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Comparative Study Between Subspecies of *Apis mellifera* for Egg Hatching and Sealed Brood Percentage, Brood Nest Temperature and Relative Humidity

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Abstract: *Apis mellifera yemenitica* (Indigenous bee), *Apis mellifera carnica* and *Apis mellifera ligustica* (Both exotic bees) were compared for percentage of egg hatching, sealed brood percentage and brood nest temperature and relative humidity during May, 2001 in Riyadh, Saudi Arabia. *Apis mellifera yemenitica* presented the highest percentage of egg-hatching (69.17) and sealed broods percentage (62.06) followed by *Apis mellifera carnica* (67.44 and 46.67) in the middle part of the comb whereas *Apis mellifera ligustica* presented maximum egg hatching and sealed brood percentages (65.5 and 59.9) in the lower part of the comb, respectively. *Apis mellifera yemenitica* also showed a good percentage of egg hatching in all parts of the comb but other two races more concentrated in middle and lower parts of the comb. A positive correlation was recorded between colony strength and egg hatching and sealed brood percentage in all three-honeybee races. Egg hatching and sealed brood percentage presented a negative correlation with temperature and a weak positive correlation was recorded for relative humidity.

Key words: *Apis mellifera yemenitica*, *Apis mellifera carnica*, *Apis mellifera ligustica*, egg hatching, sealed brood percentage, brood nest temperature, relative humidity

INTRODUCTION

The history of bee keeping in Saudi Arabia is very old. At present three honeybee races *Apis mellifera yemenitica* (Indigenous bees), *Apis mellifera carnica* and *Apis mellifera ligustica* (Both exotic bees) are reared in various regions of the Kingdom. *Apis mellifera yemenitica* is one of the major subspecies of *Apis mellifera*, which is extreme in both morphology and ecology and was taxonomically, recognized in 1975.

El-Sarrag^[1] studied some factors affecting brood rearing activities of honeybee colonies (*Apis mellifera carnica* L.) in two apiaries in Saudi Arabia (Al-Deriyah and Dirab under Riyadh conditions). The smallest area of worker brood was obtained during October in Dirab (112.7 sq. in.) and in Al-Deriyah (122.4 sq.in.). There was a single peak of worker brood during March (Al-Deriyah) and/or April (Dirab). About 93 % of drones were reared during January-June. The average rate of drone rearing activity in both sites was 10.49-2.69 sq. in./month/colony. Among the effects of the prevailing weather factors on colony activities, only sunshine showed moderate correlation with worker brood rearing (-0.3437), drone brood rearing (-0.4762) and pollen collection and storage (-0.5358). *Apis mellifera yemenitica* surpassed *Apis mellifera carnica* in the following traits: brood rearing, collection and storage of honey and pollen, wax

working, swarming, migration and superseding behavior^[2]. Al-Quarni^[3] recorded two peaks of brood rearing and pollen storing activities during March/June (major peak) and October/November (minor peak) under Riyadh conditions. The *Apis mellifera yemenitica* significantly surpassed the Carniolan bee with respect to the monthly pollen storage and worker and drone brood rates. Regarding honey production it was observed that, there was only one honey flow per year during early March until late April. Rashad and Parker^[4] mentioned that the environmental factors and the amount of honey and pollen in the hive are the main factors that affect the brood amount.

Sun light intensity, temperature and relative humidity affected the amount of honey and pollen in honeybee colonies, while brood area was mainly affected by temperature^[5]. EL-Sarrag *et al.*^[2] found that brood rearing activity had increased during autumn (late July-October) and winter (Nov. Feb) rather than in summer (March-July). The main reason behind that was pollen availability. Todd and Reed^[6] found a positive correlation between amount of pollen stored and brood reared. Kraus *et al.*^[7] compared temperatures in the brood nests of *Apis cerana* and *Apis mellifera*. In center they found similar temperatures in worker brood cells, at external temperatures between 18-33°C. Temperature in worker brood cells of *Apis mellifera* colonies was close to 35°C. Brood nest

