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DHAMKHI Lina
DIAB Khawla
MEDDOUR Imane
MAKRI Noor Elhouda

Entitled

Isolation and identification of some foodborne molds, and testing of their sensitivity to *Matraria chamomilla* L. methanol extract.

BOARD OF EXAMINERS

SELLOUM Mounir	**MAA**	**Chairperson**
HENDEL Noui	**MCA**	**Supervisor**
MEDJEKAL Samir	**Prof**	**Examiner**

Class : 2022-2023

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Dedication

First and foremost, I would like to thank God. for given me strength and encouragement throughout all the challenging moments.

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The dream wasn't close and the way wasn't clear, but I did it...

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Lina

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Abstract

The primary function of ubiquitous microorganisms like fungi is the recycling of organic material. Their diversity is enormous (there are over 1.5 million species in the entire world)., contamination caused by fungus creates a serious issue for the food and pharmaceutical industries, as well as for the public's health. The aim of our study was to isolate and identify fungi species sampled from a variety of foods, which involve cereals, fruits, vegetables, and medicinal plants To check their tolerance and estimate the antifungal efficacy in vitro using methanolic extract of the aromatic plant *Matricaria chamomilla*. using different type of methods which depend on type of strains in general, such as Direct and the Indirect plating technique using a number of media; other method used in ordre to distinguish between the deferent genera of fungi such as the microscopic method of identification. The results showed that *Penicillium expansum*, *Aspergillus niger*, and *Alternaria alternata* represent the majority of the fungal genus found in most samples while *Aspergillus flavus* is the least prevalent in the samples, with 20% in rosemary and 16% in banana and on the other hand. The antifungal activity of the medicinal plant *Matricaria* sp showed a significant inhibition with deferent efficacity of 45% and 31% and for *Penicillium expansum* and *Penicillium italicum* 25% for the tree genera *Trichoderma harzianum Aspergillus flavus* and *Fusarium culmorum*

Key words: foodborne fungi, isolation, antifungal activity. Mold inhibition, *Matricaria*,

ملخص

الوظيفة الأساسية للفطريات الدقيقة المنتشرة في كل مكان إعادة تدوير المواد العضوية حيث أن تنوع الفطريات هائل (بأكثر من 1.5 مليون نوع في جميع أنحاء العالم) كما أن العدوى الناجمة عن الفطريات مشكلة خطيرة في الصناعة الغذائية والصيدلانية، وكذلك للصحة العامة. كان الهدف من دراستنا عزل وتحديد أنواع الفطريات المأخوذة من مجموعة متنوعة من الأطعمة التي تشمل الحبوب، الفواكه والخضروات والنباتات الطبية للتحقق من تحملها وتقدير نسبة فعالية مضاد الفطريات في المختبر باستخدام نبتة *Matricaria chamomilla* و ذلك بعدة طرق منهجية في البحث العلمي والتي تعتمد على نوع السلالات بشكل عام، مثل تكنولوجيا الزرع المباشر والغير مباشر باستخدام عدة أوساط زراعية؛ وأخرى للتمييز بين أنواع الفطريات بشكل أكثر تحديدا كالطرق المجهرية. أظهرت النتائج أن *Penicillium expansum* و *Aspergillus niger* و *Alternaria alternata* تمثل غالبية الجنس الفطري الموجود في معظم العينات بينما *Aspergillus flavus* كان الأقل انتشارًا في العينات بنسبة 20٪ في إكليل الجبل و16٪ في الموز ومن ناحية أخرى، أظهر المضاد الفطري *لنبات Matricaria chamomilla* نسبة تثبيط تقدر ب31% لكل من النوعين *penicillium expasum* و *Penicillium italicum* و نسبة % 25لكل من *Trichoderma* harzium

كلمات مفتاحية: الفطريات التي تنتقل عن طريق الغذاء ، والعزلة ، والنشاط المضاد للفطريات. تثبيط العفن ، *Matricaria* ،

Résumé

La première fonction des micro-organismes ubiquitaires tels que les champignons qui ont le recyclage de la matière organique. Leur diversité est énorme (il y a plus de 1,5 million d'espèces dans le monde entier), la contamination causée par les champignons qui crée un problème sérieux pour les industries alimentaires et pharmaceutiques, ainsi que pour la santé publique. L'objectif de notre étude était d'isoler et d'identifier les espèces de champignons à partir d'une variété d'aliments, qui incluent: les céréales, les fruits, les légumes et les plantes médicinales Pour vérifier leur tolérance et estimer l'efficacité antifongique in vitro en utilisant l'extrait méthanolique de la plante aromatique *Matricaria chamomilla* . En utilisant différents types de méthodes qui dépendent du type de souches en général, telles que la technique d'ensemencement direct et indirect utilisant un certain nombre de milieux ; d'autres méthodes utilisées afin de distinguer les différents genres de champignons telles que la méthode d'identification microscopique. Les résultats ont montré que *Penicillium expansum*, *Aspergillus niger* et *Alternaria alternata* représentent la majorité des genres fongiques trouvés dans la plupart des échantillons, alors qu'*Aspergillus flavus* est le moins répandu dans les échantillons, avec 20% de romarin et 16% de banane d'autre part. L'activité antifongique de la plante médicinale *Matricaria* sp a montré une inhibition significative avec une efficacité différentielle de 45% et 31% et pour *Penicillium expansum* et *Penicillium italicum* 25% pour les genres d'arbres *Trichoderma harzianum Aspergillus flavus* et *Fusarium culmorum*

Mots clés : champignons d'origine alimentaire, isolement, activité antifongique, inhibition des moisissures, *Matricaria*,

List of abbreviations

PCR : Polymerase chain reaction.

PDA: Potato dextrose agar.

MEA: Czapek yeast extract agar.

G25N: 25% Glycerol nitrate agar.

CYA: Czapek yeast extract agar.

SDA: Sabouraud Dextrose Agar.

DMSO: Dimethylsulfoxide.

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INTRODUCTION

Introduction

Molds, or fungi, are saprophytic fungal microorganisms whose foods are generally very favorable substrates for their development, are important players in the microbial world and constitute a very important agent of deterioration. They are ubiquitous and have an extremely varied enzymatic arsenal, which allows them to grow on various substrates by altering their physical and chemical properties (Lubulwa & Davis, 1994).

Countless food products can be contaminated by a wide range of filamentous fungi when the right humidity and temperature conditions are met (Mateo et al., 2002) thus, representing a serious risk to human health since strains of certain genera such as *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium* are able to produce toxic secondary metabolites called mycotoxins (Salas et al., 2017). Ultimately, they can lead to serious problems related to human intoxications and are therefore, one of the primary causes of food borne diseases.

In this regard, several studies have focused on the isolation and identification of molds and the toxicity of their mycotoxins in the food industry (Tanaka et al., 2000) to ensure food safety that meets consumer expectations. The biological activity of different plant species is one of the solutions considered as they are known for their antimicrobial effects in general and antifungal effects in particular (El Mansouri, 2013) . Aromatic and medicinal plants are a significant natural source of fungitoxic substances that can be used in the development of biopesticides or can serve as an alternative to conventional medications.

In order to better understand the development of molds and their consequences, the main objective of our work is to isolate, and identify the different genera and fungal species obtained from samples taken from various types of foods, such as cereals, fruits, vegetables, and medicinal plants. In our study, we test the sensitivity of these foods and evaluate in vitro the antifungal activity of the methanolic extract of the aromatic plant *Matricaria chamomilla* L.

CHAPITRE I :
LITERATURE REVIEW

1. Molds

1.1. General characteristics of molds

1.1.1. Definition

The term "mold" is commonly used to describe any saprophytic, eukaryotic, immobile fungal microorganism belonging to both higher fungi (Ascomycetes, Hyphomycetes, and Basidiomycetes) and lower fungi with coenocytic hyphae (Zygomycetes)(Chapeland-Leclerc et al., 2005).

They are multicellular organisms whose vegetative apparatus, the thallus, is formed by long branched filaments, often divided (hyphae), and sometimes siphoned (Al-Bakkali, 2016). When the growth is sufficiently advanced, the whole hyphae constitute a mycelium visible to the naked eye, which appears as a kind of felting on the surface (Mahideb & Merrouche, 2022).

Fungi are heterotrophic organisms; their nutrition comes from the absorption of organic matter. As they are free of photosynthetic pigment (chlorophyll), a source of organic carbon, is therefore necessary for their development. They are unable to manufacture the organic substances necessary for the growth of their cells (El Bakkali, 2016). Molds can reproduce vegetatively (asexually) or sexually (Tabuc, 2007) and are very undemanding on the environmental conditions of the substrate. They also require oxygen because they are aerobic, usually acidophilic, develop even at low temperatures, and have a low water requirement, usually 0.65(Guiraud, 1998).

The sporulation of molds is dependent on nutrient factors as well as environmental factors, mainly light, which strongly influences the growth of some molds. However, many molds do not require light to sporulate (Barker & Worgan, 1981; Botton et al., 1990).

The eumycetes, or fifth kingdom, was the conventional classification for fungi. In the Phylogenetic tree e, they constitute a separate group within eukaryotes (Kendrick, 2017).

1.1.2. Habitat

Molds are a natural part of the environment and can be found almost anywhere that moisture and oxygen are present. Fungi live in moist places such as soil, plants, and dead or decaying matter. Outdoors, molds play a part in nature by breaking down dead organic matter such as fallen leaves, dead trees, and other debris; however, indoors, mold growth should be avoided (Growth, 2005).

It is important to point out that the optimal conditions for the growth and reproduction of molds vary considerably from one fungal species to another, resulting in a biodiversity that tends

to increase in tropical regions, some of which are specific to narrowly limited locations. Approximately 70,000 species of fungi are described. The harmful intervention of filamentous fungi manifests itself mainly in the food industry with phytopathogenic activity, especially on fruits and vegetables (Swann et al., 1999).

1.1.3. Nutrition requirement

Molds are heterotrophic microorganisms, and each type has different growth requirements. However, all molds require the presence of the three basic nutrients such as carbon, nitrogen, and mineral ions in their environment (Davet, 1996). they can utilize an incredibly diverse array of enzyme substrates. Some of them may thrive well on substrates with high sugar or salt content. Some may prefer simple sugars, while others have the ability to utilize complex ones.

1.1.3.1. Carbon and energy source

Practically all organic compounds can be used as a source of carbon and energy by molds. The majority of them can metabolize glucose and sucrose along with certain polysaccharides like starch and cellulose (Boiron & Périlleux, 1996; Nicklin et al., 2000).

1.1.3.2. Nitrogen source

Polymères d'unités flavonoïdes composées d'unités flavan-3-ol, avec un degré de polymérisation de 2 à plus de 50 unités, reliés par de fortes liaisons carbone, non hydrolysées mais oxydées un acide fort qui libère des anthocyanidines (Khanbaba et Ree, 2001).

1.1.3.3. Mineral elements

For the growth and reproduction of several fungal species, the presence of mineral and metal ions in the culture medium is essential. These include sulfate, magnesium, potassium sulfate, magnesium, potassium, sodium, and phosphorus, with concentrations that are more or less different depending on the species (Uchikoba et al., 2001).

1.2. Classification

Molds can be classified into a number of different families of microscopic fungi but do not belong to a single uniform systematic category. Their classification is based on morphological characteristics, including the mycelium's structure, and the method of reproduction (Davet, 1996). According to (Bourgeois et al., 1989), the main classes of molds, namely Zygomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes are all members of the Eumycetes (real fungus) group.

1.2.1. Zygomycetes

These molds have sexual reproductive organs and a non-partitioned mycelial thallus (Giraud et al., 1998), characterized by their sexual spores called Zygospores (Chabasse, 2002). The Mucorales are the most significant family in this class, which comprises many saprophytic mold species as well as certain species of parasitic fungi, animals, and humans (mucormycoses), and in particular, food-related contaminants (Figure1) (Andersen & Thrane, 1996; Boiron & Périlleux, 1996; Leveau & Bouix, 1993).

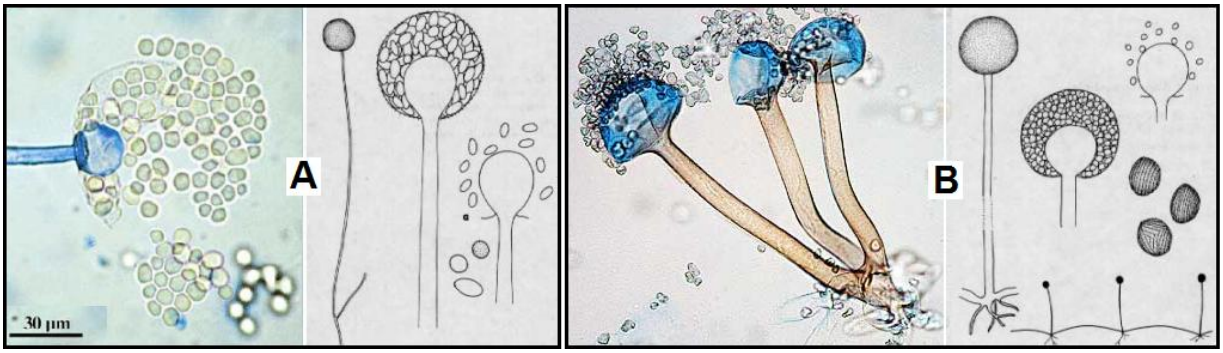

Figure 1. Some fungi of the Zygomycetes class; A) *Mucor*, B) *Rhyzopus* (Malloch, 1997).

1.2.2. Ascomycetes

Fungi classified as ascomycetes have a partitioned mycelial thallus and reproduce sexually by producing endogenous spores (ascospores). Many molds and plant parasites are both included in this class (Giraud et al., 1998). Nonetheless, the majority of them are found in the orders Eurotiales, Microscales, and Sphaeriales. Endothia and Neurospora are the most well-known genera in this class (Figure2) (Bourgeois et al., 1989).

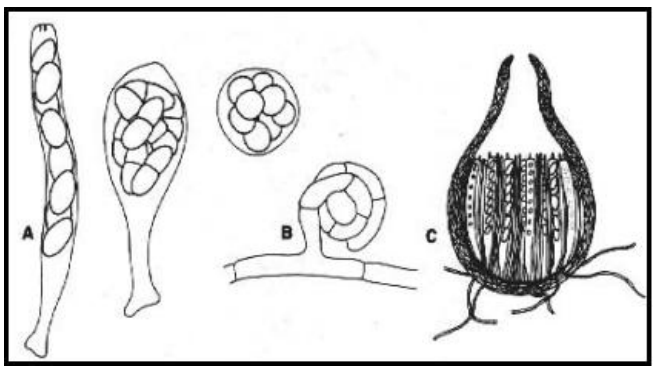

Figure 2 Ascomycetes A: three kinds of asci: cylindrical, clavate, and spherical. B: initial phase of sexual reproduction. C: cross-section of a flask-shaped perithecium bearing cylindrical asci. (Malloch, 1997).

