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Application Details	
APPLICATION NUMBER	201911001990
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	17/01/2019
APPLICANT NAME	1 . Dr. SUSHIL KUMAR UPADHYAY 2 . PROF. (Dr.) RAJ SINGH
TITLE OF INVENTION	CRUDE APITOXIN EXTRACTOR DEVICE
FIELD OF INVENTION	MECHANICAL ENGINEERING
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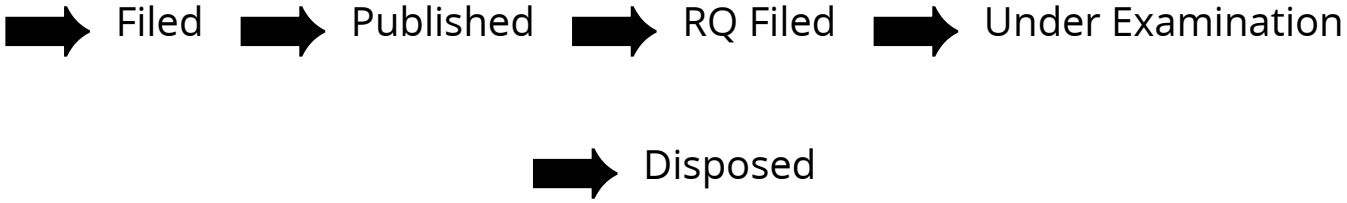
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	28/08/2020

Application Status	
APPLICATION STATUS	Awaiting Request for Examination

View Documents



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FORM 2

THE PATENTS ACT 1970

(39 of 1970)

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THE PATENT RULES, 2003

COMPLETE SPECIFICATION

(See section 10 and rule 13)

1. TITLE OF THE INVENTION: -

CRUDE APITOXIN EXTRACTOR DEVICE

2. Applicant(s)

Name	Nationality	Address
Dr. SUSHIL KUMAR UPADHYAY	**INDIAN**	**ASST PROFESSOR, DEPARTMENT OF BIOTECHNOLOGY, MAHARISHI MARKANDESHWER (DEEMED TO BE UNIVERSITY), MULLANA-AMBALA, HARYANA, INDIA.**
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3. PREAMBLE OF THE DESCRIPTION

The following specification particularly describes the invention and the manner in which it is to be performed

FIELD OF INVENTION

This invention relates to adevice for the collection or extraction of honey bee venom without any damage to the model insect, *Apis* sp.innovative, efficient, environmentally safe with lowest cost and multifold productive device

BACKGROUND OF INVENTION

Honey bees are one of the oldest forms of animal life still in existence from the Eolithic Age. Primeval humans gathered and ate the honey of wild bees, the only available sweet. Bronze age societies celebrated preindustrial triumphs by drinking mead, probably the first intoxicating beverage, fermented from honey. Like honey, beeswax has been prominent in ancient folklore, mythology and also used in writing, painting, sculpturing, and protecting works of art, as well as for illumination. Honey, beeswax, and propolis (a mixture primarily of plant resins and beeswax that bees use in nest construction) have been used extensively in pharmacopoeia.

Honey bees originated in southern Asia, probably in the region of Afghanistan and found in the form of wild colonies. There are many species of honey bees, among them *Apismellifera*, the most widely distributed of the species of *Apis* (Insecta: Hymenoptera). The scientific name, *Apismellifera*, literally means "the honey-carrying bee" or "the honey-making bee" was proposed in 1761 by Carolus Linnaeus. After the Independence of India in 1947, the importance of beekeeping was stressed by Mahatma Gandhi by including it in his rural development programmes. Initially, the beekeeping industry was under the All India Khadi and Village Industries Board which was converted to Khadi and Village Industries Commission (KVIC) in 1956. In 1962, Central Bee Research and Training Institute was established by KVIC at Pune, Maharashtra.[1] The production of honey in India increased significantly and 70% of honey production comes from informal segments. The other products of the hive include pollen, brood (still eaten by some primitive cultures), propolis (bee glue), royal jelly, venom, and of course bees wax. For making beeswax, each worker bee has four pairs of wax glands on the underside of its abdomen. The human nutritional value of pollen and queen (royal) and worker jelly has been of great interest throughout the world.

