Study of the defensive behavior of Iraqi honeybee's colonies (order: hymenoptera) in Basra province – Iraq

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Abstract

The study was carried out in three areas of Basra Province to evaluate the aggression of the Iraqi honey bee *Apis mellifera* L. using three types of stimuli to represent the natural defensive behavior of bees, Response degrees were measured due to Stort's technique. results showed the aggressiveness of honey bees in Al-Jubayla region, with a significant difference from that of Garmat Ali, the stinging time of the leather ball reached 1.300 min, the number of stings in the glove was 4.499, and the return to normal time was 17.797 min. The aggressiveness of honey bees in the Garmat Ali region was significantly different from that of Shatt al-Arab the ball sting time was 2.107 min, the number of stings in the glove was 2.777 and the return to normal time was 6.79 min. While the aggressive of honey bees in Shatt al-Arab region was quiet, the significant difference was negative in the Al-Jubyla and Garmat Ali region, as the average time to sting the leather ball reached 5.325 min., the average number of stings in the glove was 2.111 and the average normal return time was 12.442 min. The mean values of the number of stings in each glove and the leather ball for the three regions were 2.133 stings. The degree of aggression of the studied colony is quiet, frictionless, which is a good quality for breeding. This study is considered the first of its kind in Iraq.

1. Introduction

Honey bees *Apis mellifera* L. are complex social insects They live in hives that contain stock honey, pollen, and brood, which are vulnerable to a wide range of predators and parasites so the colony must defend these resources [1] Many studies have been done on bees behavior from different sides as these insects quite different from other insects The behavior that distinguishes honey bees from other insects Which includes defensive behavior House or nest defense It is colony defense is one of the most important activities This is not because bees can protect their nest, brood and stored food from enemies, but rather because stinging behavior is one of the most common obstacles to beekeeping. As all programs of genetic improvement Which carefully selects standard traits to take into account the hostile or defensive behavior of honey bees [2] Requires The defensive behavior of honey bees is a complex and multisensory integration It includes olfactory, visual, sensory and mechanical signals and thus constitutes a condition Exciting for study and interest [3]. The defensive behavior of honey bees includes two Situations, which are guarding behavior at the entrance to the hive and the state of flight and stinging. The strategies of colony defense behavior differ according to the different types of honey bees.

As for the Iraqi bee's races, there is no independent study to determine their aggressive except that mentioned by Brother Adam 1970, as he explained there are two types of models of bees in northern Iraq, Al-Ghanami which are quiet and a Sayafey bee which is brutal and attacks strongly [4,5]. The aim of the present study is to determine the degree of aggressive of honey bee colonies in Basrah, south Iraq.

2. Materials and Methods

2.1 Study areas

experiments on defensive behavior were carried out during March 2020 in Spring 2020, Three agricultural areas were chosen omitted Province, it is the Garmat Ali region, which includes apiary of the Agricultural Research Station of the College of Agriculture, University of Basra, omitted, replace by 2 Al-Jubayla region which include the apiary of the engineer Diah Essa, and the Creedland ommited in the Shatt al-Arab area. Twelve bee's colonies (four colonies for each region) were identified as experimental unit, and the Powers were united. The colonies

were reared in Langstroth hives, each containing a queen of 1-2 years old with a bee density of 7-8 frames and two stores of honey and pollen at a rate of 4-5 frames/hive. To conduct the study, away from any interference that may influence by environmental factors, experiments were carried out on sunny days with moderate temperature, replace by and humidity, quiet air currents, and healthy hives were chosen.

2.2 Impact stimuli

Three types of stimuli were used in a sequential manner [6], the visual stimulator using a tennis ball coated with black leather, a color that the bees do not like it. The mechanical stimulus using the hive tool to knock its wall regularly, and the third stimulus is by opening the outer and inner cover of the hives When the hive at its highest levels of excitement, (Figure 1). These three Stimulus similar of the natural one, which include alerting, activating attracting, and culminating (attack) the latter two methods are often used when examining colonies.

2.3 Stimulus Impact Scale

Stort's technique [7] was followed to measure the of bees to the three stimuli including the time of the first bee stings the ball, the time it took for the colony to become defensive, the number of a sting in the glove, the number of a sting in the leather ball, and the time it took for the colony to return to its normal state. A camera was installed in front of the entrance of the colony to photograph the numbers of guard bees before and after the Application experiments, Putting the leather ball in front of the entrance to the colony, then we knocked on the colony wall. and finally we opened the outer and inner cover of the hive, the period between one stimulus and another was five minutes three replicants were done before data were recorded where the time between each replicant was 30 min. Use in analyzing the multivariate data, calculating the mean, standard deviation, and confidence limits, and comparing the averages using the ready-made statistical program SPSS version 23.0. [8].

