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VARIATION OF VENOM AND THORACIC MUSCLE PROTEINS OF *VESPA ORIENTALIS* POPULATIONS IN RELATION TO GEOGRAPHICAL ISOLATION IN SOUTHERN SINAI PROTECTORATES, EGYPT

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ABSTRACT

The oriental hornet *Vespa orientalis* was surveyed in three Wadi systems in South Sinai protectorates (Wadi Mandar in Saint Katherine protectorate, Wadi Kheriza and Wadi El-Kid in Nabq protectorate) during June and July 2005. Such survey was operated to look at the variation within and among individuals in these sites using venom and thoracic muscle proteins. SDS-Polyacrylamide gel electrophoresis [PAGE] was used for the separation of protein sub-units and determination of the protein molecular weights. A total of 82 polymorphic bands were detected with molecular weights ranging between 375 to 6 KD from 72 individual wasps collected from these three localities. The data matrix of proteins pattern of venom and its related UPGMA-based dendrogram showed that there were some variations in the number and position of bands on the scale of individual comparison. However, these variations were slightly detected when comparing the different localities and sub-localities of the studied Wadis. On the other hand, the data matrix of proteins pattern of muscles and its related UPGMA-based dendrogram showed very low variation either within or between sites. It is proposed that although the three Wadis are considered as narrow Wadi beds bounded by high mountains, these mountains are not high enough to be considered as untraversing barriers for the wasps that are characterized by a very high mobility of flying which may enable them to cross the ridged mountain barriers and moving across the adjacent Wadi systems.

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Keywords: *Vespa orientalis*, venom, thoracic muscles, protein pattern, dendrogram, Southern Sinai protectorates.

INTRODUCTION

Vespa orientalis Linneaus, (1771) is a common and a wide spread species of hornets found throughout North Africa, Southeastern Europe and Southwestern Asia (Archer, 1998). The *Vespa orientalis* has different species allover the world, but according to its distribution in Egypt, only one species is recorded: *Vespa orientalis* (El-Morsy and Kamal, 1996). Such peculiarity makes this insect species a unique model for studying the variations between its individuals as it is considered an isolated species (Archer, 1998).

Vespa sting is very painful, where they have smooth stinger that can be used multiple times. Human may die from *Vespa* stings when exposed to large number of stings at the same time. Some people suffer from several allergic reactions to the inflammatory substances in the *Vespa* venom which in severe cases may cause sudden drop in blood pressure and lose consciousness (Hoffman, 1993). As scavengers, *Vespa* wasps can also transmit a number of serious diseases, where after feeding on garbage, they can feed on fruits and other human food and through this way they transport a considerable number of microbes. On the other hand, *Vespa* have some harmful effects on plants and crops through transmitting fungi and bacteria from infected to healthy plants. In addition, they cause deep cuts in the fruits by their chewing mouth parts serving fungi to invade these wounds. Finally, Vespa cause profound problems to the honeybees by attacking their hives, feeding on flower nectar and transmitting diseases from infected nest to another (Hagag and Abou Zeid, 1999).

Gel electrophoresis of protein is a widely used technique in insect molecular studies. The technique relies on the fact that identical proteins migrate the same distance under the electrical force applied to an electrophoretic gel, while non-identical proteins usually migrate different distances (Berlocher, 1984). The protein SDS-PAGE method is a reliable tool for identification, characterization and detection of the level of inter-population variation of the same investigated species (Wolff *et al*., 1997; El-Akkad and Ali, 2002).

The importance of the protein composition of the venom and its relationship with the physiological ecology of the insects, make the venom protein composition closely connected with the evolutionary aspects of phylogenetic differentiation (Leluk *et al.,* 1989). Venom protein analysis is a rich source of information of a biological function of the insect venom (Banks and Shipolini, 1986). Hymenopterans venom composition and the anatomy of their venom apparatus often correlates with their behavior (Edison, *et al*., 1982). In addition, insect venoms previously found little direct use in modern medicine, biochemistry and pharmacology, but such situation is now rapidly changing as more information is becoming available. As new techniques for isolating, identifying and especially for mass-producing individual venom components are developed, the above uses and roles of venoms will certainly increase (Zalat *et al.,* 2002).

The present study aims at providing basic information on the variation in protein composition of venom and thoracic muscles of the oriental hornet, *Vespa orientalis,* according to insect's habitat and behavior in different localities in Southern Sinai protectorates.

