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# The Suitable Diurnal Time and Synchronized Period Required to Gain High Yield of Venom from Honeybee Colonies in Egypt

**By**

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## ABSTRACT

This study was conducted during spring of 2021, (Marsh to May,) to evaluate the suitable diurnal time (early morning (8.00-9.00 am), mid-day (1.00- 2.00 pm) and before sunset (6.00-7.00 pm) and to define the synchronized period (7, 14, 21 days) required to gain high yield of venom from honeybee colonies. The first experiment was conducted to evaluate the diurnal profitable time, to gain the highest yield of dry bee venom. For this purpose, twelve colonies of Carniolan hybrid in similar strength were divided into three equal groups. The results proved that the mean highest amounts of venom collected before sunset was significantly higher (26.9± 1.9 mg/col.) than those collected in either the early morning (17.0± 3.8 mg /col.), or in mid-day (15.0± 2.6 mg/col.). The second experiment targeted suitable synchronized period spent between two successive collections of venom from the same colony. Three tested interval periods were investigated (7, 14 and 21 days, using 24 colonies from the same hybrid in homogenous strength divided to three equal groups. Each group applied in one of the mentioned synchronized periods. The results indicated that collecting venom after 14 days from the first collection gained the highest venom amounts (21.5 ±3.7 mg/col.), with non-significant difference with the 21 days period (20.7± 2.1 mg/col.). However, the collected amounts of venom after 7 days from the first harvesting were significantly less (17.7± 2.2 mg/ col.) than the other periods.

***Key words:*** *Apis mellifera***,** *honey bee*, *venom***,** *sunset*

## 1. INTRODUCTION

The use of weapons as defense against enemies was one of the major factors that favored the evolution of social life in insects (Andersson, 1984). In the eusocial Aculeate Hymenoptera, the stinging apparatus and the venom, originally evolved as devices to paralyze preys, became arms to defend the colony mainly from the attacks of predators (Starr, 1985). The primary role of defending the colony is stinging behavior using the venom secreted from the modified ovipositor apparatus. A single sting of a honeybee contains a small amount of venom, which acts as a painful deterrent for vertebrates but has lethal effect on a wide variety of invertebrates. Also, venom secretion in honey bee worker begins just prior to emergence and increases slowly towards a maximum between the tenth and sixteenth day as recorded by Autrum *et al*. (1959).

Plunkett *et al*. (2017) found that Hymenoptera venom extracts were developed using many new methods for the collection,

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purification, and production of the unique materials required for this product.

The best method and circumstances to collect the largest amount of venom from honey bee colonies were evaluated. In Portugal, Nobre (1990) described a device for the collection of honey bee (*Apis mellifera*) venom. The device consists of an electrified glass plate; when the bees sting it, they do not lose their stings and are not electrocuted. Omar *et al.* (1993) made a new modified device, which consists of a generator produces impulses of different wave forms, adjustable frequencies and amplitudes, it is energized either from 220 v AC supply or A single 12 v battery. The device developed is based on the stimulation of the defense instinct of bees by the electric stimulus signal consisting of series of impulses of complex waves were tested. Simics (1995) stated that the maximum production of venom being reached after two to three weeks, where the synthesis of different components of venom do not reach maximum rate simultaneously. Hussein (2013) determined that the best time of day to collect the honey bee venom was after Sun dawn (102 mg dry venom /colony). These changes in venom have been suggested to be related to a transition from house (young) bee activity to field (old) tasks. Beekeepers′ improper practices during harvesting and storing might be the most critical parameters that determine the quality of venom. However Meral Kekeçoğlu *et al*. (2022) demonstrated that statistically significant differences in the amounts of analyzed components were not dependent on harvesting time, collection site on the beehives or season. On the other hand, region samples significantly differed in the amounts of all three components, ranging from 1.28% to 3.81% for apamin, 19.51-64.03% for melittin and 7.22%-28.18% for PLA2. Leuter *et al*. (1939) reported that the production of a potent bee venom required good nectar, honey and pollen sources consequently, protein food mainly is required for full production of venom. Omar (2011) tested three positions for two venom collection boards attachment to honey bee hives (at hive entrance, beside the hive frames, over frames top), and the venom extraction achieved the highest amount (93. 22 mg/colony) when the venom collection board was put on the top bars inside the colony, The venom quantity increased significantly with 7.41% when compared by the position of boards at the hive entrance.

