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**Research article**
**Ερευνητικό άρθρο**

# Seasonal variation on bee venom collection. The impact on some biological aspects on *Apis mellifera*

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**ABSTRACT:** Bee venom was collected by electrical stimulation from *Apis mellifera* macedonica every six and twenty-four days respectively for two years.Collections were accomplished from April to October the first year, and from May to October the second year. The bee venom yield and the bees' behavior like the aggressiveness, the number of dead bees on the collecting device and the hoarding behavior were studied. A great variation was found among the colonies regarding the collected amount of bee venom. The production was high in spring, decreased in summer and increased again in autumn in both years. Two different tests were used to study the defensive response of honeybees. The rhythmic reflux of a leather ball in front of the hive and the test of rating assay. Both tests showed that bees' aggression did not significantly increase after collection. Furthermore, the aggressiveness of bees did not change during the period of collection. The average number of dead bees found on the wires of collecting device,was below 20 in each collection. Hoarding test indicates that no significant differences existed between before and after the stimulation of worker honey bee by electrical impulses.The collection of BV did not affect brood and adult population of bees.

***Keywords*: *Apis mellifera* macedonica, bee venom collection, seasonal variation, bee behavior, aggressiveness, hoarding behavior**

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## INTRODUCTION

Bee venom (BV) is a valuable product for the pharmaceutical industry, and its production and placing on the market are expected to be of great concern to the beekeepers. It's composition is a complex mixture of active peptides (melittin, adolapin, apamin, MCD, secapin) enzymes (phospholipase A2 and B, hyaluronidase, phosphatase and α-glucosidase), biogenic amines (histamine, dopamine, noradrenalin), amino acids (aminobutyric acid, a-amino acids), phospholipids, sugars, volatiles substances, minerals and other components (Bogdanov, 2014) which have variety of pharmaceuticals properties, such as arthritis, chronic pain, multiple sclerosis, some types of cancer and others (Orsolic et al, 2003;Gajski and Garaj-Vrhovac, 2013; Liu et al, 2008;Dantas et al, 2014; Son et al, 2007; Mirshafiey, 2007).It is also widely used in cosmetology (Kurek-Gorecka et al, 2020; Lee et al, 2014; Kim and Kim, 2010).

The collection procedure involves the stimulation of bees with electrical impulses. However, the defensive behavior of bees is an obstacle to the collection of venom since bees become particularly aggressive. There is a view that no humans and animals should be near the area of the BV collection and honeybees must be moved to a distant location for the bee venom collection (Morse and Benton, 1964). Although, this aggressiveness during the collection is very well known, limited published information documented it.

In addition to the increased defensive behavior, the collection of BV results in decreasing of the sealed brood area from 11.3% to 18.1% (Sanad and Mohanny, 2013), in the number of dead bees (Simics, 1995) in decreasing honeybee population, and in diminishing productivity of honey (Mitev, 1971; Balzekas 1978) and of royal jelly (Zhou et al., 2003). On the other hand, these adverse effects were not justified by other researchers (Rybak et al, 1995; Skubida et al, 1995;Bahreini et al, 2000). In oppose, field studies,showed that the collection of BV increased the hygienic behavior and the hoarding behavior of bees (El-Saeady et al, 2016).

Besides those discrepancies, the amount of venom collection was also a topic of discussion with diverse results mainly because it depends on many parameters such as the frequency of collection, the time of the day, the season of collection, the colony strength, the race and the age of the bees, the device of collection and other factors (Lauter and Vria, 1939;Omar, 1994;Haggag et al, 2015).

The different findings on the effect of BV collection on both their defensive behavior and their biological responses, have encouraged this investigation. We collected bee venom by electrical stimulation at regular intervals for two years, and we studied characteristics of bee colony behavior, like bee aggressiveness, mortality, growth of population and hoarding behavior. In addition we determined the amount of bee venom that is produced per colony at different seasons to compare it with studies that had been conducted in other countries.

