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Effect of Bee Population & Bee Venom Milking during Spring and Early Summer in s some of Honey Bees (*Apis Mellifera* L.) Colonies Biological Characters

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Abstract. The results of the current study conducted in the apiary of the College of Agriculture and Forestry / University of Mosul during the year 2021 showed that the highest amount mean of bee venom for the interaction reading was 0.232 mg/colony in the treatment of milking bee population 10 frames (TMV2) during the period from 1-6/5/2021 compared with the lowest mean interaction of 0.061 which was recorded during the period from 6/26-1/7 for the same treatment, while the highest mean interaction for the treatment of milking bee venom population of 5 frames (TMV1) during the period from 1-6/5 /2021 was 0.125 mg/colony compared with the lowest mean of 0.022 mg/colony during the period from 29/5 to 3/6/2021 for the same treatment. As for the treatment general mean, the highest mean was recorded by the TMV2 treatment which mounted to 0.1136 mg/colony compared with the lowest general mean of zero mg/colony by the TCM2 treatment. As for the milking treatment of TMV1, the highest mean recorded was 0.0527 mg/colony by the TMV1 treatment compared with the lowest average of zero mg/colony by the TCM1 treatment. As for the process effect of the bee venom milking on bee population, the bee population decreased by 1.12 bee frame by the TMV2 treatment which recorded a mean of 14.15 bee frames compared with the TCM2 treatment that, in turn, recorded a mean of 15.27 bee frames. As for the treatments with a bee population of five frames, the bee population decreased by 1.30 bee frames by the treatment of TMV1 which recorded the lowest mean of 8.07 bee frames compared with the highest mean of 9.37 recorded by the TCM1 treatment. As for the general mean of the sealed workers brood, the statistical analysis did not show any significant differences between the two treatments; namely, the TMV2 and the TCM2 whose means both reached to 394.89 inch^2 and 367.37 inch^2 respectively. Regarding the treatments with bee population of five frames, the highest mean was recorded by the treatment TCM1 amounting to 315.98 inch^2 compared with the lowest mean of 245.07 recorded by the TMV1 treatment. As for the pollen area in treatments with a bee population of 10 frames, the highest mean was recorded by the TCM2 treatment which mounted to 66.78 inch^2 compared with the lowest mean in the TMV2 treatment which mounted to 61.44, and the statistical analysis did not show significant differences for the effect of milking bee venom. In the pollen mean for the treatments with a bee population of five frames, the highest mean was recorded by the TCM1 treatment which reached to 65.11 inch^2 compared with the lowest mean of 54.63 inch^2, recorded by the TMV1 treatment.

* The current study is extracted from the doctoral thesis of the first researcher.

Keywords: Milking bee venom, Bee population , Sealed brood workers, Pollen.

Introduction

The honey bee *Apis mellifera* is of great importance for human life as it had preceded humanity by its presence on earth of about 10-20 million years and is one of the animal life forms that has still existed since the Stone Age. Ancient people collected wild honey and used it as a sweetener drink or intoxicating drink (Omar & Al-Ghamdi, 2010). Gupta et al. (2014) & FAO (2020) stated that bee products are of great economic and medical importance as honey and other bee products such as: wax, bee venom, propolis, royal jelly, pollen and others are which have had great importance in the field of medicine. In addition, bees contribute in pollinating various agricultural crops by collecting pollen which helps in solving the problems of food shortages on the earth. Besides, El-Seedi et al. (2020) explained that bee venom is one of the important bee products because of its great medical benefits, as it has been known since ancient times through stinging operations as previous studies indicated its uses as a treatment for various diseases such as arthritis, Parkinson's disease increasing the ability of

