Commagene Journal of Biology

Söğüt et al., (2026) *Comm. J. Biol.* 7, 1-8.
DOI: 10.31594/commagene.1808165

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

Mortality Rates in *Musca domestica* (Diptera: Muscidae) Adults after Exposure to Sound Frequencies Lower and Higher than the Ultrasonic Limits

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Received: 23.10.2025 **Revised:** 11.12.2025 **Accepted:** 17.12.2025 **Published:** 03.02.2026

Abstract: This study investigated the effects of acoustic sound frequencies including sub-ultrasonic and ultrasonic levels on the mortality of adult house flies (*Musca domestica*). A total of 240 flies were exposed to four frequencies: 6.00 kHz and 14.30 kHz (sub-ultrasonic), 20.00 kHz (ultrasonic threshold), and 25.50 kHz (ultrasonic), with three replicates per frequency. Mortality was recorded at six time points (1st, 2nd, 4th, 6th, 8th, and 24th hours). Statistical analyses (one-way ANOVA and Pearson correlation) were used to evaluate the influence of frequency and exposure duration on mortality. The highest mortality rate (76.66%) occurred at 25.50 kHz after 24 hours, whereas the lowest (26.66%) was recorded at 14.30 kHz. Mortality increased markedly over time, from 3.00% and 6.19% during the first two hours to 74.26% in 24 hours. Pearson correlation indicated significant positive associations between both frequency and mortality (r = 0.455, P<0.01) and exposure duration and mortality (r = 0.525, P<0.01). One-way ANOVA confirmed a significant frequency effect (F = 7.210, P<0.001). However, Levene's test (P<0.05) showed variance heterogeneity, warranting cautious interpretation. Overall, higher frequencies and longer exposures increased mortality in a dose- and time-dependent manner, with ultrasonic levels showing the strongest effect.

Keywords: *Musca domestica*, mortality, sound waves, ultrasonic, vector control.

Sesötesi Sınırlardan Daha Düşük ve Daha Yüksek Ses Frekanslarına Maruz Kalan *Musca domestica* (Diptera: Muscidae) Erişkinlerinde Ölüm Oranları

Öz: Bu çalışma, yetişkin ev sineklerinde (*Musca domestica*) akustik ses frekanslarının ultrasonik altı ve ultrasonik seviyeleri de dahil olmak üzere mortalite üzerindeki etkilerini araştırmıştır. Toplam 240 sinek, dört frekansa maruz bırakılmıştır: 6.00 kHz ve 14.30 kHz (ultrasonik altı), 20.00 kHz (ultrasonik eşik) ve 25.50 kHz (ultrasonik); her frekans için üç tekrar yapılmıştır. Mortalite, 1., 2., 4., 6., 8. ve 24. saatlerde kaydedilmiştir. Frekans ve maruz kalma süresinin mortalite üzerindeki etkilerini değerlendirmek için tek yönlü ANOVA ve Pearson korelasyon analizleri uygulanmıştır. En yüksek mortalite oranı (%76.66) 24. saatte 25.50 kHz'de, en düşük oran (%26.66) ise 14.30 kHz'de kaydedilmiştir. Mortalitenin zamanla belirgin şekilde arttığı görülmüştür; ilk iki saatte sırasıyla %3.00 ve %6.19 olan oranlar, 24. saatte %74.26'ya yükselmiştir. Pearson korelasyon analizine göre hem frekans ile mortalite (r = 0.455; P<0.01) hem de maruz kalma süresi ile mortalite arasında (r = 0.525; P<0.01) anlamlı pozitif ilişkiler bulunmuştur. Tek yönlü ANOVA, frekansın anlamlı bir etkisi olduğunu doğrulamıştır (F = 7,210; P<0.001). Ancak Levene testi (P<0.05) varyans heterojenliğine işaret ettiğinden sonuçların dikkatli yorumlanması gerekmektedir. Genel olarak, daha yüksek frekanslar ve daha uzun maruz kalma süreleri mortaliteyi doz- ve zaman-bağımlı şekilde artırmış; en güçlü etkiler ultrasonik seviyelerde görülmüştür.

Anahtar kelimeler: *Musca domestica*, ölüm oranı, ses dalgaları, ultrasonik, vektör kontrolü.

