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BUDAPEST

OPTIMIZING *IN VITRO* MYCELIAL GROWTH OF WOOD EAR MUSHROOM *(Auricularia auricula)*

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Used Abbreviation

PDA: Potato Dextrose Agar

PDYEA: Potato Dextrose Yeast Extract Agar

MYEA: Malt Yeast Extract Agar

MEA: Malt Extract Agar

YEA: Yeast Extract Agar

1. Introduction

Fungi have long been reported as a source of natural medicine and mushrooms used for medicinal purposes feature prominently in Traditional Asian Medicine (Hyde, et al., 2019; Hapuarachchi et al, 2018). Berbee et al, 2017; Lucking et al, 2021; Blackwell et al, 2011, reported that there are an estimated 1.5- 5.1 million species of fungi that are believed to exist in various ecosystems of the Earth, of which 150,000 species of fungi have been described. Fungi are an important and diverse component of biodiversity in various ecosystems. These organisms consist of a diverse range of major fungal groups and play the role of both flora and fauna (Gautam et al, 2022). Some fungi may cause numerous human, animal, and plant diseases, others may play an important role in the nutrient cycle.

Mushrooms are fungi. They belong to a kingdom of their own which is separate from plants and animals. Generally, plants make their food through photosynthesis, while animals eat and then internally digest, their food. Fungi do neither: their mycelium grows into or around the food source, secretes enzymes that digest the food externally, and the mycelium then absorbs the digested nutrients. Mushrooms are the reproductive structure of some fungi. It is said that mushrooms are like the fruit of the plant, except that instead of producing seeds, it produce millions of microscopic spores that form in the gills of spores underneath the mushroom's cap. The spores are blown away into the wind and if they land on a suitable substrate, spores will germinate to form a network of microscopic mycelium which penetrate into their new food source.

Currently, there are over 10,000 known types of mushrooms which may seem like a large number, but mycologists suspect that this is only a fraction of what's out there. There are about 3000 main edible fungi species, of which approximately 200 species are consumed by humans (Kalač, 2012). Mushrooms have always been appreciated by Europeans for their gastronomic value. Climatic conditions and flora diversity of northeast of Portugal made the region with higher wild edible mushroom diversity in the whole European region (Barros et al, 2006). Japanese vendors sell medicinal mushrooms on streets which are regularly used in diet to health maintenance and promote longevity (Smith and Sullivan, 2004)

Edible mushrooms contain a high amount of proteins and are an excellent source of fibers, vitamins, and minerals (Cheung, 1996; Mantilla, et al, 2002; Barros et al, 2007). The fruiting bodies of mushrooms generally contain about 56.8% carbohydrate, 25% protein, 5.7% fat, and 12.5% ash

on a dry weight basis(Ouzouni et al, 2009). Edible mushrooms also contain various polyphenolic compounds recognized as excellent antioxidants due to their ability to scavenge free radicals by single-electron transfer (Ribeiro, et al., 2006; Lee, et al., 2007; Kim et al., 2008). Mushroom extracts may modulate the response of the host immune system; in particular, various polysaccharides of mushrooms are likely to affect the progression and promotion stages of cancer and other mushroom substances may be able to interfere with tumor initiation through different mechanisms (Chatterjee et al., 2011).

Mushroom growth is highly influenced by several factors such as spawn, growing media, pH, temperature, moisture content, and light intensity (Kadiri and Kehinde, 1999). The key operation and first critical stage of successful mushroom cultivation can be done by maintaining and producing reliable pure culture mycelia, which can lead us to prepare to spawn with the required potential. Storage and maintenance of mushroom species in a pure, viable, and stable condition is essential for their use as a reference strain, both in the research and industrial scales (Bhatt et al, 2010).

2. Objectives

The aim objectives of the study were as follows:

A. The study successfully determined which among the various culture media was appropriate for propagating wood ear mushrooms *(Auricularia auricula)* under in vitro conditions.
B. The study was able to distinguish the impact of light on the growth parameters of wood ear mushrooms *(Auricularia auricula)* when culture media
C. The study determined the optimal pH for promoting the mycelial growth of wood ear mushrooms *(Auricularia auricula)* using different culture media
D. The study was able to discern the influence of varying levels of Carbon Sources on the mycelial growth of wood ear mushrooms *(Auricularia auricula)* when cultivated in different culture media.
E. The study successfully compared the impact of different culture media, sucrose, peptone, and their combinations on the mycelial growth of wood ear mushroom *(Auricularia auricula).*

3. Literature Review

Auricularia mushrooms, sometimes known as jelly fungi, are members of the *Auriculariaceae* family and are represented by *Auricularia mesenterica*. According to Lowy in 1952, they are commonly found in tropical, subtropical, and temperate climates. According to Chang and Wasser in 2017, the deliberate production of *Auricularia* mushrooms is well-documented in historical records, with a significant emphasis on their usage as a food source and a therapeutic component. Although *Auricularia auricula-judae* and *A. polytricha* are frequently grown for sale, Wu and colleagues in 2014 noted that the majority of Auricularia species are edible.

On the trunks of several broadleaf trees or on dead wood, the facultative parasite *Auricularia auricula* has been seen to proliferate. The fruiting body of this kind of mushroom has a gelatinous, stretchy, and rubbery texture. According to Hobbs in 1995, it is a key ingredient in Chinese cuisine and is widespread throughout North America, Europe, and much of Asia. As observed by Ying and colleagues in 1987, it is also significant to understand its role in traditional Chinese medicine, where it is regularly prescribed for ailments including hemorrhoids and excessive uterine bleeding due to its qualities that promote pain alleviation and coagulation.

Chinese people first started picking these mushrooms from the wild around 300–600 BC, and they later learned how to grow wood ears (as mentioned by Cheng et al., 1978, and Chang, 1993). Regis et al. (2023) and Chang (1977) cited the writings from a Ming dynasty book called “Classical Chinese Materia Medica,” which stated that *Auricularia auricula-judae* was cultivated on wood logs as early as the 7th century. This is one of the earliest records of mushrooms being grown artificially. Early Hawaiians were also aware of wood ears, and by the late 1800s, they were being sent to San Francisco, as Schenck and Dudley reported in 1999. Furthermore, Stamets claimed in 2003 that they were exported from New Zealand.

According to Schenck and Dudley in 1999, drying these mushrooms results in a considerable reduction in size, allowing them to be sent in their dehydrated state and then rehydrated for use. Notably, as reported by Sekara et al. in 2015, the commercial production of A. auricula-judae has undergone a remarkable development in rural China during the previous three decades.

Though Wu et al. first used the scientific name *Auricularia heimuer* in 2014, it is quite possible that the mushroom in question is the same one mentioned in the 2000-year-old Chinese medical text "Shennong's Compendium of Materia Medica," which is a book on traditional Chinese

medicine. According to Zhang and Chen's 2015 research, the Chinese have been regularly consuming this fungus for over 1400 years for both culinary and therapeutic uses.

According to Hu & Chen in 1991, the polysaccharides discovered in *Auricularia auricula* have been employed in medicine as immune stimulants, anticoagulants, and cholesterol reducers. It's crucial to note that these extracts have been shown to impede egg implantation in animals during the early and middle stages of pregnancy. Hu & Chen advised against these extracts being ingested by pregnant, nursing, or trying to conceive women.

Recent scientific studies have revealed that *A. heimuer* has anticancer and antioxidant effects. Several researchers, including Dai et al. in 2009, Huang et al. in 2010, Reza et al. in 2012, and Zeng et al. in 2012, revealed this important information. *A. heimuer*, a cultivated mushroom, also has a significant economic impact on China. According to Zhang and Chen (2005); and Wu et al., 2015), in 2013 its production volume reached an astonishing 4.75 billion kg (fresh weight) and was valued at over 4 billion USD.

A similar species called *Auricularia auricula-judae* has been discovered to generate the dye-decolorizing peroxidases AauDyP1 and 2, which may find use in industry. In 2010, Liers et al. showed that AauDyP may efficiently act on several nitrophenol molecules, converting them into polymers, quinones, and dinitrophenols. The Shaanxi Institute of Microbiology in China provided the A. auricula-judae strain used to study these enzymes, especially DSM 11326 (strain code: SXM9-C021). However, the exact identity of this strain is still unknown because no DNA sequence information is available for it.

Wu et al., 2015 cited *Auricularia auricula-judae* (Bull) Quél. (=*Auricularia auricula* (L.) Underw.), was first identified in Europe, is widespread in Central Europe, and has been reported to occur throughout the Northern Hemisphere (Kobayasi, 1942; Lowy, 1951; Montoya- Alvarez et al, 2011) When it was discovered in China for the first time in 1881, (Kalchbrenner and Thümen 1881) the term "Heimuer" was frequently used to describe it in Chinese newspapers (Teng 1939; Tai, 1979; Mao, 1998; Dai and Yang, 2008). However, Wu et al. (2014) cited the important discovery that the Chinese "Heimuer" was unique from the European A. auricula-judae in 2014 led them to name it a new species, A. heimuer.

The taxonomic situation was comparable in North America (Looney et al. 2013). *A. americana* Parmasto & I. Parmasto was actually the previously reported A. auricula-judae in the Southeast United States. In 2014, Malysheva and Bulakh (2014) concluded that *A. auricula-judae* is present

in the European half of Russia after looking at numerous reports of the parasite in various regions of the country. In the meantime, *A. americana* and *A. villosula* V. Malysheva were named for two species that resembled one other and were discovered in the Russian Far East. As a result, it seems that A. auricula-judae is a species complex and that the real A. auricula-judae is probably restricted to Europe.

3.1. Ecosystem, Environmental Factors, and Geographical Distribution

Usually flourishing on the wood of deciduous trees and shrubs, auricularia mushrooms, also known as wood ear fungi, exhibit a strong preference for elder trees. Interestingly, despite the widespread assumption that they only grow on elder trees, these mushrooms are discovered on elder trees about 90% of the time. According to recent studies, A. auricula has also been found in moist shola forests that are semi-evergreen to evergreen and part of the Indian Western Ghats. On decomposing tree limbs, the main tree trunk, and decaying logs, among other similar substrates, one can find these mushrooms growing singly or in groups.

As observed and reported by Harding and Patrick in 2008, they form huge clusters with overlapping caps during the monsoon season, and when subjected to high humidity, they develop particularly massive basidiomes. Although they frequently grow alone, they can also be found in tufts or groupings. Both Ingold in 1985 and Du et al. in 2011 emphasized the fact that their habitat is found in both temperate and subtropical regions of the world. According to reports from Vidyaresmi in 2008, Geetha in 2011, and Mohanan in 2011, researchers have gathered Auricularia species from the Thiruvananthapuram district in Kerala, India.

Geographically, the wild Auricularia mushroom is found in a variety of temperate and subtropical climates (Figure 1 and Table 1, as reported by Kobayasi, 1981 and Wu et al., 2021) throughout Asia, Europe, North America, and other continents. The techniques for acquiring these mushrooms have changed over time, moving from foraging in the wild to adopting techniques for artificial production. The realization of their expanding nutritional and therapeutic value has led to this change.

China (Li W. F et al., 2021; Xu et al., 2021) is currently the fourth-largest producer and exporter of black fungus in the world, with northern China serving as the main growing location for *A. heimuer*. According to Li W. F. et al. in 2021 and Xu et al. in 2021, China produced an astonishing 7,064,300 tons of *A. heimuer* in 2020, with more than 40% of this output being concentrated in northern China. The two separate ways of wood cultivation and replacement cultivation are

primarily used to grow *A. heimuer*. According to Zhao et al. in 2021, replacement cultivation is the preferred option because it is more affordable. However, unstoppable seed production and their cultivation process incorporation have caused confusion regarding nomenclature and a reduction in the quality of the mushroom despite the abundance of wild resources for *A. heimuer* in northern China and the appropriate growth climate. The genetic linkages of this species are complex as an outcome of natural selection paired with intensive artificial domestication, and successful breeding techniques depend on a thorough understanding of these interactions.

Molecular markers like RAPD and SSR are currently often used to distinguish between different fungal species. However, as highlighted by Li J. J. et al. (2021); Yin et al. (2022), and Sun et al., 2022, researchers generally concur that the stability of RAPD markers is poor and frequently necessitates numerous repeated tests to generate more reliable results. The wood ear or black fungus mushrooms, also known as auricularia mushrooms, play important ecological roles in a number of environments. They are essential decomposers and are classified as saprophytic fungi. These mushrooms are particularly adept in dissolving the lignin and cellulose present in wood, as well as other complex organic substances. The recycling of nutrients throughout forest ecosystems depends on this breakdown process. It returns important elements to the soil, allowing other species to utilize them. Key participants in the cycling of nutrients are auricularia mushrooms, which transform organic molecules into forms that are easily absorbed by plants and microbes. The productivity and health of forest ecosystems depend on this mechanism.

Additionally, many Auricularia species create mycorrhizal connections with the roots of particular tree species, such as oaks and hardwoods, to benefit both the tree and the plant. In these relationships, the mushrooms help the trees absorb nutrients, especially phosphorus and water, while the trees provide the mushrooms with sugars and carbohydrates. The development and general health of the tree are improved by this symbiotic relationship. In addition, minute animals like insects and arachnids use the malleable, ear-like structure of Auricularia mushrooms as a home and a place to lay their eggs. By establishing special microenvironments within forest ecosystems, these mushrooms promote biodiversity (Xin et al, 2022).

Table 1. Global Distribution of Auricularia (Sun et al., 2022)

Complex name	Species name	Distribution regions (country)
Auricularia cornea	*A. camposii*	Brazil
	A. cornea	China, The Republic of Benin, Brazil, Germany, Ghana, South Africa, Singapore, Sri Lanka, Vietnam
	A. eburnean	China
	A. eminii	Democratic Republic of the Congo, South Africa
	A. nigricans	Costa Rica, Mexici, USA
	A. novozealandica	New Zealand
Auricularia delicata	*A. Australiana*	Australia
	A. Conferta	Australia
	A. delicata	Cameroon, Papua New Guinea
	A. lateralis	Chins
	A. pilosa	Australia, Ethiopia, Tanzania, Zambia
	A. tremellosa	Brazil, Peru, Mexico
	A. sinodelicata	China
	A. scissa	Dominican Republic
	A. subglabra	Brazil, Costa Rica, France, Guiana
Auricularia fuscosuccinea	*A. fibrillifera*	China, Papua New Guinea, Zambia
	A. fuscosuccinea	Brazil, USA
	A. papyracea	Japan
	A. thailandica	China, Thailand
	A. xishaensis	China
Auricularia auricularia-judae	*A. americana*	Canada, USA, Mexico
	A. angiospermarum	USA
	A. auricularia- judae	Czech Republic, France, Germany, UK
	A. hainanensis	China, Japam, New Guinea
	A. heimuer	China, Japan, Russia
	A. minor	China
	A. minutissima	China
	A. tibetica	China
	A. villosula	China, Russia, Thailand
Auricularia mesenterica	*A. africana*	Kenya, Uganda
	A. asiatica	Thailand
	A. brasiliana	Brazil
	A. mesenterica	China, Czech Republic, Estonia, France, Italy, Russia, Switzerland, UK, Uzbekistan
	A. orientalis	China
	A. pusio	Australia
	A. srilankensis	Sri Lanka
	A. submesenterica	China

Figure 1. Global Distribution of Auricularia (Xin Sun et al., 2022)

A variety of animals and insects, including birds, herbivorous mammals, and even invertebrates, eat auricularia mushrooms. They sustain the complex food chain by enhancing the dietary diversity of these creatures. Auricularia species can act as markers of the health and state of a forest ecosystem by being present or absent. Changes in the abundance and distribution of these mushrooms can shed light on the dynamics of the ecosystem as a whole, as well as on how quickly things decompose and how good the soil is. As decomposers, nutrient recyclers, symbiotic partners for trees, givers of shelter and nutrition for wildlife, and important markers of ecosystem health, Auricularia mushrooms play critical ecological roles. They play a crucial role in maintaining the efficiency and stability of forest ecosystems.

3.2. Fruiting body

The fruiting body of *Auricularia auricula* has some distinctive qualities that set it apart from other species. It typically has a diameter of 3 to 8 centimeters, but on rare occasions, it can reach a diameter of 12 centimeters. As noted by Onyango et al. in 2011, it has a pretty unusual shape that can resemble a floppy ear but can also resemble a cup. In most cases, it adheres laterally to its

substrate, occasionally through a brief stalk. This species is tough, gelatinous, and elastic when it's fresh, but as it dries, it turns brittle and rigid. According to Mohanan's description from 2011 its outer surface exhibits a vivid reddish-tan hue with traces of purple and is frequently covered in fine, grayish downy hairs. Particularly in younger specimens, this surface might either be smooth or show undulations, folds, and creases. The hue tends to get darker as it gets older. According to Sterry et al. in 2009, the inner surface is lighter in color, usually a gray-brown tint, and can be smooth or show creases, folds, and even "veins," highlighting its ear-like appearance.

