Effect of lemongrass extraction on *Aspergillus niger* fungus

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Abstract

In this project we intend to find out how effective lemongrass extract is in inhibiting the growth of *Aspergillus Niger* fungus. We prepared our own lemongrass extract and Potato Dextrose Agar, cultivated *Aspergillus Niger* fungus and finally, tested the lemongrass extracts on the fungus. We found out that lemongrass extract does indeed slow down the growth of *Aspergillus niger* fungus, showing that this may be the case, too, for its cousins.

1. Introduction

Aspergillus Niger (**Fig. 1**) is one of the most common species of the genus Aspergillus. It is commonly used to produce citric acid and keep canned and jarred foods fresh over long periods of time. However, it may cause contamination of fruits and food, and can be dangerous when consumed. Nevertheless, it serves an important purpose both medically and commercially.

Fig. 1 *Aspergillus niger*

Bhuvaneshwari, L et al (2007) suggested a method to prepare plant extracts. We eventually also used this method to prepare our lemongrass extracts which we used for our experiment. It is stated that "Cold maceration of the samples were carried out by soaking 2g of the sample in 15ml solvent", allowing us to find a good concentration of the solvent we should use.

Vogel, H.J. (1956) showed us a method to grow fungi in their research paper which we used to cultivate samples of *Aspergillus niger* fungi that we needed. It states that "Place a loop of spores from a stock spore solution into the middle of a culture plate. Incubate at 30 to 37°C for 5-10 days with periodic checking." This research paper gave us the temperature we should incubate the fungi at and for how long it should be incubated.

2. Objectives and Hypotheses

We aim to investigate the effectiveness of lemongrass extract in the inhibition of *Aspergillus niger* growth.

Our hypothesis is: “Lemongrass extract helps to slow down Aspergillus niger growth”

3. Methods and Materials

i. Collection and preparation of lemongrass extract

Lemongrass leaves were purchased and the moisture was removed by leaving them to dry for 5 days. Leaves were crushed to powder with a grinder to make dissolution in ethanol easier. Following the guidelines of the research paper referenced, 2 grams of powder was mixed with 15ml of ethanol. Prior to filtering, the mixture was left to stand for 2 days with periodic shaking. The extract used in the experiments is the filtrate, while the residue was disposed of properly.

ii. Preparation of Potato Dextrose (PD) Agar

9.5 grams of PD extract was mixed with 500ml of water, following which it was autoclaved at 121ºC

iii. Cultivation of *Aspergillus niger*

First, a mixture of phosphate-buffered saline (PBS) was prepared by using commercial PBS tablets to maintain a pH level, an autoclave to control the environment and some polysorbate 80 for an emulsifier. After that, 8 ml of the PBS mixture was poured into a flask with glass beads in it and the lid was closed tightly. The flask was shaken vigorously until the spores were dislodged. The spores were removed using a Pasteur pipette and stored at 4°C. Afterwards, the spores were filtered through lens tissue to remove hyphal fragments. Then, 50 m*l* of Potato Dextrose Agar was poured (see Stage **ii** above) into each of 9 260 m*l* tissue culture plates and left it to set. Finally, a loop of spores that had been obtained earlier was placed into the middle of a culture plate.

iv. Testing of lemongrass extract on *Aspergillus Niger*

Using the culture plates prepared in Step **iii**,

- At the center of 3 culture plates, 500µl of lemongrass extract and 500µl of ethanol was dripped in each plate. These are the **treatment groups**.
- At the center of another 3 culture plates, we 500µl of ethanol only was dripped in each plate. These are the **positive control groups**.
- At the center of the remaining 3 culture plates, 500µl of water was dripped in each plate. These are the **negative control groups**.

Finally, the culture plates were incubated at 30 to 37°C for 5-10 days with periodic checking every 48 to 96 hours (i.e. 4-6 days) and were examined for contamination.

v. Collection of results

A table was constructed (**Fig. 2**) to record our results. We would measure the area of inhibition in each sample under different conditions and fill in the respective cells representing that sample. If needed, we would repeat previous steps.

Temperature ranges (°C)	Range of pH levels (pH)		
	1~6	7	8~14
14 ~ 24			
25 ~ 32			
33 ~ 40			

Fig. 2 *The table that we intend to use for data collection.*

4. Results and Discussion

Results (Assuming π = 3.14):

[Note: the area of growth was measured instead of the area of inhibition, which could not be measured.]

