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A SNAPSHOT OF FEW BIOLOGICAL AND BIONOMICAL CHARACTERISTICS OF*ANOPHELES CULICIFACIES* AND *ANOPHELES ANNULARIS* IN THREE MALARIOGENICALLY STRATIFIED DISTRICTS OF ODISHA, INDIA

By

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Abstract

Despite tremendous efforts over the past century, malaria remains the major health burden in the state Odisha, India. In view of designing a situation specific malaria control strategy, baseline data on bionomics of major *Anopheles* vectors were collected from three malariogenical stratified districts of Odisha. A comparative study of vector abundance, seasonal prevalence, habit/habitats, resting sites preference, parity rate, proportion of human blood fed vectors and Anopheline species composition was established in a high (Kalahandi), moderate (Bargarh) and a low endemic (Cuttack) districts, when malaria transmission was at its peak level. The mosquito collection showed a wide range of Anopheline fauna diversification with collection of two efficient malaria vectors i.e. *An. culicifacies*, the primary vector with a high peak value during monsoon and *An. annularis,* the secondary vector in three study districts. Both vectors was significantly more in indoor than outdoor in the three districts and among indoor collections, the density was higher in CS than HD whereas the density was more in HD than CS in Cuttack district for *annularis*. Similarly, their feeding, biting pattern as well as parity rate varies among these districts. The molecular identification of *An. culicifacies* revealed the presence of all five sibling species viz. A, B, C, D & E whereas only species A was detected out of two sibling species A & B of *An. annularis*. These entomological indicators such as vector density, distribution, biology and bionomics as well as their vectorial attributes are important parameters to measure the pattern and intensity of malaria transmission. Proper monitoring and evaluation of these indices during the peak transmission period can reduce the increasing trend of malaria.

Key words: India, Malaria, Biology, Bionomics, *Anopheles*, Odisha

Introduction

Despite tremendous expansion in the financing and malaria control interventions, the disease continues to be a global health threat (Garcia, 2010). Odisha, an eastern state of India is highly endemic for malaria and bears almost a quarter of the country's total burden (Pradhan *et al*, 2016). There was no significant decline in malaria incidences for decades in spite of several programmatic efforts. However, this hyper-endemic state is now a role model not only in the country but also globe for its fight against malaria. Since 2017, India showed an impressive reduction in malaria cases out of all the eleven highest burden countries and the credit solely went to OdishaState. The possible reason for this success included increased political support, domestic funding, strong leadership, awareness programs and highly effective control interventions (WHO, 2018). By the initiative program DAMaN (Malaria Elimination in Remote Areas) Odisha did strong efforts on prevention, diagnosis and treatment of malaria. Apart from this, partnership of Abbott with State govt. system such as NVBDCP strengthens Odisha's malaria detection and surveillance system. Using past and present experiences Odisha has pledged to eliminate malaria by 2030. Recent innovative vector/ malaria control intervention tools to increase confidence in elimination. But, to confront one of the major challenge i.e. asymptomatic malaria case detection, improved and more sensitive diagnostic methods need to be scaled up. Also, adequate health infrastructure, brief knowledge on prevalent vector species, the ecology, biology and bionomics, area specific intervention strategies as well as adequate research on parasite /vector's complex molecular biology and new drugs and vaccine development will play a pivotal role in malaria elimination. There is an uneven distribution of intensity of malaria transmission across the globe. Environmental and topography factors determine the intensity of malaria transmission expressed as hyper-, meso-, and hypo-endemicity (Giles and Warrell, 1993). Malariogenic stratification was due to variable ecological conditions and climatic diversity that create a suitable environment for malaria vector responsible for variable vectorial attributes (Marten *et al*, 1995; Rao *et al*, 2015). Millions of people died annually as a result of this mosquito borne disease though it can be completely curable if diagnosed at an early stage but to completely prevent the disease an effective vector control was highly essential which relied on understanding of vector transmission dynamics (Russell *et al*, 2013). Distribution, abundance, habit/habitat preference feeding, resting, biting behavior, gonotrophic condition, infection status, parity rate of major *Anopheles* fauna were important factors affected the risk of malaria transmission (Kent *et al*, 2007; Tchuinkam *et al*, 2010; Tainchum *et al*, 2014). In the context of malaria elimination, the value of understanding the biology and bionomics of malaria vectors as their role in malaria transmission dynamics is highly critical. The present study highlighted some of these features of major *Anopheline* vector fauna in a high (Kalahandi district), moderate (Bargarh district) and a low endemic (Cuttack district) region of the state, Odisha. Hyper endemic district Kalahandi is situated in south western region of Odisha between latitude19º 3' N to 21º 5' N and longitude82º 30’ E to 83º 74' E. The district is highly endemic for malaria, dominant with two major vector species viz. *An. fluviatilis* and *An. culicifacies* (Sharma *et al*, 2004; Sahu *et al*, 2017). But, the hypo endemic sub coastal district, Cuttack is located at 20º 31' 23" N latitude and 85º 49' 60" E longitude covering an area of3932 km^2 richer in few sibling species, *An. culicifacies,* & *An. subpictus* (Tripathy *et al*, 2010; Kumari *et al*, 2013). The moderate malaria endemic district, Bargarh is located in western Odisha at 21.33º N 82.62ºE covering an area of 5,837 km^2. Although a few reports regarding malaria vectors and their dynamics in specific areas of Odisha were available, but to the authors knowledge no systematic study was done on distribution, abundance, bionomics, attributes...etc. Last few years data showed that mortality graph continuously projected upwards, peaking in June and July, when there is arrival of monsoon season in the state. Few major vectorial features were investigated of dominant *Anopheles* mosquitoes in three malariogenically stratified districts where each of them represented a respective endemic zone during transmission peak.