Apitoxin, or honey bee venom, is a bitter colorless liquid containing proteins, which may produce local inflammation. Bee venom from honey bee has been utilized for centuries as a pain reliever, anti-coagulant and anti-inflammatory agent for chronic diseases, such as

Arthritis, Rheumatism, Tendonitis, Bursitis, Fibrosis and Multiple Sclerosis.[2-5]Apitherapywhich uses live honeybee stings has elucidated therapeutic value for piglets, calves and dairy cows with several respiratory diseases in Korea.[6] Bee venom has been reported to contain various bioactive substances including polypeptides (melittin, apamin, and mast cell degranulating peptide), amines (histamine, serotonin, dopamine, and norepinephrine), and enzymes (phospholipase, hyaluronidase, histidine decarboxylase).[7] Two major components of bee venom, melittin and phospholipase A2, are generally thought to play an important role in the induction of irritation and allergic reaction associated with the bee stings.[3]Melittin, a 26 amino acid polypeptide, has been known to have antibacterial effects.[2,8,9] Recently, melittin-loaded perfluorocarbon nanoparticles possessed the ability to safely deliver significant payloads of melittin intravenously and to target and kill tumor cells.[10] The antifungal activities of the bee venom against most common dermatophytes (*Trichophyton mentagrophytes* and *T. rubrum*) known to cause a varieties of skin infections in humans, were determined by using modified broth dilution assay. Moreover, the antifungal activities of the bee venom were much stronger than that of fluconazole, one of the commercial antifungal drugs used in the treatment and prevention of superficial and systemic fungal infections.[11]

The sting of honey bee is the modified form of ovipositor. Photomontage is showing the entire ventral surface of the sting (Fig. 1a). The two barbed lancets, in close apposition, overlay the stylet trough to form the venom channel. The ultimate abdominal segment's (seventh segment) tergal and sternal plates enclose most of the sting as comparison to segments eight to ten (Fig. 1b). The aperture through which eggs and feces pass when expelled from the body also functions as the entrance to the sting chamber. The fuzzy projections arising from the floor of the sting chamber are the tips of the sting sheath (Fig. 1c). The medial surface of the abdominal tip (ventral sclerite) is revealing myraids of stout cuticular spines. Higher magnification of the abdominal tip in the right middle micrograph is showing the setose condition of the sting sheath at its proximal origin. (Fig. 1 d)

Stinger is an effective weapon because it delivers venom that causes pain when injected into the skin. The major chemical responsible for the pain of a honey bee sting is called melittin. It stimulates the nerve endings of pain receptors in the skin. The result is a very painful sensation, which begins as a sharp pain that lasts a few minutes and then becomes a dull ache. Even up to a few days later, the tissue may still be sensitive to the touch. The stinging can lead to very large swelling around the sting site or in a whole portion of the body. When the sting is caused by a honey bee, the stinger usually remains in the skin when the insect leaves

because the stinger is barbed. Remove the stinger as quickly as possible, because venom continues to enter the skin from the stinger for 45 to 60 seconds following a sting. After the stinger is removed, wash the wound and treat it. When a person was stung many times before being able to flee from the nesting site, resulted in mass envenomation. Humans can be killed if stung enough times in a single incident. With honey bees the toxic dose (LD50) of the venom is estimated to be 8.6 stings per pound of body weight. In fact, an otherwise healthy adult would have to be stung over 1,000 times to be in risk of death.Ultimate objective of the inventors is to device new techniques for better utilization of resources to mankind by the *vision "**Lab to Land Transfer of Technology**" and mission "**Earn as You Learn**"*.

