

Original Article

Evaluation of Repellency and Lethal Effects of Ultrasonic Waves on the *Blattella germanica* (Blattodea: Blattellidae)

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Abstract

Background: The German cockroach, *Blattella germanica* (Insecta: Blattodea, Blattellidae), which occurs widely in human buildings, is a small cockroach species. Cockroach control chemical pesticides are toxic to the environment, and it is sometimes impossible to prevent them. Controlling *Blattella germanica* through ultrasonic waves can be efficient and less dangerous for the environment.

Methods: In this study, the repellency and lethal effect of ultrasonic waves on male and female German cockroaches was tested in a twin glass cubic chamber at laboratory condition. The wave frequencies tested ranged from 20 to 100kHz with 5kHz steps. A signal generator generated these frequencies, and the piezoelectric transmitter of these ultrasonic waves was positioned in the chamber's center on the upper side.

Results: Fisher's test showed that there was the greatest repellency effect in both male and female at frequencies of 35 and 40kHz. According to the results of the regression test, the most lethal effect is at the frequencies of 40 and 75kHz.

Conclusion: The operating ultrasonic frequencies investigated in this study can be used to repel and kill German cockroaches as pests endangering human health and environment.

Keywords: Repellency; Lethal effect; Ultrasonic waves; *Blattella germanica*

Introduction

Blattella germanica is one of the most critical and widespread domestic pests worldwide (1). Cockroaches have chewing mouthparts and feed on a variety of materials (omnivorous) and aiding in the mechanical transmission of various pathogenic viruses, bacteria, fungi, and protozoans to humans (2–4). There are two main methods for controlling cockroaches, including chemical and non-chemical methods. A third but not very common method of control involves the use of parasitoid wasps belonging

to the family Evaniidae, which naturally parasitize and destroy embryos inside egg capsules of cockroaches (3). The chemical control method includes residual spraying, dusting spray, mist spray, and poisoned baits (5, 6). The use of chemical insecticides to control cockroaches is the most popular method (3). Although the use of chemical insecticides in pest control, including cockroaches, has increased, these pesticides can be dangerous for environment and harmful to non-target organisms (1,

7–9). One of the unwanted consequences of the widespread use of pesticides is that the insect will become resistant to pesticides, reducing insecticides' impact (7–9). Non-chemical control methods for cockroaches include ultrasonic waves, vacuuming, trapping, and the use of heat and cold, non-toxic gases such as nitrogen and carbon dioxide (10). Using the non-chemical method with ultrasonic pest repelling seems beneficial for both humans and their environments. Cerci receptors of cockroaches receive air vibrations and can act as sound and vibration receptors. Cerci tactile hairs respond to sudden vibrations or loud noises by sending spike potentials through the cerci nerve to points of contact with long axons at the synapses of the sixth abdominal nerve node. The long axons enter uninterruptedly into the thoracic nodes, where synaptic Haas connect with the leg muscles' motor neurons. This system causes a fast transfer of stimuli and evasion maneuvers (11).

Ultrasonic Pest Repellent generates an ultrasonic frequency range that is not audible to humans but is loud for pests. To this end, some studies have examined the performance of some commercial ultrasonic devices that have been used to control German cockroaches (12–16). The results of these studies showed that these devices did not have an acceptable detrimental effect on pests. The frequency used in these studies was 2–5kHz, and the acoustic pressure in one centimeter of the sound source was 68 to 74dB. The degree of repellency depends on the ultrasound's intensity and frequency. Two main problems with previous ultrasonic devices can be considered two primary mismatches. First, the power of actual sound generated by devices was almost less than what the manufacturers claimed. Second, the sound frequency did not match the German cockroach's operational frequencies, which are 20–50kHz (17). A study confirms that to date, research on German cockroaches has not yielded an acceptable solution, and controlling this insect is still an outstanding problem.

According to the above contents, in this study we examined the lethality and repellent effects of a span range of ultrasonic wave frequencies (20–100) kHz on the specimens of German cockroaches.

Materials and Methods

In this study, ten male and female German cockroaches (5 females with capsules), were taken from the Insectarium of the Tehran University of Medical Sciences (TUMS) and bred in the Esfahan Health Research Station. German cockroaches were raised by keeping them in Pyrex Glass 2,000mL, temperature 25 ± 2 (°C), humidity 60 ± 5 (%), and, light period 12:12 (hours). They were given bread as food and water with a wet cotton wick. Vaseline oil was rubbed on the edges of Pyrex glass so that the German cockroaches could not escape from the container. Then the container lids were closed with nets.