The elongated and allantoid bisidiospores of Auricularia measure between 16 and 18 micrometers in length and 6 to 8 micrometers in breadth (Duncan et al, 1967; Kobayasi, 1981; Parmasto et al, 1987; Montoya-Alvarez et al, 2011). According to Young et al.'s description of these spores from 2005, they are often white, cream, or yellowish and have a transparent character. According to Mohanan's 2011 observations, basidia, the spore-producing structures, are roughly 60-72x47.5 micrometers in size, with well-developed lateral sterigmata measuring 34.5 micrometers in length. These spores, which have dimensions of 14–68 micrometers, have smooth surfaces, a hyaline (translucent) appearance, and a variety of forms, including reniform (kidney-shaped) and allantoid (sausage-shaped). In addition, as described by Philips et al. in 1981, they might also include tiny droplets (guttulate).

3.3. Chemical Composition and Nutritional Profile of Auricularia

Recent studies have revealed the enormous nutritional value of mushrooms, particularly those produced on plant remains. These consumable fungi are rich in protein, all the essential amino acids, unsaturated fatty acids, vitamins, macro and microelements, polysaccharides, and melanin, among other essential nutrients. They are particularly appealing for diets because they have few calories and no cholesterol. As a result, they are starting to be accepted as a nutritious dietary option in a number of countries throughout the world, including Japan, Korea, China, the USA, and France.

The *Auricularia auricula-judae*, also known as "tree jellyfish" in Japan, "wood ear" in China, and "black fungus" in Russia, is one famous example. In Asia, this fungus is highly appreciated for its culinary and therapeutic benefits. Applying fresh fungus to the problematic area, it is used to cure a number of diseases, including sore throats, eye infections, staphylococcus infections, tonsillitis, and laryngoceles, according to traditional Chinese medicine. Even its potential for decreasing cytotoxicity and blood clot formation has been revealed by recent studies.

All living things, including mushrooms, depend on water, so it plays a crucial part. It is necessary for many aspects of life, such as osmotic relationships, nourishment, perspiration, growth, and development. Except for the hard, dry fruiting body of Ganoderma lucidum, the water content of mushroom fruiting bodies is normally high—around 90%. Numerous macro- and micronutrients, such as potassium, phosphorus, magnesium, calcium, copper, and zinc, are found in *Auricularia auricula* (Shan et al, 2019). Although its organic components can vary, they frequently comprise energy, crude protein, crude fat, and carbs. The quality of mushroom protein may be determined by looking at elements such as sulfur-containing amino acids, essential amino acids, and solubility, all of which have a big impact on how well proteins are digested and absorbed.

The precise makeup of *Auricularia auricula-judae* hasn't been widely reported in the literature despite the interest it has gotten. Due to its potential as a functional ingredient and a source of biologically active compounds, the main goal of this study is to investigate the chemical makeup and nutritional value of this fungus.

3.4. Medicinal values of cultivated mushrooms

In many parts of the world, including Asia, European folk cultures, African communities, and indigenous peoples like the Maori in New Zealand and the Tzeltal in Mexico, the Auricularia species have a long history of traditional use. These mushrooms are valued for their culinary and therapeutic qualities. A. auricula was included as one of seven therapeutic mushrooms in the "Dongui-bogam" by Heo Jun, published in 1613 in Korean medicine. Early in the 17th century, herbalist Carolus Clusius discovered that certain Auricularia species might be used throughout Europe to heal sore throats. As time went on, these mushrooms gained acceptance in Europe as a method of thrombosis prevention because of the support of important individuals like Li Shih-Chen, the author of "Pen Tsao Kang Mu." (Berch et al. 2007). They were well-liked in conventional Chinese medicine as treatments for ailments like jaundice and sore throats. In Europe, they were employed as food supplements as well as medical treatments (Zięba et al, 2020, Regis et al., 2023). Numerous research has examined the therapeutic benefits and pharmacological actions of substances derived from Auricularia species, including their anti-coagulant, anti-inflammatory, anti-microbial, antioxidant, anti-tumor, anti-viral, and immunomodulatory capabilities. The ability of these mushrooms to treat hypoglycemia, and hypolipidemia, and reduce cholesterol levels has been demonstrated. Research has also shown that they can control the interstitial microbiomes, which helps the growth of good probiotics. In diabetic mice, *Auricularia auricula-judae* eating

resulted in decreased plasma glucose, total cholesterol, and triglyceride levels, demonstrating the anti-diabetic properties of this mushroom. These mushrooms contain phenolic chemicals that have antioxidative capabilities in a number of ways. The antioxidative properties of the phenolic compounds found in *Auricularia auricula* contribute to its possible health advantages (Kozarski et al, 2011; Mau et al, 2001, Bandara et al, 2019, Islam et al, 2021, Yoon et al, 2003)

With an emphasis on mushroom polysaccharides, Auricularia species show potential for the development of low-toxic, very effective medications for chronic disorders. The biological actions of these substances are being improved, and new uses are being explored, but further study, including clinical trials, is still required (Islam et al, 2021; Regis et al, 2023).

It is important to remember that a number of variables, including culture methods, climate, and environmental circumstances, might affect the production of antioxidant chemicals (Table 2) in Auricularia mushrooms. The internal stratification of various layers and the presence of abhymenial hairs on basidiomata are taken into consideration when classifying Auricularia based on physical characteristics. Auricularia mushrooms' macroscopic characteristics might change depending on the specimen's age, exposure to light, moisture content, and other environmental conditions (Bandara et al, 2017). Additionally, elements like the source of the nitrogen, temperature, pH, and light intensity can have a big impact on the growth and mycelial stage of these mushrooms. For mycelial growth, a variety of culture media have been employed, including Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and others.

The one-factor-at-a-time strategy includes altering one independent variable at a time while maintaining the other independent variables' values. However, this approach can take a while and doesn't always result in ideal circumstances. Studying the interactions between medium components and their effects on mycelial growth and exo-biopolymer synthesis can be done more effectively using the orthogonal matrix method.

The positive effects of Auricularia spp. on health present a promising solution for addressing health issues. It is crucial to develop new drugs that have low toxicity, high efficiency, and minimal side effects for treating chronic diseases. A recent and appealing approach involves the use of mushroom polysaccharides, particularly Auricularia auricula-judae polysaccharides (AAPs), which have been recognized as significant biological components (Zhang et al., 2022).

Table 2. Antioxidant potential of Auricularia species compared to some other mushroom species (Regis et al, 2023)

Antioxidant potential	Extraction method	Mushroom	Values	Reference
DPPH radical scavenging activity	Methanolic extraction EC50 of 0.08 mg/mL	*Auricularia auriculs-judae*	93.33%	Hussein et al. 2015
		Polyporus tenuiculus	90.71 %	Hussein et al. 2015
		Agaricus bisporus	85.44%	Jagadish et al. 2009
		Polyporus conchatus	46.53%	Hussein et al. 2015
	Methanolic extraction 1 mg/mL	*Auricularia auricula-judae*	100%	Gasecka et al., 2018
		Tremella fuciformis	94%	Gasecka et al., 2018
Hydroxyl radical scavenging	Polysaccharide extract	*Auricularia auricula-judae*	80%	Zou et al., 2015
		Gleostereum incarnatum	56.23%	Zhang et al., 2015
Chelatin ability on Fe2+ ions	Polysaccharide extract	*Auricularia auricula-judae*	0.43 mg/mL	Zou et al., 2015
		Ganoderma lucidum	0.59 mg/mL	Kozarski et al., 2011
		Phellinus linteus	0.91 mg/mL	
		Agaricus braseliensis	2.04 mg/mL	
		Agaricus bisporus	7.8 mg/mL	
Water-soluble phenolic contents	Digeted microwaved sample	*Auricularia polytricha*	156%	Izham et al., 2022
		Lentinula edodes	116%	
		Agaricus bisporus	48%	
		Pleurotus sajor-caju	60%	

Researchers have continuously advanced their understanding through in vivo and in vitro studies to enhance biological activities and broaden the applications of AAPs (Miao et al., 2020; Xia et al., 2019). Despite the elucidation of these compounds, their complex structures and unclear mechanisms of action require further exploration, especially in clinical studies (Islam et al., 2021). Notably, cultivation practices and environmental factors, such as climate, can influence the production of antioxidant compounds in Auricularia mushrooms (Ao & Deb, 2019; Wang et al., 2032).

Table 3. Research findings of Auricularia products against health risk factors. (Regis et al., 2023)

Risk factors for health	Findings	Reference
Stunted growth of children due to malnutrition	*Auricularia thailandica* rich in amino acid and minerals	Bandara et al., 2017
Obesity	*Auricularia* polysaccharide extracts inhibit adipocyte differentiation	Park et al., 2018
	Auricularia auricula-judae extract suppressed plasma glucose, total cholesterol, the weight of internal organs, epididyma fat, and activity of hepatic enzymes	Choi et al., 2019 ; Ganesan et al., 2018
Anemia among women	A novel *Auricularia auricula-judae* polysaccharide-iron (AAPS-iron(III)) showed highly efficiency on the treatment of iron deficiency	Liu et al., 2019
Raised blood pressure	*Auricularia* extracts absorb glucose, reduce dialyzed glucose and inhibit the activity of alpha amylase, decrease chronic hyperglycemia	Liu et al., 2021 ; Wu et al., 2014
	A dose of *Auricularia* polysaccharides led to reduction in total cholesterol and LDL levels and blood glucose compared to control diabetic rats.	Hao (2014), Lu et al. (2018), Takeuchi et al. (2004), Zhang et al. (2020), Zhang & Riskowski (2020)

3.5. Global Production of Auricularia

With more than 90% of the entire global production as of 2021, China stands out as the top producer of *Auricularia auricula* (Zhao et al, 2019). A significant 674,000 tons of dried *Auricularia auricula* were produced in China in 2018, with a total market value of 37.46 billion yuan and impressive foreign exchange earnings of 6.15 billion yuan. These mushrooms are now regarded as a unique and valuable agricultural commodity in China, greatly enhancing agricultural output, raising farmer incomes, and lowering poverty in the agricultural industry. *Auricularia auricula* cultivation is expanding, with annual production gains, but profit margins from market sales are gradually declining, signaling a change from a seller's market to a buyer's market.(Li and Bi, 2021).

Auricularia auricula polysaccharides (AAP) are in greater demand as functional and nutritional components, which is fueling market growth. Processing methods have advanced, and numerous high-value AAP derivatives have been created. These goods have not yet fully penetrated the market despite their various health advantages (Zhang et al, 2022). *Auricularia auricula* polysaccharides have a distinctive structure and a wide range of biological and pharmacological actions (Li et al, 2020). As a result of their high content, simplicity in extraction, distinctive structure, lack of side effects, and significant therapeutic potential, they are becoming more and

more well-known globally. There have been extensive investigations into the structural, biological, and pharmacological effects of AAPs from diverse A. nigra species, from which several AAPs have been isolated.

3.6. Stages in Mushroom Cultivation

According to Wang's (1999) research, there are three primary steps in the mushroom cultivation process: producing spawn (also known as inoculum), preparing the substrate for fruiting, and actually growing the mushrooms. In essence, these phases entail spreading fungal propagules across the substrate, letting the mycelium of the fungus colonize the surface, and then cultivating and harvesting the fruiting bodies or sporophores. The vegetative phase, which is characterized by mycelium growth, and the reproductive phase, which results in the formation of fruiting bodies, are both important stages in the mushroom life cycle that are covered by this cultivation method. It is crucial to select substrates for both phases that will give the ideal chemical and physical conditions for healthy mushroom growth. Additionally, supplements could be required for both phases to promote fruiting and improve development. The flowchart shown in Figure 2 shows the stages of mushroom cultivation.

3.6.1. Spawn production

After inoculation, the mycelium grows within the substrate during the spawn or mycelia running phase, as stated in the research by Zadrazil et al. in 2004. This phase also includes the mycelium's biodegradation of the substrate's constituent parts, which in turn helps fruiting bodies form. During this stage, it's critical to maintain precise temperatures and humidity levels that are appropriate for the type of growing mushrooms. According to Chang in 2001 and Chang and Miles in 2004, mushroom spawn is simply the medium in which the mycelium of a fruiting culture has developed, acting as the "seed" or inoculum for substrates in mushroom production.

Figure 2. Illustrated Steps of Auricularia Cultivation (Dang Lelamurni, 2013)

Inoculum or spawn preparation must be done in a sterile environment at all times. The vegetative stage of the mushroom life cycle is the one during which spawn is produced. The substrates used for producing spawn can be used separately or in combination, and they can be different from those used for mushroom cultivation or fruiting. According to Chang in 2001, a few regularly used substrates in the formation of spawn include different grains including wheat and sorghum. It's crucial to remember that producing spawn is thought of as a difficult and precise process, and is

frequently impracticable for the typical mushroom farmer. As Philippoussis noted in 2009, it is often created by specialized spawn producers who use microbiological sterile methods.

In accordance with the foregoing explanations, inoculum, also known as spawn, is a mixture of colonized grain and mycelium that is grown in sterile polyethylene bags to ensure appropriate gas exchange. In 1993, Stamets made it clear that the main purpose of inoculum or spawn is to increase the mycelium's vitality to the point at which it can be incorporated into bigger bulk substrates. The chosen substrate works as a source of vital nutrients as well as a medium for even mycelium dispersion. When the mycelium has completely covered the surface and penetrated the substrate, the spawn is said to be ready. It is crucial to distinguish between the growing phase (vegetative phase) and the reproductive phase (creation of fruiting bodies) based on Klebs' principles, as stated by Griffin in 1981. The growth phase can take place under a wider range of environmental conditions than the reproductive phase, even those that might prevent reproduction, as reported by Parbery in 1996.

3.6.2. Preparation of fruiting substrate

According to research by Kues and Liu in 2000 and results from Baldrian and Valaskova in 2008, the quality and yield of mushrooms as well as the growth of mycelium are greatly influenced by the substrate's nutritional composition and physical characteristics. As a result, the optimum substrate for mushroom development should be compatible with the particular species of mushroom in issue, both chemically and physically. The formulations for fruiting substrates are carefully created as the base from which mushrooms will arise in the context of mushroom production. According to Phillipoussis in 2009, these formulations are frequently enhanced with different additives to provide additional nutrition for the desired mushroom production. According to Phillipoussis, the first step in growing mushrooms is to prepare the growth media, which includes putting the substrate together and treating it.

The materials or wastes from forestry and agriculture are frequently used as substrates in mushroom production. Depending on the type of mushroom being grown, these materials can include sawdust, wheat and rice straws, or other acceptable crop leftovers. Some substrates can be inoculated directly with little to no pre-treatment, while others need both physical and microbiological pre-treatment. Wood explained in 1989 that while physical pre-treatment can include autoclaving to sterilize objects, microbiological pre-treatment often entails a controlled bulk composting process. These pre-processing stages are essential because, as Chang and Miles

pointed out in 2004, a nutrient-rich substrate does not always equate to a favorable environment for mushroom growth. It is crucial to properly prepare the substrates; otherwise, contamination by bacteria or molds may grow and impede the formation of mycelium and, eventually, mushrooms. Depending on the type of grown mushroom, several preparation techniques are used. According to Philippoussis in 2009, having an acceptable water content in the substrate is of vital importance to ensure that the water activity in the final medium is favorable to fungal development. Therefore, enabling strong mycelium growth and, subsequently, mushroom formation, requires establishing the ideal substrate conditions.

3.6.3. Mycelia growth and sporophore formation

The choice of substrate, the suitability and necessity of composting techniques, and the incorporation of nutritional supplements are only a few examples of the different variables that must be in accordance with the fungi's requirements for growth and fruiting in terms of chemical aspects. These elements should also give the fungi the ability to fend off potential competition from competing microbes that might interfere with the process. Physical elements like pH, temperature, aeration, and light are also extremely important and should be properly regulated to fulfill the unique requirements of the grown mushroom. The nutritional status and physiological state of the mycelium have a significant impact on how fruiting bodies, or sporophores, develop. Favorable climatic conditions encourage the creation of primordia once the spawn running phase is over, which is followed by the production of sporophores. Primordia can be divided into two main categories: diffuse primordia, which grow into a hemisphere of hyphae, and compact primordia, which are densely braided with hyphae. When the necessary conditions are met, these primordia eventually transform into sporophores.

