The Effect of Moisture on the Growth of Mold on Organic Bread

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Abstract:

Mold is a very common fungus that can grow almost anywhere where the conditions will stimulate its growth. Moisture content is known to be one of the most critical components in promoting mold growth such that a higher moisture content will facilitate greater mold growth Block (287). The purpose of this study was to determine if moisture levels influence the growth of mold on samples of organic bread. For analysis, the percent cover of mold on the samples of organic bread with varying moisture levels was collected over a nine day period. Moisture was introduced into the bread samples through the addition of either 5 mL of water or 10 mL of water. The addition of a higher volume of water corresponded to a higher moisture content in the said sample. The main findings of this study based on a p-value of 0.02 was that higher moisture levels will result in increased mold growth. However, this was only supported when comparing two samples. If this experiment were to be repeated in the future, it would be important to make certain changes such as including a larger sample size in order to obtain more significant results.

Introduction:

Mold is a type of fungus that is very commonly found in many different environments all across the globe. This fungus is able to grow when the environment will have the most suitable conditions that it requires for its growth and development Block (287). Some of the most critical factors in fostering conditions that result in mold growth include temperature levels, moisture levels, oxygen, pH, water activity, and nutrient availability Block (287). The presence or absence of these environmental factors will either inhibit or facilitate the growth of mold depending on the quantity at which they are present in Block (287). Although each of these conditions have a large effect on mold growth, moisture is known to be one of the most crucial components in stimulating the growth of mold Block (287).

This experiment focused specifically on how moisture influences the growth of mold. This experiment was conducted on samples of organic bread and the growth of mold was determined by percent cover. Moisture was introduced in this experiment by using two treatment levels apart from the control in which bread samples were exposed to either 5 mL or 10 mL of water. The purpose of this study was to see if higher water content which leads to higher moisture content will result in a greater percentage cover of mold given that all other conditions were consistent between the samples. The null hypothesis of this study was that moisture has no effect on the growth of mold on the samples of the organic bread such that there is no difference in the mean percent cover between the different moisture levels. The alternative hypothesis of this experiment was that there is a difference in the mean percent cover between the different moisture levels due to higher moisture content promoting a higher percent cover of mold. It was predicted that if a bread sample had higher moisture content, it will yield a greater percent cover of mold because the increased moisture would facilitate the growth of mold.

This was predicted because it is known that if there is a higher water content, it will increase the moisture of the sample since more water will be absorbed by the bread Scott (84). This increased moisture will then cause the water to be more accessible to mold which mold requires in order to develop. This is because much like all living organisms, molds also depend on water for survival and molds thrive in damp conditions with high moisture content Scott (84). Thus, a higher moisture content creates conditions which accelerate mold growth allowing the mold to grow and spread more, thereby increasing the percent cover of the mold on the bread.

Methods:

In order to conduct the experiment, water was added to samples of organic bread to increase the moisture content of the bread. Two treatment levels of 5 mL and 10 mL were used alongside a control which was not exposed to any moisture level. The sample size of (n=3) was used for each treatment type.

To begin with, three slices of organic bread were used and cut equally into four smaller pieces. Out of the 12 total pieces of bread slices, only 9 were to be used for the experiment. One slice of bread was taken and placed directly into a ziplock bag. This sample was labelled as a control and thus was not exposed to any water. This process was repeated two more times such that a total of three bread slices were to act as controls. Next, a bowl was filled with water which was to be used for adding the water to the bread samples in order to increase the moisture of the bread samples. 5 mL of water was retrieved from the bowl and sprinkled on a new slice of bread. The water was sprinkled such that half of the water was distributed on one side of the bread and the other half of water was distributed on the other side of the bread. This process was also repeated two more times so that in the end, there would be three ziplock bags with 5 mL bread samples. Then, with the remaining three slices of bread, 10 mL of water was added onto the bread and the bread was placed directly into a ziplock bag. The 10 mL of water was also distributed on both sides of the bread to ensure that the entire sample of bread had equal moisture content.

After ensuring that all the ziplock bags had been labelled appropriately and were sealed shut as shown in Figure 1, the 9 ziplock bags were placed in a large container and stored in a dark and cool environment, in an area where family members did not frequently visit.

Observations and the percent cover of mold were recorded in the lab notebook. Observations were focused on looking at the growth and the percent cover of the mold. This involved looking at how much mold had grown, the colour of the mold, where mold was present on the bread sample and the percent cover of the mold. Observations were taken every two days on the samples of bread to assess if growth of mold had occurred and once mold signified by green colonies had been initially observed, the data and observations were recorded on a daily basis.

