RESPONSIBILITY OF FAT BODIES RELATED TO ENVIRONMENTAL FACTORS ON HONEYBEE (APIS MELLIFERA L.) (HYMENOPTERA: APIDAE) STRAINS IN KURDISTAN REGION

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Abstract. This study was carried out from April 2016 to the end of October 2017 including three seasons each year: Spring, early Summer, Summer and Autumn in the apiary of College of Agriculture Science in the University of Sulaimani – Kurdistan Region – Iraq. The results showed the highest rate of unsealed brood area for *Apis mellifera* Native was 1408.500 inch in early Summer 2016 and the lowest rate was 6.250 inch for *Apis mellifera ligustica* in Spring 2017, but the sealed brood area was 5906.500 inch for *Apis mellifera* Native strain in early Summer and the lowest rate was 22.000 inch for *Apis mellifera* Native strain in summer 2017 and 2016 respectively. Laboratory test indicated that the fat bodies play an important role in supplying the tissues with essential elements for body activities. Which means those fat bodies function as a reservoir.

Keywords: Apis mellifera L., foraging, honey production, nectar, pollen grain

Introduction

Foraging activity and behavior of Apis mellifera L. is dependent on many factors working at the same time, and the animals respond to some environmental conditions in a similar manner or method wherever they occur. Foraging for nectar and pollen is a continuous process throughout the year in tropical and sub-tropical regions, where the plants are available. However, the foraging activity of honey bees for pollen are significantly affected by weather conditions and availability of pollen (Neupane and Thapa, 2005). Foraging is one of the distinctive behaviors of honey bee (Apis mellifera L.), which links the honey bee colony and the surrounding environment (Abou-Shaara, 2014). It is known that the foraging activity for honeybees begin early in the morning and end in the evening. In some studies, honey bee workers started their foraging activity at 6.17 am in April, in one of the apple orchards (situated in Cheepa) in district Nainital of Kumaon Himalayas, Uttarakhand state of India. This start time however, may also be affected by the region (Joshi and Joshi, 2010). Al-garni (2006) and Blazyte-Cereskiene et al. (2010) found that temperature has a significant impact on foraging activity, for example high temperature has a negative impact on foraging bees. Additionally, very low temperatures (below 10 °C) can prevent flight. No significant direct effect of relative humidity was reported on honeybees, including foraging activity (Joshi and Joshi, 2010).

The fat bodies are normally distributed through the body cavity of insects, mostly in the abdomen where they appear as irregular masses of a soft and usually white tissue composed of large, loosely united cells. These cell masses are known collectively as fat body, because the cytoplasm of the cells contains small droplets of oily fat (Snodgrass and Erickson, 2003). The fat bodies can be irregularly distributed in the perivascular space of the abdomen and thorax, surrounding organs (visceral fat body) or in the dorsal and ventral sinus of the abdomen, close to the tegument (parietal fat body), in the head and even in body appendixes (Chapman, 1978; Zanini and Caetano, 2003). Ayoub (2011) found that the average dimension of a fat body cell in newly emerged workers ranges between 86.71 and 86.76 μ m and in 10-days old workers the average dimension of fat body cells ranges between 89.15 and 89.95 μ m, while the average dimensions of fat body cell for foraging workers ranges between 67.33 and 69.05 μ m.

Fat body is the main storage agent of the metabolic device of insects and is responsible for the synthesis and supply of hemolymph. Fatty body is made up of cells of the mesodermal origin, which sometimes contain epidermal cells (Oliveira and Cruz-Landim, 2003). Roma et al. (2010) demonstrated that fat body consists of a mass of cells under the epidermis, and in some insects, fat body also surrounds the digestive system and the reproductive system.

The aim of this work is to shed light on the factors related to be activities, to find out the temperature most sufficient for long journeys (round trips) for each season in the study region, and to determine the adaptability of different bee races to environmental factors in different seasons of the year.

Material and methods

Preparation and arrangement of colonies

This study was carried out from April 2016 to the end of October 2017, and included three seasons per year; Spring, Summer, and Autumn. It was performed in the apiary of College of Agriculture Science in the University of Sulaimany – Iraq. We prepared four colonies of *Apis mellifera carnica*, four colonies of *Apis mellifera ligustca*, and four colonies of *Apis mellifra* Native, taking the following characteristics into account:

The queens were characterized by fertility, similar ages and being uniform in size in each race, therefore all experimental colonies included five frames each homogenous and uniform in activity. For measurements, two Langstroth frames were prepared at first, then it was modified by dividing each frame in to 17 inch in length and 8 inch in wide sections, Holes were inserted into each frame using frame fastening wires (silk), with each unit being one square inch, with a total of 136 units.

The area of the brood, honey and pollen were measured four seasons per year (spring, early summer, late summer and autumn of 2016 and 2017) using the standard Langstroth frame as described above. Also the area of unsealed and sealed broods was measured every 2 weeks until the end of the study. The measurement was taken using a typical frame with the method described by Mustafa (2003) and Targany (2008) (*Figs. 1* and 2).



Figure 1. Typical frame

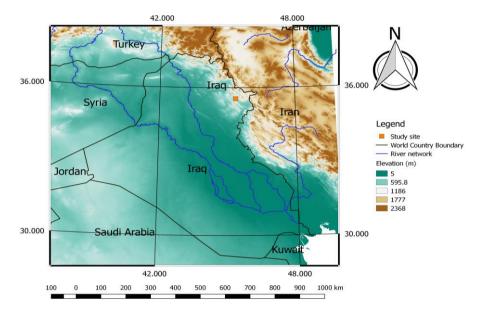


Figure 2. Map of the study site

Labeling of samples

Newly emerged workers were labeled with nail stains of different colors (Snodgrass and Erickson, 2003; Shamdin, 2003; Targany, 2008). One drop of the stain was stamped on the notum, characterized by little hairs, in a position where workers could not clean the label. The preliminary experiment was carried out in the laboratory in order to avoid any side effects of the stains on the viability of these young workers.

Dissection

The workers were fixed on the dissecting tray after the legs and wings were removed, and then were filled with physiological solution. Then the unit was transferred to the dissecting microscope, under which the head was cut off and placed on the filter paper for the measurement.

