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Investigating the Effect of Time Spent in Moisture on the Rate of Mycelial Colony Growth of Pleurotus Ostreatus (Common Oyster Mushroom) in a Nutrient Controlled Spawn

Rangan A* | Department of Environment, India

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***Corresponding Author:**
Aryan Rangan,
Department of Environment, India,
E-mail: aryanrangan@gmail.com

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RESEARCH QUESTIONS

How does changing the level of moisture given to a sample of Pleurotus ostreatus over time change the rate of growth of the mycelial colony?

PERSONAL ENGAGEMENT

The kingdom of fungi has always enticed me. When compared to other organisms, fungi prove to be unique and diverse, almost alien in nature. Yet, fungi have existed for longer than humans,and will likely persist beyond our reign. I was motivated to choose this experiment over others simply due to my love of all thing's fungi.

BACKGROUND INFORMATION

The common Oyster Mushroom, also known as Pleurotus ostreatus is well known for its abilityto grow on odd surfaces [1]. While in the wild they generally prefer dead trees, due to their role a saprotroph, they can adapt to almost any organic surface [2]. Fungi grow by spreading their white, thread-like "roots" known as mycelium [3]. This mycelium is made of fungal hyphae and are most of the organism [3]. The "mushroom" part of the fungi is actually a reproductive structure known as the "fruiting body." This structure is approximately 85–95% water, and only 10% dry matter [4]. Due to the Oyster Mushrooms ability, its mycelium can grow on dead, processed plant matter, such as cardboard [7]. This is because cardboard is made of cellulose, and fungi possess enzymes to externally digest that matter [7, 9]. An interesting note, however, is that due to the nature of mycelium, it possesses the ability to regrow itself via asexual reproduction from a cut piece of the fruiting body [6]. However, this reproduction can only occur if the cut piece of fungi can grow upon a substrate to gain nutrients, such as cardboard [7]. The benefit of using cardboard is that it is extremely controlled in nutrients and therefore can be better accounted for in trials [7]. When mycelium grows from a cut sample, it spreads in the shape of a circle, but because of the substrate, [6] it is likely to look a bit off. This is not an issue if you take the diameter using a ruler, nonetheless. However, in this experiment, there must be some feasible way to control the level of moisture that reaches any sample of P. ostreatus. Well, as is commonly known, water will slowly evaporate over time, even at room temperature. The water vapour that escapes its liquid form

rises to the air, and eventually condenses back into moisture. If we were to control the evaporation of water at room temperature and later the condensation into a plastic tub using a water bath system, we could simulate the transfer of moisture that we want to manipulate. However, there is a flaw in this design, due to the random nature of the evaporation and technological limitations, we cannot accurately measure the concentration of humidity inside of any given petri-dish. To circumvent this, we can measure moisture not as the mL of water inside the petri dish, but instead we can view the evaporation over a measure of time. For instance, we could say that any given trial is inside of the water bath for one day, and therefore the time they were exposed to moisture was one day. For the samples with only half moisture, we could swap those trials inside and out of the hot water bath on a daily basis to simulate a lower moisture content.

HYPOTHESIS

The rate of growth of a Mycelial colony will be faster for the fungi that have All Moisture (AM) levels. By proxy, the fungi with No Moisture (NM) will experience the slowest rate of growth. This is because mycelium is attempting to form a fruiting body, which is the edible part of the fungus. The composition of fungal fruiting bodies is approximately 85-90% water, which implies that high moisture is integral to fungal growth [4]. For this reason, the rate of mycelial growth will likely increase with the quantity of time spent in moisture.

VARIABLES

Apparatus:

- 6 Petri dishes
- 70% Ethanol
- Cardboard Sheet
- Fresh *Pleurotus Ostreatus*
- Scissors
- Tape
- Timer (On phone)
- Ruler (±0.05cm)
- Knife
- Goggles
- Face Mask
- Lab Coat
- 2 Large Plastic Tubs

Figure 1: The Manipulated, Responding, Controlled Variables and an explanation for each

