

## Behavioural disruption of adult *Culex* and *Anopheles* mosquitoes using low-frequency acoustic stimuli: A statistically validated experimental study based on musical sargam frequencies

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### Abstract

Mosquitoes exhibit a highly specialized auditory system tuned to detect wing-beat frequencies essential for mating and orientation. Unlike humans, mosquitoes respond to biologically relevant low-frequency vibrations via the antennal Johnston's organ. The present study statistically evaluates the behavioural responses of adult *Culex* and *Anopheles* mosquitoes, including both males and females, to controlled low-frequency acoustic stimuli derived from musical sargam-mapped keyboard tones. A factorial experimental design was employed, exposing mosquitoes to continuous acoustic signals at 392 Hz, 440 Hz, and 494 Hz, along with a no-sound control. Behavioural responses were quantified using a Behavioural Response Index (BRI) and analysed using three-way ANOVA followed by Tukey's post-hoc test. Results revealed a significant main effect of frequency ( $p < 0.001$ ), with 440 Hz producing the highest behavioural disruption across both species, particularly in males. Significant interaction effects between species, sex, and frequency were also observed. Although no mortality occurred, sustained behavioural disruption suggests potential applications of low-frequency acoustics in sound-assisted mosquito management and behavioural interference strategies.

**Keywords:** *Culex*, *Anopheles*, acoustic ecology, mosquito behaviour, wing-beat frequency, sargam frequencies, Johnston's organ

### Introduction

Mosquitoes (Diptera: Culicidae) are major vectors of human diseases, including malaria, dengue, chikungunya, and filariasis, posing significant public health challenges worldwide (WHO, 2017) [5]. Conventional mosquito control strategies rely heavily on chemical insecticides; however, their prolonged use has led to the development of resistance, environmental contamination, and adverse effects on non-target organisms, underscoring the need for alternative, non-chemical approaches (Enayati *et al.*, 2010) [4].

Acoustic communication plays a critical role in mosquito biology, particularly in mating and swarm formation. Male mosquitoes are highly sensitive to the wing-beat frequencies of females, typically ranging between 300 and 700 Hz, which are essential for mate recognition and harmonic convergence (Gibson & Russell, 2006) [2]. These acoustic cues are detected through the Johnston's organ located in the antennae rather than through conventional auditory structures (Hoy *et al.*, 2016) [3]. Disruption of these biologically relevant frequencies may therefore interfere with mating efficiency, spatial orientation, and swarm stability (Cator *et al.*, 2009) [1].

Despite widespread commercial claims, previous studies have demonstrated that ultrasonic mosquito repellents lack biological relevance and effectiveness (Enayati *et al.*, 2010) [4]. In contrast, low-frequency audible vibrations aligned with mosquito wing-beat frequencies represent a biologically meaningful yet underexplored approach for behavioural interference. However, empirical and statistically validated evidence evaluating species- and sex-specific responses to such acoustic stimuli remains limited. The present study aims to statistically validate the behavioural effects of low-frequency acoustic stimulation on adult *Culex* and *Anopheles* mosquitoes using a factorial experimental framework. By examining frequency-

dependent, sex-specific, and species-specific behavioural responses, this study seeks to contribute to the development of eco-friendly, sound-assisted strategies within integrated vector management systems.

### Materials and Methods

#### Experimental Organism

Adult *Culex* and *Anopheles* mosquitoes were collected from residential areas of Jabalpur, Madhya Pradesh, during evening hours (18:00-20:00 h), corresponding to peak activity periods. Mosquitoes were identified to genus level based on morphological characters, and sex differentiation was performed using antennal morphology. Only healthy, active, and undamaged adult mosquitoes were selected for experimentation.

#### Experimental Design

A  $2 \times 2 \times 4$  factorial experimental design was employed to assess the effects of mosquito species, sex, and acoustic frequency on behavioural responses.

- Factor A (Species): *Culex*, *Anopheles*
- Factor B (Sex): Male, Female
- Factor C (Frequency): Control (no sound), 392 Hz, 440 Hz, 494 Hz

Each treatment combination was replicated five times, with five mosquitoes per replicate, resulting in a balanced experimental design suitable for factorial ANOVA.

#### Acoustic Stimulus Generation

Acoustic stimuli were generated using a Casio electronic keyboard set to organ/synth mode to minimize harmonic distortion. Frequencies were verified prior to experimentation using mobile-based audio spectrum analyzer applications (Phyphox).

