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Investigation into the 35 Khz - 60 Khz Frequency Range of the Naturally Generated Ultrasound of the African Bat, *C. Afra* , Eliciting Optimal Evasive Response in the African *A. Gambiae S. S.*

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Abstract:

This research investigated the 35 kHz - 60 kHz frequency band of the naturally generated ultrasound of the African sheath tailed bat, *Coleura afra,* which elicited optimal evasive response in the African *Anopheles gambiae.* Recent study findings with the natural sounds of *C. afra* had shown ultrasonic components (35 kHz - 60 kHz) with capability to evoke avoidance response in the female *A. gambiae s. s.* Malaria whose vector are mated female *A. gambiae* is a health challenge in Africa and responsible for many deaths. Efforts to reverse the trend have shown low impact as manifested in the 2006 and 2008 World Health Organization statistics on Malaria. Currently, the effective vector control measures include indoor residual spraying and the long-lasting insecticide-treated nets. Therefore there was need to critically investigate the 35 kHz - 60 kHz sound of *C. afra* with a view of exploiting it as an additional vector control measure. The study therefore aimed at filtering the 35 kHz - 60 kHz frequency band, determine and analyse the acoustic transmission parameters of the sound of *C. afra* in the 35 kHz - 60 kHz frequency range; determine the activity and the behavioural response of the female *A. gambiae* to the ultrasound in the 35 kHz - 60 kHz frequency range. A set of ten 3-5 day old female *A. gambiae* bred and reared at the Kenya Medical Research Institute, Kenya were used in the bioassay study. The temperature and humidity was maintained at 25±2 ^{o}C and (60-80 %) respectively. The sound samples of *C. afra* were recorded using the Avisoft recorder from Kit-Mikayi caves, Kenya. The 35-60 kHz frequency band was filtered and analysed using the Avisoft SASLab Pro version 5.1 and Raven Pro. version 1.4 software. The mosquitoes' behavioural response to the 35-60 kHz sound of *C. afra* and associated activities were observed and noted. It was established that the 35-60 kHz sound of *C. afra* consisted of 5046 calls of FM and CF modulated harmonics. The calls were generated through tongue clicks at the rate of 493.016 calls/minute. The calls were dominated by the short duration high frequency signals with an average acoustic energy of 9.2433×10^{-4} Pa^2s which was lowest. The non-pulsate sound had a minimum and maximum amplitude of 71.21 Pa and 104.82 Pa respectively, with 2,519 calls between 90.00 - 99.00 Pa peak amplitude range. The signal power steadily declined with the increase in signal frequency. Statistically, there was a highly significant relationship between the acoustic energy with the amplitude, frequency and bandwidth. The female *A. gambiae* assumed a normal posture with the body inclined at 45^{o} accompanied by occasional rubbed wings and legs under the bioassay control experiment. There was no remarkable change in physical behavioural activities in 60 % of the sample mosquitoes on exposure to the 35 - 60 kHz sound. Only 40 % exhibited immobility and excitation tendencies. At 95 % confidence level, a paired T-test showed that the acoustic energy significantly affected the mean activities of the female *A. gambiae* ($p = 5.6477 \times 10^{-5}$) with a low positive correlation. It was established that the mean mosquito activities under the influence of 35 - 60 kHz differed significantly from the mean activities under the control (p = 0.008). Only 30 % of the mosquito samples showed significant difference in the individual total mosquito activities under the influence of the 35 - 60 kHz sound of *C. afra* and the individual total activities under the control. The mosquitoes recorded a mean rate of activities of 1.5598/minute when exposed to the 35 - 60 kHz sound of *C. afra* , 2.5195 times above the rate of activities at the control experiment. The rate of mosquito activities was significantly affected by the peak amplitude, peak frequency and the bandwidth. The low significance in evasive response was attributed the non-pulsate nature of the sound of *C. afra*, declining signal power with increase in frequency, mixed sonar and social calls, and short duration high frequency calls. These results of this study give an insight into the reasons for low evasive response in female *A. gambiae* on exposure to the 35 - 60 kHz sound of *C. afra*. The acoustic transmission parameters of the sound required modifications in order to yield improved results. The improved results would provide Ultrasound as an addition malaria vector control measure which is locally available in Africa and hence cut down on mortality and economic burden resulting from Malaria.

Key words:

Band Pass Filter, Frequency Modulation (FM), Constant Frequency (CF), Sonar Calls, Peak Amplitude, Bandwidth, Frequency, Acoustic Energy, Signal.

I. INTRODUCTION

Coleura afra is an African sheath-tailed bat belonging to the order *Chiroptera* and inhabits caves feeding on insects of which mosquitoes are part. *Coleura afra* is the smallest species of the family in Africa and is distinguished from all other Emballonuridae by the absence of a gular sack and radio carpal pouches, a body weight of 10-12 g, its deep brown fur which is paler at the base than at the tip, its smaller size (Fore Arm < 55 mm) and the three pairs of lower incisors [1, 9, 10, 28, 33, 34, 35]. The bat generates sound through tongue clicks or vocalization that occurs as paired clicks and stretches into the ultrasonic range (20 kHz – 100 kHz); essential in communication and navigation purposes [12, 17, 25]. The aerially hawking bats emit these ultrasonic probes and detect flying insect prey by the echoes that return from their bodies [29, 32]. Bats alternate the production of sound and listening to the incoming sound. Bats change their echolocation based on situation. The bat sound consists of both FM and CF with the structure of the FM signal being a broadband with a downward sweep through a range of frequencies [19, 20, 30]. The FM signal is precise in range discrimination though the distance from an FM-Bat detecting target is limited. The CF-Bat detects both target velocity and the fluttering of target wings as Doppler shifted frequencies. The FM component of the signal is excellent for hunting prey while flying in close, cluttered environment due to precise target localization. CF component is suited for bats flying in open during hunting or bats that wait on perches for their prey to appear due to excellent prey detection ability.

