Most of the Investigations on transmissible diseases were done during 19th and early 20th centuries. Japanese encephalitis (JE) is an arboviral disease, spread to humans by infected mosquitoes. JE basically is a rural disease as the major vectors of JE breed in rice fields. It is estimated that around 1.9 billion people live in rural JE prone areas of the world (Arunachalam et al., 2009). Nearly 3 billion people (about 60% of the world’s population) live in JE endemic regions (Halstead and Tsai, 2004). In India, major outbreaks of JE were reported in rural areas (Reuben and Gajanana, 1997). A study showed that nearly 36.36% of cases were reported from urban areas whereas, 63.63% from rural areas of Uttar Pradesh (Roy et al., 2006).

The incidence of JE was noticed almost every year in Andhra Pradesh (AP) in the districts of Kurnool, Ananthapur, Prakasam, Warangal etc., (Murty et al., 2000). Entomological assessment indicates that Cx. tritaeniorhynchus and Cx. gelidus are the major vectors in Kurnool district, AP based on relative abundance, and more number of virus isolations (Arunachalam et al., 2009). Male mosquitoes indirectly play a role in spread of disease by participating in mating and migration (Murty et al., 2000; Hight et al., 2003). Different mosquito species have variable potency of vector carrying capacity. This vector carrying capacity depends directly or indirectly on various biophysical and biochemical parameters of mosquito. Hence, the present study was carried out with an aim to analyze the
biophysical and biochemical parameters of Japanese encephalitis vectors, *Culex tritaeniorynchus* and *Cx. gelidus*.

**OBJECTIVES OF THE THESIS**

- Calculation of wing beat frequency in *Cx. tritaeniorhynchus* and *Cx. gelidus*.
- Design of flight surface and compute the Aerodynamic parameters of *Cx. tritaeniorhynchus* and *Cx. gelidus*.
- Determine the speed of flight in *Cx. tritaeniorhynchus* and *Cx. gelidus*.
- Calculation of power requirements for the flight of *Cx. tritaeniorhynchus* and *Cx. gelidus*.
- Comparative biochemical analysis in *Cx. tritaeniorhynchus* and *Cx. gelidus*.

**SCHEMATIC OVERVIEW OF WORK PLAN**

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Field survey
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Collection and transportation of Mosquitoes
↓
Calculation of Wing beat frequency
↓
Basic and derived parameters
↓
Speed of flight
↓
Power requirements of flight
↓
Comparative Biochemical analysis
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METHODOLOGY IN BRIEF

The suburban areas located on the margin of Kurnool were monitored for the collection, identification and transportation of Cx. tritaeniorhynchus and Cx. gelidus mosquitoes during experimental period.

Wing beat frequency of the mosquito is an important parameter not only from the point of view of aerodynamics and bio-energetics, but also from the point of view of ecological relationships. Wing beat frequency of Cx. tritaeniorhynchus and Cx. gelidus were calculated using theoretical models like Mechanical oscillator theory (Greenwalt, 1960), Dimensional analysis (Deakin, 1970), Newton’s law (Crawford, 1971) and Mass flow theory (Puranik et al., 1977). Wing beat frequency was calculated experimentally by using stroboscopic flash method (Adeel Ahmad, 1982). Observed wing beat frequency from experiment was compared with the theoretical models of wing beat frequency.

For computing moment of inertia of flight surface (wing) the wings were removed from the body, fixed on a graph sheet and were made into number of strips. The masses of the wing strips were measured using a sensitive balance (SARTORIUS ME-5) of least count 0.001 mg and the area of the strips were measured by keeping them under an enlarger, projecting on a graph sheet. This method of theoretical determination of moment of inertia of an
insect wing is similar to that of Adeel Ahmad (1984). Theoretical determination of basic and derived parameters of a flier is similar to that of Hasan et al., (1983).

In order to study the speed of mosquitoes under well defined aerodynamic conditions, a wind tunnel was designed and constructed in the laboratory (Radha krishna, 1995). The forward velocity of mosquitoes, Cx. gelidus and Cx. tritaeniorhynchus was studied under the wind tunnel.