Manipulated Variable	**Responding Variable**	
Amount of time *Pleurotus ostreatus* is grown in moisture	Rate of Mycelial Colony Growth of Pleurotus ostreatus (±0.05cm)	
Controlled Variables	**How are they controlled?**	**Why are they controlled?**
Temperature	All fungi are kept in areas at room temperature.	Temperature can potentially increase or decrease the rate of mycelial growth.
Nutritional Content	All fungi are grown on cardboard.	An imbalance in nutritional content of substrates would create unreliable data.
Source of Cardboard	All fungi were grown on cardboard that had been cut from the exact same original sheet	If fungi were grown on cardboard that had been cut from different sheets, there is an uncertainty about whether or not it had the same qualities.
Sterilized Materials	All applicable materials used in this experiment were sterilized using 70% ethanol.	If a material such as the knife or the petri dish were unsterilized, it opens the possibility of an unwanted micro-organism invading.
Light Intensity	All fungi are always kept in a dark area.	Light can potentially damage the fungi and decrease the rate of growth.
Biological Diversity	For all trials, the same colony of fungi was used to inoculate the petri-dishes.	If different fungal colonies were used, they could potentially have variation in growth that would be unaccounted.
Time between measurements	A timer was kept on a phone for exactly 24 hours after inoculation. Each day I would return and take measurements and set another timer.	This is done to minimize the uncertainty in time. This particular variable does not affect the mycelial colony diameter to a massive extent, but it is still important to minimize uncertainty.
Airflow	All trials were kept in a enclosed container that had minimal air come in or out.	If the trials were kept outside or in an area with heavy ventilation, we would have a skewed data set due to the interference.
Constant Measuring Tool (Ruler)	For all trials, the same ruler was used for all mycelial colony diameter measurements.	This was done as if a separate measuring tool had been used, the uncertainties may have been different which would've changed the results unintentionally.

METHOD

1. Thoroughly sanitize your workstation to ensure no outside contaminants (air particles, dust, molds, etc.)
2. Wear the appropriate lab equipment (goggles, mask, lab coat).
3. Sterilize your petri dish with 70% ethanol to remove all contaminants inside the dish
4. Using a ruler, measure and cut out a 3cm by 3cm cardboard square.
5. Place this cardboard sheet into a beaker of boiling water, (put water in a beaker and use a hot plate to boil it) this sterilizes it. Soak it in the beaker for a few minutes.
6. Remove your cardboard sheet and allow it to drain, but ensure it remains well moistened. Place the moist cardboard sheet into the petri dish.
7. Meticulously wash the fresh Pleurotus ostreatus to remove all contaminants. (Mushrooms are often covered in dirt; this step is extremely important).
8. Using 70% ethanol, sterilize your knife.
9. Using your knife, cut a thick strip from the stem of Pleurotus ostreatus. Ensure that the strip can sit comfortably within the previously cut cardboard square.
10. Place the strip atop the moist cardboard sheet and close the petri dish to inoculate it.
11. Repeat steps 2-9 for each petri dish. For cutting the strips.
12. Once all petri-dishes are inoculated, seal them with tape and organize them into 3 groups of 2. Label these groups: All Moisture (AM), Half Moisture (HM), and No Moisture (NM).
13. Move the AM group into a small plastic tub and fill a large plastic tub with water. This water should be at room temperature (20-22°C). Place the small tub in the water bath (do not get the Petri dishes wet) and ensure all tubs are sealed.
14. Next, take the HM group and place them in the other small tub. Fill the other large tub with water (20-22°C) and place the small tub in the large tub. (Once again, keep the petri dishes dry).
15. Take both tubs and move them into an easily accessible dark, dry location that stays room temperature. This could be inside of a container or cabinet, anywhere that doesn't fluctuate temperatures, doesn't often change airflow, and stays dark.
16. Lastly, move the NM group into the area you have chosen. This group is not exposed to moisture and as such can simply be placed next to the tubs.
17. Leave the groups in that area for 1 day, return the next day (set a timer on your phone for exactly 24 hours) and measure with a ruler (mm) the diameter of the mycelial colony. Include the cut mushroom body in your measurements as it is also part of the mycelial colony diameter.
18. Observe the petri dishes and write down any qualitative observations you see.
19. Once you have finished taking measurements and observations, return the petri dishes to their original areas, but remove the HM group from moisture. Instead, lay it like the NM group, without any moisture.
20. Return the next day at the same time yet again (using the timer on your phone) and repeat the process of taking measurements (with the same ruler). This time return group 2 to the water bath.
21. Repeat this process for 8 days to ensure sufficient data.