The objective of this investigation was to evaluate the best method and circumstances to collect the highest amount of venom from honey bee colonies.

## 2. MATERIALS AND METHODS

This work was carried out in the apiary of Economic Entomology Department, Faculty of Agriculture, Cairo University, Giza in Egypt to study some important factors related with collecting venom from colonies by electrical shock method.

### 2.1. Effect of collecting venom time

Effect of daytime collection on the quantity of dried venom produced, (in mg) from honeybee colony using electric shocks. Twelve honeybee colonies at same strength were divided to three groups

1. Four colonies used to collect venom at early morning, from 8-9 am.
2. Four colonies used to collect venom at midday, from 1-2 pm.
3. Four colonies used to collect venom before sunset, from 6-7 pm.

### 2.2. Determine the best periods time between venom collections

Effects of period between the 1st and 2nd collection time on the quantity of dried venom produced (in mg) from honeybee colony using electric shocks.

Twenty-four honeybee colonies at same strength were used in this experiment and were divided into three groups:

1. Eight colonies used to collect venom from them at two times; zero time which is the first time to collect and the other time after seven days.
2. Eight colonies used to collect venom from them at two times; zero time which is the first time to collect and the other time after fourteen days.
3. Eight colonies used to collect venom from them at two times; zero time which is the first time to collect and the other time after twenty-one days.

The bee venom was collected by the electric shock device VC- 6F model (Aptronics Services, Model 9611, Richmond, B.C., Canada), according VC- 6F model specifications for collection, and the other time after fourteen days.

**2.3. Statistical analysis**

Means and standard deviations of the data collected for each experiment were calculated using Microsoft Excel and statistical significance determined by t-test and two-way ANOVA.

## 3. RESULTS AND DISCUSSION

**3.1. Effect of collecting venom time**

The data obtained in Table (1) and illustrated in Fig. (1) showed that the best time to collect venom from honey bee colonies through the day was before sunset. There was significant difference between the third group (before sunset) and the other two groups (early morning and midday). The results showed that the highest amount of venom was 26.9±1.9 mg before sunset, while it was 17.0±3.8 and 15.0±2.6 mg at early morning and midday, respectively.

In fact, at the time before sunset almost all of forager's workers get back and this increase the dawn Sun (102 mg dry venom/ colony), Furthermore, Kaisser and Michel (1958) stated that the maximum production of venom being reached after two to three weeks. The synthesis of different components of bee venom do not reach maximum rate simultaneously. These changes in venom were suggested to be related to a transition from house (young) bee activity to field (old) bee activity, Likewise, Mitev (1971) stated that honey bee venom was collected from one group of 8 colonies, using a device which produces a week electric current at 12-12.5 V. The bees were subjected to this for 45 min in the morning and after a pause of one hour, the procedure was repeated, where there was then a rest period of 72 hrs. before the treatment was restarted. Whereas, in two years an average of 1,593 mg of venom per bee was collected. During the main flow, the collection of venom had an adverse effect on the condition of the bees; the treated colonies produced 2/08 % more bees and wax than the control colonies. Furthermore, Omar *et al.* (1993) reported a modified device consisting of a generator that produces impulses of different waveforms, adjustable frequencies, and amplitudes. The device is energized by either a 220 V AC supply, or a single 12 V battery. The device developed is based on the stimulation of