Workers were cut with a sharp scalpel into two lateral longitudinal slices of the abdomen. These were mounted on the dissecting tray by fine, stainless pins (Mahmoud, 1992; Shamdin, 2003). The workers were keep in a physiological saline with 0.9% w/v of NaCl, 0.9 g per 100 ml distilled water (Pantin, 1964). The fixed workers were dissected under 2x and 4x objective lenses of binocular. After cutting the cuticle and

removing the muscles, the fat bodies were picked up and placed on a clean slide and stained with methylene blue in normal limitation. The binocular dissecting and compound microscope for photographs and measurements was used with an eye piece graticule.

The climatic data

The data of climatic information was taken from Sulaimani General Directorate Meteorology and Seismology.

Statistical analysis

The results were analyzed statistically using factorial RCBD design with triple replicates and performed using the XLSTA program (2016). Duncan's multiple range Test was used to determine the differences between means at P = 0.05.

Result and discussion

Effect of environmental factors on bioactivity of honey bee colonies in spring

Unsealed workers brood area

Table 1 shows the average unsealed workers' brood area in spring. The highest average unsealed brood area of Apis mellifera ligustica was dated 14-5-2017 at 1316.250 inch², with an average temperature of 23.75 °C and relative humidity of 38.95%. The average unsealed brood area for Apis mellifera carnica and Apis mellifera Native were 1188.500 and 1086.500 inch. In the same period and weather conditions the lowest averages were 257.25, 268.75 and 266.500 inch² at 2-4-2016 and 253.000, 257.250 and 260.500 inch² at 2-4-2017 for A. m. carnica, A. m. ligustica and A. m. Native respectively, with an average temperature of 15.75, 16.5 °C and relative humidity of 65.75% respectively.

Period		Honey bee race	2			Relative
Spring	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	humidity %
2-4-2016	257.250 ј	268.750 ij	266.500 j	264.166	15.75	65.75
16-4-2016	292.250 hij	303.000 hij	295.000 hij	296.750	18.50	61.7
30-4-2016	350.500 gh	356.750 gh	344.750 ghi	350.666	21.50	59.75
14-5-2016	392.500 fg	381.750 g	387.000 fg	387.083	24.50	46
2-4-2017	253.000 j	257.250 ј	260.500 j	256.916	16.50	65.75
16-4-2017	487.000 e	477.750 e	456.750 ef	473.833	19.30	63.55
30-4-2017	660.000 d	655.250 d	643.250 d	652.833	21.50	48.5
14-5-2017	1188.500 b	1316.250 a	1086.500 c	1197.083	23.75	38.95
Mean	485.125	385.785	467.531		20.16	56.24375

Table 1. Effect of some honeybee races and environmental factors on unsealed brood area in spring 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

Figure 3 shows the effect of honey bee races on the general average of unsealed brood worker area. These were 485.125, 467.531 and 385.785 inch for *Apis mellifera carnica*, *Apis mellifera* Native and *Apis mellifea lugistica* race respectively. Statistical analysis showed significant differences at level 0.05 among *A. m. ligustica*, *A. m. carnica* and *A. m.* Native at different temperatures and relative humidity. The results agree with Becher et al. (2009) who found that honey bees (*Apis mellifera*) are able to regulate incubation temperatures within a narrow range between 32 and 36 °C. However, this small variation in brood temperature is sufficient to cause significant differences in the behavior of adult bees in agreement with Petz et al. (2004), who found the temperature, in particular, is very important for internal and external activities of honey bee colonies. Maintaining an appropriate degree of temperature from 33 to 36 °C within colonies is very important for honey bees.

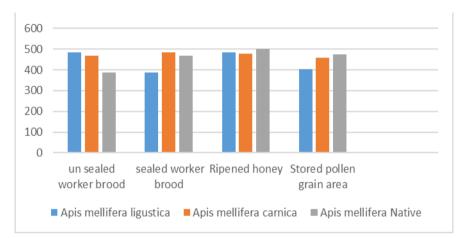


Figure 3. Effect of environmental conditions on the honeybee worker activities in spring

Sealed workers brood area

Table 2 shows the average sealed brood area of the workers in spring. The highest average sealed brood area for Apis mellefera ligustica was 1327.750 inch² with an average temperature of 23.75 °C and average relative humidity of 38.95% at 14-5-2017. Comparatively, the average sealed brood area for Apis mellifera carnica and Apis *mellefera* Native were 1137.750, 1098.500 inch² respectively during the same period and same weather conditions. Meanwhile, the lowest average of sealed brood was 261.000 inch² for A. m. ligustica at 2-4-2016, when the average temperature was 15.75 °C and the relative was humidity 65.75%. At the date of 14-5-2017 the highest general average was 1188.000 inch². Figure 3 shows the effect of the environmental conditions on A. m. carnica, A. m. Native, and A. m. ligustica. The general average of sealed brood area was 508.968, 501.812, and 514.187 inch² respectively. Statistical analysis showed significant differences at level 0.05 between races. According to the results, the sealed worker broods were in high numbers during spring, which is in accordance with Fathy (1997), who found that the results showed that the main peak of brood activity and a high rate of stored pollen were observed in May, and therefore the maximum number of bees was recorded during June and July. However, there is no reciprocal relationship. The lowest level of brood, bee and pollen production occurred during February.

Period		Honeybee race				Relative
Spring	Apis mellefera carnica	Apis mellefera ligustica	<i>Apis mellefera</i> Native	Mean	Temperature	humidity %
2-4-2016	274.750 ј	261.000 j	297.000 hij	277.583	15.75	65.75
16-4-2016	311.250 ghij	289.250 ij	326.250 ghij	308.916	18.50	61.70
30-4-2016	390.250 efg	342.500 fghij	380.250 efgh	371.000	21.50	59.75
14-5-2016	429.250 e	371.250 efghi	425.250 ef	408.583	24.50	46.00
2-4-2017	277.000 ј	267.000 j	296.500 hij	280.166	16.50	65.75
16-4-2017	539.500 d	544.750 d	506.250 d	530.166	19.30	63.55
30-4-2017	712.000 c	710.000 c	684.500 c	702.166	21.50	48.50
14-5-2017	1137.750 b	1327.750 a	1098.500 b	1188.000	23.75	38.95
Mean	508.968	514.187	501.812		20.16	56.24

