Research article

Numanoğlu Çevik, Y. and M. A. Kanat, Discrimination of two species (*Androctonus crassicauda* and *Leiurus abdullahbayrami*; Buthidae Scorpions) by MALDI-TOF MS-based PCA. International Journal of Life Sciences and Biotechnology, 2022. 5(3): p. 370-385. DOI: 10.38001/ijlsb. 1077343

Discrimination of two species (*Androctonus crassicauda* and *Leiurus abdullahbayrami*; Buthidae Scorpions) by MALDI-TOF MS-based PCA

Yasemin Numanoğlu Çevik[1]*, Mehmet Ali Kanat[1]

ABSTRACT

The venoms of the scorpions *Androctonus crassicauda* and *Leiurus abdullahbayrami*, scorpion species each of the two members of the Buthidae family, were analyzed by MALDI–TOF MS in a mass range between 1 and 50 kDa. For this, all of the scorpion venoms (n=11) were prepared to equal 2mg/mL concentration. After centrifuging the venoms at 15.000 rpm for 15 minutes at +4 ºC, the supernatants (n=11) were mixed with a matrix solution (α-CHCA) in Eppendorf tubes separately. The prepared scorpion venom-matrix (SVMx) samples vortexed. For the biomass analysis, a 1µL SVMx sample was spotted onto MALDI 96 MSP was placed in the Microflex MALDI-TOF MS. The system was operated in linear positive ion mode at a 1.000-50.000 Dalton (Da) mass range. A 60 Hz nitrogen laser was employed at 337 nm as the ion source. Interspecies differentiation was evaluated over peptide and protein molecules in this mass range. The similarities and differences between two different scorpion species were revealed with the principal component analysis study, which was conducted with spectral patterns including peptide and protein profiles. The similarity rate of the *LAB*-123 and the *LAB*-460 scorpion venoms of the same species was found as 66% while the similarity rates of venoms of the *ACR* species to the *LAB* species ranged from zero to 37%. It was demonstrated that scorpion venoms belonging to two different species from the Buthidae family can be differentiated with the help of dendrogram and gel profile, CCI color matrix, 3D or 2D-scattering profile, spectral mass loading data formed by peptide and protein spectral patterns of eleven scorpion venoms. It is anticipated that this approach, which was used for the first time with the application of MALDI-TOF MS-based PCA analysis for the differentiation of scorpion venoms, will be useful in differentiating venoms with different spectral patterns.

ARTICLE HISTORY
Received
22 February 2022
Accepted
20 May 2022

KEYWORDS
Androctonus crassicauda, *Leiurus abdullahbayrami*, Scorpion, Buthidae, MALDI-TOF MS-PCA analyse

Introduction

Scorpions are creatures that live widely all over the world, except for the polar regions and some islands. [1] It belongs to a group of arthropods whose phenotype has remained largely unchanged over the past 400 million years. The evolutionary success of these predators is largely due to their venom, which they use to deter predators and immobilize their prey. [2].

[1] Microbiology and Reference Laboratory and Biological Products Department, General Directorate of Public Health, Minister of Health, 06430, Ankara, Turkey
* Corresponding Author: Yasemin Numanoğlu Çevik cevikyasemin@yahoo.com

Scorpion venoms are a mixture of complex molecules, many of which are peptides with different biological activities. When scorpions transfer these peptides to their prey, they show neurotoxic effects by affecting ion channels in specific pharmacological target tissues [3]. The Buthidae scorpion family is the largest scorpion family, with about 96 genera and 1230 species diversity. Except for Antarctica and New Zealand, they live in all tropical, subtropical and partially temperate warm landmasses in the World. *Leiurus abdullahbayrami* (LAB), a member of the genus Leiurus [4]. It is known as the yellow scorpion, which lives endemic in Syria and Turkey (in the Southeast Anatolia region). *Androctonus crassicauda* (ACR), known as the black scorpion, is widely found in North Africa, the Middle East and Asia [5]**.** Both scorpion species have peptide structures of medical importance[3] .Analytical methods such as gel electrophoresis [6,7] and liquid chromatography [8] have already been classically used to generate venomic profiles. Most toxic peptides contain more than 100 amino acids and therefore venom screening can be performed with mass spectrometry, which makes it easy to detect [2,9,10]. In the last decade, the Matrix Assisted Laser Desorption Time of Flight (MALDI-TOF MS) method has been preferred for the analysis of peptides and proteins in venoms[8,10,11]. MALDI-TOF MS is a sensitive alternative method for biochemical and even molecular based identification approaches, since it requires fewer reagents, fewer steps [12,13]. In addition, it has emerged as a rapid, accurate, easy-to-apply and cost-effective technique for protein and peptide analysis [14]. Smith et al. established peptide profiles of some common scorpion species (*Urodacus elongatus*, *Urodacus yaschenkoi*, *Urodacus armatus* and *Lychas marmoreus obscurus*) in Australia by MALDI-TOF MS using the 1.5-diaminonaphalene matrix [15]. Schaffrath and Predel analyzed the peptide profiles of *Heterometrus cyaneus* (Vietnam) and *Buthus occitanus* in detail using MALDI-TOF MS/MS and two different matrices (2.5-dihydroxybenzoic acid; DHB and α-cyano-4-hydroxycinnamic acid; CHCA) [16].

In this study, the venoms of the scorpions *Androctonus crassicauda* and *Leiurus abdullahbayrami*, different species each other of two members of the Buthidae family and the most dangerous scorpion species, were analyzed by MALDI-TOF MS. Peptide and protein profiles of two members of Buthidae family, *Androctonus crassicauda* (n=9) and *Leiurus abdullahbayrami* (n=2), were created by MALDI-TOF MS method and it was

demonstrated that they can be differentiated from each other with the help of Principal Component Analysis (PCA).

