UNIVERSITI MALAYSIA KELANTAN

Determination Of Storage Shelf – Life For Agarwood Inducer Inoculum *Fusarium Solani* USJPCC-600 And *Aspergillus Niger*

MUHAMMAD MUHAIMIN BIN MOHD SALJI
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DECLARATION

I declare that this thesis entitled “title of the thesis” is the results of my own research except as cited in the references.

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Student’s Name : _____

Date : _____

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ABSTRAK

Fusarium solani dan *Aspergillus niger* adalah kulat filamentosa yang meluas di seluruh dunia dalam pelbagai persekitaran seperti tanah, bahan organik yang reput, dan persekitaran dalaman. Walaupun asal usul tepat mereka tidak diketahui secara pasti, kedua-dua spesies ini mempunyai taburan kosmopolitan, mencerminkan kebolehpasaran mereka kepada pelbagai habitat. *Fusarium solani* terkenal dengan peranannya sebagai patogen tumbuhan, menyebabkan penyakit seperti busuk akar dan layu pada tanaman pertanian. Demikian juga, *Aspergillus niger* adalah serba boleh, terlibat dalam penguraian bahan organik, tetapi juga boleh menjadi masalah sebagai pencemar dalam pemprosesan makanan dan persekitaran dalaman, yang boleh memberi kesan kepada kesihatan manusia. Salah satu manfaatnya untuk sesetengah budaya adalah gaharu. Ini kerana kedua-dua kulat adalah penyebar utama untuk pengeluaran resin dalam gaharu. Tujuan kajian ini adalah untuk menentukan tempoh simpan Fusarium solani dan *Aspergillus niger* pada parameter yang berbeza. Analisis mikroskop cahaya kulat untuk menentukan morfologi kulat yang diinginkan, sementara Kepekatan Optik digunakan untuk penentuan ketersediaan sel. Secara keseluruhan, kajian ini memberikan pandangan tentang keberkesanan masa penyimpanan dan suhu untuk hayat *Fusarium solani* dan *Aspergillus niger*.

ABSTRACT

Fusarium solani and *Aspergillus niger* are ubiquitous filamentous fungi found worldwide in diverse environments such as soil, decaying organic matter, and indoor settings. While their exact origins are not definitively known, both species have cosmopolitan distributions, reflecting their adaptability to various habitats. Fusarium solani is notorious for its role as a plant pathogen, causing diseases such as root rots and wilts in agricultural crops. Similarly, *Aspergillus niger* is versatile, participating in organic matter decomposition, but can also pose problems as a contaminant in food processing and indoor environments, potentially affecting human health. One of its benefits for culture is gaharu. It is because both fungi are the main infector for resin production in gaharu agarwood. Purpose of this study is to determine the shelf life of *Fusarium solani* and *Aspergillus niger* on different parameter. Light microscopy analysis of the fungi to determine the morphology of desired fungi, while Optical Density use for determination of availability of the cell. Overall, this study provides insights into the effectiveness of storage time and temperature for availability of *Fusarium solani* and *Aspergillus niger*.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Fusarium Schlechtendahl Emend. Snyder and Hansen is a filamentous fungus belonging to the Class Ascomycetes and Family Hypocreaceae (Gibson et al., 1994). It is a globally distributed soil-inhabiting fungus, with certain species confined to tropical regions, others prevalent in temperate zones, and some adapted to harsh climates in desert, alpine, and arctic areas (Francis & Burgess, 1975). While Fusarium species are commonly found in fertile cultivated and rangeland soils, they are relatively uncommon in forest soils. *Fusarium* species are often recognized as soil-borne fungi due to their abundance in soil and frequent association with plant roots, either as parasites or saprophytes (Jeschke et al., 1990). Various species within the genus are considered significant plant pathogens, and some produce mycotoxins such as fumonisins, zearalenones, and trichothecenes on plants. These mycotoxins can contaminate seeds, entering the food chain and posing risks to human and animal health. As a result, *Fusarium* species are hazardous to agricultural products, wildlife, livestock, and humans(Balali & Iranpour, 2006).

The genus *Aspergillus*, comprising over 300 recognized species, represents a diverse group of filamentous fungi with a global presence. These molds exhibit a distinctive morphology characterized by conidiophores, structures that produce conidia or asexual spores. Ubiquitous in various environments such as soil, decaying vegetation, and indoor spaces, Aspergillus species showcase adaptability to diverse climatic conditions (Baker & Bennett, 2007). Beyond their ecological role, certain species have economic importance, contributing to the production of citric acid, enzymes, and participating in fermentation processes for products like soy sauce and sake. However, some Aspergillus species, notably *A. flavus* and *A. parasiticus*, are known toxin

producers, raising concerns in agriculture due to the potential contamination of crops with mycotoxins like aflatoxins. In the medical realm, Aspergillus can cause infections in immunocompromised individuals, with *A. fumigatus* being a common culprit in respiratory conditions. Moreover, these fungi are utilized extensively in biotechnological applications, contributing to the production of various industrial compounds and serving as valuable model organisms in genetic and molecular studies. The multifaceted nature of *Aspergillus* underscores its significance in ecological, economic, medical, and biotechnological contexts (Christensen & Tuthill, 1985).

1.2 Problem Statement

Agarwood, also known as oud or aloeswood, is a fragrant resinous wood that is highly valued for its distinctive aroma. Natural *agarwood* is considered rare because the process of *agarwood* formation is a natural and unpredictable occurrence that can take many years to develop. Not all *Aquilaria* trees produce *agarwood*, and even among those that do, the formation of *agarwood* is not guaranteed. It is estimated that only about 10% of Aquilaria trees are naturally infected with the mold that produces *agarwood* (Tan et al., 2019). Over time, the techniques employed to induce *agarwood* formation have evolved, incorporating contemporary approaches (Laurence, 2013). Notably, the use of modern methods, such as fungal inoculation, has proven to be particularly effective, especially when applied to *Aquilaria* species (Mohamed et al., 2014). The deliberate introduction of fungi enhances *agarwood* production. It has been observed that naturally occurring microorganisms can play a significant role in augmenting the production of *agarwood*. Moreover, the quality of *agarwood* obtained from natural forests surpasses that of wood sourced from young plantations. This underscores the importance of employing advanced methods to stimulate *agarwood* formation, with a recognition that natural environments contribute to a higher quality yield compared to artificially established plantations. Using certain methods, *agarwood* can be produced artificially and take less time to be produce. Fungi such as *Fusarium spp., Trichoderma spp., Curvularia spp., Cunninghamella spp.,* and various *Aspergillus spp.* are frequently employed for the purpose of stimulating the production of agarwood resin in *Aquilaria* species (Blanchette, 2003; Mohamed et al., 2010). Fungi has different shelf life depending on how it can survive in certain conditions. Certain inoculation can

survive longer than the depending on its survivability such as temperature, food, oxygen, and water. Determining the method to monitor the shelf life of *Fusarium solani* and *Aspergillus niger* require a few methods and it takes quite some time to get accurate results. Also, the effect of temperature on effectiveness of *Fusarium solani* and *Aspergillus niger* is also a chellenge to be faced to get the best condition for the growth of the fungi and its storage condition preferences. As fungi can only live for three to five days, the researcher aims to increase its shelf life drastically to about one year. The best method is to use sporulation method to turn the fungi into spore as it can survive in extreme condition. The optimum condition for sporulation process to occur also need to be determined.

1.3 Objectives

The main purpose of this research is to

i. To verify shelf-life inoculum effectiveness.
ii. To verify the effect of storage temperature on effectiveness of inoculum.

1.4 Scope of Study

This research take place at one place only which is behind Universiti Malaysia Kelantan. The fungi chosen for this research is already provided which is *Fusarium solani* from USJPCC 600 and *Aspergillus Niger*. The research of *Fusarium solari* and *Aspergillus Niger* to stored consists of several tests which place at low temperature (4°C) and room temperature (37°C) to test the survivability of the fungi. The shelf life of the fungi also be tested to determine how long it can be stored. and how to increase the shelf life of the specific fungi. The fungi will be incubated in incubator at 25°C in agar plates for a week. After that, it will be transferred into Sabouraud Dextrose Broth (SDB) and incubated at 25°C and 120rpm for a week also. Both fungi will be stored for 1 week, 2 weeks, 3 weeks and 1 month in both temperatures. The availability of the fungi will be tested whether it still alive or not.

1.5 Significances of Study

From this research, it can be an opportunity to make artificial *agarwood* possible. It also more time saving as searching for natural infected agarwood consumes so much time and 4 artificial agarwood also takes less time to be produced. The *Aquilaria* tree is protected because to this research. Due to overharvesting and illegal logging, aquilaria trees, which provide *agarwood*, are currently under risk. Researchers want to lessen the reliance on natural trees and give an alternate way to produce *agarwood* by creating artificial *gaharu* inducers. This can aid in preserving the native Aquilaria tree population and safeguarding their habitats.

Additionally, it may boost the sustainable production of *agarwood*. Particularly in the perfume and incense sectors, agarwood is in high demand. Traditional ways of producing *agarwood* take a long period and rely on *agarwood* appearing naturally in trees, which might take several years. Artificial inducers may be able to provide *agarwood* production with a more controlled and sustainable method, satisfying market demand without diminishing natural resources.

It also creates new business prospects. The cultivation of *agarwood*, a valued commodity, can support regional economies. In areas where Aquilaria trees are natural or grown as a crop, researching artificial *gaharu* inducers may result in the creation of new enterprises and employment opportunities. Positive economic effects may result from this, especially in rural areas.

Quality Control and Standardisation. Since natural *agarwood* fluctuates in both quality and scent, it might be difficult to constantly meet market demands. Studying synthetic *gaharu* inducers may help scientists create processes for growing agarwood with more consistent properties. Producers may now better regulate quality and respond to certain customer preferences.

Broadening our comprehension of nature. The exact process of how agarwood forms in *Aquilaria* trees is still unclear. It is possible to learn more about the biological, chemical, and physiological processes involved in the formation of agarwood by researching artificial gaharu inducers. Broader uses of this knowledge are possible in the disciplines of plant biology, biotechnology, and related ones.

CHAPTER 2

LITERATURE REVIEW

2 This chapter contain information or idea about this topic. The information was taken from previous study related to the topic.**Introduction of agarwood**

Gaharu, commonly referred to as agarwood, is an essential non-timber forest product. It has been utilised by numerous cultures all over the world for hundreds of years as incense, perfume, and medicine. *Gaharu* is currently traded internationally between at least 18 nations for hundreds of tonnes worth of money.(Tan et al., 2019)

The resinous, fragrant, and extremely precious heartwood produced mostly by *Aquilaria* species, in the family Thymelaeaceous, is known by the name's *agarwood, aloeswood, eaglewood, and gaharu*. Agarwood trade has been documented for more than 2000 years, with the Middle East and East Asia serving as its main markets. Being supplied from sources ranging from the continental United States to the northeast of the Indian subcontinent. South-east Asia and the islands of the Indo-Malesian chain.(Tan et al., 2019)

Agarwood has traditionally been used for religious, therapeutic, and fragrant purposes in Buddhist, Jewish, Christian, Muslim, and Hindu communities. Since the 1970s, as the Middle East and consumer markets in North-east Asia have grown economically, a trade that was formerly carried out to supply very specific markets and a relatively small number of consumers has expanded considerably. Concerns have been raised that demand may exceed sustainable supply as a result of the increase in commerce (and, consequently, harvest).(Azren et al., 2019)

2.2 Natural *agarwood*

Aquilaria trees become agarwood when they sustain wounds and are subjected to biotic and abiotic stressors. The infection activates the trees' defence system, causing resin to be produced. This helps the trees, in a process known as tylosis, suppress the growth of the bacteria attacking the trees. The infection causes the tree to go through a biochemical process that yields oleoresin, which eventually causes the wood's colour to change from a lighter to a darker colour, creating what is known as agarwood. Few traders are prepared to wait the years it takes to produce *agarwood* of the wild variety. The only way to be sure that the tree contains the needed resin is to chop down the tree because only a small percentage of Aquilaria in the wild are afflicted and produce *agarwood*.(Ma et al., 2021)

2.3 Artificial *agarwood*

To stop the extinction of *Aquilaria* trees, artificial induction techniques for *agarwood* have been developed. Because of the threat to the trees, researchers have developed techniques to create *agarwood* in a controlled environment. Biological immunisation, chemical induction, and physical trauma are the three techniques employed.

