DIPLOMA THESIS

M.Sc. Horticulture Engineering

Naijuka Edwiger Ampurire

Budapest

2024

Hungarian University of Agriculture and Life Sciences
Budai Campus

M.Sc. Horticulture Engineering

EFFECTS OF LARVA'S PROTEIN AND FRASS SUPPLEMENTATION ON OYSTER MUSHROOM CULTIVATION

Supervisor:	Dr. András Geösel, (Ph.D) Institute Director
Co supervisor:	Ruth Mwangi PhD Student
Author:	Naijuka Edwiger Ampurire
Neptun code:	BPR1QB
Department:	Vegetable and Mushroom Growing
Institute:	Faculty of Horticultural Science

BUDAPEST

2024

Abstract

Oyster mushrooms are increasing for their easy cultivation technology, availability of raw materials, high yield potential as well as its high nutritional and medicinal value. supplementation is one of the key practices research projects have shown for better results in production of oyster mushrooms. Agricultural waste supplements are mostly used like rice bran, wheat bran and spent mushroom substances. The resent years sent mushroom substances have been a challenge to the mushroom farmers, however as one of the solutions is to feed the spent mushroom substrate on inspects for decomposition which is economically cheap and environmentally friendly. Meal worm's larva is one of good decomposers of spent mushroom substance, since their breeding cycle is fast. As they feed, they drop their frass and multiply easily.it is on this point of finding the best way to recycle the spent mushroom substrate and find supplements for oyster mushrooms to boost production. This study aims to find the Effects of larva's protein and frass supplementation on oyster mushroom cultivation.

The experiment was carried out in the mushroom laboratory of Hungarian University of Agriculture and Life science at the department of Vegetable and Mushroom growing Two separate supplements were tested on the growth rate of oyster mushroom mycelium for 7days at room temperature (larva additive and protein) respectively at three different levels namely 1%,5% and 10%.two different sets of control were used positive control with 10% larva additive and protein + water agar and the main control with PDA .the highest growth rate was recorded at 1% larva additive with the mean value of 78.419 mm ,followed by 5% larva additive with 76.723mm and the positive control of 10% larva additive +water agar with 76.812mm while the lowest growth rate was observed in protein10% with a mean of 50.209mm. larva additive supplement had a high growth rate as compared to protein supplements from the results , it is conclude that supplementation of both larva and protein supports growth of the oyster mycelium and can be used in cultivation to boost yield potential on a lower concentration. However, recommend testing of the treatments in vivo experiment to establish the effect of the supplements on the fruiting body and nutritional values.

Contents

1. Introduction

Oyster mushroom (*Pleurotus spp.)* is a widely cultivated mushroom worldwide because of its simple cultivation requirements. Many people admire the mushroom due to its taste, flavor, high nutritional values, and some medicinal properties (Adebayo & Oloke, 2017). Oyster mushroom have a high nutritional value, nutrients like proteins with the essential amino acids, carbohydrates and fibers (Deepalakshmi & Mirunalini, 2008). Research has indicated that oysters mushrooms are a good source of essential minerals and vitamins such as thiamine ,niacin, riboflavin ,vitamin B6 and lower fat content,(Effiong et al., 2023, Mattila et al., 2001). The presence of some bioactive substances, majorly polysaccharide-protein complex in the genus *Pleurotus* has been reported to confer some pharmacological potential such as antimicrobial, antioxidant, anticancer, anti-inflammation, anti-hypercholesterolemia, anti-hypertensive, anti-diabetic, hepato-protective, anti-allergic activities and many other related medicinal values (Adebayo & Oloke, 2017, Golak-Siwulska et al., 2018).

Oyster mushrooms can thrive on various substrates like agricultural waste, straw, or sawdust, the addition of supplements to these substrates can significantly impact their growth, yield, and quality (Hoa et al., 2015, Girmay et al., 2016). Including supplements in the preparation of the substrate improves the chemical and nutritional content of substrates thus better-quality oyster mushrooms. Supplements in mushroom cultivation refer to additional organic or inorganic materials added to the substrate to enhance its nutritional content and support optimal mushroom growth. Some commonly used supplements in oyster mushroom cultivation include wheat bran, rice bran, corn cobs, soybean meal, gypsum, and calcium carbonate.(Naraian et al., 2016, Jeznabadi et al., 2017).The effects of supplements on oyster mushroom cultivation can vary depending on several factors such as the type and concentration of the supplement, the composition of the substrate, environmental conditions, and the specific strain of oyster mushroom being cultivated. Supplements rich in nitrogen and carbon, such as wheat bran or rice bran, can stimulate mycelial growth and enhance fruiting body formation, leading to increased mushroom yield per unit of substrate(N. A. Khan et al., 2017, Nunes et al., 2012).

Certain supplements can enrich the substrate with essential nutrients, vitamins, and minerals, thereby enhancing the nutritional quality of the harvested mushrooms. This is particularly important for oyster mushrooms, which are valued for their high protein content and medicinal properties. Supplements containing readily available nutrients can accelerate the colonization of the substrate by oyster mushroom mycelium, reducing the incubation period and allowing for faster mushroom production cycles (Ashraf et al., 2013). The addition of supplements can improve the biological efficiency of oyster mushroom cultivation, which is the ratio of mushroom biomass produced to the dry weight of the substrate. This is crucial for maximizing production and optimizing resource utilization (Nunes et al., 2012). Supplements with antimicrobial properties, such as lime and gypsum act as a buffer for the mycelial expansion (Afzal et al., 2019). This helps suppress competing microorganisms and reduce the risk of substrate contamination by molds, bacteria, or other fungi thus improving the overall success rate of mushroom cultivation.

This current study is intended to analyze the effects of protein and larva additives as supplements for oyster mushroom cultivation both on the growth of the mycelium and the fruiting body of oyster mushrooms.

2. Literature review

2. 1. Oyster mushroom production

Oyster mushrooms (Pleurotus ostreatus) are a type of edible mushroom that are prized for their meaty texture and slightly sweet, nutty flavor. It is commonly known as oyster mushroom as the

fruit body of Pleurotus resembles the shape of a shell. They originated from the Greek word Pleura which means lateral position of fruit-body or pileus from its stem and named after their oyster-like shape and are widely cultivated and consumed globally (Barh et al., 2020a). Oyster mushrooms are low in fat and calories, but rich in vitamins and minerals, making them a healthy addition to a variety of dishes, including soups, stews, sauces, and stir-fries (Kalač, 2009).They can also be grilled, roasted, or fried. Oyster mushrooms have been traditionally used for their medicinal properties in some cultures. They are a source of beta-glucans, compounds with immune-boosting effects, and contain antioxidants that can help protect against cellular damage (Adebayo & Oloke, 2017). Cultivation of oyster mushrooms is relatively simple and can be done on a small-scale using waste materials, such as coffee grounds and straw, as the growing medium. Commercially, they are often grown on a large-scale using bags filled with pasteurized sawdust or straw, which are inoculated with oyster mushroom spawns and then incubated in a controlled environment. Oyster mushrooms are a versatile and healthy food that offer a variety of culinary and potential health benefits (Barh et al., 2020b).

2.2 Classification of Oyster mushrooms

Classification is an important part of the study of biology, as it helps scientists understand the evolutionary relationships between different organisms and provides a framework for organizing and understanding the diversity of life on earth (Deepalakshmi & Mirunalini, 2008) By classifying oyster mushrooms, we can better understand their place in the natural world and use that information to help conserve and manage their populations.

Oyster mushrooms (*Pleurotus ostreatus)* belong to the kingdom Fungi and the division *Basidiomycota,* Class: *Basidiomycetes***,** Order: *Agaricales***,** Family: *Pleurotaceae***,** Genus: Pleurotus**, Species**: *Pleurotus ostreatus* (Piska et al., 2017). The classification of oyster mushrooms is based on their morphological, physiological, and genetic characteristics. They are part of a larger group of fungi that includes many other edible and medicinal mushroom species. By understanding the classification of oyster mushrooms, scientists and cultivators can better understand their behavior and needs, as well as identify and distinguish them from other similar species. In the family Pleurotaceae, oyster mushrooms are part of a group of closely related species that includes *Pleurotus ostreatus, Pleurotus pulmonarius* (phoenix oyster), Pleurotus sajor-caju, Pleurotus citrinopileatus, *Pleurotus djamor,Pleurotus floria* and *Pleurotus eryngii* (king oyster mushroom). These species are differentiated from one another by differences in their physical characteristics, habitat, and genetics (Jaitly, 2011)**.**

2.3 Oyster Mushrooms Species

Oyster mushrooms belong to the genus Pleurotus and are widely cultivated and consumed around the world. There are several types of oyster mushrooms, each with its own characteristics and culinary uses,(Bao et al., 2004). They include,

Pleurotus ostreatus (Grey oyster mushroom), Pleurotus ostreatus is a wood-decaying saprophytic fungus and predominant in Europe but its occurrence is worldwide. The Pleurotus ostreatus fructifies around 15 to 20°C. The species appears with cup shaped pileus with variable color such as grey, chocolate brown, black, brown, blue, white, cream color, etc. (figure 1) The stripes and gills are white in color. which is the most cultivated oyster mushrooms (Barh et al., 2020a) It has a mild, delicate flavor and a velvety texture. Grey oyster mushrooms are versatile and can be used in various dishes such as stir-fries, soups, and pasta dishes.

Figure 1 *Pleurotus* ***ostreatus*** fruiting body. (Source, Barh et al., 2020a)

Pleurotus pulmonarius (Phoenix Oyster Mushroom), also known as the "summer oyster" or "Italian oyster," (figure 2) that is similar in appearance as Pleurotus ostreatus but with a slightly firmer texture and a more pronounced flavor. Phoenix oyster mushrooms are often used in the same way as pearl oyster mushrooms in cooking.

Figure 2 ***Pleurotus pulmonarius*** fruiting body Source, (Barh et al., 2020a)

Mushrooms Pleurotus eryngii (King Oyster Mushroom): Unlike other oyster mushrooms, the king oyster mushroom has a thicker stem and a smaller cap. (Figure 3) It has a meaty texture and a rich, earthy flavor, making it popular for grilling, roasting, or sautéing as a meat substitute.

Figure 3 ***Pleurotus eryngii*** fruiting body .Source, (Barh et al., 2020a)

Pleurotus citrinopileatus (Golden Oyster Mushroom) As the name suggests, this variety has a golden to yellowish color. It has a mild, sweet flavor and a delicate aroma. Golden oyster mushrooms are often used in Asian cuisine and can be stir-fried, added to soups, or used in salads, *Pleurotus cornucopiae* (figure 4) Also known as the "abalone mushroom," this species has a unique shell-like shape and a slightly firmer texture compared to other oyster mushrooms. It has a rich, savory flavor and is often used in soups, stews, and pasta dishes.

Figure 4 ***Pleurotus citrinopileatus*** fruiting body (Source, Barh et al., 2020a)

Pleurotus djamor (pink oyster mushroom). like the name says it has a pink color ,with curly cup that is about 2-5cm in diameter .pink oysters have short live span(one day) that is why they are rare on market and normally appear during spring and has a fishy and meaty flavour when prepared.(Jaitly, 2011). (figure5) The species is commonly found in high temperature regions and is characterized with its aggressive colonization ability (Barh et al., 2020a)

Figure 5 shows *Pleurotus djamor* (pink oyster mushroom) (Source; Barh et al., 2020a)

According to research Pleurotus spp have different yielding abilities as shown in (table 1), Moreso their morphological characteristics differ ranging from strip length, pileus length, pileus width pileus thickness margin of the fruit body and the color of fruit body as shown in (table 2) The biochemical components like total soluble sugars, reducing sugars, non-reducing sugars and crude protein also differed(Jaitly, 2011)

Table 1 shows the yield performance of different Pleurotus spp (Source Jaitly, 2011)

Sl. No.	Species	Av. No. of fruit bodies	Av. yield g/kg dry substrate	Av wt./fruit body
1.	*P. sajor caju*	21.48	742.98	34.59
2.	*P. djamor*	17.35	656.09	37.81
3.	*P. florida*	12.67	650.46	51.34
4.	*H. ulmarius*	18.71	855.52	45.73
5.	*P. citriopileatus*	16.06	681.72	42.45

Table 2 shows morphological characteristics of different Pleurotus ostreatus species.