Materials and Methods

Supply of venom, materials and preparation of venom sample

The Turkish Public Health Institute Antivenom Production Center (TPHIAPC; Ankara, Turkey) has been milking venoms from scorpions kept alive to produce antivenom routinely. For the purpose of this study, the *Androctonus crassicauda* (*ACR*, n=9) and *Leiurus abdullahbayrami* (LAB, n=2) scorpion venom samples were provided as a generous gift by TPHIAPC. The scorpion venom samples were composed of the nine different *ACR* scorpions (codes are: 47, 57, 117, 134, 168, 176, 243, 405 and 496) and two different *LAB* scorpions (codes are: 123 and 460). The MALDI matrice, α-cyano-4-hydroxycinnamic acid (CHCA), was obtained from Bruker (Germany). Acetonitrile (ACN, HPLC grade; Sigma-Aldrich), trifluoroacetic acid (TFA; Sigma-Aldrich), Ultra-pure water (UPW) with a 0.1 μm filter without DNAase and RNAase (Sigma-Aldrich) and Bruker bacterial test solution (BTS) containing Escherichia coli, RNAase, and myoglobin protein profiles were also employed.

All of the milked scorpion venoms were prepared to equal 2mg/mL concentration with UPW by measuring at 280 wavelengths with Nano Ready Touch (Life Real) instrument. Then, they were centrifuged 15.000 rpm for 15 minutes at +4 $^{\circ}$C. The supernatants were transferred to new polypropylene tubes separately. All of the scorpion venom solutions (n=11) were mixed with a matrix solution (18 mg/mL α-CHCA in a 50% ACN and 2.5% TFA mixture, 1:1; v/v) in Eppendorf tubes separately. The prepared scorpion venom-matrix (SVMx) samples vortexed at 3000 rpm for 2 minutes.

Instrumentation and analysis of scorpion venom-matrix samples

For the biomass analysis 1μL SVMx sample was spotted onto a special steel 96 micro scout plate (MSP; Bruker Daltonics). This operation was applied to every eleven SVMx samples (both ACRs and LABs) and then allowed to dry completely at room temperature. The SVMx samples loaded MALDI 96 MSP was placed in the Microflex MALDI-TOF MS (Bruker Daltonics; Bremen, Germany) device. The system was operated in linear positive ion mode at a 1.000-50.000 Dalton (Da) mass range. A 60 Hz nitrogen laser was employed at 337 nm

as the ion source. To obtain the spectra, laser pulses consisting of 40 packets of 400 were applied in the measurement of each peptide molecule. Each sample was studied in triplicate, and the highest readings were included in the analysis.

Internal quality control for MALDI-TOF MS was completed with seven peaks (m/z, 5096.39312 Da; 5381.29950 Da; 6255.88327 Da; 7274.94901Da; 10298.9927 Da; 13682.32001 Da and 16953.88117 Da) assigned with a standard deviation of 59.75 and maximum peak error of 79.20 ppm.

Mass spectrum profile and PCA analysis of scorpion venoms

The mass spectra generated from MALDI-TOF MS regarded as multivariate data, in which every mass signal represents a single molecular dimension. The mass spectrum was analyzed using the principal component analysis (PCA) supported by external MATLAB software integrated into MALDI Biyotyper software (version 3.1) [13]. Prior to further analysis based on the Biotyper pre-processing standard method (smoothing method: Savitski-Golay; baseline subtraction method; multi polygon; normalization method) was applied [17]. The PCA was based on the peaks acquired from MALDI-TOF MS to find patterns and unique peaks of the spectrum and allows the formation of clustered groups of spectra having similar variation characteristics and the visualization of the differences between them. Cluster analysis was performed by performing PCA dendrograms which represent the relationship and proximity of each spectrum to each other [12]. Accordingly, the mass spectra obtained in this study were further analysed to determine the composite correlation index (CCI) which was used to statistically determine the relationship between the acquired spectra [18].

Results

In this study, the venoms of the scorpions *Androctonus crassicauda* and *Leiurus abdullahbayrami* were analyzed by MALDI TOF MS. Peptide and protein profiles of *Androctonus crassicauda* (n=9) and *Leiurus abdullahbayrami* (n=2), were obtained by MALDI-TOF MS method. Photographs of 11 scorpion venoms studied in this study (Figure 1) and information including height and weight are presented (Table 1).

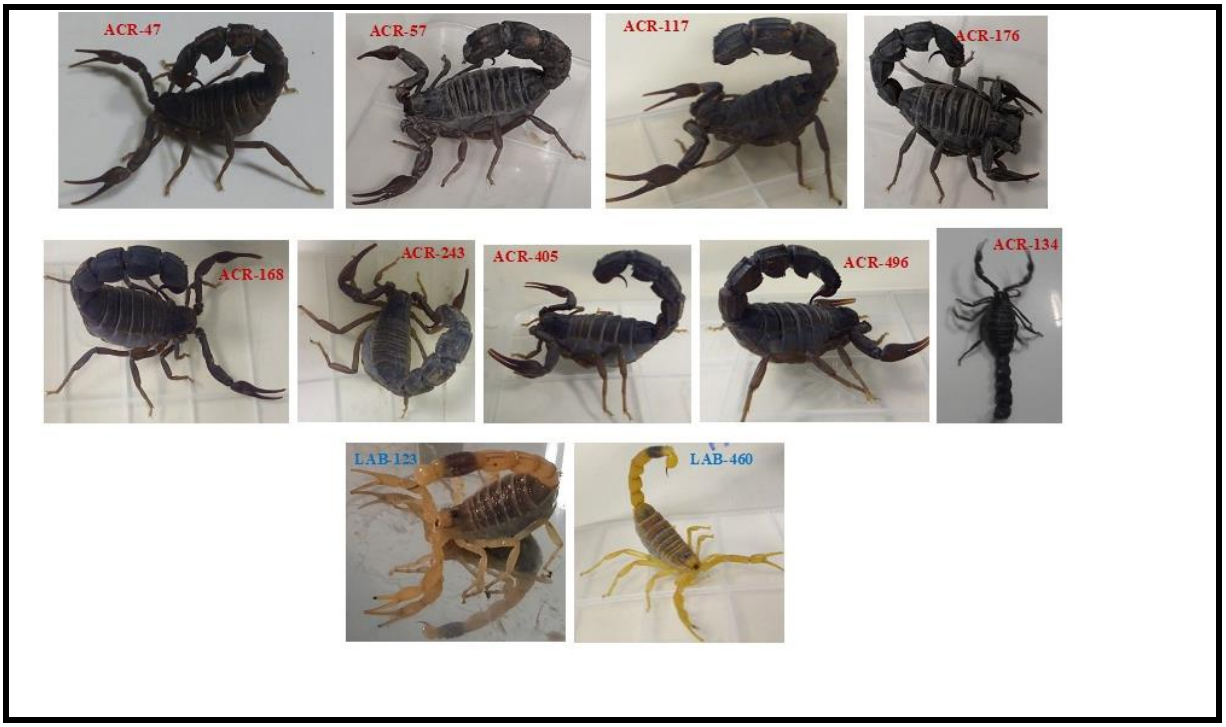
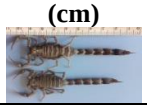


