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VISTA

Abundance of Anopheline larvae in different sites of Baghpat, Uttar Pradesh

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ABSTRACT

The aim of this study was morphological identification of malaria vectors within *Anopheles* species from four localities of district Baghpat. This study was carried out between July 2014 and September 2016, morphologically identified *Anopheles* species from four locality district in Baghpat of Uttar Pradesh State. A total of 12,220 Anopheline larvae were collected through standard dipping method with a 250 ml mosquito dipper as described by World Health Organization, collected larvae emerged into adult *Anopheles* mosquitoes and morphological characters were identified using standard identification key under the Nikon (SMZ 445) dissecting light microscope. Larvae collected from Baghpat 2295 (18.50 %), Katha 2174 (17.52 %), Khekra 2673 (21.54 %) and Baragaon 2331 (18.8%). Among these species of 38.82%*Anopheles culicifacies*, 11.8% *Anopheles stephensi*, 47.66% *Anopheles subpictus* 1.26 % *Anopheles vagus* in Baghpat. In Katha, species of 45.72% *Anopheles culicifacies*, 9.42%*Anopheles stephensi*, 43.88% *Anopheles subpictus*, 0.96% *Anopheles vagus*. *Anopheles* species such as 27.45% *Anopheles culicifacies*, 12.08% *Anopheles stephensi*, 60.45% *Anopheles subpictus* in Khekra and 42.34% *Anopheles culicifacies*, 6.04% *Anopheles stephensi*, 51% *Anophelessubpictus* were identified in Baragaon. *Anopheles culicifacies* and *Anopheles stephensi* were identified dominant malaria vectors of anopheline species.

Keywords : *Anopheles*, *subpictus, culicifacies, stephensi*, malaria vectors.

1. Introduction

Malaria is one of the vector-borne diseases causing high morbidity and mortality. Baghpat is endemic for malaria about 1112 cases were confirmed in the last five years (2012-17). Malaria infection transmits by biting of the infected female anopheline mosquitoes. Basically malaria vectors transmit different types of plasmodium parasites i.e. *P. vivax, P. falciparum, P. ovale* and *P. malariae* from human to human. About 58 *Anopheles* species are found in India, among these six species viz., *Anopheles culicifacies*, *Anopheles stephensi, Anopheles fluviatilis, Anopheles minimus, Anopheles sundaicus and Anopheles dirus* (*Anopheles baimaii)* are the primary vectors of malaria [1]. Four species viz., *Anopheles annularis, Anopheles philipinensis (Anopheles nivipus), Anopheles vagus and Anopheles varuna* are secondary vectors of malaria [2,3]. The principal vectors of malaria are *An. culicifacies*, *An. stephensi* and *An. fluviatilis* in most of the parts of India [4]. Historically, an intensive survey of arthropods including seven species of anopheline including *An. stephensi* reported from Uttar

Pradesh [5].

In India, the diversity of geo-ecological and distribution of different anopheline species are important for malaria epidemiology, the interaction between parasite-vectors-human, which contributes to the transmission of malaria [1]. *Anopheles* vectors species are capable of transmitting malaria at very low densities, *Anopheles stephensi* is important to urban malaria vector [2]. A study showed that the resting, biting behaviour and breeding habitats of the primary malaria vectors have role in malaria transmission [6,7]. However, mosquitoes differ in their preference for the type, size, turbidity, and stability of the habitats [8,9]. These factors determine the density, size and disease transmission competence of vectors [10,11]. *Anopheles* mosquitoes breed at the edges of aquatic habitat such as temporary rain pools, ponds, drainage, ditches, pits, rice fields and roadside puddles to human dwellings [12,13]. *Anopheles* mosquito breeds in areas with aquatic bodies such as ponds and waste-waters [14]. *Anopheles culicifacies* breeds in irrigation channels, ponds and fields of rice paddies while *An. fluviatilis* breeds in stagnant waters in streams and paddy fields [15,16,7].