According to Parbery in 1996, the environmental factors that favor the reproductive phase are usually different from those thatfavor the vegetative phase or spawn running. As noted by Fan et al. in 2008, the beginning of fruiting body development is closely correlated with a drop in the nutritional content of the growth substrates. This association was further highlighted by Granado et al. in 1997, who discovered that mechanical harm to existing mycelium can result in the localized stimulation of fruiting body development. Because of the link between mechanical damage and the growth of the fruiting body, mature fruiting bags are frequently painstakingly cut to encourage emergence, either horizontally or vertically using a clean knife. The proper development of primordia and fruiting bodies depends on a number of environmental parameters,

including temperature, aeration, light, and hydrogen ion concentration (pH). Depending on the species and various growth and fruiting periods, different pH values may be ideal. The range of temperatures ideal for fruiting is typically smaller than the range required for mycelial growth. Both the vegetative and reproductive phases require adequate aeration, however, the reproductive phase's requirements are more demanding.

3.7. Culture media

Culture media for mycelia (fungi) aim to provide essential nutrients, including carbon sources (e.g., sugar) (Vahidi et al, 2006) nitrogen sources (e.g., peptones)(Manu-Tawiah and Martin, 1988), and trace elements. Agar type like Sabouraud Dextrose Agar, Potato Dextrose Agar, and Malt Extract Agar are commonly used for solidifying the medium (Fletcher, 2019). pH, antibiotics, and antifungal (Vahidi and Namjoyan, 2004)are adjusted to create optimal growth conditions and prevent contaminations. The media may be selective, promoting the growth of specific fungal strains (Strauss et al, 2000). Temperature and incubation conditions are tailored to the preference of the fungal species. Culture media for mycelia are designed to support fungal growth, isolates strains, and facilitate experimental studies.

3.7.1. Potato Dextrose Agar

By offering the best conditions for fungi to flourish, Potato Dextrose Agar (PDA) is essential in promoting mycelial growth. PDA provides a rich source of nutrients, including carbohydrates, vitamins, and minerals, required for fungal metabolism and growth. It is made out of mashed potatoes and dextrose (a sugar), which has been solidified with agar. The agar component acts as a solidifier, establishing a solid foundation for the colonization and growth of fungal mycelium. For the medium to remain appropriately moist without becoming overly saturated, as is necessary for fungal metabolic processes, PDA maintains a proper moisture content. Additionally, PDA is typically set to a pH that is somewhat acidic, promoting the growth of a variety of fungus. PDA is a useful medium for the isolation, development, and preservation of fungal cultures due to its simplicity in preparation and widespread use in labs (Deacon, 2006). While allowing for the selective isolation of fungi, chloramphenicol functions as a selective agent to prevent the bacterial overgrowth of competing microorganisms from mixed specimens (Sagar, 2019).

Numerous studies have shown how well Potato Dextrose Agar (PDA) supports the mycelial development of different fungus species (Hoa and Wang, 2015). PDA is well known for its capacity to offer both the crucial nutrients and the ideal environment required for healthy mycelial

growth. Due to its dependability and adaptability, PDA is frequently used by mycologists and researchers for the isolation, culture, and maintenance of fungus. PDA is a preferred option in mycological research and fungal cultivation due to its nutrient-rich content, balanced pH, and hardening agent (agar), which make it a good medium for the proliferation and study of fungal mycelium. These results are in line with the widespread belief that PDA is a useful medium for mycelial formation, and it is frequently mentioned in scientific literature and the lab procedures for fungi research(Fletcher, 2019)

3.7.2. Potato Dextrose Yeast Extract Agar

In the sciences of microbiology and mycology, potato dextrose yeast extract agar is a frequently used culture medium. It is used to cultivate and study a range of fungi, with an emphasis on yeasts and molds in particular. The vital nutrients, carbohydrates, and energy sources required for fungi to develop are provided by this medium. Researchers use PDYA to isolate and describe several fungus species in order to examine their distinctive characteristics (Castillo t al, 2018).

3.7.3. Yeast Extract Agar

A nutrient-rich medium that supports the growth of a variety of bacteria, yeasts, and molds is yeast extract agar. According to the recipe given by Windle Taylor for plate enumeration of microorganisms in water, yeast extract agar is created. Water can include a large number of microorganisms, particularly those from soil and vegetation. Peptone, yeast extract, and other nutrients for growth include B vitamins and nitrogen molecules. Separate counting of aerobic mesophilic organisms that form colonies after three days of incubation at 220°C and after 24 hours of incubation at 370C is possible with this type of media (Dos Santos et al, 2018)

3.7.4. Malt Yeast Extract Agar

Based on the work of Wickerham (1951), Yeast Malt Agar is a specific medium designed to enable the isolation and development of yeasts, molds, and other microorganisms that flourish in acidic environments. Yeast Malt Agar contains fungistatic ingredients such sodium propionate and diphenyl to ensure its selectivity. This successfully inhibits the growth of molds and makes it possible to count the number of yeasts in mixed populations.

Wickerham suggested utilizing yeast malt broth (M425) as a method for enriching yeasts. The inoculated soup is covered with a coating of sterile paraffin oil that is around 1 centimeter thick using this technique. This method inhibits the formation of mold spores while encouraging yeast

growth. After developing, yeast cultures can be streaked onto YM Agar to produce isolated colonies of fermentative yeast species. Yeast Malt Agar's components play specialized functions in promoting microbial development, including the following: Peptone is a supply of carbon, nitrogen, long-chain amino acids, and other vital nutrients; yeast extract is a source of vitamin B nutrients and other growth factors; malt extract is a further source of carbon; and dextrose is a carbohydrate and energy source. The medium can be made more acidic by adding chemicals like 10% HCl, tartaric acid, or 10% citric acid, or by adding sterile 10% lactic acid to increase its selectivity. Alternately, antibiotics like streptomycin (final concentration of 40mcg/ml) or penicillin (20U/ml) might be added for more specificity. It is crucial to remember that the agar medium shouldn't be heated up once acidity has been administered (Wicherham, 1951).

3.7.5. Malt Extract Agar

Malt Extract Agar, often known as MEA, appears as a light cream-colored medium. Its properties are specifically created to foster the best-growing conditions for yeasts and molds while actively preventing the spread of germs. MEA has a pH that is only slightly acidic, generally 4.7 to 0.2, which is one of its major characteristics (de Valdez et al, 1997). The optimal conditions for yeasts and molds to grow and thrive are created by the pH level's careful adjustment, which also inhibits the growth of undesirable bacteria. MEA offers maltose to yeasts and molds as a valuable energy source in terms of nutrition. These microbes receive the energy they need to support their metabolic processes from the carbohydrate et maltose (Flores-Carreón al, 1969)

Dextrin, a polysaccharide generated from premium starch, and glycerol are also included in the mix. Both of these substances act as vital sources of carbon, supplying a sufficient supply for the growth and proliferation of yeasts and molds. The medium is meticulously supplemented with mycological peptone to satisfy the bacteria' need for nitrogen. This element provides a source of nitrogen, which is necessary for the synthesis of proteins and overall growth. Agar is employed as the solidifying agent to solidify the medium and offer a stable surface for the development of yeasts and molds. It encourages quick growth and enables the emergence of these bacteria' distinctive forms and pigmentation. Compounds like lactic acid, tartaric acid, or antibiotics are introduced into the formula to further improve the medium's selectivity and create a regulated environment for the growing of yeast and mold. These elements efficiently prevent bacterial development, ensuring that the medium accomplishes its intended function.

3.7.6. Compost Phase 2 Agar

A. bisporus is one of many mushrooms that are frequently produced in settings that are very different from their natural habitats. With the exception of compost agars, the growth media employed for mycelial formation in these artificial growth environments largely lack insoluble components and are abundant in simple sugars. In sharp contrast, mushrooms flourish in their natural habitats. Composted Mushroom Medium (CMM) was created expressly to match the qualities of their commercial substrates and satisfy the nutritional needs of mushrooms. The nutritional value of the insoluble components found in compost, notably the microbial biomass, was an important factor taken into account throughout its manufacture. According to Rainey P.B. (1989), this microbial biomass is essential for sustaining the growth of mushrooms.

For instance, composted wheat straw and horse dung are frequently used to grow A. bisporus. Complex, mostly insoluble plant and microbial leftovers are included in this mixture. The insoluble part of this substrate, which contains elements including lignin, cellulose, hemicellulose, proteins, and microbial biomass, appears to be preferred by the mushroom mycelium for use in its growth (Fermor et al., 1979). This emphasizes how artificial growth media's composition differs significantly from that of the mushroom's natural habitat.

According to Sparling et al. in 1982, microbial biomass makes up around 2% of the dry weight of the compost. The significance of this microbial biomass as a source of nutrients for A. bisporus has recently come to light in studies (Fermor & Wood 1981, 1982, Grant et al. 1984, Fermor & Grant 1985). According to Eddy & Jacobs in 1976 and Atkey & Wood in 1983, a dark brown, amorphous matrix made up of polysaccharides, microbial cells, and detritus builds up on the surface of the straw throughout the composting process. A. bisporus has the ability to manufacture a variety of extracellular enzymes, including muramidases, which are responsible for dissolving bacterial cell walls. The'microbial matrix' serves as a concentrated source of nitrogen, minerals, and carbon for the mushroom thanks to its enzymatic activity (Fermor & Grant 1985). This emphasizes the significance of microbial biomass as an essential element in A. bisporus's nutrition and growth.

3.8. pH

Xu, et al., (2003) reported that the ideal pH for mycelial development and exo-biopolymer synthesis was 6. Many ascomycetes and basidiomycetes have greater acidic pH requirements during submerged culture in order to achieve optimal growth conditions, according to Lee, et al.,

2013. Furthermore, according to Shin, et al. (2007), pH 6.5 created the best biomass for *Auricularia polytricha,* whereas pH 7.0 produced the best biomass for *F. velutipes*, according to Hamedi, et al. (2007), and pH 4.0 and 9.0 produced no biomass at all. According to Kozhemyakina et al. (2010), *F. velutipes* could flourish in a wide range of initial pH values between 3.0 and 7.5. Thus, pH starting values of 5.0 to 6.5 produced the most mycelium biomass.

According to Shujing (2010), pH may affect the stability and characteristics of the pigment because it improves the solubility of the pigment isolated from Auricularia. In a study on the storage quality of Auricularia, Yu-fen (2014) discovered that a pH of 4 resulted in less chemical component loss during storage.

Yang et al. (2003) found that the mycelial growth of various mushroom species is capable of occurring across a broad pH range. Imtiaj et al. (2008) similarly noted that mushrooms exhibit mycelial growth within a wide pH spectrum (from 5 to 9), with the most favorable pH falling between 6 and 7. Jo et al. (2009) revealed that the optimal pH for the growth of Ganoderma applanatum ranged from 6 to 9. Additionally, Chandana et al. (2008) observed that the G. lucidum Korean strain could thrive across pH levels ranging from 5 to 9, with the best growth achieved at pH 5. Chandra and Pukayastha (1997) and Jonathan et al. (2007) reported that A. campestris and V. esculenta exhibited robust mycelial growth at pH 6. Fasola et al. (2007) found that the mycelial growth of Volvariella speciosa occurred within the pH range of 3 to 9.

3.9. Carbon Sources

Researchers from all around the world have investigated the viability of growing mushroom mycelia in a variety of environments. According to Yong et al. (1986), changing the culture conditions, particularly the choice of carbon and nitrogen sources, can affect the aromatic properties of fungus.

There are conflicting views regarding the advantages of various carbon sources, including sucrose, fructose, glucose, and plain old table sugar, for in vitro plant development. Due to its effective absorption across the cell membrane, sucrose, which is commonly utilized at doses of 2-5%, is the most effective of these in tissue culture (Bridgen et al., 1994). The effects of glucose on in vitro plant development are complex.

Fructose was acknowledged by Kaufman et al. (1962) and Dickinson (1966) as an appropriate nutrition source for growing stem segments and pollen (Kaufman et al., 1962; Dickinson D.B, 1992). It is important to keep in mind, nevertheless, that incorporating fructose into the growth

medium might occasionally have unfavorable effects such as hyper-hydricity, which lowers chlorophyll levels and causes erratic nitrogen and sugar metabolism (Bouza et al., 1992). Table sugar has been employed as a more affordable alternative to standard medium components in the context of in vitro micropropagation of potatoes (Demo, 2008). Additionally, it has been discovered that adding plant juices or extracts from different sources, such as yeast, tomato, banana, orange, oregano, or coconut, to the culture medium can speed up the growth of numerous plant species' tissues (He, S.L. et al., 2003; Hong et al., 2003; Amo-Marco et al., 1994; Siddique et al., 1998). It is clear that various carbon sources have distinct effects on how in vitro-cultured plants grow.

3.10. Peptone/ Sucrose ratio

There are several factors to take into account when producing spawn, but mycelium growth is the primary concern. Finding methods to hasten mycelial colonization and produce robust, quickly growing mushroom mycelia is what we're all about. We examine a wide range of variables, including the type of culture, the balance between carbon and nitrogen, the sources of carbon and nitrogen, temperature, pH, plant growth regulators, and a ton of other elements, in order to make that happen (Lu, 2009).

The inclusion of glucose and peptone has now been found to be the magic ingredient in culture media and its effect on mycelial growth in mushroom strains. These chemicals serve as mycelium development's equivalent of rocket fuel, according to Pereima's (2017) research. In 2010, Nwokoye and the group made the decision to learn Pleurotus ostreatus's mycelial development secrets. They experimented with different forms of carbon and nitrogen, including the tried-and-true mixture of glucose and peptone. As it turned out, peptone served as a nitrogen source while glucose operated as a turbocharger for the mycelium's growth.

From 1956 to 2017, it was the focus of numerous peptone investigations. A protein from Pleurotus eryngii was discovered to have anti-inflammatory effects by Yuan et al. (2017). Peptone accelerated the growth of orchid embryos, according to Semiarti et al. (2017). According to Griffiths (2004), peptone is a superior nitrogen source over nitrate for the growth of Chlorella vulgaris. Tang (1956) even showed that peptone made with protease from Aspergillus was a nutritional treasure for some microorganisms. Peptone is hence capable of accelerating the growth and development of organisms in vitro.

4. Materials and Methods

4.1 Time and place of the experiment

Mycelia that had been cultivated and maintained in a clean condition since 2020 were stored at the Institute of Horticultural Science, specifically within the Department of Vegetable and Mushroom Growing at the Hungarian University of Agriculture and Life Science, Buda Campus. The experiment in question was carried out within the same mushroom laboratory during the period from November 2022 to January 2023.

4.2 Culture Media Preparation

Eight distinct culture media (Table 4) were prepared to evaluate their suitability for promoting the mycelial growth of the wood ear mushroom (Auricularia auricula). These culture media underwent sterilization for a duration of 20 minutes at 121°C and were subsequently aseptically transferred into plastic Petri dishes. An inoculum was extracted from cultures of A. auricularia-judae that had been growing for five days on PDA at a temperature of 25°C. Mycelial discs, each measuring 5mm in diameter, were then situated at the center of the plastic Petri dishes, which contained approximately 20 mL of each of the eight different media. Following the cooling of the media, Chloramphenicol BP 0.5% (100 ± 5µl) was introduced aseptically to prevent any potential bacterial contamination. Finally, the media were poured into the Petri dishes, with each plate receiving 20 mL.

Table 4. Culture media composition use for this study.

Composition (g/100 mL)	PDA	Agar	YE	ME	CP2	Peptone	Sucrose
PDA	3.9						
PDYEA	1.95	0.8	0.5				
MEA		1.6		1.5			
MEYEA		1.6	0.5	0.75			
YEA		1.6	1				
Compost Phase 2		1.6			100		
PDA + Peptone	1.95	0.8				0.25	
PDA + Sucrose	1.95	0.8					0.25

PDA: Potato dextrose Agar, PDYA: Potato Dextrose Yeast Agar, MEA: Malt Extract Agar, YE: Yeast Extract; YEA: Yeast Extract Agar, CP2: Compost Phase 2

4.2.1. Potato Dextrose Agar (PDA) Media Preparation

To prepare Biolab PDA agar, start by weighing the appropriate amount of PDA powder, typically around 39 grams per liter of distilled water. Next, add the weighed PDA powder to a container and pour in the required volume of distilled water. Thoroughly mix the solution to ensure even distribution, using a sterile stirring rod or magnetic stirrer if available. Then, transfer the mixture into containers like Petri dishes or test tubes, leaving some headspace to prevent overflow during autoclaving, and ensure proper sealing. Place these containers inside an autoclave or pressure cooker, following the manufacturer's instructions for sterilization, typically at 121°C (250°F) for 15-20 minutes to eliminate contaminants. After sterilization, allow the agar to cool to a safe handling temperature while keeping it in a liquid state. In a sterile environment, such as a laminar flow hood, pour the liquefied agar into sterile Petri dishes or tubes, taking care to avoid introducing contaminants. Let the agar cool and solidify in the containers with slightly ajar lids to facilitate gas exchange without compromising sterility. Finally, store the prepared PDA plates or tubes in a cool, dry place until needed, and refrigerate them if not using immediately. This process ensures the proper preparation and handling of Biolab PDA agar for laboratory use.