Past researches have shown that hearing in insects is the product of evolutionary adaptation to bat predation [7]. The Anopheles mosquito, predated on by bats is able to detect ultrasound. The female *A. gambiae* acts as a malaria vector during the adult stage [3, 11]. The mosquitoes mate during flight after which the female search for a blood meal. The mosquito rests with the abdomen sticking up in the air. The adult mosquitoes mate within a few days after emerging from the pupae stage. The females will feed on sugar sources for energy requiring a blood meal for the development of eggs. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed [11, 15]. This process depends on the temperature but usually takes 2-3 days in tropical conditions. The mosquito completes its lifecycle in 1.5 - 3 weeks. It is through these mosquito bites that malaria is transmitted from one person to another. The Mosquitoes communicate using their antennae which are ultrasound sensors. The pedicel at the base of the antenna has the Johnstones' organ which is a sensory organ [23]. Malaria, which is transmitted by the female *A. gambiae* , is a major health problem in the sub-Saharan Africa [5, 8, 24]. There were an estimated 247 million malaria cases among 3.3 billion people at risk in 2006, with around 881,000 deaths, most of whom were under five years of age, 91% of which occur in Africa [6]. The human malaria parasite, *Plasmodium falciparium* is common in Africa and life threatening [13]. Recent research of geographical distribution of *A. gambiae* showed that they inhabit wetter and warmer environments (24.6°C annual mean temperature; 22 cm annual mean precipitation; 2.57 frost days annually) [18]. In the year 2008, an estimated 243 million malaria cases were reported worldwide, 85% of which were in Africa. Malaria accounted for an estimated 863 000 deaths in the year 2008, of which 89% were in the African Region [36]. There are 1.4 billion people worldwide who are at risk of Malaria infection and nearly 49% of the world's population lives in malaria risk areas [8, 16]. Currently, malaria parasites have developed unacceptable levels of resistance to drugs and many insecticides are no longer useful against mosquitoes that transmit malaria [13, 26]. This was confirmed in recent research finding which have shown widespread nature of pyrethroid resistance in Sub-Saharan Africa [2]. The malaria control measures being used against adult mosquitoes and larvae include chemical, biological, environmental and personal protection; some of which have negative environmental effects. Other strategies and approaches available for preventing mosquito bites and malaria infection include the use of repellents, insecticide treated mosquito nets (ITNs) and prophylaxis. ITNs have been proven to reduce mortality rate by approximately 20% [5, 14, 31, 36]. Also, it has been established that health education interventions are effective and remain a valuable tool in community-based malaria prevention and control interventions in sub-Saharan Africa [4]. Attempts made for chemical control and eradication of mosquitoes and malaria have failed due to the buildup of resistance among both the mosquitoes and the disease agents [6, 8]. Insect control is an essential part of reducing transmission [6, 26]. To date, the two operational scale interventions include indoor residual spraying and the long-lasting insecticide-treated nets (LLINs), are effective at reducing transmission [6]. Single animal species ultrasound mimicking repellents have been proven to give 20% mosquito repulsion [5]. The reduced efficacy of current control methods, compounded by the failure to discover new drugs, insecticide replacements and effective vaccines, it became necessary to develop new control strategies. One strategy developed in recent years was to genetically manipulate insect pests such that they are unable to transmit disease-causing pathogens, and to mass release them into the environment to displace natural populations of susceptible mosquitoes [3]. The challenge with the strategy was the identification of candidate mosquito genes that confer resistance to infection [31].

Mosquitoes detect ultrasound in the range of 38 - 44 kHz, regardless of the source, initiating avoidance response since it creates stress on their nervous system. Due to the ability of the mosquitoes to detect sound in this range, it was important to investigate the sound of *C. afra* in the 35-60 kHz which is a mosquito predator. Ultrasound also jams mosquitoes' own ultrasound frequency besides immobilizing them [19, 20, 27].

Recent research with recorded ultrasound of *O. tormotus*, *C. afra* and their combination yielded repellency in the female *A. gambiae*. The average percentage of the number of the female *A. gambiae* affected by sound of *A. tormotus*, *C. afra* and their combination in the optimal frequency range was 45.88 %, 22.94 % and 38.82 % respectively [19, 20, 21]. The optimum frequency range of the sound of *C. afra*, 35-60 kHz, evoked evasive responses in an average of 22.94 % of the mosquitoes [19, 20, 21], which is 2.94 % higher than the reported 20 % effective repulsion of EMR sound [3]. Therefore, there was need to investigate the 35kHz - 60 kHz frequency range of the ultrasound of *C. afra* in order to establish reasons for low optimal evasive response in mosquitoes with a view of improving on parameters and adopting it as an environment friendly means of malaria vector control.

1.1. Statement of the Problem

Malaria is a well known health challenge which is responsible for many deaths in Africa. In the year 2006, there were 247 million malaria cases and 3.3 billion people were reported to be at risk. In the same year, around 881,000 deaths were reported, most of whom were under five years of age and 91% of which occur in Africa. The number of the death cases reduced slightly to 863 000, which is still very high despite the many efforts involved to alleviate it. Based on this enormity, malaria control, becomes paramount with successful approaches requiring efforts directed towards insect control. Reportedly, two operational scale interventions include indoor residual spraying and deployment of long-lasting insecticide-treated nets (LLINs) had been employed yet unable to tame the menace. This situation therefore requires addition mosquito control measures in order to effectively reduce the mortality and morbidity caused by malaria. Devices mimicking ultrasound from bats and male mosquito have been employed in malaria control with minimal success. The recorded natural ultrasound from the African bat, the *C. afra* which generates ultrasonic calls has been observed to startle the female *Anopheles gambiae*. The results determined from the research with recorded natural ultrasound of *C. afra* yielded 22.94 % repellency in mosquitoes in the 35 kHz - 60 kHz frequency range. The 35 kHz - 60 kHz frequency range had not so far been critically studied with a view of determining the detailed parameters and behavioural response of the female *A. gambiae* to the sound. This study investigated the naturally generated *C. afra* ultrasound in the optimal frequency range that elicited optimal evasive response in the female African *A. gambiae*. The 35 kHz - 60 kHz sound of *C. afra* was filtered and analysed using the Avisoft-SASLab Pro version 5.1 and Raven Pro 1.4 software; and a bioassay study set up with 3-4 day old female *A. gambiae* performed. The acoustic transmission parameters and the mosquito behavioural responses to the 35 kHz - 60 kHz frequency range of the sound of *C. afra* were determined. These results would provide information on the suitability and improvements of the 35 kHz - 60 kHz ultrasound of *C. afra* in malaria vector control in Africa.

1.2. Objectives

1.2.1. General Objectives

To filter the 35 kHz - 60 kHz frequency band, determine and analyse the acoustic transmission parameters of the sound of *C. afra* in the 35 kHz - 60 kHz frequency range; determine the activity and the behavioural response of the female *A. gambiae* to the ultrasound in the 35 kHz - 60 kHz frequency range.

1.2.2. Specific Objectives

- Filter the 35 kHz-60 kHz frequency band from the recorded sound of C. afra.

- Determine and analyse the acoustic transmission parameters of the sound of C. afra in the 35 kHz-60 kHz frequency range
- Determine and analyse the behavioural response and activity of the female A. gambiae in the 35 kHz - 60 kHz frequency range.

1.3. Hypotheses

- There exist no significant relationship between the acoustic energy, amplitude, frequency and bandwidth of the sound of *C. afra* in the 35 kHz- 60 kHz frequency range.
- The acoustic energy of 35 kHz - 60 kHz sound of *C. afra* did not affect the mosquito activity significantly.
- There exist no significant difference between the mean mosquito activities under control and the mean mosquito activities under the 35- 60 kHz sound of *C. afra*.
- The rate of mosquito activities was not significantly affected by the peak amplitude (mean), frequency and the bandwidth (mean) of the 35 kHz - 60 kHz sound of *C. afra*.

