# Investigating the effect of sonic stimulation on susceptibility of *Caenorhabditis elegans* to bacterial pathogens, or toxic chemicals

A dissertation thesis submitted to

Institute of Science, Nirma University

in partial fulfilment of the requirement for degree of

MASTER OF SCIENCE

In

MICROBIOLOGY


Submitted by: -

Krishna Bhanushali (21MMB002)

Khushi Kotecha (21MMB011)

Yesha Shah (21MMB027)

Under the Guidance of

Dr. Vijay Kothari

NIRMA UNIVERSITY
INSTITUTE OF SCIENCE
NAAC ACCREDITED 'A' GRADE

# CERTIFICATE

This is to certify that the thesis entitled **"Investigating the effect of sonic stimulation on susceptibility of *Caenorhabditis elegans* to bacterial pathogens, or toxic chemicals."** submitted to the Institute of Science, Nirma University in partial fulfilment of the requirement for the award of the degree of M.Sc. in Microbiology, is a bonafide record of research work carried out by **Krishna Bhanushali (21MMB002), Khushi Kotecha (21MMB011)** and **Yesha Shah (21MMB027)** under the guidance of **Dr. Vijay Kothari**. No part of the thesis has been submitted for any other degree or diploma.

**Prof. Sarat K. Dalai**
**(Director)**

Director
Institute of Science
Nirma University
Ahmedabad

**Dr. Vijay Kothari**
**(Dissertation Guide)**

INSTITUTE OF SCIENCE AHMEDABAD NIRMA UNIVERSITY

**Place:** Ahmedabad

**Date:**

Institute of Science, Nirma University
Sarkhej-Gandhinagar Highway, Ahmedabad 382 481, INDIA, Ph.: +91-02717-241900/01/02/03/04, +91-79-30642753, Fax: +91-02717-241916
E-mail: director.is@nirmauni.ac.in, Website: www.nirmauni.ac.in

## Acknowledgement

First and foremost, praise and thanks to God, the Almighty for his shower of blessings throughout our research work to complete our research successfully. We would like to extend our gratitude to our parents and family members for believing in us and making us capable enough to reach this destination.

A sincere thanks to **Nirma Institute of Science (NIS)** who greatly helped by adding the infrastructural support to this project. We would sincerely like to thank Prof. **Sarat Dalai**, **Director of Institute of Science**, Nirma University for his untiring help, great knowledge, and inspirational words.

We would like to express our special thanks of gratitude to our guide, **Dr. Vijay Kothari**, who gave us a great opportunity to work on this project and also for the patient guidance, encouragement, and advice he has provided throughout the dissertation. We have been extremely lucky to have a guide who responded to our questions and queries so promptly.

We are thankful to all the faculty members **Dr. Sriram Seshadri**, **Dr. Sonal Bakshi, Dr. Nasreen Munshi, Dr. Aarthi Sundararajan, Dr. Ravi Kant, Dr. Shruti Chatterjee, Dr. Heena Dave** and for their continuous encouragement and support.

We are indebted to **Ms. Gemini Gajera** without whose guidance and tireless efforts, this dissertation project would have never been completed. We are also thankful to **Ms. Nidhi Thakkar** and **Ms. Sweety Parmar** for providing us with continuous support and encouragement.

We would like to thank **Mr. Sachin Prajapati, Mr. Valji Desai, Ms. Arti Varma, Ms. Nisha Dave and Mr. Rajendra Patel** for being very helpful and providing us laboratory reagents, equipment, etc.

We would also like to thank our colleagues and friends who have helped us in the best possible way. We would also like to thank all M.Sc. dissertation members for their kind and helping nature. Lastly, we would like to express our deepest gratitude to all those who have not been mentioned by name but always have been there in their inscrutable ways.

| | | |
|---|---|---|
| Krishna Bhanushali | Khushi Kotecha | Yesha Shah |

# Index

## Abbreviations: -

dB- Decibel

DMSO- Dimethyl Sulfoxide

Hz- Hertz

M9 buffer- Minimal medium buffer

MTCC- Microbial Type Culture Collection, Chandigarh, India

NGM- Nematode Growth Medium

TFFT- Temporal Frequency Analysis Tool

# 1. Introduction:

For fundamental research on metazoan creatures in general, as well as drug development and repurposing, the free-living worm *C. elegans* is a promising model (Crespo et al., 2020) to investigate the nematocidal/nematostatic effects of toxins and other natural or manufactured substances (Taki et al., 2021), neurodegenerative diseases which includes Amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and Huntington's disease (Li, & Le, 2013) the mechanisms of action of anthelmintics and nematicides and other factors such as mortality, lifespan, behaviour, eating, growth, and reproduction (Martins et al., 2022). Toxic metals, organic phosphates, and pesticides are only a few of the compounds that *C. elegans* is susceptible to from a toxicological perspective (Martins, et al., 2022; Holden-Dye & Walker., 2007). This nematode has been extensively used. The worm is highly tractable at both the genetic and biochemical levels, has many fundamental biological functions similar to humans (Gupta & Gupta., 2019).

All phyla of animals, including humans, are infected by parasitic nematodes. The prevalence of parasites and parasitic illnesses is global. Significant issues with public health are brought on by their negative consequences for society's social and economic conditions. These infections can cause gastrointestinal problems, malnutrition, anaemia, allergies, and occasionally even life-threatening conditions. A collective effort is certainly required to solve these issues. Anthelmintic drugs are referred to as, the medication used to treat parasitic worm infection. These helminths are primarily categorized into trematodes (flukes), cestodes (tapeworms) and nematodes (roundworms) (Holden-Dye & Walker., 2014; Abongwa et al., 2017).

Antimicrobial resistance: Antimicrobial resistance (AMR) has been discovered to be a major issue that has to be addressed on a worldwide scale. The majority of pathogenic bacteria are capable of developing resistance to practically all antibiotics. Antibiotics' selection pressure, which causes mutation and recombination in the organism and renders the strain resistant, is the cause of this antimicrobial resistance. Then, through horizontal gene transfer, these resistance genes are spread across novel species (Huttner et al., 2013). This puts public health at risk on a worldwide scale. According to estimates, a child passes away from an antibiotic-resistant bacterium every five minutes. In India, infectious diseases account for the majority of deaths. The results of several laboratories show an increasing trend in the emergence of antibiotic resistance (Dadgostar, 2019).

Our ability to interact with one another, navigate our surroundings, and appreciate music and other kinds of art are all made possible by sound, which plays a significant part in our everyday lives. Moreover, it has a number of practical uses, including acoustics engineering, sonar technology, and medical imaging.

Several essential characteristics, such as frequency, loudness, and wavelength, define sound. The number of sound waves that pass a specific spot in one second is known as frequency and is measured in Hertz (Hz). Decibels are used to quantify amplitude, which is the strength or intensity of a sound wave (dB). The term "wavelength" refers to the distance in metres between two comparable places on adjacent waves (m).

In order to induce relaxation and aid in stress reduction, sound therapy is frequently used in conjunction with meditation and other relaxation techniques. Music therapy is another method that sound is applied to healing in addition to sound treatment. According to research, music has a strong impact on the brain and may ease tension and anxiety, uplift the spirit, and encourage relaxation (Roth & Wisser, 2004).

*C. elegans* is a small, transparent, free-living roundworm that has been widely used as a model organism in biological research due to its simplicity and ease of study. It is extensively used in various fields of research, including genetics, neuroscience, developmental biology, and aging (Hope, 1999; Iliff et al., 2021). The model host for infectious microorganisms was *C. elegans* (N2 Bristol). *Escherichia coli* OP50 was used as the meal to keep this worm in nematode growing medium (NGM). *E. coli* OP50 was procured from LabTIE B.V., JR Rosmalen, the Netherlands (Kothari et al., 2016).

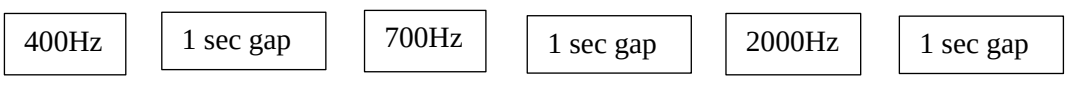
*C. elegans* behaviour has been investigated using sound. To better understand how it reacts to various stimuli, including sound waves, researchers have used sound as a tool. According to studies, they can recognise sound waves and react to them in a wide range of ways (Iliff et al., 2021; Koelsch & Stegemann., 2012).

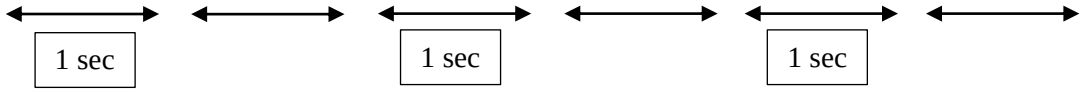
# 2. Literature Review:

## 2.1 Sounds used in our experiments:

**2.1.1 400 Hz (Mono frequency):** Our laboratory's previous studies have shown that the 400 Hz frequency is thought to be beneficial in nature since it offers the maximum survival benefits compared to other mono frequencies (Patel et al., 2019).

**2.1.2 400+700+2000 Hz (Mixed frequency):** We attempted developing a blend of these three frequencies as they had previously been utilised as mono frequencies in our lab and had shown the maximum benefits on the host *C. elegans* (400 Hz, 1 sec gap, 700 Hz, 1 sec gap, 2000 Hz) (Kothari et al., 2019).






**2.1.3 'OM':** Chanting OM has many mental, physical, and spiritual advantages, and some scientific studies have even demonstrated the advantages for one's health. OM is regarded as a strong sound therapy method. It is thought to possess a vibratory characteristic that has the power to influence both the body and the brain (Gangadhar et al., 2018). Researchers discovered that OM chanting calmed the brain, which might lessen stress by utilising a functional MRI equipment to examine brain activity. It can be used to treat depression, according to different research. According to mythology, the sound of OM resonates with the vibrations of the universe, bringing harmony and balance to the body and mind. This is due to the relaxing influence of OM's sound waves on the nerve system, which lowers tension and anxiety. The impacts of OM mantra are mostly discussed in terms of spiritual and subjective feelings, as well as their effect on biological organisms (Surlya & Jain., 2021).

**2.1.4 Classical music (*Raga Piloo*):** Music has the potential to promote general health because physical health is linked to both mental and emotional wellbeing. For many years, *Raga Piloo* has been a significant Raga in the history of Indian traditional music. It has been handed down through musical centuries and is referenced in ancient writings, treatises, and instrumental compositions. It has been adored and played by eminent classical musicians, and it is still a popular Raga today. Traditional Indian medicine has used *Raga Piloo* (Indian classical music) to treat a variety of conditions, including depression and sleeplessness. *Raga Piloo*, which is recognized for its calm and introspective features, is thought to have therapeutic benefits (Sarkar & Biswas., 2015).