## MATERIALS AND METHODS

The study was carried out from April to October in 2016 and from May to October in 2017, at the experimental apiary of the Laboratory of Apiculture-Sericulture which situated at the farm of Aristotle University Thessaloniki in Greece. Nine honey bee colonies of *Apis mellifera* macedonica, equal in both strength and population, with ten honey bee combs, housed in Langstroth hives classified into three experimental groups. In groupA bee venom was collected every six days, in group B every twenty-four days and the C group used as control. Control group was handled as the other two groups with the exception that no BV was collected from this group.

### Collection of bee venom during different periods

The venom collector was in the form of a hive frame with dimensions 23.5x43.9 cm. It consisted of two glass plates with the electric wires powered bytwo batteries AA. The current was continuous but interrupted by a microprocessor withimpulse durationof two seconds. This electric shock device was placed inside the hive on a second empty floor, for twenty-five minutes (Fig. 1). Bees that came into contact with the wires received a mild electrical shock that forced them to sting onto two glass sheets of the device (Fig. 2). The BV collector device was removed from the five without the use of smoke and the attach bees were detached by shaking. The two glass surfaces were transferred into the laboratory and the BV collected as powder by using a full face mask (Drager X-plore 6300), gloves and all the necessary precautions. Bee venom weighed,packed in dark glass jars and stored in the freezer (-20$^{o}$ C).

**Figure 1.** The bee venom collection device was placed inside the hive on a second empty floor.

**Figure 2.** Bees get in contact with the charged wire net received electrical stimulation that causes them to release of venom in the glass plate.

**Defensive response of the honeybee**

To measure the aggressiveness of worker bees, a black leather ball attached to white twine, bound to a wood (100 cm length) was swung rhythmically in front of the beehive for 1 minute as first described by Free (1961) and Stort (1974) as leather -patch assay. The rhythm of movement was about one turn per second. For each colony and each test, different ball was used to avoid the effects of remaining alarm pheromones. Measurements concerned the number of stings in ball and operator's gloves.Measurement was scheduled every six days on groups A and C and every twenty- four days on groups A B and C, just before venom collection. Bee colonies before the suspension of leather balls were not disturbed, so different components did not affect the defensive sequence and create perplexing effects as described by Collins and Kubasek(1982).

Rating assay which is the most reliable assay to test the defending behavior of honey bees,according to Guzman-Novoa et al (2003), was also used. This test was performed by two operators who evaluated the sound intensity of the bees, the tendency of workers to fly around the hive, the running on the combs, to hit the veil, and to sting the gloves of the operators during manipulations. The rating scale of the above measurements was 1-5 (Guzman-Novoa et al, 2003). The inspections were done every 7 to 10 days during the experimental period. The higher the score the higher the aggression of the bees.

**The effect of bee venom collection on the number of dead workers**

The effect on dead worker bees was recorded by counting the dead bees on the wire of the collecting devices after each collection as previously described by Sanad and Mohanny (2013)

**The effect of bee venom collection on honeybee hoarding behavior**

A number of thirty adult worker bees gathered from the brood area of each experimental bee colony and placed in cages (10x10.3x4.2 cm). Each cage supplied with a piece of a dark comb of 40 $cm^2$ surface area. The cages were placed in an incubator at $35^{o}C$ and 50% relative humidity.

After 24 hours of starving, caged bees fed with sucrose solution (1:1) which was supplied in a gravity feeding vial. The decrease of syrup from the feeder vial was recorded and refreshed daily. Measurements continued for fifteen days. Four complete replications were carried out with bees from the same colonies. Hoarding results expressed as μl sucrose solution removed from the feeder per bee in one day.

**The effect of bee venom collection on population and brood area**

During the two-year research, we kept all the bee-colonies with the adult population covered 10 frames so that the production of BV did not depend on the size of the bee colony. To achieve this we removed the frames of the experimental hives with sealed brood in cases where the bee exceeded the 10 frames population and needed extra space. The effect of bee venom collection on the brood and the bee populations was estimated in comparison with the number of brood combs that were removed from the three groups so that the bees could be kept in a population of 10 frames.