Biological, chemical, and physical processes can all play a role in the production of agarwood (Pojanagaroon and Kaewrak 2003). According to Soehartono and Newton (2000), only 10% of *Aquilaria* trees produce agarwood naturally, and the process takes more than 20 years. *Agarwood's* high economic and therapeutic worth has raised market demand, and to meet this demand, researchers have devised a variety of artificial methods to shorten the time required for *agarwood* creation and quality maintenance (Pojanagaroon and Kaewrak 2003). The next section discusses the many techniques created for synthetic agarwood generation in the Aquilaria.

Physical method is one of the methods that used to produce *agarwood*. For the artificial creation of *agarwood*, a variety of wounding techniques are employed, including cutting, cauterising, holing, nailing, axe chopping, wounding with chisels, and bark removal (Pojanagaroon and Kaewrak 2003; Liu et al. 2013). People in China

intentionally wound trees with huge knives and hammer nails into the trunks of the trees in order to make *agarwood*. According to Dai et al. (2010), trunk breaking method only resulted in fatty acid generation, whereas nail insertion and holing in the tree helped in the creation of essential oil in the form of sesquiterpenes and aromatic constituents. The drawback of the physical method is that the agarwood produced is of lower quality than agarwood obtained naturally (Persoon 2007). Additionally, Liu et al. (2013) applied various physical techniques to stimulate the production of *agarwood*, including the burning chisel drilling (BCD) method, agarwit, and partially trunk pruning (PTP). Different chemical procedures were also developed at the same time as physical methods since they could not meet the market's need for the desired quality.

Other than physical method, chemical method also used to produce the *agarwood*. The synthesis of agar oil has been induced throughout the tree using chemical techniques. Different substances have been employed to stimulate the development of *agarwood*, including methyl jasmonate, soybean oil, brown sugar, sodium chloride, formic acid, hydrogen peroxide, and salicylic acid (Chen et al. 2011; Wei et al. 2012; Ito et al. 2005). Different concentrations of these compounds have been injected into the tree's xylem. Chemicals were transported by water to various tree parts, where they caused damage to the entire plant. In Vietnam, Professor Robert Blanquette drilled plastic pipe into trees to give chemical inducers to boost oleoresin production on a regular basis. In compared to a natural process, the yield of *agarwood* was increased by combining physical and chemical approaches (Pojanagaroon and Kaewrak 2003).

A biotechnological strategy using various fungal strains with and without chemical inducers to augment stress conditions has greatly enhanced artificial infection for *agarwood* development. The benefit of biological agents is that, according to Novriyanti et al. (2010), they are progressive methods for the systematic growth of fungi that can infect *agarwood* production both continuously and intermittently. The isolation of *Epicoccum granulatum* from an infected tree branch and subsequent reporting of its relationship with the plant in the creation of resinous wood in *A. agallocha* in 1952 revealed a role for fungi and the formation of *agarwood* (Bhattacharyya 1952). Later, a large number of scientists identified numerous fungus and established the beneficial effect of fungi in causing the creation of *agarwood.*

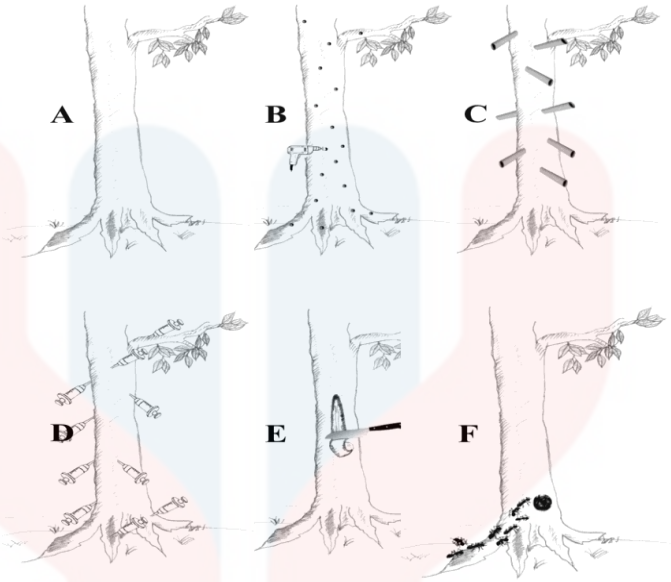


Figure 2.1: Natural and artificial method used in *agarwood.*
Source: (Akter et al., 2013)

Illustration of induction methods commonly used in *agarwood* formation. In natural maturation process, no induction or injury is required but need years to achieve considerable amount of resin synthesis (A). General practice of artificial induction is to drill the stems, roots and large brunches (B). The drilled pores were kept open to ease access of natural agents into the pores. Addition of sugary syrup or inocula to these pores attracts insects and facilitates infection. Metal or PVC tubes were installed in those pores (C) to prevent healing the pores and establish prolonged infection. Syringe with inocula and inducers (D) were also used to assist batch and continuous inoculation and induction. In some region, indigenous people peel off the bark (E) to promote infection to the core tissues and harvest the chips of woods seasonally from live tree. There are certain areas where a hole was dug in the tree to facilitate hatching of ants and snails (F), which is being believed to be responsible for *agarwood* formation. (Akter et al., 2013)

2.4 Fungus used for *agarwood*

According to the current study, the fungus *Aspergillus*, *Penicillium*, *Fusarium*, *Lasiodiploidia*, and *Chaetomium* colonised agarwood. *Aquilaria* species can be infected by fungi including *Aspergillus* species, *Botryodiplodia* species (*Lasiodiplodia* species),

Diplodia species, *Fusarium bulbiferum*, *F. laterium*, *F. oxysporum*, *Penicillium* species, *Pythium* species, and *Trichoderma* species, according to earlier investigations. In the current study, the *Aspergillus* isolate AR13 displayed the principal enzyme activity. This might make it easier for *Aspergillus* fungus to infect *agarwood* in the future (Chhipa & Kaushik, 2017). *Agarwood* had primarily been attacked by opportunistic fungi from soils in a rainforest environment, according to reports in Wiriadinata. It is thought to be a mild pathogen and does not seriously harm trees. However, the ongoing release of enzymes by this fungus may boost the *agarwood*'s defence mechanisms. Thus, the pathogenesis-related enzymes were measured in the current study. Cellulose, the main framework molecule of the plant cell wall, exists as microfibrils, and matrix molecules (glycoproteins, hemicelluloses, pectins, and lignins) fill the spaces between the microfibrils and cellulose chains. Cell walls of plants are softened and broken down by fungi's cellulolytic enzymes. Thus, the dyediffusion method was used to test the cellulase activity of all 17 fungal isolates. The research showed that *Aspergillus* isolate AR13 has the highest cellulolytic activity when compared to all other isolates (Nagajothi et al., 2016)

According to field studies on plant diseases, high relative humidity and moderate temperatures promote fungus growth, while low relative humidity and extremely hot or cold temperatures prevent growth and spore germination. Other research on soil fungi demonstrates that prevalence varies with habitats and seasons, and that it positively and negatively related to moisture and temperature. The high surface-to-volume ratio of fungi, which makes them susceptible to water loss, may be the cause of this apparent positive link between moisture and fungal growth and abundance (Talley et al., 2002).

2.5 Induction method

Numerous techniques for bio-inducer infection were discussed, including trunk surface *agarwood*-inducing technique (Agar-Sit) (Chen et al., 2018), pinhole-infusion (Tian et al., 2013) and bottle dripping (Justin et al., 2020). Bottle dripping and ChemJet use a similar method that involves placing the bottle or pressurised injection inside the hole upside down and pouring slowly into the wound. A total of 1 to 3 L of inoculant

can be provided per tree, and approximately 10 mL and 20 mL of inoculant can be delivered in each hole using bottle dripping and ChemJet tubing, respectively (Mustapa et al., 2022). It was demonstrated in that the drip technique is carried out by setting the bottle in a hole, securing the cap with parafilm to stop inoculant leaking, connecting it to the wound via the hose, and then sealing it with clay (Justin et al., 2020).

The pinhole-infusion technique is another alternative. To loosen the wood, large holes are drilled into the trunk at a width of 4 to 5 cm and a thickness of 0.5 cm. The inoculant hole was then covered with tape to help the fungus solution absorb and propagate throughout the wood cell (Tian et al., 2013). According to (Chen et al., 2018), they used an approach called Agar-Sit that is quite similar to the one described above. The open rectangular bark surface was etched with a 2 cm2 grid with a depth of 1.5–2.0 cm across the xylem. The inoculant was then applied to the grids using a brush and sprayed on. To reveal the xylem surface, the 50 cm long bark of the trunk was sliced into rectangular forms and then covered. The degradation layer of the surface was removed after six months of treatment, and the agarwood that had formed was gathered. Another *agarwood* induction was conducted after repeating the procedure.

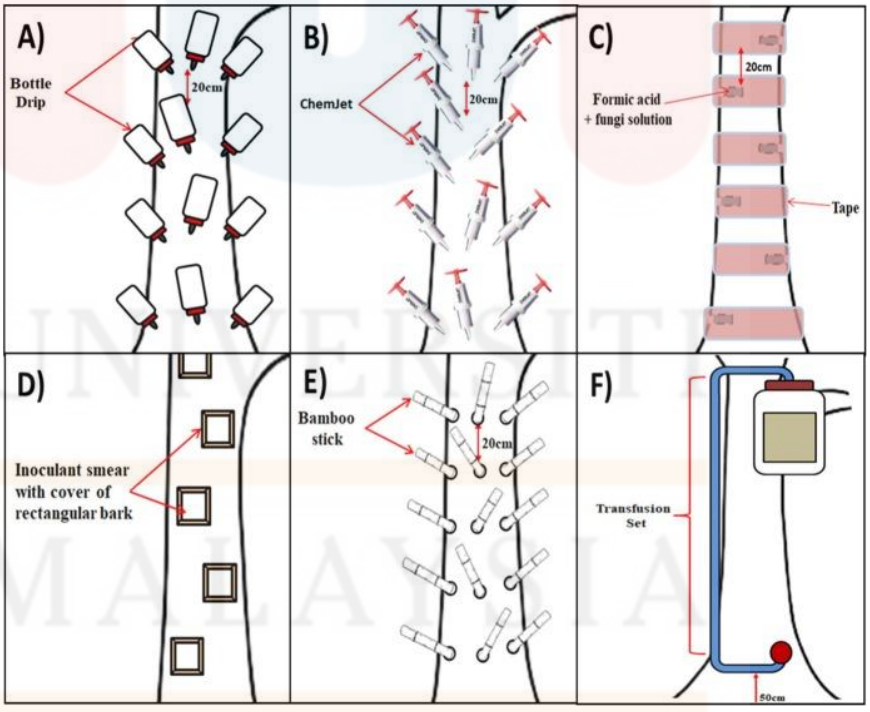


Figure 2.2: Different type of inducing technique.

Source: (Ngadiran et al., 2023)

2.6 *Fusarium* Species

Fusarium, a significant group of plant-pathogenic fungi in agriculture and horticulture, poses a threat to various crops, causing diseases such as head blight in wheat (*Fusarium graminearum*) (figure 3.1) and wilt and stem rot in crops (*Fusarium oxysporum)* (figure 3.2). These two *Fusarium* species are recognized among the top ten plant pathogens due to their scientific and economic impact in molecular plant pathology (Dean et al., 2012). An examination of plant diseases on the American Phytopathology Society website reveals that 83 out of 108 plant species suffer from Fusarium diseases, affecting agricultural and horticultural production. Beyond plant diseases, Fusarium species produce mycotoxins like trichothecenes and fumonisins, negatively impacting animal production and posing potential risks to human health (Desjardins, 2006; O'Donnell et al., 2013).

Some *Fusarium* species also cause diseases in humans, particularly those who are immunocompromised, leading to severe consequences. Maintaining a stable taxonomy for Fusarium is crucial for practitioners involved in diagnosing diseases, identifying these fungi, and developing management strategies. Unfortunately, the taxonomy of *Fusarium* has been historically unstable over its 210-plus years of history. Definitions of species and genera have undergone significant changes, ranging from over a thousand species to just nine in less than 30 years (1920s to 1950s) (Summerell & Leslie, 2011). Presently, there are over 300 phylogenetically distinct *Fusarium* species, with nearly half yet to be formally described (Summerell, 2019). The last two decades have witnessed substantial transformations in our understanding of *Fusarium*, with the introduction of molecular techniques identifying cryptic species and the acknowledgment of the necessity for a single name for fungi like *Fusarium*.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

In this chapter, material and method are briefly presented for determining the fungi inoculum and shelf life of the inoculum. The method to increase the effectiveness of the inoculum also presented in this chapter.

