

**The Effects of *Ganoderma lingzhi* and *Lentinula edodes* on the Regeneration
Rates and Longevity of *Lumbriculus variegatus***

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Abstract

Medicinal mushrooms such as *Ganoderma lingzhi* (Reishi) and *Lentinula edodes* (Shiitake) have been reported to have numerous health benefits: lower cholesterol, stimulate the immune system, reduce inflammation and increase cellular longevity. These mushrooms have been used as remedies in traditional Chinese medicine for centuries but are not considered to be official medicinal prescriptions in western medicine. *Lumbriculus variegatus* was a good model organism to show the effects of these mushrooms on regeneration because the segments of the California blackworms can be regrown under normal conditions after being severed. *G. lingzhi* and *L. edodes*, purchased from a Chinese apothecary, were double extracted with 96% ethanol and spring water. Each extract was then combined in a 1:1 ratio and applied to the *D. melanogaster* media in culture vials, and to the culture spring water for *L. variegatus* in 1:100, 1:1,000, and 1:10,000 concentrations, while controls used spring water. Results thus far have shown positive correlations with both *G. lingzhi* and *L. edodes* when compared to controls.

Introduction

The effectiveness of Eastern medicine in the evolving world and modern disease has been widely asked by many doctors in the Western world. Many Western doctors refute the use of traditional Chinese medicine and the use of natural remedies in order to fight various illnesses. *Ganoderma lingzhi* and *Lentinula edodes* are two well-known mushrooms used for their health benefits and medicinal properties in China. These mushrooms are recognized for their anti-aging, anti-cancer, regulation, and for increasing regeneration in cellular activity. Cancer is a major issue in the world and a vast topic that the Science community must prioritize learning about. These medicinal mushrooms may be the solution and a less harmful way to fight cancer, along with aiding in regeneration in cellular repair and by enhancing and modulating the immune response.

Ganoderma lingzhi, better known as Reishi mushroom, is held in high regard and considered the best mushroom with the most beneficial properties in Chinese medicine. This mushroom has been tested and displays anti-cancer properties where it triggers the apoptotic pathway in the cancer cell and leads to overall cancer cell death while still boosting the human immune system and repair of non-cancer cells. Other uses for *G. lingzhi* also include treatment for high blood pressure, high cholesterol, promotes cardiovascular and kidney health, and treats liver disease.

Another important mushroom in Eastern medicine would be the *Lentinula edodes*, also known as the Shiitake mushroom. This particular mushroom is most commonly found in diets across the world. Many people are unaware of the benefits of consuming this common mushroom. The Shiitake mushroom is known for enhancing the immune system, which may indirectly slow tumor growth in the body, contains antibacterial, antiviral, and anti-inflammatory properties. Anti-aging properties are prevalent in this mushroom since it goes against oxidative stress that can lead to signs of aging in the skin.

The purpose of this study is to find the effects of *Ganoderma lingzhi*, Reishi, and *Lentinula edodes*, Shiitake, have on the *Lumbriculus variegatus* (California Blackworms). The hypothesis is that these mushrooms will extend the lifespan of the worms along with speed up the regeneration rate of the segments in the worm.

Materials and Methodology

The materials used in this experiment included *Ganoderma lingzhi*, *Lentinula edodes*, *Lumbriculus variegatus*, Petri dishes, micropipettes, purified water. The first step in this experiment was to create a double-extraction of the two mushrooms. To make the double-extraction, the mushrooms first needed to be pulverized simply by using a blender. Then 20g of Reishi was soaked in 250 mL and 20g of Shiitake were soaked in 100 mL of 96% EtOH for 10 days and wrung out using cheesecloth for the Reishi and coffee filters for the more fine, powder-like Shiitake. These mushrooms were then put in the same volumes of purified water for

