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SOME MORPHOLOGICAL AND ANATOMICAL CHARACTERISTICS OF HONEY BEES FROM DUHOK PROVINCE, IRAQ

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ABSTRACT

In this study, some morphological and anatomical characteristics of 2000 nurse honey bees (*Apis mellifera*) collected from four apiaries at Duhok province, northern of Iraq were investigated during summer 2015. Results showed that body mass, body length, mandible length, tongue length and femur length ranged from 136.14 ± 6.12 to 145.06 ± 3.87mg, 12.23 ± 0.36 to 12.39 ± 0.14mm, 1.51 ± 0.05 to 1.56 ± 0.07 mm, 6.32 ± 0.12 to 6.58 ± 0.11 mm, and2.66 ± 0.06 to 2.78 ± 0.07mm, respectively. Concerning the anatomical characteristics, the length of the main ducts of hypopharyngeal glands, average acini dimension, and average dimension of fat body cells ranged from 12.98 ± 0.25 to 13.42 ±0.42mm, 139.12 ± 6.45 to 148.45 ± 7.39µm, and 80.26 ± 4.32 to 85.96 ± 5.22µm, respectively. The results also showed that workers collected from colonies at the mountains had longer femur, longer tongue, more body mass, bigger fat body cells, and bigger hypopharyngeal glands acini than those collected from colonies at the plains. Significant positive relationship was found between the body mass of the workers and dimensions of fat body cells as well as dimensions of the acini. Also, significant positive relationship was found between the body length and the length of main duct of hypopharyngeal gland. This study has special importance in understanding characteristics of honey bees in northern of Iraq.

***KEYWORDS*:** *Morphometric, Honeybee, Hypopharyngeal gland, Fat body.*

1. INTRODUCTION

Honey bees (Hymoneptera: Apidae, *Apis mellifera*) are social insects known as the most economically valuable insects because of their honey production and pollinating activities (Lawal and Banjo, 2010). Honey bees are considered the most economically valuable pollinators for agricultural crops worldwide (Johnson, 2010).

Morphometric approaches have been applied to separate *Apis mellifera* than the other three commonly defined species; *A. florea*, *A. cerana*, and *A. dorsata* (Ruttner, 1986). Also, these approaches have contributed in a large measure to the discrimination between different honeybees' subspecies in order to characterize their diversity (Ruttner, 1988). Standard morphometric was used in honey bee studies by measuring different wing angles, indices and distances (Ruttner, 1988). Various honey bee colonies, races and species were discriminated by employing morphometric analysis (Moradi and Kandemir, 2004; Raina and Kimbu, 2005; Farhoud and Kence, 2005; Shaibi *et al*., 2009; Rattanawannee *et al*., 2010; Nedić *et al*., 2011).

The tongue length was considered a very important character because it shows the geographical variability more accurate than all the other characters, femur length and tongue length are reported by Ruttner (1988) as highly discriminatory morphometric characteristics. The weights of honey bee workers ranged from 81-140 mg (Winston, 1987). They are mediated by genetics as well as by environmental effects such as the amount of food fed to larvae (Daly and Morse, 1991).

The important function of the fat body is the one of regulating the chemical composition of the hemolymph through the absorption, storage, synthesis and liberation of lipids, proteins and carbohydrates (Cruz-Landim, 1985a).

Hypopharyngeal glands (HPGs) are composed of a pair of ducts that are connected with more than 500 glandular acini (containing secretory cells) (Winston, 1987). The glands are located underneath the pharynx in the head capsule. They play an important role in rearing the queens and

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brood because they synthesize and secrete royal jelly (Michener, 1974). According to Deseyn and Billen (2005) the size of the hypopharyngeal glands is positively correlated with gland activity. Measuring morphological and anatomical characteristics of honey bees are very important to characterize the bees and to separate them than other subspecies.

The hypothesis of this work is that the honey bees of the study regions represent more than one distinct honey bee subspecies. The study aimed to measure some morphological and anatomical characteristics of honey bees from Duhok province, northern Iraq, which has not been well studied so far. Also, the variations between honey bees located at mountains and in the plains within the study region were investigated. Moreover, the relationships between measured characteristics were calculated.

