Modern Applied Science
www.ccsenet.org/journal.html
Vol. 3, No. 4
April 2009

Experimental Investigation on the Effects of Audible Sound to the Growth of *Aspergillus* Spp

Poopathy Muthu Karippen
School of Science and Technology, Universiti Malaysia Sabah
Lock Bag 2073, 88999 Kota Kinabalu, Sabah, Malaysia
E-mail: pups_leo@yahoo.com

Jedol Dayou
Vibration and Sound Research Group (VIBS), Universiti Malaysia Sabah
Lock Bag 2073, 88999 Kota Kinabalu, Sabah, Malaysia
Tel: 60-88-320-302 Fax: 60-88-435-324 E-mail: jed@ums.edu.my

Chong Khim Phin (Corresponding author)
School of Sustainable Agriculture, Universiti Malaysia Sabah
Lock Bag 2073, 88999 Kota Kinabalu, Sabah, Malaysia
Tel: 60-88-320-000 x5655 E-mail: chongkp@ums.edu.my

Abstract

This paper discusses the effect of sound on the growth of fungus, *Aspergillus* spp. *Aspergillus* was cultured on Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) and exposed to sound waves for five hours per day for three days. This experiment was observed for three days and data were recorded everyday after the exposure. Four parameters were used to measure the growth of fungus which were colony forming units per ml, optical density, length of germ tube and the diameter of the colony. The results showed that sound waves have effects on the growth of *Aspergillus*. When *Aspergillus* was exposed to sound waves with frequencies of 5 kHz, 10 kHz and 15 kHz, the growth was affected by the different frequency. The higher the frequency, the higher the growth inhibition found on *Aspergillus*. All the three frequencies inhibited the growth of *Aspergillus* compared to the control (No exposure to sound) and the maximum inhibition occurred at the frequency of 15 kHz.

Keywords: *Aspergillus*, Inhibition, 5 kHz, 10 kHz, 15 kHz, Sound frequency

1. Introduction

Waves are a common phenomenon in our environment. We are surrounded by sound waves, light waves, water waves and other kinds of waves, which we can control and use to convey information or transport energy from one location to another (Young and Freedman, 1996). Mechanical waves have been shown to have effect on microbes. Ultrasound for example, has been used for sterilizing and killing unwanted bacteria due to thinning of cell membranes, localized heating and production of free radicals (Piyasena et al., 2003). Ultrasound is able to inactivate bacteria and deagglomerate bacterial clusters or flocks through a number of physical, mechanical and chemical effects arising from acoustic cavitations (Joyce et al., 2003).

Sound waves have been used for different types of experiments not only on bacteria but also certain parts of plants that react to the sound waves. Some researchers reported that optimization of *Chrysanthemum* callus growth can be altered with different sound wave frequencies, strength and loading time. In their study, they have concluded that sound wave stimulation significantly affects the production of *Chrysanthemum* callus. The optimum formulation for the relation of the sound wave stimulus and callus growth could be predicted (Jiping, et al., 2003). In another experiment conducted by Bochu et al. (2004) using chrysanthemum callus treated by optimal sound waves (1.4 kHz or 0.095 kdb) they found that callus of chrysanthemum has significantly higher IAA but lower ABA levels than the untreated samples. The effect of

sound stimulation on the metabolism of chrysanthemum roots was also studied and it was found that the growth of roots was not inhibited but accelerated under suitable sound stimulation (Yi et al., 2003).

In another experiment to study the germination index, stem height, relative increase rate of fresh weight, rooting ability, root system activity and the penetrability of cell membrane in paddy found it was found that 400 Hz and 106 dB are the 'best frequency and intensity' for the best stimulation. However when the sound wave stimulation exceeds 4 kHz or 111 dB, it is harmful to the seeds. Considering the advantages that can be altered either to promote or retard the growth of bacteria or certain parts or compounds in plants, we hypothesized that the application of this concept may be appropriate for controlling the growth of fungus especially *Aspergillus* spp, in food storage. In this study, the effect of audible sound of certain frequencies to *Aspergillus was* investigated. The effect was assessed based of the comparison on their, turbidity by turbidimetric method, colony forming unit, germ tube elongation and diameter of colony with their respective control samples.

2. Materials and methods

2.1 Aspergillus spp

Aspergillus spp was originally isolated from one week old bread kept under high humidity in a natural environment. The confirmation of the isolated fungus was done based on their classical morphology. The culture was maintained at 25ºC inside an incubator until needed.