In order to determine the percent cover, a photo of the samples of bread was taken and transferred onto a laptop. A grid was created from a transparent sheet and sharpie which was then placed over on top of the photo. The number of quadrants covered in mold growth were counted and converted into a percentage to give the percent cover of the bread sample for the corresponding day. The data was then analyzed using the software GraphPad Prism. The original raw data was not normally distributed as was observed through a non-linear QQ plot. A transformation of $y = \ln(y)$ was conducted which led to the normalization of the data. Given that the assumptions of the ANOVA had now been met, a one way ANOVA was conducted. A Tukey's test was also conducted to determine where the difference in the treatment group means lies.

Figure 1. Figure 1 shows the experimental layout of the bread samples after the addition of the different moisture levels. The bread samples were kept in ziplock bags and labelled appropriately. These ziplock bags were then placed in a container and stored in a dark and cool place.

Results:

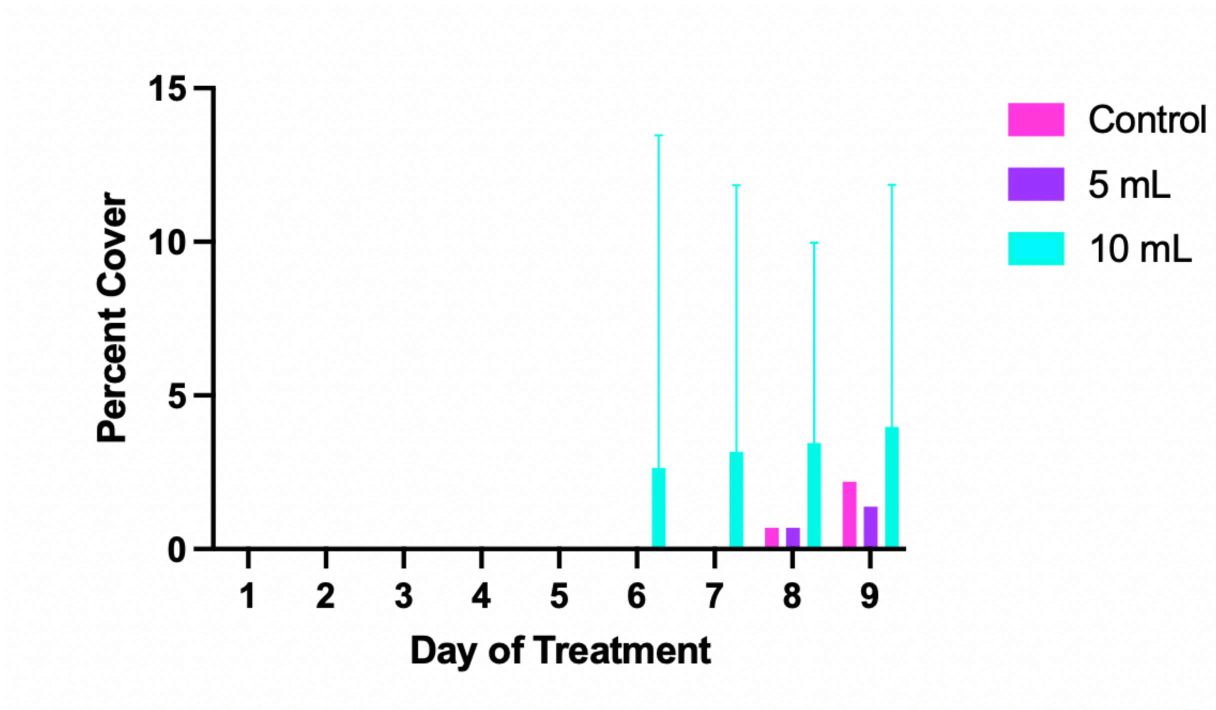

Figure 2: The percent cover of mold on the samples of organic bread over a nine day period is plotted in Figure 2. The data is presented as the means of the percent cover of the three treatment types including the control (n=3), 5 mL sample (n=3) and 10 mL sample (n=3). The mean percent cover of the control sample was 1.445, the mean percent cover for the 5 mL sample was 1.040 and the mean percent cover for the 10 mL sample was 3.314. The 95% confidence interval error bars of the mean are also plotted. The 95% confidence interval for the control sample was [-8.110, 11.00], for the 5 mL sample was [-3.364, 5.443] and for the 10 mL sample was [2.422, 4.206]. A one way ANOVA found the p-value to be 0.02. Tukey's test revealed the p-value between the 5 mL and 10 mL samples to be 0.0268, for the control vs the 5 mL sample to be 0.8283 and the p-value for the control vs the 10 mL sample to be 0.0546.