immunogenicity against SARS-COV2, anti-coated and non-coated viruses. Gaber et al. (2020) & Kasozi et al. (2020) added that bee venom has a therapeutic effect for respiratory and nervous system diseases. So it is important in the application of complementary therapy. It can also be benefit for liver cells and is anti-cancer cells. This comes from its composition which contains many active substances such as enzymes and many active proteins such as Melittin and Apamine. Haggag et al.(2015) concluded that there are factors affecting the amount of milking venom from the hive, such as the bee population of the colony, the season of Bogdanov (2016) explained that worker bees, whose ages ranged between 15-21 days, are the most productive of venom, and that about 100 micrograms of dry bee venom is released in one sting. El-Bahnasy et al. (2017) explained that the factors that could be a reason for the increase in bee venom production are the large size of the sting, the venom sac in the workers, the increase of bee population within the colonies and the degree of bee aggressiveness. Gholamian (2007) and Al-Saiegh (2021- under publication) mentioned that an increase in the average production of the milked venom in the milking device is placed on top of the frame compared with the device that is placed on the hive fly board, and they did not observe the negative effects of the characteristics of the brood area. Argena et al. (2021) found that the seasons of the year have a clear effect on the rate of bee venom production. The maximum production of bee venom was recorded in spring and production decreased in summer and then rose again in autumn. It was also found that the process of milking bee venom had no effect on the production of sealed and unsealed brood. Badawy (2022) noticed a negative effect of the milking process of bee venom on the pollen area, and recorded the highest average area of honey in milking treatment during the summer season which mounted to 172.630 inches^2/colony followed by the spring season where the average area reached 143,986 inches^2/colonies for the Italian hybrid bee for the year 2018. Iraq is considered to be one of the leading countries in keeping honey bees due to its environmental and plant diversity from the north to the south. Nineveh Governorate is considered to be one of Iraq cities with great weight in bee keeping and attention to its products. The number of apiaries within Nineveh Governorate reached 910 and about 29,000 hives on 1/11/2020 according to the latest statistics of the Plant Protection Department / Nineveh Agriculture Directorate / Nineveh Governorate (2020). Due to the lack of studies in Iraq about bee venom milking and the importance of the economic and medical issue, the current study was proposed to be the first applied academic study in the country specialized in the production of bee venom and to be the first nucleus of future scientific studies to complete the scientific research journey in this field.

The current study aimed at knowing the effect of seasons and bee population on the quantity of honey bee venom and its impact on a number of biologicall characteristics of honey bees. The device is distinguished from its counterparts that the process of venom milking is carried out on both sides of the frame, and thus it is considered the first internal venom milking device to be made on both sides of the glass plate; thus, ensuring an increase in the area where the workers are exposed to the electric current, and as a result increasing the amount of milked venom in each milking session and saving both time and efforts for beekeepers with an increase in the amount of venom dissolved.

Materials and Methods

At the beginning of spring 2021, 14 divisions of local hybrid bees were purchased from one of the licensed beekeepers. Each swarm contained five pressurized bee population frame, and all swarms had a queen age of one year reared in 2020. After transferring the swarms to Langstroth wooden hives, a balancing of the colonies was carried out up to obtaining two sets of balanced colonies in all biological characteristics, The first group contained 5 bee population frames, and the second group contained 10 bee population frames. Each group was divided into two parts representing the treatments designated for the study to form four treatments. Each treatment included 3 replicates (three colonies), which are as follows: 1- the milking control treatment given the cod (TCM1) containing five frames of bee population 2- the treatment of bee venom milking given the cod (TMV1) containing five frames of bee population to place the milking device inside each colony during the period of bee venom milking 3- the milking control treatment, given the code (TCM2) containing ten frames of bee population 4- the treatment of bee venom milking given the code (TMV1) containing ten frames of bee population to place the milking device inside each colony during the period of bee venom milking. All experiments were designed according to the randomized complete block design of factorial trials (RCBD). The results were analyzed according to Anova

table usig SAS program, and the means were tested by Duncan's test.