LAB DIAGRAMS: (Not all materials included)

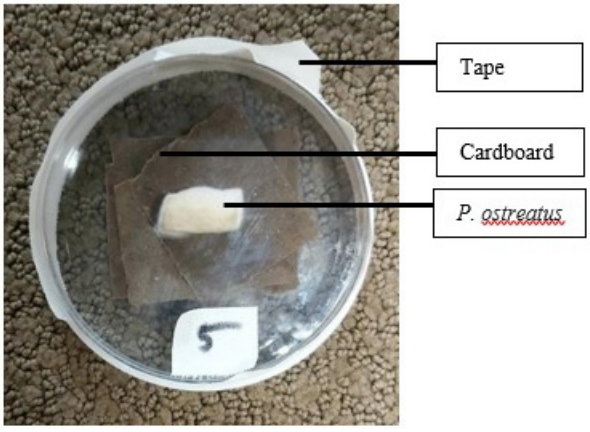

Figure 2: Setup of Petri-Dishes inoculated with P. ostreatus taken 3 days after inoculation (Author: Researcher)

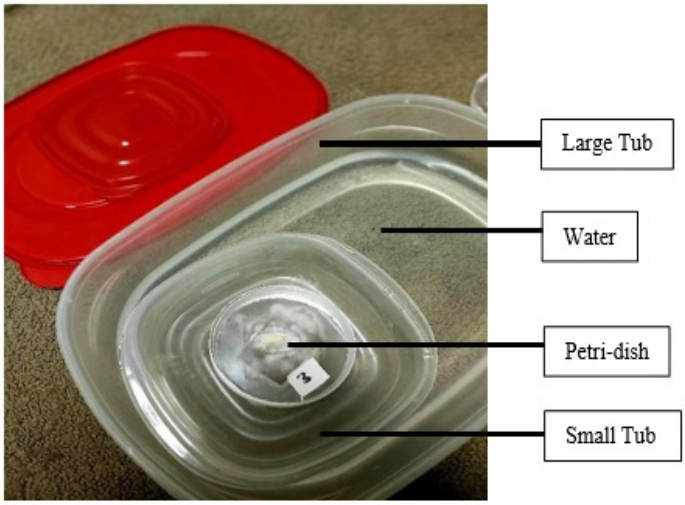

Figure 3: Setup of the two-tub water bath with an inoculated Petri-dish inside taken 8 days after inoculation (Author: Researcher)

SAFTEY AND ETHICAL ASSESSMENT

In terms of ethical considerations, this experiment has none as there are no sentient organisms being tested, only fungi. Moreover, there are no notable chemicals used that would require special disposal, however, the mycelial colony is organic material and as such should be disposed of correctly into the compost bin. Of course, during the experiment, it is important that you always wear safety goggles, and you follow the COVID-19 regulations. This includes frequently washing your hands and wearing a mask at all times.

RAW DATA:

Qualitative Data

Figure 4: Quantitative observations of the mycelial colony diameter of P. ostreatus taken every 24 hours to calculate the rate of mycelial growth for each trial and determine the effect of moisture on mycelial growth with an uncertainty of ±0.05 cm.

Moisture Levels	Trials (AM/HM/NM)	Mycelial Colony Diameter of *P. ostreatus* (cm) (±0.05cm)								
		0 DAI	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI
All Moisture	AM Trial 1	2.31	2.32	2.34	2.37	2.41	2.42	2.43	2.44	2.45
	AM Trial 2	2.12	2.14	2.17	2.21	2.51	2.62	3.14	3.22	3.43
Half Moisture	HM Trial 1	1.51	1.55	1.63	1.71	2.23	3.01	3.12	3.56	3.81
	HM Trial 2	1.62	1.65	1.73	1.84	2.12	2.74	3.56	4.03	4.21
No Moisture (control)	NM Trial 1	2.11	2.15	2.26	2.34	2.51	2.55	2.57	2.59	2.63
	NM Trial 2	2.54	2.58	2.73	2.77	2.83	2.85	2.86	2.87	2.89

Note: DAI = Days after inoculation, AM = All Moisture, HM = Half Moisture, NM = No Moisture.

Qualitative Data

Notes: HM Trial 1 looks slightly fogged up because of the mycelium growing beyond the cardboard, HM Trial 2 and NM Trial 2 are vertical when they should be pointed horizontally.