4.2.2. Malt Extract Agar (MEA) Media Preparation

To prepare Biolab Malt Extract Agar (MEA), first weigh the MEA powder according to manufacturer instructions or your specific recipe (usually 30-40 grams per liter of distilled water). Mix the powder with distilled water, ensuring even distribution. Pour this mixture into containers, leaving space for autoclaving, then sterilize at 121°C for 15-20 minutes. After cooling, pour the agar into sterile containers, maintain sterility, and let it solidify. Store the prepared MEA plates or tubes in a cool, dry place until use, or refrigerate if not using immediately. Following these steps ensures proper preparation and handling for laboratory use.

4.2.3. Yeast Extract Agar (YEA) Media Preparation

To prepare Biolab Yeast Extract Agar, start by measuring the agar powder quantity according to manufacturer instructions. Mix it with distilled water, ensuring even distribution, and transfer it to containers, leaving space for autoclaving. Sterilize at 121°C for 15-20 minutes. After cooling, pour the agar into sterile containers, maintain sterility, and let it solidify with slightly open lids. Store the prepared Yeast Extract Agar plates or tubes in a cool, dry place for later use.

4.2.4. Potato Dextrose Yeast Extract Agar (PDYEA) Media Preparation

PDYEA was prepared by blending 1.95 grams of PDA powder, 0.8 grams of Agar, and 0.5 grams of yeast extract powder in 100 mL of distilled water. Thoroughly mix the solution to ensure uniform distribution, using either a sterile stirring rod or a magnetic stirrer if available. Following this, transfer the mixture to containers like Petri dishes or test tubes, leaving some space at the top to prevent spillover during autoclaving, and ensuring proper sealing. The next step involved placing these containers in an autoclave or pressure cooker, adhering to the manufacturer's sterilization guidelines. Typically, sterilization was conducted at 121°C (250°F) for 15-20 minutes to eradicate potential contaminants. After sterilization, allow the agar to cool to a safe handling temperature while maintaining its liquid state. In a sterile environment, such as a laminar flow hood, carefully pour the liquefied agar into sterile Petri dishes or tubes, taking precautions to prevent the introduction of contaminants. Allow the agar to cool and solidify in these containers, while keeping the lids slightly open to facilitate gas exchange without compromising sterility. This process ensured the proper preparation of PDYEA for laboratory use.

4.2.5. Compost Phase 2 Agar Media Preparation

In this procedure, a portion of fresh commercially prepared mushroom compost, weighing between 5 to 10 kilograms, underwent an oven-drying process at 75°C for a duration of 2 days or until it no longer lost weight. Subsequently, this dried compost was stored at room temperature. A 50-gram sub-sample of the dried compost was taken and combined with 1000 milliliters of distilled water. This mixture was allowed to steep for 1 hour. The temperature was then raised to 100°C for 5 minutes, and vigorous stirring was employed to assist in the removal of the 'microbial matrix' adhering to the straw surfaces. Following an additional 2-hour infusion period, the cooled mixture underwent filtration through four layers of muslin, yielding approximately 800 milliliters of a dark brown infuscate. To this liquid 1.5% Bacto agar (Difco B140) was introduced, and the pH was adjusted to 7.4 using 1 N NaOH. The resulting medium was autoclaved at 121°C for 15 minutes and thoroughly mixed to ensure uniform distribution of particulate matter. Finally, the prepared agar medium was poured into Petri dishes for use in subsequent microbiological experiments.

4.2.6. Potato Dextrose + Peptone Agar Media Preparation

To prepare PDA+ peptone agar, a mixture was created by combining 1.95 grams of PDA, 0.8 grams of Agar, and 0.25 grams of peptone powder in 100 mL of distilled water. This mixture was thoroughly mixed to ensure even distribution, using either a sterile stirring rod or a magnetic stirrer

if available. Subsequently, the blend was transferred into containers like Petri dishes or test tubes, leaving some room at the top to prevent overflowing during autoclaving, and ensuring proper sealing. The next step involved placing these containers inside an autoclave or pressure cooker, following the manufacturer's sterilization instructions. Typically, sterilization was carried out at 121°C (250°F) for 15-20 minutes to eliminate any potential contaminants. Following sterilization, the agar was allowed to cool to a safe handling temperature while maintaining a liquid state. In a sterile environment, such as a laminar flow hood, the liquefied agar was poured into sterile Petri dishes or tubes, with careful attention to preventing the introduction of contaminants. The agar was then allowed to cool and solidify in these containers, while keeping the lids slightly ajar to facilitate gas exchange without compromising sterility. This process ensured the proper preparation of PDA+ peptone agar for laboratory use in the past.

4.2.7. Potato Dextrose +Sucrose Agar Media Preparation

To prepare PDA+ sucrose agar, a mixture was made by combining specific amounts of PDA, Agar, and sucrose powder in 100 mL of distilled water. This mixture was thoroughly mixed to ensure even distribution, using either a sterile stirring rod or a magnetic stirrer. Subsequently, the mixture was transferred to containers like Petri dishes or test tubes, leaving space at the top to prevent overflowing during autoclaving, and ensuring proper sealing. The next step involved sterilizing these containers in an autoclave or pressure cooker, following the manufacturer's instructions. Typically, sterilization was conducted at 121°C (250°F) for 15-20 minutes to eliminate any potential contaminants. After sterilization, the agar was allowed to cool to a safe handling temperature while maintaining a liquid state. In a sterile environment, such as a laminar flow hood, the liquefied agar was poured into sterile Petri dishes or tubes, with great care taken to prevent the introduction of contaminants. The agar was then allowed to cool and solidify in these containers while keeping the lids slightly open to facilitate gas exchange without compromising sterility. This process ensured the proper preparation of PDA+ peptone agar for laboratory use.

4.2.8. Culture media + Carbon Source

Table 5. Composition of Culture Media with Different Carbon Sources.

Composition (g/1 L)	PDA	PDYEA	MEA	MEYEA	YEA	CP2	PDA+ Peptone	PDA+Sucrose
PDA	20	10					5	5
Agar	8	4	8	16	15		6	6
YE		3		3	5			
ME			8	4				
Peptone							1.6	
Sucrose	20	20	20	20	20	20	20	21.6
Lactose	20	20	20	20	20	20	20	20
Fructose	20	20	20	20	20	20	20	20
Glucose	20	20	20	20	20	20	20	20
Maltose	20	20	20	20	20	20	20	20

PDA: Potato dextrose Agar, PDYA: Potato Dextrose Yeast Agar, MEA: Malt Extract Agar, YE: Yeast Extract; YEA: Yeast Extract Agar, CP2: Compost Phase 2

To prepare a culture medium conducive to mycelia growth (Table 4), begin by weighing and thoroughly mixing agar, a carbon source (such as glucose or malt extract), a nitrogen source (like yeast extract or peptone), mineral salts, and any optional additives. In a separate container, add distilled or deionized water and slowly incorporate the dry mix while stirring to prevent clumping. Afterward, sterilize the medium by autoclaving and allow it to cool to approximately 50-55°C. Pour the cooled medium into Petri dishes or containers for solidification. Inoculate the solidified medium with mycelium, typically obtained from a previously cultured source. Place the inoculated dishes in an incubator set at the optimal temperature for the specific fungus being cultivated. Monitor the growth of the mycelium, adjusting the medium composition as needed based on the particular requirements of the fungus. It is crucial to maintain sterile conditions throughout the process to prevent contamination. For the most accurate and effective results, refer to species-specific protocols or literature related to the fungi being cultured.

4.3. Experimental Design and Treatments

A complete randomized design (CRD) was used in this study. The data were obtained from mycelial growth under different *in vitro* culture media with 5 replicates.

4.4. Data gathered.

4.4.1. Influence of Various Growth Media on the Development of Wood Ear Mushrooms (Auricularia auricula)

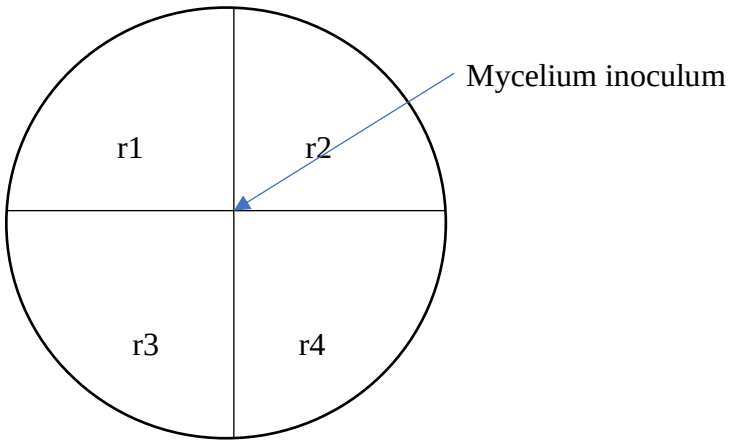

Figure 3. Illustration of four axes along which the mycelial growth was measured.

The strains were inoculated into the aforementioned media and incubated for ten days to generate solidified plates of PDA, PDYA, MEA, YEA, Compost Phase 2 Agar, PDA+ Peptone, and PDA + Sucrose. This was carried out under sterile conditions at a temperature of 25°C. Radial growth was measured using the methodology described in the study by Zharare et al. (2010). In each Petri dish, four radial lines, labeled r1, r2, r3, and r4, were marked outside the bottom, extending perpendicularly from the center (Figure 3).

The mycelium grew predominantly in concentric circles, and the radial growth of the mycelium was measured daily for 21 days along each of the radial lines (axes r1-r4) in each of the Petri dishes. The four measurements were then averaged to obtain a mean radial growth per Petri dish.

4.4.2. Assessing the Impact of Varied pH Levels on the Mycelial Growth of *Auricularia auricula* Utilizing Diverse Culture Media.

Prior to autoclaving, 1N HCL or NaOH was added to the various culture media to adjust the pH levels accordingly. The pH ranged from 4.5 to 9.0 in increments of 0.5. Following autoclaving, the Petri plates were inoculated and transferred to an incubation room for mycelial growth, maintaining a temperature range of 21°C to 25°C.

4.4.3. Impact of Light on Growth Characteristics of Wood Ear Mushroom *(Auricularia auricula)* Across Various Culture Media.

Petri dishes containing different culture media were inoculated with 4 mm diameter mycelial discs from previous axenic cultures. Four Petri dishes for each culture medium were utilized, with four placed in a dark room and four exposed to normal lighting conditions. This incubation period spanned 21 days, with the pH maintained at 6.5 and the temperature held at 24±2°C. Mycelial growth was measured on the 8th day, and spore density was determined on the 21st day.

4.4.4. Investigating the Impact of Various Carbon Source Concentrations on the Mycelial Growth of Auricularia auricula Across Different Culture Media.

Five distinct carbon sources, namely lactose (pH 6.47), fructose (pH 6.50), glucose (pH 6.63), maltose (pH 8.65), and sucrose (pH 6.68), were employed to examine the mycelial growth of Auricularia auricula. Two percent of each carbon source was added to various culture media before undergoing the boiling process. These media were subsequently poured into Petri plates, with a volume of 20 ml per plate. Following autoclaving for 20 minutes at 120°C and 1 kg/cm2 pressure, and allowing for cooling, the same inoculum size was placed at the center of each media. Subsequently, the Petri plates were transferred to an incubation room to facilitate mycelial growth within a temperature range of 21°C to 25°C.

4.4.5. Examining How Different Culture Media, Sucrose, Peptone, and Their Combination Affect the Mycelial Growth of Auricularia auricula.

Different culture media, such as sucrose agar, peptone agar, and combinations of culture media with sucrose, culture media with peptone, and culture media with both sucrose and peptone, were utilized as culture media to investigate the mycelial growth of Auricularia auricula. The basic components of the media were mixed with agar and heated to dissolve the agar. The resulting

mixture was then poured into Petri plates, autoclaved, cooled to room temperature, inoculated with A. auricula, and incubated as previously specified.

4.5. Statistical Analysis

The acquired data were subjected to statistical scrutiny using IBM SPSS Statistics software, version 29 (IBM Corp., New York, NY 10022, USA, 2020). Mean differences across factors were investigated employing the one-way analysis of variance (ANOVA) post-hoc test (Turkey's, Games' Howell). To verify the normality of error in various analyses, Kolmogorov-Smirnov ($p > 0.05$), as well as Skewness and Kurtosis (Absolute value of Skewness and Kurtosis = 2 and 4, respectively), were employed. Ensuring the precision of the analysis, Levene's test was utilized to assess the homogeneity of variances among the mean values of parameters. Homogeneity was confirmed if the p value exceeded 0.05. In cases where the p value was less than 0.05, the maximum variance/minimum variance test was executed to gauge the extent of the violation of homogeneity between parameters. Significance differences between factors were ascertained at the interval level of $p < 0.05$.

5. Results

5.1. Time and Place of the Experiment

Mycelia that had been cultivated and maintained under controlled conditions since 2020 were securely stored at the Institute of Horticultural Science, specifically within the Department of Vegetable and Mushroom Growing at the Hungarian University of Agriculture and Life Science, Buda Campus. The experiment in question was conducted within the confines of the same dedicated mushroom laboratory during the timeframe spanning from November 2022 to January 2023.

5.2. Mycelial Specimens Employed and Concise Overview

The mycelia used for this experiment was *Auricularia auricula* judae mycelia. Auricularia species share a common characteristic of producing slim, brownish, rubbery-gelatinous fruiting structures that resemble shelves or ears. These structures can reach sizes of up to 120 mm (4.7 inches) in width and 5 mm (0.20 inches) in thickness. These fruiting bodies can appear individually or in groups. Their upper surface varies from being slightly hairy to densely covered in fine hairs. The lower surface, where spores are produced, can be smooth, wrinkled, veined, or have a net-like, reticulate appearance (Wu, Fang et al., 2014). In some instances, unpigmented white variations of these fruiting bodies are also observed (Reid, DA., 1970; Bandara, AR. et al., 2020).

Table 6. Different species of wood ear (*Auricularia auricula-judae*) mushroom, location and distribution. (Internet, 2023)

Images	Scientific Names	Type/ Location	Distribution	Reference
	*Auricularia americana*	Canada	North America, China, Russia, Far East	Parmasto & I. Parmasto ex Audet, Boulet & Sirard 2003

	*Auricularia angiospermarum*	United States	North America	Y.C. Dai, F. Wu & D.W. Li 2015
	*Auricularia auricula-judae(Bull.)*	France	Europe	Quél. 1886
	*Auricularia australiana*	Australia	Australia	Y.C. Dai & F. Wu 2021
	*Auricularia cornea*	Hawaii	Africa, South Asia, North America (Mexico), South America, South Pacific Islands, Australia, New Zealand	Ehrenb. 1820
	*Auricularia fuscosuccinea(Mont.)*	Cuba	North America (Mexico, Florida), South America, Caribbean	Henn. 1893
	*Auricularia heimuer F.*	China	China, Russian Far East, Japan, Korea	Wu, B.K. Cui & Y.C. Dai 2014

	*Auricularia mesenterica(Dicks.)*	England	Europe, Uzbekistan	Pers. 1822
	*Auricularia minutissima*	China	China, Russia Far East	Y.C. Dai, F. Wu & Malysheva 2015
	*Auricularia nigricans (Sw.)*	Ciba	North America (Mexico, Florida), Central and South America, Caribbean	Birkebak, Looney & Sánchez-García 2013
	*Auricularia subglabra*	Cuba	Central and South America	Looney, Birkebak & Matheny 2013
	*Auricularia tremellosa (Fr.)*	Costa Rica	China	Pat. 1887

5.3. Cultivation of mycelium under aseptic and uncontaminated conditions.

In the study, mycelial discs have been conserved and maintained in a sterile water solution since the year 2020. This preservation method involved immersing the mycelial discs in aseptically prepared sterile water and housing them within plastic tubes. The primary subculturing of these mycelial discs was carried out on Petri dishes containing a nutrient-rich substrate known as Potato Dextrose Agar (PDA). The initial subculturing process yielded 60 Petri dishes, with a success rate of 10%, while the remaining 90% were afflicted by contamination. Subsequent subculturing

iterations were conducted to generate a total of 500 mycelial discs, which were subsequently employed in the experiments.

5.4. Effects of various culture media for propagating wood ear mushrooms (Auricularia auricula) under in vitro propagation

The choice of culture media is crucial for the mycelial growth of Auricularia, the wood ear mushroom. It impacts nutrient availability, growth rate, yield, research reliability, economic considerations, and sustainability in mushroom cultivation and research. The selection of appropriate culture media is essential for optimizing growth and productivity, advancing scientific knowledge, and supporting sustainable agricultural practices. Figure 4 shows the mycelial growth of wood ear mushroom (*Auricularia auricula-judae)* grown under eight culture media on the thirteenth day of inoculation under ambient condition.