Fig 1 The photographs of nine *A. crassicauda* and two *L. abdullahbayrami* were taken by Mehmet Ali Kanat

Table 1 Information about the physical characteristics of the eleven scorpions (nine of *Androctonus crassicauda* and two of *Leiurus abdullahbayrami*)

Scorpion	***ACR*-47**	***ACR*-57**	***ACR*-117**	***ACR*-134**	***ACR*-168**	***ACR*-176**	***ACR*-243**	***ACR*-405**	***ACR*-496**	***LAB*-123**	***LAB*-460**
Weight (g)	6.18	6.77	5.90	4.82	5.36	5.85	5.41	5.73	4.7	4.15	4.68
Dimensions (cm) 	9.00	9.00	9.00	8.50	9.00	8.50	8.40	8.00	8.00	7.50	6.00

Potential marker peptides and proteins in discrimination of scorpions

In the present study, two *LAB*s (*LAB*-123 and *LAB*-460) and nine *ACR*s (codes are; 47, 57, 117, 134, 168, 176, 243, 405 and 496) scorpion venoms were analyzed by MALDI-TOF MS. Generally, three readings were performed for each SVMx sample and the analysis of eleven scorpions spectra with the highest intensity were performed. It was observed that peptide

molecules in the range from 2.10^3 to 4.10^3 Da were present in all ten scorpion venoms except *ACR*-168. In addition, in all eleven scorpion venoms, it is seen that peptide molecules are especially concentrated in the range of both from $4 .10^3$ to 6.10^3 Da and from $7 .10^3$ to $8\ 10^3$ Da. Unlike the *ACR* species, the third high-intensity peaks were detected in the range of 14300-14500 Da in both *LAB* scorpion species. On the other hand, protein peaks with high intensity from 16000 to 18000 Da, which is not found in the *LAB* scorpion species, were detected in all *ACR* scorpion venoms. Protein peaks in the range from 22000-34000 Da were observed only in four *ACR* (*ACR*-47; *ACR*-117, *ACR*-134; *ACR*-176) scorpion species (Table 2). The top three $(M+H)^+$ molecular basic peptide's/protein's peak (BPP) have high-intensity values for each scorpion venom spectra defined in Table 2. The first highest intensity $(M+H)^+$ molecular peptide/protein peak, the second-highest intensity BPP, and the third-highest intensity BPP were defined as the (1st. BPP), (2nd. BPP), and (3rd. BPP) respectively. Accordingly, while the first three BPP masses of the *LAB* species were compatible with each other, it was observed that there were differences in the scorpion venoms of the *ACR* type (Table 2).

Table 2 Peptides and proteins detected in two *LAB* and nine *ACR* scorpion venoms and their first three main peaks that have highest intensity of $(M^+H)^+$

Scorpion Sample Code	**$(M+H^+)^+$**									
	2-4 $x10^3$ Da	**4-6 $x10^3$ Da**	**7-8 $x10^3$Da**	**13-15 $x10^3$ Da**	**16-18 $x10^3$ Da**	**20-21 $x10^3$ Da**	**22-34 $x10^3$ Da**	**1st. BPP (Da)**	**2nd. BPP (Da)**	**3rd. BPP (Da)**
LAB-123	++	+	++	++	-	+ (21)	-	3583	6492	14454
LAB-460	+	+	+	++	-	-	-	3426	6488	14345
ACR-47	+	+	++	+	+	-	+ (27-28)	16936	8472	7161
ACR-57	+	+	++	-	+	-	-	3881	7211	16932
ACR-117	++	+	++	-	++	-	++ (33)	16326	8163	7154
ACR-134	+	+	++	-	++	-	++ (33)	8468	17074	8063
ACR-168	-	+	++	++	+	-	-	8463	16923	6856
ACR-176	++	+	++	++	+	-	++ (33)	16940	8474	3183
ACR-243	+	+	+	+	+	-	-	6703	8476	3610
ACR-405	++	+	++	+	+	-	-	16326	3882	6569
ACR-496	+	+	++	-	++	-	-	16930	8266	5644

(-): not available; (+): available; (++): more available

The dendrogram profile of 11 scorpion venoms of two different species is presented in Figure 2A. Gel profile showing the distribution of peptides and protein peaks of a total of eleven scorpion venoms analyzed by MALDI-TOF MS was presented in Figure 2B. Macroscopic examination revealed both common and differential peaks in the spectra of the *LAB* and the *ACR* scorpion venoms (Figure 2B). In addition, the Composite Correlation Index (CCI) color matrix (Fig. 2C) was shown in Figure 2C. When the closeness and distance percentages of all eleven scorpion venoms were evaluated based on their mass spectra, three distinct similarity matches were evident in the CCI color matrix in Figure 2C. According to this, the most similar (CCI; 81%) venoms belong to the *ACR*-134 and the *ACR*-176 scorpions which were members of the cluster-III in the dendrogram (Figure 2 A). The third scorpion venom, showing more than 70% similarity to these two scorpion venoms (Figure 2C, α-black circle), was the *ACR*-168 scorpion venom in the cluster–I in the dendrogram profile (Figure 2A).