2. MATERIALS AND METHODS

2.1. Sampling

This study was conducted in Duhok province, Kurdistan, northern Iraq, during summer 2015. A total of two thousands nurse bees were collected from 100 colonies at four locations (apiaries). Twenty five colonies from each apiary were used to obtain 500 nurse bees randomly (20 bees per colony). The first and the second apiaries (A1, A2) were located in two villages (Kanimase and Begova) at the mountains along Turkish border, while the other two apiaries (A3, A4) were located in the plains of Duhok city and Zakho city. The distance between each apiary and the other three locations was not less than 60 kilometers.

2.2. Morphological characteristics

Worker bees were collected from the brood areas and were killed in a freezer at -20°C for two hours (Abou-Shaara and Al-Ghamdi, 2012), and then were weighted immediately. Lengths of femur, tongue, mandible and body of each worker were measured, a binocular and millimeter slide were used, and a ruler was used for measuring the body length. Then, bees were individually kept in the labeled tubes and stored in deep freezer until dissection.

2.3. Anatomical characteristics

2.3.1. Dissecting

Frozen samples were thawed at room temperature and immediately dissected to prevent tissue deterioration (Ayoub, 2011). The bees were dissected under a stereomicroscope at 40X magnification. Dimensions of fat body cells, dimensions of acini and the length of the main duct in hypopharyngeal glands of all workers were investigated.

2.3.1.1. Fat body cells

From each dissected worker, twenty fat body cells from the abdomen were randomly selected and their longest diameters were measured (Fig. 1). The average size of the fat body cells from each dissected worker was calculated (Ayoub, 2011).

2.3.1.2. Hypopharyngeal glands

A longitudinal incision was made in the top of the head. Then the hypopharyngeal glands were dissected on the surface of a clean glass slide, stained by diluted Giemsa stain and washed by physiological saline. The length of the main duct in both sides and the longest diameter of ten acini from each side (twenty from each worker) of the head were measured, and the average of right and left ducts was calculated (Fig. 2). The average size of acini for each dissected worker was calculated. These characteristics measured by micrometer slide.

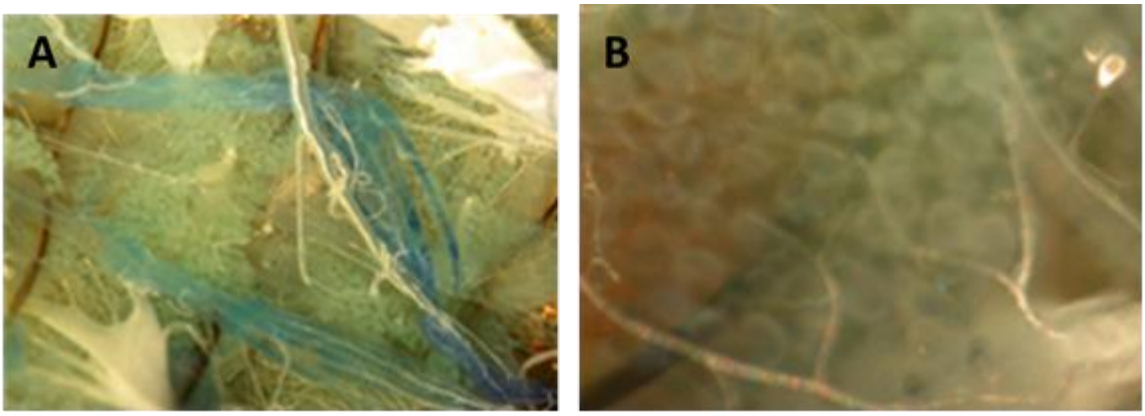

Figure (1): Fat body cells of nurse bees. A: inside the abdomen, and B: magnified cells.

