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Full length research paper

Effects of an in-built ultrasonic device on *Anopheles gambiae* s.l mosquitoes in an indoor environment

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ABSTRACT

Mosquitoes are major vectors of malaria and lymphatic *filariasis* in Nigeria. Several initiatives aimed at reducing the burden of these diseases yield little or no results. In order to make the ultimate goal of reduction or elimination of these diseases a success, there is an urgent need for alternative approaches to control mosquitoes. One of these approaches is the use of ultrasonic device for the control of *Anopheles gambiae*. The effectiveness of in-built ultrasonic device fixed in Air- Conditioner to expel and/or knock-down *An. gambiae* s.l mosquitoes was evaluated. The rate at which an ultrasonic device in an Air Conditioner (AC) expelled and knocked down *Anopheles gambiae* s.l mosquitoes was assessed in a Peet Grady (PG) chamber at the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. Mosquitoes were bred in the IAMRAT insectary under standard rearing conditions. Three to five days old adults were used for the test. An Air Conditioner (AC) with an inbuilt Ultrasonic Device (AC-UD) was mounted on the wall of the test chamber while the control chamber was without the (AC-UD). In order to determine the expel rate due to the ultrasonic device, 50 sucrose fed female mosquitoes were introduced into the test chamber with AC-UD. The rates at which the mosquitoes migrated to the adjacent chamber without AC-UD were recorded hourly for six hours and at 24 hours. In a separate experiment, the knockdown rate was determined by introducing 50-sucrose fed female mosquitoes into a test chamber with AC-UD and the windows properly locked. Knock down was recorded hourly for the first six hours, and subsequently at 24 hours post exposure by counting the number of mosquitoes on the ground. The tests were carried out on the basis of switching "ON" and "OFF" of the AC's fan and ultrasonic device to indicate the associated effects of the variables of interest. For the knockdown tests, the final mortality at 24 hours recorded was 60.7%, 15.3% and 17.3% for experiments "fan ON + ultrasonic ON", "fan ON + ultrasonic OFF", and "fan OFF + ultrasonic ON" respectively. The final mortality recorded for the control at 24 hours was 4.7%. For the expel tests, the experiment "fan ON + ultrasonic ON" had the highest proportion of mosquitoes expelled (62.7%) after 24 hours, while mosquitoes not expelled were knocked down (28.0%). This was followed by "fan OFF + ultrasonic ON" with expel of 26.0% and only 14% knockdown. For "fan ON + ultrasonic OFF" the expelled mosquitoes was 13.3% with only 1.3% knockdown after 24 hours. The expel rate for the control was 1.3% after 24 hours while 2.0% was knocked down after 24 hours. The combination of the ultrasonic device and fan on was relatively more effective in knocking down and/or expelling *Anopheles gambiae*. This suggests that the ultrasonic device in combination with the fan implanted in the AC is effective for personal protection against mosquitoes.

Keywords: Ultrasonic device, *Anopheles gambiae,* knockdown and expel test, peet grady, insectary.

INTRODUCTION

Anopheles mosquitoes are the vectors of malaria and lymphatic filariasis in Nigeria (Okorie *et al.* 2011). Nigeria has the highest burden of malaria accounting for over 30% of infections in Africa (WHO, 2010a). Nigeria also has the highest burden of lymphatic filariasis in Africa with an estimated 80 to 120 million people at risk (Hotez *et al*. 2012; WHO, 2010b). Malaria vector control aims to reduce and/or prevent contact between humans and vectors, and several control methods are available including chemicals (insecticides), bednets, environmental management and biological control (WHO, 2010a). In Nigeria, the Federal Ministry of Health (FMoH) has recently scaled up the distribution of Insecticide Treated / Long-Lasting Insecticidal mosquito nets (ITNs/LLINs) and indoor residual spraying (IRS). So far, over 50 million LLINs have been distributed across the country (PMI, 2013; The Carter Center, 2012). However, several alternatives are still needed and the prospect of using non chemical means of control such as use of ultrasonic device for personal protection is attractive.

Ultrasound comprises acoustic wave with a frequency greater than the upper limit of human hearing (Health Protection Agency, 2010). There are established range of audibility for humans and the highest sensitivity levels is between 1000-4000 Hz. Some animals are sensitive to sounds pitched higher than the hearing of human. For example: ultrasonic whistles are used when training dogs and circus animals, bats use echolation to navigate and hunt.