another 7 days and the process of wringing them out was repeated. Then, both concentrations were combined in a 1:1 ratio of 50 mL alcohol concentrate and 50mL water concentrate for Reishi, and 25 mL alcohol concentrate and 25 mL water concentrate for Shiitake. To set up experiments for longevity and regeneration rate of *Lumbriculus variegatus*, 35 worms were cut to 1cm lengths and 5 were placed into each petri dish. 10 mL of purified water was added into the control. To create the 1:100 ratio, 10mL of water was added with another 100 microliters of Reishi and Shiitake solution. For the 1:1,000 ratio, 10 microliters of solution were added into 10mL of water, and to make a 1:10,000 ratio, 10mL of water was combined with 1 microliter of solution. The immediate reaction of the *L. variegatus* to the solutions was examined. Every 4 days, worms were taken out of their Petri dishes and the previous solution was replaced with a new dose.

Results



Figure 1 (shown previous) shows survivability tests set up on December 4th, 2019. 3

Lumbriculus variegatus were placed in each petri dish along with the 1:10, 1:100, and 1:1,000 concentrations of solutions of *Ganoderma lingzhi* and *Lentinula edodes* both. The purpose of these tests was to examine the worm's reaction to these high dilutions of the mushroom solution and to decide which concentrations would be most beneficial to use for this experiment

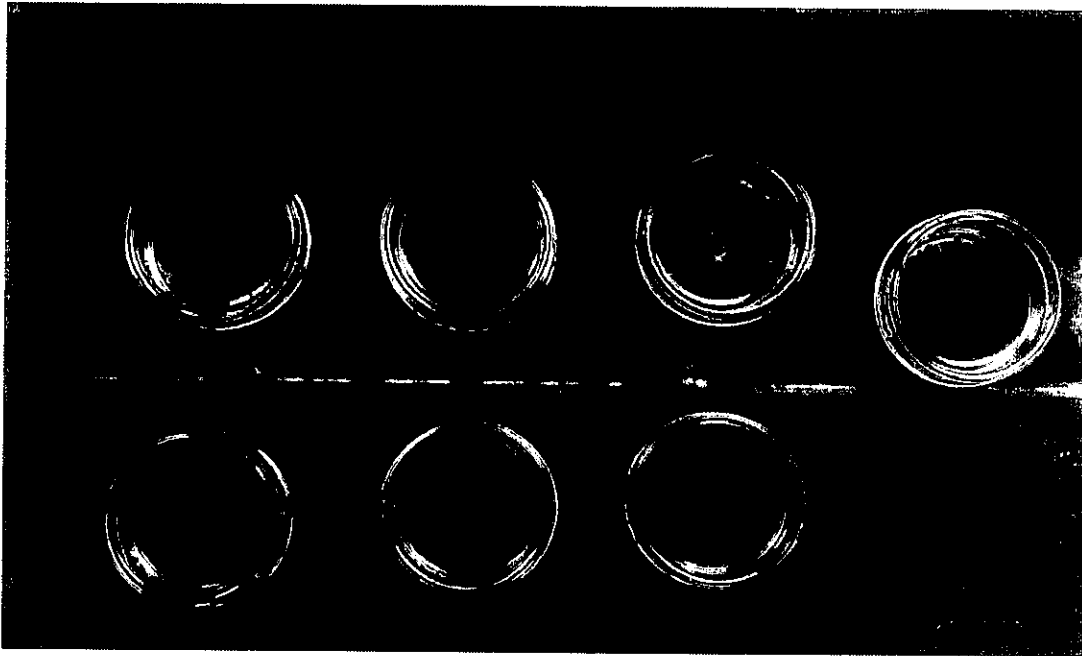


Figure 2 (shown above) shows the results of an experiment set up on December 17th, 2019. Five 1cm segments of *Lumbriculus variegatus* were placed into separate Petri dishes. Different dilutions of the *Ganoderma lingzhi* and *Lentinula edodes* were added into the Petri dishes. After

27 days, the photo above was taken. The only experimental group that remained alive was those under a 1:100 *Lentinula edodes* solution.

