# Surface morphology and morphometric analysis of sensilla of Asian tiger mosquito, *Aedes albopictus* (Skuse): an SEM investigation

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#### Abstract

*Background & objectives:* The sensilla and sensory mechanism play a significant role in hostseeking and oviposition behaviour of mosquitoes, which enable them to transmit various diseases to humans. *Aedes albopictus* (Skuse) has emerged as a major vector of Chikungunya virus in the recent epidemics in most parts of southern India. Studies on the sensory structures of dengue vector, *Aedes aegypti* (Linn) are comprehensive; whereas information on the sensillary systems of Asian tiger mosquito, *Ae. albopictus* is inadequate. Therefore, the present study has been carried out to observe various types of sensilla located on the antenna, maxillary palp, labial palp, tarsi and ovipositor of *Ae. albopictus* using scanning electron microscopy.

*Methods:* The antennae, maxillary palpi, labellum, tarsi and ovipositor of 10 different female mosquito of *Ae. albopictus* were fixed individually in 2.5% glutaraldehyde solution, washed twice and dehydrated with ascending grades of ethanol. Samples were cleared with xylene, air-dried, mounted on stubs, gold coated in an ion-sputtering unit and the sensilla were viewed between 5 and 10 KV using FEI-Quanta 400–EDAX scanning electron microscope. ANOVA revealed significant differences in the morphometric features of various sensilla.

*Results:* In the antenna Sensilla trichoidea are numerously distributed in all flagellar segments revealed four distinct subtypes. Two types of grooved peg sensilla were observed. Sensilla coeloconica was observed in the terminal flagellum of antenna and tarsomeres with large variation in diameter. Sensilla chaetica are distributed throughout the body and revealed greater variation in morphology and morphometric parameters.

*Interpretation & conclusion:* The significant difference among various types of sensilla would possibly reveal their functions. The porous sensilla are olfactory and contact chemoreceptors while the aporous sensilla would play the role of mechanoreception. Sensilla coeloconica on the antenna, tarsus showed major differences with *Ae. aegypti*. The ovipositor sensilla revealed three types of chaetica arranged in rows but has not been reported earlier with other mosquito species.

Key words Aedes albopictus – chaetica – coeloconica – grooved peg sensilla – morphometric analysis – scanning electron microscopy – sensilla trichoidea

# Introduction

The Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae), is native to southeast Asia<sup>1</sup> but has recently become established throughout the

America and in at least nine countries in Europe, Africa, and the Middle East<sup>2</sup>, disseminated by a worldwide commerce in used tyres<sup>3</sup>. Although capable of transmitting a large number of arboviruses<sup>4</sup> the species has generally been considered as a "secondary" vector because it is not host specific. *Ae. albopictus* is generally considered to have a low vectorial capacity because of its lack of host specificity. In 2006, there was a major outbreak of dengue, dengue haemorrhagic fever and chikungunya all over India with a total of 1.37 million people affected by chikungunya alone. Nevertheless, it has been one of the major vector of the Chikungunya virus in recent explosive outbreaks throughout the southern India.

The life of female mosquito is governed by its orientation responses to stimuli from different resources such as oviposition site, nectar, mate and hosts. Odors emanating from these sources are known to be important orienting stimuli for the female mosquitoes. Female receives these signals by means of sensilla present on different parts of her body and respond to the signals in different ways, depending upon their genetic background, expressed phenotype and physiological state<sup>5</sup>.

Variety of chemical signals in air or in solution exerts their influence for monitoring the behavioural responses of insects and functioning of other body parts. The structural and functional diversity of sensory system of insects is related with their habit, habitat and orientation responses<sup>6</sup>. A wide variety of sensillar types exists, with different functions and locations on the insect body, according to the habits of each insect species and the frequency of exposure to the diverse stimuli in the habitat in which it occurs<sup>7,8</sup>. Ample of information is available on the distribution of various sensilla located on the antennae, labellum and maxillary palp of haematophagous dipterans<sup>9–15</sup> but limited work has been carried out on the sensillary studies with respect to tarsi and ovipositor<sup>16–19</sup>

Antennae are the major site of the chemoreceptors that detect and discriminate between air-borne stimuli and guide the mosquitoes to suitable human host or to an oviposition site. In other words, antennal sensilla were assumed that most olfaction-driven behaviours such as host-seeking, oviposition as well as nectar-feeding site location is performed by these types of sensilla. Subsequently, other sensory structures present on other parts of the body, viz. maxillary palp, labellum, tarsi, ovipositors, etc. also play an important role in mosquito behaviour<sup>12</sup>.

