**RESEARCH PAPER SUBMITTED TO**

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**BY**

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**Title:** Evaluating how Lion's Mane, *Hericium erinaceus*, mushroom substrate affects yield

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**Abstract:**

Mushroom production is well established in the Lower Mainland of BC, The Fraser Valley being one the biggest exporters of brown and white mushrooms in Canada. This is supplemented by the wild crafted and wild foraged fungi for the gourmet mushroom industry. Further exploration into Lion's Mane, *Hericium erinaceus,* and their ability to aide in the carbon cycle, filter pollutants, increase food security, and divert waste from landfills will benefit future generations that require expedited solutions to climate change. Similar studies are already being conducted on numerous varieties of Oyster mushrooms. Researching Lion's Mane growth substrate would help expand the understanding of the growth substrate's potential.

This study was conducted at the Kwantlen Polytechnic University Seed Lab at the Richmond Campus, with the aim to assess the yield of Lion's Mane mushrooms grown on various substrates. Four substrates (oak, sphagnum peat moss, coco coir, and coffee) were tested for yield of Lion's Mane mushrooms.

No significant yield difference was detected between oak pellet, sphagnum peat moss and coffee ground-based growth substrates. No mushrooms grew on the coco coir. Further investigation on various aspects of Lion's Mane mushroom cultivation in Canada is needed to promote a sustainable industry.

**1. Introduction:**

The most cultivated mushrooms in BC are white and brown button mushrooms (Ministry of Agriculture, 2014). Mushrooms are known to be an impressive protein alternative to animal meat, but they are also grown for their substantial health-promoting properties (Bliss, 2006). Lion's Mane mushrooms are rich in nutrients, are an excellent source of antioxidants, support nerve regrowth, promote blood sugar regulation, contain immune-modulating properties, boost antiviral and antibacterial effects, possess anti-tumor properties, support gut health, and are used for anti-inflammatory purposes (Stamets, 2005).

There is room for growth in the mushroom farming industry to include new species other than the white or brown button mushroom, such as Lion's Mane, Reishi, and Shiitake, which could offer greater health benefits. Rop, Mlcek, and Jurikova discuss the glucose polymer, Beta-Glucans, in *Beta-glucans in higher fungi and their health effects,* as a soluble fiber found in oats, seaweed, algae, and mushrooms. This compound is found in the cell wall of these foods and is known to boost immune function (Rop, Mlcek, & Jurikova, 2009). It is associated with lowering a person's risk of developing Type 2 diabetes due to a reduction in body weight (Rop, Mlcek, & Jurikova, 2009).

Previous research has concentrated on growing Lion's Mane mushrooms on grain because this material is relatively cheap, easy to pasteurize, and accessible (Grace & Mudge, 2015). A potential gap in this field of research is a lack of experimentation on growth substrate with this species since it is such a high-value crop when sold to restaurants and the functional medicine mushroom industry.

Ultimately, further critical analysis of Lion's Mane mushrooms grown on different substrates compared to the control substance, oak pellets, is needed to evaluate the treatment that generates the maximum yield. This will offer data for future use by the public and justify a more detailed evaluation of responsible use of spent media.

*1.1 UN Sustainable Development Goals*

This experiment is associated with four UN Sustainable Development goals. These goals are Life on Land, Climate Action, Responsible Production and Consumption, and Good Health and Well-Being. Our rapidly disappearing forests only function with the help of fungal mycelia below the forest floor. Fungi play an important role in keeping the soil alive and fertile so native plants can flourish, attract pollinators, reduce erosion, and mitigate climate change (Sheldrake, 2021). 90% of plants have mutually beneficial relationships with fungi (Sheldrake, 2021). Carbon is stored below the soil surface in the fungal roots (Sheldrake, 2021). Mycelia can act like natural pesticides by attacking harmful insects and bacteria by trapping and consuming them, ultimately reducing harm to the surrounding ecosystem (Stamets, 2005).