The test site was two glass square cubic chambers with dimensions $1\times 1\times 1\text{m}^3$, connected by a glass rectangular cubic chamber with dimensions $1\times 0.4\times 0.5\text{m}^3$. In one of these two square cubic chambers, a sliding glass door was used to enter and exit the cockroaches (Fig. 1). The piezoelectric device, was located in the center of the upper page in first chamber (50 cm from the floor of the first chamber) (Fig. 1). The connection between the two chambers was closed by solid cardboard, and only 10cm was kept open. In fact, the shape of this chamber was taken from article (9), which was changed in this study. The time for each test was every morning, and the duration of each test was one hour. In each experiment, a bit of fresh bread was placed in the center of the bottom page in the first chamber as an attractor for German cockroaches. Temperature and humidity were equal in both glass chambers.

The experiments were performed to determine the frequency range of annoying German cockroaches. In this study, to generate and to display ultrasonic wave shapes, the signal gen-

erator model JSG106H2 and the oscilloscope model MOS620CH were used, respectively.

Experiments were performed at frequencies (20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100) kHz, and at these frequencies, the sound intensity ranged was 166 to 173dB. For each frequency, six German cockroaches (3 males + 3 females) were entered the chamber through the gate and, after one minute, the signal generator and oscilloscope that were set up earlier were turned on and the movements and behavior of German cockroaches were observed. Several German cockroaches entered the second chamber without the sound transmitter and moved away from the sound source.

For each frequency, three repetitions and one control were considered. In the control test, all conditions (temperature and humidity) were like the ultrasonic wave test, with the only difference being that the generator and oscilloscope were off. A total of 68 experiments were performed. After each test, German cockroaches were collected with an electric aspirator. Also the live German cockroaches were transferred into glass containers which was contained with a piece of bread and wet cotton, and then closed the glass with piece of net.

Statistical analysis

In this study two main statistical observations, were tested. First, the Fisher Test was applied to investigate the relationship between the repellency and lethal effects of ultrasonic waves tested on male and female German cockroaches. Second, the Logistic regression test was applied to investigate which frequency has the most repellency and lethal effect on German cockroaches examined. In all tests, a P-value of 0.05 was considered.

Results

The sample size of German cockroaches tested was 408 (306 treatment plus 102 control), equal for males and females. Several German cockroaches entered the second chamber, which

is without a sound transmitter and moved away from the sound source.

At the time of testing, some of the far-flung German cockroaches turned around and died after a short paddle. Others in the main chamber with the audio transmitter turned around and died. According to the Fisher test results (Table 1), the effect of ultrasonic wave repellency at 35 and 40kHz was significant in both males and females with $P < 0.05$. The p-value is insignificant in other 15 frequencies. At all frequencies tested, the repellency impact is more significant in males than females except at frequencies of 70 and 85kHz that females are more than males (Fig. 2). At frequencies of 20, 90, and 100kHz, no female German cockroach moved away. At 85kHz, the male ones did not move away (Fig. 2). When 95kHz was used, repellency effects were similar for male and female German cockroaches (Fig. 2).

Table 2 shows the results of the Fisher Test for mortality. It is indicated that the lethal effects of ultrasonic waves at 25 and 80kHz are significantly different between males and females with $P < 0.05$. As for the rest of the tested frequencies, there is no significant difference. The mortality rate for male cockroaches is more than or equal to the female ones except for 80 kHz. In fact, the most lethal effect of the female German cockroach is at the frequency of 80kHz (Fig. 3). Moreover, the results specify that no female cockroaches were killed at 20 kHz; however, dead male cockroaches were observed at all frequencies (Fig. 3).

The logistic regression test results can be seen in Tables 3 and Table 4. Table 3 displays that the maximum effect of ultrasonic wave repellency is at 40kHz frequency with 61.1% of repellency and with $P < 0.05$. Table 4 determines the highest mortality impact at 40 and 75 kHz, which was 72.2% and 66.7%, respectively, and in these two frequencies with $P < 0.05$.