3.2 Material

Fungi	*Fusarium solani* USJPCC-600
Chemical reagent	Antibiotic and Antifungal Agents, Potato dextrose agar (PDA), Sabouraud Dexrose Agar
Apparatus	Laminar flow hood, incubator, test tube, culture tube, petri dish, microscope, inoculation loop or needle, centrifuge, autoclave, pH meter, colony counter, pipettes and micropipettes, growth chamber, vortex mixer, timer.

3.3 Method

3.3.1 Cultural of Fusarium Solani

After obtaining a pure culture, the experiment involved the cultivation of *Fusarium* and *Aspergillus* on Potato Dextrose Agar (PDA) over a 7-day period. First, PDA agar was prepared following the manufacturer's guidelines. Each fungus was individually inoculated onto separate PDA agar plates using their respective spores or mycelial fragments. The plates were then incubated at optimal temperatures: 20-30°C

for *Fusarium* and 25-37°C for *Aspergillus*, allowing for the fungi to develop over the specified time frame. Throughout the incubation period, careful observations were made regarding the growth patterns, including colony size, color, and texture.

Morphological features of *Fusarium*, characterized by white to pinkish/orange cottony textures, and *Aspergillus*, with green/yellow-green powdery structures, were documented. Additionally, the plates were inspected for spore production, with *Fusarium* expected to produce conidia and *Aspergillus* conidiophores. Contamination checks were conducted, and any unexpected growth was recorded. Microscopic examinations were performed to analyze spores and hyphae, aiding in the differentiation of the two fungi. The suitability of PDA agar for promoting robust growth and sporulation was assessed, and detailed records were maintained for subsequent analysis and conclusions. The "Preservation and Maintenance of Living Fungi" (Smith and Onions, 1994) provides an overview of additional techniques for preserving fungal cultures. It is advised to use a variety of techniques so that cultural preservation is more likely (if one method fails).

3.3.2 Storage temperature

Preparation of four different temperature condition for *Fusarium Solani* growth storage. The experimental design involved the cultivation of two distinct fungi, *Fusarium solani* and *Aspergillus niger*, on Potato Dextrose Agar (PDA) under varying temperature conditions: 4°C and 37°C. To initiate the experiment, PDA agar was meticulously prepared in accordance with the manufacturer's specifications. Subsequently, separate PDA agar plates were inoculated with spores or mycelial fragments specific to *Fusarium solani* and *Aspergillus niger*. The plates were then subjected to two different incubation temperatures, with one set incubated at 4°C to simulate a cooler environment, and the other set at 37°C to replicate a warmer condition. These temperatures were chosen based on the known temperature preferences of *Fusarium solani* and *Aspergillus niger*, aiming to observe their respective growth behaviors under conditions resembling potential ecological niches.

Over a 7-day incubation period, meticulous observations were made to document the growth patterns, morphological features, and spore production of each fungus under the specified temperature regimes. For *Fusarium solani*, known for its versatility in temperature tolerance, the 4°C condition provided insights into its

adaptability to lower temperatures. In contrast, the 37°C condition simulated an elevated temperature to assess the response of *Aspergillus niger,* known for thriving in warmer environments.

Throughout the experiment, contamination checks were performed to ensure the purity of the cultures, and microscopic examinations were conducted to analyze spores, hyphae, and other relevant structures. Detailed records were maintained, encompassing colony characteristics, growth rates, and any notable variations observed between *Fusarium solani* and *Aspergillus niger* under the distinct temperature parameters. This experimental approach aimed to provide a comprehensive understanding of how these fungi respond to different temperature conditions, contributing valuable insights into their environmental adaptability.

3.3.3 Study on morphology of the fungi

The mycelial colour, texture, and growth characteristics of each individual fungus were examined in pure culture. In-depth analysis of *Fusarium solani* and *Aspergillus niger* was conducted by examining their mycelial characteristics, including color, texture, and growth patterns, within individual pure cultures. After the inoculation of the PDA agar plates with the respective spores or mycelial fragments of each fungus, the plates were incubated at the specified temperatures (4°C and 37°C) over the designated 7-day period. Firstly, mycelial color was meticulously observed. *Fusarium solani*'s mycelium was expected to exhibit varying colors, ranging from white to pinkish or orange, depending on the specific strain. In contrast, Aspergillus niger's mycelium typically presents a lighter color, often described as white to pale yellow. Secondly, the texture of the mycelial growth was examined. *Fusarium solani* is known for developing a cottony or woolly texture, while *Aspergillus niger* tends to display a powdery or velvety texture. These textural attributes provide valuable information for distinguishing between the two fungi and contribute to their taxonomic classification. Lastly, growth characteristics were closely monitored. *Fusarium solani*, recognized for its rapid growth and adaptability, typically manifests as spreading colonies with well-defined edges. *Aspergillus niger*, known for its prolific growth, forms colonies with a radial and often dense appearance. Throughout the experimental duration, detailed records were maintained, documenting any changes in mycelial color, texture, and growth patterns over the course of the 7-day incubation period. Microscopic examinations were also

employed to complement macroscopic observations, providing a more comprehensive understanding of the internal structures and developmental stages of the mycelium. By focusing on these specific mycelial characteristics, this method aimed to contribute insights into the distinctive features of *Fusarium solani* and *Aspergillus niger,* fostering a comprehensive comprehension of their morphology and growth dynamics in pure culture conditions.

3.3.4 Plate to plate subculture

Sub-culturing fungi from plate to plate is a meticulously executed method within microbiology, pivotal for the maintenance and propagation of fungal cultures. The procedure commences with the preparation of fresh agar plates, meticulously ensuring sterility to provide an optimal growth environment. These plates are typically composed of nutrient-rich media tailored to the specific requirements of the fungi under study. Once the agar plates are prepared, the process moves to colony selection. A key aspect here is identifying a robust and well-isolated fungal colony on the original plate, one that exhibits characteristics of purity and freedom from contamination. Sterilization of tools, often through flame-sterilization, is paramount to prevent the introduction of foreign microorganisms. Using a sterile inoculation loop or needle, a minute portion of the selected fungal colony is carefully transferred to the surface of the fresh agar plate. This transfer can be executed through various techniques, such as streaking or spreading, depending on the desired outcome and the characteristics of the fungus being cultured. The newly inoculated plate is then sealed to prevent contamination and placed in an incubator set to the specific temperature and environmental conditions conducive to fungal growth. Regular monitoring is essential to observe the development of fungal colonies. Depending on the growth rate of the fungus, visible signs of growth may become apparent within a few days to a week. Sub-culturing may need to be repeated periodically to maintain the purity of the culture or to propagate the fungus for further study. Each sub-culturing iteration involves selecting a single colony from a previous plate and transferring it to a fresh plate, perpetuating the process. This method ensures the continuity of pure fungal cultures and facilitates ongoing experimentation and research within the field of microbiology.

3.3.5 Preparation of media

In order to create suitable growth media for *Fusarium solani* and *Aspergillus niger*, Potato Dextrose Agar (PDA) was prepared with and without the addition of chloramphenicol. The incorporation of chloramphenicol, a broad-spectrum antibiotic, aimed to inhibit bacterial contamination, ensuring a pure fungal culture. The media preparation followed a meticulous procedure to facilitate optimal growth and observation of the two fungi under controlled conditions. Firstly, the standard PDA medium was prepared by dissolving commercially available PDA powder in distilled water according to the manufacturer's recommendations. The mixture was then autoclaved to sterilize the medium, eliminating any potential contaminants. Post-sterilization, the PDA was cooled to a temperature suitable for handling, poured into sterile Petri dishes, and allowed to solidify.

For the preparation of PDA with chloramphenicol, the antibiotic was added to the medium before autoclaving. A commonly used concentration of chloramphenicol in fungal media is around 0.05ml. The inclusion of chloramphenicol ensured the suppression of bacterial growth while allowing the fungi to thrive. Once both types of media were prepared, separate sets of PDA plates and PDA with chloramphenicol plates were inoculated with spores or mycelial fragments of *Fusarium solan*i and *Aspergillus niger*. The plates were then incubated at the predetermined temperatures (4°C and 37°C) for a period of 7 days.

Throughout the incubation period, careful observations were made regarding the growth patterns, mycelial characteristics, and any signs of contamination. The inclusion of chloramphenicol aimed to create a controlled environment, facilitating the assessment of fungal growth without interference from bacterial contaminants. The experiment sought to elucidate the impact of chloramphenicol on the growth dynamics and morphology of *Fusarium solani* and *Aspergillus niger*, contributing to a more nuanced understanding of these fungi in pure culture conditions. Detailed records were maintained to document any variations in growth and development under the influence of chloramphenicol in comparison to the standard PDA medium.time.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Temperature effect on *Fusarium Solani* and *Aspergillus Niger* growth

After a 7-day incubation period at temperatures of 4°C and 37°C, both fungi exhibited survival and growth, suggesting their adaptability to a broad temperature range. The mycelial characteristics, including color and texture, remained consistent with their typical profiles. These findings highlight the environmental flexibility of *Fusarium solani* and *Aspergillus niger*, providing valuable insights into their adaptability and potential implications for various applications and ecological contexts.

Fungi	Media	Incubation Temperature	Storage Temperature	Description	Result
Fusarium Solani	Potato Dextrose Agar (PDA)	25° C	25° C		
Fusarium Solani	Sabouraud Dextrose Broth (SDB)	25° C	25° C	Incubate at 120 rpm	

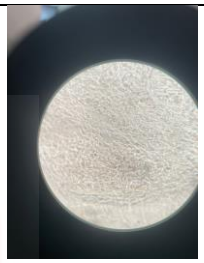
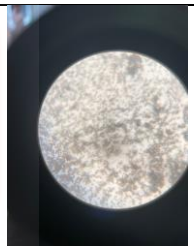
Fusarium Solani	Sabouraud Dextrose Broth (SDB)	25° C	25° C	Incubate at 120 rpm	
Aspergillus Niger	Potato Dextrose Agar (PDA)	25° C	25° C		
Aspergillus Niger	Sabouraud Dextrose Broth (SDB)	25° C	25° C	Incubate at 120 rpm	
Aspergillus Niger	Sabouraud Dextrose Broth (SDB)	25° C	25° C	Incubate at 120 rpm	

Table 4.1: Morphology of *Fusarium Solani* and *Aspergillus Niger* under microscope 10x Magnification

The remarkable observation of *Fusarium solani* and *Aspergillus niger* displaying nearly identical morphology under the microscope across the original culture, as well as at both 4°C and 37°C temperatures, adds an intriguing layer to the discussion. While the similarities in microscopic characteristics suggest a robust underlying structural consistency in both fungi, it is essential to acknowledge that slight variations were noted, influenced by a few factors.

One contributing factor to the observed microscopic differences could be the inherent adaptability of these fungi to temperature fluctuations. Although the overall morphology remained largely consistent, subtle alterations may have occurred in response to the distinct temperature conditions. Temperature-induced changes in cellular structures, such as cell wall thickness or spore production, might have

contributed to the observed variations, even though the fundamental morphology remained remarkably similar.

Furthermore, it is crucial to consider the potential influence of genetic diversity within the original culture of both *Fusarium solani* and *Aspergillus niger.* Variations in genetic makeup, even within a single species, can lead to phenotypic differences. These genetic nuances may have manifested as subtle disparities in microscopic features, contributing to the observed differences at the various temperature settings. Additionally, the experimental conditions, while controlled, might not perfectly mimic the complex and dynamic environments these fungi encounter in nature. Factors such as nutrient availability, oxygen levels, and other microenvironmental variables could have played a role in shaping the observed microscopic features.

The findings underscore the need for a comprehensive understanding of the factors influencing fungal morphology and highlight the dynamic nature of these organisms in response to environmental cues. Future investigations could delve deeper into the genetic and physiological mechanisms governing the observed variations, shedding light on the adaptability of *Fusarium solani* and *Aspergillus niger* in different temperature conditions. These insights could have implications for both basic mycological research and applied fields, providing valuable information for the study and potential manipulation of fungal behavior.