temperature in *Apis mellifera* colonies was mostly close to 35°C^[8-10]. Guanhuang and Qinghal^[11] found the brood combs temperature of *Apis mellifera* colonies having 10-frame in colony was 35.5 and for *ligustica* (3 frame in colony) was 35 when the outside temperature was 25°C to 26.5°C and humidity was 60-72%. Temperatures in the brood area during the summer are not uniform. The warmest and most uniform temperatures occur (34.1±0.8°C) in the central brood nest. Broodless regions have the lowest temperatures with the largest fluctuation (30.8±5.4°C)^[12]. Koniger^[13] found that colonies with brood, regulated the entire brood nest to a nearly constant temperature of 33±2°C. Levin *et al.*^[14] stated that brood nest temperatures were shown to be affected by several interrelated factors associated with time of year, developmental brood stage and brood nest position. Bisht *et al.*^[15] found that during warm season in India, *Apis mellifera* colonies maintained a brood nest temperature between 34 and 36°C. Accorti *et al.*^[16] stated that a constant temperature of 34.8 and 35.7°C was found in the center of the nest of the brood of *Apis mellifera ligustica*. The same thermal constancy of about 35-35.5°C was found in the corners of the combs with the drone brood the thermal constancy was about 34-34.9°C for shorter periods. Wholgemuth^[17] stated that the relative humidity normally varies between 40 and 80% in the brood area of a honey bee colony. Crane^[18] reported that most of the temperatures maintained in various parts of the colony and nest/hive are likely to differ slightly under different conditions and for bees of different genetic origin. Hauser^[19] stated that the preferred resting temperature maintained in brood nest is 35°C. Crane also stated that at very high ambient temperatures, a colony must cool the brood nest so that the temperature does not rise above 35°C.

The objective of the present study was to compare the three races for egg-hatching percentage, sealed brood and brood nest temperature and relative humidity, during year, 2001.

MATERIALS AND METHODS

The research was carried out in the apiary of the Faculty of Agriculture, King Saud University, Riyadh, Kingdom of Saudi Arabia. The research program included a comparative study of the three races of honeybees *Apis mellifera yemenitica*, *Apis mellifera carnica* and *Apis mellifera ligustica* (each having five colonies) to evaluate the percentage of egg hatching, sealed brood and brood nest temperature and relative humidity during May, 2001.

Egg sample: Both the position and the number of eggs on brood combs of each tested colony were recorded by using a Langstroth frame of wire grid (divided into sq. inches). The position of eggs was determined by the position of square inches on the wire grid. Using column and row system, the positions of the particular square inches were identified on brood comb. There are 22-hexagonal cell/inch square for *Apis mellifera carnica* and *Apis mellifera ligustica* whereas 28-cells/inch square for *Apis mellifera yemenitica*. By using this method about 10 sq. inches were identified on the brood comb and 100-200 eggs were recorded for each colony.

Eggs-hatching: Five days after counting the newly laid eggs the number of hatched eggs were counted using square grid and the percentage of hatched eggs for each colony were counted by the following equation:

$$\text{Percentage of hatched egg} = \frac{\text{Number of hatched eggs}}{\text{Total number of counted eggs}} \times 100$$

Seven days after hatching percentage determination, the number of sealed brood cells were calculated as under:

$$\text{Percentage of sealed brood cell} = \frac{\text{Number of sealed cells}}{\text{Number of counted eggs}} \times 100$$

The temperature and relative humidity was estimated inside the hive in upper, middle and lower part of the combs with Hygrometer-thermometer HD 8501 on daily basis.

RESULTS AND DISCUSSION

The average number of eggs tested in upper part of the combs in *Apis mellifera yemenitica* colony were 119.7 where the hatching percentage was recorded 49.73% and sealed brood percentage remained 41.53% (Table 1). In the middle part of the comb average number of eggs were examined 198.1 where the hatching percentage was recorded 69.17% and the sealed brood percentage remained 62.06%. In the lower part of the comb the average number of eggs were tested 64.9 where the hatching percentage was recorded 50.65% and the sealed brood percentage remained 41.25%. A detailed probe of analysis of variance ($F_{2,90}, P_{0.013}$ at $\alpha=0.5$ and $LSD= 22.34$) for egg hatching percentage in upper, middle and lower part of the comb revealed no significant difference

