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The effect of substrate selection on the cultivation of edible mushrooms

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تأثير اختيار الركائز على زراعة الفطر الصالح للأكل

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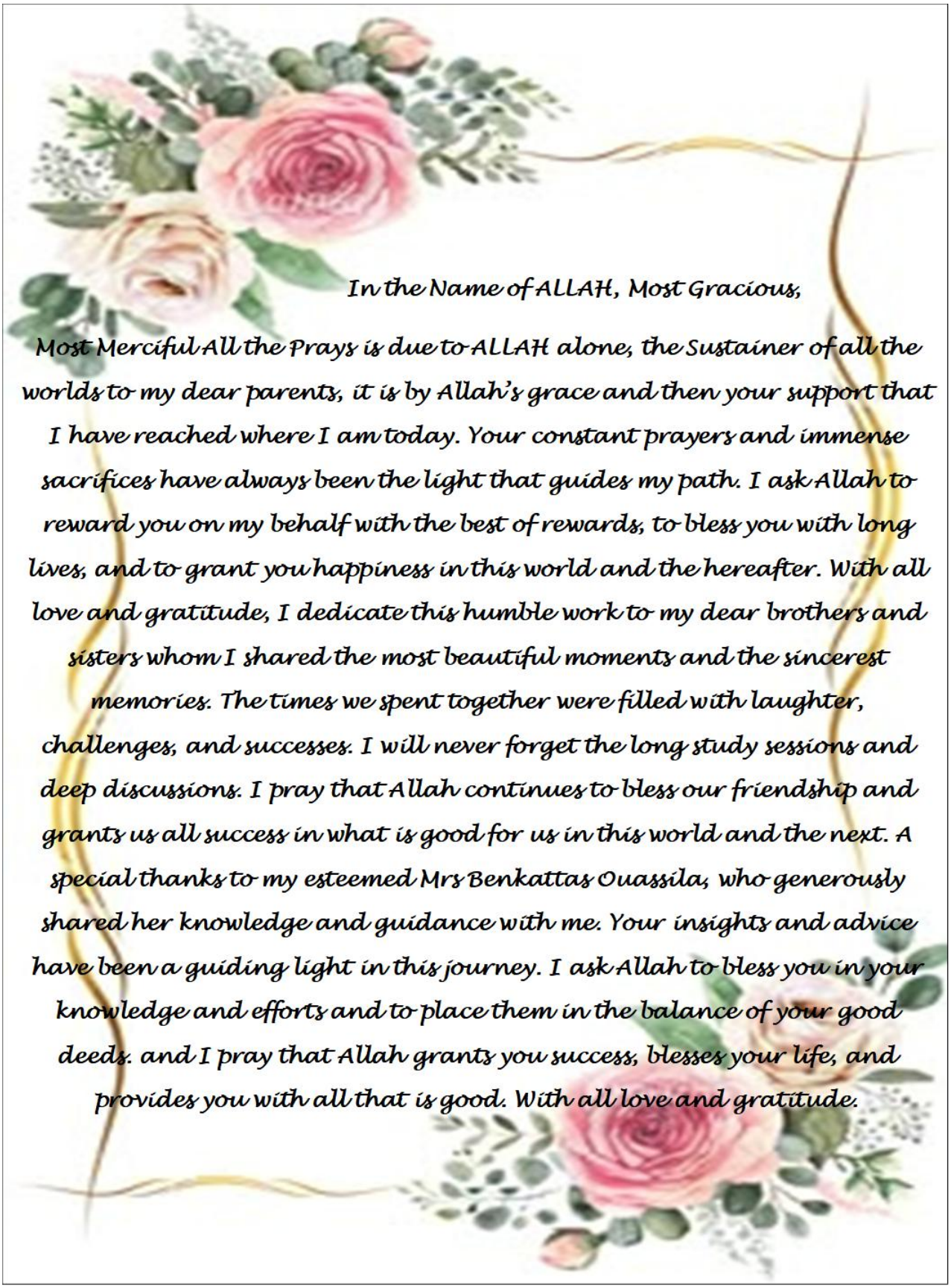

In the Name of ALLAH, Most Gracious,

Most Merciful All the Prays is due to ALLAH alone, the Sustainer of all the worlds to my dear parents, it is by Allah's grace and then your support that I have reached where I am today. Your constant prayers and immense sacrifices have always been the light that guides my path. I ask Allah to reward you on my behalf with the best of rewards, to bless you with long lives, and to grant you happiness in this world and the hereafter. With all love and gratitude, I dedicate this humble work to my dear brothers and sisters whom I shared the most beautiful moments and the sincerest memories. The times we spent together were filled with laughter, challenges, and successes. I will never forget the long study sessions and deep discussions. I pray that Allah continues to bless our friendship and grants us all success in what is good for us in this world and the next. A special thanks to my esteemed Mrs Benkattas Ouassila, who generously shared her knowledge and guidance with me. Your insights and advice have been a guiding light in this journey. I ask Allah to bless you in your knowledge and efforts and to place them in the balance of your good deeds. and I pray that Allah grants you success, blesses your life, and provides you with all that is good. With all love and gratitude.

Abstract

The effect of substrate selection on the cultivation of edible mushrooms

Mushrooms have been valued for centuries for their nutritional and medicinal properties. Rich in proteins, vitamins (B and D), minerals (selenium, potassium), dietary fiber, and bioactive compounds (polysaccharides, phenolics, terpenoids), they possess antioxidant, anti-inflammatory, immunomodulatory, and anticancer effects. In this study, we cultivated three mushroom species: a strain of the *Pleurotus ostreatus* genus and its propagation on cellulosic substrates, a strain of *Agaricus bisporus*, and ***Terfezia*** (desert truffle). The mycelium of each species was obtained after cultivation on a PDA and SDA. The results show that growth was better on PDA (good development) for ***Pleurotus*** and ***Agaricus***, while *Terfezia* exhibited weak radial growth. The inoculum preparation for *Pleurotus* was carried out on three cereal grain-based substrates (wheat, barley, and corn), while only wheat was used as a substrate for *Agaricus*. For *Terfezia*, the inoculum was produced in a honey-based liquid medium as well as another solid medium that we prefer to keep anonymous. The results were remarkable, with good mycelial dispersion on the grains and other substrates. ***Pleurotus*** *ostreatus* was cultivated on wheat straw took almost 42 days and coffee grounds almost 32 days, while *Agaricus bisporus* required a specific growth medium. ***Pleurotus*** yielded a good harvest on the coffee grounds fruiting substrate compared to the other substrates selected in this study.

Keywords: *Agaricus bisporus, Pleurotus ostreatus, Terfezia*, PDA, SDA, Honey-based liquid medium

Résumé

L'effet de la sélection du substrat sur la culture des champignons comestibles

Les champignons sont appréciés depuis des siècles pour leurs propriétés nutritionnelles et médicinales. Riches en protéines, vitamines (B et D), minéraux (sélénium, potassium), fibres alimentaires et composés bioactifs (polysaccharides, composés phénoliques, terpénoïdes), ils possèdent des effets antioxydants, anti-inflammatoires, immunomodulateurs et anticancéreux. Dans cette étude, nous avons cultivé trois espèces de champignons : une souche du genre Pleurotus ostreatus et sa propagation sur substrats cellulosiques, une souche d'Agaricus bisporus et Terfezia (truffe du désert). Le mycélium de chaque espèce a été obtenu après culture sur PDA et SDA. Les résultats montrent une meilleure croissance sur PDA (bon développement) pour Pleurotus et Agaricus, tandis que Terfezia a présenté une faible croissance radiale. La préparation de l'inoculum pour Pleurotus a été réalisée sur trois substrats à base de céréales (blé, orge et maïs), tandis que seul le blé a été utilisé comme substrat pour Agaricus. Pour Terfezia, l'inoculum a été produit dans un milieu liquide à base de miel ainsi que dans un autre milieu solide que nous préférons garder anonyme. Les résultats ont été remarquables, avec une bonne dispersion mycélienne sur les grains et autres substrats. La culture de Pleurotus ostreatus sur paille de blé a duré près de 42 jours et celle de marc de café près de 32 jours, tandis qu'Agaricus bisporus a nécessité un milieu de croissance spécifique. Pleurotus a donné une bonne récolte sur le substrat de fructification à base de marc de café, comparativement aux autres substrats sélectionnés dans cette étude.

Mots-clés : *Agaricus bisporus*, *Pleurotus ostreatus*, *Terfezia*, PDA, SDA, Milieu liquide à base de miel

ملخص

تأثير اختيار الركائز على زراعة الفطر الصالح للأكل

تعتبر الفطريات ذات قيمة غذائية وطبية منذ قرون، فهي غنية بالبروتينات والفيتامينات (خاصـــةً مجموعة B وD) والمعادن (مثل السيلينيوم والبوتاسيوم) والألياف الغذائية والمركبات النشطة بيولوجيًا (مثل السكريات المتعددة والفينولات والتربينويدات). تتميز بخصائص مضادة للأكسدة والالتهابات، كما تعزز المناعة وتُظهر تأثيرات مضادة للسرطان.

في هذه الدراســـة، قمنا بزراعة ثلاثة أنواع من الفطريات: ســـلالة من جنس *Pleurotus ostreatus* (فطر المحاري) وتم تكاثرها على ركائز خلوية، وســـلالة من *Agaricus bisporus* (فطر الزر الأبيض)، بالإضـــافة إلى فطر الكمأة الصـــحراوية (*Terfezia*). تم الحصول على الميسيليوم (الخيوط الفطرية) لكل نوع بعد زراعته على وسطَي **PDA** و**SDA**. أظهرت النتائج أن نمو *Pleurotus* و*Agaricus* كان أفضـــل على وســـط **PDA** (تطور جيد)، بينما أظهرت *Terfezia* نموًا شعاعيًا ضعيفًا.

تم تحضير اللقاح الفطري لـ*Pleurotus* على ثلاثة ركائز من الحبوب (القمح والشعير والذرة)، بينما استُخدم القمح فقط كركيزة لــ*Agaricus*. أما بالنسبة لــ*Terfezia*، فتم إنتاج اللقاح في وسط سائل يعتمد على العسل بالإضافة إلى وسط صلب آخر نفضل عدم الكشف عنه. كانت النتائج ملحوظة، حيث ظهر انتشار جيد للميسيليوم على الحبوب والركائز الأخرى.

استغرق نمو *Pleurotus ostreatus* على قش القمح حوالي 42 يومًا، بينما استغرق على تفل القهوة نحو 32 يومًا، في حين تطلب *Agaricus bisporus* وســـط نمو خاص. أظهر *Pleurotus* حصـــادًا جيدًا على ركيزة تفل القهوة مقارنةً بالركائز الأخرى التي تم اختيارها في هذه الدراسة.

الكلمات المفتاحية : *Agaricus bisporus* ،*Pleurotus ostreatus* ،*Terfezia*، الركائز، وسط سائل يعتمد على العسل.

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General Introduction

Mushrooms, the fruiting bodies of fungi, have been consumed for centuries due to their rich nutritional profile and medicinal properties. They are an excellent source of proteins, vitamins (B and D), minerals (selenium, potassium), dietary fibers, and bioactive compounds such as polysaccharides, phenolics, and terpenoids. Beyond their culinary uses, mushrooms possess antioxidant, anti-inflammatory, immunomodulatory, and anticancer properties, making them valuable in functional foods and pharmaceuticals (Alim et *al*., 2023).

In Algeria, mushrooms like ***Agaricus bisporus*** (button mushroom), ***Pleurotus ostreatus*** (oyster mushroom), and ***Terfezia*** (desert truffle) hold cultural and economic significance. While ***Agaricus bisporus*** and ***Pleurotus ostreatus*** are widely cultivated, ***Terfezia*** grows wild in arid regions, forming symbiotic relationships with desert plants. Understanding their cultivation, ecological requirements, and health benefits is crucial for sustainable production and food security (Kerfez et *al*., 2015).

Mushrooms have long been valued for their nutritional and medicinal properties, playing a significant role in both traditional and modern diets. This chapter provides a comprehensive review of the literature on edible and medicinal fungi, with a focus on three key species in Algeria: ***Agaricus bisporus***, ***Pleurotus ostreatus***, and ***Terfezia*** (desert truffle). The discussion encompasses their classification, life cycle, growth conditions, and bio-ecology, as well as their health benefits and common cultivation challenges.

Mushrooms represent one of the largest branches of the Tree of Life. Organisms belonging to the fungal lineage include: cap mushrooms, rusts, smuts, puffballs, truffles, morels, molds, and yeasts, as well as many lesser-known organisms (Alexopoulos et *al*., 1996).

Over 3,000 mushroom species are classified as major edible species, of which only about a hundred have been commercially cultivated, and around ten of these are produced on an industrial scale. The global production of cultivated edible mushrooms reached 24 million tons in 2009 (Chang & Wasser, 2012) and has been steadily increasing for the past two decades.

Recently, mushrooms have gained significant attention as functional foods due to their potential health benefits. Consequently, the food industry has shown growing interest in both cultivated and wild edible mushrooms. The most widely cultivated mushroom worldwide is Agaricus bisporus, followed by Lentinula edodes and Pleurotus ostreatus.

The genus Pleurotus (Basidiomycota, Agaricales) was first defined by Paul Kummer in 1871. It is a cosmopolitan group of mushrooms known for their high nutritional value, therapeutic properties, and a wide range of biotechnological and environmental applications (Corrêa et *al*., 2016).

The Agaricus genus includes well-known species, both wild-harvested and cultivated, valued not only for food but also for other uses. Agaricus sylvaticus (Percario et *al*., 2009), Agaricus bisporus, and particularly Agaricus subrufescens (Kozarski et *al*., 2011; Wasser & Weis, 1999; Chang & Wasser, 2012) are among the most notable medicinal culinary mushrooms. However, the biodiversity within this genus remains poorly understood, both at the interspecific and intraspecific levels.

Mushrooms, the fruiting bodies of fungi, have been consumed for centuries due to their rich nutritional profile and medicinal properties. They are an excellent source of proteins, vitamins (B and D), minerals (selenium, potassium), dietary fibers, and bioactive compounds such as polysaccharides, phenolics, and terpenoids. Beyond their culinary uses, mushrooms possess antioxidant, anti-inflammatory, immunomodulatory, and anticancer properties, making them valuable in functional foods and pharmaceuticals (Alim et al., 2023).

Mushrooms have long been valued for their nutritional and medicinal properties, playing a significant role in both traditional and modern diets. This chapter provides a comprehensive literature review on edible and medicinal fungi, focusing on three key species in Algeria: Agaricus bisporus, Pleurotus ostreatus, and Terfezia (desert truffle). The discussion covers their classification, life cycle, growth conditions, bio-ecology, health benefits, and common cultivation challenges.

Our Work

In this study, the mushroom of focus is the oyster mushroom (Pleurotus). Our research aims to cultivate Pleurotus ostreatus on different substrates and determine which substrate offers the best yield and profitability.

For Agaricus bisporus and Terfezia (desert truffle), our goal is to develop spawn production techniques for these two mushrooms.

To achieve this, we have divided our work into three parts:

1. First Part: A literature review providing essential information on mushrooms in general, followed by a detailed review of the Pleurotus genus, particularly Pleurotus ostreatus, Agaricus bisporus, and Terfezia (desert truffle)—the focus of our study. This section covers their morphology, habitats, classification, preservation techniques, and nutritional value.

2. Second Part: A description of the materials and methods used for oyster mushroom production and spawn generation from mushroom tissue cultures.

3. Third Part: Presentation and interpretation of the results obtained.

We will conclude our work with a summary and future perspectives. Through this research, we do not claim to innovate but rather aim to contribute to the field, hoping to open doors for further studies in mushroom cultivation.

Chapter I:
literature review

Chapter I: literature review

I. Generalities on fungi

Fungus, any of about 144,000 known species of organisms of the kingdom Fungi, which includes the yeasts, rusts, smuts, mildews, molds, and mushrooms. There are also many funguslike organisms, including slime molds and oomycetes (water molds), that do not belong to kingdom Fungi but are often called fungi (Kerfez et al., 2015). Many of these fungus-like organisms are included in the kingdom Chromista. Fungi are among the most widely distributed organisms on Earth and are of great environmental and medical importance. Many fungi are free-living in soil or water; others form parasitic or symbiotic relationships with plants or animals (Constantine et al., 2025).

Wild mushrooms are a vital source of income and nutrition for many poor communities and of value to recreational foragers. Literature relating to the edibility of mushroom species continues to expand, driven by an increasing demand for wild mushrooms, a wider interest in foraging, and the study of traditional foods (Acar et al., 2020)

2,000 species of mushrooms have been documented so far but very few are commercially cultivated and marketed in different parts of the world. There is significant increase in edible mushrooms cultivation among developed countries (Kaliyaperumal et al., 2019). They are the source of cholesterol-free, low in calories, rich in proteins, carbohydrates, vitamins, minerals, fibers, many secondary metabolites. These biomolecules have beneficial effects on human kind for treating many health disorders. In view of growing population and many health problems, the available known edible wild and cultivated mushrooms is yet deficient. There are numerous potential edible mushrooms with nutraceutical and health benefits, which deserve further investigations (Kerfez et al., 2015).

Extensive research has been carried out in Africa, Algeria included, to investigate the diversity of fungi, particularly macromycetes (higher fungi), with the aim of gaining a deeper understanding of the extensive array of species and variations found within the fungal kingdom. These studies have underscored the vital significance of fungi as a fundamental element of biodiversity (Alim et al., 2023).

The harvesting of wild mushrooms for own consumption is a risky activity since the correct identification of species is essential for its conscious and safe consumption. There are many references regarding edible fungi in different countries of tropical Africa and, in parallel, some authors have been focusing on the identification of toxic or inedible species (Alim et al., 2023).

I – 1 Typology of cultivable edible mushroom

Today, the science of study of mycological applications for human welfare has touched greater heights with the application of molecular biological techniques to improve useful fungal cultures of yeasts and mushrooms (Raut, 2019). The fact that certain fungi are edible has been known for many centuries, And in various European countries up to 80 distinct varieties of wild fungi are offered for sale on the market Though many edible fungi have been domesticated and are in production, the most commonly cultivated are shiitake *(Lentinula edodes),* oyster mushroom (*Pleurotus spp.),* white button mushroom (*Agaricus bisporus*), black fungus or wood-ear mushrooms (*Auricularia auricula and Auricularia polytricha*) and paddy straw mushroom *Volvariella spp*. The cultivation of shiitake by Japanese on logs dates back at least 2000 years, but button mushroom cultivation is comparatively recent. Today, the button mushroom is the most widely grown in many countries, although it is the fourth mushroom most produced in quantity, with most of the development of cultivation technology confined to improving this mushroom for reasons of its larger acceptability by the consumer (Zied & Pardo-Giménez 2017).

