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MORPHOLOGICAL IDENTIFICATION OF MALARIA VECTORS WITHIN *ANOPHELES* SPECIES AT HADEJIA AND JAHUN

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ABSTRACT
This study was carried out between May and October, 2011 to morphologically identify Anopheles species in parts of Jigawa State. Using Anopheles characteristics as described by Gilles and Coetzee (1987) using Zeiss light microscope at x 20. A total of 3027 Anopheles were collected and identified. 3027 (78.40%) were collected from Hadejia Local Government Area while 834 (21.60%) were collected from Jahun Local Government Area. Using Anopheles characters of Gilles and Coetzee (1987) under zeiss light microscope, 2605 (67.47%) were Anopheles gambiae s.l., 907 (23.49%) were Anopheles funestus and 349 (09.04%) were Anopheles maculipalpis. Hadejia Local Government Area had the higher number of Anopheles identified. An. gambiae s.l. ranked the highest among other species. Further molecular identification of sub-species complex of An. gambiae s.l. and An. funestus is strongly recommended in the area.
Keywords: Morphological, malaria, vectors, Anopheles, species.

INTRODUCTION

Malaria remains a leading cause of morbidity and mortality worldwide with an estimated 500 million cases and 2.5 million deaths annually (Stauffer, 2003). *Anopheles gambiae s.l* and *An. funestus* transmit the *Plasmodium* parasites in sub-saharan Africa among the human population. Determination of risk of malaria transmission requires quick and accurate methods of identification of *Anopheles* mosquitoes especially when targeting vector control (Maxwell *et al.,* 2003).

Anopheles mosquito transmits malaria. The most important vectors of malaria are members of *Anopheles s.l* (complex), a group of morphologically identical yet genetically and behaviorally distinct species that differ markedly in their ability to transmit the diseases (Coluzzi, 1978). Members of the species complex include *An. gambiae s.s, An. arabiensis, An. merus, An. melas, An. bwambe,* and *An. quadrimaculatus* (Coetzee *et al.,* 2000).

Anopheles mosquitoes breed in areas with water bodies such as ponds, rivers, surface water, waste-waters, well, etc (Service, 1980). Moreover, the study areas are suitable for the growth and development of various strains of mosquitoes as ponds, wells and surface water bodies of different sizes are available during rainy season, May to October and supplemented waste-water throughout the year which could also serve as breeding sites (Zakari, 2006).

Molineaux and Grammicia (1980) concluded that differences in *Anopheles* species cause the failure of insecticide application and mass drug administration in suppressing malaria transmission. The present *Anopheles* status of the study areas needs to be updated Therefore, the aim of this study is to identify various *Anopheles* species in the area for effective vector control.

MATERIALS AND METHODS

Study Area

The study was carried out in Hadejia and Jahun Local Government Areas of Jigawa State, Nigeria between May and October, 2011. The area is situated on latitude 11°31'N and longitude 9° 09' E. It falls within the Sudan savannah zone, it is semi arid region. The minimum and maximum temperature range between 15.89°C and 36°C and fall as low as 10°C during harmattarn season between December and February. Rainfall ranges form 491mm to 1186mm and starts from May and ends in October, while dry season starts in November and ends in April (Wikipedia, 2010). More water bodies that harbor survival of various strains of *Anopheles* are observed in the area.

Sample Collection

Indoor Collection

Indoor resting adult mosquitoes were collected according to the protocol of Molineaux and Grammiccia (1980). The collection was achieved by Indoor Residual Spray (IRS) technique. The selected houses were visited between 6 am and 10am every other day for ten days. A sheet of large white cloth (4mx3m) was spread on the floor in a room for easy recognition of mosquitoes and this was followed by spray of pyrethrum (Pyrethrin). After 15 minutes, the mosquitoes that fall on the sheet were collected and stored separately in Eppendorf tubes containing anhydrous calcium sulphate ($CaSO_4$) as drying agent until required (Molineaux and Grammiccia, 1980). Thirty six houses were visited in this study.

Collection and Rearing of Larvae

Larval sampling was done using the standard dipping method with a 350ml mosquito scoop (Bioquip, Gardena, CA, USA) as described by Service (1993) and this took twenty days. The larvae were immediately preserved in plastic jugs and taken to laboratory for rearing according to WHO (1975a and 1975b).

They were kept at room temperature and fed with ground fish diet powder in Aquarium. The adults that emerged (within 1-4 days) were killed by anaesthetizing using drops of Acetyl acetate placed on large Whatman's filter paper above the adults container. They were collected and stored separately in Eppendorf tubes prior to identification.

Morphological Identification of Anopheles Mosquitoes

Using morphological characters of Gilles and Coetzee (1987) under x20 Zeiss light microscope. The identification focused on dark spot at the upper margins of the wings which is common to all *Anopheles.* The palpis are elongated and segmented into three. A pale spot on second dark area, a light spot between the two dark spots on vein 6 and absence of fringes on vein 6 are features for *Anopheles funestus.* Speckles on the legs, third preapical dark area on vein 1 with a pale interruption and tersus 1-4 with conspicuous pale bands are features for *Anopheles gambiae.* Vein 1 with 2 accessory sector pale spots, hind tarsi 4 and 5 entirely pale and legs are pale are features for *Anopheles maculipalpis.*

Data Presentation

The data obtained on *Anopheles* species in the study area was expressed in percentage and presented in tables.