Manufacturing an Electric Bee Venom Milking Device, in Cooperation with Technical Center for Electronics Company:

Locally, the bee venom milking system was manufactured consisting of the following:

A- Electrical transformer: it is a box containing a number of electrical devices connected to each other that control the conversion of electrical current from continuous to alternating and control the electrical impulses that resulted in electric shock to honey bee workers that moved on wires during the process of milking bee venom through which a control was done on the period for milking (Fig. 1),

B- The milking bee venom frame:

It is a wooden frame similar to the Hoffman frame used in a Lancastroth hive. Its measurements was 48 cm length, 23 cm width and 2.6 m thickness. Several modifications were made to make it capable of milking venomand was perforated from the middle and a channel was made for the passage of the glass plate from the middle. It was also perforated for the passage of parallel positive and negative electrical wires without meeting touching each other. The distance between the positive and the negative wires was 4 mm. The distance between the wires and the glass plate was only 3-4 mm which representing approximately the length of the workers' abdomen. The glass plate was a transparent, smooth glass plate 4 mm thick, 37 cm long and 22 cm wide. The glass plate was inserted through a special channel located in the wooden frame to receive the secreted venom from the venom gland which quickly dried to turn into a white powder and then later collected by a sharp iron scraper designated for that. Before inserting the glass plate, it must be cleaned, well-wiped and then covered with a thin layer of transparent nylon paper (selufan) in order to ensure not breaking the worker's sting which was inserted in the nylon during the process of stunting, and then it was easy for the worker to withdraw it thus obtaining the highest degree of pure venom.

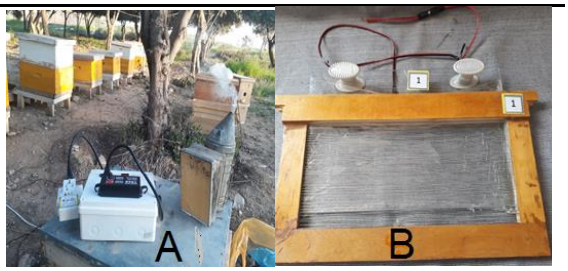

Figure 1. Components of the bee venom milking device A - the electrical transformer b - the wooden frame.

The Stage of Extracting the Venom after the Process of Milking and Electric Shock:

Six frames were manufactured for milking bee venom: three of which were designated for the five frame population treatment of milking bee venom, and the other three were designated for the ten frame population treatment of bee venom. In order to ensure obtaining the largest amount of bee venom during milking ,the frames were put in the various treatment replicates in the evening to ensure the returning of all the foraging bees into the hive; thus, achieving the goal by milking bee venom from the forager bees and the brooder bees inside the hive. The milking frames were inserted and placed in the center of the brood nest frame in the colonies designated for milking. Then the transformer was put on 30 minutes timing, and the electric pulse was set on one second, the electrical pulse was set on 3 seconds stop, and the voltage was fixed at 14 volts. The process of milking the bee venom lasted for two hours daily, starting from 5-7clock in the evening, a week of milking and a week of resting the bees, starting from Saturday and ending on Thursday during the period of conducting this study. All necessary precautions were taken, including wearing plastic cloves, as well as glasses for eye protection, and wearing medical masks to prevent inhalation of venom especially after beginning the process of scraping bee venom from glass plates using a razor (Figure ,2). After skimming and collecting the bee venom from the glass plate, the collected quantity was weighed by a sensitive Tn-Series balance. After that, the weighed venom was kept in a dark jar fixed on it the date of the collection week, the treatment number and the replicate. After the scraping and packing process were complete, all jars were transferred and placed in a large dark glass container which was kept in the freezer for preservation.

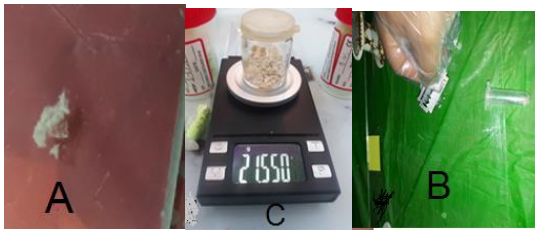

Figure 2. A - Dry bee venom B - Scraping venom method C - The sensitive balance used.