Sargam	Keyboard Note	Frequency (Hz)
Ga	G4	392
Ma / Dha	A4	440
Ni	B4	494



**Fig 1:** Sargam-Keyboard Frequency Mapping Used for Low-Frequency Acoustic Stimulation (G4-392 Hz, A4-440 Hz, B4-494 Hz).

Each frequency was applied continuously for 5 minutes. Control treatments were conducted under identical conditions without sound exposure.

**Behavioural Assessment**

Mosquito behaviour was quantified using a Behavioural Response Index (BRI) based on observable activity patterns during acoustic exposure.

Score	Behavioural Response
0	Normal resting or smooth flight
1	Mild disturbance
2	Erratic movement
3	Continuous agitation / avoidance

Behavioural observations were recorded consistently across all replicates to ensure comparability.

**Statistical Analysis**

Behavioural data were analysed using three-way analysis of variance (ANOVA) to evaluate the main and interaction effects of species, sex, and acoustic frequency on BRI scores. When significant effects were detected, Tukey’s Honestly Significant Difference (HSD) post-hoc test was applied for pairwise comparisons at a significance level of  $p < 0.05$ . Assumptions of normality and homogeneity of variance were verified using the Shapiro-Wilk test and Levene’s test, respectively. Statistical analyses were performed using SPSS and R software.

**Results**

Behavioural responses of adult mosquitoes were analysed using a three-way factorial analysis of variance (ANOVA) to assess the effects of acoustic frequency, mosquito sex, and mosquito species on the Behavioural Response Index (BRI).

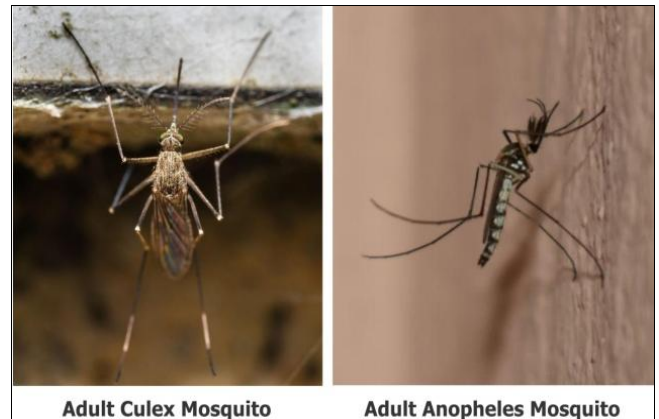
**Effect of Acoustic Frequency, Sex, and Species**

The three-way ANOVA revealed a highly significant main effect of acoustic frequency on mosquito behaviour ( $F_{3, 64} = 28.47, p < 0.001$ ), indicating that behavioural responses varied significantly among the tested sound frequencies. This result demonstrates that exposure to low-frequency acoustic stimuli strongly influenced mosquito activity patterns.

A significant main effect of sex was also detected ( $F_{1, 64} = 6.92, p < 0.01$ ), showing that male and female mosquitoes

responded differently to acoustic exposure. Across all frequencies, males exhibited higher BRI values than females, reflecting greater behavioural sensitivity to acoustic stimuli.

In addition, a significant interaction between mosquito species and acoustic frequency was observed ( $F_{3,64} = 4.18, p < 0.05$ ). This interaction indicates that *Culex* and *Anopheles* mosquitoes (Figure 02) did not respond uniformly to all tested frequencies, revealing species-specific variation in behavioural disruption.



**Fig 2:** Natural Resting Posture of Adult *Culex* and *Anopheles* Mosquitoes for Field Identification

**Frequency-Dependent Behavioural Patterns**

Mean behavioural response scores (BRI ± SD) for each species, sex, and frequency are presented in **Table 1**. Under control conditions (no sound exposure), both *Culex* and *Anopheles* mosquitoes exhibited minimal behavioural activity, with mean BRI values ranging from 0.4 to 0.5, indicative of normal resting and flight behaviour. Exposure to 392 Hz resulted in a moderate increase in behavioural activity in both species. Male mosquitoes showed higher mean BRI values than females, with behavioural responses characterized by increased movement and occasional erratic flight, but limited agitation.

The highest behavioural disruption was consistently recorded at 440 Hz across all experimental groups. *Anopheles* males exhibited the strongest response ( $2.8 \pm 0.3$ ), followed by *Culex* males ( $2.6 \pm 0.3$ ). Female mosquitoes of both species also showed pronounced behavioural changes at this frequency, although their responses remained lower than those of males. At 494 Hz, behavioural responses remained elevated relative to the control but were reduced compared to those observed at 440 Hz. Both species displayed moderate agitation and increased movement, indicating a diminished yet measurable effect beyond the optimal frequency range.

