Studies on Mosquitoes *Anopheles* Species Resting Densities Indoors in Kodok locality, Upper Nile State, South Sudan (2020-2022)

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Abstract

Malaria is a disease caused by the parasite *Plasmodium*, which is transmitted by the bite of an infected female anopheles mosquito, only the Anopheles genus transmitted malaria to human. Entomological cross sectional studied carried out over 24 months, period from April 2020 to March 2022 in four areas of the Kodok locality in Upper Nile State in South Sudan to determine indoor resting density and seasonal changes in indoor resting density, additionally to number of *Anopheles* and other mosquitoes in a given room by using pyrethrum spray sheet collection for mosquitoes collection Bi-monthly from descriptive household, eighty houses and dipper for larvae collection from eight breeding sites to establish active larvae breeding site patterns, seasonal density and seasonal change in larvae density , morphological identification and sorting out of mosquitoes species according to species morphologically characteristics by using key identification of Gillies and de Meillon 1968.

Total mosquitoes collected were 63644 within eighty houses and identified into species; *An. gambiae s.s* 68% (43084), *An. arabiensis* 26% (16840) and other mosquitoes 6% (3720). The density vary between different houses ; *An. gambiae s.s* 52-67and *An. arabiensis* 20-28 in rainy (April to October) season but in dry season (November to March) *An. gambiae s.s* 2-12 and *An. arabiensis* 0-5. During the sampling of mosquitoes

larvae, a total number 7704 larva collected; *anopheles gambiae* 88% *(*6748) and other mosquitoes 12% (956), within eight potential breeding sites, 384 numbers of visit to sites.

Keywords: Kodok locality, Upper Nile State, South Sudan, *An. gambiae s.s*, *An. arabiensis, anopheles* density, other mosquitoes, larva density, seasonal change, seasonal density

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1. Introduction:

Nearly half of the world's population was at risk of malaria, most cases and deaths occur in sub-Saharan Africa with an estimated 241 million cases and 627 000 deaths, Children under 5 years of age are the most vulnerable group affected by malaria; they accounted for about 80% of all malaria deaths in the WHO African Region (1), Malaria is a disease caused by the parasite *Plasmodium*, which is transmitted by the bite of an infected female anopheles mosquito (2).

The peak up period of transmission is during the rainy season mainly April to October, *P. falciparum* is the dominant species of parasite and responsible for more than 90% of the cases in South Sudan (3), *Anopheles* species are anthropophilic, bite in the morning or evening and at night; others feed during the day, they may bite indoors (endophagic) or outdoors (exophagic) behavior (4).

Population density of this species varies seasonally in relationship to rainfall while population density increases quickly with the first rains and the maximum density is reached at the end of the rainy-season (5). Breeding sites in Kodok locality is driven by predominantly shaded swamp and human-made (waste water) throughout the year however; *Anopheles gambiae* is considered the main malaria vector in Kodok locality and the most abundant species in larval habitats during both the dry and the rainy seasons while *An. gambiae s.*s was the principal vector in these areas of the study followed by *An. arabiensis* which are already found to be involved in malaria transmission in kodok locality as in line with (6).

Malaria transmission rates can differ depending on local factors such as rainfall patterns, the proximity of mosquito breeding sites to people, and types of mosquito species in the area. Some regions have a fairly constant number of cases throughout the year; these countries are termed "malaria endemic" in other areas; there are malaria seasons, usually coinciding with the rainy season (7).

2. MATERIALS AND METHODS

2.1 Study Area:

Kodok locality located in Upper Nile State in South Sudan at western bank of White Nile River, The estimate terrain elevation above sea level is 306 meters, Which lies between latitude 90 53' 00" N and longitude 32^0 07' 00" E and with humidity 49. The population estimated 7,709 according to National Census 2012; the natural vegetation consisted of grasses in swamps, and trees (e.g. Acacia spp)

Figure (1) Map of Upper Nile State Showing fashoda county (Kodok) map with boundaries with other counties (Localities).

2.2 Study design:

Cross sectional studied was conducted bi-weekly by using pyrethrum spray sheet collection for mosquitoes collection in eighty houses in four areas; Hai Bilpam, Hai Ochagi, Hai Nevasha and Hai Salam.