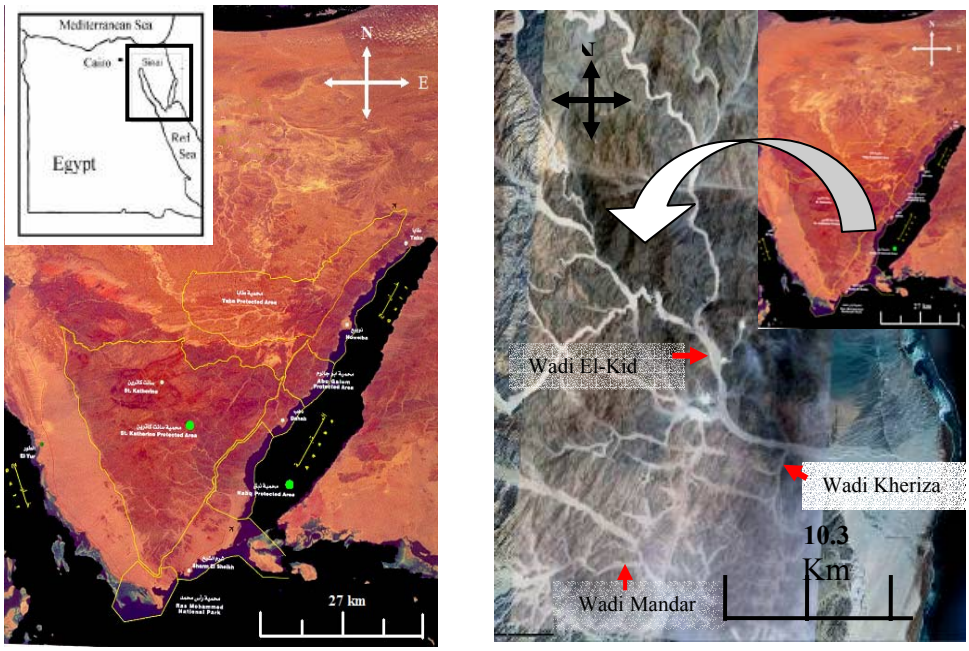
MATERIALS AND METHODS

Sample collection

During June and July 2005, samples of the oriental hornets *Vespa orientalis* were assembled from three different Wadis in Southern Sinai (Wadi Mandar, Wadi Kheriza and Wadi El-Kid) representing the mountainous environments of the protectorate which consists of different drainage systems; each made up of a number of connected Wadis. The distance between Wadi Mandar and Wadi Kheriza is about 11.2 km, while the distance between Wadi Kheriza and Wadi El-Kid is presumably 9.7 km and that separating Wadi El-Kid from Wadi Mandar was measured as 14.4 km (Fig. 1). Each locality was divided into 3 sites; the entrance, the middle and the end of the locality. A total number of 72 individuals of *Vespa orientalis* were used to extract their venom reservoirs and thoracic muscles (8 individuals were taken from each site) and both venom and thoracic muscles were analyzed.

The wasps were collected using a standard sweeping net. Trapped samples were transferred in plastic bottles, labeled with locality, site, date and time of collection and then kept in an ice box during the

remaining field work. In the laboratory, samples were preserved separately in a deep freezer with their own labels. Frozen insects were thawed; and then with a fine forceps, the venom reservoir is pulled out of the wasp's gaster and placed in an eppindorf tube containing a droplet of deionized water. All fats and other tissues were removed from the reservoir sting apparatus. The chitin of the thorax was dissected after eliminating all the insects' appendages and the thoracic muscles were removed with a fine-tipped forceps and preserved in the deep freezer.



A **B**

Figure (1): A- The National Protectorates of Southern Sinai satellite map showing both Saint Katherine and Nabq Protectorates. **B-** Satellite maps showing the two localities selected in Nabq Protectorate (Wadi Kheriza and Wadi El-Kid) in addition to Saint Katherine Protectorate site (Wadi Mandar).

Separation of venom and thoracic muscle proteins

Polyacrylamide gel electrophoresis [PAGE] was used for the separation of protein sub-units and determination of the protein molecular weights. The PAGE technique described by Hames (1990) for the discontinuous buffer system was followed in the present work.

Once fingerprint patterns have been generated and scored, the bands are assigned to specific positions in lanes to be compared. Different strategies might be followed to quantify the pair-wise similarity of the polypeptides represented in the different lanes. The gel was photographed by digital camera adjusted on the macron magnification and the evaluation of protein profiles was done visually besides the using of the Gel Documentation Advanced System program.

The characters used are the bands obtained from the biochemical analysis of venom and muscles proteins. Each number at a node or branching represents a hypothetical separated group and the number on the lines correspond to the characters were tabulated in data matrix tables. The resulting character table was analyzed using the MVSA package, and from these results, the dendrograms were constructed.

The data matrix tables built on the basis of the presence and absence of bands were used to conduct a neighbor-joining tree to the venom and muscles proteins, using Manhattan distance computed between all individuals. Thus, the relationship among these individuals could be inferred and concluded from the UPGMA-based dendrograms which group the more genetically similar individuals into separate clusters.

RESULTS

Venom proteins

The electrophoretic protein pattern, obtained from the venom of the selected individuals of *Vespa* collected from the three different wadis, is shown in figure (2). The obtained protein pattern consisted of 48 polymorphic bands with the range of molecular weight from 320 to 6 KD. The data matrix based on protein pattern of venom shows that there are some variations in the number and position of bands on the scale of individual comparison; however such variation is slightly detected when comparing the different localities and sub-localities.