Fig. 1: Degree of response to stimuli, defensive behavior in honey bees: A = before the start of the experiment, B = response to the first stimulus, C = response to the second stimulus, D = response to the third stimulus, E = the time spent by the colony to return to its normal state.

3. Results and Discussion

Results for the different aspects of studies on aggression defenses of the bees of Basrah have been shown in Tables 1,2, and 3. Tables discussed different factors that may effect on the behavior of the bees from aggressive defenses point, and that factors normally happened in nature far from human being. These factors can be summaries as, 1) Visual factors, through hanging tennis ball coated with leather, to see that started time of stinging. And 2) Mechanical factors through either knocking the wall of the hive, or opening the outer and inner cover of the hive. In order to make a comparison between these factors, in different stations, clear, we are going to discussed each factor separately, through which we can conclude finally of what station bees are the most aggression.

1- Start time of stinging the tennis ball:

From the tables it is clear that the average time of stinging in the three regions was highly significant differences (P0.05). Al-jubayla station come first with 1.30 mints after starting the experiment, coming by Garmat Ali with 2.10, and finally Shatt Al-Arab with 5.33. On the other hand, differences between colonies of the same station were significant differences for each colony 1 and 4 with colony 2 and 3. The colonies Al-jubayla station come first stinging the tennis ball this may be explained by the crowding out of the number of colony per unit area, as the distance between the colony does not exceed 25 cm and between a row and another 50 cm, which makes the guard bees alert and activate what might expose them to robbery by other colony and attack as quickly as that is the time when the hive becomes Less aggressive than the Shatt al-Arab region. As [9] explained that the guard bees showed greater aggressive to sting in some of the colony in which they were. The number of guard bees is large due to the high level of serotonin in the central nervous system, as olfactory processing takes place at the start of the alarm pheromone release, and the high response to this pheromone depends on the mechanism of total or partial conversion to serotonin, which is greatly affected in the seasons of the year [1].

2- The first time for the aggressive of the colony:

Tables mentioned above declare that the average first time for the aggressive of the colony in the three regions was significant differences (P0.05). Garmat Ali station come first with 3.12 mints, followed by Al-jubayla with 3.21, and finally Shatt Al-Arab with 9.42. The time of aggressive depends on the how much it affects the tennis ball, such that the colonies respond

quickly to sting the tennis ball will be the faster to be aggressive colony, and the most active state.

3- Number of stings in the glove:

From the table it is clear that the average Number of stings in the glove in the three regions were shown in tables 1,2, &3, differences was highly significant (P0.05). Al-jubayla station was the first gating 4.49 sting, then Garmat Ali with 2.77 Shatt Al-Arab was the last with 2.11. The colonies at this stage of stimulation, were in attack situation and stinging because of alarm pheromone secretion. Shatt al-Arab region showed insignificant difference with Garmat Ali in all types of stimulation. This may reflect of the in stimulation of the warning pheromone, that effect on the metabolic rate, and the genetic defense behavior [10].

4- Number of the sting on the ball:

Differences between the stings on the ball for the three regions, was highly significant differences (P0.05), due to different in average number of stings. Al-jubayla station was the highest number of sting with 1.91, followed by Shatt Al-Arab with 0.84, and Garmat Ali with 0.67. From the results it clear that the colony was less stimulus to the ball than glove It may be due to the glove saturated with large amounts of the alarm pheromone as a result of large numbers of bees gathered on it compared to the ball. The genetic structure of bee colonies in general, as there are at least 11 genes that control this behavior and that changes in the regulation of these genes may participate in the development of the difference in the defensive behavior of honey bees. [11]. and in the same leg he has indicated [12] that the colony-level defense response strongly correlates with colony-level allele frequency in a way that can be used to identify causative genomic regions.

5- Normal return time:

Tables also show the time for the colonies to returen to the normal situation, from which differences were clear and highly significant (P0.05). Garmat Ali station got the shortest time with 6.79 min., Shatt Al-Arab coming second with 12.44 min., and finally Al-jubayla station with 17.79. min. the more aggressive colony needs a longer time to return to their normal time. Therefore, we note that the colony of the region of Jubayla usually return to their normal time after the disappearance of the influence at a rate of 17.795 min., while the colony usually returns

to their natural time after the demise of the influence Karma Ali region with an average of 6.790 mints. defending the colony is a type of behavior at the insect, which is known as an interaction between genes and the environment. Ayyoub & Omar [4] mentioned that there is a big variation between the geographical location and the genotypes, with their relationship to defensive behavior. Diversity and density of vegetation may negatively affect the aggressiveness of the colony through their effect on collecting honey. That was observed by [7] who studies a strain of African bees and a strain of Italian bees, and to hybrid the two strains, to get more honey and a less aggressive colony. The same results obtained by [13] with the Sudanain race, which led to the hybridization the two races and to improve honey production. Aggression is a context-dependent behavior that often represents an adaptive trade-off with other energetically demanding phenotypes. Diseases can impose strong selection pressures

Table 1.: The mean, standard deviation, and confidence limits to measure the response of Iraqi honey bees to an external stimulus at Al-Jubayla region.