**2.1.5 432 Hz frequency:** Many people believe that the 432 Hz tuning frequency has mysterious and healing properties (Rosenberg, 2021). Because it aligns with the natural frequencies of the cosmos, some proponents of the 432 Hz tuning frequency think it has a calming effect on the body and mind. The idea of "tuning to the Earth" or "natural tuning" is frequently linked to the 432 Hz frequency, and some people even assert that it has special biological and spiritual meaning (Calamassi & Pomponi., 2019)

## 2.2. Test compounds:

**2.2.1 Rotenone**: - Rotenone is one of the naturally occurring plant toxins. This botanical component has been in significant use for centuries as an insecticide, pesticide, and piscicide. Depending on the product composition, rotenone is either EPA toxicity class I or III (very harmful or somewhat toxic). Chronic exposure to rotenone causes toxicity by inhibiting mitochondrial activity that interrupts respiratory complex I of the electron transport chain, followed by mitochondrial dysfunction, impaired proteostasis, degeneration of dopaminergic neurons, neuroinflammation, and finally Parkinsonian motor deficits (Dengg & van Meel 2004). It is extracted from the stems and roots of several plants, such as those found in Derris spp. and *Lonchocarpus* spp. throughout Asia, Africa, and South America. It has a molecular weight of 394.42 and the chemical formula $C_{23}H_{22}O_6$ (Gupta, 2012). Rotenone is present in

the form of colourless crystals, but it is readily degraded by heat and light, making it non-persistent in the environment.

**2.2.2 Manganese chloride: -** Manganese (Mn) is a vital element necessary for human growth, brain function, and other biological activities that is often found in trace concentrations in diets (Lucchini et al., 2017). Mn is predominantly found in $Mn^{2+}$ and $Mn^{3+}$, two oxidized forms, in the human body. Blood $Mn^{2+}$ species are complexed with low-molecular-mass species like bicarbonate and citrate as well as high-molecular-mass fractions like albumin and $\beta$-globulin as hydrated ions (Chen et al., 2015). However, cumulative exposure to abnormally high atmospheric concentrations of manganese induces neurotoxicity in the brain, with the results resembling idiopathic Parkinson's disease (PD) (Lucchini et al., 2017; O'Neal & Zheng., 2015)

**2.2.3 Benzimidazole: -** An anthelmintic, benzimidazole is often used to treat parasitic worm infections in people and animals. This medication causes the nematode to specifically bind to parasite $\beta$-tubulin with high affinity and hinder microtubule polymerization. Due to this, the parasite's cell structure is damaged, which leads to its eventual death (Holden-Dye & Walker., 2014).

**2.2.4 Ivermectin: -** Ivermectin is one of the most significant medications in veterinary and human medicine for the management of parasitic infections (Li & Le, 2013). Ivermectin's powerful paralytic activity is mediated by a family of nematode glutamate-gated chloride channel alpha subunits (GluCl), which is the target location for the drug's anthelmintic action. Due to agonistic activity on chloride channels in nerve and muscle cells, it increases plasma membrane permeability, which causes hyperpolarization and paralyzes. Ivermectin tends to cause rapid paralysis in *Ascaris suum* where as in *Caenorhabditis elegans*, a free-living nematode it causes slow-onset stiff paralysis. Therefore, *C. elegans* is a suitable model to study the mechanisms of ivermectin toxicity (Holden-Dye & Walker., 2014; McCavera et al., 2009).

## 2.3 Test organisms:

Pathogenic organisms used in our experiments were *Serratia marcescens* and *Escherichia coli.*

**2.3.1. *Serratia marcescens***: *Serratia marcescens* is a member of the genus *Serratia*, which is a part of the family *Enterobacteriaceae* (Hejazi & Falkiner., 1997). Numerous infectious illnesses have been linked to *S. marcescens*, including infections of the urinary, respiratory, and digestive tracts, peritonitis, wound infections, and infections associated with intravenous catheters that can result in potentially fatal bacteraemia (Kim et al., 2015). Depending on the age of the colonies, a few strains of *Serratia* are capable of generating a pigment known as prodigiosin, which can range in hue from dark red to pink.

**2.3.2. *Escherichia coli***: The bacteria are gram-negative, rod-shaped, non-spore

forming and motile organism. The majority of *E. coli* strains are benign, however certain serotypes (EPEC, ETEC, etc.) may severely infect their hosts' diet. A water and food borne pathogen called enterotoxigenic *E. coli* attacks the small intestine of the human gut and causes diarrhoea. The Enterobacteriaceae family contains enteropathogenic *Escherichia coli* (EPEC). Common causes of gastroenteritis include enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC) (Kaper et al., 2004; Nataro & Kaper., 1998).

# 3. Objectives

1. Effect of sonic stimulation on the virulence of pathogenic organism *Serratia marcescens* and *Escherichia coli* towards the host *Caenorhabditis elegans.*

2. To investigate the effect of sonic stimulation towards toxicity induced by certain toxic compounds on host *Caenorhabditis elegans.*

# 4. Materials and Methods

**Table 1. Test organism and related parameters**

| **Organisms** | **Strain number** | **Media used** | **Incubation temperature (˚C)** | **Incubation time (h)** |
|---|---|---|---|---|
| *Serratia marcescens* | MTCC 97 | Nutrient broth/ Nutrient broth supplemented with 3% v/v glycerol (HiMedia) | 28 | 24 - 48 |
| *Escherichia coli* | OP50 | Nutrient broth/agar | 35 | 24 |
| *Escherichia coli* | MTCC 723 | | | |
| *Caenorhabditis elegans* | N2 Bristol | Nematode Growing Media (NGM) | 22 | - |

## 4.1 Reagent preparation

- **1 M phosphate buffer**

| **Reagents** | **For 1000 mL** |
|---|---|
| $KH_2PO_4$ (HiMedia) | 108.3 g |
| $K_2HPO_4$ (HiMedia) | 35.6 g |
| Distilled water | 1000 mL |

- **NGM agar** (Stiernagle, 1999)

| **Reagents** | **For 1000 mL** |
|---|---|
| Agar (HiMedia) | 17 g |
| Peptone/ (HiMedia) | 2.5 g |
| NaCl (HiMedia) | 3.0 g |
| 1 M $CaCl_2$* (Merck) | 1 mL |
| 1 M $MgSO_4$* (HiMedia) | 1 mL |
| Cholesterol in ethanol (5 mg/ml) * (HiMedia) | 1 mL |
| 1 M phosphate buffer (pH: 6.0) * | 25 mL |
| Distilled water | 972 mL |

The (*) reagents were autoclaved separately and then added. Cholesterol should not be autoclaved

- **Bleaching solution**

| Reagents |
|---|
| 1 N NaOH (HiMedia) |
| 4% Sodium Hypochlorite |

Both reagents were added in equal volume.

- **M9 buffer**

| Reagents | For 1000 mL |
|---|---|
| $KH_2PO_4$ (HiMedia) | 3.0 g |
| $Na_2HPO_4$ (HiMedia) | 6.0 g |
| NaCl (HiMedia) | 5.0 g |
| 1 M $MgSO_4$* (HiMedia) | 1 mL |
| Distilled water | 999 mL |

The (*) reagent was autoclaved separately and then added.

- **S buffer** (Stiernagle, 1999)

[129 mL 0.05M $K_2HPO_4$ + 871 mL 0.05M $KH_2PO_4$ + 5.85g NaCl] for 1000 mL

In distilled water

***E. coli* OP50 culture**

The culture activation procedure from an *E. coli* OP50 plate was carried out in 50 mL Nutrient broth and incubated for 20 - 22 h at 35˚C. After that, activated culture was collected and stored in a microfuge tube for further use. Within the first 30 to 40 days of the period, the culture can be used. Moreover, 100 µL of culture is used to pre-seed the NGM plates needed for *C. elegans* maintenance.

**4.2. Maintenance of *C. elegans***

On agar plates made of Nematode Growth Media (NGM), *C. elegans* were kept alive in the laboratory. Pre-seeded *E. coli* OP50 was employed as a food source. The techniques are chunking and washing commonly employed for moving *C. elegans* from one sterile NGM plate to another. Moreover, the synchronisation procedure is employed to extract the eggs from adults and providing synchronous population of *C. elegans*.

**Washing:**

- Take an old NGM plate containing worms and collect the worms through micropipette (1000 µL) by using 1 mL sterile M9 buffer. Collect it in microfuge tube (1.5 mL) and allow it to settle down.
- After the worms settling down, remove the supernatant (containing NGM traces) and add 1 mL sterile M9 buffer.
- Repeat it for 3 times.

- Add 300 µL of sterile M9 buffer in microfuge tube and gently mix it.
- Then collect the washed worms through micropipette (1000 µL) and put the drops of liquid containing worms randomly on the surface of new pre-seeded *E. coli* OP50 NGM plate.
- Incubate the plate in 22 ˚C incubator till next use.

**Chunking:**

- Take an old NGM plate containing worms and cut the chunk (1 cm) by using sterile scalpel.
- Put the chunk in inverted position on the new pre-seeded *E. coli* OP50 NGM plate.
- Let the chunk be there on the plate for 2 minutes, this will allow the worms to crawl from chunk to the fresh plate.
- Remove the chunk carefully and incubate the plate in 22˚C incubator till next use.

**Synchronization:**

- This method provides synchronous population of worms that are used for experiments avoiding age differences.
- Initially worms are collected in falcon tube through pipette from an old plate containing gravid adults (worms carrying eggs) as seen in microscope.
- Washing is done as mentioned above, after that add 200 µL of 1 N NaOH and 4% sodium hypochlorite for bleaching of worms; and then vortex it for 20 - 30 seconds.
- Then centrifuge it for 1 minute at 1500 rpm to pellet down of worms.
- Discard the supernatant and add 10 - 15 mL of sterile M9 buffer to the pellet containing eggs.
- Repeat above two steps of centrifugation for 5 times.
- Add 300 µL of sterile M9 buffer and gently mix it, then put the drops (whole 300 µL content) of liquid containing eggs randomly on the surface of new pre-seeded *E. coli* OP50 NGM plate.
- Incubate the plate in 22˚C incubator till next use.
- This synchronized plate is incubated for 28 - 30 h which help the eggs to be hatched and worms attain L3 to L4 stage which can be used for survival assay.

## 4.3. Inoculum standardization and culture preparation

- A loopful culture of *Serratia marcescens* from stock was streaked on nutrient agar plate and incubated at 28˚C for 48 h
- Three to four *S. marcescens* colonies were selected, and they were dissolved in about 5 mL of normal saline (0.85% NaCl).
- OD was measured at 625 nm, and standardized to match McFarland 0.5 turbidity standard. OD of bacterial culture would be adjusted to 0.8 - 1.0.
- Inoculation of bacterial culture having OD between 0.8 - 1.0 and volume 200 µL in nutrient broth followed by incubation for 48 h
- After the incubation $OD_{764}$ of bacterial culture would be adjusted to 1.5 and it was used for *in vivo* assay.

## 4.4. Preparation for an *in vivo* assay

- The experiment was carried out on sterile, untreated 24 well polystyrene plates (HiMedia). The experiment set up is displayed in Fig.1.
- After synchronization, *C. elegans* were transferred to NGM plate on the next day devoid of *E. coli* OP50 and starved for three days.
- This plate contained L3 - L4 stage *C. elegans*, which were then viewed under a microscope (4X).
- Worms were collected in a sterile microfuge tube (1.5 mL) using M9 buffer from NGM plate.
- Worms were counted in the individual well; each well should have 10 worms.
- In 24 well plates, wells containing 10 worms in 1000 µL of M9 buffer that is *C. elegans* unchallenged and wells containing 900 µL of M9 buffer that is *C. elegans* challenged with 100 µL of *Serratia marcescens* culture.
- For the positive control, three wells were filled with 895 µL of M9 buffer, 10 worms, 100 µL of bacterial culture, and 5 µL of antibiotic (0.5 % v/v).
- Using a light microscope (4X), live and dead worms were counted daily for five days during incubation at 22˚C.

