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Contribution to the study of the effect of fungicides on the growth of fungi with agricultural interest in El-Oued region

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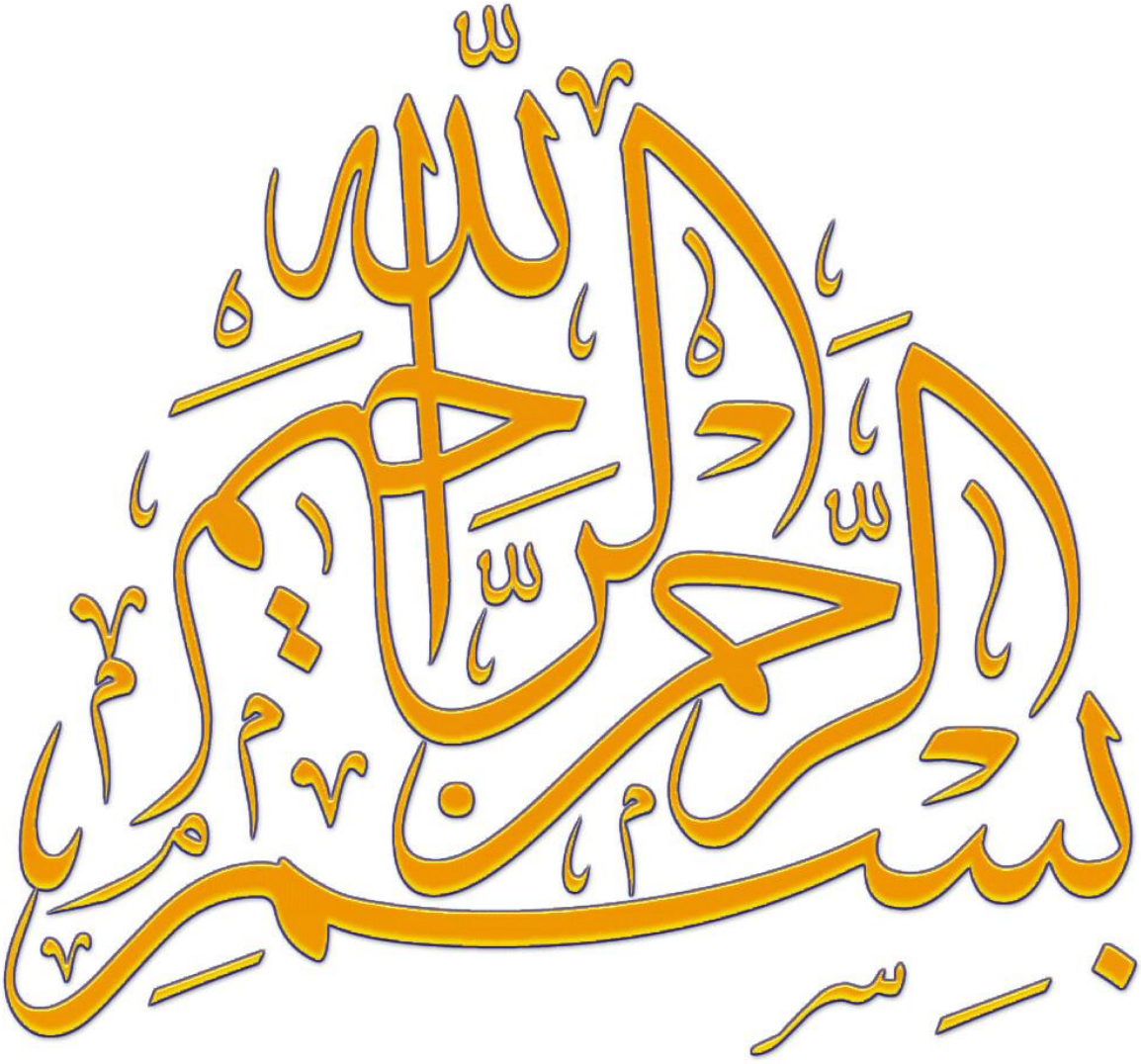
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الإهـــــــــداء

أبي العزيز..

عزي وفخري . . سبب حلمي وسعادتي . . من علمني أن الدنيا كفاح . . سلاحها العلم والمعرفة . . من سعى لأجل راحتي و نجاحي . .

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إخوتي ... سهير ، خولة ، أميرة ..

الكتابة لا تكفي لأصف كيف أحبكم . . والعمر قصير لأكتب حبكم . .

أراكم بسمتي . . وارى جمال الأيام انتم . .

أخي ... الحاج أعماره ..

أبي الثاني . . عوني بعد الله . . احبه فوق حب المحبين حبا فهو سندي . .