Figure 6: Qualitative observations of the mycelium and of the original sample to discern the effects of moisture on the mycelium of P. ostreatus.

Moisture Levels	Trials (AM/HM/NM)	Mycelium Density	Mycelium Color	Sample Color	Texture
All Moisture	**AM Trial 1**	Thin	Brown	Dark Brown	Coarse
	AM Trial 2	Very Compact	White	Light Brown	Fuzzy
Half Moisture	**HM Trial 1**	Somewhat Compact	White	White	Fuzzy
	HM Trial 2	Somewhat Compact	White	Light brown	Fuzzy
No Moisture (control)	**NM Trial 1**	Thin	White	Darkish Brown	Coarse
	NM Trial 2	Thin	White	Darkish Brown	Coarse

Note: Texture was felt after all other measurements had been taken to prevent contamination.

Observations:

- AM Trial 1, had a very odd smell emitting from it. I would compare it to the smell of rot. This is the most likely reason for it being a statistical outlier.
- Occasionally, the P. ostreatus would undergo periods of rapid growth in short amounts of time, only to return to an ordinary rate the next day. The cause of this requires further investigation.
- Overall, the P. ostreatus were very slow to grow. This is likely a result of the cardboard substrate chosen to spawn the mycelium.

ANALYZES QUANTITATIVE DATA:

Sample Calculations

Figure 7: Rate of growth, mean, and standard deviation for all measurements from the data collected by measuring mycelial colony diameter over time.

Moisture Levels	Trials (AM/HM/NM)	Rate of Growth (cm/day) (±0.05cm/day)	Mean (cm) (±0.05cm)	Trial St. Dev	Overall St. Dev
All Moisture	AM Trial 1	0.0156	2.39	0.0538	0.328
	AM Trial 2	0.146	2.62	0.518	
Half Moisture	HM Trial 1	0.256	2.46	0.922	0.098
	HM Trial 2	0.288	2.61	1.06	
No Moisture	NM Trial 1	0.0578	2.41	0.2	0.0504
	NM Trial 2	0.0389	2.77	0.129	

Sample Calculation

Rate of Mycelial Colony Growth: $\frac{(Final-Initial)}{\#\ of\ days}$ **Final** = Last measurement in Trial **Initial** = First measurement in Trial	**For HM Trial 1:** $\frac{((3.81)-(1.51))}{(9)} = 0.2555\cdots = 0.256$
Mean: $x = \frac{\Sigma xi}{n}$ xi = The Mycelial Colony diameter of P. ostreatus for a particular trial **n** = The number of days after inoculation	**For HM Trial 1:** $\frac{1.51+1.55+1.63+1.71+2.23+3.01+3}{9} = 2.458889\ \ldots = 2.46$
Standard Deviation: $s = \frac{\sqrt{\Sigma(xi-\bar{x})^2}}{n-1}$ $\bar{x}$ = The mean of all P. *ostreatus* Mycelium Colony diameters for a given trial	**For HM Trial 1:** **Step 1.** (1.51 - 2.46)2 = 0.903 (1.55 - 2.46)2 = 0.828 (1.63 - 2.46)2 = 0.689 (1.71 - 2.46)2 = 0.563 (2.23 - 2.46)2 = 0.0529 (3.01 - 2.46)2 = 0.303 (3.12 - 2.46)2 = 0.436 (3.56 - 2.46)2 = 1.21 (3.81 - 2.46)2 = 1.82 **Step 2.** (0.903+0.828+0.689+0.563+0.0529+0.303+1.21+1.82) = 6.8049 = 6.80 **Step 3:** $\frac{6.80}{y-1} = 0.85$ **Step 4:** √0.85 = 0.92195 = **0. 922**