WO2007037566A1;Sang-MiHanKwang-Gil LeeJoo-Hong YeoHae-Young KweonSoon-Ok WooMyeong-LyeolLeeMan-Young LeeChoul-Goo Kim disclose a bee venom collector device, comprising: a covering member (1); a frame (5) to which the covering member is attached, having wire guiding grooves 5 arranged at regular intervals in one side thereof; a control bar (2), having a plurality of wire grooves (2') formed at regular intervals thereon, combined with the frame (5) at a position opposite to the guiding grooves (5') in such a way that it is movable to modulate the tension of wires guided by the wire grooves; a pair of wires (3), each being connected to both a cathode and an anode supported and guided by the guiding grooves (2') and (5') formed respectively at the control bar (2) and the frame (5), repeatedly running therebetween on tracks arranged at regular intervals according to the grooves; a glass plate, fitted in the frame (5), for receiving the bee venom; a base plate (8) attached at the back of the glass plate (7) to protect the glass plate; a fixing member (9) for firmly fixing the glass plate (7) and the base plate (8) in an inner space (4) of the frame; a controller (11), electrically connected to the pair of the wires (3) supported by the frame (5), for freely controlling the supply time of currents and elevating an output voltage to a predetermined level; and a power supply (15) for supplying electrical power to the controller (11).

KR101003672B1;Inventor disclose a device for collecting apitoxin is provided, which makes movement and installation convenient by preventing a wire from being exposed to the outside of a device for collectingapitoxin. CONSTITUTION: A device for collecting apitoxin comprises: a main body(110) in which a holder is formed in both sides where a space part is formed; an electrode member in which both ends are set in a holder crossing the space part; a collecting plate which is located in the lower part of the electrode rod and in which both ends are fixed to the lower part of the holder; a rechargeable

battery capable of supplying power; a PCB in which electronic components are mounted; and a control module(180).

KR101240291B1; Inventor disclose a bee venom collector is provided to prevent the short-circuiting between electrodes and to maintain the gap between the electrodes. CONSTITUTION: A bee venom collector comprises a main body(210) which is formed with a holder in the front and rear, an electrode member in which an electrode is mounted in the holder and a positive electrode and a negative electrode are arranged with an interval, and which is connected to a positive connection rod and a negative connection rod, and a control module(280) which is assembled to one side of the main body and supplies power to the electrode or blocks the power. The control module comprises a rechargeable battery and a light collecting plate(281) for supplying electricity to the battery using sunlight.

KR200410849Y1; Inventor disclose a bee venom collecting plate structure, the past, the collection plate +, - make all winding a current line fly to collection plate to collect the venom from the collecting plate bee venom is emitted by the shock, or collected sense the current line my seolhae a separate home abacus on board, +, - applying an electrical shock by a current wire gathering plate surface Jin bee venom this current line wound around the bee venom released into the collection plate or home abacus with a sharp scraping, such as when collecting a razor When it is the scratching or picking the venom taken out of the house in Suphannaeseol collected editions situation it is that often the current line any damage, inconvenience to the house Suppan taken out separately is pointed out. The present design is +, the installation disposed at a predetermined interval to collect only - to place a current line in parallel, even if any one of the current terminals is disconnected, and so the difficulty in harvesting the bee venom, particularly Gwyn the collection plate surface existing takes +, - the current line is not extruded to form a wire groove in the glass plate used as the collecting plate +, - so as to match the current line surface with the glass plate surface, even with the scraping recovering bee venom, +, - damage to the current line and not to apply, glass plate and the +, - relates to a bee venom collecting plate structure to allow recovery of the discharge current plate bee venom neatly without remaining.

None of the prior art indicate above either alone or in combination with one another disclose what the present invention has disclosed. The objectives of present invention are to design an efficient and multifold productive device for the extraction or collection of crude aptitoxin from live honey bee without any physical, biological and behavioral damage or injury to insect.

i. To design a compact, portable, applicable inside bee box, economically affordable and environmentally safe equipment for crude bee venom collection.

ii. To augment the status, life style, employment and livelihood for sustainable development by creation of new dimension in classical bee keeping (apiculture) through the newly designed machine.

iii. To obtain and collect good quality and quantity of crude apitoxin by reducing the rate of oxidation and aerial exposure through application of deviceinside bee box.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1. Photomontage and ultra topography of honey bee sting: **a.** ventral view of sting, **b.**sclerites ties alongside of sting, **c.** entrance of sting chamber through which eggs and feces passes also, **d.** setose condition of sting sheath. (*Source: SEM atlas of the honey bee*).

Fig. 2. Chemical structure of (**a**) fluconazole, (**b**) melittin and (**c**) apamine.*(Source)*[13]

Fig. 3. Newly designed crude apitoxin extractor.