II. Materials and Methods

2.1. Materials

2.1.1. The Samples of *A. gambiae* mosquitoes

A set of ten female *A. gambiae* which were 3-5 day old were used in the bioassay study. The mosquitoes were bred and reared at the Kenya Medical Research Institute Centre for Global Health Research laboratories, Entomology department. The environmental conditions were kept at 60-80 % humidity, 25±2 ^{o}C temperature and light-day cycle of 12L: 12D hours were used in the study.

2.1.2. Sound Samples of C. afra

A set of nine sound samples were recorded using the Avisoft recorder (Model 112) at a sampling frequency of 500 kHz `from a colony of bats in Kit-Mikayi caves, Kisumu County; Kenya.

2.1.3. Equipment

A computer fitted with a sound card, hardlock key and sound output ports was used together with the Avisoft-SASLab Pro version 5.1 software and Raven Pro. 1.4. Two Panasonic 8.0 Ω external speakers were used for the playback of the externally amplified 35 - 60 kHz sound of *C. afra* directed to the bioassay cage housing the female *A. gambiae.* A stopwatch was used to determine the activity duration. The bioassay cage whose dimensions were 50 cm long, 25 cm width and 25 cm in height was made of glass, covered at the two ends with a mosquito netting.

2.2. Methods

2.2.1. Filtering of sounds samples

The 35-60 kHz frequency range was extracted from the recorded sound of *C. afra* using a band pass filter inbuilt in the Avisoft SASLab analysis software with an upper cut-off frequency, f_{uco}= 60 kHz and a lower cut-off frequency, f_{lco}=35 kHz. The 35-60 kHz sound of *C. afra* was saved in the hard disc as 35-60 kHz *Coleuraafra.wav*.

2.2.2. Determination of acoustic transmission parameters of the 35 - 60 kHz sound of C. afra

The acoustic transmission parameters of the 35 - 60 kHz sound of *C. afra* were automatically generated using Raven Pro. version 1.4 and Avisoft SASLab Pro version 5.1 software. Essential settings made to the softwares for the generation of the transmission parameters were spectrogram parameters: FFT: 512, Window: Hamming, Frame size: 100% and Overlap: 50%. The sound card was set to a sampling frequency of 500 kHz at 16 bits with a down sampling of 1. The sound was analysed using Avisoft SASLab Pro version 5.1 and Raven Pro. version 1.4 software. Also the calibration method was set to SPL with reference sound for Channel 1and at a /gain (dB) set to zero under the tools menu. The acoustic pressure level in the software was set to 20 µPa, which is the threshold for human perception and the reference signal yielded 94 dB. Similarly, the envelope was set to original waveform whereas the pulse detection was set to gate function. The signal was internally amplified and then externally amplified before getting into the external speakers, placed 5 cm from one side of the cage. The speakers were set to face the glass cage on the netting sides. The amplitude modulation constant of 35-60 kHz appended sound of *C. afra* was set to n = 0.9. It was normalized at 80% for the entire duration for the *C. afra* signal.

The acoustic transmission parameters of the 35 - 60 kHz sound of *C. afra* determined in the study were:

- The total number of calls in the 35 - 60 kHz sound of C. afra
- Call durations in the 35 - 60 kHz sound of C. afra
- Amplitudes of the pulses in the 35 - 60 kHz sound of C. afra
- Frequencies of the calls in the 35 - 60 kHz sound of C. afra
- Signal bandwidths of the 35 - 60 kHz sound of C. afra
- Signal Energy and Powers of the 35 - 60 kHz sound of C. afra

The Pearson's product moment correlation coefficient that established the relationships between the acoustic transmission parameters with the signal energy was determined statistically using the SPSS software. Similarly, a paired samples T-test option of the SPSS version 16.0 set to a significance level of 0.05 was used to determine the significance values (p-value) of parameters compared.

2.2.3. Determination of the A. gambiae Behavioural Response and Rate of Activity evoked by 35-60 kHz sound of C. afra

A bioassay study involved 3-4 day old female *A. gambiae* and the 35-60 kHz recorded sound of *C. afra* was performed using a glass cage whose design is described in.2.1.3. The transfer of the female *A. gambiae* from the rearing cage to the bioassay cage and also remove them from it was done using an aspirator. The observations made and recorded were based on behavioural response of the female *A. gambiae* to the 35-60 kHz ultrasound of *C. afra*. The landing activities of the mosquitoes were based on the mosquito flights and rests. A stopwatch was used to determine the flight and rest durations of the mosquitoes. The effect of the acoustic transmission parameters on the activity of the female *A. gambiae* was established statistically using the Paired Samples T-test at 0.05 significance level.

III. Results and Discussion

3.1. Filtering of the 35 kHz-60 kHz frequency band from the recorded sound of *C. afra*

The sound of *C. afra* appended using Avisoft SASLab Pro version 5.1 software was subjected to a band pass filter as described in 2.2.1. The band pass filter modified sound of *C. afra* shown in the spectrogram in Figure 3.1 below. The frequencies below 35 kHz and above 60 kHz were gradually attenuated (amplitude = 0 i.e. off), allowing those in the range of 35-60 kHz (amplitude = 1, i.e. on).

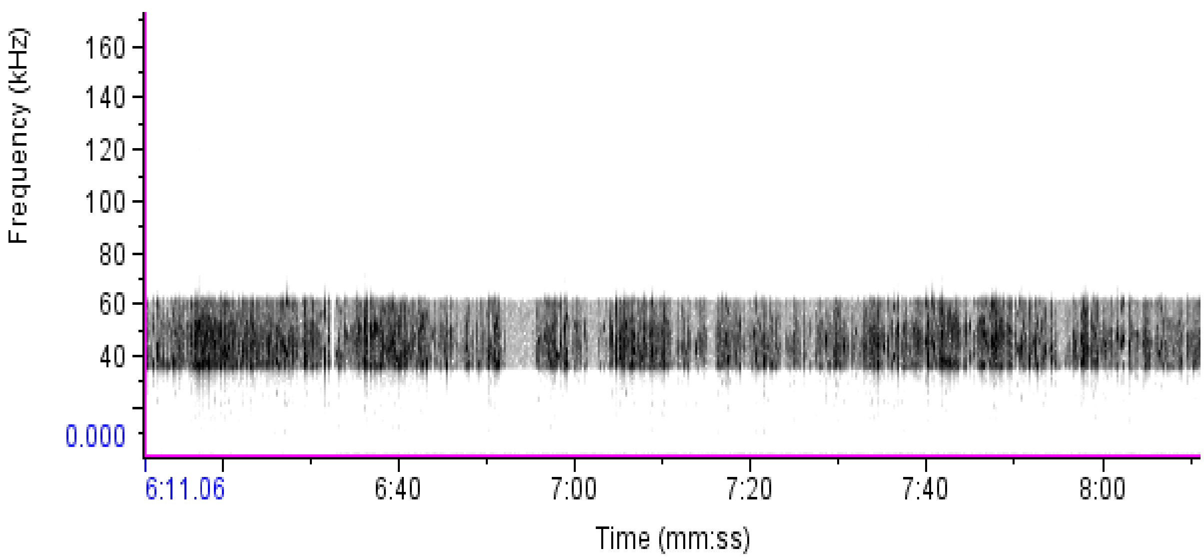

Fig3.1. *A spectrogram showing the 35-60 kHz band of C. afra*

Several different frequency emissions were observed in the sound of *C. afra* simultaneously were observed as shown in Figure 3.2, constituting the harmonics in the 35 - 60 kHz range.