2.2 Dilution Plating Using Spread Plate Technique

Aspergillus spp. was scraped once from the cultivated media and dispersed into 9 ml of sterile water. 1ml of this solution was transferred to a second tube containing 9 ml of sterile water, resulting in a 0.01 dilution of the spore mass in the original material. A 1-ml portion from the dilution was pipette to a separate Petri dish containing cooled agar medium and spread evenly throughout using a spreader. After a day of incubation, colonies appeared in varying densities, depending upon the amount of dilution from the original material. The number of spores present in the original sample was calculated by selecting the plates showing 40-100 colonies. With this information the following calculation was performed:

$$\text{Colonies per gram of original sample} = \frac{\text{Colony count}}{\text{Dilution factor}}$$

For Potato Dextrose Broth (PDB), *Aspergillus* spp was scraped once from the cultivated media and dispersed into 9 ml of sterile water. One ml of this solution was transferred directly into the bottles containing PDB which was prepared earlier. The cultured media was incubated for a day and the growth of fungus was measured using a spectrophotometer, haemocytometer and micrometer.

2.3 Sound Wave Device

An air-tight experimental sound wave chamber (MarJedLV) with the speaker in the middle to provide sound waves was used in this study (Figure 1). A generator was used to generate a different range of frequencies to the treatment. The treated groups were loaded and placed in the device with optimal sound waves conditions within three different ranges. The control treatment was placed without exposure to sound waves. The growth of fungus was observed and data recorded daily.

2.4 Optical Density

A Cary 50 UV/Vis Spectrophotometer was used for comparing the growth of *Aspergillus* spp after the treatments. The PDB was set as blank, and the turbidity among the samples were expressed as Optical Density (OD). The turbidity measurement was correlated with the growth of microbial population (Tortora, et al., 2001).

2.5 Colony Forming Unit (CFU/ml or CFU/g)

The plate count technique was performed to determine the number of cells or cells clumps that are capable of forming colonies on agar plates after exposure to sound waves. These treatments were prepared using the spread plate technique. Knowing the dilution factor, volume plated, and number of colonies on the plate (or average from the duplicate plates), count of microorganisms in the treatment was calculated using the following equation (Ahmed and Carlstrom, 2003):

$$\text{Count (CFU/ml or CFU/g)} = \frac{\text{Average number of colonies from duplicate plates}}{\text{Dilution factor x volume plated}}$$

2.6 Measurement of Germ tube using Micrometer

The elongations of the germ tube after exposed to the different treatments compared to control were measured using a micrometer attached to a Zeiss Axioplan Microscope.

2.7 Diameter of Colony

For each treatment that was prepared using the spread plate technique, and the diameter of 10 colonies for each replicate was taken. The average diameter of colony was recorded. The same method was followed for the other two replicates and data was recorded continuously for five days.

3. Results and discussion

3.1 Optical Density

Mean values of the optical density showed an increase from the start to day three for all four frequencies tested. The mean values of other frequencies showed a lower value compared to the control treatment. Figure 2 clearly shows that the control treatment had the highest growth rate compared to other treatments. There were significant differences between the OD from day one to day three for the different frequencies (except between day one and two for control, frequency 5kHz and 15kHz). There was no significant difference between the OD from the onset of the experiment to day one.

3.2 Colony Forming Units (CFU/ml or CFU/g)

The ability of the treated *Aspergillus* spp. to survive was tested based on the colony forming units per mL (CFU/mL). The control treatment showed the highest growth rate, followed by 5 kHz, 10 kHz and 15 kHz (Figure 3). The growth of fungus was inhibited when higher frequencies were used. Thus, the growth of *Aspergillus* was restricted by the sound effect. But surprisingly, there was no significant difference between the onset of the experiment and day one for both control and 10 kHz. From day two to day three, there was no significant difference between exposure to frequency of 10 kHz and the control. For other recorded data there were significant differences among the control, 5 kHz, 10 kHz and 15 kHz.

3.3 Measurement of Germ Tube Elongation

The mean *Aspergillus* spp. germ tube elongation was observed and measured using a micrometer. The mean values for sound treatments were lower when compared to control (Figure 4). The same increase in the mean values from start to day one was observed for all the frequencies. The slower growths of germ tube were only found after day one for 5 kHz, 10 kHz and 15 kHz (Figure 4). In contrast, the control treatment showed a constant increase in the mean value and had the highest mean value from day one to day three. There was no significant difference between day one and two among the control, 10 kHz and 15 kHz compared to the control. There was also no significant difference between days two and three, for the 5 kHz treatment as compared to control. For other recorded data there were significant differences among the control, 5 kHz, 10 kHz and 15 kHz.