Power requirement studies of Cx. tritaeniorhynchus and Cx. gelidus were carried out by the theoretical equations given by Puranik et al., (1976). In the present investigation, helicopter theory was considered for the calculation of induced power, while inertial power is determined by considering the differential equation related to oscillatory motion of the wing when the mosquito is in the state of its hovering flight.

Biochemical analysis was done in Cx. gelidus and Cx. tritaeniorhynchus. Sugar (glucose), lipid and glycogen were measured from mosquitoes before and after flight using Van Handel and Day method (Van Handel, 1985a, b; Kaufmann, 2008).

SUMMARY

➢ Entomological assessment indicated that Cx. tritaeniorhynchus and Cx. gelidus are the major JE vectors in Kurnool district, Andhra
Pradesh, South India based on relative abundance, and more number of virus isolations.

- The suburban localities located on the margin of Kurnool are defined as periurban areas, which are outside the municipal boundaries and thus without basic civic amenities. These areas were monitored for the collection, identification and transportation of *Cx. tritaeniorhynchus* and *Cx. gelidus* mosquitoes during experimental period.

- Wing beat frequency of the mosquito is an important parameter not only from the point of view of aerodynamics and bio-energetics, but also from the point of view of ecological relationships.

- Wing beat frequency of *Cx. tritaeniorhynchus* and *Cx. gelidus* were calculated using theoretical models like Mechanical oscillator theory, Dimensional analysis, Newton’s law and Mass flow theory.

- Wing beat frequency was calculated experimentally by using stroboscopic flash method. Observed wing beat frequency from experiment was compared with the theoretical models of wing beat frequency.

- The present study on wing beat frequencies of *Cx. tritaeniorhynchus* and *Cx. gelidus* suggest that the values of wing beat frequency computed by the theories proposed by Greenewalt,
Deakin and Crawford are not in agreement, while the mass flow theory shows significantly good agreement with the experimental values.

- The observed wing beat frequency \( (v_o) \) was more in females of *Cx. gelidus* (529Hz) than females of *Cx. tritaeniorhynchus* (403 Hz). In males also, the observed wing beat frequency \( (v_o) \) of *Cx. gelidus* (621 Hz) was higher than *Cx. tritaeniorhynchus* (465 Hz).

- Aerodynamic parameters are useful in understanding flight behaviour, flight energetics and power economy of a natural flier. When a flier (mosquito) is in the hovering state, it is said to be in the dynamical equilibrium which is achieved by the flier by generating the air induced downwards due to wing beat in turn develops a reacting force, just to balance its body weight. The flier and induced air put together is considered as a “system”. Thus the system constitutes an “action-reaction pair” in the hovering flight which helps the flier to be airborne.

- Basic and derived body parameters of flier include static parameters, dynamic parameters, dynamic parameters of induced air, and aerodynamic parameters. These parameters were calculated in both *Cx. tritaeniorhynchus* and *Cx. gelidus*. The present study on flight of *Cx. tritaeniorhynchus* and *Cx. gelidus* explains adequately the phenomenon of bio-aerodynamics in relation to hovering and forward flights. The Basic and derived
body parameters of *Cx. tritaeniorhynchus* were compared with that of *Cx. gelidus*.

- It was observed that static parameters differ considerably in male and female of both *Cx. tritaeniorhynchus* and *Cx. gelidus*, more in females than males.

- Dynamic parameters also differ in male and female mosquitoes as observed in static parameters. The moment of inertia is an important parameter, which is a measure of the resistance, a body offers to a change in the rotational motion about a given axis and hence it is very such useful in computing kinetic energy of the rotating wing. The moment of inertia in females of *Cx. tritaeniorhynchus* (0.289 x 10^{-6} gm-cm^2) was more than the moment of inertia in females of *Cx. gelidus* (0.246 x 10^{-6} gm-cm^2).

- The dynamic parameters of air induced in downward direction by a flier due to its wing beat are mass, rate of mass flow, acceleration, velocity and linear momentum. Among these parameters, the rate of mass flow of induced air, an important parameter to understand the hovering flight and it bears a linear relationship with mass of the flier. It was observed that the mass of induced air is more in females than males (in both *Cx. tritaeniorhynchus* and *Cx. gelidus*).