At frequencies 20 and 90kHz, $P < 0.05$, but P-value is not significant because, in these two frequencies, the number of German cockroaches that have not moved away is more than the

number of German cockroaches that have moved away.

At frequencies 20, 85 and 90kHz, is $P < 0.05$,

but P-value is not significant. Because the number of German cockroaches not killed is more than the number of German cockroaches killed.

Table 1. Study of the repellency effect of ultrasonic waves based on the gender of German cockroaches (Fisher test)

Row	Frequency (kHz)	DF.	Exacting Sig.
1	20	1	0.235
2	25	1	*0.008
3	30	1	0.712
4	35	1	0.681
5	40	1	0.500
6	45	1	0.167
7	50	1	0.681
8	55	1	0.500
9	60	1	0.500
10	65	1	0.319
11	70	1	0.681
12	75	1	0.066
13	80	1	*0.028
14	85	1	0.765
15	90	1	0.765
16	95	1	0.147
17	100	1	0.147

DF.= Degree of Freedom, Sig.= Significant, *= $P < 0.05$

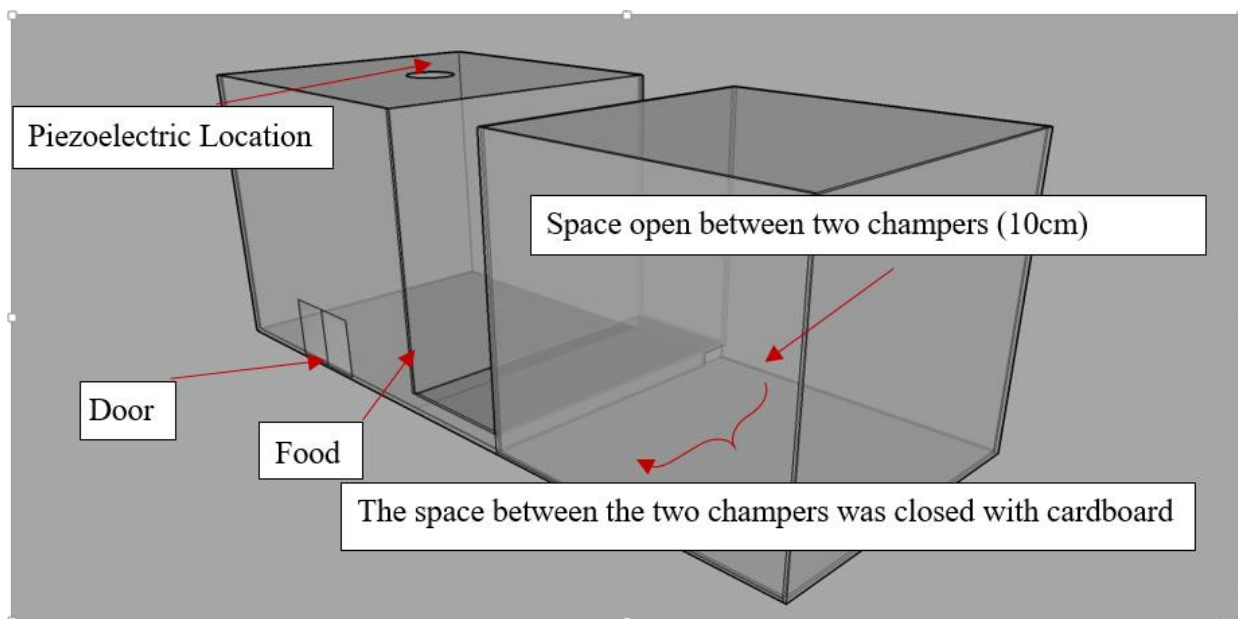


Fig. 1. Two glass square cube chambers are connected to each other by a rectangular cube, and one of the square cube chambers has a door for the entrance and exit of the safe cockroach for testing

Table 2. Study of the lethal effect of ultrasonic waves based on the gender of German cockroaches (Fisher test)

Row	Frequency (kHz)	DF.	Exacting Sig.
1	20	1	0.235
2	25	1	*0.008
3	30	1	0.712
4	35	1	0.681
5	40	1	0.500
6	45	1	0.167
7	50	1	0.681
8	55	1	0.500
9	60	1	0.500
10	65	1	0.319
11	70	1	0.681
12	75	1	0.066
13	80	1	*0.028
14	85	1	0.765
15	90	1	0.765
16	95	1	0.147
17	100	1	0.147