All biological character where measured using lancstroth being divided into inches squares as according to seaigh (1988 and 2022).

Results

The results of Table (1) showed that the highest mean of bee venom in relation to the interference readings was 0.232 mg / colony in the treatment of milking bee population of 10 frames (TMV2) during the period from 1-6/5/2021 compared with the lowest mean of interaction which was 0.061 recorded during the period from 26/6-1/7 for the same treatment. While the highest mean readings of the interaction in the treatment of bee venom milking bee population was 5 frames (TMV1) during the period from 1-6/5/2021 and rised to 0.125 mg/colony compared with the lowest average of 0.022 mg/colony during the period 5/29-3 /6/2021 for the same treatment. As for the general mean of the transactions, the highest mean was recorded by the TMV2 treatment which rised to 0.1136 mg/colony compared with the lowest general mean of 0 mg/colony by the TCM2 treatment. As for the TMV1 treatment, the highest mean recorded rised to 0.0527 mg/colony compared with the lowest mean which was zero mg/colony for the treatment of TCM1. As for the general mean of the effect of readings, the highest general mean was recorded on 1/6/2021 which mounted to 0.1787 mg/colony, compared with the lowest mean at the date of reading on 6/1/7/2021 which rised to 0.0423 mg/colony due to the increase in the amount of the milked venom during the period from 1-6/5 to the significant increase in the number of workers and the abundance of food from pollen and nectar and the appropriate weather conditions of temperature and relative humidity which reached 26.72 ° C and 41.61%, respectively (Figure 1, 2). This means that the amount of venom was clearly affected by the increase in bee population as the amount of milked venom in the treatment of bee population was 10 bee frames twice the amount of the venom of milked in the treatment of bee population 5 bee frames and in the different months of the year. The study found that June was the best month for milking bee venom. These results were consistent with what was found by Omar and Khodairy (2003)& Zhou et al (2003) studies. They found significant differences in quantity of milked venom as an effect of the months of milking. They recorded the highest mean milking of venom in June compared with March, while Mohamed's study (2017) recorded that spring and summer seasons have the highest production of bee venom compared with the rest of the seasons. Spring and summer seasons have the highest production of bee venom compared with the rest of the seasons. The general mean of the amount of venom in both spring and summer seasons was 0.230 and 0.292 mg /colony respectively. Badawy (2022) found that spring showed a significant increase in the collection of dry bee venom compared with autumn by 1.822 and 3.742 mg /colony for the 2017, and 2.445 and 3.448 mg / colony for the 2018 respectively.

Table 1. It shows the date and quantity of bee venom collected for the two seasons (spring and early summer) 2021.

History of readings	Treatments				Total mean
	TCM1	TMV1	TCM2	TMV2	
3 – 8 / 4	0	0.063 cd	0	0.098 bc	0.0795 a b
17 – 22 / 4	0	0.069 cd	0	0.120 b	0.0900 a b
1 – 6 / 5	0	0.125 b	0	0.232a	0.1785 a
15 – 20 / 5	0	0.029 d	0	0.034 d	0.1065 a
29/5 – 3 /6	0	0.022 d	0	0.143 b	0.0825 a b
12 – 17 / 6	0	0.037 d	0	0.107 bc	0.0715 a b
26/ 6 – 1 / 7	0	0.023 d	0	0.061 cd	0.0420 b
Mean	0	0.0527 b	0	0.1136 a	0.0929

* Numbers with similar letters do not significantly differ according to Duncan's multiple test for mean below 5% significance level. TMV1 and TMV2: Bee venom collection treatment 5 and 10 frames, respectively.TCM1 and TCM2 : Control Treatments 5 and 10 frames , respectively>

Effect of Bee Venom Milking on Bee Population of Honey Bees during Spring and early Summer:

The results of Table (2) showed that the highest mean of interference readings in treatments with bee population of 10 frames when reading the date 22/5/2021 by TCM2 treatment during the season rising to 20.67 bee frames at a mean temperature and relative humidity of 30.0°C and 28.50% respectively (see Figs. 1, 2). The lowest mean was recorded on

29/6/2021 by the treatment of TMV2 which was 13.00 bees frames. The reason was that bees entered the honey overflow season during which the queens were less active in laying eggs. As for the interaction readings in treatments with bee population of 5 bee frames, the highest mean was recorded in the TCM1 treatment on 22/5/2021 which rised to 12.33 bee frames compared with the lowest mean read between 6/16 and 29/6 by the TMV1 treatment. The amount is 7.00 bee frames.The reason for the decline in bee population and for all transactions after the date of 3/6/2021 was due to the end of the actual activity season of the queens and the extract of honey stored in the transactions on 2/6/2021; i.e. the high temperatures and the lack of pastures. As for the process effect of the bee venom milking on bee population, the bee population decreased by 1.12 bee frame by the TMV2 treatment which recorded a mean of 14.15 bee frames compared with the TCM2 treatment which recorded a mean of 15.27 bee frames. As for the treatments with a bee population of five frames, the bee population was decreased by 1.30 bee frames by the treatment of TMV1 which recorded the lowest mean of 8.07 bee frames compared with the highest mean of 9.37 bee frames which was recorded by the TCM1 treatment.

Table 2 it shows the effect of milking bee venom on the mean bee population of different treatment colonies for the season (spring and early summer) 2021.

History of readings	Treatments				Total mean
	TCM1	TMV1	TCM2	TMV2	
3 / 18	6.00 o	6.33 o	10.67 h– l	10.33 h– m	8.31 e
3 / 31	7.6 7 l – o	7.33 m – o	11.67 g– j	11.67 g– j	9.53 de
4 / 13	8.67 j – o	8.00 k– o	13.00 f – I	13.00 f– i	10.57 cd
4 / 26	10.00 i-n	8.00 k- o	14.33 d– g	14.33 d– g	11.65 c
5 / 9	11.00 h – k	10.00 i–n	16.67 b-d	16.33 b– e	13.48 b
5 / 22	12.33 f –i	10.33 h- m	20.67 a	19.00 ab	15.55 a
6 / 3	11.67 g – j	8.67 j– o	17.67 bc	16.33 b-e	13.53 b
6 / 16	8.67 j – o	7.00 no	17.00 b-d	13.33 e– h	11.48 c
6 / 29	8.33 k – o	7.00 no	15.33 c-f	13.00 f– i	10.9 cd
Mean	9.37 c	8.07 d	15.22 a	14.15 b	11.696

* Numbers with similar letters do not significantly differ according to Duncan's multiple test for mean below 5% significance level

As for the general mean of readings, it recorded the highest mean on the date of reading 5/22 which rised to 15.55 frames compared with the lowest mean which was recorded on the date of reading 3/18 that reached 8.31 bee frames. The study concluded certain results shown in the table below that there was a clear effect of the process of milking bee venom in reducing the bee population of different experiment treatments during the current study period. Al-Sayegh and others (2013) found the highest mean of bee population was in the white-coloured hives whose mean was10.4 bee frames, and the lowest was 9.71 bee frames in the Brown-coloured hives during spring and early summer seasons.

Effect of Bee Venom Milking on Sealed Workers Brood of Honey Bees during (Spring and early Summer) 2021.

