Research Article
Malays. j. med. biol. res.

Morphological Identification, Species Composition and Distribution of Mosquitoes in Kosti region, White Nile State, Central Sudan

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ABSTRACT

Mosquitoes are a large diverse group of insects, with members being most important as vectors of diseases. The correct identification of mosquitoes is crucial to the control of the mosquito-borne diseases. This study was designed to provide baseline data on the species composition and distribution of members of the Anophelines and Culicines genera in ten selected localities in Kosti region, White Nile State, Central Sudan. Global Positioning System (GPS) was used to select collection sites. Adult mosquitoes were collected by pyrethrum spray catch (PSC) from randomly selected houses in Allia, Alnsr, Alskahdeed, Almrabie, Alshati, Althwrat, Alraba, Block 26, Block 32 and Khadugli in Kosti region during September 2014-August 2015. The Anophelines and Culicines mosquitoes were identified using standard morphological keys. Two Anopheles species and three Culex species were morphologically identified. These were *An. gambiae* s.s.; *An. pharoensis; Cx. quinquefasciatus*; *Cx. univittatus* and *Cx. pipiens.* This is the first report of *An. pharoensis* in the study area. The Highest mosquito abundance was observed in the September followed by October, while the lowest mosquito abundance was in July. *An. gambiae* s.s and *Cx. quinquefasciatus* were the predominant species which has importance as they indicate the presence of Malaria and arboviral infections in the study areas; respectively. These findings are of importance in the planning and implementation of vector control strategy in the Kosti region, White Nile state.

Key words: Mosquitoes, Morphology, Identification, Anophelines, Culicines, Kosti, Central Sudan

Manuscript Received: 25 October 2018 - Revised: 12 December 2018 - Accepted: 05 January 2019

INTRODUCTION

Mosquitoes are among the most important disease vectors, known to transmit and maintain the circulation of pathogens that cause both global and neglected tropical diseases in humans and animals. The mosquitoes were originally all contained in four genera; *Culex*, *Anopheles*, *Aedes* and *Corethra* (Theobald, 1906). They have a significant role as vectors of many serious human and animal diseases such as malaria, yellow fever, encephalitis, lymphatic filariasis and Rift Valley fever (Anosike *et al*., 2007).

Of the 465 formally named anopheline species worldwide, about 41 (9%) are incriminated as malaria vectors (Harbach, 2013). However, Africa experiences the bulk of the global malaria burden due in part to the presence of the An. gambiae complex, which includes *Anopheles gambiae*, *An. arabiensis*, *An. merus* and *An. melas*. There are a further three, highly anthropophilic in Africa, *An. funestus*, *An. moucheti* and *An. nili* (Sinka *et al*., 2010). Nevertheless, *An. arabiensis* is considered the dominant and most dangerous malaria vector in Sudan. It is being reported from all parts of Sudan, and distributed over the dry savanna belt and semiarid parts of the country (Petrarca *et al*., 2000).

Mosquitoes of the *Culex pipiens* complex are cosmopolitan and important vectors of neglected tropical diseases, such as arbovirosis and lymphatic filariasis. Among the complex taxa, *Cx. pipiens* (with two forms pipiens and molestus)

and *Cx. quinquefasciatus* are the most ubiquitous mosquitoes in temperate and tropical regions respectively. Mosquitoes of this taxa lack of morphological differences between females, but have frank behavioral and physiological differences and have different trophic preferences that influence their vectorial status (Shaikevich *et al*., 2016).

Three genera of medically important mosquitoes are found in Sudan (*Anopheles*, *Culex* and *Aedes*). A number of 156 species, 2 subspecies of mosquitoes and 7 varieties of Culicidae have been recorded in Sudan. Forty five species of *Culex* were recorded in Sudan, the most prominent was *Culex quinquefasciatus* followed by *Cx. univittatus*. Another species of medical importance was mentioned some years ago, this was *Cx. poicilipes*. *Cx. quinquefasciatus* is the predominant species of *Culex* in Sudan. It is known to be a domestic annoying mosquito. It breeds abundantly in any collection of water such as barrels, tanks, culverts, etc. It can breed in fresh, brackish, and foul water such as cesspits and sinks. It is a vector of filariasis in Sudan and encephalitis abroad (El Rayah, 2007); also 38 species of Anopheles were recorded, and few species transmit malaria (Nugud *et al*., 1997). Mosquitoes of the genus *Culex* transmit West Nile Virus (WNV) and Rift Valley Fever (RVF). Lymphatic filariasis is caused by parasitic nematodes: *Wuchereria bancrofti*. In Sudan the disease is restricted to three regions in Kordofan, Darfur and the Blue Nile States, but now it tends to include most of the States. It is transmitted by *Culex* mosquitoes. More promising; the future potential distribution of *An. arabiensis* in Africa will be reduced as a result of climate change as revealed by the ecological niche modelling study (Drake and Beier, 2014). Although still below target levels, current malaria interventions (Insecticide treated bednet coverage (ITNs), Indoor residual spraying coverage (IRS), and Artemisinin-based combination therapy coverage (ACTs)) have substantially reduced malaria disease incidence across the continent (Malaria Atlas Project, 2017).

In order to correctly assign spatio-temporal distributions, behaviors and responses to interventions to particular anopheline species, identification of mosquitoes must be accurately made (Stevenson and Norris, 2016). The correct identification and description of mosquitos' vectors are crucial to understand the transmission of mosquito-borne diseases and to design the appropriate control strategy. Therefore, the main objective of this study was the identification of the mosquitoes' composition and the relative abundance of each species in the study areas.