Table 2. Effect of some honeybee races and environmental factors on sealed brood area in the spring season of 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

Ripened honey

Table 3 shows the average ripened honey area in spring. The highest average area for *A. m. ligustica* was 1565.750 inch² at 14-5-2017 when the average temperature was 23.75 °C and the relative humidity was 38.95%. The average area for *A. m. carnica* and *A. m.* Native were 1535.750 and 1542.750 inch² during the same period and with the same weather conditions, but the lowest average for the *A. m. carnica* was 215.750 inch² in 2-4-2017 when the average temperature and relative humidity was 16.5 °C and 65.75%. The average of ripened honey area was 476.25, 482.9688 and 499.6875 inch² for *A. m. carnica, A. m. ligustica* and *A. m.* Native respectively. The highest general average was 1548.083 inch² at the date of 14-5-2017 and the lowest was 237.166 inch² in 16-4-2016. Figure 3 shows the effect of environmental conditions on *A. m. carnica, A. m. ligustica* and *A. m.* Native. The general average of ripened honey area in spring was 476.250, 482.9688 and 499.687 inch² shows the effect of environmental conditions on *A. m. carnica, A. m. ligustica* and *A. m.* Native. The general average of ripened honey area in spring was 476.250, 482.968 and 499.687 inch² respectively. Statistical analysis showed significant differences at level 0.05 among races. According to the results, the amount of honey was high for all races in spring season due to flowering. This matches the findings of Mattu et al. (2012) who studied the times at which foraging starts and stops, the duration of the foraging and length of trips, as well as the number of flowers visited per minute.

Period		Honeybee race				Relative
Spring	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	humidity %
2-4-2016	236.500 de	234.500 de	273.000 de	248.000	15.75	65.75
16-4-2016	222.250 de	234.250 de	255.000 de	237.166	18.50	61.7
30-4-2016	232.750 de	240.250 de	277.750 d	250.250	21.50	59.75
14-5-2016	228.500 de	252.750 de	268.250 de	249.833	24.50	46.00
2-4-2017	215.750 e	237.000 de	268.750 de	240.500	16.50	65.75
16-4-2017	392.500 c	400.250 c	405.750 c	399.500	19.30	63.55
30-4-2017	746.000 b	699.000 b	706.250 b	717.083	21.50	48.50
14-5-2017	1535.750 a	1565.750 a	1542.750 a	1548.083	23.75	38.95
Mean	476.25	482.968	499.687		20.16	56.24

Table 3. Effect of some honeybee races and environmental factors on ripened honey area in spring season 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

Stored pollen grain area

Table 4 and Figure 3 show the average stored pollen grain area in spring. The highest average area for *A. m. carnica* was 1111.000 inch² at 14-5-2017 when the average temperature was 23.75 °C and the relative humidity was 38.95%. The average area for *A. m. ligustica* were 1088.750 and 972.500 inch² during the same period and the lowest average was 218.750 inch² for *Apis mellifera* carnica at 2-4-2017 when the average temperature and relative humidity was 16.5 °C and 65.75%. The average of stored pollen area was 474.062, 457.687 and 402.416 inch² for *A. m.* Native, *A. m. carnica* and *A. m. ligustica* respectively when the temperature was 20.16 °C and the average relative humidity was 56.24%. The highest general average was 1057.417 inch² at the date of 14-5-2017 and the lowest was 231.583 inch² at 2-4-2016. Statistical analysis showed significant differences at level 0.05 among all three races. The results agree with Neupane and Thapa (2005) who found that the foraging activity of honey bees for pollen area significantly affected by the weather condition and availability of pollen.

Period		Honey bee race)			Relative
Spring	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	humidity %
2-4-2016	221.000 h	216.000 h	257.750 fgh	231.583	15.75	65.75
16-4-2016	241.250 fgh	221.000 h	278.000 fgh	246.750	18.50	61.70
30-4-2016	266.250 fgh	244.250 fgh	297.000 fg	269.166	21.50	59.75
14-5-2016	303.000 ef	269.250 fgh	364.250 e	312.166	24.50	46.00
2-4-2017	218.750 h	230.250 fgh	286.500 fgh	245.166	16.50	65.75
16-4-2017	544.250 d	751.500 c	494.000 d	596.583	19.30	63.55
30-4-2017	756.000 c	751.500C	726.250 c	744.583	21.50	48.50
14-5-2017	1111.000 a	972.500 b	1088.750 a	1057.417	23.750	38.95
Mean	457.687	402.416	474.062		20.162	56.24

Table 4. Effect of some honeybee races and environmental factors on pollen grain area in spring season 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

Effect of environmental factors on bioactivity of honey bee colonies in early summer

Unsealed workers brood area

Table 5 shows the average unsealed workers brood area in early summer. The highest average unsealed brood area for A. m. Native at the date of 28-5-2017 was 1408.500 inch² when the average temperature was 26.50 °C and the average relative humidity was 36.50%. The average unsealed brood area for A. m. carnica and A. m. ligustica were 1333.250 and 1371.750 inch² during the same period and weather conditions, while the lowest average was 132.250 inch² at 9-7-2016 and 767.937, 750.937 and 770.781 inch² at 9-7-2016 for A. m. carnica, A. m. ligustica and A. m. Native respectively, as in shown in Figure 4. Statistical analysis showed significant differences at level 0.05 among studied races. Abou-Shaara et al., (2012) also found

positive correlations between foraging activities and sealed brood area as well as bee numbers.