The sensory mechanism plays a significant role in host-seeking and oviposition behaviour of mosquitoes, which enable them to transmit various diseases to humans. Extensive studies have been carried out in Ae. aegypti<sup>12,16,18,20</sup>, on the sensory structures present in the antenna, maxillary palp, labial palp, tarsi and ovipositor; whereas information on the studies of sensillary systems in the Asian tiger mosquito, Ae. albopictus is inadequate. Therefore, the present study has been carried out to observe various types of sensilla located on the antenna, maxillary palp, labial palp, tarsi and ovipositor of Ae. albopictus using scanning electron microscopy. The terminologies used in this study follow Harbach & Knight<sup>21,22</sup> to describe various body parts and ovipositor<sup>23</sup> of mosquitoes.

# Material & Methods

The test mosquitoes of Ae. albopictus used for scanning electron microscope (SEM) studies were drawn from laboratory colony maintained since 1973 in our insectary<sup>24</sup> at  $27\pm 2^{\circ}$ C and  $75\pm 5\%$  RH. The antenna, maxillary palp, labial palp, tarsi and ovipositor from 10 different Ae. albopictus mosquitoes were cut-off and fixed separately for 24 h in 2.5% glutaraldehyde solution in phosphate buffer of pH 7.4. Following fixation, the specimens were washed twice with phosphate buffer, distilled water and dehydrated with ascending grades of ethanol. After that the specimens were cleared in xylene for overnight and air-dried<sup>13</sup>. The air-dried samples were mounted on stubs and then coated with thin layer of gold in an ion-sputtering unit (JFC 1100) for 5 min; and the preparations were examined between 5 and 10 KV using FEI-Quanta 400-EDAX (The Netherlands) scanning electron microscope. The electron micrographs of various types of sensilla were viewed in Image Manager (Leica Qwin plus v 3.2.1) and their morphometric variables such as length, width and diameter were

Particulars	Mean* $\pm$ S.E.	F-value	DF	p-value
Sensilla trichoidea				
Long pointed trichoidea (STr I)	$58.6 \pm 2$			
Short pointed trichoidea (STr II)	$38.3 \pm 1.1$	77.67	27	p < 0.001
Long blunt trichoidea (STr III)	$29.3 \pm 0.9$			
Short blunt trichoidea (STr IV)	$12.7 \pm 0.6$	247.02	29	p < 0.001
Grooved peg/Capitate peg sensilla				
Long pointed grooved peg (LGPs)	$9.5 \pm 0.4$			
Short blunt grooved peg (SGPs)	$4.9 \pm 0.4$	47.46	32	p < 0.001
Capitate peg on MP (CPs)	$13.5 \pm 0.8$	52.53	50	p < 0.001
Sensilla coeloconica				
Antennal coeloconica (SCo-A)	$0.95 \pm 0.1^{\dagger}$			
Tarsal coeloconica (SCo-T)	$5.8 \pm 0.5^{\dagger}$	122.98	12	p < 0.001
Sensilla chaetica				
Antennal chaetica	$321.4 \pm 15$			
Maxillay palp chaetica	$50 \pm 2.2$	183.24	32	p < 0.001
Labial palp chaetica type-I	$16.7 \pm 0.4$			
Labial palp chaetica type-II	$28.4 \pm 1.1$			
Labial palp chaetica type-III	$51.5 \pm 2.2$	146.75	59	p < 0.001
Tarsal chaetica near claw	$36.5 \pm 1.3$			
Tarsal chaetica at joint	$34.6 \pm 2.5$			
Tarsomere chaetica	$26.3 \pm 1.1$	10.85	35	p < 0.001
Ovipositor chaetica type-I	$19.4 \pm 0.7$			
Ovipositor chaetica type-II	$42.2 \pm 1.9$			
Ovipositor chaetica type-III	$84.2 \pm 2.4$	325.52	62	p < 0.001
				1

# Table 1. Morphometric analysis based on the length of various types of sensilla observed on different body parts of Ae. albopictus Skuse

\*Values are length of respective sensillum in µm; <sup>†</sup>Values are diameter of respective sensillum in µm.

measured using the measure interactive function. The observed sensilla were classified based on their length as short and long sensilla (Table 1). There was no great variation in the width of sensilla, where as sensillum with a pore or opening to the exterior were measured and classified based on their diameter. The measured length/diameter of various types of sensilla was subjected to one way analysis of variance (Sigmastat 2.03, SPSS).