MATERIALS AND METHODS

Study area

The study was carried out in Kosti, White Nile state in Central Sudan. It is the largest city in the state at an altitude of 390 meters above the West Bank of the White Nile and is located 360 kilometers south of the capital, Khartoum. Ten different locations, Allia (13.14745°N - 32.67914°E), Alnsr (13.15724°N - 32.67572°E), Alskahdeed (13.15839°N - 32.67871°E), Almrabie (13.16475°N - 32.67227°E), Alshati (13.17278°N - 32.66313°E), Althwrat (13.16200°N - 32.64886°E), Alraba (13.14029°N - 32.65282°E), Block 26 (13.15892°N - 32.66083°E), Block 32 (13.15138°N - 32.64664°E) and Khadugli (13.15313°N - 32.63335°E) were sampled for mosquitoes fauna. The topographical features of Kosti consisted of flat land interspersed with the northwest sand dunes locally known as "gayzan". Soil is characterized as few mud spills and severe cracking in the drought period. The study region has generally hot dry climate with one long rainy season, which usually extends from June to September with small year-to-year variation and with a maximum temperature ranges between 41° C in April / May and 33° C in August and a minimum temperature ranges between 23° C in May and 17° C in January. In the period between October and May the wind blowing toward the region northeasterly and it rains an average of up to 350 mm per year. In the dry period the relative humidity rate is approximately 26%, while it is 71% in the rainy season. The main vegetation in Kosti is composed of *Acacia mellifera* (Kitir), *Acacia seyal* (Talih), *Acacia tortilis* (Sayal), *Salvador apersica* (Arak), *Commiphora africana* (Gafal) and *Maerua crassifolia* (Sareh) (Elsafori *et al*., 2012). People work as farmers, herdsmen, merchants and laborers. Domestic animals include ducks, poultry, cattle, sheep, goats, donkeys and horses with slight variation in the different collection sites. These sampling sites were selected and mapped using Global Positioning System (GPS). Houses are constructed from one / two of these materials: clay, bricks and cement with concrete roof or zinc covered with straw.

Mosquitoes Collection

Permission was obtained from the household heads before the start of the study. Adult mosquitoes were collected from both natural and man-made habitats, using pyrethrum spray catches (PSC), hand-landing technique, active search collection once per month for the period of study (September 2014 - August 2015). A collected mosquito sample was preserved in Silica gel, for morphological identification. In the hand collection method, mosquitoes were collected early in the morning. A separate cup was used for each site, and the collector was supplied with an aspirator, a torch and paper cups covered with mosquito netting. The cups were clearly labeled in pencil with the locality and date of collection. Mosquitoes were kept in the paper cups with a piece of moistened cotton on top to protect them from excessive heat and dryness until brought to the laboratory. The Spray sheet collection method was used for indoor-resting mosquitoes. The knock-down method was carried out early in the morning, usually between 7-10 am, of the day following the human-landing collection. The inhabitants of each room were first requested to vacate the room and

then the whole floor was covered with white cotton sheets. The door, the windows and all possible mosquito exits were tightly close. The room was filled with a mist of 0.25 % solution of Cypermethrin (a pyrethroid) in water, using a medium-sized hand atomizer (spray-pump). It was left closed for 15 minutes. The dead mosquitoes were then picked off the sheets and collected on Petri dishes that contained moistened cotton covered with filter papers. Subsequently, they were taken to the laboratory for morphological identification with keys.

Mosquitoes Identification

A total number of 100 of the field - collected mosquitoes were randomly selected from each location in order to be identified by external morphology following the keys of the morphological identification of mosquito species of the Afro-tropical region. The mosquitoes were examined under the dissecting microscope using adult morphological identification key for Anopheles (Gillies and De Meillon, 1968) and *Culex spp* (Harbach, 1985). The morphological characteristics useful to distinguish between Anophline and Culicine species such as wings, legs, thorax, abdomen, head and mouthparts for *Anopheles gambiae* complex and *Culex spp.* were observed using a dissecting microscope and permanent slides were prepared and photocopied in order to keep a record. The following 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 pharoensis: These groups of mosquitoes have their abdominal segments with laterally projecting tufts of scales on segments 2-7. Shaggy palps, wing with abundant pale areas, costa with at least 4 pale spots and hind tarsus 5 and about apical half of 4 are pale.

***Culex univittatus* Theobald:** These groups of mosquitoes have their wings with pale scales at base of costa. The hind tibia is with anterior pale stripe and the mid femur with complete anterior pale stripe.

***Culex quinquefasciatus* Say:** These groups of mosquitoes have their abdominal terga with basal pale bands and the fore coxa with some dark scales, usually mostly dark. The wing is entirely dark scaled and the subcosta intersects costa before level of furcating of R_{2+3}.

***Culex pipiens* Linnaeus:** These groups of mosquitoes have their abdominal terga with basal pale bands. The wing is entirely dark scaled and the subcosta intersects costa at or beyond level of furcating of R_{2+3}.

Data analysis

Chi (X^2) square test was used to analyze and compare the differences in the species of the *Anopheles* and *Culex* mosquitoes in the study sites.

RESULTS

Climatic results

The meteorological climatic conditions of the study area "Kosti" during 2015; such as the maximum and minimum temperature is illustrated in Figure 1; monthly total rain fall is illustrated in Figure 2 and the percentage of monthly relative humidity is illustrated in Figure 3.