Sl. No.	Parents	Stipe length (cm)	Pileus length (cm)	Pileus width (cm)	Margin of fruit body	Colour of fruit body
1	*P. sajor caju*	4.70	6.42	7.25	Dentate	Grey
2	*P. djamor*	0.91	4.72	7.40	Wavy	Pink
3	*P. florida*	4.80	6.71	7.90	entire, enrolled	Creamy White
4	*H. ulmarius*	3.60	5.36	7.85	entire	blue
5	*P. citriopileatus*	3.50	5.04	6.95	entire	white

Source, (Jaitly, 2011)

2.4 Nutritional value of oyster mushroom

Mushrooms as functional foods are used as nutrient supplements to enhance immunity in the form of tablets. Due to low starch content and low cholesterol, they suit diabetic and heart patients (Wani et al., 2010). Oyster mushrooms (*Pleurotus ostreatus*) are a nutritious and delicious addition to any diet. They are low in calories and fat, but high in essential vitamins and minerals that contribute to overall health (Mca et al., 2021). Oyster mushrooms are an excellent source protein more than other vegetables, (Lintzel (1941) reported the digestibility of mushroom protein to be as high as 72 to 83% and the digestibility of Pleurotus mushrooms proteins is as that of plants (90%) whereas that of meat is 99% (Wani et al., 2010). Also compared to animal protein, it contains main essential

amino acids, making them a complete protein source for vegans and vegetarians (Deepalakshmi & Mirunalini, 2008). However, the protein content of *P. ostreatus* is also significantly affected by the method of drying, as oven-dried *P. ostreatus* recorded a less protein content (24.99%) than open sun-dried mushrooms (27.14%), (Tolera & Abera, 2017) ,(table 3) shows different nutritional values found in different Pleurotus species.

Table 3 Proximate composition of different *Pleurotus* spp (Source;Barh et al., 2020a)

Species	**Moisture content**	**Protein (%)**	**Carbo-hydrate (%)**	**Crude Fibre (%)**	**Fat (%)**	**Ash content (%)**	**References**
P. eryngii	88.66	21.00	56.00	13.35	2.40	7.50	(Sardar et al. 2017)
P. ostreatus	88.51	21.00	55.20	7.35	2.00	6.35	(Patil et al. 2010)
P. florida	89.40	21.33	56.00	7.80	2.30	6.40	(Ahmed et al. 2009)
P. sajor caju	88.25	22.90	56.00	7.10	2.50	6.65	(Patil 2013)
P. euos	75.10	19.48	50.20	7.75	2.62	6.00	(Telang et al. 2010. Ritota and Manzi 2019)

Oyster mushrooms are a good source of vitamins B and D. Vitamin B complex includes thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), and biotin (B7). (Adebayo & Oloke, 2017). These vitamins help the body convert food into energy, maintain healthy skin and hair, and support brain function. Vitamin D is important for bone health and helps the body absorb calcium (Kalač, 2009). Oyster mushrooms are not considered as a significant source of essential fatty acids but contain some essential fatty acids. the main fatty acids present are polyunsaturated fatty acids(linoleic acid) (Naraian, 2017) .Oyster mushrooms are a good source of several essential minerals, such as potassium, phosphorus, zinc, magnesium and copper.(Piska et al., 2017). These minerals help in regulating blood pressure, support proper body functioning and the immune system.

Oyster mushrooms are considered a good source of carbohydrates and dietary fibers. Carbohydrates are mainly present in these mushrooms as polysaccharides or glycoproteins (M. A. Khan & Tania, 2012). The carbohydrate content of mushrooms represents the bulk of fruiting bodies accounting for 50 to 65% on dry weight basis (Wani et al., 2010). In a recent study total carbohydrate content in 100g of the fruitbodies of three different mushrooms was recorded and *Pleurotus eryngii* has carbohydrate content of 41g, while *Pleurotus sajor-caju* contains 38 g and *Pleurotus florida* has 34g (Naraian, 2017). Oyster mushrooms have high and low molecular weight carbohydrates. The high molecular weight carbohydrates are polysaccharides such as chitin and glucan. The low molecular weight carbohydrates are monosaccharides, disaccharides, and sugar

alcohols (polyols), such as arabitol, glucose, mannitol, and trehalose (Zhou et al., 2016). The carbohydrate contents of *P. ostreatus* are significantly affected by the method used for drying. Oven-dried *P. ostreatus* recorded a higher carbohydrate value (43.64%) than open sun-dried mushrooms (39.99%) (Tolera & Abera, 2017)**.** Mushrooms contain a considerable amount of fiber. The Total dietary fiber (mainly chitin) in mushrooms ranges from 10 to 31 g per 100 g dry weight (5–10) (M. A. Khan & Tania, 2012). Mushroom glucans are also components of soluble or insoluble dietary fibers. The Fiber content in Mushroom is essential in regulating digestion and promotes feelings of fullness, which can support weight management.

2.5 Medicinal values of oyster mushrooms

Oyster mushrooms have been recognized in China, Korea, and Japan for centuries, where mushrooms have been traditionally used for their medicinal properties. Currently, in some parts of the world, there is a renaissance of interest in traditional remedies. The extracts or powder of fruit bodies or mycelium of Pleurotus mushrooms have been reported to have anticancer, anti-hypercholesterolemic, antihypertensive, antidiabetic, antiobesity, hepatoprotective, antiaging, antimicrobial, and antioxidant activities (M. A. Khan & Tania, 2012). (Figure 6)

Oyster mushrooms contain antioxidants, such as phenolic and flavonoid compounds delaying and inhibiting oxidative processes. Mushrooms contain a significant amount of Ergosterol and selenium that help protect against cellular damage (Deepalakshmi & Mirunalini, 2008). Selenium plays a role in antioxidant defense and supports immune function (Adebayo & Oloke, 2017). Incorporating oyster mushrooms into the diet support heart, immune system, and digestive functions, among other benefits (Saima, 2021). Oyster mushrooms are naturally low in fat and cholesterol-free, making them an excellent food source.

Anti-cancer and anti-inflammatory. the mushrooms produce bioactive compounds such as 1,6-branched 1,3-β-glucans which have been reported to inhibit tumor growth by stimulating the immune system via activation of macrophages, balance of T helper cell populations and subsequent effects on natural killer (NK) cells and also by cytokine production (Saima, 2021). Inflammation is a natural response to injury or infection, but chronic inflammation can contribute to the development of various health conditions, including heart disease, cancer, and Alzheimer's disease (Patel et al., 2012).

Anti-viral. Some research reported that oyster mushrooms have antiviral properties. A laccase enzyme has been purified from oyster mushroom, which can inhibit the hepatitis C virus entry into peripheral blood cells and hepatoma HepG2 cells and its replication in 2010. Ubiquitin, an anti-viral protein was isolated and identified from fruiting body oyster mushroom (Saima, 2021, Piraino, F. and C.R. Brandt, 1999.).

Antidiabetic and anti-hyperlipidaemic. The combination of P. ostreatus, Murraya koenigii and Aegle marmelos produced synergistic effects have shown blood glucose-lowering effect in both insulin- dependent and insulin-independent diabetic conditions (Deepalakshmi & Mirunalini,

2008). Oyster mushrooms have been reported to have anti-hyperlipidaemic activity to the diet of normal wistar male rat and a stain with hereditary hypercholesterolaemia. Oysters showed a cooperative effect on lipid profile, liver, and kidney functions in hypercholesterolic rats.

While the evidence is promising, more studies are needed to establish the safety and efficacy of using oyster mushrooms for medicinal purposes. It is also important to note that while oyster mushrooms have been used as a traditional medicine in some cultures for centuries, their safety and efficacy as a medicine have not been fully evaluated. As with any dietary supplement or natural remedy, it's important to speak with a healthcare provider before using oyster mushrooms for medicinal purposes (Golak-Siwulska et al., 2018).

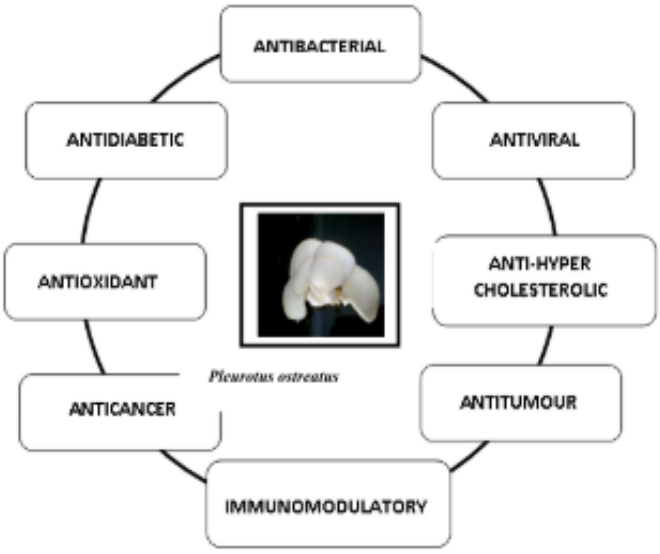


Figure 6 shows the medicinal value of oyster mushrooms. (Source Deepalakshmi & Mirunalini, 2008).

2. 6 Global cultivation of oyster mushrooms

Based on data from different sources, estimated world mushroom production in 2018-19 was 43 million tonne (MT) (figure 7) with *Lentinula edodes* (shiitake) contributing 26%, *Auricularia* spp 21% *Pleurotus ostreatus* (oyster) 16%, *Agaricus bisporus* (button) 11%, *Flammulina velutipes* 7%, *P. eryngii* (king oyster) 5%, *Volvariella volvacea* (paddy straw mushroom) 1% and others 13%. Other important contributors were *Agrocybe aegerita, Pholiota nameko, Tremella fuciformis, Hypsizygus marmoreus* (Singh et al., 2021).

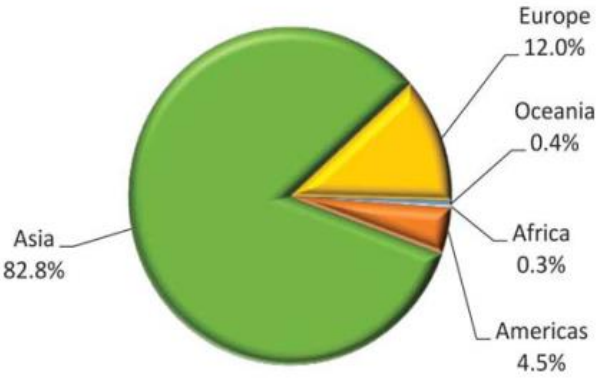


Figure 7 shows the total continental production of mushrooms in 2019. (Continent-wise total world mushroom production in 2019 as per FAOSTAT release in 2021)

Pleurotus production is largely concentrated in Asian countries such as China, Japan, South Korea, Taiwan, Thailand, Vietnam, and India. The first report on *Pleurotus ostreatus* cultivation was in 1917 (Barh et al., 2020a).as shown in (table 4) below In the beginning of this century, *Pleurotus ostreatus* was having maximum contribution to the total production in China,(figure 8) below shows production of different oyster mushroom species in China in 2018.

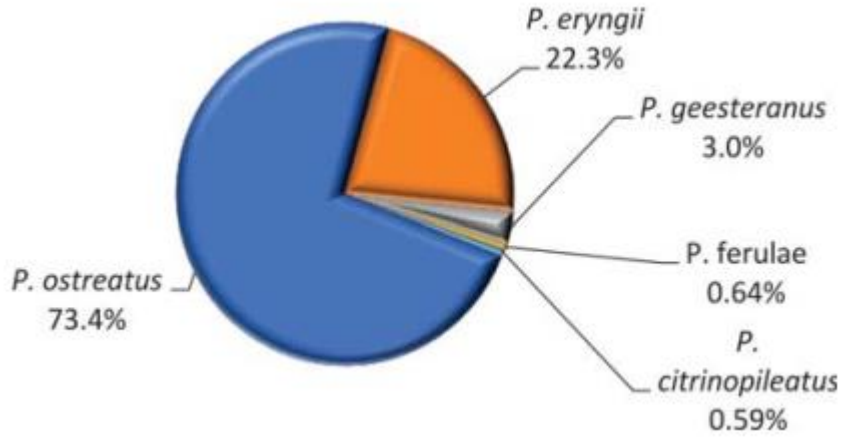


Figure 8 shows Relative share(production) of *Pleurotus* species in China in 2018. Source, (Singh *et al*., 2018).

The Oyster mushroom, paddy straw (*Volvariella volvacea*) and milky (*Calocybe indica*) mushrooms are some of the high potential cultivable mushrooms in Sri Lanka since they can be grown well under tropical and subtropical condition. *P. ostreatus* is popular in Sri Lanka as a vegetable and as an ingredient in soup.

Africa constitutes at least 25% of the total mushroom biodiversity worldwide but contributes barely 0.4% of total mushroom sales and new mushroom products on the global market (Singh et al.,

2021). So far, African mushroom growers have only succeeded in growing *Pleurotus* species (Oyster mushrooms) especially *Pleurotus ostreatus, Pleurotus sajo-caju* and *Pleurotus pulmonarius,* on corn cobs, rice husks, maize bran, and sawdust.

Mushroom production in EU for the year 2019 was 1325 million kg and here too speciality mushrooms accounted for 3-4% of the total production., For example in Italy in 2017 estimates were that 80% are button followed by *Pleurotus* (mostly *P.ostreatus* but also *P. eryngii*), *Cyclocybe aegerita* and, to less extent, shiitake (Singh et al., 2021).

