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Full Length Research Paper

Morphological Identification and Distribution of *Anopheles* Species in Gwagwalada Town, F.C.T, Nigeria

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Abstract

This Study Examined the Morphological identification and distribution* of Anopheles *Species in Gwagwalada Town, F.C.T, Nigeria. In carrying out this study a total of 2,929 specimens of the* Anopheles *mosquitoes consisting of 1,790 adults reared from larvae and pupae were identified using morphological characters. Anopheles gambiae s.l constituted the highest overall preponderance in the indoor resting adults collected, 942 (82.70 %), then An. arabiensis with 91 (7.99 %), followed by An. rufipes 62 (5.62 %), and An. funestus constituted the least population 42(3.69 %) collected. There was high significant differences at (P<0.05) among the distribution of the various Anopheles species.

Keywords: Morphological, Identification, Distribution, Anopheles Species, Gwagwalada town.

INTRODUCTION

Mosquitoes are the greatest enemies of humans because of the widespread suffering and death caused by the diseases they transmit. Such diseases are malaria, yellow fever, dengue fever and filariasis, (Adebote *et al.,* 2010). *Anopheles* is a genus of Mosquito (*Culicidae*). There are approximately 3,800 *Anopheles* species, of which 30-40 transmit four different species of parasites of the genus *Plasmodium* that cause malaria which affects humans in endemic areas. *Anopheles gambiae* is one of the best known, because of its predominant role in the transmission of the most dangerous *Plasmodium falciparum* (Coetzee and Goos, 2013). Some species of *Anopheles* also can serve as the vectors for canine heartworm *Dirofilaria immitis,* the Filariidae *Wuchereria bancrofti* and *Brugia malayi,* and viruses. Mosquitoes in other genera *(Aedes, Culex)* can also serve as vectors of disease agents (Alaba and Alaba, 2010). Like all mosquitoes, anophelines go through four stages in their life cycle: egg, larva, pupa, and imago.

The first three stages are aquatic and last 5-14 days, depending on the species and the ambient temperature.

The adult females can live up to a month (or more in captivity) but most probably do not five more than 1-2 weeks in nature (Gadzama, 2012).

This study is carried out in areas where human activities are being carried out like construction of reservoirs, ditches and in clay pots widely used for storing drinking water which acts as sources of breeding sites for mosquitoes. In Nigeria, Gwagwalada in particular, malaria is one of the most common factors of morbidity. The entire population of the area live at the risk of infection, the risk being greater in rural than urban communities. Many people are infected annually with great population develop clinical illness. Roughly 50% of the population experience at least one acute attack of malaria every year (FMOH, 2013). The disease causes considerable social and economic loss through absenteeism from work, school, etc. Apart from these deleterious effects, malaria is a major cause of mortality. Many children under the age of five years die of malaria annually (Salako, 2009). Factors leading to the death include acidosis, delirium, convulsions, coma and acute or chronic anaemia (Bruce-Chwatt, 2012),

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MATERIALS AND METHODS

The Study Area

The study was undertaken in six selected sites in some parts of Gwagwalada, an Area Council in Abuja, Nigeria. These sites are:-Passo, New Kutunku, Dukpa, Giri, Radio house and Dagiri. Gwagwalada Area Council is located about 55km away from Federal Capital City. It lies on latitude 8° 55', North and 9° 00' North and longitude east and 7°.05' east (Ishaya, 2013). The area covers a total of 65sq kilometer located at center of very fertile area with abundance of grasses (Ishaya, 2013). Figure 1

This study area falls in to the guinea savanna vegetation zone of the country which is the broadest of all the vegetation types, constituting about 50% of the land area of Nigeria. There are two seasons within this vegetational zone, dry season that lasts between four to seven months and a rainy season that lasts between four to five months. The rainfall ranges between 1016mm and 1524mm with relative humidity of between 60% and 80%.

The guinea savanna is divided into two vegetation zones: - the northern and the southern guinea savanna (Ishaya, 2013). The northern guinea savanna is characterized by mainly grasses like *Hyperrhenia Andropogon, Schizachyrium* species with interspersing trees *of lsobelina doka, Albizia Zygai, Anthoesta virgelli, Annona senegalensis (*Ishaya, 2013).

High fall grasses of about 5 -10m eg. *Andropogon gayanus, Tectorum* species and more densely trees characterize the southern guinea Savanna, hence the name transition woodland. The trees are thick - barked of up to 40-50feet.