It is evident from the constructed dendrograms that all individuals of the different wadis are genetically similar regardless to the site from which they were collected except for some single cases. The individuals from different sites tend to cluster together regardless to the site from which they were collected. Such similarity is very clear from the obtained dendrogram of venom (Fig. 3), which delineated 4

different clusters. Cluster (1) included the closely related individuals nearly representing the three studied wadis which are all wasps of the 3 sites of Wadi Mandar except M10, M11, M18, M20 and M21; together with the 3 sites of Wadi El-Kid except K1, K3, K4, K6, K11, K17, K18, K20 and K21; in addition to only two sites of Wadi Kheriza (site 1 and site 3) except Kh6 and all individuals of site 2. Cluster (2) included the individuals of M10, M20, K1, K3, Kh8, Kh9, Kh10, Kh11, Kh12, and Kh13, and Kh14 that are related to each other and joined to other individuals of cluster (1). The individuals of M11, M21, M18, K4, K6 and K11 were clustered within one group-comprising cluster (3). Finally cluster (4) contained the remaining individuals, which are Kh6, K17, K18, K20, and K21. The dendrogram also reveals that wasps with the symbols K18, K17 and M20 are key individuals from which the main branches of the neighbor-joining tree originated.

Thoracic muscles proteins

The electrophoretic pattern protein, obtained from the thoracic muscles of the same individuals of *Vespa* subjected to venom proteins analysis is shown in figure (4). The number of bands obtained from thoracic muscles was the same as the venom (48 polymorphic bands) with molecular weight ranging from 375 to 6 KD, whereas, 13 common bands have been detected to share between venom and thoracic muscles with molecular weights: 97, 44, 38, 33, 31, 30, 28, 27, 25, 22, 20, 18 and 6 KD. The data matrix based on protein pattern of thoracic muscles shows very low variation either between individuals or localities, within or between sites.

The muscle dendrograms consists of two major groups, one comprising M6, M7, M9,K4, K5, K6, K12, K16, Kh7, Kh9, and Kh20, while the other included all the remaining individuals. Conclusively, a mixed dendrogram between both venom and muscles was constructed to make an overview on all the studied variations between wasps of all sites (Fig. 5). The dendrogram delineated four main clusters included the individuals M5, M6, K3, K4, K5, K15 and K 16 (cluster 1); M18, Kh16, Kh18, Kh19 (cluster 2); M11, M21, K6, K11, K17, K18, K20, K21, and Kh6 (cluster 3), while all the remaining individuals in cluster (4). From this dendrogram, five wasps appear to be the key individuals from which the main branches of the neighbor-joining tree originated. These samples are: M18, M20, Kh16, Kh18 and Kh19.