### Statistical analysis

Results are presented as the Mean±SD. Means and standard deviations were calculated using Microsoft Excel. Experimental results were statistically analyzed using Duncan's *t*-tests and one-way Analysis of Variance (ANOVA) (IBM SPSS Statistics ver. 25.0). For all analyses, the differences with p-values ≤ 0.05 were considered statistically significant.

## RESULTS

### The amount of bee venom collection

The mean values (± SD) of BV collected from each colony are shown in Table 1. The differences among the colonies were not significant (p-value group A = 0.230, p-value group B = 0.183), although there was a great variation among them as indicated by the high SD and the range. Some colonies yield very little or no BV and others produced as much as four times the mean.The total amount of BV collected from a single colony variedbetween 753.2 to 879.7 mg when it was collected every 6 days and between 131.7 to 240.6 mg when collected every 24 days. The higher the frequency of collection the more BV was collected. Climate factors may influence the amount of BV but not significantly as indicated by the results of 2016 and 2017.

Figure 3 shows the fluctuation of the amount of BV collected every 6 and 24 days respectively in 2016. The BV production was high in spring (average 48 and 38 mg respectively) decreased in summer (average 13 and 26 mg respectively) and increases again in fall (19 and 28 mg respectively). The tendency of higher production BV during spring was apparent in 2017 too (figure 4).

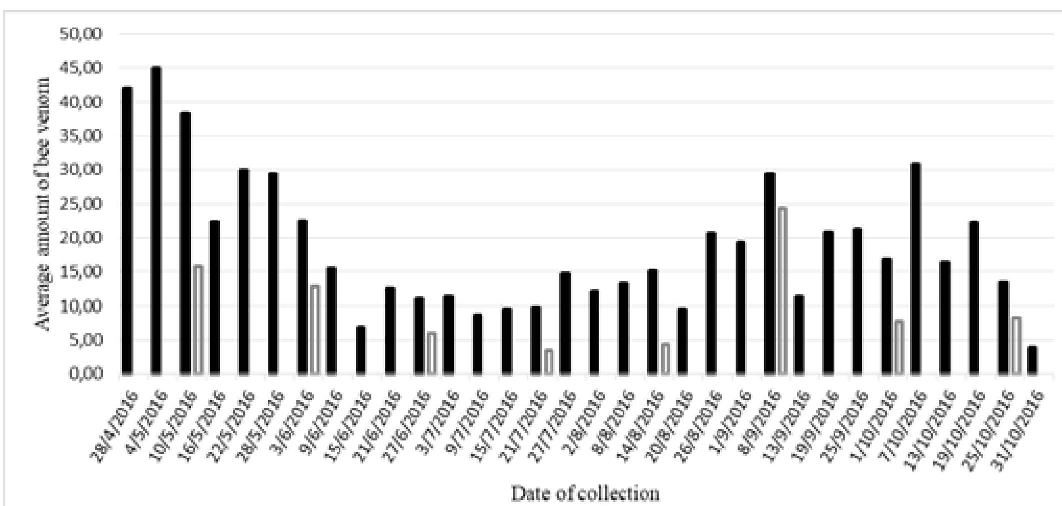


**Figure 3.** The average amount of bee venom (mg) collected during 2016. ▮ Collection every 6 days, ▯ collection every 24 days