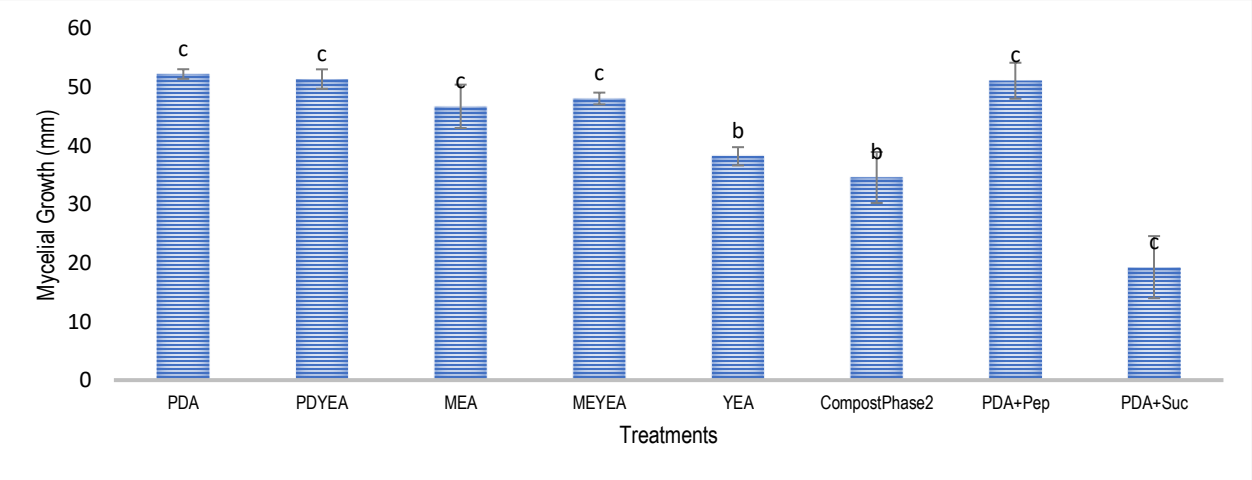

Figure 4. Mycelial growth of wood ear mushroom (*Auricularia auricula-judae)* under eight (8) different culture media stored under different light conditions after thirteen (13) days of inoculation.*(PDA-Potato Dextrose Agar; PDYEA- Potato Dextrose Yeast Extract Agar; MEA- Malt Yeast Extract Agar; MEYEA- Malt Extract Yeast Extract Agar; YEA- Yeast Extract Agar, PDA+Pep- 50%Potato Dextrose Agar + 50%Peptone; PDA+Suc- 50% Potato Dextrose Agar + 50% Sucrose.*

In the present study, eight culture media namely*:* Potato Dextrose Agar (PDA); Potato Dextrose Yeast Extract Agar (PDYEA), Malt Extract Agar (MEA), Malt Yeast Extract Agar (MYEA), Yeast Extract Agar (YEA), Compost Phase 2 Agar, PDA + Peptone and PDA +Sucrose were evaluated. Mycelial growth of *Auricularia auricula-judae* was determined after 13 days of inoculation. Data show that PDA (52.02 mm) and the combination of PDA and Peptone (51.48) (Table 5) were the

best for the mycelial growth of *Auricularia auricula-judae* due to the highest growth rate of both culture media. Potato Dextrose Yeast Extract Agar (51.37mm); Malt yeast Extract Agar (48.05 mm); Malt Extract Agar (46.72mm); Yeast Extract Agar (38.19mm); Compost Phase 2 (34.57 mm) followed the first two culture media (PDYEA and PDA+Peptone) as best culture media for wood ear mushroom mycelial growth respectively and having the combination of PDA and Sucrose (PDA+Sucrose) as the least recommended culture media used in this experiment with an average mycelial density of 19.28. Without the first mentioned culture media used in the experiment, MEA would have been the best culture media which have been proven in an experiment conducted by Priya,RU, and Geetha, D (2016).

Table 7. Effect of different culture media on the mycelial growth of wood ear mushroom *(Auricularia auricula-judae)* thirteen (13) days after inoculation sotred under ambient conditions.

Treatments	Mycelial Growth (mm) 13 DAI
Potato Dextrose Agar	52.03±0.82 c
Potato Dextrose Yeast Extract Agar	51.37±1.66 c
Malt Extract Agar	46.72±3.70 b
Malt Yeast Extract Agar	48.05±1.00 c
Yeast Extract Agar	38.19±1.56 c
Compost Phase 2 Agar	34.57±4.35 b
Potato Dextrose Agar + Peptone	51.48±2.88 c
Potato Dextrose Agar + Sucrose	19.28±5.30 a

Mean within the same column followed by the same letters are not significantly different at $p<0.05$. Each value is expressed as standard error (SE) (n=5).

Table 7 presents data on mycelial growth (measured in mm) on the final observation day (Day 13) across various culture media. Notably, statistically significant differences in the average mycelial diameter were observed among all different culture media employed in the experiment. All of these culture media demonstrated effectiveness in supporting the growth of mushroom mycelia, resulting in larger mycelial growth. Based on Figure 5, the pairwise comparisons of the treatments involving Malt Yeast Extract Agar, Potato Dextrose Agar, and PDA+Sucrose indicate a high level of statistical significance ($p<0.05$) when compared to PDA+Peptone. Mycelial density of wood ear

mushrooms under different cultures media (PDA< PDYEA, MEA, YEA, Compost Phase 2, PDA+Peptone) were much compact compared to MYEA and PDA+Sucrose.

The graphical representation (Figure 5) provides a detailed analysis through Pairwise Comparison of Treatments, elucidating the influence of diverse culture media on the mycelial growth of wood ear mushrooms (Auricularia auricula-judae) after thirteen days of inoculation under ambient conditions. The data reveals notable distinctions among treatments, specifically highlighting the significant differences between Compost Phase 2- PDA; PDYEA-PDA+Sucrose; PDA+Sucrose-PDA; PDA+Peptone-PDA+Sucrose, as indicated by a p-value less than 0.05. This statistical significance underscores the impact of the chosen culture media on the observed mycelial growth variations in the wood ear mushroom.

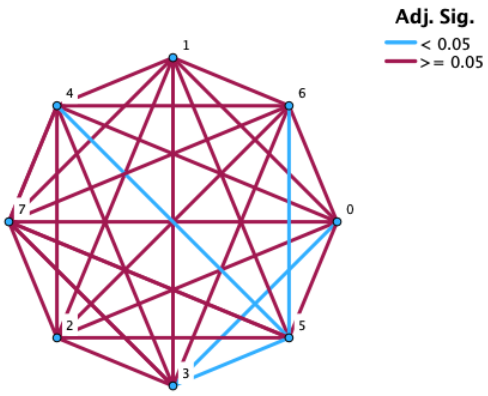

Figure 5. Pairwise Comparison of Treatments on the effect of different culture media on the mycelial growth of wood ear mushroom (*Auricularia auricula-judae*) thirteen days after inoculation in an ambient condition. *Each node shows the sample average rank of treatments.0-Compost Phase 2; 1-MEA; 2-MEYEA; 3-PDA; 4-PDA+Peptone; 5-PDA+Sucrose; 6-PDYEA; 7-YEA*

These findings suggest that the mycelial growth and the species under examination exhibit variations depending on the specific culture media employed. This conclusion aligns with the results reported by Kibar and Peksen in 2011, who similarly observed differences in mycelial growth based on the culture media used. The substantial divergence in the mycelial growth responses can likely be attributed to the particular mushroom species being studied. The

composition of the culture media was thoroughly analyzed, and the robust mycelial growth observed in the Potato Dextrose yeast Extract Agar can be attributed to the nutritional richness of this culture medium. The combination of PDA and YEA is shown to be an effective culture medium for promoting mycelial growth due to its abundance of nutrients, well-balanced nutritional composition, favorable pH levels, fungal-enhancing properties, and a potential track record of supporting healthy mycelial development.

In a comparative investigation into growth characteristics and physicochemical analysis of *P. ostreatus* and *P. florida*, it was found that dextrose emerged as the most favorable carbon source for achieving the highest mycelium yield. Dextrose, being an isomer of glucose, has the capacity to convert into glucose during the metabolic processes (Neelam, S. et al., 2013). Fungi necessitate both nitrogen and carbohydrates as essential elements. Nitrogen is responsible for the synthesis of nitrogen-containing compounds, while carbohydrates serve as structural and storage components within the cells (Hoa et al., 2015).

Potato Dextrose Agar (PDA) supplemented with yeast extract had a notable positive impact on mycelium morphology. This observation aligns with a study conducted by Neelem et al. in 2013, which endorsed the use of growth media supplemented with yeast extract as an effective organic source of nitrogen for enhancing mycelium growth in *P. ostreatus*. In contrast, Hoa et al. in 2015 contested this concept and proposed that growth media containing ammonium chloride (NH4Cl)represented the optimal culture medium, particularly the nitrogen concentration, and serves as a means of physiologically controlling and regulating microorganisms. A high nitrogen concentration can result in a low carbon-to-nitrogen ratio (C: N), thereby limiting the mycelium growth of *P. osteatus.*

Conversely, Potato Dextrose Agar combined with Sucrose exhibited the lowest mycelial growth among the treatments, primarily due to the elevated sucrose concentration in the medium. This outcome was attributed to the potential negative impact of osmotic pressure resulting from the increased sucrose concentration, which may hinder metabolite biosynthesis. On the contrary, Bai et al. in 2012 reported that an excessively high carbon concentration could also lead to an elevated C: N ratio, thereby inhibiting mycelium growth. Nonetheless, its effectiveness may vary based on the specific mushroom strain and the conditions of the research.

5.5. Impact of light on the growth parameter of wood ear mushrooms (Auricularia auricula) under different culture media

The significance of light in mycelial growth varies among fungal species and environmental conditions. The influence of light on mycelial growth is species-specific and critical for ecological understanding and cultivation practices (Tisch et al., 2010; Yu et al., 2019). Over the years, extensive research has been dedicated to exploring the regulatory influence of light on various facets of fungal development, encompassing vegetative growth, sexual reproduction, and the biosynthesis of natural compounds. A multitude of investigations have elucidated the distinct effects of light exposure on the asexual growth of macrofungal mycelium.

Prior research by Arjona et al. (2009), revealed that the use of highly-intensity fluorescent light sources had a pronounced inhibitory effect on the expansion of mono- and binucleated mycelium in *Pleurotus ostreatus.* The impact of various light qualities on mycelial growth in *Cordyceps militaris* displayed notable diversity. Mycelia grown under white light exhibited an accelerated growth rate in contrast to culture in the dark, whereas the application of blue light significantly curtailed colony expansion. However, it is noteworthy that regardless of the specific light quality employed, the aerial mycelia density of all strains consistently remained lower in comparison to growth in dark conditions. These findings collectively emphasize the intricate and species-dependent responses of fungal development to varying light conditions.

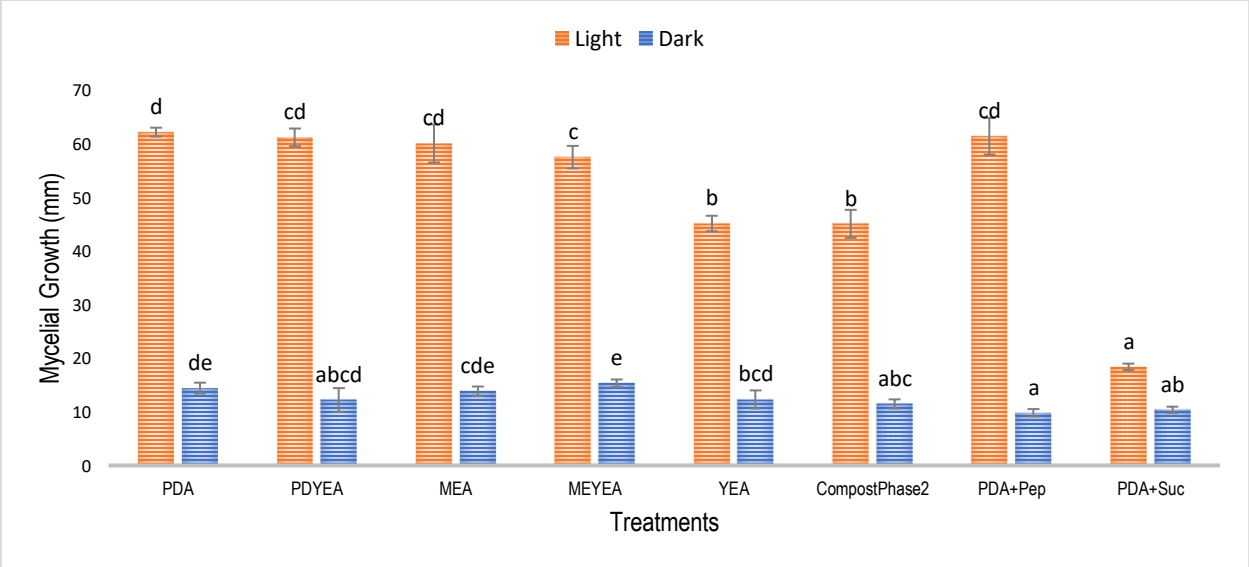

Figure 6. Comparison of mycelial growth of wood ear mushroom (*Auricularia auricula-judae*) under eight (8) different culture media stored under different light conditions after thirteen days of inoculation. *Light Conditions: 24/7 Light Condition, Dark Condition*.

PDA-Potato Dextrose Agar; PDYEA- Potato Dextrose Yeast Extract Agar; MEA- Malt Yeast Extract Agar; MEYEA- Malt Extract Yeast Extract Agar; YEA- Yeast Extract Agar, PDA+Pep- 50%Potato Dextrose Agar + 50%Peptone; PDA+Suc- 50% Potato Dextrose Agar + 50% Sucrose.

Figure 6, presents a comprehensive analysis of the mycelial growth of wood ear mushrooms, scientifically known as *Auricularia auricula-judae*, cultivated in eight distinct culture media exposed to varying light conditions, specifically 24/7 light and complete darkness. Notably, mycelial growth thrived significantly more under continuous illumination, recording a growth increase of 44.26% in comparison to growth in the absence of light. When cultured under dark condition, the culture medium MEYEA stood out as the most conducive, with mycelial growth reaching 15.35 mm. Following MEYEA in order of effectiveness were PDA, MEA, YEA, PDYEA, Compost Phase 2, and PDA+Sucrose displaying mycelial growth measurements of 14.44 mm, 13.87 mm, 12.30 mm, 12.22 mm, 11.47 mm, and 10.37 mm, respectively. It's noteworthy that PDA+peptone exhibited the least mycelial growth among treatments in dark conditions, with a diameter of 9.83 mm.

Conversely, in 24/7 light condition, PDA culture media proved to be the most effective, showcasing a mycelial growth diameter of 62.20 mm. It was succeeded by PDA+Peptone (61.42 mm), PDYEA (61.21 mm), MEA (60.07 mm), MEYEA (57.53 mm), YEA (45.15 mm), Compost Phase 2 (45.09 mm), and PDA+ Sucrose with the lowest recorded mycelial growth, measuring 18.39 mm. These findings illuminate substantial variations in mycelial growth across different culture media and underscore the influence of light conditions on the growth patterns of *Auricularia auricula-judae.*

When subjected to continuous 24/7 light exposure (Table 6), a conspicuous divergence in mycelial growth emerged among treatments, with PDA+Sucrose, Compost Phase 2, YEA, MEA, and PDA demonstrating highly significant distinctions. The pairwise comparison analysis (see Figure 7, highlights substantial variations in mycelial growth, with nearly all treatments exhibiting high significance when compared to each other. Nevertheless, upon closer examination, specific treatment pairs such as PDYEA-PDA, MEA-PDA, YEA-Compost Phase 2, MEYEA compared to MEA, and PDYEA, PDA+Peptone compared to MEYEA, MEA, PDYEA, and PDA revealed no significant differences between them, as indicated by a p-value > 0.05.

Table 8. Effect of different light conditions (24/7 light condition; dark condition) on the mycelial growth of wood ear mushroom *(Auricularia auricula-judae)* thirteen (13) days after inoculation stored under ambient condition.

Treatments	Mycelial Growth (mm) 13 DAI	
	Dark Condition	24/7 Light Condition
PDA	14.44±1.01 de	62.20±0.82 d
PDYEA	12.22±2.21 abcd	61.21±0.62 cd
MEA	13.87±0.85 cde	60.07±3.55 cd
MEYEA	15.35±0.69 e	57.53±2.10 c
YEA	12.30±1.70 bcd	45.15±1.44 b
Compost Phase 2	11.47±0.86 abc	45.09±2.61 b
PDA+Peptone	9.83±0.68 a	61.42±3.42 cd
PDA+Sucrose	10.37±0.59 ab	18.39±0.62 a

Mean within the same column followed by the same letters are not significantly different at $p<0.05$. Each value is expressed as standard error (SE) (n=5).

