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Extraction of venom from honey bee in district swat, Khyber Pakhtunkhwa, Pakistan

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Abstract
This part of work was performed to identify honey bees, extract and analyzed its venomat Agriculture Research Institute Center Swat, Pakistan during the period of June 2015 to August 2015.Honey bees were collected both in dry and wet conditions, preserved in 70% ethanol which was further used for the microscopic identification. Results including microscopic analysis and scientific identification key revealed that the collected specimens were identified as *Apis millifera*. Honey bee venom is one of the defensive toxin containing a wide range of pharmacologically active compounds. Two methods are reported for collecting the bee venom includingglandular venom (GV) and venom extracted through the use of electrical stimulation (ESV). In the present work latter, ESV was used to extract the venom from honey bees using artificial collector frames containing series of wires, which stimulates bees to sting for releasing the venom from the venom sac in response to electric shocks. Venom were preserved in dark bottles at 4 ^{O}Cin order to provide proper protection against light and temperature denaturation. The outcome generated from the present work will provide the phylogenetic analysis of the honey bees.

Keywords: *Apis millifera*, Phospholipase A2 (PLA2), gland venom (GV), electric stimulating venom (ESV), Venom sac

1. Introduction
Bees belong to the order Hymenoptera contains almost approximately 30,000 different species. Honey bee is one of the most important economical insect [1] that belong to the genus *Apis* that consists of eight species. The most common and typical honeybee is the western honeybee, *Apis mellifera* which comprises of 24 different races [2, 3].Honey bees are reported to live in colonies in which each group including drones (fertile males), workers (sterile female) and queen (fertile female) are responsible for their specific role [4].

Fig 1: Morphology of Apis mellifera; A. Individual Apis mellifera and B. Different colony members of honey bees

The body of honey bees are composed of three major parts i.e. head, thorax and abdomen. Head usually contains pair of compound eyes for flight orientation with reference to UV rays of sunlight, recognition of colors. Each compound eye consists of about 3000 to 5000 ommatidia (visual processing units). It also contains simple eyes (ocelli) for recording wavelength, duration and intensity of light. In addition, antennae responsible for perceiving sound, odor, taste and air movement were also reported by JeanProst [5]. The thorax is comprised of wings and three pair of legs. There is variation in the venation pattern of each species of honey bees which might be helpful in comparing the same species from different castes. Two different wings including forewings and hind wings exists in honey bees that comprises of about 20 small hooks located along the front border of the hind wings. Beating rate of wings of honey bees were reported as nearly 200 times per second [5].

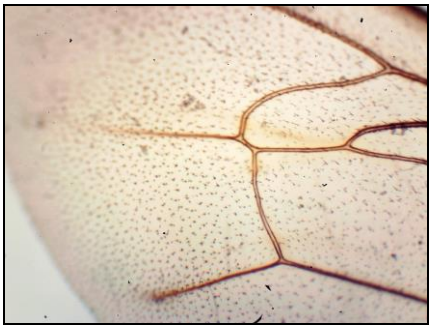

Fig 2: Microscopic analysis of the honey bee's wing

Tarsi are present at the end of each leg that is responsible to sense touch. In addition, first pair of leg possess a notch that is responsible for cleaning the antennae. Middle pair contains spines on one side for removal of pollen masses while the third pair possess corbicula that keep the pollen masses [5]. Abdominal region contain seven partitions in which the first portion is much narrower and forms the bee's waist while the seventh segment of the female workers and queens include the sting. In addition, the abdomen of workers possess certain glands responsible for the secretion of wax for the formation of honeycomb [5].The sting

maintainsthe scalpel sharp point with two lancets on each sides. The allergic response in a sting victim changes from irritation around the site of sting and potentially critical allergic affects i.e. anaphylactic shock [6, 7]. In addition, the sting also contains venom bulb which contain certain chemical substances like melittin, mostly strong cardio-toxic and hemolytic peptide, damages tissues and causes pain [8-10]. Muscular abdominal plates push the stinger into the flesh. The sting and the venom apparatus get avulsed from the abdomen and left at the site of the stinging event, hence single sting against victim may lead the honey bee to die [4]. In response of venom administration, the body of the stung organism secretes histamine that causes localized irritation. Phospholipase A2 (PLA2) and hyaluronidase possess strong anti- HIV activities by blocking the HIV-1 entry into the host cells via mechanism related with PLA2 binding to the cells. In addition, the alarm pheromone is also secretedduring the stinging period that attract other bees for further defense to the site [11].

