# Determining the Optimal Temperature for *Pleurotus ostreatus* Mycelium Growth

**Paniz Ghavimi, Ravi Soni, Maria Caray, Abdullah Khawaja**
Faculty of Sciences, University of British Columbia
BIOL 342: Integrative Biology Laboratory
Dr. Celeste Leander
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**ABSTRACT**

*Pleurotus ostreatus*, a widely cultivated mushroom, possesses various economic, nutritional, and environmental values and has been extensively used in the medical field in antioxidants and antitumors. The mycelium, a root-like structure of the mushroom, grows by creating a network of thread-like branching fibres, also known as hyphae, and is mainly composed of chitin, proteins, and cellulose. This study aimed to investigate the optimal temperature for mycelium growth of *P. ostreatus* between 20℃, 25℃, and 30℃ in agar medium. Circular gills from oyster mushrooms were incubated in these three different temperatures, and the diameter of mycelium expansion was tracked and measured over the span of two weeks. The results indicated that 25℃ was the most optimal temperature for *Pleurotus ostreatus* mycelium network growth for this experiment. However, several challenges and sources of error were faced throughout the study, such as potential contamination, moisture loss, and light sensitivity, which may have impacted the results. Future studies should consider preventing these sources of error to improve the accuracy of their results. By understanding the most suitable temperature for mycelium growth, their mass production for nutritional or medical uses will be more successful.

**INTRODUCTION**

The oyster mushroom or *Pleurotus ostreatus* belongs to the family of Tricholomataceae, and globally it is the second most cultivated mushroom (Kües & Liu, 2000). These species are essential degraders and possess various economic, nutritional, and environmental values (Hoa & Wang, 2015). Additionally, they have been extensively used in the medical field as antioxidants and antitumors (Chorváthová et al., 1993).

Mycelium is a root-like structure of a mushroom, and grows by creating a network of thread-like branching fibres, also known as hyphae, which contain elongated cells (Haneef et al., 2017). Mycelium is mainly composed of chitin, proteins, and cellulose (Rathore et al., 2019). Parameters such as nutritional conditions, humidity, moisture, pH, and temperature determine and play a key role in mycelium growth (Drais et al., 2022).

*Pleurotus ostreatus* has the ability to perform and develop in a wide range of temperatures. Various types of research have been conducted on determining the most efficient temperature for mycelium growth; nevertheless, each reported a different temperature, ranges 22℃-28℃ (Ezrari et al., 2021; Hoa & Wang, 2015; Fletcher et al., 2019). By understanding the most suitable temperature for the network of hyphae, their mass production for nutritional or medical uses will be more successful.

In this research study, we investigated the optimal temperature for mycelium growth of *P. ostreatus* between 20℃, 25℃, and 30℃ in agar medium by tracking and measuring the diameter of mycelium expansion over the span of two weeks. We hypothesized that mycelium growth will be affected by different temperatures over time. According to the previous literature as described

above, we predicted that we will observe the most mycelium growth at 25℃. Consequently, our null hypothesis was that mycelium will not be affected by different temperatures over time.

## MATERIALS AND METHODS

All materials and equipment were provided by the BIOL 342 laboratory.

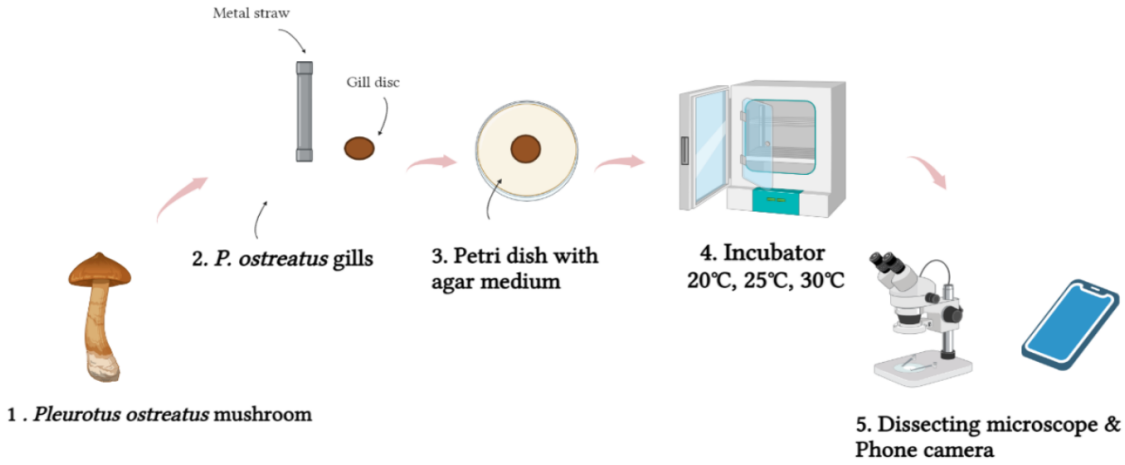


**Figure 1**. Demonstrates project design.

A box of *Pleurotus ostreatus* was purchased at a local Save-On-Foods grocery store on the day of the experiment. Under a fume hood and with sterilized forceps, the gills of the mushrooms were removed and placed on an empty sterile petri dish.

Fifteen, 10-cm Petri plates were filled with 20 mL of autoclaved yeast agar medium by the teaching team. As displayed in Figure 1., by using a sterilized metal straw, circular gills were collected from *Pleurotus ostreatus* and a gill disc was placed onto the middle of the solidified agar medium using sterilized forceps under sterile conditions. 5 plate replicates per temperature (20℃, 25℃, 30℃) were prepared, and placed in the respective incubators. Mycelium growth was measured every two days for the span of two weeks, by illuminating them with a dissecting microscope and taking images with a smartphone at 0.7X and 4X for each sample. Next, the growth of the network expansion was tracked by uploading the images onto ImageJ version 1.53t, which traced and analyzed the length of mycelium in cm following the physical measurement of the Petri dishes (in cm).

All statistical analyses were performed using RStudio version 1.3.1073. Two-way ANOVA tests were performed to determine independent relationships between time and temperature on mycelium growth. Tukey-HSD tests were performed to determine significant differences in mycelium growth between conditions in temperature and days in time.

## RESULTS

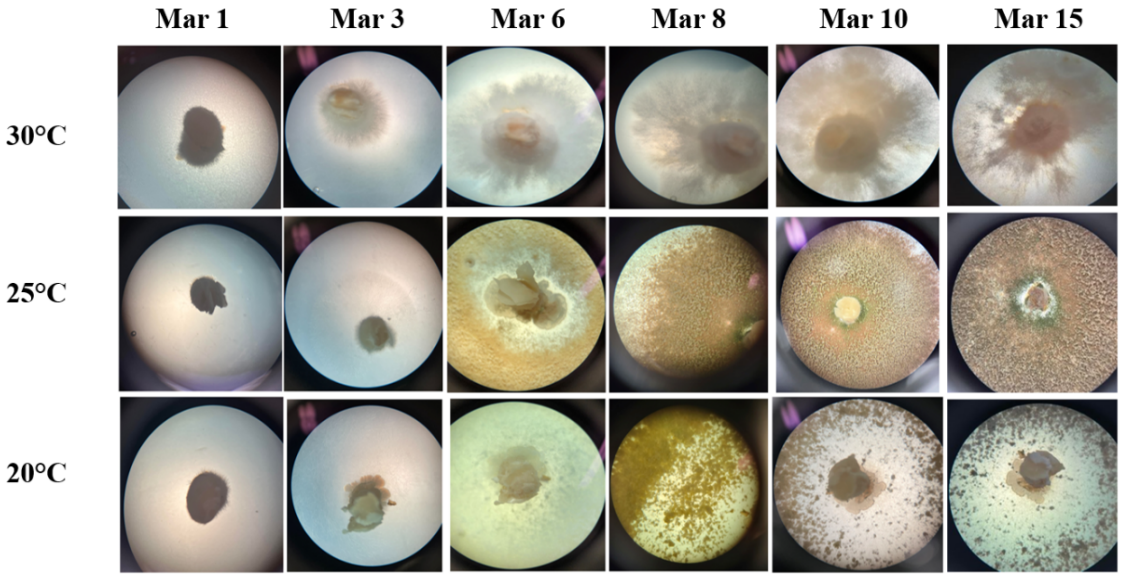


**Figure 2**. Displays mycelium growth at 0.7X over the span of two weeks.

Observation of the trends in visual mycelium growth over time between the varying temperatures revealed that *Pleurotus ostreatus* mycelium network growth was greatest at 25℃ for this experiment.

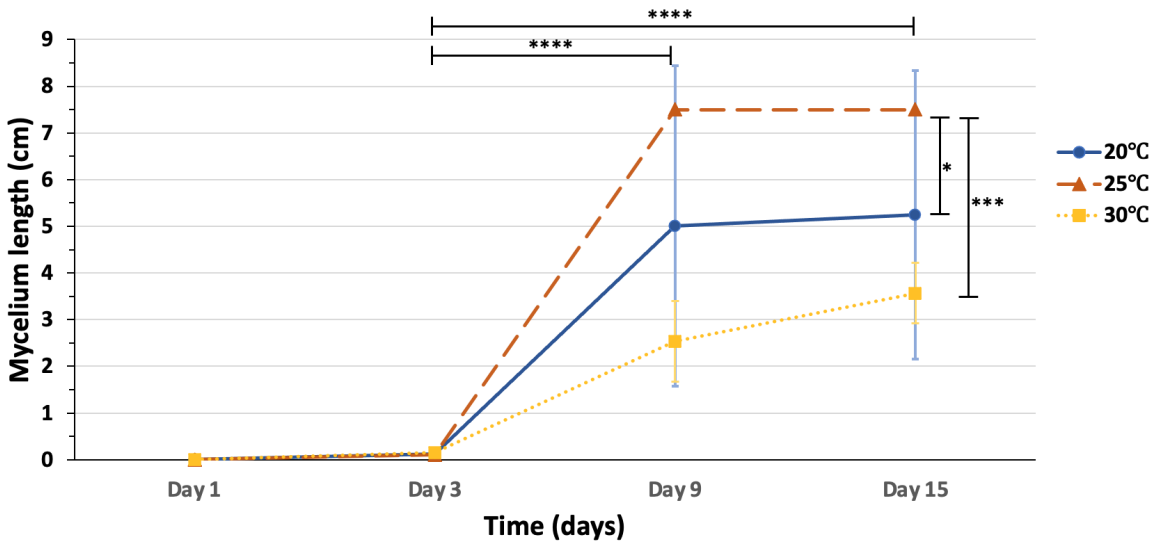


**Figure 3.** The mean mycelium growth of *Pleurotus ostreatus* in 20℃ (n=5), 25℃ (n=5) and 30℃ (n=5) on days 1, 3, 9 and 16 (n=15) with error bars represent the standard deviation of each mean. A two-way ANOVA test on the data resulted in a F(temperature) = 41.74, $p<0.0001$ and F(time) = 10.43, $p<0.001$. Significance between the means from a Tukey-HSD test are shown as <0.05 = *, <0.01 = **, <0.001 = ***, <0.0001= ****.

As seen in Figure 3., day 1 was the day of the experiment, thus the growth rate was set at 0 cm. On day 3, there was minimal recorded mycelium growth of similar means throughout the three temperatures (Mean ± standard deviation cm: 20℃: 0.12 ± 0.02, 25℃: 0.11 ± 0.01, 30℃: 0.15 ± 0.05).On day 9, the samples at each temperature produced distinct average lengths (Mean ± standard deviation cm at 20℃: 5.25 ± 3.43, 25℃: 7.50 ± 0.00, 30℃: 2.54 ± 0.86). On day 15, the distinction between the three temperatures remained the same (Mean ± standard deviation cm at 20℃: 5.25 ± 3.10, 25℃: 7.50 ± 0.00, 30℃: 3.57 ± 0.65). When testing for association between variables, both temperature and time had significant independent correlations with mycelium growth ($p<0.0001$ and $p<0.001$, respectively).

When testing for mycelium growth between temperature conditions, significant differences were found when comparing 20℃ vs. 25℃ ($p<0.05$) and 20℃ vs. 30℃ ($p<0.001$). However, no significant differences in mycelium growth were found in 25℃ vs. 30℃ ($p=0.1$). When testing for mycelium growth between days, significant differences were found from Day 3 to Day 9 (<0.0001) and Day 3 to Day 16 (<0.0001). However, no significant differences in mycelium growth were found from Day 9 to Day 15 ($p=0.8$).

**DISCUSSION**

There were several challenges and sources of error faced throughout the study. While starting the experiment, no technique was used to sterilize the mushrooms to prevent the potential loss of spores. This may have introduced potential contaminants to the mushrooms. Future experimenters are advised to use larger-sized discs that will enable sterilization without the large risk of spore loss. In addition, the Petri dishes were not sealed at any point during the duration of the study, due to the presence of condensation in the dishes. This may have been another source of contamination as the dishes were not completely isolated from any other material kept in their respective incubators. To avoid this, experimenters should leave ample time for the agar to completely cool down from the autoclave machine before adding any mushroom spores into the agar. The aforementioned sources of contamination may have caused the growth of mold and bacteria within the samples in the 20℃ and 25℃ incubators, making it difficult to assess whether the mycelium growth was consistent throughout the Petri dishes. For these samples, mold and bacteria growth extended to the edges of the Petri dishes, thus it was assessed that mycelium also grew to the edges of the Petri dishes (5 cm). Along with preventing the sources of contamination, it is suggested to use larger Petri dishes to better assess the maximum length of mycelium growth in these samples.

