

Statistical Analysis of Growth of a Fungal Species under Different Conditions

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1. Introduction

The aim of this study was to identify the colonial diameters of a fungal species under different conditions.

1.1 Study Design

Since last century, there have been many reports about the decline of growth rate of grapevine around the world. Some growers tried to replant grapevines in the same area, but vines were growing slowly (1). Thus, growers were concerned about the decline in growth of grapevine.

A fungal species, *Phaeoacremonium* species, was found in the xylem vessels of infected grapevines, and the xylem vessels became dark brown after the grapevines were infected (1). The fungi grow inside the xylem vessels and block the vessels as the diameters of the colonies increase, which resulted in decline growth of grapevines (1). If the fungi parasite inside the vascular system, the fungi must depend on the water and the nutrients from the host (1). Thus, water potential has a direct impact on the growth of the fungal colony.

The subject of this study is to test if the fungi grow the same in different temperatures, different pH value, and different water potential with different solutes in order to find effective strategies to control the infection.

1.2 Variables

Concentration of sucrose, KCl, NaCl, and glycerol in water effects were tested separately in different experiments. Each experiment had two trials, and four replications for each level of treatment. Under different conditions, Diameters of the fungal colonies after seven days were recorded in centimeters as response variables.

In the water potential experiments with sucrose, KCl, NaCl, and glycerol concentration, there were five treatment levels for each experiment, which were the concentrations for each solute at -0.3, -0.8, -2.3, -4.3, -6.3 MPa.

2. Methodology

Statistical analysis of this study was performed using statistical software R3.5.1. Two statistical procedures were used in this analysis. Analysis of variance table was used to test trials and treatment effects on the fungal diameters with temperature, pH value and water potential. Regression was used to show how colonies change with temperature, pH value, and water potential.

2.1 Analysis of Variance Table

Analysis of variance table was used to analyze variance within each treatment group, and treatment levels and trials variance within the overall mean to detect if there is a significant trials effect and treatment effect.

2.2 Regression

For all experiments, we are interested in how colonies' diameters change as conditions change. Thus, regression model was used to analyze the data if trials effect or treatment effect are significant. The model is as follow:

$$Y = \beta_0 + \beta_1 X + \beta_2 X^2 + \dots \beta_n X^n + \beta_{n+1} * Z_1 + \beta_{n+2} * Z_2 + \beta_{n+3} * Z_3 + \varepsilon$$

Where:

Y = diameter of fungal colony

β_i = the regression of coefficient X_i

$Z_1 = 1$ if KCl, 0 otherwise

$Z_2 = 1$ if NaCl, 0 otherwise

$Z_3 = 1$ if Sucrose, 0 otherwise

All $Z_i = 0$ if Glycerol for ($i = 1, 2, 3$)

ε = model's residuals

3 Water Potential Effect

First, we combined all data of water potential with four different solutes under six concentration, which were sucrose, KCl, NaCl, and glycerol. Plot of fungal diameters versus concentration is shown below.

According to the plot, it seems that there is a cubic relationship between fungal diameter and solute concentration. We also compare the residuals between the quadratic model and cubic model to check if improvement is significant. According to the output 1 from the appendix, p-value of the F test equal to 1, which mean that there is almost no improvement in the residuals. Then, we also test between the quadratic model and the linear model. P-value of the F test is 0.067, which is larger than $\alpha = 0.05$. There is no significant improvement on residuals. However, there is a significant improvement between the linear regression and the cubic regression. We also test the between the cubic model and the model to power of 5, but the p-value is 1. Thus, we decided to use the cubic regression model. The residual plots of the model are shown below.

According to the residual plot, constant variance assumption seems to be violated. According to the output 2 from the appendix, p-value of the independent test is less than 0.001, and the p-value of the constant variance is 0.0469. Independent assumption and constant variance assumption are violated. Thus, we decided to check if transformation can fix the assumptions. According to the Box Cox plot below, mean of λ is 0.6. Thus, we decide that the λ value is 0.6.

After the transformation, we fit a cubic regression model with $(Y_i)^{0.6}$. Residual plots of the model after transformation are shown as below.

According to the residual plots, residuals deviated from normal; independent and constant variance assumptions also seem to be violated. According to the output 3 from the appendix, p-value of all tests are less than 0.001. All assumptions are violated. There is no improvement in residuals. Thus, we decided to use the cubic model with the original data. The estimates of parameters are shown below.

$$Y = 8.022462 - 2.013397X - 0.01014X^2 + 0.020324X^3 - 0.03333*Z_1 - 0.185417*Z_2 + 0.270833Z_3 + \varepsilon$$

Where:

Y = Diameter of fungal colony

X = water potential

$Z_1 = 1$ if KCl, 0 otherwise

$Z_2 = 1$ if NaCl, 0 otherwise

$Z_3 = 1$ if Sucrose, 0 otherwise

All $Z_i = 0$ if Glycerol for ($i = 1, 2, 3$)

4. Conclusion

Since water potential usually will not exceed 0 MPa, and the diameters of the fungal colonies are 0 when water potentials of all solutes are -6.3 MPa. Thus, it is meaningless to interpret the diameters of the fungal colonies when water potential is greater than 0 MPa or less than -6.3 MPa. According to the model, the diameter of the fungal colony is largest when water potential is close to 0. However, as concentration of solutes increase, diameters of the fungal colonies decrease at a rate of $0.060972X^2 - 0.02028X - 2.0134$ by taking the derivative of the original regression in term of X. When compare to diameter of the fungal colony when glycerol is presented, diameter of the fungal colony is 0.27cm larger overall when sucrose is presented in the water, 0.033cm smaller when KCl is presented, and 0.1854cm smaller when NaCl is presented. However, all four experiments are run independently, and interaction effects among four treatments have not been tested. Future study about the interaction effects is needed.

Work Cited

1. Whiting, E. C., et al. "Effect of Temperature and Water Potential on Survival and Mycelial Growth of *Phaeomoniella Chlamydospora* and *Phaeoacremonium* Spp." doi:10.1094/pdis.2001.85.2.195.

Appendix

Output 1: F test for model determined

Output 2 Residual tests for cubic regression model with original data

Output 3 Residual tests for cubic regression model after transformation