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Ecology and Behaviour of *Anopheles culicifacies sensu lato* (s.l.) – A General review

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A B S T R A C T

Anopheles culicifacies, a complex of five isomorphic sibling species, is a key primary vector of malaria in India with nearby countries. The five species are provisionally designated as species A, B, C, D and E. Our understanding of the bionomics of all the five sibling species would positively facilitate, keeping in mind the climatic changes. *An. culicifacies* prefers to like to rest indoors having biting activity throughout the night at variable frequencies. Irrigation tanks, wells, streams and river side water bodies are major breeding sites for *An. culicifacies*. Knowledge about ecology and behaviour of *An. culicifacies* would support authorities for appropriate management of the disease in a changing ecological set up.

Introduction

Malaria is one of the major public health problems in India and it is a barrier to the development of the nation (Sharma et al., 2006). In India, the primary rural vector of malaria is *An. culicifacies* (Ghosh et al., 2008; Mishra et al., 2012). India contributes for transmission of 60-70% of the 2-3 million malaria cases reported every year. (Manonmani et al., 2007).

Anopheles culicifacies Giles 1901, having a hierarchy under Order-Diptera, Sub-order-Nematocera, Family-Culicidae, Sub-family-Anophelinae, Genus- *Anopheles* and Subgenus-*Cellia*, Type form available at the

British Museum of Natural History, London, is a wide spread species. It mimics *Culex fatigans* (*Culex quinquefasciatus*) when resting.

It is also the best known anopheline and widely distributed in most parts of the Indian subcontinent and has been recorded in all mainland zones including Kashmir and high elevations in the Himalayas (up to 3000 meters) except islands of Andaman & Nicobar and Lakshadweep (Fig.1) (Nagpal & Sharma, 1995; Subbarao, 1998 and Rao, 1984) and also extending to Ethiopia,

Yemen, Iran, Afghanistan and Pakistan in the West and Bangladesh, Myanmar, Thailand, Laos and Vietnam in the East. It is also found in Nepal and Southern China to the North and extends to Sri Lanka in the South (Rao, 1984) and the most important vector in plains of rural India contributing 60-70% of reported cases annually (Sharma, 1996).

Biology and genetics of *An. culicifacies* complex has been extensively studied in India and is shown to comprise of five reproductively distinct species, designated as A, B, C, D and E. These five sibling species are spread across India (Fig.1) with distinct biological characteristics and role in malaria transmission. It is recognized as a primary vector of malaria, a disease of great socio-economic importance, in different parts of the world (Russell and Rao, 1940; Russell et al., 1963; Ferreira and Breda, 1963; Reid, 1968; Hearsh et al., 1983 & Chatterjee and Chandra, 2000).

A review on diverse aspects like-distribution, identification of sibling species, bionomics (resting habit, man-hour density, blood meal analysis, man-biting habit, seasonal prevalence), larval habitat, association with other anopheline species, role as vector of human diseases and ecological parameters using Remote Sensing (RS), a Global Positioning System (GPS) and a Geographic Information System (GIS), control measures taken against this species which is still lacking, as a ready reference on this widely distributed, highly prevalent and medically important mosquito species.

Density

The per man hour densities (PMHD) of *An. culicifacies* were found 3.8, 1.4, 4.8; during winter, summer and rainy seasons respectively during the construction of an

irrigation canal in an endemic district of Odisha, India (Panigrahi & Mahapatra, 2013). A study in Gujarat, India showed the importance of irrigation water release in maintaining high *An. culicifacies* adult density during the dry season (Konradsen et al., 1998). The mean prevalence of *An. culicifacies* during the study period was in the range of 8–120 per man per hour (PMH) (Sharma et al., 2014).