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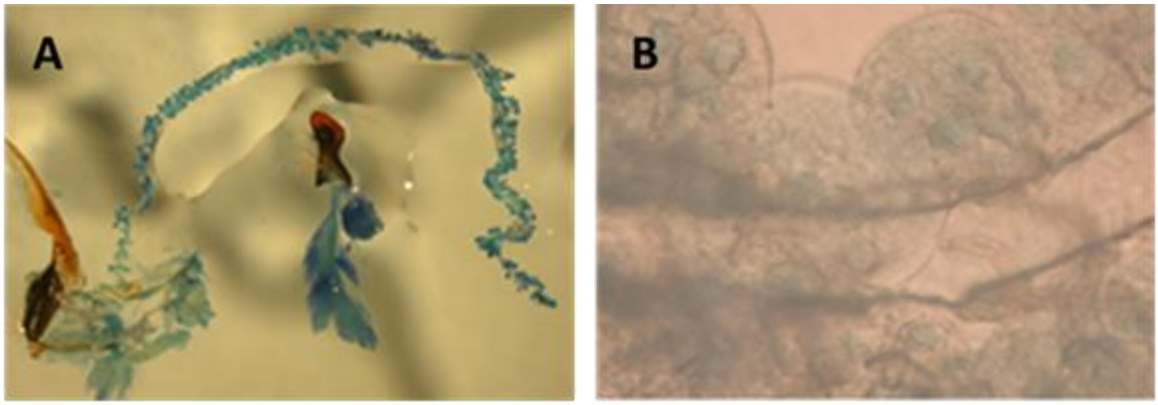

Fig. (2): Hypopharyngeal gland of bee worker. A: Hypopharyngeal gland with the mandible, and B: Magnified acini with part of the main duct of hypopharyngeal gland.

2.4. Statistical Analysis

Analysis of variance was performed, and Duncan's multiple range tests were used to detect significant differences among measured characteristics of workers collected from different apiaries at significance level 0.05. All data were processed with Origin 7.0 software. Linear regression was applied and the data of each worker were used to detect the relationships between studied characteristics.

3. RESULTS

3.1. Morphological characteristics

Means of measured characteristics for workers collected from 4 apiaries (A1, A2, A3 and A4) are presented in table 1 and 2. Body mass of bee workers ranged from136.14 ±6.12 mg (A4) to 145.06 ±3.87 mg (A2) with difference of 8.92 mg. Body length varied from 12.23 ± 0.36 mm (A4) to 12.39 ±0.14 (A1) with difference of 0.16 mm. Length of the mandible ranged from 1.51± 0.05 mm (A4) to 1.56 ± 0.07 mm (A1) with difference of 0.05 mm. Length of tongue varied from 6.32 ±0.12 mm (A4) to 6.58 ± 0.11 mm (A1) with difference of 0.26 mm, while length of the femur was varied from 2.66 ± 0.06 mm (A4) to 2.78 ± 0.07mm (A2) with difference of 0.12 mm (Table 1).

No significant differences were detected between mountain apiaries (A1 and A2) and plain apiaries (A3 and A4) in body length and mandible length. Mountain apiaries were significantly higher than plain apiaries in body mass, tongue length, and femur length (Table 1).

Table (1): Means± S.D. of measured morphological characteristics of workers collected from four apiaries(A1 and A2: apiaries at the mountains, and A3 and A4: apiaries in the plains).

Characteristics	Apiary 1 (A1)	Apiary 2 (A2)	Apiary3 (A3)	Apiary 4 (A4)
Body mass (mg)	139.82 ± 4.22 ab	145.06 ± 3.87 a	136.72 ± 4.05 b	136.14 ± 6.12 b
Body length (mm)	12.39 ± 0.14 a	12.28 ± 0.35 a	12.25 ± 0.21 a	12.23 ± 0.36 a
Mandible length (mm)	1.56 ± 0.07 a	1.53 ± 0.06 a	1.53 ± 0.06 a	1.51 ± 0.05 a
Tongue length (mm)	6.58 ± 0.11 a	6.55 ± 0.08 a	6.36 ± 0.09 b	6.32 ± 0.12 b
Femur length (mm)	2.76 ± 0.08 a	2.78 ± 0.07 a	2.70 ± 0.05 ab	2.66 ± 0.06 b

Means with the same letter for each characteristic are not significantly different.

