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ORIGINAL ARTICLE

The effect of conductor wires of different metals on the amount of honey bee (*Apis mellifera*; Hymenoptera: Apidae) venom collected and its chemical content*

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Key words. Venom collector, electrical conducting wires, venom production, venom content

Abstract. Researchers have been interested in honey bee, *Apis mellifera* L., venom for many years, but the previous studies focused mainly on its pharmacological properties and the factors affecting its production and chemical content were less well evaluated. This research aims to investigate the potential effect of the method of collecting bee venom on its chemical content. To achieve this, brass, steel, aluminium and copper wire conductors were used. This research was carried out at Van YYU Beekeeping Application and Research Centre, where the venom was collected dry on glass plates from 16 Caucasian F1 honey bee colonies. The quantity produced and its composition in terms of melittin, apamin and phospholipase A2 were determined after storage. Duncan and LSD Multiple Comparison tests on the amounts of venom collected using aluminium, copper, steel and brass wires for conducting electricity, revealed it was 21.03 ± 3.50, 15.08 ± 0.32, 22.38 ± 6.35 and 30.35 ± 3.66 mg, respectively. Although these values differed significantly ($p < 0.05$), the chemical content of the bee venom was the same. The colour of the venom was associated with the material of the electrical conductor used. It was concluded that it would be beneficial to carry out further studies using more colonies along with using residue analysis.

INTRODUCTION

Honey bee venom (BV), which is soluble in water (Abdela & Jilo, 2016; Eze et al., 2016), has a clear appearance, a sharp smell and a sour taste. It is acidic (pH = 4.5–5) (Krell, 1996) and dries quickly in air and crystallizes (Owen & Sloley, 1988; Abdela & Jilo, 2016). BV is 88% water and has a complex peptide structure including 12% melittin, adolapin, apamin and the Mast Cell Degranulating (MCD) Peptide along with enzymes and bioactive amines, the most important of which is phospholipase A2 (PLA2) (Sig et al., 2019; Wehbe et al., 2019).

Melittin is a small protein consisting of 26 amino acids (Dotimas & Hider, 1987; Chen et al., 2016) which makes up 40–60% of dry BV; the contents is higher in bees 12–21 days old (Bachmayer et al., 1972; Huh et al., 2012).

The apamin in BV is a neurotoxin and consists of 18 amino acids (Habermann, 1984). Dry bee venom contains 2–3% apamin (Son et al., 2007). MCD Peptide, which has a similar structure to apamin, due to its disulphide bond, contains 22 amino acids. Similarly, it makes up 1–3% of dry venom (Banks et al., 1990). Adolophine, which makes up 1% of dry venom (Cherniack & Govorushko, 2018), contains 103 amino acids (Shkenderov & Koburova, 1982).

The phospholipase found in bee venom is more effective than that in snake venom or the pancreas of mammals. Melittin in bee venom increases the cell-lysis ability of phospholipase (Dotimas & Hider, 1987). PLA2, which makes up 12–15% of the dry weight of the BV, consists of 128 amino acids in a single polypeptide chain (Wehbe et al., 2019; Carpena et al., 2020).

Hyaluronidase, which makes up 0.5–1.5% of the dry weight of BV, consists of 349 amino acids with a total molecular weight of 40,746 Da. The amino acid sequence of hyaluronidase is unknown, it is less robust than PLA2 and difficult to purify without changing its molecular structure (Gmachl & Kreil, 1993).

Worker honey bees produce most venom when they are about two weeks old (Hasan et al., 2023) and cease producing venom when 3 weeks old. A worker bee can produce 0.15–0.3 mg of venom (Carpena et al., 2020) and the amount that can be obtained from a colony varies (El-Bahnasy et al., 2017).

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The venom glands are located in the abdomen of worker honey bees (Shimpi et al., 2016). In traditional medicine, manual methods were used to collect venom, which involved the removal of the venom sac or squeezing the area around the sting until the venom exudes. However, these methods are very time consuming and unpopular and were replaced by an electroshock method after 1960 (Hsiang & Elliot, 1975).