China is the main producer of cultivated, edible mushrooms (Figure 1). Over 30 billion kg of mushrooms were produced in China in 2013 (CEFA, 2014) and this accounted for about 87% of total production. The rest of Asia produced about 1.3 billion kg, while the EU, the Americas, and other countries produced about 3.1 billion kg

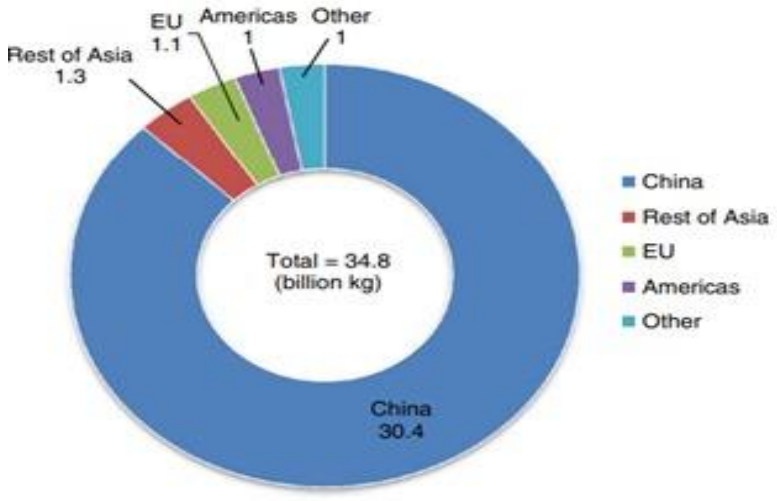


Figure 1: Cultivated mushroom production in China and selected regions of the world, 2013 (billion kg) (CEFA, 2014).

Five main genera constitute around 85% of the world's mushroom supply (Figure 2 and 3). *Lentinula* is the major genus, contributing about 22% of the world's cultivated mushrooms. *Pleurotus*, a close second, with five or six cultivated species, constitutes about 19% of the world's output while *Auricularia* contributes around 17%. The other two genera, *Agaricus* and *Flammulina*, are responsible for 15 and 11% of the volume, respectively (CEFA, 2014)

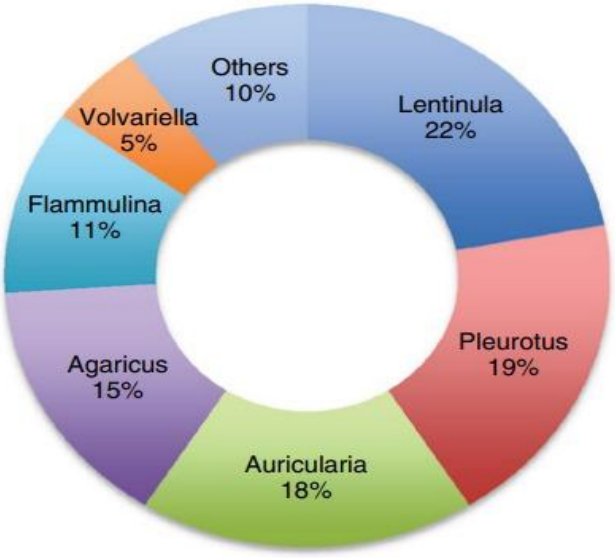


Figure 2*: World edible mushroom production* (% of total) (CEFA, 2014)

in 2013 *Lentinula* is the most widely grown mushroom accounting for over 7 billion kg. This represents a 106.8% increase in volume from 2010. The second most widely grown mushroom in China is now *Auricularia*. Production of this genus (with two main species) has increased nearly 92% since 2010. *Pleurotus* is the third most widely grown genus in China 2013 accounting for nearly 6 billion kg (a 10.8% increase since 2010) (CEFA, 2014).

Figure 3: Six genera of edible and most commonly cultivated mushrooms

I.1 Taste and medicinal qualities

In many areas of human life, including food, health, culture, and religion, mushrooms have had a significant impact. Most people eat mushrooms for their flavor and texture. Recently, they have gained popularity as a protein source and a drug research tool. According to the phyla *Ascomycota* and *Basidiomycota*, mushrooms are fungi that produce spongy fruiting bodies, particularly those that possess a stalk and an envelope top. Mushrooms are composed of 90% water and 10% dry material. Additionally, it has a physicochemical composition that is important for nutrition. Edible mushrooms have been shown to offer therapeutic benefits, including anti-cancer, cardiovascular, hepatoprotective, neuroprotective, hypolipidemic, antiviral, antibacterial, and anti-diabetic actions (Ambhore et al., 2024).

Mushrooms are an excellent source of nutrition, rich in proteins, minerals, complex sugars, unsaturated fatty acids, and secondary metabolites. Their nutritional composition and benefits have been extensively studied, as have their potential therapeutic alternatives. Their bioactive components, such as polyphenolic compounds and antioxidants, have also been studied (Ambhore et al., 2024).

I.2 Level of difficulty in cultivation

Some of the most popularly grown mushrooms globally include *A. bisporus, L. edodes, Pleurotus spp., Auricularia auricula, V. volvacea*, and *Flammulina velutipes*. (Valverde et al., 2015; Dimopoulou et al., 2022). One of the most important features of producing mushrooms for commercial purposes is the capacity to maintain a consistent supply for selected market outlets. Some of the initial problems that mushroom producers encounter include determining the best mushroom to cultivate and finding a spawn provider, organizing available resources to build a growing system, and determining criteria for supplying various marketing outlets (Raut, 2019). However, the mushroom sector is facing substantial obstacles as a result of mushrooms limited shelf-life and the related packaging and storage issues.

Other concerns include an increasing price of raw substrates, unpredictable and unstable selling price, lack of proper marketing channels, spawn quality, mushroom cultivation policies, lack of better farm management practices, limited post-harvest processing techniques, lengthy period of compost preparation, quality control and certification, lack of transportation facilities, poor knowledge about financial assistance, and inadequate scientific research on mushroom farming. Major challenges in mushroom industry are summarized in figure 4 (Valverde et al., 2015; Dimopoulou et al., 2022)

Figure 4: Challenges and issues in mushroom industry (Raut, 2019)

At various spatio-temporal scales, ecological impacts to climate change have been recorded for a variety of mushroom species (Gange et al., 2007; Kauserud et al., 2008). Many researchers have investigated the inter-annual and long-term patterns of mushroom fruiting body production in response to changing land use and pollution levels (Hall et al., 2003). Temperature and precipitation have a considerable influence on mushroom yield and its phenology (Taye et al., 2016). The productivity of mushrooms is influenced by climate, which is further influenced by the interaction of site and substrate quality, along with forest stand structure (Boddy et al., 2014; de-Miguel et al., 2014; Tomao et al., 2017). Because humid and warm conditions promote fungal fruiting, recent temperature spikes due to climate change have shown higher yield and earlier emergence in the humid temperate locations, while drier Mediterranean conditions lowered mushroom output (Boddy et al., 2014). The projected future conditions of increased temperature and reduced precipitation, as predicted by multiple climate change models, are expected to enhance the levels of drought stress and aridity in forest ecosystems. Consequently, these changes are likely to have a significant negative impact on mushroom productivity (Agreda et al., 2015; Buentgen et al. 2015).

Significant quantities of agricultural crop residues, livestock waste, and agro-industrial by-products are annually generated, containing substantial amounts of cellulose, lignin, and hemicellulose. Furthermore, nutrition and substrate composition significantly influence the overall mushroom yield. Several studies have revealed that the protein content of mushrooms' fruiting bodies is significantly influenced by the chemical composition as well as the C/N ratio of substrates. Therefore, these residues are potential source for the mushroom cultivation (de-Miguel et al., 2014).

Agricultural residues can be categorized into two distinct groups. Crop-based residues are produced in the field, and processing-based residues are generated during wood and industrial processing, which are both important sources of biomass (Hall et al., 2003).

I.3 The best way to cultivate mushrooms

Mushrooms are easily cultivable in hilly regions due to abundant moisture but can also be grown in artificial environment with proper temperature and humidity control. (Ramamurthi et al., 2025).

The basic requirements for mushroom cultivation are manure/compost, spawns, right temperature and humidity. Favorable growing conditions involve 80%-90% of relative humidity, ample ventilation, a temperature range of 20-280 C during spawn run and 12-180 C for reproductive growth. Initially for a week temperature must be maintained at 23 ± 20 C and then it can be reduced to 16 ± 20 C for subsequent weeks. The CO2 concentration should be 0.08-0.15 % (NHB, 2025). If the above stated conditions are maintained appropriately the pin heads start to appear within few days and progressively mature into button stage. Apart from these insecticides, nutritional supplements like nitrogen, vermiculite, water are also required for a healthy harvest. The following steps are to be followed for mushroom cultivation:

The compost (synthetic or natural) used for mushroom growth usually comprises of wheat straws, horse manure, poultry manure, rice bran, gypsum etc. (Mohan, 2009**)**; (TIME IS, 2010). Utmost care is taken to protect the raw compost against rain or external moisture, as it might introduce undesirable microbes. The chopped wheat straws or rice bran are mixed with horse dung, sprinkled with water and are heaped in a pile to allow fermentation. The fermentation process along with heat development breaks down the chemical compounds in small components. Frequent turnings and watering are done at a specific interval so as to avoid the drying up of compost. Gypsum is sometimes added to the compost to reduce greasiness et allow more aeration **(**Beyer, 2016). Within 15 to 20 days the compost gets all set to be used as bed, it is then spread onto wooden trays and sowed with spawns (Mohan, 2009**); (**Beyer, 2016).

Spawns refers to the mycelium carefully propagated on agars or grains. Spawning is a process of sowing or mixing spawns in compost. Although mushroom produces spores which acts as a seed for further propagation but are not used generally due to uncertain germination and growth (MSM, 2013). The spawns are thoroughly mixed with the compost, are covered with newspaper and is watered sufficiently to maintain the moisture. Throughout the cultivation period humidity is kept high to avoid loss of moisture. Gradually they grow into white cottony mycelium growth (Mohan, 2009**);** (MSM, 2013).

Casing is a kind of sterilized soil or dressing containing cow manure which is spread onto the spawn mixed compost. It is applied when the mycelium growth commences on the compost surface. After 15 to 20 days of its application mushroom head or pins start becoming visible on the surface. They are allowed to mature for a specific time period and are harvested before opening of

the cap. Mushrooms with opened cap (looks like an umbrella after opening of cap) are undesirable and are considered of menial quality (Mohan, 2009**); (**Beyer, 2016).

I.3.1 The three most well-known edible mushrooms in Algeria (*Agaricus bisporus*, *Pleurotus ostreatus*, *Terfezia*)

Algeria is very rich of a large number of species, among which some edible and excellent qualities. The objective of this work is to succeed the cultivation of *Agaricus bisporus*, after having obtained the mycelium inoculated on the nutrient medium that will be transferred on different source of blanc, the success of culture will be completed after having selected the most suitable substrate. We present the culture of a Kit, then using techniques inoculation on the Agar, and the transfer of the mycelium to cereals (Kerfez et al., 2015).

Algeria's fungal flora is extremely rich in many locations, with certain parts being of excellent quality and commercially available for long periods. Mushroom culture was introduced during the colonial era in Spain and France. Caves and tunnels were exploited for the production of Parsee mushrooms (Chang, 2004).

Agriculture is a driver of growth and economic diversification; it represents the primary source that can ensure food security and achieve self- extraordinary success throughout the country. It is true that each region has its own sufficiency. The agricultural sector is of great importance; it contributes to economic development, creates jobs, and helps reduce the unemployment rate (Kerfez et al., 2015).

In Algeria, agriculture is a valuable activity; it is the support that can lead our state to economic and social prosperity. In recent years, agriculture has achieved techniques, methods, and climatic conditions; but the goal is unique; each wilaya seeks to secure agricultural products and meet the needs of consumers, first and foremost at the local level, and secondly at the national level. Algerian agriculture is located in certain wilayas which must ensure agricultural production for all national markets, this pressure requires the use of pesticides to increase the annual production rate, endangering the health of farmers and consumers, and thus reducing soil fertility it is time to think about modern agriculture, to protect the environment, to reduce water consumption, to cultivate above ground and requires small spaces, including mycoculture which requires only a substrate

(wheat straw, coffee grounds ...) to have organic mushroom production (Ramamurthi et al., 2025). After harvest, the recovery of substrate (mushroom growing medium) is a super organic fertilizer produced 100% by mycelium containing nitrogen, potassium and other organic substances, beneficial for soil fertilization and environmental protection (Kerfez et al., 2015).

Some studies in Algeria aimed to cultivate edible oyster mushrooms (*Pleurotus ostreatus*) and process them into flour for use as a raw material in nutritional supplements. To produce oyster mushroom flour, two essential steps were carried out: drying and grinding.

The production and consumption of fresh mushrooms in Algeria, particularly *Pleurotus ostreatus*, is not yet widespread in the Algerian market. It has only recently begun to gain popularity among consumers (Tahir, 2021).

Desert truffles, locally known as Eterfès or Al-Kamaa, are nutritious seasonal and socioeconomically important edible mushrooms that grow in Southern Algeria. They are distributed naturally around the Mediterranean (Turkey, Italy), North Africa (Tunisia, Morocco, Egypt) and the Middle East (Saudi Arabia, Kuwait, Iraq, Iran, Lebanon, Syria, Jordan). The people of the latter region are considered the most frequent truffle consumers and the truffle commodity is regarded as a costly delicacy. The term desert truffles are mainly used to describe species from the genera *Terfezia* and *Tirmania,* but also lesser-known species of the genera *Picoa, Mattirolomyces*, and *Loculotuber* (Bradai et al., 2014).

Desert truffles are a type of obligate hypogeous Ascomycetes symbiotic ectomycorrhizal fungi formed in association with host roots of *Helianthemum spp*. And the soil inhabiting fungi *Terfezia* or *Tirmania spp*. The type of mycorrhizal fungus ramifies through the soil, absorbing nitrogen and other minerals that are transported back to the host plant. Desert truffles have high economic value and, among all other mushrooms, are the world's most expensive mushrooms (Al-Ruqaie, 2002). The truffles usually appear

in the Algerian deserts after the rainy season between February and April, as well as in many other countries.The importance of truffles as a traditional food and medicine was recently surveyed by Mandeel and Al-Laith. Truffles are a rich source of crude fiber (7%–13%), proteins (20%–27%), fat (3%–7.5%), carbohydrates (approximately 60%), ascorbic acid (AA) (2%–5%), and minerals. Therefore, truffles are of considerable interest for ecological, agroforestry, and commercial purposes (Bradai et al., 2014). Truffles are considered to be one of the oldest foodstuffs

known for their nutritional value and delicious taste, especially compared with meat and fish. In addition to truffles' well-known nutritional importance and their unique aroma and flavor, their reported biological activities have also drawn scientific attention as they are believed to have positive effects in the development of value-added truffles or truffle-related products. Some of their bioactivities include antiviral and antimicrobial activities, hepatoprotective activity, antimutagenic properties, as well as anti-inflammatory effects.1,4 pounds, and glutathione (Al-Ruqaie, 2002). Although almost all organisms possess antioxidant defense and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to prevent the damage entirely. However, exogenous antioxidants, usually found in foods, can delay or inhibit the initiation or propagation of oxidative chain reactions. The most commonly used synthetic antioxidants in foods are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertbutylated hydroxyquinone (TBHQ). However, BHA and BHT were suspected to be carcinogenic for animals in recent years (Chehma, 2005).

Therefore, the search for and research on natural antioxidants have received much attention. Wild and cultivated mushrooms are well known to contain various polyphenolic compounds, which are recognized as an excellent antioxidant due to their ability to scavenge free radicals by acting as reducing agents, hydrogen-donating antioxidants, and singlet oxygen quenchers.3,7–9 Despite several studies focused on the therapeutic effects of the truffles, a few reports are available on antioxidant properties.3,4,6,10 There is, however, no thorough report on this aspect of Algerian desert truffles. Therefore, the study was to evaluate the antioxidant and antiradical properties of methanolic extract of the most popular wild edible desert truffles marketed in South Algeria *Tirmania nivea* (white truffle), *T. pinoyi* (brown truffle), and *Terfezia leonis* (black truffle) including total antioxidant activity, superoxide anion radical scavenging assay, reduction power and free radical scavenging activity, and metal chelating activity. The determination of carotenoids, anthocyanins, and total phenolic compounds as the potential antioxidant's components were also described (Bradai et al., 2014).

II.Classification, lifecycle, reproduction, and growth of mushrooms

II-1 The *Agaricus bisporus* mushroom

The bispore agaric is a species of *basidiomycete* mushrooms of the *Agaricaceae* family, it is the most cultivated mushroom in mushroom farms because it is simple and easy to cultivate. This mushroom grows naturally in early summer or autumn on fatty soils, manure, gardens, in cypress hedges, pastures, courtyards but always outside forests.

Kingdom: *Fungi*

Division: *Basidiomycota*

Class: *Agaricomycetes*

Subclass: *Agaricomycetidae*

Order: *Agaricales*

Genus: *Agaricus*

Species: *Agaricus bisporus*

Life cycle of the button mushroom, homobasidiomycetes are characterized by the fact that they are haploid during most of their life cycle. Fusion of nuclei only takes place in basidial cells just before spores are produced. Each diploid nucleus produces four haploid nuclei after meiosis and these are distributed to the four spores formed by each basidial cell (Sonnenberg et al., 2011).

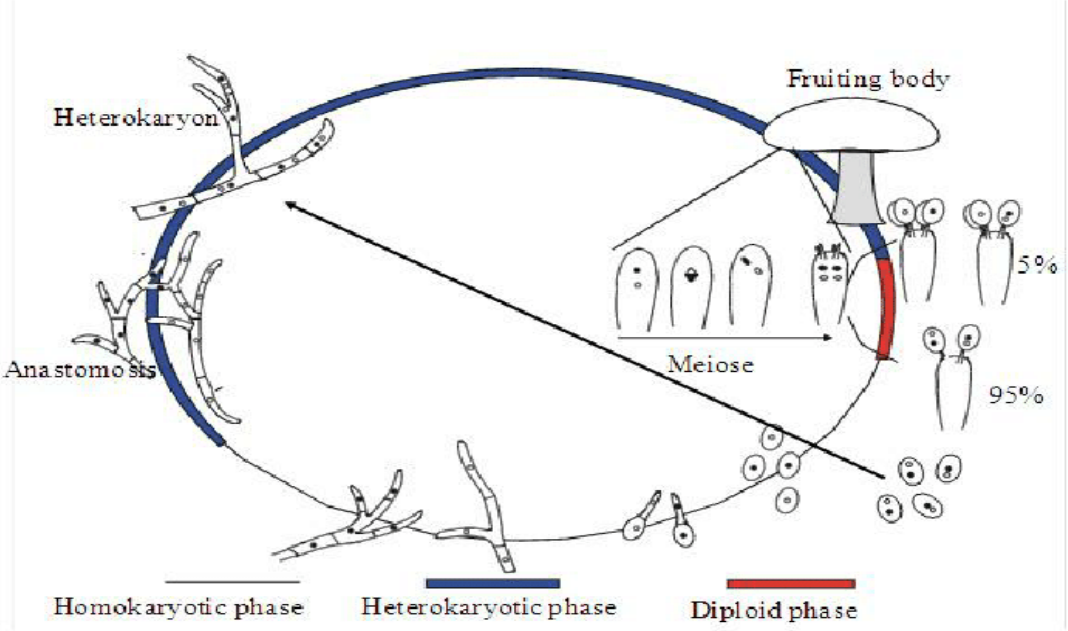


Figure 5: Typical life cycle of *Agaricus bisporus.* Most basidia produce 2 spores, each receiving non-sister nuclei. Due to the low recombination frequency between homologous chromosomes, these spores retain (almost) all alleles of the parental nuclei. The homologous chromosomes have an altered distribution over the constituent nucle (Nesrine, 2017).