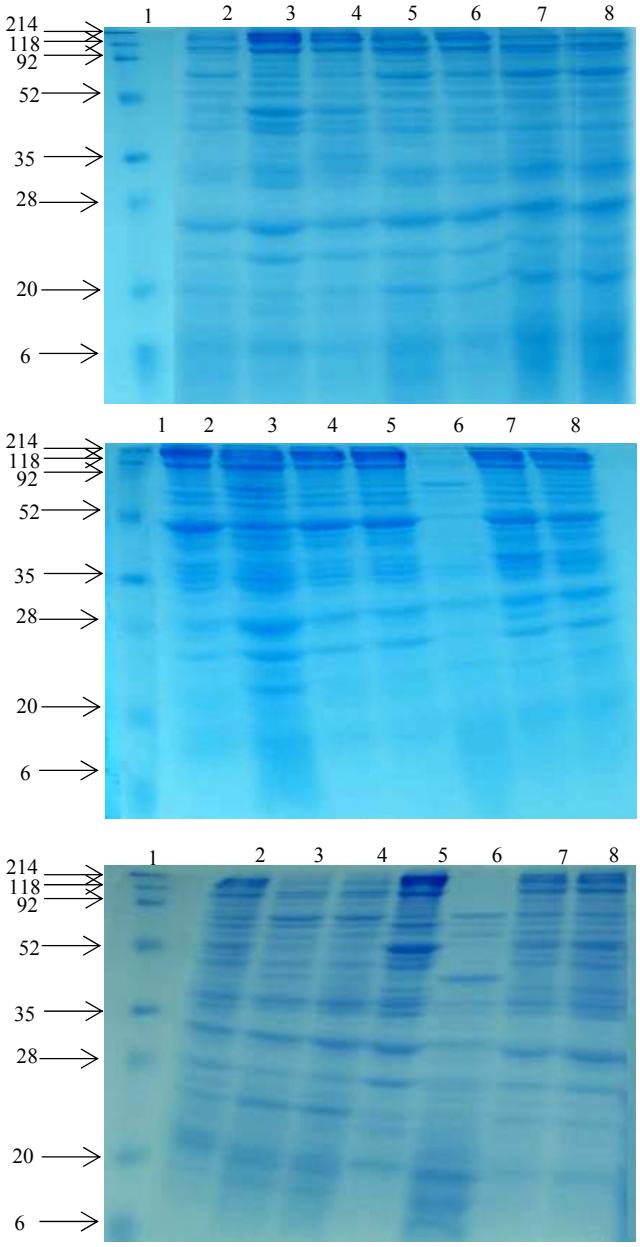


Figure (2): Electrophoresis protein pattern of the venom of the selected individuals of *Vespa* of the three localities: (A) Wadi Mandar, (B) Wadi Kheriza and (C) Wadi El-Kid. Lane (1): High molecular weight standard (from top to bottom), 214, 118, 92, 52, 35, 28, 20 and 6 KD. Lanes (2-8): The protein bands of analyzed individuals.

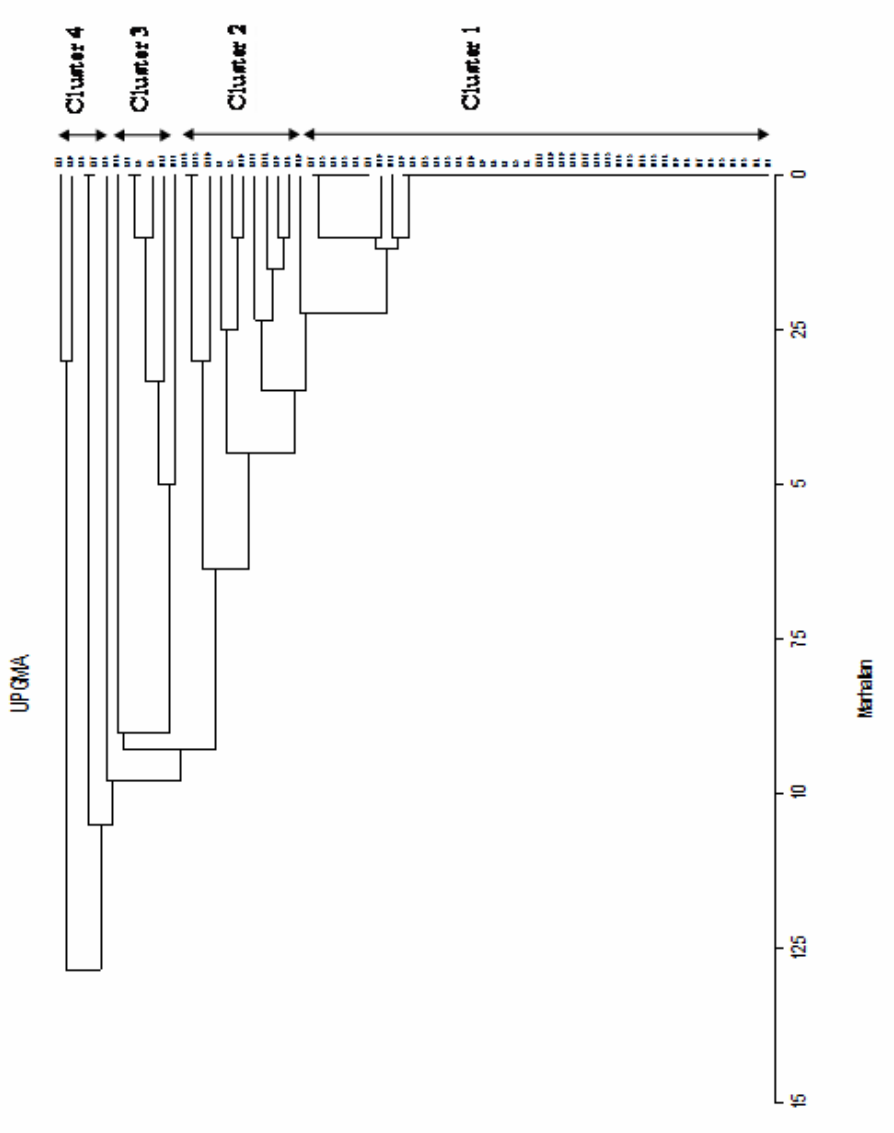


Figure (3): Dendrogram showing the variation of the population of *Vespa orientalis* according to venom proteins analysis in three different wadis in St. Katherine and Nabq Protectorates. M = Individuals of Wadi Mandar, Kh = Individuals of Wadi Kheriza, K = Individuals of Wadi El-Kid.