Fig3.2. *A spectrogram showing the 35-60 kHz band of C. afra*

Figure 3.3 showing the variation of frequency with time confirms the existence of both FM and CF modulation components in the 35 - 60 kHz sound of *C. afra*. It can be observed from the portion of the spectrogram that some call components had their frequency varying with time, hence frequency modulation (FM). Some components of calls had their frequencies invariant over time, hence constant frequency (CF). The calls and modulations are essentially for sonar and social purposes. The mixture of calls therefore compromised on the quality of the sound of *C. afra* for purposes of repellency in mosquitoes.

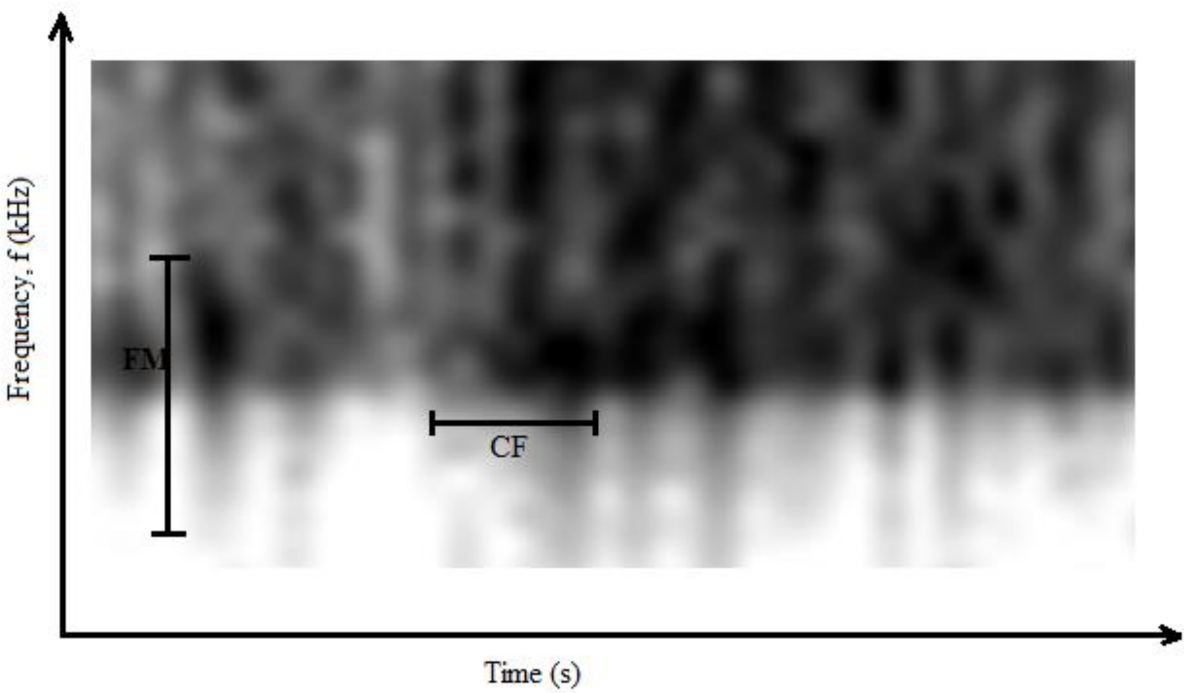

Fig3.3.*Modulation components in the 35-60 kHz band of C. afra*

3.2. Determination and analysis of the acoustic transmission parameters of the sound of C. afra in the 30 kHz-60 kHz frequency range

The study determined the following acoustics transmission parameters that affected signal acoustic energy and power; essential in mosquito repellency:

- Number of calls in the sound of C. afra.
- Duration of each pulse
- Amplitude of the signal
- Frequency of the signal
- Signal bandwidth

There were 5046 calls studied in the 35 - 60 kHz optimal frequency range for the sound of *C. afra*. The colony of the *C. afra* generated calls at the rate of 493.016 calls/minute. The sound of *C. afra* was dominated by short duration calls constituting 24.11 % of the total calls each lasting 0.0005 second. The short duration calls possessed an average energy of 9.2433 x 10^{-4} Pa^2s which was 13,331.8 times less than the maximum energy of 12.3229 Pa^2s recorded for the sound sample. Only one call lasted for a duration of 2.889 second which was the longest. Notably, the maximum energy was recorded by the 5046th call that lasted for the maximum duration of 2.8892 s. The sound of *C. afra*, none pulsate in nature, shown in Figure 3.4, had a minimum and maximum mean peak amplitudes of 71.21 Pa and 104.82 Pa respectively. As observed in recent studies, high acoustic energy yielded greatest repellency of the female *A. gambiae* [19, 20].

The change in wave in energy $\Delta E = \frac{1}{2}\Delta m\omega^2 A^2$ .... (1)

Also,

But $\omega^2 = 4\pi^2 f^2$..(2)

[37].

Equations (1) and (2) give the relationships between energy E, amplitude A and frequency f summarised as $\boldsymbol{E \propto A^2}$ and $\boldsymbol{E \propto f^2}$ respectively.

The distal elongated flagellum of the mosquito antenna mechanically filters and resonantly tunes itself in response to particle oscillations as a forced damped harmonic oscillator dependent on signal energy [38]. The ultrasonic frequencies and amplitude heavily determined the acoustic energy which evokes evasive response in the female *A. gambiae*. The low acoustic energy contributed by the lowest duration calls yielded low evasive response of a paltry, 22.94 %. The acoustic signal power in this frequency range declining steadily from a maximum of about 80.0 dB to 30.0 dB with increase in frequency as shown in Figure 3.4 below. It was observed that calls of frequencies about 60.0 kHz lasted for only 0.0005 second. The 5046th call had a high frequency of 59.5 kHz and recorded the highest duration of 2.8892 second with maximum entire peak amplitude of 110.89 Pa. It is evident that long call duration with the corresponding high amplitude and frequency yielded maximum signal power.

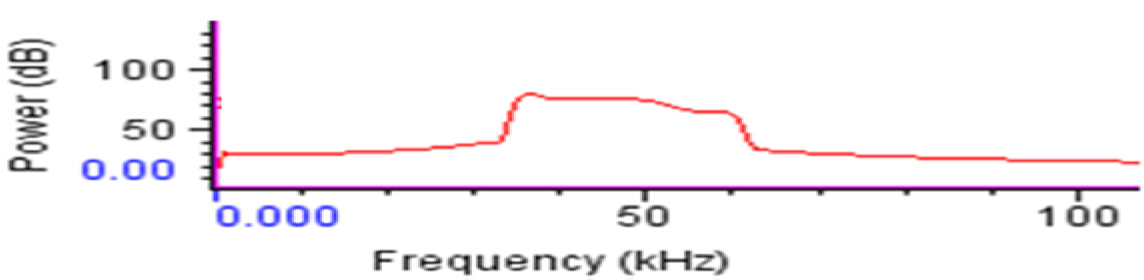

Fig3.4. *Signal Power Variation with Frequency*

It was noted that only one call recorded maximum mean peak amplitude of 104.82 Pa, translating to 0.02 % of the entire sound calls studied. The sound of *C. afra* was dominated by calls with mean peak amplitudes in the 90.00 - 99.99 Pa range as shown in Table 3.1.