Mushroom cultivation could help prevent climate change. Fungi filter pollutants out of the environment, support regenerative farming practices, and are a vital part of the carbon cycle (Sheldrake, 2021). Mushrooms can be grown on various substrates, but what is amazing is that they can grow out of spent waste. Mushrooms require a basal substrate (sawdust, live wood, spent substrate like coffee) humidity, fresh oxygen, light, and a food source (wheat bran) to grow (Sayner, 2022). This project focuses on responsible consumption of spent products such as straw, coffee grounds, and coco coir that would otherwise be composted or sent to the landfill to decompose and release greenhouse gasses such as methane. Assessing what

treatments in the experiment allow for the highest yield will help to determine which material could be used for sustainable mushroom production when testing for yield.

**2. Methodology:**

*2. 1 Experimental Design*

The experiment used a completely randomized design with four treatments and three replicates. An initial trail using grow bags as experimental units was terminated 2 weeks after inoculation due to *Trichoderma spp* infection. The second trial used Mason jars as experimental units. Jars were randomly assigned to either oak, peat, coco coir, or coffee ground treatments (Figure 1).

| | | | |
|---|---|---|---|
| 1<br>CocoCoir3 | 4<br>CocoCoir2 | 7<br>Coffee3 | 10<br>Peat3 |
| 2<br>Control2 | 5<br>Control1 | 8<br>Coco Coir1 | 11<br>Coffee2 |
| 3<br>Control3 | 6<br>Peat2 | 9<br>Coffee1 | 12<br>Peat1 |

**Figure 1.** Completely Randomized Design of a 12-plot experiment (Control, Peat, Coco Coir, Coffee as Treatments)

Twelve 12 oz glass Mason jars were sanitized in a boiling water bath for 35 minutes. They were emptied and placed “mouth”-up on a paper towel. The jar lids were sprayed with 90% alcohol and left to dry face up.

Oak pellets (control substrate) were softened by adding 10 cups of pellets to 2.8 L of water. Each substrate material (oak pellets (10cups), sphagnum peat moss (4 cups), coco coir (4 cups), coffee (4 cups), vermiculite (1 cup), wheat bran (2 cups)) was put in its own oven safe container, and stirred with 1 cup of distilled water, and pasteurized in an oven at 200°F for 2.5 hours.

Pasteurized substrates were cooled at room temperature for 1 hour then measured into the sterilized Mason jars. Nitrile gloves and a medical-grade face mask was worn to reduce contamination while measuring, and a separate, clean measuring device was used for each jar. The recipe for each jar is as follows:

- 1 cup oak pellets
- 1 cup treatment (oak pellets, sphagnum peat moss, coco coir, or coffee grounds)
- 1 Tbsp of vermiculite
- 2 tsp of wheat bran
- 1/3 cup distilled water (this was added to weigh down the jars in the water bath and to add back a bit of moisture to the substrate that may have been lost in the oven)
- 3 ml liquid Lion's Mane Mushroom Culture (Grow Mushroom Canada, Vancouver Island, BC)

Each jar rim was wiped with a fresh 90% alcohol wipe to remove any debris that could interfere with the vacuum seal. A lid was then placed on each jar and shut tightly. Closed jars were placed back in the water bath and brought to a rolling boil for 35 minutes.

Once the vacuum seal process was finished, the jars were taken out of the water bath and placed on paper towel to cool overnight (8+ hours). Three milliliters of Lion's Mane mushroom culture (Grow Mushroom Canada, Vancouver Island, BC) were added to each jar with a syringe, which required opening the jar slightly. The experimenter wore Nitrile gloves and a medical face mask during inoculation to reduce the chances of contamination.

Each jar was labeled (name, date, replicate, signature) and place in an air-conditioned closet maintained at 21°C until July 26$^{th}$ (two days after inoculation) when the jars were covered by a thick towel to protect from UV radiation and transported to a growth chamber (Conviron, Winnipeg MB) in the Seed Lab at the KPU Richmond Campus. Growth of fungi was observed and photographed every 3-5 days.