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استاذي الفاضل : " عمار العايش التوهامي ". . .

قُمْ للمعلّمِ وَفِّهِ التبجيلا . . كادَ المعلّمُ أن يكونَ رسولا . .

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ضـــو عائـشة

Abstract

The aim of this scientific research is to study the effects of antifungals and their effectiveness to eliminate the widely spread fungi that threaten the products of El-OUED State, especially Alternania, Botrytis, and Rhizoctonia.

We have selected many samples from El-Oued, which are: potatoes, beans, peas, zucchini, salad, carrots, and tomatoes. We have grown it in the media PDA and Sabouraud, and we selected samples that showed results in the first stages of cultivation, which are: potatoes, carrots, tomatoes, and salad.

They contain a variety of fungal species, with fungi in particular: Alternaria, Rhizoctonia, and Botrytis.

We use for this study and in order to prove the effectiveness of the fungicide used and the optimal and least harmful concentration on plants and on human health, later on, the method of diffusion and the method of tablets, so that we conclude later that the most effective of the resistance is the fungal-anti TACHIGAZOLE + VALETTE, followed by TACHIGAZOLE and lastly by VALETTE. The reduction in fungal growth ranges from 50 to 100 %.

We conclude that to reduce the fungi that affect the growth of vegetables and fruits in our agricultural stores, we need means of protection and prevention, with the use of fungicides with the aforementioned notifications and instructions without excessive use of them.

Keywords: fungi, anti-fungal, El-Oued, reproduction, plants.

Résumé

Le but de cette recherche est d'étudier les effets des antifongiques et leur efficacité pour éliminer les champignons largement répandus qui menacent les produits dans la Wilaya .OUED, en particulier Alternania, Botrytis et Rhizoctonia-d'El

Nous avons sélectionné de nombreux échantillons, qui sont : des pommes de terre, des haricots, des petits pois, des courgettes, de la salade, des carottes et des tomates. Nous l'avons cultivé dans les médias PDA et Sabouraud, et nous avons sélectionné des échantillons qui ont montré des résultats dans les premiers stades de culture, à savoir : .pommes de terre, carottes, tomates et salade

Ils contiennent une variété d'espaces fongique , avec des champions en particulier : Alternania, Botrytis, et Rhizoctonia.

Nous utilisons pour cette étude et afin de prouver l'efficacité des médicaments utilisés et la concentration optimale et la moins nocive sur les plantes et sur la santé humaine, par la suite, la méthode de diffusion et la méthode des disques,que nous de sorte concluons plus tard que la le plus efficace des résistances est l'antifongique .TACHIGAZOLE + VALETTE, suivi de TACHIGAZOLE et enfin de VALETTE La réduction de la croissance fongique varie de 50 à 100 % .

ignons qui affectent la croissance des Nous concluons que pour réduire les champ légumes et des fruits dans nos magasins agricoles, nous avons besoin de moyens de protection et de prévention, avec l'utilisation de fongicides avec les notifications et .ci-cessive de ceuxinstructions susmentionnées sans utilisation ex

Oued, reproduction, plante-champignons, antifongique, El **: Mots clés**

الملخص :

الهدف من هذا البحث هو دراسة تأثير المضادات الفطرية و فعاليتها للقضاء على الفطريات المنتشرة بكثرة والتي تهدد منتوجات ولاية الوادي خاصة هي من نوع *Alternania* و *Botrytis* وأيضا *Rhizoctonia* .

لقد إخترنا العديد من العينات المنتقاة من ولاية الوادي ألا و هي : البطاطا ، الفول ، البسباس ، الكوسة ، الخس (السلطة) ، الجزر و الطماطم . و لقد قمنا بزراعتها في الوسطين PDA و Sabouraud ؛ و إنتقينا العينات التي أظهرت نتائج في مراحل الزرع الأولى و هي : البطاطا ، الجزر ، الطماطم والخس (السلطة) .

أنها تحتوي على مجموعة متنوعة من الأنواع الفطرية ، مع الفطريات على وجه الخصوص : *Alternania* و *Botrytis* وأيضا *Rhizoctonia*

نستخدم لهذه الدراسة و لكي نثبت فعالية الأدوية المستخدمة و التركيز الأمثل و الأقل ضررا على النباتات وعلى صحة الإنسان فيما بعد ؛ طريقة الإنتشار و طريقة الأقراص . بحيث نستنتج فيما بعد أن الأكثر فعالية للمقاومة هو مضاد الفطريات تشازول + فاليت و يليها تشازول و آخرها الفاليت ؛ يتراوح الإنخفاض في نمو الفطريات من 50 إلى 100 % .