3.2. Anatomical characteristics

The length of the main ducts of hypopharyngeal glands ranged from 12.98 ± 0.25 mm (A3) to 13.42± 0.42 mm (A1) with difference of 0.44 mm . The average acini dimension of both sides of the gland varied from 139.12 ± 6.45 µm (A4) to 148.45 ± 7.39 µm (A2) with difference of 9.33 µm. The average dimension of fat body cells from the abdomen of each worker ranged from 80.26 ± 4.32 µm (A4) to 85.96 ±5.22 µm (A2) with difference of 5.7 µm (Table 2). No significant differences were detected between

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mountain apiaries (A1 and A2) and plain apiaries (A3 and A4) in length of main duct. Mountain apiaries were significantly higher than plain apiaries in dimension of acini and dimension of fat body cells (Table 2).

Table (2): Means± S.D. of measured anatomical characteristics of workers collected from four apiaries (A1 and A2: apiaries at the mountains, and A3 and A4: apiaries in the plains).

Characteristics	Apiary 1 (A1)	Apiary 2 (A2)	Apiary3 (A3)	Apiary 4 (A4)
Length of main duct (mm)	13.42 ± 0.42 a	13.24 ± 0.32 a	12.98 ± 0.25 a	13.25 ± 0.36 a
Dimension of acini (µm)	144.15 ± 5.33 ab	148.45 ±7.39 a	141.23 ± 6.26 b	139.12 ± 6.45 bc
Dimension of fat body cells (µm)	85.73 ±3.08 a	85.96 ± 5.22 a	81.75 ± 3.25 b	80.26 ± 4.32 b

Means with the same letter for each characteristic are not significantly different.

Significant positive relationship was found between the body mass of the workers (as independent variable) and both dimensions of fat body cells (Figure 3) and dimensions of the acini in hypopharyngeal glands (Figure 4); (as dependent variables); (r^2 = 0.049, P = 0.000, n = 400); and (r^2 = 0.054, P = 0.000, n = 400), respectively. Workers with more body mas had bigger fat body cells and bigger acini in hypopharyngeal glands.

Significant positive relationship existed between the body length of the worker (as independent variable) and the length of main duct of hypopharyngeal gland (as dependent variables) in nurse bees. (r^2 = 0.061, p = 0.000, n = 400); Workers with longer body had longer main duct of hypopharyngeal gland (Figure 5).

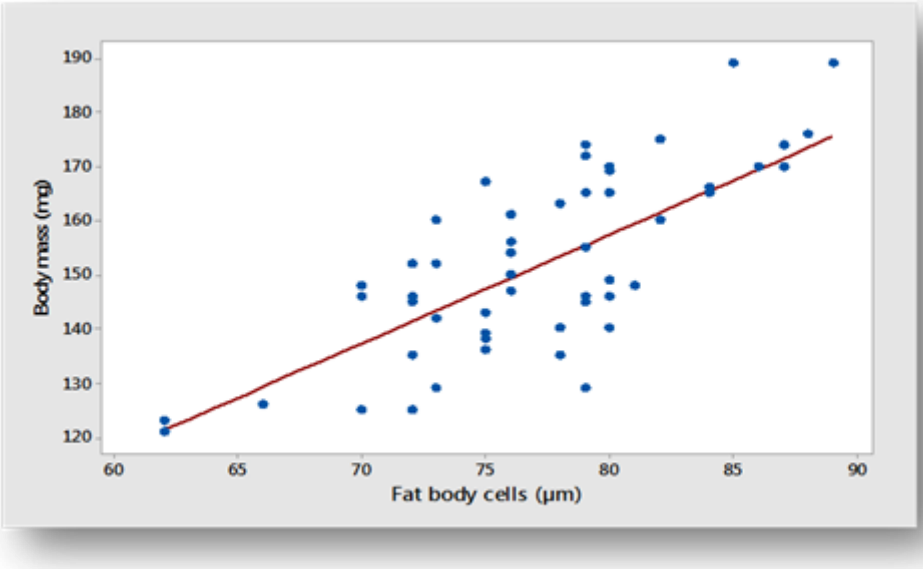

Figure (3): Relationship between the body mass and the dimensions of fat body cells.