Growth of mycelia, pin-head formation (primordial hyphae formation), and mature fruiting bodies were observed over 83 days, between July 24$^{th}$, and October 15, 2022. After the 83 days, the mushrooms that had grown in the jars were harvested, counted, weighed (fresh and dry weight (g)) on a digital analytical balance. The mushrooms were then oven dried at 170°F until completely dehydrated (35-120 minutes, depending on size). The dry mushroom yield of each jar was recorded.

*2.2 Statistical Analysis*

Data was square root transformed to satisfy assumptions of normality and homogeneity of variance. Transformed data were analysed by one-way ANOVA in the ANOVA module of the Jamovi interface for R (The Jamovi project 2022, R core team 2021).

**3. Results**

Growth substrate did not have a significant effect ($p>0.05$) on Lion's Mane yield (Figure 2). No difference was detected between the treatments ($p>0.05$). No growth was observed on the Coco Coir treatment due to infection. All Coco Coir jars were removed from the study prior to analysis.

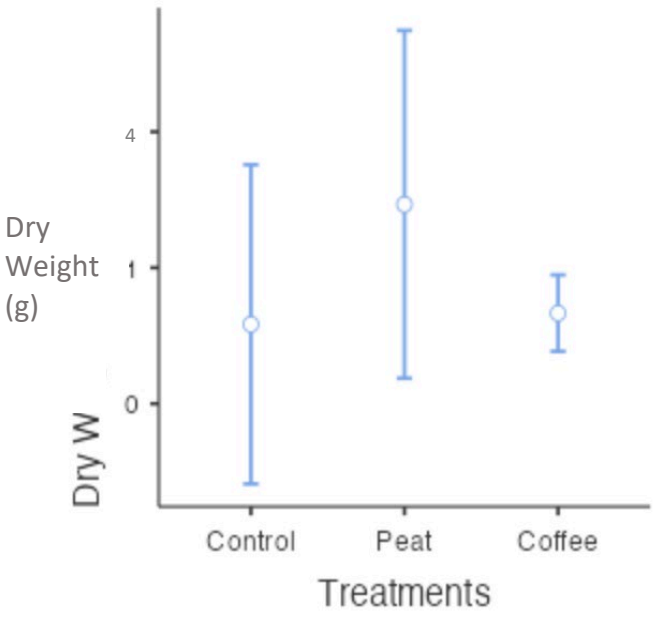


**Figure 2.** Dry weight of Lion's Mane mushroom by growth substrate. Error bars denote 95% confidence interval around mean of square root transformed values (*n*=3) to satisfy assumptions of normality and homogeneity of variance. Note non-linear y-axis scale due to back transformation. No significant differences were detected between means.

**Table 1**

*One-Way Anova (Fisher's) square root transformed values of dry weight (g), (p>0.05).*

One-Way ANOVA (Fisher's)

| | F | df1 | df2 | p |
|---|---|---|---|---|
| Dry Weight (g) - Transform 4 SQRT | 4.26 | 2 | 6 | 0.070 |

**Table 2**

*Fresh and Dry Weight (g) of Harvested Mushrooms from 12 Jars*

| Jar | Fresh weight (g) | Dry weight (g) |
|---|---|---|
| Control 1 | 1.656g | 1.112g |
| Control 2 | 0.101g | 0.012g |
| Control 3 | 0.565g | 0.345g |
| Peat 1 | 2.145g | 1.978g |
| Peat 2 | 4.653g | 4.032g |
| Peat 3 | 1.118g | 0.967g |
| Coco Coir 1 | 0 | 0 |
| Coco Coir 2 | 0 | 0 |
| Coco Coir 3 | 0 | 0 |
| Coffee 1 | 0.698g | 0.459g |
| Coffee 2 | 0.363g | 0.300g |
| Coffee 3 | 0.722g | 0.598g |