DF.= Degree of Freedom, Sig.= Significant, *= P< 0.05

Table 3. Determining the most effective frequency repelling German cockroaches using logistic regression test

Row	Frequency (kHz)	Score	DF.	Exacting Sig.
1	20	41.306	16	0.010
2	25	1.693	1	0.193
3	30	1.693	1	0.193
4	5	341	1	0.068
5	40	8.300	1	*0.004
6	45	0.078	1	0.780
7	50	1.774	1	0.183
8	55	0.61	1	0.804
9	60	3.341	1	0.068
10	65	0.078	1	0.780
11	70	1.693	1	0.193
12	75	0.601	1	0.804
13	80	0.600	1	0.439
14	85	0.649	1	0.421
15	90	5.690	1	0.017
16	95	3.455	1	0.063
17	100	5.690	1	0.017

DF.= Degree of Freedom, Sig.= Significant, *= P< 0.05

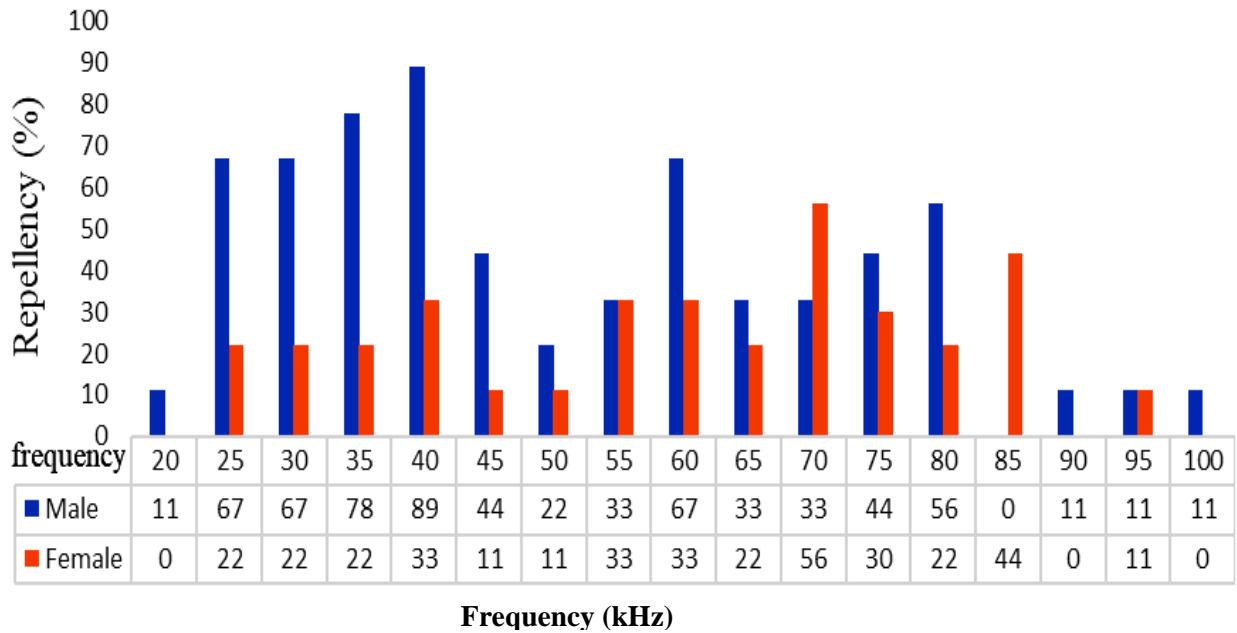


Fig. 2. The repellency rate of ultrasonic wave frequencies (20–100) kHz on *Blattella germanica*