Common trees, such as *Daniella Oliveri, Afzelia africanus* among other species, are common. The temperature of this area is highly influenced by the Niger-Benue trough where heat is trapped. The highest diurnal temperature ranges between 27^{0}C and 37^{o}C in the months of November-April (dry season). The rainy season comes between the months of April to October with temperature range of 23^{o}C and 36^{o}C. It is pertinent to observe that, this area has a higher temperature than any other Area Council in the Federal Capital Territory throughout the year (Ishaya, 2013).

Sample Collection

All *Anopheles* immature stages, comprising the four larval instars and pupae were collected from natural breeding sites in shallow temporary puddles and sunlit pools on land as a result of manmade activities, like gutters or rock surfaces, Excavation ditches and uncompleted pit / soak away.

Larval and pupal samples were collected in all the study sites using the scooping net methods which involve the technique such as shallow skim, collection of immature from partial submersion, complete submersion, dipper as a background, flow-in-method, scraping, simple scoop and salt marsh method.

The Shallow Skim

Anopheles larvae found at the surface of the water among aquatic vegetation or floating debris were collected with a shallow, skimming stroke along the surface, with one side of the dipper pressed just below the surface. End the stroke just before the dipper is filled, to prevent overflowing.

Partial submersion

Anopheles larvae found around emergent vegetation, logs and tree stumps, larvae were drawn into the dipper by submerging one edge so that the water flows rapidly into the dipper.

Complete submersion

Certain Culicine larvae (such as species *of Aedes* and *Psorophora*) are very active and usually dive below the surface when disturbed. In this case, a quick plunge of the dipper below the surface of the water was done and the dipper was brought up to avoid losing the larvae with overflowing current.

Dipper as a Background

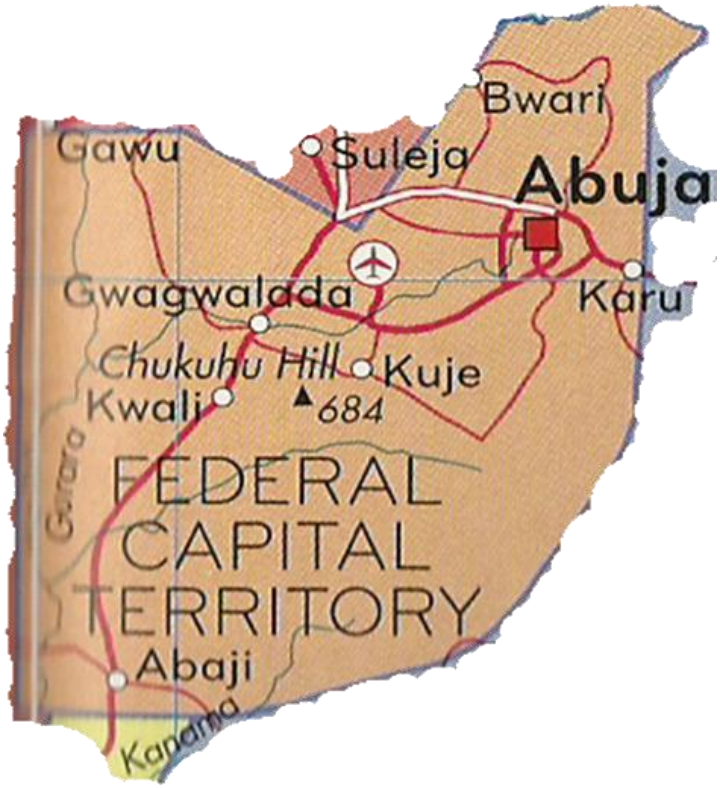
This is an especially useful technique in woodland pools, for early season species. A white coloured dipper was submerged completely into woodland pools, for early season Sspecies so that the larvae and pupae can be spotted which was brought up carefully to avoid losing its contents.

Flow-In-method

This method is useful in situations where the water is shallow, with mud, leaf litter or other debris on the substrate. The immature *Anopheles* were collected by pushing the dipper down into the material on the bottom and letting the shallow surface water and mosquito larvae flow directly into the dipper.

Scraping

This method is used in permanent or semi-permanent habitats containing clumps of vegetation, such as



Source: Abuja Geographical Information System

Figure 1. Map of Gwagwalada, FCT-Abuja

tussocks. The larvae and pupae were collected by dipping into the water tussock, and using the dipper to scrape up against the base of the vegetation to dislodge any larvae present.