Experiment 1:

6.68 ml of lemongrass extract was mixed in 50.1 ml of ethanol.

	Treatment	Positive Control	Negative Control
Average Area of Growth / cm^2 (3.s.f.)	20.3	17.6	27.0

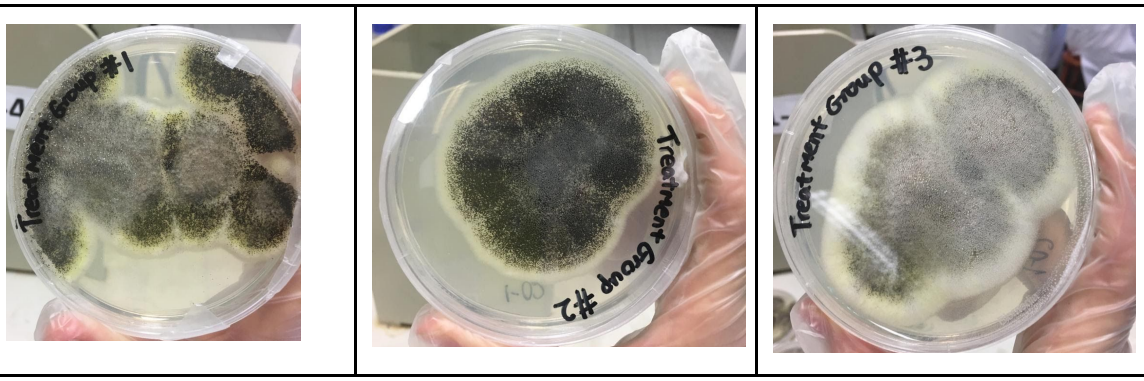

Fig. 3(a) *Treatment groups for first attempt*

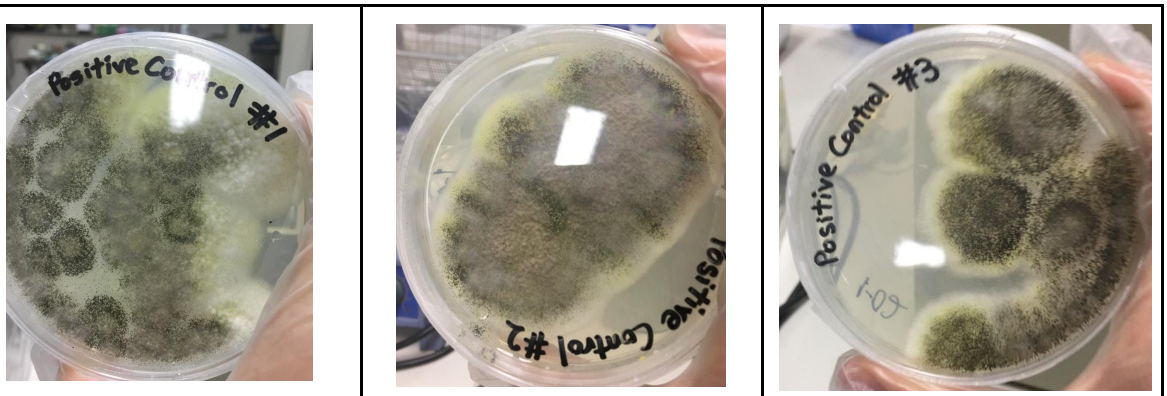

Fig. 3(b) *Positive control groups for first attempt*

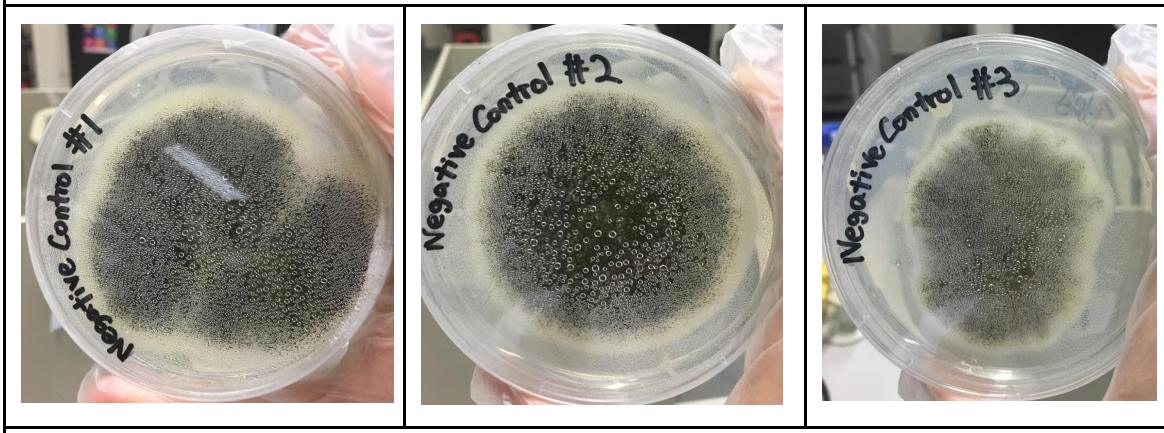

Fig. 3(c) *Negative control groups for first attempt*

Experiment 2:
We put 7.56g of lemongrass in 50.1m*l* of ethanol. The ratio of lemongrass to ethanol to make the extract was increased to 1:6.6. No results were able to be obtained.