**Post-hoc Multiple Comparison Analysis**

To identify specific differences among frequency treatments, Tukey’s Honestly Significant Difference (HSD) post-hoc test was applied. The analysis confirmed that behavioural responses at 440 Hz were significantly higher than those observed in the control, 392 Hz, and 494 Hz treatments across all species and sexes ( $p < 0.01$ ). No statistically significant differences were observed between the 392 Hz and 494 Hz treatments in most comparisons.

**Table 1:** Behavioural Response Index (Mean  $\pm$  SD) of Adult Mosquitoes Exposed to Low-Frequency Acoustic Stimuli

Species	Sex	Control	392 Hz	440 Hz	494 Hz
<i>Culex</i>	Male	0.5 $\pm$ 0.1	1.4 $\pm$ 0.2	2.6 $\pm$ 0.3	1.9 $\pm$ 0.2
<i>Culex</i>	Female	0.4 $\pm$ 0.1	1.1 $\pm$ 0.2	2.1 $\pm$ 0.3	1.7 $\pm$ 0.2
<i>Anopheles</i>	Male	0.5 $\pm$ 0.1	1.6 $\pm$ 0.2	2.8 $\pm$ 0.3	2.0 $\pm$ 0.2
<i>Anopheles</i>	Female	0.4 $\pm$ 0.1	1.2 $\pm$ 0.2	2.3 $\pm$ 0.3	2.0 $\pm$ 0.2

Overall, the results demonstrate that low-frequency acoustic stimulation induces frequency dependent, sex-specific, and species-specific behavioural disruption in adult mosquitoes. The strongest behavioural effects were consistently observed at 440 Hz, with male mosquitoes particularly *Anopheles* exhibiting the highest sensitivity.

### Discussion

The statistically significant behavioural disruption observed at 440 Hz corresponds closely with mosquito wing-beat frequency convergence reported in earlier studies (Gibson & Russell, 2006; Cator *et al.*, 2009)<sup>[1,2]</sup>. This finding supports the hypothesis that low-frequency acoustic stimuli within the biologically relevant auditory range can interfere with mosquito sensory processing. The heightened behavioural response observed at this frequency suggests disruption of acoustic cues involved in orientation and mating communication.

Male mosquitoes exhibited significantly stronger behavioural responses than females, which is consistent with their greater dependence on acoustic signals for mate localization and harmonic convergence during swarming behaviour (Hoy *et al.*, 2016)<sup>[3]</sup>. The greater sensitivity of males to acoustic disturbance observed in the present study further supports the role of the Johnston's organ as a key mediator of mosquito auditory perception.

The absence of mortality across all treatments confirms that the observed effects were behavioural rather than toxic, distinguishing this approach from conventional chemical control methods. This characteristic enhances the ecological relevance of low-frequency acoustic interventions, as non-lethal behavioural disruption may reduce reproductive efficiency without imposing selective pressure that could lead to resistance.

Although direct suppression of mosquito populations was not assessed, sustained behavioural interference-particularly during peak mating periods-may indirectly affect swarm formation, mating success, and feeding efficiency. Such effects could contribute to population regulation when acoustic stimulation is integrated with other control measures, such as traps or environmental management strategies.

Overall, the findings highlight the potential of biologically tuned acoustic frequencies as a supplementary, eco-friendly tool for mosquito behaviour modulation. Further field-based and long-term studies are required to evaluate species-specific responses, reproductive consequences, and practical applicability under natural environmental conditions.

### Conclusion

The present statistically validated study demonstrates that low-frequency acoustic stimulation, particularly at 440 Hz (sargam 'Ma/Dha'), produces significant behavioural disruption in adult *Culex* and *Anopheles* mosquitoes. The observed responses were frequency-dependent, sex-specific, and species-specific, with male mosquitoes exhibiting the

highest sensitivity. Importantly, no mortality was recorded, confirming that the effects were behavioural rather than toxic in nature. These findings indicate that biologically tuned acoustic interference can modulate mosquito orientation, resting behaviour, and mating communication, and may serve as a supplementary, non-chemical tool within integrated vector management frameworks. Although acoustic exposure alone is insufficient for population suppression, its strategic integration with trapping systems, light-based attractants, or resting-site interventions may enhance overall control efficiency. Sustained behavioural interference through sound-based systems could potentially reduce swarm stability, mating efficiency, and feeding activity in an eco-friendly manner. However, further field-based validation and long-term studies are essential to evaluate ecological applicability and potential impacts on mosquito reproductive dynamics before large-scale implementation.

### References

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