Mushrooms can be grown in any dark hole or building, successful commercial mushroom growing requires special houses equipped with ventilation systems. While mushrooms are usually grown in the absence of light, darkness is not a requirement. Mushrooms have been grown in unused coal and limestone mines, old breweries, basements of apartment houses, natural and man-made caves, rhubarb sheds, and many other unusual structures. Mushrooms were reportedly grown in an old dairy barn, which was so damp that cows living in it had died of pneumonia. In 1894, the first structure specifically designed to grow mushrooms was built in Chester County, Pennsylvania, which is usually referred to as the mushroom capital of the world. Growing mushrooms is a waste-recycling activity (Taye et al., 2016). Mushroom farms benefit the environment by using many tons of mulch hay, straw-bedded horse manure, and poultry manure. These products are considered agricultural waste products and would not have a home if it were not for mushroom production. Mushroom production is both an art and a science with many complex and distinct stages. This fact sheet will outline the overall mushroom production cycle and give a brief description of each of the production stages. Phase I and Phase II composting, spawning, spawn colonization (Phase

III), casing, case run, pinning, and harvesting are the primary stages of the mushroom production cycle (de-Miguel et al., 2014).

The specific criteria (temperature set points, carbon dioxide concentrations, and so forth) involved in each stage will change depending on different mushroom crops and different mushroom growers, but the basic concepts and methods of mushroom production remain constant. Although a written description of mushroom growing may seem simple, the process of preparing a composted substrate and its pasteurization is quite complex (Sonnenberg et al., 2011).

II-2 The Pleurotus ostreatus mushroom

Pleurotus ostreatus also known as the Oyster Mushroom, is an edible mushroom intended for human consumption. It belongs to the phylum *Basidiomycota* According to mushrooms represent one of the most abundant groups of eukaryotic living organisms on Earth. The systematics of the Oyster Mushroomare (Hibbet et al., 2014)

Division: *Basidiomycota*
Class: *Agaricomycetes*
Subclass: *Agaricomycetidae*
Order: *Agaricales*
Family: *Pleurotaceae*
Genus: *Pleurotus*
Species: *P. ostreatus*

Basidiomycota are a group of fungi that reproduce primarily sexually. The biocycle of the Oyster Mushroom is shown in Figure 6. It includes two phases:

The first phase is the vegetative phase. It corresponds to the growth and development of the primary monokaryotic mycelium resulting from the germination of a basidiospore. Subsequently, two compatible primary mycelia fuse by plasmogamy and give rise to a secondary dikaryotic mycelium characterized by the formation of anastomotic loops during its multiplication and growth phase

The second phase is the fruiting phase. It corresponds to the formation of the carpophores. When environmental conditions become unfavorable, this dikaryotic mycelium aggregates and forms primordia that evolve into carpophores, which the basidia, the seat of karyogamy, are

individualized. The latter is followed by meiosis. Four haploids mononuclear basidiospores form at the end of sterigmata. Subsequently, they detach and germinate when living conditions are favorable) and the biocycle resumes. The in vitro culture of the Oyster mushroom is done by multiplication of the dikaryotic mycelium (Saar et al., 2014)

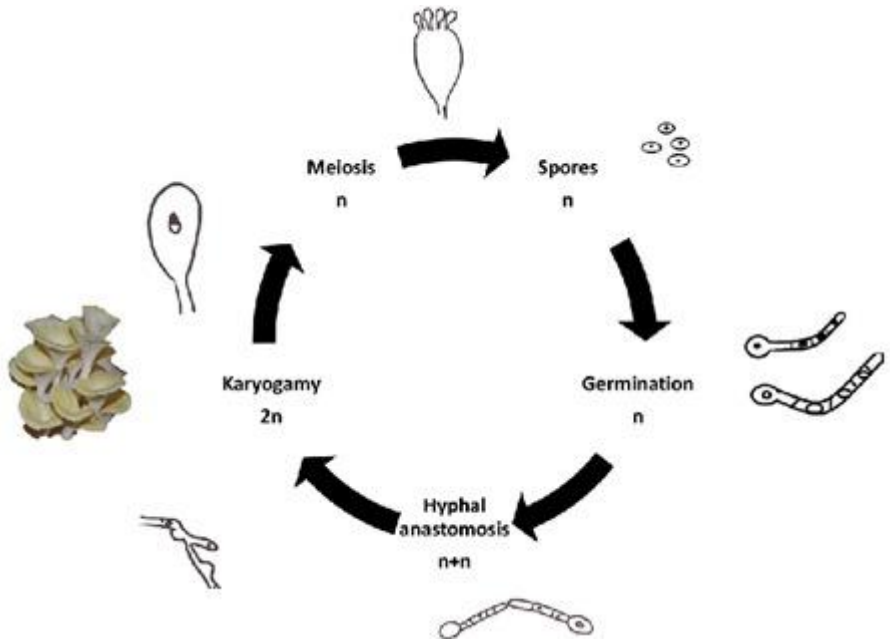


Figure 6: The biocycle of the Oyster Mushroom

The industrial cultivation of Oyster mushrooms, saprophytic, lignocellulolytic fungi, has developed through the use of substrates consisting of agricultural by-products such as wheat straw and agro-industrial by-products, such as olive pomace, coffee grounds, used alone or in mixtures Wheat straw is a co-product of wheat grain harvesting and wheat bran is a co-product of wheat grain milling Olive pomace is the solid residue of the olive oil industry (de-Miguel et al., 2014). Coffee grounds, on the other hand, are the final residue of the coffee processing industry, generated after the preparation of the coffee beverage (Chuck, 2019). As for sawdust, it refers to all the residues and shavings produced by sawing wood In addition to the quality of the edible mushroom strain which must be efficient, the formulation of a substrate is also a crucial step in the cultivation of this strain and significantly influences the yields and quality of the carpophores (mushrooms) produced Due to their carbon heterotrophy, the culture substrate must provide the mushroom with all the elements necessary for its development, nitrogen, carbon, vitamins, and mineral elements (Amrane et al., 2017).

II-3 The *Terfezia* mushroom

Morphological analyses and the similarities in shape encountered in hypogeous ascocarps have led to the classification of truffles in a separate order from the *Pezizales* known as the *Tuberales* (Riousset et al., 2001).

Truffles are fungi belonging to the families *Pezizaceae, Helvellaceae, Tuberaceae, Morchellaceae, Discinaceae, Pyronemataceae, Glaziellaceae, and Carbomycetaceae* (LæssØe and Hansen., 2007).

(Trappe, 1971) added the genus Amylascus to the *Tuberales*, but in 1979, he transferred the genus *Mukagomyces* from the *Terféziaceae* to the *Tuberacea* family and the genus Amylascus to the *Pezizaceae* family. (Trappe, 1979) considered the order *Tuberales* to be "artificial and anachronistic" and included the *Tuberaceae* family in the order *Pezizales*. More recent studies by (Dannell, 1996) and (Percudani et al., 1999), based on molecular biology studies of the orders *Tuberales* and *Pezizales* and focusing on the 18S and 28S regions of ribosomal DNA, confirmed (Trappe, 1979) conclusions, showing that the order *Pezizales* had no reason to exist. Currently, the genus Tuber is part of the family *Tuberaceae* and the order *Tuberales*. Taxonomically, the truffle belongs to:

- *Kingdom: Fungi.*
- *Phylum: Septomycota.*
- *Subphylum: Ascomycotina.*
- *Class: Euascomycetes (Eutunicates).*
- *Subclass: Discomycetidae.*
- *Order: Tuberales.*

- *Family: Tuberaceae.*
- *Genus: Tuber*

According to (Korf, 1973), there are 80 to 100 species of ascomycetes that are considered truffles, but (Trappe, 1979) assigns about sixty species to the genus Tuber, including the best known: Tuber *melanosporum* (black truffle), Tuber albidum (white truffle or blanquette), Tuber *oestivum* (summer white truffle), Tuber *borchii* (blanquette), Tuber *brumale*, Tuber *indicum* (Chinese truffle), Tuber *magnatum* (Italian white truffle), Tuber *mesentericum* (mesenteric truffle), Tuber *rufum* (dog-nose truffle), Tuber *uncinatum* (grey truffle). The life cycle of Tuber is not well known (Chavalier and Grente 1979).

(Delmas, 1983) proposed a probable life cycle for truffles; this cycle generally comprises three phases: a saprotrophic phase, a symbiotic phase, and a reproductive phase (Figure 7).

The saprotrophic phase is characterized by spore germination in the soil and the development of the mycelium. This could form either the fruiting primordium or the mycorrhizae. Thus, the hyphae develop until they come into contact with the roots to enter the symbiotic phase (Callot et al., 1999; Ricard et al., 2003).

During the symbiotic phase, the mycelium makes contact with the plant roots and colonizes them, forming the typical ectomycorrhizal structures, including the mantle and Harting's network. Around the mycorrhizal apices, the extra-matrix mycelium develops, exploring the surrounding soil (Murat-Fuminieux, 2004). The reproductive phase begins with the association of two hyphae to form the ascocarp. Since these two mycelia are identical (autogamy), the truffles are homothallic. During this phase, karyogamy and ascospore formation occur (Callot et al., 1999; Ricard et al., 2003).

The intense heat of July and the thunderstorms of August (water supply) stimulate the biological cycle of truffles through spore germination, provided that the quantities of water and heat are optimal (Murat-Fuminieux, 2004).

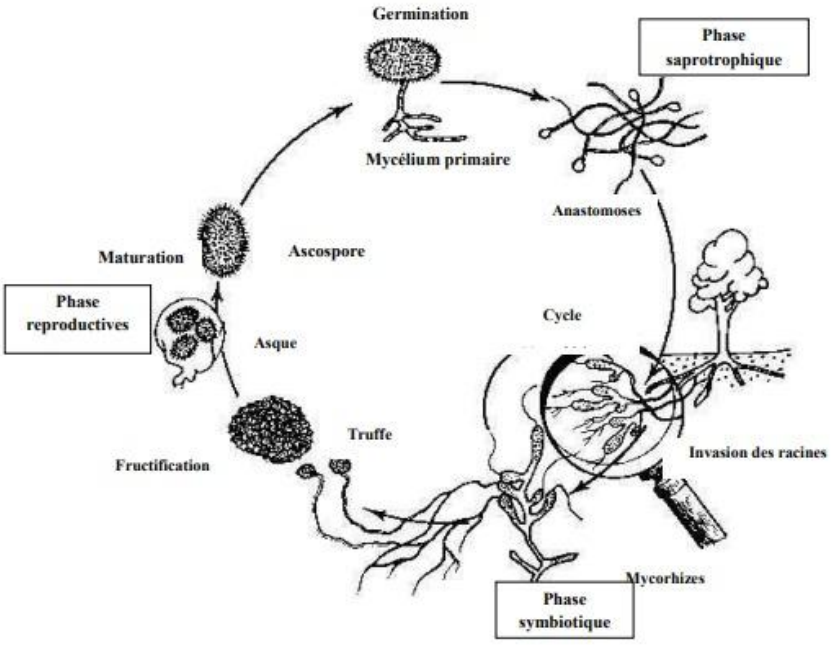


Figure 7: Life cycle of the black truffle (Tuber melanosporum) (Delmas, 1983) Desert truffles, or *Terfez,* are highly valued, edible, tuberous ascocarps. They have a specific geographic distribution, particularly limited to semi-arid and arid regions.

Terfez live in mycorrhizal associations with *Cistaceae*, particularly rockroses (Trappe, 1971; Fortas and Dib, 2005; Kagan-Zur and Roth-Bejerano, 2008).

The genre's truff is in a sympathetic association with races plants hôtes (Chevalier et Grente, 1979 ; Le Tacon et al., 1988 ; Chevalier et Frochot, 1997 ; Riousset et al., 2001 ; Selosse et al., 2004 ; Smith et al., 1997).

The parts of the genre of the ectomycorrhizes. The numbers Compatible ones from the plants contain “ectomycorrhiziennes” (Chênes, Charmes, Hêtres, Pins, main forest trees, their associates are connected to: Tuber *melanosporum*, Tuber *uncinatum*, Tuber *magnatum*, Tuber *indicum* (Le Tacon et al., 1988; Callot et al., 1999; Ricard et al., 2003; Murat-Furminieux, 2004).

Desert truffles are adapted to the hot climates of semi-arid and arid areas. They thrive in climates with dry summers (no rain from June to September) and wet winters (Trappe, 1971)

The regions where desert truffles thrive are generally characterized by annual rainfall between 50 and 380 mm. Furthermore, their development and distribution are particularly linked to autumn and winter rainfall (Trappe et al., 2001; Kagan-Zur and Roth-Bejerano 2008). indicate that Terfez

development requires a minimum of 180 mm of rainfall, distributed throughout the truffle fruiting season (October to March).

Desert truffle soils are relatively homogeneous. *Terfez* species thrive on flat, sandy soils, either gypsum or gravelly-gypsum, relatively rich in limestone and poor in organic matter and phosphorus. The majority of *Terfez* species thrive in soils with an alkaline or near-neutral pH. In Algeria, *Terfez* grows on sandy, calcareous soil, poor in organic matter and phosphorus, well-supplied with potassium and rich in magnesium (Fortas, 1992).

III. Bio-ecology of mushrooms (Agaricus bisporus, Pleurotus ostreatus, Terfezia)

Although we often think of fungi as organisms that cause disease and rot food, fungi are important to human life on many levels. As we have seen, they influence the well-being of human populations on a large scale because they are part of the nutrient cycle in ecosystems. They have other ecosystem roles as well. As animal pathogens, fungi help to control the population of damaging pests (Fortas, 1992). These fungi are very specific to the insects they attack, and do not infect animals or plants. Fungi are currently under investigation as potential microbial insecticides, with several already on the market. For example, the fungus *Beauveria bassiana* is a pesticide being tested as a possible biological control agent for the recent spread of emerald ash borer (Adebayo et al., 2017). It has been released in Michigan, Illinois, Indiana, Ohio, West Virginia and Maryland the mycorrhizal relationship between fungi and plant roots is essential for the productivity of farm land. Without the fungal partner in root systems, 80–90 percent of trees and grasses would not survive. Mycorrhizal fungal inoculants are available as soil amendments from gardening supply stores and are promoted by supporters of organic agriculture (Carter, 2014).

We also eat some types of fungi. Mushrooms figure prominently in the human diet. Morels, shiitake mushrooms, chanterelles, and truffles are considered delicacies. The humble meadow mushroom, *Agaricus campestris,* appears in many dishes. Molds of the genus *Penicillium ripen* many cheeses. They originate in the natural environment such as the caves of *Roquefort,* France, where wheels of sheep milk cheese are stacked in order to capture the molds responsible for the blue veins and pungent taste of the cheese (Adebayo et al., 2017).

Many secondary metabolites of fungi are of great commercial importance. Antibiotics are naturally produced by fungi to kill or inhibit the growth of bacteria, limiting their competition in

the natural environment. Important antibiotics, such as penicillin and the cephalosporins, are isolated from fungi. Valuable drugs isolated from fungi include the immunosuppressant drug cyclosporine (which reduces the risk of rejection after organ transplant), the precursors of steroid hormones, and ergot alkaloids used to stop bleeding. Psilocybin is a compound found in fungi such as *Psilocybe semilanceata* and *Gymnopilus junonius,* which have been used for their hallucinogenic properties by various cultures for thousands of years (Carter, 2014).

Mycorrhizae are the mutually beneficial symbiotic association between roots of vascular plants and fungi. A well-accepted theory proposes that fungi were instrumental in the evolution of the root system in plants and contributed to the success of Angiosperms. The bryophytes (mosses and liverworts), which are considered the most primitive plants and the first to survive on dry land, do not have a true root system; some have vesicular–arbuscular mycorrhizae and some do not (Fortas, 1992).

They depend on a simple rhizoid (an underground organ) and cannot survive in dry areas. True roots appeared in vascular plants. Vascular plants that developed a system of thin extensions from the rhizoids (found in mosses) are thought to have had a selective advantage because they had a greater surface area of contact with the fungal partners than the mosses and liverworts, thus availing themselves of more nutrients in the ground. Fossil records indicate that fungi preceded plants on dry land. The first association between fungi and photosynthetic organisms on land involved moss-like plants and endophytes (Baldrian, 2017).

These early associations developed before roots appeared in plants. Slowly, the benefits of the endophyte and rhizoid interactions for both partners led to present-day mycorrhizae; up to about 90 percent of today's vascular plants have associations with fungi in their rhizosphere. The fungi involved in mycorrhizae display many characteristics of primitive fungi; they produce simple spores, show little diversification, do not have a sexual reproductive cycle, and cannot live outside of a mycorrhizal association. The plants benefited from the association because mycorrhizae allowed them to move into new habitats because of increased uptake of nutrients, and this gave them a selective advantage over plants that did not establish symbiotic relationships (Carter, 2014).

III-1 The *Agaricus bisporus* mushroom conditions (Edaphic, Climatic, Biotic)

Mushrooms are heterotrophic organisms that are commonly classified into three main categories depending on their mode of nutrition, we find: Edible mushrooms and *Agaricus bisporus* (Nesrine, 2017).

- Saprophytes: which feed on dead organic matter.
- Parasites: which feed on living organic matter.
- Symbiotics: they have developed an obligatory association with plants to ensure their survival in the natural environment.

Agaricus is a typical heterotroph that feeds upon biomass produced by other living organisms. They secrete enzymes into their environment that break down organic matter into simple forms that can be absorbed into the hyphae and then they re-assemble these materials to make new fungal biomass (Taye et al., 2016). Fungi are considered 'decomposers', but what is not often appreciated is that their nutrition is the same as predators, herbivores and omnivores (including humans). All are heterotrophs and obtain nutrition by breaking down (decomposing) organic material produced by other organisms (Nesrine, 2017). As a result of their activities, they make more of themselves (they could be considered a 'producer') but because they break down much more material than they produce they are net 'decomposers'' Like most organisms, interactions between *Agaricus* and other organisms and the physical environment are extremely important to its success. This is reflected in the links below that describe how mushrooms are commercially grown. As described above, *Agaricus* is known as a 'secondary decomposer — it feeds on material after it has been eaten by 'primary decomposers'; this is similar to the interaction of cows with the microorganisms in their stomachs. Cows cannot digest grass; they need the microbes to act on the grass (in one of their stomachs) and produce something that they can utilize. *Agaricus* is also very strongly affected by (i.e. interacts with) physical conditions, in particular temperature, humidity and the concentration of carbon dioxide. These conditions both control the mycelium to growth rate and also the initiation of fruiting bodies (Adebayo et al., 2017).

III-2 The Pleurotus ostreatus mushroom conditions (Edaphic, Climatic, Biotic)

These mushrooms grow horizontally out of the dead and dying wood of deciduous trees, especially beech. They are a saprobe, which means they live off decaying organic matter, and are specialists in breaking down some of nature's toughest materials - cellulose and lignin. In the process, they release vital nutrients back into the ecosystem. This oyster starts out a beautiful grey blue color with a cap edge that rolls slightly inward, gradually opening out turning grey brown and wavy with age. Look underneath, and you'll find they have crowded whitish gills that are decurrent - meaning they run right from the cap edge and down the stem. In this case, the stem is rudimentary, a short often fluffy number that's only a few centimeters long. A handful of other oyster mushrooms exist, but are often much paler in color. If they're much smaller, they're likely to be the oystering family. A similar, all white version known as angel's wings is a great find as it's quite rare and (poisonous) (Baldrian, 2017).