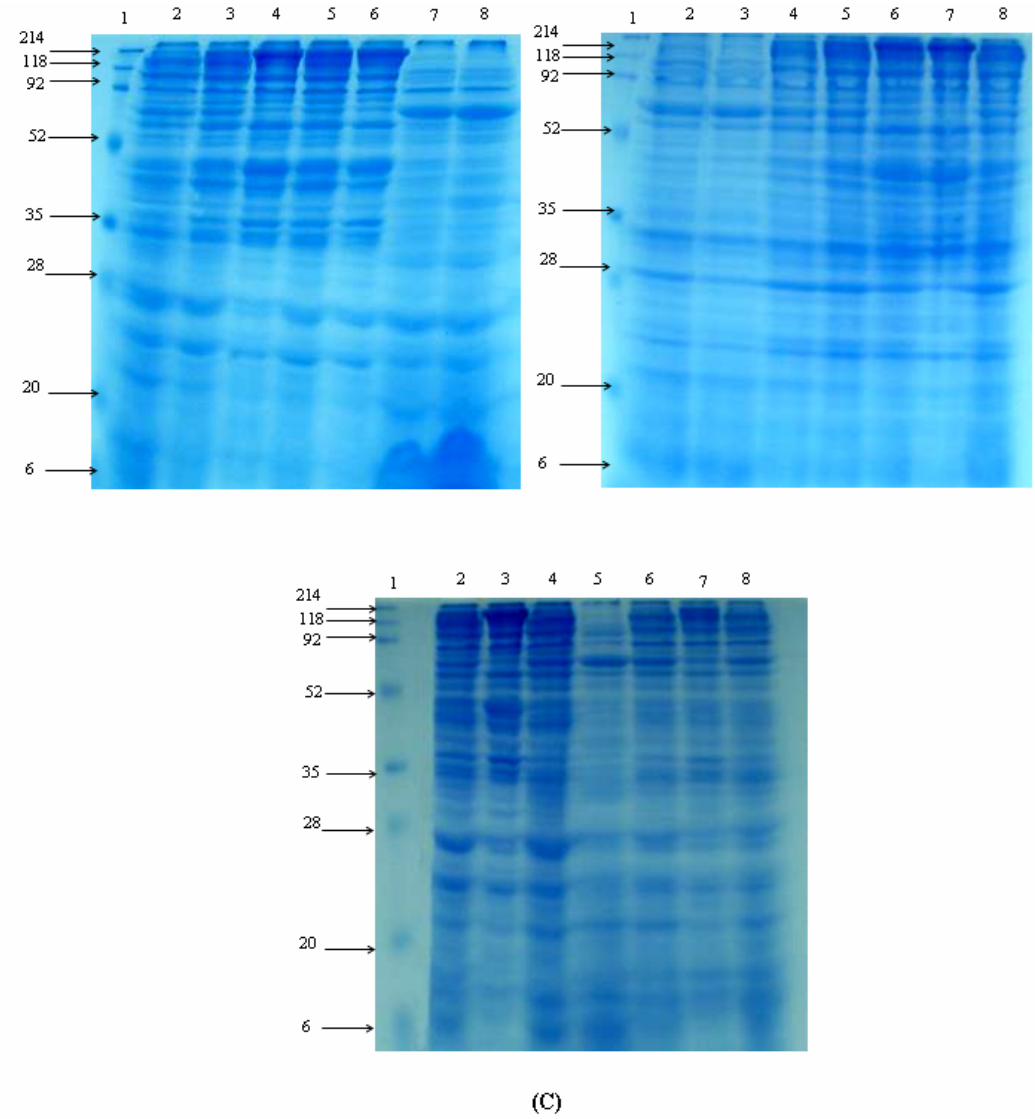


Figure (4): Electrophoresis protein pattern of the thoracic muscles of the selected individuals of *Vespa* of the three localities: (A) Wadi Mandar, (B) Wadi Kheriza and (C) Wadi El-Kid. Lane (1): High molecular weight standard (from top to bottom), 214, 118, 92, 52, 35, 28, 20 and 6 KD. Lane (2-8): The protein bands of analyzed individuals.