1.2.3. Basidiomycetes

They exclusively include a specific class of parasitic molds. In the case of Agaricus and Coprinus, they are distinguished by a thallus with septate mycelium and sexual reproduction accompanied by the production of exogenous spores (basidiospores) (Botton et al., 1990)."fungus of the cap " is a prevalent name for basidiomycetes (Figure 3).

Figure 3. Some fungi of the Basidiomycetes class (Malloch, 1997) ; A) *Coprinus*, B) *Agaricus* .

1.2.4. Deuteromycetes

Deuteromycetes, sometimes known as imperfect fungus, are characterized by a partitioned mycelium and vegetative reproduction by asexual spores or by straightforward mycelium fragmentation (Figure 4) (Boiron & Périlleux, 1996).

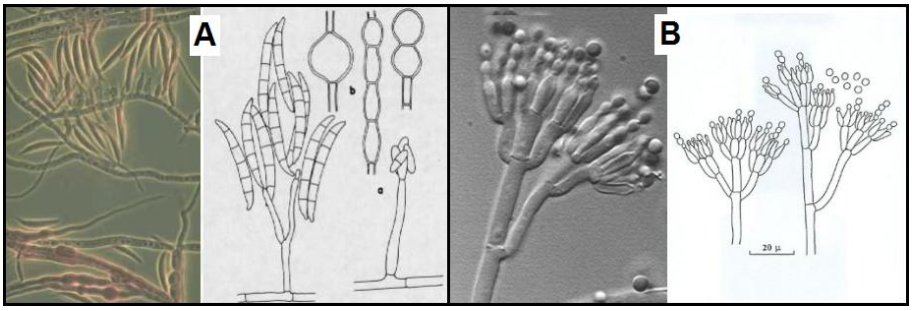

Figure 4. Some fungi of the class Deuteromycetes (Malloch, 1997) ; A) *Fusarium* ,B), *Penicillium* .

These molds make up the majority of the Hyphales and are divided into several groups based on the properties of the conidial organs and how the hyphae are grouped. *Trichoderma*, Cephalosporium, *Fusarium*, Geotrichum, and other members of this class like *Penicillium* and *Aspergillus* are among the many plant and food product contaminants that belong to the Deuteromycetes group (Collins & Lyne, 1970; Ramirez, 1982).

1.3. Growth conditions

1.3.1. Physicochemical factors

Molds have an active metabolism in relation to their mode of nutrition by absorption (Moreau, 1996), and their growth is influenced by the types of substrates that are available with regard to nutrient requirements as well as by physicochemical conditions like temperature, water activity (aw), oxygen, and PH (Gibson et al., 1994; Reboux, 2006). Although they are relatively undemanding, filamentous fungi need a number of physicochemical parameters that have a significant impact on their germination and development.

1.3.2. Temperature

In general, mold can develop at temperatures ranging from minus zero to more than 50 °C (Proctor,1995). The most common molds are mesophilic; they develop between 15°C and 30°C, with an optimum between 20°C and 25°C. However, some species are psychrophilic and can grow at -6°C. Other thermophiles strains can grow at very high temperatures above 45°C (Guiraud, 1998), which significantly contributes to mycelial development. Moreover, it participates in sporulation and spore germination.

1.3.3. PH

Most fungi prefer acidic pH conditions, they typically grow between 4.5 and 8, with an ideal range of 5 to 6. Some species have a growth range of 1.7 to 2. Moreover, fungi often modify the pH of the medium by selectively absorbing and exchanging ions, producing CO_2 or NH_3, or by producing acids (Boiron & Périlleux, 1996).

1.3.4. Water activity (Aw)

The amount of water available in the substrate and the surrounding atmosphere are very important for mold growth. Generally, molds are metabolically more xerotolerant than other microorganisms (bacteria, yeasts). Most molds grow well in water activities close to 0.85, which means they can grow on water-poor nutrients (Castegnaro & Pfohl-Leszkowicz, 2002).

1.3.5. Oxygen

An important development component for molds is the amount of oxygen that is available to them. Fungi are mostly aerobic microorganisms. However, some of them can tolerate relatively low oxygen levels (facultative anaerobes), and other species can endure extremely stringent anaerobiosis (strict anaerobes) (Bourgeois et al., 1989).

1.4. Mycotoxins

Mycotoxins are molecules produced as secondary metabolites by filamentous fungi and developed by various molds under certain environmental conditions. Their biosynthesis depends on several factors, including temperature, light intensity, carbon dioxide in the air, available nutrients, and the presence of other competing species (Hendey et al., 1993). They are naturally present in the ambient air, soil, and on crops (Yiannikouris & Jouany, 2002).

Nowadays, contamination of foods with fungi and their mycotoxins is the most crucialo concern with regard to food safety as they pose a serious health risk. They are considered to be among the most significant food contaminants in terms of impact on public health, food safety, and the economies of many countries (Pitt et al., 2000).

Mycotoxins and their metabolites (aflatoxin, zearalenone, trichothecene, ochratoxin A, citrinin, patulin, penicillic acid, tenuazonic acid, cytochalasins, deoxynivalenol, fumonisins, fusarin C, fusaric acid, etc.) produced by specific fungi are truly important for human and animal health because of their frequency or their toxicity (Bennett & Klich, 2003).

1.5. Mold identification

The identification of fungi is based on macroscopic, microscopic and molecular criteria after isolation and culture on culture media.

The macroscopic criteria are determined through an examination of the colonies' front and back colors, diameter, relief, aspect (filamentous, sticky), transparency (opaque, transparent), shape appearance, and pigmentation.

The microscopic criteria are based on the appearance and morphology of the different fungal structures: the type of thallus (septate or not), the color of the hyphae (dark or light), the shape of the spores, their origin (endogenous or exogenous), and the shape of the heads.

Figure 5 illustrates how the macroscopic and microscopic characteristics of the genera *Penicillium* and *Aspergillus* differ There are now molecular criteria that enable accurate species identification in addition to morphological ones (Boudih, 2011).

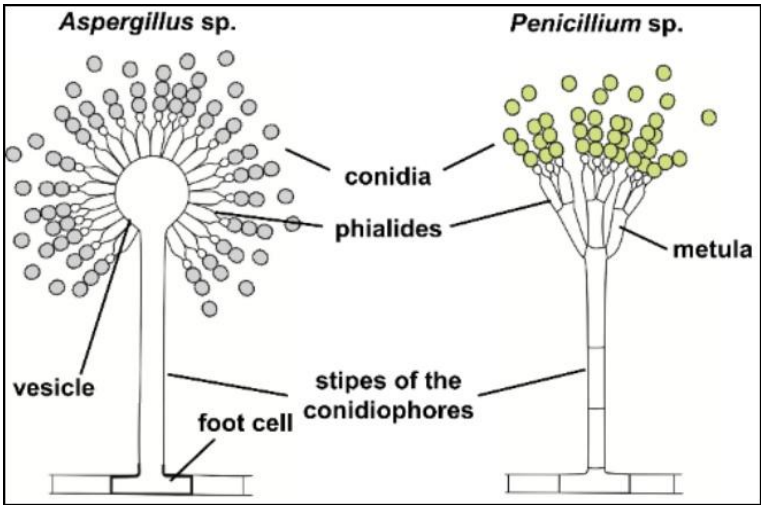

Figure 5. The structure of an *Aspergillus* on the left and that of a *Penicillium* on the right (Chabasse, 2002).

1.6. Isolation methods

1.6.1. Serial dilution agar plate

The standard plate count is one of the most reliable methods for counting fungi. A series of successive dilutions are created; a sample of each one is added to a liquefied agar medium, and the medium is subsequently poured into a Petri dish. As the agar solidifies, the fungus is locked inside.

1.6.2. Single spore isolation methods

Single-spore isolation is one of the best methods to obtain a pure culture of the fungi. Individual spores from a spore suspension could be transferred onto a glass slide using a capillary pipette. On the other hand, spores might be manually extracted under a microscope.

1.7. Identification methods

1.7.1. Molecular Methods

Investigated the three major kinds of molecules:

- the primary metabolites, composed of substances required for an organism's essential functions
- the secondary metabolic products, such as terpenes or alkaloids, that serve no essential functions.
- semantophoretic molecules carry genetic information include proteins, acid deoxyribonucleic acid (ADN), and acid ribonucleic acid (ARN), which are also used in taxonomy, PCR amplification (Verscheure et al., 2002).

2. *Matricaria* L.

Due to the plant's long history of use in treating female pathologies, the term "*Matricaria*" is derived from the Latin word "mater" or perhaps from "Matrice" (uterus). However, plants with the genus name *Chamomilla* are today considered within the genus of *Matricaria* (Barbosa & Capellari Júnior, 2019).

The plants of this genus are herbaceous, annual, aromatic, with an erect stem, are much branched and not very hardy, the leaves are segmented into many segments, alternate, and narrow. The flowers are radially symmetrical, collected in compact greenish-yellow, semi-spherical and hermaphrodite chapters.

They are widely used by various cultures in North and South Africa, temperate regions of Europe, Asia, and America, with some naturalization in Australia as teas and tisanes, both in ethnobotanical and ethnomedical contexts.

The biological activity of the *Matricaria* plant has been studied, and the initial results are promising. It has been demonstrated to have antioxidant, anti-inflammatory, anti-tumor, antimicrobial, anti-platelet, anti-cancer, anti-genotoxic, and hypocholesterolemic activity.

Apigenin, a chemical component of the plant, is thought to be largely responsible for its anti-cancer properties. According to future research, the heart, liver, gastrointestinal, and central nervous systems of the body may benefit chamomile.

CHAPITER II : MATERIALS & METHODS

1. Materials and methods

1.1. Fungal isolates origin

1- Some samples of medicinal plants, cereals and fruits were collected from M'sila city markets (Tables 1, 2, 3);

2- and Three mold isolates from the microbiology laboratory fungal collection of the department of microbiology & biochemistry. These isolates are: Isolate F , isolate V, and isolate R

Table 1. Medicinal plant samples.

Sample type	Sample source	Sample conditioning	Sampling date	Sample weight
Rosmarinus officinalis	Market in the M'sila center-city at the 5 July neighborhood.	In a cardboard box, then into a polyethylene bag.	25-03-2023	44 g
Laurus nobilis	Market in the M'sila center-city at the 1000 neighborhood	In a cardboard box, the into a polyethylene bag.	02-03-2023	10 g
Ziziphus lotus	Market in the M'sila center-city at the Wawa'a Al-Madani neighborhood	In a cardboard box, then into a polyethylene bag.	11-03-2023	15 g
Thymus vulgaris		In polyethylene bags.	11-03-2023	20 g

Table 2. Fruit and vegetable samples.

Sample type	Sample source	Sample conditioning	Sampling date	Fruits
Orange	Market in the M'sila center-city at the Djaafra neighborhood	In a polyethylene bag.	22-02-2023	2 fruit
Tomatoes		In a polyethylene bag.	02-03-2023	1 fruit
Lemon	Market in the M'sila center-city at the 1000 neighborhood	In a polyethylene bag.	25-02-2023	1 fruit
Bananas		In a polyethylene bag.	03-03-2023	1 fruit
Strawberries	Market in the M'sila center-city at the 5 July neighborhood	In a polyethylene bag.	27-02-2023	2 fruits
Pepper		In a polyethylene box.	10-03-2023	2 fruits
Apples	Market in the M'sila center-city at the Wawa'a Al-Madani neighborhood	In a polyethylene bag.	02-03-2023	5 fruits

Table 3. Grain samples.

Sample type	Sample source	Sample conditioning	Sampling date	Sample weight
Maize	Market in the M'sila center-city at the 1000 neighborhood	Polyethylene containers, bags	28-02-2023	30 g
Wheat	Market in the M'sila center- city at the Wawa'a Al-Madani neighborhood	In a plastic container, then into a plastic bag.	11-03-2023	22 g

1.2. Methods

1.2.1. Aseptic technique

- The bench should be clean and surface disinfected.
- The Bunsen burner must be working to eliminate any airborne mold spores; it is important to create a sterile zone using the Bunsen burner flame.
- The isolation process will be carried out in sterile conditions to avoid contamination; the inoculating needle is flamed in the Bunsen burner flame until it turns bright red (it will be allowed to cool for about 15 seconds).
- The Petri dish is opened and, with the sterilized needle, a small portion of the mold colony is cut out, transferred to the center of a sterile plate agar medium (Figure 6).
- The inoculated plate is incubated at 25°C for 7 days.

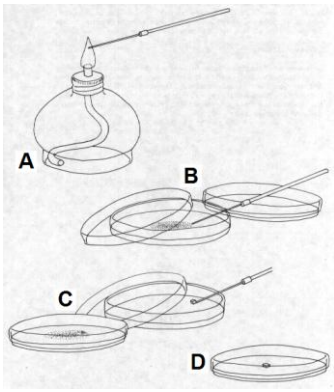

Figure 6. Sterile technique steps. A: Inoculating needle sterilized with Bunsen burner B: Small piece of colony is removed from Petri dish C: and transferred to a new one D: a plate containing a piece of the old culture at its centre. (Malloch, 1997).

1.2.2. Isolation method

The isolation method was conducted depending on the type of food sample, either directly or indirectly:

- **Direct seeding:** food particles are placed directly on the growth agar medium (figure 7).
- **Indirect seeding**: This technique was utilized while working with cereals and medicinal plants; after the surface disinfection with water with 1% sodium hypochlorite added (washing for 1min), the samples are rinsed with sterile distilled water, and dried on absorbing paper. The disinfected grains are placed, aseptically, on the growth potato dextrose agar (PDA) medium (Figure 8; 9).
- **Incubation**: The plates are incubated for 7 days at 25 °C and maintained upside-down.

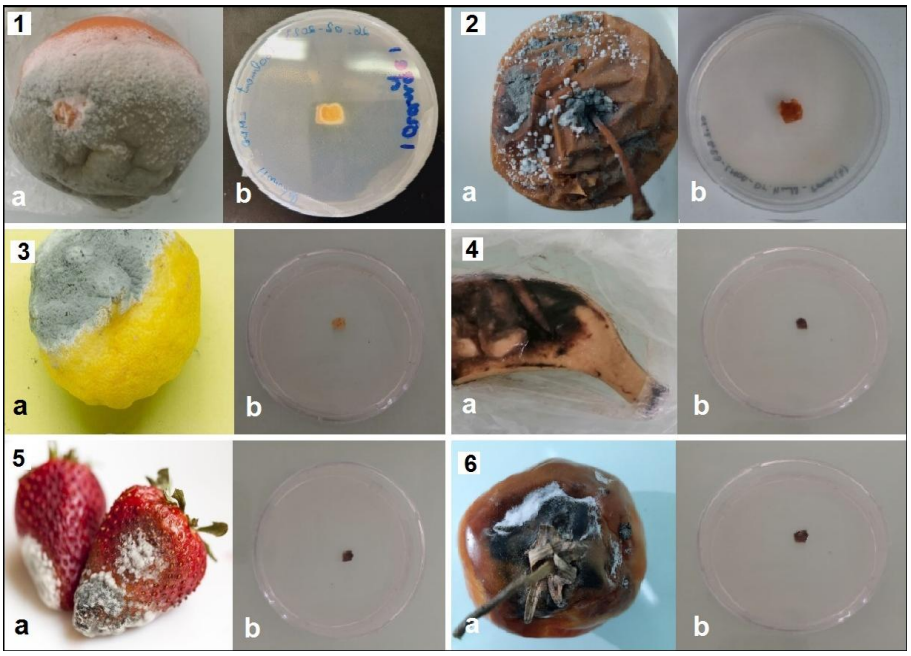

Figure 7. Infested fruits and vegetables (a), and direct cultivation on PDA plate medium (b).

