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Bence Boinitzer

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Utilization of the Protein and Waste of Larvae Bred on Spent Mushroom Compost in the Cultivation of Button Mushroom

Insider consultant: dr. Szabó Anna

Senior Lecturer

Ruth Wambui Mwangi

PhD Student

Insider consultant's
Institute/department: Vegetable and Mushroom

Created by: **Bence Boinitzer**

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1. Introduction and objectives

Mushrooms, and *Agaricus bisporus*, the white button mushroom, are a valued part of the food industry across the globe, for their nutritional values and versatility. *The button mushroom* is one of the key players of the global mushroom market as one of the most cultivated mushrooms worldwide. The name *bisporus* comes from its unique feature – it produces two spores for every basidium – which makes it quite unique among the other species of the genus. (Callac et al., 1993).

The white button mushrooms for culinary and medicinal uses and their cultivation can be traced back to the 17th century. During this time lot of innovative and unique mushroom cultivation methods emerged, especially in France. (Thakur, 2020).

Originally the mushroom itself was harvested from its natural habitats. Later horticulturists began to develop underground cultivation experiments in caves near Paris. The caves provided the constant humidity and temperature necessary for successful mushroom growth, setting a foundation for the development of systematic mushroom farming. This breakthrough in cultivation technology allowed for increased production and set the stage for the species' global commercialization (Chang & Miles, 2004).

Today, the white button mushroom is cultivated worldwide, with major production hubs in the United States, China, and the Netherlands. With the introduction of controlled-environment agriculture (CEA) systems, button mushroom cultivation has significantly advanced. Composting techniques, which involve pasteurizing and pre-colonizing the substrate with mycelium before introducing it into growing chambers, have revolutionized the efficiency and consistency of mushroom production. These methods minimize the risk of contamination and optimize the colonization rate, enabling faster production cycles and higher yields (Royse, 2014).

Moreover, the integration of automated monitoring systems and environmental controls has allowed growers to maintain ideal conditions for mushroom growth. Parameters such as temperature, humidity, light exposure, and CO2 levels are precisely regulated to maximize growth potential and ensure uniformity in production. This technological advancement enables year-round cultivation, enhancing the reliability and scalability of button mushroom farming (Wood, 2008).

One of the key factors in mushroom cultivation is the substrate itself. Traditional substrates, such like straw-bedded horse manure are the most widely used in white button mushroom cultivation. Apart from using totally novel substrates, one of the developing research fields is the different chemical and natural additives, which ones introduced can increase substrate nutritional quality and optimize mushroom growth (Kumar et al, 2020).

After the mushrooms are harvested, the remaining substrate, known as "spent mushroom substrate" (SMS), can be further repurposed as a soil amendment, animal feed, or biofuel feedstock. SMS is rich in nutrients and organic matter, making it an excellent fertilizer for enhancing soil health and crop yields in conventional agriculture (Othman et al., 2020).

This opens up an interesting new method for sustainable practices and production. One of the key factors in button mushroom production is the nitrogen content of the cultivation substrate. The SMS itself – while today most of it gets to be burned or used for soil enhancement – can be an excellent feed for multiple different type of insect species. The excrement and the insects themselves later could be used as substrate additives, with their high-protein (which means high nitrogen) content, achieving nearly perfect recycling – at least at this aspect of the mushroom production.

In this work the focus is on the effects of three protein additives used: Mealworm excrement, Mealworm protein and Black soldier fly larva protein. These substances are integrated into to growing substrate in various concentrations to examine effects on the yield and quality of the white button mushroom. We're testing the effects and functionality of these proteins. A Company specialized to make protein sources from SMS feed Mealworms & Black Soldier fly larvae. We supplemented button mushroom compost with the proteins and studied the effects on button mushroom production.

Hopefully this work will provide insights into the effectiveness of integrating these supplements in mushroom cultivation. The aim of our study is to examine the applicability of these mushroom supplementation methods, to reach our final goal which is sustainability and the reduction of agricultural waste by the usage of agricultural byproducts.

2. Literature Review

2.1 Commercial Uses of Mushrooms

Edible mushrooms have become a staple in global cuisine due to their distinct flavor profiles, rich texture, and nutritional value. They contain essential nutrients such as proteins, fiber, vitamins (B-complex, D2), minerals (selenium, potassium), and antioxidants. *Agaricus bisporus*, commonly known as the button mushroom, is a dominant species in the edible mushroom market, constituting about 38% of global mushroom production (Usman et al., 2021). Other popular varieties include shiitake, oyster, and enoki mushrooms, which are particularly valued in Asian cuisines.

Mushrooms are also a source of umami, a savory taste that enhances the flavor of various dishes without the need for additional salt, thus aligning with health-conscious dietary trends. Their texture and versatility make them ideal for plant-based diets, which are rising in popularity due to environmental and health concerns (Sun et al., 2020).

Beyond fresh consumption, mushrooms are used in the development of numerous value-added products. Dehydrated mushrooms, mushroom powders, and mushroom-based food supplements have become prevalent in health food markets. These products retain the nutritional benefits of fresh mushrooms while providing extended shelf life and versatility. For example, mushroom powders can be incorporated into soups, sauces, and functional beverages, contributing to both flavor and health (Jahan et al., 2019).

As a novelty on the market, the food industry also uses mushrooms as a base for mycoprotein, a sustainable protein alternative. Mycoprotein, produced from the fungal species *Fusarium venenatum* and is praised for its low environmental footprint compared to animal-based proteins (Khan et al., 2024).

Mushrooms play an important role in industrial and environmental applications. Their ability to degrade complex organic molecules, such as lignin and cellulose, has led to the use of fungi in bioremediation, the process of using living organisms to clean contaminated environments. *Pleurotus ostreatus* which is a species within so called white-rot fungi has the ability to degrade polycyclic aromatic hydrocarbons and some other pollutants like pesticides, and industrial dyes (Pozdnyakova et al., 2020).

In addition to bioremediation, mushrooms are also utilized in biotechnological processes such as enzyme production. Enzymes derived from mushrooms, such as cellulases and laccases, are employed in industries ranging from textile manufacturing to biofuel production, enhancing the economic value of mushroom cultivation (Elkhateeb et al. 2022).

2.2 Medicinal Uses of Mushrooms

Mushrooms have a long history in traditional medicine, particularly in East Asia, where species like *Ganoderma lucidum* (Reishi) and *Lentinula edodes* (Shiitake) have been used for their health-promoting properties. These fungi were believed to enhance vitality, boost the immune system, and treat various ailments, including liver diseases, hypertension, and infections (Wasser, 2017).

The bioactive compounds found in mushrooms are diverse and have been the subject of extensive research. The list includes polysaccharides - particularly β-glucans - triterpenoids, phenolic compounds and other secondary metabolites and their roles have been explored in many conditions from immune suppressed states to bacterial infections and neurodegenerative diseases (Chen & Seviour, 2007).

The immunomodulatory effects of mushrooms are particularly well-documented. Studies have shown that β-glucans from mushrooms such as *Lentinula edodes* enhance the production of cytokines and increase the activity of immune cells, offering protection against infections and possibly cancer (Yehia et al., 2022). In clinical settings, mushroom-derived supplements are being explored as adjunct therapies in cancer treatment, with some evidence suggesting they can reduce the side effects of chemotherapy and improve patient outcomes (Fonseca et al., 2024).

Numerous studies have demonstrated the potential of mushrooms in cancer prevention and treatment. For instance, polysaccharides from *Ganoderma lucidum* have been shown to inhibit tumor growth and metastasis in animal models by enhancing the host immune response (Sohretoglu et al., 2018). Furthermore, triterpenoids from Reishi mushrooms exhibit direct cytotoxic effects on cancer cells through the induction of apoptosis and inhibition of angiogenesis (Chen et al., 2022).

2.3 Global Market of Mushrooms

<u>Current State of the Mushroom Industry</u>

In the past few years the market for mushrooms globally have been in a growth phase, as the demand for medicinal and edible mushrooms continuously on the rise. Figure 1. shows the market value associated with it reached 50 billion USD by 2021 and it's compound annual growth rate is projected as 9% for the next 5 years, reflecting the rising consumer interest in plant-based diets, functional foods, and sustainable agricultural practices (Grand View Research, 2022). Edible mushrooms like the button mushroom continue to dominate production, followed by shiitake and oyster mushrooms.