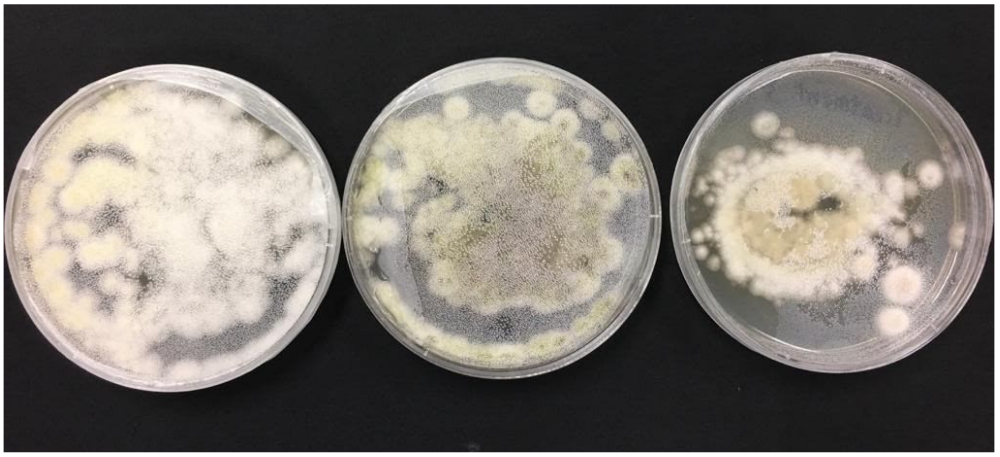

Fig. 4(a) *Treatment groups for second attempt*

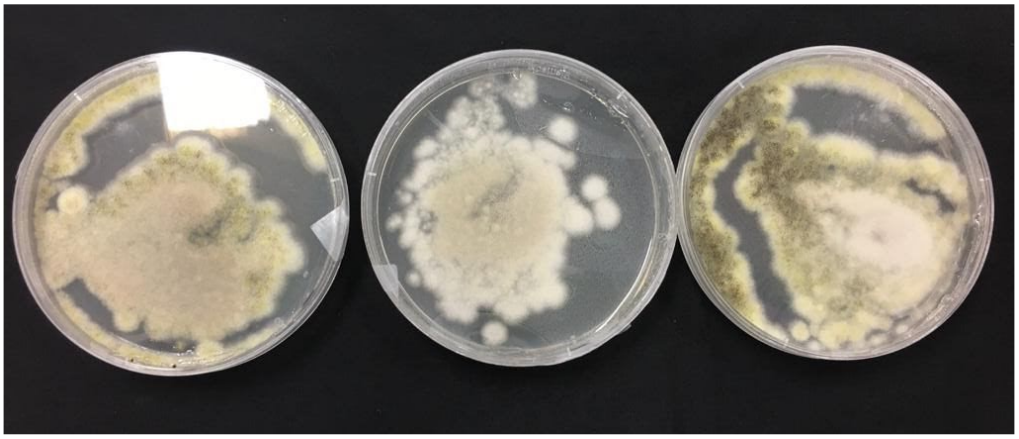

Fig. 4(b) *Positive control groups for second attempt*

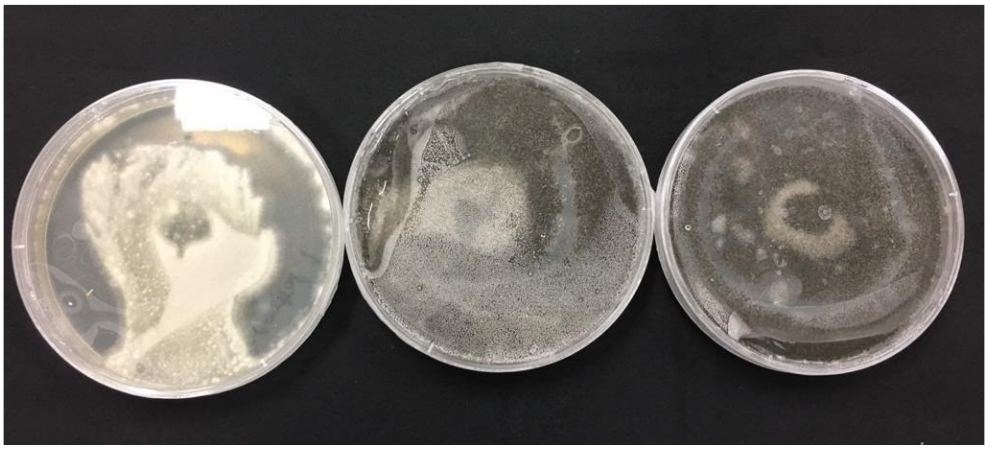

Fig. 4(c) *Negative control groups for second attempt*

Discussion:

First attempt:

- In small amounts, lemongrass extract is unable to completely stop the growth of the *Aspergillus niger* fungus. It was only successful in slowing down the growth of the fungus
- We were unable to record the diameter of growth for most of the samples Since they were of irregular shape, and this was likely due to human error when placing the spores in the culture plate.

Second attempt:

- The area of growth of the fungus seems to be about the same specifically for the treatment groups. However, there was larger patches of grown fungus in the first experiment, showing that larger amounts of lemongrass extract could further slow down the growth of the bacteria
- However, the area of growth of the fungus in positive controls was larger in experiment 1 than in experiment 2, hence it might be possible that the fungus

grew more as a whole, and the larger amount of lemongrass did stop the growth of some fungus
- Aside from that, the area of growth in treatment groups are smaller than in negative control groups, showing that the adding lemongrass extract helped kill some of the fungus

This shows that the growth of deadlier cousins of *Aspergillus niger*, such as *Aspergillus flavus* which may cause aspergillosis in immunocompromised individuals, may be slowed down by lemongrass extract too as they have similar cell structures. Hence, lemongrass extract may be a possible solution to curing aspergillosis.

5. Conclusion and Recommendations for future work

From the experiment, we found out that lemongrass does indeed help to slow down the growth of *Aspergillus niger*, however it is unable to completely kill it.