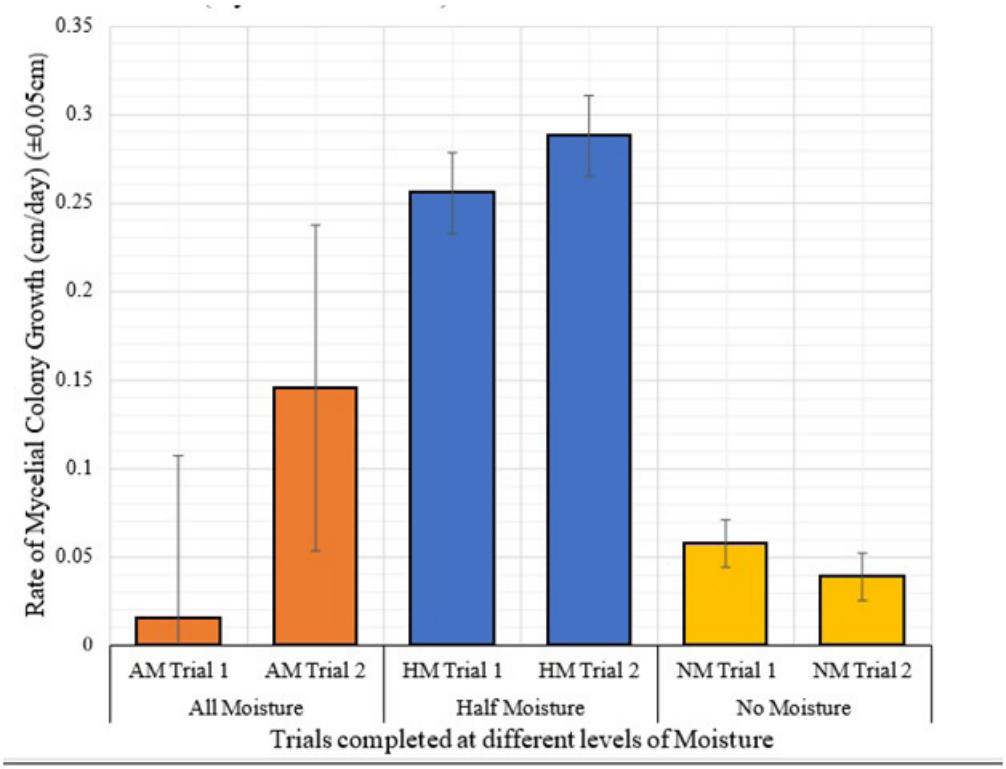

Figure 8: The effect of time spent in different levels of moisture of the rate of Mycelial Colony Growth for Pleurotus ostreatus (Oyster Mushroom) with ±1 Standard Deviation

Note: The error bars for the AM Trials are odd because of the abnormal data, as will be explained later. (See evaluation and conclusion section).

Strengths and Limitations

Limitation	Result of Limitation	Possible Improvement
AM Trial 1 Outlier (random error)	As I mentioned, AM Trial 1 ended up with an excessive amount of moisture and started to rot, which severely limited the amount of mycelial growth that could be accomplished.	While it is not true to say that this error was unpreventable; the current equipment at my disposal is not sophisticated enough to eliminate random errors such as these. To improve upon this, a superior lab set-up may be needed, such as changing the substrate or having more controlled moisture concentrations.
Limited number of trials (systemic error)	Due to external restrictions, 6 trials over the course of 9 full day was all that was recorded. This leaves a large margin of error, especially for the AM Trials that had external difficulties.	Plan ahead of time the exact number of trials that will be done over a set time period and have a preplanned data sheet so as to record exactly what you need.
Research may not be applicable to real life scenarios (systemic error)	Due to implicit biases, the conclusion reached in this study may not represent how P. ostreatus mycelial colonies behave in real life scenarios.	There is not a solid way to improve upon this, short of simply conducting more research into the topic to gain further insight.
Not controlling the size of *P. ostreatus* sample (systemic error)	Due to this shortcoming, it is entirely likely that nearly every aspect of the lab was influenced in some way. The mycelial colony diameter was semi influenced by the original sample and this likely caused some incorrect assumptions on my end.	The simple solution to this limitation would be to better control the size of the original sample, possibly by using a potato corer or by specifying parameters such as a 2 cm by 2 cm cube.
Human Error (random error)	There was a small error in the timing between measurements. While I set a timer for 24 hours to take measurements each day, the time ultimately varied by a few minutes or so each day. Not enough to influence results in any meaningful way, but a limitation nonetheless.	Due to the nature of this error as entirely random and unavoidable, there is little I can do to rectify this error unless I were to change the measurement time gaps to something more manageable such as 1 hour.

ANOVA TEST

To effectively determine a significant variation between these three levels of moisture and the

mycelial colony growth, a Single Factor: ANOVA Test was employed using Microsoft Excel.