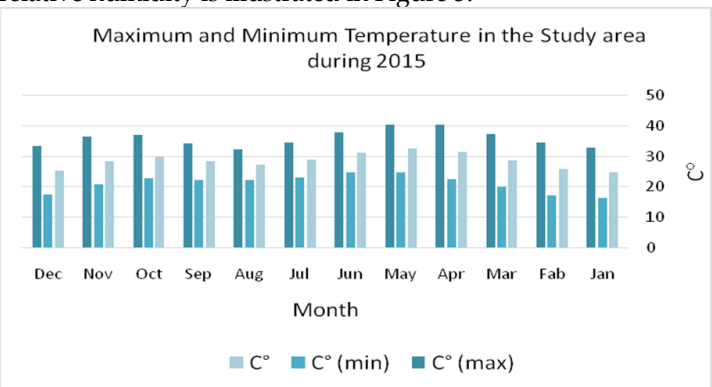

Figure 1: Maximum and Minimum Temperature in the Study area during 2015

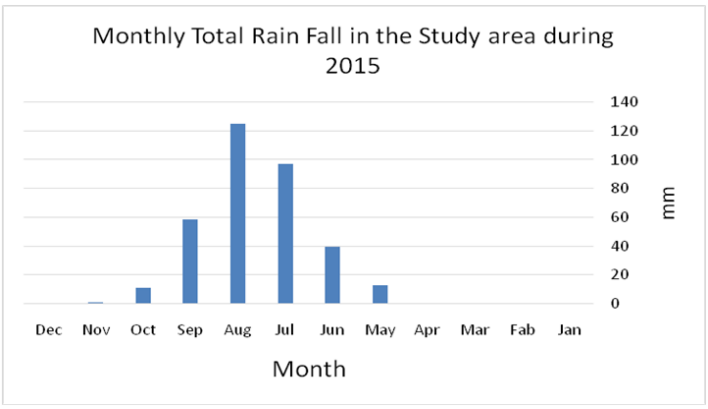

Figure 2: Monthly Total Rain Fall in the Study area during 2015

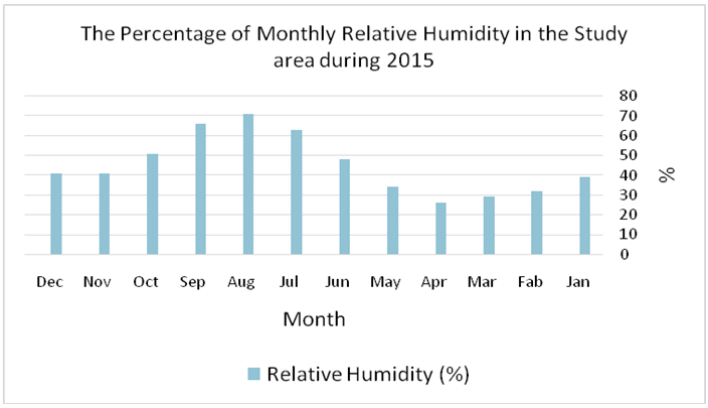

Figure 3: The Percentage of Monthly Relative Humidity in the Study area during 2015

*The average annual relative humidity is 45.1% and average monthly relative humidity ranges from 26% in April to 71% in August