- The aerodynamic parameters are aspect ratio, wing loading, disc loading, wing mass/body mass and wing beat frequency. Wing loading, the body mass of a flier supported by its wing area, is an
essential parameter of the flight of natural fliers. In both males and females, the wing loading of *Cx. tritaeniorhynchus* was less than that of *Cx. gelidus*, suggesting that *Cx. tritaeniorhynchus* has relatively high aerodynamic efficiency than *Cx. gelidus*.

- The mechanism of flight of mosquito is very similar as seen in majority of insects. The speed of flight of mosquitoes, *Cx. gelidus* and *Cx. tritaeniorhynchus* was studied using the wind tunnel experiment.

- Speed of *Cx. tritaeniorhynchus* in females was slightly higher than males, whereas in *Cx. gelidus*, males have higher speed of flight than females.

- Power requirement studies on bio-energetics of fliers is useful in understanding the “mechanical efficiency” of flight muscles, “dynamic efficiency” of flight surfaces and “aerodynamic efficiency” of fliers during their flights. Power requirements of the flier include induced power, inertial power, aerodynamic power and dynamic efficiency.

- Induced power, an important parameter for the understanding of the energetics of hovering flight, is the power required to accelerate the air in downward direction, which is the basic requirement for the hovering flight of a flier. The present study suggests that the induced power was considerably high in female mosquitoes when compared to males (in both *Cx. tritaeniorhynchus* and *Cx. gelidus*).
Inertial power is the energy required per second to oscillate the wings. In *Cx. tritaeniorhynchus*, inertial power was more in females than in males where as in *Cx. gelidus*, inertial power is almost similar in both males and females.

During the hovering flight, mosquitoes consumed a large quantity of inertial power to maintain wing vibrations when compared to induced power and aerodynamic power.

Aerodynamic power is the power required to move the flier in forward direction. Aerodynamic power is sex dependent and it is more in females than in male mosquitoes. However, females of *Cx. tritaeniorhynchus* required more aerodynamic power when compared with females of *Cx. gelidus*.

The flight energy of mosquitoes is derived from the polysaccharide glycogen stored in fat body and flight muscle, and the disaccharide sucrose (or its components glucose and fructose) obtained from nectar and fruit juices and stored in the crop. In the present study, biochemical analysis of *Cx. tritaeniorhynchus* and *Cx. gelidus* was carried out and role of carbohydrates and lipids in the flight was discussed.

In sugar fed *Cx. tritaeniorhynchus*, more carbohydrate content was utilized for flight and less for survival of non flown controls. In contrast, *Cx. gelidus* utilized less carbohydrate content for flight and more for survival of non flown controls.
In both *Cx. tritaeniorhynchus* and *Cx. gelidus*, blood fed mosquitoes utilized more percentage of carbohydrate content for flight than sugar fed mosquitoes.

**CONCLUSIONS**

- The present study is the first initiative to determine the wing beat frequency of Japanese encephalitis disease vectors, *Cx. tritaeniorhynchus* and *Cx. gelidus*.

- It was observed from the present study that the values of wing beat frequency computed by the theories proposed by Greenewalt, Deakin and Crawford are not in agreement, while the mass flow theory has shown significantly good agreement with the experimental values.

- Mass and observed wing beat frequency of the study has shown that in both *Cx. tritaeniorhynchus* and *Cx. gelidus*, mass of the mosquitoes is inversely proportional to the wing beat frequency.

- The present study on basic and derived parameters of *Cx. tritaeniorhynchus* and *Cx. gelidus* explained adequately the phenomenon of bio-aerodynamics in relation to hovering and forward flight. It was observed that static parameters differ considerably in males and females of both *Cx. tritaeniorhynchus* and *Cx. gelidus*. 
The kinetic energy of the flight surface of *Cx. tritaeniorhynchus* was less than that of *Cx. gelidus* (in both males and females).

It was evident from the present study that many of the dynamic parameters of induced air of *Cx. tritaeniorhynchus* and *Cx. gelidus* are sex dependent and these parameters are more in females and less in males.

It was observed from the present study that due to relatively less aspect ratio, species of *Cx. tritaeniorhynchus* have more maneuverability and power economy than species of *Cx. gelidus*.