**Table (1): Effect of daytime collection on the quantity of dried venom produced (mg) from honeybee colony using electric shocks.**

| Replicate | Early Morning 8.00-9.00 am | Mid-day 1.00-2.00 pm | Before Sunset 6.00-7.00 pm | Total (mg) Collected/Day |
|---|---|---|---|---|
| **1** | 24.7 | 13.1 | 22.6 | 60.4 |
| **2** | 21.2 | 22.6 | 27.4 | 71.2 |
| **3** | 14.6 | 14.0 | 25.6 | 54.2 |
| **4** | 7.3 | 10.4 | 31.9 | 49..6 |
| **Mean ±SE** | **17.0±3.8 b** | **15.0±2.6 b** | **26.9±1.9 a** | **58.9±4.6** |

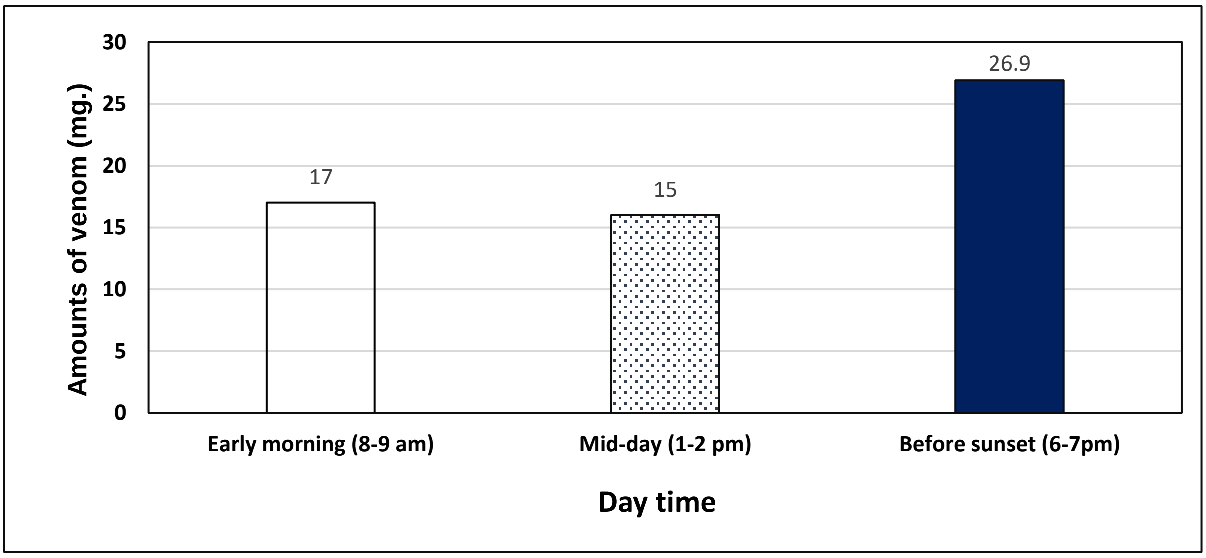


**Fig. (1): Effect of daytime collection on the quantity of dried venom produced (mg) from honeybee colony using electric shocks.**