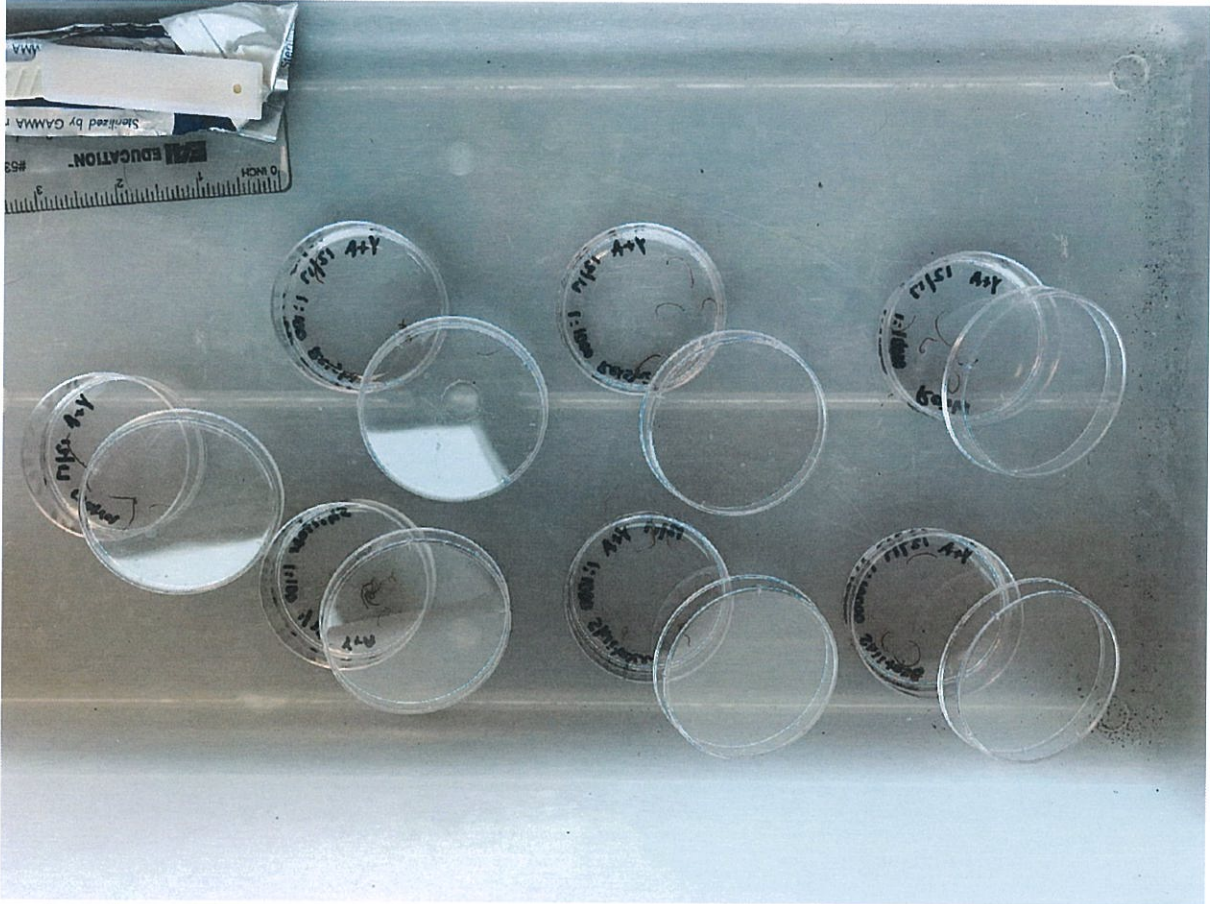


Figure 3 (shown above) taken on January 21st, 2020 shows the 2nd experiment to test *Lentinula edodes* and *Ganoderma lingzhi* effects on longevity and regeneration of *Lumbriculus variegatus*. Experiments were set up on January 23rd, 2020