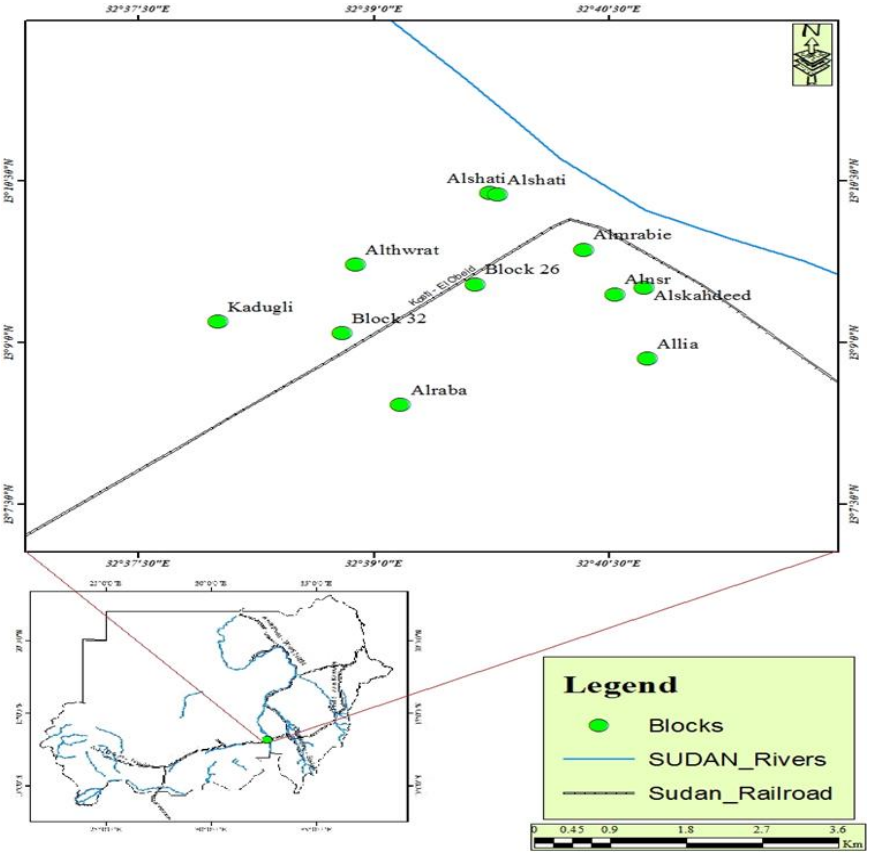

Figure 4: Map showing the Sampling sites in Kosti region used in this study

Morphological identification results

Selected wing, leg, abdomen, and head characteristics were observed and analyzed on 1000 adult mosquitoes from 10 populations (Figure 4) using a dissecting microscope. All of the recognized features for both Anopheline and Culicine mosquitoes are illustrated in Figures 5 -17. All the morphologic terms and abbreviations used in these figures follow those of Gillies and De Meillon (1968) for *Anopheles spp.* and (Harbach (1985) for *Culex spp.* In the field collected mosquitoes, *Anopheles* was identified as having spotted wing and long palps, while *Culex* as having transparent wings and short palps. The male was distinguished from the female by the presence of plumose antennae. *An. pharoensis* is illustrated in Figures 5 and 6. *An. gambiae s. s.* is illustrated in Figures 7 – 10. *Cx. univittatus* is illustrated in Figure 11. *Cx. quinquefasciatus* is illustrated in Figures 12 – 14. *Cx. pipiens* is illustrated in Figure 15.

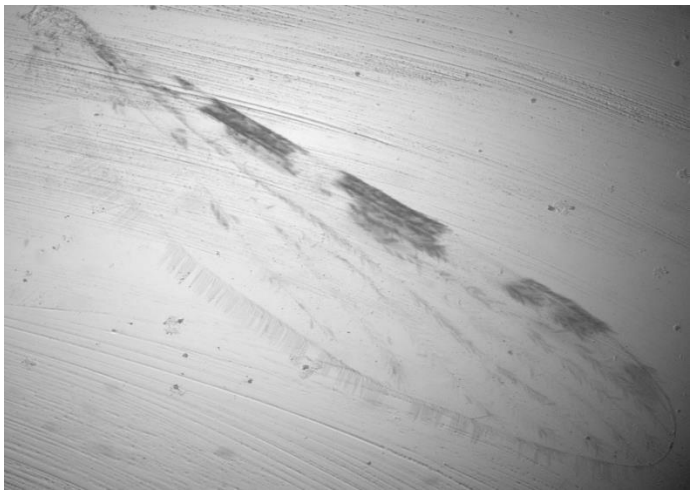

Figure 5: Wing (costa) with 4 pale spots in *An. pharoensis*

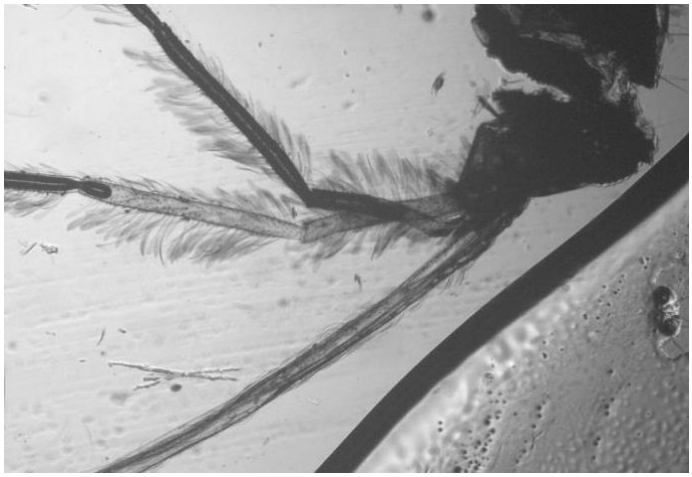

Figure 6: Shaggy palp in *An. pharoensis*

Figure 7: Palps with 3 pale bands in *An. gambiae s. s.*

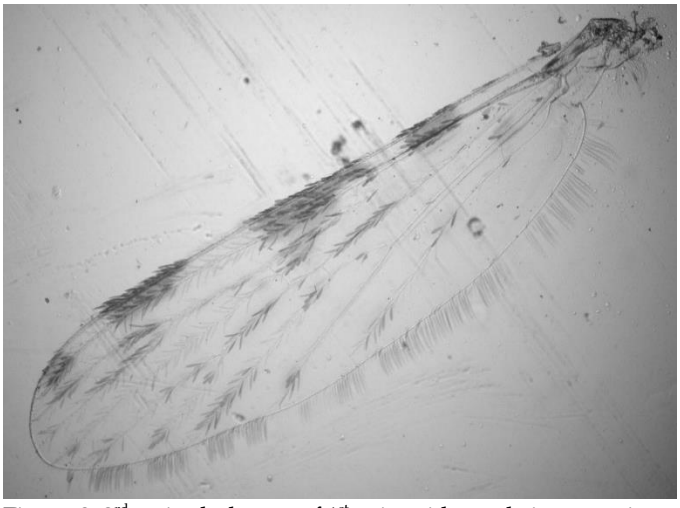

Figure 8: 3rd main dark area of 1st vein with a pale interruption sometimes fused with preceding pale area in *An. gambiae s. s.*

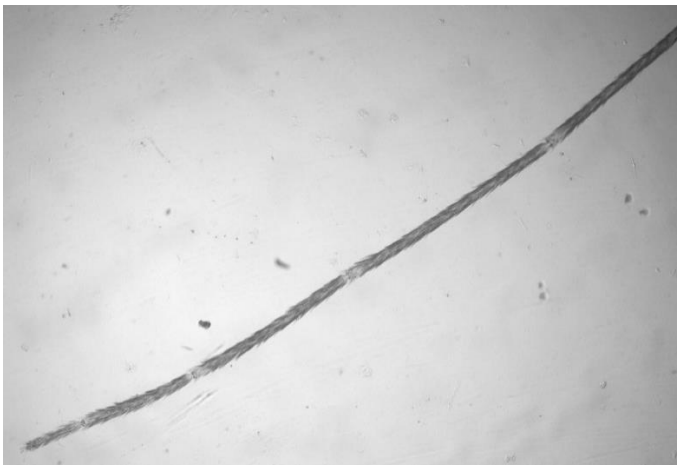

Figure 9: Tarsi l-4 with conspicuous pale bands on at least the apices in *An. gambiae s. s.*