During the final measurements on day 16, the agar for all three temperatures was dried out, with the edges of the agar shrivelled due to the significant loss of moisture. Additionally, the incubators that were used did not provide dark conditions for the entire duration of the two weeks. Instead, the incubators rotated in light and dark conditions every four hours. As mycelium is sensitive to moisture loss and light, this may have decreased the potential network

growth for all three temperatures. To prevent this, it is suggested that future studies use a different composition of agar that will be less susceptible to moisture loss and use an incubator that provides dark conditions at all times, or wrap Petri dishes with aluminum foil to prevent light coming through to the mycelium. During the photo-taking process to track mycelium growth, there were challenges in taking clear quality images with no glare, and that captured the entire view of the microscope, making it difficult to process with ImageJ. This may be fixed in the future with the use of a digital microscope or a camera attachment for a dissecting microscope.

Our experiment rejected the null hypothesis and we found that various temperatures over time did in fact impact mycelium growth. We believe that an optimal growth rate, if we were to replicate this experiment again, would likely be between 20℃ and 25℃ - reasonably at 23℃. This is due to the high density and rapid growth rate at 25℃ and the two samples at 20℃ with minimal bacterial production.

Previous literature indicated mycelium proliferation between the temperature ranges of 22℃-28℃ (Ezrari et al., 2021, Hoa & Wang, 2015, Fletcher et al., 2019). However, this study has narrowed down the temperature range to 22℃-25℃.

## CONCLUSION

The most optimal temperature for *Pleurotus ostreatus* mycelium network growth was identified to be at 25℃ followed by 20℃ and then 30℃ for this specific experiment. Growth with respect to time as well as the interaction between temperature and time was found to be statistically significant. However, we believe the optimal temperature to likely be around 23℃. By understanding and determining the most suitable temperature for mycelium or network of hyphae, their mass production for economic, nutritional or medical uses will be more successful as optimal temperatures will result in more rapid growth with fewer odds of bacterial growth.

## ACKNOWLEDGEMENTS

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**APPENDIX A - Data Tables from Image J:**

- **March 1**
  - 20℃

| | Area | Mean | Min | Max | Angle | Length |
|---|---|---|---|---|---|---|
| 1 | 3.145E-4 | 186.853 | 99.000 | 230.717 | -56.310 | 0.113 |
| 2 | 2.552E-4 | 183.660 | 113.250 | 211.985 | -64.983 | 0.091 |
| 3 | 4.621E-4 | 213.454 | 110.500 | 230.667 | 148.392 | 0.116 |
| 4 | 4.933E-4 | 193.751 | 96.000 | 230.167 | -175.533 | 0.124 |
| 5 | 5.381E-4 | 186.998 | 138.413 | 219.857 | -72.719 | 0.158 |

    - The length on the right side indicates the growth of the mycelium in cm - average length = 0.120 cm for 20
  - 25℃

| | Area | Mean | Min | Max | Angle | Length |
|---|---|---|---|---|---|---|
| 1 | 4.144E-4 | 182.685 | 108.396 | 219.683 | -42.166 | 0.120 |
| 2 | 2.783E-4 | 151.564 | 100.000 | 189.875 | 33.433 | 0.091 |
| 3 | 3.535E-4 | 177.566 | 99.000 | 202.816 | -19.026 | 0.102 |
| 4 | 3.461E-4 | 183.165 | 106.833 | 207.309 | 123.690 | 0.095 |
| 5 | 4.377E-4 | 200.698 | 134.667 | 230.500 | -101.659 | 0.117 |

  -
  - Average length = 0.105 cm for 25
  - 30℃

| | Area | Mean | Min | Max | Angle | Length |
|---|---|---|---|---|---|---|
| 1 | 5.324E-4 | 200.352 | 142.167 | 233.500 | 87.979 | 0.105 |
| 2 | 4.300E-4 | 169.881 | 110.333 | 227.333 | -74.827 | 0.112 |
| 3 | 8.469E-4 | 199.692 | 133.625 | 238.708 | 39.036 | 0.171 |
| 4 | 3.907E-4 | 167.443 | 112.000 | 203.667 | 10.408 | 0.138 |
| 5 | 6.737E-4 | 187.592 | 126.833 | 213.602 | -33.007 | 0.215 |

  -
  - Average length = 0.148 cm for 30
- **March 8**
  - 20℃
    - #1, #2, #4, at 7.5cm (edge of plate)
    - #3 = 1 & #5 = 2

| | Area | Mean | Min | Max | Angle | Length |
|---|---|---|---|---|---|---|
| 1 | 0.136 | 211.519 | 176.509 | 229.160 | -155.674 | 1.863 |
| 2 | 0.054 | 176.872 | 135.111 | 187.019 | -93.945 | 0.676 |

    - However both 3 and 5 indicate no signs of mold growth
    - Average length = 5.01 cm for 20

- 25℃
    - All growth to 7.5cm (the edge of the plate)
    - All samples have mold growth but at the edges of the sample
    - Average length = 7.5 cm for 25
- 30℃

| | Area | Mean | Min | Max | Angle | Length |
|---|---|---|---|---|---|---|
| 1 | 0.061 | 212.211 | 198.333 | 220.593 | 55.008 | 1.209 |
| 2 | 0.137 | 180.042 | 125.333 | 209.433 | 47.586 | 2.717 |
| 3 | 0.135 | 184.066 | 127.389 | 209.842 | -88.939 | 2.675 |
| 4 | 0.125 | 194.865 | 149.667 | 226.169 | -38.928 | 2.483 |
| 5 | 0.182 | 200.412 | 142.167 | 215.910 | -146.746 | 3.614 |

    - Average length = 2.54 cm for 30

- **March 15**
    - 20℃
        - #1, #2, #4, at 7.5cm (the edge of the plate)
            - These samples show signs of mold growth in between the growth phase through rings
        - #3 = 1 & #5 = 2

| | Area | Mean | Min | Max | Angle | Length |
|---|---|---|---|---|---|---|
| 1 | 0.062 | 187.393 | 132.687 | 224.167 | -0.356 | 2.213 |
| 2 | 0.042 | 135.448 | 116.500 | 171.500 | 167.471 | 1.520 |

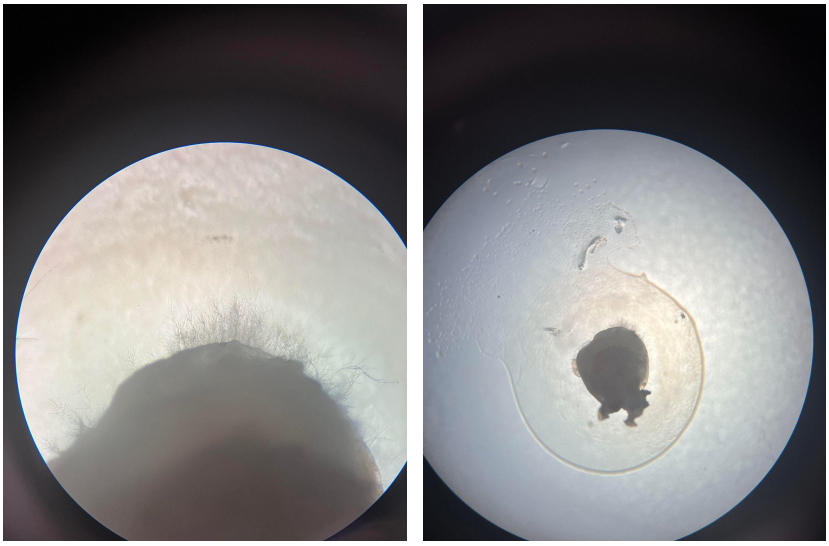
        - However both 3 and 5 indicate no signs of mold growth
        - Average length = 5.24 cm for 20
    - 25℃
        - All growth to 7.5cm (the edge of the plate)
        - All samples have mold growth but at the edges of the sample
        - Average length = 7.5 cm for 25
    - 30℃

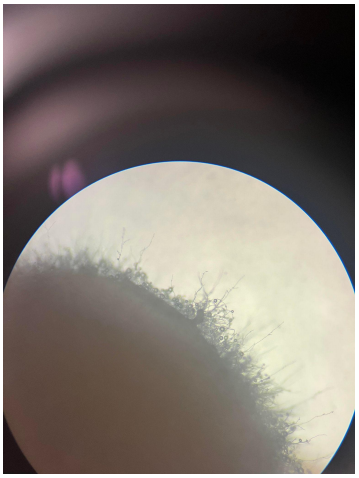
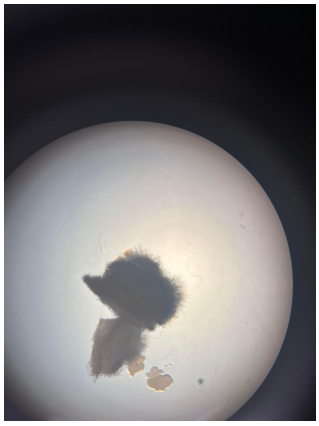
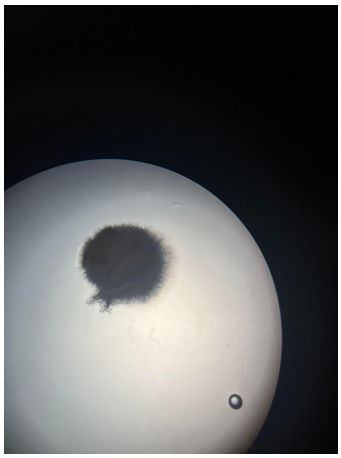
| | Area | Mean | Min | Max | Angle | Length |
|---|---|---|---|---|---|---|
| 1 | 0.326 | 175.759 | 132.870 | 221.000 | 99.866 | 3.848 |
| 2 | 0.265 | 165.172 | 92.000 | 206.636 | -38.660 | 3.166 |
| 3 | 0.258 | 176.680 | 89.667 | 220.667 | 59.300 | 3.067 |
| 4 | 0.265 | 173.538 | 118.000 | 194.316 | -8.973 | 3.170 |
| 5 | 0.387 | 193.038 | 68.667 | 222.139 | 17.784 | 4.587 |

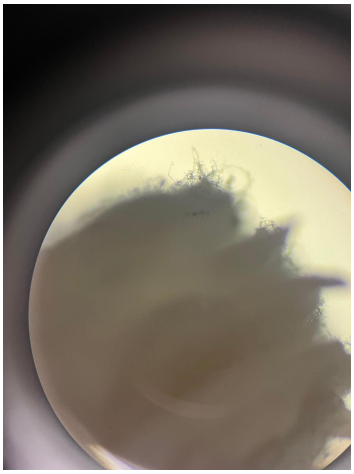
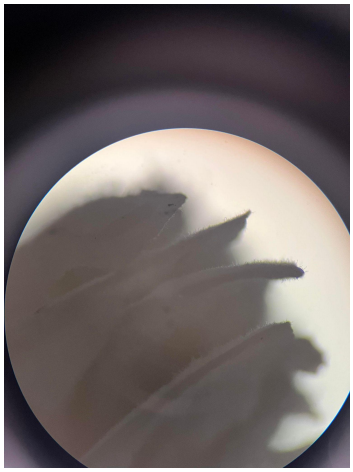
- Average length = 3.57 cm for 30

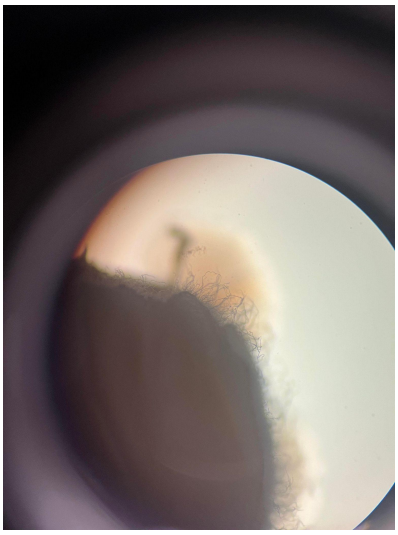
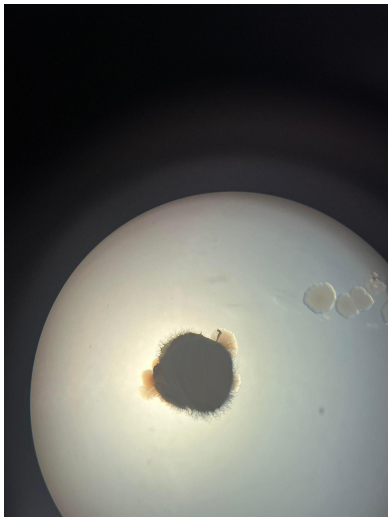
**APPENDIX B: Sample Images of Mycelium Growth:**