Period		Honeybee race	•			Relative
Early summer	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	humidity %
28/5/2016	438.000 e	413.750 e	439.500 e	430.416	26.75	44.00
11/6/2016	446.500 e	422.250 e	428.750 e	432.500	28.00	35.50
25/6/2016	405.750 e	393.750 e	386.750 e	395.416	30.50	31.75
9/7/2016	138.750 f	133.000 f	132.250 f	134.666	34.50	27.30
28/5/2017	1333.250 ab	1371.750 a	1408.500 a	1371.167	26.50	36.50
11/6/2017	1332.000 ab	1313.500 ab	1229.000 b	1291.500	32.40	30.65
25/6/2017	1019.250 cd	936.500 d	1024.750 cd	993.500	33.50	23.50
9/7/2017	1030.000 cd	1023.000 cd	1116.750 c	1056.583	34.85	22.60
Mean	767.937	750.937	770.781		30.87	31.47

Table 5. Effect of some honeybee races and environmental factors on unsealed brood area in early summer 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

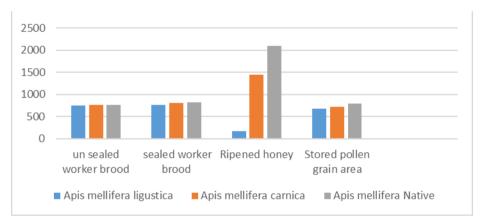


Figure 4. Effect of environmental factors on different the activities of honeybee races in early summer

Sealed workers brood area

Table 6 and Figure 4 show the average sealed brood area of the workers in early summer. The highest average sealed brood area for A. m. Native 1514.750 inch² at 28-5-2017 when the average temperature was 26.50 °C and the average relative humidity was 36.50%. The average sealed brood area for A. m. carnica and A. m. ligustica were 1404.250 and 1319.750 inch² during the same period and weather conditions, while the lowest average of sealed brood was 101.500 inch² for A. m. Native at 9-7-2016, when the average temperature and relative humidity were 34.50 °C and 27.30%. The average of sealed workers brood area was 823.843, 803.625 and 770.312 inch² for A. m. Native, A. m. carnica and A. m. ligustica respectively, when the average temperature was 30.87 °C and average relative humidity was 31.47%). The highest general average was

1412.917 inch² in 28-5-2017 and the lowest was 107.000 inch² at 9-7-2016. Statistical analysis showed significant differences at level 0.05 among races. Harbo (2015) found that the bees used a total of 121 grams of honey to produce 1,000 cells of the brood (eggs, larvae, and virgins in the normal brood nest) and about 163 mg of honey to rear the working bees to the virgin stage. In colonies containing incubation of all stages, brood weight was approximately equal to (about 25% less than) the weight of the honey used in its production.

early summ	early summer 2010-2017									
Period		Honeybee race	•							
Early summer	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	Relative humidity %				
28/5/2016	473.250 f	417.750 f	475.250 f	455.416	26.75	44.00				
11/6/2016	484.500 f	434.000 f	462.500 f	460.333	28.00	35.50				
25/6/2016	459.000 f	412.750 f	438.750 f	436.833	30.50	31.75				
9/7/2016	110.000 g	109.500 g	101.500 g	107.000	34.50	27.30				

1514.750 a

1404.250 ab

1143.750 c

1050.000 cde

823.843

1412.917

1406.917

1111.750

1002.917

26.50

32.40

33.50

34.85

30.87

36.50

30.65

23.50

22.60

31.47

Table 6. Effect of some honeybee races and environmental factors on sealed brood area in 2016 2015

770.312 Different letters mean significant difference (p<0.05) based on Duncan test.

1319.750 b

1427.500 ab

1081.750 cde

959.500 e

Ripened honey area

28/5/2017

11/6/2017

25/6/2017

9/7/2017

Mean

1404.250 ab

1389.000 ab

1109.750 cd

999.250 de

803.625

Table 7 shows the average ripened honey area in early summer. The highest average area for A. m. Native was 5906.500 inch² at 25-6-2017 when the average temperature was 33.50 °C and the relative humidity was 23.50%. In comparison, the average area for A. m. carnica and A. m. ligustica were 4813.750 and 3781.000 inch² during the same period and weather conditions. The lowest average for A. m. ligustica and A. m. Native was 243.000 inch² at 9-7-2016 when the average temperature and relative humidity were 34.50 °C and 22.60%. The average of ripened honey area was 2103.000, 1793.156 and 1453.375 inch² for A. m. Native, A. m. carnica and A. m. ligustica respectively as show in Figure 4.

Table 7. Effect of some honeybee races and environmental factors on sealed honey area in early summer 2016-2017

Period	Honey bee race					Relative
Early summer	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	humidity %
28/5/2016	244.250 g	272.000 g	283.250 g	266.500	26.75	44.00
11/6/2016	288.000 g	296.500 g	315.250 g	299.916	28.00	35.50
25/6/2016	288.000 g	316.000 g	324.000 g	309.333	30.50	31.75
9/7/2016	247.000 g	243.000 g	243.000 g	244.333	34.50	27.30
28/5/2017	3143.500 d	2186.000 e	3387.750 cd	2905.750	26.50	36.50

11/6/2017	3785.000 c	2966.750 d	4821.500 b	3857.750	32.40	30.65
25/6/2017	4813.750 b	3781.000 c	5906.500 a	4833.750	33.50	23.50
9/7/2017	1535.750 f	1565.750 f	1542.750 f	1548.083	34.85	22.60
Mean	1793.156	1453.375	2103.000		30.87	31.47

Different letters mean significant difference (p<0.05) based on Duncan test.

The highest general average was 4833.750 inch² in 25-6-2017 and the lowest was 244.333 inch² at 9-7-2016. Statistical analysis showed significant differences at level 0.05 among *A. m. carnica*, *A. m.* local and *A. m. ligustica*. The results agree with Tirado et al. (2013) who indicated that Climate change is associated with a marked disparity in the abundance of honey bees and honey yield. Foraging activities of social insects are affected by climate.

Stored pollen grain area

Table 8 shows the average stored pollen grain area in early summer. The highest average area for A. m. Native was 1575.500 inch² at 25-6-2017, when the average temperature was 33.50 °C and relative humidity was 23.50%. The average area for A. m. *carnica* and A. m. ligustica were 1401.000 and 1268.750 inch² during the same period and weather conditions. The lowest average was 78.500 inch² for A. m. Native at 9-7-2016 when the average temperature and relative humidity were 34.50 °C and 27.30%. The average of stored pollen area was 789.000, 716.281 and 673.906 inch² for A. m. Native, A. m. carnica and A. m. ligustica, with a temperature of 30.87 °C and an average relative humidity of 31.47% as shown in Figure 4. The highest general average was 1415.083 inch² at 25-6-2017 and the lowest was 87.500 inch² at 9-7-2016. Statistical analysis showed significant differences at level 0.05 among A. m. carnica, A. m. Native and A. m. ligustica. The result agree with Mesbah et al. (2017), who showed that the largest quantity of pollen was in August and the summer season, while the lowest was in May and the spring season. Also the highest mean area of brood and honey sealed in trap colonies and without a trap was in September, while the lowest was in May in colonies both with and without traps.