2.2.1 Houses

An eighty houses was been examined /day/section, houses were randomly selected from a random list, choosing a first block follow by the third as follow and within block three houses are selected, first house then the fourth house.

2.2.2 Breeding Habitats:

Larval survey was carried out in eight breeding habitats in the areas to collect larvae from two breeding sites per each area and to investigate the permanent and seasonal ones by using dipper.

2.3 Entomological sampling method

Entomology studies were conducted over 24 months period from April 2020 to March 2022 in four areas (Hai Bilpam, Hai Ochugi, Hai Nevasha and Hai Salam) in the Kodok locality, for mosquitoes collection bi-monthly chosen the first and third week each month to surveyed descriptive household by using pyrethrum spray sheet collection for indoor resting, collection started from 7:30 to 11:00 a.m. after consent of house occupant. All mosquito collected species was preserved in paper cups covered with netting for each house and room labeled and mosquitoes' larvae survey sampled by using dipper in swamp and manmade, at the end all species were Sorting and identifying into species by using key identification of Gillies and de Meillon 1968, Gillies and Goetzee 1987, with light microscope and lens, all anopheles larvae are consider as anopheles gambiae ss.

2.4 Data analysis:

was done using Package for the Social Sciences (SPSS, version 16).Descriptive analysis was done to obtain the Means or density and species composition by using ANOVA and Statistical variables investigated were; seasonality (dry versus rainy season) and spatial composition.

3. Result:

3.1 Distribution and spatial relative abundance of anopheles species in study areas:

The distribution of identified members of the mosquitoes species by studied sites indicated that total number 63644 mosquitoes identified to species; *An. gambiae s.s* 68% (43084), *An. arabiensis* 26% (16840) and other mosquitoes 6% (3720) and after that mosquitoes were sorting in to group according to the areas and species composition Hai Bilpam(*An.gambiae* s.s 65% , *An. arabiansis* 28%),Hai Ochugi (*An.gambiae* s.s 71%, *An. arabiansis* 24%), Hai Salam (*An.gambiae* s.s 68% , *An. arabiansis* 27%) and Hai Nevasha (*An.gambiae* s.s 68% , *An. arabiansis* 26%) and other mosquitoes species are respectively (7%, 5%, 5%, 6%). The highest number of *An.gambiae* s.s is in Hai Ochugi 71% whereas the lowest in Hai Bilpam 65% and *An. arabiansis* is the highest in Hai Bilpam 28% whereas the lowest in Hai Ochugi 24% as in table (1) that observed each of the *An. gambiae* and *An. arabiensis* was prevalent over the other mosquito's species in the study area.

3.2 The density of anopheles gambiae complex per houses through the year:

The density of anopheles per houses (rooms) during the study period vary between different houses *An. gambiae s.s* 52-67 and *An. arabiensis* 20-28 in rainy season but in dry season *An. gambiae s.s* 2-12 and *An. arabiensis* 0-5 table (2), *An. gambiae* predominated comparing to total indoor resting mosquitoes species table (1). These species were most abundant in the rainy season, annual indoor resting density for *An. gambiae* were 661.19 mosquitoes per house. The high density in April every year as rain started with *anopheles gambiae* is a lead through the year.

3.3 Total number mosquitoes larvae collected and spatial relative distribution:

during the sampling period April 2020 – March 2022, a total larvae were 7704; *anopheles gambiae* 88% *(*6748) and other mosquitoes 12% (956), within 8 potential breeding sites (water locations), 384 number of visits and number of *anopheles gambiae* was recorded in the different areas of the study; Hai Ochugi 87% (1778), Hai Bilpam 87% (1581), Hai Salam 89% (1942) and Hai Nevasha 87% (1447). Mosquitoes species showed that the locality was infested with Anopheles gambiae s.s 88% and other mosquitoes species 12%. Overall, An. gambiae was the predominant in all breeding sites. The density of anopheles gambiae in swamp is high then waste water during the study period.