Use of ultrasonic bat-cry signals has the potential to disrupt behaviour of night-flying insects. This is because studies have shown that many species of insects will dive to the ground or move away when they detect ultrasonic signals that resemble echolocation calls so as not to be predated upon by bats (Mankin, 2012). Two theories have been used to explain the repellent action of sound on mosquitoes: (1) that inseminated females are repelled by the wing beat of males in flight and (2) that mosquitoes avoid the ultrasonic cries of bats (Foster and Lutes, 1985). Ultrasound has been identified as a key factor in some predator / prey relationships (Koehler *et al.* 1986). The capability to detect ultrasonic signals has evolved primarily in insects that are preyed upon by bats such as moths, crickets, locusts and praying mantis (Walker, 1996). Thus, numerous studies have reported that ultrasonic repellers have not been effective in controlling insects such as cockroaches, mosquitoes, fleas and dragonflies that typically are not normally preyed upon by bats (Mankin, 2012).

Various sonic, electronic and ultrasonic devices in various forms are advertised world-wide as repelling mosquitoes. The use of ultrasonic devices to control mosquitoes has not received significant attention in Nigeria. Therefore, the aim of this study was to evaluate the effectiveness of in-built ultrasonic device (fixed in LG™ Air- Conditioner) to expel and/or knock-down *An. gambiae* s.l mosquitoes in a controlled laboratory setting.

MATERIAL AND METHODS

Construction of Peet Grady chambers

Peet grady chambers were constructed in a room measuring 4460 cm (w) x 4360 cm (d) x 1800 cm (h). Aluminum frame and panel, including glass materials were used for the construction. Power connectors and fans (outlets) were fitted in the chambers. The peet grady chambers measure 1890 (w) X 1890 (d) X 1900 (h) cm.

Description of Air Conditioner

The air conditioner system used was the Titan LG air conditioner. The ultrasonic device was implanted in the front of the system with options of switching the ultrasonic device on or off. All experiments were conducted without the cooling system of the air conditioner.

Mosquito Larval Collection and Rearing

Mosquito larvae were collected from various breeding sites in Ibadan and transported to IAMRAT insectary. The mosquitoes were morphologically identified as *An. gambiae* s.l and reared under standard insectary conditions (Temp: 28±2^{o}C and 72±5% RH).

Experimental Test

The test was carried out using a prototype air conditioner with an inbuilt ultrasonic device (AC-UD) that produces frequency range of 20 – 100 KHz. A locally made volume amplifying device was used to monitor the waves generated from the air conditioner. An oscilloscope was calibrated and used to test the frequency emitted by the ultrasonic device. The test was conducted in the Peet Grady Chamber constructed for the purpose. It comprised of:

(1) Test chambers (contained the ultrasonic device in-built in an air conditioner) while the

(2) Control chambers (were without ultrasonic device in-built in air conditioner).

Clean white cotton cloth was laid on the floor of the chambers for easy identification of knocked-down mosquitoes. Every opening in the chambers that could provide escape routes for the mosquitoes in the chambers were adequately sealed using industrial masking tape. Good housekeeping was strictly observed. Fifty sucrose-fed adult *An. gambiae* s.l mosquitoes were Released into the chambers and the number of

mosquitoes which fell after five minutes were observed. The test was continued if less than 5% of the *Anopheles gambiae* mosquitoes fell down. Subsequently all experiments were carried out using 50 mosquitoes per test and three replicates were carried out per test and a control.

Experimental Design

Knock-down and Expel test were carried out on the basis of switching "ON" and "OFF" of the AC's fan and ultrasonic device to show the associated effects of the variables of focus as listed below:

Experiment 1: Knock-down tests

Fan ON + ultrasonic ON
Fan OFF+ ultrasonic ON
Fan ON + ultrasonic OFF
Control [Fan OFF + Ultrasonic OFF]

Experiment 2: Expel tests

Fan ON + ultrasonic ON
Fan OFF+ ultrasonic ON
Fan ON + ultrasonic OFF
Control [Fan OFF + Ultrasonic OFF]

The air conditioner was fitted with an ultrasonic device apart from a fan which normally circulates cold air. However, the fan and ultrasonic device had had different controls for the purposes of the study. The fan in the air conditioner had to be disconnected (switched off) to conduct experiments in the fan OFF mode.