4.1.2 Morphology of original stock freezer *Fusarium Solani* and *Aspergillus Niger* under microscope.

Fungi	Under 10x Magnification	Under 100x Magnification
Fusarium Solani (Original)		

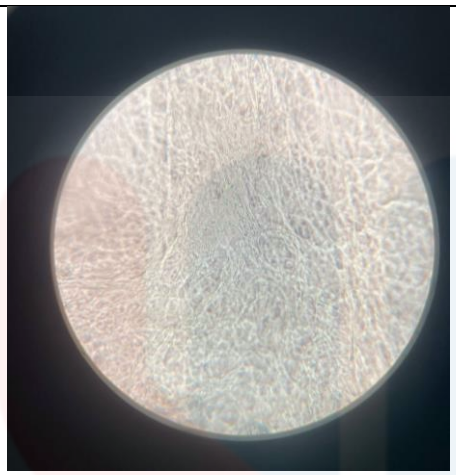
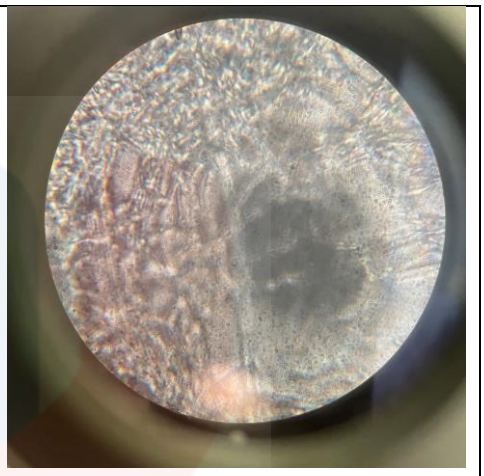
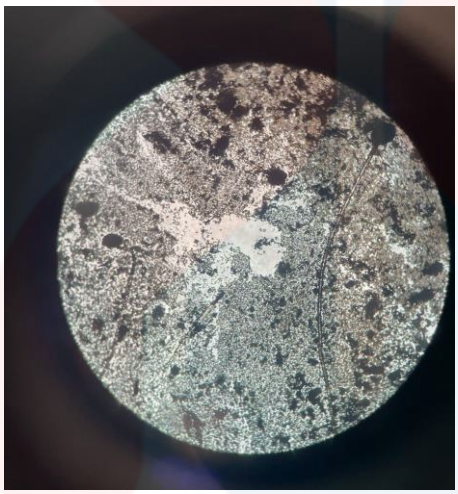
		
Aspergillus Niger (Original)		

Table 4.2: Morphology of *Fusarium Solani* and *Aspergillus Niger* under microscope

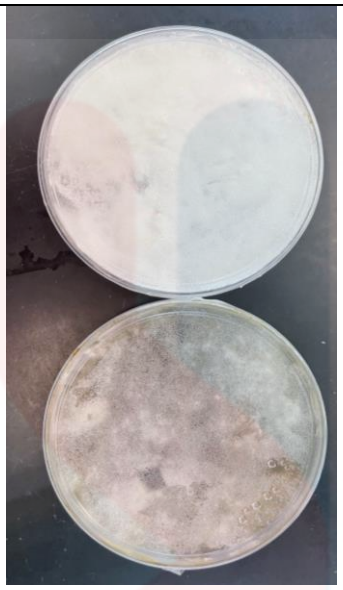
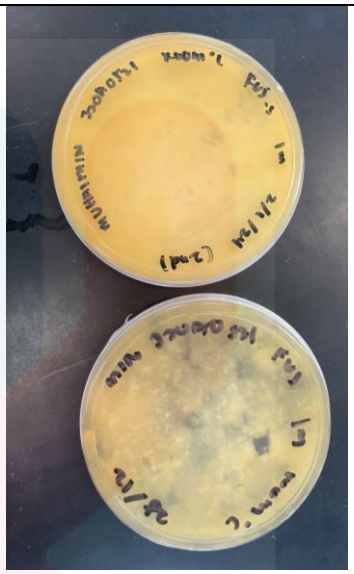
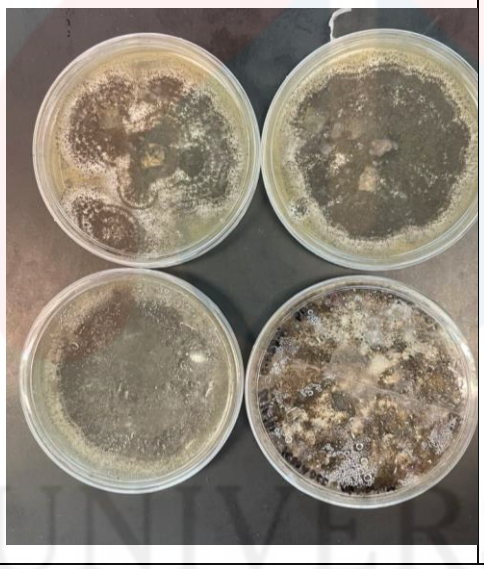
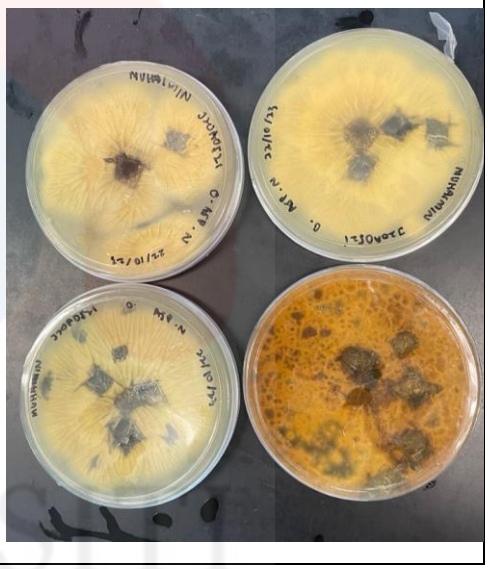
Fungi	Front	Back
Fusarium solani		
Aspergillus niger		

Table 4.3: Visual morphology of *Fusarium solani* and *Aspergillus niger*

In *Fusarium solani*, long rod-like structures observed under the microscope likely refer to the hyphae of the fungus. *Fusarium solani* is a filamentous fungus, meaning it consists of elongated, thread-like structures known as hyphae. These hyphae can vary in length and diameter but generally appear as long, slender rods when viewed under the microscope. The hyphae of *Fusarium solani* are septate, meaning they have cross-walls called septa that divide the hyphae into distinct compartments. These septa give the hyphae a segmented appearance under the microscope.

The hyphae of *Fusarium solani* play a crucial role in nutrient absorption, growth, and reproduction. It's important to note that while the hyphae are the

predominant structures observed in *Fusarium solani*, other structures such as conidiophores and conidia may also be present. Conidiophores are specialized structures responsible for producing asexual spores (conidia), and they may appear as elongated structures arising from the hyphae. Conidia are typically single-celled and may be arranged in chains along the length of the conidiophore.

When describing the long, rod-like structures in *Fusarium solani* under the microscope, it's essential to emphasize the septate nature of the hyphae and their role as the primary vegetative structures of the fungus.

In examining *Fusarium solani* under a microscope, the observation of branched conidiophores provides valuable insights into its morphological characteristics. *Fusarium solani*, a filamentous fungus, exhibits a complex reproductive structure that includes these specialized branching structures. The presence of branched conidiophores signifies a key aspect of its reproductive strategy, allowing for increased conidia production and dispersal.

The branched conidiophores emerge as elongated projections from the hyphae, branching at various points to give rise to multiple arms or branches extending outward. This branching pattern creates additional sites for conidia formation, enhancing the fungus's reproductive capacity. The conidia themselves, typically single-celled and cylindrical to ellipsoidal in shape, are often arranged in chains along the length of the conidiophore branches.

The septate nature of *Fusarium solani* hyphae and conidiophore branches is evident under the microscope, with septa forming transverse partitions across the structures. This compartmentalization contributes to the structural integrity of the fungus and facilitates nutrient transport. Furthermore, when grown on agar media, colonies of *Fusarium solani* with branched conidiophores typically display a cottony or woolly texture, with variations in coloration over time. Overall, the observation of branched conidiophores in *Fusarium solani* underscores its morphological diversity and adaptive reproductive strategies, offering valuable insights into its biology and ecology.

The microscopic observation of small black dots, conidiophores with black structures at their tips, and black-colored conidia in *Aspergillus niger* provides valuable insights into the morphology and reproductive characteristics of this fungus. The small black dots correspond to conidiophores, which are specialized structures responsible for the production and dispersal of asexual spores known as conidia. These conidiophores typically emerge as elongated stalks from the hyphae and bear conidiopores at their tips.

The black coloration of these conidiophores indicates the presence of mature conidia ready for dissemination.

Furthermore, the black structures observed at the tips of the conidiophores represent the conidiopores, the sites where conidia are formed and released. Conidiopores are crucial components of the reproductive process in *Aspergillus niger*, serving as the outlets for the asexual spores. The black coloration at the conidiopores suggests the accumulation of spores, indicating the fungus's reproductive activity. Moreover, the conidia themselves exhibit a distinctive black coloration. These conidia are the primary means of reproduction and dispersal for *Aspergillus niger*. The black pigmentation of the conidia serves various functions, including protection against environmental stressors such as UV radiation and desiccation.

In summary, the observed morphology of *Aspergillus niger* under the microscope, including small black dots (conidiophores), conidiopores with black structures at their tips, and black-colored conidia, highlights the fungus's reproductive strategies and adaptations for survival in diverse environments. Understanding these morphological features is essential for accurate identification and characterization of *Aspergillus niger*, particularly in clinical and research settings.

Based on the result gained, *Fusarium solani* exhibited distinct morphological features when cultured on agar plates. Colonies appeared as cottony or woolly masses with various shades of coloration, ranging from white to gray or even pinkish hues. Underneath the microscope, Fusarium solani displayed characteristic structures such as long, septate hyphae, giving rise to a dense network of mycelium. Additionally, the presence of branched conidiophores bearing chains of conidia further confirmed the identity of *Fusarium solani*. These conidia, typically single-celled and cylindrical to ellipsoidal in shape, were arranged in chains along the length of the conidiophore branches. The visual observation of *Fusarium solani* morphology on culture plates corroborated previous descriptions of its typical characteristics. Furthermore, the uniformity and consistency of its morphological features across replicate plates indicated the reproducibility of our experimental conditions.

It also produces sourly and stinky smell. The observation of a sour and stinky smell associated with *Fusarium solani* culture plates is an intriguing finding that may provide additional insights into its growth dynamics and metabolic activities. While *Fusarium solani* is not typically known for producing strong or distinctive odors, variations in smell can occur due to factors such as medium composition, microbial

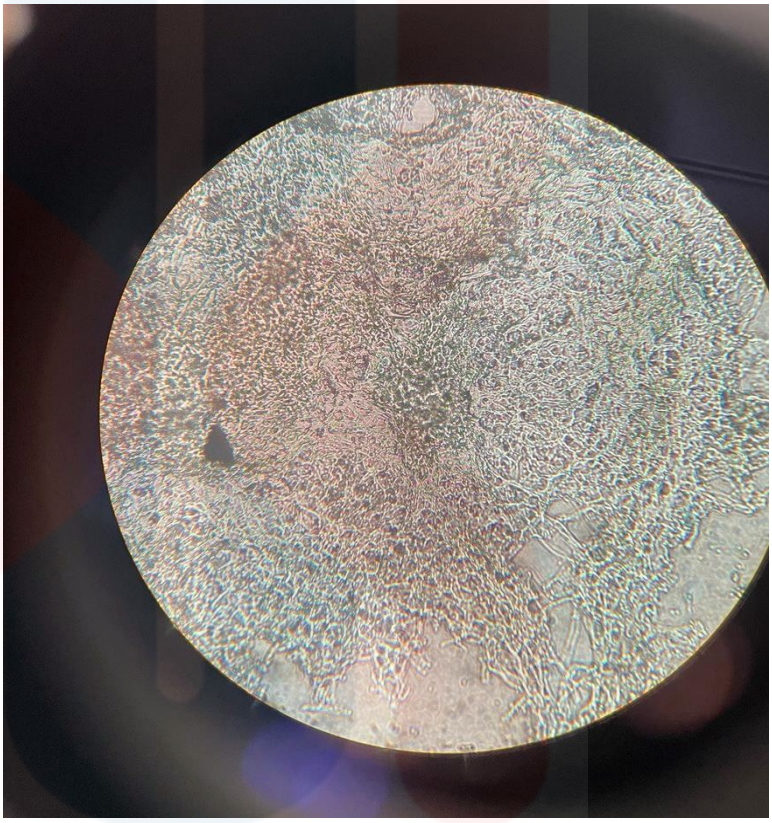
interactions, and environmental conditions. The soury and stinky smell could potentially be attributed to the production of volatile organic compounds (VOCs) by *Fusarium solani* during its growth and metabolism. These VOCs may result from metabolic processes such as fermentation or the breakdown of organic substrates present in the culture medium. Additionally, the presence of other microorganisms in the culture, whether as contaminants or as part of a mixed culture, could contribute to the overall odor profile observed. Further investigation into the specific compounds responsible for the sour and stinky smell, as well as the underlying metabolic processes driving their production, could provide valuable insights into the physiology and ecology of *Fusarium solani* in laboratory settings.