BV production and quality are influenced by a variety of factors, including method of collection, source of nutrition (flora/region), age, race of honey bee, colony strength and defensive behaviour of the bees (Haggag et al., 2015).

Around the world, various automatic ways of collecting venom have been developed. Modern collectors are easy to build and use and generally operate in the same way. The apparatus consists of a power source, a piece of glass and wires of different gauges for conducting the electricity.

Previous studies on collecting BV (Rybak et al., 1995; Bogdanov, 2016; Maulana et al., 2018) generally used the impulse frequency of 50–1000 Hz, the average impulse time about 3–6 s, the voltage range of 12–30 V / 1 A[this is current, not voltage; do you mean batteries?], and the grid consisting of stainless steel wires 0.5 cm apart. However, exact properties of the stainless steel conducting wires were not given.

Most previous research has been mainly on the general nature of BV and its use as a medicine for treating human ailments. Little research has been done on collection techniques and no standard has been established for the conductor wire cross-sections used in collection apparatus. However, conductors with a cross section around 2 mm^2 are frequently used. Wires made of different metals have different conductivity and resistance values. This study aimed to determine the effect of different conductors on the amount of venom production and melittin, phospholipase A2 and apamin contents.

MATERIAL AND METHODS

Material

The province of Van, where this research was done, is between longitudes 42°40′ and 44°30′E and latitudes 37°43′ and 39°26′N. This province, with a central altitude of 1725 m, is within the closed basin of Lake Van in the Upper Murat-Van Section of the Eastern Anatolia Region in Turkey. The climate is continental, with harsh and long winters and the number of days above 30°C in summer is around 20. Annual precipitation varies between 370 mm and 570 mm. Summers are very hot and dry. A large part of the area is a high plateau of steppe structure. In addition, only 23% of the land in this province is used for plant production, while approximately 70% is in the form of meadows and pastures (Karaca et al., 2019). Therefore, Van has great potential for beekeeping.

Fig. 1. Conductor wires in collectors.

The honey bees used in this research for collecting BV in September 2022, consisted of 16 Caucasian F1 hybrid honey bee colonies. At the beginning of this study, the colonies each contained 12 frames with bees, three of which contained closed brood cells and were randomly assigned to four groups. In this study, 16 collectors made of wood with the dimensions of 35 × 26 cm were used. The collectors were divided into 4 different groups based on whether the 2 mm^2 conducting wire was either brass, steel, aluminium or copper. A 12 V / 7 Ah rechargeable battery was used as the source of the electrical energy. Each collector had a glass plate for venom harvest.

The different types of conductor wires in each collector were connected in parallel and directly supplied with DC from the 12 V / 7 Ah battery. Thus, the same voltage was present on all conductor wires of the collector. The resistance and conductivity values of the conductive wires in the collector were calculated (the resistance value refers to a single 23-cm-long wire). The resistance of each 23-cm wire was also measured with an ohmmeter (resistance meter).

The resistance of the wires used to conduct the electricity was determined using the formula $R = L/K*S$ (R – resistance, L – conductor length, K – conductivity, S – conductor cross section) and the results are in Table 1. The conductor wires used in the collectors are depicted in Fig. 1.

Methods

The 4 different types of collectors used in the study were randomly distributed among a total of 16 colonies of the same strength and placed inclined inside the hive. Collectors were placed in a hive for 45 min and collected 15 min after the electrical power was turned off. Entire collecting apparatus in hive is shown in Fig. 2.

The dried venom on the glass plates was harvested by scraping it off the glass. The dry venom was weighed on electronic scales with a sensitivity of 0.001 g and then stored in amber coloured glass bottles at 4°C until analysed.

Table 1. Resistance of conducting wires.