the defense instinct of bees by the electric stimulus signal consisting of series of impulses of complex waves, twenty-three waveforms belonging to four groups, namely square, sine, triangular and exponential waves were tested. Also, Omar (2011) compared the venom quantities collected from aggressive colonies with calm one. The venom production negatively correlated with colony aggressive behavior, where the bee stingers were unstable on venom collection boards during time of colony excitation by electrical impulses. He also tested three positions for two venom collection boards attachment to honey bee hives (at hive entrance, beside the hive frames, over frames top), the venom extraction achieved the highest amount (93.22 mg/ colony) when the venom collection board was attached over the colony frames. The venom quantity increased significantly with 37.41% when compared with the position of boards at the hive entrance. Hussein (2013) determined the best time of the day to expose the honeybee venom from experimental colonies, which was divided into three groups, each composed of 3 colonies. These three groups, exposed to the device colonies bee venom collector (After dawn, during noon, and after sunset). The preliminary data showed that exposure of bees to venom collector device produced the least extractable amount at noon (87.23 mg dry venom), whereas, the highest amount of bee venom (102 mg dry venom/ colony) after the dawn Sun. The venom quantity increased significantly (+48.07%) when compared with collecting the venom at noon. The suitable time for exposing the honey bee workers of the experimental colonies to bee venom collector device without causing high damage to the bees was during two seasons 2011/12012. Moreover, Metwally (2016) mentioned that the highest quantity of collected bee venom was in the first time of collection (Morning) 40.0, 65.0 and 85.0 mg in 4, 5 and 6 broad combs, respectively. This represented 47.06, 63.11 and 69.11% of total collected bee venom; the highest quantity of collected bee venom was in the first time of collection 90.0, 100.0 and 120.0 mg in 4, 5 and 6 broad combs, respectively. This represented 62.07, 50.0 and 60.3% of total collected bee venom, the highest quantity of collected bee venom was in the first time of collection (night) 100.0, 180.0 and 220.0 mg in 4, 5 and 6 brood combs, respectively. This represented 90.09, 83.72 and 90.16% of total collected bee venom, The highest bee venom quantity than afternoon and morning collection time were 40, 65, 85 mg and 90.0, 100.0, 120 mg and 100.0, 180.0, 220.0 mg in 4, 5 and 6 brood combs at morning, afternoon and night day time, respectively.

worker population in colony, which gives a rather high chance to collect more amount of venom. Hussein (2013) also determined the best time of day to extract the honey bee venom was after the

dawn Sun (102 mg dry venom/ colony), Furthermore, Kaisser and Michel (1958) stated that the maximum production of venom being reached after two to three weeks. The synthesis of different components of bee venom do not reach maximum rate simultaneously. These changes in venom were suggested to be related to a transition from house (young) bee activity to field (old) bee activity, Likewise, Mitev (1971) stated that honey bee venom was collected from one group of 8 colonies, using a device which produces a week electric current at 12-12.5 V. The bees were subjected to this for 45 min in the morning and after a pause of one hour, the procedure was repeated, where there was then a rest period of 72 hrs. before the treatment was restarted. Whereas,

**Table (2): Effects of the period between the 1st and 2nd collection on the quantity of dried venom produced (mg) from honeybee colony using electric shocks.**

| Replicate | Treatment 1 | | Treatment 2 | | Treatment 3 | |
|---|---|---|---|---|---|---|
| | **Zero time** | **After 7 days** | **Zero time** | **After 14 days** | **Zero time** | **After 21 days** |
| **1** | 14.2 | 17.0 | 24.7 | 15.0 | 17.3 | 18.6 |
| **2** | 22.6 | 12.0 | 14.0 | 17.0 | 13.2 | 15.8 |
| **3** | 25.5 | 23.0 | 23.0 | 31.8 | 21.7 | 22.9 |
| **4** | 20.2 | 18.9 | 22.6 | 22.0 | 27.4 | 25.5 |
| **Mean ± SE** | **20.6±2.4 c** | **17.7±2.2 c** | **21.1±2.4 a** | **21.5±3.7 a** | **19.9±3.0 b** | **20.7±2.1 b** |