1. Introduction

Musca domestica L., commonly called the house fly, is a significant household, medical, and veterinary pest. House flies are holometabolous insects, often completing their development processes in animal waste. Commonly, house flies feed on human food and waste (Malik et al., 2007; Işık & Kırkpınar, 2017).

Vector-borne organisms remain a major global public health challenge and house flies are recognized as mechanical carriers of more than 100 pathogenic microorganisms including bacteria, viruses, protozoa, and helminths (Graczyk et al., 2005; Nazni et al., 2005). Their movement between waste, animal facilities, and food surfaces enables the dissemination of enteric pathogens such as *Escherichia coli*, *Salmonella spp*., *Shigella spp*., and antimicrobial-resistant bacteria, posing serious risks for human and veterinary health (Zurek & Ghosh, 2014; Onwugamba et al., 2020). Vector-borne diseases collectively account for more than 17% of all infectious diseases and cause over 700,000 human deaths annually (World Health Organization, 2020).

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A variety of control methods are used against *Musca domestica* including chemical insecticides, botanical extracts, and entomopathogenic fungi such as *Beauveria bassiana* (Barson et al., 1994; Malik et al., 2007; Pavela, 2016). The widespread and prolonged use of chemical insecticides in house fly management has raised substantial environmental and public-health concerns. Conventional insecticides can contaminate soil, water, and animal waste habitats, leading to bioaccumulation of toxic residues and exposure of non-target organisms including beneficial insects, livestock, and humans (Damalas & Eleftherohorinos, 2011). Environmental contamination is particularly problematic in intensive agricultural and livestock production systems where repeated applications are common. In addition, several active ingredients have been associated with endocrine-disrupting effects, ecotoxicological hazards, and reductions in biodiversity. Moreover, the increasing development of insecticide resistance in vector species further reduces the long-term effectiveness of chemical interventions (Sparks & Nauen, 2015). These concerns highlight the necessity of sustainable and integrated vector management strategies that include environmentally safe alternatives for controlling medically important insect species such as *Musca domestica*.

Non-chemical approaches have gained increasing importance within integrated pest management programs due to growing concerns about insecticide resistance, environmental contamination, and risks to non-target organisms. In this context, physical methods such as acoustic stimulation, light traps, and appropriate environmental management and biological methods including entomopathogenic fungi or other natural enemies are being integrated into control strategies to help reduce both adult and larval populations of house flies (Pavela, 2016; Xu et al., 2019). Although some early laboratory studies explored the potential larvicidal effects of *Bacillus thuringiensis* (Bt) on house flies, the results have remained inconsistent and species-specific. Importantly, Bt-based formulations are not recommended by the World Health Organization or other major public-health authorities for routine *Musca domestica* control, primarily due to the lack of reliable, reproducible efficacy under field conditions. Therefore, current integrated pest management programs prioritize sanitation, mechanical control, targeted chemical use, and biological agents with demonstrated field performance (Cetin et al., 2019; Nerio et al., 2010).

Physical control tools such as acoustic stimulation and specific light wavelengths are being explored as complementary strategies for reducing adult house fly activity or survival. Laboratory studies have reported that high-frequency sound or ultrasonic stimuli can alter insect behavior, disrupt communication, or reduce mobility, although results vary significantly among species and experimental conditions (Aflitto & De Gomez, 2015; Rajendran & Hajira Parveen, 2005). Similarly, the visual system of *Musca domestica* exhibits defined spectral sensitivity peaks, suggesting that certain LED wavelengths may influence orientation or attraction behaviors (Xu et al., 2019). These emerging technologies do not replace conventional control methods but provide potential supplementary tools that may contribute to environmentally safer vector management.

Sound is a mechanical vibration wave propagated in a medium composed of matter. Anything that can produce sound is a source of the sound. Sound waves are longitudinal waves with the same direction of vibration and the same direction of propagation. It is one of the types of physical energy and vibration energy. Sound is characterized by the transmission of small, vibrational changes in air pressure. One of the most important sound sizes is sound pressure. This pressure causes changes in air pressure over a given time during the propagation of sound. Another important size of sound is the sound velocity [v (t)]. This size also varies according to time (Ozkurt & Altuntas, 2018).