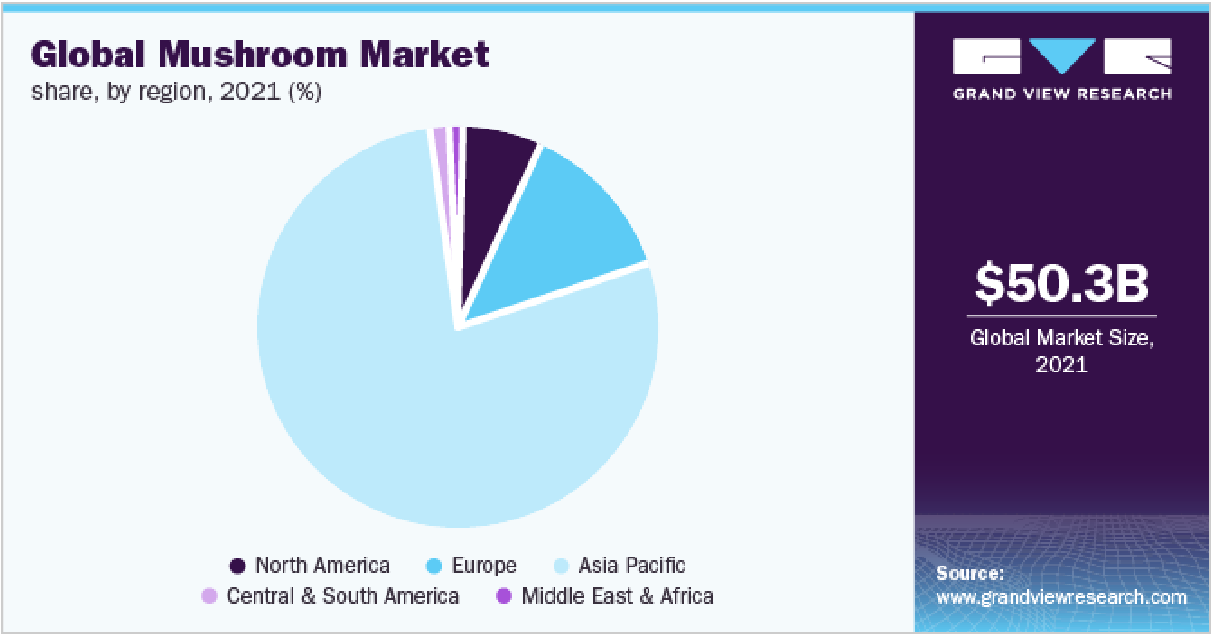

Figure 1. – Size of global mushroom market

(source: https://www.grandviewresearch.com/industry-analysis/mushroom-market)

Key market drivers include the growing awareness of mushrooms' nutritional and medicinal properties, increasing disposable incomes in emerging economies, and advancements in mushroom cultivation techniques. North America, Europe, and Asia-Pacific remain the largest consumers, while on the top of the producer list is China, who provides well over 70-75% of the worlds production. (Royse et al., 2017).

Leading Mushroom-producing Countries

China is the undisputed leader in mushroom cultivation, producing over 38 million tons annually, a significant portion of which is consumed domestically (Li et al., 2022). Other leading producers include India, the United States, the Netherlands, and Poland. European countries are major exporters of mushrooms, particularly to North America, where demand for gourmet and medicinal mushrooms continues to grow.

India has emerged as a fast-growing producer, primarily focusing on button and oyster mushrooms, with large-scale initiatives aimed at promoting mushroom farming as a sustainable livelihood for rural populations. In recent years, technological innovations and government support have helped Indian mushroom producers enhance production volumes and export capacity (Raman et al. 2018).

Economic Impact of Mushroom Farming and Trade

Mushroom cultivation has proven to be a highly lucrative agricultural activity due to its high yield per unit area, low water requirements, and the ability to utilize agricultural waste as a growth substrate. The economic impact of mushroom farming extends to small-scale farmers in developing countries, who benefit from low start-up costs and the growing demand for organic and sustainable produce. In developed countries, large-scale commercial farms generate substantial revenue through the sale of fresh mushrooms and value-added products like mushroom powders and supplements (Bhat et al., 2020).

The mushroom trade has also created significant employment opportunities, especially in regions where labor-intensive farming practices are predominant. Furthermore, the industry's shift towards sustainable and organic practices has opened new markets for premium products, enhancing profit margins for producers.

Global Market Trends and Projections

Figure 2. indicates the global mushroom market is expected to witness steady growth in the coming years due to increasing demand for plant-based proteins, functional foods, and natural medicine. Consumer preferences are shifting towards organic and non-GMO (genetically modified organism) products, which has spurred innovation in cultivation practices and product development (Chatterjee et al., 2022).

In addition, the medicinal mushroom market is poised for significant expansion. Mushrooms like *Ganoderma lucidum* (Reishi), *Hericium erinaceus* (Lion's Mane), and *Cordyceps sinensis* have gained popularity as dietary supplements, touted for their immune-boosting, neuroprotective, and anti-aging effects (Venturella et al., 2021). The nutraceutical industry is capitalizing on these trends by incorporating mushroom extracts into a wide range of products, from functional beverages to skincare.

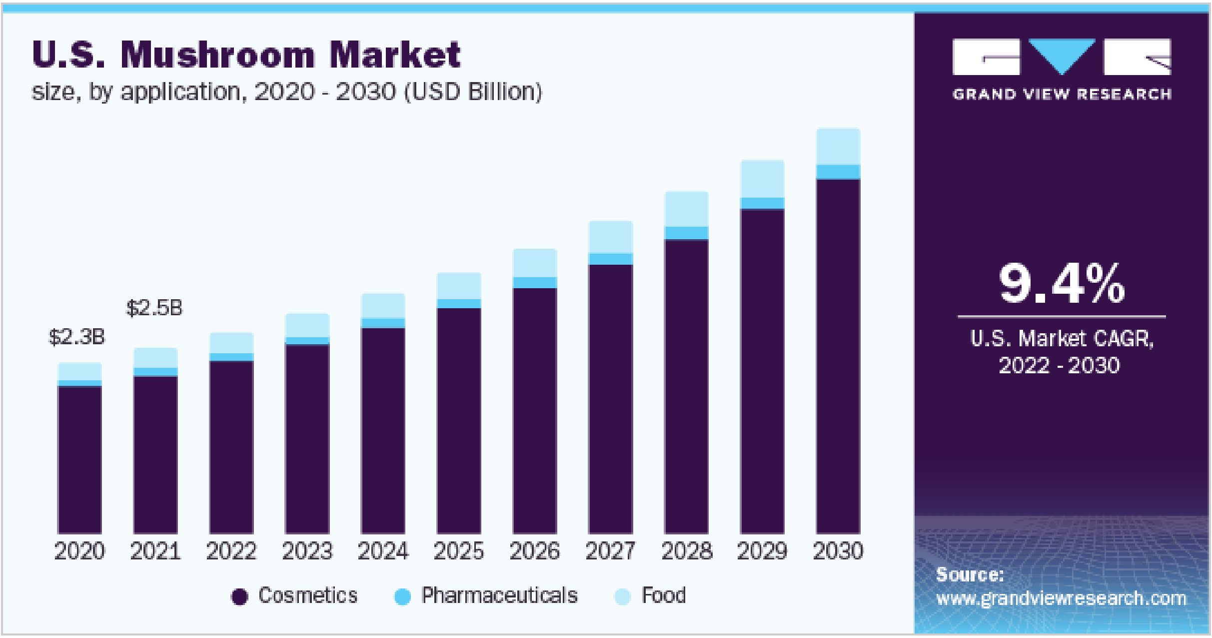

Figure 2. – U.S. mushroom market size prediction 2020-2030

(source: https://www.grandviewresearch.com/industry-analysis/mushroom-market)

Challenges and Opportunities in the Global Mushroom Trade

Despite its promising growth, the global mushroom industry faces several challenges. These include fluctuations in demand due to the perishable nature of fresh mushrooms, competition from synthetic meat alternatives, and the need for improved post-harvest processing technologies. Additionally, the COVID-19 pandemic temporarily disrupted supply chains, causing short-term declines in production and trade (Haryati et al., 2021).

However, the market also presents numerous opportunities. Technological advancements in cultivation, such as vertical farming and automated growing systems, are expected to enhance productivity and reduce costs. Furthermore, the rising interest in mushrooms' environmental benefits, such as their role in sustainable agriculture and bioremediation, could further drive market growth in the coming years.

2.4 Mushroom Cultivation

Overview of Cultivation Methods

Mushroom cultivation can be broadly categorized into two main approaches: traditional outdoor cultivation and modern indoor cultivation. Each method has its advantages and challenges, with the choice of cultivation technique depending largely on the species being grown, available resources, and desired scale of production.