Simple scoop

This is the technique which is the most commonly used by personnel into larval surveillance and is the one referred to in much of the literature as "the standard dipping procedure." The technique was used by simply scooping a dipperful of water out of a habitat. This method is useful in a wide variety of habitats, especially for collecting *Culex.*

Salt marsh

This procedure was used in conducting salt marsh larval surveillance. In salt marsh potholes, dipping was done around the edge of the pothole. The middle of the pothole, was either skimmed or scooped with strokes. The precaution taken was that since there were different techniques which was used in different habitat types, dipping for immature mosquitoes, regardless of the technique used, it should be important to look for the actual presence of larvae before dipping, and to proceed carefully and pay attention to what one is doing.

Collection was carried out by scooping and where this was not possible; collection was facilitated by the use of a plastic pipette or dropper. The larvae and pupae collected were placed in 25ml plastic cups and screened at the top by polystyrene netting of mesh size 0.02cm for the purpose of rearing them to adulthood.

Rearing of Mosquito Larvae and Pupae to Adults in the Laboratory

The larvae and pupae placed in plastic cups were allowed to develop in the field and transported to the laboratory in Biological Sciences Department, University of Abuja where they were maintained according to WHO (2013). A total of 1,790 larvae were placed in a plastic cups cover with polysty netting and held by rubber bands and kept at room temperature. The larvae were fed with ground fish diet powder. The set up was monitored daily; the adults that emerged (within one to four days depending on the larvae and pupae stages) were killed by anesthetizing using a drop of Acetyl Acetate placed on Whitman's filter paper. The paper was placed on the netting covering the cup and covered with a Petri dish for five minutes. The dead mosquitoes were removed individually and identified using the taxonomic keys of (Gilles and Coeteze, 2013). Non – members of the

Anopheles species were discarded.

Collection of Adult Mosquitoes from Indoors

Collections of adult mosquitoes were made during the rainy season with few being collected during the onset of the dry season in some selected humid environments. The indoor resting adult collections were made at dawn between 6 a.m and 9 a.m by aspiration using a pooter (WHO, 2013).

The harvesting was done early in the morning because many *Anopheles species* rest indoor to have their blood in take digested. The *anopheline* were distinguished from the other mosquitoes by examining the angle they rest. Anopheline rest at a 45° perpendicular to the surface on which they rest whereas, culicinae rest parallel to the surface.

The mosquitoes were aspirated from ceilings on clothing, and on the walls in consenting households. The mosquitoes collected were transferred into plastic or paper cups through a slit made on the side of the cup, which was covered on its top with polystyrene netting of size 0.02cm was used, held in place by a rubber band. The mosquitoes collected were kept in a cool box and taken to the laboratory for morphological identifications. The individual species were identified rightly in to species level, where they were counted and their numbers recorded.

They were individually, kept in an eppendort tube according to date of collection and locality and dried over anhydrous calcium sulphate (CaS04) in a zip lock polyethylene bag and kept for sporozoites determination.

Sample Analysis

Morphological identification

Each adult raised from the larvae reared or caught indoors was individually identified to species and species complex level, using morphological characters of Gillett, (2012) and Gillies and Coetzee (2013) under 20x Zeiss light microscope within 24hour of post emergence. The following respective characteristics were observed:-

Anopheles gambiae *s.s*

These groups of mosquitoes have their abdomen without laterally projecting tufts of scales, the scaling on the abdomen was scanting and confined to the 8th or rarely 7th tergum. Legs are speckled with tarsi 1-4 having conspicuous pale bands on the apices. The palps were smooth or shaggy with pale bands and the 3rd preapical dark area on vein 1 with a pale interruption, which sometimes fused with proceeding pale spots.

Anopheles arabiensis

These groups of mosquitoes have their abdomen without laterally projecting tufts of scales, their hind tarsi 4 and 5 were entirely pale and palps with 3 pale bands usually with some specklings while veins I with 2 have accessory pale spots with speckled legs.

Anopheles rufipes

This group of mosquitoes has their abdomen without laterally projecting tufts of scales and their legs are not speckled. Hind tarsal segments 4 and 5 are entirely white while their fore tarsus 1-3 have no distinct apical pale bands but their palps were smooth (i.e. not shaggy) with the 2 outer ones broad and are rarely fused having the 2nd main dark area (Median dark area) on vein 1 with well defined with 2 pale interruptions while the 3rd main dark area (preapical dark area) on Vein I have no pale interruption.