The other member of the cluster-III the *ACR*-405 scorpion venom was most similar to the venoms of the scorpions *ACR*-134 (74%), later *ACR*-47 (68%) and finally *ACR*-176 (52%). The last member of cluster-III, the *ACR*-47 scorpion venom has been included in the cluster with a separate branch in the dendrogram profile (Figure 2A) and its similarity to the *ACR*-496 is 68% (Figure 2C; in a β-black circle) and the *ACR*-134 (CCI; 55%) scorpion venom's similarity is moderate, but it was too dissimilar to the *ACR*-176 scorpion venom with a CCI percentage below 20%.

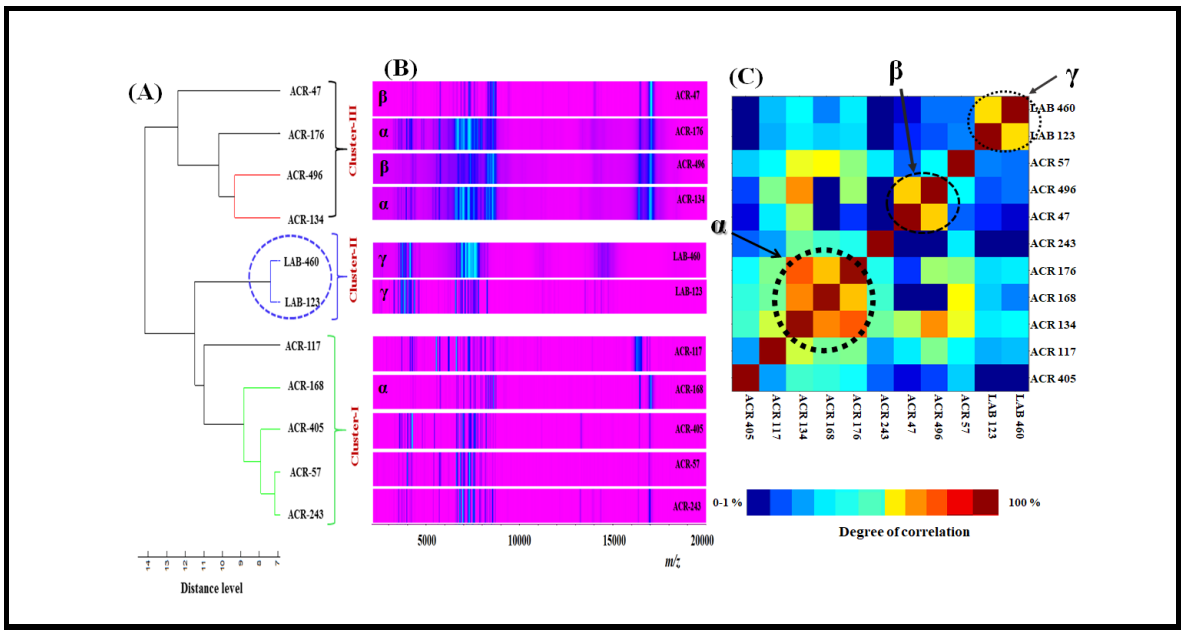


Fig 2 (A) Dendrogram profile, (B) gel profile and (C) CCI color matrix of the two *LAB* and the nine *ACR* scorpion venoms. The degree of similarity between pair mass spectra comparisons ranging from red (very similar) to blue (very dissimilar). Three scorpions (*ACR*-134, *ACR*-176 and *ACR*-496) which is best resemble each other in the big black **α**-circle (C)

The distribution of $(M+H^+)^+$ peptide and protein molecules found in scorpion venoms confirms each other with both the values in Table 2 and the spectral mass projections in the gel profile (Figure 2B). Accordingly, it is seen that both the first two BPPs in the *ACR*-134 and the *ACR*-176'scorpion venom as well as $(M+H^+)^+$ molecules in general were quite similar. Two of the three clusters formed in the dendrogram profile belong to the *ACR* scorpion venoms (cluster-I and cluster-III). It was determined that the similarity between the scorpions in the cluster-I was lower than the one in the cluster-III. The closest of each other in cluster-I are the scorpions the *ACR*-57 and the *ACR*-168 (Figure 2B, yellow color). The CCI values of the *LAB*-123 and the *LAB*-460 scorpion venoms were calculated against both each other and against the *ACR* scorpion venoms were also shown in figures 3A and 3B and 3C respectively. The similarity rate of the *LAB*-123 and the *LAB*-460 scorpion venoms of the same species was found as 66 % and the CCI color matrix matching was shown in Figure 2C (γ-black circle). In contrast, the similarity rates of venoms of the *ACR* species to the *LAB* species ranged from zero to 37 % (Figures 3B and 3C). On the other hand, when the first three main base peaks in its spectrum were compared with the other nine *ACR* scorpion

venoms and both *LAB* scorpion venoms, the peptide and protein peaks of the *ACR*-57 scorpion venom appear to partially resemble the peptide and protein profiles of both of the *LAB* scorpion venoms (Table 2). In contrast, when the CCI values of the *ACR*-57 scorpion venom were calculated according to the *LAB* scorpion species were examined, it was seen that the similarity was too low (for the *LAB* 123 is 24 % and the *LAB*-460 CCI value is 23%) (Figure 3B and 3C). It is clear that projections of LAB species of all biomarker peptides (m/z; 3426, 3583, 6488, 6492 Da) and proteins (m/z; 14345 and 14454 Da) are continuous (Figures 3D and 3E).