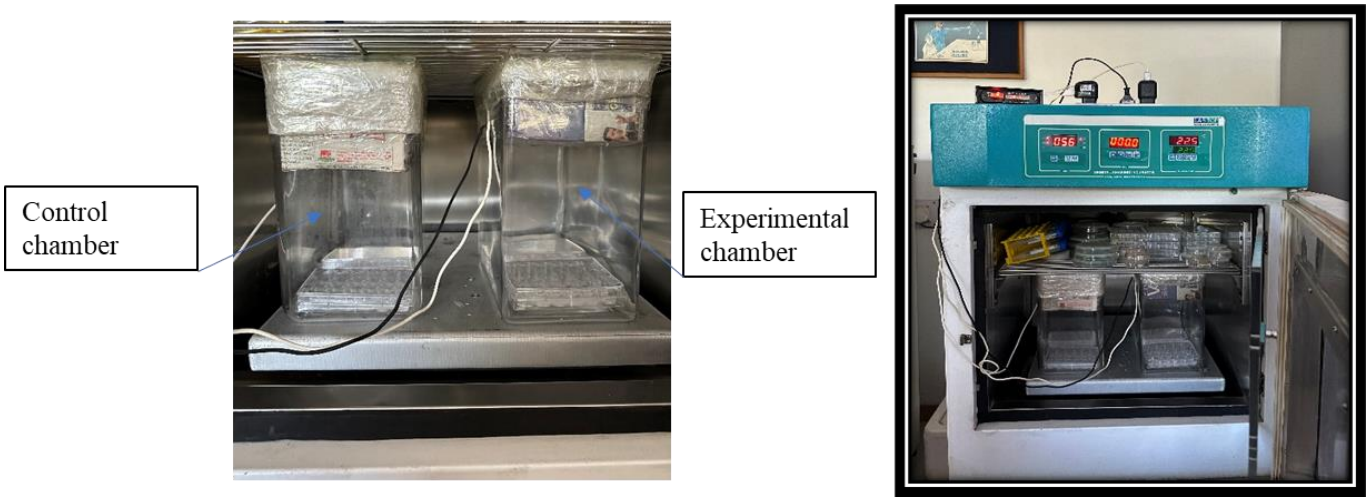



**Fig.1 Experimental set up in 22˚C incubator (Labtop)**

- For the *in vivo* assay 2 different well plates were prepared and kept in separate glass chambers.
- In both chambers, speakers were fixed above the plates. Distance between speaker and 24 well plate was 15 cm.
- The speaker and amplifier were connected in the experimental glass chamber and sound was constantly provided. In the control chamber sound was not provided.
- After the incubation of 24 h, plates were rotated 180˚ and there positions were altered so that sound can reach each well equally.
- To prevent sound leaking from inside and to shield from outside disturbances, these glass chambers were densely packed with polystyrene sheets and capped with glass lids.
- Positions of experimental and control chambers were inter-changed after every 24 h.

## 4.5. Important Parameters for experimental set up

- **Glass chambers:** Actira glass chambers measuring L: 250 x W: 150 x H: 250mm were used.
- **24 well plates:** For the experiment, sterile 24 well plates (HiMedia) were used.
- Croma Bric Bluetooth speakers were used as the sound source.
- **Memory card and card reader:** A memory card with a test frequency was connected to the amplifier using a card reader for sound treatment.
- **FM player:** For the experiments, an Exceed Magic box USB-digital FM receiver and a Multi sonic Magic box digital FM player were used.
- **Sound level metre:** A digital sound level metre KM929MK-1 (Kusam-Meco Import Export Pvt. Ltd., Navi Mumbai) was used to measure sound intensity (Fig 2). This sound level metre detects sound intensity ranging from 35 dB to 130 dB.
- **Imaging software:** Magvision


**Fig. 2 Sound level meter**

**Table 2: Experimental set up**

| Experimental set up position | Speaker used for set up | Vertical distance between speaker and plate (edge to edge; cm) | Background noise (dB) | |
|---|---|---|---|---|
| | | | Experimental chamber | Control chamber |
| Labtop 22˚C incubator | Croma Bric Bluetooth speaker | 15 | 59.0 ± 2.4 | 57.14 ± 1.68 |

**Table 3: Sound intensity in chambers**

| Frequency | Chamber | Sound intensity (dB) |
|---|---|---|
| Mono-frequency | Control | 60.5 ± 1.68 |
| | Experimental | 85.5 ± 0 |
| Poly-frequency (400+700+2000) | Control | 60.5 ± 1.68 |
| | Experimental | 60.8 - 95.9 ± 5.60 |
| Poly-frequency (OM) | Control | 60.5 ± 1.68 |
| | Experimental | 60.3 - 86.1 ± 2.70 |
| Poly-frequency (*Raga Piloo*) | Control | 60.5 ± 1.68 |
| | Experimental | 60.5 - 99.4 ± 8.62 |

## Statistical analysis*:

All the experiments were performed in duplicate and triplicates, measurements are reported as mean ± standard deviation (SD). Statistical significance of the data was evaluated by applying t-test (two tailed, paired) using Microsoft Excel®.

P values less than 0.05 were considered to be statistically significant. For in vivo analysis, GraphPad Prism version 8.0.1 (244) software was used.

*Applicable for all results.

# 5. Chapter 1

Effect of sonic stimulation on the virulence of pathogenic organism *Serratia marcescens* and *Escherichia coli* towards the host *Caenorhabditis elegans.*

## 5.1 NCH software used for generation and analysis of desired frequency

For the generation of poly frequency NCH Software was used. 400 Hz, 700 Hz, 2000 Hz poly frequency was generated using NCH® tone generator software and Wavepad software (Wavepad v 7.13) and TFFT (temporal frequency analysis) tool was used to analyze frequencies as shown in Fig. 3.

In between these selected frequencies, after each beep there was a gap of 1-second included using Wavepad software.

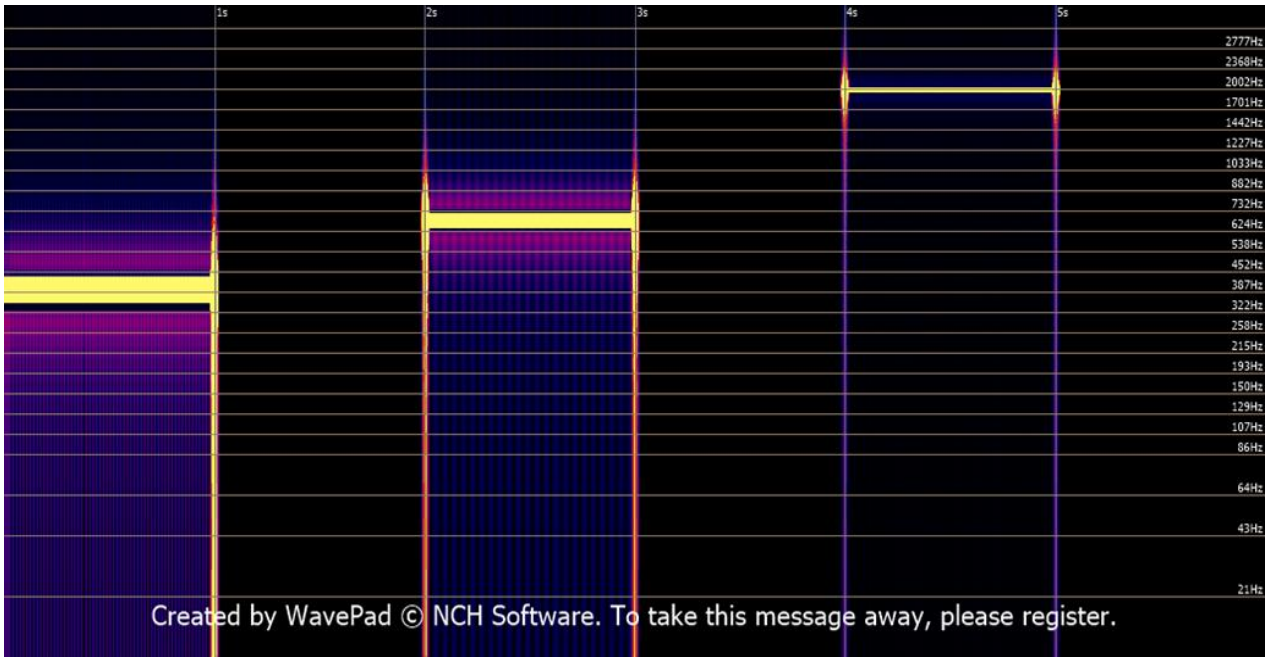



**Fig. 3 Frequency generated through NCH® tone generator software**

**RANGE:**

**400Hz: 322-452Hz**

**700Hz: 624-732Hz**

**2000Hz: 1701-2002Hz**

## 4.2 Result and discussion

- **Optimization of Optical density**

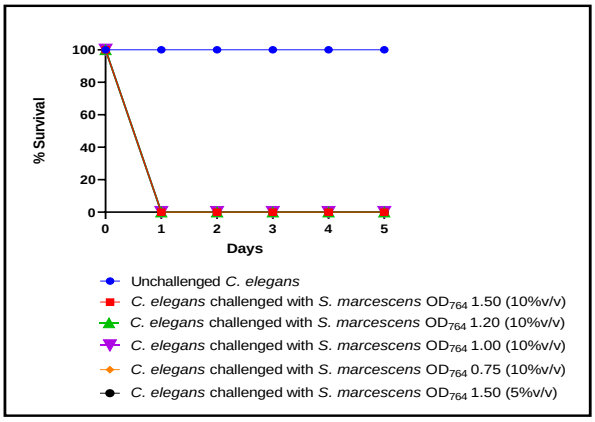



**Fig 4. *Serratia marcescens* cultured in glycerol supplemented with nutrient broth kills *C. elegans* equally irrespective of OD.**

Standardization of OD was carried out using this experiment. Here we used different volume of bacterial culture and different OD were used.

*C. elegans* challenged with *Serratia marcescens* killed 100% worms within 24 h at different OD in nutrient broth supplemented with 3% v/v glycerol**.**

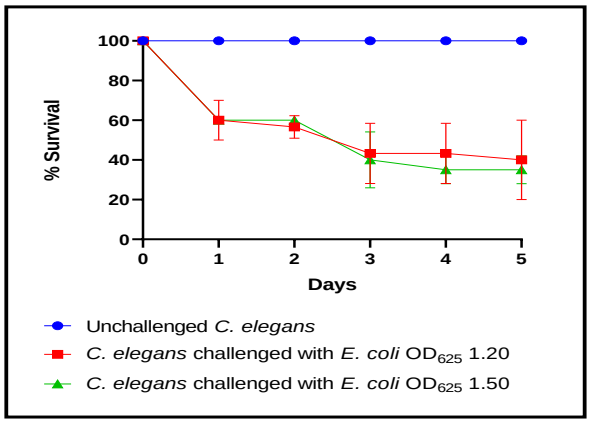



**Fig 5. Maximum infectious dose was optimized of *E. coli* infection in *C. elegans***

*C. elegans* challenged with *E. coli* has shown 40% ± 20** and 35% ± 7** survival at $OD_{625}$ 1.20 ± 0.05 and 1.50 ± 0.05 respectively. As the maximum killing of worms was observed at OD 1.5. Therefore, it was considered for the further experiments.