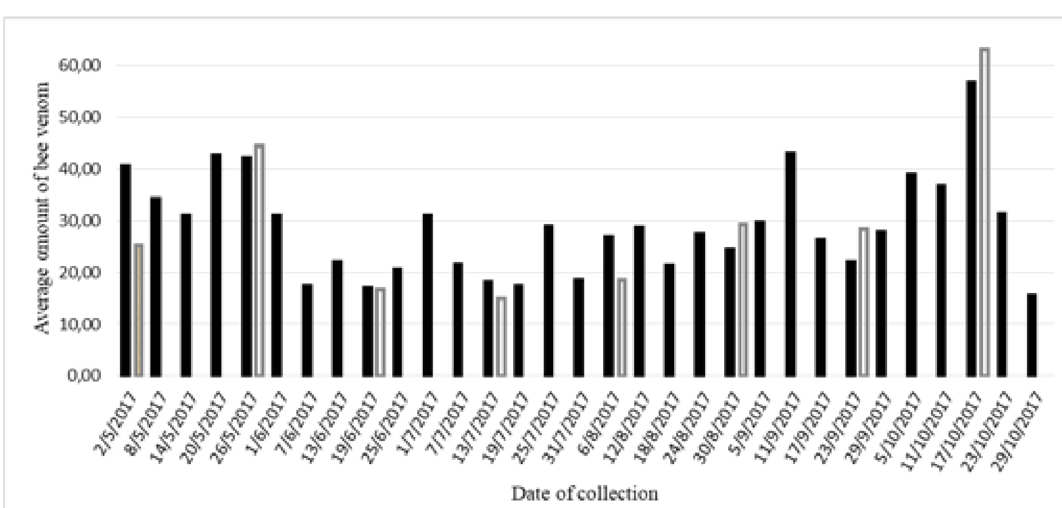


**Figure 4.** The average amount of bee venom (mg) collected during 2017. ▮ Collection every 6 days, ▯ collection every 24 days

**Table 1.** Bee venom production from bee colonies from April to October of two years (mg)

| Frequency of collection | Mean ± SD | min & max | Total amount per colony |
|---|---|---|---|
| **Every 6 days (A)** | | | |
| **2016** | 22.15[a] ± 16.2 | 0.3-100.7 | 753.2 |
| **2017** | 28.38[a] ± 11.52 | 4.7-115.0 | 879.7 |
| **Every 24 days (B)** | | | |
| **2016** | 14.63[a] ± 13.62 | 0.6-55.1 | 131.7 |
| **2017** | 30.08[a] ± 15.32 | 0.0- 82.7 | 240.6 |

*The statistical analysis was performed between the two experimental periods for each group

**Table 2.** The average number of stings (±SD) in leather ball measuring the defensive behavior of bees

| | Collection | | |
|---|---|---|---|
| **Year** | **Every 6 days (A)** | **Every 26 days (B)** | **Control (C)** |
| **2016** | 3.4[a] ± 13.3 | 3.9 [a] ± 12.8 | 5.32 [a] ± 11.22 |
| **2017** | 1.0[a] ± 2.7 | 0.9[a] ± 3.26 | 5.37[b] ± 15.57 |

*The statistical analysis was performed between groups for each experimental period

**Table 3.** The tendency of defensive behavior of honey bees (scale 1-5)

| | | Collection | | |
|---|---|---|---|---|
| **Rating assay** | **Year** | **Every 6 d (A)** | **Every 26 d (B)** | **Control (C)** |
| Sound intensity | 2016 | 1.38[a] | 0.78[b] | 1.28[a] |
| | 2017 | 3.05[a] | 2.58[a] | 2.71[a] |
| Flying around the hive | 2016 | 1.80[a] | 1.15[b] | 1.49[c] |
| | 2017 | 2.97[a] | 2.65[a] | 2.86[a] |
| Running on combs | 2016 | 1.70[a] | 1.22[b] | 1.42[ab] |
| | 2017 | 3.08[a] | 2.73[a] | 2.63[a] |
| Hitting operator's veil | 2016 | 0.31[a] | 0.17[a] | 0.33[a] |
| | 2017 | 2.68[a] | 2.00[a] | 2.40[a] |
| Stinging operator's hands | 2016 | 0.45[a] | 0.12[a] | 0.33[a] |
| | 2017 | 0.98[a] | 0.62[a] | 0.99[a] |

*The statistical analysis was performed between groups for each experimental period and for each parameter

### Defensive response of the honeybees

Applying the leather -patch assay we found that the collection of BV did not increase the defensive responseof the bees. No statistically significant differences were observed between colonies collected BV and controlsin 2016 (p-value = 0.481).In the second year the bees of the control hives appeared to be more aggressive than bees used to collect BV but this is misleading as the number of stings in the leather ball was small and cannot be considered as aggressive behavior (Table 2).