نستنتج أن للحد من الفطريات التي تؤثر على نمو خضر و الفواكه الخاصة بمحالينا الزراعية نحتاج لسبل الحماية والوقاية لها مع استخدام المبيدات الفطرية بالإعلامات و الإرشادات المذكورة دون الاستخدام الجائر لها .

الكلمات المفتاحية : الفطريات ، مضاد الفطريات ، الوادي ، التكاثر ، النباتات .

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Abbreviation list

% : indicate that the number preceding it should be understood as a proportion multiplied by 100

°C: degrees Celsius.

µL :microliter, unit of volume measurement outside the International System. Symbol for microliter (microlitre), an SI unit of fluid measure equal to 10−6 liters (litres).

ATP : Adenosine triphosphate .

ATPases :Adenosine 5'-TriPhosphatase, adenylpyrophosphatase,

C : C sign up for an anti-fungal that is a mixture of TRACHIGAZOLE + VALETTE.

cm :centimeter

CMI : Concentration Minimal Inhibitrice .

D : Days.

DNA: Deoxyribonucleic acid .

g : grams

L: liter

min: minutes .

ml :Unit of volume equal to one thousandth of a liter

mm:unit of length derived from the meter. thousandth part of a meter.

PDA : Potato-Dextrose-Agar

q.s.p:Quantité suffisante pour

RNA: Ribonucleic acid

T : T sign up for an anti-fungal TRACHIGAZOLE .

um : Unit code.

V : V sign up for an anti-fungal VALETTE .

Summary

INTRODUCTION

Introduction

History is littered with instances serious food shortage caused by plant disease. Yield losses due to disease vary between crops and regions and are often 10–20%. However, under favorable conditions for fungal growth, crop yield may be more severely reduced, threatening food security and the livelihoods of farming communities **(HEWITT, 2000) .**

Plant diseases are caused by both infectious (fungi, bacteria, viruses, and nematodes) and non-infectious (a mineral deficiency, sunburn, etc.) agents. Infectious plant diseases are caused by living organisms that attack and obtain their nutrition from the plant they infect. The parasitic organism that causes the disease is called a pathogen and the plant invaded by the pathogen and serving as its food source is referred to as a host. A favorable environment is critically important for disease development - even the most susceptible plants that are exposed to huge amounts of a pathogen inoculum will not develop the disease unless the environmental conditions are favorable. Fungi account for about 85 percent of plant diseases, followed by viruses, bacteria, and nematodes. Environmental factors are important in the evolution of plant diseases **(ANONYMES 01, 2022) .**

Disease control has been accepted at least since the times of the Greek and Roman Empires and the various ways, some more spiritual than practical, that were then developed to control crop loss laid the foundations of plant protection technology and the search for new and more effective fungicides. The last 40 years, however, have witnessed a revolution in pesticide discovery that is particularly evident in the quest for new modes of fungicide action. **(Anonymes 01 ; 2022).**

Vegetables are the main food item other than cereals in Algeria and around the world. It plays a major role in the world food system. El-Oued State is one of the regions distinguished in the cultivation of vegetables of various kinds (tomatoes, beans, Fennel, zucchini, salad, carrots).

However, these crops are affected by many diseases, whether bacterial, viral, fungal, or even insects. Black Rhizoctonia. Alternaria. Gray mold is considered one of the most common diseases affecting these crops. It is the main pathogen of the subfamily Rhizoctonia; Botrytis; dual.) which spreads through the soil and infects tubers, stems, fruits, roots, and leaves.

Identify fungal diseases correctly before selecting anti-fungal fungal pathogens require different anti-fungal, so taxonomy is important! anti-fungal can be first applied when the fungus is at very low levels. This gives the most effective control. It is very difficult to control a foliar fungal disease when it is well established **(HEWITT, 2000)**

Fungal pathogens can develop resistance to some anti-fungal, rendering them ineffective. It is important to minimize the risk of the development of resistant strains by minimizing the quantity per season of a anti-fungal. Since fungal diseases have become one of the major problems facing the world, fungi are evolving much faster than the rate of development of new fungicides and antifungals.

The aim objective of our work is to determine the optimal and effective concentration to eliminate the fungi and be less harmful and affect us later.

In this work we will discuss two chapters:

- first chapter in which we will talk about what fungi are, their way of living, their reproduction, and their life chain, and we will mention some of the most prevalent species in our region.

- Chapter Two is about the fungicides we discussed in Chapter One, how to eliminate them or prevent the possibility of infection and how to stop their effect.

✓ In our research, we will discuss the validity of some hypotheses, which are:

1- What are the most effective and most commonly used medicines to eliminate fungi?

2- What is its effectiveness? And what is the ideal concentration to use?