III-3 The desert truffle mushroom conditions (Edaphic, Climatic, Biotic)

desert truffle species with a morphometric characterization schow three species of family *Terfeziaceae*: *Terfezia arenaria*, *Terfezia claveryi* and *Tirmania nivea*. These hypogeous ascomycetes live in mycorrhizal association with *Helianthemum lippii* (Cistaceae). Desert truffles grow in heterogeneous soils of sandy texture, moderately calcareous, slightly alkaline, with low organic matter and slight phosphorus contents. The truffles colonize desert depressions "Dayas" and beds of wadis, since these geomorphological units accumulate rainwater, which promotes the development of both truffles and host–plant. Desert truffles are edible hypogenous fungi that are very well adapted to conditions of aridity. The average production was 785.43 ± 743.39 g / ha, closely related autumnal precipitations occurring during October–December, which is the critical pre-breeding period for both desert truffles and host-plant species (Bradai et al., 2014).

The soils where *Tirmania nivea* grow have a typically sandy texture with a very loose particle structure. This gives these soils a porous character and ensures excellent drainage but low water retention. Chemically, the comparison of average pH values with the standard alkalinity scale of an aqueous extract of 1/5 (Morand., 2001).

reveals that the soils where *Terfez* grow have a moderately alkaline pH, varying between 8.10 and 8.57. As for organic matter, which varies between 0.38 and 0.44%, the soils of three prospected sites are poor in organic matter. On the mineral level, the measured elements (P, Ca++, Mg++, K+ HCO3–, SO4–2) show that the soils of desert truffles are poor in minerals (AFNOR, 1996). The values of physicochemical analyses of the soil representing the biotope of *Terfez* are reported on average in the Table 1 (Awameh et al., 1979).

Table 1: Average physicochemical parameters of soils sampled at the same white truffle harvest point in Oued M'ya (Ouargla, Algeria):

Soil Parameters	Sampled Sites		
	Site 1	Site 2	Site 3
pH	8,10	8,10	8,57
Texture type	Sandy		
Organic matter (%)	0,38	0,44	0,42
Total limestone (%)	7.19	4.81	4.35
Total phosphorus (%)	0.11	0.09	0.08
Ca++ (cmol+kg–1)	0,48	0,32	0,40
Mg++ (cmol+kg–1)	0,01	0,08	0,02
K+ (cmol+kg–1)	0,084	0,092	0,097
HCO3-(cmol+kg–1)	0,25	0,23	0,28
SO4-2 (cmol+kg–1)	0,37	0,49	0,36

The development and growth of desert truffles require the presence of certain specific ecological conditions. From a edaphic perspective, truffles of the genus *Terfezia* and Tuber require well-structured, aerated soils that allow for good circulation of mineral elements (Ozenda, 2004). The *Terfez* biotopes in Algeria are characterized by calcareous, sandy soils poor in organic matter (Fortas, 1990). It is important to note that in the Oued M'ya region, truffle-producing areas are mainly depressions and wadi beds. These geomorphological formations are characterized by their ability to accumulate rainwater, which favors the development of *Terfez* and Rock Roses. According to (Awameh et al., 1979). desert truffles prefer flat, sandy terrain or depressions where rainwater accumulates. Indeed, wadi beds are among the most favorable habitats for vegetation growth in Saharan regions (Ozenda, 2004).

During 2009, the average rainfall was 70.60 mm (ONM, 2010) and the truffle yield in the studied sites was estimated at 4.3 kg per hectare, given that during 2007, no production was obtained because rainfall did not exceed 46.4 mm (ONM, 2008). However, truffle production can be disrupted by excessive or poorly distributed rainfall, prolonged periods of cold or intense heat, or even periods of prolonged drought (Chafi et al., 2004).

According to our findings, the development and distribution of desert truffles in the Northern Sahara of Algeria are particularly linked to the existence of climatically favorable biotopes, particularly autumn and winter rainfall. Indeed, *Terfez* thrive in warm climates, provided that rain falls in autumn and/or winter, followed by periods of drought. These rainfalls, even in small amounts, play several roles, notably in the transport, dispersal, and germination of truffle spores, but also in the germination and growth of symbiont plants (rock flowers) (Awameh et al., 1979).

IV. Nutritional value of mushrooms

IV.1. Effects of mushrooms on human health

Mushrooms have been considered as ingredient of gourmet cuisine across the globe; especially for their unique flavor and have been valued by humankind as a culinary wonder. More than 2,000 species of mushrooms exist in nature, but around 25 are widely accepted as food and few are commercially cultivated. Mushrooms are considered as a delicacy with high nutritional and functional value, and they are also accepted as nutraceutical foods; they are of considerable interest because of their organoleptic merit, medicinal properties, and economic significance (Chang et al., 2008); Ergönül et al., 2013). However, there is not an easy distinction between edible and medical mushrooms because many of the common edible species have therapeutic

properties and several used for medical purposes are also edible (Guillamón, 2010). The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Lentinus edodes*, *Pleurotus spp*., and *Flammulina velutipes.* Mushrooms production continuously increases, China being the biggest producer around the world (Chang et al., 2008); (Aida et al., 2009); (Patel et al., 2012). However, wild mushrooms are becoming more important for their nutritional, sensory, and especially pharmacological characteristics (Ergönül et al., 2013). Mushrooms could be an alternative source of new antimicrobial compounds, mainly secondary metabolites, such as terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolones, but also of some primary metabolites like oxalic acid, peptides, and proteins. *Lentinus edodesis* the most studied

species and seems to have an antimicrobial action both gram-positive and gram-negative bacteria (Alves et al., 2012) They have a great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fiber, poor fat but with excellent important fatty acids content

IV.2. Antioxidant properties

development and inflammatory processes (Chang et al., 2012); (Zhang et al., 2011). Numerous molecules synthesized by macro fungi are known to be bioactive, and these bioactive compounds found in fruit bodies, cultured mycelium, and cultured broth are polysaccharides, proteins, fats, minerals, glycosides, alkaloids, volatile oils, terpenoids, tocopherols, phenolics, flavonoids, carotenoids, folates, lectins, enzymes, ascorbic, and organic acids, in general. Polysaccharides are the most important for modern medicine and β-glucan is the best known and the most versatile metabolite with a wide spectrum of biological activity (Patel et al., 2012); (Chang et al., 2012); (Finimundy et al., 2013); (Chen et al.,2007). A balanced diet is the supporting treatment for the prevention of illness and especially against oxidative stress. In this context, mushrooms have a long history of use in the oriental medicine to prevent and fight numerous diseases

IV.3. Anti-cancer benefits

A large variety of mushrooms have been utilized traditionally in many different cultures for the maintenance of health, as well as in the prevention and treatment of dis eases through their immunomodulatory and antineoplastic properties. In the last decade, the interest for pharmaceutical potential of mushrooms has been increased rapidly, and it has been suggested that many mushrooms are like mini-pharmaceutical factories producing compounds with miraculous biological properties (Patel et al., 2012) ;(Ferreira et al., 2010). In addition, the expanded knowledge of the molecular basis of tumorigenesis and metastasis has given the opportunity for discovering new drugs against abnormal molecular and biochemical signals leading to cancer More than 100 medicinal functions are produced by mushrooms and fungi and the key medicinal uses are antioxidant, anticancer, antidiabetic, antiallergic, immunomodulating, cardiovascular protector, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxification, and hepatoprotective effects; they also protect against tumor

IV.4. Immune system enhancement

The antitumor polysaccharides isolated from mushrooms are acidic or neutral, with strong antitumor action and differ significantly in their chemical structures. A wide range of glycans extending from homopolymers to highly complex heteropolymers exhibits antitumoral activity. Mushroom polysaccharides have antitumor action by activation of the immune response of the host organism, in other words, mushroom polysaccharides do not directly kill tumor cells.

These compounds prevent stress on the body and they may produce around 50% reduction in tumor size and prolong the survival time of tumor bearing mice (Zhang et al., 2007); (Wasser et al 2002). β-glucans are the main polysaccharides found in mush rooms and around half of the fungal cell wall mass is constituted by β-glucans. This is important for the industry because many of them are excreted into the cell growth medium, making their recovery, purification and chemical characterization very simple (Klis et al 2001); (Schmid et al., 2001). β-glucans are responsible for anticancer,

immunomodulating, anticholesterolemic, antioxidant, and neuroprotective activities of many edible mushrooms. Also, they are recognized as potent immunological stimulators in humans, and it has been demonstrated their capacity for treating several diseases. β-glucans bind to a membrane receptor and induce these biological responses (Falch et al., 2000) ;(Khan et al., 2013).

Natural products with fungal β-glucans have been con summed for thousands of years and they have long been considered to improve general health (Mayell, 2001). β-glucans are not synthesized by shumans and they are not recognized by human immune systems as self-molecules; as a result, they induce both innate and adaptive immune responses (Vetvicka et al., 2004). Fungal β-glucans are notably beneficial to humans; they markedly stimulate the human immune system and protect from pathogenic microbes and from harmful effects of environmental toxins and carcinogens that impaired immune systems. They also protect from infectious diseases and cancer and aid patients' recovery from chemotherapy and radiotherapy. Besides, these compounds are also beneficial to middle-age people, people with active and stressful lifestyles, and athletes. (Chen et al.,2007); (Manzi et al., 2000).

IV.5. Digestion

Moreover, edible mushrooms provide a nutritionally significant content of vitamins (B1, B2, B12, C, D, and E) (Heleno et al., 2010); (Mattila et al., 2001). Thus, they could be an excellent source of many different nutraceuticals and might be used directly in human diet and to promote health for the synergistic effects of all the bioactive compounds present (Barros et al., 2007); (Vaz et al., 2010).

Nutritional Value. The nutritional value of edible mush rooms is due to their high protein, fiber, vitamin and mineral contents, and low-fat levels (Mattila et al., 2001); (Barros et al., 2008). They are very useful for vegetarian diets because they provide all the essential amino acids for adult requirements; also, mushrooms have higher protein content than most vegetables. Besides, edible mushrooms contain many different bioactive compounds with various human health benefits (Flegg et al., 1997); (Gruen et al., 1982). It is important to remark that the growth characteristics, stage and postharvest condition may influence the chemical composition and the nutritional value of edible

V. Common diseases affecting mushroom cultivation (*Agaricus bisporus, Pleurotus ostreatus*)

• Competitor molds (Trichoderma spp., Neurospora spp.)

The study focused on identification of Trichoderma species that are the causal agents of green mould disease on mushroom farms, based on morpho-physiological features, molecular characteristics, as well as virulence. Green mould disease of mushrooms *Agaricus bisporus* is characterised by dense white mycelia of fast-growing colonies on casing or compost that changes colour into green after extensive sporulation. Spots on fruiting bodies of A. *bisporu*s are early and accompanying symptoms. In serious outbreaks, no fruiting bodies are produced (Seaby, 1996). Until the 1980s, Trichoderma species had been considered as a minor problem, causing losses on mushroom farms occasionally. First, green mould epidemics caused huge losses in Great Britain and the Republic of Ireland during1985–1986 (Seaby, 1996). Soon after the mould was found in The Netherlands in 1994 (Geels, 1997), and Spain and France in 1997–1998. in Europe and North America (Kredics et al., 2010).

A regular method of pathogen control on mushroom farms worldwide is application of various fungicides. Only a few fungicides have been officially recommended in mushroom industry: prochloraz in Europe and worldwide, and chlorothalonil, thiabendazole and thiophanatemethyl in North America (Kredics et al., 2010).

It has been reported that prochloraz and benzimidazole fungi-cides display high selectivity between A. bisporus and its pathogens, which means that they are not toxic to host mushrooms while being highly toxic to pathogenic fungi (Chrysayi-Tokousbalides et al., 2007). Initially, benzimidazole fungicides gave good disease control, but resistance has emerged on farms in North America, suggesting improved hygiene as the only option for pathogen control (Romaine et al., 2005).

Many natural compounds have been tested as alternative agents against pathogens of edible fungi and their strong fungistatic effect has been demonstrated. (Sokovic et al., 2006) reported that essential oils of oregano (Origanum vulgare L.) and common thyme (Thymus vulgaris L.) had very strong activity against T. aggressivum f. europaeum, T. harzianum and T. atroviride. Likewise, strong to totalinhibition of T. harzianum was caused by the essentialoil of tea tree (Melaleuca alternifolia L.), while essential oil and its components of peppermint (Mentha piperita L.)

inhibited the growth of Trichoderma viride (Seaby, 1996). On the basis of the results of (Romaine et al., 2005), which demonstrated inhibitory effect of Bacillus species to T. aggressivum growth, examined ´ the effectiveness of lactonase-producing bacteria, such as Bacillus subtilis (Ehrenberg) Cohn, in prevention of green mould disease. Therefore, biofungicides based on tea tree

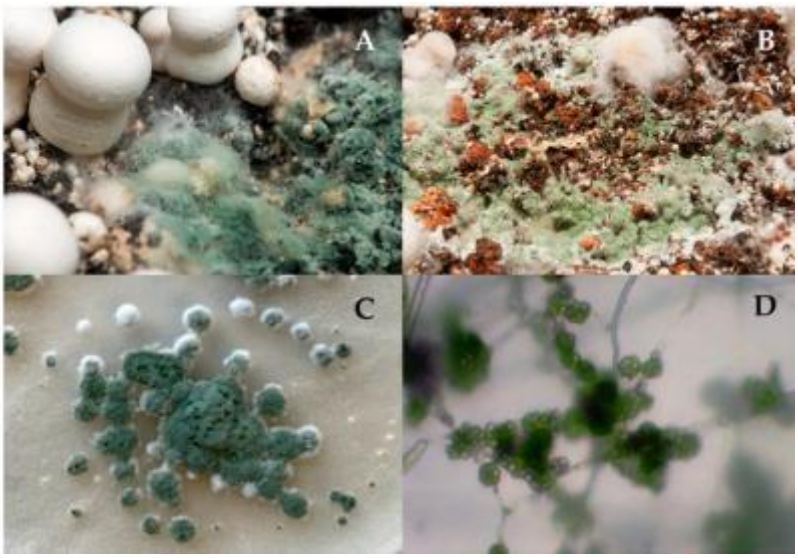


Figure 8: Green mould on the casing layer and mushroom (A, B). Isolate of T. aggressivum f. europaeum TAET1 : Culture (C) and conidiophores (D) (Sánchez-Montesinos et al., 2021)

In recent years, the threat of mold contamination in the mushroom industry has intensified due to climate change and rising temperatures. Competitor molds, like green and orange mold significantly hinder the colonization of mushroom mycelium on the substrate. Green mold outbreaks, caused primarily by Trichoderma, and sporadically by other fungi such as *Penicillium* and *Aspergillus*, have been a persistent issue in oyster mushroom cultivation across the globe (Allaga et al., 2021; Ahedo-Quero et al., 2024). Similarly, orange mold, while a more recent concern, poses a serious threat to the mushroom industry. This issue has been notably problematic during the spawn running stage, where distinctive orange color contamination rapidly overwhelms the farms, often within a week. The problem is exacerbated during the moist summer months, with temperatures exceeding 32°C and relative humidity reaching 70–80%. in 1956 asserted that orange mold on mushroom beds is caused by the obligatory aerobes *Neurospora spp*., which naturally thrive in moist tropical or subtropical climates, as their latent ascospores are activated by high temperatures. Despite being a contaminant in the mushroom industry, Neurospora spp. has been utilized in the food industry, particularly as a pigment producer in the traditional Indonesian dish oncom merah (Nout and Aidoo, 2010). Interestingly, no evidence has been obtained that *Neurospora* is the causal agent of any disease or infection in humans and animals (Perkins and Davis, 2000).

The quick colonization of lignocellulose substrates by orange mold, along with its ability to produce profound spores within a short time at an ambient temperature, underscores the urgency of controlling its spread before causing irreparable damage (Collier et al., 2020).

In mushroom cultivation, sterilization, and pasteurization are commonly employed to eliminate competitive mold from substrates; however, these methods do not prevent the reintroduction of new inoculum after treatment. Chemical control using fungicides is another approach, but this raises environmental and health concerns, especially since mushrooms have a short cropping cycle and are known to bioaccumulate toxic metals (Sharma et al., 2007).

Furthermore, the choice of fungicides must be highly selective, given that both molds and mushrooms are fungi. Beyond the substrate treatment methods, mold contamination also depends on substrate qualities including carbon content, protein levels, and the C:N ratio (Osunde et al., 2019).

Research has demonstrated that the gene expression pattern of Neurospora crassa during its asexual growth stages is regulated by both external environmental stimuli and internal signals (Wang et al., 2019). Notably, genes associated with carbon and nitrogen metabolism are particularly sensitive to changes in nutrient availability, adapting their expression to fluctuating conditions (Wang et al., 2019). This interplay between nutritional factors and Neurospora morphogenesis is crucial for developing strategies to optimize mold control in mushroom cultivation systems.

The present study sets out to address the challenges posed by orange mold through a series of multifaced objectives. Accordingly, the research focused on identifying and characterizing the organisms responsible for orange mold and assessing their pathogenicity in relation to Pleurotus ostreatus. In addition, the research explored diverse control measures, including applying natural plant extract, in vitro analysis of different carbon and nitrogen sources, and evaluating substrate properties. The findings revealed the importance of substrate physiochemical characteristics, particularly the C:N ratio, in controlling orange mold. Importantly, this study represents the first comprehensive report on managing orange mold in the mushroom industry, offering valuable insight into controlling Neurospora sitophila, with potential applications extending beyond mushroom cultivation to the broader food industry (Zied et al.,2014).