Table3.1. *Distribution of Calls of C. afra per Mean Peak Amplitude Range*

Mean Peak Amplitude Range, Pa	**Total Pulses or Calls**
70.00 - 79.99	403
80.00 - 89.99	2074
90.00 - 99.99	2519
100.00 - 110.99	50

A Pearson's product moment correlation coefficient, ***r*** was determined statistically using SPSS 16.0 statistical software to establish the relationship between the acoustic energy for the sound of *C. afra* and the amplitude. This comparison was determined at a 99 % confidence and significance level of 0.01. The results are shown in Table 3.2 below:

Table 3.2. *The Correlation and Significant level between the Acoustic energy and Mean Peak Amplitude*

		Acoustic Energy	**Mean Peak Amplitude**
Acoustic Energy	Pearson Correlation	1	-0.0450
	Sig. (2-tailed)		0.001
	N	5046	5046
Mean Peak Amplitude	Pearson Correlation	-0.0450	1
	Sig. (2-tailed)	1.3813 x10-3	
	N	5046	5046

The acoustics energy and the amplitude were related negatively with r = -0.0450. Also, at 1 % level of significance, there was great evidence to show significant relationship between the acoustic energy for the sound of *C. afra* and the amplitude with $p = 1.3813 \times 10^{-3}$ <<<< 0.01. Also, a Pearson's product moment correlation coefficient, ***r*** was determined statistically using SPSS statistical software to establish the relationship between the acoustic energy for the sound of *C. afra* and the mean peak frequency. These results give a clear approval of the equations (1) and (2) that gives the relationship between acoustic energy, frequency and amplitude. The results are shown in Table 3.3 below:

Table 3.3. *The Correlation and Significant level between the Acoustic energy and Mean Peak Frequency*

		Acoustic Energy	**Mean Peak Frequency**
Acoustic Energy	Pearson Correlation	1	-0.068
	Sig. (2-tailed)		1.1650×10^{-6}
	N	5046	5046
Mean Peak Frequency	Pearson Correlation	-0.068	1
	Sig. (2-tailed)	1.1650×10^{-6}	
	N	5046	5046

The acoustics energy and the mean peak frequency indicated a very weak negative correlation at r = -0.068. There was great evidence to show significant relationship between the acoustic energy for the sound of *C. afra* and the mean peak frequency with $p = 1.1650 \times 10^{-6}$ <<<< 0.01.

Out of the 5046 calls studied, composed of harmonics, the maximum fundamental frequency (mean) determined was 50.70 kHz. The frequencies were limited to 35 kHz and 60 kHz, using a band pass filter discussed in 2.2.1. The minimum and maximum mean bandwidth of the sound were 3.90 kHz and 28.30 kHz. The maximum mean bandwidth of the sound of *C. afra* is equal to that of *O. tormotus* though the minimum band width was lowest in this case [22]. In earlier studies, *O. tormotus* recorded the highest evasive response in female mosquitoes which are vectors of malaria. Similarly, a Pearson's product moment correlation coefficient, ***r*** was determined statistically using SPSS statistical software to establish the relationship between the acoustic energy for the sound of *C. afra* and the mean bandwidth. Table 3.4 below shows the results:

Table 3.4. *The Correlation and Significant level between the Acoustic energy and Mean Bandwidth*

		Acoustic Energy	Mean Bandwidth
Acoustic Energy	Pearson Correlation	1	0.126
	Sig. (2-tailed)		2.6622×10^{-19}
	N	5046	5046
Mean Bandwidth	Pearson Correlation	0.126	1
	Sig. (2-tailed)	2.6622×10^{-19}	
	N	5046	5046

There existed a weak positive correlation between the acoustic energy and the mean bandwidth of the sound of *C. afra*. The relationship between the acoustic energy and the mean bandwidth of the sound of *C. afra* was therefore highly significant ($p = 2.6622 \times 10^{-19}$). Hence the bandwidth of the ultrasound also determines the extent of repellency in the female *A. gambiae*. The trend line in Figure 3.5 showed the linear relationship between the acoustic energy and the bandwidth. An increase in bandwidth led to a corresponding increase in acoustic energy at the rate of 0.004 Pa^2s^2 as given in Figure 3.5. The results of the parameters are summarised in Table 3.5.

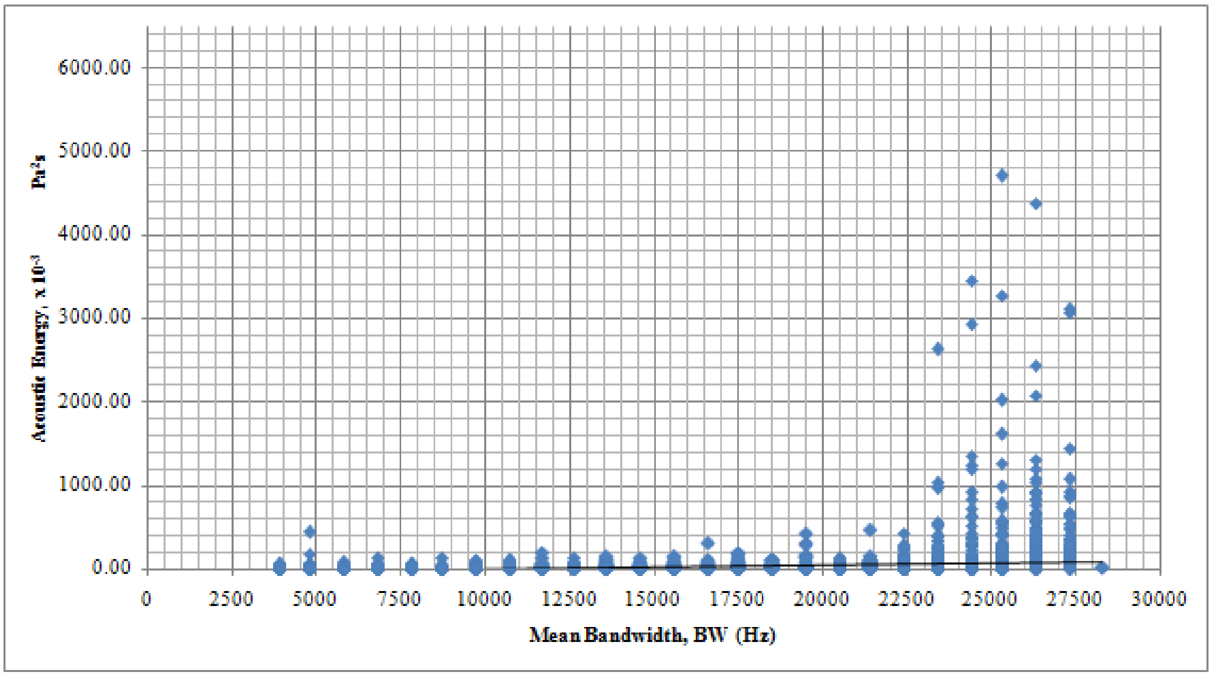

Fig3.5. *Trend relationship between Acoustic Energy and Bandwidth*

Table 3.5. *The Acoustic Transmission Parameters of the 35-60 kHz Sound of C. afra*