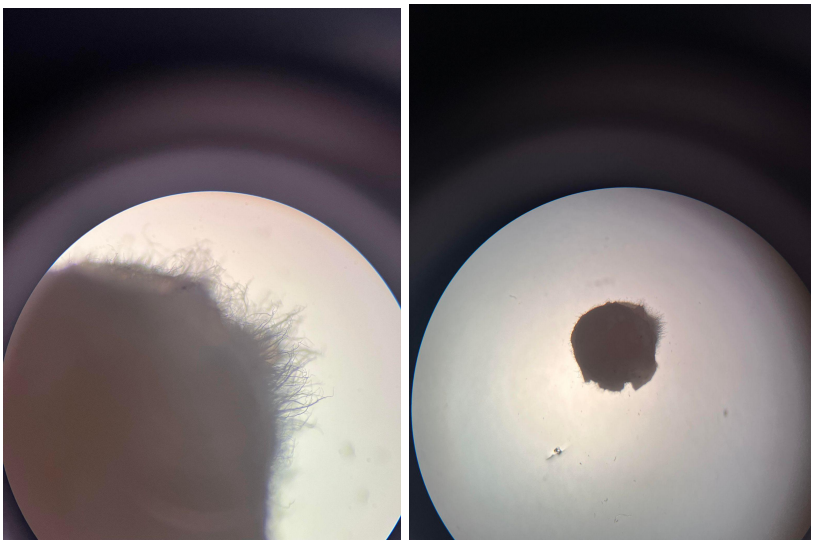
**March 1, 2023**

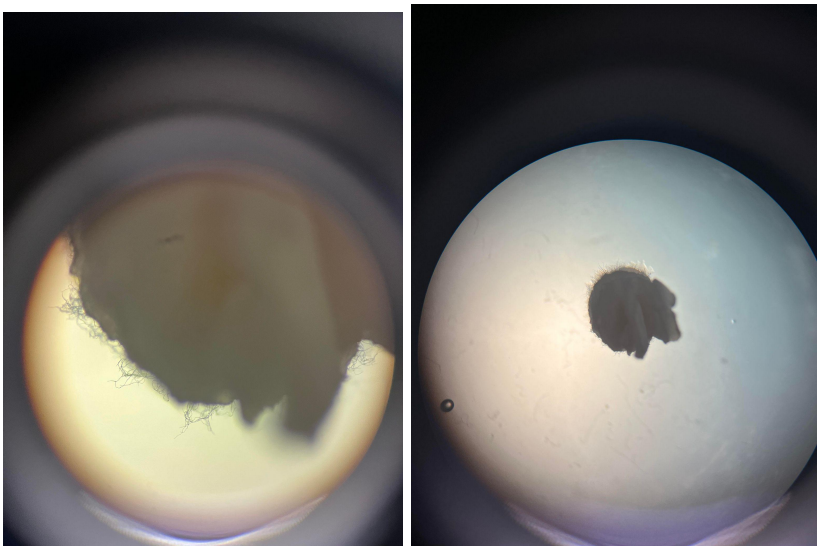
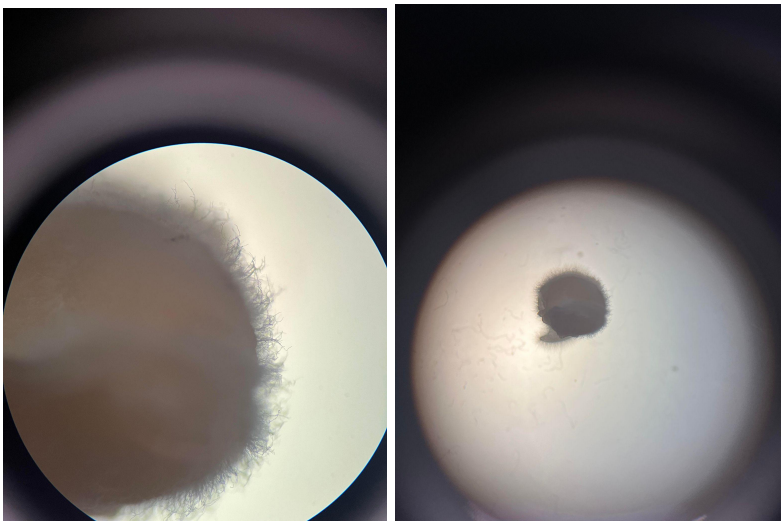
- At 30:
  - Photo 1:
  - 
    - Many hyphae
    - Circle of bacteria surrounding the fungi
    - Photo at 1.0 magnification
    - Photo also at 4.5 magnification
  - Photo 2:

-  
    - Many hyphae all the way around
    - Hyphae are long with bubbles in between
- Photo 3:
-  
- Photo 4:
-  
- Photo 5:

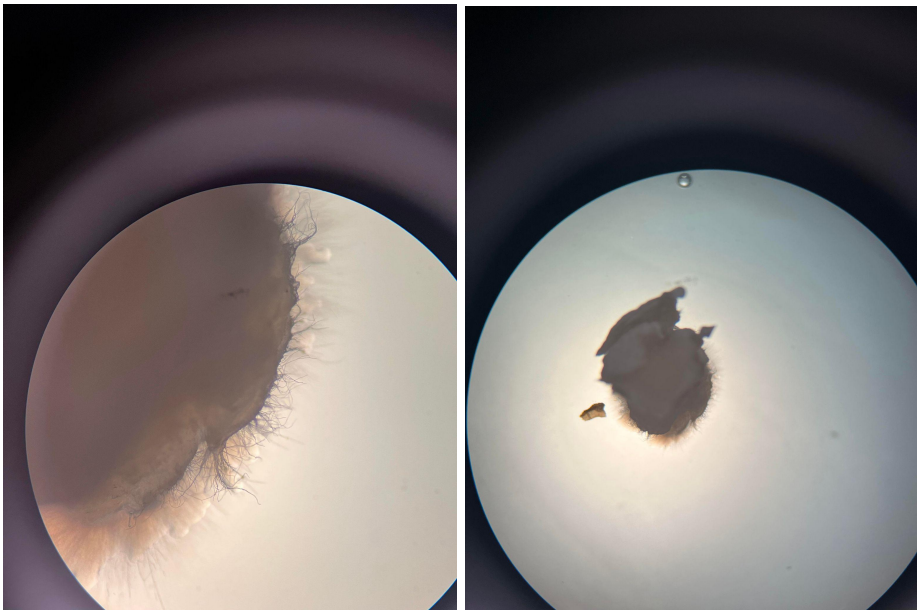
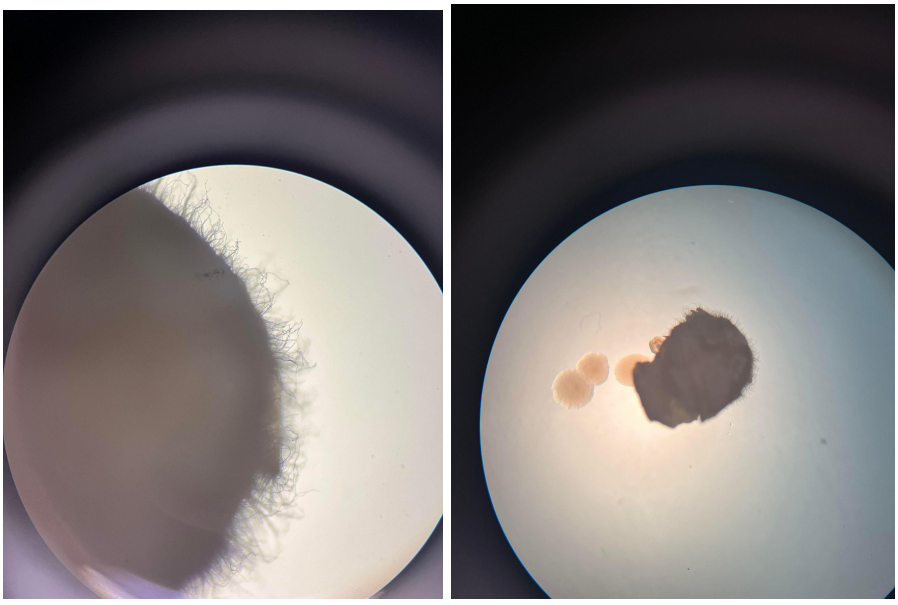
-  
- 
    - Hyphaewere very dense and many different layers

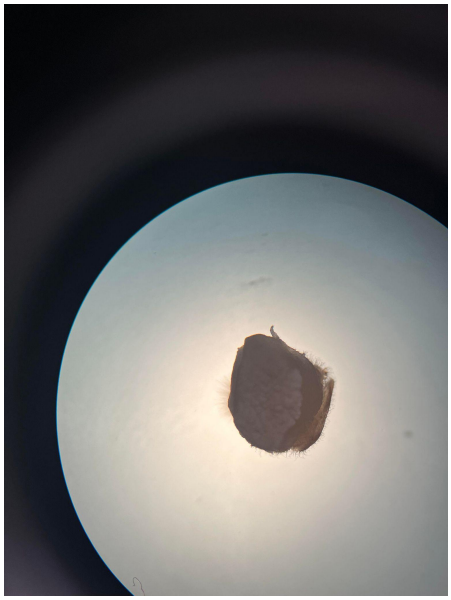
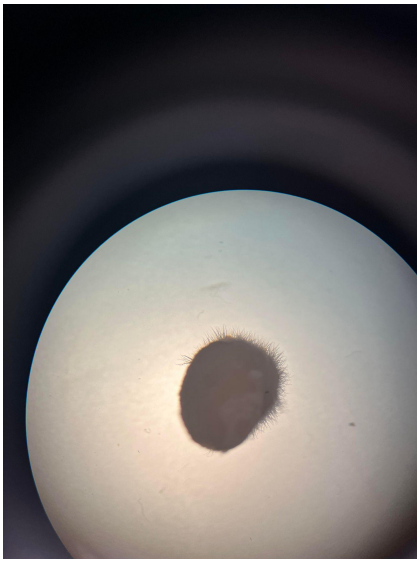
- At 25:
    - Bacteria has spread to different regions of the agar plate
    - Photo 1:
    -  
    - Photo 2:

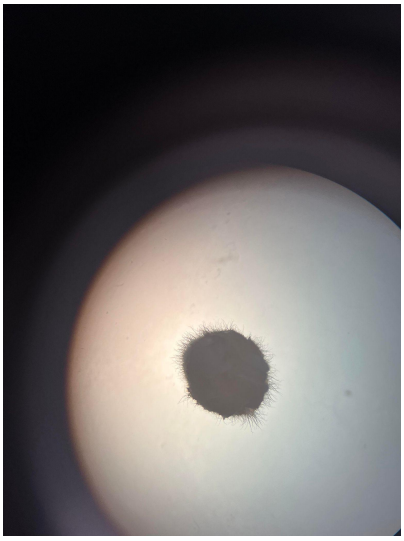
- 
- Photo 3:
- 
- Photo 4:

- 
- Photo 5:
- 

- At 20:
  - Much easier to see the layers and were more separated
  - Photo 1:

- 
- Photo 2:
- 
- Photo 3:

-  
- Photo 4:
-  
- Photo 5:

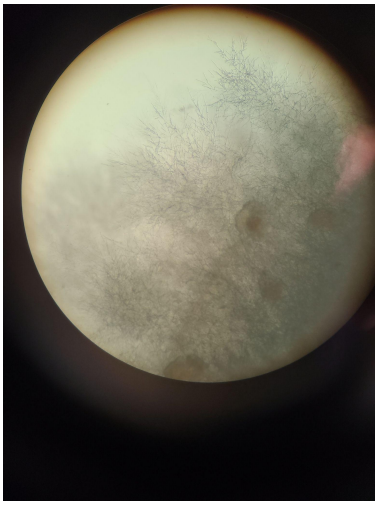
-  

- Orange portions are likely bacterial

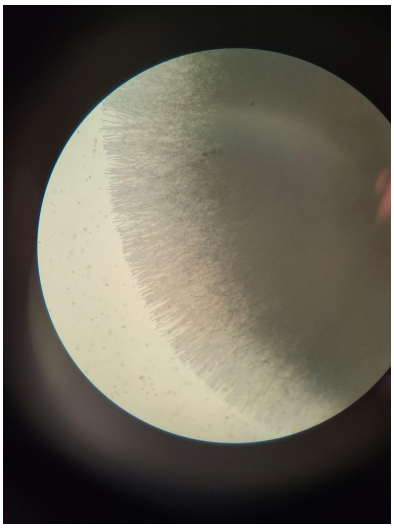
**March 8, 2023:**

30 Degrees:

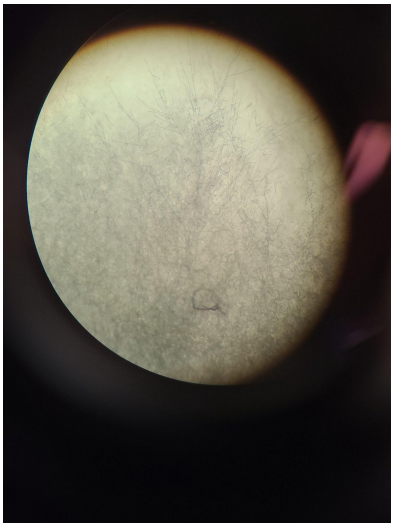
#1:

| 0.7X | 1X | 4X |
| --- | --- | --- |
|  |  |  |

#2:

| 0.7X | 1X | 4X |
| --- | --- | --- |
|  |  |  |

#3:

| 0.7X | 1X | 4X |
| --- | --- | --- |
|  |  |  |

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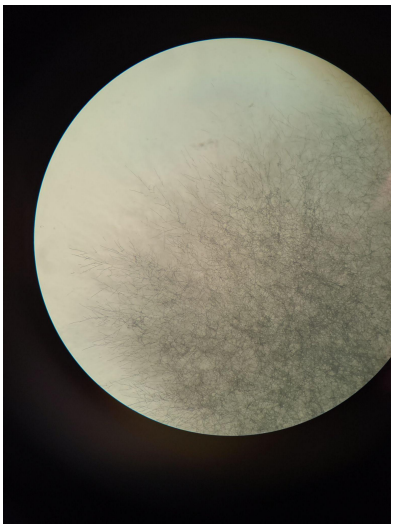
|  |  |  |