Early summer	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	Relative humidity %
28/5/2016	332.250 gh	307.500 gh	390.000 g	343.250	26.75	44.00
11/6/2016	352.000 gh	294.500 gh	380.500 g	342.333	28.00	35.50
25/6/2016	303.250 gh	244.250 h	323.000 gh	290.166	30.50	31.75
9/7/2016	86.000 i	97.250 i	78.500 i	87.250	34.50	27.30
28/5/2017	1047.250 ef	967.500 f	979.750 f	998.166	26.50	36.50
11/6/2017	1027.500 ef	982.000 f	1102.500 de	1037.333	32.40	30.65
25/6/2017	1401.000 b	1268.750 c	1575.500 a	1415.083	33.50	23.50
9/7/2017	1181.000 cd	1229.500 c	1482.250 ab	1297.583	34.85	22.60
Mean	716.281	673.906	789.000		30.87	31.47

Table 8. Effect of some honeybee races and environmental factors on pollen grain area in early summer 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

Effect of environmental factors on bioactivity of honey bee colonies in late summer

Unsealed workers brood area

Table 9 shows the average unsealed workers brood area in summer. The highest average unsealed brood area for A. m. Native at 23-7-2017 was 920.250 inch² when the average temperature was 32.75 °C and the average relative humidity was 24.15%. The average unsealed brood area for A. m. carnica and A. m. ligustica was 773.500 and 753.000 inch² during the same period and weather conditions, while the lowest average of unsealed brood area was 86.250 inch² for A. m. Native at 6-8-2016 when the average temperature and relative humidity were 43.95 °C and 24.30%. The average of unsealed workers brood area was 382.250, 328.781 and 289.875 inch² for A. m. Native, A. m. *carnica* and, A. m. ligustica respectively with an average temperature of 36.11 °C and average relative humidity of 27.31% as shows in *Figure 5*. The highest general average was 815.583 inch² at 23-7-2017 and the lowest was 104.583 inch² at 6-8-2016. Statistical analysis showed significant differences at level 0.05 among A. m. carnica, Apis mellifera Native and A. m. ligustica. Reddy et al. (2012) also found that at the same ambient temperature, outgoing A. mellifera foragers and workers who were sampled from the brood nest had a higher chest temperature, much higher than leaving A. cerana foragers and brood nest workers. A. mellifera colonies also maintained a brood temperature significantly higher than A. cerana. Our findings indicate that the larger A. mellifera foragers require higher chest temperature to be able to forage in the apiary in Kunming, China.

Summer		Honey bee race	e			Dalation
Period	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	Relative humidity %
23/7/2016	115.250 g	112.250 g	105.750 g	111.083	34.05	25.85
6/8/2016	115.250 g	112.250 g	86.250 g	104.583	43.95	24.3
20/8/2016	130.250 g	116.500 g	102.250 g	116.333	42.50	26.95
3/9/2016	152.000 g	139.750 g	121.750 g	137.833	34.50	35.7
23/7/2017	773.500 b	753.000 bc	920.250 a	815.583	32.75	24.15
6/8/2017	529.000 d	406.000 e	688.000 c	541	36.70	24.5
20/8/2017	398.750 ef	326.500 f	505.250 d	410.166	34.60	26.44
3/9/2017	416.250 e	352.750 ef	528.500 d	432.5	29.90	30.65
Mean	328.781	289.875	382.250		36.11	27.317

Table 9. Effect of some honeybee races and environmental factors on unsealed brood area in summer 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

Sealed workers brood area

Table 10 and Figure 5 show the average sealed brood area of the workers in summer. The highest average sealed brood area for A. m. Native 948.250 inch² at 23-7-2017, when the average temperature was $32.75 \,^{\circ}$ C and the average relative humidity was 24.15%. In comparison, the average sealed brood area for A. m. carnica and A. m. ligustica were 939.500 and 795.500 inch² during the same period and weather conditions, while the lowest average of sealed brood was 61.250 inch² for A. m.

ligustica at 20-8-2016, when the average temperature and relative humidity were 42.50 °C and 26.95% respectively. The average of sealed workers brood area was 397.968, 372.187 and 315.031 inch² for *A. m.* Native, *A. m. carnica* and *A. m. ligustica* respectively, when the average temperature was 36.11 °C and the average relative humidity was 27.31%. The highest general average was 894.41 inch² at 23-7-2017 and the lowest was 62.916 inch² at 20-8-2016. Statistical analysis showed significant differences at level 0.05 among *A. m. carnica*, *A. m.* Native and *A. m. ligustica*. Hossam et al. (2012) and Contreras et al. (2013) also found, that all daily activities and patterns of forager honeybees are under the control and/or change with weather conditions.

Table 10. Effect of some honeybee races and environmental factors on sealed brood area in summer 2016-2017

Summer		Honeybee race	•			D.1.4*
Period	Apis mellifera carnica	Apis mellifera ligustica	Apis mellifera local	Mean	Temperature	Relative humidity %
23/7/2016	90.250 g	93.000 g	84.500 g	89.250	34.05	25.85
6/8/2016	76.250 g	72.250 g	84.500 g	77.660	43.95	24.30
20/8/2016	61.750 g	61.250 g	65.750 g	62.916	42.50	26.95
3/9/2016	77.000 g	76.500 g	83.500 g	79.000	34.50	35.70
23/7/2017	939.500 a	795.500 b	948.250 a	894.410	32.75	24.15
6/8/2017	710.000 b	560.500 cd	715.750 b	662.080	36.70	24.50
20/8/2017	497.250 def	421.000 f	588.250 cd	502.160	34.60	26.44
3/9/2017	525.500 cde	440.250 ef	613.250 c	526.330	29.90	30.65
Mean	372.187	315.031	397.968		36.11	27.31

Different letters mean significant difference (p<0.05) based on Duncan test.