At first glance, it may seem that a higher amount of lemongrass extract does not affect its antibacterial properties, but seeing that the fungus in the negative control groups grew more in the second attempt than the first attempt, it could be that the lemongrass extract did indeed stop some growth of the fungus.

Future Work:
We were unable to finish the research questions due to the lack of time. Hence, it would be a good option for future work. For instance, we could find out how the pH level affects the growth of the *Aspergillus niger* fungus and the effectiveness of lemongrass extract for killing the fungus

However, it would be unlikely to test how temperature worked on the extract, since too high or too low temperatures would have a huge impact on the growth of the fungus, and make the difference between the treatment, negative control and positive control groups negligible.

Also, due to the lack of time, we were unable to use a different concentration of lemongrass, and had to increase the amount of lemongrass extract used in the experiment. This could have affected the results and we could instead use lemongrass extract with a higher concentration of lemongrass next time.

Finally, we should have carried out both experiments simultaneously to ensure that the area of growth was only affected by the concentration or amount of lemongrass used, and not by other environmental factors that could have been different at the time of the 2 experiments.

References

- Picture of *Aspergillus Niger* (**Fig. 1)** retrieved from Mold Busters Website (https://www.bustmold.com/resources/mold-library/aspergillus-niger/) on 12 July 2020
- Bhuvaneshwari, L et al (2007) Phytochemical analysis & Antibacterial activity of Nerium oleander. Retrieved on 27 January 2020.
- Vogel, H. (1956). A convenient growth medium for Neurospora crassa. Microbial Genet Bull. Retrieved on 26 January 2020.