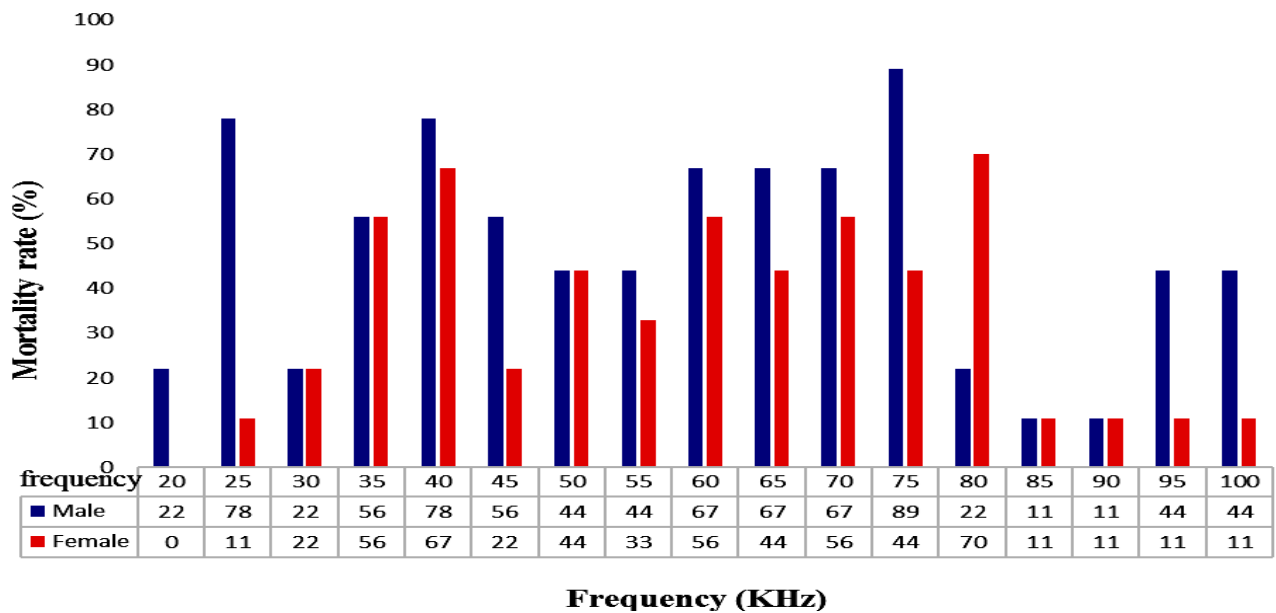


Fig. 3. The mortality rate of ultrasonic wave frequencies (20–100) kHz on *Blattella germanica*