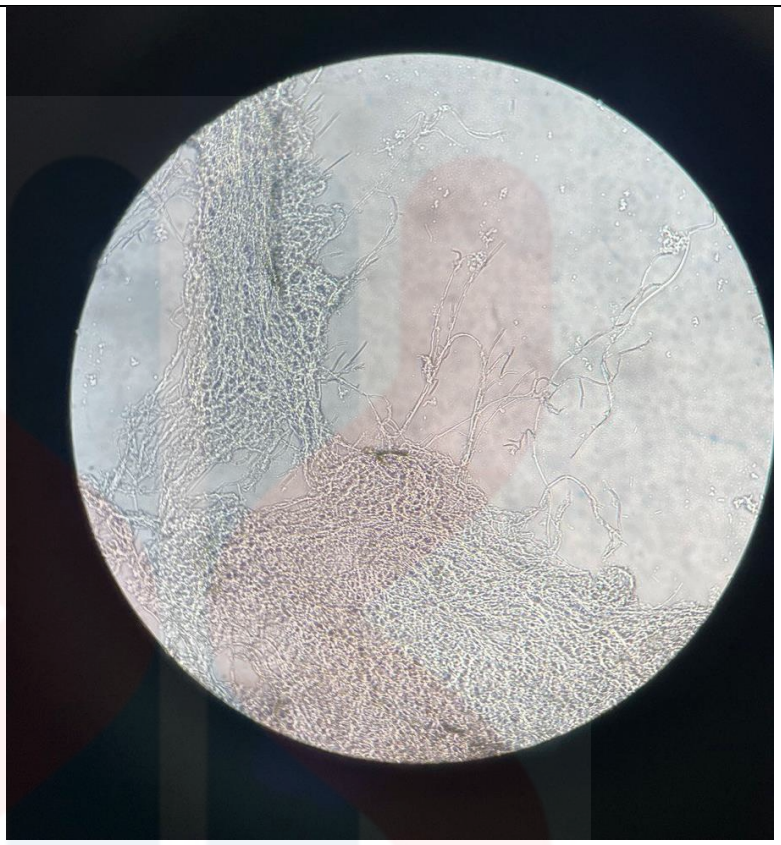
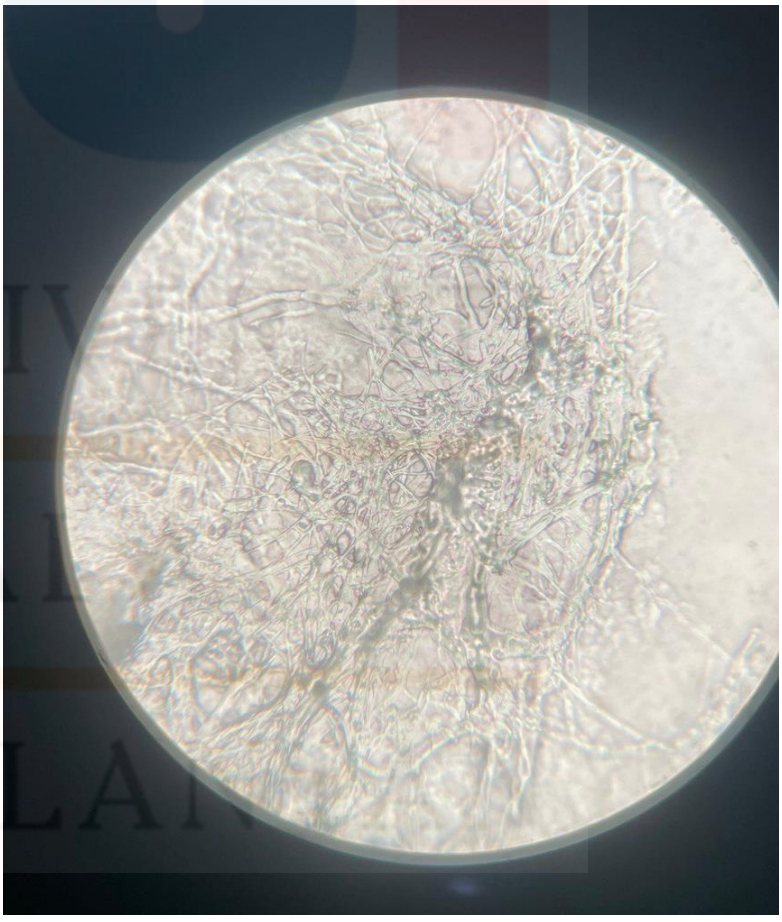
The visual morphology of *Aspergillus niger* on culture plates revealed distinct characteristics. Colonies appeared as velvety or powdery masses with a dark green to black coloration, often exhibiting a radial growth pattern. In addition to its visual appearance, *Aspergillus niger* colonies may emit a characteristic musty or earthy odor. This smell, reminiscent of damp soil or decaying organic matter, can be noticeable upon close inspection of the culture plates.

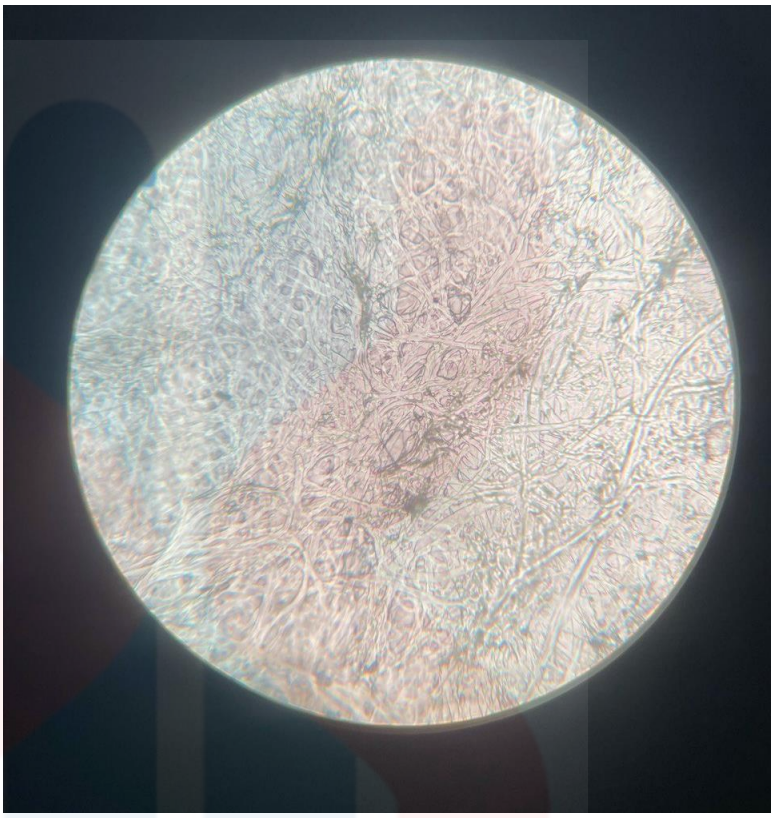
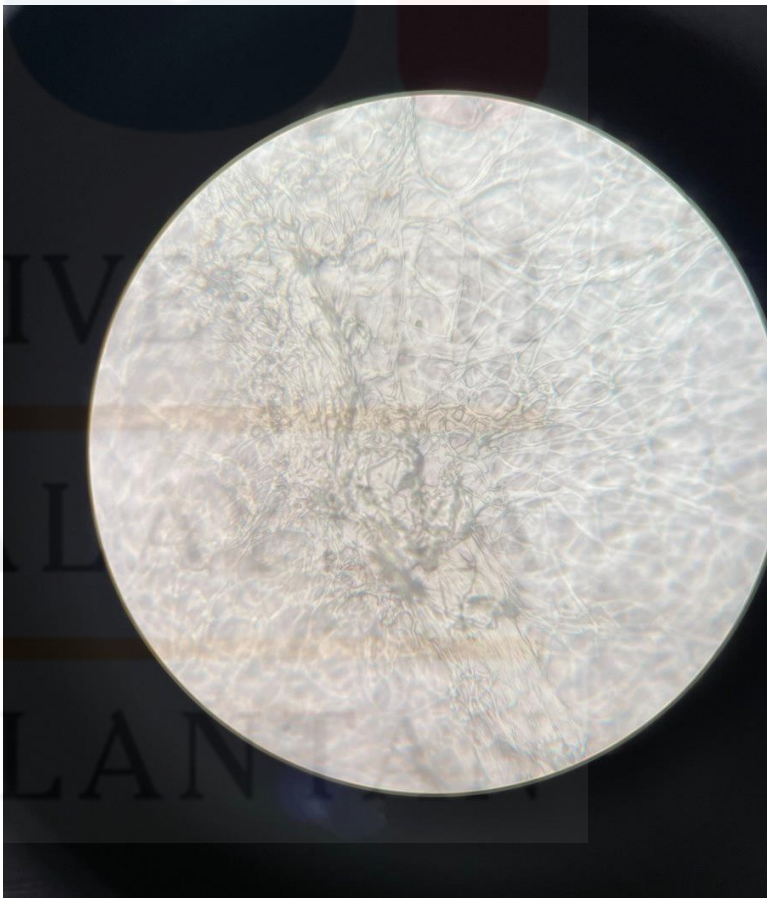
4.1.3 Storage effect on *Fusarium Solani* and *Aspergillus Niger* growth

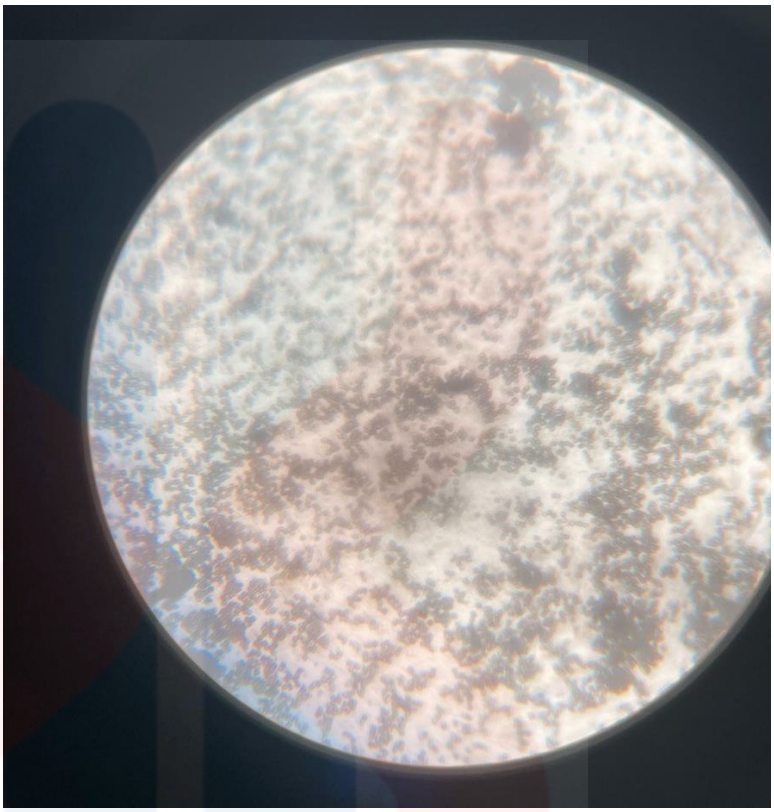
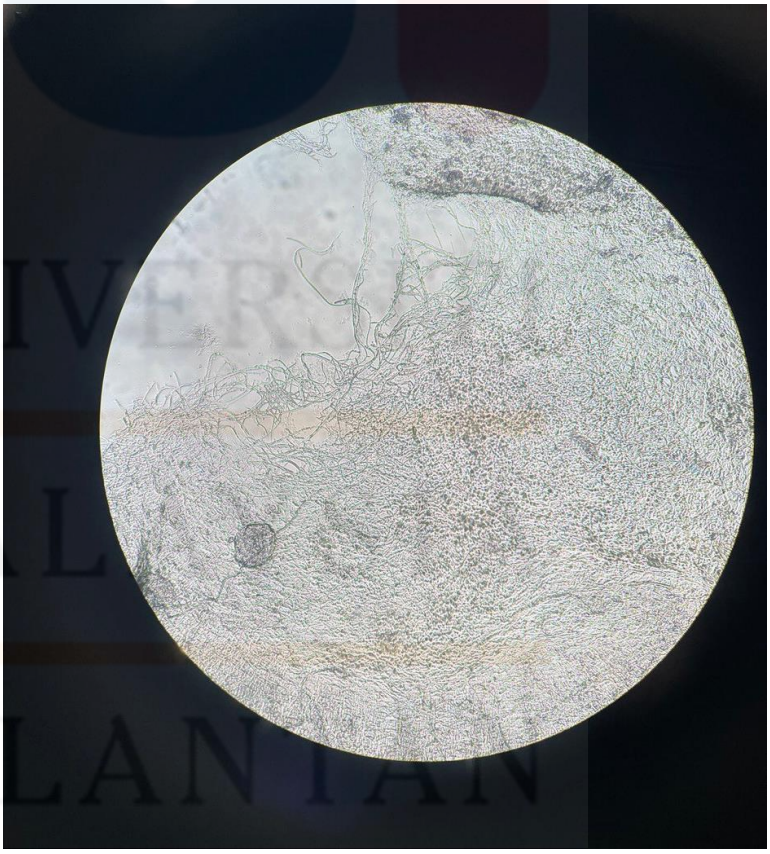
Both fungi were stored in 4°C and 37°C for 1 week, 2 weeks, 3 weeks and 1 month to monitor the availability of each of it.