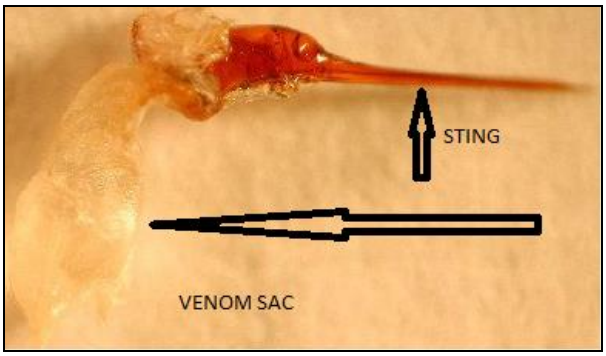

Fig 3: Honey bee venom sac (Adapted from "An introduction to bee biology by David Stone"

1.1 Chemistry of venome

Venom extracted from social animals (Wasps, bees and Ants) is used as an efficient and significant chemical weapon for specific member or colony defense which can be attain by venom administration into an enemy in very low concentration reaching the bloodstream in few minutes [12]. Bee venom contains about 88 % water and 0.1µg dry venom. During one sting, about 50–140 mcg of venom are released. Various researchers reported that the dry venom is chemically composed of a complex mixture of active peptides (melittin, mast cell degranulating), enzymes (PLA2, hyaluronidase, acid phosphatase) and biogenic amines (vasoactive amines, histamine) [2, 13, 14].

There are consistent studies for the pharmaceutical uses of the honey and other bee products as a curative agents in the treatment of certain pathological conditions including arthritis, rheumatism, skin diseases, and cancerous tumors [15-18]. In addition, bee venom also possess anti-microbial activities including anti-bacterial, anti-viral, anti-parasite[19], anti-tumor [20] due to the presence of anti-microbial peptides that makes it appropriate to be utilize for the treatment strategies [21].

There are two methods for the extraction of honey bee venom including electrical stimulation (ESV) and direct extraction from glands (GV) method [6, 7]. Glandular venom extraction (GV) is thought to be the most effective method as the venom extracted via this method contains more biochemical components [6] based on the quantitative and qualitative comparison of venom from both methods. To elucidate the pharmacological purpose, the basic understanding about the selection and proteins of venom are essential.

The present study was performed to collect, identify the honey bee species from a local farm at District Swat. In addition, venom were also extracted from bees which could be further utilize for biochemical analysis.

2. Materials and Methods

2.1 Study area

Study site for the present study was selected the Swat valley (5,337 km^2) located at the northern side of Khyber Pakhtunkhwa, Pakistan. The average annual rainfall and temperature was reported as about 878 mm and 19 ℃. Honey bees were identified at Agriculture Research Institute Center Swat through microscopic observation and using the key (The Asian Honey Bee, A guide to identification). In addition, extraction of bee venom was also performed, followed by preserving the extracted venom in dark bottle.

Fig 4: Study site (Swat Valley) map

2.2. Collection of honey bees

The honey bee specimens were collected from a local bee keeping site using Aerial net. The honey bees were killed in the killing jars and preserved in 70% ethanol. The specimens were identified using key (The Asian Honey Bee, A guide to identification), followed by the microscopic analysis of the honey bees.

2.3 Extraction of honey bee venom

Venom was extracted from the honey bees using venom collector. The collector frame was placed at the entrance of the hive as well as near their feeding places, followed by connecting with the electrical device for electrical impulses to stimulate sting. The deposited venom between the glass and the protective material were dried and scrapped off using blade, collected in powder form in dark bottle and was preserved at4^0C.