Figure 4 shows the 2D-scatter profile of all eleven scorpion venoms and their matching spectral $(M+H^+)^+$ mass projection values. Consistent with the dendrogram profile (Figure 2A), cluster formations are similar in the 2D-scattering profile. It can be seen that *LAB* scorpion venoms (cluster II) are very clearly separated from cluster-I with 20% variance (PC2) (Figure 3A). In the PCA scattering profile, each dot represents the spectrum of scorpion venoms, while in the spectral loading projection, each dot represents a peptide or protein $(M+H^+)^+$ molecule. The high-intensity peptide molecules (m/z; 3582-4310 Da and 6492-6500 Da) found in the spectra of the *LAB*-123 and the *LAB*-460 matched their projections in the 2D-scattering profile, confirming its position. A similar situation is seen in Figure 4B, where the projections of high-intensity peptide and protein molecules of the *ACR* scorpion venoms (cluster-I and cluster-III) are consistent with their positions in Figure 4A.

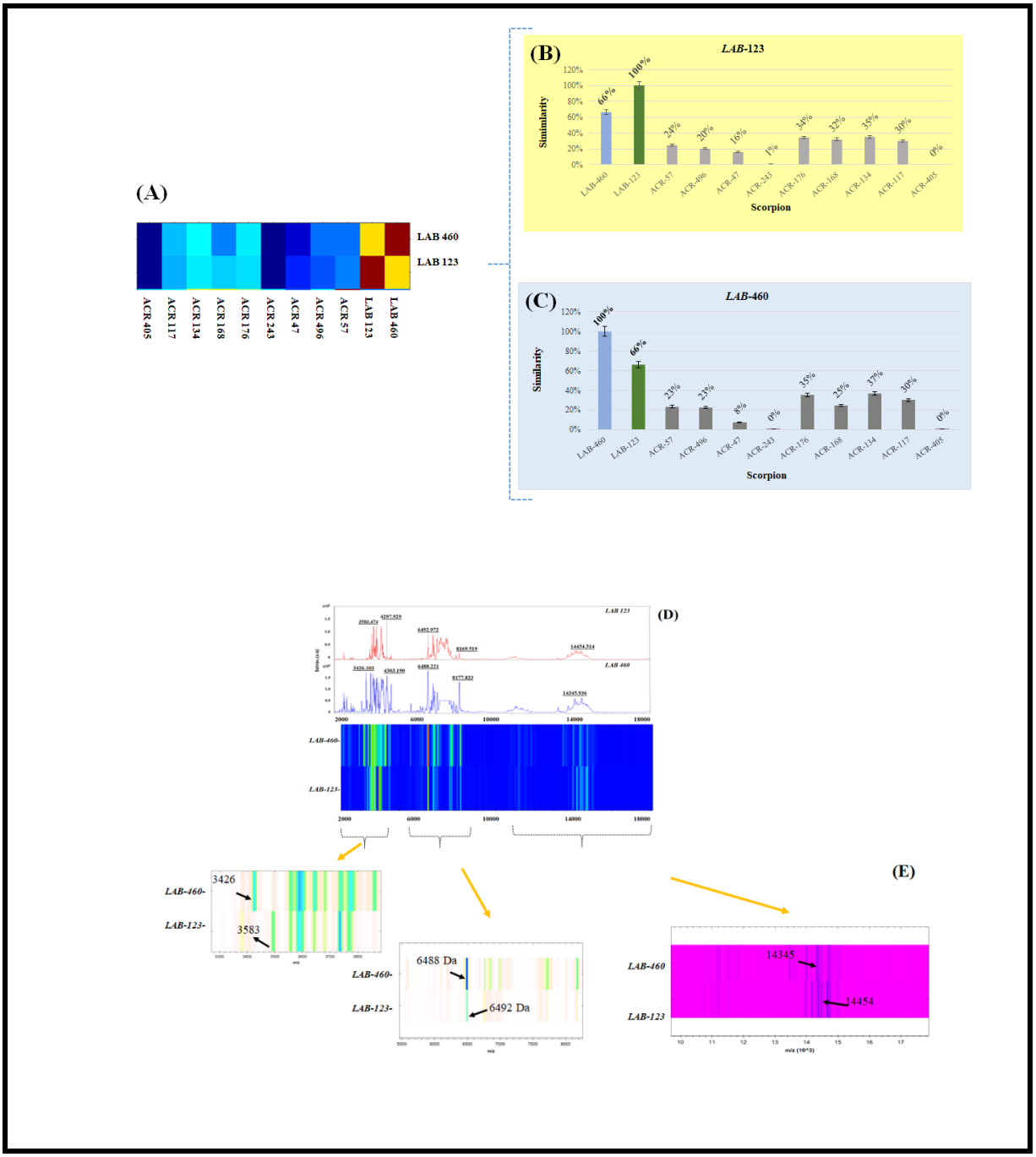


Fig 3 The CCI color matrix (A) and similarity graph for the *LAB*-123 (B) and the *LAB*-460 (C) scorpion venoms for both the nine *ACR* scorpion venoms and for the *LAB* strains. (D) Representative of whole venom MALDI-TOF MS spectra in the range from 2000 to 18 000 *m/z* and spectral gel images of the *LAB*-123 and the *LAB*-460 in the range from 2000 to 18 000 m/z. (E) The three of the BPPs of two *LAB*s scorpion venom gel profiles were detailed