4.1.3.1 Fusarium Solani

Temperature	Duration	Morphology
4°C	1 week	

37°C	1 week	
4°C	2 weeks	

37°C	2 weeks	
4°C	3 weeks	

37°C	3 weeks	
4°C	4 weeks	
37°C	4 weeks	

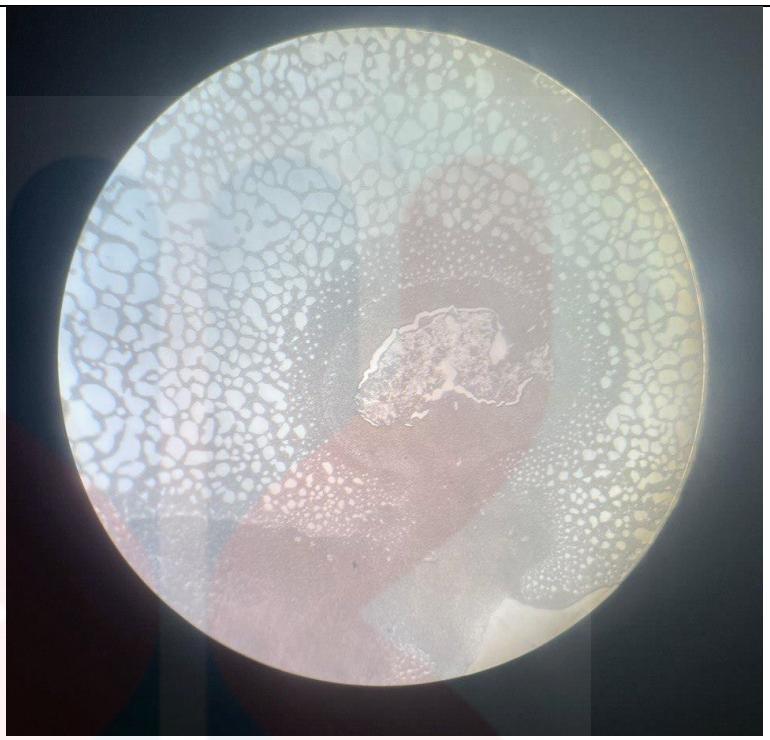
		

Table 4.4: Morphology of *Fusarium solani* under 10x Magnification on 4°C and 37°C

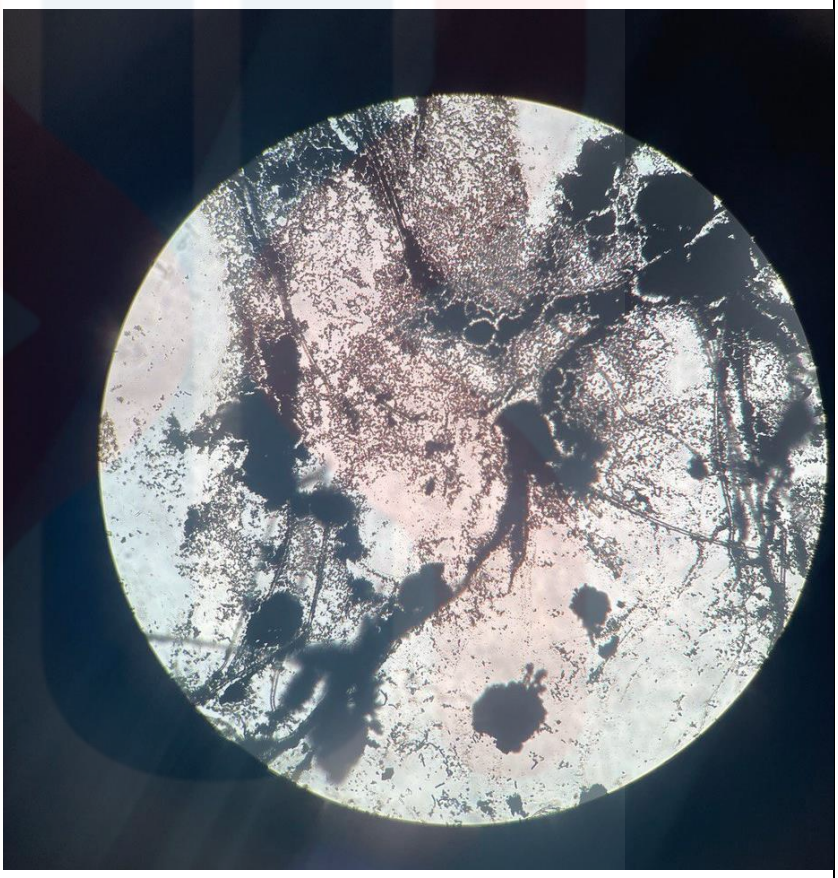
The experimental investigation into the storage of *Fusarium solani* samples at 4°C and 37°C for varying durations yielded significant results regarding fungal growth and morphology. In the initial weeks (1 and 2), both temperatures exhibited the expected morphology consistent with *Fusarium solani*, characterized by long rod-like and thread-like structures observed under the microscope, indicative of hyphae, along with the presence of branched conidiophores. These findings suggest that *Fusarium solani* can maintain its typical morphology under a range of storage temperatures for at least two weeks.

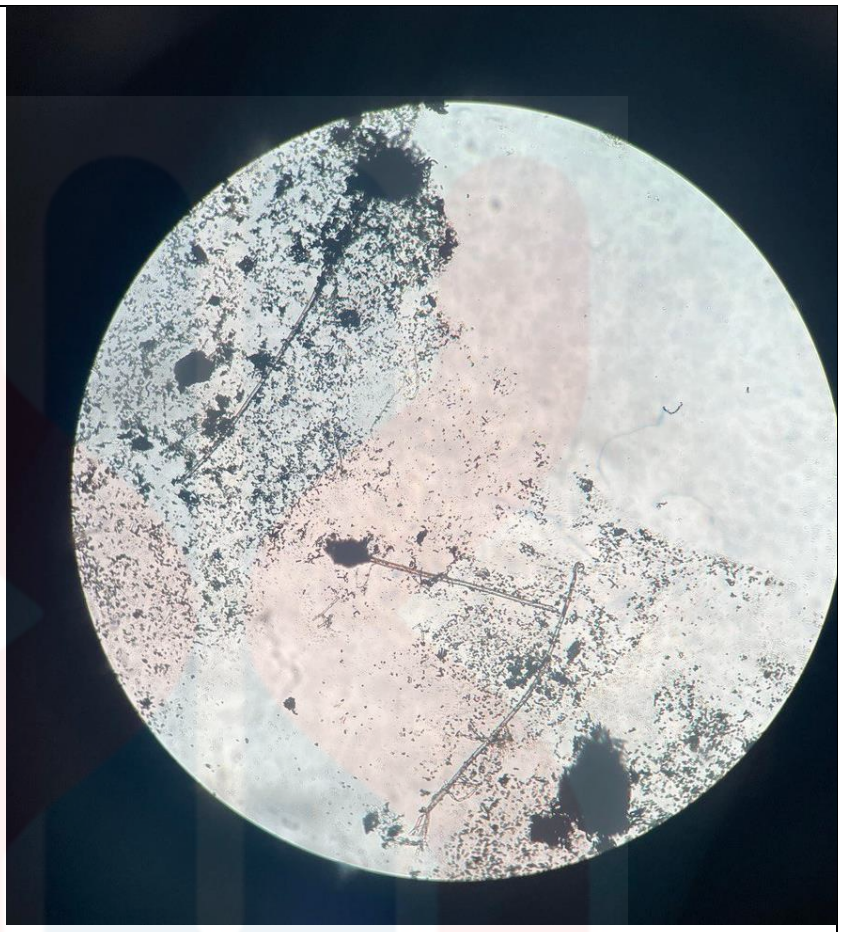
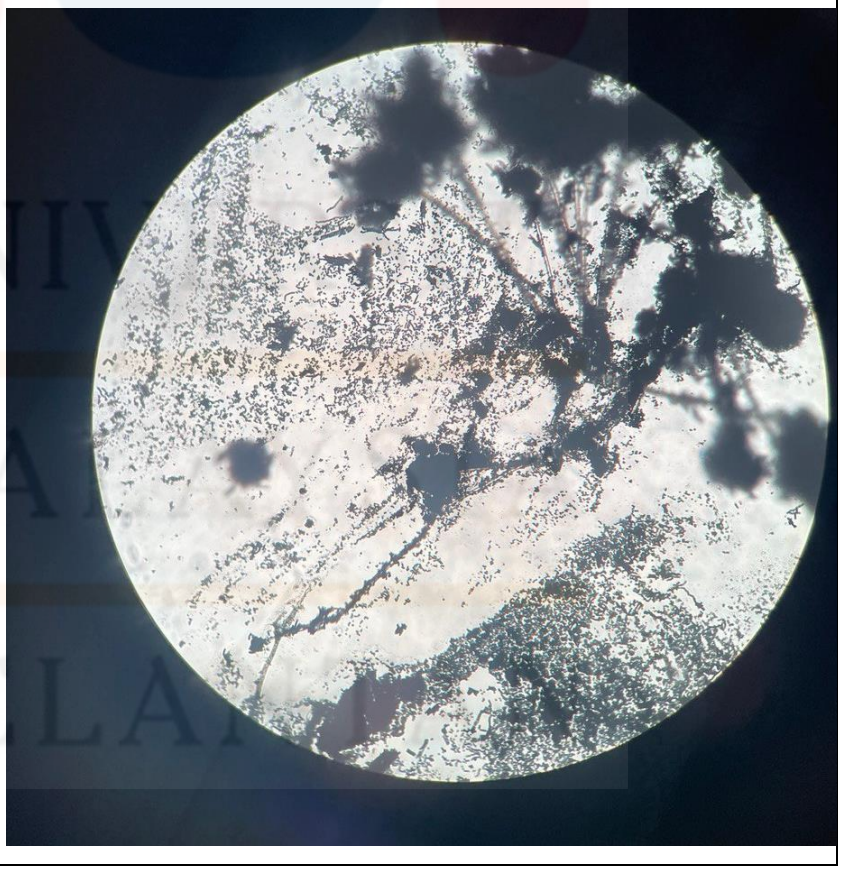
However, by the third week, a notable discrepancy arose. Samples stored at 4°C continued to display the characteristic morphology of *Fusarium solani*, with no significant deviation observed. In contrast, samples stored at 37°C showed a concerning absence of Fusarium-like structures. Instead, small black dots resembling cocci were observed under the microscope, indicating potential contamination. This discrepancy became more pronounced by the fourth week, with samples stored at 4°C still maintaining the expected *Fusarium solani* morphology, while those stored at 37°C exhibited further deviation and were suspected to be contaminated. These findings highlight the temperature sensitivity of *Fusarium solani* morphology and its