Materials and Method

Study area and period**:** Kalahandi, Bargarh and Cuttack are the three districts of Odisha confined to south-western, western and eastern region of the state respectively (Fig. 1). On the basis of malariogenic stratification, thes three districts differ from each other in relation to API (Annual Parasite Incidence). The study was done over three years from January 2015 to December 2017.

Seasonal features**:** Odisha, lying just south of the Tropic of Cancer has a tropical climate. It experiences three well defined meteorological seasons: Summer (March-May), rainy (June-October) and winter (November-February). The average rainfall is 150cm directly influenced by the south-west monsoon and the month of July is the wettest period of the year. The monsoon shower during that period creates breeding ground for all sorts of mosquitoes including *Anopheles* species.

Mosquito collections: *Anopheles* species were collected from ten randomly selected malaria prone villages from each of the three districts. Eighteen houses from each village were selected and geographical information system (GPS) coordinates of each one were listed using differential GPS. Indoor resting mosquito collection was carried out twice /day in each village during morning (6:30-

8:30 AM) and evening (6:30-8:30 PM) hours from cattle sheds (CS) & human dwellings (HD). Majority of the houses were made of mud walls and thatched roofs. The indoor resting collection was performed by Pyrethrum Spray Catch early in the morning from 7:00 to 9:00hrs in 5-6 houses. Equal time was spent for collection of mosquitoes from different habitat and resting sites of each house/site using an oral aspirator and a flashlight. Outdoor collection from variety of environments, such as in dry pots, waste broken containers, water pipes, tree bases, tree holes, cracks and holes in the ground etc. Also, overnight collection was carried out using dry cell battery operated CDC light traps placed inside houses (near bed of occupants slept under insecticide treated nets) and cattle sheds operated from 18:00 to 6:00hrs. Next morning, mosquitoes were collected using an aspirator. All mosquitoes were transported to laboratory for identification and molecular processing.

Mosquito identification: Female *Anopheles* were identified using standard keys (Barraud, 1934; Habarch *et al,* 2007), sorted out based on abdominal conditions in field & laboratory and dissected for parity status (WHO, 1975).

Sibling species of *Anopheles*: PCR identified primary Anopheline vector (*An. culicifacies* & *An. annularis*), followed by methods standardized (Das *et al,* 2013; 2014).