Human perception limits include different levels of sound intensity. The characteristic that enables the sound produced by the same source to be perceived differently at varying distances is known as the sound intensity. Briefly, it is the level of sound or noise the ear hears. Sounds have different intensities. Decibel [dB] units measure volume or noise level. dB is a logarithmic unit employed to measure sound intensity and represent the ratio between two physical quantities. The loudness is directly proportional to the frequency. At low-frequency values, the sound is bass, but at high frequency, it is treble (Ozkurt & Altuntas, 2016).

Sound vibrations with more than 20000 vibrations per second (over 20 kHz) are called ultrasonic. Some sources indicate that the limit for this vibration frequency ranges from 16,000 to 20,000 (16-20 kHz). These frequencies cannot be heard by humans and they may be perceptive only by specific animals. Ultrasonic sound is used in many industries and technology such as medical and veterinary applications, prevention of microbial growth in the food industry, water and wastewater treatment, and so on. The ultrasonic sound cannot be heard by the human ear (Mahvi, 2009; Ulusoy & Karakaya, 2011; Stiawan et al., 2019).

Beyond house flies, recent studies on other pest insects have demonstrated measurable biological effects of ultrasonic frequencies. For instance, exposure of *Aedes aegypti* larvae to 18–30 kHz ultrasonic waves for 180 seconds resulted in complete mortality even at 60 cm distance from the transducer, likely due to the physical disruption of internal larval structures such as the dorsal tracheal system, thorax, and abdomen (Kalimuthu et al., 2020). Non-target aquatic organisms, such as the copepod *Megacyclops formosanus*, exhibited no observable adverse effects, suggesting species-specific selectivity (Kalimuthu et al., 2020). Additionally, ultrasonic exposure (43–45 kHz, sinusoidal/cosine waves) reduced survival and body weight in *Ephestia kuehniella* larvae and pupae, while eliciting avoidance behavior (Salehi et al., 2016). These findings indicate that ultrasonic waves can suppress pest populations both directly, through mortality, and indirectly, via behavioral and developmental disruption, highlighting their potential as environmentally safer supplementary control tools.

Ultrasonic devices have occasionally attracted attention as potential tools for pest management; however, scientific evaluations indicate that their effectiveness against *Musca domestica* is limited. House flies possess mechanosensory structures adapted to detect low-frequency airflow rather than ultrasonic frequencies (>20 kHz) and they lack the peripheral sensory adaptations necessary to perceive typical ultrasonic emissions (Smallegange et al., 2008; Tuthill & Wilson, 2016; Göpfert et al., 1999). Laboratory studies have shown that exposure to ultrasonic frequencies induces only minimal behavioral changes or avoidance responses in adult *Musca domestica* (Ryu et al., 2014; Ma et al., 2023; Apryandana et al., 2024). Independent evaluations by regulatory institutions support these findings. Similarly, the World Health Organization (2024) states that electronic or acoustic repellent devices have not demonstrated consistent or operationally meaningful reductions in vector density or human–vector contact. Overall, the limited research on the effects of ultrasonic frequencies on house flies has created a scientific gap.

Despite their conceptual potential as supplementary tools, ultrasonic devices currently lack validated, reproducible, and field-effective outcomes particularly for *Musca domestica*, whose sensory physiology does not support ultrasonic perception. Future research may clarify dose response relationships, species-specific thresholds, or combined acoustic visual approaches but ultrasonic technologies currently should be regarded as experimental rather than operational vector control strategies.

To address this knowledge gap, the present study investigated the biological responses of adult *Musca domestica* to acoustic frequencies both below and within the ultrasonic range and quantified the statistical relationships between frequency, exposure duration, and mortality outcomes. By providing empirical data on how different sound frequencies influence adult survival, this study aims to clarify the potential and current limitations of acoustic stimulation as a supplemental tool in house fly management.

2. Material and Method

2.1. Ethics Committee Approval

This study did not involve human participants or animal subjects. Therefore, ethical approval was not required. Ultrasonic sound refers to acoustic waves above 20 kHz, which are generally beyond the range of human hearing. Many insects, including moths, crickets, and bush crickets, can detect frequencies within this range and exhibit behavioral responses to ultrasonic cues (Aflitto & De Gomez, 2015). Ultrasonic devices have been explored as physical pest-management tools, yet their efficacy varies widely and remains insufficiently supported for routine vector-control applications.