Additionally, the MEYEA study revealed a noteworthy and statistically significant difference in mycelial growth under dark conditions, particularly when compared to PDA+Peptone. A more detailed examination through pairwise comparisons of treatments figure 7, unveiled highly significant differences, specifically observed between PDA+Peptone and PDA+Sucrose when compared to MEA, MDA, and MEYEA. Moreover, distinctions were evident in comparisons between Compost Phase 2 and PDA, as well as MEYEA with YEA and MEYEA; and PDYEA with MEYEA, all demonstrating a p-value < 0.05.

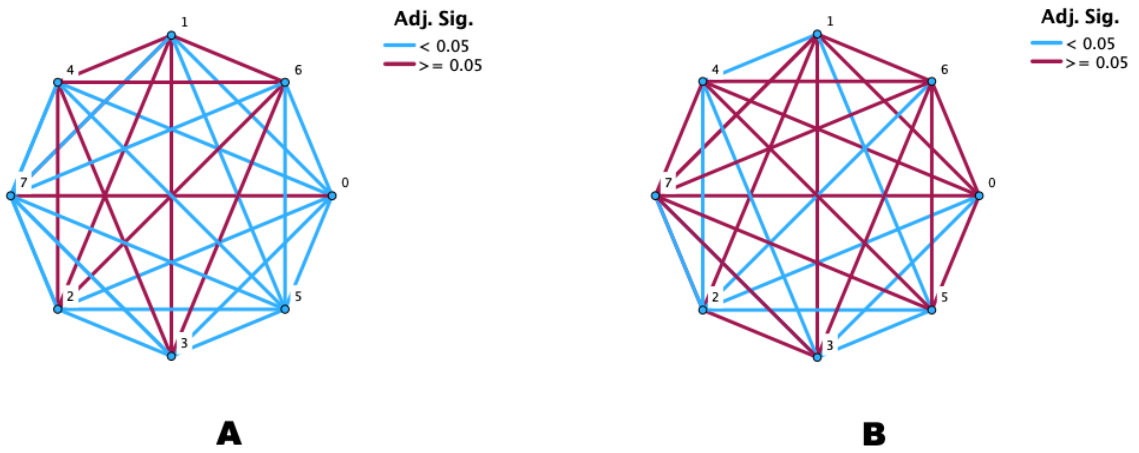

Figure 7. Pairwise Comparison of Treatments on the effect of different light conditions on the mycelial growth of wood ear mushroom *(Auricularia auricula-judae)* grown under different culture media thirteen days after inoculation under ambient conditions. *A: 24/7 light condition; B: dark condition. .0-Compost Phase 2; 1-MEA; 2-MEYEA; 3-PDA; 4-PDA+Peptone; 5-PDA+Sucrose; 6-PDYEA; 7-YEA. Each node shows the sample average rank of treatments.*

The outcomes of these experiments align with findings from Girmay et al. (2016), who observed that the presence of light resulted in a greater than 100% increase in the production of fruiting bodies compared to instances where fully developed fruiting bodies were cultivated under limited light conditions. The variations in the quantity of fruiting bodies produced under restricted light conditions suggested distinctive dynamics in mycelium development. In accordance with this, Priya and Geetha (2016) noted in their investigation that artificial light is more efficacious than darkness in promoting the mycelial growth of wood ear mushrooms. Additionally, Vidyaresmi (2008) reported that artificial or room light led to the highest mycelial growth and biomass production of Auricularia spp. when compared to dark conditions.

Bermudes et al. (1990) discovered that exposure to ambient fluorescent lighting resulted in reduced growth and diminished bioluminescence in *P.stipticus.* Lingle (1993) similarly observed that ambient light conditions had an impact on *P. stipticus.* In the current investigation, a distinct concentric mycelial growth zone for *Auricularia auricula-judae* under continuous 24-light conditions was observed. Research has demonstrated that light does indeed affect the growth rate

of fungi (Tan K.K., 1978), although it has been advised to interpret the effects of light on fungal growth with caution. (Griffin D.H., 1994). In contrast, Berliner (1961) found that diurnal periodicity in *A. mellea* and *P..stipticus* remained unaffected regardless of whether the culture was incubated under continuous 24-hour darkness, uninterrupted 24-hour light, or a 12-hour light/12-hour dark cycle. One possible explanation for the disparities between this study and those employing ambient light conditions is the marked difference in the intensity and spectral characteristics of the light used.

5.6. Identifying the optimal pH for promoting mycelial growth of wood ear mushrooms (Auricularia auricula) using different culture media

The pH of the culture media plays a crucial role in influencing the mycelial growth of fungi, as highlighted by Kibar and Peksen in their 2011 study. Generally, mushrooms exhibit a wide tolerance for pH variations within the culture medium. As demonstrated in Table xx., the mycelial diameter varies at different pH levels within the culture media. It is evident that various macrofungi species can thrive at varying pH levels, encompassing slightly acidic and neutral conditions. Notably, a majority of mushroom species achieve optimal mycelial diameter and substantial mycelial density when cultivated in a pH range that spans from slightly acidic to neutral (approximately pH 6.0- 7.0).

When Auricularia mycelia were grown in various culture media with a pH of 5.0, the data presented in Table X indicates that PDA, adjusted to pH 5.0, resulted in the most substantial mycelial growth among the tested treatments, with an average diameter of 52.23 mm. At pH 5.5, the culture media, particularly PDYEA, exhibited significant mycelial growth, reaching the highest average diameter at 52.58 mm. At a pH of 6.0, MEYEA emerged as the most favorable culture medium for mycelial growth, displaying an average diameter of 52.32 mm.

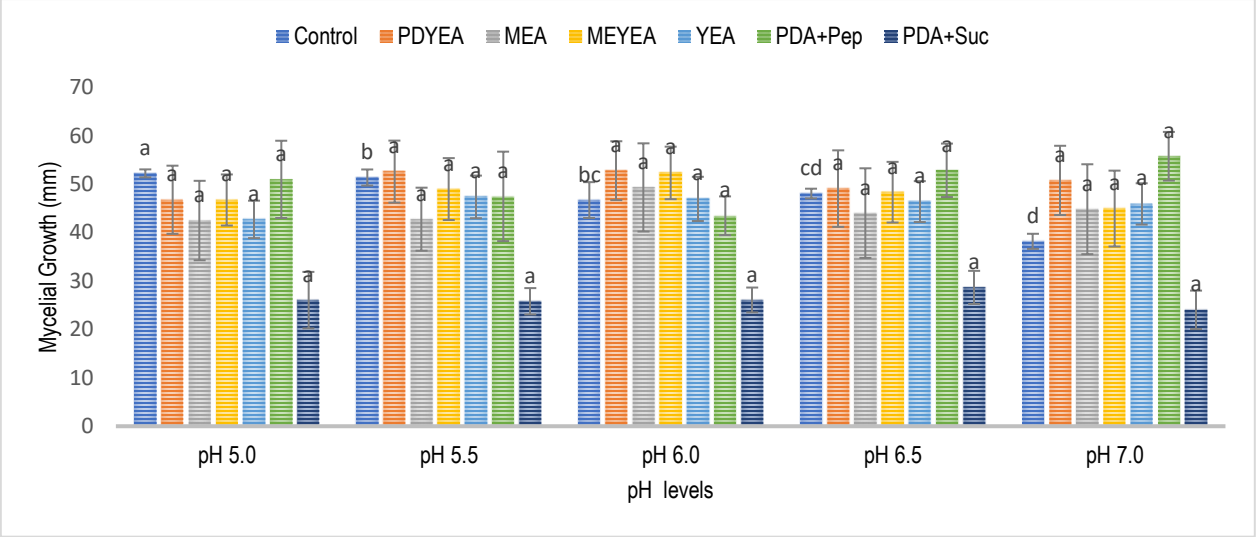

Figure 8. Comparison of mycelial growth of wood ear mushroom (*Auricularia auricula-judae*) under eight different culture media stored under different pH levels after thirteen days of inoculation.*PDA-Potato Dextrose Agar; PDYEA- Potato Dextrose Yeast Extract Agar; MEA- Malt Yeast Extract Agar; MEYEA- Malt Extract Yeast Extract Agar; YEA-Yeast Extract Agar, PDA+Pep- 50%Potato Dextrose Agar + 50%Peptone; PDA+Suc-50% Potato Dextrose Agar + 50% Sucrose.*

For culture media with a pH of 6.5, mycelial growth varied from 28.67 mm to 52.85 mm. Notably, PDA+Peptone consistently showed the lowest mycelial growth, averaging a diameter of 28.67 mm. A similar trend was observed at a pH of 7.0, where PDA+Peptone consistently exhibited the lowest mycelial density, with an average diameter of 24.03 mm. In this scenario, the other culture media demonstrated mycelial growth ranging from 38.19 mm to 55.76 mm, with Compost Phase 2 being the most effective, displaying an average mycelial growth of 55.76 mm. Statistical analysis revealed significant differences (p-value < 0.05) among all culture media with pH values of 5.00, 5.5, and 7.0. However, no significant difference was observed between treatments with PDA and PDA+Sucrose at pH 6.0 and pH 6.5 with p-value >0.05.

The data (Figure 9) indicates a highly significant difference (p-value < 0.001) when comparing PDA+Sucrose with MEA, YEA, PDA, Control, MEYEA, PDA+Peptone, and PDYEA. Additionally, through pairwise comparisons of treatments, it is evident that Compost Phase 2 exhibits a highly significant difference (p-value < 0.001) when compared with Control, MEYEA, PDA+Peptone, and PDYEA. Our results also align with the research of Yang et al. (2003), indicating that mycelia from various mushroom species can thrive across a broad pH spectrum.

Imtiaj et al. (2008) noted that mushrooms exhibit adaptability to pH levels between 5 and 9 for mycelial growth, with an optimal range typically falling between pH 6 and 7.

Table 9. Effect of different pH levels on the mycelial growth of wood ear mushroom *(Auricularia auricula-judae)* thirteen (13) days after inoculation stored under ambient condition.

Treatments	Mycelial growth 13 DAI				
	pH 5.0	pH 5.5	pH 6.0	pH 6.5	pH 7.0
PDA	52.23±0.82 a	51.37±1.66 b	46.72±3.70 bc	48.05±1.00 cd	38.19±1.56 d
PDYEA	46.78±7.04 a	52.58±6.41 a	52.77±6.08 a	49.08±7.90 a	50.77±7.15 a
MEA	42.48±8.21 a	42.76±6.51 a	49.32±9.11 a	44.02±9.26 a	44.82±9.29 a
MEYEA	46.73±5.27 a	48.98±6.39 a	52.32±5.43 a	48.33±6.26 a	44.95±7.83 a
YEA	42.71±3.87 a	47.36±4.40 a	46.95±4.57 a	46.39±4.23 a	45.91±4.27 a
Compost Phase 2	50.98±7.98 a	47.47±9.24 a	43.47±4.00 a	52.85±5.52 a	55.76±5.02 a
PDA+Peptone	26.03±5.84 a	25.81±2.72 a	26.08±2.57 a	28.67±3.42 a	24.03±3.98 a
PDA+Sucrose	26.03±5.84 a	25.81±2.71 b	26.08±2.57 bc	49.08±7.90 cd	50.77±7.15 d

Mean within the same column followed by the same letters are not significantly different at p<0.05. Each value is expressed as standard error (SE) (n=5).

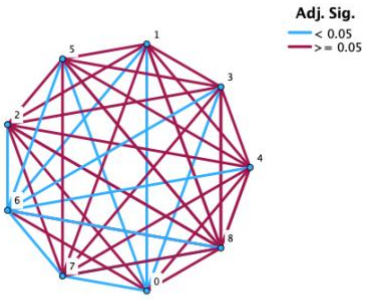

Figure 9. Pairwise Comparison Of Treatments On The Effect Of Different pH Levels On The Mycelial Growth Of Wood Ear Mushroom (*Auricularia Auricula-Judae)* Grown Under Different Culture Media Thirteen Days After Inoculation At Ambient Condition. *.0-Compost Phase 2; 1-MEA; 2-MEYEA; 3-PDA; 4-PDA+Peptone; 5-PDA+Sucrose; 6-PDYEA; 7-YEA. Each Node Shows The Sample Average Rank Of Treatments.*

Jo et al. (2009) found that pH levels of 6 to 9 were suitable for the growth of *Ganoderma applanatum.* Similarly, Chandana et al. (2008) reported that the Korean strain of G. lucidum could flourish within a wide pH range from 5 to 9, with the best growth occurring at pH 5. Other studies by Chandra and Pukayastha (1997) and Jonathan et al. (2007) confirmed that *A. campestris* and *V. esculenta* exhibited high-quality mycelial growth at pH 6. Lastly, Fasola et al. (2007) demonstrated that the mycelial growth of *Volvariella speciosa* was viable across a pH range of 3 to 9.

According to Guiling and Fuwen (1988), that the best pH for the mycelial growth of Jews ear mushroom was found to be between pH 5.0- pH 6.0. Sing et al. (2000) identified pH 6.0 as the appropriate pH level for Auricularia polytricha and other edible mushrooms. However, Keun Yang et al. (2002) found that the optimum pH for Auricularia polytricha is pH 4.0. The pH of the medium is crucial, as indicated by studies conducted by Garasiya et al. (2007), which revealed that Auricularia polytricha cannot thrive in an alkaline substrate. Therefore, in cultivation, both the substrate and water pH should be maintained within the range of pH 6.0 to pH 6.5.

5.7. Influence of different types of Carbon Sources on the mycelial growth of wood ear mushrooms (Auricularia auricula) under different culture media

The choice of carbon source in the growth medium of Auricularia can significantly influence mycelial growth. Variations in carbon sources affect growth rate, biomass yield, morphological characteristics, nutritional quality, and susceptibility to contamination. Experimentation is often conducted to determine the most suitable carbon source and associated growth conditions for optimal mycelial development.

Sucrose as a disaccharide comprised of glucose and fructose, stands out as a preferred carbon sources due to its capacity to deliver readily accessible energy. It can be efficiently metabolized by mushroom mycelia, facilitating robust growth. Maltose is another disaccharide composed of two glucose molecules, it’s a suitable carbon source since it readily breaks down into glucose, the primary energy source for mycelial development. Lactose, a disaccharide composed of glucose and galactose, served as a carbon source, albeit with variation in its suitability across mushroom species. Its utilization hinges on the specific fungus’s enzymatic capabilities. Fructose, a monosaccharide commonly found in fruits and honey, is less frequently employed as a principal carbon source for mushroom mycelial growth. Some mushroom species may not efficiently metabolize fructose, reflecting differences in their metabolic pathways. Glucose, a monosaccharide

represents a primary energy source for mushroom mycelium and is often regarded as a dependable and effective carbon source for promoting mycelial growth.

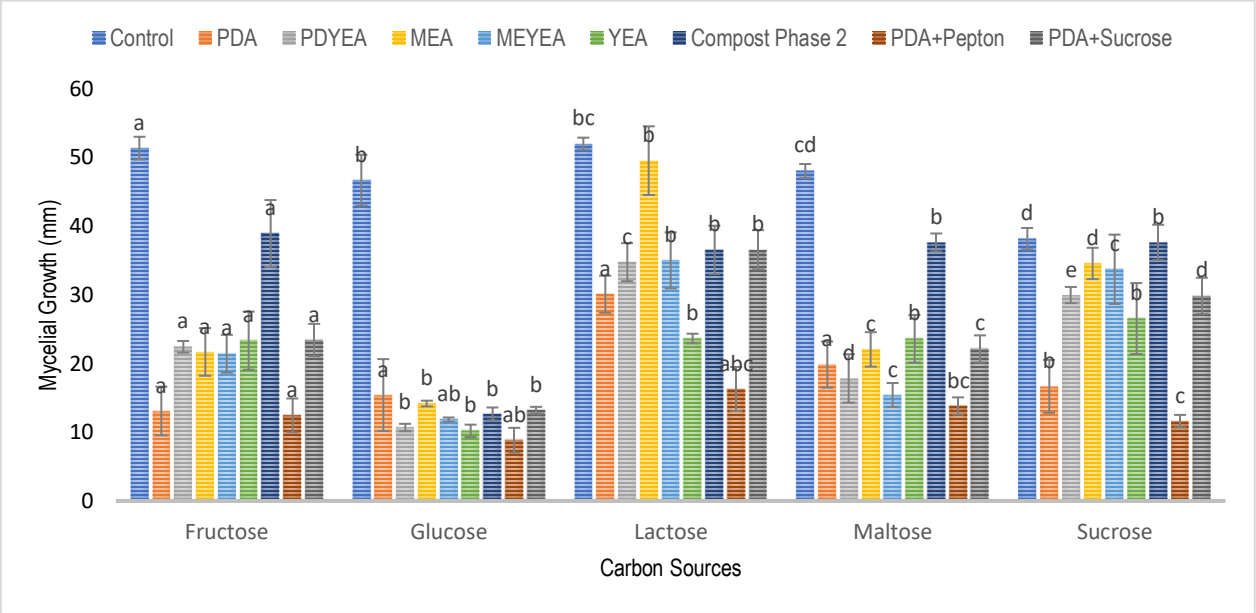

Figure 10. Comparison of mycelial growth of wood ear mushroom (*Auricularia auricula-judae)* grown under different culture media with different types of Carbon Sources after fifteen days of inoculation. (*PDA-Potato Dextrose Agar; PDYEA- Potato Dextrose Yeast Extract Agar; MEA- Malt Yeast Extract Agar; MEYEA- Malt Extract Yeast Extract Agar; YEA- Yeast Extract Agar, PDA+Pep- 50%Potato Dextrose Agar + 50%Peptone; PDA+Suc- 50% Potato Dextrose Agar + 50% Sucrose.*

The mycelial growth of various mushroom species demonstrates adaptability to a broad spectrum of carbon sources. To identify the optimal carbon source, diverse carbohydrates, including lactose, fructose, glucose, maltose, and sucrose, were introduced into different culture media, and the outcomes are detailed in Table 10. The results shed light on the suitability of different carbon sources for fostering mycelial growth in wood ear mushrooms. Statistical analysis reveals a significant variance in mycelium growth across mushrooms cultivated on distinct carbon sources.