- **Traditional Cultivation**: Historically, mushrooms were cultivated outdoors on natural substrates such as compost, logs, or wood chips. This method, still practiced for some species like *Lentinula edodes* (shiitake), relies on seasonal environmental conditions and requires large areas of land.
- **Modern Indoor Cultivation**: With advancements in technology, controlled-environment farming has become the standard for most commercially cultivated mushrooms. Indoor farms allow for year-round production by regulating temperature, humidity, light, and carbon dioxide levels to optimize growth conditions (Ten et al., 2021). Figure 3. shows how a complex indoor farm usually looks like.

Figure 3. – Indoor mushroom cultivation

(source: https://jeevanmushroom.com/the-art-of-button-mushroom-cultivation/)

Substrate Preparation and Sterilization Methods

Substrates play a critical role in mushroom cultivation as they provide the necessary nutrients for fungal growth. The choice of substrate varies depending on the mushroom species but generally includes materials like straw, sawdust, or agricultural waste. For instance, button mushroom is commonly grown on a composted mixture of straw and manure, while oyster mushroom thrives on straw or hardwood sawdust (Shah et al., 2004).

Compost preparation involves multiple phases, including pre-conditioning, pasteurization, and supplementation with nutrients like gypsum to balance pH levels and optimize microbial activity. Current research emphasizes the importance of microbial communities in substrate decomposition, as these microorganisms help break down complex lignocellulosic materials into simpler compounds that the mushroom can utilize (Suwannarach et al., 2022). Moreover, innovations in substrate preparation involve using alternative agricultural waste products, such as corncobs and coffee grounds, which promote sustainability and reduce waste disposal costs (Krishnappa, 2022).

Proper pasteurization of the substrate is essential to eliminate competing microorganisms that could hinder mushroom growth. Heat treatment is the most used method, either through steam pasteurization or autoclaving. Recent advances have introduced chemical sterilization techniques using substances like lime or formaldehyde, though concerns over environmental impact have limited their use (Claeys et al., 2009).

As in any organic system, diseases and pests can cause significant decreases in yields. Common threats include *Trichoderma* mushrooms and mushroom flies, especially in closed, indoor cultivation sites. (Rinker et al., 2017). While it is a hard challenge to manage these negative factors, in the recent years new, biological disease control methods surfaced, such as using predatory insects and the introduction of beneficial microbes. The first and strongest line of defense, however, is still the maintenance of proper sterilization and hygiene protocols.

Different competitive fungus, such as molds and yeasts, can disrupt the cultivation and their presence can reduce yields. Here proper operational hygiene, the prevention of the contamination is the number one preventative method. New technologies like antifungal coatings on substrates are being developed to reduce the risk of contamination. The proper management and understanding of microbial ecology can also help to reduce risks. (Ghimire et al., 2021).

The Importance of Substrate in Mushroom Cultivation

The substrate has a double role as a protective environment and nutrition source for the fungal mycelium. Usually it is created from straw, sawdust, corn stalks or other agricultural waste products. (Royse et al., 2017). In many cases, however these can lack in various micro- and macro nutrients, which warrants the use of so-called substrate additives. They can be organized into three categories.

- **Nutritional Supplements:** Usually complex organic (and sometimes inorganic) products, high in nitrogen - for example soybean meal, wheat bran, and corn gluten meal (Carrasco et al., 2018; Zhang et al, 2020).
- **Biological Additives:** These are living microorganisms introduced to the substrate, for example Glutamicibacter. These microbes have various effects, like increasing the nutrient absorption or suppressing various other – not beneficial – organisms. (Kumari et al., 2021; Braat et al., 2022).
- **Chemical Additives:** These have a role in adjusting the pH or providing minerals to the substrate. Examples include lime or calcium carbonate. (Radzi et al., 2021).

These additives can have a profund effect, increasing the yield of mushrooms by up to 30% (Mamiro et al., 2007). Similarly, the use of soybean meal as a substrate additive has been associated with enhanced protein content and improved amino acid profiles in mushrooms, making them more nutritious for consumers (Chaudhary et al., 2024).

One of the most important additives for white button mushroom production is nitrogen and nitrogen-rich substances. Increasing the available nitrogen content in the substrate can lead to significantly increased yields.

Moreover, the use of biological additives has been shown to increase the resilience of mushroom crops against diseases, reducing reliance on chemical pesticides and promoting more sustainable farming practices (Smetana et al., 2018).

Environmental Parameters for Optimal Mushroom Growth

The successful cultivation of mushrooms requires precise control of environmental parameters, including temperature, humidity, and light. Most edible mushrooms thrive in temperatures ranging from 15°C to 25°C, with higher humidity levels (70-90%) being crucial for fruiting body development. Light is not required for the vegetative growth of most species, but exposure to specific wavelengths can influence the morphology and pigmentation of certain mushrooms (De Bonis et al., 2024).

Carbon dioxide concentration also plays a significant role, with elevated levels are beneficial during the vegetative growth phase and promoting the formation of elongated stems in some species, which may be desirable in gourmet markets (e.g., *Pleurotus* species), in many cases lower concentrations are required to initiate pinning and the formation of fruiting bodies

Maintaining optimal air circulation and gas exchange is critical to prevent the accumulation of excess CO_2 and maintain healthy growth.

Sustainability

The concept of a circular economy is particularly relevant to mushroom farming, where waste from other agricultural sectors (e.g., spent grain, coffee grounds) can be repurposed as substrates. The use of agricultural by-products as substrates is a cornerstone of sustainable mushroom farming. In addition to reducing waste, these substrates provide a cost-effective alternative to synthetic fertilizers and reduce the need for non-renewable resources. For instance, the cultivation of *Pleurotus ostreatus* on wheat straw or rice bran has been shown to yield mushrooms of comparable quality to those grown on more traditional substrates, with the added benefit of lower environmental impact (Sharma, 2013).

Furthermore, research is ongoing to identify new by-products that can be used as substrates, with a focus on materials that are widely available in specific regions. This localization of substrate sourcing not only enhances sustainability but also reduces the cost of production, making mushroom farming more accessible to small-scale and urban farmers (Sharma et al., 2013).

Mushrooms are sensitive to environmental conditions, particularly temperature and humidity. In regions with extreme weather patterns, maintaining optimal growing conditions can be resource-intensive, requiring significant energy inputs for heating, cooling, and humidity control. Water scarcity is another challenge in certain parts of the world, as mushroom cultivation requires a consistent supply of moisture to support mycelial growth and fruiting. Sustainable water management practices, such as rainwater harvesting and recycling systems, are becoming increasingly important in addressing these limitations (Jayaraman, 2024).

Spent Mushroom Substrate (SMS), the remaining compost left after the mushroom harvest, is currently reused in multiple industries for its high nutrient composition. The most common usage of SMS is in agriculture as an organic fertilizer and soil conditioner, it helps to improve soil structure, water retention, and provides essential nutrients. It is also used in landscaping, erosion control, and as a natural compost in gardening. However, due to the high amounts produced, disposal is still a challenge. Mushroom farms often either send it to landfills, burn them, compost it further for reuse, or distribute it to local farms, but these techniques are not always sustainable or large scales. Future research in SMS usage are exploring its potential in bioremediation, biofuel production, and a base for generating bioplastics, that would reduce the waste and create additional income to mushroom producers. Improvement in SMS management and innovative approaches could transform this byproduct from waste to a valuable source for various industries (Phan C. et al. 2012).

3. Materials and Methods

3.1 Materials

The following materials were utilized in the experiment:

- Plastic bags for substrate casing
- Two Cornwall Electronics humidifiers (6-liter tank capacity, 24-hour operating duration)
- Mertens thermometer (analog, maximum and minimum recording)
- Mertens barometer (analog)
- Supplemental protein sources:
    - Mealworm excrement
    - Mealworm protein
    - Black soldier fly larvae protein
- 1-degree division substrate thermometers for monitoring temperature
- Kern EG industrial scale (precision: 0.1g)
- Phase III compost (procured from BioFungi Kft., pre-colonized and prepared for fruiting)

3.2 Methods

3.2.1 Cultivation experiment

<u>Substrate Preparation</u>

Phase III compost, pre-colonized with mycelium, was obtained from BioFungi Kft. The experiments have been carried out the Hungarian University of Agriculture and Life Sciences, Institute of Horticultural Studies, Department of Vegetable and Mushroom Growing, experimental cultivation unit. The compost was brought to the experimental growth room, where the supplemental protein sources were integrated. The compost was mixed thoroughly with three different supplemental protein sources: mealworm excrement, mealworm protein, and black soldier fly larvae protein. These protein sources were incorporated to investigate their influence on mushroom yield and growth.