1) orange, 2) apple, 3) lemon , 4) banana, 5) strawberry, 6) tomato.

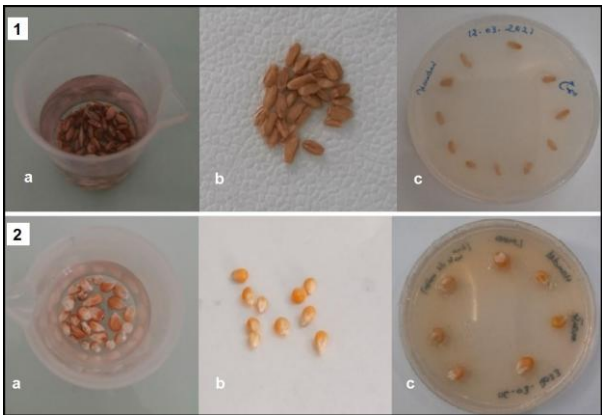

Figure 8. Isolation procedure from grains (1- wheat, 2- maize): surface disinfection (a, b), and cultivation on PDA plate medium (c).

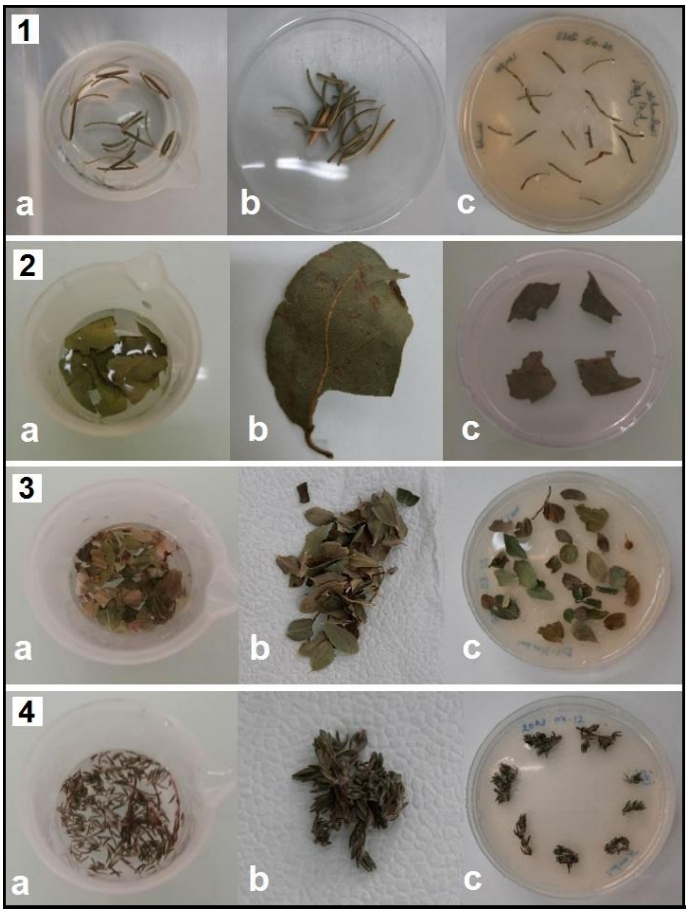

Figure 9. Isolation procedure from plants : surface disinfection (a, b), and cultivation on PDA plate medium (c).

1) *Rosmarinus officinalis*, 2) *Laurus nobilis*, 3) *Ziziphus lotus* , 4) *Thymus vulgaris*.

1.2.3. Purification of molds strains

The pure fungal isolates are obtained after multiple PDA subcultures. The procedure needs using a sterile needle to cut a little mycelial in the agar medium. The resulted cultures are incubated at 25°C for 4 to 7 days. The resultant fungal vegetative devices are macroscopically examined and distinguished with the naked eye on the basis of phenotypic characterization.

The isolations including several vegetative devices with different looks, colors, and textures are transferred to PDA slants and incubated at 25 °C for 5-7days and then are kept at 4°C.

In the case of contamination by another fungal strain, the strains were purified by subculturing in the same medium and under the same incubation conditions until the pure strains were obtained.

1.2.4. Identification techniques

1.2.4.1. Identification media method

The identification is done by taking fragments from the pure with a sterilized inoculating needle and inoculating specific identification media; PDA, SDA, MEA, G25N, and CYA, according to Pitt and Hocking (2009).

As seen in figure 10 , a single culture is inoculated into three points on CYA and MEA Petri dishes for incubation at 25 °C, equidistant from the plate's edge and center, as well as from one another. Cultures in the media G25N and different temperature incubation (5 °C, 37 °C) of CYA are inoculated with two points.

In some cases, it is important to minimize colonies from stray spores from some fungi, especially *Penicillium* and *Aspergillus*. The most effective technique to suspend them is to inoculate plates using semi solid agar (0.2%).

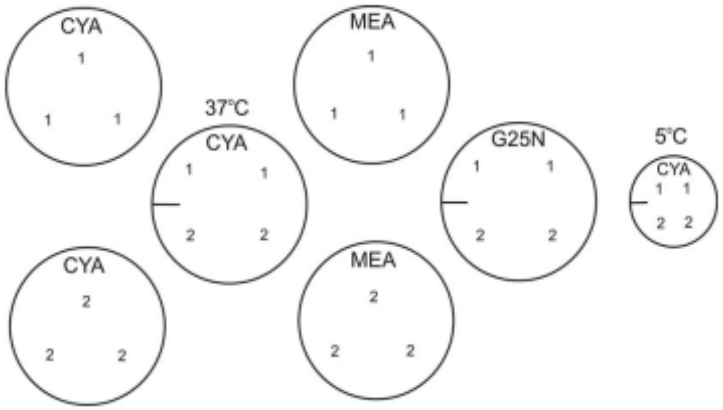

Figure 10. Schematic of regimen used for culturing fungal isolates for identification (Pitt & Hocking, 2009).

1.2.4.2. Macroscopic identification

The study of the macroscopic morphological characters was made by observation with the naked eye of the surface and the back of the pure fungal strains obtained. The characters studied were the mycelium (color and texture of the thallus, color of the underside of the vegetative apparatus contour of the vegetative apparatus, and speed of apical growth), spores (density on the thallus, aspect of the spores: granular or powder), the uniformity of spore color, the presence of diffusible pigments and exudates

Morphological and cultural characters are studied by observation with the naked eye of the surface and the back of the pure fungal strains obtained, and after the cultures are obtained according the Pitt and Hocking procedure. The characters studied were the growth rate (fast, medium, slow), colony diameter, the mycelium (color and texture of the thallus, color of the underside of the vegetative apparatus contour of the vegetative apparatus, and speed of apical growth), spores (density on the thallus, aspect of the spores: granular or powder), the uniformity of spore color, the presence of diffusible pigments and exudates

1.2.4.3. Microscopic identification

1.2.4.3.1. Wet mount slide

A wet mount slide is made by transferring a small amount of mycelium with a scalpel or dissecting needle to a drop of lactophenol cotton blue. The specimen is then covered with a coverslip and examined with the low-power objective (hyphae that have spore structures are searched). To discern more detail about the spores, the high- power objective is used.

1.2.4.3.2. The cellophane tape method

One to 2 drops of lactophenol cotton blue are placed on a microscope slide. Using a piece of clear cellophane tape, the surface of the fungal colony is gently touched with the sticky side of the tape. The tape containing the material from the fungal colony is transferred to the lactophenol cotton blue stain and the tape is pressed onto the slide, taking in consideration that the culture material is in the stain (Figure 11). The preparation is then microscopically observed.

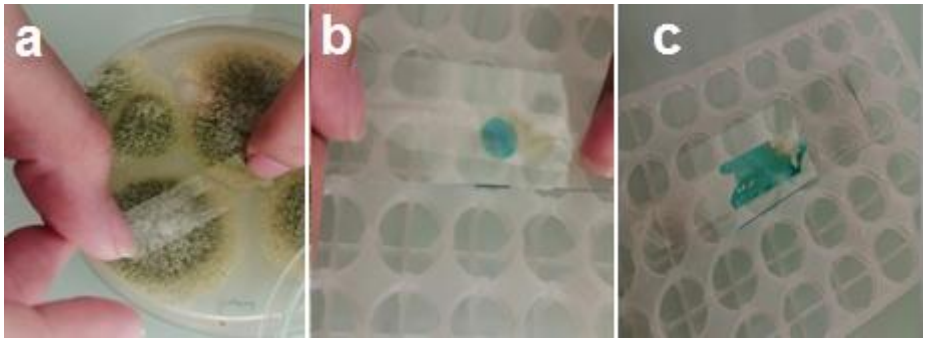

Figure 11. The cellophane tape method: a) Touching the mycelium , (b,c) Placing on the slide and pressing.

1.2.4.3.3. Slide culture

A circular plastic slide holder is placed at the bottom of the sterile Petri dish containing a piece of blotter paper. A sterile microscope slide with an attached small square of agar is placed

on the plastic holder. The margins of the agar square are inoculated with the fungus to be examined, and covered with a sterile coverslip. The box is moistened by adding some drops of sterile distilled water to the paper and incubated at 25°C for a few days (Figure 12).

After a sufficient incubation, the slide can be mounted on a microscope, and the undisturbed mold structures will be viewed as they are growing. Later, the agar block will be removed from the slide and cover slip, and two typical slide mounts will be made and microscopically examined.

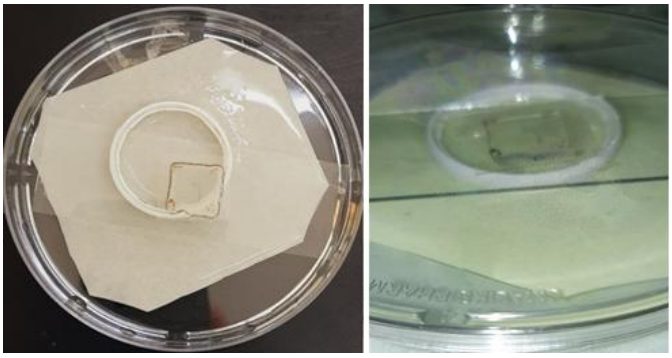

Figure 12. A prepared slide culture.

1.2.5. Photomicrographs and identification guides

Photographs and measurements of the various microscopic structures were obtained using Motic Images 2000 version 1.3 microscopy software. Identification guides that were used include: Pitt & Hocking, 2009 ; Les moisissures d'intérêt médical (Cahier de formation- BIOFORMA), Lin et al.(2012), Navi et al. (1999), Rahman et al. (2011), and Malloch (1997).

1.2.6. Antifungal activity of *Matricaria chamomilla* methanol extract

The antifungal activity of *Matricaria chamomilla* methanol extract was determined using the contact assay according to Hendel et al. (2021). The *Matricaria chamomilla* methanol extract (ME), dissolved in dimethyl sulfoxide (DMSO), was incorporated into melted PDA to obtain a final concentration of 1%. After solidification in a Petri dish, the medium was aseptically seeded at its center by a fungal disc (6 mm). The control plates were supplemented with DMSO instead of the ME. Each test was performed in triplicate. Colony diameters were recorded daily up to the 7th day. Growth inhibition was calculated according to formula:

I% = (DC-DT) / DC ×100

Where: DC: Diameter of the control fungal colony (mm)
DT: Diameter of the treated fungal colony (mm)

CHAPITRE III: RESULTATS & DISCUSSION

1. Mold identification

1.1. Macroscopic identification

Macroscopic characteristics are described on different identification media; PDA, MEA, CYA, G25N, SDA at 25°C. Weak growth was observed at 37°C and 5°C for almost all fungi.

- ***Alternaria alternata***

On the PDA plate, the colonies are between 65 and 77 mm; the mycelium developed air hyphae on grayish-white colonies that turned olivaceous-black or brown in seven days; and white cottony mycelium on the margins. The reverse is dark brown to almost black, but the edges are grayish white. Colonies on CYA and MEA 51– 64 mm, or covering the whole Petri dish, plane, of deeply floccose off-white to grey brown mycelium with some orange aspect on the colonies of MEA. The reverse is brown to nearly black on both mediums. On SDA, the colonies are 25 – 30 mm diameter; floccose and the mycelium developed air hyphae on olivaceous grey isolates, and the margins' colors range from pale white to grey; the reverse is brown to nearly red. On G25N medium, colonies up to 10 mm, similar in appearance to those CYA low-density colonies but more white; reverse white with an almost black center (Figure 13).

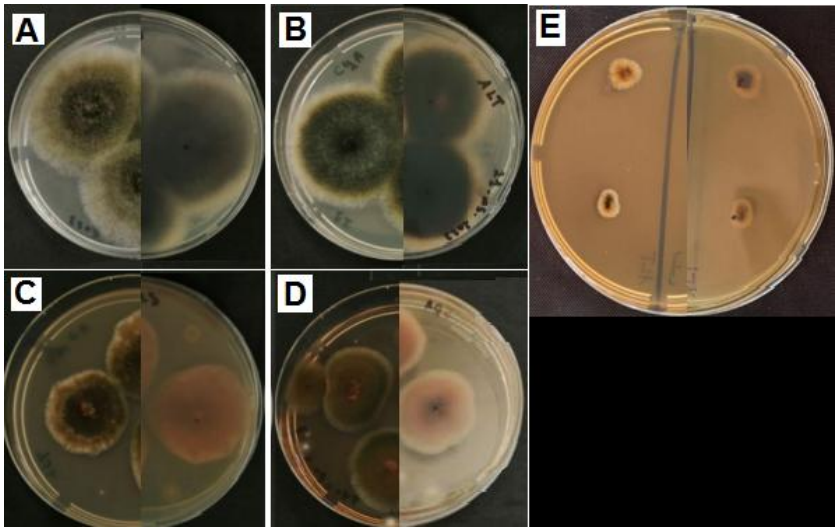

Figure 13. *Alternaria alternata* on deferent medias at 25 °C (A) PDA; (B) MEA; (C) CYA; (D) SDA; (E) G25N.