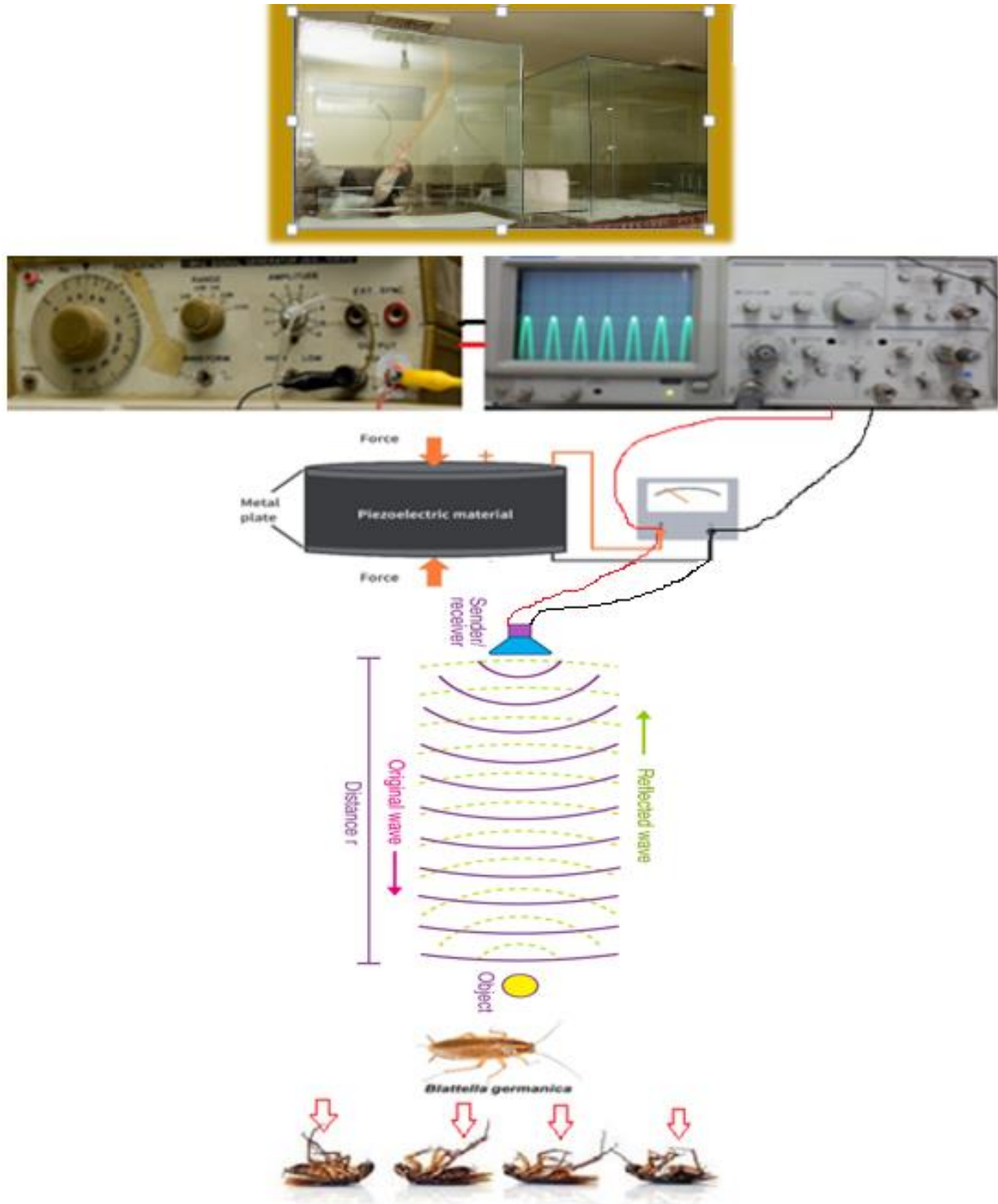


Fig. 4. Graphical abstract of ; two glass cubic chambers with dimensions $1 \times 1 \times 1$ m³ , Piezoelectric device in the center of the upper page, signal generator model JSG106H2 and the oscilloscope model MOS620CH, Schematic of ultrasonic waves; Shape of a live German cockroach, and the German cockroaches which died from ultrasonic waves

Table 4. Determining the most effective frequency killing German cockroaches using logistic regression test

Row	Frequency (kHz)	Score	DF.	Sig
1	20	459.957	16	0.001
2	25	0.102	1	0.749
3	30	2.746	1	0.097
4	35	1.712	1	0.191
5	40	7.790	1	*0.005
6	45	0.030	1	0.862
7	50	0.102	1	0.749
8	55	0.030	1	0.862
9	60	3.249	1	0.071
10	65	1.712	1	0.191
11	70	1.712	1	0.191
12	75	5.276	1	*0.022
13	80	0.663	1	0.416
14	85	7.000	1	0.008
15	90	7.000	1	0.008
16	95	1.352	1	0.245
17	100	1.352	1	0.245

DF.= Degree of Freedom, Sig.= Significant, *= P< 0.05

Discussion

Cockroaches, which are abundant in most human places, are known vectors of about 150 species of bacteria, 60 species of fungi, six species of yeast, 90 species of protozoa, 45 species of pathogenic ringworms, and several hookworms and whipworms, as well as they are significant sources of allergens (17). Nowadays, all people know the dangers of using various types of chemical insecticides that are used to control cockroaches and especially the German cockroach inside and outside human places. The use of simple, low-cost, and risk-free physical control methods is a choice that should be seriously considered. One of those methods is the use of ultrasonic waves.

Ultrasound refers to mechanical waves with a frequency higher than human hearing (more than 20kHz or 20,000 cycles per second). Ultrasonic waves are generally longitudinal and

originate from the vibrations of an elastic body. They always require a medium to diffuse and use pressure changes to diffuse. These waves are rapidly absorbed by the air. As a result, they are not transmitted over very long distances (18, 19). The frequency range of ultrasounds is very wide and depends on their use (20, 21).

Humans are exposed to ultrasonic waves in different ways, through air as well as through direct contact with a vibrating solid or liquid coupling medium. Air exposure to ultrasound is used in many areas of life, such as dog whistles, bird, rodent and insect repellants. Air ultrasound mainly affects the external organs of the body such as ears and eyes (in case of long-term exposure). Adverse effects reported in people exposed to airborne ultrasound include temporary threshold changes in sound perception and hearing loss, changes in blood sugar levels, electrolyte imbalances, fatigue, headache, dizziness, nausea, tinnitus, and irritation. Also, in the second method, ultrasound works in the liquid environment of the body based on tissue heating (thermal effect) or cavitation phenomenon. Liquid exposure occurs mainly in medicine, and in diagnosis, treatment, and surgery. When used at higher frequencies, the effect of ultrasound on tissue is based on heating. The thermal effect usually becomes dominant as the frequency increases from kilohertz (kHz) to megahertz (MHz) levels (22–24).