Figure 4. – Filled and labeled bag
(source: own picture)

For the supplementation trials:

- **Mealworm excrement (MWE)** was tested at five different concentrations: 0%, 1%, 3%, 5%, and 10% (w/w). Six replicates were prepared for each concentration.
- **Mealworm protein (MWP)** was supplemented at two concentrations: 1% and 3% (w/w). Six replicates were prepared for each concentration.
- **Black soldier fly larvae protein (BSP)** was also tested at concentrations of 1% and 3% (w/w), with nine replicates per concentration.

Each experimental unit consisted of 2 kg of compost mixed with the corresponding protein source. The substrate was placed into plastic bags with care to minimize air content, thereby reducing the risk of contamination. After thoroughly mixing and packing, the casing process was initiated. Each bag was labeled with the corresponding protein source, concentration, replicate number, and the date of casing as seen on Figure 4.

Environmental Monitoring

Temperature and humidity were monitored daily using analog maximum-minimum thermometers and barometers. The room conditions were maintained to provide a controlled environment, optimal for mushroom growth. Specifically, temperature was measured in the substrate and surrounding environment, ensuring conditions were conducive to mycelial colonization and fruiting. Light conditions were kept constant throughout the experiment to avoid introducing variability. While light was not considered a variable, consistent illumination was provided to ensure even environmental exposure across all experimental units.

Incubation and Fruiting Conditions

To stimulate fruiting, specific conditions favorable to mycelium pinning were established. The room humidity was increased to approximately 90% by regularly sprinkling water on the ground and operating two Cornwall Electronics humidifiers, seen on Figure 5. These humidifiers ran continuously to maintain the required humidity levels, ensuring a stable microenvironment. Ventilation was also controlled but not altered as a variable; a consistent airflow was maintained to support mycelial respiration and overall growth.

Incubation continued until the appearance of pinheads, indicating the onset of fruiting. During this period, environmental parameters were consistently monitored and adjusted as necessary. The humidifiers were refilled daily to sustain the humidity levels, and care was taken to ensure uniform moisture distribution across the room.

Figure 5. – The humidifier used in the experiment

(source: own picture)

Harvesting Procedure

Mushrooms were harvested three times per week throughout the experiment, which spanned a total of nine weeks. Harvests were conducted for all experimental replicates. The mushrooms from each bag were carefully collected and weighed using a Kern EG industrial scale with a sensitivity of 0.1 g. Both the total weight and number of mushrooms were recorded to evaluate the impact of each protein source on yield and mushroom quality.

3.2.2 Statistical Methods and Data Analysis

Data collected from the experiment were analyzed using a combination of descriptive and inferential statistical methods to assess the effect of the supplemental protein sources on mushroom yield and quality. The key variables measured included the total yield (weight in grams), average weight (in grams) and the number of mushrooms produced per treatment group.

Experimental Design

The experiment followed a completely randomized design (CRD), where different protein sources (Mealworm excrement, Mealworm protein, and Black soldier fly larvae protein) and their respective concentrations were treated as the independent variables. Each treatment group was replicated multiple times (as detailed in the Materials and Methods), allowing for sufficient sample size to support robust statistical analysis.

The dependent variables measured were:

- Mushroom yield (total weight of mushrooms in grams)
- Mushroom weight (average weight of harvested individual mushrooms)
- Mushroom count (total number of mushrooms harvested)

Data Normalization and Pre-processing

Prior to statistical analysis, the dataset was checked for normality using the Shapiro-Wilk test, ensuring that the data conformed to the assumptions required for parametric statistical tests. Outliers were identified and evaluated a removed only if they represented measurement errors or extreme biological anomalies.

Correlation Analysis

Correlation coefficients were calculated to examine potential relationships between the mushroom yield (weight) and mushroom count across all treatment groups. This helped identify whether higher yields were consistently associated with higher mushroom counts or if the two variables behaved independently.

Analysis of Variance (ANOVA)

In order to see if there is any effect on mushroom yield, number and weight, an Analysis of Variance (ANOVA) was conducted. The factors included:

- **Protein source** (Mealworm excrement, Mealworm protein, Black soldier fly larvae protein)

- **Concentration** (Mealworm excrement: 0%, 1%, 3%, 5%, 10% Mealworm and Black Soldier Fly proteins: 0%, 1%, 3%)

We examined the effects of these factors on both the average weight and number of the mushrooms produced during the cultivation cycle.

In addition to the F-value and F-critical difference, a significance level of $p < 0.05$ was used to assess the statistical significance of the results. Post-hoc tests, such as Tukey's Honest Significant Difference (HSD), were conducted to identify specific differences between treatment groups if the ANOVA results indicated significant effects.

t-Test:

After the completion of the ANOVA tests we also used a t-test which is an inferential statistic used to determine if there is a significant difference between the means of two groups and how they are related. A significance level of $p < 0.05$ was used to assess the statistical significance of the results. We used this test between each concentration and the control group individually for both the average weight and number datasets.

Results Visualization

The results were graphically represented using tables, line and scatter plots. These visualizations provided a clear comparison of the mushroom yields and counts between the different treatment groups, as well as illustrating the distribution of the data within each group.

Software

All statistical analyses were performed using statistical software IBM SPSS Statistics and Microsoft Excel ensuring reliable and reproducible results. Graphical outputs were generated using Microsoft Excel for data visualization.

By utilizing these statistical methods, the data was thoroughly examined to determine the significant effects of supplemental protein sources on mushroom production, and to provide a solid foundation for the interpretation of the results presented in subsequent sections.

4. Results

4.1 Weight and number of mushrooms

Over the course of 20 days, our experiment yielded an average of 5,369.89 grams of mushroom per cultivation cycle, with individual mushrooms weighing an average of 136.37 grams. Across each cycle, approximately 226 mushrooms were harvested. Normality tests on all data revealed no significant outliers, confirming the integrity of our dataset for further analysis.

A distinct growth pattern emerged in the mushroom cultivation cycles, characterized by two flushes in harvest-ready mushrooms—observed on days 3 and 15, displayed on Figure 6. This bimodal distribution was consistent across all treatment groups, including the control, suggesting that the natural growth cycle of the mushrooms remained unaltered by the protein supplementation as shown on Figure 7.