Fig. 4. The two arms of wooden frame at a distance of 1.5cm with electric circuit/ panel.

Fig. 5. An efficient power pack (stabilizer) with 25 to 50mV capacity.

Fig. 6. Plane glass sheet used in crude apitoxin extractor (3mm).

Fig. 7. The dimensions of wooden frame of newly desingned crude apitoxin extractor.

Fig. 8. An onsite methodology cum hand's on training program on crude bee venom extraction by inventor: **a,b.** spotting or identification of crude bee venom spot on glass sheet, **c.**collection of scratched/collected crude apitoxin in eppendorf tube, **d.** inventor with trainee in bee keeping farm.

Fig. 9. Crude apitoxin in inventor hand (a,b) and inventor onsite (c).

DETAILED DESCRIPTION OF INVENTION

In present invention an efficient "Crude Apitoxin Extractor" is designed, after facing several constrains in bee venom collection by the application of available devices during year to year research on the "Apiculture for capacity building and women empowerment for livelihood and sustainability". After a decade, by continuous uphill struggle round the clock and exhaustive literature survey, authors are now in situation to design and propose a new innovative, efficient, environmentally safe with lowest cost and multifold productive device for the collection or extraction of honey bee venom without any damage to the model insect, *Apis* sp. (Fig. 3).

The designed device is a compact wooden frame with two adjacent hanging arms at a distance of 1.0cm. The two arms of the wooden frame have unique copper wire panel for the supply of electricity (25 to 50mV) throughout device (Fig. 4). The electric current will passed through an efficient power pack (stabilizer) with 25 to 50mV capacity to the device. It has regulator for voltage and the pulse duration (Fig. 5). The top and bottom of frame are nicely designed and linked together, for the support of glass sheet in between two hanging arms of the frame. The space between arms of frame and architecture of frame itself allowed the placement of glass sheet during appliance application for apitoxin extraction as well as glass plate can be removed or taken out after the completion of task immediately for further action. Thus the glass sheet may be taken in and out through the wooden frame as per requirement (Fig. 6). Because of the application of newly designed device inside bee box, the dimension of the device, wooden frame, glass sheet, distance between hanging arms in which glass plate to be fitted is depends on the size of honey bee box used by bee keepers for bee keeping or apiculture. A stipulated standard size or dimension of newly invented proposed device, "Crude Apitoxin Extractor" given in Fig. 7.

The extraction or collection of venom with the help of newly designed device is an art that can be performed by anybody of any age group. The apitoxin extraction based upon device is not a very technical process. The bee keepers were suggested to dress up you with gloves, mask, hood, goggle, shoes and suit (Fig. 9c). During the onsite (Fig. 8d) operation of device for venom collection following steps taken in consideration.

1. At very fist, check the electric pannel of device to avoid any shot circuit during operation (Fig. 4).
2. The clean and dry glass sheet (Fig. 6) between to hanging arms of wooden frame was placed and locked carefully with the help of attached adjustable screw/clip.
3. The device is now ready (Fig. 3) to connect the wooden framed electric pannel with stabilizer of 25 to 50mV capacity that has regulator for voltage and pulse duration (Fig. 5).
4. The crude apitoxin extractor now placed in honey bee box (between frames with hive/ honey comb) and the top of bee box was closed by the cotton cloth. (Note: stabilizer should be out of box).
5. Now the stabilizer was allowed to connect with main electric board.
6. An electric shock of weak strength was applied in venom extractor. For same switch on the button of electric board for fraction of minute. (Note: Keep the

electric volt at minimum at first). Resultantly honey bee irritated and left a drop of bee toxin on glass plate attached to venom extractor.