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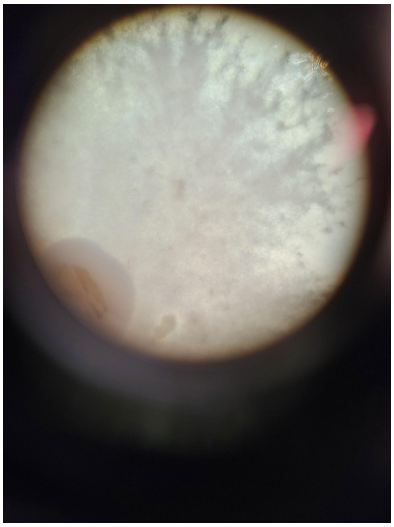
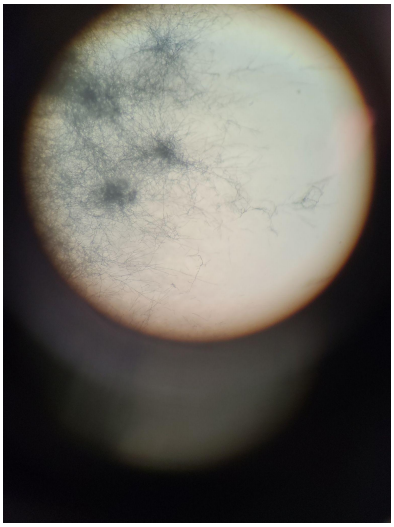
|  |  |  |

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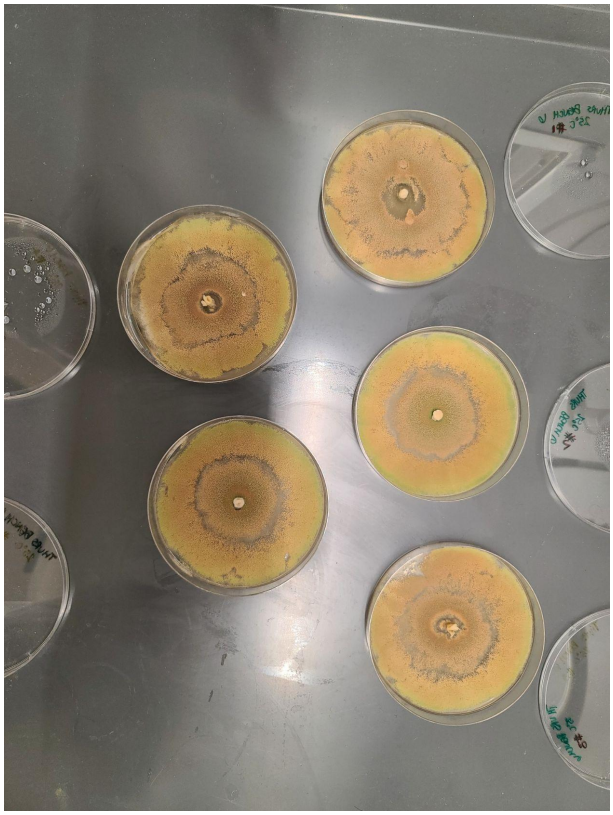

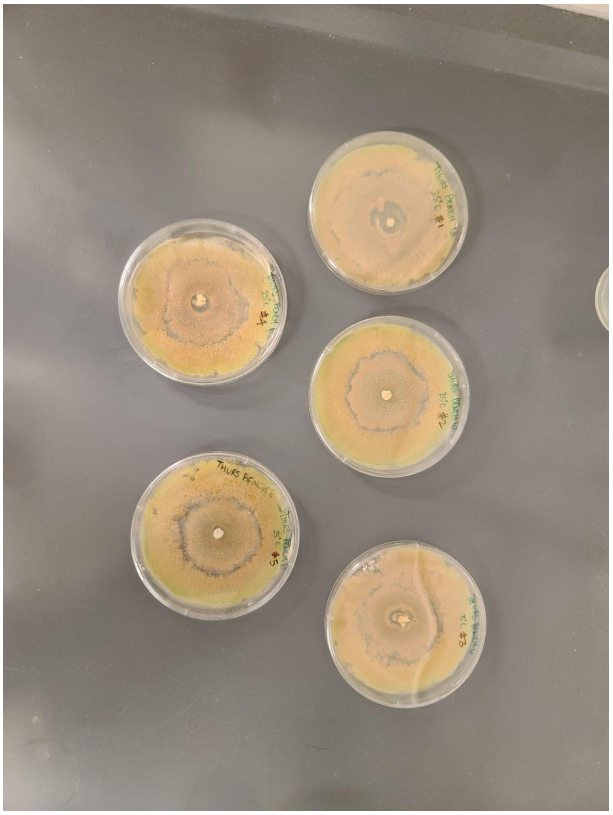

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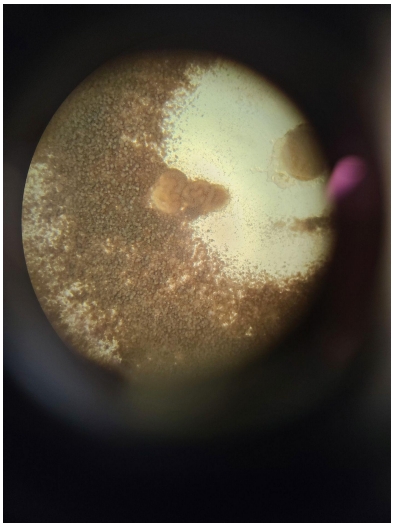
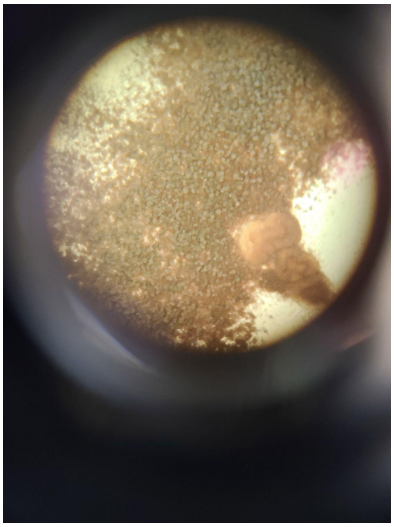
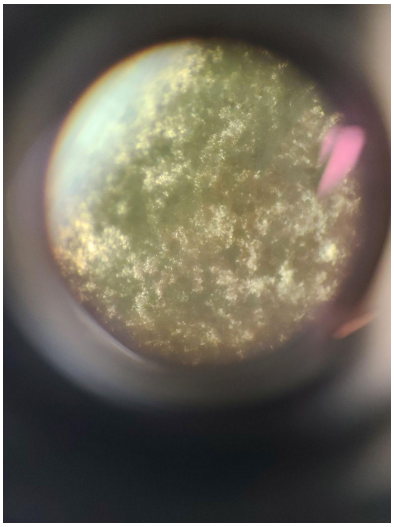
|  |  |  |

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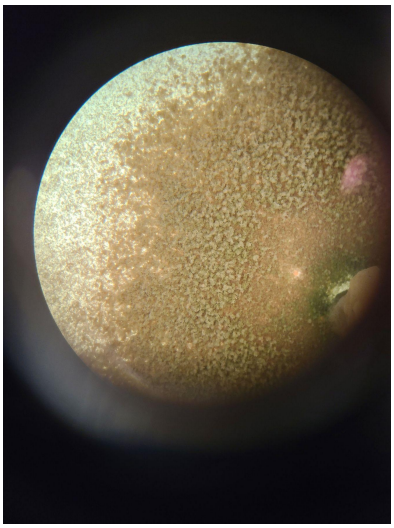
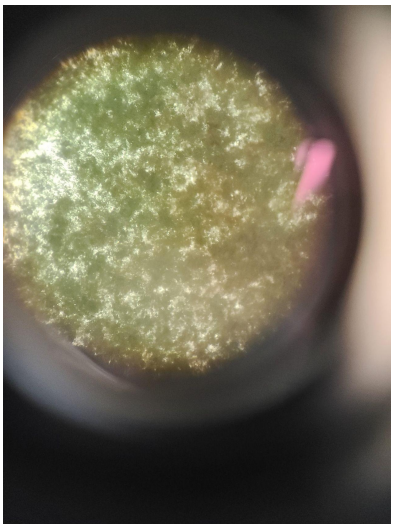
|  |  |  |

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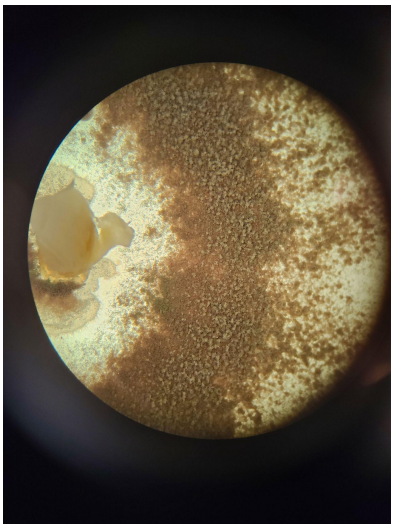
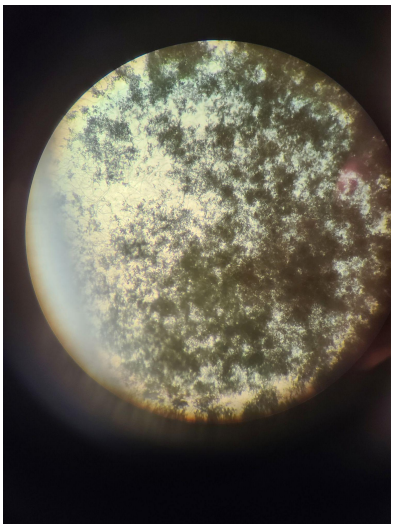
|  |  |  |

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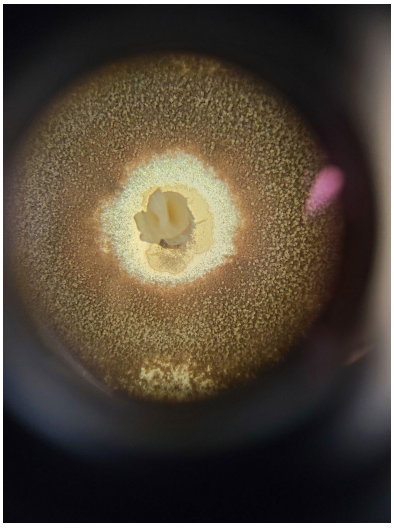
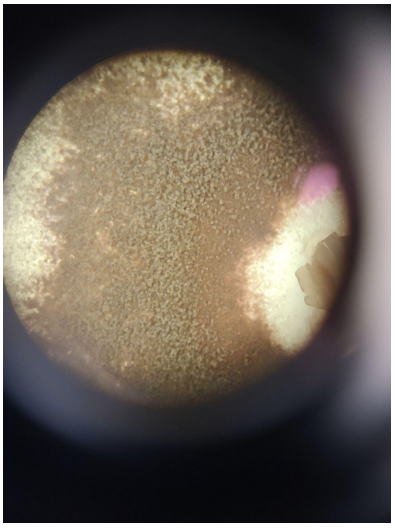
|  |  |  |