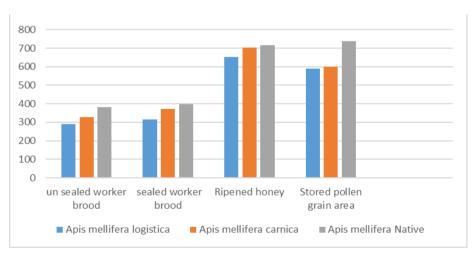


Figure 5. Effect of environmental factors on different the activities of honeybee races in summer

Ripened honey area

Table 11 shows the average ripened honey area in early summer. The highest average area for *A. m.* Native was 1431.000 inch² at 23-7-2017, when the average temperature was 32.75 °C and relative humidity was 24.15%. In comparison, the average area for *A. m.*

carnica and *A. m. ligustica* were 1420.500 and 1370.750 inch² during the same period and weather conditions. The lowest average for *A. m. ligustica* was 257.000 inch² at 23-7-2016, when the average temperature and relative humidity were 34.05 °C and 25.85%. The average of ripened honey area was 715.000, 704.343 and 651.468 inch² for *A. m.* Native *A. m. carnica* and *A. m. ligustica* respectively as shown in *Figure 5*. The highest general average was 1290.917 inch² at 6-8-2017 and the lowest was 54.833 inch² at 20-8-2016. Statistical analysis showed significant differences at level 0.05 among races. The results agree with Ali (2011), who found a high foraging rate of Yemeni honeybee in comparison to honey bees in Carniola during June and August and at different times of observation: 6-7 am, 11 to 12 am, and 4 to 5 pm.

Summer		Honeybee race	2			Dolotimo
Period	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	Relative humidity %
23/7/2016	264.500 d	257.000 d	269.500 d	263.666	34.05	25.85
6/8/2016	275.500 d	271.250 d	275.500 d	274.083	43.95	24.30
20/8/2016	55.000 e	46.000 e	63.500 e	54.833	42.50	26.95
3/9/2016	84.750 e	87.000 e	87.000 e	86.250	34.50	35.70
23/7/2017	1420.5000 a	1370.750 a	1431.000 a	1407.417	32.75	24.15
6/8/2017	1333.250 a	1186.250 b	1353.250 a	1290.917	36.70	24.50
20/8/2017	1165.750 b	1020.000 c	1198.750 b	1128.167	34.60	26.44
3/9/2017	1035.500 c	973.500 c	1041.500 c	1016.833	29.90	30.65
Mean	704.343	651.468	715.000		36.11	27.31

Table 11. Effect of some honeybee races and environmental factors on sealed honey area in summer 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

Stored pollen grain area

Table 12 and Figure 5 show the average stored pollen grain area in summer. The highest average area for A. m. Native was 1582.750 inch² at 23-7-2017, when the average temperature was 32.75 °C and the relative humidity was 24.15%. The average area for A. m carnica and A. m ligustica were 1205.250 and 1191.500 inch² during the same period and with the same weather conditions. In comparison, the lowest average was 7.290 inch² for the A. m Native at 3-9-2016 when the average temperature and relative humidity was 34.50 °C and 35.70%.

Table 12. Effect of some honeybee races and environmental factors on pollen grain area in summer 2016-2017

Summer	Honey bee race					Relative
Period	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	humidity %
23/7/2016	69.500 ij	86.250 i	60.250 ij	72.000	34.05	25.85
6/8/2016	76.000 ij	62.000 ij	51.750 ij	63.250	43.95	24.30
20/8/2016	55.000 ij	46.000 ij	63.500 ij	54.833	42.50	26.95
3/9/2016	22.500 ij	38.750 ij	7.290 j	22.846	34.50	35.70
23/7/2017	1205.250 de	1191.500 de	1582.750 a	1326.500	32.75	24.15

6/8/2017	1178.250 ef	1163.500 ef	1511.500 b	1284.417	36.70	24.50
20/8/2017	1116.250 fg	1094.000 g	1374.750 c	1195.000	34.60	26.44
3/9/2017	1065.500 gh	1027.000 h	1248.250 d	1113.583	29.90	30.65
Mean	598.531	588.625	737.505		36.11	27.31

Different letters mean significant difference (p<0.05) based on Duncan test.

The average of stored pollen area was 598.531, 588.625 and 737.505 inch² for *A. m* carnica, *A. m ligustica* and *A. m.* Native respectively, when the temperature was $36.11 \,^{\circ}$ C and the average relative humidity was 27.31%. The highest general average was $1326.500 \,\text{inch}^2$ at 23-7-2017 and the lowest was $22.846 \,\text{inch}^2$ at 3-9-2016. Statistical analysis showed significant differences at level 0.05 between all races. These results show a low amount of pollen, while Pernal and Currie (2010) found, that in the absence of pollen or with poor pollen quality, colonies of honeybees increase the proportion of pollen foragers without increasing the rate of foraging.

Effect of environmental factors on bioactivity of honey bee colonies in autumn

Unsealed workers brood area

Table 13 shows the average unsealed workers brood area in autumn. The highest average unsealed brood area for *A. m. carnica* at 29-10-2017 was 487.000 inch², when the average temperature was 19.50°C and the average relative humidity was 52.75%. The average unsealed brood area for *A. m. ligustica* and *A. m.* Native was 477.750 and 456.750 inch² during the same period and weather conditions, while the lowest average of unsealed brood area was 18.500 inch² for *A. m.* Native at 29-10-2016 when the average temperature and relative humidity were 22.55 °C and 44.4% respectably.