Table 1: Average number of eggs tested, eggs hatching percentage and sealed cells percentage in upper, middle and lower parts of the Comb in three honeybee races *Apis mellifera yemenitica*, *Apis mellifera carnica* and *Apis mellifera ligustica*

Honeybee (Species)	Upper part of the comb			Middle part of the comb			Lower part of the comb		
	Av. No. of eggs tested	Hatching (%)	Cells sealed (%)	Av. No. of eggs testes	Hatching (%)	Cells sealed (%)	Av. No. of eggs tested	Hatching (%)	Cells sealed (%)
<i>Apis mellifera Yemenitica</i>	119.7	49.73	41.53	198.10	69.17	62.06	64.9	50.65	41.25
<i>Apis mellifera Carnica</i>	35.5	25.54	23.57	205.70	67.44	46.67	26.4	36.06	29.37
<i>Apis mellifera Ligustica</i>	39.5	13.35	11.31	192.25	54.50	50.12	142.5	65.50	59.9

Table 2: Comparison of egg hatching percentage in upper, middle and lower parts of *Apis mellifera yemenitica*, *Apis mellifera carnica* and *Apis mellifera ligustica* combs

Comb part	<i>Apis mellifera yemenitica</i>	<i>Apis mellifera carnica</i>	<i>Apis mellifera ligustica</i>
Upper part	49.73a	25.54bc	13.35c
Middle part	69.17a	67.44a	54.5ab
Lower part	50.65a	36.06b	65.50a
LSD	22.34	23.73	11.79

$\alpha = 0.05$

between treatment means (Table 2). The egg hatching percentage showed a strong positive correlation in upper and middle parts of the comb while a strong negative correlation existed in lower part on the comb (1.00T, 1.00H; 0.974T, 1.00H; 0.998T, -0.993H in upper, middle and lower parts of the comb, respectively) with temperature and relative humidity, respectively in side the hive.

The average number of eggs examined in upper part of the combs in *Apis mellifera carnica* colony were 35.5 where the hatching percentage was recorded 25.54% and sealed brood percentage remained 23.57%. In middle part of the combs the average number of eggs were tested 205.7 where the hatching percentage was recorded 67.44% and the sealed brood percentage remained 46.67%. In the lower part of the comb the average number of eggs were recorded 26.4 where the hatching percentage was recorded 36.06% and the sealed brood percentage remained 29.37%. Analysis of variance ($F_{10,07}$, $P_{0.012}$ at $\alpha=0.5$ and $LSD= 23.73$) for egg hatching percentage in upper, middle and lower part of the comb revealed a significant difference between means of egg hatching percentage between middle, upper and lower part while non-significant difference between upper and lower parts of the comb. The egg hatching percentage showed a strong positive correlation (0.997T, 0.997H; 1.00T, 0.982H; 1.00T, 1.00H in upper, middle and lower parts of the comb, respectively) with temperature and relative humidity, respectively in side the hive.

The average number of eggs examined in upper part of the combs in *Apis mellifera ligustica* colony were 39.5 where the hatching percentage was recorded 13.35% and sealed brood percentage remained 11.31%. In the middle

part of the comb average number of eggs were tested 192.25 where, the hatching percentage was recorded 54.5% and the sealed brood percentage remained 50.12%. In the lower part of the comb the average number of eggs were examined 142 where the hatching percentage was recorded 65.5% and the sealed brood percentage remained 59.9%.

A detailed study of analysis of variance ($F_{65,13}$, $P_{0.00}$ at $\alpha=0.5$ and $LSD= 11.79$) for egg hatching percentage in upper, middle and lower part of the comb revealed a non-significant difference between middle and lower part while significant difference between upper and both middle and lower parts of the comb (Table 2). The egg hatching percentage showed a strong negative correlation (-0.993T,-1H; -0.998T, 0.99H; -0.99T, -0.99H in upper, middle and lower parts of the comb, respectively) with temperature and relative humidity, respectively in side the hive.

A detailed probe of analysis of variance ($F_{10,18}$, $P_{0.012}$ at $\alpha=0.5$ and $LSD= 20.8$) for egg hatching percentage in three honeybee races (upper part of the comb) revealed a significant difference between treatment means of *Apis mellifera yemenitica* and *Apis mellifera carnica* and *Apis mellifera ligustica* while *Apis mellifera carnica* and *Apis mellifera ligustica* remained statistically similar for egg hatching percentage in upper part of their respective combs (Table 3).