Anopheles funestus

These groups of mosquitoes have their abdomen without laterally projecting tufts of scales and their legs were not speckled. Their costa have at least I pale spot on their basal half with hind tarsus 4 and 5 not entirely pale and where pale banding exists on hind tarsus is narrow and apical. There is no pale spot interruption on the 3rd preapical dark area on vein I but on the 3rd preapical dark area, it is broader than sub costal pale spot.

Population Analysis

Curbet, (1976) Techniques was applied to analyze the population and the following were carried out.

a. Visual count- The number of *Anopheles species* harvested were physically counted and recorded at different times of harvest in various sites.

b. As stated above, the sporozoites were also observed, counted and recorded.

Data Analyses

Chi ($_X^{2)}$ square test was used to analyse and compare the differences in the species of the *Anopheles* mosquitoes in the study sites.

RESULT

Mosquito survey

A total of 2,929 specimens of the *Anopheles* mosquitoes

consisting of 1,790 adults reared from larvae and pupae were identified using morphological characters. *Anopheles gambiae s.l* constituted the highest overall preponderance in the indoor resting adults collected, 942 (82.70%), then *An. arabiensis* with 91 (7.99%), followed by *An.rufipes* 62 (5.62%), and *An. funestus* constituted the least population 42(3.69%) collected. There was high significant differences (P<0.05) among the distribution of the various *Anopheles* species.

Table 1 showed the various species of anopheles mosquitoes raised from immature stages. From the distribution, only *An. arabiensis* and *An. gambiae* were observed as 317 (17 .71%) were *An. arabiensis* and 1,473 (82.29%) were *An. gambiae.* There was however no significant difference between the site of collection and Anopheles mosquitoes species in New Kutunku (x^2 = 7.86 df = 3; P>0.05) and Dukpa (x^2 = 5.32; df = 3; P>0.05).

An. funestus, a total of 64(5.62%) were *An.rufipes,* while *An. arabiensis* made up a total of 91(7.99%) and *An.gambiae s.s* constituted the highest number of 942(82.70%). There was a high significant difference between the population of *Anopheles* mosquitoes and the site of collections in the calculated values obtained in Dagiri (36.01), New Kutunku (12.63) and Radio House (6.97), Passo (125.11), Giri (44.19) and Dukpa (23.51) showed significant difference at P< 0.05 levels at degree of freedom 15 which is 24.99. *An. gambiae s.s* was significantly higher while *An.funestus* was significantly lower.

DISCUSSION

The absence of the adult *An.funestus* and *An.rufipes* in some study sites reflects their zoophilic and zoophagic nature, (White and Rosen, 1972), while its presence in some study sites in relatively low proportions in adults indoor collections reflects the presence of abundant cattle which could be seen within the vicinity of the studied sites where the females which are zoophagic and endophilic feeds on both cattle and wild animals in animals sheds but could bite human close to cattle White and Rosen, (2010).

The presence of *An.arabiensis* in relatively high proportion reflects its wide feeding range. It is also reported that *An. arabiensis* posses high ecophenotypic plasticity. The females are endophagic and could remain endophilic for 1 or 2 days (White, 2012). However, *An.arabiensis* readily bites outdoors and the exophagic - *An.arabiensis* readily bite out doors and the exophagic - engorged females enter house to rest (Gilles and Demeillon, 2012). It can also endure drought and they can be seen even at the bed of a drying streams.

Identification of the Anopheline collected in the selected study sites in Gwagwalada Area Council had a total of four *Anopheles* species which were based on their morphological characters. They were *An. funestus, An. rufipes, An. arabiensis* and *An. gambiae s.l,* though the two species, *An. gambiae s.s,* and *An. arabiensis* were identified as siblings of *An. gambiae s.l.*The result of morphological examination of adults reared from larvae has revealed the presence of predominantly *An. gambiae s.s.* This observation is important because, it reveals that *An. gambiae s.s,* were the main vectors of malaria which were breeding in the study area and secondly, that the breeding sites were associated and created by human activities in close proximity to human habitation which, serve as focal points of transmission of malaria and other diseases. Human activities generally constitute a threat in the transmission of malaria.