Type and method of stimula	mean, standard deviation, and confidence limits of aggression				
	Colony 1 rate	Colony 2 rate	Colony 3 rate	Colony 4 rate	General Average
Time to occur the first sting in the leather ball (min)	0.707 ± 0.614 (-2.668 - 4.081)	1.717 ± 1.189 (-1.658 - 5.091)	1.757 ± 0.593 (-1.618 - 5.131)	1.020 ± 1.000 (-2.354 - 4.394)	1.300 ± 0.890 (-0.387- 2.987)
The first time for the aggressive of the colony/ min	4.470 ± 4.811 (1.096 - 7.844)	2.727 ± 1.207 (-0.648 - 6.101)	1.433 ± 0.595 (-1.941 - 4.808)	4.217 ± 5.008 (0.842 - 7.591)	3.211 ± 3.276 (1.524 - 4.899)
Number of sting in the glove	2.000 ± 2.000 (-1.374 - 5.374)	4.333 ± 2.081 (0.959 -7.708)	3.333 ± 0.577 (-0.041 - 6.708)	8.333 ± 1.527 (4.959 -11.708)	4.499 ± 2.844 (2.813 - 6.187)
Number of sting in the ball	0.667 ± 0.577 (-2.708 - 4.041)	3.000 ± 2.645 (-0.374 - 6.374)	2.667 ± 2.886 (-0.708 - 6.041)	1.333 ± 0.577 (-2.041 - 4.708)	1.916 ± 1.975 (0.229 - 3.604)
Normal return time / minute	21.667 ± 2.886 (18.292 - 25.041)	12.677 ± 8.740 (9.302 - 16.051)	16.847 ± 2.782 (13.472 - 20.221)	20.000 ± 13.228 (16.626 - 23.374)	17.797 ± 7.838 (16.110 - 19.485)

Table 2.: The mean, standard deviation, and confidence limits to measure the response of Iraqi honey bees to an external stimulus at Garmat Ali region.

Type and method of stimula	mean, standard deviation, and confidence limits of aggression				
	Colony 1 rate	Colony 2 rate	Colony 3 rate	Colony 4 rate	General Average
Time for starting sting in the leather ball (min), after introduce	3.123 ± 1.073 (-0.251 - 6.498)	1.443 ± 0.000 (-3.374 - 3.374)	$< 0.00 \pm 0.000$ (-3.374 - 3.374)	1.757 ± 2.043 (-1.618 -5.131)	2.107 ± 1.687 (-0.467 - 2.907)
The first time for the aggressive of the colony/ min	4.133 ± 0.952 (0.759 - 7.508)	1.140 ± 1.093 (-2.234 -4.514)	2.707 ± 2.556 (-0.668 -6.081)	4.507 ± 2.093 (1.132 - 7.881)	3.121 ± 2.070 (1.434 - 4.809)
Number of sting on the glove	4.667 ± 2.081 (1.292 -8.041)	0.333 ± 0.577 (-3.041 - 3.708)	$< 0.00 \pm 0.000$ (-3.374 - 3.374)	3.333 ± 1.527 (-0.041 - 6.708)	2.777 ± 2.353 (0.396- 3.771)
Number of sting on the ball	1.667 ± 0.577 (-1.708 -5.041)	$< 0.00 \pm 0.000$ (-3.374 -3.374)	$< 0.00 \pm 0.000$ (-3.374 -3.374)	1.000 ± 1.000 (-2.374 - 4.374)	0.666 ± 0.887 (-1.021 - 2.354)
Normal return time (min)	13.050 ± 6.489 (9.676 - 16.424)	1.333 ± 1.154 (-2.041- 4.708)	1.667 ± 1.527 (-1.708 - 5.041)	11.110 ± 1.922 (7.736 - 14.484)	6.79 ± 6.328 (5.103 - 8.477)

Table 3.: The mean, standard deviation, and confidence limits to measure the response of Iraqi honey bees to an external stimulus at Shatt al-Arab region.