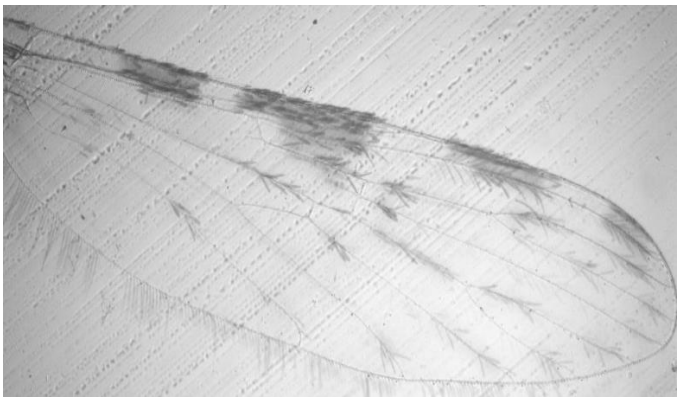

Figure 10: wing with pale fringe spots opposite all veins up to 5b or 6 in An. gambiae s. s.

Figure 11: Wing with pale scales at base of costa in Cx. univittatus

Figure 12: Abdominal terga with basal pale bands in Cx. quinquefasciatus

Figure 13: Forecoxa with some dark scales, usually mostly dark in Cx. quinquefasciatus

Figure 14: Wing entirely dark-scaled. Subcosta intersects costa before level of furcating of R2+3 in *Cx. quinquefasciatus*

Figure 15: Subcosta intersects costa at or beyond level of furcating of R2+3 in *Cx. pipiens*

Mosquitoes species abundance and distribution in the study areas

Out of 1009 mosquitoes sample collected in this study, 80.67% are *Culex* species and 19.33% are *Anopheles* species. Out of the 195 Anopheles 187 (95.90%) are *Anopheles gambiae* complex that includes *Anopheles gambiae s.s.* and *An. arabiensis*. The variation in the sibling species composition of *An. gambiae* complex was significant ($p < 0.05$). *Anopheles gambiae s.s.* was predominant in almost all localities and the only exception was Kadugli (Figure 18).

Out of the 814 *Culex* 403 (49.51%) are *Cx. quinquefasciatus* and 398 (48.89%) are *Cx. univittatus* and only 13 (1.60%) are *Cx. pipiens*. The species composition in each locality is illustrated in Figure 18. While the species distribution in the study areas during the period from September 2015 to August 2016 is illustrated in Figure 19. Female distribution is illustrated in Figure 20 and the male distribution is illustrated in Figure 21.

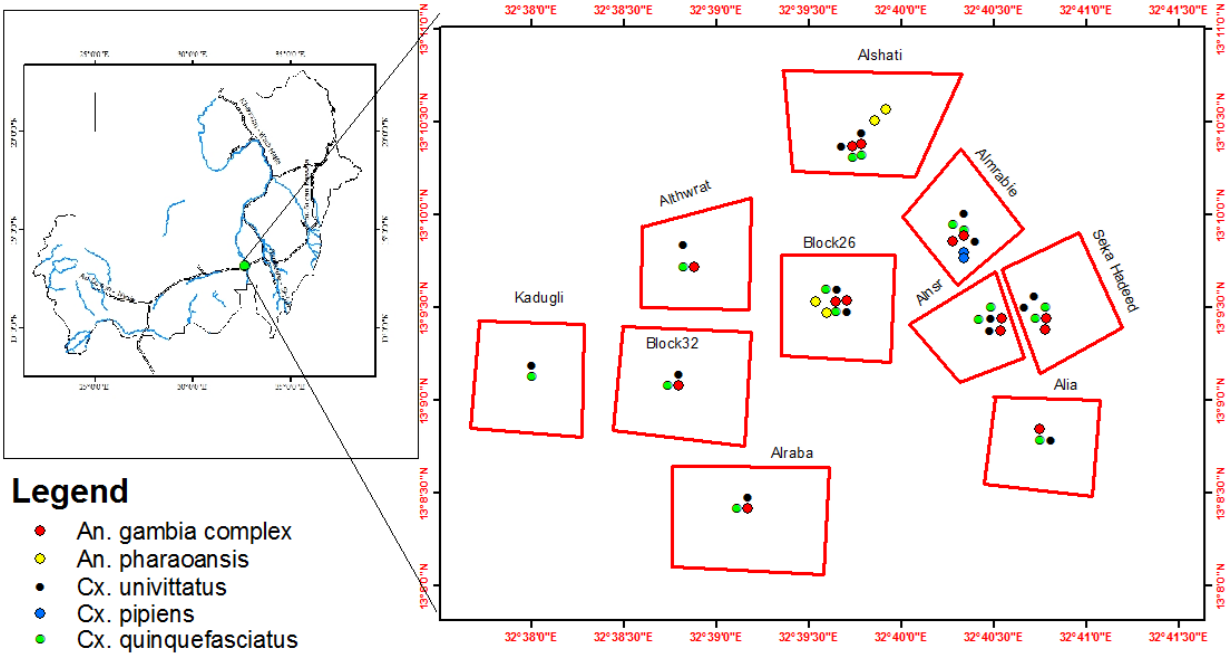

Figure 16: Map showing the Mosquitoes species distribution in sampling sites using Global Positioning System (GPS) in Kosti District