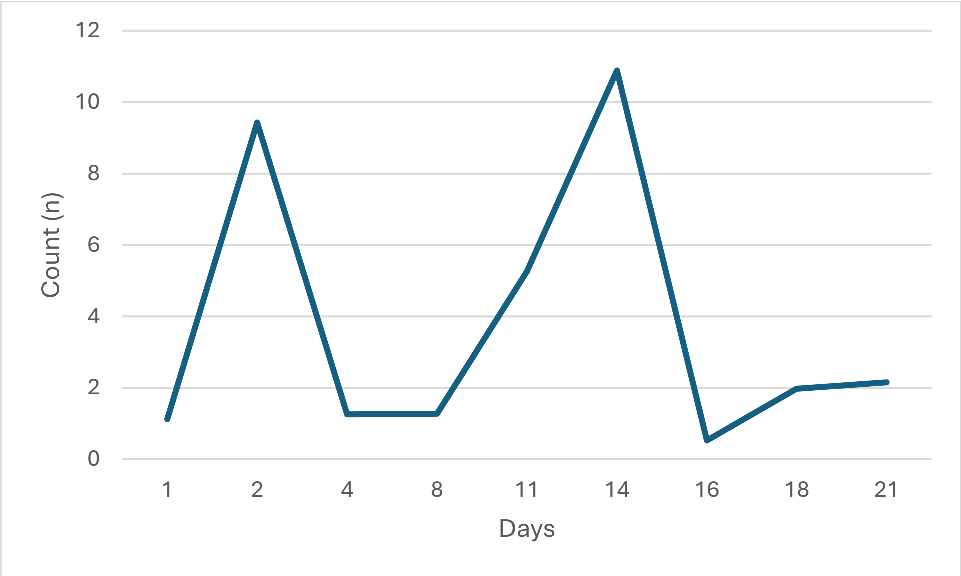

Figure 6. – number of mushrooms ready to be harvested

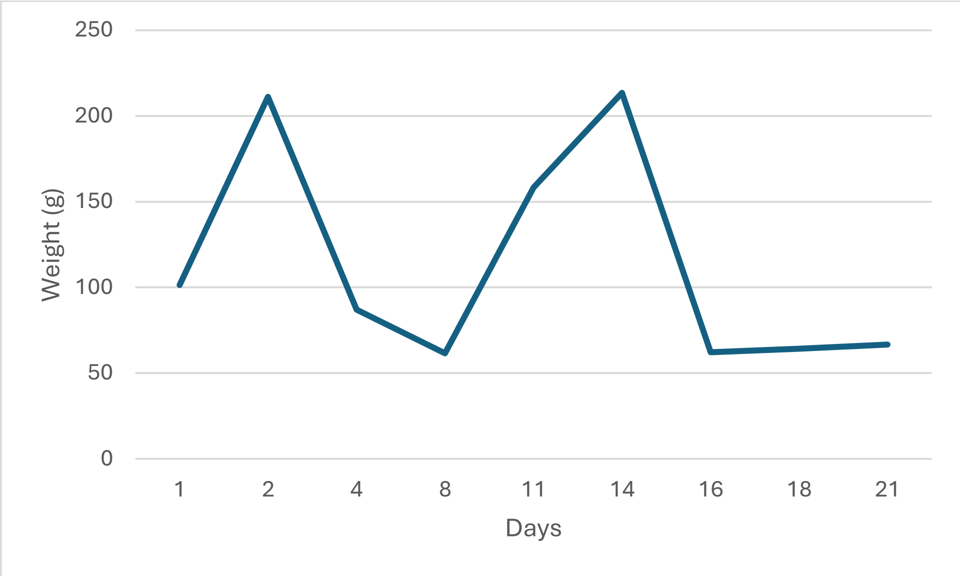

Figure 7. – weight of mushrooms ready to be harvested

To investigate the relationship between the number of harvest-ready mushrooms and their average weight at each time point, we performed a correlation analysis. On Figure 8. the results indicated a strong positive correlation, suggesting a direct relationship between mushroom yield and individual weight under varying conditions.

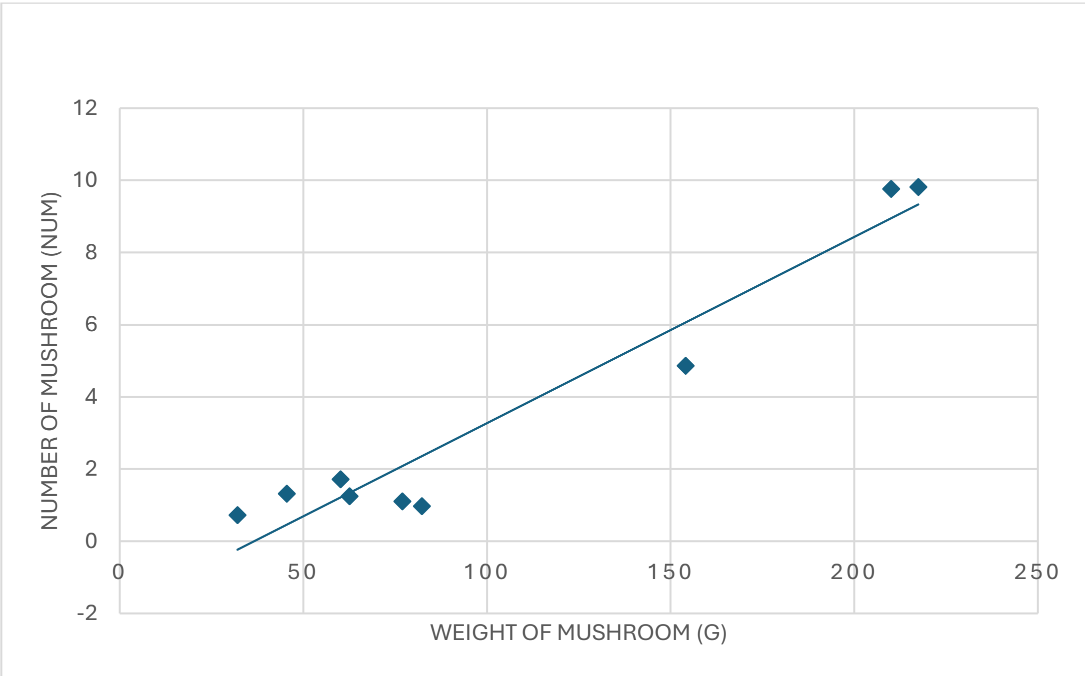

Figure 8. – correlation between weight and number of mushrooms

Following the data visualization, we completed a correlation analysis, which showed a positive correlation between to variables with a strength of 0.9682.

4.2 Impact of Protein Supplementation

Mealworm Excrement

An assessment of mealworm excrement supplementation at various concentrations revealed that total mushroom yield increased across all concentrations seen on. However, there was no evident trend correlating higher concentrations with increased yield. Conversely, the average weight per mushroom remained relatively constant regardless of mealworm excrement concentration, indicating a limited impact on individual mushroom growth characteristics. The results are displayed in Table 1.

Moreover, as Figure 10. & 12. shows, mushroom count was generally higher in the mealworm excrement-supplemented groups compared to the control. Nevertheless, this increase did not display a clear dependency on concentration levels visible on Figure 9. & 11.

%	Total Weight (g)	Avg. Weight (g)	Count (num)
0	4237,19	151,33	177
1	4680,64	130,02	203
3	4275,2	194,33	205
5	4485,17	144,68	202
10	4661,02	129,47	219

Table 1. – Effects of meal worm excrement

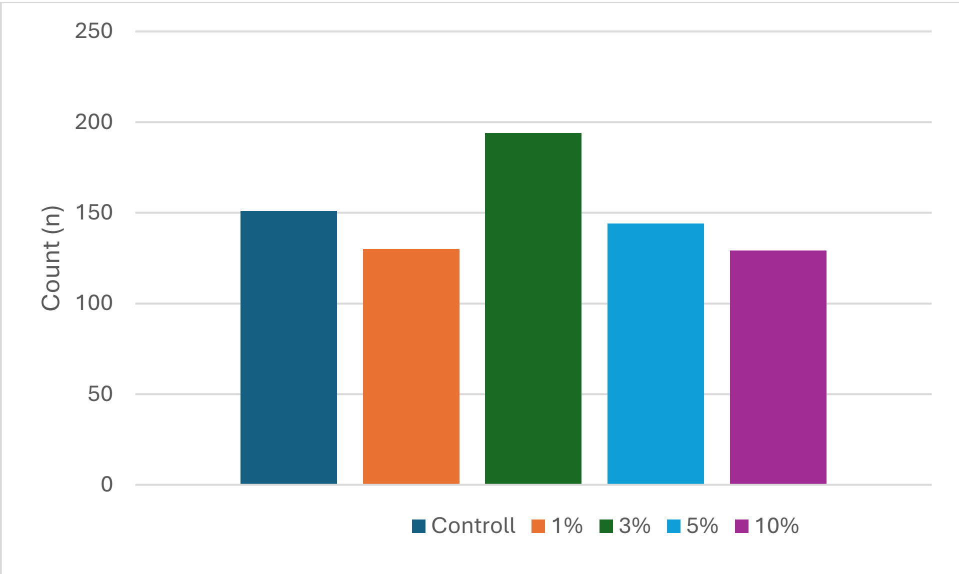

Figure 9. – Average weight of mushrooms harvested during the experiment

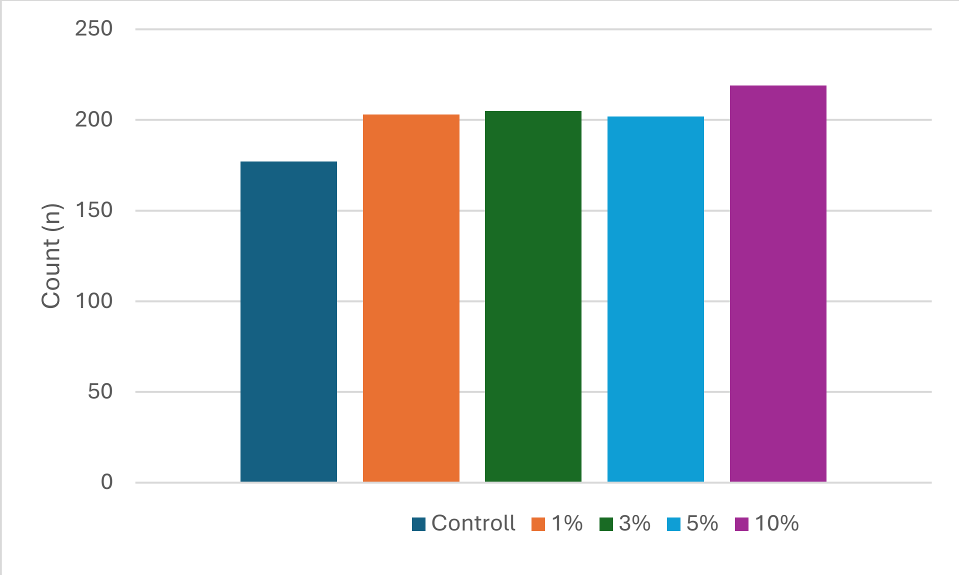

Figure 10. – Number of mushrooms harvested during the experiment

An ANOVA analysis indicated no statistically significant differences among treatment groups or between any treatment and the control, as reflected in F-values well below the critical threshold.