**Table 3**

*Number of Mushrooms Harvested from Each of the 12 Jars*

| Jar | Number of Mushrooms |
|---|---|
| Control 1 | 1 med (3cm)<br>5 small (<1cm) |
| Control 2 | 3 (<1cm) |
| Control 3 | 3 (<1.5cm) |
| Peat 1 | 1 small (<1cm)<br>2 med (2cm) |
| Peat 2 | 5 small (<1cm)<br>1 large (6cm) |
| Peat 3 | 8 small (<1cm) |
| Coco Coir 1 | 0 |
| Coco Coir 2 | 0 |
| Coco Coir 3 | 0 |
| Coffee 1 | 3 small (<1cm) |
| Coffee 2 | 3 small (<1cm) |
| Coffee 3 | 3 small (1-1.5cm) |

**4. Discussion:**

*4.1 Problems with this experiment*

*4.2.1 Trichoderma spp*

A fungal infection, *Trichoderma spp.,* that took over the 1st trial of bags, and later, one of the coco coir jars. Trichoderma is a green fungus species that is part of the mushroom world, rather than a bacteria or algae (Sheldrake, 2021). It's a widespread filamentous fungi found in soils, on plants roots, and decaying plant matter (Sheldrake, 2021).

*Trichoderma spp.* is water and air borne and is what makes sanitization so problematic when cultivating mushrooms. There are about 250 different species in the Trichoderma genus, and this type of fungi has been used for quite some time in agriculture and horticulture as a plant growth enhancer, biostimulants and can be used as a biocontrol agent to fight other

pathogens (Bissett et al., 2015). As exceptional as these fungi sounds, it was quite detrimental to this trial as it competes with mushroom mycelium.

*4.2.2 Gas exchange*

The original thought was that the introduction of air at the beginning of the study would be enough to sustain the growth of the mycelium, not considering that as the mycelium grew, it would use up the fresh oxygen and expel carbon dioxide.

On August 23, 2022, three mid-sized nail holes were hammered into each lid, taking care to sanitize the nail each time. As of Aug 30, the mycelium took off and primordial growth could be observed. There was a new flush of fresh oxygen in the jars for the mushrooms to use, but the holes that had been hammered a week prior were now filling up with primordial hyphae. The Lion's Mane seems to want to grow where was the highest concentration of fresh oxygen, but effectively suffocated itself by sealing over the holes.

*4.2.3 Relative humidity (RH)*

The relative humidity of the growth chambers posed a serious issue. RH is a measure of how much water vapour is in the air (Chandler, 2021) and Lion's Mane require anywhere between 80-90% RH to fruit. This variable was difficult to control in the greenhouse at home. In trying to keep the RH between 80-90%, the mushrooms were requiring a new flush of fresh oxygen every two hours. This was near impossible to keep up and some days the RH gauge in the green house was reading at 98%, and this would have caused the mushrooms to mold and ruin the study.

The second trial of mushroom jars were transported to the Conviron growth chambers on July 26$^{th}$ to keep the variables consistent. On October 3, 2022, the lab technician sent a

notification to say the chamber was leaking, and the jars would need to be moved to the second chamber. The second chamber shorted out over the weekend and along with it, the temperature gauge, so neither had been correctly reading all weekend. By Monday, the chamber had effectively dehydrated the mushrooms before harvest.

*4.2.4 Temperature*

Initially, the Conviron growth chamber set temperature was 21°C. Four weeks in, with little to no mycelial growth change, the temperature was adjusted to 19°C. On August 30, 2022, the first few strands of mycelium appeared.

While this experiment was conducted under a rigorous process, it was possible that error occurred in this experiment. There could have been a human counting error when counting the number of mushrooms in each jar. There also could have been error in reading the scale as it was very sensitive. This type of random error may have been caused by the extreme sensitivity of the scale. It is important to acknowledge that this study had several limitations and researchers may find it beneficial to conduct this study in an airborne infection isolated or negative pressure isolation lab rather than a household kitchen to keep contamination low.

In conclusion, these findings do not support the hypothesis that growth substrate affects yield of Lion's Mane mushrooms. Future investigations should explore these results and may want to determine whether these findings are similar when using different substrates.

**5. Acknowledgements:**

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