7. Wait for 2 to 3 minutes and switch on the button of electric board for fraction of minute. (Note: Adjust the electric volt as per the response of honey bee against stimulus).
8. Repeat the step 7 for 3 to 5 times. After that the device were taken out from bee box.
9. Allowed the drop of apitoxin on glass sheet for drying within fraction of minutes (Note: Don't blow air and protect the glass sheet containing apitoxin from sun light because the sun light may damage the quality of apitoxin)
10. The dried drop of apitoxin was spotted on glass sheet through hand lens and marked carefully (Fig. 8a, b).
11. With the help of clean, dry and sterilized knife or blade scratched out and collected in eppendorf tube carefully (Fig. 8c).
12. The eppendorf tube right now allowed to seal air tight and place in the ice pack (to avoid any loss in quality), brought to laboratory and store at -18ºC. The collected bee venom was lyophilized that gave rise powdery form of it, known as crude bee venom (Fig. 9a, b).

Results

The crude apitoxin collected with the help of newly designed device "Crude ApitoxinExtractor" is now ready for further commercial, medicinal and academic-cum-research application (Fig. 9a, b).

We claim:

1. A device of Crude Apitoxin Extractor comprises a compact wooden frame with two adjacent hanging arms at a distance of 1.0 cm.

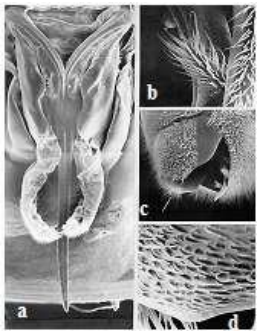
2. The device as claimed in claim 1, wherein said two arms of the wooden frame have unique copper wire panel for the supply of electricity (25 to 50mV) throughout device; and the electric current will passed through an efficient power pack (stabilizer) with 25 to 50mV capacity to the device; and it has regulator for voltage and the pulse duration.

3. The device as claimed in claim 1, wherein the top and bottom of frame are nicely designed and linked together, for the support of glass sheet in between two hanging arms of the frame; and the space between arms of frame and architecture of frame itself allowed the placement of glass sheet during appliance application for apitoxin extraction as well as glass plate is removed or taken out after the completion of task immediately for further action.

4. The device as claimed in claim 1, wherein the glass sheet may be taken in and out through the wooden frame as per requirement.

Dated this January 17, 2019

(Ashish Sharma)
Authorized Agent for the Applicant
Indian Patent Agent Regn No. IN/PA-3021



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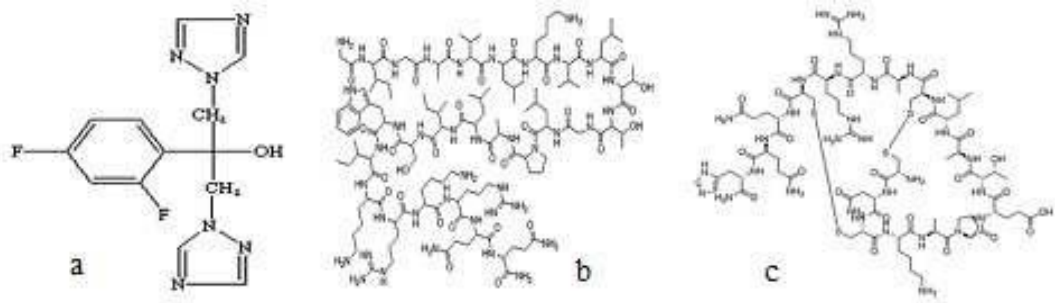

Fig. 2. Chemical structure of (**a**) fluconazole, (**b**) melittin and (**c**) apamine.*(Source)*[13]

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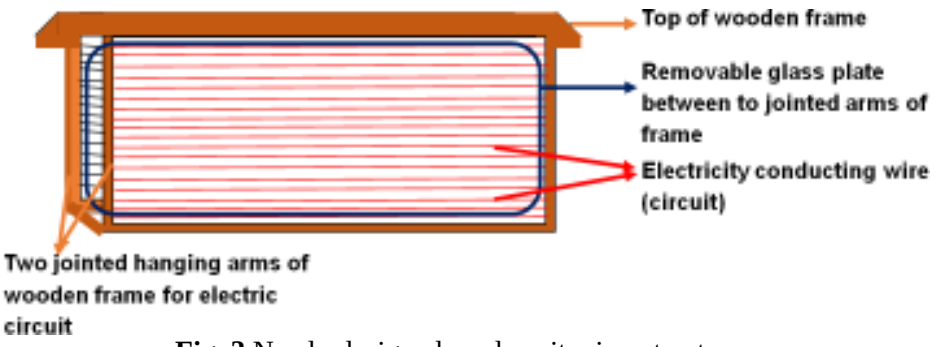

Fig. 3. Newly designed crude apitoxin extractor.