The raw data was found to have a non-normal distribution as determined by a nonlinear QQ plot. In order to use ANOVA testing, the assumption that the data had a normal distribution had to be met. Due to this, the data was transformed using the y=ln(y) transformation which then showed a normal distribution of the data. The percent cover of mold on the samples of organic bread over a nine day period is plotted in Figure 2. The data was recorded until one of the bread samples (in this case the 10 mL sample one) had reached 100% cover of mold. Figure 2 shows the data as the means of the percent cover of the three treatment types. The mean of the control sample was 1.445, the mean for the 5 mL sample was 1.040 and the mean for the 10 mL sample was 3.314. The mold observed in the 10 mL sample which had the highest mean percent cover was completely covered with a dark green mold on both sides of the bread sample. The 95% confidence interval error bars of the mean are also plotted. The 95% confidence intervals were found to be [-8.110, 11.00] for the control sample, [-3.364, 5.443] for the 5 mL sample and for the [2.422, 4.206] for the 10 mL sample. A one way ANOVA found the p-value to be 0.02 showing statistical significance in the results. To determine where the difference in the means of the percent covers between the varying moisture levels lies, Tukey's test was conducted. This revealed that the difference in the means of the treatment groups lies in the 5 mL and 10 mL treatments where the p value was found to be 0.0268. The p-value for the control vs the 5 mL samples was 0.8283 and the p-value for the control vs the 10 mL samples was 0.0546.

Discussion:

In this study, the influence that moisture content had on the growth of mold was assessed and it was discovered that moisture content influences mold growth. ANOVA testing provided a p-value of 0.02 which indicated that these results were considered statistically significant. This led to a rejection of the null hypothesis thereby providing support for the alternative hypothesis. This experiment thus provided more support for the idea that moisture levels do affect the growth of mold as assessed on samples of organic bread.

The results obtained from this study support those that have been documented in literature for years. Sian and Broadbent studied how moisture and humidity influence mold growth on rice straw (759). They found that at increased moisture levels the rate of the growth of the microorganisms will also be greater Sian and Broadbent (761). This is because at a higher moisture content, more kinds of molds and microorganisms will become active and be able to grow. Since microorganisms have different moisture requirements, higher moisture levels will help support the growth of more microorganisms Sian and Broadbent (761). These results can be applied to this study as it was found that at higher moisture content, the growth of mold was also higher since the percent cover of the mold was higher. Although Sian and Broadbent conducted the experiment on rice straw which is different from the organic bread used in this study, the fundamental idea of a microorganism needing a source of moisture to grow is applicable.

In another study, it was found that fairly dry samples of bread will inhibit the production of mold due to the lack of moisture Paul (6). Having a moist bread sample means that molds would have increased accessibility to enter the bread since the bread has been softened from the addition of water and high moisture content Paul (6). Since the bread samples in the 5 mL treatment group and 10 mL treatment group were completely dampened with water, it would have been expected that these breads would have a higher percent cover of mold. This is also somewhat consistent with the results obtained from this study. This is because although a higher mean percent cover of mold was observed in the 10 mL sample, the same was not found in the 5 mL sample which had a lower percent cover than the control.

Although ANOVA testing allowed the rejection of the the null hypothesis indicating that there is a difference in the mold growth based on moisture level, Tukey's test showed that the difference was only present in one comparison of the samples. There was a significant difference observed in the percent cover between the 5 mL group and the 10 mL group since Tukey's test provided a p-value of 0.0268. There was no difference was observed in the mean percent cover between the control and 5 mL group (p=0.8283) and the control with the 10 mL group (p=0.0546). Although the null hypothesis was still rejected, confounding variables could have affected the ability of the Tukey’s test to show a difference between the means in each treatment group. This could have arisen because moisture is not a single factor in determining whether mold will grow or not. Other factors that influence mold growth include oxygen, temperature, pH and water activity Axel et al. (3528). Based on this, just increasing the moisture content might not have been enough to have a large effect on mold growth, if the other factors were present at levels that inhibit mold growth. In a study that looked at mold growth in books, in some cases with high moisture content, mold growth was not observed which was said to be caused by other factors preventing growth Block (291). This means that in this study, there might not have been a difference observed between all of the varying moisture levels due to other variables that inhibited mold growth. The presence of the other factors might have been at levels where they were more powerful than the addition of moisture such that mold growth did not occur even though it was expected.