Anopheline larval breeding and adult survival enhance the malaria transmission, increases number of human blood meal taken by adult mosquitoes, increases the frequency of eggs laying and the survival rate of adult mosquitoes [17,18]. The role of *An. subpictus* as a vector of malaria is ambiguous. But in view of the ongoing process of climate change; *An. subpictus*, may also act as a primary vector of malaria in India [19]. Another species such as *An. subpictus* has also been incriminated as a malaria vector in India [20]. *An. subpictus* is widely distributed and found in abundance in the Oriental region. It is also found in Sri Lanka in south and China in north of India. In India, it is found throughout the mainland [2]. Moreover, these habitats are suitable for the growth and development of various mosquitoes as ponds, wells and surface water-bodies of different sizes are available during the rainy season. Therefore, the aim of this study is to identify various *Anopheles* species in the district for effective vectors control. The aim of this study was to describe the occurrence of anopheline species, such type study has not been done earlier in the district Baghpat (Fig. 1).

2. Material And Methods

This study was carried out with following subsections.

2.1 Study area

The study was undertaken in district Baghpat of Uttar Pradesh. According to census 2011, Baghpat district has total population of 13,03,048. Geographically the district covers 1321 sq km area and is located between 28°57' North Latitude and 77°13' East Longitude. It is above sea level 253 meter near to the bank of Yamuna River. It is surrounded by the Muzaffarnagar district in north, Ghaziabad district in south, Meerut district in east and Haryana in west and closely connected to state highway-57 from Delhi to Saharanpur. It is most important for cultivation of wheat and sugarcane crops. Average temperature was found in district during summer in the range of 30° to 40°C, during winter from 10° to 20°C and during rainy seazen temprature in the range of 25° to 35°C. The annual average rainfall ranged from 500 to 800mm.

2.2 Sites selection

Malaria is an endemic disease in district Baghpat. Primary Health Centre (PHC) wise Epidemiological data was obtained from District Malaria Officer (DMO) to understand the malaria situation. In Baghpat district four villages namely main Baghpat, Katha, Khekra and Baragaon were selected (Fig. 1) to collect the anopheline larvae from different type permanent and temporary breeding habitats.

2.3 Breeding habitats

Larval survey of anopheline mosquito was done to understand the presence of anopheline species. A variety of possible anopheline breeding habitats were identified as ponds, pools, pits, ditches, drains, rice fields, cemented and agriculture channels. Breeding habitats like cemented channels, drains and pond