Results in Table (3) showed that the highest average recorded for the interference readings in the treatments with bee population in 10 frames in the TMV2 treatment on 13/4/2021 which was 692.33 inch^2 at the mean temperature and relative humidity of 18.28 ° C and 51.92%, respectively (see Figs. 1, 2) measured by the lowest average recorded on 29/6/2021 which was 165.67 inch^2 for the same treatment. As for the treatments with a bee population of 5 frames, the highest mean for the interference readings was recorded on 13/4/2021 by the TCM1 treatment which rised to 499.00 inches^2 compared with the lowest mean which was 132.33 inche^2 recorded on 2021/6/29 of reading for the same treatment and at an mean temperature and relative humidity of 34.44 °C and 18.74% respectively (see both Figs. 1,2). The reason behind this decrease in the area sealed workers brood after 26/4/2021 was due to the high external temperatures that affected the pastures and as a result the lack of food sources for bees represented by pollen and nectar. As for the general mean of the sealed workers brood, statistical analysis did not show significant differences between the two treatments TMV2 and TCM2 whose mean reached 394.89 inch^2 and 367.37 inch^2, respectively. As the treatments in bee population of five frames, the highest mean was recorded by the TCM1, which mounted to 315.98 inch^2 compared with the lowest mean of 245.07 inch^2 recorded by TMV1. The statistical analysis of the general mean of the readings showed the superiority of the reading on 13/4 in all readings which recorded the highest mean of 502.92 inches^2 compared with the lowest mean recorded when read on 29/6 which mounted to 177.00 inch^2. The study concluded that there was no effect in the process of bee venom milking on

the sealed brood area in the treatments with a bee population of 10 frames, while the milking of bee venom had a significant effect on the sealed brood area in the treatments with a bee population of 5 frames. Mohamed (2017) found that the highest mean area of the sealed brood workers was in April 730.33 inches^2 /colony followed by May to reach 143.3 inch^2 /colony during the year 2015. Badawy(2022) noticed during her study that there was a effect of collecting bee venom on the sealed brood area, and that the highest mean of the sealed brood area of workers recorded in summer reaching 135,396 and 106,647 inch^2 in the Italian hybrid and Carniolan hybrid bees during 2018 respectively.

Table 3 It Shows the Effect of Milking Bee Venom on the Mean Sealed Brood of Workers in Square Inches for the Different Study Colonies for the Two Seasons (Spring and early Summer) 2021..

History of readings	Treatments				Total mean
	TCM1	TMV1	TCM2	TMV2	
3 / 18	401.33 b – h	397.67 b – h	438.00 b – f	582.33 ab	54.83 ab
3 / 31	331.00 c– j	408.00 b – g	288.00 e- j	559.00 a-c	396.50 bc
4 / 13	499.00 a – e	288.67 e – j	531.67 a-d	692.33 a	502.92 a
4 / 26	440.33 b– f	235.00 f– j	228.00 f – j	265.67 e –j	292.25 dc
5 / 9	377.00 b – I	217.67 f-j	314.33 d – j	297.00 d – j	301.50 dc
5 / 22	202.00 f – j	206.67 f – j	338.33 c – j	350.00 b – j	274.25 be
6 / 3	300.00 d – j	156.00 i – j	435.67 b – f	372.00 b – i	315.92 dc
6 / 16	170.00 g – j	164.33 i – j	409.00 b – g	270.00 b – j	253.33 de
6 / 29	123.33 j	131.67 j	287.33 e – j	165.67 i – j	177.00 e
Mean	315.98 b	245.07 c	363.37 ab	394.89 a	392.82

* Numbers with similar letters do not significantly differ according to Duncan's multiple test for mean below 5% significance level

Effect of Bee Venom Milking on Pollen of Honey Bees during Spring and early Summer:

The results in Table (4) showed that the highest recorded mean of the pollen area for the interference readings in treatments with bee population was 10 frames by the TMV2 when read on 05/22/2021 which was 127.33 inch^2 and at mean of temperature and relative humidity of 30.00° C. 28.50% (Fig. 1, 2) compared with the lowest mean recorded when read on 29/6/2021 which was 15.00 inch^2 for the same treatment . As for treatments with a bee population of 5 frames, the highest average of the interference readings was recorded when read on 5/9 by the TMV1 which mounted to 108.33 inch^2 compared with the lowest mean, which was 2.67 inch^2 recorded on 29/6 for the same treatment. As for the general mean of treatments, the highest mean was recorded by the TCM2 which mounted to 66.78 inch^2 compared with the lowest mean by TMV2 which mounted to 61.44 inch^2, and the highest mean was recorded by TCM1 which mounted to 65.11 inche^2 compared with the lowest mean of 54.63 inch^2 which was recorded by the treatment of TMV1. As for the general mean of readings, the reading on 5/9 exceeded all the readings with mean of 102.63 inch^2 compared with the lowest mean which was 26.80 inch^2 recorded on 29/6 .We conclude from the results that the process of bee venom milking had a clear negative significant effect on the pollen area within the milking colonies as a result of the double consumption of pollen to fill the shortage of proteins and other substances due to the secretion of the venom during milking .

Table 4. It Shows the Effect of Milking Bee Venom on the Mean Pollen Area in Square Inches for the Different Study Colonies for the Two Seasons (Spring and early Summer) 2021.

History of readings	Treatments				Total mean
	TCM1	TMV1	TCM2	TMV2	
3 / 18	59.00 d– g	57.31 c – g	27.67 e – h	46.33 d – g	47.48 cd
3 / 31	44.30 e – h	64.00 b – g	38.33 d – h	43.00 d – h	47.40 cd
4 / 13	61.33 c – g	40.00 d – h	72.00 b – f	63.00 b– g	58.73 c
4 / 26	29.00 e – h	47.00 d – h	69.33 b – g	32.33 e – h	44.40 cd
5 / 9	82.67 a – e	108.33 a–c	116.60 ab	112.00 ac	102.63 a
5 / 22	74.33 b – f	72.67 b–f	116.00 ab	127.33 a	97.55 ab
6 / 3	78.00 a –f	58.00 c–g	77.00 a– f	71.67 b– f	71.15 bc
6 / 16	93.00 a – d	41.67 d-g	58.33 c– g	42.33 d– g	58.80 c
6 / 29	64.00 b – g	2.67 h	25.67 f – h	15.00 gh	26.80 d
Mean	65.11a	54.63 a	66.78a	61.44 a	61.98

* Numbers with similar letters do not significantly differ according to Duncan's multiple test for mean below 5% significance level.

This was consistent with Mohamed's findings (2017) that the highest mean of pollen area was 140.00 inch^2/colony on spring of 2015 which reached 54.72 inch^2 /colony on spring 2016. Badawy (2022) noticed a negative effect of the

process of bee venom milking on the pollen area, and recorded the highest mean area of pollen collected bee venom treatments during summer mounted to 127,808, 172,630 inch^2/colony in the Italian bee breed between 2017 and 2018 respectively.

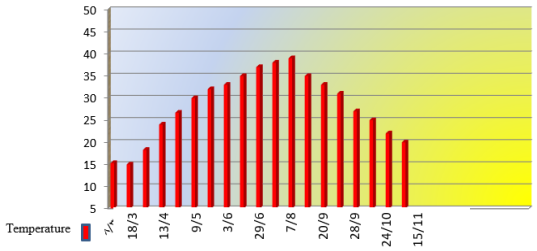

Figure 3. Shows the Celsius temperature of Nineveh Governorate for the year 2021**.**

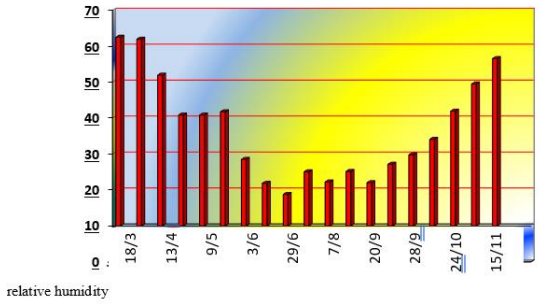

Figure 4. Shows the degrees of relative humidity in Nineveh Governorate for the year 2021**.**

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