Conducting wire	Conductor length (m)	Conductor cross section (mm^2)	Conductivity (m/Ω mm^2)	Resistance (Ω)
Aluminium	0.23	2	35	0.00329
Copper	0.23	2	56	0.00205
Steel	0.23	2	6	0.01917
Brass	0.23	2	16	0.00719

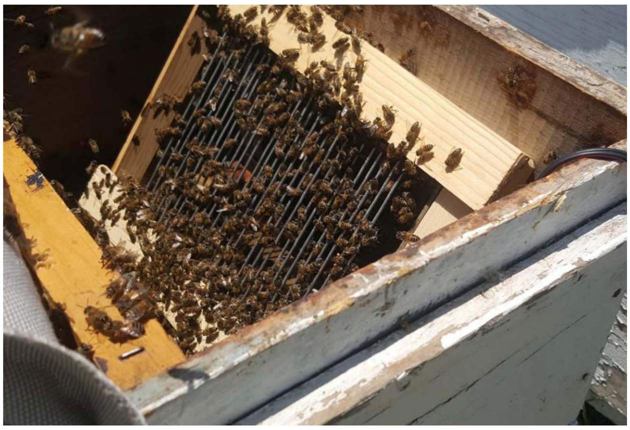

Fig. 2. Entire collecting apparatus in hive.

The determination of the content of bee venom was done using the method developed by Güven Gökmen et al. (2023). Details of the HPLC used in this method are given in Table 2.

In summary, HPLC was used to analyse the components apamin, phospholipase-A2 and melittin in bee venom, which were separated on a Poroshell C18 column. The optimum separation temperature was 30°C, and the column flow rate was 1.2 ml/min. Standard solutions of apamin, phospholipase A2 and melittin were prepared. The calibration curve was based on the peaks determined using the prepared standard solutions.

In BV content analysis, data could not be obtained from one sample. Hence, the evaluation was based on a total of 15 samples.

In order to determine whether the different collectors have an effect on honey bee worker mortality, light coloured pieces of cardboard was placed in front of each hive when the collection devices were placed in the hives. These boards were left in front of the hive for 5 h and the dead worker bees on them were counted and recorded.

One-way analysis of variance was used to compare bee venom production and contents (melittin, PLA2 and apamin), and Duncan Multiple Comparison Test was used to evaluate whether the differences in the variance analysis were significant. Mann-Whitney test was used to determine whether the differences revealed by the Kruskal-Wallis analysis of honey bee deaths were significant.

For statistical analysis, the GLM procedure in the SAS (2022) Package Program was used.

RESULTS

Average BV productions recorded are given in Table 3. Collectors using aluminium, copper, steel or brass wires for conducting electricity produced an average 21.03 ± 3.50, 15.08 ± 0.32, 22.36 ± 6.35 and 30.35 ± 3.66 mg of BV, respectively, with the most venom collected by the collector with a brass wire for conducting electricity. In terms of production, the amounts were statistically significant ($p < 0.05$), with the results recorded for the copper and brass electrical conductors in separate groups and those for the aluminium and steel electrical conductors very close to each other.

In the chemical content analyses that constitute the next stage of this study, melittin, apamin and PLA2 contents were recorded and the values are in Table 4. The highest value for melittin in dry BV was 66.46% (steel electrical conductor) and the lowest was 26.18% (brass electrical conductor). For apamin the highest mean was recorded for those with an aluminium electrical conductor (2.89 ± 0.21) and the lowest for those with a copper electrical conductor (1.24 ± 1.21).

For PLA2 values, the group with aluminium electrical conductors had the highest mean (10.19 ± 0.58), while those with copper electrical conductors had the lowest mean (6.53 ± 1.87), indicating a similar situation to apamin. For all features, the difference between groups was not statistically significant.