in two years an average of 1,593 mg of venom per bee was collected. During the main flow, the collection of venom had an adverse effect on the condition of the bees; the treated colonies produced 2/08 % more bees and wax than the control colonies. Furthermore, Omar *et al.* (1993) reported a modified device consisting of a generator that produces impulses of different waveforms, adjustable frequencies, and amplitudes. The device is energized by either a 220 V AC supply, or a single 12 V battery. The device developed is based on the stimulation of the defense instinct of bees by the electric stimulus signal consisting of series of impulses of complex waves, twenty-three waveforms belonging to four groups, namely square, sine, triangular and exponential waves were tested. Also, Omar (2011) compared the venom quantities collected from aggressive colonies with calm one. The venom production negatively correlated with colony aggressive behavior, where the bee stingers were unstable on venom collection boards during time of colony excitation by electrical impulses. He also tested three positions for two venom collection boards attachment to honey bee hives (at hive entrance, beside the hive frames, over frames top), the venom extraction achieved the highest amount (93.22 mg/ colony) when the venom collection board was attached over the colony frames. The venom quantity increased significantly with 37.41% when compared with the position of boards at the hive entrance. Hussein (2013) determined the best time of the day to expose the honeybee venom from experimental colonies, which was divided into three groups, each composed of 3 colonies. These three groups, exposed to the device colonies bee venom collector (After dawn, during noon, and after sunset). The preliminary data showed that exposure of bees to venom collector device produced the least extractable amount at noon (87.23 mg dry venom), whereas, the highest amount of bee venom (102 mg dry venom/ colony) after the dawn Sun. The venom quantity increased significantly (+48.07%) when compared with collecting the venom at noon. The suitable time for exposing the honey bee workers of the experimental colonies to bee venom collector device without causing high damage to the bees was during two seasons 2011/12012. Moreover, Metwally (2016) mentioned that the highest quantity of collected bee venom was in the first time of collection (Morning) 40.0, 65.0 and 85.0 mg in 4, 5 and 6 broad combs, respectively. This represented 47.06, 63.11 and 69.11% of total collected bee venom; the highest quantity of collected bee venom was in the first time of collection 90.0, 100.0 and 120.0 mg in 4, 5 and 6 broad combs, respectively. This represented 62.07, 50.0 and 60.3% of total collected bee venom, the highest quantity of collected bee venom was in the first time of collection (night) 100.0, 180.0 and 220.0 mg in 4, 5 and 6 brood combs, respectively. This represented 90.09, 83.72 and 90.16% of total collected bee venom, The highest bee venom quantity than afternoon and morning collection time were 40, 65, 85 mg and 90.0, 100.0, 120 mg and 100.0, 180.0, 220.0 mg in 4, 5 and 6 brood combs at morning, afternoon and night day time, respectively.

### 3.2. Determine the best period time between venom collections

The data obtained (Table 2) and illustrated in Fig. (2), showed that the best period time

**Table (2): Effects of the period between the 1st and 2nd collection on the quantity of dried venom produced (mg) from honeybee colony using electric shocks.**

| Replicate | Treatment 1 | | Treatment 2 | | Treatment 3 | |
|---|---|---|---|---|---|---|
| | **Zero time** | **After 7 days** | **Zero time** | **After 14 days** | **Zero time** | **After 21 days** |
| **1** | 14.2 | 17.0 | 24.7 | 15.0 | 17.3 | 18.6 |
| **2** | 22.6 | 12.0 | 14.0 | 17.0 | 13.2 | 15.8 |
| **3** | 25.5 | 23.0 | 23.0 | 31.8 | 21.7 | 22.9 |
| **4** | 20.2 | 18.9 | 22.6 | 22.0 | 27.4 | 25.5 |
| **Mean ± SE** | **20.6±2.4 c** | **17.7±2.2 c** | **21.1±2.4 a** | **21.5±3.7 a** | **19.9±3.0 b** | **20.7±2.1 b** |

between 1st and 2nd collection time were 14 days which target 21.5±3.7 mg with significant difference between the other two groups, after 7 days, after 21 days which target 17.7±2.2, 20.7±2.1 mg, respectively.

hours (when foraging was occurring), with the collection frames in the upper body. Mid-July was the best period for venom collection. The final apparatus developed consisted of an electro stimulator (generator) which passes current