2.2. Colony of Adults House Flies

Adult house flies were initially collected from the stables of the Faculty of Agriculture, Department of Zootechnics, at the Central Campus of Cukurova University using mouth aspirators and light traps. The collected individuals were transferred to the Insectarium Laboratory (Department of Medical Biology, Faculty of Medicine, Cukurova University) and used to establish a laboratory colony under controlled environmental conditions. After the colony was stabilized, experimental trials were conducted using subsequent generations (F1–F2 adults) rather than directly field-captured flies in order to minimize variation related to age, physiological condition, prior environmental stress, and natural mortality (Fig. 1).

Figure 1. Treatment groups of houseflies (*Musca domestica*) exposed to ultrasonic sound frequencies.

Specimens were identified by examining morphological characters including the head, thorax, abdomen, and other structures under a stereomicroscope (Borror & White, 1970). Wing morphology was also considered as a part of the identification process (Dodge, 1953; Pratt, 1976). All specimens were verified by an experienced entomologist to ensure accurate identification. Although molecular confirmation was not performed, the use of multiple morphological characters combined with expert verification minimized the risk of misidentification. A total of 300 adults (20 individuals * 4 frequencies * 3 repetitions - including 60 untreated controls) were obtained from the established colony for three independent repetitions of each frequency treatment. Control groups were maintained under identical physical conditions as the treatment groups but without exposure to sound.

2.3. Experimental Setup

In this experiment, an adjustable frequency oscillator (signal generator) capable of generating frequencies between 5 and 35 kHz, including ultrasonic ranges, was used together with a 20 W audio amplifier to apply different sound pressure levels. Acoustic signals were emitted through a plastic-enclosed transducer. The output frequencies were verified using a frequency meter, and sound intensity levels were measured with a sound level meter. All experimental procedures were conducted inside a cabinet containing both the house flies and the transducer. During the operation of the experimental setup, the transducers were placed inside the cabinet covered with a plastic coating to prevent *Musca domestica* from hitting the vibrating transducer. Also, we used a piezo transducer, a device that converts a form of energy to other forms of energy. By using the piezoelectric property, electrical energy can be converted into mechanical energy or vice versa. In short, the transducer is a converter. In this experiment, we applied square wave electrical energy at specific frequencies to the piezo disc material (which can be regarded as an electronic circuit component), causing it to vibrate inside the chamber (Fig. 2). All exposure trials were conducted in 5-L plastic containers with perforated lids to ensure adequate ventilation. Throughout the experiment, environmental conditions inside the laboratory remained stable with an average temperature of 28 °C and relative humidity around 50%. A 16:8 (L:D) photoperiod was maintained which is commonly used for the maintenance and handling of adult *Musca domestica*. These controlled environmental conditions considerably reduced the likelihood that external abiotic factors could influence fly behavior, stress levels, or mortality during sound exposure.

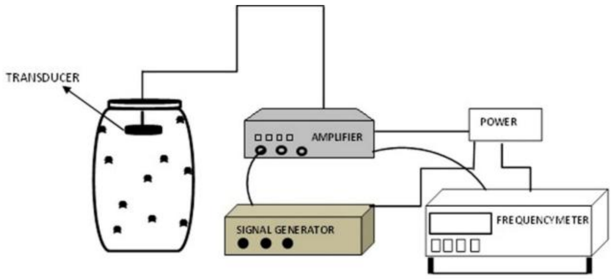

Figure 2. Experimental setup

2.4. Exposure Trials

In our study, mortality of adult house flies was recorded at the 1st, 2nd, 4th, 8th, and 24th hours for all repetitions. Four sound treatments were applied by transmitting acoustic waves at 6.00 kHz, 14.30 kHz, 20.00 kHz, and 25.50 kHz into the experimental chamber using a simple transducer mounted on the cabinet door. A control group was kept under the same physical conditions as the treatment groups but was not exposed to any sound. The experiment examined the lethal effects of sound waves generated at frequencies both below and above the ultrasonic threshold. Sound intensity in the chamber was monitored with a dB meter and a 20-watt amplifier was used to ensure that all applied frequencies whether sub-ultrasonic or ultrasonic were delivered at low and comparable intensity levels. Therefore, sound levels were maintained between 50 and 55 dB in all treatment chambers.