Longevity of *Lumbriculus variegatus*

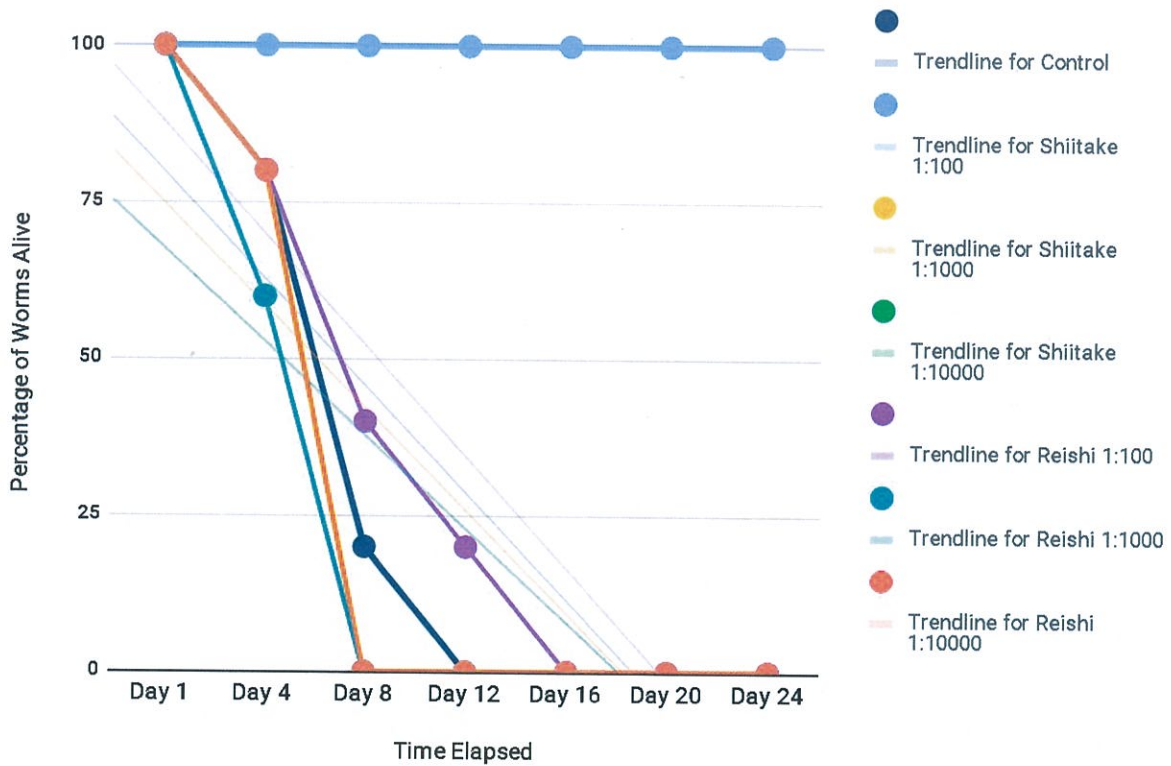


Figure 4 (shown above) shows the longevity of *Lumbriculus variegatus* in 4 day increments.

Every Petri dish had 5 *Lumbriculus variegatus* and on average there was a loss of about 25% of *Lumbriculus variegatus* in each time span of 4 days. The longest living experimental group was the group under a 1:100 concentration of *Lentinula edodes* solution which is still alive January 29, 2020.