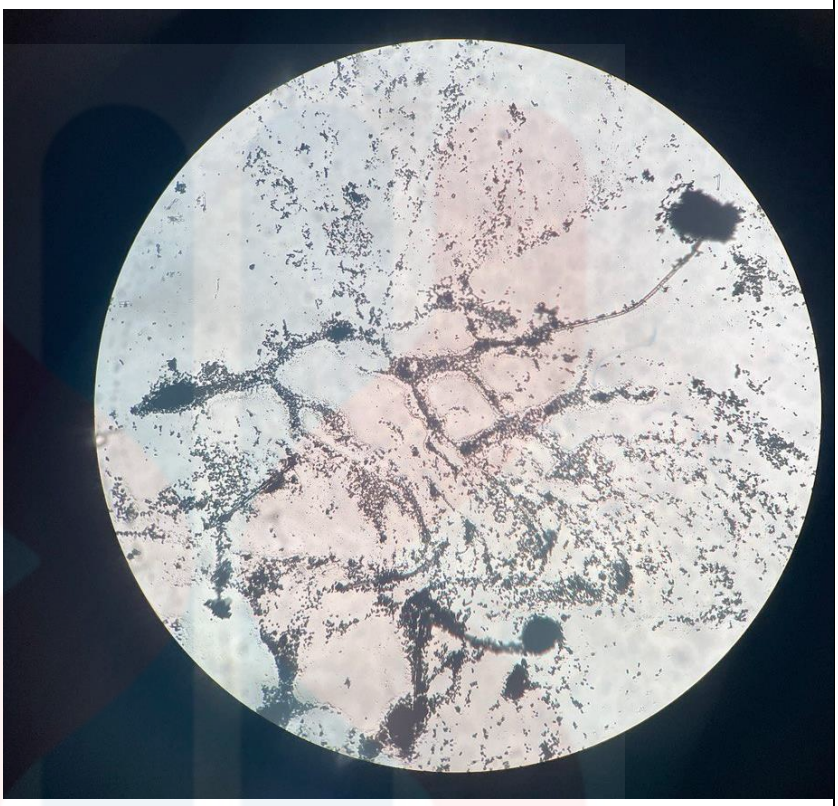
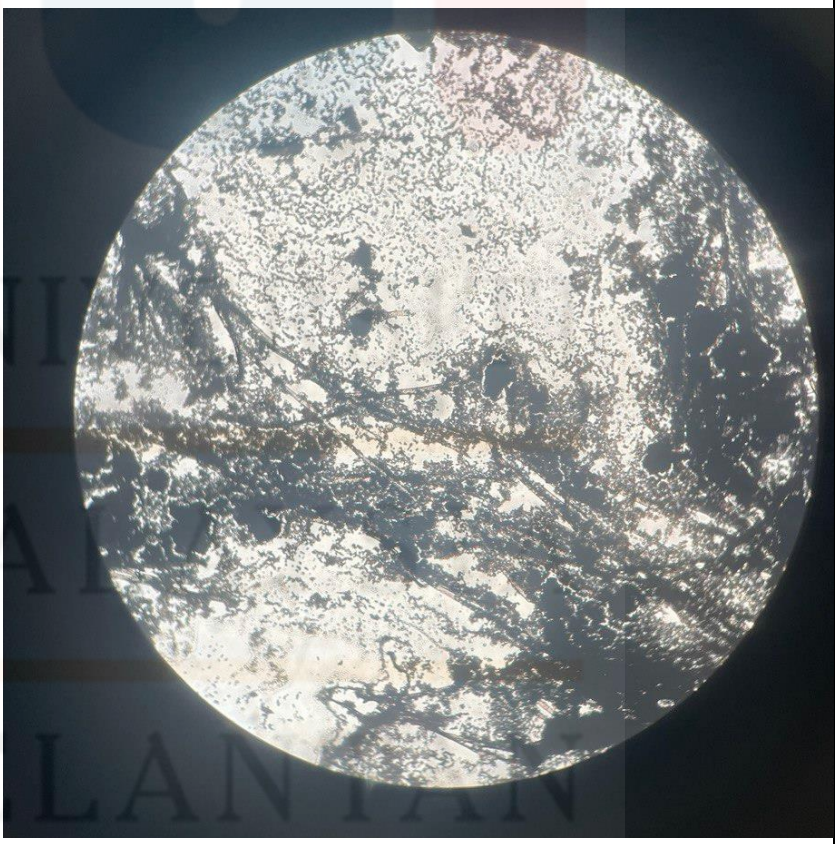
susceptibility to external influences, such as temperature fluctuations and contamination.

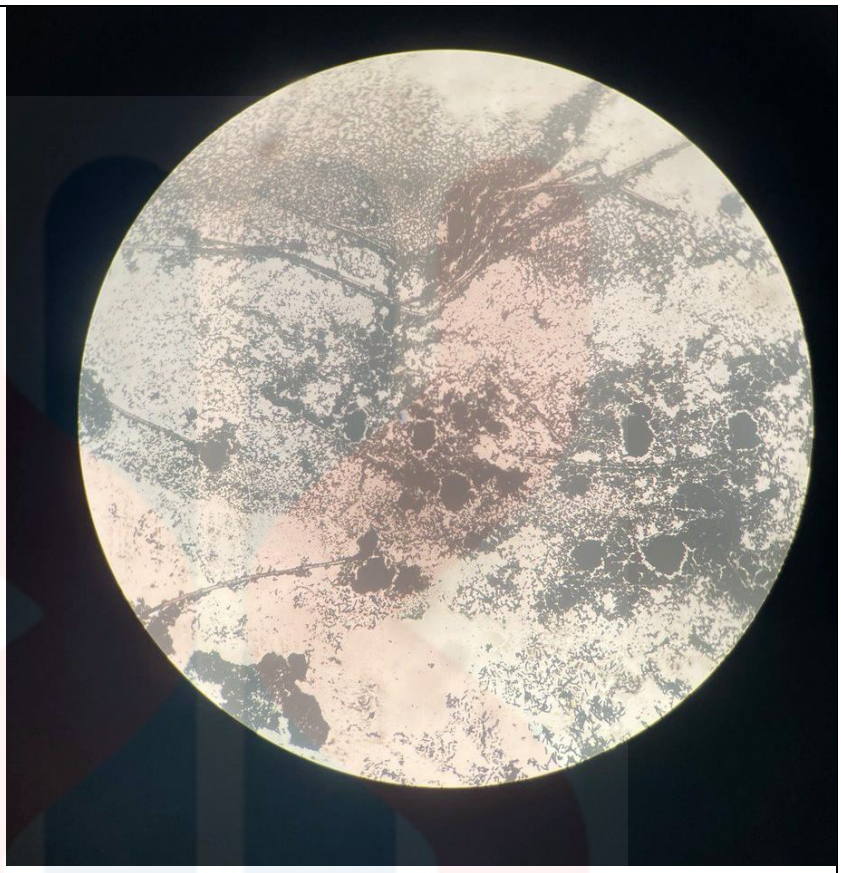
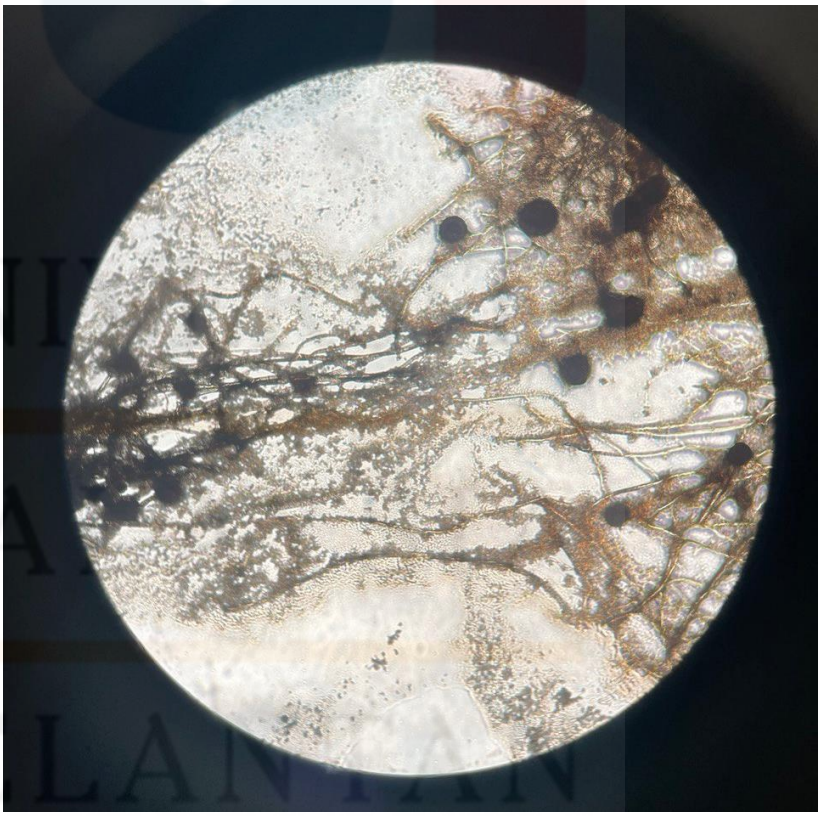
The consistent maintenance of thread-like structures at 4°C suggests that lower temperatures are conducive to preserving the integrity of the fungus and its typical morphology. In contrast, the absence of thread-like structures and the presence of suspected contaminants at 37°C indicate a disruption in fungal growth and morphology under higher temperatures. Overall, these results underscore the importance of proper storage conditions in maintaining fungal integrity and morphology. They also emphasize the need for careful monitoring and quality control measures to ensure the reliability of fungal cultures in research and diagnostic settings. Further investigation into the mechanisms underlying the temperature-dependent effects on *Fusarium solani* morphology and susceptibility to contamination is warranted to elucidate these observed discrepancies fully.

4.1.3.2 Aspergillus Niger

Temperature	Duration	Morphology
4°C	1 week	
37°C	1 week	

		
4°C	2 weeks	

37°C	2 weeks	
4°C	3 weeks	
37°C	3 weeks	

		
4°C	4 weeks	
37 °C	4 weeks	

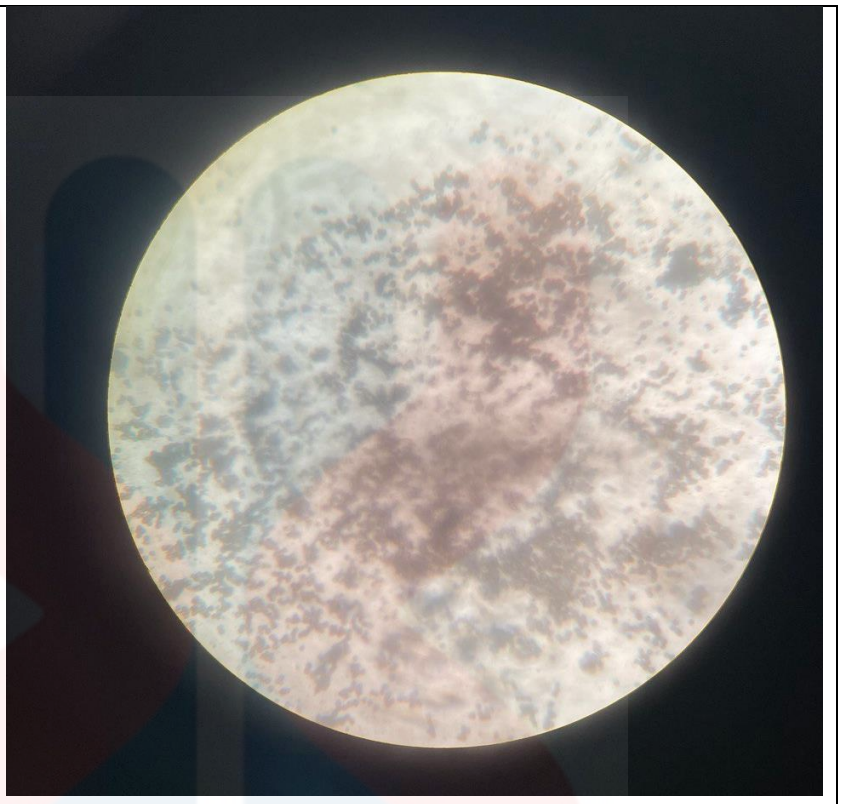
		

Table 4.5: Morphology of *Aspergillus niger* at 10x Magnification on 4°C and 37°C

Based on the observation done for storage of *Aspergillus niger* samples at 4°C and 37°C for varying durations revealed notable findings regarding fungal growth and morphology. In the initial weeks (1 and 2), both temperatures exhibited similar morphological characteristics consistent with *Aspergillus niger*. Small black dots, conidiophores with black structures at their tips, and black-colored conidia were observed on plates from both temperature conditions. However, there was a noticeable difference in the presence of conidiospores, with a higher abundance observed on plates stored at 4°C compared to those at 37°C.

This discrepancy may be attributed to factors such as temperature-dependent spore production rates or variations in metabolic activity under different temperature regimes. By the third week, the morphology of *Aspergillus niger* remained consistent with earlier observations for both temperature conditions. The presence of conidiospores and small black dots persisted, indicating ongoing fungal growth and reproduction. However, by the fourth week, a significant deviation was observed in samples stored at 37°C. While samples stored at 4°C continued to exhibit the expected morphology of *Aspergillus niger*, those stored at 37°C showed signs of contamination and failed to assemble the original morphology of the fungus. This discrepancy suggests

that higher temperatures may have facilitated the growth of contaminants or induced changes in the fungal population, leading to altered morphology and compromised viability.