Another aspect studied is the colour of the BV harvested from glass plates. The colour of dry venom on light-coloured paper is shown in Fig. 3. The palest venom colour was recorded for the group with steel electrical conductors, while that of the groups with brass or aluminium electrical conductors was darker. The colour was the same for the same wire types.

Table 2. The conditions of the HPLC.

HPLC	Conditions
HPLC Model	Agilent 1260 with a variable wavelength detector (VWD)
Column	InfinityLab Poroshell C18 EC-C18
Column size	4.6 × 50 mm, 2.7 microns
Optimum separation temperature	30°C
Column flow rate	1.2 ml/min
Standard solutions concentrations	25, 50,100 and 125 µg/ml
Mobile phase	Buffer A: 0.1% TFA in H_2O Buffer B: 0.1% TFA in acetonitrile-HPLC Grade
Absorbance measurement	218 nm

Fig. 3. Colour of dried honey bee venom collected using the aluminium, brass, cooper and steel electrical conductors.

Table 3. Production of honey bee venom (mg).

Conducting wire	n	$\bar{X} \pm S_{\bar{X}}$	Lowest value	Highest value
Aluminium	4	21.03 ± 3.50^{ab}	13.00	28.80
Copper	4	15.08 ± 0.32^{b}	14.50	15.60
Steel	4	22.36 ± 6.35^{ab}	6.50	37.50
Brass	4	30.35 ± 3.66^{a}	22.50	39.90

[a, b] Differences between group means indicated by different letters are statistically significant ($P < 0.05$).

Table 4. Percentage content of melittin, apamin and PLA2.

Conducting wire	n	Melittin			Apamin			PLA2		
		$\bar{X} \pm S_{\bar{x}}$	Min.	Max.	$\bar{X} \pm S_{\bar{x}}$	Min.	Max.	$\bar{X} \pm S_{\bar{x}}$	Min.	Max.
Aluminium	4	50.94 ± 5.36	38.43	63.89	2.89 ± 0.21	2.51	3.30	10.19 ± 0.58	8.75	11.50
Copper	3	38.46 ± 7.24	30.32	52.89	1.24 ± 1.21	0.23	2.58	6.53 ± 1.87	3.46	9.93
Steel	4	46.39 ± 7.76	33.78	66.46	2.47 ± 0.72	1.29	4.45	8.76 ± 1.19	6.50	11.77
Brass	4	33.26 ± 3.20	26.18	40.19	1.64 ± 0.33	0.94	2.53	7.20 ± 1.21	4.11	9.63

Worker honey bee deaths during the collection of venom was also evaluated. For this, the number of dead worker bees on the white cardboard at the front of the hives was recorded (Table 5). Since this data consists of counts, non-parametric analyses were used and as a result the Kruskal-Wallis analysis indicated that the difference between the groups was statistically significant ($p < 0.05$), and that between the brass and copper groups in particular was significant (Mann-Whitney test).

DISCUSSION

In the present study the amount of BV produced by the different groups varied between 6.50 mg/colony and 39.90 mg/colony. Although these amounts are lower than the average values of 0.17 and 0.20 g/colony reported over two years by Nowar (2016), they are similar to the value of 0.0185 g/day reported by Sanad & Mohanny (2013). On the other hand, Hussein et al. (2019) report the highest yield in May of 99.9 mg and lowest of 11.1 mg in October. These studies carried out in different regions, report the highest and lowest productions, respectively, in June and November.

However, there are many factors that affect the production and quality of BV, such as, the race of honey bee, age of the bees, colony strength, season, food source (flora/region), defensive behaviour and method of collection (Haggag et al., 2015). Taking into consideration the amount produced and period of previous studies, the results are similar to those of the current study. The research was carried out during a period marked by a decrease in the availability of nectar and pollen, and change in weather that occurs from late summer to early autumn. This partly accounts for the decrease in the production of venom.

The differences in production of BV associated with the different conductor wires are not easy to explain. The venom production is highest when the conductor wire is brass (having lower conductivity than copper and aluminium but higher than steel). Further studies of more colonies and carried out in different periods are required.