Analysis of variance ($F_{5,77,18}$, $P_{0.040}$ at $\alpha=0.5$ and $LSD= 11.55$) for egg hatching percentage in three honeybee races (middle part of the comb) revealed a non-significant difference between treatment means of *Apis mellifera yemenitica* and *Apis mellifera carnica* while a significant difference was recorded between treatment means of *Apis mellifera yemenitica* and *Apis mellifera ligustica* and also between treatment means of *Apis mellifera carnica* and *Apis mellifera ligustica* (Table 3).

A detailed study of analysis of variance ($F_{3,91}$, $P_{0.082}$ at $\alpha=0.5$ and $LSD= 25.75$) for egg hatching percentage in three honeybee races (lower part of the comb) revealed a non-significant difference between treatment means of

Table 3: Comparison between subspecies of *Apis mellifera* for egg hatching percentage in upper, middle and lower parts of the comb

Honeybee species	Upper part	Middle part	Lower part
<i>Apis mellifera yemenitica</i>	49.73a	69.17a	50.65ab
<i>Apis mellifera carnica</i>	25.54b	67.44ab	36.06bc
<i>Apis mellifera ligustica</i>	13.35bc	54.50c	65.50a
LSD	20.80	11.55	25.75

$\alpha = 0.05$

Table 4: Temperature and relative humidity recorded for Yemenitica, Carnica and Italian honeybee colonies in upper, lower and middle parts of the comb

Honeybee (Races)	Temperature (°C)			Relative Humidity (%)		
	Upper	Middle	Lower	Upper	Middle	Lower
<i>Apis mellifera yemenitica</i>	34.43	33.63	32.73	33.33	34.66	34.66
<i>Apis mellifera carnica</i>	34.82	34.94	34.32	34.00	37.80	32.80
<i>Apis mellifera ligustica</i>	33.75	35.05	32.85	46.00	43.50	46.50

Apis mellifera yemenitica and *Apis mellifera carnica* and *Apis mellifera yemenitica* and *Apis mellifera ligustica*, while a significant difference was recorded between treatment means of *Apis mellifera carnica* and *Apis mellifera ligustica* (Table 3).

The egg hatching percentage was also correlated with colony strength that showed a strong positive correlation ($r = 1.00$). A correlation between the temperature and relative humidity also revealed a strong positive correlation 0.779, 0.910 and 0.971 in *Apis mellifera ligustica*, *Apis mellifera carnica* and *Apis mellifera yemenitica*, respectively in side the hive.

The egg-hatching percentage was studied in upper, middle and lower part of the comb because one of the objectives was to ascertain the coverage of honeybee queen for egg laying in different parts of the comb. The results of the studies showed that *Apis mellifera yemenitica* queen covered nearly whole comb whereas the *Apis mellifera carnica* and *Apis mellifera ligustica* queens preferred middle parts of the comb for egg laying. The highest egg hatching and sealed brood percentage was recorded in middle part of *Apis mellifera yemenitica* and *Apis mellifera carnica* comb whereas in *Apis mellifera ligustica* the maximum egg hatching percentage and sealed brood percentage was recorded in lower part of the comb.

Apis mellifera yemenitica during May, 2001 maintained an average temperature 34.43, 33.63 and 32.73°C at the relative humidity 33.33, 34.66 and 34.66% in upper, middle and lower parts of the comb, respectively (Table 4). The *Apis mellifera carnica* maintained an average temperature 34.82, 34.94 and 34.32°C at the relative humidity 34, 37.8 and 32.8% in upper, middle and lower parts of the comb, respectively (Table 4). *Apis mellifera ligustica* maintained average temperature 33.75,

35.05 and 32.85°C at the relative humidity 46, 43.5 and 46.5% in upper, middle and lower parts of the comb, respectively when average outside temperature was 42°C (Table 4). The results were consistent with those of Kraus^[7], Koniger^[13], Bisht *et al.*^[15] and Wholgemuth^[17].

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