** $p < 0.01$

➢ **Optimization of media composition**

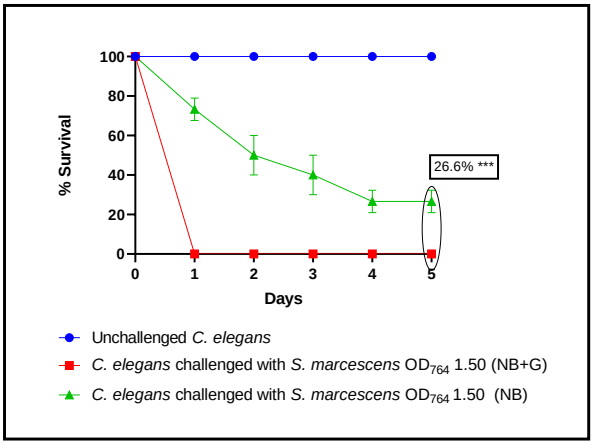



**Fig 6. Glycerol supplemented in media enhance the virulence of *S. marcescens.***

This graph compares the percent survival of *C. elegans* infected with *S. marcescens*, cultured in nutrient broth has shown 26.6% ± 5.7*** survival of infected worms on 5$^{th}$ day, whereas bacteria cultured in nutrient broth supplemented with 3% glycerol has shown 0% survival of infected worms within 24 h.

Although the effects of sound therapy can vary depending on individual factors, some studies have suggested that the therapy can have prolonged effects. Therefore, to achieve delayed death model in the host *C. elegans* challenged with *S. marcescens* cultured in nutrient broth was considered for further experiments.

*** $p < 0.001$

➢ **Selection of positive control**

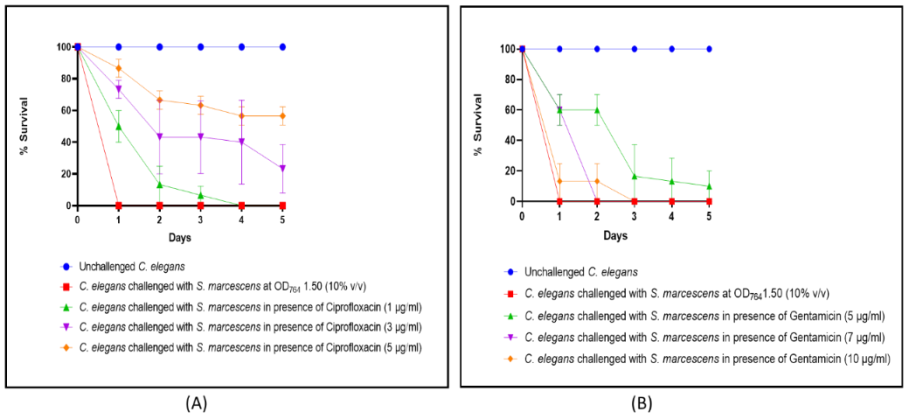



**Fig 7. Ciprofloxacin is an appropriate positive control than Gentamicin for *C. elegans* infected with *S. marcescens*.**

Below table shows percent survival of *C. elegans* challenged with *S. marcescens* in presence of different antibiotic with different concentrations.

| Days | Ciprofloxacin | | | Gentamicin | | |
|---|---|---|---|---|---|---|
| | 1µg/ml | 3 µg/ml | 5 µg/ml | 5 µg/ml | 7 µg/ml | 10 µg/ml |
| 1 | 50 ±10 *** | 73.33 ± 5.77*** | 83.67 ± 5.77*** | 60 ± 10*** | 60 ± 10*** | 66.67 ± 5.77*** |
| 2 | 13 ± 11.54 | 43.33 ± 23.09* | 66.67 ± 5.77*** | 60 ± 10 | 0 | 13.37 ± 11.55 |
| 3 | 6.66 ± 5.77 | 43.33 ± 23.09* | 63.33 ± 5.77*** | 16.67 ± 20.82 | 0 | 11.33 ± 11.55 |
| 4 | 0 | 40 ± 26.46* | 56.67 ± 5.77*** | 13.33 ± 15.28 | 0 | 0 |
| 5 | 0 | 23.33 ± 15.28* | 56.60 ± 5.77*** | 10 ± 10 | 0 | 0 |

Ciprofloxacin (5 µg/ml) tends to be the most sensitive towards *S. marcescens*. Therefore, this concentration was considered as positive control for the further experiments.

*p < 0.05, ** p <0.01, *** p <0.001

Previous data from our lab showed the effect of sonic stimulation (400 Hz, 700Hz, 2000 Hz; 85.5 dB) on *C. elegans* challenged with *S. marcescens* (Kothari et al., 2019).

It showed when sound with mono-frequency of 400 Hz (with a 1-sec gap) was given to *C. elegans* infected with *S. marcescens* and it was compared with *S. marcescens* unexposed to sound, 30.83% survival benefit with significant effect was observed.

Similarly, when 700 Hz and 2000 Hz mono-frequency was used it gave 27% and 11% survival benefit respectively (Kothari et al., 2019). So, we took these three frequencies and combined them: 400 Hz – 1 sec gap – 700 Hz – 1 sec gap – 2000 Hz – 1 sec gap and used it for our experiments.

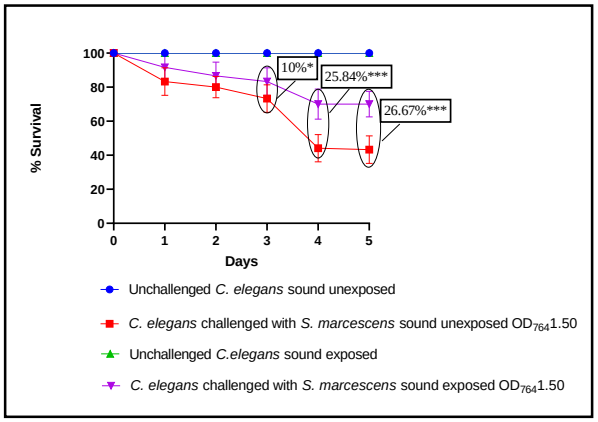


**Fig 8. Sonic stimulation (400+700+2000 Hz) (60.8 - 95.9 dB) reduces *S. marcescens* virulence towards the host *C. elegans.***

*C. elegans* challenged with *S. marcescens* exposed to sound has shown survival benefit of 10%* ± 8.16, 25.84% ± 8.94*** and 26.67% ± 8.01*** on 3rd, 4th and 5th day respectively. The unchallenged *C. elegans* with and without sound treatment showed 100% survival. Progenies were observed on the 3rd day

Here, two independent experiments were performed in triplicate and the raw data for the outlier is mentioned in appendix.

*p < 0.05, *** p <0.001

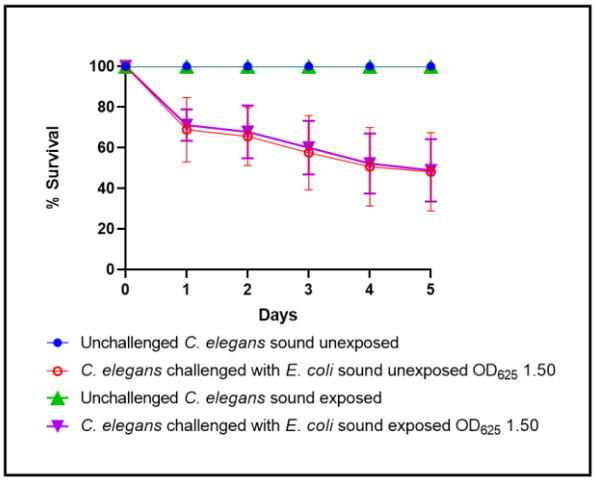



**Fig 9. Sonic stimulation (400 Hz, 85.5dB) did not alter *E. coli* virulence towards *C. elegans*.**

*C. elegans* challenged with *E. coli* exposed to sound has shown no significant difference between *C. elegans* exposed to sound and *C. elegans* unexposed to sound. Progenies were observed on the 3rd day.

Here, three independent experiments were performed in triplicate and the raw data for the outlier is mentioned in appendix.

*p < 0.05, ** p <0.01, *** p <0.001

# 6. Chapter 2

To investigate the effect of sonic stimulation towards toxicity induced by Rotenone (cytotoxic, Mytotoxic), $MnCl_2$ (neurotoxicity), Ivermectin (anthelmintic) and Benzimidazole (anthelmintic) on host *Caenorhabditis elegans.*

## 6.1 $MnCl_2$ preparation: -

- 1 M $MnCl_2$ was prepared in sterile distilled water. When $MnCl_2$ was added in S buffer precipitates were formed. So, the solution was stored at 22˚C for 24 h.
- Precipitates were separated using centrifugation at 8000 rpm for 10 minutes. Precipitates were dried at 70˚C in hot air oven.
- Then actual concentration was calculated and used.

## 6.2 Preparation for an *in vivo* assay

- The experiment was carried out in sterile untreated 24 well polystyrene plates (HiMedia).
- Synchronized and gnotobiotic worms in there L3 - L4 stage were collected in a sterile microfuge tube (1.5 mL) using S buffer from NGM plate.
- After adding 5 to 10 µL S buffer containing worms, the plates were examined under microscope (4X magnification) while the lid was still on.
- Worms were counted in the individual well; each well should have 10 ± 1 worms.
- $MnCl_2$ concentration 25 mM and 50 mM were added to 24 well plate containing S buffer. S buffer was used as control (wells without worms).
- Using a light microscope (4X magnification), live and dead worms were counted daily for 5 days during incubation at 22˚ C.

## 6.3 Rotenone (Merck)

## Preparation for an *in vivo* assay: -

- From main stock, working stocks of concentration 2 µM, 3 µM, 4 µM and 5 µM were prepared.
- Working stocks of Rotenone (Merck) were the stored at -80˚C
- DMSO (0.5% v/v) was used as vehicle control.
- After the preparation of working stocks, 24-well plate was prepared for different concentrations of rotenone.

- Each well contains 10 ± 1 worms. In 24-well plate, three wells contain 10 worms in 1000 µL M9 buffer that is unchallenged *C. elegans.*
- Experiment was performed in triplicates. Three wells contain 10 worms in 995 µL M9 buffer mixed with DMSO (0.5% v/v).
- Three wells contain 10 worms in 995 µL M9 buffer mixed with rotenone (0.5% v/v) having different concentration.
- Incubation was carried out at 22˚ C and live and dead worms were counted till five days under the light microscope (4X magnification).