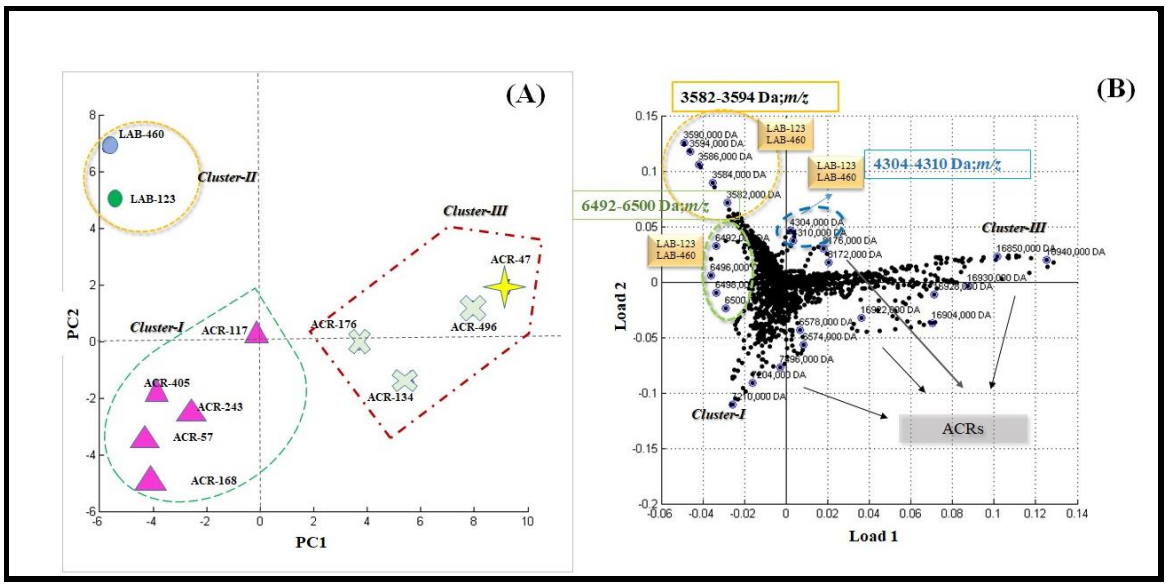


Fig 4 (A) The 2D scatter profile (B) and spectral mass loading projections of two *LAB* and nine *ACR* scorpion venoms. Each spot (triangle, ✕ and star) represents one spectrum. The black plot represents $(M+H^+)^+$ peptide or protein peak. Both profiles were generated by PCA. The three of BPPs' of two *LAB* scorpion venoms (m/z; 3426, 3583, 6488, 6492, 14345, 14454 Da) were clearly visible in both spectra and gel profile

The 3880±5 Da $(M+H^+)^+$ molecule was high in abundance in the *ACR*-57, the *ACR*-134, the *ACR*-176, and the *ACR*-405 scorpion venom (Figure 5A), while low in the *ACR*-168, the *ACR*-243 scorpion venom was not available in the *LAB*-123, the *LAB*-460, the *ACR*-47. The 6725±5 Da $(M+H^+)^+$ peptide molecule was also present in other nine scorpion venoms except for the *ACR*-176 and the *ACR*-405. The 7210±10 Da peptide molecule was present in all eleven scorpion venoms. The 8170±10 Da peptide was predominant in the *LAB*-123, the *LAB*-460, the *ACR*-176, the *ACR*-405 and the *ACR*-496, low abundance in the *ACR*-47 and the *ACR*-168, while other *ACR*s (57, 117, 134) scorpion venoms did not have this peptide molecule. When we focused on protein molecules (above 10 000Da), protein peaks between 14000-14500 Da were observed in both LAB-123 and LAB-460 scorpion venoms. In contrast, protein molecules were found in high abundance in all *ACR* scorpion venoms, particularly between 16,300 and 16,999 Da (Fig. 5C). These protein peaks were observed as the most significant difference between *ACR* and *LAB* poisons. This difference was also clearly seen in the gel profile in Figure 3A. In addition, the projections of the first 3 BPPs of the *LAB* scorpion venoms in their spectra and gel profile were shown in Figure 3E in detail.