Table 10. Effect of different carbon sources on the mycelial growth of wood ear mushroom *(Auricularia auricula-judae)* fifteen (13) days after inoculation stored under ambient conditions.

Treatments	Mycelial growth (mm) 15 DAI				
Statistic	Sucrose	Maltose	Glucose	Fructose	Lactose
Control	38.19±1.56 d	48.05±1.00 cd	46.72±3.70 b	51.37±1.66 a	52.02±0.88 bc
PDA	16.69±3.85 b	19.86±3.36 a	15.42±5.23 a	13.09±3.54 a	30.10±2.70 a
PDYEA	29.98±1.18 e	17.84±3.52 d	10.69±0.54 b	22.44±0.85 a	34.77±2.77 c
MEA	34.58±2.28 d	22.07±2.51 c	14.18±0.41 b	21.70±3.47 a	49.55±5.00 b
MEYEA	33.73±5.05 c	15.42±1.75 c	11.85±0.31 ab	21.43±2.76 a	35.02±0.31 b
YEA	26.56±5.16 b	23.62±3.47 b	10.21±0.90 b	23.34±4.23 a	23.65±0.70 b
Compost Phase 2	37.64±2.28 b	37.63±1.32 b	12.66±0.95 b	38.95±4.86 a	36.57±3.51 b
PDA+Peptone	11.61±0.92 c	13.83±1.25 bc	8.84±2.47 ab	12.46±2.47 a	16.30±3.21 abc
PDA+Sucrose	29.87±2.63 d	22.15±1.96 c	13.20±0.50 b	23.42±2.37 a	36.58±2.86 b

Mean within the same column followed by the same letters are not significantly different at $p<0.05$. Each value is expressed as standard error (SE) (n=5).

The incorporation of diverse carbon sources into the culture media exerted discernible effects on mycelial growth, as evidenced by the findings presented in Table XX. Notably, the Control exhibited the highest mycelial growth in this experiment. However, within the same table, it is evident that Compost Phase 2 with lactose concentration, Compost Phase 2 with maltose, PDA with glucose, Compost Phase 2 with fructose, and MEA with lactose exhibited the most substantial mycelial growth 15 days post-inoculation.

Sucrose and fructose, when employed as carbon sources in various culture media, yielded favorable results, with all media displaying highly significant differences between treatments (p-value < 0.05). Conversely, the addition of maltose to the culture media resulted in PDA+Peptone showing no significant difference among treatments, similar to the outcomes observed with glucose in MEYEA and lactose in PDA+Peptone, all with a p-value > 0.05. Statistical analysis employed Pairwise Comparison of Treatments to determine the optimal carbon source. Figure X revealed that, in comparisons such as Glucose versus Fructose, Sucrose, Lactose, and Maltose, as well as

Fructose with Lactose and Lactose with Maltose, there were highly significant differences between treatments, as indicated by a p-value<0.05.

Upadhyay (2003) identified glucose and fructose as outstanding carbon sources for promoting the growth of Auricularia polytricha. Carbon is a crucial factor influencing the growth of all microorganisms, including the mycelial growth of Auricularia polytricha. Garasiya et al. (2007) conducted studies indicating that the highest dry mycelial weight of Auricularia polytricha was observed in starch. In 2003, Upadhay also discovered glucose and fructose as excellent carbon sources for fostering the growth of Auricularia spp.

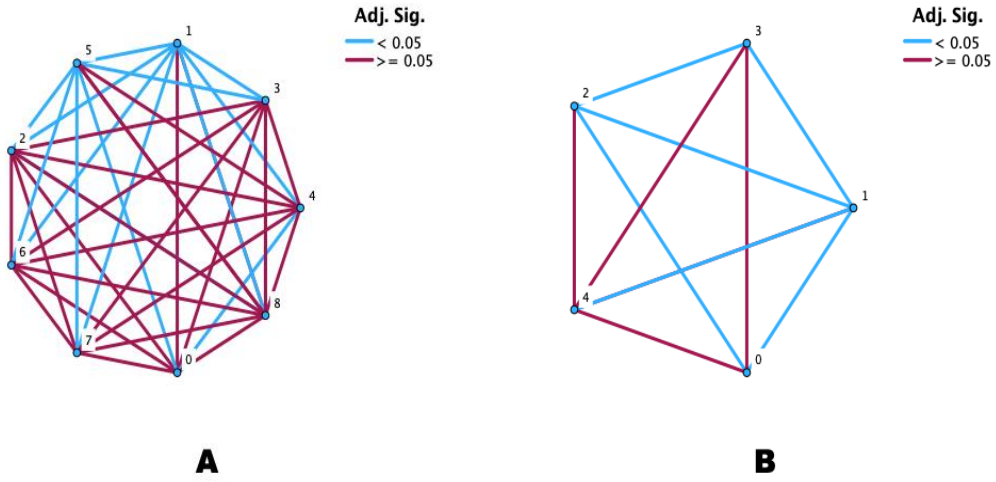

Figure 11. Pairwise Comparison Of Treatments On The Effect Of Different Carbon Sources On The Mycelial Growth Of Wood Ear Mushroom (Auricularia Auricula-Judae) Grown Under Different Culture Media Fifteen Days After Inoculation At Ambient Condition.*A- Comparison of Treatments; B-Comparison of Carbon Sources (A) .0-Compost Phase 2; 1-MEA; 2-MEYEA; 3-PDA; 4-PDA+Peptone; 5-PDA+Sucrose; 6-PDYEA; 7-YEA (B).0-Fructose; 1-Glucose; 2-Lactose; 3-Maltose; 4-Sucrose. . Each Node Shows The Sample Average Rank Of Treatments.*

5.8. Mycelial Growth of Wood ear Mushroom *(Auricularia auricla-judae)* under different culture media with a combination of sucrose and peptone.

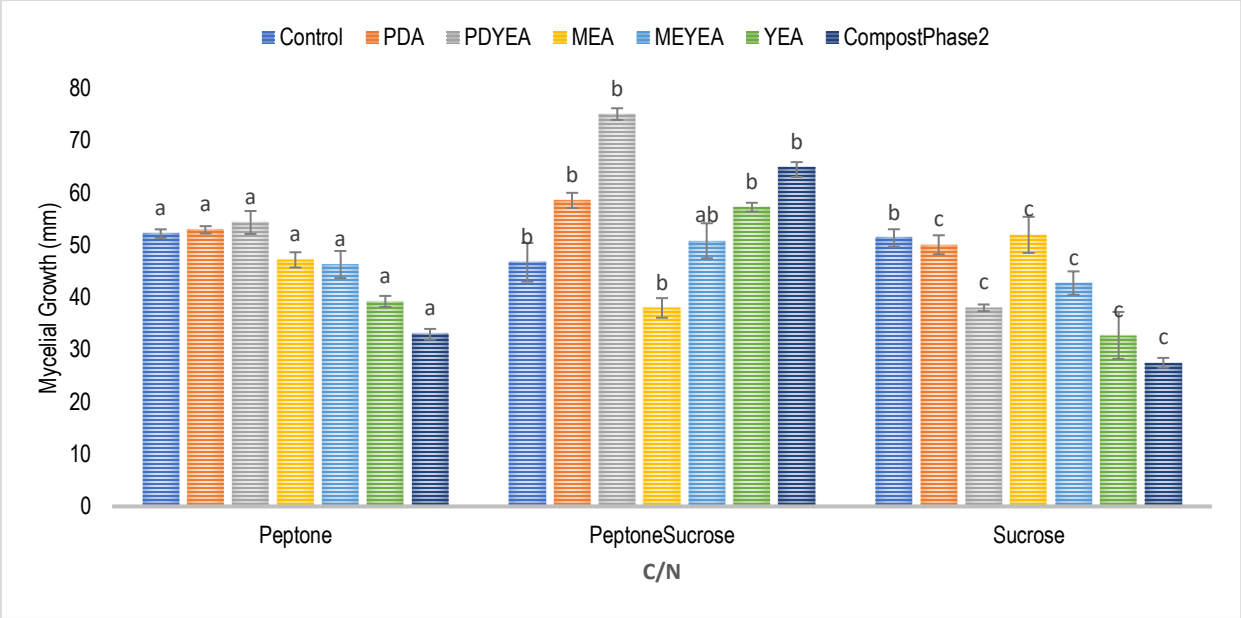

Figure 12. Comparison of mycelial growth of wood ear mushroom (*Auricularia auricula-judae)* grown under different culture media with peptone, sucrose and their combination after eight days of inoculation.(*PDA-Potato Dextrose Agar; PDYEA- Potato Dextrose Yeast Extract Agar; MEA- Malt Yeast Extract Agar; MEYEA- Malt Extract Yeast Extract Agar; YEA- Yeast Extract Agar, PDA+Pep- 50%Potato Dextrose Agar + 50%Peptone; PDA+Suc- 50% Potato Dextrose Agar + 50% Sucrose.*

In this investigation, we utilized sucrose as the source of carbon and peptone as the nitrogen source, employing five repetitions. The culture media were formulated with a 50% concentration of each component; one set involved a combination with 50% sucrose, while the other set involved a combination with 50% peptone. Additionally, a novel parameter was introduced, where a 50% concentration of culture medium was blended with 25% sucrose and 25% peptone, again with five repetitions each. The findings, as delineated in Table XX, illustrate the growth of Auricularia mycelia under different combinations of carbon sources, nitrogen sources, and culture media. PDYEA exhibited the most substantial mycelial growth, with an average diameter of 54.33 mm, when integrated with peptone. Conversely, MEA emerged as the most effective culture medium when paired with a 50% concentration of sucrose. Notably, the combination of 25% sucrose and

25% peptone with YEA resulted in the most significant mycelial growth among the treatments, with an average diameter reaching 57.25 mm.

Table 11. Effect of different Peptone, Carbon and Their Combination on the mycelial growth of wood ear mushroom *(Auricularia auricula-judae)* eight (8) days after inoculation stored under ambient conditions.

	Mycelial Growth 8 DAI		
Treatments	Peptone	Peptone:Sucrose	Sucrose
Control	52.20±0.82 a	46.72±3.70 b	51.37±1.66 b
PDA	52.92±0.70 a	58.55±1.45 b	50.06±1.82 c
PDYEA	54.33±2.19 a	75.06±1.12 b	38.00±0.64 c
MEA	47.17±1.46 a	37.98±1.87 b	51.96±3.45 c
MEYEA	46.28±2.60 a	50.82±3.36 ab	42.74±2.23 b
YEA	39.22±1.06 a	57.25±0.85 b	32.72±4.48 c
Compost Phase 2	33.03±1.10 a	64.94±1.91 b	27.49±0.93 c

Mean within the same column followed by the same letters are not significantly different at p<0.05. Each value is expressed as standard error (SE) (n=5).

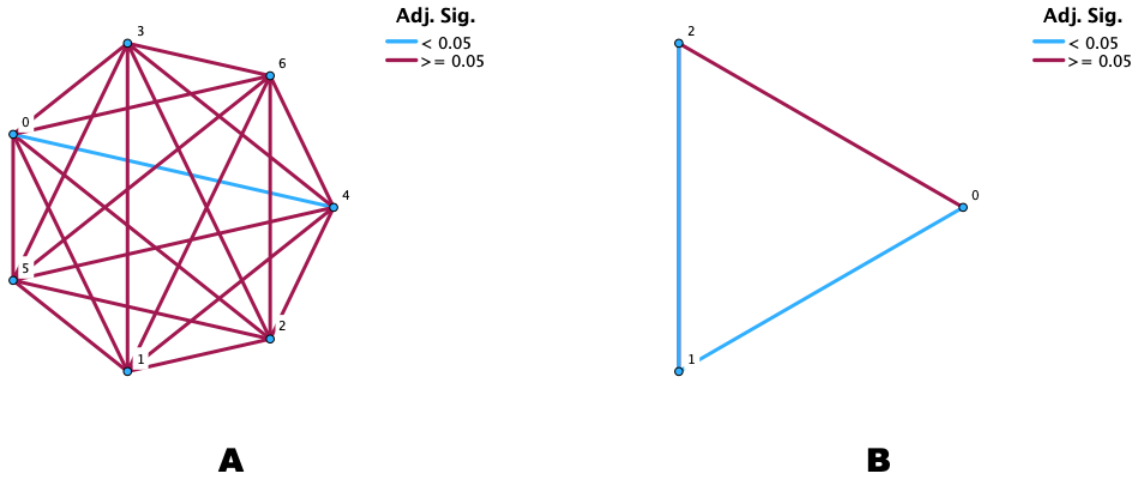

Figure 13. Pairwise Comparison Of Treatments On The Effect Of Different Carbon Sources On The Mycelial Growth Of Wood Ear Mushroom (*Auricularia Auricula-Judae)* Grown Under Different Culture Media Fifteen Days After Inoculation At Ambient Condition.A- Comparison of Treatments; B-Comparison of Carbon Sources. (A) .0-Compost Phase 2; 1-MEA; 2-MEYEA; 3-PDA; 4-PDA+Peptone; 5-PDA+Sucrose; 6-PDYEA; 7-YEA (B).0-Fructose; 1-Glucose; 2-Lactose; 3-Maltose; 4-Sucrose. B).0-Peptone; 1-Peptone:Sucrose; 2-Sucrose . Each Node Shows The Sample Average Rank Of Treatments.

6. CONCLUSION

Intriguingly, the study uncovered the profound influence of light conditions on mycelial growth, suggesting a hirherto underexplored aspects of fungal cultivation. The revelation that continuous light exposure significantly enhances mycelial production underscores the importance of optimizing environmental factors for maximizing the yield of this valuable mushroom species.

The pH sensitivity of the wood ear mushroom adds a layer of complexity to cultivation. While the study identified the range of pH conducive to ideal mycelial density, the unique response of PDA+Peptone at different pH levels raises questions about the interplay between culture media composition and pH in fungal development. This nuanced understanding could pave the way for tailored cultivation practices based on specific strains and desired outcomes.

The synergy between carbon and nitrogen sources emerged as a critical factor, with Lactose and MEA standing out as a potent combination for mycelial growth. The suggestion to explore a broader spectrum of nitrogen sources opens up possibilities for fine-tuning nutrient ratios, potentially enhancing the efficiency and reproducibility of wood ear mushroom cultivation.

Moreover, the study's call for further investigations into temperature variations adds a dynamic dimension to the research. Delving into the effects of different temperature ranges on mycelial growth could uncover valuable insights into the adaptability and resilience of Auricularia mesenterica, offering practical guidance for cultivation in diverse climatic conditions.

In essence, this comprehensive study not only provides practical recommendations for optimizing wood ear mushroom cultivation but also invites the scientific community to delve deeper into the intricacies of fungal biology. The potential applications of this research extend beyond the realms of agriculture, with implications for pharmaceuticals, nutraceuticals, and sustainable food production. The wood ear mushroom, once relegated to traditional medicine and culinary practices, now stands at the forefront of scientific exploration, promising a myriad of benefits for both human health and ecological sustainability.

7. SUMMARY

The study aimed to investigate the effects of various culture media on the in vitro propagation of wood ear mushrooms (Auricularia auricula). Eight different culture media were evaluated, including Potato Dextrose Agar (PDA), Potato Dextrose Yeast Extract Agar (PDYEA), Malt Extract Agar (MEA), Malt Yeast Extract Agar (MYEA), Yeast Extract Agar (YEA), Compost Phase 2 Agar, PDA + Peptone, and PDA + Sucrose. The mycelial growth of Auricularia auricula-judae was measured after 13 days of inoculation, revealing that PDA and PDA + Peptone were the most effective culture media, with the highest growth rates.