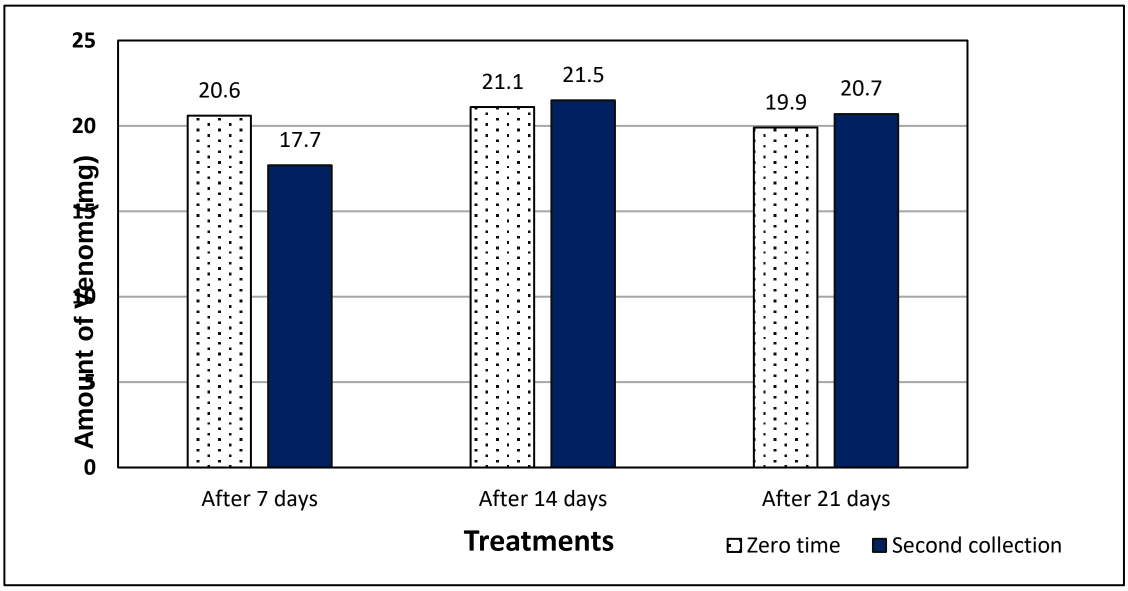


**Fig. (2): Effects of the period between the 1st and 2nd collection on the quantity of dried venom produced (mg) from honeybee colony using electric shocks.**

In fact, 14 days is good enough time to make recovery to workers from whom venom was collected, and stored more venom in their venom sac. In addition, more generation of workers were added to the colony population, therefore 14 days interval was a suitable time to collect venom. These findings are in agreement with those reported by Zhou *et al.* (1992) who studied the effects of venom collection periods on the defensive behavior of Italian bees (*Apis mellifera ligustica*). Counting the numbers of stings left in a black cotton ball moved in front of the hive. When venom was collected in the hive once every 3 days or once a day, the numbers of stings delivered immediately after venom collection were 68.2 % and 63.6 % less than the number of stings delivered by the control. Furthermore, at Pulawy, Poland, Rybak *et al.* (1995) described the techniques used for honey bee venom collection, during 1989-1994. The results showed that the optimal electrical parameters were: Impulse frequency, 1 kHz; voltage, 25 V; impulse duration, 1 second; interval between impulses, 2 seconds. The best results were obtained when venom collection was carried out every 14 days, for 1 hour (early morning, before bee flight) or 2