Melittin, PLA2 and apamin are essential quality criteria of BV. Bogdanov (2016) reports the contents of melittin, PLA2 and apamin as 40–50%, 10–12% and 2–3%, respectively, which is accepted by researchers. In addition, Chen et al. (2016) state that the contents of melittin is 40–60%. However, these values, are dependent on genetics and many environmental factors (Haggag et al., 2015), which will be resolved by future research.

Table 5. Worker honey bee deaths (number).

Conducting wire	n	$\bar{X} \pm S_{\bar{x}}$	Average rank	Total deaths
Aluminium	4	3.5 ± 1.19^{bc}	6.75	14
Copper	4	7.7 ± 0.67^{a}	12.67	27
Steel	4	6.0 ± 0.91^{ab}	10.63	24
Brass	4	1.0 ± 0.71^{c}	3.13	4

$^{a, b, c}$ Differences between group means indicated by different letters are statistically significant ($P < 0.05$).

In this study, the means for melittin using aluminium and steel wire conductors was 50.94 ± 5.36 and 46.39 ± 7.76%, respectively, whereas for copper and brass wire conductors it was lower. A similar situation was recorded for percentage of apamin for which the averages for aluminium and steel wire conductors was 2.89 ± 0.21 and 2.47 ± 0.72%, respectively, that is between 2–3%. For PLA2 Şirin et al. (2016) report a value, which can be used to determine whether a mixture is bee venom or not, and only the percentage for the steel wire conductor group (10.19 ± 0.58%) is within the range of 10–12% stated by Bogdanov (2016).

The results of this study for melittin, PLA2 and apamin are generally low when the copper and brass conductor wires were used compared to previous studies. However, the relatively low number of young worker bees in the colonies during the study might account for this.

According to Roat et al. (2006), venom production of adult worker bees increases in the first two weeks of their lives and reaches its maximum level when they start foraging outside the hive. During this period, while the level of histamine in the venom increases, other ingredients, especially melittin, decrease. For this reason, it is likely that colony age distribution is an important determinant of the results recorded in this study. In order to obtain more definite results, future studies should be repeated at different times of the year and include more colonies. Products with different biochemical compositions can be used for different purposes.

BV, which is dry and powdery white in colour, can become darker due to oxidation and contamination, which may affect its pharmacological properties (Bogdanov, 2016). For this reason, colour can be used as a criterion of the quality of BV. In this study, precautions were taken to minimize the possibility of oxidation of BV and the risk of contamination. However, as can be seen in Fig. 3, there are significant differences in the colour of the BV collected using the different conducting wires. This difference in colour is associated with the contact of the body parts of the worker bees with the device and glass plate in a hot and humid environment. However, in future studies, with the use of residue analysis it will be possible to obtain clearer results. Since this research was conducted under the same environmental conditions and using the same honey bee race, the current results indicate that the finding of a previous study (Samancı & Kekeçoğlu, 2019) that the difference in colour of BV is associated with the race of honey bee used is not the only possible explanation.

The prevailing belief is that mortality among worker bees during the collection of BV is mostly due to compression resulting from contact with the conductor wires, as well as the heat generated by the clustering of worker bees on the apparatus. In the present study, the mortality of worker bees is compared between the groups, and although the differences are statistically significant, the mortality is nevertheless very low compared to that reported in other studies (Sanad & Mohanny, 2013; Hussein et al., 2019). Moreover, it is not possible to state that the main cause of death is due to venom collection.In future studies, the determination of the effect of venom collection on worker bee deaths, recorded during the pre-harvest, harvest and post-harvest periods will make a significant contribution to reducing the level of uncertainty.