## 6.4 Results and discussion

➢ **'OM'**

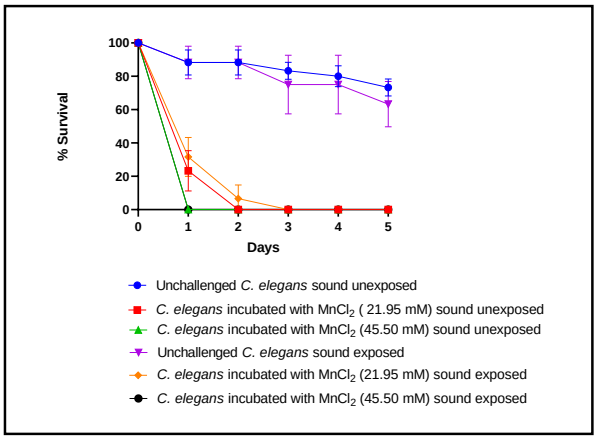



**Fig 10. Sonic stimulation (OM, 60.3 - 86.1 dB) did not alter the toxicity of $MnCl_2$ towards *C. elegans.***

$MnCl_2$ concentration 45.5mM is more toxic towards *C. elegans* as compare to 21.95 mM concentration of $MnCl_2$.

Here, two independent experiments were performed in triplicate and the raw data for the outlier is mentioned in appendix.

➢ ***Raga Piloo***

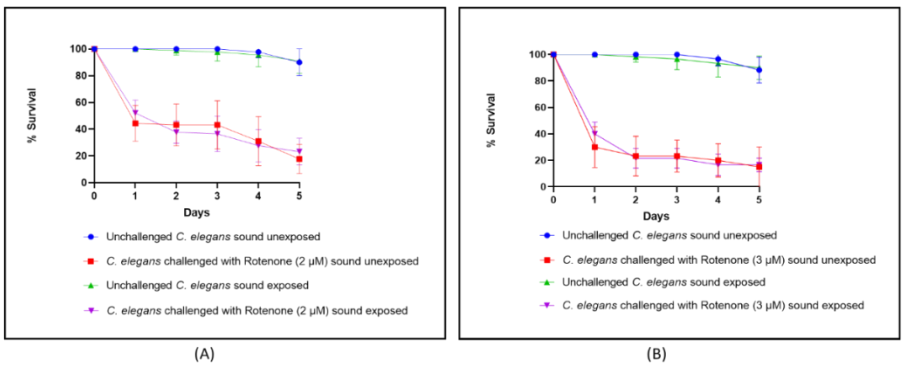


**Fig 11. Sonic stimulation (*Raga Piloo*, 60.5 - 99.4 dB) did not alter the toxicity of Rotenone towards *C. elegans*.**

*Raga Piloo* with intensity 60.5 - 99.4 dB was used as test frequency. This graph is mean of 3 independent experiments showing no therapeutic effect on challenged *C. elegans*.

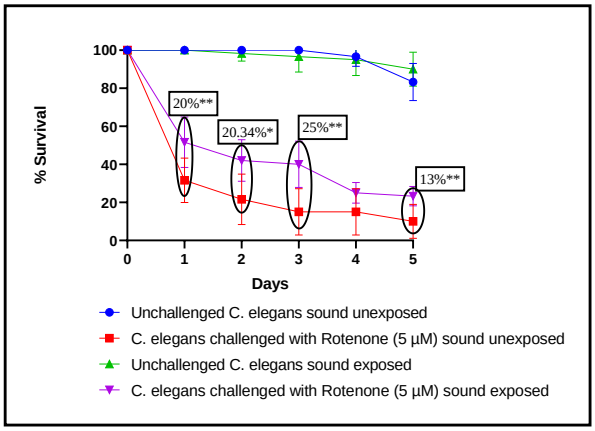



**Fig 12. Sonic stimulation (*Raga Piloo*, 60.5 - 99.4 dB) reduces the toxicity of Rotenone towards *C. elegans*.**

In presence of sonic stimulation (*Raga Piloo*), *C. elegans* challenged with Rotenone has shown significant survival benefit.

Below table represents mean and standard deviation of C. *elegans* challenged with Rotenone unexposed and exposed to sound.

| Sound type | Day | Control | Experimental | % difference |
|---|---|---|---|---|
| *Raga Piloo* | 1 | 31.6 ± 11.6 | 51.66 ± 13.2 | 20 ± 13.3** ↑ |
| | 2 | 21.6 ± 13.2 | 42 ± 10.9 | 20.34 ± 10.9* ↑ |
| | 3 | 15 ± 12.2 | 40 ± 12.2 | 25 ± 12.2** ↑ |
| | 4 | 15 ± 12.2 | 25 ± 5.4 | 10 ± 5.4 ↑ |
| | 5 | 10 ± 8.9 | 23.33 ± 5.1 | 13 ± 5.1** ↑ |

Here, two independent experiments were performed in triplicate and the raw data for the outlier is mentioned in appendix.

* p <0.05, ** p <0.01

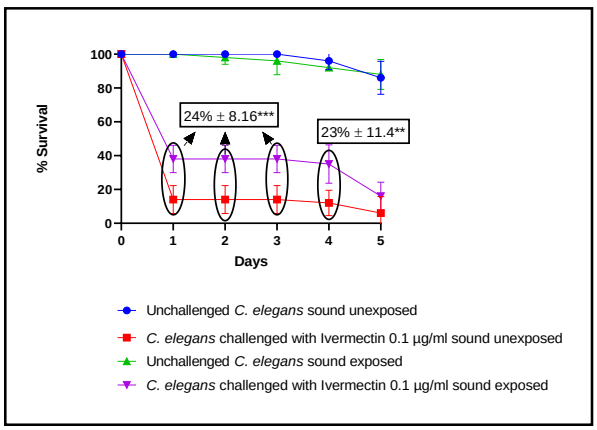


**Fig 13. Sonic stimulation (*Raga Piloo*, 60.5 – 99.4 dB) did reduce the toxicity of Ivermectin towards *C. elegans.***

In presence of sonic stimulation (*Raga Piloo*), *C. elegans* challenged with Ivermectin has shown significant survival benefit of 24% ± 8.1 on 1st, 2nd, 3rd day and 23% ± 11.4 on 4th day as compared to *C. elegans* challenged with Ivermectin unexposed to sound

| Sound type | Day | Control | Experimental | % difference |
|---|---|---|---|---|
| *Raga Piloo* | 1 | 14 ± 8.3 | 38 ± 8.16 | 24 ± 8.1*** ↑ |
| | 2 | 14 ± 8.3 | 38 ± 8.16 | |
| | 3 | 14 ± 8.3 | 38 ± 8.16 | |
| | 4 | 12 ± 7.5 | 35 ± 11.4 | 23 ± 11.4** ↑ |
| | 5 | 6 ± 9.8 | 16 ± 8.3 | 10 ± 8.3 ↑ |

Here, two independent experiments were performed in triplicate and the raw data for the outlier is mentioned in appendix.

* p < 0.05, ***p < 0.001

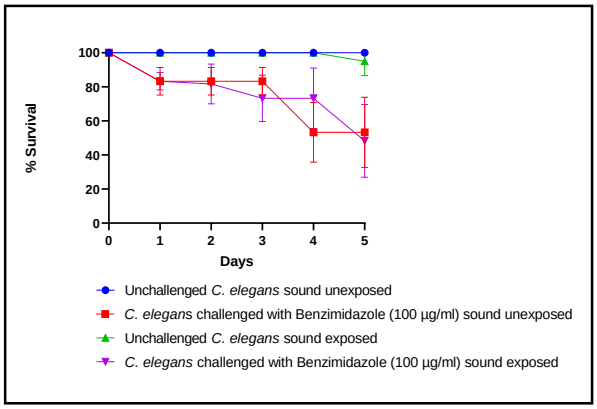



**Fig 14. Sonic stimulation (*Raga Piloo*, 60.5 – 99.4 dB) did not alter the toxicity of Benzimidazole towards *C. elegans.***

Sonic stimulation of *Raga Piloo* with intensity 60.5 – 99.4 dB was use

d as test frequency. This graph is mean of 3 independent experiments showing no therapeutic effect on challenged *C. elegans*.

➢ **432 Hz**

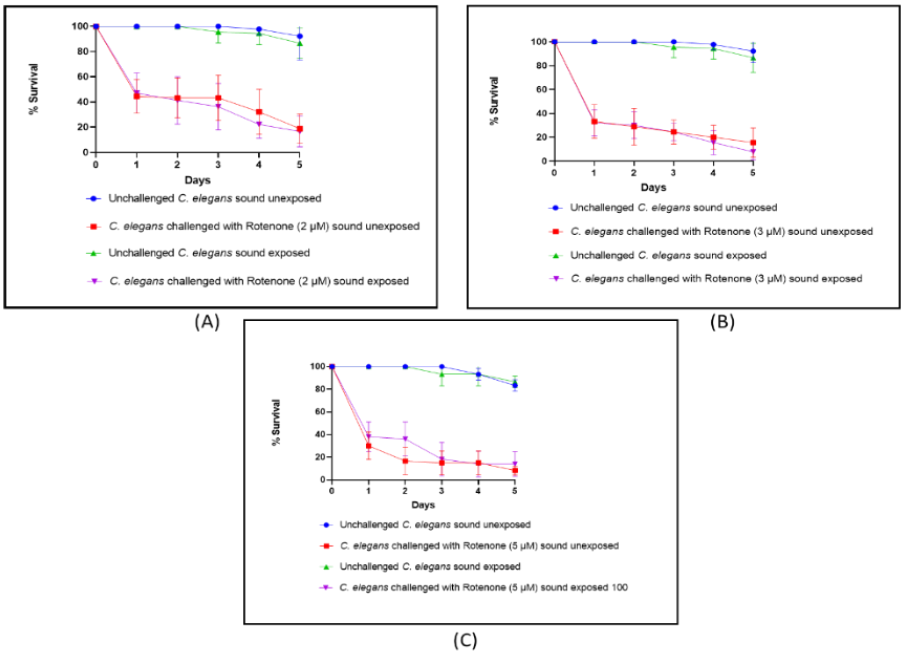



**Fig 15. Sonic stimulation (432 Hz, 85.5dB) did not alter the toxicity of Rotenone towards *C. elegans.***

Sonic stimulation of 432 Hz with intensity 85.5 dB was used as test frequency. This graph is mean of 3 independent experiments showing no therapeutic effect on challenged *C. elegans*.