Statistical analysis demonstrated significant differences in mycelial diameter among the various culture media. Pairwise comparisons highlighted specific treatments, such as Malt Yeast Extract Agar, Potato Dextrose Agar, and PDA + Sucrose, showing high statistical significance compared to PDA + Peptone. The study also explored the impact of light conditions on mycelial growth, revealing that continuous illumination significantly enhanced growth compared to complete darkness.

Furthermore, the study investigated the influence of different pH levels on mycelial growth, indicating that PDA at pH 5.0, PDYEA at pH 5.5, and MEYEA at pH 6.0 were optimal for mycelial growth. The results aligned with previous studies, emphasizing the importance of maintaining a pH range of 6.0 to 6.5 for successful cultivation. Finally, the study explored the impact of various carbon sources on mycelial growth, with sucrose and fructose proving to be effective carbon sources. Overall, the findings provide valuable insights into optimizing culture media for the in vitro propagation of wood ear mushrooms, contributing to advancements in scientific knowledge and sustainable agricultural practices.

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Lastly, but certainly not least, this achievement is dedicated to my mom. Your unwavering belief in my capabilities has been my anchor throughout this academic journey. Mom, this is for you—thank you for being my constant source of inspiration and strength.

As I reflect on this significant milestone, I am filled with gratitude for the collective support, love, and opportunities that have shaped my postgraduate experience. To everyone who has been a part of this journey, thank you from the depths of my heart.

To every believer, may "HIRAYA MANAWARI" be granted.

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10. ANNEX: TABLES AND FIGURES

Table 12. Pairwise Comparison of Treatments on the effect of different culture media on the mycelial growth of wood ear mushroom (*Auricularia auricula-judae*) thirteen days after inoculation in an ambient condition.

Pairwise Comparisons of Treatments

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
PDA+Suc-CompostPhase2	6.400	7.394	.866	.387	1.000
PDA+Suc-YEA	-8.600	7.394	-1.163	.245	1.000
PDA+Suc-MEA	18.200	7.394	2.462	.014	.387
PDA+Suc-MEYEA	19.400	7.394	2.624	.009	.243
PDA+Suc-PDA+Pep	27.800	7.394	3.760	<.001	.005
PDA+Suc-PDYEA	-28.600	7.394	-3.868	<.001	.003
PDA+Suc-PDA	31.000	7.394	4.193	<.001	.001
CompostPhase2-YEA	-2.200	7.394	-.298	.766	1.000
CompostPhase2-MEA	-11.800	7.394	-1.596	.110	1.000
CompostPhase2-MEYEA	-13.000	7.394	-1.758	.079	1.000
CompostPhase2-PDA+Pep	-21.400	7.394	-2.894	.004	.106
CompostPhase2-PDYEA	-22.200	7.394	-3.003	.003	.075
CompostPhase2-PDA	-24.600	7.394	-3.327	<.001	.025
YEA-MEA	9.600	7.394	1.298	.194	1.000
YEA-MEYEA	10.800	7.394	1.461	.144	1.000
YEA-PDA+Pep	19.200	7.394	2.597	.009	.263
YEA-PDYEA	20.000	7.394	2.705	.007	.191
YEA-PDA	22.400	7.394	3.030	.002	.069
MEA-MEYEA	-1.200	7.394	-.162	.871	1.000
MEA-PDA+Pep	-9.600	7.394	-1.298	.194	1.000
MEA-PDYEA	-10.400	7.394	-1.407	.160	1.000
MEA-PDA	-12.800	7.394	-1.731	.083	1.000
MEYEA-PDA+Pep	-8.400	7.394	-1.136	.256	1.000
MEYEA-PDYEA	-9.200	7.394	-1.244	.213	1.000
MEYEA-PDA	-11.600	7.394	-1.569	.117	1.000
PDA+Pep-PDYEA	-.800	7.394	-.108	.914	1.000
PDA+Pep-PDA	3.200	7.394	.433	.665	1.000
PDYEA-PDA	2.400	7.394	.325	.745	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .050.
a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 13. Pairwise Comparison of Light Conditions on the mycelial growth of wood ear mushroom *(Auricularia auricula-judae)* grown under different culture media thirteen days after inoculation under ambient conditions.

Pairwise Comparisons of Treatments				
Light Conditions	Sample 1-Sample 2	Test Statistic	Sig.	Adj. Sig.[a]
Dark	PDA+Pep-PDA+Suc	.400	.527	1.000
	PDA+Pep-CompostPhase2	3.600	.058	1.000
	PDA+Pep-YEA	3.600	.058	1.000
	PDA+Pep-PDYEA	3.600	.058	1.000
	PDA+Pep-MEA	10.000	.002	.044
	PDA+Pep-PDA	10.000	.002	.044
	PDA+Pep-MEYEA	10.000	.002	.044
	PDA+Suc-CompostPhase2	3.600	.058	1.000
	PDA+Suc-YEA	3.600	.058	1.000
	PDA+Suc-PDYEA	3.600	.058	1.000
	PDA+Suc-MEA	10.000	.002	.044
	PDA+Suc-PDA	10.000	.002	.044
	PDA+Suc-MEYEA	10.000	.002	.044
	CompostPhase2-YEA	.400	.527	1.000
	CompostPhase2-PDYEA	.400	.527	1.000
	CompostPhase2-MEA	3.600	.058	1.000
	CompostPhase2-PDA	10.000	.002	.044
	CompostPhase2-MEYEA	10.000	.002	.044
	YEA-PDYEA	.400	.527	1.000
	YEA-MEA	3.600	.058	1.000
	YEA-PDA	3.600	.058	1.000
	YEA-MEYEA	10.000	.002	.044
	PDYEA-MEA	3.600	.058	1.000
	PDYEA-PDA	.400	.527	1.000
	PDYEA-MEYEA	10.000	.002	.044
	MEA-PDA	.400	.527	1.000
	MEA-MEYEA	3.600	.058	1.000
	PDA-MEYEA	3.600	.058	1.000
Light	PDA+Suc-CompostPhase2	10.000	.002	.044
	PDA+Suc-YEA	10.000	.002	.044
	PDA+Suc-PDYEA	10.000	.002	.044

PDA+Suc-MEA	10.000	.002	.044
PDA+Suc-PDA	10.000	.002	.044
PDA+Suc-MEYEA	10.000	.002	.044
CompostPhase2-PDYEA	10.000	.002	.044
CompostPhase2-MEA	10.000	.002	.044
CompostPhase2-PDA	10.000	.002	.044
CompostPhase2-MEYEA	10.000	.002	.044
YEA-PDYEA	10.000	.002	.044
YEA-MEA	10.000	.002	.044
YEA-PDA	10.000	.002	.044
YEA-MEYEA	10.000	.002	.044
PDYEA-PDA	.400	.527	1.000
MEA-PDA	3.600	.058	1.000
PDA+Suc-PDA+Pep	10.000	.002	.044
YEA-CompostPhase2	.400	.527	1.000
YEA-PDA+Pep	10.000	.002	.044
CompostPhase2-PDA+Pep	10.000	.002	.044
MEYEA-MEA	.400	.527	1.000
MEYEA-PDYEA	3.600	.058	1.000
MEYEA-PDA	10.000	.002	.044
MEYEA-PDA+Pep	3.600	.058	1.000
MEA-PDYEA	.400	.527	1.000
MEA-PDA+Pep	.400	.527	1.000
PDYEA-PDA+Pep	.400	.527	1.000
PDA-PDA+Pep	.400	.527	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 14. Pairwise Comparison of Treatments on the effect of different light conditions on the mycelial growth of wood ear mushroom *(Auricularia auricula-judae)* grown under different culture media thirteen days after inoculation under ambient conditions.

Pairwise Comparisons of Treatments

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
PDA+Suc-CompostPhase2	46.620	18.412	2.532	.011	.408
PDA+Suc-MEA	84.340	18.412	4.581	<.001	.000
PDA+Suc-YEA	-95.920	18.412	-5.210	<.001	.000
PDA+Suc-PDA	102.680	18.412	5.577	<.001	.000
PDA+Suc-Control	109.500	18.412	5.947	<.001	.000
PDA+Suc-MEYEA	113.220	18.412	6.149	<.001	.000
PDA+Suc-PDA+Pep	125.840	18.412	6.835	<.001	.000
PDA+Suc-PDYEA	-127.920	18.412	-6.948	<.001	.000
CompostPhase2-MEA	-37.720	18.412	-2.049	.040	1.000
CompostPhase2-YEA	-49.300	18.412	-2.678	.007	.267
CompostPhase2-PDA	-56.060	18.412	-3.045	.002	.084
CompostPhase2-Control	-62.880	18.412	-3.415	<.001	.023
CompostPhase2-MEYEA	-66.600	18.412	-3.617	<.001	.011
CompostPhase2-PDA+Pep	-79.220	18.412	-4.303	<.001	.001
CompostPhase2-PDYEA	-81.300	18.412	-4.416	<.001	.000
MEA-YEA	-11.580	18.412	-.629	.529	1.000
MEA-PDA	-18.340	18.412	-.996	.319	1.000
MEA-Control	25.160	18.412	1.367	.172	1.000
MEA-MEYEA	-28.880	18.412	-1.569	.117	1.000
MEA-PDA+Pep	-41.500	18.412	-2.254	.024	.871
MEA-PDYEA	-43.580	18.412	-2.367	.018	.646
YEA-PDA	6.760	18.412	.367	.714	1.000
YEA-Control	13.580	18.412	.738	.461	1.000
YEA-MEYEA	17.300	18.412	.940	.347	1.000
YEA-PDA+Pep	29.920	18.412	1.625	.104	1.000
YEA-PDYEA	32.000	18.412	1.738	.082	1.000
PDA-Control	6.820	18.412	.370	.711	1.000
PDA-MEYEA	10.540	18.412	.572	.567	1.000
PDA-PDA+Pep	-23.160	18.412	-1.258	.208	1.000
PDA-PDYEA	-25.240	18.412	-1.371	.170	1.000
Control-MEYEA	-3.720	18.412	-.202	.840	1.000
Control-PDA+Pep	-16.340	18.412	-.887	.375	1.000

Control-PDYEA	-18.420	18.412	-1.000	.317	1.000
MEYEA-PDA+Pep	-12.620	18.412	-.685	.493	1.000
MEYEA-PDYEA	-14.700	18.412	-.798	.425	1.000
PDA+Pep-PDYEA	-2.080	18.412	-.113	.910	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 15. Pairwise Comparison Of Treatments On The Effect Of Different pH Levels On The Mycelial Growth Of Wood Ear Mushroom *(Auricularia auricula-judae)* Grown Under Different Culture Media Thirteen Days After Inoculation At Ambient Condition.

Pairwise Comparisons of Treatments			
Sample 1-Sample 2	Test Statistic	Sig.	Adj. Sig.[a]
PDA+Pepton-PDA	9.411	.002	.078
PDA+Pepton-MEYEA	10.101	.001	.053
PDA+Pepton-PDYEA	14.108	<.001	.006
PDA+Pepton-MEA	18.783	<.001	.001
PDA+Pepton-YEA	13.878	<.001	.007
PDA+Pepton-PDA+Sucrose	13.878	<.001	.007
PDA+Pepton-Compost Phase 2	17.936	<.001	.001
PDA+Pepton-Control	44.160	<.001	.000
PDA-MEYEA	1.007	.316	1.000
PDA-PDYEA	4.608	.032	1.000
PDA-MEA	4.608	.032	1.000
PDA-YEA	5.576	.018	.656
PDA-PDA+Sucrose	8.712	.003	.114
PDA-Compost Phase 2	16.333	<.001	.002
PDA-Control	45.158	<.001	.000
MEYEA-PDYEA	.720	.396	1.000
MEYEA-MEA	.080	.777	1.000
MEYEA-YEA	.521	.471	1.000
MEYEA-PDA+Sucrose	.521	.471	1.000
MEYEA-Compost Phase 2	12.745	<.001	.013
MEYEA-Control	35.280	<.001	.000
MEA-PDYEA	.000	1.000	1.000
MEA-YEA	.019	.891	1.000
PDYEA-YEA	.019	.891	1.000

PDYEA-PDA+Sucrose	.521	.471	1.000
PDYEA-Control	42.320	<.001	.000
MEA-PDA+Sucrose	.019	.891	1.000
MEA-Compost Phase 2	5.889	.015	.549
MEA-Control	18.000	<.001	.001
PDYEA-Compost Phase 2	17.153	<.001	.001
YEA-PDA+Sucrose	.364	.546	1.000
YEA-Compost Phase 2	17.076	<.001	.001
YEA-Control	39.637	<.001	.000
PDA+Sucrose-Compost Phase 2	17.076	<.001	.001
PDA+Sucrose-Control	32.615	<.001	.000
Compost Phase 2-Control	19.654	<.001	.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 16. Pairwise Comparison Of Treatments On The Effect Of Different Carbon Sources On The Mycelial Growth Of Wood Ear Mushroom *(Auricularia auricula-judae)* Grown Under Different Culture Media Fifteen Days After Inoculation At Ambient Condition

Pairwise Comparisons of CarbonSource

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Glucose-Maltose	-55.733	13.189	-4.226	<.001	.000
Glucose-Fructose	58.488	13.431	4.355	<.001	.000
Glucose-Sucrose	-78.826	13.266	-5.942	<.001	.000
Glucose-Lactose	-104.530	13.347	-7.832	<.001	.000
Maltose-Fructose	2.755	13.503	.204	.838	1.000
Maltose-Sucrose	-23.093	13.340	-1.731	.083	.834
Maltose-Lactose	48.797	13.420	3.636	<.001	.003
Fructose-Sucrose	-20.337	13.579	-1.498	.134	1.000
Fructose-Lactose	-46.042	13.658	-3.371	<.001	.007
Sucrose-Lactose	25.704	13.496	1.905	.057	.568

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 17. Pairwise Comparison Of Treatments On The Effect Of Different Carbon Sources On The Mycelial Growth Of Wood Ear Mushroom *(Auricularia auricula-judae)* Grown Under Different Culture Media Fifteen Days After Inoculation At Ambient Condition

Pairwise Comparisons of Treatments

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
CompostPhase2-YEA	-4.567	11.121	-.411	.681	1.000
CompostPhase2-MEA	-6.700	11.121	-.602	.547	1.000
CompostPhase2-MEYEA	-11.467	11.121	-1.031	.302	1.000
CompostPhase2-Control	-21.267	11.121	-1.912	.056	1.000
CompostPhase2-PDYEA	-30.467	11.121	-2.740	.006	.129
CompostPhase2-PDA	-36.133	11.121	-3.249	.001	.024
YEA-MEA	2.133	11.121	.192	.848	1.000
YEA-MEYEA	6.900	11.121	.620	.535	1.000
YEA-Control	16.700	11.121	1.502	.133	1.000
YEA-PDYEA	25.900	11.121	2.329	.020	.417
YEA-PDA	31.567	11.121	2.839	.005	.095
MEA-MEYEA	-4.767	11.121	-.429	.668	1.000
MEA-Control	14.567	11.121	1.310	.190	1.000
MEA-PDYEA	-23.767	11.121	-2.137	.033	.684
MEA-PDA	-29.433	11.121	-2.647	.008	.171
MEYEA-Control	9.800	11.121	.881	.378	1.000
MEYEA-PDYEA	-19.000	11.121	-1.709	.088	1.000
MEYEA-PDA	-24.667	11.121	-2.218	.027	.557
Control-PDYEA	-9.200	11.121	-.827	.408	1.000
Control-PDA	-14.867	11.121	-1.337	.181	1.000
PDYEA-PDA	5.667	11.121	.510	.610	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 18**.** Pairwise Comparison Of Carbon On The Mycelial Growth Of Wood Ear Mushroom *(Auricularia auricula-judae)* Grown Under Different Culture Media Fifteen Days After Inoculation At Ambient Condition

Pairwise Comparisons of C:N Ratio

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Sucrose-Peptone	13.757	7.280	1.890	.059	.176
Sucrose-PeptoneSucrose	36.000	7.280	4.945	<.001	.000
Peptone-PeptoneSucrose	-22.243	7.280	-3.055	.002	.007

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .050.
a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

ANNEX III: DECLARATION

DECLARATION

Me, as the undersigned **KENNETH AVILA RENDORA**; Code-Neptun:**RFN07Q,** declare, that the Diploma Thesis entitled “Optimizing In Vitro Mycelial Growth of Wood Ear Mushroom (Auricularia auricula)” submitted on 13 November 2023 is my own intellectual property.

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signature of the student

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