For the first time speed of flight of *Cx. tritaeniorhynchus* and *Cx. gelidus* was studied. Speed of *Cx. tritaeniorhynchus* in females was slightly higher than males, whereas in *Cx. gelidus*, males have higher speed of flight than females.

The present study is the first initiative to determine the power requirements of *Cx. tritaeniorhynchus* and *Cx. gelidus*.

Induced power is directly proportional to the body mass of the fliers in both *Cx. tritaeniorhynchus* and *Cx. gelidus*.

It was observed from the present study that during the hovering flight, mosquitoes consumed large quantity of inertial power to maintain wing vibrations when compared to induced power and aerodynamic power.
It was observed that females of *Cx. tritaeniorhynchus* required more aerodynamic power when compared with females of *Cx. gelidus*.

In the present investigation, in absolute terms, both sexes of *Cx. tritaeniorhynchus* carried over twice the amount of glycogen and one and half times of lipid than *Cx. gelidus* at their eclosion.

In conclusion, the present study provided the valuable information on biophysical and biochemical parameters of JE vectors, *Cx. tritaeniorhynchus* and *Cx. gelidus*.

**SIGNIFICANCE OF THE WORK**

Mosquito flight has a meaning and purpose either for feeding or breeding. The present study is focused on how aerodynamic parameters are directly related to the flight of mosquito that will give a lead to understand the flight mechanism. Many aerodynamic parameters of mosquito have direct or indirect relation with the flight and its associated factors. For example, aspect ratio is an index of maneuverability and power economy. If aspect ratio values are high, the mosquito can hover longer period in the air during swarming and insemination, which in turn promote the production of fertilized eggs. Ultimately population dynamics can also be estimated by using Aspect ratio.

Wing beat frequency (WBF) gives an over view about the distance travelled by the mosquito from breeding place to target place.
If the WBF is high, the consumption of kinetic energy (KE) to maintain such high frequency is also high. Once KE is high, the individual can become fatigue very quickly and it can travel shorter distance. On the other hand if WBF is low, distance travelled by the individual is more since the KE consumption is less. It is an energy conservation mechanism where WBF has a direct role to play. India is badly experienced with many outbreaks and in depth analysis is required to find out the actual factors responsible for the menace. Once the aerodynamic parameters are analyzed, it is very easy to estimate the distance and dispersal of mosquito so that number of villages covered during mosquito flight will be known well in advance. The identified risk villages can be treated with both vector control and drug administration to reduce the mortality and morbidity. Hence the present study has a significant role in reducing the outbreaks of vector borne diseases by understanding the flight mechanism of JE vectors, Cx. tritaeniorhynchus and Cx. gelidus.

FUTURE PERSPECTIVES

Japanese encephalitis (JE) is an arboviral disease, spread to humans by infected mosquitoes. JE disease can affect central nervous system (CNS) and cause severe complications even leading to death. It is estimated that around 1.9 billion people live in rural JE prone areas of the world. Apart from India, JE cases were also reported in other non-Asian countries like, Torres Strait of Australia mainland. Nearly 3
billion people (about 60% of the world's population) live in JE endemic regions.

The present study gives valuable information on biophysical and biochemical parameters of Cx. tritaeniorhynchus and Cx. gelidus, which are directly or indirectly responsible for spread of JE disease. This valuable information is very much useful for the researchers who are working in the area of Japanese encephalitis. Further studies are required to know the trehalose levels in the haemolymph of JE vectors. Studies are needed to be done on flight hormones of JE vectors. Investigations are required to understand the adipokinetic hormone (AKH), which plays a main role in the flight of mosquitoes. Identification and sequencing of AKH genes needed to be done through bioinformatics studies. The present study is expected to egress more biotechnological contributions in the future research.

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**Wing Beat Frequency of Japanese Encephalitis Vectors, Culex tritaeniorhynchus and Cx. gelidus in Andhra Pradesh**

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**Flight Performance Studies on Culex Gelifus (Diptera: Culicidae) Mosquito, a Vector of Japanese Encephalitis**

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**Aerodynamic Parameters and Design of Flight Surface in Japanese Encephalitis Vector, Culex tritaeniorhynchus (Diptera: Culicidae) in Andhra Pradesh**

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