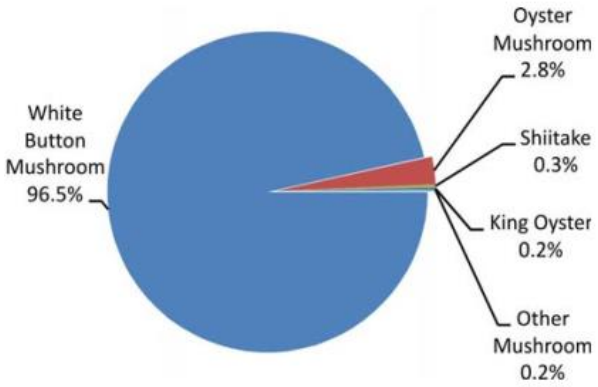


Figure 9 shows Share of different species in total mushroom production in EU in 2016

Table 4 Shows, The chronology of *Pleurotus* cultivation.

Events	Year	Reference
Cultivation of *Pleurotus ostreatus*	1917	Falck,1917
Cultivation of *Pleurotus ferulae*	1958	Mou & Cao (1986)
Cultivation of *Pleurotus flabellatus*	1962	Bano & Srinvatava (1962)
Cultivation of *Pleurotus cystidiosus*	1969	Miller (1969)
Cultivation of *Pleurotus sajor-caju*	1974	Jandaik (1974)
Cultivation of *Pleurotus citrinopileatus*	1981	Shen (1981)

Source, (Barh et al., 2020a).

2.7 Substrates of oyster mushrooms cultivation.

Cultivation and Production of mushrooms is completely different from growing green plants. Mushrooms are relatively fast-growing organisms, thus, mushroom cultivation as a short return agricultural business can be of immediate benefit to the community. Mushrooms do not contain chlorophyll and therefore depend on other plant material (the "substrate") for their food (Uide et

al., 1999). In mushroom cultivation, the substrate provides the food source and physical structure for the growing mycelium, which eventually produce the fruiting bodies, or mushrooms (Sayner & Jong, 2018). The common substrates are agricultural residues and agro industrial by-products, such as straw of different grains, corn wastes, hard wood chips, sawdust, cotton seed hulls, rice straw, barley straw, coffee grounds, spent mushroom substrates, bagasse, and/or a combination of these ingredients (Jeznabadi et al., 2017, Jongman et al., 2018, Satankar et al., 2020).

Sawdust is a common substrate used in mushroom cultivation. sawdust of Mango (Mangifera indica), Jackfruit (Artocarpus heterophyllus), Coconut (Cocos nucifera), Kadom (Anthocephalus sinensis), Mahogony (Swietonia macrophylla), Shiris (Albizzia spp), Jam (Syzygium spp) plants have been reported to be more effective in mushroom growing (table 5.) below shows the Effect of different sawdust substrates on growth and yield of Mushroom. Sawdust provides a good balance of nutrients and water-holding capacity, and it is also readily available in many regions (M. Z. Rahman & Hafiz, 2009).

Table 5. The effect of different sawdust substrates on growth and yield of Mushroom.

Characters	Substrates (Sawdust)							CV (%)
	Mango	Jackfruit	Kadom	Coconut	Mahogony	Jam	Shiris	
No. of primordia	41.00	34.65	43.00	32.60	44.00	39.00	39.89	9.91
No. of Fruiting bodies	31.00	27.75	29.00	29.61	34.00	30.00	32.00	6.27
Pileus diameter (cm)	7.00	6.11	6.20	4.00	6.16	5.50	6.90	15.56
Pileus thickness (cm)	0.65	0.53	0.59	0.47	0.63	0.54	0.60	12.17
Diameter of stalk (cm)	0.98	0.82	0.87	0.64	0.99	0.77	0.92	13.47
Length of stalk (cm)	3.0	2.98	3.59	2.20	3.36	3.01	3.34	13.42
Fresh weight of mushroom (gm/packet)	150	97	136	83	148	114	146	20.04
Dry weight of mushroom (gm/packet)	15.98	10.10	13.55	8.21	14.33	11.29	14.21	20.27

Source (M. Z. Rahman & Hafiz, 2009)

Straw is one of the common substrates used in mushroom cultivation, particularly for species like oyster mushrooms. Straw is nutrient-rich and produces a good amount of oyster mushrooms. Rye straw, oats, wheat, and rice are all great substrates for oyster mushroom growth. (Table 6) shows the effect of rice and wheat straw on *P.sajor-caju* cultivation The good news is that purchasing the substrate doesn't have to be very expensive. Straw is widely available and reasonably priced. Straw fibers are easily broken down by oyster mushrooms, allowing them to absorb nutrients (Zhang et al., 2002)

Table 6. Effect of rice and wheat straw on *P. sajor-caju* cultivation.

Test run	Straw type	Mechanical method	Size (cm)	Initial substrate weight (dry weight, g)		Spawn level (%)	Mushroom Yield (g)			Biological efficiency	Substrate dry master loss (%)
				Straw	Spawn		First flush	Second flush	Total		
1	Rice straw	Ground	0.5	146.4	27.5	18	112.7[A] (20.0)[B]	45.9 (10.5)	158.6 (33.4)[a]	108.0 (9.6)[a]	32.3 (2.5)[a]
2			2.5				118.3 (21.2)	70.0 (18.4)	188.3 (36.9)[b]	128.8 (13.8)[b]	33.2 (4.1)[b]
3		Chopped	2.5	146.4	27.5	18	114.2 (18.5)	42.0 (11.0)	156.2 (28.8)[c]	106.8 (6.2)[c]	30.7 (2.0)[c]
4			5.0				114.0 (19.1)	38.1 (8.5)	152.1 (25.7)[c]	104.0 (4.9)[c]	30.1 (1.9)[c]
5	Rice straw	Ground	2.5	146.4	17.9	12	105.6 (20.1)	43.5 (10.2)	149.1 (28.5)[d]	101.8 (5.6)[d]	36.2 (2.6)[d]
6					23.6	16	122.1 (32.8)	56.6 (13.4)	178.7 (30.0)[b]	122.1 (6.5)[b]	39.4 (4.1)[b]
7					27.5	18	124.6 (31.7)	67.2 (15.2)	191.8 (33.7)[b]	131.0 (9.7)[b]	36.7 (2.3)[b]
8		Chopped	2.5	146.4	17.9	12	82.0 (19.2)	28.6 (5.3)	110.6 (27.1)[e]	75.5 (5.4)[e]	29.0 (0.5)[e]
9					23.6	16	92.1 (21.2)	45.1 (12.5)	142.2 (22.0)[c]	97.1 (3.0)[c]	29.1 (5.2)[c]
10					27.5	18	94.1 (25.6)	60.9 (18.3)	155.0 (32.0)[c]	105.9 (8.3)[c]	30.4 (0.8)[c]
11	Rice straw	Chopped	2.5	146.4	27.5	18	114.0 (21.4)	38.1 (10.5)	152.1 (22.0)[c]	104.0 (2.9)	44.1 (3.6)[c]
12	Wheat straw						94.7 (20.8)	47.2 (14.6)	141.9 (19.1)[c]	97.0 (2.8)[c]	34.8 (2.0)[c]
13	Mixture						114.0 (18.5)	34.4 (8.9)	148.4 (26.0)[c]	101.2 (5.0)[c]	42.5 (4.0)[c]
14	Mixture						108.0 (19.2)	50.2 (12.8)	158.2 (30.4)[c]	108.3 (6.7)[c]	44.3 (3.1)[c]

source(Zhang et al., 2002)

The choice of substrate will depend on the species of oyster mushroom, the availability of materials in a particular region, and the desired outcome of the cultivation process. Substrates must be sterilized to kill off any competing microorganisms and to create a clean environment for the growing mycelium. The sterilized substrate is then inoculated with mushroom spawn, which is the start of the growing process.

2.8 Supplements in oyster mushroom cultivation

Oyster mushrooms *(Pleurotus spp.)* are known for their ability to grow on a variety of substrates, including agricultural waste products. However, to improve the composition of substrate different supplementary materials are used. Supplements in oyster mushroom cultivation are additional nutrients or substrates added to the growing medium to enhance the nutrient content, growth, and yield of the mushrooms (Salama et al., 2019). Supplements improve the nutritional status of most waste substrate which result in better mushroom growth and productivity by providing easily degradable carbohydrates, more protein and nitrogen (N. A. Khan et al., 2017, Oseni et al., 2012).

Various supplementary materials including wheat bran, rice bran and gram flour in cotton waste are utilised in the production of oyster mushroom. Soybean meal provides the most reliable nitrogen-rich supplement and enhance the nutritional content of the substrate and many more supplements such as corn meal, cotton seed meal, fish meal, peanut meal rice bran (Tikdari & Bolandnazar, 2012). Cereals bran are rich source of protein such as wheat bran, rice bran have significant results in improving mushroom yield, biological efficiency and improving mycelium growth (N. A. Khan et al., 2017)

Spent mushroom substrate (SMS) is another good supplementation of the Oyster mushroom substrate, which is the biomass produced by mushroom production that remains after harvesting a

mushroom crop. Reusing or recycling could serve as an effective method in mitigating this solid waste, providing new economic opportunities and positive environmental consequences. Since SMS is an available material, containing organic matter (C and N), macro and micronutrients, a viable option for destination is the addition to the substrate to produce a new cycle of mushrooms (Lucas de Jesus et al., 2023, Adi et al., 2023)

Mineral elements such as Gypsum and Calcium carbonate are mostly used in substrate mixture to help improve its structure and regulate the pH of the substrate mixture to the optimal range for oyster mushroom growth.

These supplements are often used in combination with a primary substrate, such as straw or sawdust, to create a nutrient-rich growing medium for oyster mushrooms. The specific supplements used may vary depending on factors such as substrate availability, cost, and the desired characteristics of the final mushroom product.

2.9 Cultivation technologies of oyster mushroom

oyster mushroom cultivation depends on finding a unique growing technology version and an effective cooperation strategy by which the success of the whole supply chain can be maintained (Gyenge et al., 2016) oyster mushrooms can be cultivated extensively and intensively using different methods such as plastic bag method ,cover method ,bottled method and the outdoor method (Barh et al., 2020a ; Gyenge et al., 2016)over years technology has advised and new methods are being introduced ,first climate computers were introduced to Dutch mushroom-growing and now are widely used in the industry (Sánchez, 2004). This method is commonly known as the Dutch technology. Several steps are followed in the cultivation of oyster mushrooms ranging from selection of the species (strain), spawn preparation, substrate preparation spawning or inoculation, incubation and fruiting as shown in the figure 10 below.

.

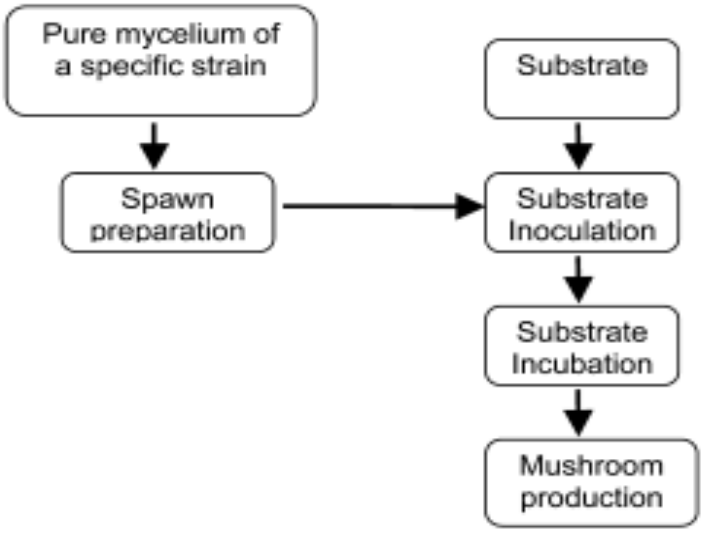


Figure 10. General steps in oyster mushroom cultivation (source: Sánchez, 2004)

Spawn preparation, Spawn as mushroom seed is defined as a living ramified mycelium of a mushroom, multiplied on a suitable sterile base material or carrier under aseptic techniques. There are several carriers that can be used for spawn production including grains of wheat, millet and sorghum (Jongman et al., 2018)The quality of spawn is one of the most decisive factors for successful crop, growers need to use qualified spawn for commercial production. Spawns can be grain spawns, plug spawns, and synthetic spawns (Sullivan, n.d.) S The potential of a high yielding strain largely depends on the quality of spawn, stage of the spawn (i.e. its age and maturity) type of multiplication, free from contaminants, etc. The 20-30 days old spawn is considered ideal for Pleurotus mushroom cultivation (Barh et al., 2020a).general step of spawn preparation are cleaning the grain, cooking the grain ,filing the bottle or bags sterilization ,cooling ,inoculation with the mycelium and colonization storage and transportation for commercial purposes,

Substrate preparation Oyster mushrooms can grow on a variety of substrates, including straw, sawdust, coffee grounds, cotton seed hulls (IQBAL et al., 2005) Substrate preparation is known to be the most critical stage in the production process ,to achieve better yield potential competitors such as *Trichoderma* spp . of *Pleurotus* spp. must be eliminated, therefore, substrate like grasses, sugarcane bagasse is pasteurized beforehand. Pasteurization can be done by using steam or by using hot water treatment at 60 -70 degrees cereous or dry heat treatment at 100 degrees cereous. (Barh et al., 2020a) prolonged soaking of substrates in water and treatment with benomyl (0.06g/L) have the ability to lessen/suppress the risk of contaminants such as *Trichoderma* spp (Jongman et al., 2018)

Spawning or Inoculation. Once the substrate is cooled after pasteurization inoculation is the process where mushroom spawns are added to the pasteurized substrate. Spawn can be grain spawn, plug spawn synthetic spawn and they can be cultured or purchased from suppliers as commercial spawn or obtained from a previous batch. Distribute the spawn evenly throughout the substrate, either by mixing it thoroughly , layering it and top spawning (T. Rahman et al., 2019).