- ***Curvularia pallescens***

Colonies on PDA are 53 mm; gray in color and black on the backside, irregular borders, cottony growth with concentric zones, and produced exudates. On CYA, they are 45–65 mm diam; those on MEA are smaller, plane, of low to floccose mycelium, grey to white. Red deep reverse and orange margins. Colonies on SDA are smaller (10 mm in diameter) with same characteristics. Colonies are 2 to 5 mm in diameter, brown and grey on G25N medium (Figure 14).

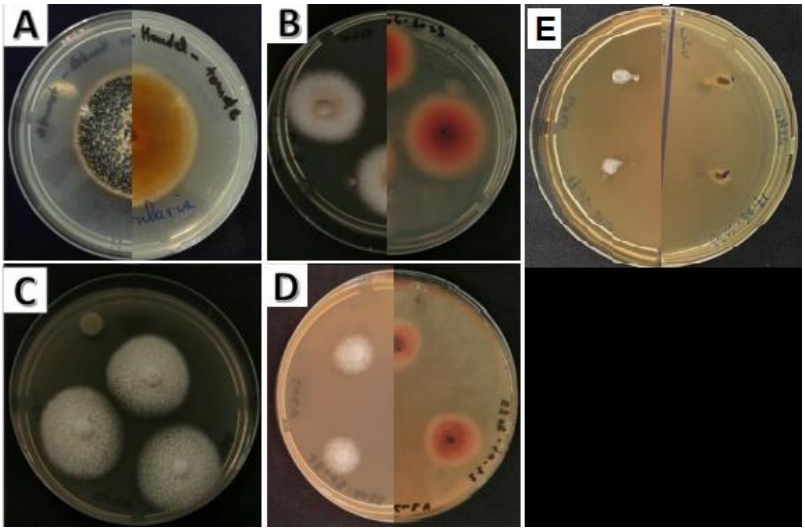

Figure 14. *Curvularia pallescens* on deferent medias at 25 °C (A) PDA; (B) MEA; (C) CYA; (D) SDA; (E) G25N.

- ***Aspergillus flavus***

Olive-green colony on PDA with 40 to 50 mm diameter. Flat at the borders and raised in the middle. On PDA, the *A. flavus* isolates produced exudates (droplets) that are colorless. On CYA, colonies are 42–66 mm diam, plane and sparse to moderately dense, velutinous margins, frequently floccose in the middle, and deeply white mycelium is only conspicuous in floccose areas. They are olive yellow, and turns greenish with age; reverse uncolored to pale yellow. Colonies on MEA are 50–60 mm diam, similar to those on CYA, and more velutinous. On SDA, colonies are 40 mm or more. As on CYA, colonies are 25–40 mm diam on G25N, floccose with little conidial production, and pale to orange or salmon reverse color (Figure 15).

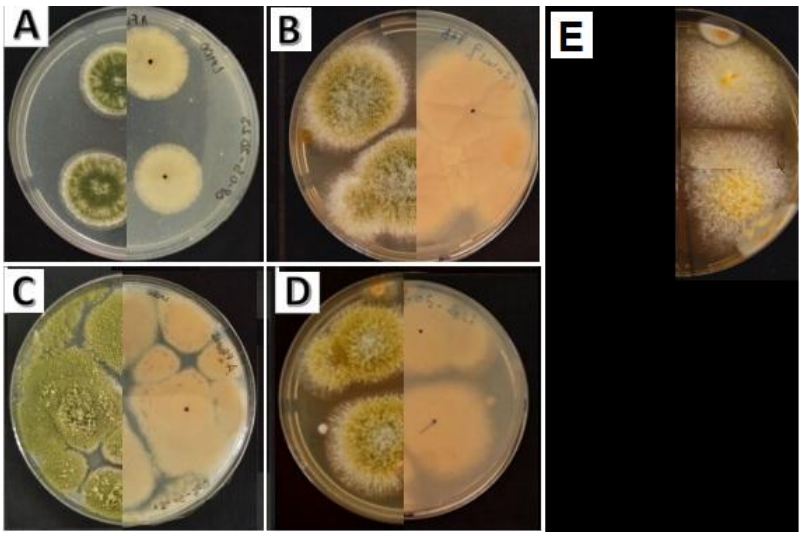

Figure 15. *Aspergillus flavus* on deferent medias at 25 °C (A) PDA; (B) MEA; (C) CYA; (D) SDA; (E) G25N.

- ***Aspergillus niger***

The colony diameter 50 mm on PDA, initially of white color, and acquired black after spore's production with velutinous to powdery appearance. Colorless to pale reverse. Colonies on CYA are 44 mm or more in diameter, velutinous, mostly subsurface, white mycelium, surmounted by a layer of closely packed, dark brown to black conidial heads that are just a few millimeters high. Bright yellow reverse with a pleated aspect. Colonies on MEA vary from 20 to 30 mm in diameter. Colonies on SDA are 30 mm or more in diameter with white center. Plane colonies on G25N, velutinous, with white or pale yellow mycelium at the margins. Pale reverse with pale brown areas (Figure 16).

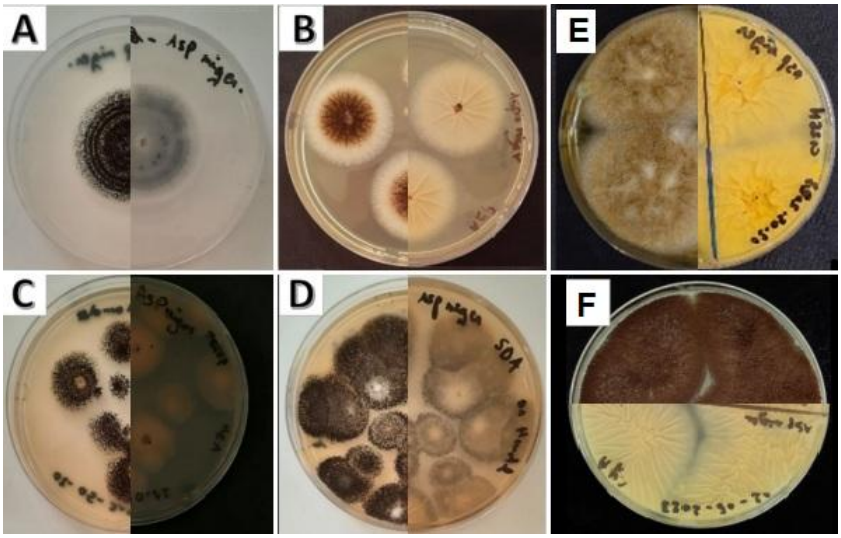

Figure 16. *Aspergillus niger* on deferent medias at 25 °C (A) PDA; (B) MEA; (C) CYA; (D) SDA; (E) G25N, (E) CYA at 37°C.

- ***Bipolaris* sp**

The colony diameter on PDA up to 30 mm in just three days, production of abundant conidia colored from brown to black, with white cottony mycelium. The reverse is dark brown with white margins. Colonies on CYA and MEA are 30-35 mm in diameter, plane, deeply floccose, and mycelium from light to dark grey; the reverse is dark brown. Floccose colonies on SDA, white mycelium with grayish spots and a deep black reverse. On G25N, 2–6 mm colonies with unremarkable growth (Figure 17).

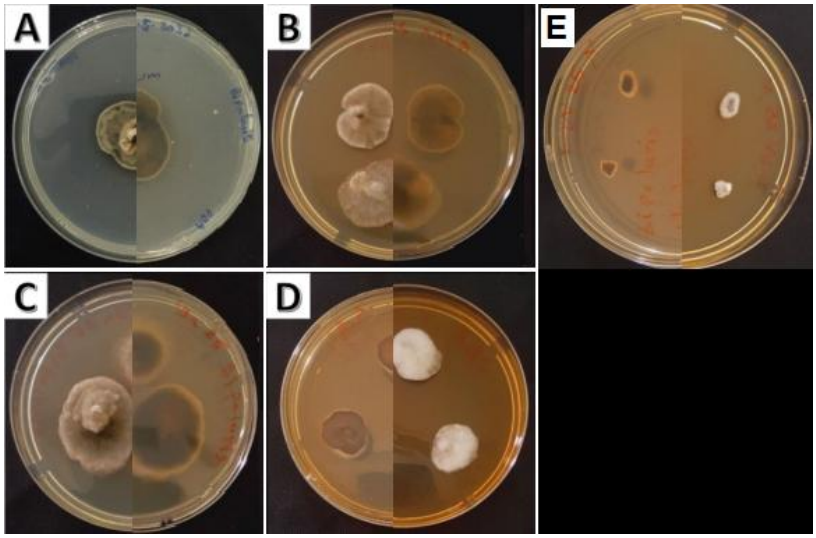

Figure 17. *Bipolaris* sp on deferent medias at 25 °C (A) PDA; (B) MEA; (C) CYA; (D) SDA; (E) G25N.

- ***Botrytis cinerea***

On all media, the colonies filled the entire Petri dish within 3-4 days, compact, cottony, warty, and powdery. Colonies are white, dirty white, grayish white, or hyaline at first but soon became light gray, dark gray to dark brown, soiled. Depending on their abundance, conidia are typically produced all over the medium's surface. Colonies on CYA and MEA are floccose, growth occasionally irregular, and mycelium starts out white turns grey to dark grey as conidiogenesis develops, reverse pale to grey. The SDA colonies they are less dense in the center (Figure 18).

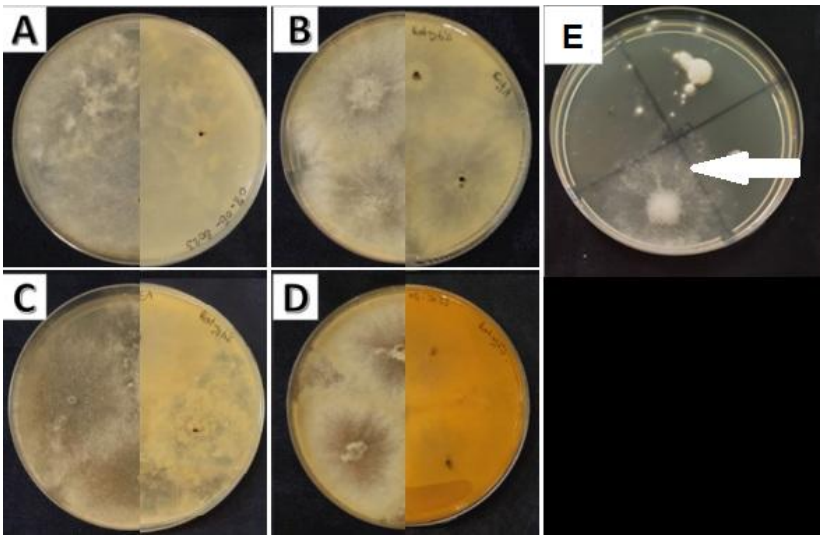

Figure 18. *Botrytis cinerea* on deferent medias at 25 °C (A) PDA; (B) MEA; (C) CYA; (D) SDA; (E) CYA at 5°C.

- ***Fusarium culmorum***

On PDA, colonies covering the whole plate with dense to floccose mycelium, pale red and pale yellow brown. Reverse pale to deep yellow with red center. Colonies on CYA are 60 to 65 mm of dense felty mycelium, frequently covered in white floccose, reaching the Petri dish rim, combined with pale red to pastel red; the reverse is pastel red to deep red. Colonies on MEA cover the whole plate, floccose, in age often reaching the Petri dish lid, pale red to pastel red, commonly with a greyish orange to yellowish brown overlay; the reverse is brown to reddish brown. The colonies are 46 mm and have an identical appearance to CYA in the absence of red spots; the reverse is reddish white (Figure19).

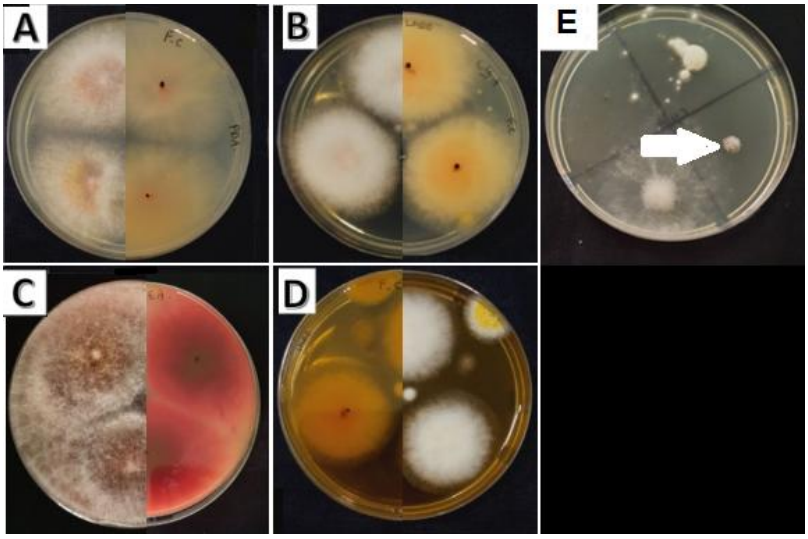

Figure 19. *Fusarium culmorum* on deferent medias at 25 °C (A) PDA; (B) MEA; (C) CYA; (D) SDA; (E) CYA at 5C°.

- ***Fausarium oxysporum***

Colonies on PDA cover the entire Petri dish with white, thick floccose mycelium. Reverse white to pale yellow in the center. Colonies on CYA 55– 60 mm diam, covering the Petri dish, moderately deep, of floccose white to pale red mycelium; orange to pale yellow reverse. On SDA, colonies are about 40 to 46 mm, while on MEA are smaller at 20 to 30 mm overall alternatively, is similar to CYA, but the reverse is orange. Colonies on G25N have a 20 mm, a less dense white mycelium, and a pale to white reverse (Figure 20).

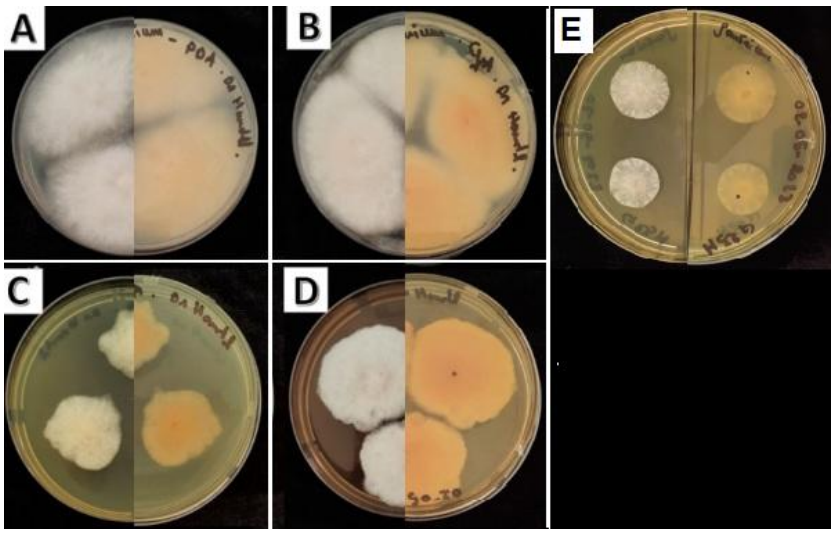

Figure 20. *Fusarium oxysporum* on deferent medias at 25 °C (A) PDA; (B) MEA; (C) CYA; (D) SDA; (E) G25N.

