

The effects of vector control interventions; long Lasting Insecticidal Nets plus Indoor Residual spraying (LLIN+IRS) and Long Lasting Insecticidal Nets (LLIN) alone on *anopheles arabiensis* Patton (Diptera: Culicidae) population densities, resting behaviors in central and eastern, Sudan

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ABSTRACT

Introduction: Appropriate Understanding species composition, distribution and behaviours of malaria vectors are of great importance in vector control operations and they must be well understood to formulate specific and focused intervention strategies. In light of this it is important to investigate the distribution and behaviours of disease's vectors especially in rural areas of Sudan where the malaria is a pig health problem. This study was aimed to determine the species composition, abundance, densities and resting habits of vectors among the two interventions (study arms) LLINs alone and a combination of LLINs plus IRS in 30 clusters from four areas Alhoosh, Alhagabdalla, Galabat and New Halfa in central and eastern Sudan .

Study design: A cluster randomized controlled trial, with two interventions (study arms) LLINs alone and a combination of LLINs plus IRS.

Methodology: Mosquitoes samples were collected indoors using pyrethrum spray catches (PSC) from human dwellings and light trap collection method to determine density, resting habits, and species composition using Polymerase Chain Reaction Assays PCR to confirm the presence of sibling species of the *Anopheles gambiae* complex in four study areas in central and eastern Sudan.

Results: Species identifications showed that 94.7 % (682 / 720) specimens were *anopheles arabiensis* patton species. The overall mean vector density was (7.9 ± 1.3± SE1.17 female / room / day) for LLIN study arm, while was (8.8 ± 1.7± SE1.53 female / room / day) for LLIN+IRS study arm. There was no significant difference between mean vector densities in the two study

arms ($P > 0.05$). The species had tendency to rest indoor (endophilic) than resting outdoor (exophilic) the pool ratio was 1:2 for the two interventions, the ratio of fed to half- gravid / gravid females in the study arm LLIN was found 1: 1.9 and in the study arm LLIN+IRS was found 1: 2 in favour of fresh fed. While there is no difference between densities and resting place of *anopheles arabiensis* in study arms LLIN and LLIN+IRS, study areas and the study period .

conclusion: The study revealed that *anopheles arabiensis* is the dominant vector of malaria in Sudan, tendency to rest indoor (indophilic) and there are no advantages of combining LLIN with IRS relative to use LLIN alone.

Keywords: *anopheles arabiensis*; LLIN+IRS combination; LLIN alone; resting habits; Sudan

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INTRODUCTION

Malaria is the most common and devastating disease in the tropics. 247 million among 3.3 billion people at risk in 2006. It has been recently estimated that 200 million people (24.6% of the total population in Africa) live in urban setting where at risk of contracting malaria [1]. *Anopheles gambiae* complex have been known as the most efficient malaria vectors in Afro - tropical region [2, 3]. *Anopheles arabiensis* is the major malaria vector reported from all parts of Sudan [4, 5, 6] [7, 8, 9]. Co- existing with *Anopheles gambiae* sensu stricto (s.s). The siblings of *Anopheles gambiae* are morphologically similar at all levels of their different developmental stages, the adults are different in their biology, biting and resting behaviours. *Anopheles funestus* Giles also contributes to malaria transmission in southern and southeastern Sudan [10]. Based on many reports analyzed, it seems that at least in some cases, there are advantages of combining ITNs