Incubation After inoculation, the substrate needs to be kept in a dark, humid environment with temperatures around 20-24°C (68-75°F) for the mycelium to colonize the substrate (Sayner & Jong, 2018) This process usually takes 2 weeks, depending on various factors such as temperature and humidity (Katel et al., 2022)

Fruiting also known as cropping Once the substrate is fully colonized by the mycelium, it's time to initiate fruiting. This is typically done by exposing the substrate to fresh air, light, and slightly lower temperatures (around 15-20°C or 59-68°F) (Sayner & Jong, 2018) signals are sent to the colonized mycelium that it's time to start producing mushrooms and small pins begin to emerge with the necessary environmental conditions the pins will rapidly grow to full size mushrooms within 5-7days.

Harvesting, harvesting can be done 4-5day after pinning when the caps are fully grown but haven't yet started to flatten out (Kumar Suresh, 2021) .harvest by gently twisting the fruiting body substrate, being careful not to damage the mycelium. 3-4 flushes of fruit bodies could be harvested from different Pleurotus species. The total cropping period is extended from 30 to 45 days.

Cultivating mushrooms requires attention to cleanliness and environmental conditions to prevent contamination and ensure a successful harvest. With practice and experimentation, you can refine your techniques and increase productivity.

2.10 Value addition and products of oyster mushrooms.

Oyster mushrooms are mostly used in fresh conditions. It is very promising in its agribusiness trend but has constraint low shelf life (Afifa Jahan et al., 2019)to overcome this constrain valve addition is done to improve their shelf life. Preservation and processing can extend the shelf life of mushrooms for 3-6 months or more Barh et al., 2020a) Forms like frozen, canned, pickled, dried which increases value of the product.

Refrigeration, fresh mushroom can be refrigerated for about 3-5days at 220c for at least(Afifa Jahan et al., 2019) according to research the most common method of mushroom preservation by the cryogenic method is blast freezing. Chill storage will preserve perishables for days or weeks and frozen storage (deep freezing) will preserve for months or even years (Rai & Arumuganathan, 2008)

Canning is another method of mushroom preservation which can increase the storage period up to 2 years with storage costs being relatively low. Even though usage of this process has dropped over the last years, about 38% of them are canned these days, holding a major share in world trade (Afifa Jahan et al., 2019) . canning requires a series of procedure starting from selection of good quality mushroom, and followed by splicing, immersion in KMS solution (0.05%), blanching, addition of hot brine solution (1-2% salt + 0.1% citric acid), filing in cans, sealing of cans, removal of air, sterilization (118 °C, 10-15lbs,15-20 min), cooling, labeling and storage (Barh et al., 2020a)

The oldest and yet one of the most important preservation methods is drying/Dehydration. Methods for dryingmushrooms include the conventional hot air drying, thin layer drying, vacuum drying, freeze-drying, microwave drying, and the more recently introduced fluidized bed and microwave-vacuum drying. Drying ensures the removal of moisture from the mushrooms so that microbial and biochemical activities can be ceased to the great extent. The dried product can be saved for 6 months without losing its flavor (Afifa Jahan et al., 2019, Barh et al., 2020a)

Mau and Huang in his research reported that preservation by radiation i-e using gamma or x-rays at the dose of100–150Krad has been found to restrict the postharvest growth and discoloration/deterioration of mushrooms, yet decrease with increase the level of irradiation dose the level of octo-carbon aromatic compounds (1-octen-3-ol, 3-octanone, etc.) present in the mushrooms (Afifa Jahan et al., 2019).

Many novel value-added products can be prepared with mushrooms like soup powder, pickles, chips, paste, ketchup, noodles, pasta, biscuits, nuggets, mushroom-based flavour enhancers, as an additive in beverages and beauty products (Rai & Arumuganathan, 2008) other than being used in the food industry , mushrooms are also used in the pharmaceutical industry and cosmetic industry ,according to research the market value of dietary supplements made from mushrooms is quickly growing and estimated over U.S. $15 billion (Afifa Jahan et al., 2019)

A number of post- harvesting activities and processes are practiced and ensured to achieve the best value added products of mushroom and these include harvesting ,cleaning (removal of base portion on having straw with clean knife and washing)grading ,pre-cooling at 4-5°C UV light treatment to increase vitamin D2,processing (drying ,canning ,freezing) , packaging and transportation to the market (Barh et al., 2020a)

Value addition of oyster mushrooms can help to increase their market value and make them more appealing to consumers which helps to reduce food waste by increasing the shelf life of the mushrooms and making them more accessible to consumers year-round(Rai & Arumuganathan, 2008)

2.11 Utilization of the oyster spent mushroom substance

Spent mushroom substrate (SMS) is the residual biomass generated after harvesting the fruitbodies of edible/medicinal fungi and it is the main by-product of the mushroom industry .the composition and properties of spent mushroom substrate are mainly associated with the type of raw materials and supplements used to prepare the initial mushroom substrate (Martín et al., 2023) martin further show that 3-5 kg of SMS is generated per kg of fresh mushrooms .In total, ca. 64 million tons of SMS were generated worldwide by the mushroom industry in 2018, and this figure could escalate to above 100 million tons by 2026.

Environmental regulations have put pressure on mushroom farmers in recent years, necessitating the development of a better method for getting rid of SMS. SMS can be disposed of in several ways, such as feeding it to fish and animals or directly applying it to the soil as a bioremediation agent. And a ton more recycling and reuse techniques

Growing medium, spent mushroom substrates have a simpler form of a protein-rich constituent, formed by a modification of agricultural resources by the fungus after few cycles of cultivation, can be used as a good source of soil conditioners for the cultivation of vegetables, fruits, flower, and foliage crops (Maurya et al., 2020)

Substrate is the material used as a growing medium for mushrooms in cultivation. It can be made from a variety of organic materials, including straw, sawdust, and grain. To make the substrate more environmentally friendly and sustainable, it can be recycled and reused in several ways, The spent substrate can be composted and used as fertilizer for crops (Maurya et al., 2020). This reduces waste and returns the nutrients back to the soil.

Spent mushroom substrate are source energy ,According to (Vasilakis et al., 2023) ,the depletion of fossil fuels and the acceleration of climate change brought on by greenhouse gas emissions, research into sustainable alternative forms of bioenergy production is important. The demand for energy is a key problem in modern society. In contrast to conventional fuels, "second generation" biofuels, produced from lignocellulosic feedstocks (like the spent mushroom substrate) or typically organic-rich industrial, agricultural, and municipal wastewaters, offer a viable substitute to produce clean energy, including biogas (biohydrogen, biomethane), bioethanol, lipids as a platform for biodiesel production, and other products.

Animal feeds, Mushrooms are proteinaceous, and the substrate formulations may include cereal straws and various grains that are components of animal diets. Thus, investigations have pursued the recycling of SMS as a feedstock. *Pleurotus* spp. spent substrate has been evaluated extensively as a feed stock cattle/ruminant feed, sugarcane bagasse compost in a dietary blend for ruminants, buffaloes, chicken, elk, goats lambs and sheep and degradation/silage studies.

Some other species spent substrate trials include *Volvariella volvacea* grown on rice straw or banana leaves for sheep, *Coprinus fimetarius* grown on rice and oat straws for goats Flammulina *velutipes* for steer *Ganoderma balabacense* to assist milk production in dairy cattle (Rinker, 2017)

The main raw materials used in mushroom cultivation are rich in cellulose, hemicelluloses.
and lignin, while their protein content is generally low, during solid-state fermentation by mushroom-forming fungi, the substrate polymers are enzymatically degraded, and the digestibility of plant residues is considerably improved. Concomitantly, the growth of mycelial biomass upgrades the substrate by increasing its content in proteins and bioactive compounds, e.g. polysaccharides and ergosterol (Martín et al., 2023)

The spent mushroom substrate can be used in substrate formulation for new cycles of mushroom cultivation provided that suitable lignocellulosic materials are employed, the fungal strain is appropriately selected, and the environmental conditions are optimally regulated. Supplemented cereal straw and wood sawdust are the most common substrates in commercial mushroom cultivation due to their composition, availability, and relatively low cost. (Martín et al., 2023)

Mushroom spent substrate is not considered totally exhausted from the production of mushrooms. Thus, considerable research and practice have attempted to recycle the “spent” material in further mushroom production (Giménez, 2008; Rinker, 2017)

Pest and disease management Uncomposed materials, composts, manures, and compost extracts have developed as alternatives to chemicals in the management of insects and diseases in agricultural and horticultural crops, spent substrate fits naturally into potential applications for pest control. SMSs have principally been investigated in disease management. against Colorado potato beetles and as an organic alternative to methyl bromide in strawberries, *Pleurotus* spent substrate has been screened against various diseases of cucumbers and nematodes (Rinker, 2017)

It is important to note that the best way to recycle the substrate depends on several factors, including the type of substrate used, the type of mushroom grown, and the desired end-product. Growers should experiment with different methods to find the best approach for their specific needs. More research is ongoing on spent mushroom substrates even in other areas outside agriculture like construction, industrials design to mention but a few. Additionally, some spent substrate may not be suitable for reuse or recycling. For example, if the substrate was contaminated with diseases or chemicals, it may need to be disposed of properly to prevent further contamination.

3.0 Materials and Methods

The experiment was to test the rate of growth of the oyster mycelium on two different supplementations (protein and the larva additive supplement) and the rate of growth was monitored seven days under the two treatments supplanted at different concentrations.

3.1 Tools and materials

- PDA (Potato Dextrose Agar) for microbiology manufactured by VWR CHEMICALS
- Petri dishes (90ml, plastic and sterilized were used)
- Measuring cylinder
- Mother culture
- Water
- Agar (Biological Agar) was used.
- Weighing scale
- Glass jars
- Labels
- Autoclave

3.2 Culturing of the mycelium

Culturing of the oyster mushroom mycelium was done in the laboratory for 7days to get fresh mycelium. (Figure 11 below) shows the growing mycelium after 4days This was done by using PDA in a Petri dish, then added spawn of oyster mushroom and placed in the incubator for 7days**.**

Figure 11 shows the Growing mycelium after four days.

3.3 Treatments.

The medium was prepared (table 6) with the supplement of protein and the larva additive treatments of 1%, 5% and 10% as follows. Nine treatment sets were established for the mycelium growth experiment. The test treatments included mealworm larvae wastes (larva additive) and mealworm larvae protein(protein). The treatments with supplements were all based on the widely used PDA (Potato Dextrose Agar) medium, which was likewise utilized in the control set. The material that was the subject of the experiment was combined in concentrations of 1%, 5%, and 10% with the PDA culture media. In addition to this, water agar with 10% supplements (larva additive and protein) in place of PDA in the treatment which served as the positive control while PDA alone served as the negative control. The latter treatment aimed to determine the amount of nutrients that the fungus receives just from the additive and the protein which were the main objectives of the study. The inoculation was done in a laminar hood, under sterile conditions in the mushroom laboratory of the MATE Department of Vegetable and Mushroom Cultivation. A 5mm plug obtained from a fresh fully colonized oyster mushroom culture was inoculated separately on the petri dishes containing the different concentrations of the treatment. This ensured that the same quantity of mycelium was added to each Petri plate using a corkscrew after the media with varying concentrations were prepared. The samples were then kept at room temperature and allowed to colonize the media for seven days. Six replicates were made for each of the treatments and the colony growth was measured daily using an electric Vanier caliper.

Table 6 shows the measurements of different treatments and the media.