In summary this study evaluated the effect of different conductor wires on the amount and content of the honey bee venom collected. It was concluded that the use of brass wire conductors resulted in collecting more venom than the use of aluminium, copper and steel wire conductors, and the difference in the conductor wire did not have a significant effect on the content on the venom, but did affect its colour. However, in order to be able to generalize, more colonies need to be studied at different times of a year in the future along with the use of residue analysis.

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CONFLICTS OF INTEREST. The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS. CE conceived this research and designed experiments. NT and CE performed experiments and analysis. NT and CE wrote the paper. Both authors read and approved the final manuscript.

DATA AVAILABILITY. The datasets generated during and/or analysed during the current study are available from the corresponding author on request.

REFERENCES

Abdela N. & Jilo K. 2016: Bee venom and its therapeutic values: a review. — *Adv. Life Sci. Technol.* **44**: 18–22.

Bachmayer H., Kreil G. & Suchanek G. 1972: Synthesis of promelittin and melittin in the venom gland of queen and worker bees: Patterns observed during maturation. — *J. Insect Physiol.* **18**: 1515–1521.

Banks B.E.C., Dempsey C.E., Vernon C.A., Warner J.A. & Yamey J. 1990: Anti-inflammatory activity of bee venom peptide 401 (mast cell degranulating peptide) and compound 48/80 results from mast cell degranulation in vivo. — *Br. J. Pharmacol.* **99**: 350–354.

Bogdanov S. 2016: *Bee Venom: Production, Composition, Quality.* URL: https://www.researchgate.net/publication/304012422_Bee_Venom_Production_Composition_Quality

Carpena M., Nunez-Estevez B., Soria-Lopez A. & Simal-Gandara J. 2020: Bee venom: An updating review of its bioactive molecules and its health applications. — *Nutrients* **12**(11): 3360, 27 pp.

Chen J., Guan S.M., Sun W. & Fu H. 2016: Melittin, the major pain-producing substance of bee venom. — *Neurosci. Bull.* **32**: 265–272.

Cherniack E.P. & Govorushko S. 2018: To bee or not to bee: The potential efficacy and safety of bee venom acupuncture in humans. — *Toxicon* **154**: 74–78.

Dotimas E.M. & Hider R.C. 1987: Honeybee venom. — *Bee World* **68**: 51–70.

El-Bahnasy S.A., Mahfouz H.M., El-Shibiny A.A. & El-Bassiony M.N. 2017: Effect of some factors on honey bee venom production from different strains. — *Sinai J. Appl. Sci.* **6**: 59–66.

Eze O.B.L., Nwodo O.F.C. & Ogugua V.N. 2016: Therapeutic effect of honey bee venom. — *J. Pharm. Chem. Biol. Sci.* **4**: 48–53.

Gmachl M. & Kreil G. 1993: Bee venom hyaluronidase is homologous to a membrane protein of mammalian sperm. — *Proc. Natl. Acad. Sci. U.S.A.* **90**: 3569–3573.

Güven Gökmen T., Yazgan H., Sevİn S., Turut N., Karahan Ş., Eşkİ F., Kivrak İ., Sezer O. & Ütük A.E. 2023: The antibacterial effect of bee venom on subclinical mastitis agents: an alternative for local treatment. — *J. Appl. Anim. Res.* **51**: 323–332.

Habermann E. 1984: Apamin. — *Pharmacol. Ther.* **25**: 255–570.

Haggag S.I., Abed Al-Fattah M.A., Ewies M.A. & El-Feel M.A. 2015: Effect of honeybee venom collection from different races on honey area. — *Acad. J. Entomol.* **8**: 190–192.

Hasan A., Qazi J.I., Tabssum F. & Hussain A. 2023: Increased bee venom production in *Apis mellifera* workers on the provision of probiotics and organic acids. — *Biocatal. Agric. Biotechnol.* **48**(4): 102616, 7 pp.

Hsiang H.K. & Elliot W.B. 1975: Differences in honeybee (*Apis mellifera*) venom obtained by venom sac extraction and electrical milking. — *Toxicon* **13**: 145–148.