2.5. Statistical Analysis

Statistical analysis of the data was carried out using SPSS 25.0 software. Descriptive statistics were applied to summarize the characteristics of the sample or dataset such as the mean and standard deviation of a variable. A kurtosis value within ±1.0 is considered ideal for most psychometric purposes, though values between ±2.0 are often acceptable depending on the specific application (George & Mallery, 2019). The lethal effects of ultrasonic sound frequencies and exposure duration on *Musca domestica* adults were assessed using a two-way analysis of variance (ANOVA) followed by Tukey's test for significant differences.In the two-way ANOVA test, the dependent variable was mortality and ultrasonic frequency range and time were determined as independent variables. Non-linear regression analysis was conducted to explore the relationship between sound frequencies, mortality rate (number of fatalities), and exposure time of *Musca domestica* individuals.

3. Results

In our trials, 240 adult house flies were exposed to four different sound frequencies in 3 repetitions used in the study conducted. The maximum number of fatalities observed in house flies exposed to ultrasonic sound frequencies was recorded 24 hours after exposure for all repetitions.

A total of 60 adult house flies were used for each frequency after 3 replicates. The highest mortality was observed in individuals exposed to the 25.5 kHz frequency at the end of the 24-hour period (76.66%). The lowest mortality was found in individuals exposed to the 14.3 kHz frequency (26.66%) (Fig. 3). In all replicates, the highest number of dead individuals was recorded between 8-24 hours of exposure. The lowest mortality was observed during the 1st (3.00%) and 2nd (6.19%) hours of exposure (Fig. 4). While the sound frequency value was above the ultrasonic limit, it was observed that when the exposure time of the house flies to these frequencies increased, the deaths also increased ($P<0.05$). At the 4th hour, mortality reached 13.12% and it continued to rise to 25.10% at the 6th hour. By the 8th hour, the mortality rate increased to 41.88%. Finally, at the end of the 24th hour, the highest mortality rate was observed, reaching 74.26% (Fig. 4). In the control groups, no mortality was observed at any of the recorded time points, confirming that deaths occurred only in response to the applied sound frequencies.

According to the statistical results of our findings, comparing the four frequencies employed in our trials, it was found that there was a statistically significant relationship. Additionally, the result showed that data were normally distributed as skewness and kurtosis (+2.0 -2.0). Also, statistically significant relationships were revealed among the four frequencies employed in our experiments. The correlation matrix presented in the Table 1 displays Pearson correlation coefficients among three variables: Frequencies, Hours, and Mortality. The results indicate that there is a statistically significant positive correlation between Frequencies and Mortality ($r = 0.455$, $P<0.01$) as well as between Hours and Mortality ($r = 0.525$, $P<0.01$). These findings suggest that an increase in the frequency of a certain factor and the number of hours is associated with a corresponding increase in mortality. However, the correlation between Frequencies and Hours ($r = 0.000$, $P=1.000$) is negligible, indicating no linear relationship between these two variables. The statistical significance ($P<0.01$) confirms that the observed correlations are unlikely to have occurred by chance. One -Way ANOVA results showed that the mortality rate increases with frequency, reaching the highest mean value at 25.50 kHz, suggesting a dose-dependent effect. Also the Levene's test for homogeneity of variances assesses the assumption of equal variance across groups. The test results ($P<0.05$ for all measures) indicate that variances are significantly different, suggesting heteroscedasticity in the data. This violation of homogeneity should be considered when interpreting ANOVA results. The ANOVA test reveals a highly significant effect of frequency on mortality (F = 7.210, P<0.001) with a large between-group sum of squares (51.933) compared to within-group variance (Table 2). This suggests that frequency significantly influences mortality and post-hoc analyses (e.g., Tukey HSD) would be required to determine specific group differences.