- **H_0 (Null)** = There is no significant variation in the growth of a P. ostreatus mycelial

colony that has been grown under differing levels of moisture.

- **H_1 (Alternate)** = There is a significant variation in the growth of a P. ostreatus mycelial

colony that has been grown under differing levels of moisture.

ANOVA Formula= $\frac{\text{Between group variation}}{\text{Within group variation}}$

The p value found was 8.20 x 10-6 which is significantly smaller the Alpha value (0.05).

Moreover, the F value was 6.79 which is greater than the F-critical value of 2.152133. With both

of these being true, we can reject the H_0 and support the H_1

EVALUATIUON AND CONCLUSION

The hypothesis reiterated: "The rate of growth of a mycelial colony will be faster for the fungi that have All Moisture (AM). By proxy, the fungi with No Moisture (NM) will experience the slowest rate of growth. This is because mycelium is attempting to form a fruiting body, which is the edible part of the fungus. The composition of fungal fruiting bodies is approximately 85-90%water, which implies that high moisture is integral to fungal growth [4]. For this reason, the rate of mycelial growth will likely increase with the quantity of time spent in moisture."

As understood from the statistical analysis (ANOVA) and the graph, it appears that the hypothesis was not necessarily supported. While it is true that the NM Trials did experience the slowest rate of growth and as a result, the smallest mycelial colony diameter, the AM trials did not experience the greatest rate of growth, nor the largest mycelial colony diameter. It appears that the HM Trials experienced the greatest increase in both rate of growth, and colony diameter.

There are several reasons as to why this could be the case. The first likely reason is based around the observations made regarding AM Trial 1. As I mentioned, there was an odd smell emitting from the petri-dish that was not present in any other Trial. Moreover, the mycelial growth was qualitative extremely low, especially when compared to AM Trial 2, which had a rate of growth over 9 times that of AM Trial 1. There is also the colour to consider, AM Trial 1 was the only Trial in which the P. ostreatus sample became entirely dark brown. Coupled with the smell and lack of mycelial growth. I believe it is safe to assume that AM Trial 1 was a massive outlier and should not be treated as representative of the AM data. It is likely that the abundance of moisture caused the sample to rot, which caused the smell, colour, and lack of mycelial growth. That said, however, AM Trial 2 also has a significantly lower rate of mycelial growth when compared to the HM Trials. It seems likely that increasing the level of moisture will not increase the rate of mycelial growth forever. A balance between humidity and dehydration must be reached for optimal mycelial growth.

However, looking again at (Figure 6), we can see that AM Trial 2 was the only trial in which the mycelium was as dense. This could potentially be a benefit of growing mycelium in high moisture environments, however due to inconclusive data it is difficult to tell whether this is simple biological variance or a relationship. If we assume it is a relationship, that would imply that AM is best for thick mycelial colonies, HM is best for long mycelial colony diameters at a fast rate, and NM for short, thin mycelial colonies.

While that is true for this database, my sample size was extremely small, so it is unclear at the moment whether there exists a positive correlation between HM and the rate of mycelial growth. Moreover, the error bars I chose for my graph are small and nicely fit the HM and NM Trials, but due to the mentioned circumstances behind the AM Trials, the error bars are skewed heavily.

STRENGTHS AND LIMITATIONS

One strength of this lab would be that the data taken was more accurate as a result of the controlled variables. An example of this would be controlling temperature, as that variable affects the mycelial colony growth.

- Another strength of this lab is that all the materials are extremely accessible, practically all needed equipment you could find in a standard lab or even at home.

SUGGESTIONS FOR FURTHER RESEARCH AND CONNECTIONS TO THE ACTUAL WORLD

My suggestion for further research on the topic of moisture and mycelium is rather blatant. It would be to test the growth rate of mycelial colonies that are not P. ostreatus. My immediate suggestion would be to test a different species of fungi that still belongs to the same genus. Pleurotus cystidiosus (Brown Oyster) seems like a good candidate for further study. The implications of such research could have far reaching connections to the real world, for instance,

if a specific moisture concentration can be identified for several different types of mushrooms, all types of industrial farming can increase their output. Of course, this is great of the industry [11]. but studies have also shown that P. ostreatus may also have several health benefits. An increase in their production could lead to a wider spread of their benefits. The most important of those being their high concentration of niacin, which has been shown to improve levels of High-Density Lipoprotein (HDL) [10].

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