Treatment	**PDA**	**Supplement**	**Agar**	**Water**
Larva additive 1%	9.75g	2.5g	-	250ml
Larva additive 5%	9.75g	12.5g	-	250ml
Larva additive 10%	9.75g	25g	-	250ml
LA positive control	-	25g	4g	250ml
Protein1%	9.75g	2.5g	-	250ml
Protein 5%	9.75g	12.5g	-	250ml
Protein10%	9.75g	25g	-	250ml
Protein positive control	-	25g	4g	250ml
Control	20g	-	-	500ml

Figure 12 shows Treatments cooling after sterilization in the autoclave

Figure 13 Mycelium growth on day one

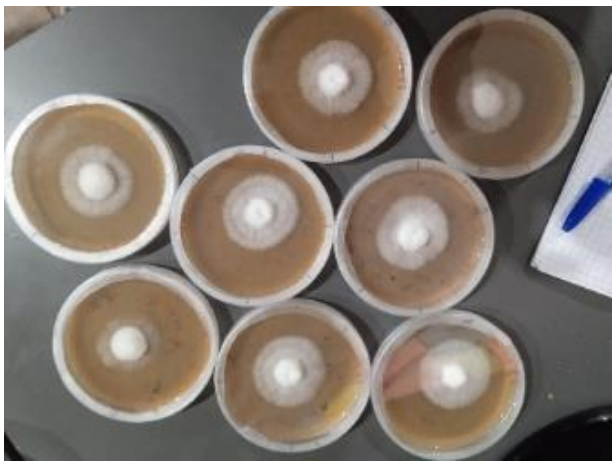


Figure 14 Mycelium growth on day three

Figure 15 Mycelium growth on Day five

Figure 16 mycelium growth on day seven

3.4 Statistical analysis

Data were analysed with statistix 8.0 using factory design with 2 supplements ,9 treatments and 6 replications in each treatment and LSD All-Pairwise Comparisons Test was used to determine the differences between all the experimental groups and was significance among treatments.

4.0 Results and discussion

4.1 Results

The graphs represent the effect of different supplements on growth rate of *P. ostreatus* cultivation.

Day one

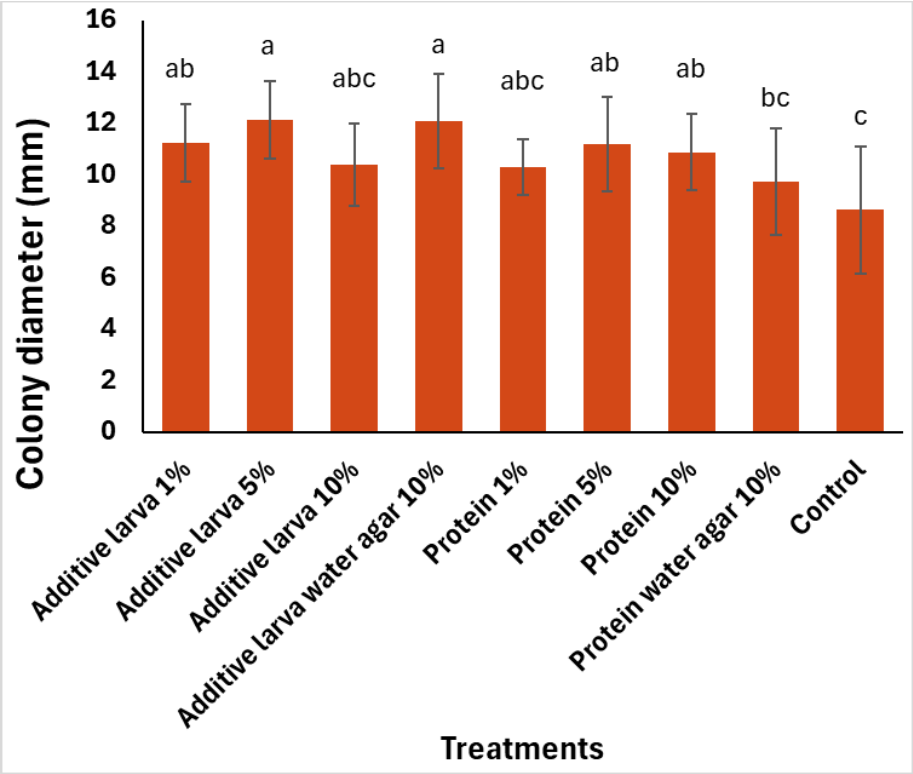


Figure 17 shows the effect supplements have on the growth rate of mycelium on day 1.

From the results above, (figure 17) above the growth of mycelium was higher with larva 5% and larva additive water agar 10% as compared to other treatments where the growth rate was relative with no but rather a low growth rate was observed in the control, All the concentrations were significantly different from the control except for 1o% additive, 1% protein and 10% protein in water agar.

Larva additive

Larva additive treatments (1%,5%and 10%) had ahigh growth rate as compared to the control with a significant difference. larva additive 1%and larva additive 10% performed better than the positive control larva additive with water agar at 10% as compared to larva additive 5% that was growing at a relative rate with the control with no significance.

Protein

There was a high growth rate observed in the protein treatments (1%5%and 10%) as compared to the control, also the treatments grew faster than the positive control (protein water agar at 10%.

Day 2

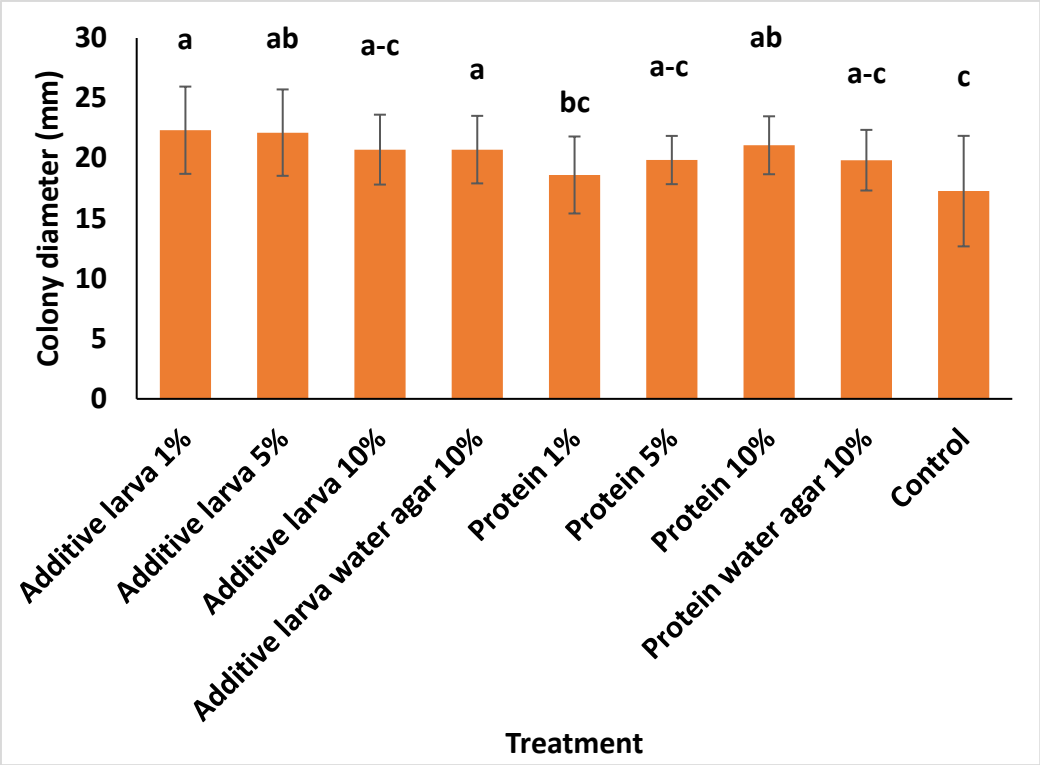


Figure 18 shows the effect of supplements on the growth rate of mycelium on day 2.

From the results in (figure 18) above, the growth of mycelium was relatively growing at the same rate among the treatments with a significant difference this could be mycelium cells were gaining nutrients for development. All concentrations showed similar growth rates as control sets except for 1%, 5% and 10% water agar additive as well as 10% protein.

Larva additive

According to the observed data, the larva additive treatments(1%,5%and10%) had ahigh growth rate as compared to the control, however the larva additive 1%5%and 10% had a lower growth rate as compared to the positive control larva additive with water agar at 10%.

Protein

The protein treatment (1%,5%and 10%) also grew faster than the control, while protein 10% and 5% had ahigh growth rate than the positive control protein with water agar 10%as compared to protein 1% that had slightly lower growth rate.

Day 3

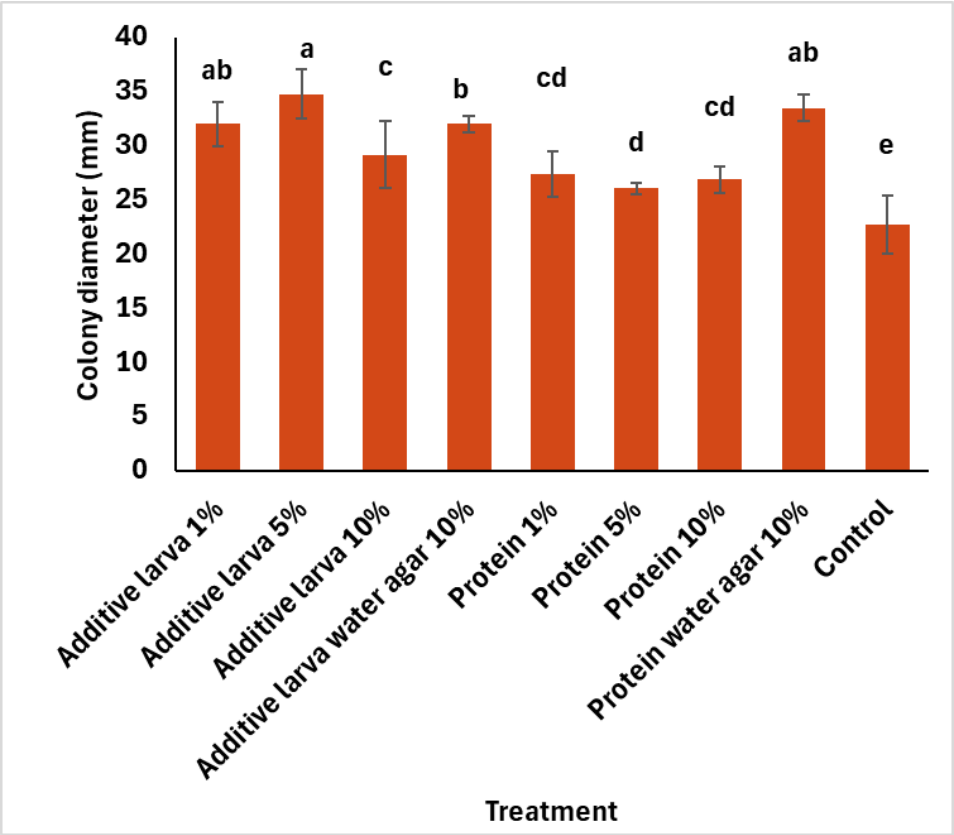


Figure 19 shows the effect supplements on the growth rate of mycelium at day 3.

From the results in (figure 19) above, the growth of mycelium was higher with larva additive 5% and control protein water agar at 10% with a significant differences ,followed by larva additive 1% and control larva additive water agar 10% that have no significant difference as compared to other treatments on the same day with a relative similar growth rate larva additive 10%,protein 1% and protein 10% have no significant differences while protein 5% is significantly different from them. However, the control shows a lower growth rate at a significant difference from all the treatments. All treatments were found to perform noticeably better than the control, and as a result, the two supplements can be used in place of PDA to develop mycelium to produce spawn.

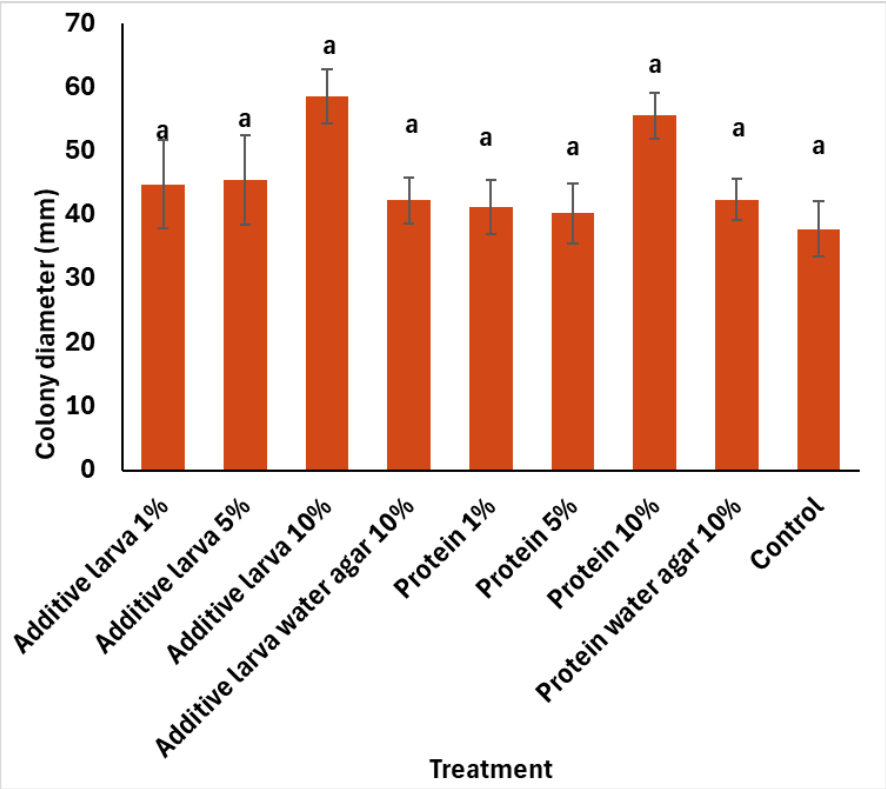
Larva additive

According to the observed data, the larva additive treatments(1%,5%and10%) had ahigh growth rate as compared to the control. larva additive 1%and larva additive 5%perfomed better than the positive control larva additive with water agar at 10% as compared to larva additive 10% that was growing at a relative rate with the control with no significance.