An. funestus and *An. rufipes* were not obtained at all at fee study sites during larvae and pupae collection, andfeis indicates that the species exhibit breeding sites preference. Small pools of brownish water logged in marshy rice field and a cocoa yam farms characterized the sites.

The breeding sites characteristics of *An. funestus An. rufipes* as observed by Molineaux and Gramiccia, (2013) and Service, (2014); include breeding in rice fields and cocoa yam farm where continuous pockets of water exist for several months in the dry season, similar to those observed in this study.

The absence of *An. funestus* and *An. rufipes* in the adult indoor collection during the study, in some study sites, reflects its zoophilic and exophagic nature as described by Mahande (2008). The results revealed the preponderance of *An. gambiae* s.s in larval and adult collections compared to *An. arabiensis,* in all sites during the study. It is the most abundant of all the species encountered and this confirms earlier reports that *Anopheles gambiae* s.s is the most widely distributed species in sub Saharan Africa, due to its genetic heterogeneity which enables it to adapt to many ecological zones (Service, 2014). The overall preponderance of *An. gambiae s.s* shows that it was the most abundant in all the study sites is explained in the light of its ability to adapt to nearly all habitats. This is in line with fee notion that this ability is conferred by genetic inversion polymorphism, which determines it heterogeneous behaviours culminated in distribution as observed in this study.

The abundance of *An. funestus* and *An. rufipes* in relatively low proportions in the adult indoor collections though, not found in the larval collections in all the study sites, suggested that both *An. funestus* and *An. rufipes* breed in more permanent larger bodies of waters like ponds, rivers and lakes (Molineux and Gramiccia, 2013).

The result obtained in this study; also suggest that the adults emerging from the larval population might be the same population resting indoors in these localities. However, a detailed marked- release recapture study would shed more light for this proposition. Their abundance of adult forms of both sexes could be related to the abundance of cattle, which were seen within the vicinity of the sites, most especially in the northern

Table 1. Distribution of adult *anopheles species* collected indoor from different study sites.

	Anopheles species					
Study Sites	***Anopheles funestus***	***Anopheles rufipes***	***Anopheles arabiensis***	***Anopheles gambiaes.s***	***Total***	***Calculated x^2***
Passo	25	41	0	152	218	125.11
New Kutunku	9	18	05	212	244	12.63
Dukpa	0	0	07	168	175	23.51
Giri	o	01	34	143	178	44.19
R/House	02	02	04	97	105	6.97
Dagiri	06	02	41	170	219	36.01
Total	42	64	91	942	1,139	

guinea savanna. Generally the results obtained during this study compares well with those reported by (Onyabe and Conn, 2008). These researchers reported on the distribution of *Anopheles* in Nigeria based on larval collections. Unlike Onyabe and Conn, (2008), this study used larval and indoor adult collection methods and was relatively intensive with collections made in several sites within a locality.

CONCLUSION AND RECOMMENDATIONS

The study has revealed that, four different species of *Anopheles* mosquitoes abound in the study area with *An. gambiae s.s* showing the highest preponderance over other species while *An. rufipes* was the lowest in population. As it was observed in the study, the degree of anthropophily by malaria vectors is directly proportional to the area of land and collection of water being a potential strategy for high population densities of mosquitoes. These factors are further dependent on local ecological factors such as climatic conditions, topography, water table occurrence, diversity of larval habitats and human life styles (Rwegoshora, 2010). By pooling together available literatures and the findings in this study, it can be inferred that integrated system of vector management and zooprophylaxis should be the potential control strategy for the vectors of malaria in the study area. Their susceptibility to various insecticides needs to be determined especially in the savanna zones of Nigeria in which the study sites belong.

Most efforts of government towards the controlling of the vector of malaria are yet to yield a positive result, and to achieve this, a detailed ecological knowledge of these vectors will be of great assistance especially at the local levels.

The following steps should also be taken:

a. Identifying the morpho-species of the *Anopheles* mosquitoes.

b. Carefully locating and identifying their breeding sites.

c. Ecological knowledge and behaviour of the vectors of malaria would greatly assist in their total eradication. This is because the source from which malaria vectors disperse may change and between years as breeding sites dry out or are created, (Carter and Coluzzi, 2013). This information calls for all stake holders involved in the concerted effort of malaria vector control to pay particular attention to the indigenes of Gwagwalada Area Council in order to come up with measures that will ensure little risk of exposure to malaria infection.

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