- ***Penicillium italicum***

Colonies on PDA are 30 and 36 mm diam, dense, velutinous, with numerous greyish-green conidia and white mycelium at the margins, the colony's reverse is colorless. Colonies on CYA 30–40 mm, either plane or radially sulcate, low and dense, velutinous to granular; mycelium is white on the margins; conidia are numerous and greyish green; some isolates produce clear exudate that is greyish brown in reverse with a pleated aspect. Colonies on MEA range in size from 30 to 35 mm, are plane and sparse, and are usually strictly velutinous. Conidial formation is moderate to heavy and colored similarly to that on CYA, but the reverse is gray. Colonies on SDA are smaller with 20 mm in diameter, more deeply green and grayish-brown in the center, and share the same traits as those on CYA (Figure 21).

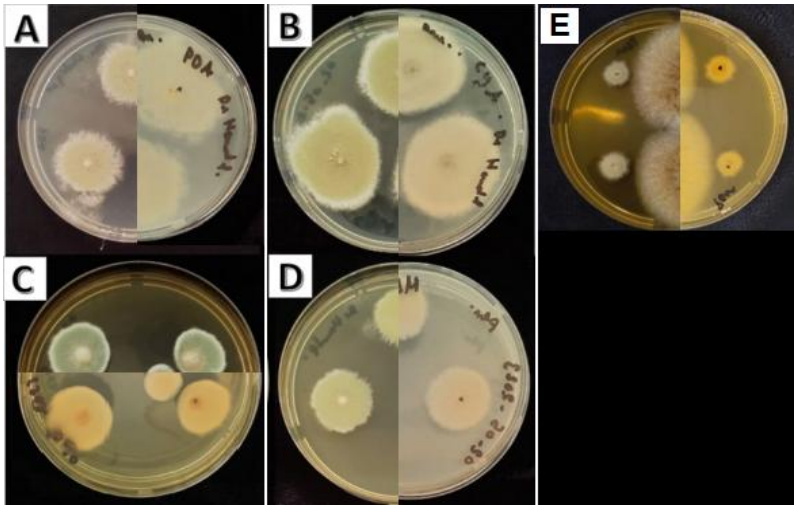

Figure 21. *Penicillium italicum* on deferent medias at 25 °C (A) PDA; (B) MEA; (C) CYA; (D) SDA; (E) G25N.

- ***Penicillium expansum***

On PDA, round colonies with a 20 mm diameter that are velutinous to granular with white mycelium in the margins,clear to pale orange exudate ; reverse is yellow. Colonies on CYA are 37–40 mm in diameter with circular bands. In adjacent areas, there is velutinous to floccose growth, white mycelium, moderate production of conidia that are dull green with wide white edges, and a pale to deep brown reverse. Colonies on MEA are variable, ranging from 30 to 40 mm. plane; the isolates are velutinous, and there is some coremial growth on the plane; mycelium is often entirely subsurface; conidial production is heavy, colored as on CYA or slightly greyer; reverse pale orange. Similar in appearance on SDA to that on MEA with a brown center and white edges in some isolates. Colonies of G25N are 20 mm in diameter, dense, with velutinous to granular surfaces; yellowish-white produce a brown soluble pigment; reverse pale (Figure 22).

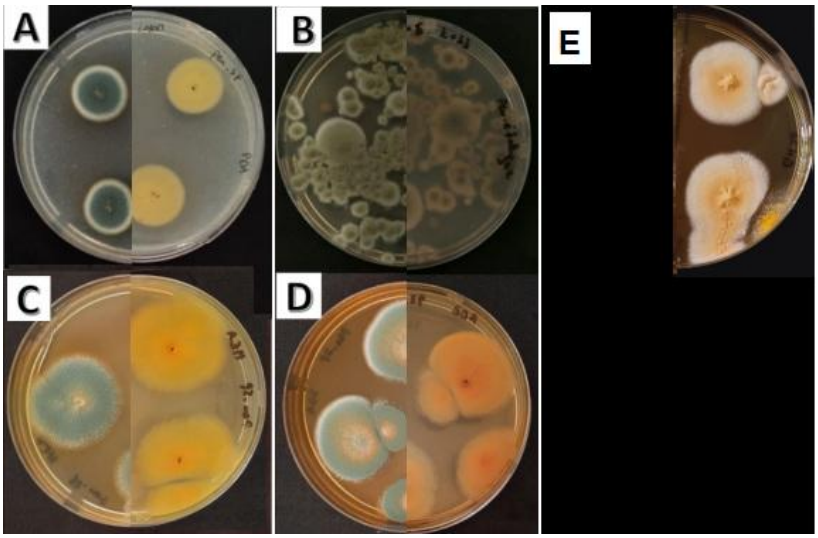

Figure 22. *Penicillium expansum* on deferent medias at 25 °C (A) PDA; (B) MEA; (C) CYA; (D) SDA; (E) G25N.

- ***Trichoderma Harzianum***

Colonies on PDA covered all Petri dishes; conidia production was denser in the center than towards the margins, with green conidia distributed throughout. Some white pustules were also found growing on the green mat of conidia. Colonies on CYA and MEA covering the entire Petri dish, frequently irregular in outline or with isolated tufts evident, with white to yellow mycelium and bright to dull yellow green conidia developing on the entire surface or in patches or tufts; the reverse is pale or yellowish. *T. harzianum* on SDA produces lengthy cottony white mycelium covers all Petri dishes. Colonies on G25N with poor growth, less than 20 mm in diameter (Figure 23).

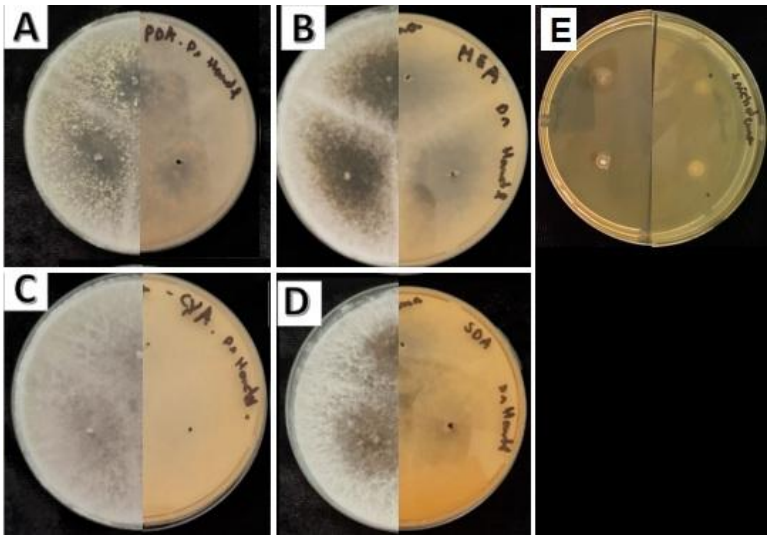

Figure 23. *Trichoderma Harzianum* on deferent medias at 25 °C (A) PDA; (B) MEA; (C) CYA; (D) SDA; (E) G25N.

1.2. Microscopic identification

- ***Alternaria alternata***

Alternaria alternata produces a melanin pigment and causes structures to range in color from brown to black on PDA plates. It has dark septate hyphae (21–27 µm) and large septate conidiophores that can be simple or branched. The conidia (poroconidia) (15x23; 24x43) are brown, muriform, ovoid, or obclavate with an elongated "beak-shaped" apical particle that forms a solitary or acropetally chaining. The conidial chain is one of the most important features that defines its type (Figure 24).

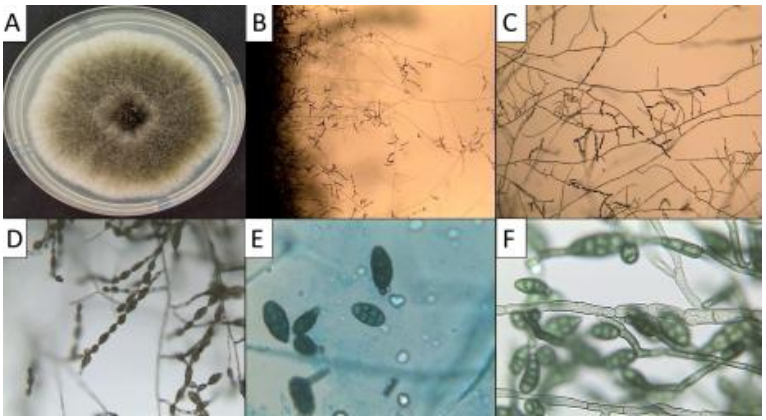

Figure 24. *Alternaria alternata.* (A) colony on PDA, (B) slide culture ×4, (C) slide culture objectif ×10, (D) conidia in situ ×40, (E) conidia ×100, (F) position of conidia.

- ***Aspergillus flavus***

Aspergillus flavus has segregated hyphae with relatively long conidiophores (45–55 µm) that are rough or spiny, in particular underneath the vesicle. Three-quarters of the surface of the vesicle from which the phialides are formed is covered by the metulae (8–10 x 5-7 µm). Metulae provide support for the phialides, which form the biseriate structure. Conidia have globose to ellipsoidal (22; 24 µm) shapes, and their walls are either smooth or finely rough (Figure 25).

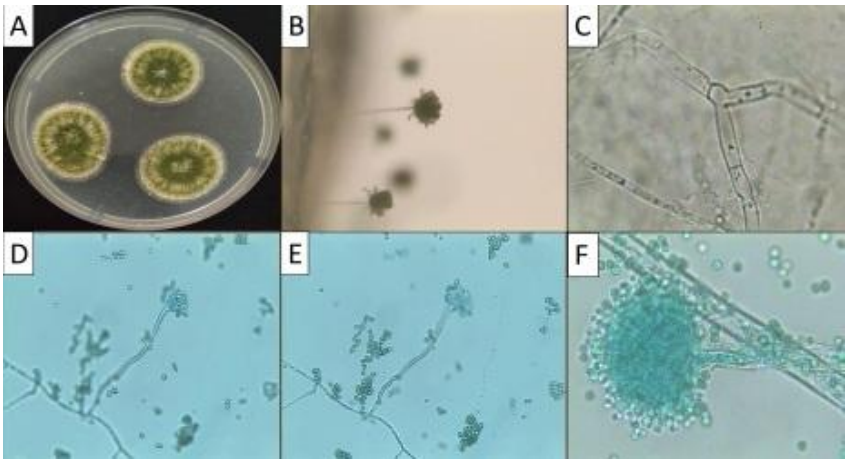

Figure 25. *Aspergillus flavus* ; (A) colonies on PDA, (B) Slide cultures ×10, (C) hyphe ×10, (D and E) the metulae and the phialides ×40, (F) Conidia, metulae and phialides ×100 .

- ***Aspergillus niger***

Aspergillus niger has microscopic septate, hyaline (clear) hyphae. Conidiophores (Stipes) are long (22.5–38 µm) with spherical vesicles at the apex measuring 30–75 µm. The metulae cover the majority of the surface where the phialides extend. Conidia are globose, 3.5-4.5 µm in diameter, black, and have a spiky exterior (Figure 26).

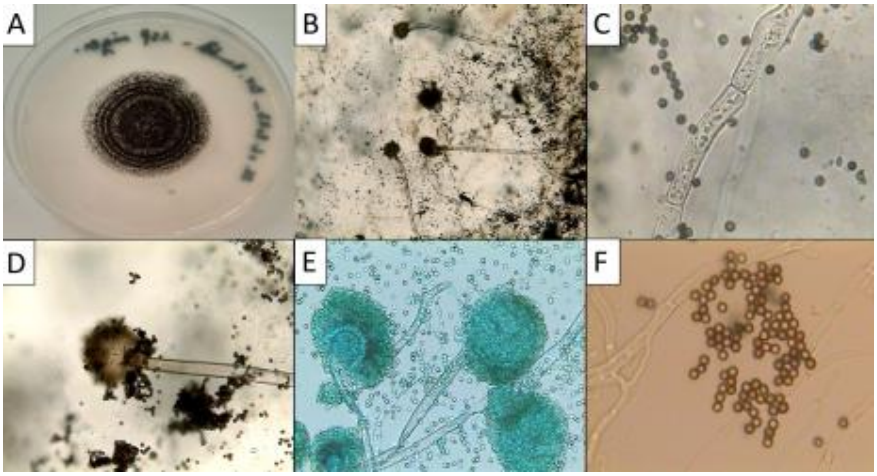

Figure 26. *Aspergillus niger* (A) colony on PDA, (B) slide cultures ×10, (C) hypha ×100, (D) conidiophores ×40, (E) conidiophores ×40, (F) conidia objectif ×100.

- ***Bipolaris sp***

In the PDA petri dish, *Bipolaris* has a brown to gray color. Has a septate and forked filament under the microscope (Figure 27B). Figure 27C demonstrates the formation of a hyphal bridge, which is one of the characteristics that determine its type. The thickness of the filament is 4.5–6 µm, and the conidiophores are simple, brown, geniculate, and sympodial in growth. The conidia (14 x 6; 40 x 11 µm) have a cylindrical shape and come out from the filament in a branched way (Figure 26E; F).

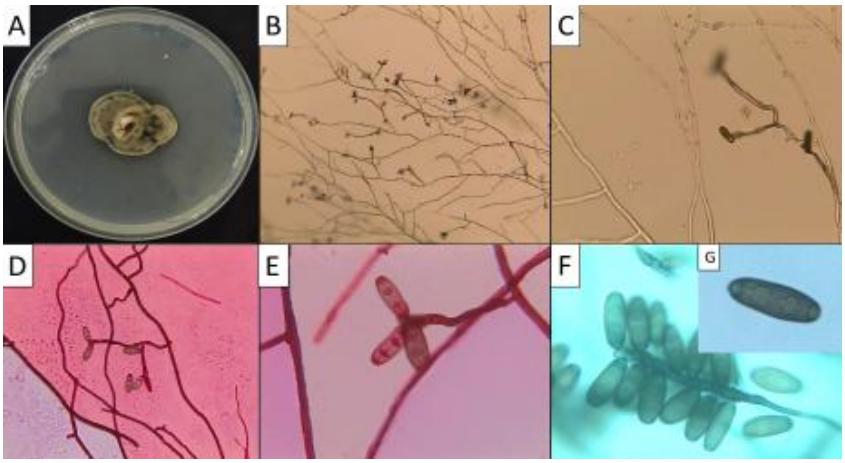

Figure 27. *Bipolaris* sp. (A) colony on PDA, (B) Slide cultures ×10, (C) hyphal bridge ×40, (D) hyphe ×40, (E) the position of conidia ×100, (F) conidia in situ ×100, (G) conidia ×100 .