with IRS relative to use either method alone, but that this outcome may be different in certain situations, since there are numerous confounding factors that can affect the results. It is therefore certain that evidence to support or refute this strategy for combinations remains inconclusive and any generalizations for optimal strategies cannot be made [11]. Universal coverage with long lasting insecticide - treated nets (LLINs) or IRS is actively promoted as the main prevention strategy under the WHO endorsed malaria control and elimination plan [12]. The LLINs + IRS combination strategy is mostly recommended for accelerating control in high transmission areas, where either IRS alone or ITNs alone may not be adequate[13,14]. The use of both IRS and LLIN has increased over the last decade as part of the drive towards covering all human populations at risk, saving millions of lives. Combined IRS and LLIN have also been suggested as a means of delaying the emergence of insecticide resistance by using different classes of insecticide for IRS and LLINs [14]. Understanding the behaviour of the malaria vectors and their abundance are essential for malaria control operations. Furthermore, understanding the resting habits of the vectors important for planning, implementing and monitoring vector control measures. This study aimed to determine the species composition, abundance, densities and resting habits of vectors among the two interventions (study arms) LLINs alone and a combination of LLINs plus IRS in 30 clusters from four areas Alhoosh, Alhagabdalla, Galabat and New Halfa in central and eastern Sudan.

MATERIAL AND METHODS

Study design: The study was a cluster randomized controlled trial, it was conducted at 30 clusters from four areas Ahoosh, Alhagabdalla, Galabat and New Halfa in central and eastern Sudan with two interventions (study arms) LLINs alone and a combination of LLINs plus IRS.

Study area: The study was carried out at 4 areas in Sudan. 2 areas (Ahoosh and Hag Abdullah) are located in Gezira agriculture scheme this is one of the biggest irrigation schemes in the world (National Pesticide Council, unpublished data). The main occupation of the inhabitants is agriculture cultivating cotton. Climatically the areas have hot dry summer from March to June and cool dry winter from October to February; the average annual rainfall is 225 mm per annum, mainly in July to September. Housing consists of a mixture traditional mud walls with thatched roof construction, and modern brick built houses. Galabat area belongs to Gadarif state, 80 km

from Gadarif town and bordering Ethiopia. The area is within the dry savannah region, with a short rainy season during June to September followed by a dry season from October until the end of May. The houses are made of local materials (grass and mud) . New Halfa area is located in the semi-arid belt of the Sudan approximately 500 km east of Khartoum in the middle of an agricultural scheme. the area is classified as dry savannah with rainfall from July to early October ranging between 300 to 411 mm per annum. Temperatures range between 16°C and 45°C. Housing is composed of mixture of grass, mud and brick (Administrative Units report - four localities) (see figure1).

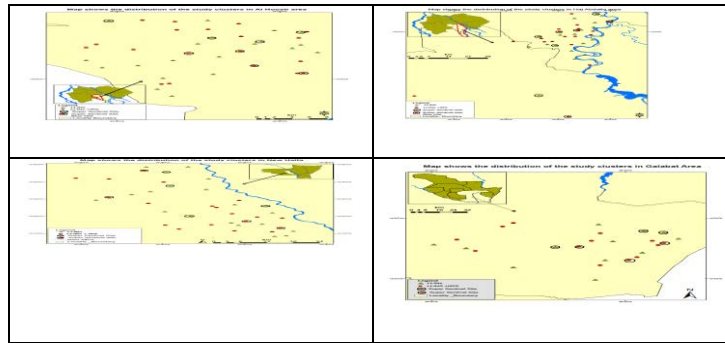


Figure1: Map showing location of clusters in the four study areas.

Materials: During the study, the following equipment and materials were used: White floor sheets (size 2m x 2m) made of cotton, window trap, light traps, clay pots, hand lenses, pyrethrum solution, small Petri dishes, paper cups with net covers , forceps , plastic containers, adhesive tapes, cotton wool, filter papers , a torch and batteries, readymade aerosol pyrethrum solution at a concentration of 0.2-0.3% in kerosene , mouth aspirators, hammers, nails , a towel, , silica gel, eppendorf tubes, recording sheets, pens and pencils

Mosquito collection: Pyrethrum spray and light trap collection methods were used to make monthly collections of indoor resting mosquitoes during the period 2012, 2013 and 2014. Pyrethrum spray 0.2- 0.3% pyrethrum in kerosene was sprayed and knocked-down mosquitoes were collected from 07:00 to 09:00 hours. Collection by light trap capture was carried out quarterly (3 hours) from 19:00 to 07:00 hours. The collections of mosquitoes were done in 30 clusters; three houses were randomly selected for adult mosquito collections. To cover 30 clusters; four teams worked for a total of 8 days per month for three months (September-November) in Alhoosh, Hag Abdllah, and New Halfa and 6 days in Galabat during the transmission season and one month (March) in dry season in each year.