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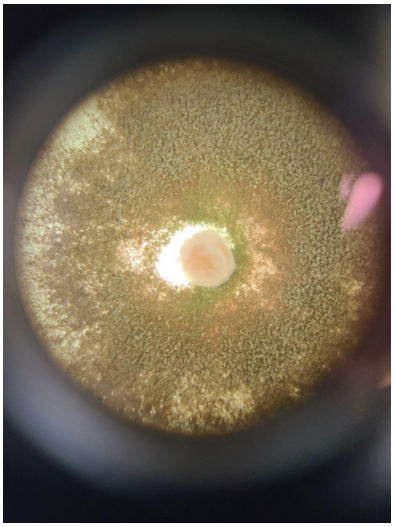
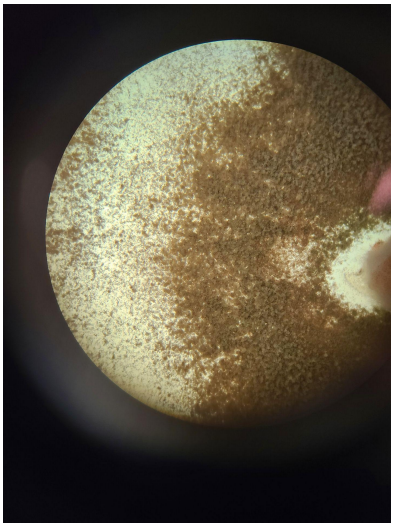
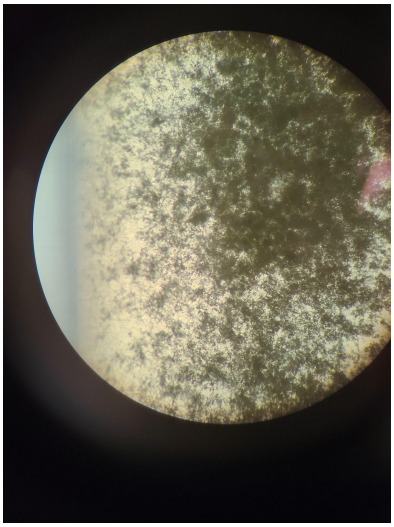
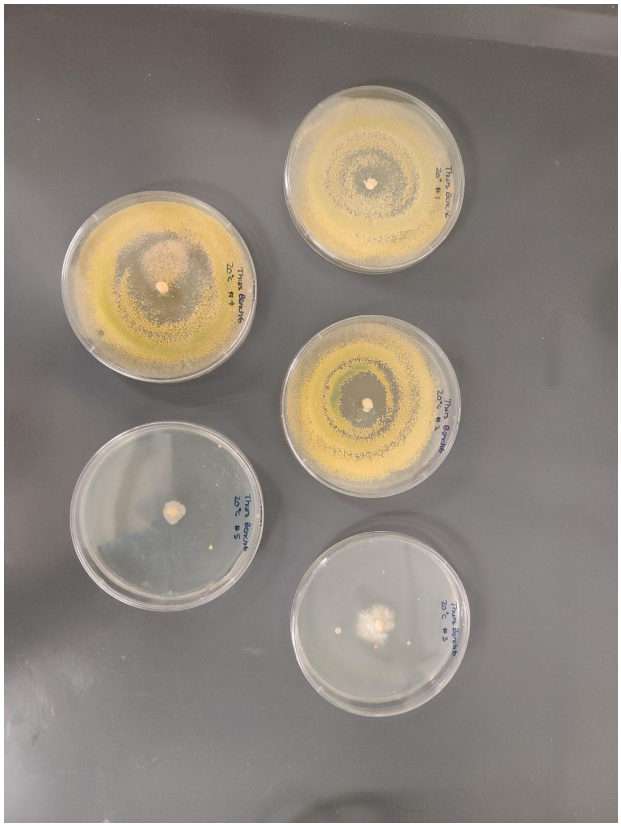
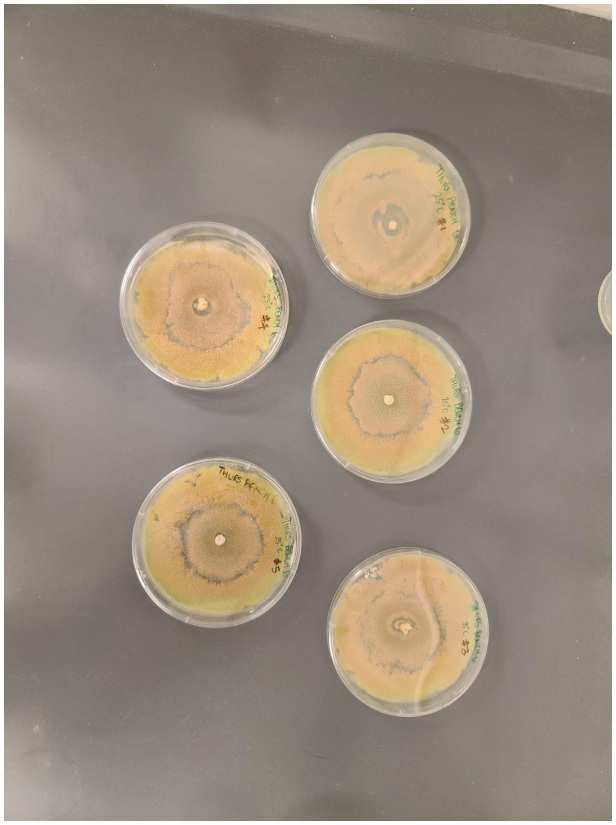
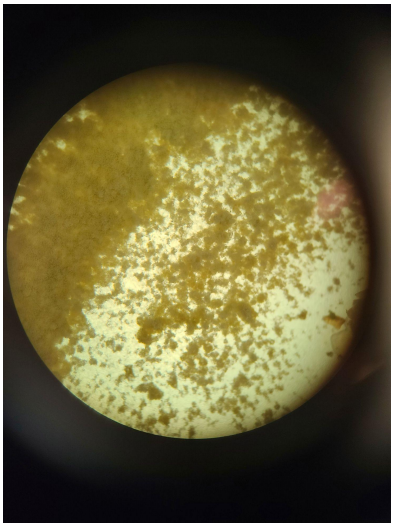
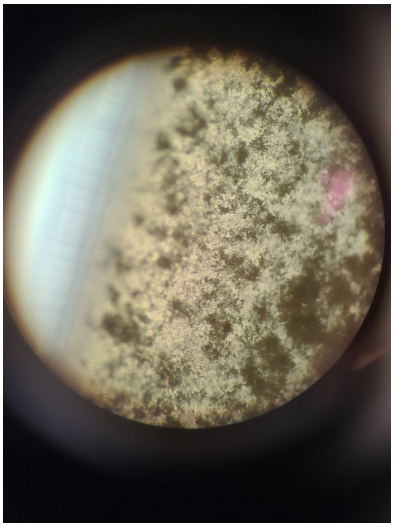
|  |  |  |

20 Degrees:





#1:

| 0.7X | 1X | 4X |
| --- | --- | --- |
|  |  |  |

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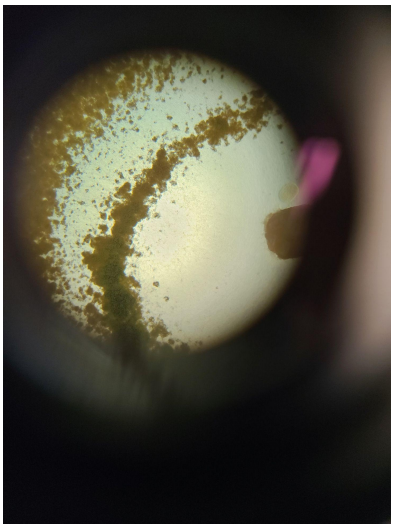
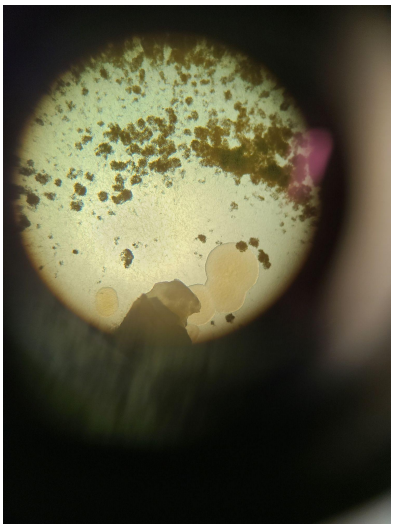
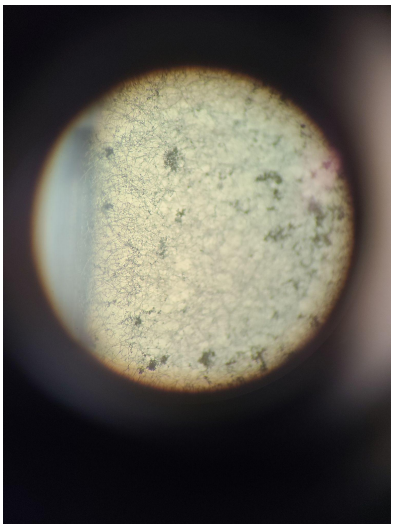
| 0.7X | 1X | 4X |
| --- | --- | --- |
|  |  |  |

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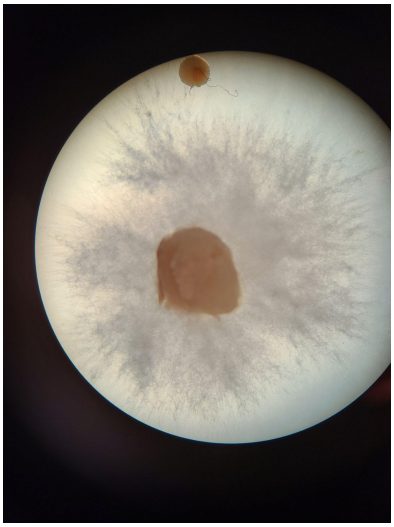
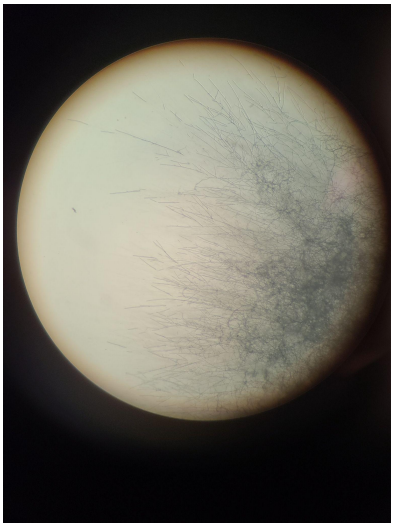
|  |  |  |

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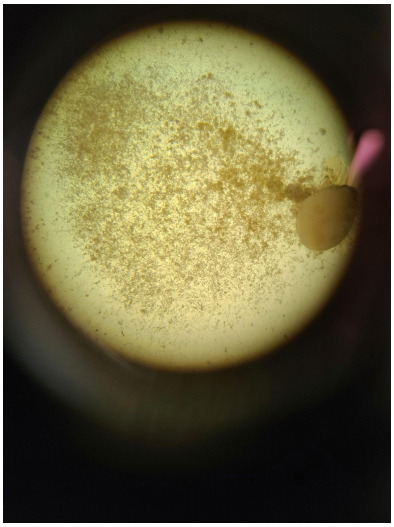
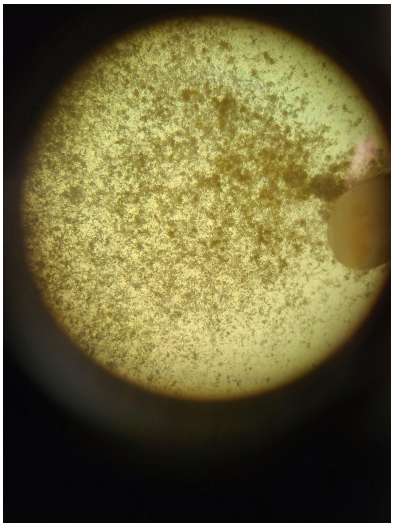
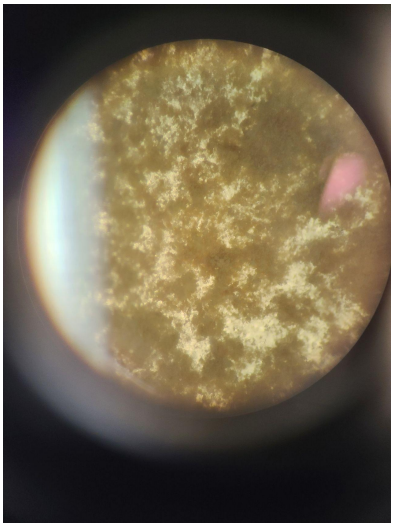
|  |  |  |