- ***Botritys cinerea***

Botrytis cineria has conidiophores derived from aerial hyphae, stipes of indeterminate length, each having an irregular bunch of short branches at the tip, 10–30 µm long, with enlarged spherical apices 8–10 µm in diameter, and conidia borne individually from these apices on denticles (small pegs), ellipsoidal, 8–12 µm long, with smooth walls (Figure 28).

- ***Curvularia pallescens***

Curvularia pallescens has a dark brown color tending to black with white edges in a PDA petri dish (Fig. A), and it has forked septa filaments with diameters between 36 and 45 µm. The curvularia conidia are cylindrical or slightly curved, with one of the central cells being larger and darker than the others. It three curved septa, with the penultimate cell being asymmetrical and measuring 58–150 µm in diameter (Figure 29).

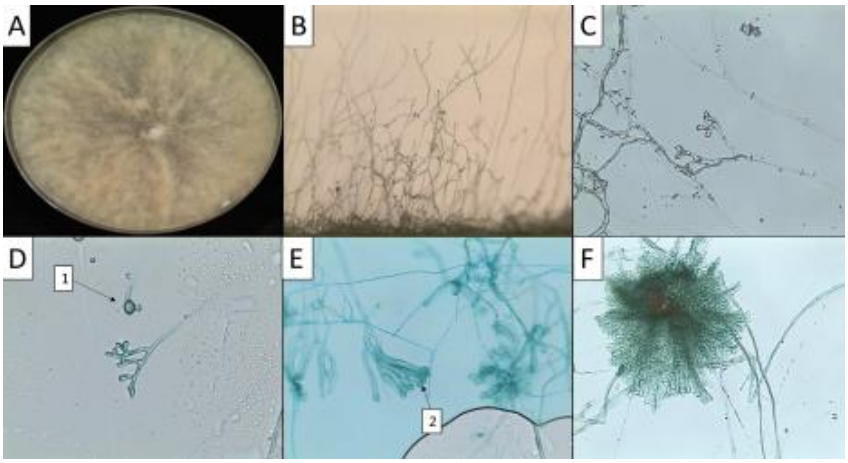

Figure 28. *Botritys cinerea* ; (A) colony on PDA, (B) Slide cultures ×10, (C and D) ×40, (E and F) ×100 , (1)conidia , (2) conidia formation area.

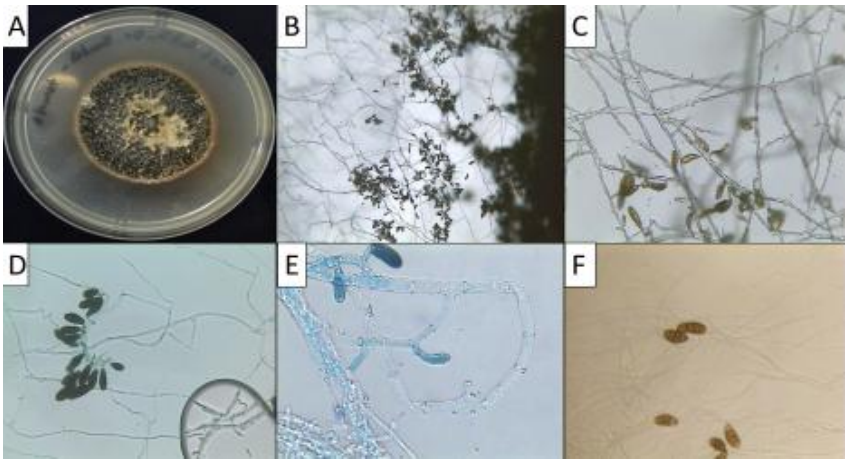

Figure 29. *Culvlaria pallescens* ; (A) colony on PDA, (B) Slide cultures ×10, (C and D) hyphe ×40, (E) the position of conidia (F) conidia ×100.

- ***Fusarium culmorum***

Fusarium culmorum has short, frequently branched conidiophores that come out of the vegetative thallus. They produce conidia through the production of phialides, which may have one or more budding sites (polyphialids). There are two types of conidia produced by phialides: Microconidia: unicellular (or bicellular) conidia, 4 to 8 m long, elongated, oval or cylindrical, isolated, or grouped, arranged in whorls or more rarely in chains. Macroconidia: multicellular conidia with only transverse divisions. They often come bundled together and range in length from 18 to 80 m. They are fusiform, curved, and quite pointed at the ends, with a podal cell forming a sort of more or less visible heel (Figure 30).

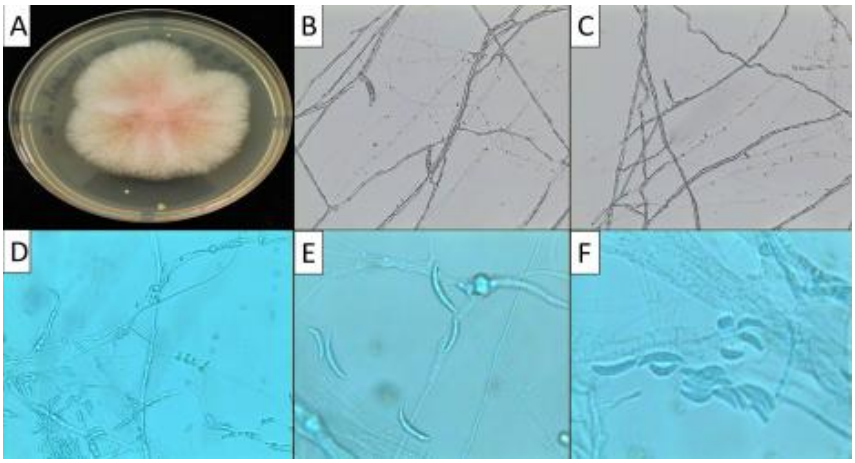

Figure 30. *Fusarium culmorum* ; (A) colony on PDA, (B, C, D) filaments and spores ×40, (E) chlamydospore ×40, F) macroconidia ×40.

- ***Fusarium oxysporum***

Fusarium oxysporum has septate hyphae (they show divisions or walls within the hyphae). Conidiophores are rather short (8 - 14 µm) and usually non-septate when compared to other *Fusarium* species. The conidiophores' sides are not parallel and slightly extend out in the middle, giving them the appearance of being somewhat inflated. As they extend from the aerial mycelium, these conidiophores (phialides or more precisely, monophialides) are produced singly. Since microconidia (5 x 12; 2.3 x 3.5 µm) are abundantly produced from the tip of these phialides, they are typically non-septate, ellipsoidal, and straight or slightly curved in shape; they are never produced in chains; they are only produced individually (Figure 31).

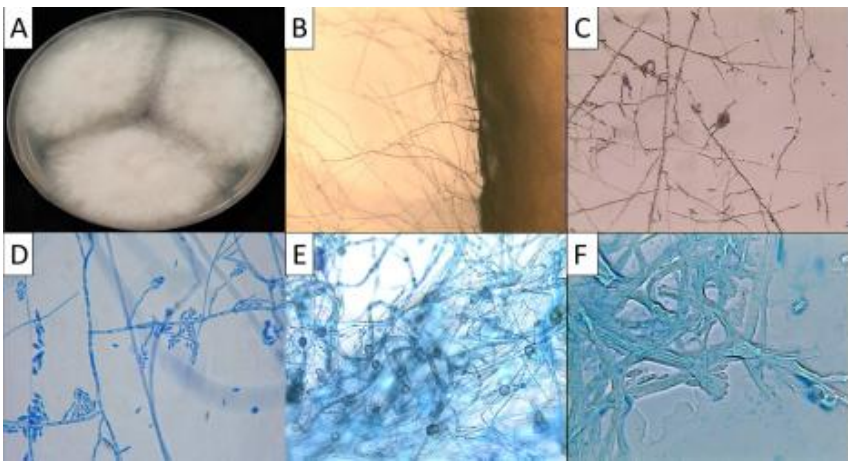

Figure 31. *Fusarium oxysporum* ; .(A) colonies on PDA, (B) slide cultures ×10, (C) hypha ×10, (D) microconidia ×40, (E) chlamydospores ×40 (F) filaments and microconidia×100.

- ***Penicillium italicum***

Penicillium italicum conidiophores borne from the surface or subsurface hyphae, stipes commonly 200–400 µm long, with fragile, smooth walls and bearing large, regular to irregular terminal terverticilla penicilli; rami 1–2 per penicillus. The metulae are frequently apically inflated; phialides 10 to 14 µm long, roughly cylindroidal in shape, then tapering abruptly to long cylindroidal collula conidia held up as cylinders, rounding with maturation; ellipsoidal to short cylindroidal, 3.0–5.0 µm long, with smooth walls carried in lengthy, irregular chains (Figure 32).

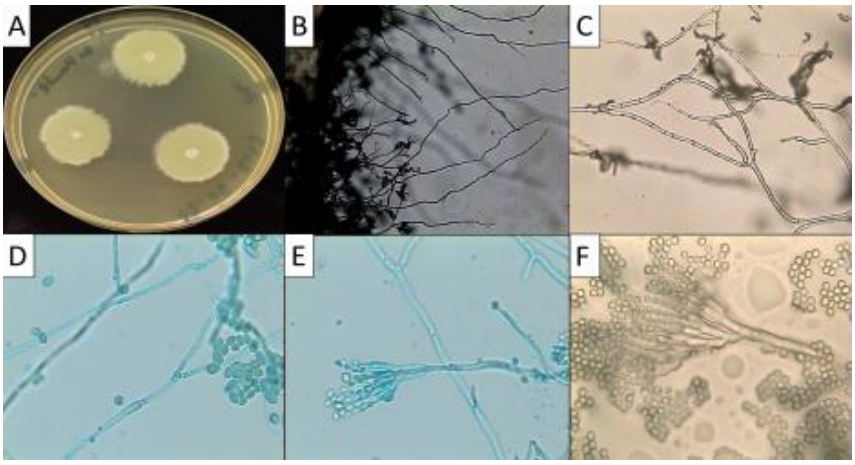

Figure 32. *Penicillium italicum* ; .(A) colonies on PDA, (B) slide cultures ×10, (C) hypha ×10, (D) conidia ×40, (E) penicillus ×40 (F) penicillus and conidia×100.

- ***Penicillium expansum***

Penicillium expansum conidiophores developed from surface or subsurface hyphae, alone, in fascicles, or in definite coremia, Stipes 200–500 µm long, smooth-walled, bearing terminal penicillin that are typically terverticillate but less frequently biverticillate; branches borne singly; occasionally visible to the unaided eye. Conidia are ellipsoidal, 3.0-3.5 µm long, smooth walled, and borne in long, densely packed, irregular chains, and the phialides are closely packed, ampulliform to almost cylindroidal, 8–11 µm (Figure 33).

- ***Trichoderma harzianum***

Trichoderma produces septate, hyaline hyphae. The conidiophores frequently have a pyramidal appearance, are relatively short, and are branched at wide positions. Phialides, which extend from the conidiophore, are flask- or ampule-shaped (inflated at the base). Conidia range in shape from being elliptical to round. At the tips of the phialides, slimy balls of single-celled conidia (2x3; 2.5x5 µm) accumulate. These conidia are frequently green in color (Figure 34).

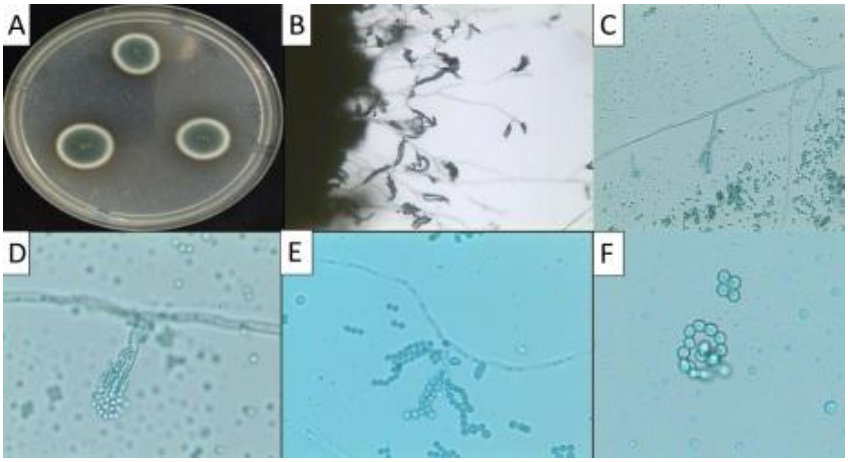

Figure 33. *Penicillium expansum* ; .(A) colonies on PDA, (B) slide culture ×10 (C) colored filaments ×10 , (D) penicilli ×40 (E)conidia ×40, (F) conidia ×100

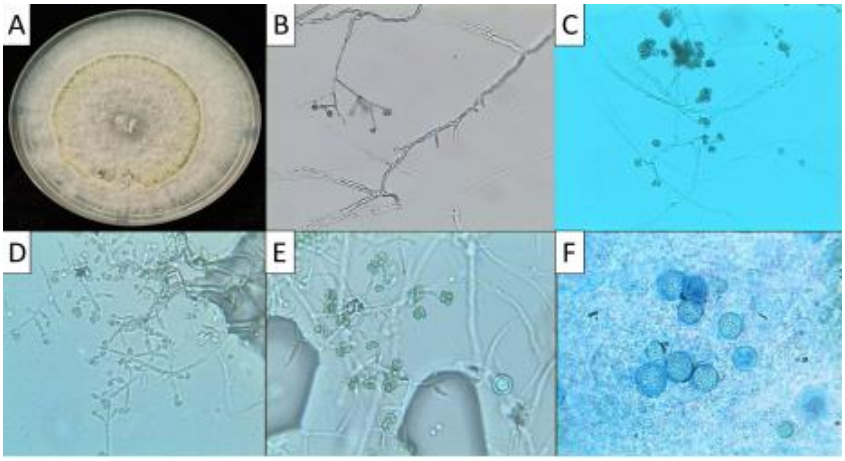

Figure 34. *Trichoderma harzianum* ; (A) colonies on PDA, (B and C) filaments and conidia ×10 , (D and E) phialides and conidia ×40, (F) chlamydospore ×100 .

2. Mold isolation and purification

For the isolation and purification of molds, we used 13 samples isolated from various fruits and vegetables, medicinal plants, and cereals, and by applying the methods described above, a group of 8 fungi from the genera *Alternaria*, *Aspergillus*, *Botrytis*, *Curvularia*, *Fusarium*, and *Penicillium* have been assembled (Table 4).

Table 4. Isolated molds and their origins of isolation.