Females collected were classified according to their blood meal stages unfed, fed, half- gravid / gravid. Females collected were kept in separate paper cups until identification (morphologically and PCR assay) .

Morphological identification: All the mosquitoes caught were counted, recorded and identified using morphological features to species with the aid of identification Gillies and Meillon, 1968 manuals[15].

Species molecular identification: A subsample of mosquitoes collected resting indoors were identified to species level using the species-specific PCR assay [16] using genomic DNA extracted following the procedure described by Livak, 1984 [17] . DNA was re-suspended in 100 µl molecular biology water. Positive controls obtained from known colonies maintained in the insectary at Prof Elgaddal National Malaria Research and Training Centre- Sennar. The *Anopheles gambiae* amplification was done in thermal cycle in 94° C for 5 minutes and 30 cycles of denaturation 94° C for 30 seconds, 50° C for 30 seconds of annealing and final extension at 72° C for 30 seconds the end cycle at 72° C for 10 minutes and incubates at 10° C forever. The samples were run on 2% agarose gel which prepared by adding 2 grams agarose gel to 100 ml TBE buffer and 5 µ Ethidium bromide show the result as follow, *Anopheles arabiensis* 315 base pair and *Anopheles gambiae* 390 base pair under Gel documentation system instrument.

Data analysis Data: Data were analyzed using the Microsoft Excel program and SPSS version 16.0 . ANOVA was used to evaluate the difference in the density, resting places of female in the two study arms (LLIN and LLIN+ IRS) in the four study areas during the study period (2012- 2013 and 2014). The P value less than 0.05 considered significant.

RESULTS

Anopheles Species Identification: A total of 720 member of morphologically identified as *anopheles gambiae* complex subsamples were randomly selected from collected mosquitoes and subjected to species specific polymerase chain reaction PCR test. The majority 94.7 % (n=720) was successfully identified as *anopheles arabiensis*. (Figure 2&3)

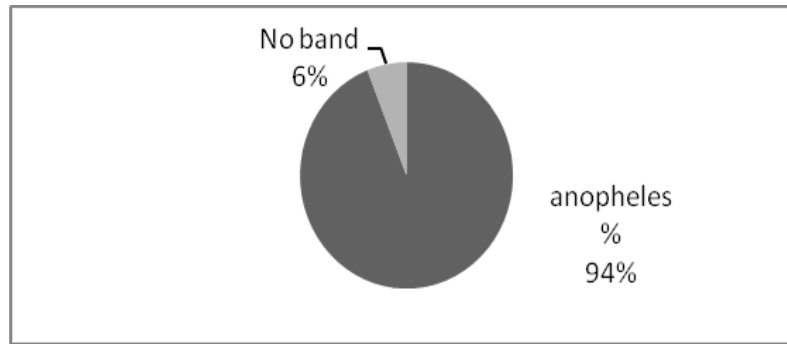


Figure2: PCR analysis results for anopheles gambiae per study areas in 2012- 2013 and 2014.