#### Results

Among the morphological features, length emerged as a prominent attribute to differentiate various types of sensilla. On the basis of size, shape and structural features, various types of sensilla have been identified and described.

# Antenna

The antenna of female *Ae. albopictus* mosquito comprises of three parts, namely scape, pedicel, and flagellum. The flagellum consists of 13 flagellomeres. The following types of sensilla were observed on the flagellar region of the antenna.

1. Sensilla trichoidea (STr): These are hair-like structures, numerously distributed on each segment of antennal flagella. These sensilla are of different sizes measuring 13–57  $\mu$ m in length and are primarily olfactory sensilla which are pointed or blunt, arising from sockets and are classified into following types based on their morphology: (a) *Pointed trichoidea* sensilla are hair like, tapering at the end and further classified into two types based on the length: (a-i) long pointed sensilla trichoidea [STr I] 58.6  $\pm$  2 µm in length; and (a-ii) short pointed sensilla trichoidea [STr II] with 38.3  $\pm$  1.1 µm length (Fig.1a); and (b) *Blunt trichoidea* are hair like, the tip of the sensillum is slightly blunt and based on their length further classified into a (a-iii) long blunt-tipped sensilla [STr III]  $29.3 \pm 0.9 \,\mu\text{m}$  long; and (a-iv) short blunt tipped sensilla [STr IV]  $12.7 \pm 0.6 \,\mu\text{m}$  in length (Fig. 1a).

2. *Sensilla chaetica:* Sensilla chaetica are thickwalled and externally grooved sturdy bristles arising from a socket with fine serrations along the edge of grooves with sharp-pointed tip occur as two distinct



Fig. 1: Scanning electron micrographs showing sensilla on the antenna of *Ae. albopictus*: (a) Antennal flagellomeres with sensilla chaetica (SCh) at the joint, long pointed sensilla trichoidea (STr I), short pointed trichoidea (STr II), long blunt sensilla trichoidea (STr III), and short blunt sensilla trichoidea (STr IV) x 500; (b) Two sensilla coeloconica (SCo) at the tip of a tubular structure protruding from the terminal flagellomere of antenna x 5000; (c) Long pointed grooved peg sensilla (LGPs) set on raised cuticular ring x 25000; and (d) Short blunt grooved peg sensilla (SGPs) set into a pit with two cuticular ring x 30000

types-large and small. Six sensilla chaetica are arranged in a whorl at the base of each flagellomeres 2-13 and distributed evenly around the circumference of all flagellomeres in the antenna. These are the largest sensilla measuring  $321.4 \pm 15 \mu m$  in length  $2 \mu m$  width at base (Fig. 1a). Smaller chaetica were found in less numbers in the first two antennal segments.

3. Sensilla coeloconica (SCo-A): Sensilla coeloconica are small, thick-walled sensilla present at the terminal flagellar segment and are commonly called pit sensilla. They appear as round openings with  $0.95 \pm 0.1 \,\mu$ m diameter in the cuticle with one peg set within the pits. In *Ae. albopictus* two coeloconic sensillum are protruding from the tip of distal flagellum as a tubular structure measuring  $10.38 \pm 0.3 \,\mu$ m with a diameter of 1  $\mu$ m opening to the exterior (Fig. 1b).

4. *Grooved pegs (GPs):* These are short, typical pegshaped, thick-walled and deeply grooved. Grooves are parallel and found on the antennal flagella of *Ae. albopictus*. Grooved pegs are classified in two subtypes: long pointed grooved peg sensilla (LGPs) measuring approximately  $9.5 \pm 0.4 \mu m$  in length (Fig. 1c) and short blunt tipped grooved peg sensilla (SGPs) with  $4.9 \pm 0.4 \mu m$  in length (Fig. 1d). These grooved pegs have  $2 \mu m$  width at the base and a less than  $1 \mu m$  pointed tip.