3.4 Diameter of colony

The control treatment showed the highest increase in the mean diameter of colonies when compared to other sound treatments (Figure 5). But surprisingly, there was no significant difference in the growth among the different frequencies in comparison with the control.

4. Conclusion

In general, the findings suggest that sound wave have effects on the growth of *Aspergillus* spp. The higher the frequency used, the higher the chance to inhibit the growth of this fungus. The frequencies of 5 kHz, 10 kHz and 15 kHz showed inhibition on the growth of *Aspergillus*. The maximum inhibition was found at 15 kHz. It is recommended that future research consider using higher frequencies to verify the possibility for a faster inhibition on the growth of fungus. The findings may benefit the food industry whereby food expiry can be slowed down. Future research should also consider some other internal factors that may contribute to inhibiting the growth of fungus.

References

Ahmed E. Yousef and Carlstrom, C. (2003). *Food Microbiology: A Laboratory Manual*. John Wiley & Sons, Inc. Hoboken, New Jersey.

Bochu, W., Jiping, S., Biao, L., Jie, L. and Chuanren, D. (2004). Sound wave stimulation triggers the content change of the endogenous hormone of the Chrysanthemum mature callus**.** *Colloids and Surfaces B: Biointerface*, 37 (3-4): 107-112.

Jiping, S., Bochu, W., Meisheng, L., Hongyang, Z., Xin, C. and Chuanren, D. (2003). Optimal designs for sound wave stimulation on the growth conditions of Chrysanthemum callus. *Colloids and Surfaces B: Biointerfaces*, 30(1-2): 93-98.

Joyce, E., Phull, S.S., Lorimer, J.P. & Mason, T.J. (2003). The development and evaluation of ultrasound for the treatment of bacterial suspensions. A study of frequency, power and sonification time on cultured bacillus species. *Ultrasonics Sonochemistry*, 10(6): 315-318.

Piyasena, P., Mohareb, E. & McKellar, R.C. (2003). Inactivation of microbes using ultrasound: A review. *International Journal of Food Microbiology*, 87(3): 207-216.

Tortora, G. J., Funke, B. R. and Case, C. L. (2001). *Microbiology: An introduction*. 7th edition. Addison Wesley Longman, Inc. United States.

Yi, J., Bochu, W., Xiujuan, W., Daohong, W., Chuanren, D., Toyama, Y.and Sakanishi, A. (2003). Effect of sound wave on the metabolism of chrysanthemum roots. *Colloids and Surfaces B: Biointerface*, 29 (2-3): 115-118.

Young, H. D. and Freedman, A. (1996). *Extended Version with Modern Physics: University Physics*. 9th Edition. Addison-Wesley Publishing Company, Inc., United States.

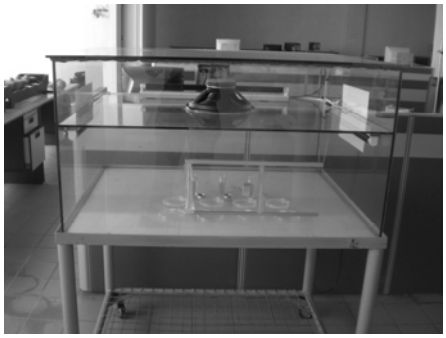

Figure 1. The sound wave chamber (MarJedLV)

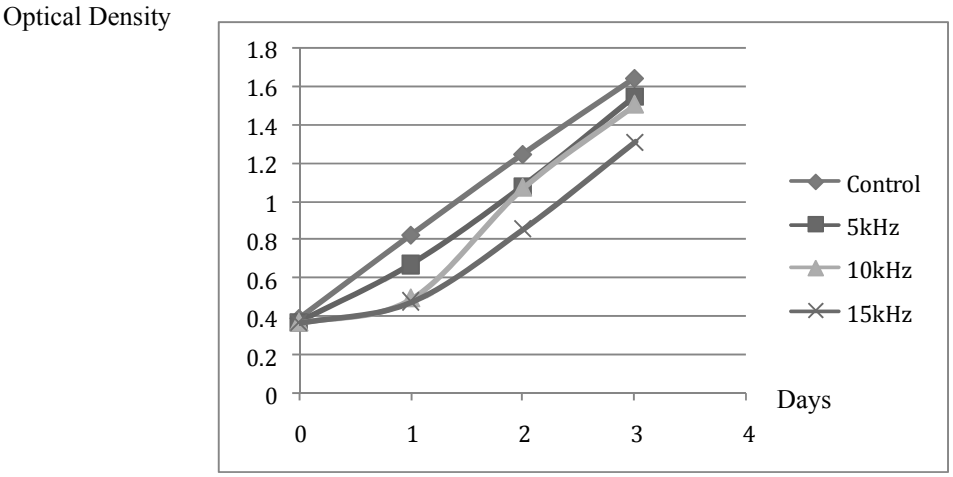

Figure 2. Effect of the different frequencies on the optical density of *Aspergillus* spp