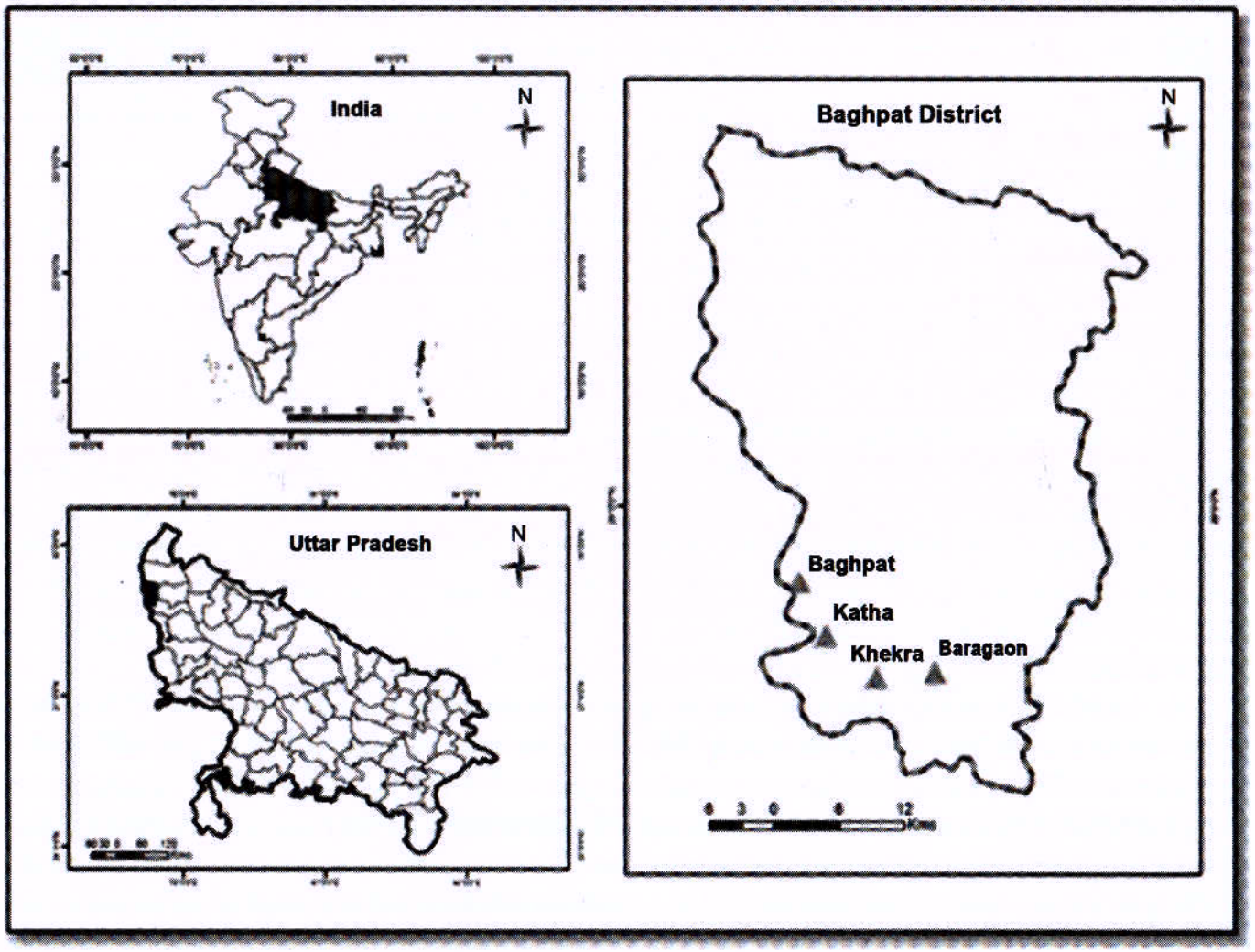

Fig. 1. Map showing the study sites of district Baghpat, Uttar Pradesh

known as permanent while ditches, pools, pits, rice field and agriculture channel were classified as temporary. A standardized data sheet was prepared pertaining to the presence of anopheline larva. After hatching of eggs, anopheline larvae float horizontally on water surface while *Aedes* and *Culex* larvae hang downward from the water surface.

2.4 Larval sampling of anopheline

Fortnightly, anopheline larvae were collected by dipping method from July 2014 to September 2016 [21]. The anopheline larvae and pupae were sampled and the stage of larval development recorded as either1st-2nd instar (early stages) or 3rd-4th instar (late stages). Sampling was done by using a 250 ml capacity standardized dipper (made by aluminum bowl with one meter handle) which was dipped quickly in the breeding habitats where the water surface was covered with dense floating vegetation at an angle of 45°. The dipper was immerged out slowly and pours into the enamel tray for counting of the larval number. A wide mouth pipette was used for collecting larvae and pupae from dipper. Larvae were collected surrounding a kilometer from selected sites. Initially, 2-4 dips were taken for presence of larvae and at interval time of 2-3 minutes, more number of dips were required depending on density, size of habitat.

2.5 Samples transported to insectary

Collected samples were transferred to the plastic container which was well labeled, brought to the laboratory for further analysis and covered with cotton

net. Dog biscuits and Brewer's yeast powder was provided in ratio of 60:40 and water was changed on each alternate day. Larvae reared at optimum temperature and humidity was maintained in insectory. Reared pupae were kept in small cages with cotton cloth for emerging into the adults. Newly emerged adult anopheline mosquitoes were transferred from cages to the test tubes, plugged with cotton, adult mosquitoes were anesthetized and morphologically identified under light dissecting microscope (Nikon, SMZ 445) and with the help of standard identification key [22].