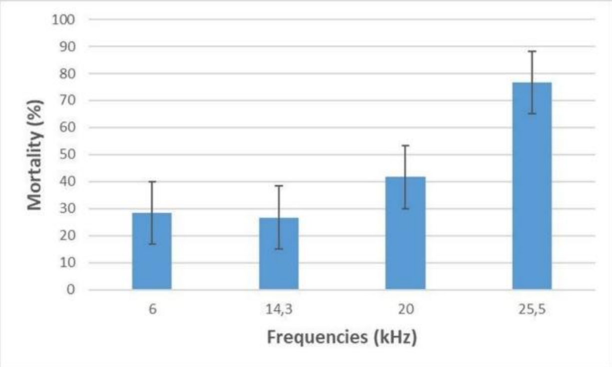

Figure 3. Mortality rates (%) of *Musca domestica* exposed to different ultrasonic sound frequencies (6, 14.3, 20, and 25.5 kHz) over 24 hours. Error bars indicate standard deviation.

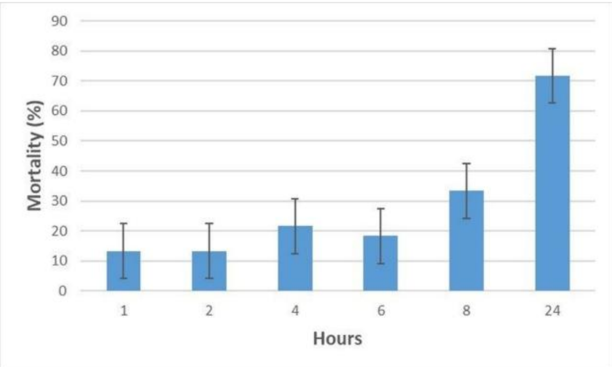

Figure 4. Mortality rates (%) of *Musca domestica* at different exposure durations (1, 2, 4, 6, 8, and 24 hours) under ultrasonic sound frequency conditions. Error bars indicate standard deviation.

Table 1. Pearson correlation coefficient between ultrasonic sound frequencies, number of dead individuals, exposure times, and life stages of *Musca domestica*

		Frequencies	Hours	Mortality
Frequencies	Pearson Correlation	1	,000	,455**
	Sig. (2-tailed)		1,000	,000
	N	90	90	90
Hours	Pearson Correlation	,000	1	,525**
	Sig. (2-tailed)	1,000		,000
	N	90	90	90
Mortality	Pearson Correlation	,455**	,525**	1
	Sig. (2-tailed)	,000	,000	
	N	90	90	90

**. Correlation is significant at the 0.01 level (2-tailed).

Overall, the findings indicate that higher frequencies are associated with increased mortality, with statistically significant differences between groups. However, the heterogeneity of variances suggests that additional robust statistical approaches may be needed for a more precise evaluation.

Table 2. One-way ANOVA testing the effect of frequencies on *Musca domestica* adults (d.f. = degree of freedom, significance level P<0.05)

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	51.933	4	12.983	7.210	0.000
Within Groups	153.056	85	1.801		
Total	204.989	89			

The one-way ANOVA analysis reveals a significant effect of treatment duration on mortality, with mean mortality increasing over time, reaching its highest at 24 hours (M = 3.47). Levene's test indicates heterogeneity of variances (P<0.05) suggesting caution in interpreting ANOVA results. The ANOVA test confirms a significant difference among groups (F = 6.147, P<0.001) and post-hoc Tukey HSD analysis identifies significant differences particularly between the 24-hour group and shorter durations (Table 3). These findings suggest a time-dependent mortality response, emphasizing the need for further investigation into the underlying biological mechanisms.

Table 3. One-way ANOVA testing the effect of exposure time on *Musca domestica* adults (d.f. = degree of freedom, significance level P<0.05)

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	89.467	5	17.893	6.147	.000
Within Groups	244.533	84	2.911		
Total	334.000	89			

4. Discussion and Conclusion

In our study, which tested the effects of ultrasonic sound frequencies that we thought could be used as an alternative in the control of vector arthropods such as *Musca domestica*, we found that 25.50 kHz caused the highest mortality. Also, two sound frequencies above the ultrasonic limit and two sound frequencies below the ultrasonic limit were used.