Isolated molds	Origin of isolation
Alternaria alternata	Banana, Wheat, Thyme, Laurel, Pepper .
Aspergillus niger	Rosemary, Jujube, Thyme, Maize, Pepper.
Aspergillus flavus	Banana, Rosemary .
Botrytis cenarea	Strawberry, Paper, Lemon
Curvularia pallescens	Tomato.
Fusarium oxysporum	Maize, Thyme, Paper.
Penicillium expansum	Jujube, Thyme, Wheat, Maize, Banana, Apples, Paper.
Penicillium italicum	Wheat, Maize , Orange, Lemon.

According to fungal strains percentage (Figure 35), *Penicillium expansum*, *Aspergillus niger*, and *Alternaria alternata* represent the majority of the fungal genus found in most samples.

Penicillium expansum is the only isolate in apples and is present in 30% of pepper.

Alternaria alternata is present in thyme with a percentage of 70.58%, 50, 33% in banana, 50% in wheat, and 100% in Laurel.

Aspergillus niger is present in 80% of rosemary, 40% of jujube, and a low percentage of maize, thyme, and pepper.

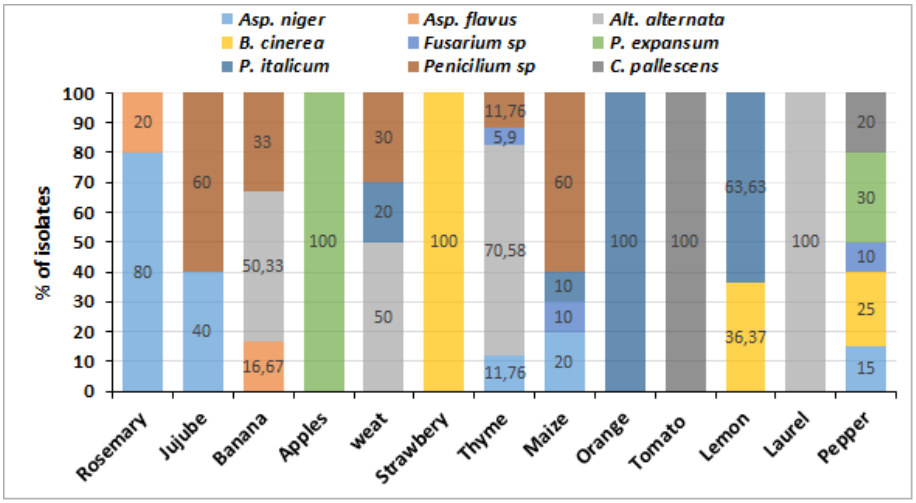

Figure 35. Fungal strains percentage compared to samples

Botrytis cinerea, *Curvularia pallescens*, and *Penicillium italicum* are the only isolates found in strawberry, tomato, and orange in that order with 100% and present in other samples in different proportions.

Aspergillus flavus is the least prevalent in the samples, with 20% in rosemary and 16% in banana.

The predominant isolated mold was *Alternaria alternata* followed by *Penicillium* isolates (*Penicillium* sp, *P. italicum*, *P. expansum*), *A. niger*, *B. cinerea*, *C. pallescens*, *A. flavus*, and *Fusarium* species (Figure 36).

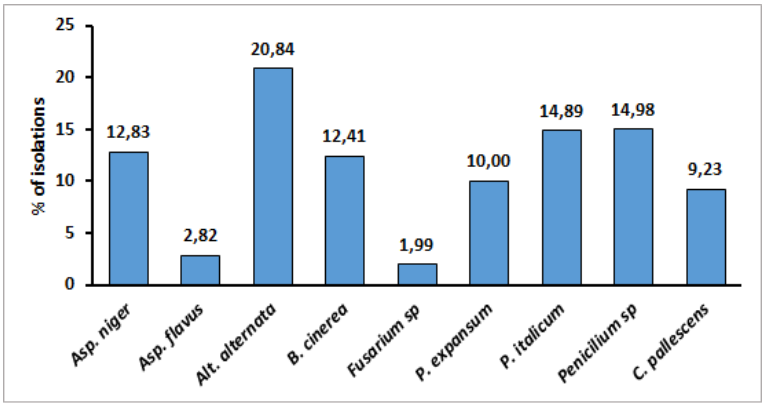

Figure 36. Fungal strains percentage compared to total isolations.

After transplanting, purifying and identifying 3 laboratory strains (F), (V), and (R), we obtained the following results:

- The strain F obtained the genus *Fusarium culmorum*.
- The strain R gave the genus *Trichoderma harzianum*.
- The strain V obtained the genus *Bipolaris* sp.

3. Antifungal activity of *Matricaria chamomilla* methanol extract

The antifungal activity of the medicinal plant *Matricaria chamomilla* was evaluated in the form of the methanolic extract (ME) incorporated into the culture medium. The radial growth of each fungus was measured daily and compared with that of the control. The results were expressed in terms of the percentage of the growth inhibition on the 5th and 7th days, and are presented separately for each of the tested fungi.

- ***Aspergillus flavus***

The growth of *Aspergillus flavus* in the first 3 days was almost similar to that of the control, with an inhibition of (5%). From the 4th day until the 7th day, there were inhibitions around 25% (Figure 37) more frequently than the previous days, depending on the effect of the extract.

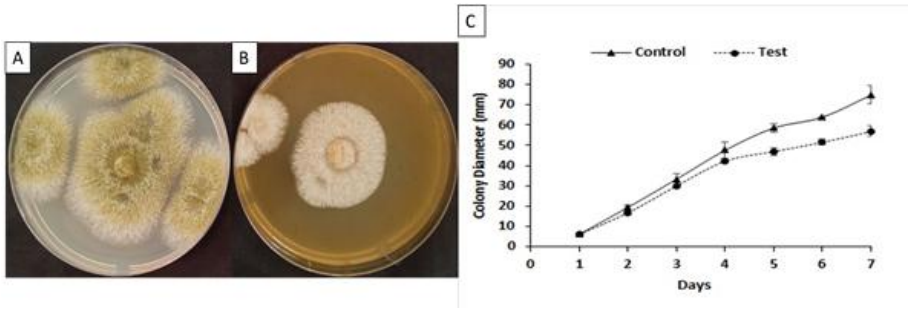

Figure 37. Antifungal test result of *Aspergillus flavus*. (A): control, (B): test, (C) effect of the extract on the radial growth of tested fungi..

- ***Aspergillus niger***

Aspergillus niger growth was inhibited for 3 days, and we notice that the effectiveness of the extract is constant. On the 4th day, the effect decreased, and the diameter of the control exceeded the diameter of the mold tested with almost no inhibition (-10%) (Figure 38) over the remaining days.

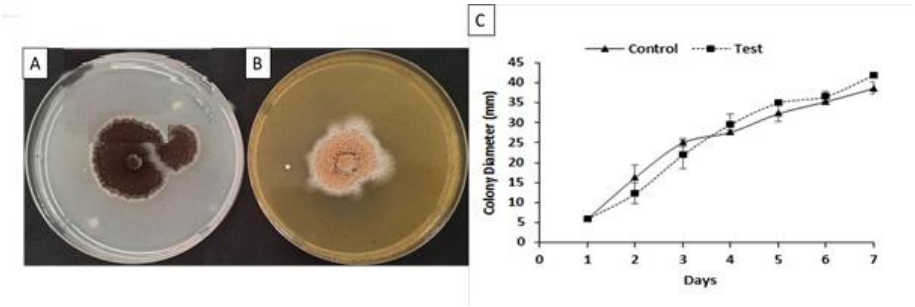

Figure 38. Antifungal test result of *Aspergillus niger*. (A): control, (B): test, (C) effect of the extract on the radial growth of tested fungi.

- ***Botrytis cinerea***

Except for 7th day, the extract reduced Botrytis cinerea growth by (30%) (Figure 39) throughout the entire incubation period, with a partial increase each day. The inhibition reached its maximum in the 2nd and 3rd days, with a 30 mm difference compared to the control. While we notice a decline in the extract's effect on the 5th day and continue their growth until it returns to normal on the 7th day.

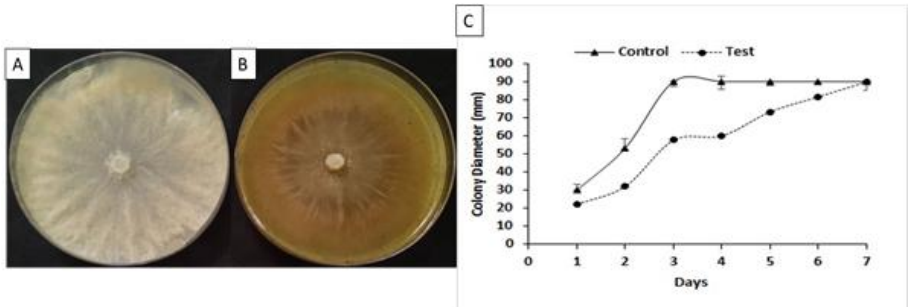

Figure 39. Antifungal test result of *Botrytis cinerea*.(A): control, (B): test, (C) effect of the extract on the radial growth of tested fungi.

- ***Fusarium culmorum***

The extract inhibited *Fusarium culmorum* by (20%) to (25%) (Figure 40) during all 7 days of incubation, where we noticed a modest but significant difference in the growth between the test and the control.

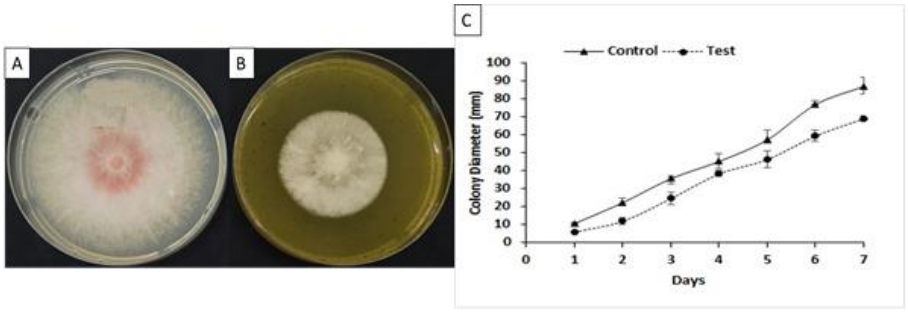

Figure 40. Antifungal test result of *Fusarium culmorum*. (A): control, (B): test, (C) effect of the extract on the radial growth of tested fungi..

- ***Penicillium expansum***

Each of the control and tested molds showed close growth over the first two days. Starting on the third day, we begin to observe the extract effectiveness in slowing the growth of *Penicillium expansum*. Moreover, at the end of the incubation, this effect causes a significant inhibition of 45% (Figure 41), reaching its peak in the sixth and seventh days.

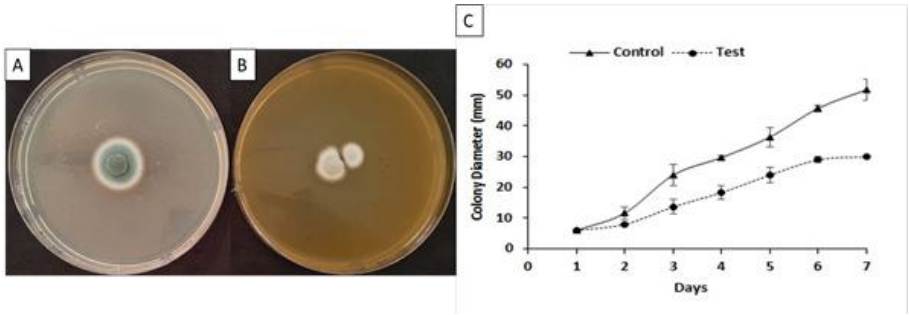

Figure 41. Antifungal test result of *Penicillium expansum* .(A): control, (B): test, (C) effect of the extract on the radial growth of tested fungi.

- ***Penicillium italicum***

Penicillium italicum was unaffected by the extract until the second day, when the inhibition began to increase and then persisted throughout the incubation period, particularly during the 5 and 7 days, at a rate of (25%) to (31%) (Figure 42).

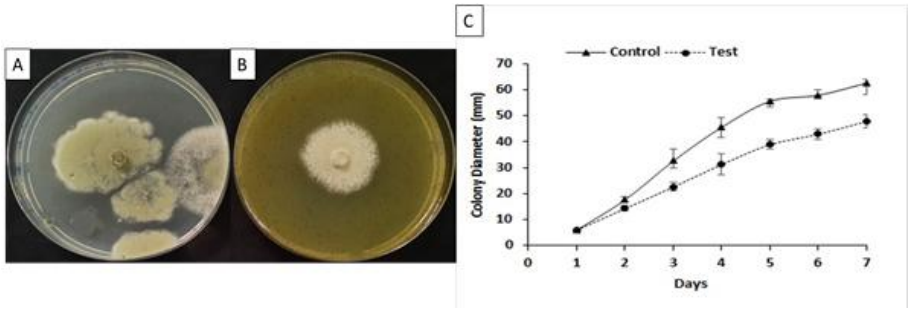

Figure 42. Antifungal test result of *Penicillium italicum* . (A): control, (B): test, (C) effect of the extract on the radial growth of tested fungi.

- ***Trichoderma harzianum***

The extract inhibited *Trichoderma harzianum* at a rate of (25%) (Figure 43) during the first 5 days of incubation until the 6th day, when a great affinity was observed. This effect stopped on the seventh day and returned to normal growth, which was explained by the disappearance of the test effect on the mold.

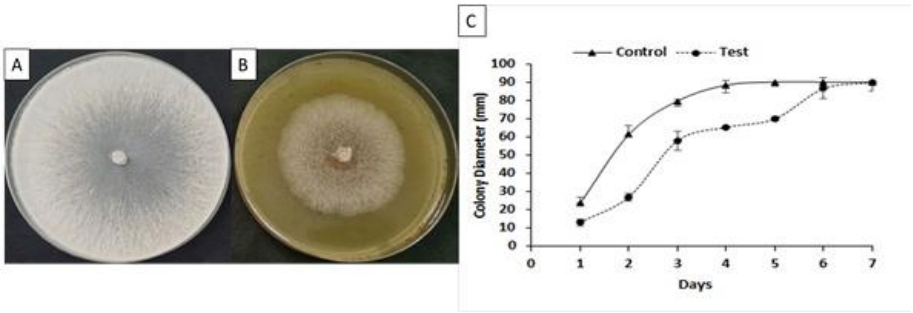

Figure 43. Antifungal test result of Trichoderma harzianum. (A): control, (B): test (growth at the 3rd day), (C) effect of the extract on the radial growth of tested fungi.

Generally, the fungal inhibition was remarkable at the 5th day and almost the total fungi were inhibited at nearly 20 to 40%. But at the 7th day, only 4 molds (57.14%) were remained inhibited (Figure 44).