3. Results

A total of 12,220 *Anopheline* larvae were collected from different sites of Baghpat district between July 2014 to September 2016. Among these collection 9,473 emerged in to adult anopheles mosquito from larvae. These larvae were collected from different types of breeding habitats i. e. agriculture channel, cemented channel, ditch, drain, pond, pool, pit and rice field. *An. culicifacies* and *An. stephensi* are dominant malaria vectors of selected sites of district Baghpat. Figure 1 shows that range of collected larvae from Baghpat site 2295 (18.50%), Katha 2174 (17.52 %), Khekra 2673 (21.54 %) and Baragaon 2331 (18.8%).

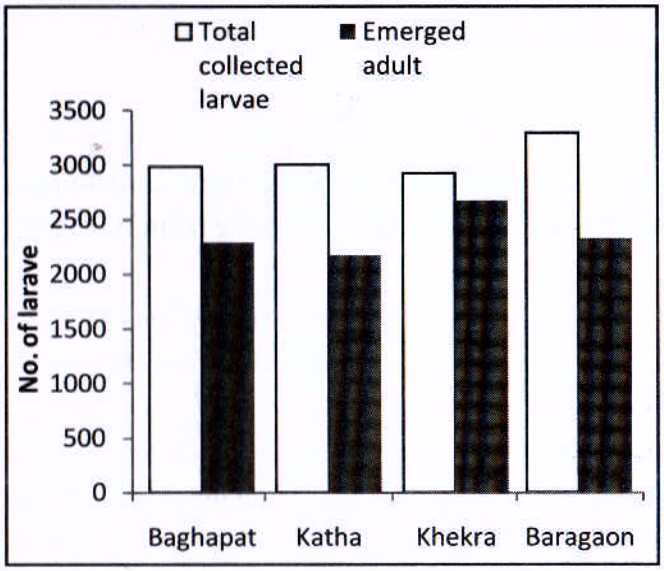

Fig. 2. No. of total larvae collected and emerged adult from different study sites

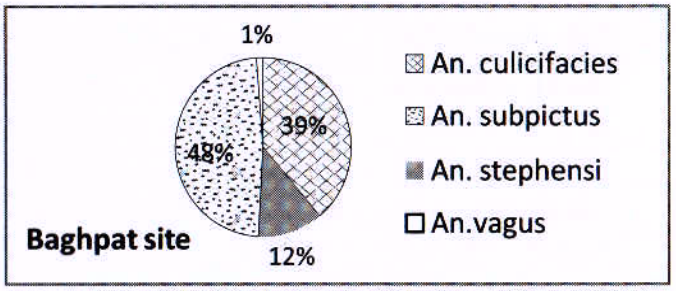

Fig. 3. Distribution of Anopheles species adults collected larvae from Baghpat site

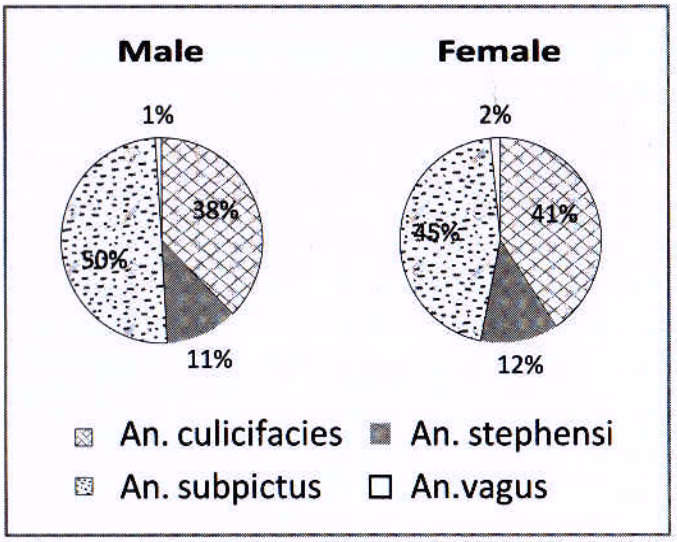

Fig. 4. Distribution of adult male and female Anopheles species collected larvae from Baghpat

Figures (3, 4) show the range of emerged *Anopheles* mosquito in Baghpat, *Anopheles culicifacies* of 38.82%, *An. stephensi* 11.8%, *An. subpictus* 47.66%, *An. vagus* 1.26% of which were males *An. culicifacies* 38%, *An. stephensi* 11% and *An. subpictus* 50% and *An. culicifacies* 41%, *An. stephensi* 12% and *An. subpictus* 45% and *An. vagus* 2 % were females.