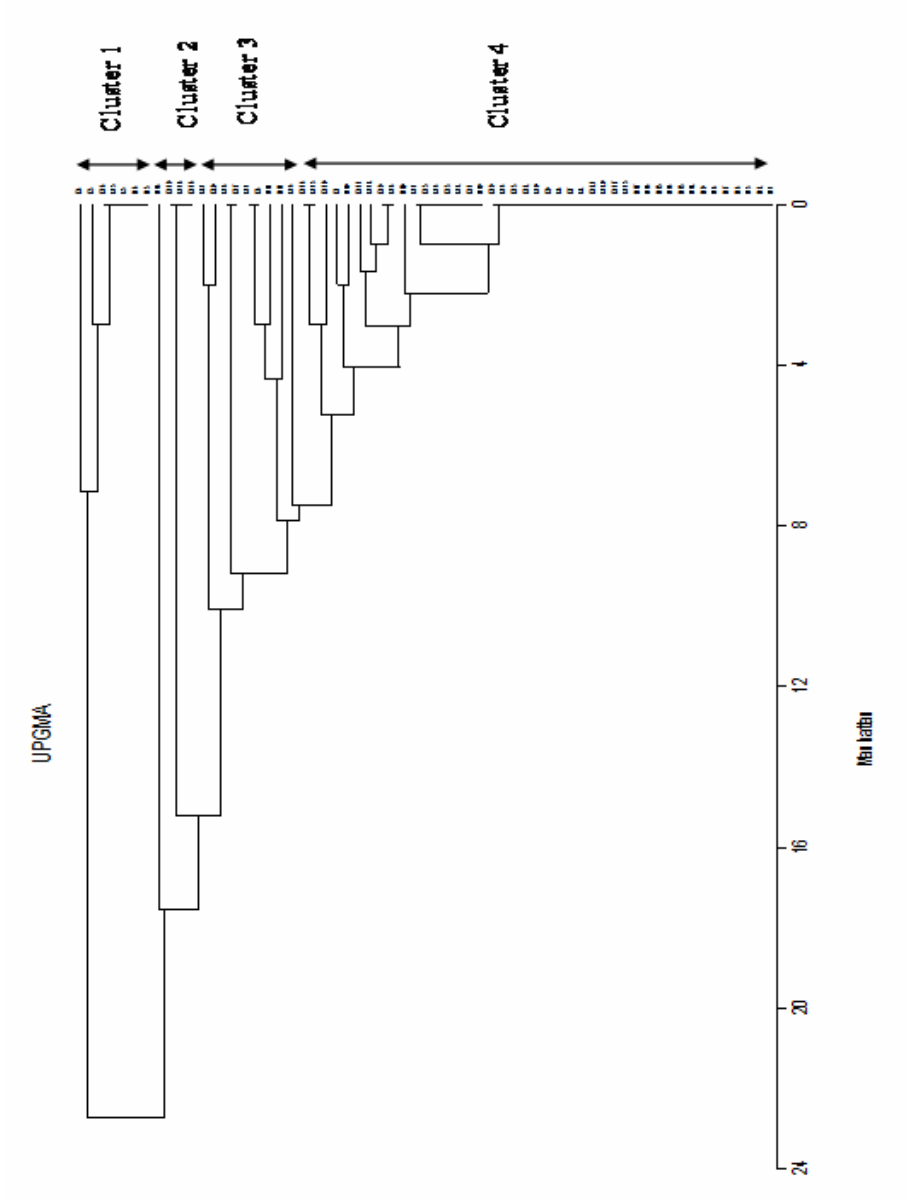


Figure (5): Dendrogram showing the variation of the population of *Vespa orientalis* according to both venom and muscle proteins analysis in three different Wadis in St. Katherine and Nabq Protectorates.

Table (1): Data matrix for dendrogram analysis of venom of *Vespa orientalis*

Sites		individuals	Characters (Venom protein patterns)
Wadi Mandar	Site 1	M1	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M2	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M3	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M4	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M5	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M6	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M7	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
	Site 2	M8	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M9	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M10	0 1 0 1 0 0 0 0 0 0 0 0 1 0 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 0 0 1 0 1 1 1 1 1 1
		M11	0 1 0 1 0 0 0 0 0 0 0 0 1 0 1 0 0 0 1 1 0 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1
		M12	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M13	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M14	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
	Site 3	M15	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M16	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M17	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M18	0 1 0 1 0 0 0 0 0 0 0 0 1 1 0 1 0 0 1 1 1 0 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 0 1 1 1 1 1
		M19	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 1 0 1 0 1 0 1 1 1 1 1 1
		M20	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 1 0 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		M21	0 1 0 1 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 1 0 1 1 1 1 0 1 0 1 1 1 1 1 1 1 0 1 1 1 1 1 1 0 0 1 1 1 1 1
Wadi Kheresa	Site 1	Kh1	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh2	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh3	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh4	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh5	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh6	1 1 0 1 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 1 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 0 1 0 1 0 0 0 1 1 1 1 1 1 1
		Kh7	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
	Site 2	Kh8	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 0 1 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh9	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 0 1 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh10	0 1 0 1 0 0 0 1 0 1 0 0 1 1 0 1 1 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 1 0 0 1 1 1 1 1 1
		Kh11	0 1 0 1 0 0 0 0 0 1 0 0 1 1 0 0 1 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh12	0 1 0 1 0 0 0 0 0 0 0 0 0 1 1 0 0 1 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1 1
		Kh13	0 1 0 1 0 0 0 0 0 1 0 0 1 0 0 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 1 0 0 1 1 1 1 1 1
		Kh14	0 1 0 1 0 0 0 0 0 1 0 0 1 0 0 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 1 0 0 1 1 1 1 1 1
	Site 3	Kh15	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh16	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh17	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh18	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh19	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh20	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		Kh21	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
Wadi El kid	Site 1	K1	0 1 0 0 1 0 0 0 0 0 0 0 0 1 0 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 1 0 0 0 1 0 1 1 1 1 1 1
		K2	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		K3	0 1 0 1 0 0 0 1 0 0 0 0 0 1 0 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 1 0 0 0 1 0 1 1 1 1 1 1
		K4	0 1 0 1 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 1 0 0 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1
		K5	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		K6	0 1 0 1 0 0 0 0 0 0 0 0 0 1 1 0 1 0 0 1 0 0 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1
		K7	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
	Site 2	K8	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		K9	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		K10	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		K11	0 1 0 1 0 0 0 0 0 0 0 0 0 1 1 0 1 0 0 1 0 0 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1
		K12	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		K13	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		K14	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
	Site 3	K15	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
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		K17	1 1 0 1 0 1 0 0 0 0 0 1 0 1 0 1 0 0 1 0 1 1 1 1 0 0 1 1 0 1 0 0 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		K18	1 1 0 1 0 1 0 0 0 0 0 1 0 1 0 1 0 0 1 0 1 1 1 1 0 0 1 1 0 1 0 1 0 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		K19	0 1 0 1 0 0 0 1 0 0 0 0 0 1 1 0 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 1 0 1 0 1 1 1 1 1 1
		K20	0 1 1 1 0 0 1 0 0 0 1 0 0 1 0 1 0 1 1 0 1 1 1 1 0 1 1 0 1 1 1 1 0 0 0 0 0 0 0 1 0 1 0 1 1 1 1 1 1
		K21	0 1 1 1 0 0 1 0 0 0 1 0 0 1 0 1 0 1 1 0 1 1 1 1 0 1 1 0 1 1 0 0 0 1 0 0 0 0 1 0 1 0 1 1 1 1 1 1 1