Human blood meal screening: Each individual female mosquito was stored in a 1ml labeled micro-centrifuge tube with unique identifier. Genomic DNA was extracted after protocol of Barik *et al.* (2013), and PCR screened human blood (Rath *et al,* 2017).

Data analysis: Density of mosquitoes was expressed as number of female *Anopheles* collected/man/hour. One way ANOVA compared the mean density of total predominant Anopheline fauna among three districts during three different seasons. A two sample t-tests compared habit and habitat preference of two vector species and the quantitative distribution of human blood fed *Anopheles* vectors species between three districts. Also, parity statuses of major vectors were compared by the Mann-Whitney U-test. Kruskal-Wallis test determined significant variation among Anopheline vectors in Odisha districts, and analyzed by Microsoft Excel 2010 spread-sheets (Microsoft Corp., Redmond, WA, USA). P-value < 0.05 was significant.

Results

As during monsoon period transmission rate was high in the state, some of the vector dynamics showed highlighted during such a period. Although *An. subpictus* was abundant in the three districts, but not find any vectorial potential/features. During the study quantitatively little no. of *An. fluviatilis*, *An. minimus*, *An. stephensi* and *An. sundaicus,* were collected even in high endemic Kalahandi district. But, the study focused only on *An. culicifacies* and *An. annularis.*

Seasonal distribution pattern of major Anopheline fauna in three districts: Adult mosquito collection from three areas showed a wide range of Anopheline fauna diversification with collection of two efficient malaria vectors i.e. *An. culicifacies*, the primary vector and *An. annularis,* the secondary vector. The per man hour density of *An. culicifacies* was higher in Kalahandi district as compared to other two study districts. One-way ANOVA data showed the density variations between seasons were highly significant during monsoon period ($p<0.05$) in Kalahandi without significant density variation between Bargarhand-Cuttack district. *An. annularis* did not show significant difference per man hour density related to season and area.

Resting habit and habitat of *An. culicifacies* and *An. annularis* (indoor vs. outdoor & human dwelling vs. cattle shed): After seasonal prevalence, resting behavior of major *Anopheles* vectors during peak transmission period being an important entomological parameters supported transmission dynamics. T-test showed that density of both vectors was significantly more in indoor than outdoor ($p<0.05$) in all three districts. Density indoor collections was significantly higher in

CS than HD in Kalahandi district for *An. culicifacies* ($p<0.05$). *An. annularis* density was more in CS than HD in Kalahandi and Bargarh without significant variation among both biotopes. A significant difference was between the two habitat in Cuttack district where it was more in HD than CS ($p <0.05$).

Biting activities of major *Anopheles* species: Each species nocturnal biting was expressed as number of mosquitoes collected from CS/HD. *Anopheles culicifacies* showed a peak biting rhythms between 12:00 AM to 3:00 AM in all three selected areas which indicated that the biting activity of *An. culicifacies* was not area dependent rather than slight variation in nocturnal biting preference between CS and HD. In *An. annularis* there was quite variation in biting preference, which ranged between 10:00PM to 4:00 AM with significant variation in CS and HD. The biting activity was higher in CS based on density. In human dwelling, peak biting time was during the wee hours in Kalahandi and Cuttack district, but in Bargarh district was between 9:00 to 10:00 PM.

Human blood feeding preference of major *Anopheles* species: Anthropophagic activity of *Anopheles* vectors (N=50) was expressed by human blood meal screening. A significant difference was between monsoon and summer/winter ($p<0.05$). Human blood fed of *An. culicifacies & An. annularis*) was more in monsoon season as compared to other two seasons. Anthropophagic vectors were more in Kalahandi district in all seasons. While highlighting peak transmission period was among Kalahandi-Bargarh and Kalahandi-Cuttack district for *An. culicifacies*. For *An. annularis,* significant variation in anthropophagic vectors was among Kalahandi-Bargarh and Cuttack-Bargarh District ($p<0.05$).