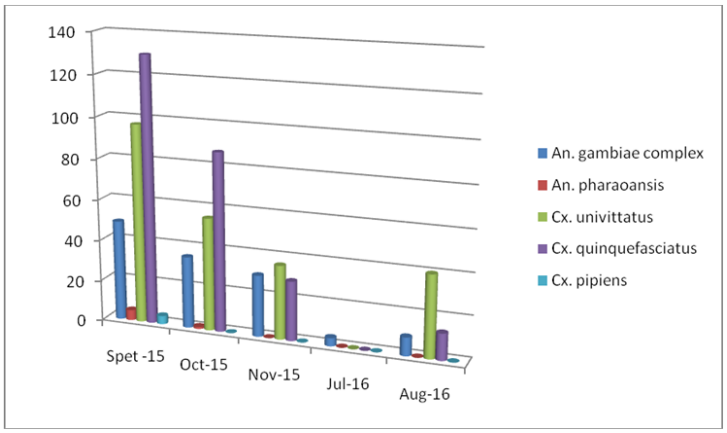

Figure 17: Mosquitoes species distribution during the period September 2015 – August 2016 in Kosti District, Central Sudan

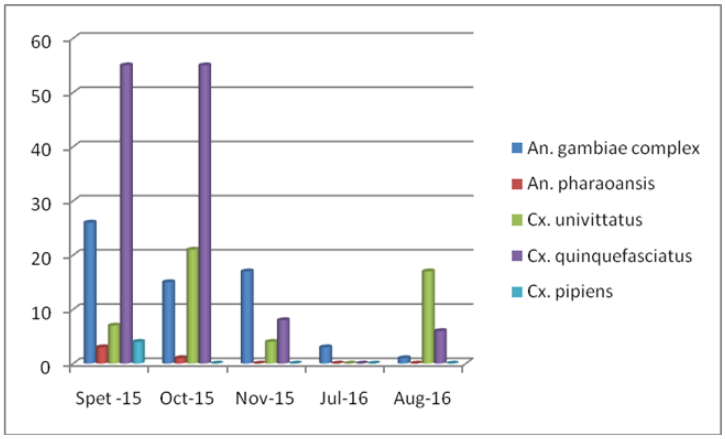

Figure 18: Female Mosquitoes species distribution during the period September 2015 – August 2016 in Kosti District, Central Sudan

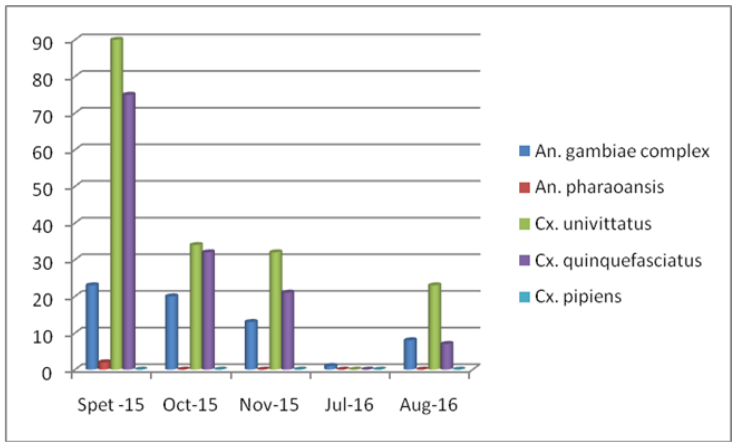

Figure 19: Male Mosquitoes species distribution during the period September 2015 – August 2016 in Kosti District, Central Sudan

Statistical Results

The program "An interactive calculation tool for chi-square tests of goodness of fit and independence" (Preacher, 2001) was used for statistical analysis among mosquitos' populations with N 1009. The chi-square statistic for all mosquitos was 368.669. The p-value was 0.00; the result is significant at $p < .05$. The chi-square statistic for female's mosquitos was 295.339. The p-value was 0.00; the result was significant at $p < 0.05$. The chi-square statistic for male's mosquitos was 114.923. The p-value was 0.00; the result was significant at $p < .05$. This result indicates that there was high significant variation among the distribution of the various mosquitos' species within the 10 different localities in Kosti district.

DISCUSSION

Identification of Anopheline and Culicine in the selected study areas in Kosti district had a total of two Anopheles species and three Culex species which were based on their morphological characters. These were *An. gambiae* s.s.; *An. pharoensis; Cx. quinquefasciatus*; *Cx. univittatus* and *Cx. pipiens.*

The report of *An. gambiae* s.s in Kosti district, Central Sudan during this study is in agreement with the previous reports from Africa, which stated that the most important members of the complex involved in malaria transmission are *An. gambiae* s.s., *An. gambiae* s.l., *An. arabiensis*. *An. quadriannulatus*, *An. melas*, *An. merus*; *An. bwamba* and *An. funestus* (Coluzzi *et al*., 1979; White, 1985; Gillies, 1987; Gillies and Coetzee, 1987; Coetzee *et al*., 1993; Hunt *et al*., 1998; Coetzee *et al*., 2000; Pock *et al*., 2003; Coetzee, 2004; Moreno *et al*., 2004). However, some of them are of minor importance. These species vary in relative frequency and distribution from one geographical location to another; distribution is influenced by prevailing climatic conditions (Lindsay *et al*., 1998).