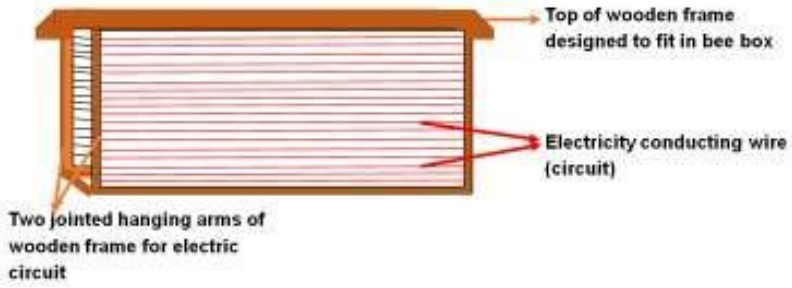

Fig. 4. The two arms of wooden frame at a distance of 1.5cm with electric circuit/ panel.

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Fig. 5. An efficient power pack (stabilizer) with 25 to 50mV capacity.

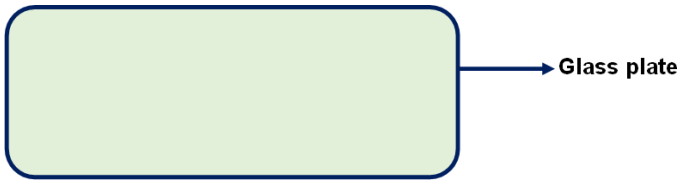

Fig. 6. Plane glass sheet used in crude apitoxin extractor (3mm).

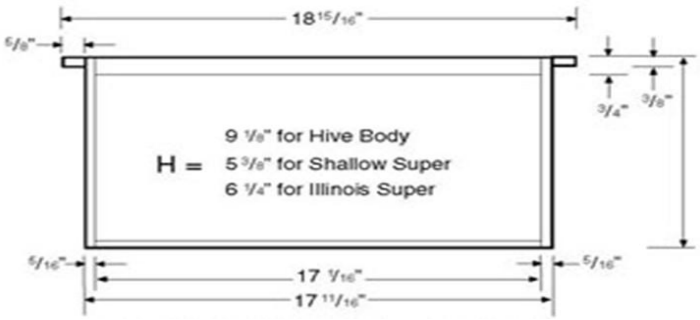

Fig. 7. The dimensions of wooden frame of newly desingned crude apitoxin extractor.

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Fig. 9. Crude apitoxin in inventor hand (a,b) and inventor onsite (c).

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ABSTRACT

CRUDE APITOXIN EXTRACTOR DEVICE

This invention relates to a device for the collection or extraction of honey bee venom without any damage to the model insect, *Apis* sp. The device is innovative, efficient, environmentally safe with lowest cost and multifold productivity. Research shows that the bee venom affects on many diseases and on some have proven complete healing. Apitoxin is used in the treatment of rheumatic diseases, multiple sclerosis, rheumatic fever, high blood pressure, sciatica, psoriasis, hematomas, hemorrhoids, prostate, for various skin diseases, burns. Therefore, to overcome the problems related to apitoxin collection, present invention propose a design of an efficient "Crude Apitoxin Extractor". Device proposed is a new innovative, efficient, compact and portable size, applicable inside bee box, environmentally safe with lowest cost and multifold productive device for the collection or extraction of honey bee venom/ apitoxin without any damage to the model insect, *Apis* sp. (Hymenoptera: Apidae). The newly designed device "**Crude Apitoxin Extractor**" containing an electric panel is made up of wooden frame (two hanging arms) with copper wires. In between these two arms with electric circuit/ panel there is a removable glass plate of 3mm, on which honey bees sting after the application of mild electric shock through a power pack and leave the apitoxin on it that later take off with the help of blade or knife and collected in eppendorf tube. This type of advances in apiculture may help in capacity building and women empowerment through self-help group, employment to youth for livelihood and sustainability.