Sibling species and their identification

Species complexes are common in Class Insecta (Subbarao and Sharma, 1997; Subbarao, 1988a). Morphologically similar and reproductively isolated populations are known as sibling, cryptic or isomorphic species. *An. culicifacies* exist as species complexes having five (A, B, C, D and E) sibling species (Goswami et al., 2006; Green and Miles, 1980; Subbarao et al., 1983; Suguna et al., 1989; Vasantha et al., 1991 & Kar et al., 1999). Laboratory and malaria epidemiologic studies, correlated with cytologic identification, have incriminated species A, C, D, and E as vectors of malaria in India and have shown that species B is a very poor vector or a non-vector (Subbarao et al., 1980, 1988, 1988, 1992). Recent developments in vector biology have revealed that the vectorial capacity and competence of each sibling species is different, including their behavioral characteristics, breeding habitats, host specificity and susceptibility to malarial parasites and insecticides (Table:1) (Ghosh et al., 2008; Barik et al., 2009). Differences in the vectorial capacities and distribution patterns among sibling species are responsible for the wide variation in the endemicity of malaria in an area. Species B is a non-vector (Subbarao et al., 1999).

At the present, DNA-based techniques are available to differentiate members of the *An. culicifacies* complex. A DNA probe

hybridization assay (Gunasekera et al., 1995) distinguishes species B from species A and also species A from species B and C when DNA from a single mosquito is diluted 200-fold. To differentiate cryptic members of many species complexes, ribosomal DNA (rDNA) has been one of the preferred candidates to develop diagnostic assays. A polymerase chain reaction (PCR) assay developed from the ITS-2 region, which differentiated species A from B, has been reported (Curtis & Townson, 1998). The assay has not been evaluated with the other species, and even for species A and B, it has not been evaluated on field specimens.

A species-specific PCR assay developed from the D3 region of the 28S rDNA cistron differentiates species A and D from species B, C, and E (Singh et al., 2004). A recently developed ITS2 PCR-RFLP assay, like the D3 assay, grouped five sibling species into two categories (Goswami et al., 2005). Thus, attempts to find variation in rDNA among all the five species have not been successful thus far. Therefore, variation in the mitochondrial cytochrome oxidase subunit 2 (COII) sequences was considered (Goswami et al., 2006).

The identification of sibling species, mitochondrial DNA has been widely used in various insect taxa, (Salazar et al., and Hazel et al., 2002; Carew et al., 2003) as well as in some anophelines (Mitchell et al., 1992; Narang et al., 1993).

Recently a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique, using variation in the COII region, distinguished species E from species B/C (Goswami et al., 2005). Although these techniques have been helpful in identifying the members of this complex, all five members cannot be identified using any single one of the methods developed

thus far. Thus, a DNA-based technique, preferably a PCR assay, to distinguish all the members is still needed for this important complex. Sequencing of the mitochondrial COII region of the sibling species and their alignment showed species-specific differences. In this paper, we report two PCR assays designed from the COII region, which, when used in association with the rDNA D3-PCR assay. Singh et al., (2004) were able to differentiate all the five members of the *An. culicifacies* complex.

Resting behaviour

Knowledge on resting habitat of mosquitoes plays a significant role to control malaria disease by using different vector control strategies. It has been found that *An. culicifacies* s.l. prefers to like to rest indoors after taking a blood meal principally in the cattle sheds relatively human dwellings (Sahu et al., 2011) and also observed to rest outdoors in natural shelters and artificial pit shelters. During study in Sri Lanka, Rao (1984) observed that *An. culicifacies* preferred to rest on walls below 1.8 meter while Bhatia et al (1957) found that 72% of *An. culicifacies* s.l. rested on the ceiling and walls above 1.8m from the floor in villages around Delhi, India. About 70% were resting on the underside of roofs of village houses whereas only 30% rested on the vertical walls and surface of furniture, vessels, grain bins, etc. in Maharashtra. *An. culicifacies* s.l. has been regarded as mainly endophilic (Rao, 1981), with outdoor-resting behaviour reported recently in some parts of India (Tewari et al., 1990). In this study, pyrethrum space-spray indoors was the most productive sampling method for *An. culicifacies* s.l.

Biting behaviour

The information on biting activity of different vector species differed during

different phases of night and seasons which could help in choosing personal protection measures that would prevent human-mosquito contact (Nandini et al., 2012). Several studies have indicated that feeding pattern of disease vectors varies widely and is dependent on climatic factors (Bhatt et al., 1994; Devi & Jauhari, 2006). The circadian rhythm of biting cycles is of great epidemiological significance. Earlier studies had however, shown that the feeding patterns of *An. culicifacies* were highly variable and dependent on local ecology, particularly the climatic factors (Brooke, 1953, Bhombore et al., 1956; Rao, 1984).