In our study, all experimental periods included control groups, in which no mortality was observed, confirming that the lethal effects recorded at 25.50 kHz were not due to random factors. A total of 240 adult house flies were exposed to four frequencies 6.00 kHz and 14.30 kHz (sub-ultrasonic), 20.00 kHz (ultrasonic threshold), and 25.50 kHz (ultrasonic) with three replicates per frequency and mortality was recorded at six time points (1, 2, 4, 6, 8, and 24 h). Although detailed measurements of sound pressure levels and distances from the emitter were not included in the analyses, the experiments were conducted under controlled conditions, with flies exposed for 24 h at an average temperature of 28 °C and relative humidity of 50%. Frequencies below the ultrasonic range, such as 6 kHz, were included and labeled as "sub-ultrasonic"; any potential biological effects at such low frequencies would require very high sound intensities, which were not assessed here. Our results indicate that prolonged exposure to 25.50 kHz can induce significant mortality in *Musca domestica*, consistent with the notion that species-specific sensitivity and exposure duration are critical factors in ultrasonic pest control. Nevertheless, given that literature reports limited evidence for direct lethal effects at low-intensity ultrasonic frequencies, these findings are context-specific and should be interpreted with caution, highlighting the need for further studies to systematically assess acoustic parameters and their impact on insect mortality.

A large number of chemical insecticides are used in the control of house flies and other vector arthropods, damaging nature and especially non-target organisms. Biological control methods and novel control methods, including physical barriers such as ultrasonic sound and light, are being studied worldwide to reduce the use of chemical agents (Malik et al., 2007; Ryu et al., 2014). Novel methods, including ultrasonic sound and light, are used as a repellent especially in controlling house flies.

In addition to ultrasonic sound, microbial agents are also used for controlling *Musca domestica* populations. For instance, five strains of *Beauveria bassiana* have been found to cause up to 90% mortality in house flies. However, some microbial agents may also pose risks to non-target organisms. Since different species exhibit varying sensitivity to ultrasonic sound frequencies, using this method for pest control could be a more environmentally friendly alternative (Lecuona et al., 2005).

Previous studies have shown that sound frequencies in the 10-20 kHz range (below the ultrasonic limit) exhibit a strong repellent effect on house flies (Ryu et al., 2014). In contrast, our study demonstrated that a frequency of 25.50 kHz (above the ultrasonic limit) induced a mortality rate of 75% within 24 hours. This suggests that long-term exposure to ultrasonic frequencies could have a lethal effect on house flies, rather than just a repellent one.

Research conducted by (Rashid et al., 2017) developed a device operating in five different modes for various species, revealing that sound frequencies between 31 kHz and 44 kHz were highly effective as repellents against house flies. These findings suggest that higher frequencies may act as repellents over shorter exposure durations, while lower ultrasonic frequencies such as those used in our study can lead to mortality over prolonged exposure periods.

Additional studies have shown that sound frequencies of 10 kHz and 20 kHz exert repellent effects on flies, likely due to their impact on reproductive processes. Specifically, ultrasound exposure has been found to cause DNA damage in house fly larvae, leading to avoidance behavior. Consistent with this, we observed that even house flies that survived exposure to 25.50 kHz avoided flying near the transducer emitting the ultrasonic waves. This further supports our conclusion that this frequency not only induces mortality but also has a strong repellent effect (An et al., 2005).

Previous research in our laboratory examining the impact of ultrasonic sound on *Culex pipiens* larvae found that among six tested frequencies (ranging from 10.8-26.5 kHz), the most lethal effects were observed at 10.8 kHz and 14.8 kHz. Interestingly, the most effective frequency for house flies in our study, 25.50 kHz, did not exhibit the same effect on mosquitoes, likely due to differences in the habitats of house fly adults and *Culex pipiens* larvae (Ozkurt & Kavur, 2019a; Ozkurt & Kavur, 2019b).

Similarly, studies on *Aedes aegypti* larvae have shown that the effectiveness of sound frequencies in the 18-30 kHz range depends significantly on both the distance to the sound source and the exposure time. Our findings align with these results, as we observed that exposure time played a significant role in *Musca domestica* mortality (Kalimuthu et al., 2020).

In our study using 20-watt amplifiers, we found that 25.50 kHz caused 75% mortality in house flies. Fredregill et al. (2015) determined 100% mortality in *Culex* mosquito larvae using frequencies ranging from 18-36 kHz with 400-watt amplifiers. Studies on these two members of the Diptera order show the mortal effect of ultrasonic sound frequencies.