# Maxillary palp

In the maxillary palp, four types of sensilla were observed—(i) *Capitate peg sensilla* (*CPs*): These are the pegs, club-shaped sensilla broadened at the tip and arising from a circular depression. These were spoon/ spatula shaped at the distal end (Fig. 2a). Capitate pegs are of  $13.5 \pm 0.8 \,\mu\text{m}$  in length and are possibly olfactory in function; (ii) *Sensilla campaniformia* (*SCa*): Campaniform sensillum is dome shaped, located on the distal end of third segment of maxillary palp (Fig. 2b), having an inner and outer diameter of 4.7 and 6.4  $\mu\text{m}$  respectively; (iii) *Sensilla basiconica* (*SB*): These are smooth surfaced, broadened at the base, tapering at the distal end,  $3.6 \pm 0.3 \,\mu\text{m}$  in length, located on the third segment surrounding the sensilla campaniformia (Fig. 2b); and (iv) *Sensilla chaetica (SCh)*: These are also thick walled, sturdy, longitudinally grooved and of  $50 \pm 2.2 \,\mu\text{m}$  in length, observed on third and fourth segment of the maxillary palp (Fig. 2c).

# Labial palp

At the tip of the labial palp different size of sensilla chaetica were observed and are classified as sensilla chaetica type I—16.7  $\pm$  0.4 µm, type II—28.4  $\pm$  1.1 µm, and type III—51.5  $\pm$  2.2 µm based on their length. These sensilla are surrounded by several microtrichia (Fig. 2d).

# Tarsus

Tarsus is the distal part of an insect's leg. The tarsus of mosquito consists of five tarsomeres ( $T_1$  to  $T_5$ ). At every joint of tarsal segment two thick spine like sturdy grooved sensilla were observed. In addition the following types of sensillary structures were observed.

1. Sensilla chaetica (SCh): These sensilla are present on all the five tarsomeres as well as at the joints of tarsomeres. These are thick, sturdy, grooved with porous tip. They are of  $34.6 \pm 2.5 \,\mu\text{m}$  in length;  $3.6 \pm$  $0.4 \,\mu\text{m}$  in width (Fig. 3a). Some chaetica are of  $26.3 \pm$  $1.1 \,\mu\text{m}$  in length with  $2.0 \pm 0.1 \,\mu\text{m}$  width located near the joints, which are similar to those found near the claws. Along the length of every tarsomeres a row of small spiny chaetica with grooves at the base extending to the smoothened sharp tip was observed (Fig. 3b).

2. Sensilla coeloconica (SCo-T): These are located on the tarsal segments in a raised pit and were hidden beneath the scales. Externally each sensillum appears as a round convex cap surrounded by a ring of raised cuticle. Two sets of coeloconica were observed on the tarsomere ( $T_1$ ) [Figs. 3c (i) and 3c (ii)]. Each set is situated at a distance of 125–150 µm and



Fig. 2: Scanning electron micrographs showing sensilla on the maxillary palp and labial palp of *Ae. albopictus:* (a) Capitate peg (Cp) sensilla on 4th segment of maxillary palp x 10000; (b) Sensilla companiformia (SCa) with cuticular ring and raised inner dome flanked by sensilla basiconica and microtrichia on 3rd segment x 20000; (c) Grooved sensilla chaetica (SCh) on 4th segment with capitate peg (Cp) sensilla x 4000; and (d) Labial palp with three types of smooth sensilla chaetica at the tip of labellum x 1600

the adjacent two SCo are set in 20  $\mu$ m. These sensilla are of 5.8 ± 0.5  $\mu$ m in diameter which are five times larger than the one observed at the tip of antenna. The terminal tarsomere has a densely grown, thick cuticular fringes located between the claws (ungues) of tarsus (Fig. 3d). These are external processes grown at the joint of ungues with glued and slightly bulbous tip used for attachment on the substrate for the resting mosquito.