Type and method of stimula	mean, standard deviation, and confidence limits of aggression				
	Colony 1 rate	Colony 2 rate	Colony 3 rate	Colony 4 rate	General Average
Time to occur the first sting in the leather ball (min)	4.800 ± 1.562 (1.426 -8.174)	9.343 ± 8.152 (5.969 - 12.718)	$< 0.00 \pm 0.000$ (-3.374 -3.374)	7.160 ± 0.970 (3.786 -10.534)	5.325 ± 5.082 (3.639 - 7.013)
The first time for the aggressive of the colony/ min	4.533 ± 0.511 (1.159 - 7.908)	11.170 ± 1.740 (7.796 -14.544)	16.823 ± 2.933 (13.449 -20.198)	5.190 ± 1.063 (1.816 - 8.564)	9.429 ± 5.435 (7.742 - 11.116)
Number of sting in the glove	2.667 ± 0.577 (-0.708 -6.041)	1.000 ± 1.000 (-2.374 - 4.374)	$< 0.00 \pm 0.000$ (-3.374 - 3.374)	2.667 ± 0.577 (-0.708 -6.041)	2.111 ± 1.311 (-0.104- 3.271)
Number of sting in the ball	1.667 ± 0.577 (-1.708 - 5.041)	0.333 ± 0.577 (-3.041 - 3.708)	$< 0.00 \pm 0.000$ (-3.374 -3.374)	1.333 ± 0.577 (-2.041 - 4.708)	0.833 ± 0.834 (-0.854 - 2.521)
Normal return time / minute	22.467 ± 2.478 (19.092 - 25.841)	7.947 ± 2.344 (4.572 -11.321)	4.917 ± 0.663 (1.542 -8.291)	14.437 ± 1.185 (11.062 -17.811)	12.442 ± 7.204 (10.754 - 14.129)

Generally, and for declaring the aggressive grade of the Iraqi bees, mean number of a spines in each of the glove and the leather ball for the three regions can be followed, according to the scale mentioned by [4]. with some modification to be agreed with the Iraqi bees (Table 4).

Table 4.: Description of the degrees of defensive behavior of Iraqi honey bees.

Scale number	Status of the bees	Degree of aggressive	number of stings
1	Quiet, never stinging	Excellent	0
2	Quiet, inclined to friction	very good	1 - 3
3	Quiet, inclined to friction Much	good	4 - 6
4	Attacks and stings few	Average aggressive	7 - 10
5	Attacks and stings a large number	aggressive	11-15
6	It attacks hard and collects on the hands and around the body	Very aggressive	more than16

Since the mean number of spines in both the glove of the beekeeper and the leather ball was 2.133 spines, the general description of the degree of aggressive of the colony is to be quite and tends to be in friction, which is a good character through which colonies can be examined and conservative, it had little response to the visual stimulus of the leather ball, which was the start point for stinging and then the release the alarm pheromone, Wafa [15] mentioned that the Syrian race showed strong defensive behavior in the studied areas, but with moderate response for Carniolan race, which was more quieter, the colony of the first hybrid was less defended in comparing with the Syrian race, and in the same leg he has indicated [16] *Apis.m. adansonii* was found to be the most defensive form *A. m. scutellata* and Variations in the agro-ecological zones, colony strength, and mean elevation were found to significantly influence the defensive behaviour of the honeybees in Uganda.

4. Conclusions

Above results showed clearly the differences between the station's bees aggressive grade, from all factors explained in tables, Al-jubyala was the station in which bees need just 1.3 min. to starting sting, followed by Garmat Ali and finally Shatt Al-Arab last. These results were repeted with other factors, from which it Reflects that Al-jubala station was the most aggressive, followed by Garmat Ali and Shatt Al-Arab finally.

References

[1] N. Morgane, R. Judith, G. Martin, The defensive response of the honeybee *Apis mellifera*. J. Expert. Bio. 219 (2016).3505-3517

[2] A. Cecilia, M.A. Palacio, E.M. Rodriguez, E. Figini, M.T. Dominguez, E. Bedascarrasbure, Evaluation of the defensive behavior of two honeybee ecotypes using a laboratory test. Genetics and Molecular Biology, 25 (2002) 57-60

[3] U. Aleksandra, C. Cecilia, P. Beata, M. Marina, K. Per, H. Fani, M. Bouga, S. Andonov, M. Bienkowska, Y. Conte, J. Wilde, D. Gerula, H. Kiprijanovska, J. Filipi, P. Petrov, L. Ruottinen, H. Pechhacker, S. Berg, W. D., E. Ivanova & R. Büchler. Swarming, defensive and hygienic behavior in honey bee colonies of different genetic origin in a Pan-European experiment. J. Api. Res. 53 (2014) 248-260

[4] A. M. Ayyub, M. A. Omar, Introduction to beekeeping, Food, and Agriculture Organization of the United Nations. Iraq. first ed, Agricultural program of the Security Council resolution, 2003. (in Arabic).