Table (1): Number of anopheles mosquitoes collected in indoor resting over 24 months in different four areas

area	Total of *An. gambiae s.s*		Total of *An. arabiansis*		Total of other Mosquitoes		Total of Mosquitoes
Hai Bilpam	12405	65%	5324	28%	1324	7%	19053
Hai Nevasha	10679	68%	4058	26%	896	6%	15633
Hai Ochugi	9165	71%	3175	24%	624	5%	12964
Hai Salam	10835	68%	4283	27%	876	5%	15994
Total	43084	68%	16840	26%	3720	6%	63644

Other mosquitoes included *An. pharoensis* .Culex and Aedes.

Table (2): Seasonal change indoor resting density of mosquitoes species over 24 months during rainy and dry season with P-Value

	seasonal changes	N	Mean	Std. Deviation	Std. Error Mean	T-Test	P-Value
Total of *An. gambiae s.s*	dry	48	236.40	248.161	35.819	-3.95	0.00*
	rainy	48	661.19	702.107	101.340		
Total of *An. arabiansis*	dry	48	78.75	71.520	10.323	-4.39	0.00*
	rainy	48	272.08	295.941	42.715		
Total of other Mosquitoes	dry	48	14.38	15.067	2.175	-4.36	0.00*
	rainy	48	63.13	75.937	10.961		
Total of Mosquitoes	dry	48	397.21	395.755	57.122	-3.83	0.00*
	rainy	48	1291.10	1564.597	225.830		

* Significant different at the 0.05 level.

Table (3): The larvae density of *anopheles gambiae* at different spatial breeding sites over 24 month

Location	Breeding site	N. of site visit	N. of dip	Density	
				Anopheles	Other mosquitoes
Hai Ochugi	Swamp1	24	240	3.8042	0.6333
	Swamp2	24	240	3.6042	0.5000
Hai Bilpam	Waste water	24	240	3.0500	0.4250
	Swamp	24	240	3.5375	0.5292
Hai Salam	Swamp	24	240	4.6375	0.5667
	Waste water	24	240	3.4542	0.4542
Hai Nevasha	Waste water1	24	240	3.2375	0.4083
	Waste water2	24	240	2.7917	0.4667
Total		192	1920	3.5146	.4979

Waste water: water leaking from tape in distribution point.

Table (4): A total number of *anopheles gambiae* larvae per location in different breeding site over 24 months

Location	Breeding site	N. of dip	Anopheles gambiae s.l		Other mosquitoes		Total
Hai Ochugi	Swamp	96	1778	87%	272	13%	2050
Hai Bilpam	Swamp +waste water	96	1581	87%	229	13%	1810
Hai Salam	Swamp +waste water	96	1942	89%	245	11%	2187
Hai Nevasha	waste water	96	1447	87%	210	13%	1657
Total		384	6748		956		7704

The high percentage of *anopheles gambiae* was found in Swamp +waste water.