Autumn		Honey bee race	e			Relative
Period	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	humidity %
17/9/2016	90.250 hijk	133.250 gh	77.000 ijkl	100.166	29.75	38.50
1/10/2016	103.500 ghij	148.250 g	77.000 ijkl	109.583	27.50	40.05
15/10/2016	59.000 ijklm	109.500 ghi	40.000 klm	69.500	24.75	42.00
29/10/2016	30.250 lm	54.500 jklm	18.500 m	34.416	22.55	44.40
17/9/2017	364.250 bc	320.250 cd	473.500 a	386.000	27.45	33.50
1/10/2017	307.500 de	270.750 ef	373.750 b	317.333	22.50	36.75
15/10/2017	253.000 f	257.250 f	260.500 ef	256.916	20.65	40.05
29/10/2017	487.000 a	477.750 a	456.750 a	473.833	19.50	52.75
Mean	211.843	221.437	222.125		24.33	41.00

Table 13. Effect of some honeybee races and environmental factors on unsealed brood area in autumn 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

The average of unsealed workers brood area was 222.125, 221.4375 and 211.8438 inch² for *A. m.* Native, *A. m. ligustica* and *A. m. carnica* respectively, when the average temperature was 24.33°C and the average relative humidity was 41.00% as shown in

Figure 6. The highest general average was 473.833 inch² at 29-10-2017 and the lowest was 34.41667 inch² at 29-10-2016. Statistical analysis showed significant differences at level 0.05 among *A. m. carnica, A. m.* local and *A. m. ligustica.* The results agree with Taragany (2008), who found that the highest means of the unsealed brood area and sealed brood area can be detected in autumn, when they are fed extra sugar solution and vice versa.

Sealed workers brood area

Table 14 and *Figure 6* show the average sealed brood area of the workers in autumn. The highest average sealed brood area for *A. m.* Native 563.000 inch² at 17-9-2017, when the average temperature was 27.45 °C and the average relative humidity was 33.50%. In comparison, the average sealed brood area for *A. m. carnica and A. m. ligustica* was 480.500 and 396.500 inch² during the same period and weather conditions, while the lowest average of sealed brood was 22.000 inch² for *A. m.* Native at 29-10-2017, when the average temperature and relative humidity were 19.50 °C and 52.75%. The average of sealed workers brood area was 160.468, 151.437 and 141.625 inch² for *A. m.* Native. *A. m. ligustica* and *A. m. carnica* respectively, when the average temperature was 24.33 °C and the average relative humidity was 31.78333 inch² at 15-10-2016. Statistical analysis showed significant differences at level 0.05 among races. These result agree with Harbo (2015), who showed that the average number of adult bees declined steadily from 20 800 in November to 12,000 in March. The brood in the colonies was small before the winter solstice, but soon increased.

Autumn		Honey bee race	e		Temperature	Relative humidity %
Period	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean		
17/9/2016	84.750 fg	142.500 e	82.250 fg	103.166	29.75	38.50
1/10/2016	52.750 fg	110.000 ef	52.000 fg	71.583	27.50	40.05
15/10/2016	22.350 g	48.000 fg	25.000 g	31.783	24.75	42.00
29/10/2016	22.750 g	51.000 fg	23.000 g	32.250	22.55	44.40
17/9/2017	480.500 b	396.500 cd	563.000 a	480.000	27.45	33.50
1/10/2017	424.500 c	359.500 d	492.000 b	425.333	22.50	36.75
15/10/2017	22.650 g	54.000 fg	24.500 g	32.383	20.65	40.05
29/10/2017	22.750 g	50.000 fg	22.000 g	32.916	19.50	52.75
Mean	141.625	151.437	160.468		24.33	41.00

Table 14. Effect of some honeybee races and environmental factors on sealed brood area inautumn 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

Ripened honey area

Table 15 shows the average ripened honey area in early summer. The highest average area for *A. m.* Native was 991.500 inch² at 17-9-2017 when the average temperature was 27.45 °C and the relative humidity was 33.50%. The average area for *A. m carnica* and *A. m ligustica* was 957.750 and 917.000 inch² during the same period and weather conditions. In

comparison, the lowest average for *A. m.* Native was 106.750 inch² at 17-9-2016, when the average temperature and relative humidity were 29.75 °C and 38.50%. The average of ripened honey area was 400.437, 387.281 and 383.406 inch² for *A. m* Native, *A. m ligustica* and *A. m carnica* respectively as shown in *Figure 6*. The highest general average was 955.416 inch² at 17-9-2017 and the lowest was 127.000 inch² at 17-9-2016. Statistical analysis showed significant differences at level 0.05 *among A. m. carnica, A. m.* Native and *A. m. ligustica.* Results agree with Bas (2013) who recorded that the highest rate of stored pollen area in autumn was 9.83 inch² when fed with multi- vitamin and vice versa.

Autumn	Honey bee race					Relative
Period	Apis mellifera carnica	Apis mellifera ligustica	<i>Apis mellifera</i> Native	Mean	Temperature	humidity %
17/9/2016	136.500 hi	137.750 hi	106.750 i	127.000	29.75	38.50
1/10/2016	180.000 fghi	177.500 fghi	156.250 ghi	171.250	27.50	40.05
15/10/2016	200.250 efgh	219.250 defg	205.750 efgh	208.416	24.75	42.00
29/10/2016	240.000 def	276.750 de	295.000 d	270.583	22.55	44.40
17/9/2017	957.750 ab	917.000 abc	991.500 a	955.416	27.45	33.50
1/10/2017	912.500 bc	874.000 c	947.500 abc	911.333	22.50	36.75
15/10/2017	200.250 efgh	219.250 defg	205.750 efgh	208.416	20.65	40.05
29/10/2017	240.000 def	276.750 de	295.000 d	270.583	19.50	52.75
Mean	383.406	387.281	400.437		24.33	41.00

Table 15. Effect of some honeybee races and environmental factors on ripened honey area in autumn 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

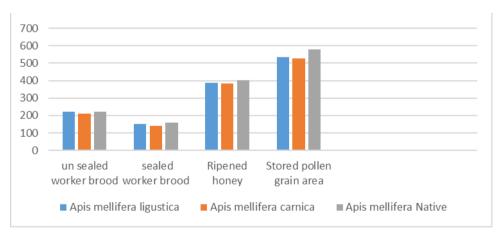


Figure 6. Effect of environmental factors on different the activities of honeybee races in autumn

Stored pollen grain area

Table 16 and *Figure 6* show the average stored pollen grain area in autumn. The highest average area for *A. m.* Native was 1196.250 inch² at 17-9-2017, when the average temperature was 27.45 °C and relative humidity was 33.50%. The average area for *A. m. carnica* and *A. m. ligustica* was 1017.250 and 982.500 inch² during the same period and with the same weather conditions. In comparison, the lowest average was

32.000 inch² for *A. m.* Native at 15-10-2016, when the average temperature and relative humidity were 24.75 °C and 42%. The average of stored pollen area was 580.937, 533.812 and 527.250 inch² for *A. m.* Native, *A. m. ligustica* and *A. m. carnica* respectively, when the temperature was 24.33 °C and the average relative humidity was 41.00%. The highest general average was 1065.333 inch² at 17-9-2017 and the lowest was 63.833 inch² at 15-10-2016. Statistical analysis showed significant differences at level 0.05 between all races. The results are in agreement with Tan et al. (2012), who found that *A. cerana* began foraging earlier and at lower temperatures than *A. mellifera* did. *A. cerana* foraging (departures per minute) reached its peak early and at a lower temperature than *A. mellifera* foraging did.