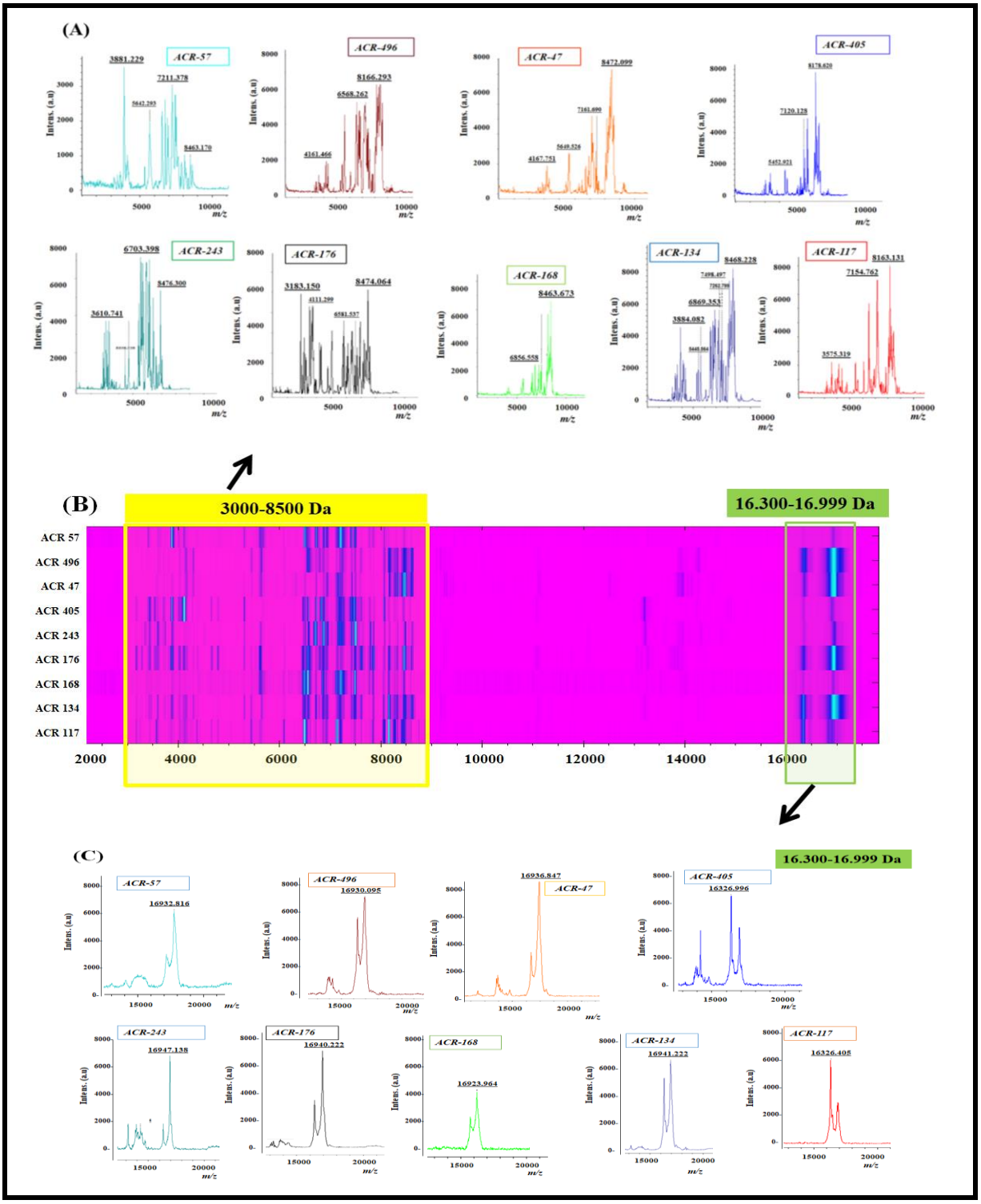


Fig 5 (A) Representative the *ACR* scorpion venoms MALDI-TOF MS spectra mass ranging from 3000-8500 Da and (C) mass ranging from 16.300-16.999 Da (B) Spectral gel images of the nine *ACR* scorpion venoms separately

Discussion

MALDI-TOF mass spectrometry has been preferred for many years as a powerful tool for precise molecular mass mapping of complex peptide mixtures [18]. In this study, a mass range between 1 kDa and 50 kDa was studied. Interspecies differentiation was evaluated over peptide and protein molecules in this mass range. Among the eleven Buthidae member scorpion venoms, *LAB*-460 had the lowest mass of peptide molecules (2029 Da), while the protein molecules with the highest mass value m/z, 33938 Da for *ACR*-176 and 33976 Da for *ACR*-134 scorpion venoms were found. While the protein peaks between 14000-14500 Da were observed in the *LAB* scorpion venoms, the protein molecules were found in high abundance in all *ACR* scorpion venoms, particularly between 16300 and 16999 Da). These protein peaks were observed as the most significant difference between the *ACR* and the *LAB* venoms. This difference was also clearly seen in the gel profile.

The peptide toxin molecules found in the venom of scorpions exert neurotoxic effects on the cells of their prey. Small compounds consisting of short polypeptide chains (*m/z*, 3000-4300 Da) that have been shown to be effective on potassium channels are present in the venom content, albeit in small amounts. On the other hand, peptide toxins known as the main components of scorpion venoms and having a relatively large molecular mass (6000-8000 Da) are active on sodium channels and are abundant [19]. As expected, roughly detected the short polypeptide chains were observed to be less than large molecular mass proteins in the eleven scorpion venoms we studied.

In this study, it was demonstrated that scorpion venoms belonging to two different species from the Buthidae family can be differentiated with the help of dendrogram and gel profile, CCI color matrix, 3D or 2D-scattering profile, spectral mass loading data formed by peptide and protein spectral patterns of eleven scorpion venoms. It is anticipated that this approach, which was used for the first time with the application of MALDI TOF MS-based PCA analysis for the differentiation of scorpion venoms, will be useful in differentiating venoms with different spectral patterns.

In the literature, very valuable analytical studies have been made and continue to be done on both species (*ACR* and *LAB*) belonging to the Buthidae family [20–22]. The contribution of

each study to this field is very important and valuable. For example, microfluidic capillary electrophoresis and LC-ESI-TOF-MS by Erdes et al. performed an extensive analysis of the *L. abdullahbayrami* scorpion venom. According to their results, a total of 45 peptide masses were identified in the *L. abdullahbayrami* scorpion venom[22]. When the results of Erdes et al.'s study and our results were compared, it was observed that 3590 Da peptide was present in both *LAB* (123 and 460) scorpion venoms, among the mass values they detected in *L. abdullahbayrami* scorpion venoms.

Conclusion

This study demonstrated the peptide and protein profiles of some Buthidae family's scorpions by using MALDI-TOF MS for the first time. In addition, using MALDI-TOF mass fingerprint data with multivariate PCA analysis, *Androctonus crassicauda* species and *Leiurus abdullahbayrami* species were discriminated. Using the peptide and protein profiles of scorpion venoms with a fast, practical and low-cost device such as MALDI-TOF MS, revealing the dramatic differences between different species and within the same species will make a very important contribution to this field. Beyond identification, analytical evaluation of the spectral patterns of each species was also performed with PCA-based data and a series of data analyses (dendrogram, clustering, CCI and spectral loading). This comprehensive study, in which this approach has been evaluated for the first time, is open to further development, supported by further analysis and spectral improvement studies. It is also anticipated that the use of mixtures of different scorpions, whose peptide and protein profiles are defined by MALDI-TOF MS, will contribute to more effective antivenom production by using them in the production of scorpion antivenom.

Data availability

The authors confirm that the data suppoting the findings of this study are available within the article.

Geolocation information

The scorpions were collected from Şanlıurfa province (GCP coordinates of 39° 56′ 0.109” N and 32° 51′ 35.07” E) which is located in the eastern part of Turkey. This study was done in Ankara which is the capital of Turkey with GPS coordinates of 39° 55′ 28.1280"N and 32° 51′ 16.6788" E.

Acknowledgments

The scorpions of the *A. crassicauda* and *L. abdullahbayrami* species were collected from Şanlıurfa province in the eastern part of Turkey for the purpose of antivenom production by Turkish Public Health Institute Antivenom Production Center. We are very grateful to the Turkish Public Health Institute Antivenom Production Center for gifting scorpion venoms.