All experiments were conducted using 3 - 5 day old female *An. gambiae* s.l mosquitoes. For each experiment, 50 female mosquitoes (depending on the number of mosquitoes available) were introduced into the chamber. Each experiment was repeated three times.

Procedures for Knockdown Test

To determine the number of mosquitoes knocked down in each experiment, 50 sucrose fed females were released into the chamber with locked windows and observed knockdown was recorded hourly for the first six hours, and subsequently at 24 hours post exposure by picking and counting the number of mosquitoes on the floor.

Percentage knockdown was calculated as = $\frac{\text{Number knocked down x100}}{\text{Total number exposed}}$

Procedures for Expel Test

To study the rate at which mosquitoes were expelled for each experiment, fifty sucrose-fed female mosquitoes were introduced into the test chamber with the windows open to allow free movement of the mosquitoes. The rate at which the mosquitoes migrated to the adjacent chamber (without ultrasonic device) was observed. Six windows connecting the test chamber and the adjacent chamber were opened prior to the introduction of the mosquitoes. The number of mosquitoes knocked down was recorded in both chambers hourly for the first six hours, and subsequently at 24 hours post exposure. After 24 hours, the windows were closed and the mosquitoes in each chamber were collected and counted.

The expel rate was calculated as =
$\frac{\text{(No. of mosquitoes introduced – no of mosquitoes present in the test chamber at 24 hrs) x 100}}{\text{Total number exposed}}$

While the expel test was being conducted, the number of mosquitoes that were not expelled but knocked down in the test chamber was also recorded hourly for the first six hours, and subsequently at 24 hours post exposure.

Percentage knockdown was calculated as = $\frac{\text{Number knocked down x 100}}{\text{Total number exposed}}$

RESULTS

Knock-down test

Results of the knock-down tests are presented in Table 1. For "fan ON + ultrasonic ON" experiment, mosquito knock-down increased progressively from 8.7% after 1 hour to 48.7% after 6 hours exposure to ultrasound. Mortality at 24 hours was 60.7% as shown in Table 1.
For experiment "fan ON + ultrasonic OFF", the knock-down rate increased from 1.3% at 1 hour to 10.0% at 6 hours. The final mortality recorded at 24 hours was 15.3% (Table 1). However, for experiment "fan OFF + ultrasonic ON", the knock-down rate increased from 2.7% after 1 hour to 16.7% after 6 hours exposure to ultrasound. The final mortality recorded at 24 hours was 17.3% (Table 1). For the control, the knock-down rate increased from 0% at 1 hour to 4% at 6 hours and the final mortality recorded at 24 hours was 4.7% (Table 1).

Expel Test

The results of the knock-down and expel tests are presented in Tables 2a – 2d. The experiment "fan ON +

Table 1. Knock-down (KD) of *An. gambiae* s.l mosquitoes exposed to the air-conditioning system with an ultrasonic device in a Peet Grady Chamber.

	Fan ON + Ultrasonic ON			Fan ON + Ultrasonic OFF			Fan OFF + Ultrasonic ON			Control		
Time (hrs)	**Total KD**	**Mean KD**	**% KD**	**Total KD**	**Mean KD**	**% KD**	**Total KD**	**Mean KD**	**% KD**	**Total KD**	**Mean KD**	**% KD**
1	13	4	8.7	2	1	1.3	4	1	2.7	0	0	0.0
2	17	6	11.3	6	2	4.0	11	4	7.3	1	0	0.7
3	27	9	18.0	9	3	6.0	15	5	10.0	1	0	0.7
4	36	12	24.0	10	3	6.7	17	6	11.3	4	1	2.7
5	64	21	42.7	12	4	8.0	22	7	14.7	5	2	3.3
6	73	24	48.7	15	5	10.0	25	8	16.7	6	2	4.0
24	**91**	**30**	**60.7**	**23**	**8**	**15.3**	**26**	**9**	**17.3**	**7**	**2**	**4.7**
Total no exposed	150			150			150			150		