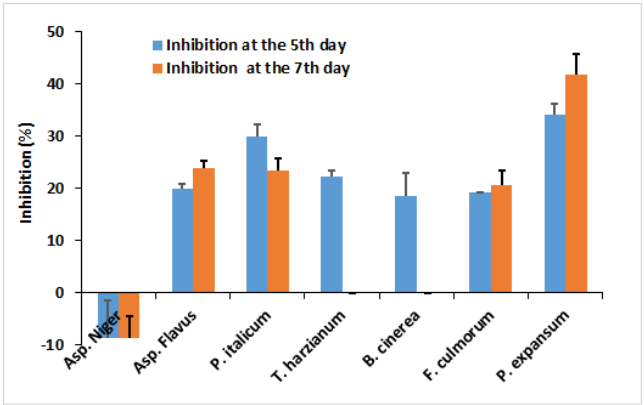

Figure 44. Fungal growth inhibition caused by *Matricaria chamomilla* ME, after 5 and 7 days.

4. Discussion

In light of the results, we have identified eight genus based on the results of the isolation and identification we applied to our samples, which are *Fusarium oxysporum*, *Botrytis ceneria*, *Alternaria alternata*, P*enecillium expancum, Penecillium italicum, Aspergillus niger, Aspergillus flavus, and Penecillium niger* from thirteen different food products, namely

According to the results obtained, the most common genera in food products are *Alternaria alternata*, *Penicillium expansum*, and *Penicillium italicum*. The genus *Aspergillus niger*, *Curvularia pallescens*, and *Aspergillus flavus* are frequent but less important; on the other hand, *Fusarium oxysporum* is even rarer.

These findings stem from a general study that looked at the percentage of each type of fungus in all isolated samples, where we observed that *Alternaria alternata* is present in 22% of banana, wheat, thyme, Laurel, and paper. In addition, *Penicillium expansum* is present in 25% of jujube, Thyme, wheat, corn kernel, banana, apples, paper, and *Penicillium italicum* with 15% in wheat, corn kernel, orange, and lemon. *Aspergillus niger* is 12% in Salvia rosmarinus, Jujube, Thyme serpyllum, maize, and pepper, and the same as *Botrytis ceneria*, which is 12% (strawberry, lemon, and pepper). *Curvularia pallescens* 7, 7% (tomato) *Aspergillus flavus* 2, 9% (banana, rosemary), and *Fusarium oxysporum* 2% (maize, Thyme, pepper).

In terms of bibliography, the literature reveals a number of significant studies that are pertinent to our research.

Penicillium expansum has been isolated from a wide range of fruits proving that it is a pathogen with a wide host range (Pitt & Hocking, 2009). According to Aziz et al. (2006), isolates have been found in corn, wheat, rice, and barley.

Alternaria alternate, has been found in a very diverse range of foods (Pitt & Hocking, 2009).

Penicillium italicum prefers the fruit of *Citrus* species as its natural habitat, where it causes a destructive rot that has a considerable economic impact (Pitt & Hocking, 2009).

Botrytis cinerea is a virulent pathogen that causes the spoilage of many types of fresh fruits (Pitt & Hocking, 2009). Especially in berries such as strawberries, blueberries, raspberries, and blackberries (Tournas & Katsoudas, 2005)

Curvularia pallescens is commonly found in tropical regions. This species is not known to produce mycotoxins (Pitt & Hocking, 2009)

The current studies were conducted to isolate and identify the seed-borne pathogenic fungus from the selected tomato using morphological and molecular methods based on the sequencing of

the 18S rDNA internal transcribed spacer (ITS) region. According to the colony and conidial features, the fungus was identified as *Curvularia* sp (Billah et al., 2021).

The major known source of *Curvularia pallescens* is sorghum. However, it has also been recorded on rice solitary conidia and causing rot in melons (Pitt & Hocking, 2009).

Aspergillus niger is very frequently isolated from sun-dried products (Valero et al., 2007).

Aspergillus flavus is capable of causing spoilage of some kinds of fresh fruit and vegetables, including citrus, tomatoes, peppers, litchis, pineapples, and pomegranates, but it is not usually very significant (Pitt & Hocking, 2009).

Fusarium oxysporum is a dangerous pathogen that causes wilt in many crop plants. It is geographically widespread, it occurs in cereals, including corns (Pitt & Hocking, 2009).

In one study, *Alternaria alternata*, which was found in more than 85% of the cereals analyzed, was the most prevalent fungus, another investigation into Egyptian wheat grains revealed the isolation of 77 fungi species from 26 genera, including 16 *Aspergillus* species and 21 *Penicillium* species (Pitt & Hocking, 2009).

In the majority of instances, the results of these studies show a similarity with those of our research, as the following genera are listed as the primary fungi that contaminate food: *Penicillium expansum and Alternaria alternate.* Also, in food products exposed to fungus contamination, cereals are considered one of the most exposed materials.

For the rest, minimal differences appear from one study to another, which can be explained by either growth conditions or nutrition requirements necessary depending on the variety of samples. As opposed to us, they come from fruits such as *Curvularia pallescens,*

Following our testing of *Matricaria chamomilla's* methanolic extract on seven fungi we had isolated and identified, we discovered the following outcomes:

The extract has the efficacy to inhibit each of *Aspergillus niger, Aspergillus flavus,* and *Penicillium italicum. Penicillium expansum, Tricoderma harzianum, Fusarium culmorum,* and *Botrytis cinerea.*

The difference between them lies in the duration of inhibition. For example; *Aspergillus niger* is only inhibited for 2 days at a rate of 5%, while *Penicillium expansum* and *Penicillium itallicum* are inhibited for 7 days of the treatment period at a rate of 45% and 30% on the 7th day, respectively.

In order to verify the validity of our study's findings, we reviewed the bibliographical aspect.

A study by (McKay & Blumberg, 2006) indicates that several phenolic compounds, primarily flavonoids and coumarins, are the main components of *Matricaria* sp. flowers. It has been proven that quercetin prevents the growth of various bacteria, fungi, and viruses. Along with caffeic acid has also been shown in other studies to have antioxidant and antifungal properties (Batiha et al., 2020).

Subsequently, our work is a preliminary study that opens the door to numerous other studies on the antifungal efficacy of *Matricaria chamomilla* L.

CONCLUSION

Conclusion & prescriptive

At the end, this study was carried out on numerous food products distributed in M'sila city markets. We noted that the isolation and purification of molds on different media cultures, and the correlation between the macroscopic and microscopic characteristics allowed us to identify 11 genera from 13 different samples. The isolates belonging to these genera are: *Alternaria alternata*, *Aspergillus flavus, A. niger, Bipolaris sp, Botritys cinerea, Culvlaria pallescens, Fusarium culmorum, F. oxysporum, Penicillium italicum, P. expansum,* and *Trichoderma harzianum*.

The results collected showed that among these fungi, *Alternaria alternata*, *Penicillium italicum*, *Penicillium expansum,* and *Aspergillus niger* are the most prevalent and can cause significant deterioration, especially in the fields of food and health safety. Namely, it is because the majority of these strains are toxigenic and secrete mycotoxins.

Following this, we tested the sensitivity of seven molds vis-a-vis the methanolic extract of the medicinal plant *Matricaria chamomilla*. The results obtained during this study revealed an interesting inhibitory effect on the growth of the tested molds, particularly *Penicillium expansum* and *P. italicum*. This activity is probably related to the abundance of secondary metabolites in this plant, including coumarins and flavonoids.

To put our study in perspective and elucidate certain points, it will be interesting to develop our subject through the following studies:

- Molecular identification of the strains obtained.
- Study the antifangal activity of *Marticaria chamomilla* at various concentrations, and the effect it has on other germs.
- Identify active metabolites that have an inhibiting effect on mold growth.
- Increase public awareness of the toxinogenesis of the identified strains.

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ANNEXES

Annexes

Culture media

Potato dextrose agar (PDA):

Potatoes	250 g
Glucose	20 g
Agar	15 g
Water, distilled	1 l

Compared to commercially prepared media, PDA made from raw components is more satisfying.

- After washing, we dice the potatoes and add 500 ml of water (30 to 45 minutes of boiling). We also melt the agar in 500 cc of water at the same time.
- Using a cloth, we strain the potato into the flask holding the melted agar.
- The glucose is added, well combined, and, if required, made up to 1 liter with water.
- By autoclaving for 15 minutes at 121 °C, we sanitize.

Czapek concentrate: included in components of CYA and G25N

NaNO3	30 g
KCl	5 g
MgSO4.7H2O	5g
FeSO4.7H2O	0.1 g
Water, distilled 100 ml	100 ml

- Without sterilizing, czapek concentrate can be kept for an unlimited period.

Trace metal solution: included in components of CYA.

CuSO4.5H2O	0.5 g
ZnSO4.7H2O	1g
Water, distilled	100 ml

- Keeps indefinitely without sterilisation.

Czapek yeast extract agar (CYA):

K2HPO4	1 g
Czapek concentrate	10 ml
Trace metal solution	1 ml
Yeast extract, powdered	5 g
Sucrose	30g
Agar 15 g	15 g
Water, distilled	1 l

- We mix all the ingredients until they homogenize by magnetic agitator under the influence of heat, and we sterilize by autoclaving at 121 °C for 15 minutes. The final pH is 6.7.

Malt extract agar (MEA)

Malt extract, powdered	20 g
Peptone	l g
Glucose	20 g
Agar	20 g
Water, distilled l l	l l

- We mix all the ingredients until they homogenize by magnetic agitator under the influence of heat, and we sterilize by autoclaving at 121 °C for 15 minutes. The final pH is 5.6.

25% Glycerol nitrate agar (G25N) :

K2HPO4	0.75 g
Czapek concentrate	7.5 ml
Yeast extract	3.7 g
Glycerol, analytical grade	250 g
Agar	12 g
Water, distilled	750 ml

- We mix all the ingredients until they homogenize by magnetic agitator under the influence of heat, and we sterilize by autoclaving at 121 °C for 15 minutes. The final pH is 7.0.

2% agar water:

Agar	20 g
Water, distilled	1000 ml

Poor medium, 2% agar water favors sporulation for many molds.

Crop dyes:

Lactophenol :

Phenol crystals	20g
Lactic acid	20ml
glycerol	40ml
Water, distilled	20ml

Lactophenol cotton blue :

cotton blue	0,075g
Lactophenol	100ml

Lactofuchsin:

Acid fuchsin	0,1g
Lactic acid	100ml

ملخص

الوظيفة الأساسية للفطريات الدقيقة المنتشرة في كل مكان إعادة تدوير المواد العضوية حيث أن تنوع الفطريات هائل (بأكثر من 1.5 مليون نوع في جميع أنحاء العالم) كما أن العدوى الناجمة عن الفطريات مشكلة خطيرة في الصناعة الغذائية والصيدلانية، وكذلك للصحة العامة. كان الهدف من دراستنا عزل وتحديد أنواع الفطريات المأخوذة من مجموعة متنوعة من الأطعمة التي تشمل الحبوب، الفواكه والخضروات والنباتات الطبية للتحقق من تحملها وتقدير نسبة فعالية مضاد الفطريات في المختبر باستخدام نبتة *Matricaria chamomilla* و ذلك بعدة طرق منهجية في البحث العلمي والتي تعتمد على نوع السلالات بشكل عام، مثل تكنولوجيا الزرع المباشر والغير مباشر باستخدام عدة أوساط زراعية؛ وأخرى للتمييز بين أنواع الفطريات بشكل أكثر تحديدا كالطرق المجهرية. أظهرت النتائج أن *Penicillium expansum* و *Aspergillus niger* و *Alternaria alternata* تمثل غالبية الجنس الفطري الموجود في معظم العينات بينما *Aspergillus flavus* كان الأقل انتشارًا في العينات بنسبة 20٪ في إكليل الجبل و16٪ في الموز ومن ناحية أخرى، أظهر المضاد الفطري *لنبات Matricaria chamomilla* نسبة تثبيط تقدر ب31% لكل من النوعين *penicillium expasum* و *Penicillium italicum* و نسبة % 25لكل من *Trichoderma harzium*

كلمات مفتاحية: الفطريات التي تنتقل عن طريق الغذاء ، والعزلة ، والنشاط المضاد للفطريات. تثبيط العفن ، *Matricaria* ،

Abstract

The primary function of ubiquitous microorganisms like fungi is the recycling of organic material. Their diversity is enormous (there are over 1.5 million species in the entire world)., contamination caused by fungus creates a serious issue for the food and pharmaceutical industries, as well as for the public's health. The aim of our study was to isolate and identify fungi species sampled from a variety of foods, which involve cereals, fruits, vegetables, and medicinal plants to check their tolerance and estimate the antifungal efficacy in vitro using methanolic extract of the aromatic plant *Matricaria chamomilla*. using different type of methods which depend on type of strains in general, such as Direct and the Indirect plating technique using a number of media; other method used in ordre to distinguish between the deferent genera of fungi such as the microscopic method of identification. The results showed that *Penicillium expansum*, *Aspergillus niger*, and *Alternaria alternata* represent the majority of the fungal genus found in most samples while *Aspergillus flavus* is the least prevalent in the samples, with 20% in rosemary and 16% in banana and on the other hand The antifungal activity of the medicinal plant *Matricaria* sp showed a significant inhibition with deferent efficacity of 45% and 31% and for *Penicillium expansum* and *Penicillium italicum* 25% for the tree genera *Trichoderma harzianum Aspergillus flavus* and *Fusarium culmorum*

Key words: foodborne fungi, isolation, antifungal activity. Mold inhibition, *Matricaria*,

Résumé

La première fonction des micro-organismes ubiquitaires tels que les champignons qui ont le recyclage de la matière organique. Leur diversité est énorme (il y a plus de 1,5 million d'espèces dans le monde entier), la contamination causée par les champignons qui crée un problème sérieux pour les industries alimentaires et pharmaceutiques, ainsi que pour la santé publique. L'objectif de notre étude était d'isoler et d'identifier les espèces de champignons à partir d'une variété d'aliments, qui incluent: les céréales, les fruits, les légumes et les plantes médicinales Pour vérifier leur tolérance et estimer l'efficacité antifongique in vitro en utilisant l'extrait méthanolique de la plante aromatique *Matricaria chamomilla* . En utilisant différents types de méthodes qui dépendent du type de souches en général, telles que la technique d'ensemencement direct et indirect utilisant un certain nombre de milieux ; d'autres méthodes utilisées afin de distinguer les différents genres de champignons telles que la méthode d'identification microscopique. Les résultats ont montré que *Penicillium expansum*, *Aspergillus niger* et *Alternaria alternata* représentent la majorité des genres fongiques trouvés dans la plupart des échantillons, alors qu'*Aspergillus flavus* est le moins répandu dans les échantillons, avec 20% de romarin et 16% de banane d'autre part. L'activité antifongique de la plante médicinale *Matricaria chamomilla* a montré une inhibition significative avec une efficacité différentielle de 45% et 31% et pour *Penicillium expansum* et *Penicillium italicum* 25% pour les genres d'arbres *Trichoderma harzianum Aspergillus flavus* et *Fusarium culmorum*

Mots clés : champignons d'origine alimentaire, isolement, activité antifongique, inhibition des moisissures, *Matricaria*,