In Sudan, there are 29 anopheline mosquito species that have so far been recorded (Lewis, 1958) they are widely distributed throughout the country, but more often are localized in the humid areas. *An. arabiensis* and *An. gambiae* are the only species of the *An. gambiae* complex reported in Sudan (Zahar, 1985; Petrarca *et al*., 2000). The identification of *An. gambiae* complex in Kosti district, White Nile state during this study is a confirmation of a previous study that reported *An. gambiae arabiensis* as the most frequent species (80.7%) in White Nile state (Seufi and Galal, 2010). This result is in agreement with previous ones that *An. 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 entomological results showed that *An. gambiae* complex is most predominant species in all localities except Kadugli one, the only other Anopheline species in the study areas was *An. pharoensis* Theobald. These findings are in agreement with those of Lewis (1958) and El Gadal *et al*. (1985) who´s found, *An. pharoensis* from Gezira area, but this species play no role in malaria transmission because of their largely zoophilic behaviour (Lewis, 1958; El Gadal *et al*., 1985).The *An. pharoensis* was reported from three localities; Block-26, Alshati and Alnsr. The finding of *An. pharoensis* (Theobald, 1901) in these localities could be explained by the influence of modern means of transport in the distribution of mosquitoes species in the country, since these three localities lie in close proximate to the railway line (Kosti – El Obeid). We suggest that this species is imported from other places to Kosti region.

The record of *An. pharoensis* in Kosti district, White Nile state was the first report of occurrence of this species of *Anopheles* in this area and this species was only reported previously with low density (0.1%) in Eastern Sudan (Himeidan *et al*., 2004) and in Gezira in Central Sudan (el Safi and Haridi, 1986). The low numbers of *An. pharoensis* in

the indoor – resting collections may be attributed to the zoophilic nature of this species since there were considerable numbers of domestic animals that includes cattle, sheep, goat, donkeys, horses and poultry in the study areas.

The confirmation of *An. gambiae* s. s. as the predominant Anopheles species is important because it reveals the presence of the Malaria vector in the study areas. Another observation was that, *An. gambiae* s. s in all collections which means that an expected Malaria transmission may occur all over the year in the study areas.

The reporting of both *Cx. quinquefasciatus* and *Cx. univittatus* as the main *Culex* species in Kosti district was in consistence with the previous statement (El Rayah, 2007), that there were 45 species of *Culex* in Sudan, the most prominent was *Cx. quinquefasciatus* followed by *Cx. univittatus*. However, *Cx. quinquefasciatus* was absent only in the July collection,. It is known to be a domestic annoying mosquito. It breeds abundantly in any collection of water such as barrels, tanks, culverts, etc. It can breed in fresh, brackish, and foul water such as cesspits and sinks. This species is widely distributed in Asia, Africa and America. It is a vector of filariasis in Sudan and encephalitis abroad (El Rayah, 2007). It has been shown recently (Samy *et al*., 2016) that the highest stability of *Cx. quinquefasciatus* with climate change is observed between 30°S and 30°N and the finding of this mosquitoes species in Kosti district which lies in 13°10′N 32°40′E during this study confirms this statement.

The entomological results showed that *Cx. quinquefasciatus* followed by *Cx. univittatusis* most predominant species in all sites, the only other *Culex* species in the study area *Cx. pipiens* was the species found just in Almrabie. The presence of *Culex* in relatively high proportion reflects its wide feeding range. The observation that *Cx. quinquefasciatus* is the predominant Culex species is important as it reveals the presence of the Arboviruses vector in the study areas.

Overall, the September collection was the richest one in terms of the abundance of the 5 identified mosquitos' species from Kosti district with the highest density of *Cx. quinquefasciatus*. While, July collection was the poorest one. The climatic conditions clearly influence the species distribution in the area. Variation in the annual distribution of the both sexes was observed. The houses construction, human activities, presence of broken pipes, water sources and type of soil, all had direct effect on the amount of the collections.

CONCLUSION

The study has revealed that, 2 different species of *Anopheles* mosquitoes found in the study area with *An. gambiae s.s* showing the highest prevalence over other species while *An. pharoensis* s was the lowest in population. In addition 3 different species of *Culex* mosquitoes were identified with *Cx. quinquefasciatus* and *Cx. univittatus* showing the highest predominance while *Cx. pipiensis* was the lowest in population. The observation of *An. gambiae* s.s in Kosti district is of importance in malaria epidemiology. These findings are of importance in the planning and implementation of malaria vector control strategy in the Kosti region, White Nile state. We concluded that the morphological technique is suitable for preliminary field survey, particularly in a country of low setting resources.

ACKNOWLEDGEMENTS

The cooperation of the people of the 10 localities from Kosti region is gratefully acknowledged. The help of the Public Health workers and the field assistants from Kosti is appreciated. This work is supported by a research grant from Ministry of Higher Education and Scientific Research, Republic of the Sudan.

REFERENCES

Andrews FW. (1948). Vegetation of the Sudan. In: Agriculture in the Sudan (ed. J. D. Tothil), Oxoford University Press. Oxford.

Anosike J, Nwoke B, Okere A, Oku E, Asor J, Emmy-Egbe I and Adimike D. 2007. Epidemiology of tree-hole breeding mosquitoes in the tropical rainforest of Imo State, South-east Nigeria. Ann. Agric. Environ. Med., 14, pp. 31-38.

Coetzee M, Hunt RH, Brack LEO, Davidson G. Distribution of mosquitoes belonging to the *Anopheles gambiae* complex including malaria vector, south of latitude, 15°S. South Afr J Sci 1993; 89: 227–31.