Protein

The protein treatment (1%,5%and 10%) also grew faster than the control, protein with water agar 10% (positive control) had ahigh growth rate as compared to protein 1%, protein 5% and 10% that had slightly lower growth rate.

Day 4



Graph 20 shows the effect supplements on the growth rate of mycelium at day 4.

From the results above, the growth of mycelium was higher with larva and protein supplements at 10% as compared to other treatments on the same day. However, the data shows no statistical significance among treatments, there is a numerical difference between the observed value. The high growth rate observed with larva and protein supplements at 10% could be attributed to cells utilizing nutrients very well for their development.

Larva additive

According to the observed data, the larva additive treatments(1%,5%and10%) had ahigh growth rate as compared to the control. However, larva additive 10% had a higher growth rate than the positive control larva additive with water agar at 10% as compared to larva additive 1% and5%.

Protein

The protein treatment (1%,5%and 10%) also grew faster than the control, protein 10% had ahigh growth rate protein with water agar 10% (positive control) as compared to protein 1%, protein 5% that had lower growth rate.

Day 5

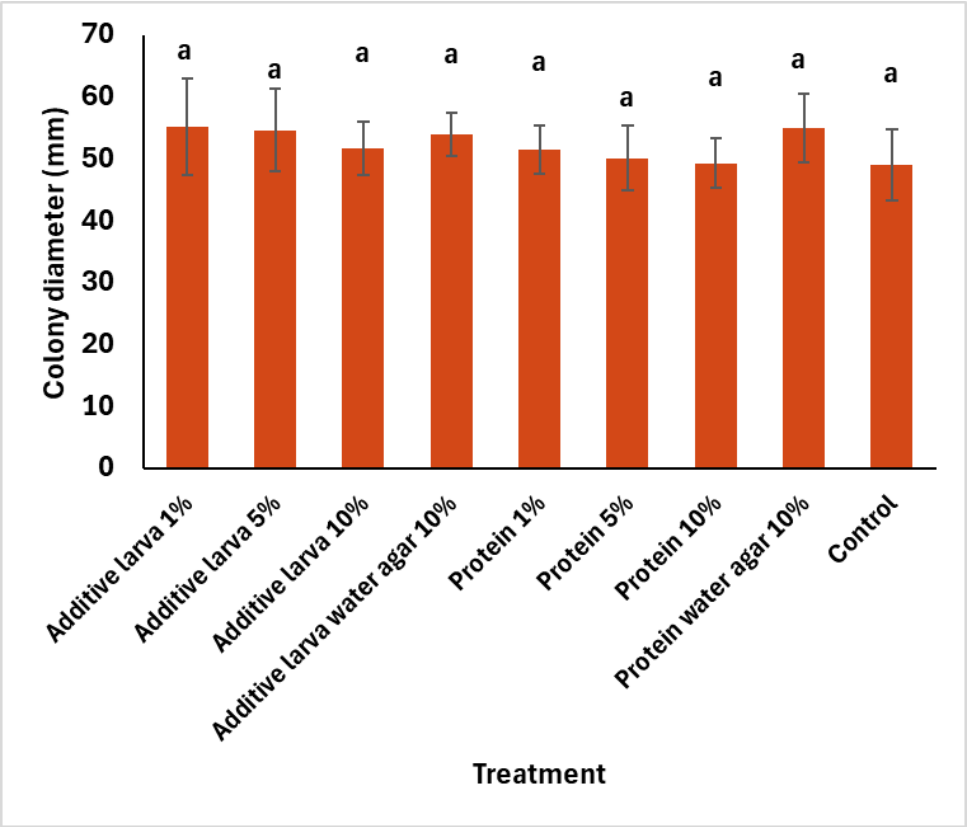


Figure 21 shows the effect supplements on the growth rate of mycelium at day 5.

From the results in figure (21 above), the growth of mycelium was relatively at same level with all the treatments and shows no statistical significance among treatments, there is a slight numerical difference between the observed value. The high growth rate observed with protein water agar control at 10%.

Larva additive

According to the observed data, the larva additive treatments(1%,5%and10%) had ahigh growth rate as compared to the control, larva additive 1% and5% had a high growth rate than the positive control larva additive with water agar at 10% as compared to larva additive 10%.

Protein

The protein treatment (1%,5%and 10%) where growing faster than the control, protein with water agar 10% (positive control) had ahigh growth rate as compared to protein 1%, protein 5% and 10% that had slightly lower growth rate.

Day 6

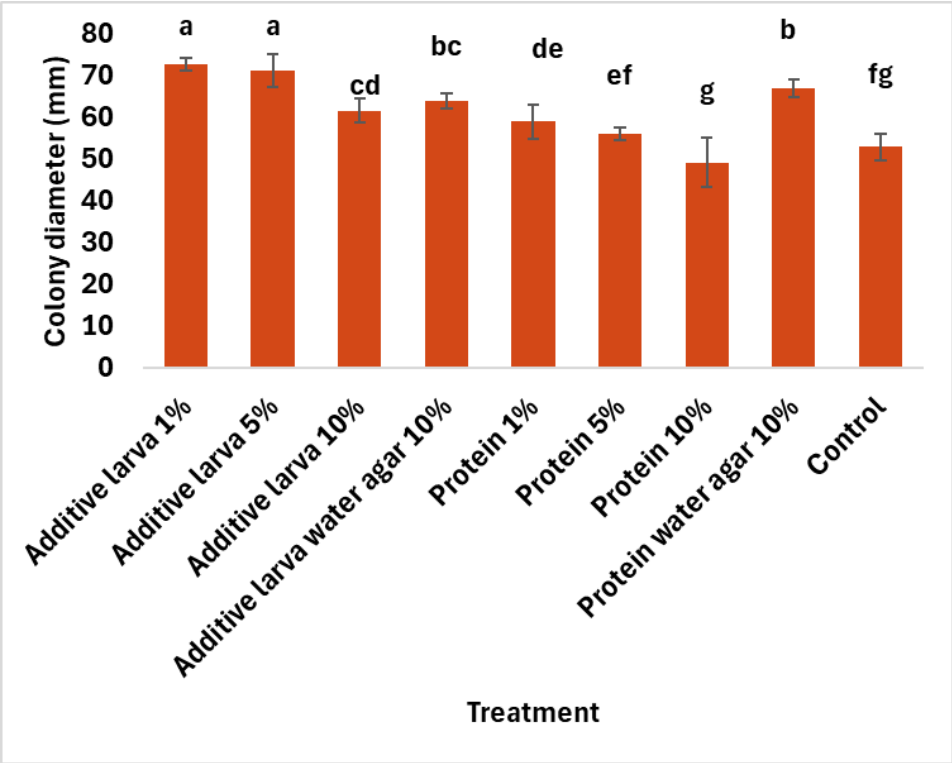


Figure 22 shows the effect of supplements on the growth rate of the mycelium on day 6.

Accordingly, the observed results in (figure 22) show a high growth rate of mycelium at 1% and 5% of larva additive, followed by protein water agar 10% as compared to the control. There is a close relationship between the growth rate of mycelium at larva 10%, larva water agar 10%, protein 1% and 5%, whereas protein 10% showed the lowest growth rate compared to the control experiment.

Larva additive

The according to the observed data, the larva additive treatments(1%,5%and10%) had ahigh growth rate as compared to the control larva additive 1% and5% had a high growth rate than the positive control larva additive with water agar at 10% as compared to larva additive 10%.

Protein

The protein treatment (1%,5%) had a high growth rate faster than the control as compared to protein 10%, protein with water agar 10% (positive control) had ahigh growth rate as compared to protein 1%, protein 5% and 10% that had lower growth rate.

Day 7

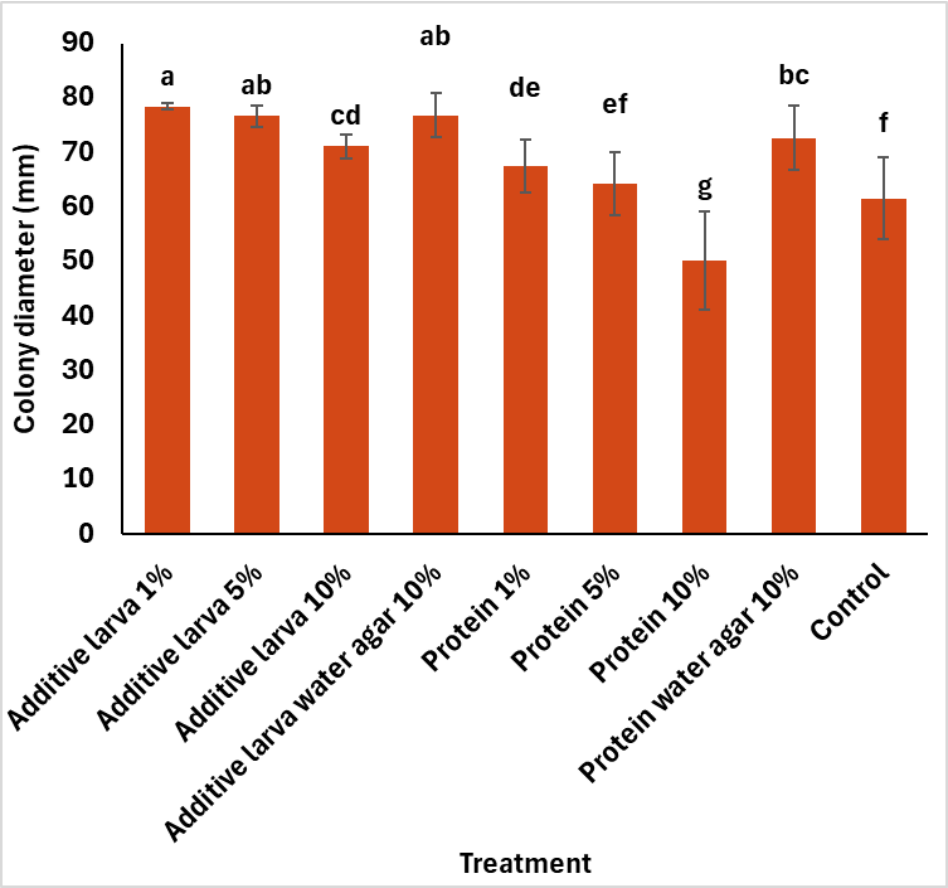


Figure 23 shows the effect of supplements on the growth rate of the mycelium on day 7.

Accordingly, the observed in (figure 23) results show a high growth rate of mycelium at 1% and 5% of larva additive with no significance in the mean value followed by protein water agar 10% and larva water agar 10% as compared to other treatments with a relative growth rate. the control had a high growth rate as compared to protein 10%.

Larva additive

According to the observed data, the larva additive treatments(1%,5%and10%) had ahigh growth rate as compared to the control. larva additive 1% and5% had a higher growth rate than the positive control larva additive with water agar at 10% as compared to larva additive 10%.

Protein

The protein treatment (1%and 5%) had a higher growth rate than the control as to compared to protein 10%, protein with water agar 10% (positive control) had ahigh growth rate as compared to protein 1%, protein 5% and 10% that had slightly lower growth rate.

The general observations of the results showed in figure 24 and 25 below show that the growth rate of mycelium was high in the larva additive supplement treatments as compared to the protein supplement treatments on all the experimental days except on day 4 where the growth rate was relatively high in larva additive 10% and protein 10% as compared to all the treatments and their

controls. The positive control of water agar larva, and protein at 10% high growth rate as control which was PDA. The mycelium was growing at high rate in low concentrations as compared to high concentration.