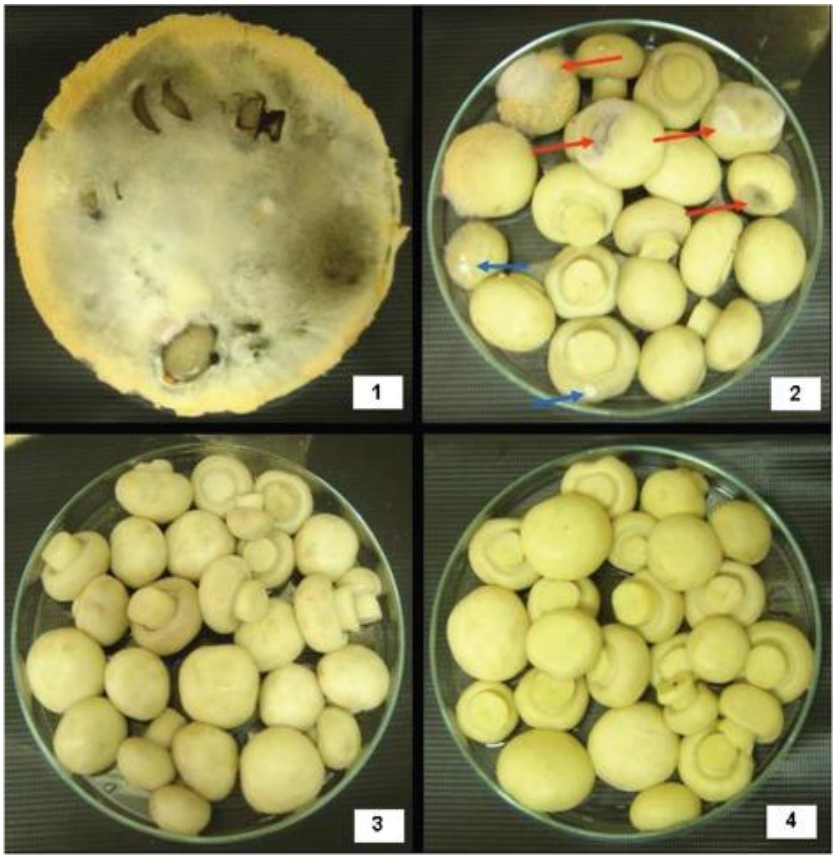


Figure 9: White button mushrooms (*Agaricus bisporus)* assessed after 10 days of storage, processed by methods 1–4, showing fruit bodies contaminated by *Neurospora spp.* and *Rhizopus spp* in panel 1; fruit bodies contaminated by fungi (blue arrows) and bacteria (red arrows) in panel 2 and non-contaminated fruit bodies in panels 3 and 4 (Zied et al., 2014)

- **Bacterial pathogens *(Pseudomonas tolaasii)***

rown blotch disease of cultivated mushrooms is caused by *Pseudomonas tolaasii,* which secretes the bacterial toxin, *tolaasin. Tolaasin* is a peptide toxin that causes pore formation in the plasma membrane of mushroom cells. Forty-two strains of pathogenic bacteria causing brown blotch or similar diseases were isolated from mushrooms showing disease symptoms. To characterize these bacteria, the genes of 16S rRNA were sequenced and analyzed. Thirty-three strains were identified as five different species of Pseudomonas. Of these, 23 were identified as *P. tolaasii* and named as P1-type pathogens. Because the strains identified as P. *tolaasii* were major pathogens that cause the brown blotch disease, figure (Yun et al., 2013).

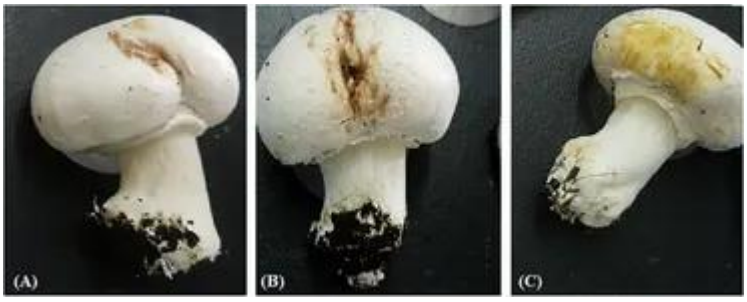


Figure10: Symptomatic mushroom tissue naturally infected with bacterial blotch pathogen, (A*) P. tolaasii*, (B) unknown Pseudomonas species formerly identified as P. *tolaasii* and (C) *"P. gingeri*" (Yun et al., 2013)

Pseudomonas tolaasii is a Gram-negative, soil-dwelling, motile bacterial pathogen, which causes bacterial brown blotch disease in several varieties of cultivated mushrooms (Soler-Rivas et al. 1999). *P. tolaasii* is an endemic bacterium of the compost and the casing layer required for mushroom cultivation. The bacterial cells migrate towards the nutrient of the casing layer *or A.bisporus* hyphae (Samson et al. 1987) and strongly attached to the mycelium. In an infection stage, *P. tolaasii* provokes breakdown of the intercellular matrix and disrupts the hyphal cells (Cole and Skellerup 1986). Symptoms of brown blotch on the cap surface appear as yellowish or light brown to dark brown discoloration. Tolaasin, an extracellular toxin produced by the pathogenic form of P. tolaasii is a compound involved in the production of brown blotch symptoms. Other compounds such as proteinase, lipase, and volatile compounds produced by *P. tolaasii* are possibly involved in the infection process and blotch symptoms (Shirata 1996; Soler-Rivas et al. 1999). The main restriction in the control of *P. tolaasii* infection is that some bacterial species present in the casing layer,e.g. Pseudomonas putida, are necessary for promoting mushroom growth). As a result, the casing layer cannot be sterile, and broad spectrum of antibiotics and chemical compounds cannot be used during the mushroom growing process. Several studies have explored the antagonistic activity of beneficial bacteria against *P. tolaasii.* (Soler-Rivas et al. 1999).

Many bacteria can inhibit the growth of pathogenic bacteria from a distance, which raises the possibility that these bacteria must produce invisible volatile compounds with antibacterial activity. The are low molecular weight and high vapor pressure compounds produced by microorganisms through glycolysis, proteolysis and lipolysis pathways (Schulz and Dickschat 2007). the studies revealed that bacteria employ during interactions with other organisms and some emitted by antagonistic bacteria have antibacterial properties Themain objective of study was to evaluate the effects of invisible volatile compounds produced by these endofungal bacteria against *P. tolaasii*. Effect of invisible volatile compounds on the growth rate, structural change and

pathogenicity properties such as motility, chemotaxis and biofilm formation were evaluated. Other objective was to identify the major invisible volatile compounds produced by endofungal bacteria against *P. tolaasii* (Yun et al., 2013).

Chapter II:

Materials and methods

Chapter II: Materials and methods

II- Materials

II-1 Biological material

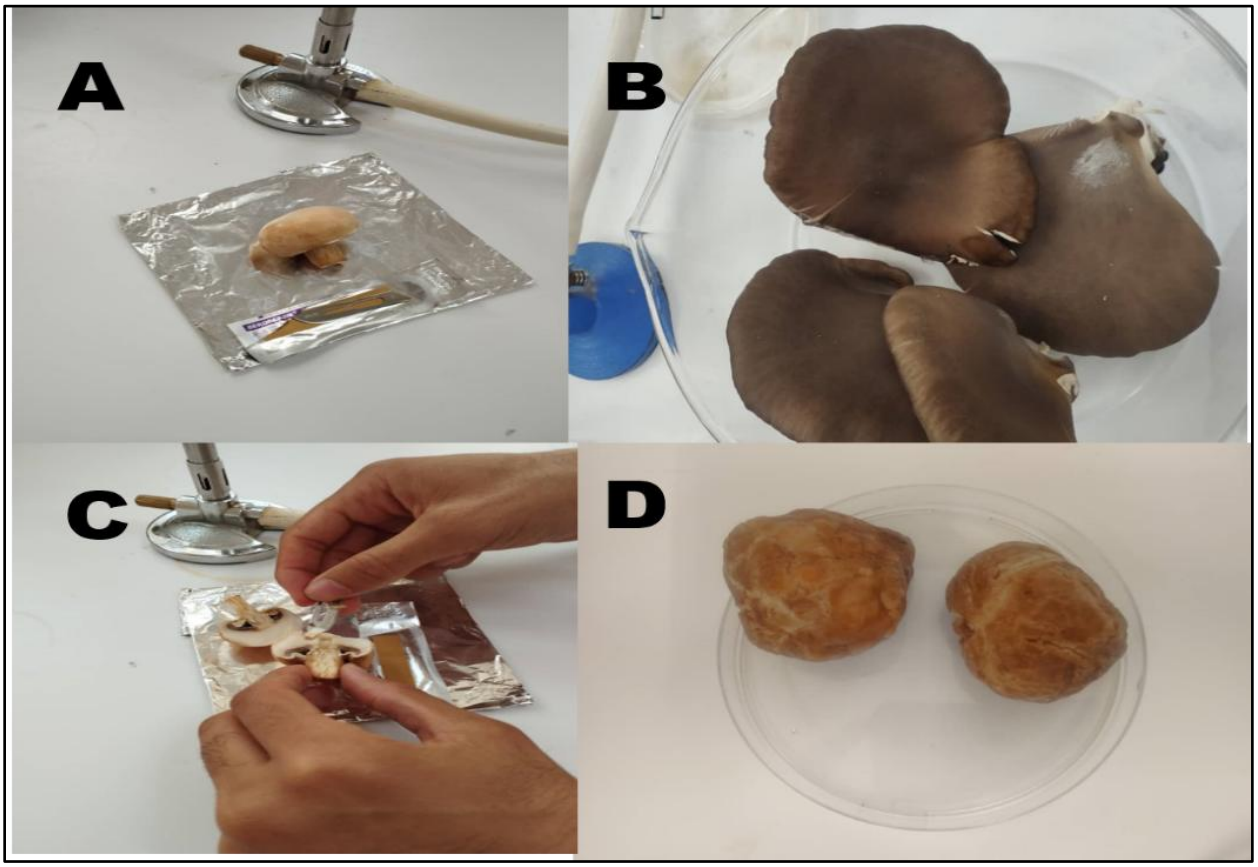
Our study was conducted in the Microbiology Laboratory of the Department of Agricultural Sciences, Faculty of Science, Amar Tellidji University-Laghouat.

In this study, the biological material represented was the white button mushroom *(Agaricus bisporus),* the oyster mushroom *(Pleurotus ostreatus)*, and truffles.

All of these species were investigated in the province of Laghouat, particularly truffles. All the studied samples are presented in the following figure11.

Table 2: List of studied carpophore samples: species, harvest year, and geographic origin and the plant material

genus	Species	Harvest Year	Geographical Origin
Agaricus	*Agaricus bisporus*	2025	Ghardaïa Mushroom Factory
Pleurotus	*Pleurotus ostreatus*	2025	Laghouat
Terfezia	*Terfezia arenaria (Moris) Trappe*	2025	Laghouat



Source: Original (2025)

Figure11 : shows the methods of exctracting spores (A-*Agaricus bisporus).* (B- *oyster*). (D-*Terrfazia)*

Plant material:

We chose Rock Roses, taking into account the strong dominance of Rock Roses in truffle-producing areas. According to the literature, the majority of host plants for desert truffles (Terfezia, Tirmania, Picoa) belong to the Cistaceae family, and in arid regions, these are mainly the genus Helianthemum. Based on these data, we hypothesized that plants of the genus Helianthemum would be the most likely to associate with truffle fungi in the northern Algerian Sahara, harvested in 2025 in Assafia

II.1.2. Oyster mushroom (*Pleurotus ostreatus*)

Methods of mycelium isolation from fruiting bodies:

There are several methods for extracting mycelium in the laboratory, including: The first method is by spore extraction. This is done by placing the oyster mushroom cap on aluminum foil and moistening it with drops of water to allow the mushroom to release spores. We leave it for 24 hours figure 12

Source: Original (2025)

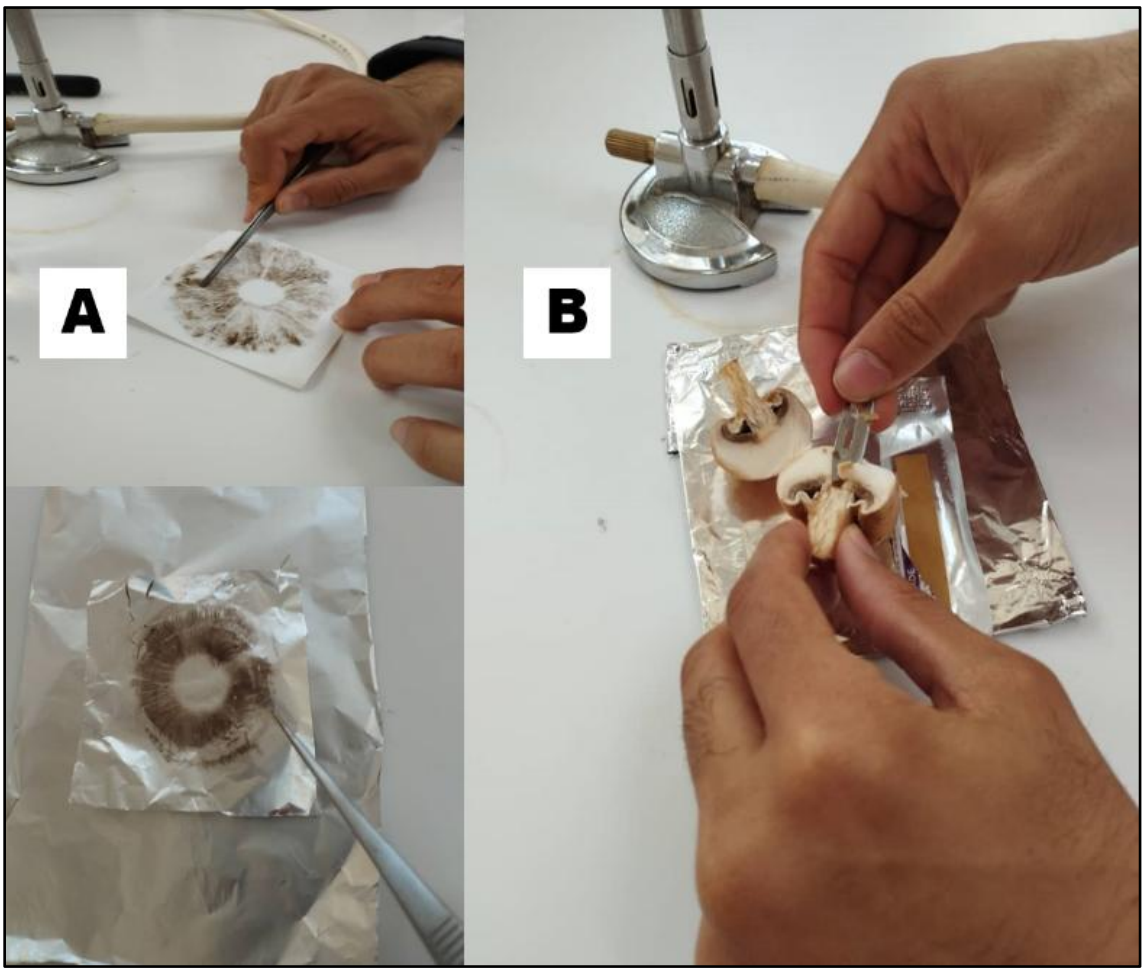
Figure 12: represents the method of extracting oyster mushroom spores.

The second method: is by sterilely lifting a piece of mycelium from inside the oyster mushroom. This is the best method because it doesn't show much contamination, and it's the method we've approved (McCracken., 1972).

II.1.3. Button mushroom (*Agaricus bisporus*)

To extract the mycelium from button mushrooms, we used the two methods mentioned above (the same as for oyster mushrooms). The first is to take a Mushroom Spore Prints. The culture is then grown in a Petri dish containing PDA or SDA medium. A sample is also taken to be grown in a liquid medium containing honey. The second method involves sterilely removing

a fragment from the inside of the mushroom and placing it in the three-culture media mentioned above. figure 13 (Nieuwenhuijzen., 2007).



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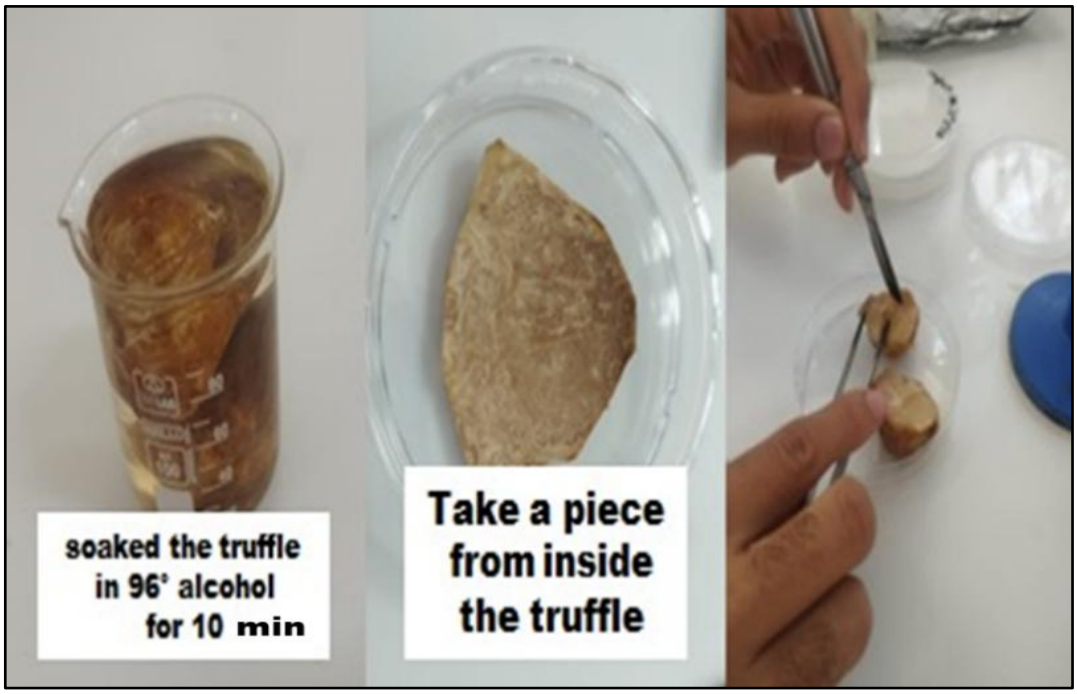
Figur13: shows the method of extracting the mycelium of the button mushroom by spores (A) and by using a piece of the inside of the mushroom (B).

II.1.4. Desert Truffle (*Terfezia*)

in order to obtain the mycelium of the truffle fungus, we isolated a piece from inside the truffle and placed it in a petri dish containing PDA medium Figure15. for reasons not entirely understood, many truffle spores do not germinate following dispersal, leading to inconsistent and often disappointing yield, so we isolated a piece from inside the truffle and placed it in a

medium containing distilled water and honey in order to stimulate the spores to release mycelium.To avoid bacterial and fungal contamination, we soaked the truffle in 96° alcohol for 10 minutes, then removed the outer shell because it was contaminated Figure14. Then, we cut pieces from inside the truffle to obtain the spores (Zambonelli et al., 2012).



Source: Original (2025)

Figure14: The figure represents the sterilization of truffles in 96° for 10 minutes and their preparation for cultivation and mycelium of *Terfezia arenaria*

Source: Original (2025)

Figure15: The figure represents the method of extracting the mycelium of the truffle mushroom.

II-2 Preparation of the culture medium

Preparation of agar media: the isolation is carried out on PDA medium (cooking filtrate of 200 g potato in 1 L of demineralized water + 20 g agar + 20 g Dextrose, increased to 1 L) or SDA (cooking filtrate of 18 g of rice in 1 L Demineralized water +18 agar + 18 g Dextrose, increased to 1 L). The two prepared media are heated on plate for 15 min for homogenization and dissolution of the ingredients. Finally, they are distributed in clean bottles and sterilized at 120° C during 20 min. Isolation and incubation: after cooling, the PDA and SDA were poured into Petri dishes and used for Pleurotus inoculation. To obtain pure culture a small piece of hat of the mushroom, face hymeneal was placed on the Petri dishes contained sterilized PDA or SDA media under aseptic condition. It was then kept for 7-10 days in an incubator at 20°C for sufficient growth. This pure culture was used for the entire experiment figure 16 (Almi et al., 2017).