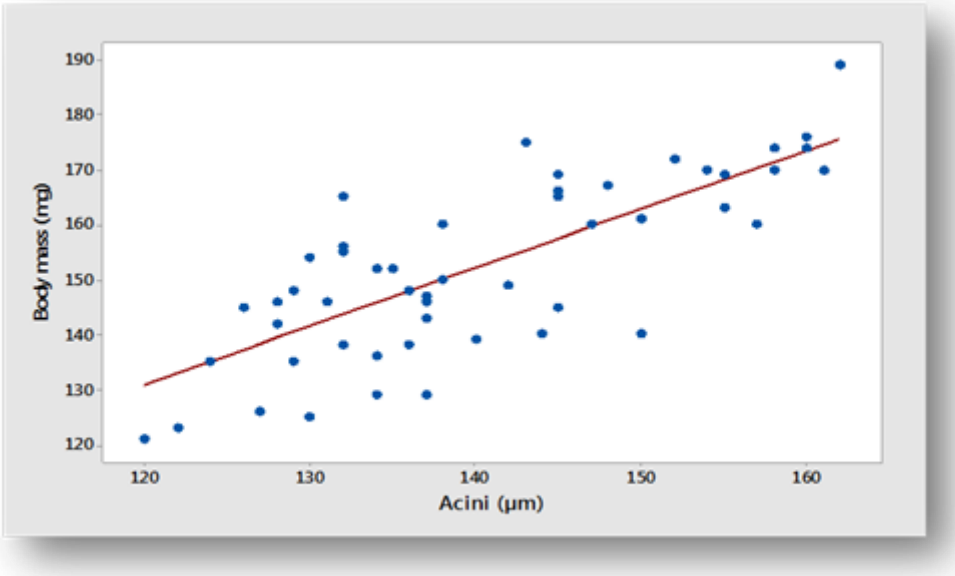

Fig. (4): Relationship between the body mass and the dimensions of hypopharyngeal gland acin

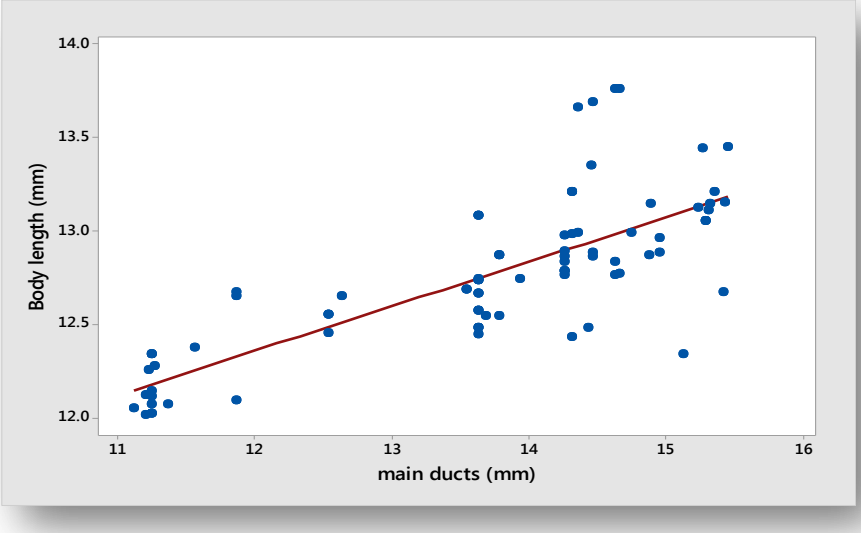

Fig. (5): Relationship between the body length of the worker and the length of the main duct of hypopharyngeal gland.