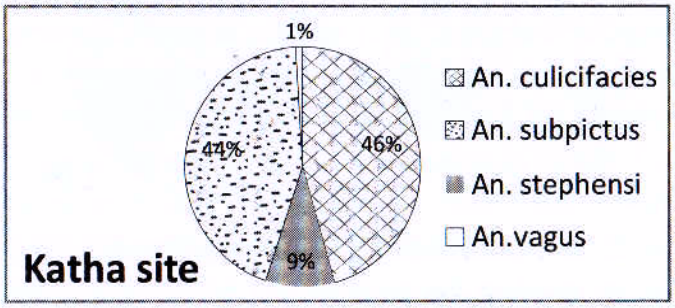

Fig. 5. Distribution of Anopheles species adults collected larvae from Katha site

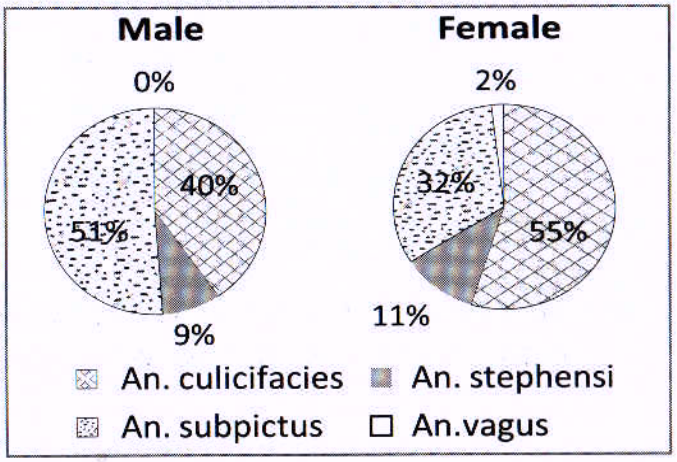

Fig.6. Distribution of adult male and female Anopheles species collected larvae from Katha

Figures (5, 6) show the range of *Anopheles* species such as *An. culicifacies,* 45.72%, *An. stephensi* 9.42 %, *An. subpictus* 43.88%, *An. vagus* 0.96% were identified from Katha of which were males *An. culicifacies* 40%, *An. stephensi* 9% and *An. subpictus* 51% and *An. culicifacies* 55%, *An. stephensi* 11% and *An. subpictus* 32% and *An. vagus* 2 % were females.

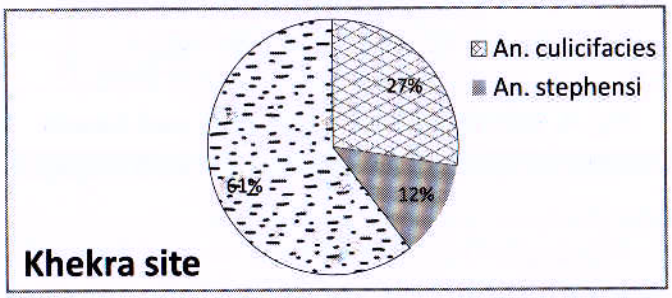

Fig. 7. Distribution of Anopheles species adults collected larvae from Khekra site

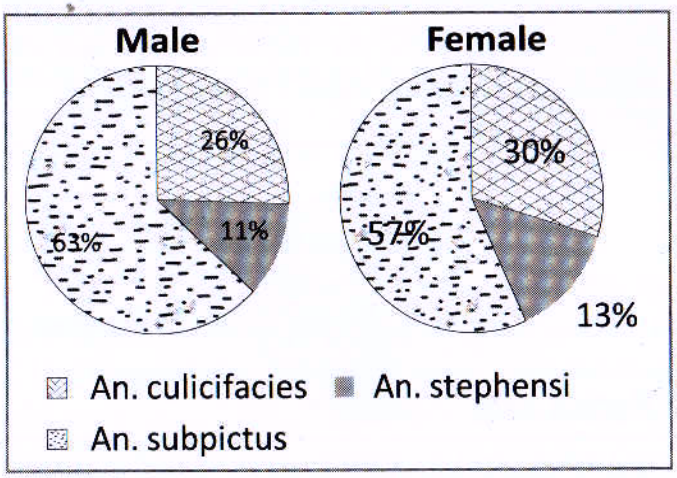

Fig. 8. Distribution of adult male and female *Anopheles* species collected larvae from Khekra