A study on the biting rhythm of mosquitoes (Reisen & Khan, 1978) postulated the possibility of genetic factors influencing the biting behaviour of mosquitoes and variations in the degree of anthropophily and endophagy among different populations of anophelines. They also found that biting of *An. culicifacies* s.l. took place mostly in the first segment of the night in the cooler months (November–March) whereas it shifted to second and third segments of the night in the hot months (September–October) and biting was entirely arrhythmic and occurred throughout the night in mid-summer.

A few studies in Delhi and east central India observed that *An. culicifacies* bite principally around midnight (Afridi and Puri, 1940) However, in Hazaribagh central India, it was found that night prevalence gradually decrease from 23.00 to 05.00 h supporting the vision that the period of chief activity was before 23.00 h. Biting activity was positively correlated with temperature during January ($r = 0.762$; $p < 0.001$) and February ($r = 0.888$; $p < 0.001$) months and negatively correlated with the relative humidity during these months ($r = -0.734$ and -0.895 ; $p < 0.001$) and or the rest of the months of the year, the biting activity was

negatively correlated with both the parameters (Bionomics of malaria vectors in India, 2002).

In Gujarat, biting activity of *An. culicifacies* starts in early part of the night from January to April, shifting by one hour from between 1800 and 1900 hrs in January to 2100 and 2200 hrs in April. About 70–90% of *An. Culicifacies* population caught during the whole night was found to feed prior to midnight during these months. Bimodal activity was seen during June and July indicating a further shift towards the second and third quarters of the night months (Bionomics of malaria vectors in India 2002). The activity picks up only at the end of the first quarter (1800 to 2100 hrs) of the night and Continues till dawn. More activity was observed in indoors (19.8/man/night) than in outdoors (11.7/man/ night). In monsoon more activity was observed in indoors (23.5/man/night) than in outdoors (11.9/man/ night) (Bionomics of malaria vectors in India, 2002).

An. culicifacies was found biting throughout the night at variable frequencies. There was no definite biting trend shown by the species although during the post-monsoons, maximum biting occurred before 0200 h. Maximum number of landing *An. culicifacies* mosquitoes were collected during post monsoon (78.1%) which were significantly more than the other two seasons (Nandini et al., 2012). In plains, the outdoor landing rate was 0.4/man/night while in forest and dam area it was 0.95 and 0.2/man/night respectively. *An. culicifacies* s.l. exhibits bimodal biting rhythm and shows a seasonal shift in its peak biting activities (Rao, 1984).

Host Preferences

Studies of its feeding behaviour show that *An. culicifacies* is predominantly zoophilic,

feeding mainly on cattle (anthropophilic index less than 10%), but where cattle are scarce, its anthropophilic index can reach in excess of 20% (Rao, 1984). In certain situations where there were few cattle to divert the vectors, its anthropophilic index has been observed to be considerably high (12.3%). In Kheda district, Gujarat, its anthropophilic index was found to be 0.62% which shows little variation between various physiographic areas and in Orissa, it was 1.6% (Parida et al., 2006). According to Bhatt et al (2008) the AI was highest (22.7%) among the specimens collected from human dwellings and least in the animal sheds. In the forest area of Uttaranchal its human blood index was 0.01%. Anthropophilic index (AI) of *An. culicifacies* in the riverine zone was 3.7% and 2.7% in the non-riverine area. Maximum AI was 5.7% in Mukundpur and 4.8% in Rithala locality of riverine and non-riverine zones respectively (Bionomics of malaria vectors in India, 2002). Transmission in forested and deforested ecosystems revealed that in the former, species B (27.96%), C (71.1%), and B/C heterozygotes (0.94%) were present and were highly zoophagic. In deforested riverine villages species A (0.48%), B (21.1%), C (77.94%), and B/C heterozygotes (0.48%) were present (Nanda et al., 2000).