#5:

| 0.7X | 1X | 4X |
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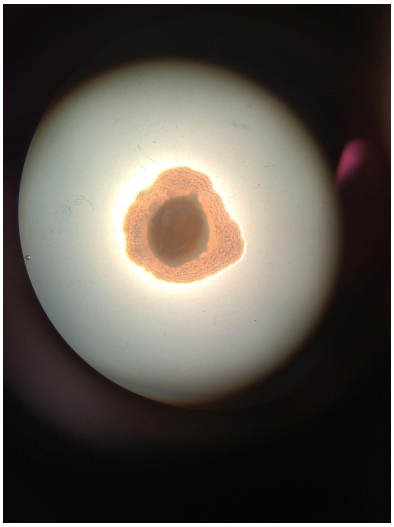
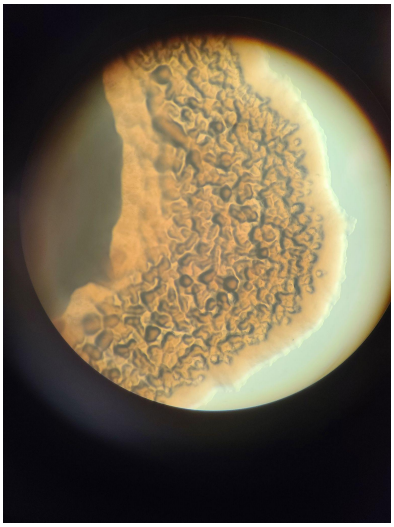
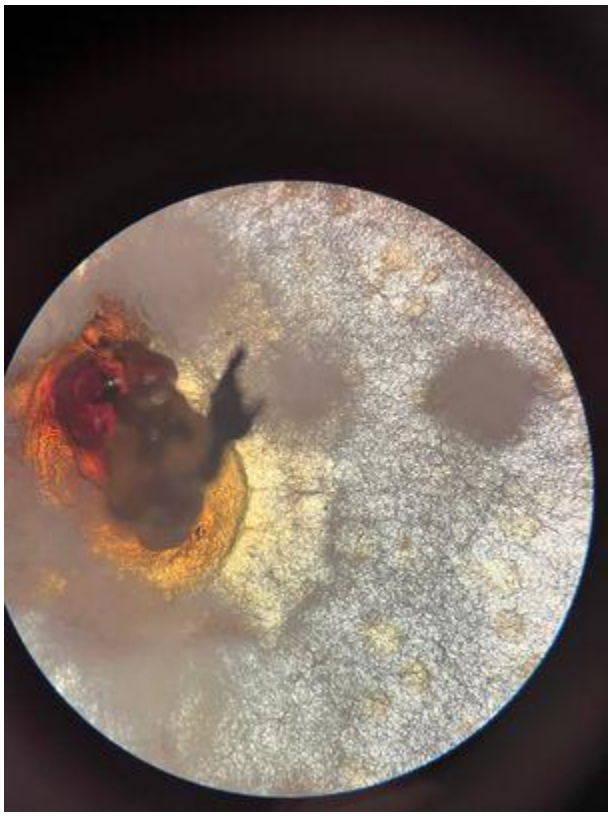
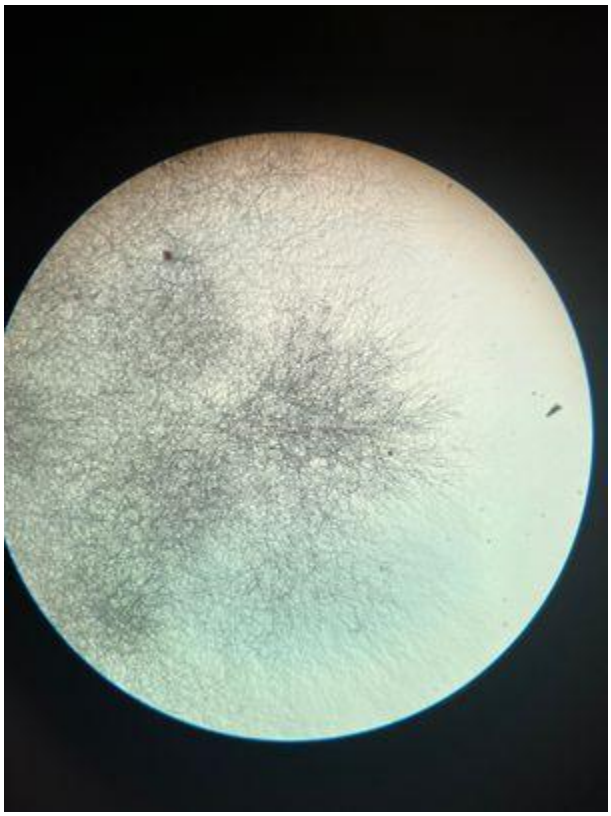
|  |  |  |