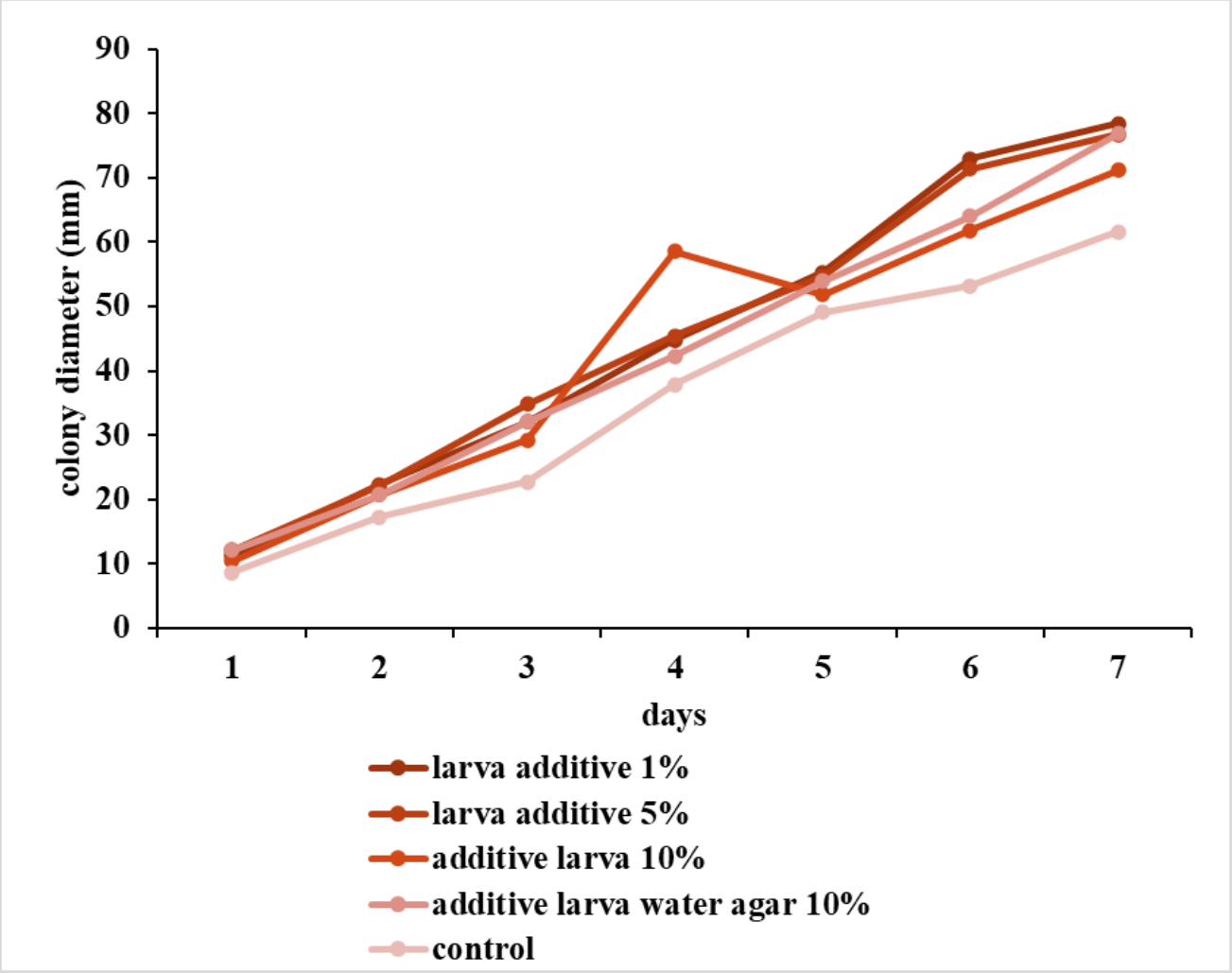


Figure 24 shows growth rate of the mycelium for 7days with larva additive supplementation.

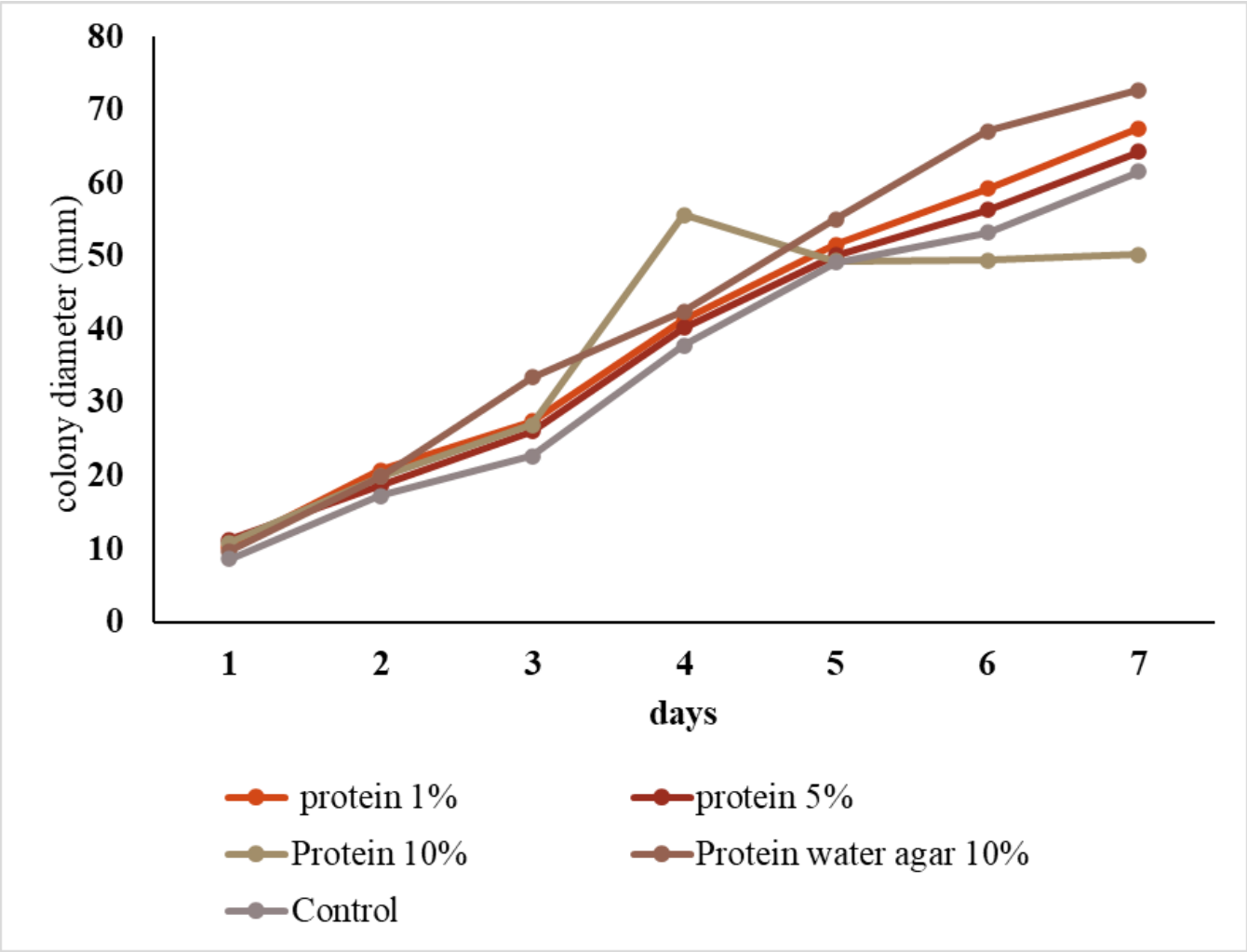


Figure 25 shows growth rate of the mycelium for 7days with protein supplementation

4.2 Discussion

Supplementation is ideal in oyster mushroom cultivation for various reasons based on the objective of the farmer, Supplements improve the nutritional status of most waste substrate which result in better mushroom growth and productivity by providing easily degradable carbohydrates, more protein and nitrogen ((N. A. Khan et al., 2017, Oseni et al., 2012). Research has shown various supplements of oyster mushrooms such as wheat bran, rice bran ,gram flour ,corn meal, fish meal ,peanut meal (Tikdari & Bolandnazar, 2012).in the recent experiment two supplements (larva additive and protein) where tested on the growth rate of mycelium , the results supports the growth of the mycelium which has a high effect on the yield and nutrition value of the fruiting body like any other supplement ,according to (Banik & Nandi, 2004) ,The addition of supplements to mushroom substrate is very important especially for substrates having low protein content to enhance the growth and yield of mushrooms, substrate .(Oseni et al., 2012). Biogas residual slurry

manures are rich in mineral nutrients and are very effective for increasing yield of oyster mushroom and (Nunes et al., 2012) found that Nitrogen supplementation enhanced mushroom BE, especially when organic sources were used , The supplementation of the substrates with various sources of organic nitrogen, such as wheat bran, rice bran, maize wastewater, soya cake powder and rice, has increased the BEs of various species of basidiomycetes. more so in an experiment called out by (Si, 2011)The growth and yield of oyster mushroom, Pleurotus ostreatus recorded significantly earlier and better as the gram powder was increased and that the application of gram powder also reduced the contamination of substrate and increases the number of flushes at possible lower interval of the harvestings.

The level of concentration of the supplement has an effect on the growth rate of the mycelium as well as the fruiting body of oyster mushroom ,according to the finds of (Oseni et al., 2012) ,Sawdust supplemented with 15% wheat bran produced mushrooms with the longest stipe and pileus diameter, respectively and followed by substrate supplemented with 20% wheat bran, while sawdust supplemented with 5% wheat bran produced mushrooms with least stipe length and pileus diameter which is in contradiction with results of the current experiment where high growth rate was observed at low concentrations for both supplements larva additive and protein respectively .

The highest mycelium running time was observed on coconut shell without any supplement in an experiment to find out the growth and yield performance of Pleurotus sajor-caju (Fr.) Singer on coconut shell supplemented with wheat bran and rice bran. (Pal et al., 2017) this result contradicts with the current study the mycelium growth rate was low in the control without any treatment.

The lowest mycelium running time was observed on coconut shell with 50% level of supplement closely preceded by 40% level of supplement. (Pal et al., 2017)this is result agrees with the current study on larva additive supplementation the lowest growth rate is observed at 10%.

5. Conclusion

From the present study it can be concluded that all the supplements (larva additive and protein) support growth of the mycelium in vitro conditions and can be used by mushroom farmers for supplementation. However lower concertation (1% and 5%) supplementation of larva additive and protein resulted into high growth rate of the oyster mushroom mycelium and high concentration (10%) of larva additive and protein supplementation have a lower growth rate of the oyster mycelium. Therefore, concentration dosage of both supplements may depend on the mushroom farmers according to farmers set objectives. Moreso different supplements may show different results with different substrates for more effective and reliable results conducting a vivo experiment to establish the effect of the supplements on the fruiting body, biology efficiency and nutritive value with different substrate should be done.

6. References

Adebayo, E. A., & Oloke, J. K. (2017). Oyster mushroom (pleurotus species); A natural functional food. *Journal of Microbiology, Biotechnology and Food Sciences*, *7*(3), 254–264. https://doi.org/10.15414/jmbfs.2017/18.7.3.254-264

Adi, M. R. M., Izhar, T. N. T., Ibrahim, N. M., Aziz, N. A., Hadiyanto, H., & Matei, M. (2023). The Effect of Chemical Treated Spent Mushroom Substrate (SMS) on Lignocellulosic Content. *IOP Conference Series: Earth and Environmental Science*, *1216*(1). https://doi.org/10.1088/1755-1315/1216/1/012015

Afifa Jahan et al., A. J. et al. . (2019). Mushroom Value Chain and Role of Value Addition. *International Journal of Botany and Research*, *9*(1), 5–14. https://doi.org/10.24247/ijbrjun20192

Afzal, R., Akram, A., Qureshi, R., Akram, Z., & Sultana, K. N. (2019). Efficacy of Grain Spawn and Lime / Gypsum Ratio on Mycelial Growth of Oyster Mushroom. *Juniper PUBLISHERS*, *13*(5), 2474–7637. https://doi.org/10.19080/AIBM.2019.13.555871

Ashraf, J., Ali, M. A., Ahmad, W., Ayyub, C. M., & Shafi, J. (2013). Effect of Different Substrate Supplements on Oyster Mushroom (Pleurotus spp.) Production. *Food Science and Technology*, *1*(3), 44–51. https://doi.org/10.13189/fst.2013.010302

Banik, S., & Nandi, R. (2004). Effect of supplementation of rice straw with biogas residual slurry manure on the yield, protein and mineral contents of oyster mushroom. *Industrial Crops and Products*, *20*(3), 311–319. https://doi.org/10.1016/j.indcrop.2003.11.003

Bao, D., Kinugasa, S., & Kitamoto, Y. (2004). The biological species of oyster mushrooms (Pleurotus spp.) from Asia based on mating compatibility tests. *Journal of Wood Science*, *50*(2), 162–168. https://doi.org/10.1007/s10086-003-0540-z

Barh, A., Annepu, S. K., Sharma, V. P., & Upadhyay, R. (2020a). *Growing oyster mushroom- A technical guide*. *March*.

Barh, A., Annepu, S. K., Sharma, V. P., & Upadhyay, R. (2020b). *Growing oyster mushroom- A technical guide*. *March 2023*.

Deepalakshmi, K., & Mirunalini, S. (2008). Pleurotus. *Westcott's Plant Disease Handbook*, *5*, 516–516. https://doi.org/10.1007/978-1-4020-4585-1_845

Effiong, M. E., Umeokwochi, C. P., Afolabi, I. S., & Chinedu, S. N. (2023). Assessing the nutritional quality of Pleurotus ostreatus (oyster mushroom). *Frontiers in Nutrition*, *10*(January), 1–13. https://doi.org/10.3389/fnut.2023.1279208

Golak-Siwulska, I., Kałuzewicz, A., Spizewski, T., Siwulski, M., & Sobieralski, K. (2018). Bioactive compounds and medicinal properties of Oyster mushrooms (Pleurotus sp.). *Folia Horticulturae*, *30*(2), 191–201. https://doi.org/10.2478/fhort-2018-0012

Gyenge, B., Kozma, T., Almádi, B., Szarvas, J., Villás, G., & Urvölgyi, M. (2016). Technology innovation in sustainable growing and distribution of king oyster mushroom. *Hungarian Agricultural Engineering*, *7410*(29), 5–10. https://doi.org/10.17676/hae.2016.29.5

IQBAL, S. M., RAUF, C. A., & SHEIKH, M. I. (2005). Yield Performance of Oyster Mushroom on Different Substrates. *International Journal of Agriculture & Biology*, *7*(6), 900–903.