4. DISCUSSION

4.1. Morphological characteristics

The results showed variations between the studied four apiaries in some morphological characteristics. Two apiaries at the mountains had higher body mass, tongue length and femur length than two apiaries in the plains. The variations between mountain and plain apiaries can be attributed to ecological factors or even to some beekeeping practices according to factors impacting morphological characteristics as reviewed by Abou-Shaara et al., 2013. In a similar way, Marghitas et al. (2008) found that worker proboscis were longer in the mountain regions than that in lower regions. Each of body length and mandible length can not be considered as discriminant characteristics due to the lacking of significant variations. The highest body mass of the workers in the mountains compared to the plains in the present study was probably associated with the food availability, because both larval and adult nutrition have important effects on

honey bee body mass (Hoover et al. 2005). It was suggested that the amount of food fed to honey bee larvae had effects on adult body mass (Daly and Morse, 1991). During the first 5 or 6 days of adult life, workers consume a large amount of pollen to obtain the proteins and amino acids required to complete their growth and development of body structures (Chapman, 1978). The lowest body mass of workers observed in the plains may be caused by the shortage of nectar and pollen stores inside the hives. This could be attributed to the limited foraging activates due to the high temperature and shortage of flowers during hot days in the plains in spite of long photoperiod times, in contrast to the plant diversity and relatively moderate temperature in the mountains area.

Also, the study showed some morphological characteristics of investigated honey bees in northern Iraq were similar to that of neighboring countries. Namely, tongue length and femur length were similar to those of Iranian honey bees (Adl *et al*.,2007). However, means of body mass were higher than means determined for Yemeni or hybrids of Carniolan honey bees in Saudi Arabia (Abou-Shaara, 2013). The variations between measured characteristics and characteristics of bees from other countries are expected due to the presence of different ecotypes of honey bees. It is known that European honeybee have a remarkable regional differentiation (Engel, 1999; Sheppard and Meixner, 2003), with sensitivity to environmental selection pressures (Franck et al., 2000b).

4.2. Anatomical characteristics

Measured characteristics of bees from apiaries at the mountains were higher than those from apiaries in the plains except length of main duct. Such variations can be indirectly explained by differences in the ecological factors of the two regions (mountains and plains). Relatively low temperature with availability of food sources at mountains compared to plains. The big fat body cells of nurse bees was explained by Brian (1983) who found that in the eusocial hymenoptera, nursing workers may store considerable amounts of colony's nutritional resources in the form of body fat or storage proteins for feeding larvae. Deseyn and Billen (2005) found that the acini in hypopharyngeal glands increased in size with the gland activity, they also suggested that the size of the gland is positively correlated with gland activity. Both temperature and nutrition affect the development of these structures including ovary development (Lin, 1999). Dimensions of fat body cells, dimensions of acini in hypopharyngeal glands and the body mass of the young adult worker were smaller than those recorded to Carniolan honey bees in south Poland by Ayoub (2011). This can be attributed to availability of variety of flowering plants in Europe for at least four to five months during summer seasons compared to dearth summer in the study regions.

The overall variations in the measurements of characteristics of the bees of our area probably resulted from adaptation of these insects for long period of time to dry hot summer and freezing winter at the mountains. The studied structures play prominent roles in the activities of the worker bees. For example fat body cells are important in regulating the chemical composition of the hemolymph, and they act as storage of lipids, proteins and carbohydrates (Cruz-Landim, 1985a). Hypopharyngeal glands play an important role in rearing queens and brood by secreting royal jelly (Michener, 1974), additionally the role of mouth parts (mandible and tongue) and the legs for the movement and brood rearing. These features are frequently used irrespective of period and season of the year and are thus developed as a result of constant use and adaptation to the environment. This indicates to the fact that constant use of certain biological features of organisms helps growth, development and adaptation to the environment (Eischen *et al.,* 1982; Milne *et al.,* 1986). This method is a simple way for the discovery of variations between species of different environmental conditions and it requires inexpensive materials compared with molecular methods.

It can be concluded that the presence of significant variations in morphological and anatomical characteristics suggests that the honey bees of the study regions may represents more than one distinct honey bee subspecies.

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پوختە

ل ڤی ڤە کولینیدا هندەك ساخلەتین شیوەیی و تیوری بین **2000** شولکەرین میشا هنگڤینی (**Apis mellifera**) ئەوین هاتینە کومکرن ژ چار جهین جودا ل پاریزگەها دهوکی، بکوری عیراقی هتتە تاقیکرن ل هاڤینا **2015** .