Parity: Parity rate of female *Anopheles* vectors (N= 50) assessed age and longevity. A significant difference was between *An. culicifacies* parity rate in all districts (Kalahandi-Bargarh: U=0, z=2.506, p= 0.012; Kalahandi-Cuttack: U=0, z=2.506, p=0.0128; Bargarh-Cuttack: U=1, z= 2.29 p=0.021). A significant variation in parity rate of *An. annulais* was detected between Kalahandi- Bargarh and Kalahandi-Cuttack district where as no significant difference was between Bargarh-Cuttack District (Kalahandi-Bargarh: U =2.5, z= 1.98, p=0.047; Kalahandi-Cuttack: U=2.5, z= 1.984, p=0.04;Bargarh-Cuttack: U= 12.5, z= 0.10 p=0.920).

Species composition of *An. culicifacies* & *An. annularis*: Molecular identification of *An. culicifacies* (N=30) showed five sibling species viz. A, B, C, D & E. Species B & D was detected in all districts. Prevalence of species B was more in Kalahandi followed by Bargarh and Cuttack as prevalence of species D was observed in alternate manner i.e. more in Cuttack followed by Bargarh and Kalahandi district. Kruskal-Wallis test showed a significant difference among B & D species among all districts (Sp B: H=9.05, p=0.0107; Sp D: H=10.33, p=0.0056). Sibling species C & E were only in Kalahandi and Bargarh district. *Anopheles annularis* (N=20) showed only sibling species A in all districts without significant difference in relation to abundance (H= 4.22, p=0.121). Some vectors were not detected by PCR, and categorized as unidentified ones.

Discussion

Malaria is a vector-borne endemic disease msinly in tropical and subtropical ecosyst- ems which endemicity changes with change in ecological, climatic, and socio-developmental conditions (Sutherst, 2004; Patza *et al*, 2016). India accounts for about two-thirds of all malaria cases in Southeast Asia regions (WHO, 2011). The state, Odisha is high malaria transmission zone compared to other states of India (Das *et al*, 2006). Despite tremendous expansion in malaria control operations, it remained a long-lasting global health threat (Cohen *et al*, 2010). Its elimination efforts were hampered due to lack of proper transmission dynamics information (De Silva *et al*, 2012). A highly reliable and accurate transmission intensity measuring tool in endemic region was a must (Drakeley *et al*, 2003). Though, key determinants of malaria transmission included vector profile, environmental, ecological, socio-economic and climatic factors that affected control efficacy (Sutherst, 2004; Louise *et al*, 2009). Malaria epidemicity is preventable by regular monitoring and screening of Anopheline vector(s), distribution and biology/ bionomics during the active transmission.

In Odisha, major primary vector species included *An. fluviatilis, An. culicifacies* and *An. minimus* whereas secondary vectors viz. *An. annularis, An. subpictus,* and *An. varuna* were the majority (Sahu *et al*, 2011; Dash, 2014; Sharma *et al*, 2015). *An. culicifacies* high prevalence in hyper-endemic Kalahandi area was in rainy season, which agreed with Pampana (1969) and Nagpal *et al*. (1983). But, secondary vector *An. annularis* did not show any seasonal prevalence in all districts. Density was visibly more during monsoon period, without significant variation as species didn't associate with seasonal rhythms in such areas. Also, statistical significance alone cannot uncloud the complex biological dynamics of vectors and climate factors. Majority of villages were surrounded by open field and dense forest, mosquito species were distributed widely and was very difficult for outdoor collection. This reason might be due to lower vector density in outdoor than indoor in the three districts. *An. culicifacies* and *An. annularis* were predominantly zoophlic and preferred cattle sheds (Waite *et al*, 2017). In the present study, *An. culicifacies* were found in greater densities in cattle sheds (CS) rather than human dwelling (HD) in all districts. The availability of eaves and crevices of thatched cattle sheds provide a preferential environment for vector species and easy availability of hosts without any repellent pressure. Targeting the zoophagic vectors provided a cost effective for efficient vector control to eliminate malaria (Waite *et al*, 2017). *An. annularis* was more dominant in CS than in HD except in Cuttack area, as some species might alter their behavior to the anthropophlic ones. A species specific endogenous circadian rhythm regulated the mosquitoes daily activities that explained why several *Anopheles* species in same habitat exploit their attributes at different times (Beck, 1968). Few studies declared possibility of genetic factors in influencing mosquito biting rhythms (Reisen *et al*, 1978). Accurate data on biting pattern of *Anopheles* vectors was a prerequisite for mounting any control intervention. In the present study, the peak biting activities of *An. culicifacies* & *An. annularis* were evaluated by hourly collection from 18:00 to 6:00hrs in both CS and HD. Biting peak rates of *Anopheles* species synchronize with the inhabitants activities. *Anopheles culicifacies* generally prefers biting at midnight but few found that the activities increase during night early hours in human dwelling (Singh *et al*, 1995). *An. culicifacies* exhibited a typical nocturnal biting pattern followed by appearance of a sharp peak in midnight. The biting rate for *An. annularis* was more in CS than HD which revealed the behavioral plasticity of that species tended to avoid any intervention, protective measures or repellent pressure in HD. Further, area wise variation in the peak period indicated the adaptive feature of these species to avoid competition for hosts. The success of malaria elimination depends on all entomological indices to better understanding biology and bionomics of mosquito vectors. One of such parameter is the human blood fed proportion, more commonly anthropophagic vectors to determine host preferences and vectorial importance. Greater frequency of feeding on humans increased species vectorial capacity. *Anopheles* fauna in Odisha displayed the high anthropophlic index (AI) of secondary vector *An. annularis* as compared to primary vector *An. culicifac ies* (Parida *et al*, 2007).