Parameter	Minimum	Maximum	Mean	Standard Deviation	Total Pulses
Duration (s)	0.0005	2.8892	0.0263	0.0760	5,046
Energy, Pa^2s	0.0002	12.3229	0.0501	0.2504	5,046
Peak Frequency (mean), Hz	34,100.0000	51,700.0000	39,707.9000	4,830.4000	5,046
Peak Amplitude (mean), Pa	71.2099	104.8200	89.0849	5.8882	5,046
Minimum Frequency (mean), Hz	32,200.0000	47,800.0000	34,695.2000	2,791.2800	5,046
Maximum Frequency (mean), Hz	38,000.0000	61,500.0000	55,067.2000	4,781.5100	5,046
Bandwidth (mean), Hz	3,900.0000	28,300.0000	20,341.6000	6,379.6400	5,046
Peak frequency (minimum entire), Pa	33,200.0000	50,700.0000	36,343.6000	3,638.9300	5,046
Minimum frequency (minimum entire), Hz	900.0000	46,800.0000	34,258.1000	2,406.4200	5,046

Maximum frequency (minimum entire), Hz	900.0000	60,500.0000	29,119.7000	22,881.6000	5,046
Bandwidth (minimum entire), Hz	900.0000	24,400.0000	6,227.5000	7,103.9800	5,046
Peak frequency (maximum entire), Hz	34,100.0000	58,500.0000	45,923.0000	5,952.6900	5,046
Peak amplitude (maximum entire), Pa	93.0299	113.0290	98.2109	3.8348	5,046
Minimum frequency (maximum entire), Hz	33,200.0000	247,000.0000	119,353.0000	101,382.0000	5,046
Maximum frequency (maximum entire), Hz	37,100.0000	194,300.0000	55,476.6000	5,110.0100	5,046
Peak frequency (mean entire), Hz	33,600.0000	51,500.0000	40,549.9000	3,552.9200	5,046
Peak amplitude (mean entire), Pa	77.2099	105.5700	92.1137	3.9654	5,046
Minimum frequency (mean entire) , Hz	33,200.0000	220,200.0000	61,524.0000	41,618.2000	5,046
Maximum frequency (mean entire), Hz	7,500.0000	60,500.0000	44,038.1000	10,822.7000	5,046

3.3.

(i). Analysis of the acoustic transmission parameters and the behavioural response of the female *A. gambiae* in the 35-60 kHz sound of *C. afra*.

The bioassay study involved ten female *A.gambiae* which were normal and active as observed under the control experiment. The filtered sound of *C. afra* was played using Avisoft SASLab Pro version 5.1 software through amplifications as discussed in 2.2.2 above and observations made. Each of the female *A. gambiae* was placed in the bioassay cage using an aspirator at a time. The control experiment was characterised by normal posture of rest where the body was inclined at 45^o from surface of rest with wings along body. There were occasional rubbings of wings and legs under the control experiment as observed recent findings [19]. The behaviour of the female *A. gambiae* under the influence of the 35-60 kHz sound of *C. afra* was observed and recorded in Table 3.6. It was noted that 60 % of the mosquitoes did not exhibit any remarkable change in behaviour and activity compared to that under the control experiment. However, excitation and immobility was observed in 40 % of the samples studied. Excitation was noted based on increased activity or body movements whereas immobility was based on reduced activity or docility under the influence of the 35-60 kHz sound of *C. afra*. The none-pulsate nature, declining signal power with frequency and short duration high frequency calls evoked minimal startle effect to the female *A. gambiae.* This sound did not evoke extreme responses such as exhaustion, collapsing, raising of antennae and physical injuries as observed in the *O. tormotus* in the same frequency range [19, 20, 22]. However, the response behaviour and activities observed in 40 % of the sample mosquitoes in this frequency range are in agreement with recent studies [19, 27]. It had recently been reported that Mosquitoes detected ultrasound in the range of 38 - 44 kHz, regardless of the source, initiating avoidance response since it created stress on their nervous system [19, 27].

Table 3.6. *The Chronological Behaviour of the female A. gambiae Elicited by the 35-60 kHz Sound of C. afra*

Mosquito Number.	Control Study (No sound)	Mosquito Behaviour under 35-60 kHz Sound of *C. afra*
1.	- Normal posture, body at 45^o from surface of rest with wings along body. - Normal flight	- Normal posture, body at 45^o from surface of rest with wings along body. - Normal flight
2.	- Normal posture, body at 45^o from surface of rest with wings along body. - Normal flight	- Normal posture, body at 45^o from surface of rest with wings along body. - Normal flight
3.	- Normal posture, body at 45^o from surface of rest with wings along body. - Normal flight	- Normal posture, body at 45^o from surface of rest with wings along body. - Normal flight
4.	- Normal posture, body at 45^o	- Normal posture, body at 45^o from surface of rest with

	from surface of rest with wings along body. - Normal flight	wings along body. - Normal flight
5.	- Normal posture, body at 45° from surface of rest with wings along body. - Normal flight	- Normal posture, body at 45° from surface of rest with wings along body. - Normal flight
6.	- Normal posture, body at 45° from surface of rest with wings along body. - Normal flight	- Normal posture, body at 45° from surface of rest with wings along body. - Normal flight
7.	- Rubbing hind legs	- Raised abdomen - Movement of hind legs - Pulling of forelegs on floor of cage - Shaking of body by moving up and down. - Mosquito immobilized (Attempts to move but unable). - Mosquito rests on net accompanied with fast body shake.
8.	- Normal posture, body at 45° from surface of rest with wings along body. - Normal flight	- No body movement at all. - Squeezes on surface of rest - Left hind leg raised and shaking.
Mosquito Number.	**Control Study (No sound)**	**Mosquito Behaviour under 35-60 kHz Sound of** *C. afra*
9.	- Legs and proboscis rest on net. - Mouth and antennae collinear - Body at 45° from surface	- Pulling downwards along net. - Shaking body. - Rests in corner - Squeezes in cavities - Open wings with widened legs. - Rests on the floor with abdomen on the floor. - Lay by side
10.	- Rests facing net - Rubbing wings - Rubbing legs	- Pushes mouth through net - Pulling along the net surface - Shaking body.