Previous studies have demonstrated that ultrasonic sound frequencies can influence insect behaviour differently depending on species and environmental context. For example, experimental and neuroethological work shows that many nocturnal moths detect bat echolocation and alter flight (e.g. erratic flight, power dives) in response to ultrasonic calls, while taxa that lack ultrasonic hearing are less responsive. This implies that ultrasonic sound as a pest-control tool will differ in effectiveness across insect groups and must be tailored to the sensory ecology of the target species. (Dunning & Roeder, 1965; Yager, 1999; Fullard et al., 2003).

Similarly, Khan-Ahmadi et al. (2023) evaluated the response of mosquitoes (*Culex*) and German cockroaches (*Blattella germanica*) to ultrasonic sound emitted from a commercial pest control device. The study concluded that randomly generated ultrasonic signals had limited effectiveness in repelling these insects, suggesting that factors such as frequency stability, amplitude, and exposure duration significantly influence ultrasonic deterrence efficiency. These findings align with our results which indicate that *Musca domestica* mortality was directly influenced by frequency and exposure duration with 25.50 kHz proving most effective over prolonged exposure.

Recent advancements in acoustic pest management have demonstrated the potential of ultrasonic technology in controlling insect populations. For example, Agah-Manesh et al. (2021) found that ultrasonic tones at 37.5 kHz significantly reduced survival, weight, and fecundity of the sugarcane pest *Sesamia cretica*. This aligns with our findings which suggests that exposure to 25–50 kHz ultrasonic sound significantly increases *Musca domestica* mortality while potentially serving as a repellent. Additionally, Kalimuthu et al. (2020) investigated the effects of ultrasonic technology on mosquito larvae and found that exposure to frequencies in the range of 18–30 kHz significantly affected larval survival with mortality rates increasing based on both frequency and exposure duration. These findings further support our results reinforcing the notion that higher ultrasonic frequencies, particularly at prolonged exposure durations, have lethal effects on various insect species. Furthermore, these studies emphasize the importance of optimizing ultrasonic frequency parameters to enhance efficiency and minimize unintended effects on non-target organisms. Given these results, ultrasonic pest control methods present a promising avenue for integrated pest management strategies, warranting further research to refine their application for broader ecological and agricultural use.

This study has several limitations that should be considered. First, detailed measurements of sound pressure levels and distances from the emitter were not included which may affect the reproducibility and interpretation of the effects of different frequencies. Second, while temperature and relative humidity were monitored during experiments, these parameters were not incorporated into the statistical analyses. Third, only adult house flies were tested, so the effects on other life stages or insect species remain unknown. Finally, the study was conducted under laboratory conditions which may not fully reflect field conditions. These limitations highlight the need for further research to systematically evaluate acoustic parameters, exposure durations, and environmental factors to optimize ultrasonic pest control strategies.

In conclusion, chemical pesticides contribute to air and soil pollution posing risks to human health and non-target organisms. Novel ultrasonic sound devices do not produce such pollution. Many insects are sensitive to ultrasound and tend to avoid areas where it is present. Sound frequencies below and above the ultrasonic threshold are commonly used as repellents against ectoparasitic insects, such as house flies and mosquitoes, and prolonged exposure can induce mortality particularly in house flies. In this study, 25.50 kHz, above the ultrasonic threshold, caused the highest mortality over 24 hours under the experimental conditions. These findings suggest that ultrasonic sound frequencies could be integrated into pest management strategies as an environmentally friendly alternative to chemical insecticides.

Overall, while ultrasonic sound can serve as an effective pest control method, its efficacy is species-specific. Further research is needed to optimize frequency parameters, exposure duration, and device design to ensure that ultrasonic pest control technologies are both efficient and sustainable.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declare that there is no conflict of interest.

Author Contributions: Conception – H.Ö., H.K.; Design – H.Ö.; Supervision – D.A.; Fund – F.S.; Materials – H.K., D.A.; Data Collection or Processing – F.S., H.K.; Analysis Interpretation – F.S., H.K.; Literature Review – H.Ö., H.K.; Writing – H.Ö., H.K.; Critical Review – D.A.

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