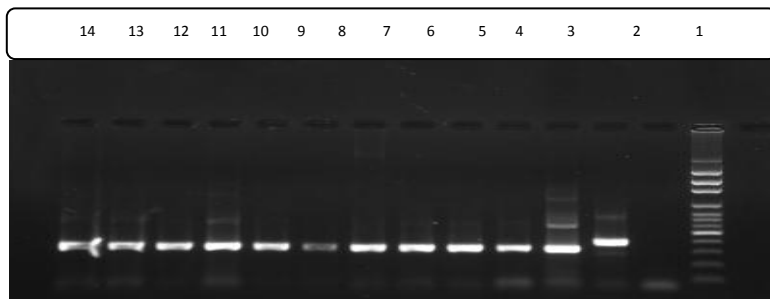


Figure3: Amplified fragments using the species-specific PCR assay for the identification of members of the Anopheles gambiae complex. Lanes 1 kb molecular markers from right to left: 2 negative control, 3 *Anopheles gambiae* control, 4 *Anopheles arabiensis* control and then the samples from (5-14) are *Anopheles arabiensis* which are the DNA ladder sizes are 315 bp and 390 bp for *Anopheles arabiensis* and *Anopheles gambiae* respectively.

Population abundance and density

Density of anopheles arabiensis species / room / day: During the study period, a total of 7444 female, of which 6297(84.6 %) were collected from indoor of the dwelling by the pyrethrum spray, while 1147 (15.4%) were collected by Light Taps from four study areas. 3573 (48 %), and 3871 (52 %) specimens collected from LLIN and LLIN+IRS. 2064 (27.7 %), 3466 (46.6%) and 1914 (25.7 %) specimens were collected in 2012, 2013 and 2014 respectively while it were

2541(34.1%), 2735(36.7%), 693(9.4%) and 1475 (19.8%) collected from Alhoosh, Alhagabda , Galabat and New Halfa study areas respectively.

The overall mean vector density was ($7.9 \pm SE1.3$ females / room / day) for LLIN study arm, while it was ($8.8 \pm SE1.7$ females / room / day) for LLIN+IRS study arm (Table 1). The mean vector density was $6.1 \pm SE 1.2$, $9.2 \pm SE 2.0$ and $8.3 \pm SE 3.5$ for LLIN, while it was $5.2 \pm SE 1.4$, $9.3 \pm SE 3.2$ and $11.9 \pm SE 3.5$ for LLIN+IRS in 2012, 2013 and 2014 respectively (Table 2). The mean vector density was $13.0 \pm SE 3.1$, $9.0 \pm SE 1.1$, $4.9 \pm SE 1.8$ and $4.6 \pm SE 1.4$ for LLIN, while it was $9.9 \pm SE 2.0$, $15.1 \pm SE 3.8$, $2.1 \pm SE 0.6$ and $8.0 \pm SE 1.8$ for LLIN+IRS for Alhoosh, Alhagabda , Galabat and New Halfa study areas respectively (Table 3).

Table1: Total number of adult *Anopheles arabiensis* specimens collected during the study period by Light trap LT and pyrethrum spray sheet PSC and mean density per room per day per study arms LLIN and LLIN+ IRS per study areas in 2012- 2013 and 2014

Study arm	Pyrethrum spray collection PSC		Light trap collection LT		Total	
	No. of anopheles collected	Mean \pm SE density No. / room / day	No. of anopheles collected.	Mean \pm SE density No. / room / day	No. of anopheles collected	Mean \pm SE density No./ room / day
LLIN	2891	6.4 ± 1.2	682	1.5 ± 0.5	3573	7.9 ± 1.3
LLIN+IRS	3406	7.7 ± 1.5	465	1.1 ± 0.3	3871	8.8 ± 1.7
Total	6297	7.0 ± 0.9	1147	1.3 ± 0.3	7444	8.3 ± 1.1

Table2: Total number and mean vector densities of anopheles collected per years

Year	LLIN		LLIN+IRS		Total	
	No. of anopheles collected	mean \pm SE	No. of anopheles collected	mean \pm SE	No. of anopheles collected	mean \pm SE
2012	1073	6.1 ± 1.2	991	5.2 ± 1.4	2064	5.6 ± 0.9
2013	1710	9.2 ± 2.0	1756	9.3 ± 3.2	3466	9.2 ± 1.8
2014	790	8.3 ± 3.5	1124	11.9 ± 3.5	1914	10.1 ± 2.4
Total	3573	7.9 ± 1.3	3871	8.8 ± 1.7	7444	8.3 ± 1.1