During the experiment, we found differences in the defensive response among the bee colonies. Most of the colonies did not react to the leather ball swinging in front of their hive. The increased aggression of some colonies was considered as random and unrelated to the existing weather conditions as bees from other hives on the same day showed calm behavior.In addition, their aggression decreased in the subsequent assays.

Table 3 indicates that the rating test yielded low values in all cases that never reached the upper levels of aggression. Statistically significant differences were found between the three experimental groups only in 2016 and were restricted in the sound intensity (p-value group B-group C = 0.001), the flying of bees (p-value group A-group C = 0.007, p-value group B-group C = 0.004) and the running on combs (p-value group A-group C = 0.008). The application of the assay during the full collection period showed that the bees did not change their behavior and did not become more aggressive as the project progresses.

### The effect of collecting bee venom on the number of dead bees

As Table 4 indicates, the average number of dead bees found on the wire of collecting device was higher in colonies collected BV every 6 days to those collected every 24 days in both years. Differences between the two protocols were significant in 2016 (p-value=

0.001). The maximum numbers of 70 and 86 dead bees found in one collection were considered random and outliers, thatthey did not participate in the estimation of the mean value. The number of dead bees in group collecting BV every 6 days that were below 25 constituted 91.2% in 2016 and 96.7% in 2017. In group collecting BV every 24 days, the number of dead bees in most cases was below 10.

**The effect of collecting bee venom on honeybee hoarding behavior**

Table 5 presents the daily consumption of syrup by caged bees. There were nosignificant differences (p-value group A 2016= 0.130, p-value group B 2016= 0.895, p-value group A 2017= 0.423, p-value group B 2017= 0.472) in syrup consumption before and after BV collection in both years. Substantial variation exists between cages indicating that other factors such as the age of the bees, the number of remaining bees, and time of the year may be involved.

**Population and brood area during the collection of bee venom**

During the experiment, the bee colonies became overcrowded and we withdrew frames with sealed brood, indicating that the collection of BV did not affect their development. We removed 3, 2 and 10 frames of sealed brood respectively from groups A, B and C in 2016 and 12, 9 and 5 frames during 2017. The removal of brood frames seems to stop the swarming impulse.

**Table 4. Number of dead bees found in the wire of collecting device**

| | Collection every 6 days (A) | | Collection every 24 days (B) | |
|---|---|---|---|---|
| | mean | Min-max | mean | Min-max |
| **2016** | $13.45^{a} \pm 16.2$ | 0-70 | $3.8^{b} \pm 5.2$ | 0-17 |
| **2017** | $19.29^{a} \pm 19.47$ | 0-86 | $9.27^{a} \pm 8.2$ | 0-25 |

*The statistical analysis was performed between groups for each experimental period

**Table 5. Consuming syrup in vivo μl/bee**

| Hive | | Collection every 6 days (A) | | Collection every 24 days (B) | | Controls (C) | |
|---|---|---|---|---|---|---|---|
| | | Before | After | Before | After | Before | After |
| **1** | **2016** | 80±30 | 40±60 | 50±20 | 40±50 | 60±30 | 40±40 |
| | **2017** | 60±70 | 60±30 | 50±30 | 80±60 | 60±40 | 60±30 |
| **2** | **2016** | 60±30 | 60±90 | 50±20 | 40±10 | 100±50 | 70±80 |
| | **2017** | 50±20 | 60±30 | 80±60 | 80±40 | 70±40 | 60±30 |
| **3** | **2016** | 50±20 | 40±40 | 20±10 | 40±70 | 40±20 | 20±20 |
| | **2017** | 60±40 | 70±40 | 70±30 | 70±30 | 40±50 | 80±30 |
| **Mean** | **2016** | $60^{a}$**±30** | $50^{a}$±70 | $40^{a}$±20 | $40^{a}$±50 | $70^{a}$±40 | $40^{b}$±60 |
| | **2017** | $60^{a}$±50 | $60^{a}$±40 | $70^{a}$±40 | $80^{a}$±40 | $60^{a}$±50 | $70^{a}$±30 |