Table (2): Data matrix for dendrogram analysis of thoracic muscles of *vespa orientalis*

Sites		individuals	Characters (Muscle protein patterns)
Wadi Mandar	Site 1	M1	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M2	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M3	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M4	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M5	0 1 0 0 1 0 0 1 0 1 0 1 0 1 1 0 0 1 0 0 0 1 1 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1
		M6	0 1 0 0 1 0 0 1 0 1 0 1 0 1 1 0 0 1 0 0 0 1 1 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1
		M7	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
	Site 2	M8	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M9	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M10	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M11	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M12	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M13	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M14	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
	Site 3	M15	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M16	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M17	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M18	1 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0 1 0 1 1 1 1 1 0 0 0 0 1 1 1 1 1 0 1 1 0 0 1 1 0 1 1 1 1 0 1 1 1
		M19	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M20	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		M21	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
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		Kh2	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh3	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh4	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh5	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh6	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh7	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
	Site 2	Kh8	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh9	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh10	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh11	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh12	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh13	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh14	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
	Site 3	Kh15	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh16	0 0 0 1 0 1 0 1 0 0 1 0 1 0 1 0 1 0 0 0 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1
		Kh17	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh18	0 0 0 1 0 1 0 1 0 0 1 0 1 0 1 0 1 0 0 0 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1
		Kh19	0 0 0 1 0 1 0 1 0 0 1 0 1 0 1 0 1 0 0 0 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1
		Kh20	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		Kh21	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
Wadi El kid	Site 1	K1	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K2	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K3	0 1 0 0 1 0 0 1 0 1 0 1 0 1 1 0 0 1 0 0 0 1 1 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1
		K4	0 1 0 0 1 0 0 1 0 1 0 1 0 1 1 0 0 1 0 0 0 1 1 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1
		K5	0 1 0 0 1 0 0 1 0 1 0 1 0 1 1 0 0 1 0 0 0 1 1 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1
		K6	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K7	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
	Site 2	K8	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K9	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K10	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K11	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K12	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K13	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K14	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
	Site 3	K15	0 1 0 0 1 0 0 1 0 1 0 1 0 1 1 0 0 1 0 0 0 1 1 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1
		K16	0 1 0 0 1 0 0 1 0 1 0 1 0 1 1 0 0 1 0 0 0 1 1 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1
		K17	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K18	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K19	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K20	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
		K21	0 0 1 0 0 0 1 0 1 0 0 0 1 0 1 1 1 0 1 1 1 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1

DISCUSSION

The results obtained from the dendrograms suggested that most of the populations of *Vespa orientalis* have similar genetic make-up without any remarkable variations either of the venom proteins or that of muscle proteins. In this approach, Zalat (1997) reported that the comparison between venom proteins of different *Vespid* species composition detected by gel electrophoresis reveals strong similarities between the individuals of both studied species of genus *Vespa* (*mandarina* and *tropica*) using the cladistic analysis method. Archer (1994) found that *Vespa orientalis* was an isolated species with no clear phylogenetic relationship with any other *Vespa* species.