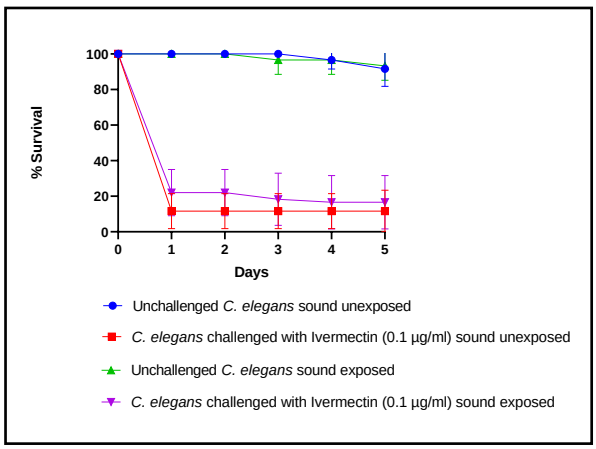
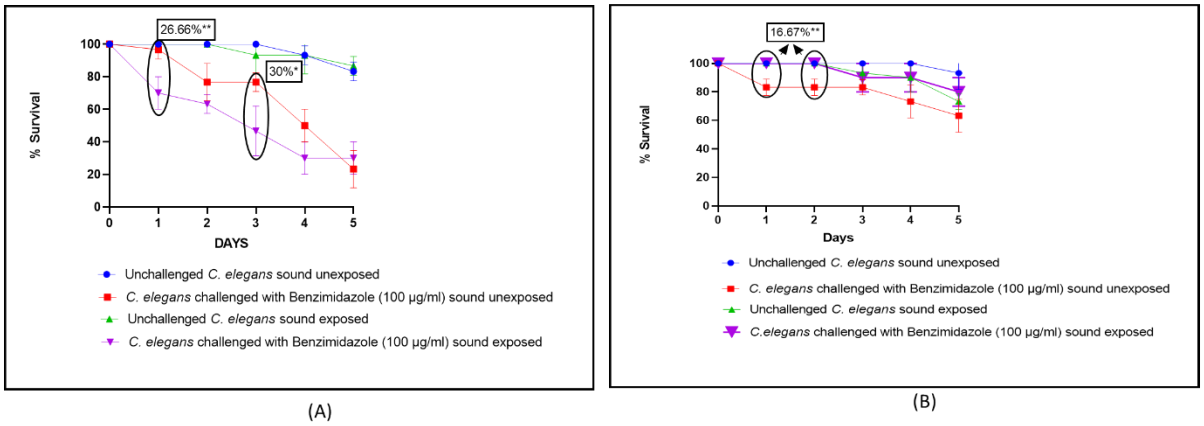



**Fig 16. Sonic stimulation (432 Hz, 85.5dB) did not alter the toxicity of Ivermectin towards *C. elegans.***

Sonic stimulation of 432 Hz with intensity 85.5 dB was used as test frequency. This graph is mean of 2 independent experiments showing no therapeutic effect on challenged *C. elegans*.




(A) (B)

**Fig 17. Sonic stimulation (432 Hz, 85.5dB) did alter the toxicity of Benzimidazole towards *C. elegans*.**

This graph represents two independent experiments where *C. elegans* were challenged with Benzimidazole. Graph (A) shows negative significant difference and Graph (B) shows positive significant difference. Therefore, this experiment needs to be reproduced

## Experiments which has shown positive and negative result

| | *Serratia marcescens* | *Escherichia coli* | Rotenone | $MnCl_2$ | Ivermectin | Benzimidazole |
|---|---|---|---|---|---|---|
| 400Hz | Yes | No | - | - | - | - |
| Mixed frequency | Yes | - | - | - | - | - |
| OM | | - | - | No | - | - |
| *Raga piloo* | - | - | Yes | - | Yes | No |
| 432Hz | - | - | No | - | No | Yes |

## Discussion:-

Regardless of microorganisms that don't have any auditory cell components or organs, there are few studies suggesting microbial growth and metabolism getting influenced by sound (Kothari et al., 2016; Aggio et al., 2012; Sarvaiya & Kothari., 2015; Bourdeau et al., 2018). Also, there are reports suggesting two Ragas:- *Malhar* and *Piloo* to affecting growth, metabolism and antibiotic susceptibility to the different organisms (Bourdeau et al., 2018).

When OM sound at intensity 60.3 - 86.1 dB was given to *C. elegans* challenged with $MnCl_2$ and compared with *C. elegans* challenged with $MnCl_2$ unexposed to sound, no significant difference was observed (Figure 10).

When *Raga Piloo* at intensity 60.5 – 99.4 dB was given to *C. elegans* challenged with Rotenone (5 µM) and compared with *C. elegans* challenged with Rotenone (5 µM) unexposed to sound, significant survival benefit of 20%, 20.34% and 25% was observed on $1^{st}$ ,$2^{nd}$ and $3^{rd}$ day respectively (Figure 13).

*Raga Piloo* also shows survival benefit of 24%, 24% and 18% on $1^{st}$, $2^{nd}$ and $3^{rd}$ day respectively when *C. elegans* were challenged with Ivermectin (0.1 µg/ml) (Figure 13).

Similarly, when mono-frequency of 432 Hz was given to *C. elegans* challenged with different chemical compounds (Rotenone and Ivermectin) no significant difference was observed between *C. elegans* challenged with chemical compounds exposed to sound and *C. elegans* challenged with chemical compounds unexposed to sound. But when *C. elegans* were challenged with Benzimidazole two different significant results were observed (Figure 17). Reproducibility for this experiment needs to be done.

## Conclusion

Above results illustrate that not all sonic stimulations are capable of exerting any sort of effect on microorganisms or certain toxic compounds challenged to worms. This study indicated that certain sonic frequencies (e.g., 400 Hz, 700 Hz, 2,000 Hz) can exert some significant therapeutic effect on worms challenged with pathogenic bacteria. *S. marcescens* appear to be a sound-responsive bacterium. Here, mixed frequency was used and it gives survival benefit to *C. elegans* challenged with *S. marcescens*. It is still unknown whether the same protective effect of sonic stimulation can be shown in more complicated model organisms.

When *C. elegans* were challenged with different chemical compounds, we observed that mono-frequency like 432 Hz and poly-frequency like *Raga Piloo* has some significant therapeutic effect on specific concentration. *C. elegans* challenged with Rotenone (5µM) has shown therapeutic effect in presence of sonic stimulation *Raga Piloo* whereas it did not show any therapeutic effect when worms were challenged with other rotenone concentrations (2µM, 3µM).

Test sound *Raga piloo* can exert significant positive effect on worms challenged with ivermectin while test sound 432Hz frequency did not show any positive effect on worms challenged with ivermectin.

## 7. References


1. "The 432 Player – Music the Natural Way https://www.432player.com/
2. Abongwa, M., Martin, R. J., & Robertson, A. P. (2017). A brief review on the mode of action of antinematodal drugs. Acta veterinaria, 67(2), 137-152.
3. Aggio, R. B. M., Obolonkin, V., & Villas-Bôas, S. G. (2012). Sonic vibration affects the metabolism of yeast cells growing in liquid culture: a metabolomic study. *Metabolomics*, *8*, 670-678.
4. Bourdeau, R. W., Lee-Gosselin, A., Lakshmanan, A., Farhadi, A., Kumar, S. R., Nety, S. P., & Shapiro, M. G. (2018). Acoustic reporter genes for non invasive imaging of microorganisms in mammalian hosts. *Nature*, *553*(7686), 86-90.
5. Calamassi, D., & Pomponi, G. P. (2019). Music tuned to 440 Hz versus 432 Hz and the health effects: a double-blind cross-over pilot study. *Explore*, *15*(4), 283-290.
6. Chen, P., Chakraborty, S., Peres, T. V., Bowman, A. B., & Aschner, M. (2015). Manganese-induced neurotoxicity: from *C. elegans* to humans. Toxicology research, 4(2), 191-202
7. Dadgostar, P. (2019). Antimicrobial resistance: implications and costs. *Infection and drug resistance*, 3903-3910.
8. Dengg, M., & van Meel, J. C. (2004). Caenorhabditis elegans as model system for rapid toxicity assessment of pharmaceutical compounds. *Journal of pharmacological and toxicological methods*, *50*(3), 209–214. https://doi.org/10.1016/j.vascn.2004.04.002
9. Gangadhar, P., Chougala, B., & Laxmeshwar, B. (2018). How to chAnt Om mAntRA And its benefits.
10. Gupta, R. C. (2012). Rotenone. In Veterinary Toxicology (pp. 620-623). Academic Press.
11. Gupta, R. K., & Gupta, R. C. (2019). Biomarkers of Blood–Brain Barrier Dysfunction. In Biomarkers in toxicology (pp. 997-1012). Academic Press.
12. Hejazi, A., & Falkiner, F. R. (1997). Serratia marcescens. *Journal of medical microbiology*, *46*(11), 903-912.
13. Holden-Dye L. and Walker R.J. Anthelmintic drugs and nematicides: studies in *Caenorhabditis elegans* (December 16, 2014), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.143.2, http://www.wormbook.org.
14. Holden-Dye, L., & Walker, R. J. (2007). Anthelmintic drugs. WormBook, 1.
15. Hope, I. A. (Ed.). (1999). *C. elegans: a practical approach* (Vol. 213). OUP Oxford.
16. Huttner, A., Harbarth, S., Carlet, J., Cosgrove, S., Goossens, H., Holmes, A., ... & Pittet, D. (2013). Antimicrobial resistance: a global view from the 2013 World Healthcare- Associated Infections Forum. *Antimicrobial resistance and infection control*, *2*(1), 1-13.
17. Iliff, A. J., Wang, C., Ronan, E. A., Hake, A. E., Guo, Y., Li, X., ... & Xu, X. S. (2021). The nematode C. elegans senses airborne sound. *Neuron*, *109*(22), 3633-3646.

18. Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic escherichia coli. *Nature reviews microbiology*, *2*(2), 123-140.
19. Kim, S. B., Jeon, Y. D., Kim, J. H., Kim, J. K., Ann, H. W., Choi, H., ... & Kim, J. M. (2015). Risk factors for mortality in patients with Serratia marcescens bacteremia. *Yonsei medical journal*, *56*(2), 348-354.
20. Koelsch, S., & Stegemann, T. (2012). The brain and positive biological effects in healthy and clinical populations.
21. Kothari, V., Gajera, G., Kalla, A., Zaveri, H., Sanandiya, P., & Patel, P. (2019). Sonic stimulation at certain frequencies can confer limited protection on nematode host infected with *Serratia marcescens*.
22. Kothari, V., Patel, P., Joshi, C., Mishra, B., Dubey, S., & Mehta, M. (2016). Quorum sensing modulatory effect of sound stimulation on Serratia marcescens and Pseudomonas aeruginosa. *bioRxiv*, 072850.
23. Li, J., & Le, W. (2013). Modeling neurodegenerative diseases in Caenorhabditis elegans. *Experimental neurology*, *250*, 94-103.
24. Lucchini, R., Placidi, D., Cagna, G., Fedrighi, C., Oppini, M., Peli, M., & Zoni, S. (2017). Manganese and developmental neurotoxicity. Neurotoxicity of Metals, 13-34.
25. Martins, Airton Cunha et al. "Caenorhabditis elegans as a Model to Study Manganese-Induced Neurotoxicity." Biomolecules 12 (2022): n. pag.
26. McCavera, Samantha et al. "An ivermectin-sensitive glutamate-gated chloride channel from the parasitic nematode Haemonchus contortus." *Molecular pharmacology* vol. 75,6 (2009): 1347-55. doi:10.1124/mol.108.053363
27. Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic escherichia coli. *Clinical microbiology reviews*, *11*(1), 142-201.
28. O'Neal, S. L., & Zheng, W. (2015). Manganese toxicity upon overexposure: a decade in review. *Current environmental health reports*, *2*, 315-328
29. Patel, P., Patel, H., Vekariya, D., Joshi, C., Patel, P., Muskal, S., & Kothari, V. (2019). Sonic Stimulation and Low Power Microwave Radiation Can Modulate Bacterial Virulence Towards Caenorhabditis elegans. *Anti-Infective Agents*, *17*(2), 150-162.
30. Rosenberg, R. E. (2021). Perfect pitch: 432 hz music and the promise of frequency. *Journal of Popular Music Studies*, *33*(1), 137-154.
31. Roth, E. A., & Wisser, S. (2004). Music therapy: the rhythm of recovery. *The case manager*, *15*(3), 52-56.
32. Sarkar, J., & Biswas, U. (2015). Indian classical ragas to cure diseases. Int. J. Adv. Sci. Res, 1(1), 9-13.
33. Sarvaiya, N., & Kothari, V. (2015). Effect of audible sound in form of music on microbial growth and production of certain important metabolites. *Microbiology*, *84*, 227-235.
34. Sepúlveda-Crespo, D., Reguera, R. M., Rojo-Vázquez, F., Balaña-Fouce, R., & Martínez-Valladares, M. (2020). Drug discovery technologies: Caenorhabditis elegans as a model for anthelmintic therapeutics. Medicinal research reviews, 40(5), 1715-1753.
35. Stiernagle, Theresa. "Maintenance of C. elegans." (1999): 51-67.
36. Surlya, B. K., & Jain, M. (2021). To evaluate the effect of OM Mantra chanting along with Anulom Vilom pranayama on medical and paramedical students. *Scholars international journal of anatomy and physiology 4*(3), 38-43
37. Taki, A. C., Byrne, J. J., Boag, P. R., Jabbar, A., & Gasser, R. B. (2021). Practical high-throughput method to screen compounds for anthelmintic activity against Caenorhabditis elegans. Molecules, 26(14), 4156.