Average Number of Segments Regrown in Days

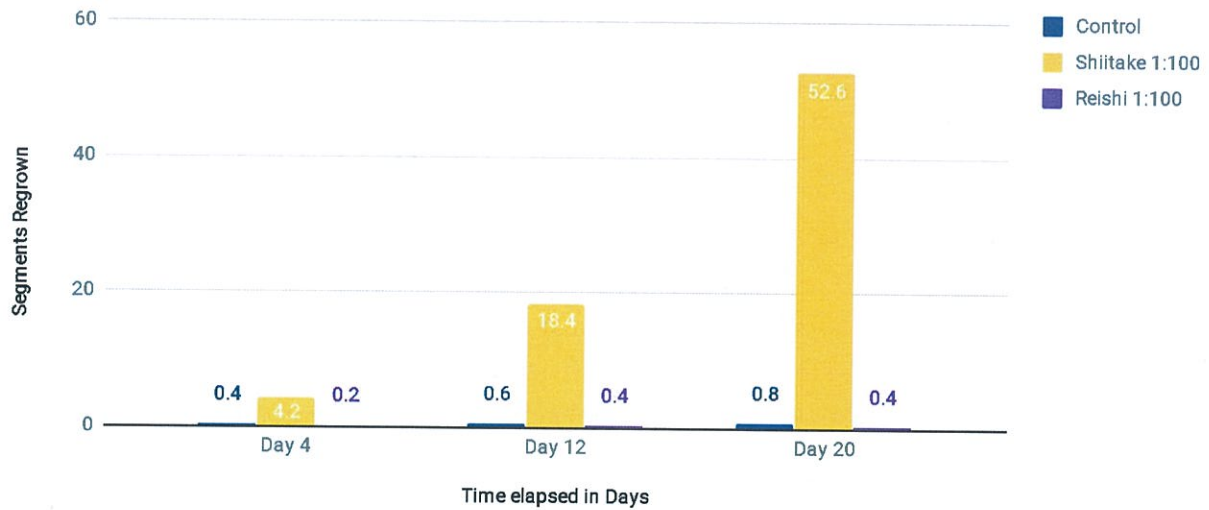


Figure 5 (shown above) shows the average number of segments regrown in the control, Shiitake 1:100, and Reishi 1:100 experimental groups. Shiitake showed the greatest regeneration rate at about 2.6 segments per day with Reishi and the control showing almost no regeneration



Figure 6 (shown to the left) shows a *Lumbriculus variegatus* under 40x magnification. This worm was under a 1:100 Shiitake solution that showed signs of regeneration. There are 12 regrown segments present, the photo was taken 12 days after the experiment began

Discussion

During the survivability testing beginning, December 4th, 2019, the *Lumbriculus variegatus* in the 1:10 concentration of both Reishi and Shiitake showed extreme immediate discomfort to the solutions. This may have been because of the high concentration of alcohol present in these low dilutions. However, after 14 days, all the *Lumbriculus variegatus* had died except for those in the 1:100 ratio solution of *Ganoderma lingzhi*. In the first experimental trial to test longevity and regeneration rates, 1:100, 1:1000, and 1:10000 concentrations were used. There were little to no immediate reactions to the solutions but after 7 days most of the *Lumbriculus variegatus* had died except for those in the 1:100 solution of *Lentinula edodes*. The 5 worms in this solution showed signs of regenerating segments at a faster rate than the others in the experimental and they also lived for much longer than the others. After 12 days, the worms in the *Lentinula edodes* 1:100 solution even segmented off so that there were a total of 6 healthy worms in the petri dish that started off with 5. These 6 worms are still living on January 26th, 2020. Figure 3 shows an ongoing experiment that was set up on January 21st, 2020. Data has yet to be collected. Results from the Shiitake 1:100 solution coincides with the hypothesis by showing a longer life span and far faster regeneration rates than the control.

Future Work

The knowledge gained from this experiment can be expanded by using *Drosophila melanogaster* as another model organism for longevity and reproduction cycle studies. The *D. melanogaster* would be given the mushroom extracts in their food to test the longevity and

reproduction rates. A large-scale future experiment would be using squamous cell carcinoma in a professional laboratory and apply the *Ganoderma lingzhi* and *Lentinula edodes* onto the cancer cells to truly view the effects these medicinal mushrooms have on cancerous human cells.

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