In future experiments it may be of interest to change the experimental design to ensure higher precision of the results. One source of error in the results could have been that not all the bread samples were the same size which meant that there could have been discrepancies in the calculation of the percent cover. Human error could have caused more or less water to be added on to bread samples and even contamination of the bread beforehand which would have also disrupted the results. Also, this study used a fairly small sample size, which contributed to the non-normal distribution of the data. A higher sample size would have led to a normal distribution of the data such that no transformation would have been needed. This means that more accurate results could have been obtained from raw data without the need of a transformation which would have impacted the ANOVA and tukey's test. Further, more treatment levels could have also been included such that the experiment looked at more moisture levels and how they impacted mold growth. Since this study only looked at two moisture levels, it might not have been enough to showcase a difference in all treatment groups. These changes might allow for a more accurate analysis of how moisture influences mold growth.

Conclusion:

Based on the statistical analysis which determined a p-value of 0.02, it can be concluded that moisture plays a large role in influencing the growth of mold. Even though the null hypothesis was rejected, the tukey's test revealed that the difference in the mean percent cover was only present in the 5 mL and 10 mL treatment groups. This could be explained by other factors that were acting on the bread samples that inhibited mold growth even in high moisture conditions. In the future, it would be best to use an increased sample size to obtain more significant results.

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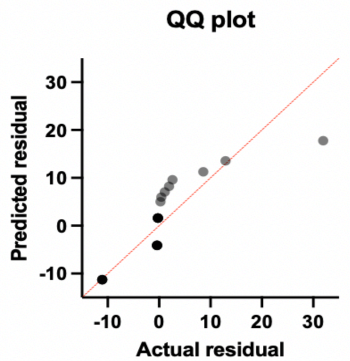
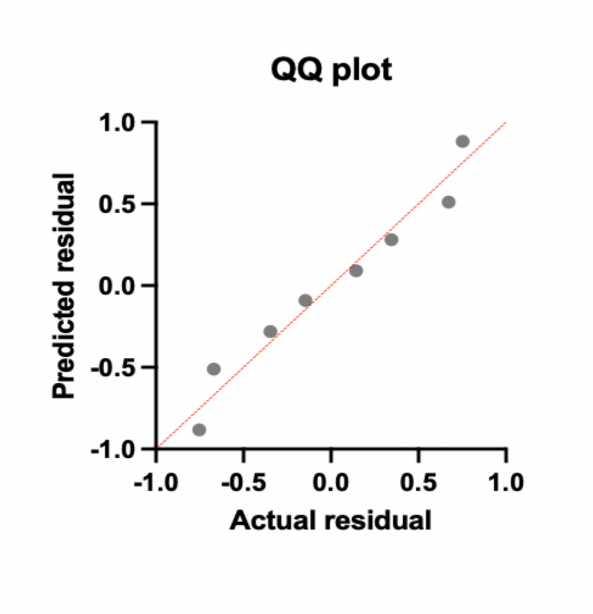
Appendix:

The following is the raw data which includes the percent cover of each bread sample. These values were transformed using y=ln(y) in order to conduct the ANOVA testing.

Day	Control Sample 1	Control Sample 2	Control Sample 3	5 mL Sample 1	5 mL Sample 2	5 mL Sample 3	10 mL Sample 1	10 mL Sample 2	10 mL Sample 3
1	0%	0%	0%	0%	0%	0%	0%	0%	0%
2	0%	0%	0%	0%	0%	0%	0%	0%	0%
3	0%	0%	0%	0%	0%	0%	0%	0%	0%
4	0%	0%	0%	0%	0%	0%	0%	0%	0%
5	0%	0%	0%	0%	0%	0%	0%	0%	0%
6	0%	0%	0%	0%	0%	0%	33%	0%	6%
7	0%	0%	0%	0%	0%	0%	47%	0%	12%
8	0%	2%	0%	2%	0%	0%	53%	0%	19%
9	0%	9%	0%	4%	9%	0%	100%	0%	29%



This QQ plot shows the raw data which did not have a normal distribution. A transformation of y=ln(y) was applied to this data in order to conduct the ANOVA.



This QQ plot shows the normalized data. The transformation of y=ln(y) transformed the data such that it now had a linear distribution. Since the assumptions of the ANOVA were now met, statistical analysis using ANOVA was conducted.