Table 2a. Fan ON + Ultrasonic ON

Fan ON + Ultrasonic ON						
Time (hrs)	**Total KD**	**Total Expel**	**Mean KD**	**Mean Expel**	**% Total KD**	**% Total Expel**
1	12	11	4.0	4	8.0	7.3
2	19	19	6.3	6	12.7	12.7
3	25	22	8.3	7	16.7	14.7
4	27	30	9.0	10	18.0	20.0
5	30	30	10.0	10	20.0	20.0
6	33	36	11.0	12	22.0	24.0
24	42	94	14.0	31	28.0	62.7
Total no exposed		150				

Table 2b. FAN OFF + ULTRASONIC ON

Fan OFF + Ultrasonic ON						
Time (hrs)	**Total KD**	**Total Expel**	**Mean KD**	**Mean Expel**	**% Total KD**	**% Total Expel**
1	6	5	2	2	4.0	3.3
2	9	7	3	2	6.0	4.7
3	11	10	4	3	7.3	6.7
4	10	11	3	4	6.7	7.3
5	13	13	4	4	8.7	8.7
6	14	16	5	5	9.3	10.7
24	21	39	7	13	14.0	26.0

Table 2c. Fan ON + Ultrasonic OFF

Fan ON + Ultrasonic OFF						
Time (hrs)	**Total KD**	**Total Expel**	**Mean KD**	**Mean Expel**	**% Total KD**	**% Total Expel**
1	0	1	0	0	0.0	0.7
2	0	4	0	1	0.0	2.7
3	0	5	0	2	0.0	3.3
4	0	7	0	2	0.0	4.7
5	0	8	0	3	0.0	5.3
6	2	14	1	5	1.3	9.3
24	2	20	1	7	1.3	13.3

Table 2d. Control

Control						
Time (hrs)	**Total KD**	**Total Expel**	**Mean KD**	**Mean Expel**	**% Total KD**	**% Total Expel**
1	0	0	0	0	0.0	0.0
2	0	0	0	0	0.0	0.0
3	0	0	0	0	0.0	0.0
4	0	1	0	0	0.0	0.0
5	0	1	0	0	0.0	0.0
6	2	1	1	0	2.0	1.3
24	2	2	1	1	2.0	1.3

ultrasonic ON" had the highest proportion of mosquitoes expelled (62.7%) while mosquitoes not expelled were knocked down (28.0%) (Table 2a). This was followed by "fan OFF + ultrasonic ON" with expel of 26.0% (Table 2b) and only 14% knocked down after 24 hours. For "fan ON + ultrasonic OFF", the expelled mosquitoes was 13.3% with only 1.3% knocked down after 24 hours (Table 2c). The expel rate of the control was 1.3% after 24 hours (Table 1d) while 2.0% was knocked down after 24 hours.

DISCUSSION

Numerous insect species produce and receive acoustic signals for a variety of functions including alarm, courtship, and avoidance (Dryden *et al.* 1989). Ultrasonic device inbuilt in an air conditioning system is one of the latest generations of mosquito control devices, and our results suggests that the ultrasonic device was effective in knocking down or repelling mosquitoes when used with the fan. Previous studies have reported that ultrasonic devices were not effective at controlling insect pests such as mosquitoes, cockroaches, bed bugs, cat fleas and rat fleas (Belton, 1981; Foster and Lutes, 1985; Koehler *et al*. 1986; Dryden *et al.* 1989; Schreck *et al.* 1984; Schreiber *et al.* 1991; Jensen *et al.* 2000; Ahmad *et al.* 2007; Mankin, 2012; Yturralde and Hofstetter, 2012). Most of these studies used ultrasonic devices with frequencies in the range of 2–60 kHz against mosquitoes. Similarly, the effect of ultrasound at 20 – 100 kHz frequency range was tested against mosquitoes and German cockroaches and this was found to be ineffective (Ahmad *et al.*, 2007). In some experiments, ultrasound resulted in significant differences in distribution of mosquitoes within a chamber compared to a chamber without ultrasound (Ahmad et al., 2007). Therefore, the practical application of ultrasound has not been found convincing.