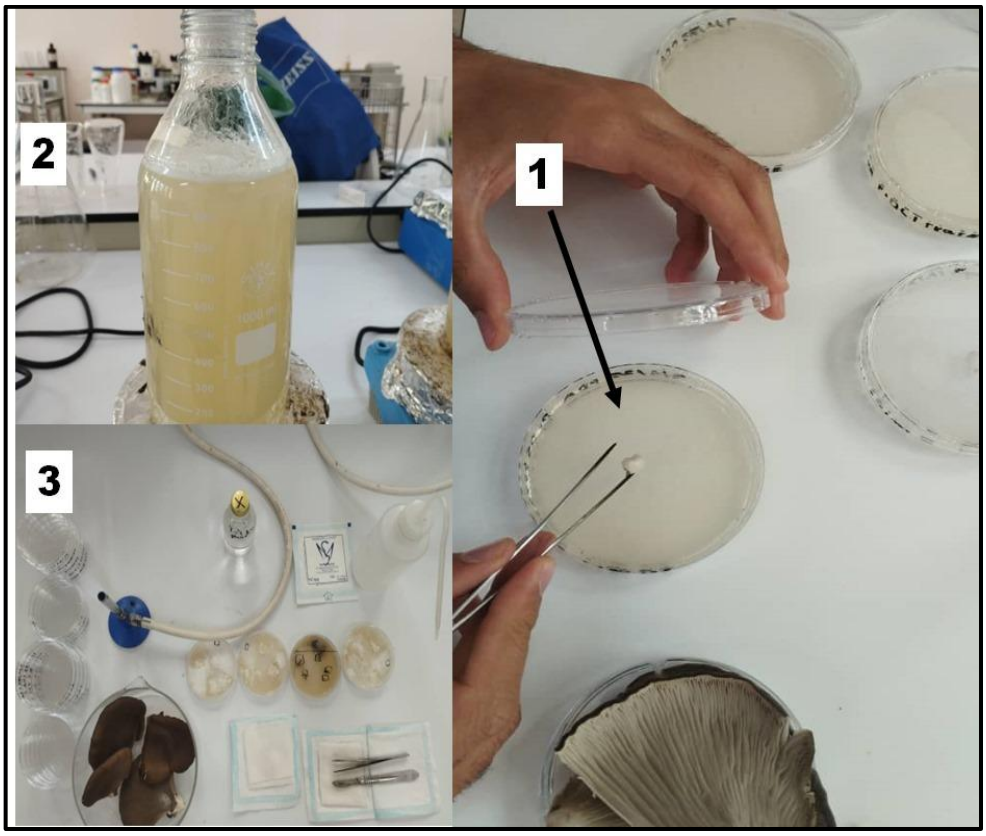


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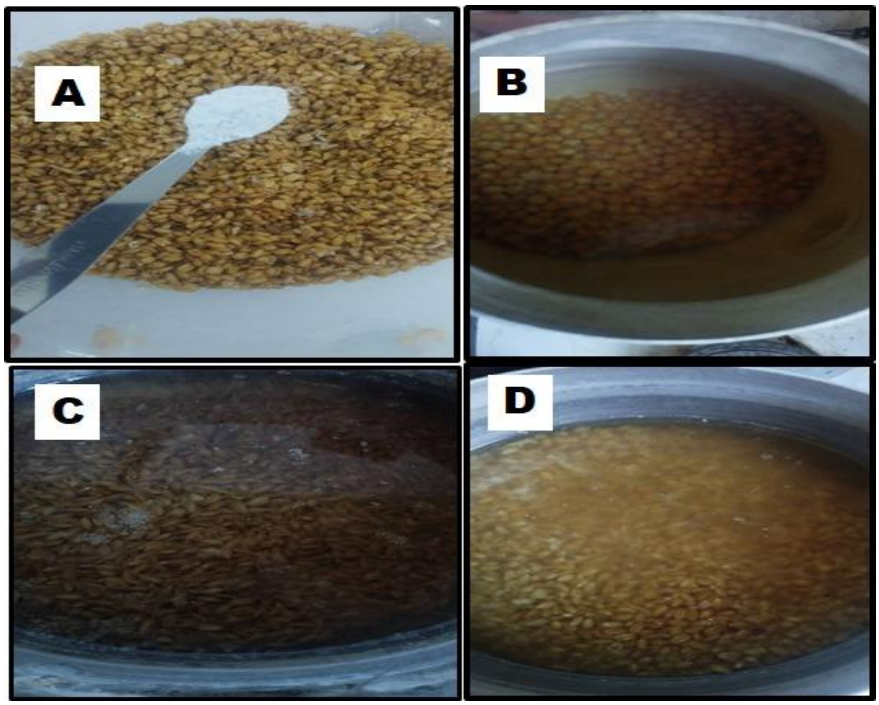
Figure16: represents the preparation of oyster mushroom culture medium (**1** A piece of the inside of an oyster mushroom, **2** SDA medium, **3** Preparing and sterilizing oyster mushrooms before planting).

After preparing the mycelium of oyster mushrooms and button mushrooms in PDA and SDA media, we prepare the culture medium (mushroom seeds) and treat it to make it suitable for feeding the mushrooms and to support mycelium.

II.2.1 Culture medium and fruiting substrates for oyster mushroom

Seedling Substrate Preparation: Three substrates were used to prepare the mother culture: (A corn grains, (B wheat grains, and (C barley grains). The substrates were first soaked separately in distilled water for 24 hours, then cooked for 30 minutes in a water bath, and then drained in a colander. They were then distributed into 9 x 16.8 cm bags, 250 g each. The bags were coated with a mixture of calcium sulfate (2 g), glucose (2 g), and calcium carbonate (1

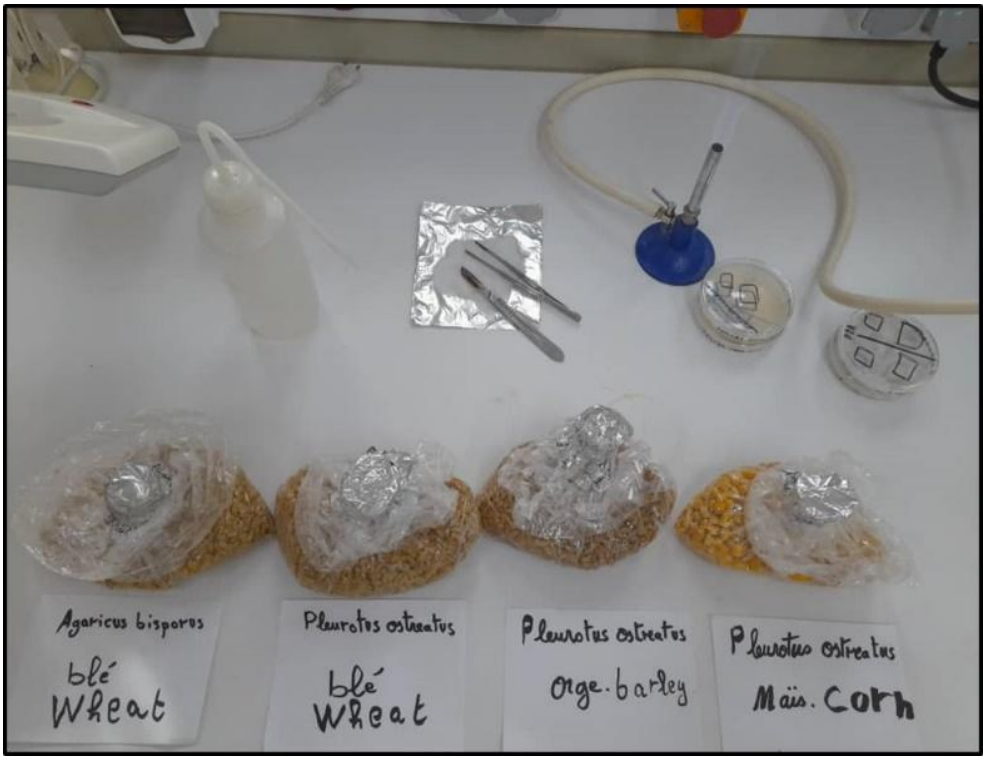
ml) before being sealed and sterilized at 120°C for 20 minutes. The process is illustrated in Figure17



Source: Original (2025)

Figure 17: represents the preparation of mushroom seed culture (A calcium carbonate, B corn grains, C barley grains, D wheat grains).

Inoculation and Incubation: A cut piece of sterile pure culture was placed through the opening of the mother culture bag, and the bag was then sealed with cotton. It was placed in a growth basket at 25°C in the dark. The latter are called oyster mushroom seeds after the mycelium spreads throughout all previously selected seed types (wheat, barley, and corn) (McCracken., 1972).



Source: Original (2025)

Figure 18: represents inoculation and incubation of different seeds (corn, wheat, barley).

II.2.2 Culture medium for button mushroom

For seedling preparation, we used wheat as a substrate for the A. *bisporus* fungus and a support for the mycelium. Wheat quality is very important. The grain must be freshly harvested, contain very few breakages, and be clean to avoid any contamination. Seedling preparation is carried out and developed in the microbiology laboratory of the Department of Agricultural Sciences at the University of Laghouat. 1 kg of wheat grains is placed in a container and washed with water to remove impurities (stubble and glumes, etc.). After washing, the seeds are placed in a clean steel sieve and allowed to dry. The washed seeds are mixed with water at a rate of 1 liter per kilogram of grain, then cooked, stirring occasionally, until the water is completely absorbed. The water-soaked seeds are then cooked and drained in a sieve for one day. A quantity of (CaCO3) was then added to the seeds at a rate of 20 g/kg of wheat. The prepared substrates were distributed into autoclavable glass bottles at a rate of 250 g/bottle to facilitate gas exchange. The bottles were then sterilized by autoclaving at a temperature of 120°C for 1 hour

30 minutes. Note that this sterilization time was used by Bisht Harch and Plant (1998) (Bisht et al., 1998).

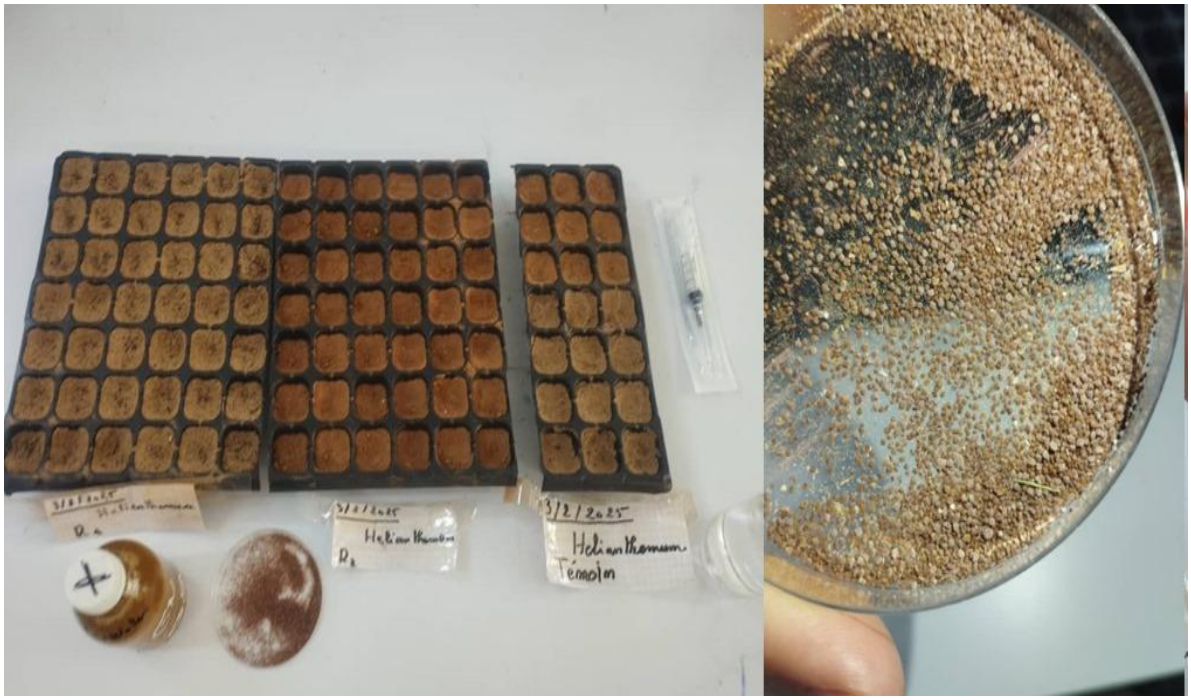
Source: Original (2025)

Figure 19: Represents the process of isolating button fungus mycelium in a Petri dish containing PDA medium.

II.2.3 Culture medium for desert truffle (Terfezia)

Soil should be prepared with the following characteristics: Well-drained soil with a high limestone content, such as calcareous (chalky and calcium-rich) or alkaline soil. It should also be low in salinity for truffle cultivation and suitable for mycelium propagation (Zambonelli et al., 2012) in the Figure 20

Helianthemum seeds were planted in this soil and left in a warm, humid place until the seeds germinated. After 15 days, they were watered with a solution containing truffle mycelium prepared in the laboratory.



Source: Original (2025)

Figure 20: Represents the process of preparing the soil for planting Helianthemum.

II.3. Oyster mushroom cultivation

Four steps were used in the mushroom cultivation procedure: straw preparation, inoculation, growing, and harvesting. Straw preparation includes particle size re- duction and sterilization. To determine the effects of different straw preparation procedures on the mush- room growth, two size reduction methods (grinding and chopping) and two particle sizes for each method (0.5 and 2.5 cm for ground straw, and 2.5 and 5.0 cm for chopped straw) were tested (Oei, 1993).

II.3.1 Preparation of culture medium I (wheat straw)

Pasteurization

We chose only wheat straw for this culture medium. First, the substrate was left in boiling tap water for a prolonged period (over 12 hours) in a humidifier and deep humidifier. This

humidification promotes fungal growth in this substrate. After draining the water (using a humidifier at a concentration of 60-65%), 2 grams of calcium sulfate Pasteurization Finally, it is pasteurized over a fire in a large pot. The wheat straw is cooked for an hour and a half, and the water is boiled. Then, it is left for 24 hours to drain off the excess water, so that the water content becomes approximately) 60-65%) figure 21(Oei, 1993).

Source: Original

(2025)

Figure 21: represents wheat straw after pasteurization and cooling process.

Inoculation

Filling the bags (Larding): larding consists of lifting the caps of the bags to deposit a small amount of spawn. Once treated with hot water and drained, the substrate was piled in layers in plastic bags. Each layer is covered with a sufficient amount of spawn. The larding rate represents approximately 10% of the weight of the substrate figure 22. Once the filling and larding were done, the bags of approximately 3.5 kilos were incubated in separate rooms. The

incubation process was carried out at a temperature of 25º C, in the dark for three weeks. Once the bags were completely colonized by the mycelium, they were cut and perforated to ensure the necessary aeration for development and fruiting (Oei, 1993).

Source: Original

(2025)

Figure 22: filling the bags larding consists of lifting the caps of the bags to deposit a small amount of spawn.

Fruiting

Stimulation of fruiting: the process of stimulating fruiting is done by: 1. Inducing a thermal shock by suddenly lowering the temperature until it reaches less than 16°C. This shock can be carried out using ice bags. 2. Lighting between 8 and 10 hours per day with white light or with natural light. 3. Continuous spraying of the bags to maintain humidity between 80 and 90%. The verification of the temperature degree and the humidity rate to maintain them is ensured by a thermo-hygrometer. The incubation period (post-harvest) can vary from 2 to 3 weeks under the conditions mentioned above (Oei, 1993).

II.4. Preparation of culture medium II (Coffee Grounds)

- **Preparing the growing medium with coffee grounds and wheat straw**

Finally, in the second pillar, coffee grounds and wheat straw are used. The coffee grounds used must be good and uncontaminated. They are treated to reduce the acidity, but 2 grams of agricultural gypsum are added to 1 kg, and then sterilized for 1 hour at 120°C. Then the pasteurized straw is mixed in the first mixture with coffee grounds at a ratio of 25% (Mansour-Benamar et al., 2014).

- **Incubation**

After thoroughly mixing the substrate, place a layer of this mixture in a plastic container. Then, spread the oyster mushroom seeds over it and cover with another layer, up to a height of 30 cm. Ensure the final layer is the mixture. The plastic containers are stored in a dark, humid place for 15 days at a temperature of approximately 25°C, Figure23 (Mansour-Benamar et al., 2014).

- **Fruiting**

After 20 days, expose the pots to high humidity and a low temperature of 20°C, and expose them to light for 8 hours, but not direct sunlight (Mansour-Benamar et al., 2014).

Source: Original

(2025)

Figure23: represents the cooling of wheat straw after pasteurization and reducing its acidity, and placing the bags in a warm, dark place.

II.4.1 Preparation of growing medium

II.4.2. Preparing of growing kits

A mushroom growing kit is essentially a pre-colonized "fruiting mass" that has not yet been prepared for mushroom production. To do this, the wheat straw is ground and sterilized, and its pH is lowered by adding gypsum. The bags are filled with seeds, sealed, and left in a dark, humid place for 15 days. The containers are then refrigerated for sale. The mycelium-covered mass is usually stored in a mushroom growing bag, which can remain dormant for a long time, especially if kept cool in the refrigerator.

Oyster mushrooms are highly resistant to contamination, produce consistently, and can grow on a wide range of substrates. They are extremely hardy and can produce mushrooms even in less-than-ideal conditions—making them an excellent choice for a growing kit.

Oysters also grow very quickly, increasing the chances of success. Most growing kits require a few simples' steps and minimal maintenance, although the chances of success and potential harvest size are greatly increased if the mushroom mass is properly prepared and placed in ideal conditions (Talib et al., 2024).

Source: Original

(2025)

Figure24: represents a growing kit

Edible mushroom cultivation encounters some competing fungi which cause loss in the product and quality of the seeds made in the laboratory. Therefore, we isolated some of the fungi contaminating the seeds in a Petri dish containing PDA medium in order to identify some diseases that hinder the growth of the seedlings of the button mushroom.

In order to inform the farmers, we conducted an investigation at a mushroom production farm about the problems facing this type of crop in the mushroom field.