**March 15, 2023:**

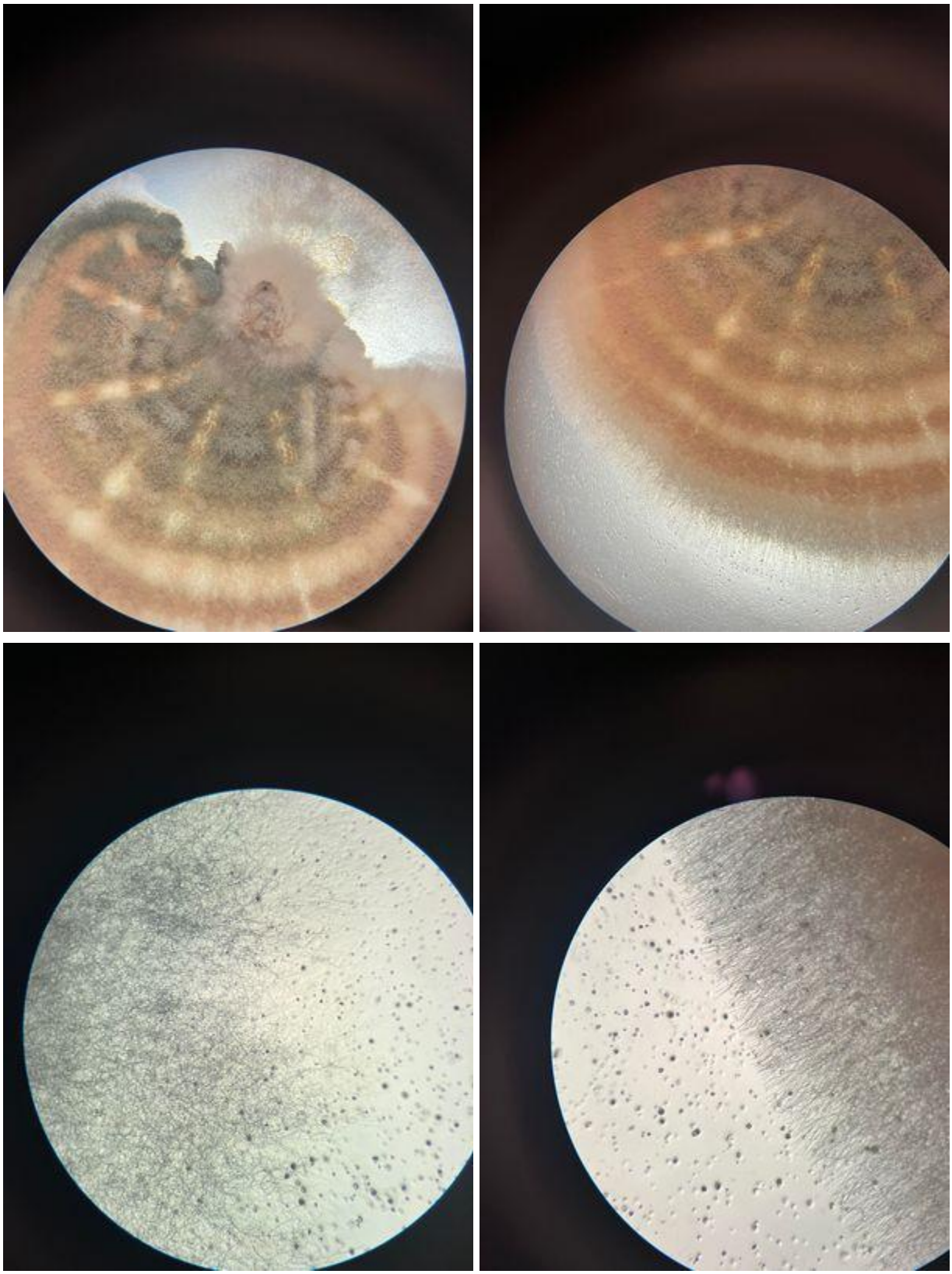
30 degrees

#1



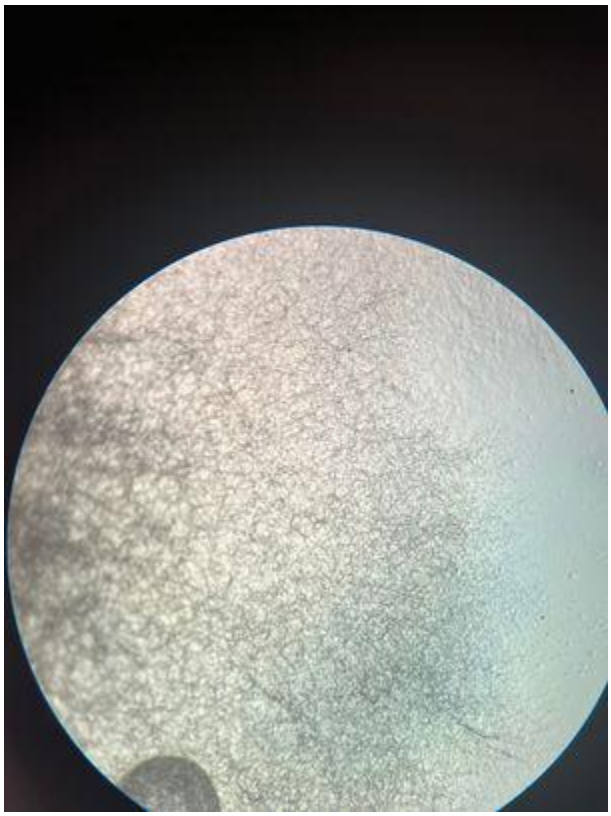


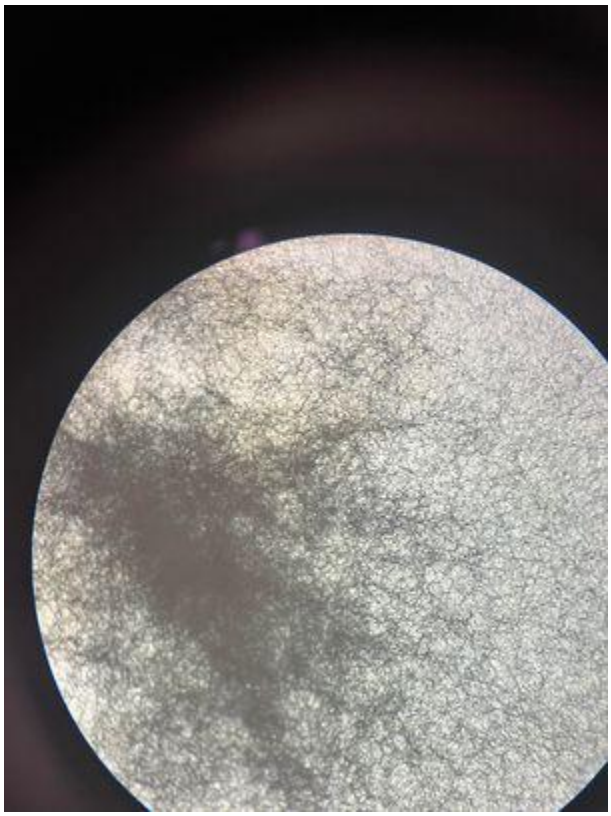
#2



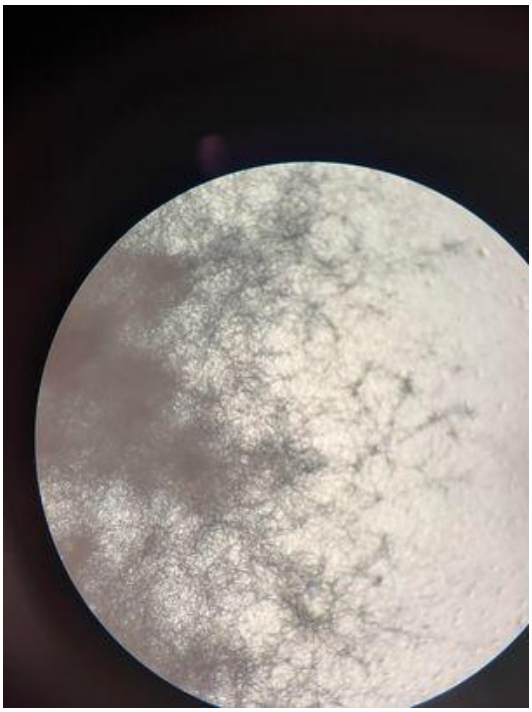
#3

#4

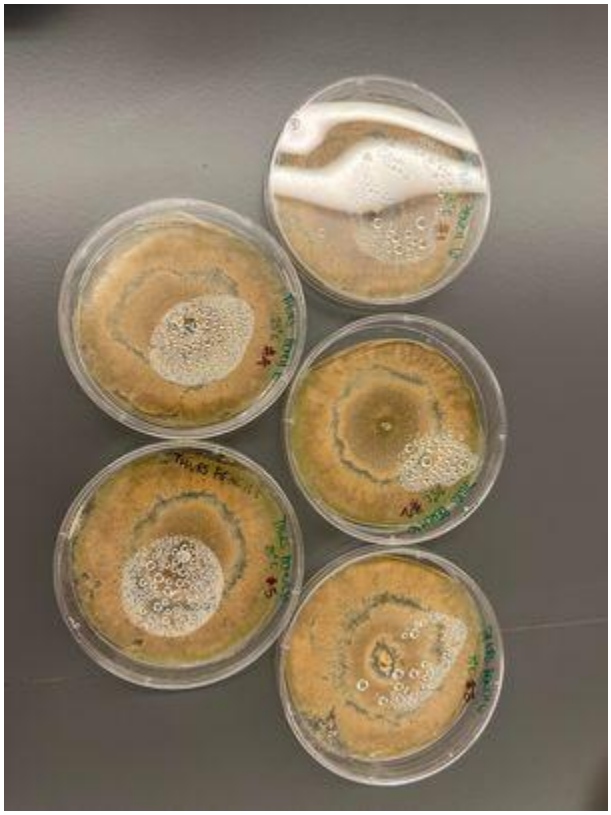




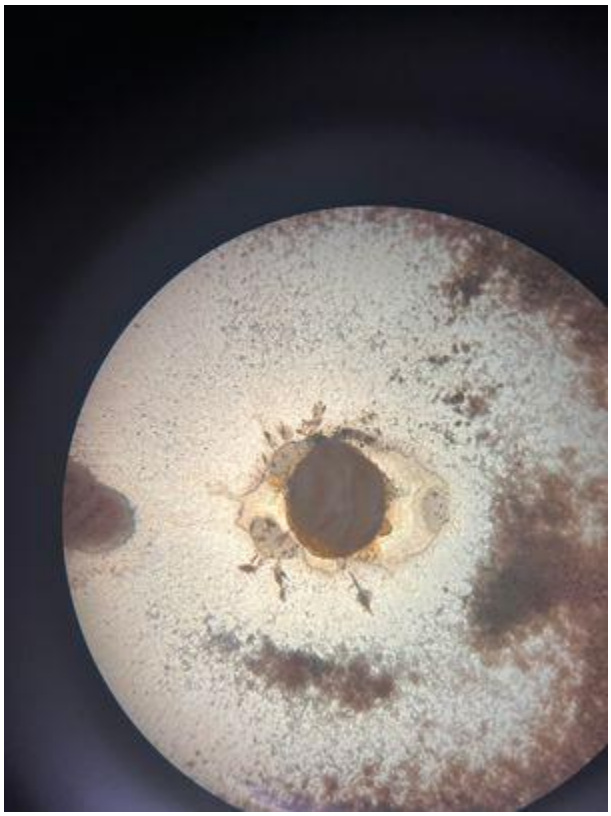
#5

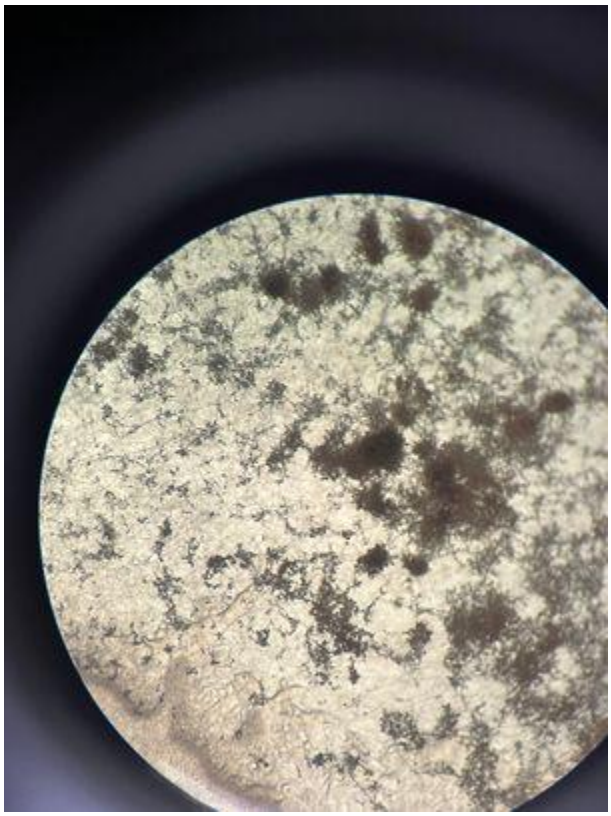



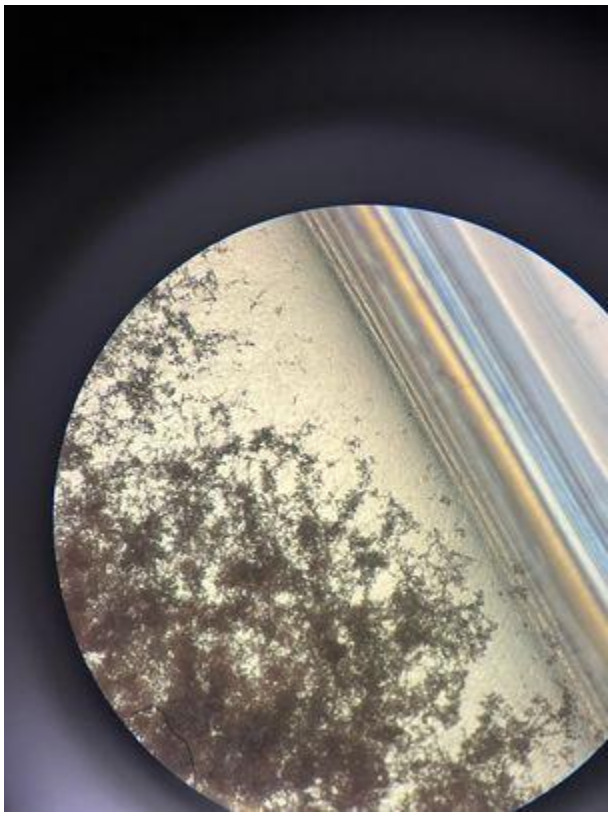
25 degrees



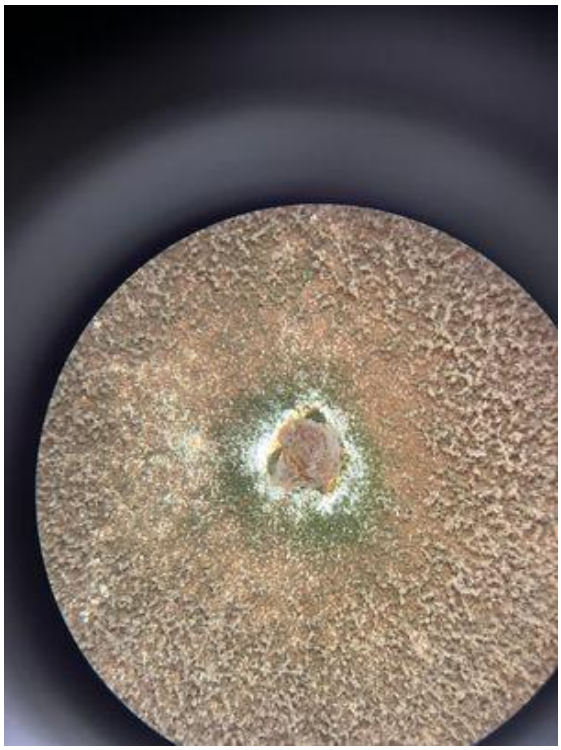
#1

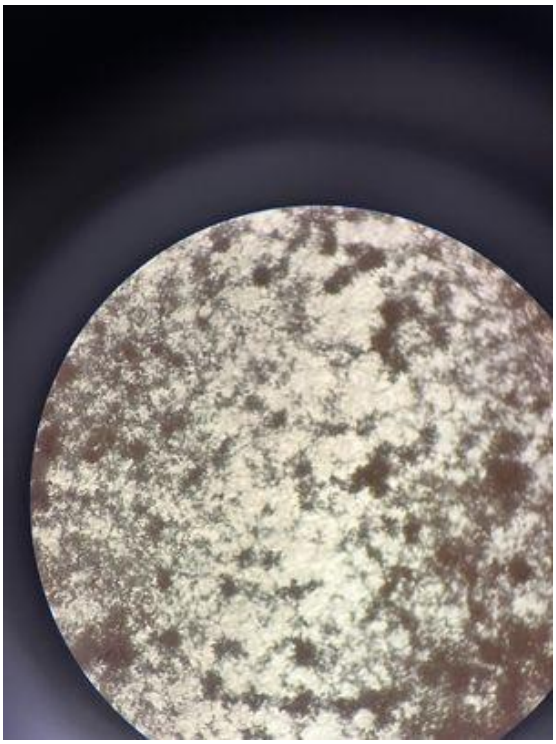






#2





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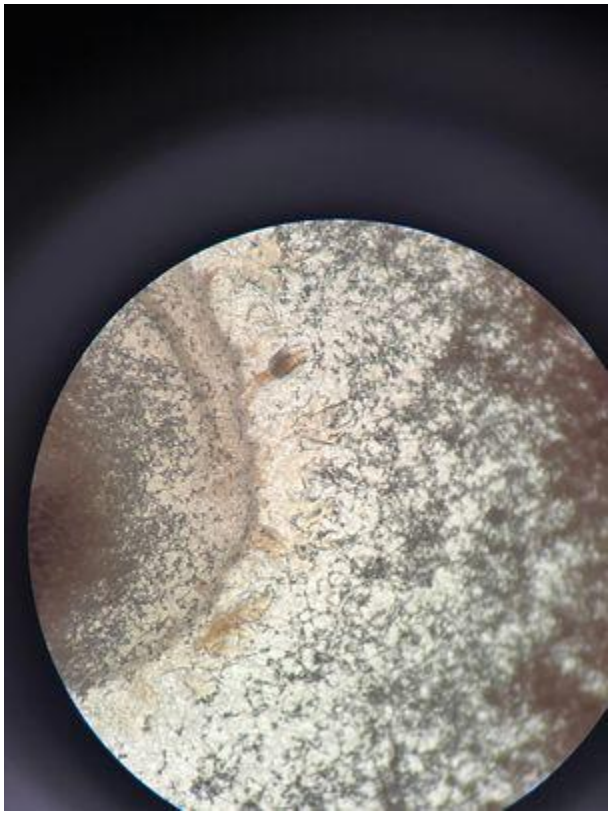
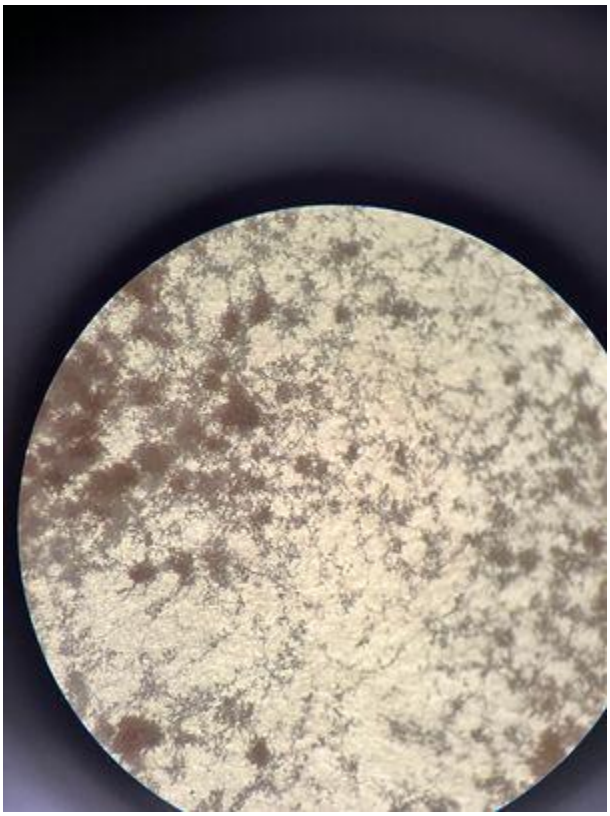