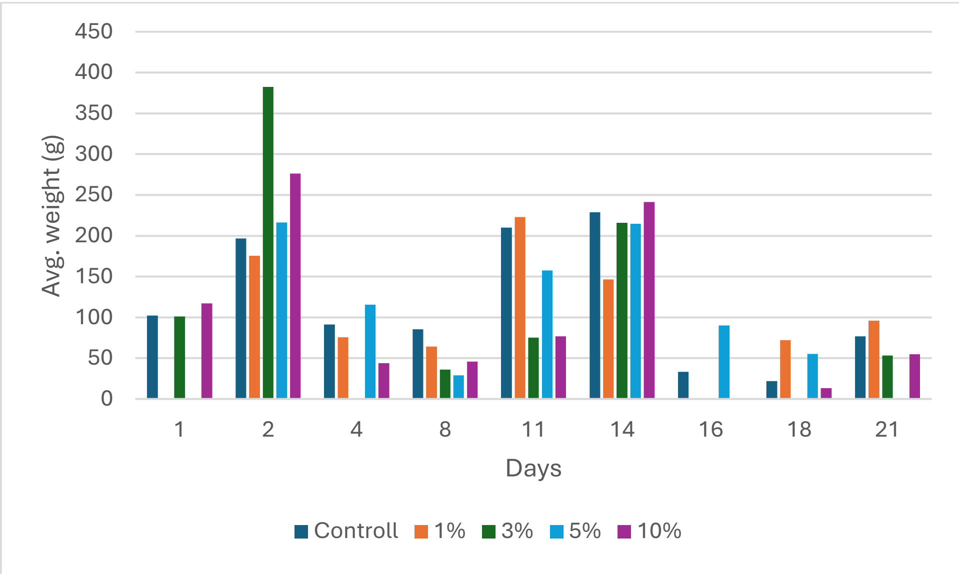

Figure 11. – Weight of mushrooms harvested each harvest day

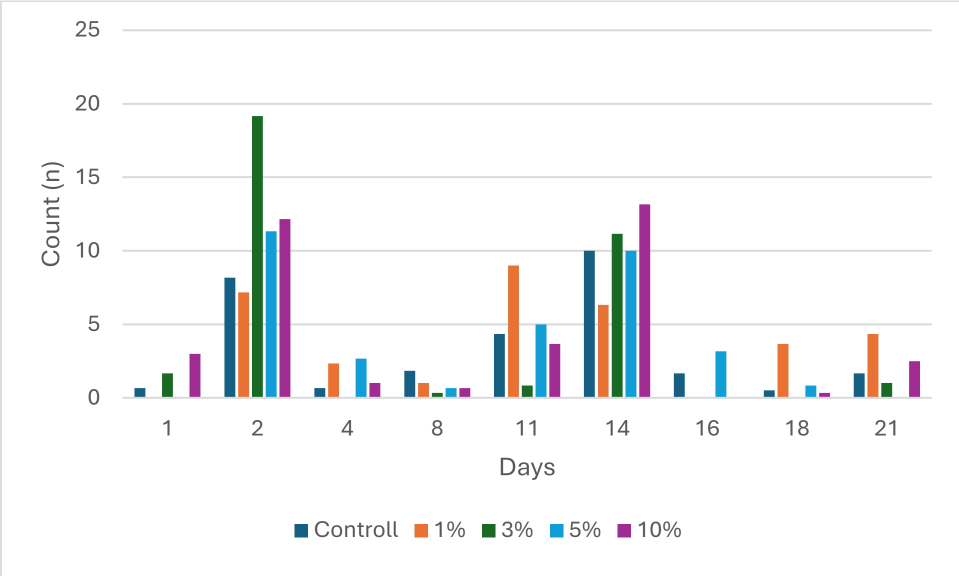

Figure 12. – Number of mushrooms harvested each harvest day

Subsequent t-tests confirmed that no significant effects were observed when comparing individual treatment groups to the control group, as none of the pairs demonstrated p-values below the 0.05 significance level.

Black Soldier Fly Protein Supplementation

When using supplementation via black soldier fly protein, Table 2. shows that both total and average mushroom weights, as well as mushroom counts, were consistently lower across treatment groups compared to the control. While supplementation influenced these metrics, no clear pattern emerged between protein concentration levels and specific growth outcomes. To have a better understanding Figure 14. & 16. shows the number of mushrooms at each concentration and the difference in groups at different harvest dates, while Figure 13. & 15. displays the difference in weight at each concentration and harvesting date.

%	Total Weight (g)	Avg. Weight (g)	Count (num)
0	6556,58	121,41	275
1	3056,93	92,84	132
3	5989,47	115,18	265

Table 2. – Effects of black solider fly protein

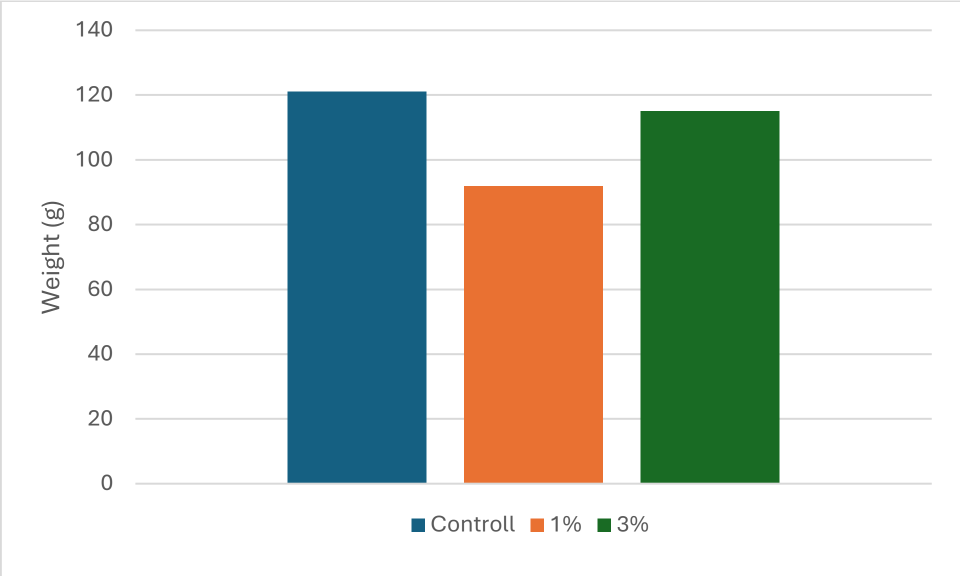

Figure 13. – Average weight of mushrooms harvested during the experiment

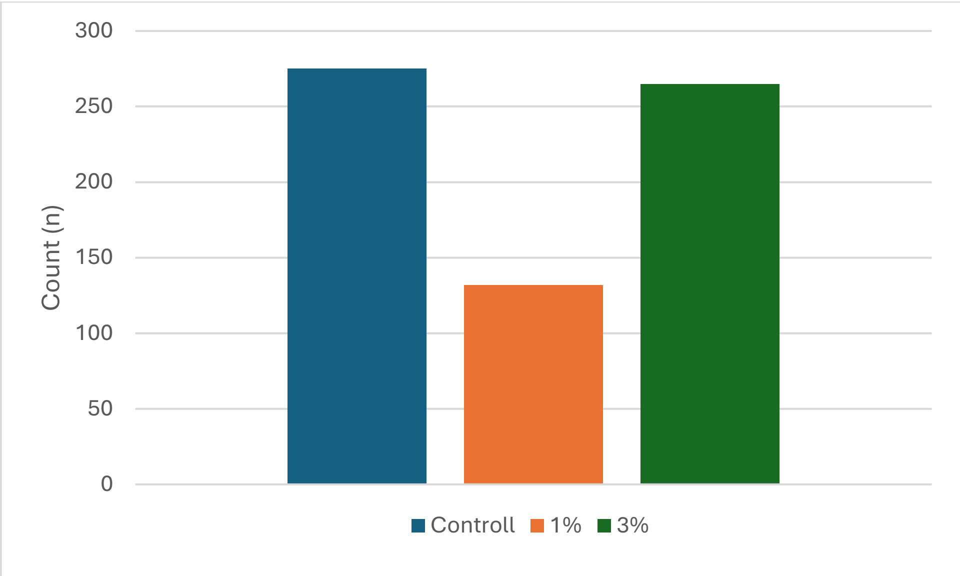

Figure 14. – Number of mushrooms harvested during the experiment

ANOVA tests confirmed no statistically significant differences between treatment groups, as both average weight and mushroom count datasets displayed F-values below the critical level, indicating comparable characteristics across the groups.