According to their frequency, there are three sources of ultrasound production, low frequency (10–100kHz), with many applications from an industrial perspective, medium frequency (100 kHz-1MHz), for use in therapeutic applications, and high frequency (1–10MHz), mainly used for medical purposes and non-destructive control devices (20, 24).

The potential of ultrasound to cause adverse effects in laboratory animals, plants, and cell culture has been well established, but whether similar effects occur in humans and in sensitive human tissues needs further investigation (16, 24–26). Also, despite many animal studies, no human research has been recorded to date

that shows the major physiological consequences of ultrasound exposure to imaging (13). It is a proven fact that the human body absorbs ultrasonic waves, and the effects of this absorption are unknown depending on the form of application and the dose and frequency of ultrasonic waves, either kHz or MHz, in the medium to long term, and can be it varies from positive to very negative (27).

To find out the proper instructions for using ultrasound emitting equipment to protect the health of workers, health professionals, patients, and all people and in general for better occupational safety, indoor environment quality and environmental health; it is necessary to formulate regulations by government departments (27). Therefore, it is recommended to conduct more studies on the effect of ultrasonic waves on the people who receive these waves so that the negative consequences of these incomprehensible sounds on human health can be prevented. It must be accepted that mankind is increasingly exposed to these sound waves, but its consequences are ignored by most people (13).

Previous studies have shown that ultrasonic waves cannot have a repellent effect on German cockroaches. In this study we investigated the effects of ultrasonic waves on male and female German cockroaches. The 20–100kHz waves were tested in 5 to 5 sine waves to reach this objective. All experiments were performed in two glass chambers with no extra noise in the environment during the test. With regular feeding and standard laboratory conditions, all the control cockroach specimens survived for more than 5 days after testing. Furthermore, the ultrasonic wave exposure cockroach specimens (treated cockroaches) started to die after one hour of rest, and all of them died within three days after testing. The results of this study show that the effects of these ultrasound frequencies are more effective on male than female of German cockroaches, and can repel, seriously injure, or kill them (Fig. 4).

In this study, where the use of ultrasonic sound waves was performed on the German cockroach, its laboratory effectiveness was confirmed. Therefore, it is recommended that the country's health and environmental officials pay special attention to this German cockroach method controlling which is nature friendly too. In addition, in cases where there are people sensitive to chemical insecticides or there are insects' resistant to chemical insecticides, it is recommended to use this alternative control method. Of course, our recommendation until formulate regulations by government departments, use this control method outside of human places. It should be known that ultrasonic waves should be periodically tested by researchers on the cockroaches of that country, and if they are effective, they can be used again.

In future studies, if these German cockroaches are exposed to higher frequencies with waveforms such as square, triangular, noise, or broom, the effects them may be significantly more intensive and deadly. Identifying these appropriate frequencies, will enable electronic devices to control different insects in future more efficiently.

Conclusion

It can be concluded that these ultrasound frequencies can repel, injure, or kill German cockroaches. This study showed that ultrasonic waves could have both repellent and lethal effects on German cockroaches. These effects were more effective on males than females. We found that there is a repellent effect of 30.7% (10.7% female + 19.9% male) in general. Its lethal effects were 40.8% overall (17% female + 23.8% male).

The highest repellency rate and death rate were observed at 40kHz and both 40kHz and 75kHz, respectively. Identifying these appropriate frequencies will enable future electronic devices to control these insects more efficiently. If these insects are exposed to higher frequencies with waveforms such as square,

triangular, noise, or broom, insects' effects may be significantly better. However, further studies are required to reveal safety of ultrasonic waves on the people who receive these waves before we could recommend this method.

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Ethical considerations

This experiment was carried out under the guidance of the Ethics Committee of the Tehran University of Medical Sciences (IR.TUMS.SPH.HCR EC.1397.294).

Conflict of interest statement

The authors declare there is no conflict of interests.

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