Figures (7, 8) show the results *Anopheles* species were identified An. *culicifacies,* 27.45%, *An. stephensi* 12.08 %, *An. subpictus* 60.45% from Khekra which were males *An. culicifacies* 26%, *An. stephensi* 11% and *An. subpictus* 63% and *An. culicifacies* 30%, *An. stephensi* 13% and *An. subpictus* 57% were adults female emerged from Khekra.

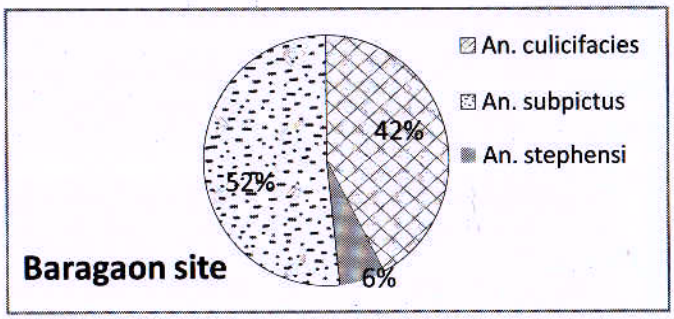

Fig. 9. Distribution of Anopheles species adults collected larvae from Baragaon site

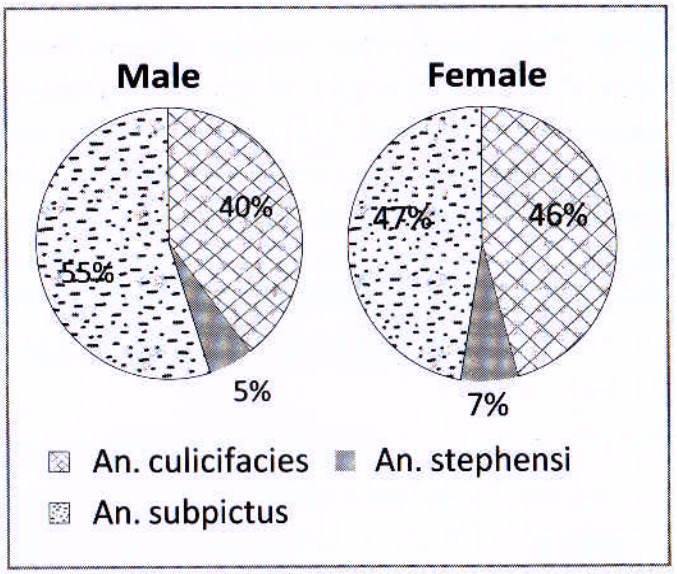

Fig. 10. Distribution of adult male and female Anopheles species collected larvae from Baragaon

An. culicifacies, 42.34%, *An. stephensi,* 6.04%, *An. subpictus* 52% were identified from Baragaon. Figure 9 and 10 show that the *Anopheles* species were identified which were males *An. culicifacies* 40%, *An. stephensi* 5% and *An. subpictus* 55% and *An. culicifacies* 46%, *An. stephensi* 7% and *An. subpictus* 47% were adult female which emerged from Baragaon. *An. culicifacies* and *An. stephensi* were identified dominant malaria vectors from anopheles species.

During study period, maximum percentage of males and females were observed *An. subpictus* of sites such as Khekra (61%), Baragaon (52%), Katha (44%), and Baghpat (48%). Percentage of males and females *An. culicifacies* were observed less than *An. subpictus* in all sites as Katha (46%), Baragaon (42%), Baghpat (39%) and Khekra (27%), whereas low percentage of males and females were observed *An. stephensi* like Baghpat (12%), Khekra (12%), Katha (9%) and Baragaon (6%). Very low percentage of *An. vagus* was observed only at Baghpat (1%) and Khekra (0.96%) sites.