Overall, these results underscore the temperature sensitivity of *Aspergillus niger* morphology and its susceptibility to environmental conditions. While lower temperatures appear conducive to maintaining the integrity of the fungus and its typical morphology, higher temperatures may lead to deviations and potential contamination. These findings emphasize the importance of proper storage conditions and quality control measures in maintaining fungal cultures for research and diagnostic purposes. Further investigation into the specific factors influencing fungal growth and morphology under different temperature regimes is warranted to elucidate the observed discrepancies fully.

4.2 Quantitative assessment of fungal availability using Optical Density (OD)

Optical density (OD) measurements are commonly used to check the growth and biomass production of fungi in broth cultures. By measuring the turbidity of the broth, which is directly related to the biomass of fungal cells present, OD provides a quick and quantitative assessment of fungal availability.

For optical density measurements, 1300µl of Sabouraud Dextrose Broth containing *Fusarium solani* and *Aspergillus niger* inoculum was pipetted using micropipette into cuvette. The wavelength of each of the measurement was set at 600nm. The sample taken was from 4°C since all of the sample are still viable based on the result earlier.

4.2.1 Fusarium solani

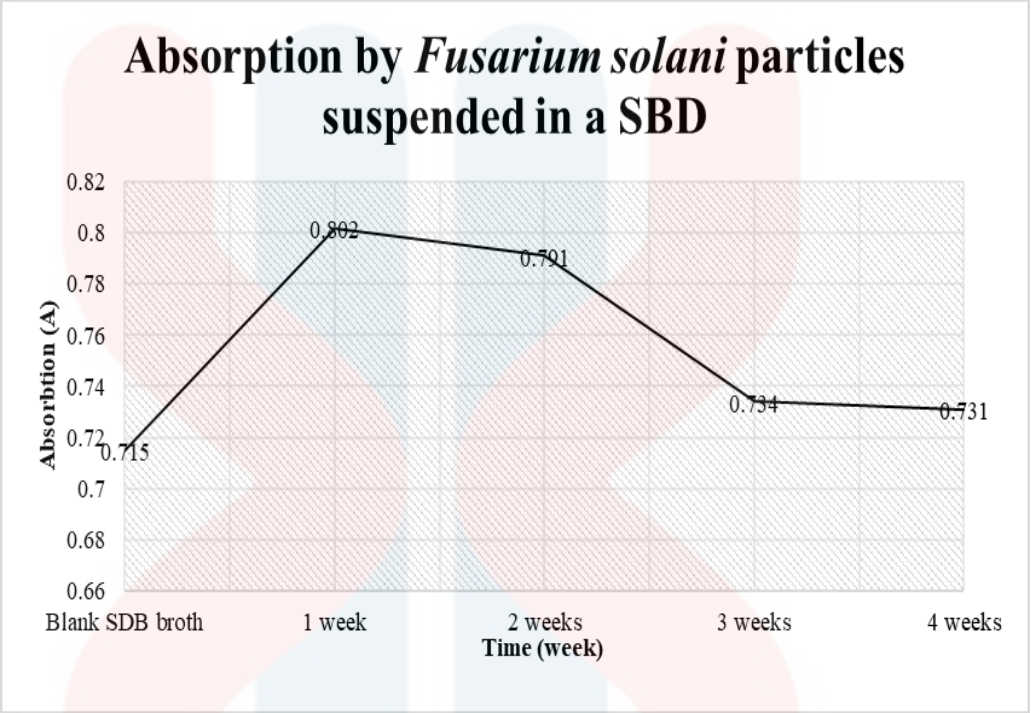


Figure 4.1: Absorbance of *Fusarium solani* particles in Sabouraud Dextrose Broth

Sample/Time	Absorption Unit (AU)
Blank SDB broth	0.715
1 week	0.802
2 weeks	0.791
3 weeks	0.734
4 weeks	0.731

Table 4.6: Reading of absorbance of *fusarium solani* particle absorption

The optical density (OD) measurements taken to assess the availability of *Fusarium solani* cells suspended in Sabouraud Dextrose Broth (SDB) at a wavelength of 600 nm provided valuable insights into the dynamics of fungal growth over time. The initial OD reading of the blank SDB broth was 0.715, representing the baseline absorbance contributed solely by the broth without fungal cells. Subsequent readings at weekly intervals showed fluctuations in OD values, indicating changes in the turbidity of the broth due to fungal growth. Notably, the OD reading increased to 0.802 after one week of incubation, suggesting a proliferation of *Fusarium solani* cells and an increase in biomass. However, by the end of the second week, the absorbance slightly decreased

to 0.791, indicating a potential stabilization or reduction in fungal biomass. This trend continued, with further observations at three weeks and 4 weeks showing gradual declines in absorbance to 0.734 and 0.731, respectively. Several factors may contribute to these observed fluctuations in OD readings.

Initially, the rapid increase in OD after one week could be attributed to the utilization of available nutrients in the broth, promoting fungal growth. However, as the culture aged, nutrient depletion may have occurred, leading to a plateau or decline in fungal biomass. Additionally, *Fusarium solani* cells might have entered a stationary phase, where growth rates are balanced by cell death or reduced metabolic activity, contributing to the observed stabilization or decline in biomass. Competition for resources among fungal cells or interactions with other microorganisms present in the broth could further influence growth dynamics.

Accumulation of toxic metabolites produced by *Fusarium solani* or other microorganisms may inhibit further growth or lead to cell death, contributing to the observed decline in OD readings over time. Overall, the fluctuations in OD readings reflect the dynamic nature of fungal growth and the complex interplay of various environmental and biological factors influencing *Fusarium solani* availability in SDB. Further investigation into these factors could provide deeper insights into fungal physiology and inform strategies for optimizing culture conditions and fungal biomass production.

4.2.2 Aspergillus niger

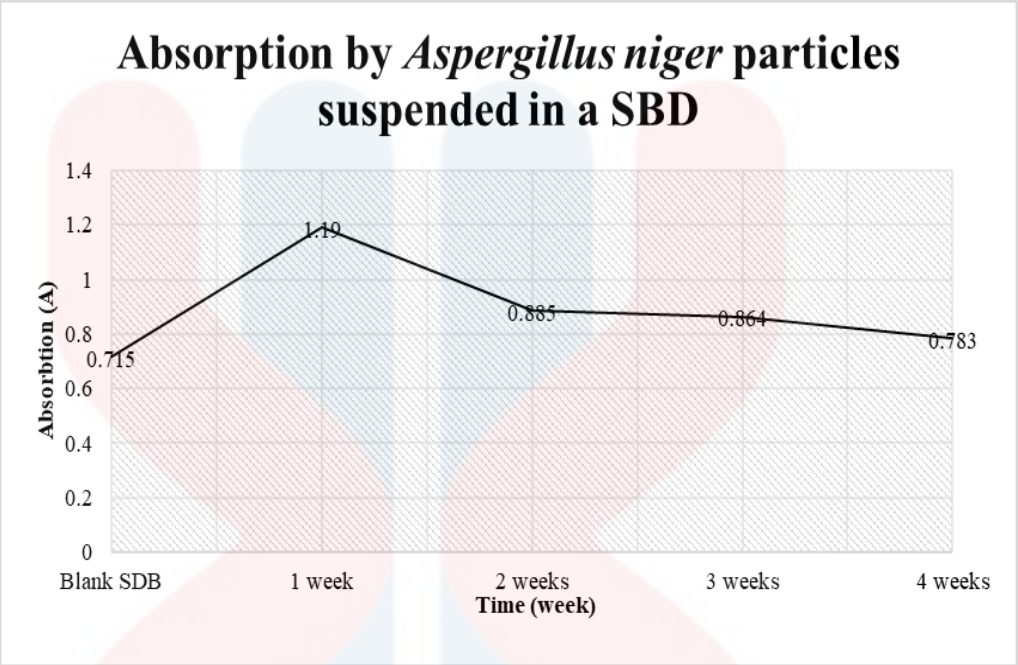


Figure 4.2: Absorbance of *Aspergillus niger* particles in Sabouraud Dextrose Broth

Sample/Time	Absorption Unit (AU)
Blank SDB	0.715
1 week	1.190
2 weeks	0.885
3 weeks	0.864
4 weeks	0.783

Table 4.7: Reading of absorbance of *fusarium solani* particle absorption

Subsequent measurements at weekly intervals showed notable fluctuations in OD values, reflecting changes in the turbidity of the broth due to fungal growth. After one week of storage, the OD reading increased substantially to 1.190, suggesting a robust proliferation of *Aspergillus niger* cells and a considerable increase in biomass. However, by the end of the second week, the absorbance markedly decreased to 0.885, indicating a potential stabilization or reduction in fungal biomass. This trend continued, with further observations at three weeks and four weeks showing gradual declines in absorbance to 0.864 and 0.783, respectively.

Initially, the rapid increase in OD after one week could be attributed to the favorable growth conditions provided by the SDB broth, promoting vigorous fungal

proliferation. Also same as *Fusarium solani*, as the culture aged, nutrient depletion may have occurred, leading to a plateau or decline in fungal biomass. Additionally, *Aspergillus niger* cells also might have entered a stationary phase, where growth rates are balanced by cell death or reduced metabolic activity, contributing to the observed stabilization or decline in biomass.

Comparing the availability of *Fusarium solani* and *Aspergillus niger* based on the optical density (OD) measurements in Sabouraud Dextrose Broth (SDB) reveals distinct growth dynamics between the two fungal species over time. For *Fusarium solani*, the OD readings initially showed an increase after one week, indicating a surge in fungal biomass, followed by a slight decline in subsequent weeks. Despite fluctuations, *Fusarium solani* maintained relatively stable biomass levels over the course of the experiment, as evidenced by comparable OD readings at one month compared to earlier time points.

In contrast, *Aspergillus niger* exhibited a different pattern of growth. After an initial rapid increase in OD readings at one week, suggesting robust proliferation, *Aspergillus niger* experienced a notable decline in biomass over the following weeks, with OD values consistently decreasing until 4 weeks. These observations suggest that *Fusarium solani* may have better adapted to the SDB culture conditions, maintaining relatively stable biomass levels over time compared to *Aspergillus niger*, which showed a decline in availability. Factors such as nutrient utilization efficiency, metabolic activity, and tolerance to environmental fluctuations may contribute to the differential growth dynamics observed between the two fungal species.

Overall, while both *Fusarium solani* and *Aspergillus niger* demonstrated growth in SDB culture, *Fusarium solani* appeared to exhibit more stable availability compared to *Aspergillus niger* over the duration of the experiment. Further investigations into the underlying mechanisms driving these differences in growth dynamics could provide valuable insights into fungal physiology and inform strategies for optimizing culture conditions for biomass production.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In conclusion, the experimental results revealed a fascinating degree of adaptability and resilience in both *Fusarium solani* and *Aspergillus niger* across varied temperature conditions. The ability of these fungi to survive and maintain nearly identical morphology under the microscope, whether at 4°C, 37°C, or in their original culture, underscores their robust nature and inherent adaptability. While slight variations were noted, likely influenced by factors such as temperature-induced changes and genetic diversity within the cultures, the fundamental similarities in morphology remained striking.

These findings contribute valuable insights into the ecological adaptability of *Fusarium solani* and *Aspergillus niger*, suggesting a versatility that may play a crucial role in their survival in diverse environmental niches. The observed adaptability has implications for understanding the behavior of these fungi in natural ecosystems, agricultural settings, and industrial applications. The experiment's-controlled conditions provided a foundational understanding of how temperature influences the microscopic features of these fungi.

However, further research could explore the underlying genetic and physiological mechanisms contributing to the observed variations. Additionally, investigating these fungi in more complex and dynamic environmental settings may offer a more comprehensive perspective on their adaptive strategies. In practical terms, the knowledge gained from this study could have implications for fields such as biotechnology, agriculture, and mycology. Understanding the factors influencing fungal morphology and adaptability can inform strategies for fungal management and exploitation in various applications. in emulgel.

5.2 Recommendations

The observed adaptability of *Fusarium solani* and *Aspergillus niger* prompts several key recommendations. Firstly, delve into the genetic and molecular aspects to identify underlying mechanisms. Extend incubation periods beyond 7 days for a comprehensive understanding of long-term changes. Explore these fungi in more complex environmental conditions to simulate natural ecosystems. Undertake comparative studies with a broader range of fungal species to identify common traits in response to temperature variations. Investigate practical applications in agriculture and biotechnology, leveraging their adaptability for solutions in bioremediation and industrial processes. Validate findings through field studies and optimize growth media for enhanced control and manipulation. Implementing these recommendations will deepen our understanding and unlock innovative applications of *Fusarium solani* and *Aspergillus niger* in various scientific and industrial contexts.

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