Coetzee M. Distribution of the African malaria mosquitoes belonging to the Anopheles gambiae complex. Parasitol Today 2004; 16: 74–7.

Drake JM and Beier JC. Ecological niche and potential distribution of Anopheles arabiensis in Africa in 2050. Malar J. 2014 Jun 3;13: 213.

Elgadal AA, Haridi AM, Hassan FT and Hussein H. (1985). Malaria control in the Gezira. Manigal irrigated Scheme of the Sudan. J.Trop. Med. Hyg.Vol. 88; pp. 153-159.

El-Rayah EM. 2007. Mosquitoes of the Sudan. Sudan Notes & Records, 6, pp.153-187.

Elsafori AK, Gumma AN and Nour MAE. Contribution to the flora of Um Rimmitta area, White Nile State, Sudan. Agric. Biol. J. N. Am., 2012, 3(10): 427-434.

Gillies MT and Coetzee M. (1987). A Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region). Johannesburg, South Africa: South African Institute for Medical Research 1987; Pub. No. 55.

Harbach RE. The phylogeny and classification of *Anopheles*. In: Manguin S, editor. Anopheles Mosquitoes—New Insights into Malaria Vectors. Rijeka, Croatia: InTech; 2013. pp. 3–55.

Himeidan YE, Dukeen MY, El-Rayah el-A, Adam I. Anopheles arabiensis: abundance and insecticide resistance in an irrigated area of eastern Sudan. East Mediterr Health J. 2004 Jan-Mar; 10(1-2):167-74.

Hunt RH, Coetzee M, Fettene M. The *Anopheles gambiae* complex: A new species from Ethiopia. Trans R Soc Trop Med Hyg 1998; 92: 231–5.

Lindsay SW, Parson L, Thomas CJ. Mapping the ranges and relative abundance of the two principal African malaria vectors, *Anopheles gambiae* s.s. and *An. arabiensis*, using climate data. Proc R Ent Soc Lond 1998; 265: 847–54

Malaria Atlas Project Mosquito Malaria Vectors. http://www.map.ox.ac.uk/explore/mosquito-malaria-vectors Available at. Accessed January 15, 2017.

Moreno M, Cano J, Nzambo S, Robuakasi L, Buatiche JN, Ondo M, *et al*. A Malaria panel assay versus PCR: Detection of naturally infected *Anopheles melas* in a coastal village of Equatorial Guinea. Malar J 2004; 3: 20.

Nugud, A., Eltayeb, R. and Abd-EL Nur, O. 1997. Vectors of malaria in Sudan. Joint workshop on scientific cooperation, The Federal Ministry of Agriculture and Forestry, 1997. Sudan, ICIPE, Kenya, Khartoum 6-7 Dec. 1997.

Petrarca V, Nugud AD, Ahmed MAH, Haridi AM, Di Deco MA, Coluzzi M. Cytogenetics of the *Anopheles gambiae* complex in Sudan with special reference to *An. arabiensis*: relationships with East and West African populations. Med Vet Entomol. 2000; 14: 149–164

Pock TJ, Duchemin JB, Marrama L, Rabarison P, LeGoff, Rajaonarivelo V, *et al*. Distribution of the species of the *Anopheles gambiae* complex and first evidence of *Anopheles merus* as a malaria vector in Madagascar. Malar J 2003; 2: 33

Preacher KJ. (2001, April). Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence [Computer software]. Available from http://quantpsy.org.

Safi SH, Haridi AM. Field trial of the insect growth regulator, Dimilin, for control of Anopheles pharoensis in Gezira, Sudan. J Am Mosq Control Assoc. 1986 Sep;2(3):374-5.

Samy AM, Elaagip AH, Kenawy MA, Ayres CF, Peterson AT, Soliman DE. Climate Change Influences on the Global Potential Distribution of the Mosquito *Culex quinquefasciatus*, Vector of West Nile Virus and Lymphatic Filariasis. PLoS One. 2016 Oct 3; 11(10):e0163863. doi: 10.1371/journal.pone.0163863.

Service MW (2014). *Anopheles gambiae*: Africa principal malaria vector 1902 —1984. Bulletin of Entomological Society of America, pp 77-94.

Seufi AM, Galal FH. Role of Culex and Anopheles mosquito species as potential vectors of rift valley fever virus in Sudanoutbreak, 2007. BMC Infect Dis. 2010 Mar 11;10:65.

Shaikevich EV, Vinogradova EB, Bouattour A, Gouveia de Almeida AP. Genetic diversity of *Culex pipiens* mosquitoes in distinct populations from Europe: contribution of *Cx. quinquefasciatus* in Mediterranean populations. Parasit Vectors. 2016 Jan 27;9:47.

Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley WH, Gething PW, Kabaria CW, Okara RM, Van Boeckel T,Godfray HC, Harbach RE, Hay SI. The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. Parasit Vectors. 2010 Dec 3;3:117. doi: 10.1186/1756-3305-3-117.

Stevenson JC and Norris DE. Implicating Cryptic and Novel Anophelines as Malaria Vectors in Africa. Insects. 2016 Dec 22;8(1). pii: E1. doi: 10.3390/insects8010001.

Theobald, F. 1906. Second report on the mosquitoes or Culicidae of the Sudan. Report on Economic Entomology, pp. 67-83.

Tolle MA. Mosquito-borne diseases. Curr. Probl. Pediatr. Adolesc. Health Care. 2009 Apr; 39(4):97-140.

White GB. *Anopheles bwambae sp*: A malaria vector in Semliki valley, Uganda and its relationships with other siblings. Syst Entomol 1985; 10: 501–22.

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