#4



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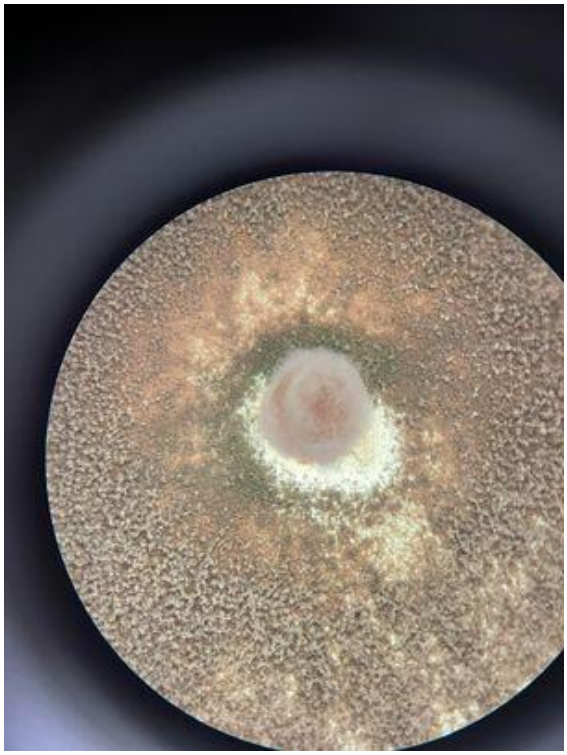
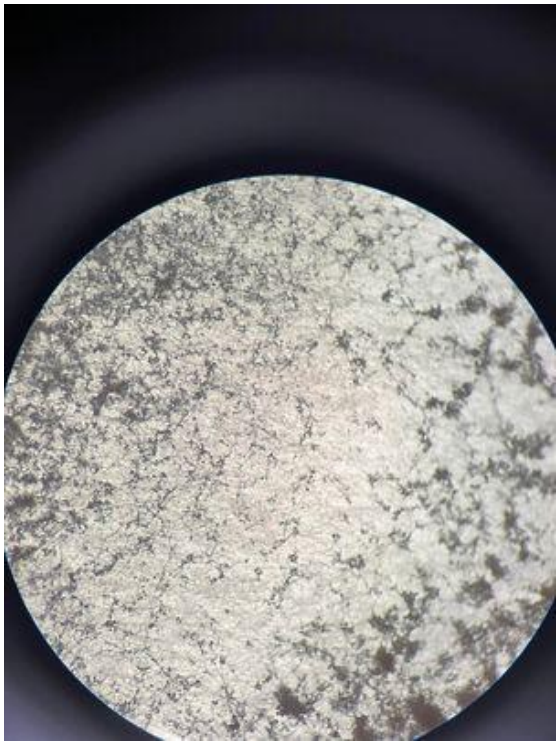
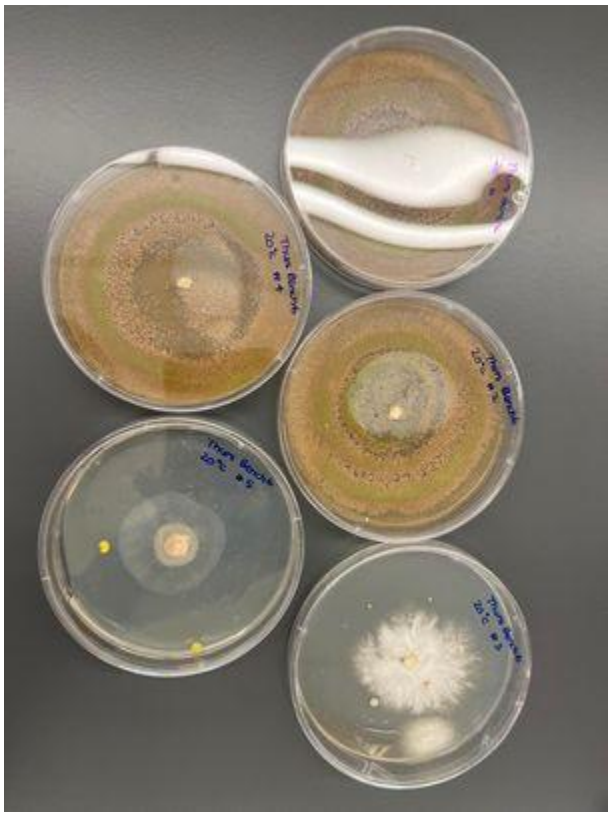
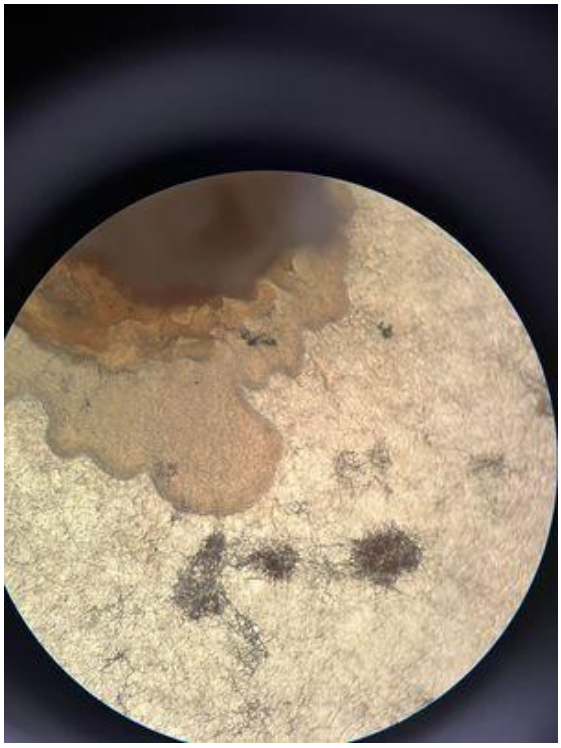




20 degrees



#1



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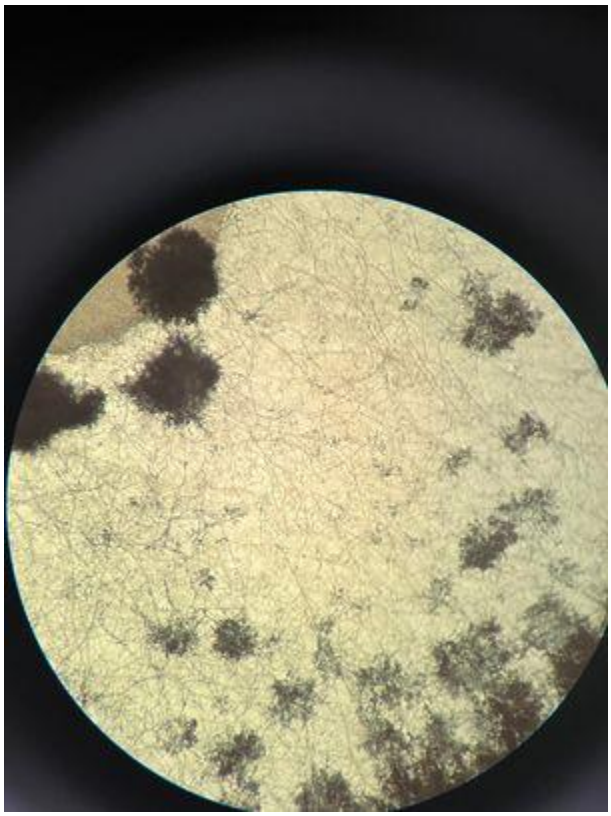

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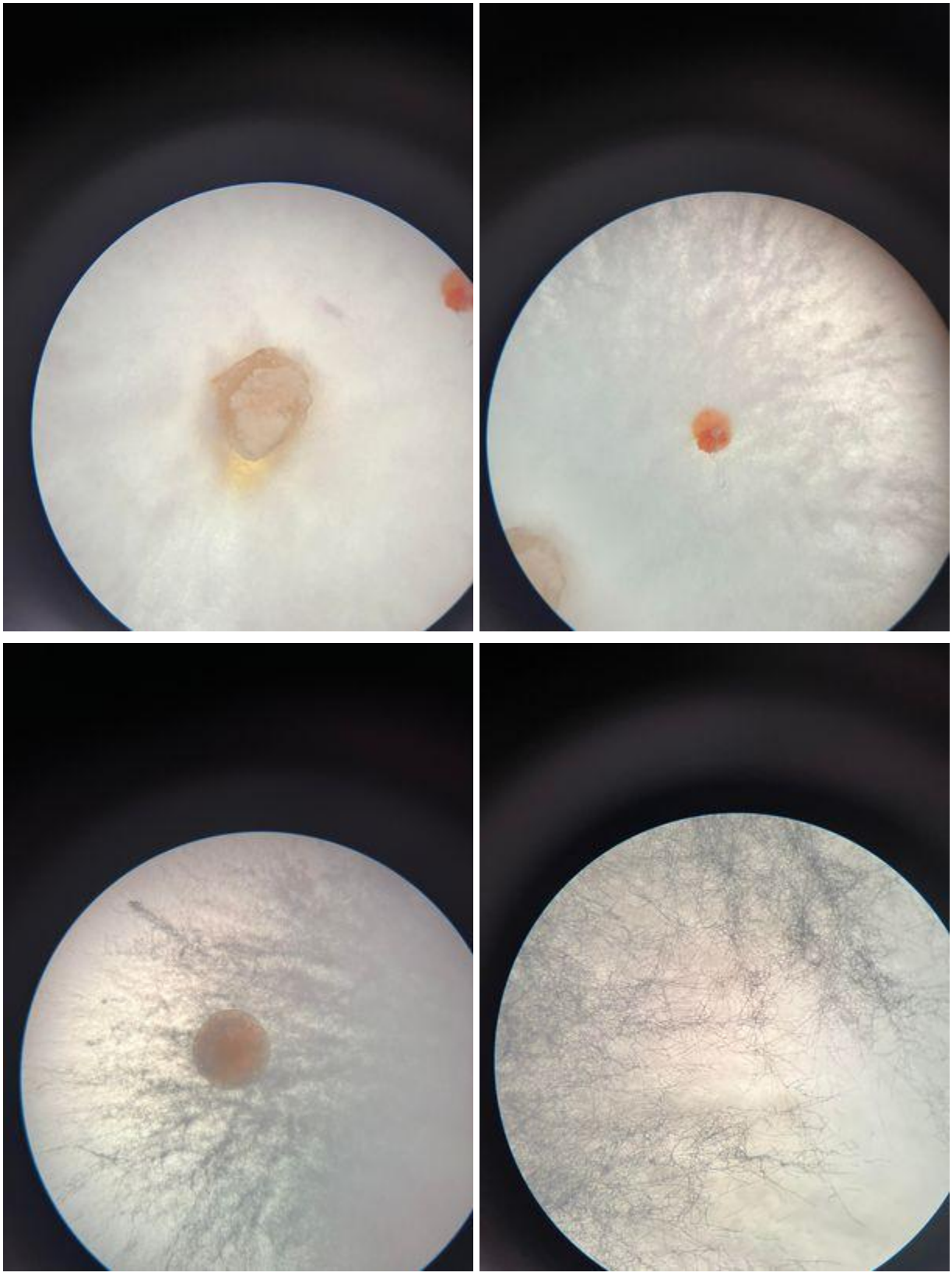

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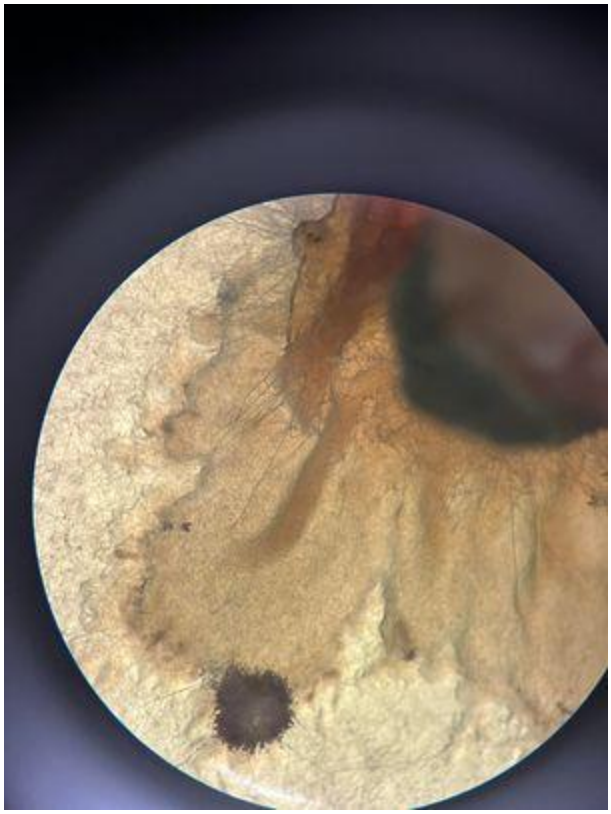

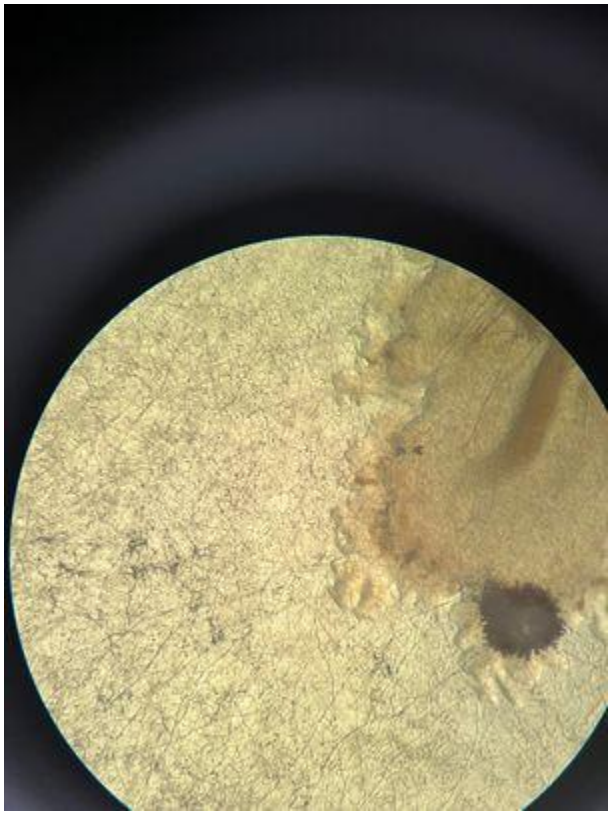

#5