*The statistical analysis was performed on the average consumption of each group before and after the experiment for each experimental period

**Table 6.** Comparison of the amount of venom collection from different bee races

| a/a | Race of bees | Period of collection | Amount of BV (mg/colony/ collection) | | References |
|---|---|---|---|---|---|
| | | | Average | Range | |
| 1 | *Carniolan* and *Caucasian* breeds | June-July | 37.3 | 20.8-53.0 | Rybak, 2008 |
| 2 | Not mentioned | Mar-Nov. | 115.7 | 24.0-383.0 | Sanad and Mohanny, 2013 |
| 3 | *Carniolan* | Jan-Dec. | 39.0 | 32.0-45.0 | Omar et al, 2014 |
| | *Italian* | | 33.0 | 26.0-40.0 | |
| 4 | *Apis ligustica* | July-Oct. | 31.9 (2014) | 20-42 | Nowar, 2016 |
| | | 2014-2015 | 35.6 (2015) | 20-50 | |
| 5 | *Carnional* | Feb.-Oct. | 123.3 | 96,6-150,0 | Omar, 2017 |
| | *Italian* | Feb.-Oct. | 130.0 | 96,6-150,0 | |
| 6 | *Apis macedonica* | April - Oct. | 22.15 | 0.3-100.7 | Current study |
| | | | 28.38 | 4.7-115.0 | |

## DISCUSSION

It is difficult to compare the results of BV collection with those of other studies because different collecting devices in a different climate and vegetation, with variable bee races and seasons of collection were used. In most studies, BV was collected in a different frequency, using a different number of colonies and duration of collection and finally, they presented the total amount of BV. In order to have compatible results with other studies, we calculated the amount of BV per colony that was collected after a single treatment by different authors. As Table 6 shows, the average amount of BV that was collected by a colony in one treatment in our research is close to those found by Rybak (2008), Omar et al (2014) and Nowar (2016) and lower than those of Sanad and Mohanny (2013) and Omar (2017).

One dissimilarity between our results and the published information is the differences between the lowest and the highest values (range). In our work, we found values with a wide range from near zero to values that were higher than 100 mg, while most of the other researchers, with the exception of Sanad and Mohanny (2013), gave a narrow range. These different results may be due to the number of observations, the number of hives, the frequency and the duration of collection. The low yields of venom production found in this work probably were due to the extremely low yields of specific bee colonies that were not substantially stimulated to sting in order to leave their venom on the glass plate.

The widely accepted notion that the procedure of collecting BV significantly increases their aggression was not confirmed in this study by either of the two tests used. Furthermore, the aggressiveness of bees remained stable and did not increase during the seven months weekly collection manipulations in both years of experiment. The concept that collection devices when placed inside of the hive triggered alarm pheromones that cause great stress on the bees and that they are very damaging to the health of the bees was not also verified. The gentle behavior of the Macedonian bee (*Apis mellifera* macedonica) may have played an important role. The significant variation among colonies in assays measuring the defensive behavior of honey bees was also noted by Guzman-Novoa et al (1999).

An average number of dead bees found on the wires of collecting device as a side effect of the collection were below 20 in each collection but it is likely to reach in some cases extremely higher numbers. In one case we counted 273 dead bees in only one hive, but it was a single event that did not repeat in the same or another bee colony. The bee losses during BV collection did not have any consistency during the year. In opposition to our results, other authors (Sanad and Mohanny, 2013) found significant differences among the death of workers within the months of BV collection. They recorded a higher number of dead bees in summer (50.3 workers/day) and lower in autumn (31.7 workers/day).The death of the bees probably resulted from the stress imposed on the bees from the collecting device, the electric currents, and fights between bees that occasionally occur in colonies during collection.