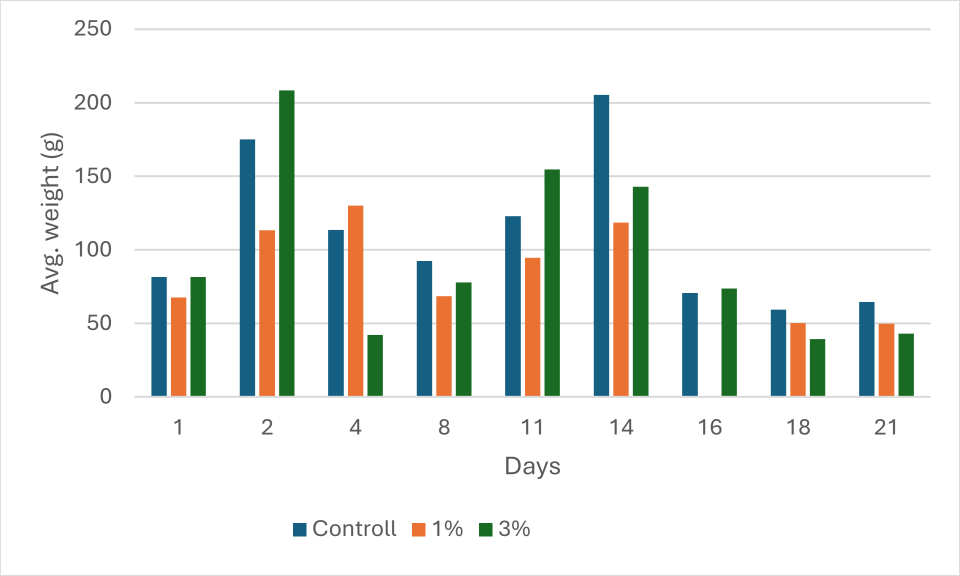

Figure 15. – Weight of mushrooms harvested each harvest day

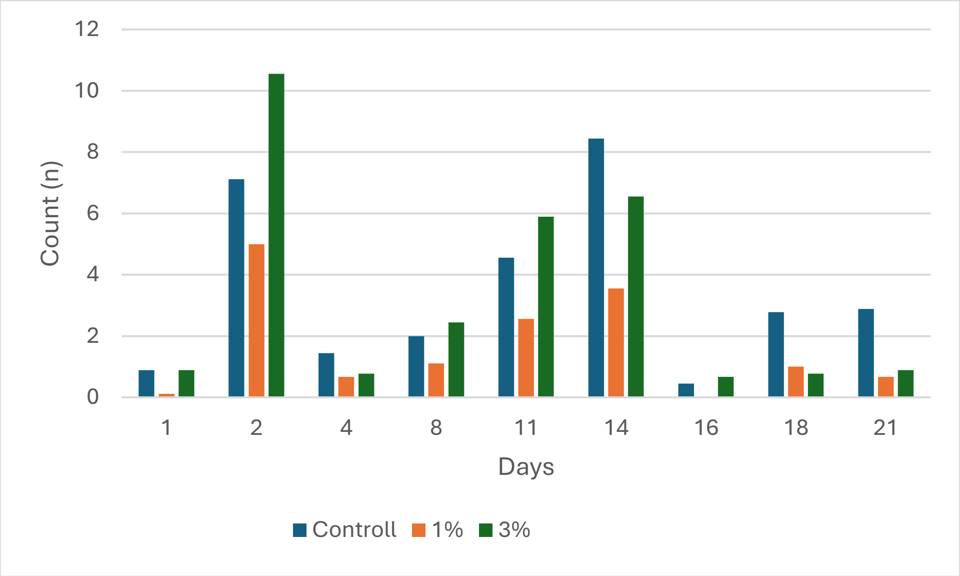

Figure 16. – Number of mushrooms harvested each harvest day

To further validate findings, t-tests were conducted on each protein supplementation condition relative to the control. The analysis is displayed in Table 3. & 4. revealed a statistically significant reduction in both mushroom count and average weight from the control to the 1% black soldier fly protein concentration.

Weight	0%	1%
Mean	109,5241689	76,94447
Variance	2603,593189	1714,627
Observations	9	9
Pearson-correlation	0,756627126	
Hypothesized Mean Diff.	0	
df	8	
t Stat	2,919314337	
P(T<=t) one-tail	0,009655773	
t Critical one-tail	1,859548038	
P(T<=t) two-tail	0,019311546	
t Critical two-tail	2,306004135	

Table 3. – t-Test for avg. weight of mushrooms between control and 1% group

Number	0%	1%
Mean	3,395061728	1,62963
Variance	7,753429355	2,925926
Observations	9	9
Pearson-correlation	0,929451003	
Hypothesized Mean Diff.	0	
df	8	
t Stat	3,920015643	
P(T<=t) one-tail	0,00220915	
t Critical one-tail	1,859548038	
P(T<=t) two-tail	0,0044183	
t Critical two-tail	2,306004135	

Table 4. – t-Test for number of mushrooms between control and 1% group

Mealworm Protein Supplementation

With mealworm protein supplementation, increases in total yield, average weight, and mushroom count were observed at 3% concentration, distinguishing it from the 1% supplementation level as seen in Table 5. To have a better visual demonstration Figure 17. & 20. shows the number of mushrooms at each concentration and the difference in groups at different harvest dates, while Figure 18. & 19. displays the difference in the weight of mushrooms at each concentration and harvesting date.

%	Total Weight (g)	Avg. Weight (g)	Count (num)
0	4237,19	151,33	177
1	4360,08	140,68	203
3	5634,18	181,74	248