4. Discussion

A total of 9,473 *Anopheline* larvae emerged into adult *Anopheles* mosquito, larvae were collected from different breeding habitats of the site of Baghpat district during July 2014 to September 2016. These larvae collected from different types of breeding habitats i.e. agriculture channels, cemented channels, ditches, drains, ponds, pools, pits and rice fields. Early (1^{st}-2^{nd}) and late instars (3^{rd}-4^{th}) of *Anopheles* mosquitoes were collected and reared into adult at optimum condition and adults morphological identified. Anopheline species were identified at four sites namely Baghpat, Katha, Khekra and Baragaon. There was a significant variation in larval abundance from one site to others. Breeding habitats such as temporary and permanent was found in selected sites. Permanent and temporary breeding habitats were indentified viz. ponds, pools, pits, ditches, cemented channels, agriculture channels and rice fields in selected sites. In all sites, maximum density of *An. subpictus* was observed. Only *An. subpictus* was found in rainy season from June to September. Temporary and permanent dry habitats filled with rainy water in rainy seasons such as ponds, pools, pits, ditches and agriculture channels. Breeding habitats are most productive malaria vectors population, ecology of breeding habitat is important for malaria control programs. Larval density was highly variable in different habitat types and during the seasons. The peak of larval production was associated with rainfall. Khekra and Katha was more rice cultivated area, anopheline species were associated in rice growing sites as permanent and temporary larval habitats [23, 24]. Breeding of *Anopheles* culicifacies was found in the rice field channels [25]. Baghpat and Katha were situated near edge of Yamuna River while Baragaon was located near irrigation channel. The predominant species was *An. culicifacies*, *An. stephensi*, *An. subpictus* and *An. vagus*. Out of *An. culicifacies* and *An. stephensi* are main vectors of malaria reported from selected site. Anopheline species as *An. subpictus* is the most abundant in most parts of India which can breed in almost every type of breeding habitat. *An. subpictus* has also been incriminated as a malaria vector [20].

In this study, maximum percentage was observed *An. subpictus* of sites such as Baghpat, Katha, Khekra and Baragaon. Previously, *An. subpictus* was recognized as an important vector of malaria in Malaysia and Maldives and Sri Lanka. *An. subpictus* has important role in malaria transmission as a secondary vector in Odisha of south India. *An. subpictus* mosquito species is a widely distributed in a variety of freshwater breeding habitats. Breeding habitats like ponds, pools, pits, ditches, cemented and agriculture channels are important for vectors breeding which need to be focused from time to time. Breeding habitats support the immature development of anopheline species. *An. subpictus* was incriminated as a vector of malaria in Maldives Islands, Celebes, South Java, Portuguese Timor and Malaysia [26]. It is known as secondary vector of malaria in Sri Lanka [27]. While the role of *An. subpictus* as a vector of malaria is doubtful in India but *An. subpictus*, may also act as a primary vector of malaria [19]. Quick and accurate identification of *Anopheles* mosquitoes is required for vectors control and malaria transmission [28]. Keeping in view, the review has been prepared on the basis of work reported in India as there is limited research work available on this species in India. Climatic factor has main role in the development of malaria vector. Rainfall helps in creation of new breeding habitats and it is important for humidity when eggs are laid and

survival of adult mosquitoes. Permanent and temporary aquatic habitats support anopheline larvae. Larval control may be necessary for the reduction of the adult mosquito population, subsequently limiting malaria transmission [12]. Malaria transmission depends on the number of vectors survival and the presence of suitable breeding habitats for the development of anopheline larvae [29].

5. Conclusion

The result of morphological examination of adults from larvae has revealed the presence of predominantly malaria vectors species like *An. culicifacies* and *An. stephensi*. Further studies from different geographical areas are required to find out the morphological and molecular characterization of the vectors of sibling species of *An. culicifacies and An. stephensi.*

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