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ل ئەنجامدا دیار بی کو کیشا جەستەی ، دریژیا لغافی، دریژاییا خەرتومی، دریژاییا رانی گە هشتە **136.14 ± 6.12** بو **145.06 ± 3.87** ملغ، **12.23 ± 0.36** بو **12.39 ± 0.1** ملم ، **1.51 ± 0.05** بو **1.56 ± 0.07** ملم ، **6.32 ± 0.12** بو **6.58 ± 0.11** ملم ، **2.66 ± 0.06** بو **2.78 ±0.07** ملم لدویڤ ئیك.

دەرباری ساخلەتین تیوری، دریژییا کەنالی سەرەکی بیی ڤەریژا بن گەروی ، هەروەسا پانیا دنکین ڤەریژی و پانیا شانین چەوریی گە هشتنە ناڤبەرا**98. 12 ±0.25** بو **13.42 ± 0.42** ملم ، **139.12 ± 6.45** بو **148.45 ± 7.39** مایکرون، **80.26 ± 4.32** بو **85.96 ± 5.22** مایکرون لدویڤ ئیك.

ل ئەنجامدا دیسا دیار بی کو میشین شولکەر بین هاتینە کومکرن ژ چیای، رانی دریژ تر، خەرتومی دریژ تر، کیشا جەستەی پتر، شانیت چەوری مەزنتر و دنکیت ڤەریژا بن گەروی مەزنتر ژ بیت وان شولکەرین هاتنیە کومکرن ل دەشتی. پەیوەندیا پوزەتیڤا کاریگەر دیار بی دناڤبەرا کیشا جەستەی یا میشا شولکەر و پانیا شانین چەوری هەروەسا پانیا دنکین ڤەریژا بن گەروی. پەیوندیەکا دیتر یا پوزەنیڤا کاریگەر هاتە دیتن دناڤبەرا دریژیا جەستەی یا میشا شولکەر و دریژیا کەنالی سەرەکی یی ڤەریژا بن گەروی.

دڤی ڤەکولینیدا گرنگییە کا تایبەت هەیە ژبو تیگەهشتنا ساخلە تین میشا هنگڤینی ل باکوری عیراقی.

الخلاصة

في هذه الدراسة تم التحري عن بعض الخصائص المظهرية والتشريحية ل **2000** نحلة عسل حاضنة ***(Apis mellifera)*** والتي تم جمعها من اربع مناحل ضمن محافظة دهوك ، شمال العراق خلال صيف **2015** .

اظهرت النتائج بان وزن الجسم، طول الجسم، طول تافك، طوا الخرطوم و طول الفخذ تراوحت من **136.14 ± 6.12** الى **145.06 ± 3.87** ملغ، **12.23 ± 0.36** الى **12.39 ± 0.1** ملم ، **1.51 ± 0.05** الى **1.56 ± 0.07** ملم ، **6.32 ± 0.12** الى **6.58 ± 0.11** ملم ، **2.66 ± 0.06** الى **2.78 ±0.07** ملم على التوالي.

اما بخصوص الصفات التشريحية، طول القناة الرئيسية للغدة تحت البلعومية، معدل ابعاد الفصوص لنفس الغدة و مدل ابعاد خلايا الجسم الدهني فقد تراوحت من **12.98 ±0.25** الى **13.42 ± 0.42** ملم ، **139.12 ± 6.45** الى **148.45 ± 7.39** مايكرون، **80.26 ± 4.32** الى **85.96 ± 5.22** مايكرون على التوالي.

اظهرت النتائج ايضا بان الطوائف الموجودة في الجبال كانت شغالاتها تمتلك فخذ اطول، خرطوم اطول، وزن اكثر، خلايا الجسم الدهني اكبر وغدد تحت البلعومية لها فصوص اكبر من تلك الشغالات التي جمعت من الطوائف الموجودة في السهول.

وجدت علاقة ايجابية معنوية بين وزن جسم الشغالة و ابعاد خلايا الجسم الدهني للشغالة و كذالك ابعاد فصوص الغدة تحت البلعومية وكذالك وجدت علاقة ايجابية معنوية اخرى بين طول جسم الشغالة و طول القناة الرئيسية للغدة تحت البلعومية.

هذه الدراسة لها اهمية استثنائية لفهم خصائص نحل العسل في شمال العراق.