Hoarding test indicates that no significant differences existed between before and after the alarming or stimulation of worker honey bee by electrical impulses. Similarly, in field experiments, the mean yield of honey, obtained from colonies in which venom was collected, was not significantly different than controls (Rybak, 2008). El-Saeady et al (2016) indicated that bee colonies used for the collection of BV in field studies (fed ad libitum) consumed more syrup after the collection than before, although the differences were not significant. They attributed the increase in feed conception to the stimulation of worker honey bees to collect more food to compensate for the loss of secreted protein (venom).

Similar to our results Skubida et al. (1995) found that the use of stimulation of honeybees with electrical impulses for honeybee venom collection, had no adverse effects on colony strength, brood rearing and productivity. Also Rybak (2008) found that collecting bee venom three times during the season did not reduce significant the yield of honey, while the mean mass of venom collected from one honeybee colony did not differ significantly between the years, but was differ the amount of collected venom between the examined colonies.

In our work, it has been shown that collecting venom from bees does not cause aggression. Since this result is different from others studies in other countries, it would be interesting to look at the effect of the BV collection on bee behavior for the same period, the same flora and the same methodology (device type, time, ambient temperature, etc) with different races. If we were able to conduct experiments under the same conditions of collection and environment in different countries at the same time, we may be able to explainthe great variability observed in the research results of various studies.

## CONCLUSIONS

In this study, we concluded that bee venom collection can take place for a long period with the highest yields being achieved in spring and autumn.No negative effects found on sealed brood, the number of dead bees, the honeybee population, and the productivity of honey. Furthermore, the aggressiveness of bees remained stable and did not increase during the collection period.

## CONFLICT OF INTEREST

None declared.

## REFERENCES


Bahreini R, Fakhimzadeh K, Nowzary J, Nehzati GA (2000) Design and construction of a venom collecting electric cage and its effects on honey production in honeybee colonies.Iranian J AgricSci 31(2): 333-339

Balzekas JA (1978) Effect of bee venom collection on the productivity of honeybee colonies.LietuosZemdirbystesMakslinoTyrimoInstitutoDarbac 22: 78-87

Bogdanov S (2014) Bee Venom:Composition, Health, Medicine: A ReviewBee Product Science, www.bee-hexagon.net, February

Collins AM, Kubasek KJ (1982) Field-test of honey bee (Hymenoptera, Apidae) colony defensive behavior.Ann EntomolSoc Am 75: 383-387

Dantas CG, Nunes TLGM, Nunes TLGM, Paixao AO, Reis FP, Junior WL, Cardoso JC, Gramacho KP, Gomes MZ (2014) Pharmacological evaluation of bee venom and melittin. Rev Bras Farmacogn 24:67-72

El-Saeady AA, Diab A, Shehata IAA, Nafea EA, Metwaly AAA, (2016) Effect of bee venom collecting on the behavior of honeybee colonies.J Plant Prot and Path 7(6): 347-361

Free JB (1961) The stimuli releasing the stinging response of honeybees. AnimBehav 9(3-4): 193-196

Gajski G, Garaj-Vrhovac V (2013) Melittin: A lytic peptide with anticancer properties. Environ Toxicol Pharm 36: 697-705

Guzman-Novoa E, Page REJr, Spangler HG, Erickson EHJr, (1999) A comparison of two assays to test the defensive behavior of honey bees (*Apismellifera*).JApic Res 38(3-4): 205-209

Guzman-Novoa E, Prieto-Merlos D, Uribe-Rubio JL, Hunt GJ (2003) Relative reliability of four field assays to test defensive behaviour of honey bees.J Apic Res 42(3): 42-46