Table3: Total number and mean vector densities of anopheles collected per study areas

study areas	LLIN		LLIN+IRS		Total	
	No. of anopheles collected	mean± SE	No. of anopheles collected	mean± SE	No. of anopheles collected	mean± SE
Alhoosh	1448	13.0±3.1	1093	9.9±2.0	2541	11.4±1.8
Alhagabdala	1053	9.0±1.1	1682	15.1±3.8	2735	12.0±2.2
Galabat	503	4.9±1.8	190	2.1±0.6	693	3.5±1.1
Newhalfa	569	4.6±1.4	906	8.0±1.8	1475	6.3±1.3
Total	3573	7.9±1.3	3871	8.8±1.7	7444	8.3±1.1

The highest density in LLIN (PSC and LT collection methods) was recorded (17.5 female *anopheles arabiensis* /room / day) in Alhoosh study area in 2014 while the lowest density was scored (1.6 female *anopheles arabiensis* / room / day) in Galabat study area in 2014. The highest density for LLIN+IRS was recorded (20.3 female *anopheles arabiensis* / room / day) in Alhagabdala study area in 2014, while the lowest density was recorded (1.4 female *anopheles arabiensis* / room / day) in Galabat study area in 2013. The highest density in LLIN was recorded (13.4 females / room / day) in Alhoosh study area in 2013 while the lowest density was scored (1.1 females / room / day) in Galabat study area in 2014. The highest density for LLIN+IRS was recorded (18.2 females / room / day) in Alhagabdala study area in 2014, while the lowest density was recorded (1.4 females / room / day) in Galabat study area in 2013 (Figure 4).

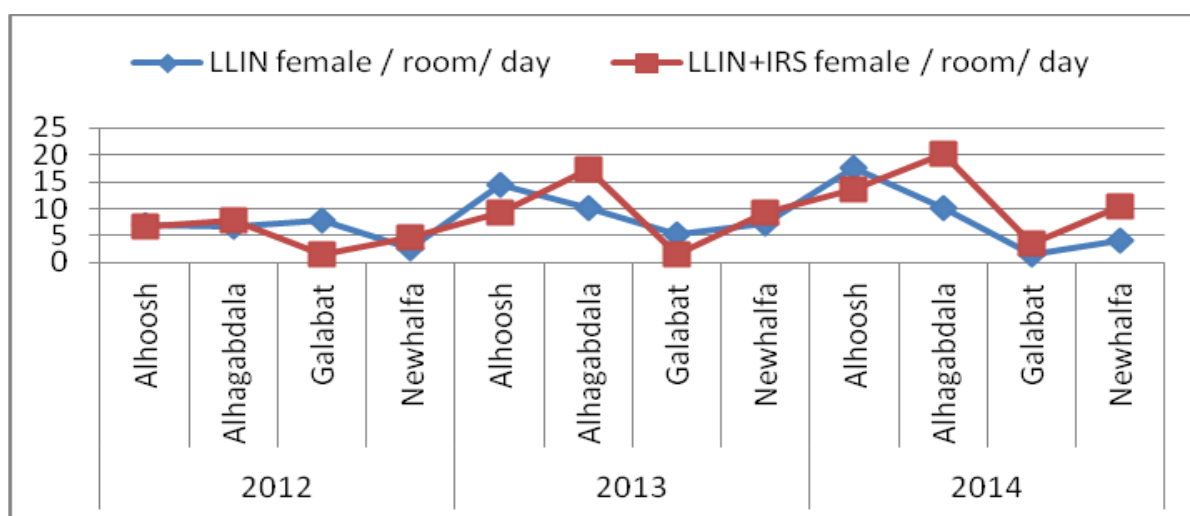


Figure 4: Densities of females *anopheles arabiensis* per study arms LLIN and LLIN+ IRS per study areas in 2012- 013 and 2014.