Sporozoite rate and vectorial capacity

The sporozoite rate of *An. culicifacies* were found 0.5% in riverine zone and 0.53% in non-riverine zone in Delhi. A total of 59 *An. culicifacies* were assayed from the riverine area by IRMA technique and one specimen of *An. culicifacies* was found positive for CS antigen of *P. vivax*, giving a sporozoite rate of 1.69%. *An. culicifacies* plays a role in malaria transmission in areas adjoining the River Yamuna and in south Delhi according to previous studies but no infected

specimens were detected in the non-riverine area of northwest Delhi while *An. culicifacies* plays an important role in malaria transmission in the non-riverine area too. Such type of results showed that, in the riverine zone, *An. culicifacies* played a greater role in malaria transmission in only the north part of the zone where water pollution is at minimal level. The overall sporozoite rate was found to be 0.6% (10/1568) during study in 1991–92 in Galteshwar (Gujarat) but in October the observed rate was 3.25 (4/125) (Bionomics of malaria vectors in India, 2002).

The sporozoite rates were found to be 0.51%, 0.04%, 0.3%, 0.4% and 20% in species A, B, C, D and E respectively. In another study in Uttaranchal, the sporozoite rates were 0.79%, 2.4% and 6.0% during the months of September, October and November 1982 respectively (IDVC Project, 2007). The average annual sporozoite index of *An. culicifacies* s.l. in forest and plain areas of Sundargarh district, India was 0.015% and 0.023% respectively (Sharma et al., 2006).

During a study, *An. culicifacies*, 13 of 716 specimens were positive for malaria sporozoites (10 for *P. falciparum* and 3 for *P. vivax*) and showed sporozoite rate of 1.8%. Malaria sporozoites positive specimens of *An. culicifacies* were found during rainy and winter seasons (Kumari et al., 2009). Sporozoite rates of *An. culicifacies* A and C were found 1.1 and 0.5% respectively, during the construction of an irrigation canal in an endemic district of Odisha, India (Panigrahi & Mahapatra, 2013). During a study, When 860 *An. culicifacies* females tested, 2 samples were infected by Plasmodium spp., a sporozoite rate of 0.25% (Vatandoost et al., 2011). According to Sahu et al (2011), none of the *An. culicifacies* dissected (n=185) was found positive either for gland or for gut infection.

The vectorial capacity (VC) estimates for *An. culicifacies* ranged between 0.0005 and 0.5649 for *P. vivax* and between 0.00001 and 0.3928 for *P. falciparum*. It was highest during November and lowest during January for both parasites. The combined VC for Pv and Pf showed positive correlation with the slide positivity rate ($r = 0.0928$; $df = 10$; $p < 0.05$). In a study carried out in Uttaranchal the sporozoite rates were recorded as 0.79, 2.4 and 6.0% during the months September, October and November 1982, respectively (Bionomics of malaria vectors in India, 2002).

Breeding preferences

Water is an important component of ecosystem and its quality in the breeding site is an important determinant of whether or not the female mosquitoes will lay their eggs and the resulting immature stages will successfully complete their development to the adult stage (Piyaratne et al., 2005a). Several studies examined the habitat characteristics of mosquito larvae specially the anopheline vectors of malaria (Piyaratne et al., 2005b in Sri Lanka, Fillinger et al., 2009 in the Gambia). The knowledge of ecological features of the mosquito breeding sites is a potential key element for implementing efficient and effective larval control measures (Killeen et al., 2002; Sattler et al., 2005; Sherif et al., 2013). Survey of breeding habitats have shown that *An. culicifacies* breeds in wide range of habitats and prefers to breed mostly in the streams, canals, rivers, irrigation channels, river bed pools, tree holes filled with rainwater and freshly inundated paddy fields (Yadav et al., 1989, 1997).

Irrigation tanks, wells, streams and river side water bodies (Fig: 2) (Tyagi et al., 2013, Tiwari et al., 2001), rain water flowing in canal and its seepage from canal (Joshi et

al., 2005) were identified as potential habitats for the development of *An. Culicifacies*. Gunathilaka et al (2013) reported that The most productive breeding site for *An. culicifacies* was drains filled with waste water in remote areas; the second highest productivity was found in built wells and indicated that *An. culicifacies* has adapted to breed in a wide range of water bodies including waste water collections although they were earlier considered to breed only in clean and clear water.