RESULTS

A total of 3861 *Anopheles* were identified of which 3027 (78.40%) and 834 (21.60%) were collected from Hadejia and Jahun Local Government Areas respectively.

Anopheles gambiae s.l were 2605 (67.47%), *An. funestus* were 907 (23.49%) and *An. maculipalpis* were 349 (09.04%). The adults collected indoors from Hadejia were 1812 (59.86%) while 1215 (40.14%) were reared from larvae. Adults collected indoors from Jahun were 603 (72.30%) while 231 (27.70%) were adults reared from larvae.

Table 1 shows the number of adult *Anopheles* species collected from Hadejia Local Government Area. In this, 3027 (78.40%) *Anopheles* species were identified of which 1812 (59.86%) were collected indoors; of this 116 (61.59%) were *An. gambiae s.l,* 495 (27.32%) were *An. funestus* and 201 (11.09%) were *An. maculipalpis* while 1215 (40.14%) were adults reared from larvae; 902 (74.24%) were *An. gambiae s.l,* 231 (19.01%) were *An. funestus* and 82 (6.75%) were *An. maculipalpis.*

Table 2 shows the *Anopheles* distribution collected from Jahun Local Government Area. The overall collection was 834 (21.60%). Of this, 603 (72.30%) were adults collected indoors; 401 (66.50%) were *An. gambiae s.l,* 143 (23.72%) were *An. funestus* and 59 (09.78%) were *An. maculipalpis* while 231 (27.70%) were adults reared from larvae; 186 (80.52%) were *An. gambiae s.l.,* 38 (16.45%) were *An. funestus* and 07 (03.03%) were *An. maculipalpis.*

Table 1: Distribution of Adult *Anopheles* species collected at Hadejia Local Government Area

***Anopheles* species**	**Adults collected indoors**	**Adults reared from larvae**	**Overall**
An. gambiae s.l	1116	902	2018
An. funestus	495	231	726
An. maculipalpis	201	82	283
Total	1812	1215	3027

Table 2: Distribution of Adult *Anopheles* species collected at Jahun Local Government Area.

***Anopheles* species**	**Adults collected indoors**	**Adults reared from larvae**	**Overall**
An. gambiae s.l	401	186	587
An. funestus	143	38	181
An. maculipalpis	59	07	66
Total	603	231	834

DISCUSSION

Identification of *Anopheles* species like all other mosquitoes is changing where taxonomists are rather describing new species and subspecies or redescribing existing one. Many techniques used in the identification of *Anopheles* mosquitoes have been published including Faran (1981) and Gillet (1972).

This study had revealed the abundance of *An. funestus, An. gambiae s.l* and *An. maculipalpis* in a large number (Table 1&2). Most of the *Anopheles* reported in this study was *An. gambiae s.l.* The most important vector of malaria in sub-Saharan Africa is *An. gambiae s.l* and it exhibits extreme heterogeneity *(Coluzzi et al.,* 2002).

The result of morphological examination of adults reared from larvae and collected indoors has revealed the presence of predominantly *An. gambiae s.l* (Table 1 and 2). This observation is important; as it reveals that *An. gambiae* and other species are breeding in the study area. Spraying insecticides can reduce vector infectivity by reducing the vector survival rate and increasing the length of the sporogonic cycle (Anonymous, 1991). For example, when indoor resting mosquitoes are forced to rest outside, where ambient temperature is suboptimal for parasite maturation and eventual vector survival (Lines *et al.,* 1991).

Hadejia Local Government area had higher number of *Anopheles* 3027 (78.40%) (Table 1); this may be due to the fact that it has more mash areas as when compared with Jahun. Service (1980); as some of the environmental management practices to include reduction and or management of breeding sites by filling container receptacles, water storage jars, village pot, tyres, canoes, and abandoned cans. Breeding sites like ponds burrow pits, fresh and salt mashes can be drained or impoundments built, which could lead to permanent control.

The study had also revealed the abundance *An. funestus* and *An. maculipalpis* in relatively low proportion in Jahun Local Government Area (Table 1) than in Hadejia Local Government Area. Similarly, the adults collected indoors, 2415 (62.55%) is higher than the adults reared from larvae, 1446 (37.45%) and this had agreed with findings of Ahmed *et al.* (2011) whereby 592 *Anopheles* were collected indoors while 579 were reared from larvae and this may be attributed to errors associated with larval rearing.

CONCLUSION

The result of morphological identification of malaria vectors within *Anopheles* species in this study justifies the presence of *An. funestus, An. maculipalpis* and *An. gambiae s.l* in the study area.

RECOMMENDATION

Further molecular characterization of the *Anopheles* identified into *An. arabiensis, An. gambiae s.s,* M and S forms, the species complex of *An. gambiae* s.l and same on An. *funestus* species need to be carried out, since morphological identification is a preliminary stage.

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