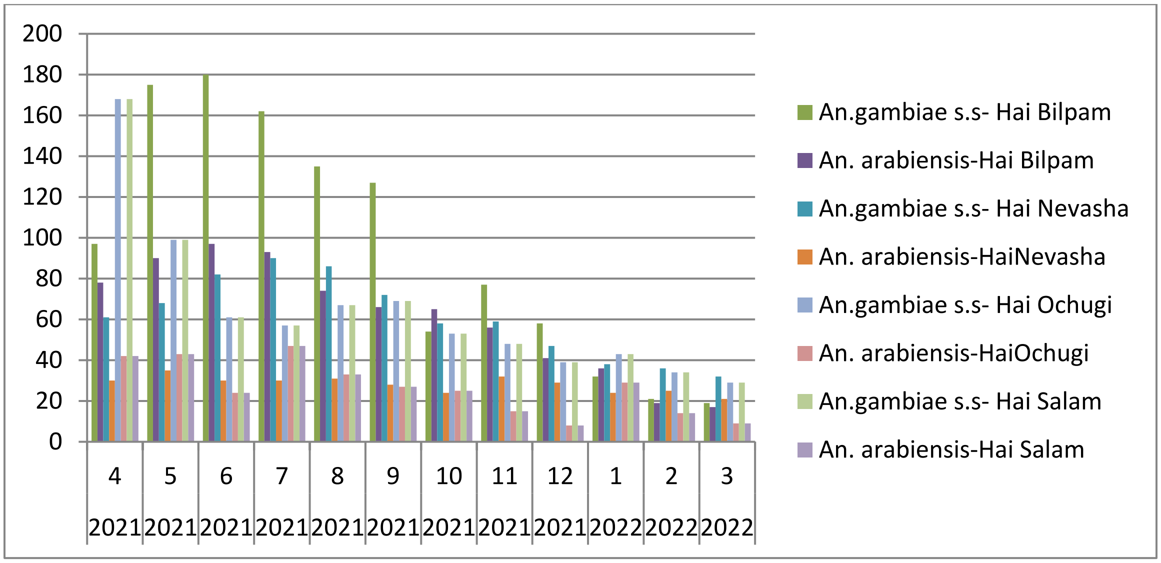

Figure (1): the composition of *An. gambiae s.s* and *An.arabiensis* indoor resting at different areas over 12 months.

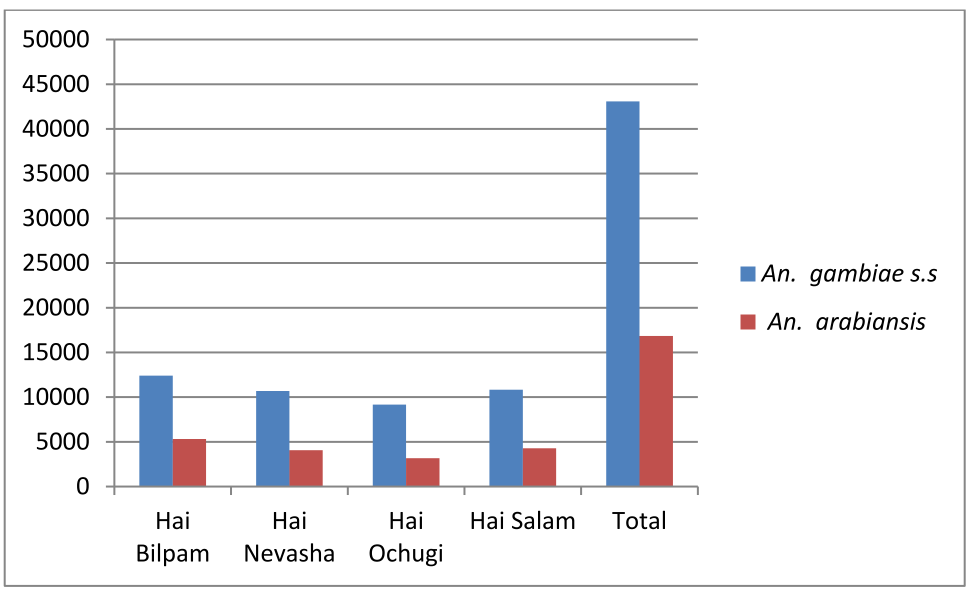

Figure (2): the species composition at the study sites.