Table 5. – Effects of meal worm protein

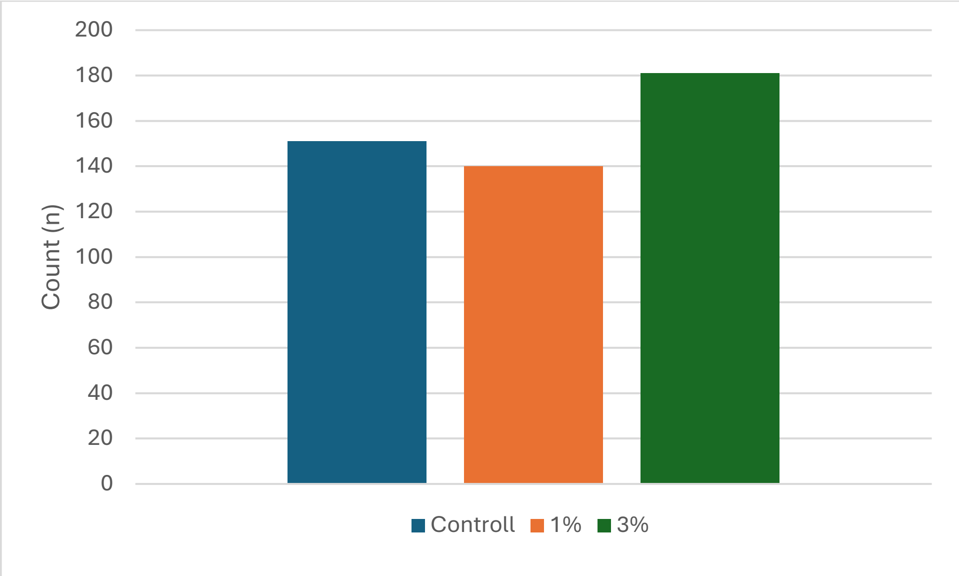

Figure 17. – Number of mushrooms harvested during the experiment

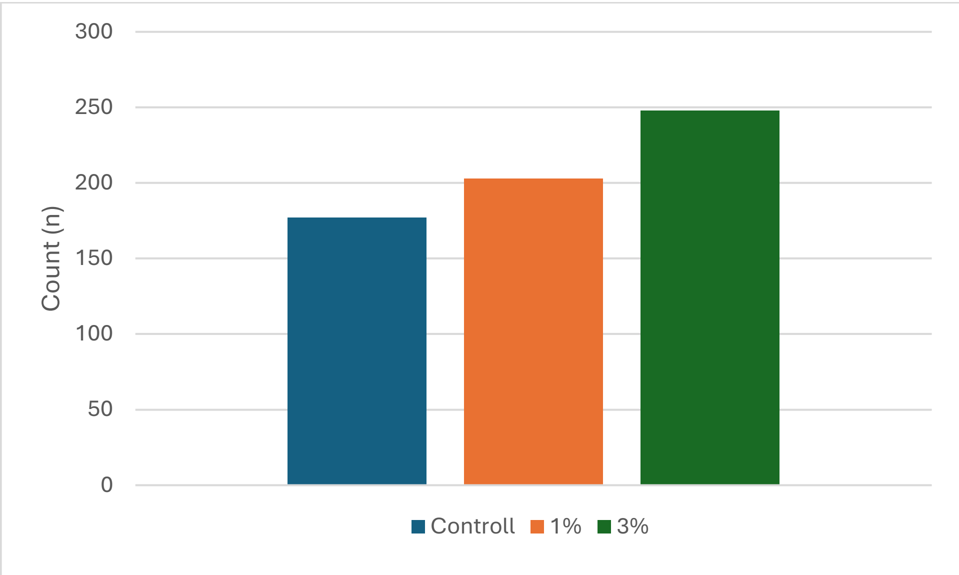

Figure 18. – Average weight of mushrooms harvested during the experiment

However, ANOVA tests revealed no statistically significant differences, suggesting equivalent dataset properties across treatment and control groups.

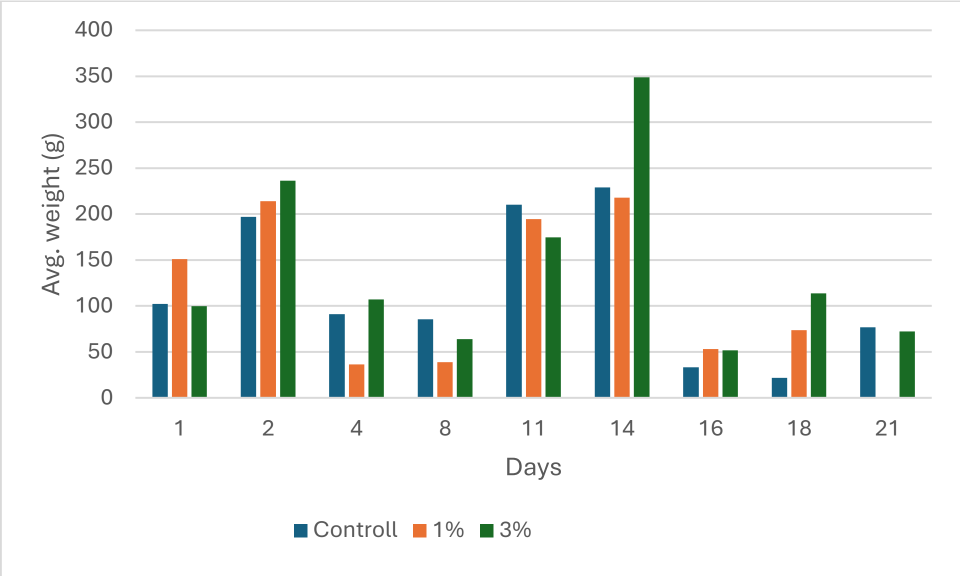

Figure 19. – Weight of mushrooms harvested each harvest day

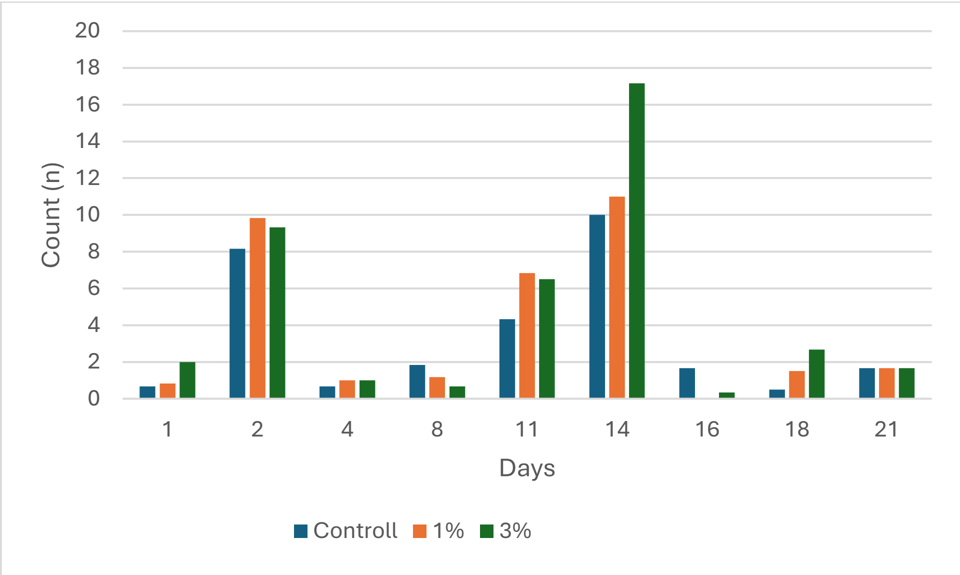

Figure 20. – Number of mushrooms harvested each harvest day

Further t-tests corroborated these findings, showing no significant differences in mushroom number or average weight between the control and treatment groups, maintaining p-values above the 0.05 threshold.

5. Discussion

5.1 Protein Supplementation in Mushroom Cultivation

Protein supplementation in mushroom cultivation has gained attention for its potential to improve substrate quality, increase yield, and reduce reliance on traditional additives. Proteins provide essential nitrogen and other growth-stimulating nutrients that promote mycelial development, potentially enhancing both the quantity and quality of mushroom yields. As global interest in sustainable agriculture grows, the use of alternative protein sources in mushroom substrates has become an important area of exploration, with a focus on organic, renewable materials that contribute to environmentally friendly farming practices.

In this study, three distinct protein supplements—mealworm excrement, mealworm protein, and black soldier fly larvae protein—were chosen based on their nutrient profiles, availability, and alignment with sustainability principles. These supplements represent diverse approaches to integrating proteins into substrates, each with its unique composition and interaction with mushroom growth.

While we found some effects of these different supplementations, direct, statistically robust findings eluded us. This can be explained by a lot of factors, limited repetitions and scale, high levels of naturally present fluctuation inherently present in mushroom cultivation, potential negative environmental factors etc. However, combining our real-life results with the available literature, one thing is clear – protein and other supplementation in mushroom cultivation is a vital part of this industry and if properly utilized it can produce significant positive results.

5.2 Limitations and Future Research Directions

This study has several limitations that should be addressed in future research. Firstly, environmental factors were kept consistent to isolate the effects of protein supplementation, but real-world cultivation often involves fluctuating conditions. Additionally, while mealworm excrement and protein showed potential benefits, understanding the specific biochemical mechanisms underlying these effects could reveal more about how these supplements interact with fungal physiology. Further studies could investigate larger sample sizes, broader concentration ranges, and diverse environmental conditions to refine recommendations on the ideal supplementation strategies.

The data suggests that while specific protein supplements appear to influence mushroom yield and other growth metrics, these effects do not consistently correlate with concentration levels. The use of mealworm excrement increased overall mushroom count without affecting individual weight, while black soldier fly protein tended to reduce both metrics at higher concentrations. Statistical analyses, including ANOVA and t-tests, confirm that these variations are not uniformly significant across all treatments, underscoring the complex interaction between protein type, concentration, and mushroom growth.

In conclusion, this research adds to the growing body of knowledge on sustainable mushroom cultivation by demonstrating that protein-rich substrates can enhance productivity without adverse effects on growth cycles. The nuanced impact of different protein sources, however, reinforces the need for careful selection and dosage optimization to maximize yield and maintain crop quality.

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9. Declarations

DECLARATION

the public access and authenticity of the thesis

Student's name:	Bence Boinitzer
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Title of thesis:	"Utilization of the Protein and Waste of Larvae Bred on Spent Mushroom Compost in the Cultivation of Button Mushroom"
Year of publication:	2024
Name of the consultant's institute:	Institute of Horticulture
Name of consultant's department:	Department of Vegetables and Mushroom growing

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