The obtained similarity could be explained as follows: although the three wadis are considered as narrow wadi beds bounded by high mountains, these mountains are not high enough to be considered as barriers for *Vespa* that is characterized by a very high mobility of flying which may enable them to cross the ridged mountain barriers and to move across the adjacent wadi systems (Zalat *et al*., 2001). On the other hand, such explanation could be supported by the suggestion that the high mountains diminish the action of winds that gives the *Vespa* a high opportunity to move freely. In addition to these reasons, it is important to mention that the collected individuals are all of workers only, not of other casts like queen or males, which actually would represent a specific hives. Otherwise, the dissimilarity in some cases were thought to be due to some individuals come from different hives even collected from the same place, thus it could be recommended to find the nests in further studies to ensure gathering individuals from the same nest.

The results of the present study revealed that the venom proteins show more variation than that of the thoracic muscle proteins. This may be due to the specialization of venom according to insect behavior, where insects from Wadi El-Kid seem more aggressive than those of Wadi Mandar and Kheriza, because Wadi El- Kid is the least crowded wadi with Bedouins community, thus wasps are not familiar with human existence. On the contrary, the *Vespa* of Wadi Kheriza and Mandar seem to be more peaceable (Abdel-Ghany, 2006). In addition, some of the venom contents could change according to the type of food on which the wasps depend mainly, besides the condition of the hive, which consequently affect its amount and composition (Hoffman,

1993). This is totally in agreement with Edison *et al*. (1982) who reported that the hymenopterans venom composition and anatomy of venom apparatus often correlate with the behavior of the venomous insects.

The electrophoretic pattern shows that the molecular weights of the venom protein range from 320 to 6 KD. Such range is relatively higher than that obtained from different species of Vespid wasps studied by Zalat (1997) who detected molecular weights ranging from 61 to 18 KD. Such difference could be due to use of different way of venom extraction, where in the present study, the whole venom sac was grinded with its venom content, while in the other works, venom was obtained in a pure form by rupturing the venom reservoir and the venom was squeezed out and dehydrated. So, these high molecular weight bands may be the protein detected in the tissues of the venom sac rather than the venom itself.

It is also clear that, although 48 bands appear in both venom and muscle, they only share the bands with molecular weights of 97, 44, 38, 33, 31, 30, 28, 27, 25, 22, 20, 18, and 6 KD. This could be due to the fact that some enzymes have different roles either in venom or muscles to adapt their functions. These bands could be common proteins or enzymes.

In conclusion, results of the present study shed lights on the variations between populations of some *Vespa* species which open the way to do further studies in these high mountains, where it is highly recommended to focus on aquatic or terrestrial fauna to study genetic variability between and within wadi systems. For flying social insects such as *Vespa*, it is also recommended to study individuals within specific hives and locate the genetic variability between and within different hives in different wadi systems.

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اختلاف بروتين السم والعضلاتِ الصدرية لزنبور البلح (فسبا أورينتاليس) وعلاقته بالإنعزال الجغرافى في محمياتِ جنوبِ سيناء، مصر

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تم حصر زنبور البلح (فسبا أورينتاليس) خلال شهري يونيو و يوليو عام 2005م وذلك في ثلاث أودية في محميات جنوب سيناء بجمهورية مصر العربية (وادي مندر فى محمية سانت كاثرين بالإضافة الى وادي خريزة ووادي الكيد فى محمية نبق). وقد تم أجريت الدراسة لحصر الاختلافات بين وضمن الأفرادِ في هذه المواقع عن طريق مقارنة بروتينَات السم و العضلاتِ الصدرية للزنبور. و لقد تم التحليل بطريقة التفريد الكهربي للبروتين حيث تم تسجيل 82 شريطاً بروتينياً تراوحت أوزانها الجزيئية ما بين 375 و 6 كيلو دالتون فى كلا من بروتينات السم و العضلات في عدد 72 زنبور جمعت من هذه المواقع.

ولقد أظهرت مصفوفة البيانات المعتمدة على نمط بروتين السم بعض الاختلافات فى عدد وموقع الشرائطُ البروتينية على أساس مقياس المقارنة بين الأفراد. وعلى صعيد أخر ، كانت هذه الاختلافات ملحوظة بشكل طفيف عند الأماكن المختلفة المتواجدة فى الوديان محل الدراسة. و من ناحية أخرى ، أظهرت مصفوفة البيانات التى تم الحصول عليها من تحليل بروتينات العضلات اختلافات ضئيلة جداً سواء على مستوى الأفراد أو مواقع التجميع. وهكذا يعتقد أنه بالرغم من أن الثلاث وديان تعتَبَر ودياناً ضيقة محاطة بالجبال العالية إلا أن هذه الجبال ليست عالية بالقدر الكافى لكي تعتبر موانع لعبور الزنابير التي تتميز بقدرة عالية على الحركة والطيَران والتي قَد تمكنها من عبور هذه الموانع بسهولة والإنتقال عبر الأودية المجاورة.