In the present study, high proportions of anthropophagic vectors were collected from Kalahandi district. Due to greater abundance of human blood fed vectors, Kalahandi district exhibited a higher incidence of malaria than other two districts. The high density of anthropophagic *annularis* vectors in Cuttack district during monsoon season may favor disease transmission in some endemic pockets. But, meso-endemic Bargarh district was not significantly influenced by this parameter that showed some other factors like climatic, ecological, socio-economic, demographic that might regulate the endemicity in this district.

Estimation of parity rate of female *Anopheles* vectors reflected the epidemiological importance of vector populations (Barbosa *et al*, 2016). In this study, the parity rate data showed a defined pattern where the rate was high in hyper-endemic Kalahandi followed by moderate-endemic Bargarh and low endemic Cuttack district for *An. culicifacies*. Though, *An. annularis* showed the similar pattern, slight deviation occurred almost in Bargarh and Cuttack district. The high parity rate indicated that majority of mosquitoes in high malarigenous areas obtain blood meal and complete at least one or more gonotrophic cycles and have high survival rate and vectorial capacity. The high parity rate also highlighted the abundance of older female vectors in that area due to failure of any vector control strategy. The monsoon season is usually the malaria transmission season in the state, and this high parity rate was behind high survival index of vectors and disease transmission.

In the present study, *An. culicifacies* predominante in these districts, and fewer *An. fluviatilis* were identified during that period, but predominant primary malaria vectors in southern districts (Sahu *et al*, 2008). The sibling species (A, B, C, D, & E) of Culicifacies complex were prevalent in Odisha with B the predominance species, but E was an efficient vector (Tripathy *et al*, 2010; Das *et al*, 2013). All sibling species of *culicifacies* in Kalahandi district with predominated species B. Species A & C were absent in Bargarh and Cuttack respectively. Species D was predominant in Bargarh district. Anthropophlic species E was prevalent in Kalahandi followed by Bargarh district due to anthropophlic species complexes which slowly increased endemicity in Bargarh district to hyper-endemicity in Kalahandi district. *An. annularis* and *An. culicifacies* were predominant all year around. During high transmission period among *annularis* sibling species, only A was abundant in all districts. This agreed with Das *et al*. (2014) who detected *An. annularis* A with potential vectorial capacity from 13 endemic districts of Odisha. New cryptic species emerged, it becomes increasingly apparent to study their genetic diversity to identify them and vectorial attributes for planning effective vector control strategies (Stevension and Norris, 2017).

Conclusion

The present data showed that malaria transmission did not change its pattern and intensity rather the ruling vectors and their attributes were the key determinants associated with transmission. Feasible control activities must be based on understanding epidemiology of malaria in the targeted area, vector species involved transmission dynamics and their behavior. This provided a baseline for evidence based planning and implementation of malaria control strategies.