Chapter III:

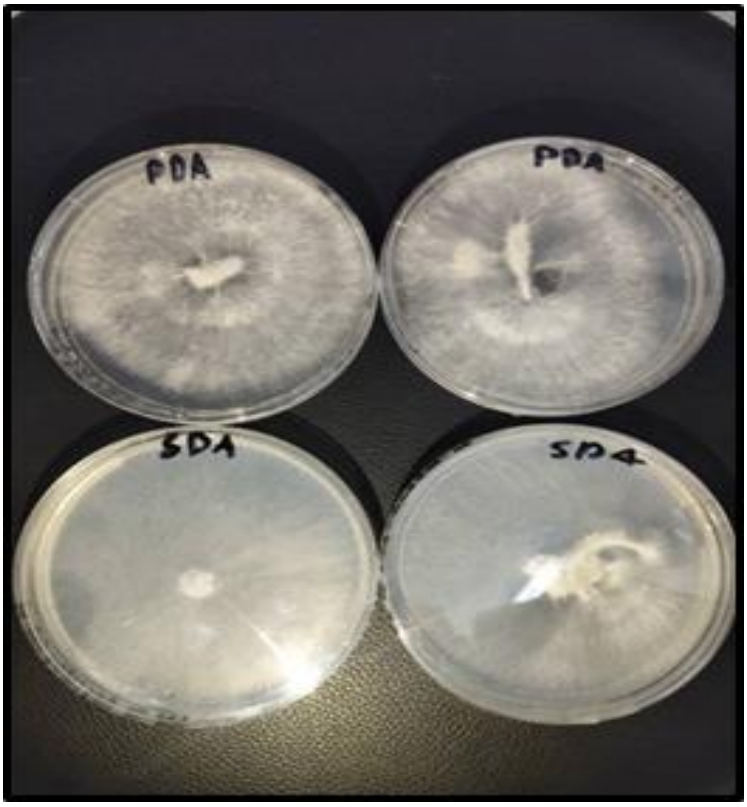
Results and Discussion

Chapter III: Results and Discussion

III.1. Oyster mushroom (Pleurotus ostreatus) cultivation

III.1.1 Comparing of mycelial colonization

As mentioned in the previous chapter, we used two types of culture media: PDA and SDA to extract oyster mushroom mycelium. We also compared them in terms of mycelium proliferation rate, taking into account the best medium for oyster mushroom growth to prepare the mycelium. The figure 24 shows the results of culturing oyster mushroom mycelium. 7 days after planting, demonstrating that the mycelium proliferation rate improved in a Petri dish containing SDA medium. However, we note that the quality of the oyster mushroom mycelium was better in PDA medium.



Source: Original (2025)

Figure 25: represents the difference between the two media in terms of the growth of oyster mushroom mycelium after 7 days of cultivation.

There are many studies on the use of PDA medium to isolate oyster mushroom mycelium and to obtain high-quality mycelium for use in seed production. Like (Katel,2020). This study provided information on the quality of fungal mycelium and its relationship to fungal growth on a variety of culture media and agar formulas, both natural and synthetic, depending on the organism and the purpose of cultivation **(**Katel et al., 2022).

For example (Almi et al., 2017), a study was conducted using SDA medium to produce high-quality mycelium with high yield for seed and fruit production.

- **Results of inoculation and incubation of different seeds (corn, wheat, barley).**

The results showed good mycelium proliferation in the various seedbeds, without any bacterial or fungal contamination. This is due to the proper treatment of the seedbed, which allowed the fungus to feed and spread over the grains, in the figure 25

Source: Original (2025)

Figure 26: Oyster mushroom seeds in different types of grains (wheat, corn, barley)

Some studies (Khare et al., 2013) have shown that several types of seeds, such as wheat, barley, and corn, have been used to prepare substrates for oyster mushroom and button mushroom seedlings. Sorghum grains demonstrated superior propagation quality in terms of fungal growth, grain colonization time, substrate germination time, and fungal production. Data showed that sorghum may be a better vector than wheat and barley.

Heat treatment of the substrate (boiling and steaming) significantly reduced substrate contamination and improved fungal production compared to an unheated control (Khare et al., 2013).

The (Kasoha,2020) results showed that grain kinds had clear effect on spawn on grain substrate, wheat substrate, and productivity as spawn completed growth after 5.8 days on 50 % barley + 50 % pea and pea substrates which was the shortest and significantly differences from the other grain kinds. Also, they were the fastest on spawn growth on wheat substrate as they reach 9.97 and 10.27 days, respectively, and they achieved the highest production of 404.61 and 383 g/kg, respectively. They also gave biological efficiency of 101.15 % and 95.76 %, respectively.

Results also showed that spawn growth on substrate with two grain types took shorter time in comparison with growing in substrate consist of one type of grain, which made the two grain kinds substrate enter production stage early and gave higher oyster mushroom production (Kasoha, 2020).

III.1.2 Fruiting body formation (pinning and fruitification)

Mushroom fruiting bodies are formed from mycelium. After the growth phase of the mycelium, the fruiting body formation phase will take place in appropriating conditions. The formation of the fruiting body is started by the tight entwining of the mycelium to form the germs of fruiting body and mushroom pin head. The mushroom pin head are differentiated into the main parts of the fruiting body such as stem, cap, and lamina (Vuong et al., 2023).

Oyster mushrooms (Pleurotus) have basidia containing basidiospores that are produced on the surface of the laminae. The basidium is made up of the top of the mycelium. The basidiospores form a layer on the surface of the lamina, so the spores also form a coating on the surface of the lamina. Spores are sexual, they germinate into primary mycelium in appropriate conditions. The primary mycelium forms secondary hyphae and develops into a mycelium network spreading throughout substrate or the culture medium (figure 26).

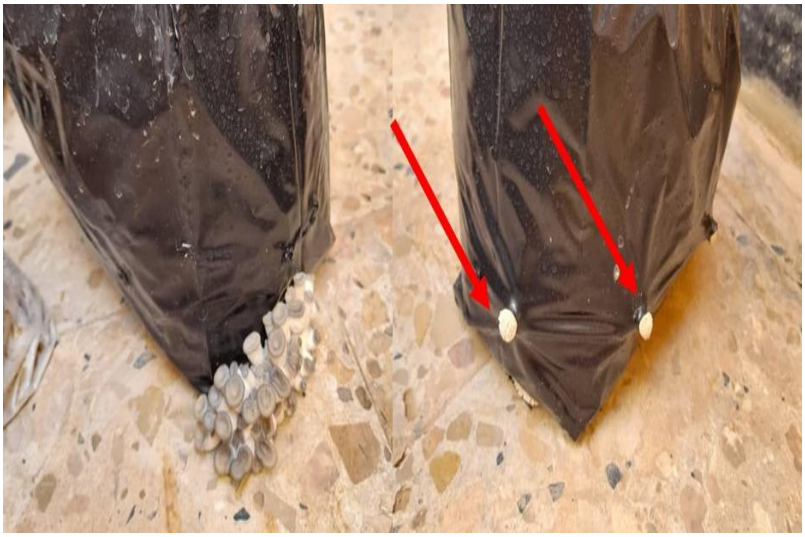


Figure 27: Early fruiting of the mushrooms in a coffee grounds substrate with straw on day 12 of planting.

The results of oyster mushroom cultivation were after 21 days of cultivation, where the agricultural conditions were prepared for the mushroom in order to obtain the best fruiting result, as the temperature was 20°C, good lighting for about 8 hours, and high humidity. The fruiting production was good. As for the mixture of coffee grounds with straw, it was faster and more productive, as it was able to 5 kg in every kg and 1.5 kg of the agricultural medium (coffee grounds with straw) and 2 kg in every 1.5 kg of (straw only). This is due to the fact that coffee grounds are a good nutrient and substrate for oyster mushrooms. Many studies have been found on the use of coffee grounds in good germination of oyster mushrooms, as they were based on Days of primordial formation days of primordial formation of oyster mushroom had significant differences within the treatment's inoculation. The remaining treatments were intermediate between the fastest and slowest in primordial formation after substrate inoculation. The length of days taken for primordial initiation for this experiment was in line with the length of days

reported by (Sharma et al., 2013) for substrates of wheat straws, waste paper and rice straws taken 4days to 5.6 days 26.4 to 31.6 days from substrate inoculation to primordia formation. As the investigation of (Zenebe et al., 2016)

Source: Original (2025)

Figure 28: The picture shows the yield of oyster mushroom cultivation in coffee grounds and straw.

Source: Original (2025)

Figure 29: The difference is clear between the fruits of the agricultural medium (B-coffee grounds and wheat straw) and the fruits of the agricultural medium (A-wheat straw only).

5 head formation varied with substrates ranging from 17 to 33 days after spawn seeding. Primordial formation occurred on cotton seed took 17days followed by sawdust 29 days while wheat straw took relatively longer incubation of 32.66 days. The remaining treatments were taking intermediate days between the shortest and the longest days from substrate inoculation to the first harvest. In this experiment, the recorded results were closely related with report of (Sharmila et al., 2015)

P. ostreatus cultivated on areca nut husk + topsoil initiated in 28 days, Bamboo shoot + topsoil took 30 days as well as areca palm leaves + topsoil that initiated after 34 days. The first maturation of Pleurotus ostreatus cultivated on coffee husk and wheat bran was in line with the days of first harvest that took 32.4 to 37.8 days figure 29 (Sharma et al., 2013).

days of mycelium fully colonization, primordia formation and first maturation number of fruits, Bunches and Aborts the effect of coffee husk supplemented with wheat bran on numbers of fruiting bodies, The maximum number of fruits (52)

The fungal growth period in our study on coffee grounds and wheat straw ranged from 15 to 32 days from the appearance of fruit heads, unlike the wheat straw-only substrate, which was from 19 to 39 days from the appearance of fruit heads figure 30

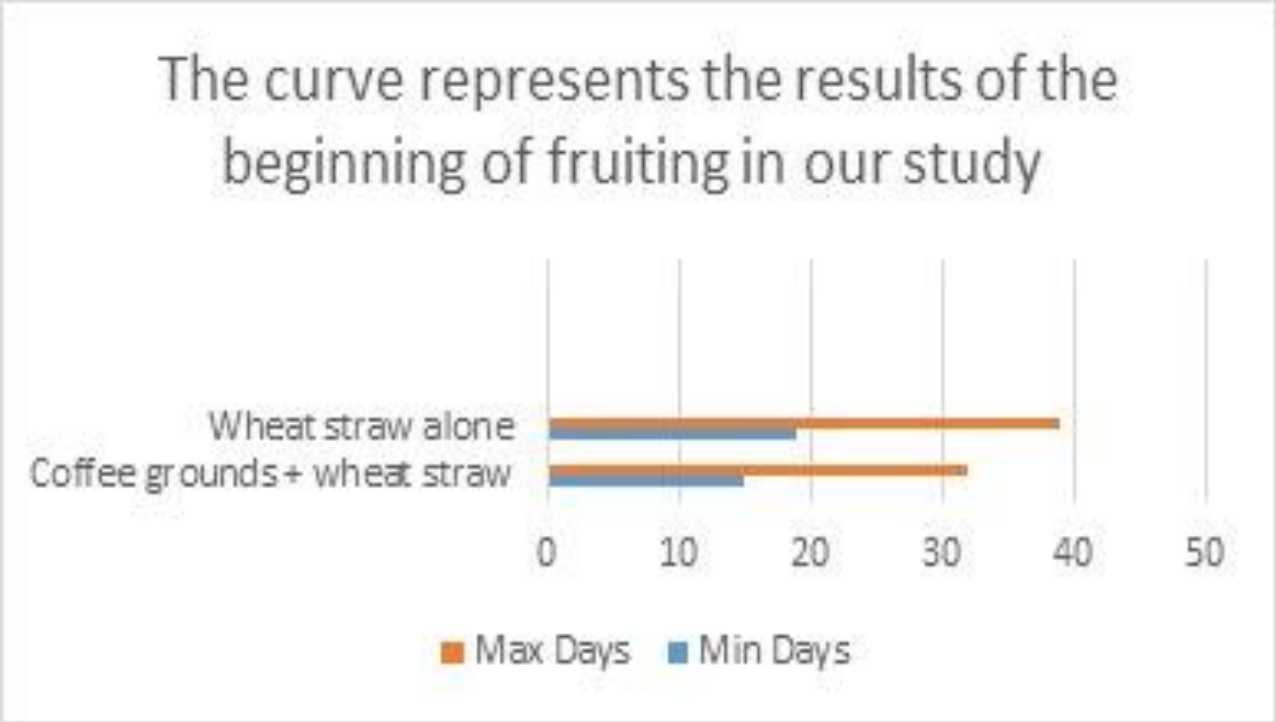


Figure 30: Fruiting body formation

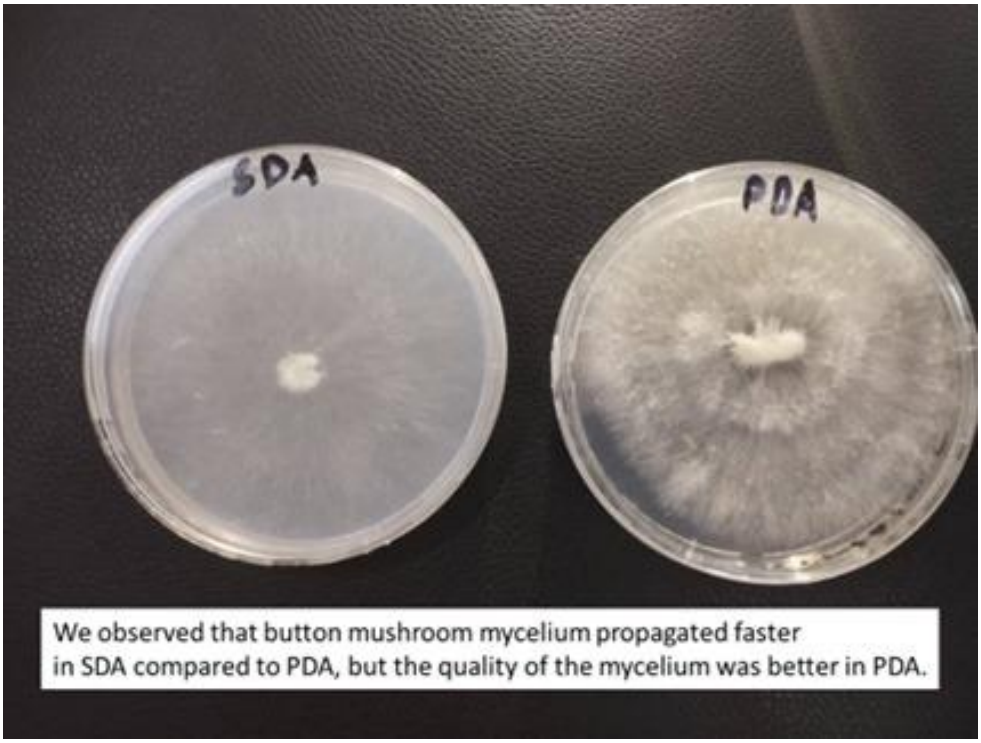
III.1.3 Harvesting protocols

Harvesting is one of the main operations at commercial mushroom level and has a significant impact on quality of mushrooms, yield obtained from the crop, and production costs. Mushrooms intended for the fresh market are solely harvested by hand. Manual harvesting ensures excellent quality mushrooms with an adequate shelf-life and appearance, regardless of the importance of compost and casing qualities. Once picked into the containers fresh mushrooms are chilled to temperatures of 4-5°C and in relative humidity of above 90%. Mechanical harvesting of mushrooms allows the producer to harvest in minutes the same crop area that would take days to manually harvest completely. The technical feasibility of automatic harvesting systems has been assessed since the 1990s. The high microbial load present in fresh mushrooms does not represent a food safety concern. Washing and thermal treatment (canning) are viable processes to extend the shelf-life of mushrooms) (Valverde, 2017)

III.2 Button mushroom cultivation

III.2.1 Comparing of mycelial growth on PDA (Potato Dextrose Agar) and SDA (Son Dextrose Agar) media

This study aimed to compare the PDA and SDA culture media for cultivating button mushroom mycelium and its propagation rate. We observed that button mushroom mycelium propagated faster in SDA compared to PDA, but the quality of the mycelium was better in PDA.



Source: Original (2025)

Figure31: Agaricus bisporus mycelium on PDA and SDA media

Ahmadi and Farsi, (2017) also reported positive significant correlation between filamentous mycelium type and yield, in this case, isolates with filamentous mycelium growth type and high mycelium growth rate produced higher yield. Faster mycelium growth rate considers as a desire characteristic in mushroom cultivation due to reduce the contamination risk of other micro-organisms. Conclusion In conclusion, there was highly significant differences between white button mushroom isolates in terms of mycelium growth rate and yield.

Isolates with faster mycelium growth rate on solid media produced higher yield. Highly positive correlation was observed between mycelium growth rates on pda medium and compost, so it could be used as an appropriate medium to comparison of mycelium growth rate in vitro. Hence, recognition of these differences could be profitable in regards to this point that white button mushroom is among the most cultivated and marketing sales (Ahmadi-Lahijani et al., 2017).

The quality of the seed used is a key step in achieving good mushroom performance. The seed quality depends specifically on obtaining and maintaining good mushroom cultures. These

cultures are obtained based on spores or mushroom tissues and they must be free from contaminating micro-organisms; in other words, they must be pure cultures.

Source: Original (2025)

Figure32: Photograph shows the invasion of wheat by the mycelium of *Agaricus bisporus*

Seed quality is illustrated in the following figure 32. The image shows seeds free of mold and bacteria, which cause seed spoilage in button mushrooms. Some studies have confirmed the role of good seeds and their content in the growth of button mushroom mycelium.

The physical structure of the granules is effective as a substrate for fungal growth, a suitable element for mycelium growth, and a source of lignocellulose. The additional nutrients required for mycelium formation and growth are obtained from lignin, cellulose, hemicellulose, etc. as

a carbon source. The grains are mainly composed of protein (nitrogen), other chemical elements, and vitamins, which promote the growth of the mycelium.

III.3 Results of mushroom growing kits

Results of growing oyster mushrooms in containers for self-cultivation at home. We notice the spread of oyster mushroom mycelium threads inside the containers 15 days after planting. Then we move to the fruiting stage after 21 days.

Source: Original (2025)

Figure 33: Results of mushroom growing kits

III.3.1 Microscopic spore characterization

The picture figure shows the difference between button mushroom spores and oyster mushroom spores.