References

1. Rincón-Cortés, et.al., Structural and Functional Characterization of Toxic Peptides Purified from The Venom of The Colombian Scorpion Tityus Macrochirus. Toxicon, 2019. 169: p.5–11. https://doi.org/10.1016/j.toxicon.2019.07.013.
2. Schaffrath, S., and Predel, R.A., Simple Protocol for Venom Peptide Barcoding in Scorpions. EuPA Open Proteomics. 2014. 3: p. 239–245. https://doi.org/10.1016/j.euprot.2014.02.017.
3. Oukkache, N. et.al., New Analysis of The Toxic Compounds from The Androctonus Mauretanicus Mauretanicus Scorpion Venom. Toxicon, 2008. 51: p. 835–852. https://doi.org/10.1016/j.toxicon.2007.12.012.
4. Yağmur, E.A, Koç, H., and Kunt, K.B., Description of A New Species of Leiurus Ehrenberg, 1828 (Scorpiones: Buthidae) from Southeastern Turkey. Euscorpius, 2009: p.1–20. https://doi.org/10.18590/euscorpius.2009.vol2009.iss85.1.
5. Ozkan, O., and Alcigir, M.E., A Comparative Pathomorphological Findings between Leiurus abdullahbayrami and Androctonus crassicauda (Scorpion: Buthidae) envenomation in rabbit animal model. J. Arthropod. Borne Disease, 2019. 13: p. 104–115. https://doi.org/10.18502/jad.v13i1.937.
6. Borges, A., and Rojas-Runjaic, F.J.M., Tityus Perijanensis González-Sponga (Scorpiones, Buthidae): Molecular Assessment of Its Geographical Distribution and Venom Lethality of Venezuelan Populations. Toxicon, 2007. 50: p. 1005–1010. https://doi.org/10.1016/j.toxicon.2007.07.019.
7. Estrada-Gómez, S. et.al., MS/MS Analysis of Four Scorpion Venoms from Colombia: A descriptive approach. J. Venom. Anim. Toxins Incl. Tropical Disease, 2021. 27: p. 1–13. https://doi.org/10.1590/1678-9199-JVATITD-2020-0173.
8. Bringans, S. et.al., Proteomic analysis of the venom of Heterometrus longimanus (Asian black scorpion). Proteomics, 2008. 8: p. 1081–1096. https://doi.org/10.1002/pmic.200700948.
9. Numanoğlu Çevik, Y. et.al., Salih, B. Identification of Snake Venoms According to their Protein Content Using the MALDI-TOF-MS Method. Anal. Chemistry Letters, 2021. 11: p. 153–167. https://doi.org/10.1080/22297928.2021.1894974.
10. Martin-Eauclaire, M. F et.al., Achieving Automated Scorpion Venom Mass Fingerprinting (VMF) in The Nanogram Range.Toxicon, 2013. 69: p. 211–218. https://doi.org/10.1016/j.toxicon.2013.03.001.
11. Favreau, P. et.al., Mass Spectrometry Strategies for Venom Mapping and Peptide Sequencing from Crude Venoms: Case Applications with Single Arthropod Specimen. Toxicon, 2006. 47: p. 676–687. https://doi.org/10.1016/j.toxicon.2006.01.020.
12. Cheng, K. et.al., Recent development of mass spectrometry and proteomics applications in identification and typing of bacteria. Proteomics- Clin. Application, 2016. 10(4): p. 346-357. https://doi.org/10.1002/prca.201500086.
13. Taban, B.M., and Y. Numanoglu Cevik, The Efficiency of MALDI-TOF MS Method in Detecting Staphylococcus Aureus Isolated from Raw Milk and Artisanal Dairy Foods. CyTA-J. Food, 2021. 19: p. 739–750. https://doi.org/10.1080/19476337.2021.1977392.
14. Sauget, M., et.al., Can MALDI-TOF Mass Spectrometry Reasonably Type Bacteria? Trends Microbiology, 2017. 25: p. 447–455. https://doi.org/10.1016/j.tim.2016.12.006.
15. Smith, J.J, Jones, A., and Alewood, P.F., Mass Landscapes of Seven Scorpion Species: The First Analyses of Australian Species with 1,5-DAN matrix., J. Venom Researche, 2012. 3: p. 7–14.
16. Schaffrath, S. and Predel, R., A Simple Protocol for Venom Peptide Barcoding in Scorpions. EuPA Open Proteomics, 2014. 3: p. 239–245. https://doi.org/10.1016/j.euprot.2014.02.017.
17. Jeong, Y.S., Lee, J., and Kim, S.J. Discrimination of Bacillus Anthracis Spores by Direct in-situ Analysis of Matrix-Assisted Laser Desorption/İonization Time-of-Flight Mass Spectrometry, Bull. Korean Chemical Society, 2013. 34: p. 2635–2639. https://doi.org/10.5012/bkcs.2013.34.9.2635.

18. Samad, R.A, and Al Disi, Z., The Use of Principle Component Analysis and MALDI-TOF MS for The Differentiation of Mineral Forming Virgibacillus and Bacillus Species Isolated from Sabkhas. R. Soc. Chemistry, 2020. 10: p. 14606–14616. https://doi.org/10.1039/d0ra01229g.
19. Romi-Lebrun, R. et.al., Characterization of Four Toxins from Buthus Martensi Scorpion Venom, Which Act on Apamin-Sensitive Ca2+-Activated K+ Channels. Eur. J. Biochemistry, 1997. 245: p. 457–64. https://doi.org/10.1111/j.1432-1033.1997.00457.x.
20. Caliskan, F. et.al., Characterization of Venom Components from The Scorpion Androctonus Crassicauda of Turkey: Peptides and genes. Toxicon, 2006. 48: p. 12–22. https://doi.org/10.1016/j.toxicon.2006.04.003.
21. Caliskan, F. Scorpion Venoms, Springer Netherlands, Dordrecht, 2015. Section:14 p: 327-349. https://doi.org/10.1007/978-94-007-6404-0.
22. Erdeş, E. et.al., Characterization of Leiurus abdullahbayrami (Scorpiones: Buthidae) venom: peptide profile, cytotoxicity and antimicrobial activity, J. Venom. Anim. Toxins Incl. Trop. Dis., 20. 2014: p. 48. https://doi.org/10.1186/1678-9199-20-48.