through electrodes mounted every 5 seconds in venom-collecting frames fitted in one of the hive bodies. The frames included a glass screen on which the venom is deposited, Khodairy and Omar (2003) determined the relationship between bee venom produced by electrical impulses, and certain characters of honey bee colonies (*i.e.* bee population, brood, stored pollen, stored honey areas and yield and foraging activity). And, the variability of venom quantity collected from colonies at different periods of active season, and found significant variations in the amount of collected venom at different periods of active season. In addition, they reported that the amount of venom was high in June compared with that collected in May and July, finally they found positive correlation between venom production and each of the bee population, bee brood, stored pollen, uncapped and capped honey areas and foraging activity. Also, at Karaj, Iran Bahreini *et al*. (2000) described a venom collecting apparatus. The device was a 42 x 50 x 58 cm cage like box, with inner walls that were equipped with electric wires, which would be sequentially charged and discharged. Bees that would come in contact with two adjacent wires would receive an electric shock of 21 V for 3 seconds. After a lapse of 7 seconds, the wire is recharged and ready for the next electric shock. This 10 second cycle continues for duration of 5 minutes, during which bees are made to sting on the plastic covering of a glass plate. Venom deposited on the glass laboratory. In a 6-month plates are scraped off by a sharp lancet in the 6 experiments, venom was extracted from 16 colonies of bees (8 treatments and 8 controls) every 15 days. Throughout the experiment, 838 mg of venom per colony was obtained. No diverse effect was observed in the production of honey. In addition, Nenchev (2001) found that the annual yield of bee venom from a bee colony for 15 sessions at 14-day intervals between March and October was 3.804 g. Also, El-Shaarawy (2008) used two collection periods for the bee venom production. The collection was done every 4 days and every 7 days intervals using different honeybee races and hybrids during the different collection seasons of the year. The means of venom weights was higher in the 7 days collection period, but in the other hand the 4 days collection period were higher in the total of the venom's weights during all the season of bee venom collection.

## Authors' contributions

All authors contributed in conceptualization, methodology, software, validation, formal analysis investigation, resources, data curtain, writing the original draft preparation, writing, review, editing, supervision and funding acquisition. All authors have read and agreed to the published version of the manuscript.

## Competing interests

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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# أنسب وقت خلال النهار وأنسب فواصل الفترات الزمنية للحصول على أعلى كمية سم من طوائف نحل العسل في مصر

**محمد عبد الوهاب عبد الفتاح[1] محمود عنتر الفيل[2] والسيد إبراهيم حجاج[2]**

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## ملخص

أجريت هذه الدراسة خلال فترة الربيع لعام 2021 (مارس الى مايو) لتقييم أنسب موعد لجمع أكبر كمية من السم في نهار اليوم ( من 8-9 صباحا , 1-2 مساء, 6-7 مساء)، وأيضا لتحديد أنسب فترة كفاصل زمني ( 7 و 14 و21 يوم) بين أول وثاني مرة للجمع من نفس الطائفة. أجريت التجربة الأولى لتحديد أنسب موعد لجمع أكبر كمية من السم في نهار اليوم. استخدمت عدد 12 طائفة من طوائف نحل العسل الكرنيولي الهجين المتساوية في القوة وقسمت إلى ثلاث مجموعات. أوضحت النتائج المتحصل عليها أن أعلى كمية سم تم الحصول عليها كانت قبل غروب الشمس ( 26.9 ±1.9 مللجم\الطائفة) وبفارق معنوي عن موعد الصباح (17 ± 3.8 مللجم\ الطائفة) أو عند الظهيرة ( 15 ± 2.6 مللجم\ الطائفة). ولاختيار أنسب فترة كفاصل زمني (7, 14 و21 يوم) بين أول وثاني مرة للجمع من نفس الطائفة, أجريت التجربة الثانية حيث اختيرت 24 طائفة من نفس الهجين المتماثلة في القوة وقسمت إلى 3 مجموعات متماثلة. أوضحت النتائج أن جمع السم عند 14 يوم كفاصل زمني أعطت أكبر كمية سم مجموعة ( 21.5 ± 3.7 مللجم\ الطائفة) بدون فروق معنوية عن 21 يوم ( 20.7 ± 2.1 مللجم\للطائفة). بينما كانت كمية السم المجموعة من الجمع الأول عند7 أيام ( 17.7 ± 2.2 مللجم\ الطائفة) ذات فروق معنوية أقل عن المدد الأخرى.

**المؤتمر الرابع عشر لإتحاد النحالين العرب، 13-15 نوفمبر (2023) القاهرة- مصر**
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