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Conflict of interest: The authors have no conflicts of interest.

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Explanation of figures

Fig. 1: Odisha map showed study areas viz. Cuttack, Bargarh and Kalahandi district.

Fig. 2: Seasonal distribution of two major Anopheline fauna in Kalahandi, Bargarh and Cuttack district. Error bar= standard error of mean.

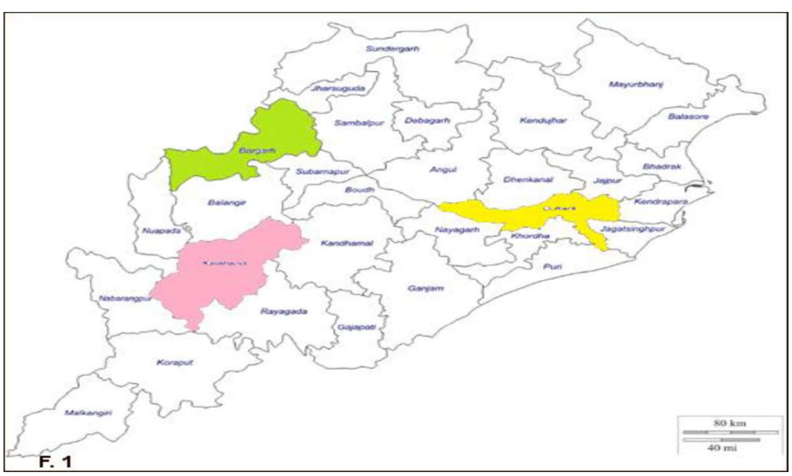
Fig. 3: Habit and habitat preference of major *Anopheles* species in Kalahandi, Bargarh and Cuttack district of Odisha. Error bar= standard mean error.

Fig. 4: Biting activity of major Anophelin*e* vectors in Kalahandi, Bargarh and Cuttack district of Odisha. CS-cattle shed; HD-human dwelling, mosquito density in number.

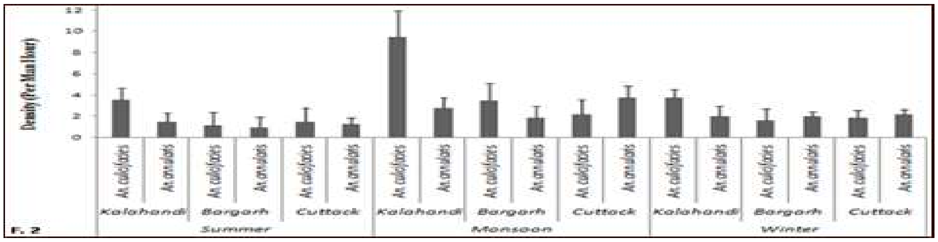
Fig. 5: Human blood fed *An. culicifacies* and *An. annularis* density in summer, monsoon and winter season. Error bar= standard mean error.

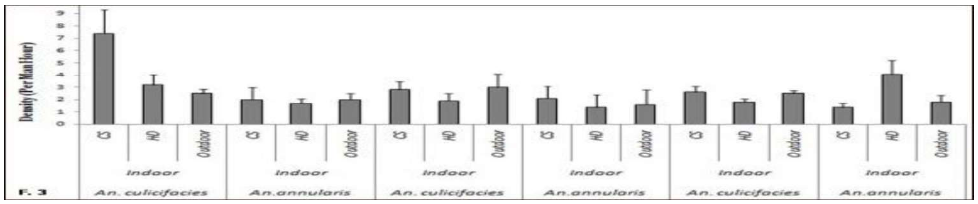
Fig. 6: Parity rate of *An. culicifacies* and *An. annularis* in Kalahandi, Bargarh and Cuttack district. Error bar= standard mean error.