(ii). Analysis of the acoustic transmission parameters and the Activity of the female *A. gambiae* in the 35 - 60 kHz sound of *C. afra*

The behaviour of the female *A. gambiae* discussed in 3.3 (i) was associated with movements (Flights - F) and Rests (or Landings - R), considered as mosquito activities. The mean activities for ten mosquitoes for various time duration ranges under the 35 - 60 kHz sound of *C. afra*, shown in Table 3.15, is compared to the acoustics Energy, Bandwidth (mean) and Mean Peak amplitude. The mean activity for ten mosquitoes for various time duration ranges under the control is also given in Table 3.14. A paired samples T-test at 95 % confidence level which was used to compare the mean activities of the ten mosquitoes under various time durations and the acoustics energy established that the mean activities of the female *A. gambiae* were significantly affected by the acoustic energy at t = -7.718, df =8, p = 5.6477 x 10^{-5} as shown in Table 3.8. In this range of sound frequency, the mean, minimum and maximum acoustic energy was 0.0501 Pa^2s 0.00017 Pa^2s and 12.3229 Pa^2s respectively. It is vividly clear that there existed a low positive correlation between the Acoustic energy and the Mean Mosquito Activity with a Pearson's product moment correlation coefficient, ***r*** = **0.353** as shown in Table 3.7 due to the none-pulsate nature of the signal, low declining signal power and low call duration. An increase in acoustic energy yielded a corresponding increase in activity as established in recent studies [19, 20, 21]. However, in some cases, immobility was observed as exhibited some mosquito sample whose rate of activity under control was reduced when they were exposed to the 35-60 kHz sound of *C. afra* [19, 27]

Table 3.7. *Paired Samples Correlations of the Acoustic energy-Mean Mosquito Activity*

Comparison	**N**	**Correlation, r**	**Sig.**
Acoustic Energy and Mean Mosquito Activity	9	0.353	0.351

Table 3.8. *Paired Samples T-test of the Acoustic Energy Compared with Mean Mosquito Activity*

Comparison	**Paired Differences**					**T**	**Df**	**Sig. (2-tailed)**
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Acoustic Energy - Mean Mosquito Activity	-5.0210	1.9516	0.6505	-6.5211	-3.5208	-7.718	8	5.6477×10^{-5}

Also, a paired samples T-test at 95 % confidence level for the comparison between the mean activity for ten mosquitoes under the Control experiment and the 35-60 kHz sound showed that there existed difference between the mean mosquito activities under control experiment and the 35-60 kHz sound of *C. afra* which was statistically significant at ***t = -3.473, df =8, p = 0.008*** as shown in Table 3.9. In conclusion, there was a significant change in mosquito activity under the 35-60 kHz sound of *C. afra*. Also, there existed low positive correlation at a Pearson's product moment correlation coefficient, ***r*** = 0.291 between the Mean Mosquito Activity under control experiment and under the 35-60 kHz sound of *C. afra*; which was not statistically significant *(p = 0.447 >> 0.05)* as shown in Table 3.10.

Table 3.9. *Paired Samples T-test of the Mean Mosquito Activity under Control and the 35-60 kHz sound of C**. afra***

	Paired Differences					**T**	**Df**	**Sig. (2-tailed)**
	Mean	Std. Deviation	Std.Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Control and 35-60 kHz Sound	-3.022	2.611	0.870	-5.029	-1.0153	-3.473	8	0.008

Table 3.10. *Paired Samples Correlations of the Mean Mosquito Activity Under Control and 35-60 kHz Sound of C. afra*

Paired Samples Correlations			
Control and 35-60 kHz Sound	N	Correlation	Sig.
	9	0.291	0.447

A paired samples T-test of the individual mosquito total activities under control and the 35-60 kHz sound of *C. afra* showed significant difference in Mosquito activities in a paltry 30 % of samples of mosquitoes observed as given in Table 3.13. Uniquely, mosquito sample 10 exhibited highly significant startle response to the sound of *C. afra* with $p = 1.38 \times 10^{-4}$. The mosquito was peculiarly active even under the control experiment. The results show that the evasive response of the mosquitoes to the 35 - 60 kHz sound of *C. afra* was not significant as explained in 3.3 (i). The female *A. gambiae* exhibited a mean rate of activities of 1.5598 /minute when exposed to the 35-60 kHz sound of *C. afra* which was above control results rated at 0.6191 activities/minute. The mean rate of mosquito activities under the influence of the sound of *C. afra* was 2.5195 times greater than the activities under the control, analysed from Table 3.16. A further comparative study on individual mosquito sample Rate of activities per minute under the influence of 35-60 kHz sound of *C. afra* and under control experiment using paired samples T-test showed a high significance, at *p = 0.148* in the change of the rate of mosquito activity at 95 % confidence interval, shown in Table 3.11. The comparison showed high positive correlation *(r = 0.574)* as given in Table 3.12 which was not statistically significant at 5 % significance level. The mosquito antennae resonated at the frequency of incoming steadily declining acoustic energy which initiated minimal activity and sometimes immobility due to some nervous stress and fear for predation [5, 19, 20, 23, 27 38]. A comparison of the Rate of mosquito activities under 35-60 kHz Sound of *C. afra* with Bandwidth, Peak Frequency (mean) and Amplitude was generated using the SPSS programme and the results presented in Table 3.17. The rate of mosquito activities was significantly affected by the peak amplitude (mean), frequency and the bandwidth (mean) of the sound of *C. afra*. As earlier observed, acoustic energy $E \alpha A^2$, an increase in amplitude leads to a corresponding increase in acoustic energy at which the antennae of the mosquito resonates [23, 37]. The comparison between the rate of mosquito activities with the peak amplitude (mean) and the bandwidth (mean) yielded a positive correlation which was not statistically significant as shown in Table 3.18. However, a negative correlation was established between the rate of mosquito activities with the Peak Frequency (mean) and it was not statistically significant.

Table 3.11. *Paired Samples T-test of the Rate Mosquito Activity under Control and the 35-60 kHz sound of C. afra*

Comparison	**Paired Differences**					**t**	**df**	**Sig. (2-tailed)**
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Rate of Activities under Control- Rate of Activities under 35-60 kHz Sound	-0.94	1.88	0.59	-2.28483	0.40483	-1.581	9	0.148

Table 3.12. *Paired Samples Correlations of the Rate Mosquito Activity under Control and the 35-60 kHz sound of C. afra*

	N	Correlation	Sig.
Rate of Activities under Control and Rate of Activities under 35-60 kHz Sound	10	0.574	0.082

Table 3.13. *A Paired Samples T-test of the Individual Total Mosquito Activities under the Control and the 35-60 kHz sound of C. afra*