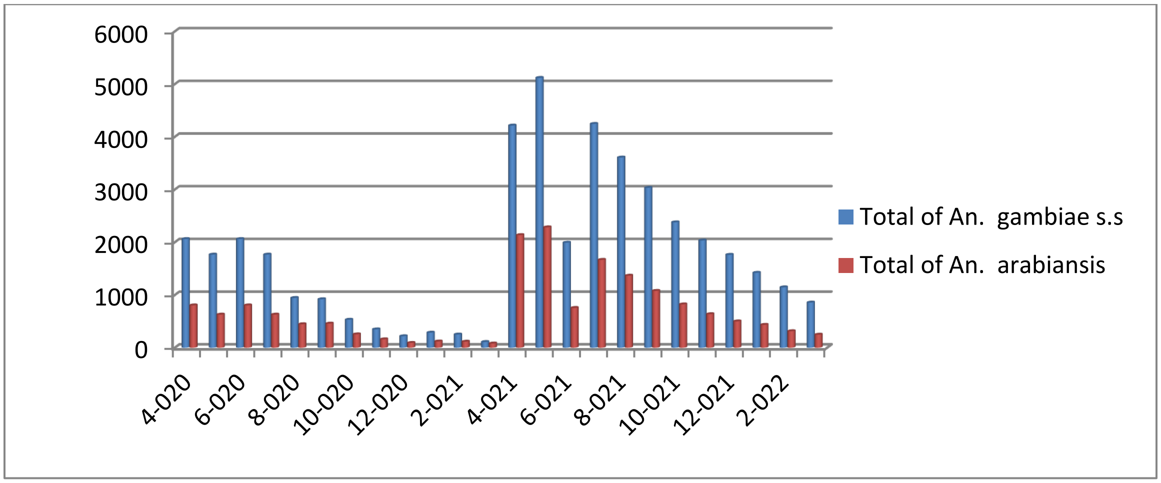

Figure (3): Change in anopheles density per month.

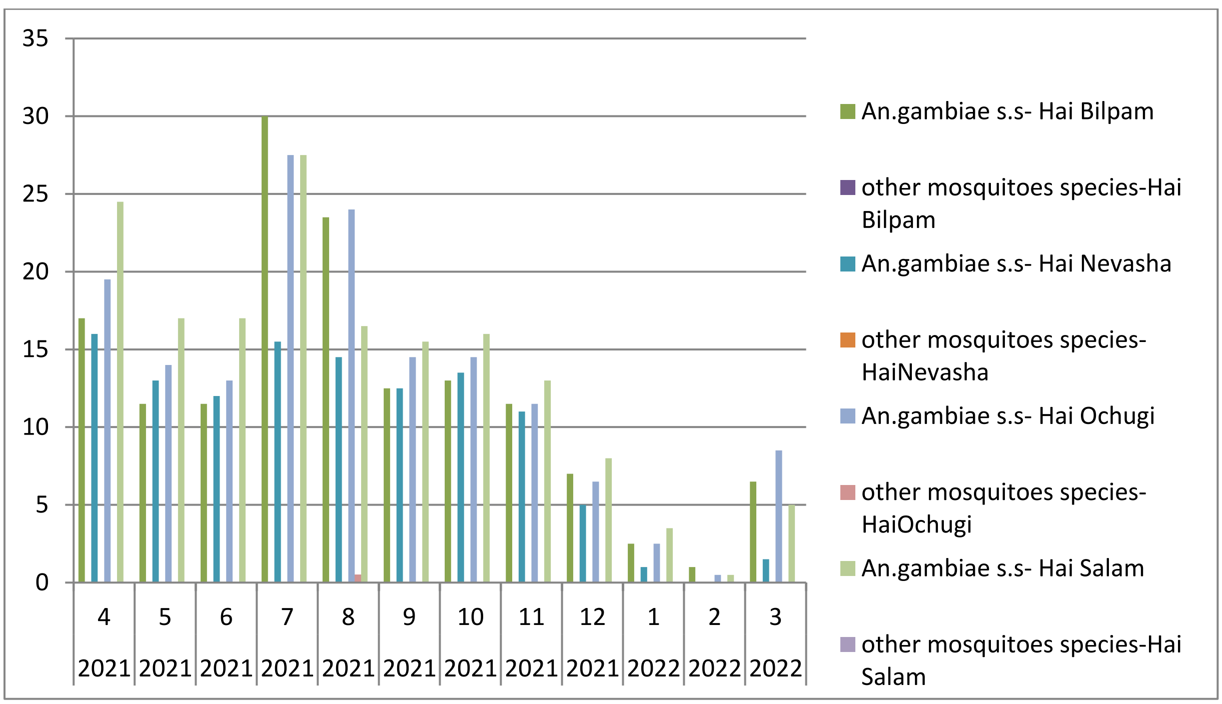

Figure (4): the mean composition of *An. gambiae s.s* and other mosquitoes species in breeding over 12 months of second year.

4 Discussion:

The findings of this study provide solid data that can make this expectation a reality in Kodok locality and other semi-areas with similar ecological conditions. The abundance of Anopheles species in Kodok locality could be attributed to a number of factors, one of which is that some communities in Kodok locality are located along the bank of White Nile River and it experiences seasonal flooding which usually provides favorable temporary and permanent breeding sites for Anopheles gambiae, as in line with (8). In the first and the second years which mean over 24 months in different four areas, *An. gambiae s.s* is generally considered to be an efficient vector, due to its very high number and density indoor resting.

