2.99^\circ\text{C}$. This indicates that these months (i.e, July to August) are the best period to produce propolis by *A. m. jemenitica* in the area studied.

Table 2. The mean \pm SD of temperature and relative humidity throughout six months in 2014.

Year 2014		
Month	Mean \pm SD of Temperature	Mean \pm SD of Relative Humidity%
April	35.74 \pm 2.70	85.12 \pm 12.12
May	35.67 \pm 3.47	66.38 \pm 18.77
June	37.33 \pm 1.43	69.41 \pm 13.45
July	37.21 \pm 2.99	53.81 \pm 16.23
August	36.47 \pm 2.66	65.47 \pm 16.55
September	34.5 \pm 3.23	86.37 \pm 7.34

1.3.4 Plant sources:

In the study area (Feeg village), there are only three sources for resins, including *J. procera*, *F. palmata*, and *A. tortilis* based on the investigator's observation. Throughout the year, from April to June 2014 and from April-June 2015 observed *Apis mellifera jemenitica* visited only three plant species (*J. procera*, *F. palmata*, and *A. tortilis*) to produce propolis, as shown in Figures 9, 10, and 11. With regard *J. procera*, honeybee workers have been observed collecting resin materials from aerial parts of trees; these resins are transparent and have a good fragrance and dark brown color.

Propolis samples were collected and kept in vials and deposited in a refrigerator at -20 °C for further experiment.



Figure 8. *Apis mellifera jemenitica* workers collect liquid resins from aerial parts of *Juniperus procera* trees in Al-Baha province, Saudi Arabia.

Moreover, in April 2014 and April 2015, honeybee workers of *A. m. jementica* collected resin substances from stems of *A. tortilis* to produce propolis. The same propolis samples were kept and deposited in a refrigerator at -20 °C for further experiment.

Figure 10.



Figure 9. *Apis mellifera jemenitica* workers collect dark red resins from stems of *Acacia tortilis* trees in Al-Baha province, Saudi Arabia.

In May 2014, workers of honeybee *A. m. jemenitica* were observed scraping the lower surface of *F. palmata* leaves (Fig. 10). This type of propolis had a green color. Honeybee workers continued to collect materials from the lower surface of *F. plamata* leaves from May to September 2014, and from July to September 2015. These samples of propolis were kept in vials and stored in a refrigerator at -20 °C for further experimentation. Regarding *F. palmata*, this study is the first to record this plant species as a source of propolis.

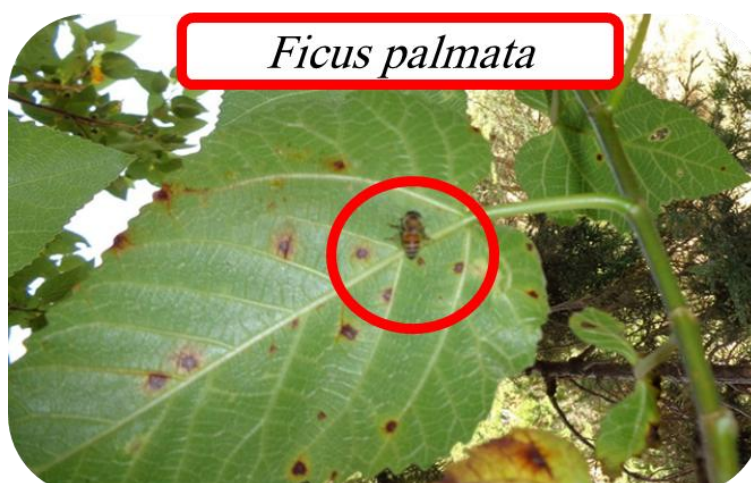


Figure 10. *Apis mellifera jemenitica* workers collect lipophilic materials from the lower surface of *Ficus palmata* leaves in Al-Baha province, Saudi Arabia.

1.5. Conclusion:

Despite using different types of modified hives to stimulate *Apis mellifera jemenitica* colonies to produce more propolis, the amounts obtained it were very low. Therefore, selecting colonies with a specific queen that tends to collect more propolis is very critical. Although temperature is considered the main factor in life for bee colonies, the results of the study didn't find a correlation between the yield of propolis

and temperature. This means other factors play a critical role in propolis production. Moreover, the geographical site and diversity of plants are more important in propolis production. Although, modified hives, especially wall roughness, producing more propolis than others. Using other traps more effectively is better.

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التأثير المحفز لخلايا النحل المعدلة لسلالة النحل اليمني لإنتاج البروبوليس وعلاقته بديناميكية العشيرة وبعض العوامل البيئية في منطقة الباحة في المملكة العربية السعودية

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الملخص

لتقييم إنتاجية خلية النحل من البروبوليس وتحديد الأنواع النباتية التي يزورها النحل لإنتاج البروبوليس نفذت الدراسة في منطقة الباحة بالمملكة العربية السعودية باستخدام أربعة خلايا معدلة. أظهرت النتائج أن الخلايا ذات الجدار الحشن كانت الأفضل في تحفيز طوائف النحل في جمع كميات من البروبوليس بفارق معنوي عند مستوى ($P < 0.05$) حيث كانت الأشهر يوليو وأغسطس الأفضل التي جمع فيها كميات من البروبوليس بفارق معنوي عند مستوى ($P < 0.05$) وقد كانت درجات الحرارة تراوحت في تلك الأشهر ما بين $36.47 \pm 2.66 - 37.21 \pm 2.99$ °C. أثبتت الدراسة إن شغالات سلالة النحل المحلية *Apis mellifera jemenitica* تزور ثلاثة أنواع نباتية تجمع منها المواد الراتنجية لإنتاج البروبوليس *Ficus palmata*, *Acacia tortilis*, *Juniperus procera*، اختلفت ألوان البروبوليس تبعاً للمصدر النباتي حيث لون البروبوليس من التين البري كان أخضر بينما كان بني من نبات العرعر واسود محمر من نبات السمرة. وأظهرت النتائج وجود ارتباط ما بين الكثافة العددية لطوائف النحل ومحصول البروبوليس بينما كانت العلاقة سلبية ما بين درجات الحرارة ومحصول البروبوليس ($r = -0.19557$) في 2014.

الكلمات المفتاحية: التين البري، بروبوليس الباحة، سلالة النحل اليمني

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