[5] A. A. Muhammad, Beekeeping is a science work and hobby, first ed, The Iraqi National Library and Archives, 2011. (in Arabic).

[6] M. C. Anita, E. R. Thomas, W. T. Kenneth, S. Allen, J. L. James, A Model of Honeybee Defensive Behaviour, J. Api. Res. 19 (1980) 224-231

[7] A.C. Stort, Genetic study of aggressiveness of two subspecies of *Apis mellifera* in Brazil. Some tests to measure aggressiveness. J. Api. Res. 13 (1974) 33-38

[8] IBM company. Statistical program SPSS version 23.0.
https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-23/2013 (accessed 6 September 2013 3:18AM).

[9] R. Prasad. why do bees sting? bee culture the magazine of American beekeeping, march 19,2018.

[10] E.E. Southwick, R.F. Moritz, Metabolic response to alarm pheromone in honey bees. J. Ins. Phy. 31 (1985). 389–392

[11] A. Cedric, S. Saurabh, H. Linda, J. Greg, G. Ernesto, D. Gloria, et al. Honey bee aggression supports a link between gene regulation and behavioral evolution. PNAS. 106 (2009) 15400–15405

[12] A. Avalos, M. Fang, H. Pan, A. R. Lluch, A. E. Lipka, S. D. Zhao, T. Giray, G. E. Robinson, G. Zhang, and M. E. Hudson, Genomic regions influencing aggressive behavior in honey bees are defined by colony allele frequencies. PNAS. (2020), 1-7

[13] N. S. Kamel, B. Syed, A. M. Saeed, A study on the emotional behavior of Sudanese and Carniolan honey bees. Arab Beekeepers Union on 1/10/2013.

(in Arabic).

[14] H. M. Carr, J. H. Palmer, C. C. Rittschof. Honey bee aggression: evaluating causal links to disease-resistance traits and infection. Behavioral Ecology and Sociobiology 74 (2020)1-14

[15] Y. Wafa, Study of defense behavior and infection rate of *Varroa* among some races of honey bee and evaluation of the effect of some factors on stinging behavior. Third Syrian beekeepers conference. faculty of Agriculture. Damascus University. 14/12/2010. (in Arabic).

[16] K. Patrice, N. N. Gideon, N. N. Paul, A. Christopher, and K. Robert, *Apis mellifera adansonii* Is the Most Defensive Honeybee in Uganda. Psyche. Volume (2018) 6

الخلاصة

أجريت الدراسة في ثلاث مناطق من محافظة البصرة لتقييم درجة الشراسة نحل العسل العراقي *Apis*
mellifera باستخدام ثلاثة أنواع من المحفزات التي تمثل محفزات السلوك الدفاعي الطبيعي للنحل، تم
حساب درجة الاستجابة للمحفزات الثلاثة بتقنية ستورت. أظهرت النتائج شراسة نحل العسل في منطقة
الجبيلة واختلافها المعنوي عن موقع كرمة علي اذ بلغ زمن لسع الكرة الجلدية 1.300 دقيقة وكان عدد
اللسعات في القفاز 4.499 شوكة وزمن العودة إلى الوضع الطبيعي 17.797 دقيقة ، واختلفت شراسة
نحل العسل في موقع كرمة معنويا موقع شط العرب عن اذ بلغ زمن لسع الكرة 2.107 دقيقة، وكان عدد
شوكات اللسع في القفاز 2.777 شوكة وزمن العودة إلى الحالة الطبيعي 6.79 دقيقة ، بينما كانت
الفروقات معنوية سالبة في موقع شط العرب مع كل من الجبيلة وكرمة على الذي كان النحل فيها هادئ اذ
بلغ زمن لسع الكرة الجلدية 5.325 دقيقة وعدد شوكات اللسع في القفاز 2.111 وزمن عودة الطائفة الى
الوضع الطبيعي 12.442 دقيقة ، ومن حساب قيم متوسطات عدد الشوكات في كل من قفاز الفاحص
والكرة الجلدية في المقياس الموضوع للمواقع الثلاثة والتي بلغت 2.133 شوكة تكون درجة شراسة الطوائف
بصورة عامة هو هادئ يميل الى الاحتكاك وهي صفة جيدة للتربية، تعتبر هذه الدراسة الأولى من نوعها في
العراق.