# Ovipositor

Ovipositor of *Ae. albopictus* is a tubular structure with two short and broad cerci with densely planted sensilla chaetica along the rim, the post genital lobe is a shallow notch and covered from the dorsal side by a trapezoidal 9th abdominal tergite (Fig. 4a). The rim of cerci comprises of sensilla chaetica located in three distinct lines (i.e. inner, middle and outer lines)



Fig. 3: Scanning electron micrographs showing sensilla on the tarsi of Ae. albopictus: (a) Two sturdy grooved chaetica at the joint of 1st & 2nd tarsomere x 2600; (b) Row of grooved spines on the 3rd tarsomere x 800; (c-i). Sensilla coeloconica on 1st tarsomere x 1000; (c-ii) SCo x 5000; (d) Cuticular fringes located at the base of two claws x 3000; T<sub>1</sub>-T<sub>3</sub>—Tarsomeres 1, 2 & 3; Sc—Scales; SCh I, II, III—Sensilla chaetica type I, II, III



Fig. 4: Scanning electron micrographs showing sensilla on the ovipositor of Ae. albopictus: (a) Ventral aspect of female ovipositor showing 8th sternite (Stn), 9th tergite (Trg), cerci (Cr I & II), post-genital lobe (PGL) and genital opening (Go) x 500; and (b) Scanning micrograph illustrating the internal sensory structures (i.e.) three types of Sensilla chaetica (SCh I, II, III) on the rim of cerci arranged in circles x 3000

planted in random. The outer line composed of long grooved sensilla cheatica; middle line sensilla are smooth surfaced, shorter in length and arising from a thick socket, while the inner line abode structures with a bulbous base and pointed tip, which are set into a socket similar to sensilla basiconica. On the ovipositor of *Ae. albopictus* different sizes of bristle like chaetica are observed which are long, grooved, smooth surfaced, with varying length and so classified as sensilla chaetica type I ( $19.4 \pm 0.7 \mu m$ ), type II ( $42.2 \pm 1.9 \mu m$ ) and type III ( $84.2 \pm 2.4 \mu m$ ) (Fig. 4b). Some of these sensilla possess a pore at the tip and may be contact chemosensory in nature, playing important role prior to oviposition to test the chemistry of water.

### Discussion

The present study describes various types of sensilla on different body parts of *Ae. albopictus* with distinct morphological characters and morphometric features. In general, five types of stimuli are used by mosquitoes to locate hosts, namely visual cues, water vapours, heat,  $CO_2$  and body odour. The respective sensilla responding to these stimuli would be the compound eyes, grooved pegs, sensilla coeloconica, capitate pegs and sensilla trichoidea<sup>20</sup>. Once a female mosquito has landed the texture and perhaps taste of the host's surface would be perceived first by the tactile setae and contact chemosensilla on the tarsi and subsequently labellum sensilla. Labial sensilla probably respond to cues in the blood during probing.

Sensilla trichoidea are the most abundant sensilla observed in the present investigation, with other types like sensilla coeloconica, grooved peg sensilla and sensilla chaetica on the antennae of *Ae. albopictus*. Similar types of sensilla were described earlier in other species of Aedinine, Culicine and Anopheline mosquitoes<sup>9,10,12</sup>. In the present study, we report two sensilla coeloconica protruding from the terminal tip of antennal flagella of *Ae. albopictus* in a tubular structure; whereas in *Ae. aegypti* and *Anopheles* mosquitoes these sensilla coeloconica were observed at tip of the flagellum; but any protrusion was not observed<sup>12,25</sup>. In the previous studies<sup>10,26,27</sup>, two subtypes of grooved peg sensilla were mentioned, i.e. long and short grooved peg sensilla in some *Aedes* and *Culex* species, however, we have found not only the difference in length but also in tip structure (one is pointed and the other is blunttipped). The highly significant difference in the length of these two types of grooved peg sensilla would indicate a possible difference in the perception mechanism of odour molecules. Bowen<sup>10</sup> has reported that short grooved pegs house sensory cells that are excited by lactic-acid whereas in *Ae. aegypti*, *Aedes atropalpus* (Coquillett), *Ae. epactius* Dyar and Knab and *Culex pipiens* Linnaeus, the lactic acidexcited cells are absent.

Maxillary palps consist of five segments in mosquitoes<sup>12</sup>. On the maxillary palp in addition to sensilla chaetica, the other sensory structures like, capitate pegs, sensilla basiconica, sensilla campaniformia and some non-innervated structures such as microtrichia, cuticular projections were also observed. Similar structures were reported in other mosquitoes<sup>28,29</sup> and in biting insects<sup>11,15</sup>. The capitate pegs in *Ae. aegypti* has been reported to respond to n-heptane, amyl-acetate and acetone<sup>30</sup>.