Haggag SI, Abed Al-Fattah MA, Ewies MA, El-feel Mahmoud A (2015) Effect of honeybee venom collection from different races on honey area.Aca J Entom 8(4): 190-192

Kim JM, Kim YB (2010) The Effect of Bee Venom Therapy on Skin Aging. J. Korean Med.Ophthalmol. Otolaryngol. Dermatol. 23(2): 27-40

Kurek-Gorecka A, Gorecki M, Rzepecka-Stojko A, Balwierz R, Stojko J (2020) Bee products in dermatology and skin care. Molecules 25, 556

Lauter WM, Vria VL (1939) Factors influencing the formation of the venom of the honey bee.J Econ Entomol 23: 806-807

Lee H, Bae SK, Pyo MJ, Heo Y, Kim CG, Kang C, Kim E (2014) Anti-wrinkle effect of PLA2-free bee venom against UVB-irradiated human skin cells. Journal of Agriculture & Life Science 49(1): 125-135

Liu S, Yu M, He Y, Xiao L, Wang F, Song C, Sun S, Ling C, Xu Z (2008) Melittin prevents liver cancer cell metastasis through inhibition of the Rac1-dependent pathway. Hepatology 47(6): 1964-1973

Mirshafiey A (2007) Venom therapy in multiple sclerosis. Neuropharmacology 53: 353-361

Mitev B (1971) Collection of bee venom using a weak electric current - its effect on the condition and the performance of the colony.ZhivotNauki 8(1): 103-108

Morse RA, Benton AW (1964) Notes on venom collection from honeybees.Bee World45(4): 141-143

Nowar EE (2016) Venom glands parameters, venom production and composition of honeybee *Apismellifera* L. affected by substitute feeding. Middle East JAgric Res 5(4): 596-603

Omar MOM (1994) Some factors affecting bee venom extraction from honey bee colonies.Assiut J AgricSc25: 139-148

Omar RE (2017) Effect of bee venom collection on the measurement of brood rearing activity of honey bee colony *Apismellifera* L.Middle East J Agric Res 6(2): 409-414

Omar RE, Khattab MM, El-Lakwah FA, El-Ashhab KA (2014) Bee venom collection and its effect on royal jelly production in honey bee colonies.J Plant Prot and Path, Masoura Univ. 5(2): 279-286

Orsolic N, Sver L, Verstovsek S, Terzic S, Basic I (2003) Inhibition of mammary carcinoma cell proliferation in vitro and tumor growth in vivo by bee venom. Toxicon 41: 861-870

Rybak M (2008) Effect of venom collection using the method of coupled electrical and sound stimulation on honey yield in bee colonies.J ApicSci 52(2): 91-94

Rybak M, Muszynska J, Skubida P, Marcinkowski J (1995) A technology for bee venom collection.PszczelnZeszNauk 39(2): 223-231

Sanad RE, Mohanny KM (2013)Theefficacy of a new modified apparatus for collecting bee venom inrelation to some biological aspects of honeybee colonies.J Am Sci 9(10): 177-182

Simics M (1995) Bee venom collection for medical use.Canadian - Bookkeeping 18(6):140

Skubida P, Muszynska J, Rybak M, MarcinkowskiJ (1995) Bee venom collection and its effect on the general output of the apiary and wintering.PszczelnZeszNauk 39(2): 209-221

Son DJ, Lee JW, Lee YH, Song HS, Lee CK, Hong JT (2007) Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. PharmacolTherapeut 115:246-270

StortCA (1974) Genetic study of aggressiveness of two subspecies of *Apismellifera* in Brazil 1. Some tests to measure aggressiveness,J Apic Res 13(1): 33-38

Zhou B, Zhang S, Su C, Zhou G, Zhou BF, Zhang SJ, Su C, Zhou GH (2003) Effect of collection of venom by electric shocking on honeybee population, production of royal jelly and honey.Acta Agriculture UniversitatisJiangxiensis 25(1): 141-145