Autumn		Honeybee race	e		Temperature	Relative humidity %
Period	Apis mellifera carnica	Apis mellifera ligustica	Apis mellifera Native	Mean		
17/9/2016	109.250 fgh	130.500 f	64.500 ghij	101.416	29.75	38.50
1/10/2016	93.500 fghi	115.750 fg	50.250 ij	86.500	27.50	40.05
15/10/2016	65.250 ghij	94.250 fghi	32.000 j	63.833	24.75	42.00
29/10/2016	87.000 fghi	98.000 fghi	55.750 hij	80.250	22.55	44.40
17/9/2017	1017.250 c	982.500 cd	1196.250 a	1065.333	27.45	33.50
1/10/2017	976.500 cd	952.750 d	1133.750 b	1021.000	22.50	36.75
15/10/2017	978.750 cd	957.750 d	1116.250 b	1017.583	20.65	40.05
29/10/2017	943.000 d	886.500 e	998.750 cd	942.750	19.50	52.75
Mean	533.812	527.250	580.937		24.33	41.00

Table 16. Effect of some honeybee races and environmental factors on pollen grain area in autumn 2016-2017

Different letters mean significant difference (p<0.05) based on Duncan test.

Stages of fat bodies during the life of honey bees

This study also aimed to explain the stages of fat bodies throughout the life of honey bees as shown in *Plate 1*:

- a. Perivisceral fat bodies in one day old workers appeared with a dense inclusion, dark color and a thick wall with invisible nucleus.
- b. Perivisceral fat bodies in seven day old workers of *Apis mellifera carnica*: A new generation of fat bodies, slightly larger with vacuolization features inside, and course, thick walls as result of newly emerging in spring.
- c. Perivisceral fat bodies in fourteen day old worker of *Apis mellifera carnica* in spring: The fat cell was very large, it vacuolated various shapes, and had thick walls, light blue in color.
- d. Perivisceral fat bodies in twenty one day old worker of *Apis mellifera* Native in spring: The fat bodies were similar to twenty one day old workers of *Apis mellifera ligustica* in spring, but were larger in size.
- e. Perivisceral fat bodies in twenty eight day old worker of *Apis mellifera* Native in spring: These showed very obvious and clear features, loaded with a dense inclusion, some nucleus and variously shaped, dark purple, vacuolated cells.

Rukhosh et al.: Responsibility of fat bodies related to environmental factors on honeybee (*Apis mellifera* L.) (Hymenoptera: Apidae) strains in Kurdistan region - 1376 -

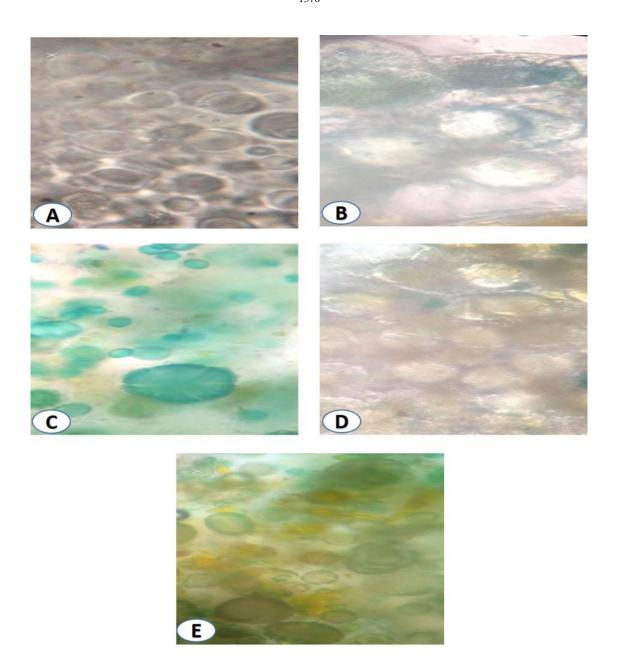


Plate 1. Stages of fat bodies during the life of honey bees (40X)

Conclusion

The results of the field experiment revealed the direct effect of environmental conditions (Temperature and relative humidity) on three races of honeybees. It was conducted for two years in different seasons. Comparing the two races (*Apis mellifera carnica* and *Apis mellifera ligustica*) native to the country with *Apis mellifera* Native during the first year, *Apis mellifera* Native are more affected by environmental factors than the other two races (*Apis mellifera carnica* and *Apis mellifera ligustica*). The results showed the highest rate of unsealed brood area of *Apis mellifera ligustica* in spring 2017. The sealed brood area of the *Apis mellifera* Native strain was the highest in early summer, and the lowest rate of *Apis mellifera* Native strain was in autumn 2017. The

highest and the lowest rate of pollen grain area for *Apis mellifera* Native strain were detected in the summer season of 2017 and 2016 respectively. We also studied the fat body difference through the stages of life for honeybees.

The highest and the lowest rate of Pollen grain area were (1582.750 and 7.290) inch for *Apis mellifera* Native strain in summer 2017 and 2016 respectively.

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ELECTRONIC APPENDICES

Appendix 1: Pollen 2016

Appendix 2: Pollen 2017

Appendix 3: Pollen 2016-2017

Appendix 4: Ripened honey 2016

Appendix 5: Ripened honey 2017

- Appendix 6: Ripened honey 2016-2017
- Appendix 7: Sealed brood 2016
- Appendix 8: Sealed brood 2017
- Appendix 9: Sealed brood 2016-2017
- Appendix 10: Unsealed brood 2016
- Appendix 11: Unsealed brood 2017
- Appendix 12: Unsealed brood 2016-2017