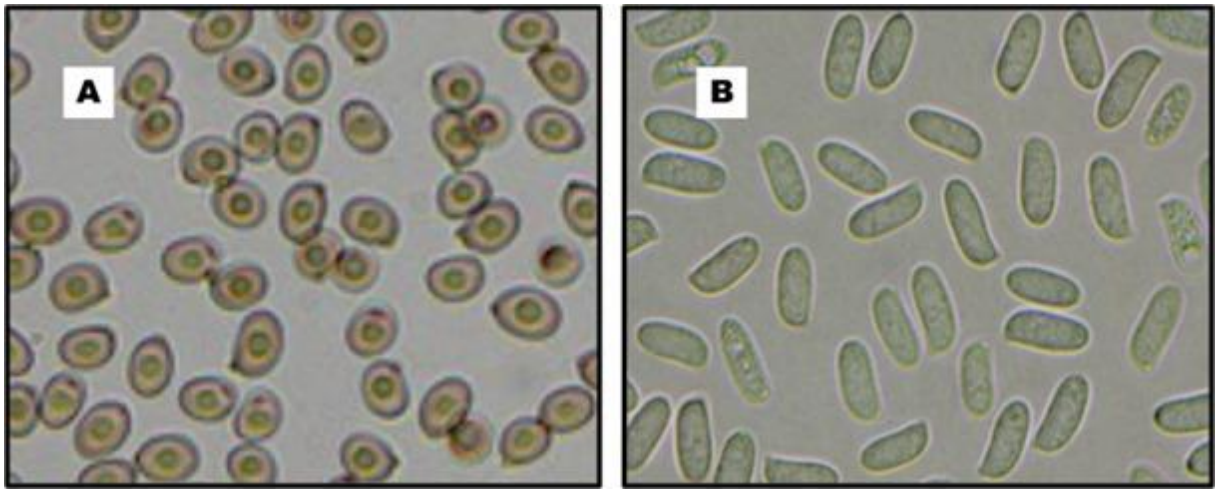


Figure 34: difference between button mushroom A spores and oyster mushroom spores B (100x magnification**)**

We notice that Spores of agaricus bisporus are:

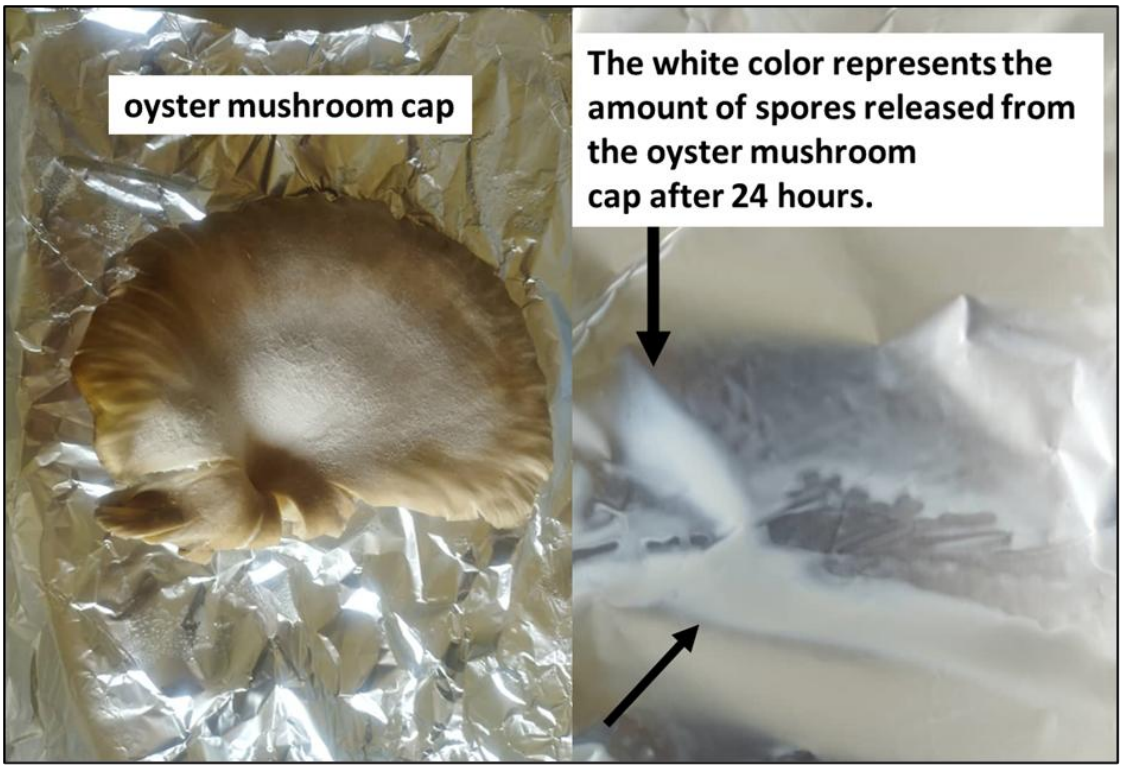
- Oviod to subglobose, smooth, 4-7.5 x 4-5.5µm.
- Deep chocolate brown

the Pleurotus ostreatus Spore Print:

- White to faintly yellowish
- Microscopic Features: Spores 7–11 x 2–4 µm; cylindric-ellipsoid; smooth

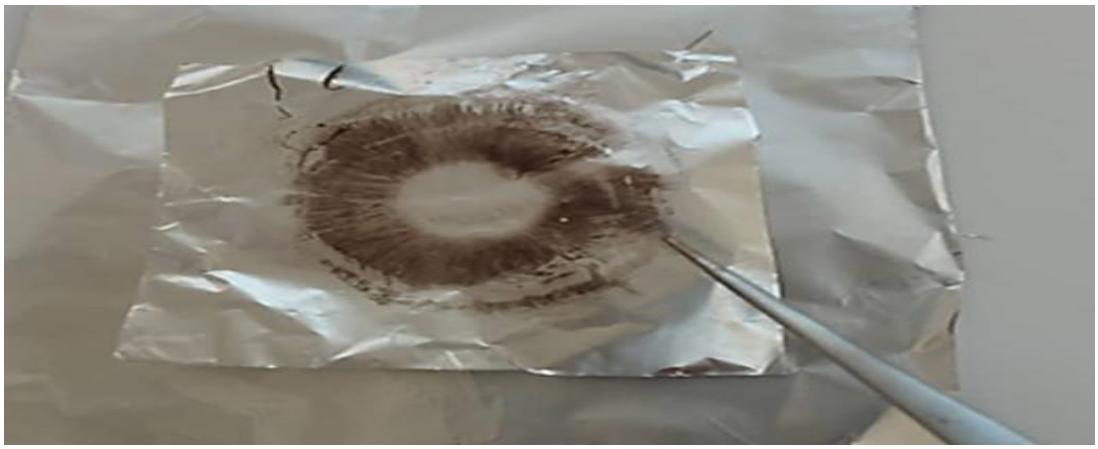
III.3.2 Spore preservation through spore printing

Store your spores in a cool, dark place. Place the aluminum foil in a resealable bag and add silica gel to absorb any moisture from the surrounding air. As long as they stay dry, your spores will remain highly fertile for at least two years. If they get wet, they will activate and die during storage, so humidity is not your friend. They can still work for more than five years, but after two years, you will see a gradual reduction in the rate of successful inoculations.



Source: Original (2025)

Figure 35: oyster mushroom print

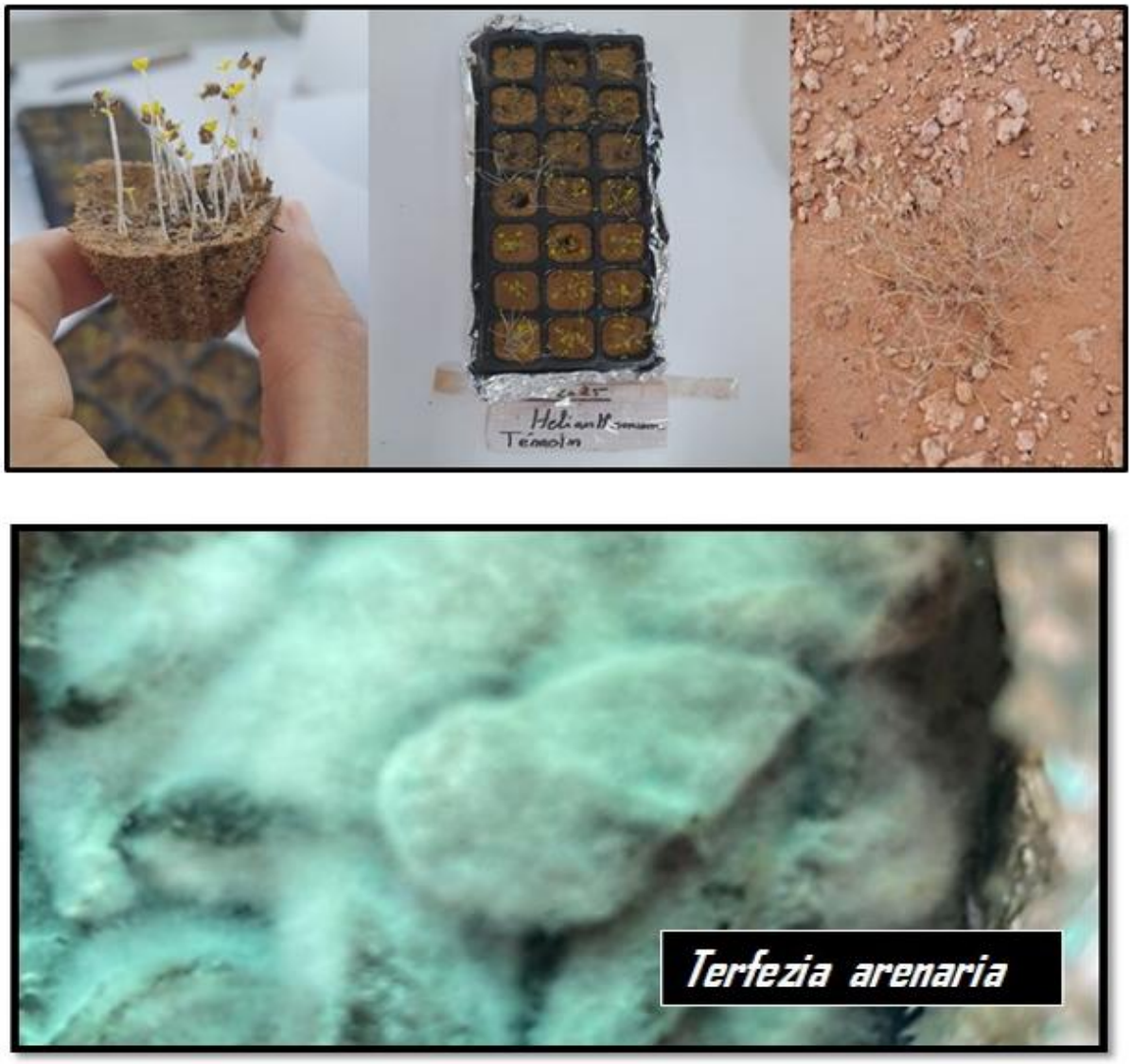


Source: Original (2025)

Figure 36: ***Agaricus bisporus*** mushroom print

III.4 Helianthemum root system analysis

A sample of Helianthemum was taken from a farm and its roots were studied. Results of growing Helianthemum in the laboratory. Figure 38



Source: Original (2025)

Figure 37: Results of growing *Helianthemum* and cultivation mycelium of *Terfezia arenaria*

III.4.1 Root development monitoring with mycorrhizal association assessment

Development at the cellular level, which only changed with the synthesis conditions but not with the fungal species. In field conditions, root colonization was mainly intracellular, forming an endomycorrhiza with large, septate and moniliform hyphae. Colonization only concerned the cortical cells of the roots. Intercellular hyphae were rarely observed. In no case was a sheath observed around the root; only in some cases did isolated hyphae appear around the root.

III.4.2 Common fungal pathogens and sporulation-related disorders in mushroom cultivation

Commercially grown Agaricus bisporus, is exposed to many different types of pathogens during its cultivation cycle. However, there are increasingly fewer chemical options available today to control pathogens and flies on farms Our study investigated some of the diseases facing farmers in Algeria. We found that the most common pests affecting farms are:

The Trichoderma, the presence of Trichoderma, in compost can make great loss of mushroom production. Trichoderma's mycelium growing, during incubation of mushroom mycelium, until compost became overgrown by mushroom mycelium.

Because in full incubated compost Trichoderma does not growing. Because of this, the most dangerous faze in mushroom growing, is period of end of pasteurization, after that, beginning of Phase II, beginning of Phase III. During this period of time, Trichoderma can infected compost and can start to growing. Infection in this fazes, is very dangerous, because if Trichoderma start to growing in compost in bags, or in blocks, there temperatures are higher, and it promoting growing of Trichoderma. Often, compost producers, gives responsibility of Trichoderma infections, to buyers, when they have mushrooms production lost, and explain to them that infection is consequence of bad sanitation, or lack of hygienic measures on mushroom farms. But, if we consider infection of Trichoderma, in period of spawning, or mycelium incubation, with infection in period of mushroom fructification, we will see, that loss of mushrooms is slight bigger, in first part than, appearance of infection in period of spawning and incubation. Trichoderma appearance in compost is consequence of bad quality of compost, and disbalance of C/ N, when this relation is around 20, at the end of pasteurization of compost, end of Phase II). Appearance of anaerobic products during composting in fazes 1 and 2, will be followed by appearance of Trichoderma, in compost. During anaerobic fermentation of compost

are produced and accumulated materials, what became food for Trichoderma: succinic acid, lactic acid, Analytical control of compost, and quantitative analyses of these acids, can give us, possibility to prevue possibility of Trichoderma appearance in compost. If pH value, in compost is low, this can be indicator, about Trichoderma appearance in compost. If Trichoderma is present in compost in many growing cycles, regardless of very good hygienic measures, and very good sanitation, in the clean zone, and tunnels, and where is spawning zone.

Figure 38: trichoderma green mold sporoliting on casing (Ghardaia Mushroom Farm, 2025).

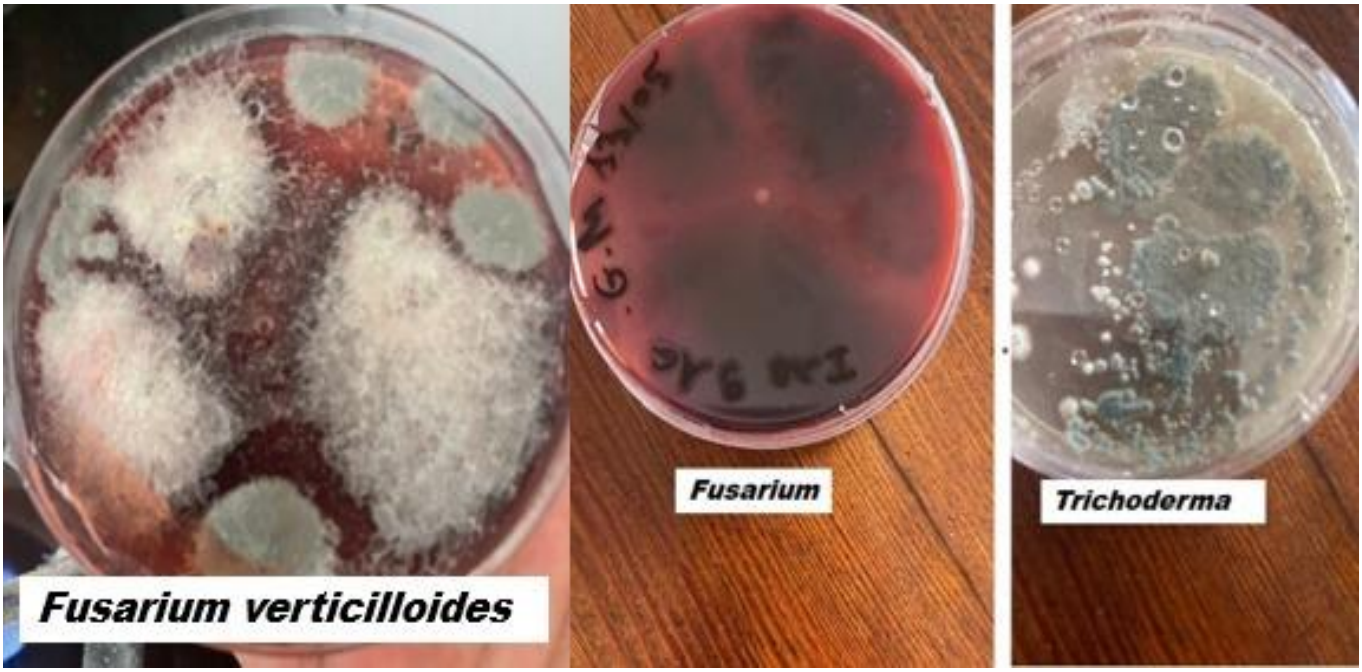


Figure39: The picture represents some of the fungi that contaminate our mushroom cultivation, ***Fusarium*** and ***Trochoderma.***

Mouchettes in Mushroom Growing Flycatchers, particularly attracted by the smell of mushrooms, can quickly become a scourge for growers. These small insects, often fruit flies, lay their eggs on mushrooms, which can lead to an infestation in no time. Once the larvae hatch, they begin feeding on the fungi's tissues, causing significant damage. This infestation leads to a reduction in the quality and quantity of crops. Second and third harvests are particularly affected, as damage from larvae can weaken the fungi and reduce their production potential. Additionally, the presence of speckles can promote the development of secondary diseases, making the final product less attractive to consumers. Therefore, controlling these insects is crucial to ensuring the viability and profitability of your mushroom crop**.**

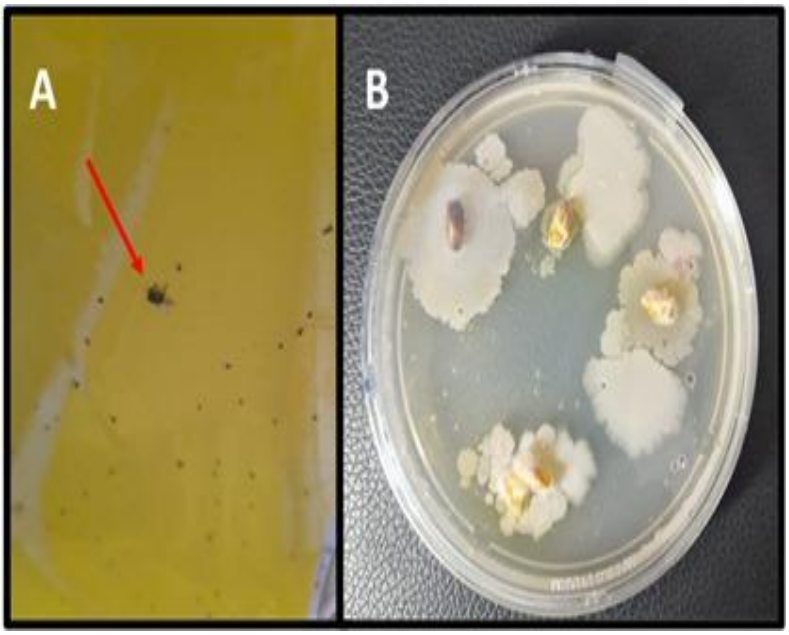


Figure40: (A) Phorid fly and (B) the results of some of the germs that infect button mushroom seeds and affect the quality of seeds produced in the laboratory

Commercial mushroom fly pests include three dipteran families: Sciaridae, Phoridae, and Cecidomyiidae (figure 40) Each family can cause significant yield or quality loss and vector mites, nematodes, and diseases on commercial farms. The dominant problem-atic species display regional, annual, and seasonal variations

Conclusion

In this study, a comparative analysis of the culture medium revealed that while SDA promoted faster fungal colonization, potato dextrose agar (PDA) produced higher mycelial quality. Substrate experiments showed that mixed substrates (coffee grounds with wheat straw) significantly enhanced oyster mushroom yields, achieving a 15% increase compared to substrates containing only straw, while reducing the initial establishment period.

This reinforces the importance of substrate optimization. For button mushroom cultivation, sprout quality and proper substrate treatment were critical factors in preventing contamination, as rapid mycelial growth was associated with higher yields. Furthermore, this study highlighted the feasibility of indoor cultivation using grow kits, with clear mycelial growth emerging within 15 days and fruiting by day 21.

However, significant challenges were identified, such as Trichoderma contamination and Phoridium infestations, underscoring the need for strict hygiene and pest management.

Preliminary results of ***Helianthemum lippii*** root analysis also indicated potential associations with mycorrhizal fungi associated with desert truffle (Tervisia) cultivation, warranting further research.

Practical recommendations include the use of SDA for rapid bud production and PDA for fungal quality, the adoption of coffee ground and coffee straw blends to increase oyster mushroom yields, the implementation of integrated pest management, and the exploration of home cultivation clusters for small-scale production. This research contributes to sustainable mushroom production in Algeria, with implications for food security, agricultural diversification, and the development of medicinal mushrooms. Future studies should focus on cost-benefit analyses of raw materials and the scaling up of the most effective methods for commercial use.

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