Belton (Belton, 1981) tested the repellent effect of four ultrasonic devices with fundamental frequencies between 1.95 and 5 kHz and peak harmonic frequencies ranging from 4 to 15 kHz on laboratory bred and wild *Aedes aegypti* and *Culex pipiens* and reported that the four devices were ineffective. This ineffectiveness was ascribed the high fundamental frequencies from the devices. Also two commercially available ultrasonic devices (UD-1 and UD-2) evaluated for their efficacy in repelling *Blatella germanica* cockroaches and *Aedes aegypti* and *Anopheles quadriannulatus* mosquitoes were found to be ineffective in a controlled laboratory environment. One of the ultrasonic device was found to emit sound intensities above 90 dB SPL which could be potentially harmful to man (Schreck et al., 1984). In another study, nine ultrasonic devices were found to be ineffective in repelling the cockroach, *Blatella germanica* and did not deter rat fleas, *Xenopsylla cheopis* from mating, oviposition, larval development and pupation (Koehler et al., 1986). The authors argued that the ultrasonic devices will not be effective on household insects such as the German cockroach and flees because they have not been subjected to the evolutionary pressure that moths have undergone thereby evolving tympanic organs that allows it to detect the ultrasonic frequencies generated by bats.

Our results are consistent with some of these findings when the ultrasonic device was used alone; however, we noticed significant increase in performance when the ultrasonic device was used in combination with a fan. The results of this study show that the harmonic frequencies, of specific range(s), inclined on sweep/switch system has effect on the mosquitoes when used in combination with the fan in the air conditioning system. The knock down effect was observed at higher frequencies suggesting that these may have more deterrent effect on the mosquitoes. It is therefore, probable that the fan in the system works in a way that produces complimentary effects with the ultrasonic device to knock down the mosquitoes. In addition, in the expel test, it was noted that the majority of mosquitoes that were not expelled were knocked down suggesting that the ultrasonic device, in combination with the fan, had an additional effect on the mosquitoes, suggesting the dual effects on the mosquitoes.

In a recent study, use of ultrasound was found to be effective in knocking down and expelling *Ae. aegypti* mosquitoes (Hadi *et al.* 2009). One of the personal protection methods against mosquito bites that has been suggested of recent is to sleep in air-conditioned rooms (Freedman, 2008; Kakkilaya, 2011). However, there are no reviews or trials that have stated the efficacy of this measure in reducing mosquito bites and the incidence of malaria is indisputable (Freedman, 2008; Kakkilaya, 2011). The use of air conditioner has an added advantage in that it encourages people to stay indoors to avoid the heat and thereby decreases their exposure to the bites of mosquitoes (Freedman, 2008; Gahlinger *et al*, 1986). The use of fan has also been suggested to be effective in preventing mosquitoes from biting by (1) preventing mosquitoes from landing on a person (2) diluting and dispersing the carbon dioxide exhaled since carbon dioxide attract mosquitoes and (3) reducing the amount of sweat produced by cooling the individual and thereby making the person less attractive to the mosquitoes (Hoffmann and Miller, 2003). Carbon dioxide attracts mosquitoes and is generally used either alone or in combination with octenol, lactic acid, etc to enhance the capture rates of mosquito traps (Hoel *et al.*, 2007). Volatile organic compounds present in human sweat such as short-chain carboxylic acids, ammonia, and L-lactic acid have been found to be attractive to mosquitoes (Smallegange, , 2010).

It has been suggested that there is potential in control-

lling the timing and frequency of ultrasonic signals using modern technology to control insects such as mosquitoes, as the higher frequency/intensity of the ultrasound may increase the degree of repellency (Ahmad et al., 2007; Foster and Lutes, 1985; Mankin, 2012). This study revealed appreciable effects that the frequency changes of the ultrasonic device tested here, which occurs in designed steps had on the knockdown and expel rate of the mosquitoes when used with the fan. It further suggest the need for integrated or complimentary approach in the use of designed devices, be it physical, electrical, mechanic or chemical for the control of *An. gambiae* s.l.

The results of this study indicates that the ultrasonic device when used in combination with an air - conditioner will be a good intervention for the personal protection against mosquitoes. However, since the air conditioner with an inbuilt ultrasonic device (ACUD) can practically be affordable by only the elite group in the society we acknowledge that economic status may be a limiting factor to its use. Air conditioners with inbuilt ultrasonic device could help reduce the vector-human contact and could subsequently reduce the burden of malaria in homes where this can be afforded. It is concluded that ultrasound alone at the frequency used had effect on the mosquitoes. However, the performance of the device is enhanced when a fan was used along with the ultrasound in the device.

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