Huh J.E., Kang J.W., Nam D., Baek Y.H. & Choi D.Y. 2012: Melittin suppresses VEGF-A-induced tumor growth by blocking VEGFR-2 and the COX-2-mediated MAPK signaling pathway. — *J. Nat. Prod.* **75**: 1922–1929.

Hussein E.A., El-Ansari M.K. & Zahra A.A. 2019: Effect of the honeybee hybrid and geographic region on the honey bee venom production. — *J. Plant Prot. Path.* **10**: 171–176.

Karaca S., Bulut S. & Türkmen F. 2019: Investigation of some land and soil properties by geographical information system analysis: Van province land and soil properties. — *Turk. J. Agric. Res.* **6**: 199–205.

Krell R. 1996: *Value-Added Products from Beekeeping*. Food and Agriculture Organization of the United Nations, Rome, 223 pp.

Maulana E., Nurussa'adah Wardana A.K., Khuzain M., Prasetyo G. & Anwarudin M.E. 2018: Bee venom harvesters device integrated with solar cell. In: *Electrical Power, Electronics, Communications, Controls and Informatics Seminar EECCIS, 9–11 October 2018, Batu, Indonesia.* Institute of Electrical and Electronics Engineers, pp. 123–126.

Nowar E.E. 2016: Venom glands parameters, venom production and composition of honeybee. — *Middle East J. Agric. Res.* **5**: 596–603.

Owen D.M. & Sloley B.D. 1988: 5-hydroxytryptamine in the venom of the honeybee (*Apis mellifera* L.): variation with season and with insect age. — *Toxicon* **26**: 577–581.

Roat T.C., Nocelli R.C.F. & Cruz-Landim C. 2006: Systematics, morphology and physiology: ultrastructural modifications in the venom glands of workers of *Apis mellifera* L. (Hymenoptera: Apidae) promoted by topical application of juvenile hormone. — *Neotrop. Entomol.* **35**: 469–476.

Rybak M., Muszynska J., Skubida P. & Marcinkowski J. 1995: A technology for bee venom collection. — *Pszczel. Zesz. Nauk.* **39**: 223–231.

Samanci T. & Kekeçğlu M. 2019: Comparison of commercial and Anatolian bee venom in terms of chemical composition. — *Uludag Bee J.* **19**: 61–68.

Sanad R.E. & Mohanny K.M. 2013: The efficacy of a new modified apparatus for collecting bee venom in relation to some biological aspects of honeybee colonies. — *J. Am. Sci.* **9**: 177–182.

SAS *Institute Inc.* 2022: *SAS/STAT Software*. URL: www.sas.com.

Shimpi R., Chaudhari P., Deshmukh R., Devare S., Bagad Y. & Bhurat M. 2016: A review: Pharmacotherapeutics of bee venom. — *World J. Pharm. Sci*. **5**: 656–667.

Shkenderov S. & Koburova K. 1982: Adolapin – a newly isolated analgetic and anti-inflammatory polypeptide from bee venom. — *Toxicon* **20**: 317–321.

Sig A.K., Güney M., Öz Sig Ö. & Şan H. 2019: Bee venom: A medical perspective. — *Turk. J. Clin. Lab.* **10**: 414–421.

Son D.J., Lee J.W., Lee Y.H., Song H.S., Lee C.K. & Hong J.T. 2007: Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. — *Pharmacol. Ther.* **115**: 246–270.

Şirin Y., Çakir H.E., Can Z., Yildiz O. & Kolayli S. 2016: The investigation of usage of SDS-Page electrophoresis in identification of honey bee venom. — *Uludag Bee J*. **16**: 49–56.

Wehbe R., Frangieh J., Rima M., El Obeid D., Sabatier J.M. & Fajloun Z. 2019: Bee venom: Overview of main compounds and bioactivities for therapeutic interests. — *Molecules* **24**(16): 2997, 13 pp.

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