- Raw data pretaining to figure-4

| *C. elegans* challenged with *Serratia marcescens* at different OD | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DAY 1 | | | DAY 2 | | | DAY 3 | | | DAY 4 | | | DAY 5 | | |
| Unchallenged *C. elegans* | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *C. elegans* challenged with *Serratia marcescens* at $OD_{764}$ 1.50 (10% v/v) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. elegans* challenged with *Serratia marcescens* at $OD_{764}$ 1.20 (10% v/v) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. elegans* challenged with *Serratia marcescens* at $OD_{764}$ 1.00 (10% v/v) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. elegans* challenged with *Serratia marcescens* at $OD_{764}$ 0.75 (10% v/v) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. elegans* challenged with *Serratia marcescens* at $OD_{764}$ 1.50 (5% v/v) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

- Raw data pretaining to Figure-5

| *C. elegans* challenged with *Serratia marcescens* in presence of different antibiotics concentration | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DAY 1 | | | DAY 2 | | | DAY 3 | | | DAY 4 | | | DAY 5 | | |
| Unchallenged *C. elegans* | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *C. elegans* challenged with *S. marcescens* at $OD_{764}$ 1.50 (10%v/v) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. elegans* challenged with *S. marcescens* in presence of Ciprofloxacin (1 µg/ml) | 50 | 60 | 40 | 0 | 20 | 20 | 0 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. elegans* challenged with *S. marcescens* in presence of Ciprofloxacin (3 µg/ml) | 70 | 70 | 80 | 70 | 30 | 30 | 70 | 30 | 30 | 70 | 20 | 30 | 40 | 10 | 20 |
| *C. elegans* challenged with *S. marcescens* in presence of Ciprofloxacin (5 µg/ml) | 90 | 80 | 90 | 70 | 70 | 60 | 70 | 60 | 60 | 60 | 50 | 60 | 60 | 50 | 60 |
| *C. elegans* challenged with *S. marcescens* in presence of Gentamicin (5 µg/ml) | 60 | 70 | 50 | 60 | 70 | 50 | 40 | 10 | 0 | 30 | 10 | 0 | 20 | 10 | 0 |
| *C. elegans* challenged with *S. marcescens* in presence of Gentamicin (7 µg/ml) | 50 | 70 | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. elegans* challenged with *S. marcescens* in presence of Gentamicin (10 µg/ml) | 70 | 70 | 60 | 20 | 20 | 0 | 20 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

- Raw data pretaining to Figure-6

| Glycerol supplemented in media enhance the virulence of *S. marcescens*. | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DAY 1 | | | DAY 2 | | | DAY 3 | | | DAY 4 | | | DAY 5 | | |
| Unchallenged C.elegans | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *C.elegans* challenged with *S.marcescens* (NB+G) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C.elegans* challenged with *S.marcescens* (NB) | 80 | 70 | 70 | 40 | 50 | 60 | 30 | 40 | 50 | 30 | 30 | 20 | 30 | 30 | 20 |

- Raw data pretaining to Figure-7

| *C. elegans* challenged with *E. coli* at different OD | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DAY 1 | | | DAY 2 | | | DAY 3 | | | DAY 4 | | | DAY 5 | | |
| Unchallenged *C. elegans* | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *C. elegans* challenged with *E. coli* at $OD_{625}$ 1.20 | 70 | 50 | 60 | 60 | 50 | 60 | 40 | 30 | 60 | 40 | 30 | 60 | 40 | 20 | 60 |
| *C. elegans* challenged with *E. coli* at$OD_{625}$ 1.50 | 60 | 60 | 0 | 60 | 60 | 0 | 30 | 50 | 0 | 30 | 40 | 0 | 30 | 40 | 0 |

- Raw data pretaining to Figure-8

| *C. elegans* challenged with *Serratia marcescens* exposed to (400+700+2000 Hz) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Outlier | DAY 1 | | | | | | DAY 2 | | | | | | DAY 3 | | | | | | DAY 4 | | | | | | DAY 5 | | | | | |
| | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *C. elegans* challenged with *Serratia marcescens* sound unexposed $OD_{764}$ 1.50 | 80 | 90 | 70 | 90 | 90 | 80 | 80 | 80 | 70 | 90 | 80 | 80 | 70 | 80 | 60 | 80 | 70 | 80 | 50 | 45 | 40 | 30 | 50 | 50 | 40 | 50 | 30 | 50 | 40 | 50 |
| Unchallenged *C. elegans* sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *C. elegans* challenged with *Serratia marcescens* sound exposed $OD_{764}$ 1.50 | 80 | 100 | 80 | 90 | 90 | 100 | 80 | 80 | 80 | 90 | 90 | 100 | 70 | 80 | 80 | 90 | 90 | 90 | 60 | 80 | 70 | 80 | 60 | 70 | 60 | 80 | 70 | 80 | 65 | 70 |

- Raw data pretaining to Figure-9

| *C. elegans* challenged with *E. coli* exposed to 400hz | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Outlier | DAY 1 | | | | | | | | | DAY 2 | | | | | | | | | DAY 3 | | | | | | | | | DAY 4 | | | | | | DAY 5 | | | | | |
| | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *C. elegans* challenged with *E. coli* sound unexposed $OD_{625}$ 1.50 | 60 | 60 | 60 | 90 | 70 | 90 | 60 | 60 | 70 | 60 | 60 | 40 | 80 | 70 | 90 | 60 | 60 | 70 | 30 | 50 | 40 | | 60 | 90 | 60 | 60 | 70 | 30 | 40 | 35 | | 60 | 90 | 40 | 50 | 60 | 30 | 40 | 35 | | 50 | 90 | 40 | 40 | 60 |
| Unchallenged *C. elegans* sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *C. elegans* challenged with *E.coli* sound exposed $OD_{625}$ 1.50 | 60 | 70 | 70 | 70 | 80 | 80 | 60 | 70 | 80 | 40 | 70 | 60 | 70 | 80 | 80 | 60 | 70 | 80 | 40 | 70 | 40 | 70 | 50 | 70 | 60 | 70 | 70 | 50 | 50 | 20 | 60 | 50 | 70 | 50 | 50 | 70 | 40 | 40 | 20 | 60 | 50 | 60 | 40 | 60 | 70 |

- Raw data pretaining to Figure-10

| *C. elegans* challenged with $MnCl_2$ exposed to OM sound | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DAY 1 | | | | | | DAY 2 | | | | | | DAY 3 | | | | | | DAY 4 | | | | | | DAY 5 | | | | | |
| | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | |
| Unchallenged *C. elegans* sound unexposed | 80 | 90 | 90 | 80 | 90 | 100 | 80 | 90 | 90 | 80 | 90 | 100 | 80 | 90 | 80 | 80 | 90 | 80 | 80 | 70 | 80 | 80 | 90 | 80 | 80 | 70 | 70 | 80 | 70 | 70 |
| *C. elegans* incubated with $MnCl_2$ 25mM sound unexposed | 20 | 40 | 30 | 30 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. elegans* incubated with $MnCl_2$ 50mM sound unexposed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unchallenged *C. elegans* sound exposed | 90 | 70 | 90 | 90 | 100 | 90 | 90 | 70 | 90 | 90 | 100 | 90 | 100 | 60 | 80 | 60 | 90 | 60 | 100 | 60 | 80 | 60 | 90 | 60 | 80 | 50 | 60 | 80 | 50 | 60 |
| *C.elegans* incubated with $MnCl_2$ 25mM sound exposed | 40 | 30 | 50 | 20 | 30 | 20 | 20 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. elegans* incubated with $MnCl_2$ 50mM sound exposed | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

- Raw data pretaining to Figure-11

| *C. elegans* challenged with Rotenone exposed to *Raag piloo* sound | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (A) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | DAY 2 | | | | | | DAY 3 | | | | | | DAY 4 | | | | | | DAY 5 | | | | | |
| | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *C. elegans* challenged with Rotenone (2 µM) sound unexposed | 50 | 60 | 50 | 50 | 60 | 40 | 20 | 30 | 40 | 50 | 50 | 70 | 40 | 60 | 40 | 20 | 30 | 30 | 50 | 60 | 70 | 40 | 60 | 40 | 20 | 20 | 30 | 30 | 50 | 70 | 30 | 20 | 30 | 20 | 20 | 10 | 10 | 30 | 10 | 30 | 20 | 30 | 20 | 0 | 10 |
| Unchallenged *C. elegans* sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 80 | 90 | 80 | 100 | 100 | 90 | 100 | 100 |
| *C. elegans* challenged with Rotenone (2 µM) sound exposed | 50 | 50 | 40 | 50 | 70 | 60 | 50 | 40 | 60 | 50 | 30 | 30 | 40 | 50 | 30 | 40 | 30 | 40 | 50 | 50 | 30 | 30 | 50 | 40 | 30 | 10 | 40 | 30 | 20 | 10 | 30 | 40 | 40 | 30 | 10 | 40 | 30 | 30 | 20 | 30 | 40 | 20 | 20 | 10 | 10 |
| | (B) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | DAY 1 | | | | | | DAY 2 | | | | | | DAY 3 | | | | | | DAY 4 | | | | | | DAY 5 | | | | | | | | | | | |
| | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | | | | | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 100 | 100 | 100 | 90 | 80 | 80 | 100 | 80 | 100 | | | | | | |
| *C. elegans* challenged with Rotenone (3 µM) sound unexposed | 20 | 20 | 10 | 40 | 40 | 50 | 30 | 10 | 0 | 30 | 40 | 30 | 10 | 10 | 30 | 40 | 30 | 20 | 10 | 10 | 10 | 40 | 20 | 30 | 10 | 0 | 0 | 40 | 20 | 20 | | | | | | |
| Unchallenged *C. elegans* sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 80 | 80 | 100 | 100 | 100 | 80 | 80 | 90 | 90 | 100 | 100 | | | | | | |
| *C. elegans* challenged with Rotenone (3 µM) sound exposed | 50 | 40 | 30 | 50 | 30 | 40 | 30 | 20 | 30 | 20 | 10 | 20 | 30 | 20 | 30 | 20 | 10 | 20 | 20 | 10 | 30 | 10 | 10 | 20 | 20 | 20 | 20 | 10 | 10 | 20 | | | | | | |