In the present study one campaniform sensilla was observed in third segment of maxillary palp, which consists of a domed cap that is hinged to the surrounding ring of raised cuticle. Similar structure was reported in Anopheles stephensi Liston<sup>29,31</sup>, however, in Aedes and Culex mosquitoes such structure is not reported. On the labellum, sensilla chaetica were observed surrounded with microtrichia in circular manner. On the basis of their size these sensilla were classified into Ch1, Ch2 and Ch3. Our observations were supported by earlier findings<sup>32,33</sup> in Ae. aegypti and An. stephensi. The other sensilla located on the tarsi and ovipositor would be playing an important role in oviposition site selection/in testing the water chemistry prior to oviposition. It has been observed that once the water is located, a female Ae. *aegypti* then checks the oviposition substrate just above the waterline with tactile setae<sup>18</sup> on the 8th sternite

Among blood feeding dipterans, authors have as-

sumed that the spines on the tarsi might be playing the role in contact chemoreception in black flies, *Simulium vittatum* Zetterstedt and *Similium venustum* Say<sup>17,19</sup>. The leg sensilla of tsetse flies contain taste neurons that respond to uric acid and certain amino acids, which are also positive stimuli for feeding<sup>34</sup>.

A paired sensilla coeloconica is present on the tarsi of the Ae. albopictus females. In Ae. aegypti a pair of sensilla campaniformia at the tarsal margin has been reported<sup>16</sup>, whereas the structure observed in Ae. albopictus does not conform to the morphology of sensilla campaniformia present in the third segment of maxillary palp with a inner dome. The sensillum coeloconicum on the tarsi of mosquito might be playing the role of short-range olfaction in addition to thermo and hygroreception. Tarsal chemoreceptors may respond to vapours and the most noticeable effect of repellent vapours on flight posture of Ae. aegypti is that, the metathoracic legs curled more steeply upwards. In addition, we have observed grooved chaetica of various size and shapes. These sensilla chaetica are presumed to function as contact chemoreceptors while coming in contact with water before deposition of the eggs in addition to mechanotactile function.

Three types of sensilla on the tarsi of *Ae. aegypti*, i.e. campaniform sensilla, spines and hairs<sup>16</sup> have been previously reported which were contact chemoreceptors and having mechanoreceptive neuron in addition to chemoreceptive function. It has been shown that tarsal sensilla can perceive and distinguish concentrations of salts: salinity is a well-known factor in the selection of an oviposition site<sup>35</sup>. However, a possibility that tarsal contact chemoreceptors might respond to vapours should not be ignored.

We observed three lines of sensilla chaetica on the ovipositor of *Ae. albopictus* in this work and it is presumed that these sensilla may function as contact chemoreceptor in addition to mechanotactile function. The sensilla campaniformia on the insula of *Ae. aegypti*, which we have not observed in the present study and the chaetica earlier referred as hair sensilla in *Ae. aegypti*<sup>18</sup>, as well as the distinct morphological attributes of female genitalia of *Aedes* genus in the Indian mosquito species<sup>36</sup> would furnish added information to compare the ovipositor of *Ae. albopictus* with *Ae. aegypti*. In other dipterans, such as *Lucilia cuprina* (Weidemann) ovipositor sensilla plays role of both taste and smell, in *Chrysomya nigripes* Aubertin and *C. megacephala* (Fabricius) ovipositor<sup>37,38</sup> bears contact and mechanotactile sensilla.

This study will be useful in explaining the mechanism of odour perception and chemoreception by these sensilla and could possibly help in assessing the vectorial capacity of female mosquito. The results of sensillary study by several workers on other mosquitoes together with our preliminary investigation on the sensillary structures distributed on various body parts of *Ae. albopictus*, using electron microscope makes it possible to outline the types of sensory structures in the Asian tiger mosquito to understand their possible role in mosquito behaviour.

#### Acknowledgement

We gratefully acknowledge the help and encouragement from Dr R. Vijayaraghavan, Director, Defence Research and Development Establishment, Gwalior and all the members of Entomology Division for technical assistance in maintaining the mosquito culture and the financial support from Project DRDE-175 of our Establishment.

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Received: 12 November 2008 Accepted in revised form: 26 March 2009