Generally, it is regarded to be intolerant of salinity (Russell & Rao, 1942; Sabesan et al., 1986), preferring to breed in newly-dug freshwater pits (Russell & Rao, 1942), domestic wells and pits used for plantation of coconuts and casurina (Sabesan et al., 1986) in India but in Sri Lanka, it was reported first time that species E of the *An. Culicifacies* complex have the ability to breed in brackish water (Jude et al., 2010) and also very much confined to breed in streams (Hoek et al., 2003). It had earlier been reported that the population of *An. culicifacies* of Shri Lanka is very sensitive to minute qualitative change in water, as prefers to breed in clear, open stagnant water pools (Carter, 1930).

The artificial ponds and seepage pools of canal are the major breeding sites for *An. culicifacies*. Thus, in the canal command area, control of malaria transmission requires use of insecticide treated bed-nets and use of bio-larvicides (seepage pools) and larvivorous fish (artificial ponds) wherever feasible (Panigrahi & Mahapatra, 2013).

Effect of water quality on breeding

Various chemical properties of the larval habitat related to vegetation, ranging from pH, optimum temperature, concentration of

ammonia, nitrate and sulphate have been found to affect larval development and survival (Mutero et al., 2004; Rydzanicz and Lonc 2003). Tyagi et al (2013) observed that *An. culicifacies* abundance was significantly associated with dissolved oxygen and P^H value. Maximum occurrence of larvae was found from the water body of higher OD value and P^H value in the range of 6-7. It naturally breeds in clear water (Mehta, 1934) and when given an opportunity to select waters with different amounts of free ammonia and ammonium carbonate, lays eggs indiscriminately, even in water containing 6.6 ppm of saline ammonia (Barik et al., 2009). Abundance of species E was positively correlated with concentration of dissolved oxygen (DO) in water and physical quality of water may not play a significant role in the propagation of immature stages of species E in Tonigala, a rural village in the Puttalam district of Sri Lanka (Surendran and Ramasamy, 2005). Concentration of salinity of water up to 12 ppm does not affect the larvae of *An. culicifacies* s.l. (Rao, 1984).

Association with other Anopheline species

An. culicifacies is associated with *An. annularis* in ponds, paddy fields and small pools, and with *An. stephensi* and *An. subpictus* in domestic containers (Bhatt et al., 1990). It was also found breeding in association with other anopheline species like, *An. barbirostris*, *An. nigerrimus* and *An. aconitus* in areas of Shahjahanpur, India (IDVC Project, 2007). Positive breeding association of *An. culicifacies* was observed with *An. annularis* in ponds and small pools, with *An. stephensi* and *An. barbirostris* in irrigation channels and with *An. stephensi* in paddy fields in canal-irrigated areas of Kheda district. (Bionomics of malaria vectors in India, 2002). Among habitat characteristics, *An. culicifacies* s.l. is

positively associated with light and vegetation and negatively associated with the presence of potential predators like odonates, carnivorous hemipterans and fish (Piyaratne et al., 2005a, b)

Seasonal Prevalence

An. culicifacies s.l. was prevalent throughout the year in areas where, there is no cold weather especially from June to November (Russell and Rao, 1941). In the canal-irrigated areas, its density starts to build up from February and reaches peak in March and thereafter it declines gradually till July. The rise during February is associated with the cultivation of the first crop of paddy. The second rise in the density though less pronounced, is associated with the onset of monsoon and it gets stabilized from August to October and thereafter it further declines in December. In the noncanal-irrigated areas, the density of *An. culicifacies* remains low throughout the year with less wider fluctuations (Bionomics of malaria vectors in India 2002). Very negligible densities of less than 0.5 per man hour occurred in the month of April and rose to 40–50 per man hour during the irrigation season. The marked upward trend in June and July was entirely due to a great increase in the number of available breeding places. Its activity started in June, reached a peak in July and then gradually decreased. Subsequently, its relative density again increased from September in the mountainous and plain regions, with a second peak in October. The density of *An. culicifacies* in mountainous areas was higher in the autumn. There was low activity during the cold winter and hot summer periods (Vatandoost et al., 2011). In most areas the *An. culicifacies* s.l. populations have 2 main seasonal peaks of relative density, during the spring and autumn (Zahar, 1990; Reisen, 1978).