Comparison	**Paired Differences**					**t**	**df**	**Sig. (2-tailed)**
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Control Mosquito Sample 1 - Ultrasound Mosquito sample 1	-1.7778	2.1082	0.70273	-3.39827	-0.15728	-2.530	8	0.035
Control Mosquito Sample 2 - Ultrasound Mosquito sample 2	1.1111	4.5123	1.50411	-2.35737	4.57959	0.739	8	0.481
Control Mosquito Sample 3 - Ultrasound Mosquito sample 3	-1.3333	2.8723	0.95743	-3.54116	0.87450	-1.393	8	0.201
Control Mosquito Sample 4 - Ultrasound Mosquito sample 4	-2.0000	3.5355	1.17851	-4.71765	0.71765	-1.697	8	0.128
Control Mosquito Sample 5 - Ultrasound Mosquito sample 5	3.0000	7.7782	2.59272	-2.97883	8.97883	1.157	8	0.281
Control Mosquito Sample 6 - Ultrasound Mosquito sample 6	-7.4444	13.0203	4.34009	-17.45272	2.56383	-1.715	8	0.125
Control Mosquito Sample 7 - Ultrasound Mosquito sample 7	-3.4444	5.8973	1.96576	-7.97749	1.08860	-1.752	8	0.118
Control Mosquito Sample 8 - Ultrasound Mosquito sample 8	2.2222	3.9616	1.32054	-.82295	5.26739	1.683	8	0.131
Control Mosquito Sample 9 - Ultrasound Mosquito sample 9	-2.4444	2.6977	0.89925	-4.51811	-.37078	-2.718	8	0.026
Control Mosquito Sample 10 - Ultrasound Mosquito sample 10	-18.1111	7.9913	2.66377	-24.25378	-11.96844	-6.799	8	1.38×10^{-4}

Table 3.14. *Total Mosquito Sample Activities under Control for various Time Duration*

Mosquito Sample	**Mosquito Activity Duration (s)**								
	0 - 200	201 - 400	401 - 600	601 - 800	801 - 1000	1001 - 1200	1201 - 1400	1401 - 1600	1601 - 1800
Mosquito 1	0	0	0	0	0	0	0	0	2
Mosquito 2	13	0	0	0	0	0	0	0	1

Mosquito 3	0	0	0	1	0	0	0	0	1
Mosquito 4	0	0	0	1	0	0	0	0	1
Mosquito 5	2	2	22	8	0	0	0	0	1
Mosquito 6	11	16	32	0	0	0	0	0	1
Mosquito 7	0	0	2	0	0	1	0	0	1
Mosquito 8	0	2	10	8	1	0	0	0	1
Mosquito 9	8	0	0	1	0	0	0	0	1
Mosquito 10	4	2	6	12	6	0	0	0	1
MeanActivity	4	2	7	3	1	0	0	0	1

Table 3.15. *Total Mosquito Sample Activities under influence of 35-60 kHz Sound of C. afra for various Time Duration*

Mosquito Sample	**Mosquito Activity Duration (s)**								
	0 - 200	201 - 400	401 - 600	601 - 800	801 - 1000	1001 - 1200	1201 - 1400	1401 - 1600	1601 - 1800
Mosquito 1	2	6	0	2	4	0	0	2	2
Mosquito 2	0	0	0	0	2	0	0	1	1
Mosquito 3	4	8	0	0	0	0	0	1	1
Mosquito 4	11	1	2	0	2	2	1	0	1
Mosquito 5	2	0	0	0	0	2	0	3	1
Mosquito 6	18	15	11	15	11	16	19	20	2
Mosquito 7	2	17	11	0	0	0	3	0	2
Mosquito 8	0	0	0	0	0	0	0	0	2
Mosquito 9	7	8	3	3	5	2	2	1	1
Mosquito 10	28	28	22	32	18	23	21	21	1
MeanActivity	7	8	5	5	4	5	5	5	1

Table 3.16. *The Rate Activities of Mosquito Samples under Control and the 35-60 kHz Sound of C. afra*

Mosquito Sample	1	2	3	4	5	6	7	8	9	10
Total Number of Activities Under Control	2	14	2	2	35	59	4	22	10	31
Total Number of Activities Under the 35-60 kHz	18	4	14	20	8	127	37	2	32	194
Rate of activity per Minute under Control	0.07	0.48	0.07	0.07	1.20	2.02	0.14	0.75	0.34	1.06
Rate of activity per Minute under 35-60 kHz Sound	0.62	0.14	0.48	0.68	0.27	4.34	1.27	0.07	1.09	6.64
Activity Factor relative to Control	9.00	0.29	7.00	10.00	0.23	2.15	9.25	0.09	3.20	6.26

Table 3.17. *Comparison of the Rate of activity per Minute under 35-60 kHz Sound of C. afra with Mean Bandwidth, Peak Frequency (mean) and Mean Peak Amplitude*

	Paired Differences					T	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Rate of Activities - Bandwidth (mean)	-2.4248×10^{4}	4.6410×10^{3}	1.4676×10^{3}	-2.7568×10^{4}	-2.0928×10^{4}	-16.522	9	4.859×10^{-8}
Rate of Activities - Peak Frequency (mean)	-4.2058×10^{4}	1.8899×10^{3}	5.9764×10^{2}	-4.3410×10^{4}	-4.0706×10^{4}	-70.374	9	1.1938×10^{-13}
Rate of Activities - Mean Peak Amplitude	-8.2441×10^{1}	6.5165	2.0607	-8.7103×10^{1}	-7.778×10^{1}	-40.007	9	1.89551×10^{-11}

Table 3.18. *Paired Samples Correlations of the Rate of activity per Minute under 35-60 kHz Sound of C. afra Mean Bandwidth, Peak Frequency (mean) and Mean Peak Amplitude*

Comparison	N	Correlation	Sig.
Rate of Activities - Bandwidth (mean)	10	0.315	0.375
Rate of Activities - Peak Frequency (mean)	10	-0.292	0.412
Rate of Activities - Mean Peak Amplitude	10	0.151	0.677

IV. Conclusion

There were 5046 calls studied in the 35 - 60 kHz optimal frequency range for the sound of *C. afra* that were generated at the rate of 493.016 calls/minute through tongue clicks. The sound of *C. afra* was dominated by short duration calls constituting 24.11 % of the total calls each lasting 0.0005 second and possessed an average energy of 9.2433 x 10^{-4} Pa^2s. The acoustic energy in this frequency range declined steadily from a maximum of about 80.0 dB to 30.0 dB with increase in frequency and recorded a positive correlation with the bandwidth (mean). However, a negative correlation was recorded for the comparison between acoustic energy with peak amplitude (mean) and frequency. There existed a highly significant relationship between the acoustic energy with amplitude, frequency and bandwidth of the sound of *C. afra*. The acoustic energy of sound of *C. afra* significantly affected the mosquito activities. However, the none-pulsate nature, declining acoustic energy with increase in frequency, mixed calls and short duration high frequency calls evoked low startle effect to the female *A. gambiae.* Also, the nature of the calls of sound of *C. afra* yielded low significant difference between the mean mosquito activities under control and the mean mosquito activities under the 35-60 kHz frequency range. The rate of mosquito activities was significantly affected by the peak amplitude (mean), frequency and the bandwidth (mean) of the sound of *C. afra*. The use pulsated sound of *C. afra* and long duration high frequency calls would yield better evasive response in mosquitoes.

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