Fig 5: Venom collector device at field

3. Results

3.1. Identification of honey bees

Takhtaband field was selected for the separation of bee venom where bees were kept for the production of honey for commercial and medicinal purposes. Honey bees were collected for identification and microscopic analysis. The collected bee samples were preserved in both dry and wet conditions. The collected preserved samples were investigated for the exact identification using identification key and microscopic examination at Agricultural Research Center Swat. The identified specimen of bees was *Apis Mellifera*, although it possess certain similarities with *Apis cerana* species. Honey bees were categorized on the basis of hind wing venation (extra vein in *Apis cerana*).

3.2. Extraction of honey bee venom

Venom was extracted from the honey bees at the collection site. Initially the venom collector was kept below the bee hives, however, the collection of venom was not proper. Hence the collector device was kept away from the hives, followed by placing the syrup (mixture of water and sugar) to attract the bees. After feeding on the syrup, the bees cleared their bodies via sitting on the herbs or collector wires that produced electric shocks and stimulates the bees to sting on the wire for releasing the venom. The collector was disconnected from the electric supply and the bees scattered. The venom present on the surface of the glass was removed slowly and gently via the blade, preserved in dark bottle at4^0C.

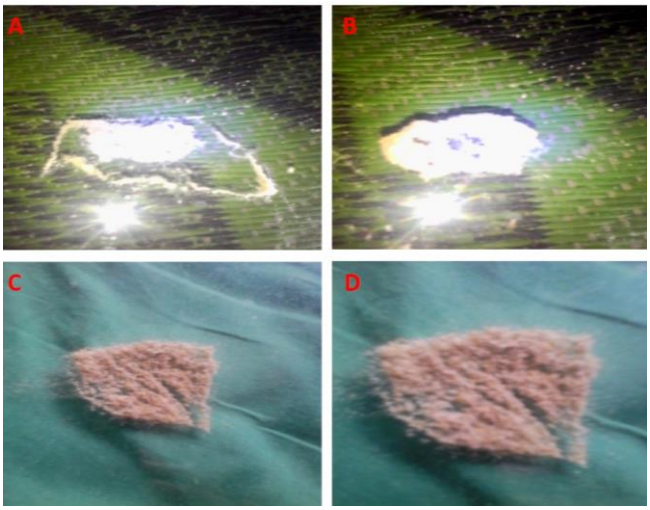

Fig 6: Various samples of venom extracted from the honey bees

4. Discussion

The current study was conducted for the identification of honey bee species and venom extraction from the specimen collected from a local bee culturing area at District Swat, Pakistan. Scientific identification of the honey bee was performed in Agricultural Research Institute Swat. The species was successfully identified as *Apis mellifera* using particular identification key and microscopic analysis of the specimens. More than 30000 species of honey bee have been reported that exists either in small or large colonies. In the current study, the honey bee was kept as small colonies in special boxes. Different forms of the honey bees were identified on the basis of morphological characters as reported by Jean Prost (1994) [5]. Various researchers proposed the anti-microbial i.e. anti-bacterial and anti-viral activities activities [19-21], anti-HIV activity [10, 11] and treatment of various diseases like arthritis, rheumatism, skin diseases and cancerous tumors [17].Dotimas and Hider (1987) reported that bee venom contains 88% water and 0.1 µg dry venom[13]. There are consistent studies to elucidate that the dry venom isa complex mixture of active peptides, enzymes, and amines[2, 3, 13]. The venom collected in this part of work was in dry form.Two different types including glandular venom (GV) and electric shock venom (ESV) extraction have been reported for the extraction of venom from the honey bees[6, 7]. The present work reflects the venom extraction via ESV. For that purpose, an electrical collector device was prepared for the extraction of venom from honey bees, which is consistent with the model devised by Ali (2012)[6], who reported that the bees in close contact with the collector wires activatethe electric shocks that can stimulate the bees to sting and release the venom on the glass surface of the collector frame, followed by collection and preservation of venom in dark bottlesat4^{o}C for proper protection against light and temperature denaturation. However, the venom extracted via GV method can be more effective as it contains more chemicals than the venom collected through ESV.Further HPLC analysis of the bee venom willbe require to elucidate the biochemical composition of venom.

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