Table.1 Biological variations among members of *An. culicifacies* sibling species complex
(Source: Barik et al., 2009)

Biological behaviour	Sibling species complex				
	A	B	C	D	E
Anthropophilic index ^a	0–4	0–1	0–3	0–1	80
Breeding Preference	Rain water, clean irrigation water	Riverine ecology	Rain water, clean irrigation water	Rain water, clean irrigation water	Riverine ecology
Biting activity	All night	All night	All night	Until midnight	-
Peak biting time	22:00–23:00	22:00–23:00	18:00–21:00	18:00–21:00	-
Resting behaviour	Endophilic	Endophilic	Endophilic	Endophilic	Endophilic
Vector potential ^b	Moderate	Poor	Moderate	Moderate	Very high
Sporozoite rate (%)	0.51	0.04	0.3	0.4	20

^a Proportion of mosquitoes biting the human population.

^b *P. vivax* and *P. falciparum* malaria.

Fig.1 Distribution of *An. culicifacies* sibling species in India (Subbarao, 1998)
(Source: Barik et al., 2009)

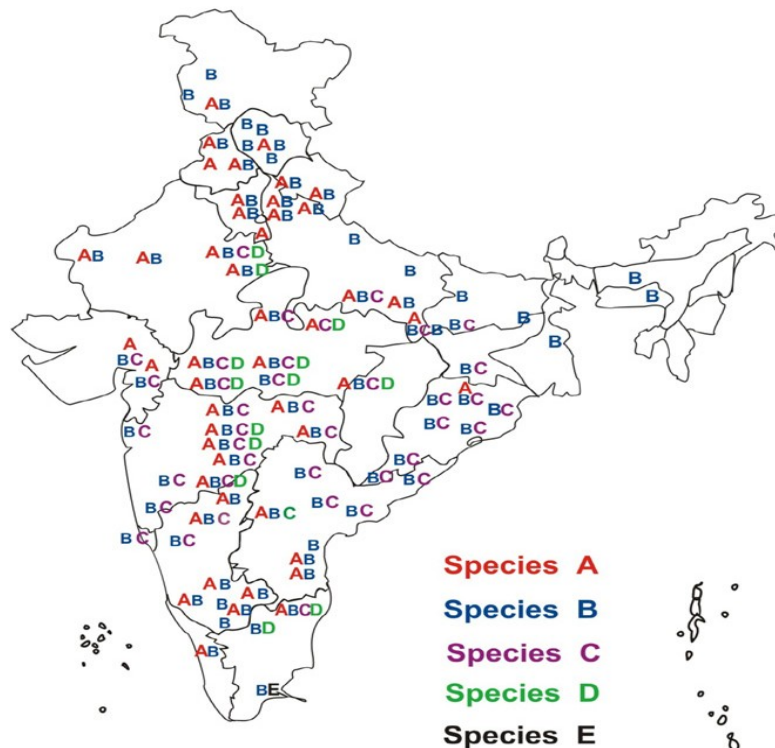
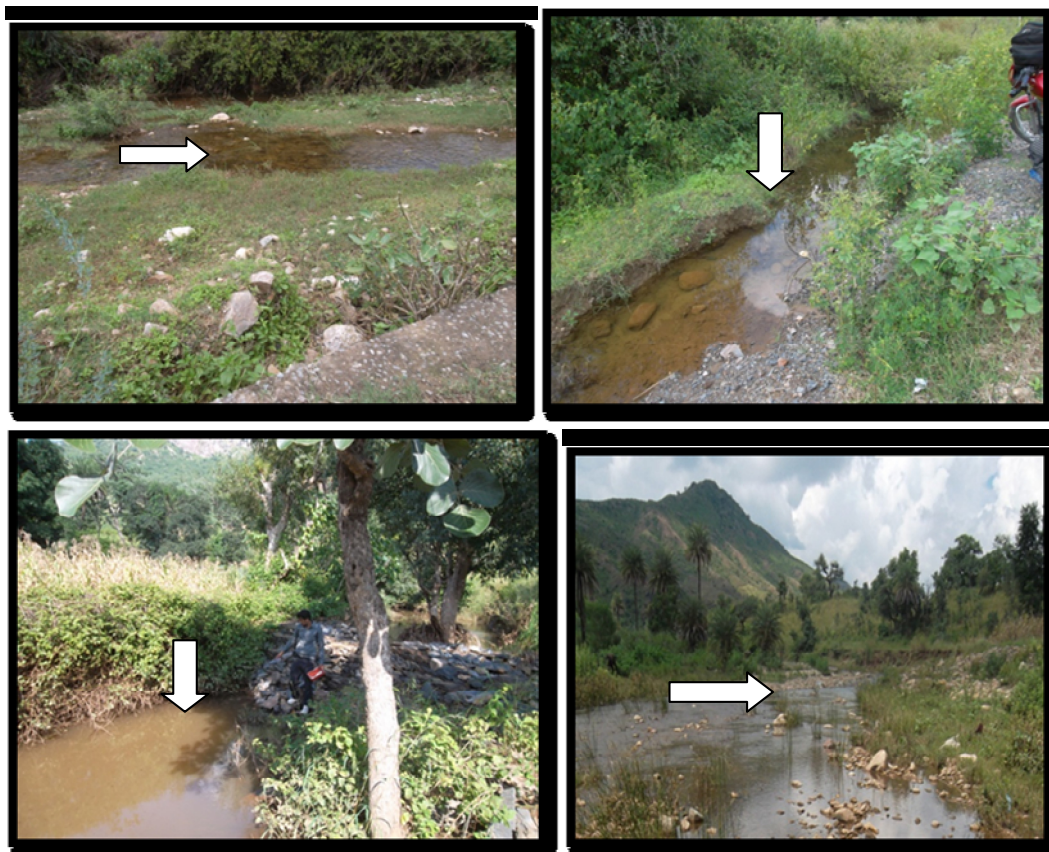