Resting behavior

Of 2891 females collected from indoors resting sites in LLIN study arm, 6.4 %, 32.1% and 61.6 % were unfed, fed and half-gravid/gravid respectively and 3406 females collected in LLIN+IRS , 9.4 %, 30.3% and 60.2 % were unfed, fed and half-gravid /gravid. The overall ratio of fed to half-gravid / gravid females in the two study arms LLIN and LLIN+IRS together was 1:2. Ratio of fed to half- gravid / gravid females *anopheles arbiensis* in the study arm LLIN was 1: 1.9 and in the study arm LLIN+IRS was 1: 2 in favour of fresh fed. This indicates that this species had tendency to rest indoor (endophilic) than resting outdoor (exophilic) (Table 4).

Table 4: The ratio of fed to gravid / half - gravid females in LLIN study arm in four study areas in 2012- 2013 and 2014

Study arm	UF	FF	HG	G	Total	UF %	FF %	HG & G	HG & G / FF
LLIN	184	927	937	843	2891	6.4	32.1	61.6	1.9
LLIN+IRS	320	1033	1098	955	3406	9.4	30.3	60.2	2.0
Total	504	1960	2035	1798	6297	8.0	31.1	60.9	2.0

UF = unfed, FF = freshly fed, HG = half- gravid, G = gravid of female *anopheles arbiensis* . [26]

DISCUSSION

The study showed that *Anopheles arabiensis* was the principal malaria vector species in the study areas (94.7 % *Anopheles arabiensis* patton species). Our results similar with that previous studies showed that, the predominate vector species which has been reported in Northern, Eastern and Central Sudan [9, 18, 19, 20, 21]. This study confirmed that *anopheles arabiensis* is the only member of the *Anopheles gambiae* complex present. The densities of *Anopheles arabiensis* were indifference between study arm LLIN alone and LLIN plus IRS, study areas and between years $P > 0.05$.

Many previous studies revealed that the combination of LLIN plus IRS had no additional impact on *Anopheles arabiensis* densities a possible explanation for this contradiction is that exposure to treated bed nets [22]. 92.5% coverage of household by IRS with bendiocarb and LLINs (PermaNet 2.0) 87% ownership (one LLIN per 2 person) after the LLIN universal coverage campaign in 2012. (National Malaria Control Programme, unpublished data).

This study has shown that the large proportion of population of *Anopheles arabiensis* found resting indoors after feeding “endophilic” compare to outdoor “exophilic” in the study arms LLIN

and LLIN+IRS, study areas and years. This finding was supported by many studies in Northern Sudan and Elsewhere in Africa [4]. However *Anopheles arabiensis* is known to have a wide range of feeding and resting patterns and behaviours depending on geographical location [28]. There is evidence that long-term use of DDT induced the exiting behaviour of *Anopheles arabiensis* in South Africa has been shown resting outdoor this is probably depending on host availability [23]. In contrast *Anopheles arabiensis* was found resting outdoor in Eastern and Central Sudan as reported by many authors [24, 2, 6, 25]. Another Study was carried out in Kenya showed the same findings [26]. However, it is likely to increase the success of indoor house – spraying with residual insecticides as measure of vector control.

CONCLUSION

The study showed that, the *Anopheles arabiensis* is the dominant vector of malaria in the Sudan, tendency to rest indoor (indophilic). The LLIN+IRS as combination control method doesn't add any advantage for vector density and resting habits.

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ETHICAL APPROVAL

Permission was sought from head of the houses to perform collections in their rooms. Community consent had been obtained beforehand in clusters. This study approved by the National Health Research Ethics committee, Federal Ministry of Health.

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