- Raw data pretaining to Figure-12

| *C. elegans* challenged with Rotenone exposed to *Raag piloo* sound | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Outlier | DAY 1 | | | | | | DAY 2 | | | | | | DAY 3 | | | | | | DAY 4 | | | | | | DAY 5 | | | | | |
| | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 100 | 100 | 100 | 90 | 80 | 80 | 100 | 80 | 100 |
| *C. elegans* challenged with Rotenone (5 µM) sound unexposed | 10 | 30 | 40 | 30 | 40 | 40 | 0 | 30 | 20 | 20 | 20 | 40 | 0 | 20 | 20 | 0 | 20 | 30 | 0 | 20 | 20 | 10 | 10 | 30 | 0 | 10 | 10 | 0 | 20 | 20 |
| Unchallenged *C. elegans* sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 80 | 80 | 100 | 100 | 100 | 80 | 80 | 90 | 90 | 100 | 100 |
| *C. elegans* challenged with Rotenone (5 µM)sound exposed | 60 | 40 | 30 | 60 | 60 | 60 | 60 | 40 | 30 | 40 | | 40 | 60 | 40 | 30 | 40 | | 30 | 20 | 30 | 30 | 30 | 20 | 20 | 20 | 20 | 30 | 30 | 20 | 20 |

- Raw data pretaining to Figure-13

| *C. elegans* challenged with Ivermectin exposed to *Raag piloo* sound | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Outlier | DAY 1 | | | | | | DAY 2 | | | | | | DAY 3 | | | | | | DAY 4 | | | | | | DAY 5 | | | | | |
| | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 100 | 100 | 100 | 90 | 80 | 80 | 100 | 80 | 100 |
| *C. elegans* challenged with Ivermectin (0.1 µg/ml) sound unexposed | 20 | 10 | 0 | 20 | 20 | 20 | 20 | 10 | 0 | 20 | 20 | 20 | 20 | 0 | 10 | 20 | 20 | 20 | 20 | 0 | 10 | 20 | 10 | 10 | 20 | 0 | 0 | 10 | 0 | 20 |
| Unchallenged C. elegans sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 80 | 80 | 100 | 100 | 100 | 80 | 80 | 90 | 90 | 100 | 100 |
| *C. elegans* challenged with Ivermectin (0.1 µg/ml) sound exposed | 30 | 40 | 30 | 50 | 40 | 30 | 30 | 40 | 30 | 50 | 40 | 30 | 30 | 40 | 30 | 50 | 40 | 30 | 30 | 20 | | 50 | 40 | 30 | 30 | 20 | 10 | 10 | 10 | 10 |

- Raw data pretaining to Figure-14

| *C. elegans* challenged with Benzimidazole exposed to *Raag piloo* sound | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DAY 1 | | | | | | DAY 2 | | | | | | DAY 3 | | | | | | DAY 4 | | | | | | DAY 5 | | | | | |
| | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 80 | 100 |
| *C. elegan* s challenged with Benzimidazole (100 µg/ml) sound unexposed | 90 | 90 | 70 | 80 | 80 | 90 | 90 | 90 | 70 | 80 | 80 | 90 | 90 | 90 | 70 | 80 | 80 | 90 | 30 | 50 | 40 | 60 | 60 | 80 | 30 | 50 | 40 | 80 | 60 | 80 |
| Unchallenged *C. elegans* sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 90 | 100 | 100 |
| *C. elegans* challenged with Benzimidazole (100 µg/ml) sound exposed | 80 | 80 | 90 | 80 | 90 | 80 | 80 | 80 | 90 | 100 | 70 | 70 | 80 | 70 | 50 | 90 | 80 | 70 | 85 | 75 | 40 | 90 | 80 | 70 | 40 | 30 | 20 | 70 | 60 | 70 |

- Raw data pretaining to Figure-15

| *C. elegans* challenged with Rotenone exposed to 432 Hz | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (A) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Outlier | DAY 1 | | | | | | DAY 2 | | | | | | DAY 3 | | | | | | DAY 4 | | | | | | DAY 5 | | | | | |
| | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 80 | 80 | 100 | 100 | 100 | 100 | 80 | 100 |
| *C. elegans* incubated with Rotenone (2 µM) sound unexposed | 50 | 60 | 50 | 50 | 60 | 40 | 20 | 30 | 40 | 50 | 50 | 70 | 40 | 60 | 40 | 20 | 30 | 30 | 50 | 60 | 70 | 40 | 60 | 40 | 20 | 20 | 30 | 30 | 50 | 70 | 30 | 30 | 30 | 20 | 20 | 10 | 10 | 30 | 10 | 30 | 30 | 30 | 20 | 0 | 10 |
| Unchallenged *C. elegans* sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 80 | 90 | 80 | 90 | 90 | 100 | 100 | 100 | 70 | 70 | 80 |
| *C. elegans* incubated with Rotenone (2 µM) sound exposed | 50 | 60 | 20 | 70 | | 50 | 40 | | 40 | 50 | 60 | 20 | 70 | | 50 | 30 | 30 | 20 | 50 | 60 | 20 | 50 | | 50 | 30 | 10 | 20 | 30 | 30 | 20 | 40 | 10 | 10 | 30 | 10 | 20 | 30 | 20 | 0 | 30 | 10 | 0 | 30 | 10 | 20 |
| | (B) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | DAY 1 | | | | | | DAY 2 | | | | | | DAY 3 | | | | | | DAY 4 | | | | | | DAY 5 | | | | | |
| | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | | SET 1 | | | SET 2 | | | SET 3 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 80 | 80 | 100 | 100 | 100 | 100 | 80 | 100 |
| *C. elegans* incubated with Rotenone (3 µM)sound unexposed | 20 | 20 | 10 | 30 | 40 | 50 | 40 | 40 | 50 | 30 | 10 | 0 | 30 | 40 | 50 | 30 | 40 | 30 | 10 | 10 | 30 | 30 | 20 | 30 | 40 | 30 | 20 | 10 | 10 | 10 | 20 | 20 | 20 | 40 | 20 | 30 | 10 | 0 | 0 | 20 | 10 | 20 | 40 | 20 | 20 |
| Unchallenged *C. elegans* sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 80 | 90 | 80 | 90 | 90 | 100 | 100 | 100 | 70 | 70 | 80 |
| *C. elegans* incubated with Rotenone 3 µM sound exposed | 30 | 10 | 30 | 40 | 30 | 50 | 30 | 30 | 40 | 30 | 10 | 30 | 40 | 30 | 50 | 30 | 20 | 30 | 30 | 10 | 30 | 30 | 20 | 20 | 30 | 20 | 30 | 10 | 0 | 20 | 10 | 10 | 10 | 30 | 20 | 30 | 0 | 0 | 10 | 0 | 10 | 10 | 20 | 10 | 10 |
| | (C) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Outlier | DAY 1 | | | | | | DAY 2 | | | | | | DAY 3 | | | | | | DAY 4 | | | | | | DAY 5 | | | | | |
| | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 100 | 90 | 90 | 90 | 80 | 80 | 90 | 80 | 80 |
| *C. elegans* challenged with Rotenone (5 µM) sound unexposed | 10 | 30 | 40 | 10 | 20 | 30 | 0 | 30 | 20 | 10 | 10 | 30 | 0 | 20 | 20 | 10 | 10 | 30 | 0 | 20 | 20 | 10 | 10 | 30 | 0 | 10 | 10 | 10 | 10 | 10 |
| Unchallenged *C. elegans* sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 80 | 100 | 100 | 100 | 80 | 100 | 100 | 80 | 100 | 80 | 90 | 90 | 80 | 90 | 90 |
| *C. elegans* challenged with Rotenone (5 µM) sound exposed | 30 | | 30 | 30 | 60 | 40 | 30 | 0 | 20 | 30 | 60 | 40 | 30 | 0 | 30 | 20 | 30 | 0 | 10 | 0 | 10 | 20 | 30 | | 20 | 0 | 20 | 20 | 30 | |

- Raw data pretaining to Figure-16

| *C. elegans* challenged with Ivermectin exposed to 432 Hz | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Outlier | DAY 1 | | | | | | DAY 2 | | | | | | DAY 3 | | | | | | DAY 4 | | | | | | DAY 5 | | | | | |
| | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | | SET 1 | | | SET 2 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 100 | 100 | 100 | 90 | 80 | 80 | 100 | 100 | 100 |
| *C. elegans* challenged with Ivermectin (0.1 µg/ml) sound unexposed | 0 | 0 | 0 | 20 | 20 | 20 | 0 | 10 | 0 | 20 | 20 | 20 | 0 | 0 | 10 | 20 | 20 | 20 | 0 | 0 | 10 | 20 | 20 | 20 | 10 | 0 | 0 | 30 | 20 | 10 |
| Unchallenged *C. elegans* sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 80 | 90 | 90 | 100 | 100 | 100 |
| *C. elegans* challenged with Ivermectin (0.1 µg/ml) sound exposed | 20 | | 0 | 30 | 30 | 30 | 20 | | 0 | 30 | 30 | 30 | 20 | 0 | 0 | 30 | 30 | 30 | 0 | 0 | 10 | 30 | 30 | 30 | 0 | 0 | 10 | 30 | 30 | 30 |

- Raw data pretaining to Figure-17

| *C. elegans* challenged with Benzimidazole exposed to 432 Hz | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Outlier | (A) | | | | | | | | | | | | | | |
| | DAY 1 | | | DAY 2 | | | DAY 3 | | | DAY 4 | | | DAY 5 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 90 | 80 | 80 |
| *C. elegans* challenged with Benzimidazole (100 µg/ml) sound unexposed | 100 | 100 | 90 | 90 | 70 | 70 | 70 | 80 | 80 | 50 | 40 | 60 | 10 | 30 | 30 |
| Unchallenged C. elegans sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 100 | 100 | 80 | 100 | 80 | 90 | 90 |
| *C. elegans* challenged with Benzimidazole (100 µg/ml) sound exposed | 70 | 60 | 80 | 70 | 60 | 60 | | 60 | 50 | 20 | 30 | 40 | 20 | 30 | 40 |
| | (B) | | | | | | | | | | | | | | |
| | DAY 1 | | | DAY 2 | | | DAY 3 | | | DAY 4 | | | DAY 5 | | |
| Unchallenged *C. elegans* sound unexposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 |
| *C. elegans* challenged with Benzimidazole (100 µg/ml) sound unexposed | 80 | 80 | 90 | 80 | 80 | 90 | 80 | 80 | 90 | 80 | 60 | 80 | 80 | 60 | 80 |
| Unchallenged *C. elegans* sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 100 | 80 | 90 | 70 | 70 | 80 |
| *C. elegans* challenged with Benzimidazole (100 µg/ml) sound exposed | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 80 | 100 | 80 | 90 | 100 | 80 | 70 | 90 |