Jaitly, S. S. and A. K. (2011). Journal of phytology. *Journal of Phytology*, *3*(8), 18–20. https://scienceflora.org/journals/index.php/jp/article/view/2302/2280

Jeznabadi, E. K., Jafarpour, M., Eghbalsaied, S., & Pessarakli, M. (2017). Effects of Various Substrates and Supplements on King Oyster (Pleurotus eryngii). *Compost Science and Utilization*, *25*(January 2018), S1–S10. https://doi.org/10.1080/1065657X.2016.1238787

Jongman, M., Khare, K. B., & Loeto, D. (2018). Oyster mushroom cultivation at different production systems : A review European of Biomedical AND Pharmaceutical sciences. *EUropean Journal Of Biomedical and Pharmaceutical Sciences*, *5*(5), 72–79.

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, *113*(1), 9–16. https://doi.org/10.1016/j.foodchem.2008.07.077

Katel, S., Mandal, H. R., & Sharma, R. (2022). Chapter-3 Oyster Mushroom Cultivation. *Chief Editor Dr. RK Naresh*, *August*. https://www.researchgate.net/profile/Rk-Naresh-2/publication/362684642_Research_Trends_in_Agriculture_Vol_30/links/62f86e96b8dc8b4403df7b23/Research-Trends-in-Agriculture-Vol-30.pdf#page=43

Khan, M. A., & Tania, M. (2012). Nutritional and Medicinal Importance of Pleurotus Mushrooms: An Overview. *Food Reviews International*, *28*(3), 313–329. https://doi.org/10.1080/87559129.2011.637267

Khan, N. A., Amjad, A., Binyamin, R., Rehman, A., & Hafeez, O. B. A. (2017). Role of various supplementary materials with cotton waste substrate for production of Pleurotus ostreatus an oyster mushroom. *Pakistan Journal of Botany*, *49*(5), 1911–1915.

Kumar Suresh. (2021). 62. Fundamental Cultivation Technology Of Oyster Mushroom. *Just Agriculture Multidisciplinary E-Newsletter*, *2*(3).

Lucas de Jesus, G., José Lavoranti, O., Schafer, G., Dias de Oliveira, G., Scheffer de Andrade Silva, R., & Lorena Cuquel, F. (2023). Nutrient uptake in supplemented substrate by oyster mushroom. *World Journal of Microbiology and Biotechnology*, *39*(7), 1–18.

https://doi.org/10.1007/s11274-023-03622-3

Martín, C., Zervakis, G. I., Xiong, S., Koutrotsios, G., & Strætkvern, K. O. (2023). Spent substrate from mushroom cultivation: exploitation potential toward various applications and value-added products. *Bioengineered*, *14*(1), 2252138. https://doi.org/10.1080/21655979.2023.2252138

Mattila, P., Könkö, K., Eurola, M., Pihlava, J. M., Astola, J., Vahteristo, L., Hietaniemi, V., Kumpulainen, J., Valtonen, M., & Piironen, V. (2001). Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *Journal of Agricultural and Food Chemistry*, *49*(5), 2343–2348. https://doi.org/10.1021/jf001525d

Maurya, B., Verma, D., Didwana, V., & Maurya, B. (2020). Spent mushroom substrate: a potential sustainable substrate for agriculture. *International Journal of Grid and Distributed Computing*, *13*(2), 104–109. https://www.researchgate.net/publication/347489805

Mca, G., Dauner, L., Madawala, S., & Sc, K. (2021). Nutritional and medicinal benefits of Oyster (Pleurotus) mushrooms: a review. *Fungal Biotec*, *1*(2), 65–87. https://doi.org/10.5943/funbiotec/1/2/5

Naraian, R. (2017). Nutritional Value of Three Different Oyster Mushrooms Grown on Cattail Weed Substrate. *Archives of Biotechnology and Biomedicine*, *1*(1), 061–066. https://doi.org/10.29328/journal.hjb.1001006

Naraian, R., Singh, M. P., & Ram, S. (2016). Supplementation of Basal Substrate to Boost up Substrate Strength and Oyster Mushroom Yield: An overview of Substrates and Supplements. *International Journal of Current Microbiology and Applied Sciences*, *5*(5), 543–553. https://doi.org/10.20546/ijcmas.2016.505.056

Nunes, M. D., Da Luz, J. M. R., Albino Paes, S., Oliveira Ribeiro, J. J., Soares da Silva, M. de C., & Megumi Kasuya, M. C. (2012). Nitrogen Supplementation on the Productivity and the Chemical Composition of Oyster Mushroom. *Journal of Food Research*, *1*(2), 1–8. https://doi.org/10.5539/jfr.v1n2p113

Oseni, T., Dube, S., Wahome, P., Masarirambi, M., & Earnshaw, D. (2012). Effect of Wheat Bran Supplement on Growth and Yield of Oyster Mushroom (Pleurotus Ostreatus) on Fermented Pine Sawdust Substrate. *Experimental Agriculture & Horticulture*, 30–40. http://www.bapress.ca/eah/v1n2/Tajudeen O. Oseni.pdf

Pal, J., Sharma, R., Lal, M., & Suman, B. C. (2017). Effect of different spawn rates and substrate supplementation on yield of Indian Oyster mushroom, Pleurotus pulmonarius (Fr.) Quel. *Journal of Applied and Natural Science*, *9*(3), 1406–1410. https://doi.org/10.31018/jans.v9i3.1375

Patel, Y., Naraian, R., & Singh, V. K. (2012). *Medicinal Properties of Pleurotus Species (Oyster Mushroom): A Review*. *3*(1), 1–12. https://doi.org/10.5829/idosi.wjfpb.2012.3.1.303

Piska, K., Sułkowska-Ziaja, K., & Muszyńska, B. (2017). Edible mushroom pleurotus ostreatus (Oyster mushroom) – Its dietary significance and biological activity. *Acta Scientiarum Polonorum, Hortorum Cultus*, *16*(1), 151–161.

Rahman, M. Z., & Hafiz, M. H. (2009). Cultivation of Oyster Mushroom (Pleurotus flabellatus) on Different Substrates. *Int. J. Sustain. Crop Prod*, *4*(1), 45–48. https://www.researchgate.net/publication/242421716

Rahman, T., Akoijam, B., Singh, R., Paul, P., Talang, H., Kumar, B., & Hazarika, S. (2019). Spawn production and mushroom cultivation technology. In *ResearchGate* (Issue May). https://www.researchgate.net/profile/Samarendra-Hazarika/publication/342082516_Spawan_Production_Mushroompdf/data/5ee11b24299bf1b17a8b66ed/Spawan-Production-Mushroom.pdf

Rai, R. D., & Arumuganathan, T. (2008). *POST HARVEST TECHNOLOGY OF MUSHROOMS National Research Centre for Mushroom*. *213*, 84.

Rinker, D. L. (2017). Spent mushroom substrate uses. *Edible and Medicinal Mushrooms: Technology and Applications*, *August*, 427–454. https://doi.org/10.1002/9781119149446.ch20

Saima, N. (2021). *Medicinal Properties Of Oyster Mushroo*. *2*(1). file:///C:/Users/NIKKI/Downloads/74. Medicinal Properties Of Oyster Mushroom.pdf

Salama, A., Abdou, A., Helaly, A., & Salem, E. (2019). Effect of different nutritional supplements on the productivity and quality of oyster mushroom (Pleurotus ostreatus). *Al-Azhar Journal of Agricultural Research*, *44*(2), 12–23. https://doi.org/10.21608/ajar.2019.101167

Sánchez, C. (2004). Modern aspects of mushroom culture technology. *Applied Microbiology and Biotechnology*, *64*(6), 756–762. https://doi.org/10.1007/s00253-004-1569-7

Satankar, V., Sardar, A., Jagajanantha, P., & Mageshwaran, V. (2020). Effect of substrates (cotton stalks and cotton seed hulls) on growth, yield and nutritional composition of two oyster mushrooms (Pleurotus ostreatus and Pleurotus florida). *Central Institute for Research on Cotton Technology*, *34*(1), 135–145. https://www.researchgate.net/publication/338421192

Sayner, A., & Jong, E. (2018). How To Grow Oyster Mushrooms : The Ultimate Step By Step Guide. *GroCycle*, *September*, 1–22. https://grocycle.com/how-to-grow-oyster-mushrooms/

Si. (2011). *No 主観的健康感を中心とした在宅高齢者における 健康関連指標に関する共分散構造分析Title*. *16*(22), 35–41.

Singh, M., Kamal, S., & Sharma, V. (2021). Status and trends in world mushroom production-III-World Production of Different Mushroom Species in 21st Century. *Mushroom Research*, *29*(2), 75. https://doi.org/10.36036/mr.29.2.2020.113703

Sullivan. (n.d.). *0Ÿ 1234*. *57*, 3–4.

Tikdari, M. M., & Bolandnazar, S. (2012). *Application of organic nitrogen supplementations increases the yield of oyster mushroom (Pleurotus florida)*. *2*(3), 10–15.

Tolera, K. D., & Abera, S. (2017). Nutritional quality of Oyster Mushroom (Pleurotus Ostreatus) as affected by osmotic pretreatments and drying methods. *Food Science and Nutrition*, *5*(5), 989–996. https://doi.org/10.1002/fsn3.484

Uide, H. O. P. R. G., Beetz, B. A., & Greer, L. (1999). M Ushroom C Ultivation and. *Agriculture*, 1–14.

Vasilakis, G., Rigos, E. M., Giannakis, N., Diamantopoulou, P., & Papanikolaou, S. (2023). Spent Mushroom Substrate Hydrolysis and Utilization as Potential Alternative Feedstock for Anaerobic Co-Digestion. *Microorganisms*, *11*(2), 1–17. https://doi.org/10.3390/microorganisms11020532

Wani, B. A., Bodha, R. H., & Wani, A. H. (2010). Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plants Research*, *4*(24), 2598–2604. https://doi.org/10.5897/jmpr09.565

Zhang, R., Li, X., & Fadel, J. G. (2002). Oyster mushroom cultivation with rice and wheat straw. *Bioresource Technology*, *82*(3), 277–284. https://doi.org/10.1016/S0960-8524(01)00188-2

Zhou, S., Ma, F., Zhang, X., & Zhang, J. (2016). Carbohydrate changes during growth and fruiting in Pleurotus ostreatus. *Fungal Biology*, *120*(6–7), 852–861. https://doi.org/10.1016/j.funbio.2016.03.007

7. Acknowledgement

I am grateful to my thesis supervisor András Geösel, Ph.D. and my co-supervisor, who gave me valuable guidance and feedback since the beginning of my thesis journey.

Am grateful for the support of GINOP_PLUSZ-2.1.1-21-2022-00183 program „Research and development of recycling mushroom cultivation waste using insect larvae" for making the experiment a success.

Also, I would like to thank my family and friends who have always been there with my side.

Without them, I could never have completed this task.

8. Attachments

DECLARATION

on authenticity and public assess of mater's thesis

Student's name: Naijuka Edwiger Ampurire

Student's Neptun ID: BPR1QB

Title of the document: The Effects of larva's protein and frass supplementation on oyster mushroom cultivation.

Year of publication: 2024

Department: Vegetable and Mushroom department.

I declare that the submitted master's thesis is my own, original individual creation. Any parts taken from an another author's work are clearly marked, and listed in the table of contents.

If the statements above are not true, I acknowledge that the Final examination board excludes me from participation in the final exam, and I am only allowed to take final exam if I submit another master's thesis.

Viewing and printing my submitted work in a PDF format is permitted. However, the modification of my submitted work shall not be permitted.

I acknowledge that the rules on Intellectual Property Management of Hungarian University of Agriculture and Life Sciences shall apply to my work as an intellectulal property.

I acknowledge that the electric version of my work is uploaded to the repository sytem of the Hungarian University of Agriculture and Life Sciences.

Place and date: Budapest , 2024 year 04 month 22 day

Student's signature

STATEMENT ON CONSULTATION PRACTICES

As a supervisor of Naijuka Edwiger Ampurire (Student's name) BPR1QB (Student's NEPTUN ID), I here declare that the master's thesis has been reviewed by me, the student was informed about the requirements of literary sources management and its legal and ethical rules.

I recommend the master's thesis to be defended in a final exam. YES

The document contains state secrets or professional secrets: yes (no*[1])

Place and date: Budapest 2024 year 04 month 22 day

Internal supervisor
Dr. Andras Gosel PhD
associate professor
Department of Vegetable and Mushroom Growing

[1] Please underline applicable.