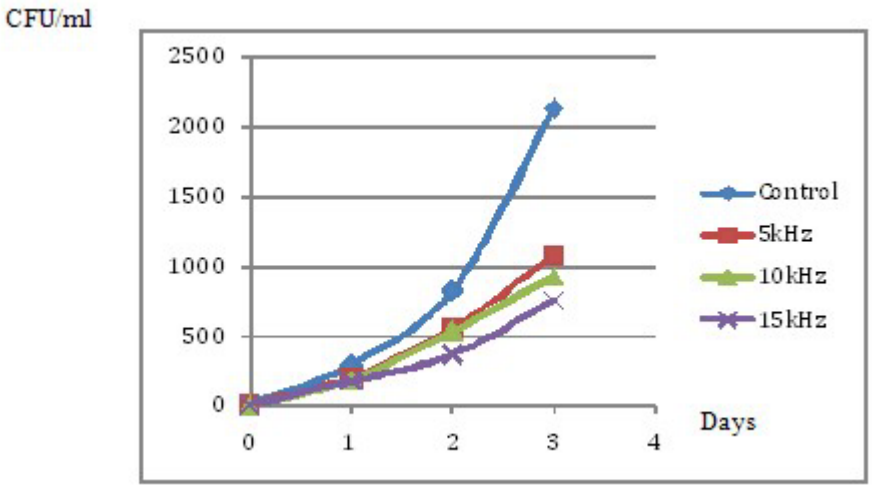

Figure 3. The effect of different frequencies on the colony forming units of *Aspergillus* spp

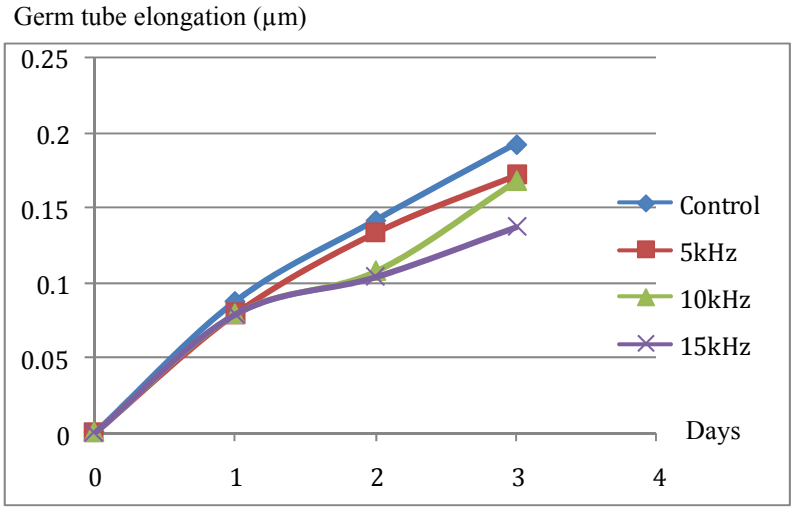

Figure 4. The effect of the different frequencies on germ tube elongation of *Aspergillus* spp

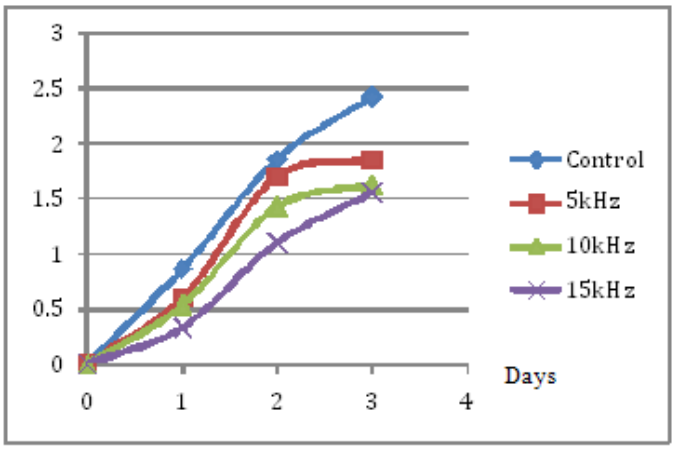

Figure 5. The effect of different frequencies on the mean diameter of colonies of Aspergillus spp