Fig.2 Breeding sites (stream, river bed and pools) of *An. culicifacies* s.l.



In Bhabar area of Uttarakhand in northern India, *An. culicifacies* density remains low during January to June and October to December. It increases during monsoon reaching a peak in August. In terai, its density picks up in March, remains high during April–August (Shukla et al., 2007). In northern Orissa, *An. culicifacies* density shows a small peak in March and another in July (Bionomics of malaria vectors in India, 2002).

Conclusion

For malaria control, Vector control is an essential component which has become less effective in current years, partially due to poor use of alternative control tools, improper use of insecticides, lack of an

epidemiological basis for interventions, insufficient resources and infrastructure, and weak management. Changing environmental conditions, the behavioural characteristics of certain vectors and resistance to insecticides have added to the difficulties. *An. culicifacies* is a complex of five species A, B, C, D and E, information on the individual genetic species is inadequate and available from India and Sri Lanka so, a broad range of behavioral, ecological and epidemiological indices has been attributed as it is available in the literature cited throughout the world.

An. culicifacies, an abundant breeder, is a dominant cattle-shed frequenting mosquito having wide distribution. It has a great adaptability to survive with many other

Anopheline mosquito species in almost all type like- streams, canals, rivers, irrigation channels, river bed pools, tree holes of breeding habitats. Its man-hour density was higher in rainy season than other summer and winter season. Although the cattle blood is the first choice, its anthropophilic index was higher in riverine zone compare than non riverine zone. Development of sporozoites in the salivary glands both by experimental and natural infection is of great epidemiological importance to establish itself as a malaria transmitter. It can play a significant role in malaria transmission, especially during rainy season, in endemic areas.

Knowledge on the breeding habits of the sibling species can help in designing optimal Vector control strategies (Surendran and Ramasamy, 2005) and the feeding preferences of mosquito larvae may guide the development and use of biocontrol measures. On the other hand climate variability is said to be one of the many factors which affect the incidence of vector borne diseases. With the onset of rise in global temperature in the next century there is bound to be alterations in the distribution of the vector as well as the prevalence of the disease (Barik et al., 2009).

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