The result reveal that a number of An. gambiae is high then other mosquitoes species (*An funestus, Culex and Aedes*) indoor resting which are clear in table (1), Hai Ochugi taken the lead overall in high percentage follow by two areas Hai Salam and Hai Nevash and the lowest is Hai Bilpam , also we can found *Anopheles arabiensis* accounted being a second vector along with *An. gambiae s.s.* as in line with (9).

An. arabiansis have highest number and percentage then other mosquitoes species collected indoor resting in Hai Bilpam ,Hai Salam, Hai Nevasha, Hai Ochugi respectively, and other mosquitoes species take less consideration in number and percentage comparing to An. gambiae s.l in Hai Bilpam, Hai Nevasha respectively and two other areas are equally in number and percentage as in line with (10).

On the seasonal abundance and density of *An. gambiae* species have improved understanding on vector and its role in malaria transmission, the result confirmed that density of *An. gambiae s.s* was influence by rain and increased at the begging of the rainy season between April and September as in figure (4) , as in line with (11), the peak density were observed toward the end of the rainy season, the highest percentage *An. gambiae s.s* found in Hai Ochugi 71% and *An.arabiensis* 28%, a competition between different areas show that the density of *An. gambiae s.s* rising up during the two seasons dry and rainy in all areas follow by *Anopheles arabiensis* as in line with (12).

The spatial distribution of *An. gambiae* was aggregated throughout the year, but the degree of aggregation increased during the rainy season as in line with (13), The high-density houses persisted throughout the season, the high-density houses during the dry season differed from those of the rainy season, Only a few highest-density houses during the dry season persisted across two years, finally, the locations of the hotspots within each period (rainy season, and dry season) were similar between years as in appendix table as in line with (14).

Results of ANOVA significant differences test showed counts of *An. gambiae* s.s in Kodok locality are significantly higher in houses especially in areas near to swamp and *An. arabiensis* follow it, that have relation to long-term contribution to malaria cases, *An. gambiae* are more important because they have role in malaria transmission.

The availability of permanent water sources complements vector survival by ensuring species that are best Adapted to these kinds of habitats such as members of the Anopheles gambiae are able to breed and sustain malaria transmission.

Large permanent habitats with emergent vegetation are known to favor proliferation of Anopheles, the result showed that the locality was infested with Anopheles gambiae s.s larvae 88% and other mosquitos' species 12%. , An. gambiae was the predominant in all the population densities of the larvae peaked in April and the lowest larvae densities occurred in November to March.

The Anopheles gambiae s s larvae are the most abundant and most infective breeding site found in kodok locality; in Hai salam have highest percentages then three other areas Hai Nevasha, Hai Ochugi and Hai Bilpam but the lowest percentage of other mosquitoes species found in Hai Salam in all breeding sites as in table (6).

The density of anopheles gambiae in swamp in three areas are high then waste water during the study period especially in Hai salam and An. gambiae population density per swamp were higher for the all sites, The density of larvae in Swamp Breeding sites can contribute strongly to the adult An. gambiae population density as in line with (15).

Species composition and abundance of mosquitoes larvae are positively identified are Anopheles gambiae s.l constituted high percentage then other mosquitoes species. The study showed that mosquito larvae were abundant after rainy seasons due to the formation of larval habitats, particularly at the edges of White Nile River which served to sustain Anopheles populations during the dry seasons and it was observed that habitats that contained growing grass and other vegetation had more Anopheles larvae than habitats without vegetation as in line with (16).

The mean larval densities were significantly higher in the rainy season than in the dry season, *An. gambiae* s.s also has wide variations with the highest peaks in April up to September as in line with (17).

Results of ANOVA significant differences test showed counts of late instars of anopheline larvae in Kodok are significantly higher in swamp and lower in waste water in relation to long-term contribution to larval productivity, swamp are more important because they have water available for anopheline larval development long after most of the other habitats have dried up.

Further effects could have been provided by other permanent water sources located outside the study areas but from where no sampling was carried out, A good example is found in River Nile located less than 1 km away from the furthest breeding site in the three areas; well within the flight range of gravid Anopheles gambiae females as in line with (19).

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