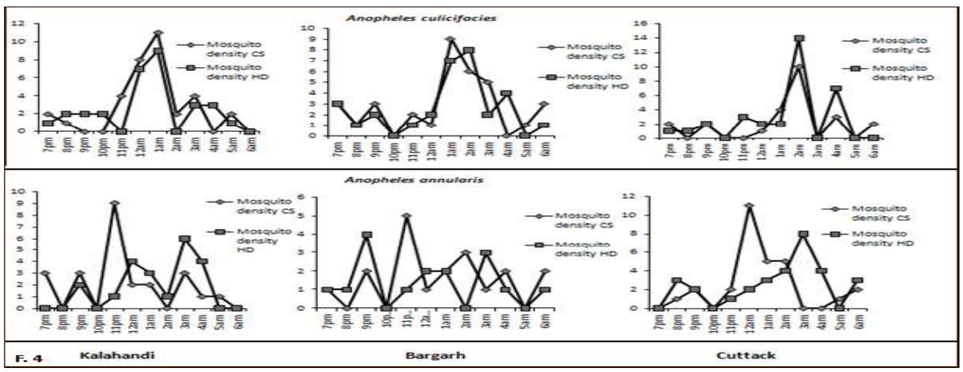
Fig.7: Species composition and distribution of major Anopheline fauna in Kalahandi, Bargarh and Cuttack district. Error bar= standard mean error.

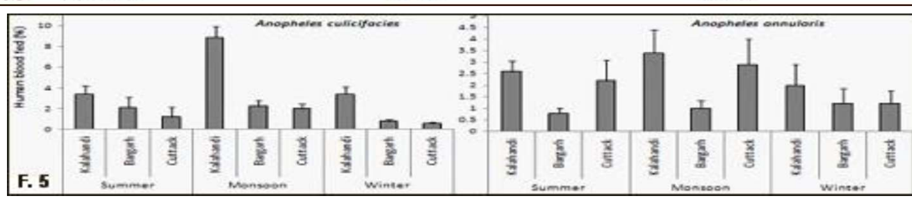


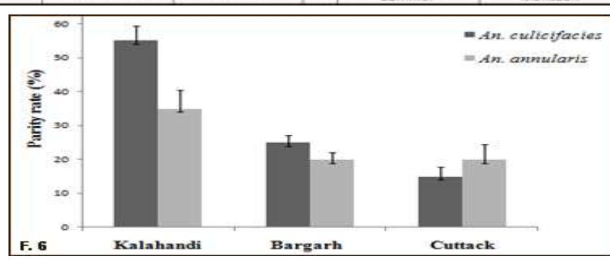
F. 1

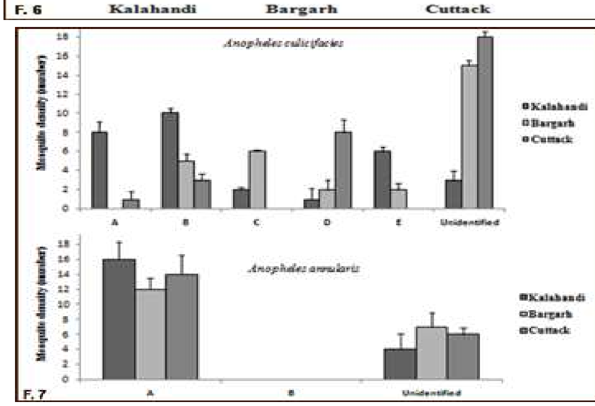

Density (Per Man Hour)
An. culicifacies
An. annularis
Kalahandi
Bargarh
Cuttack
Summer
Monsoon
Winter
F. 2


Density (Per Man Hour)
CS
HD
Outdoor
Indoor
An. culicifacies
An. annularis
F. 3


Anopheles culicifacies
Anopheles annularis
Mosquito density CS
Mosquito density HD
Kalahandi
Bargarh
Cuttack
F. 4


Human blood fed (%)
Anopheles culicifacies
Anopheles annularis
Kalahandi
Bargarh
Cuttack
Summer
Monsoon
Winter
F. 5


Parity rate (%)
An. culicifacies
An. annularis
Kalahandi
Bargarh
Cuttack
F. 6


Anopheles culicifacies
Anopheles annularis
Mosquito density (number)
Kalahandi
Bargarh
Cuttack
A
B
C
D
E
Unidentified
F. 7