Chapter I: Fungi

I – Generalities of fungi

I - 1 - Definition

Fungi are a group of eukaryotic unicellular (yeasts) or multicellular filamentous (filamentous fungi or mould) microorganisms **(BOUDERAOUNE, 2013).**That are heterotrophic by absorption that may take the form of reproduction, parasitism or symbiosis **(NARSAOUI et LEPOIVER , 2003)**

Fungi, also called mycetes, are uni- or multicellular eukaryotic organisms, including macroscopic species (macro fungi) and other microscopic (micromycetes) **(CHABASSE *et al.,* 2002).** But on the contrary form a very heterogeneous group whose common essential characteristic is heterotrophic nutrition by absorption, which can take the form of saprophytism, parasitism or symbiosis **(ABDELKADER, 2012).**

Fungi are characterized by chitinous walls, devoid of chlorophyll pigments, they are heterotrophic **(FANIT *et al.*,2008) .**

The number of fungal species ranges from 60 to 100 thousand. They are found everywhere in our environment. Most are phytopathogenic and develop as plants shed in the soil and on plants or plant debris during the rotting process, and are found in the air as well as on land and surfaces, in food and sometimes in water **(MEGHAZI, 2015).**

I - 1 - 1 - Parasitic fungi: Parasites that are present in all groups of fungi, some of which are plant parasites, all regular groups of which can be attacked at the level of their different members, whether in the air, in the soil or in the aquatic environment, fresh or marine water **(LAGGOUNE et *al* , 2012) .**

I - 1 - 2 - Saprophytic (free-living) fungi: They participate in the processes of decomposition of organic matter, immobilization of mineral elements and establish neutral interactions with the plant **(KLEIN and PASCHKE, 2004).**

I - 1 - 3 - Phytopathogenic fungi: They establish antagonistic interactions with plants **(VANDER, 2003).**

Fungi are characterized by chitinous walls, devoid of chlorophyll pigments, they are heterotrophic, fungi grow by a system of hyphae all of this hyphae form the hyphae or fungi, or yeast cells **(FANIT *et al.*,2008) .**

I - 2 - Fungal cell structure

I - 2 - 1 - fungal cell wall

The fungal cell wall is the external organelle, which protects the plasma membrane and cell compartment from mechanical shock, environmental stresses and the host's immune system. It is in direct contact with the environment and thus plays an important role at the interface between the cell and its environment. The fungal cell wall is viewed as a structurally rigid envelope that determines the morphology of the cell, which can nevertheless adapt and change its composition depending on external factors. Indeed the cell wall is constantly being reshaped to be mechano-resistant, and it is a highly dynamic structure with great flexibility. The cell wall is essential for fungal survival, morphogenesis, and pathogenesis; This structure is one of the few targets of antifungal therapy **(KUKHALEISHVILI, 2021) .**

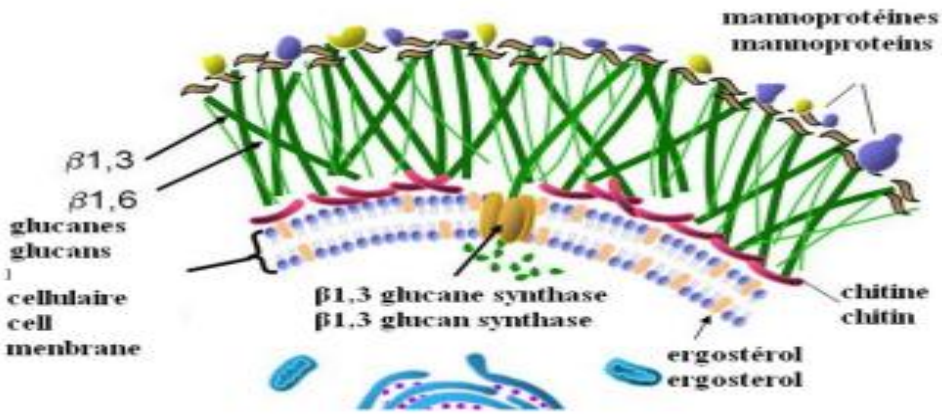


Figure 01: composition of the fungal cell membrane and cell wall(F. LAGROUH, 2017) .

I - 2 - 2 – fungal cell nucleus

The nucleus is in fungi with eukaryotes. The only notable differences with other organisms and the more complex are its small size in the fungi, the small amount of DNA and histones, and certain details of meiosis **(BOIRON, 1996).**

I - 2 - 3 - Mitochondria

Mitochondria exist in the cytoplasm of fungal cells. They appear circular, oval or elongated, but are often branched. Their size, shape and number may vary during the cell cycle and in response to environmental conditions. Fungal cells may contain few highly branched mitochondria or many, more than twenty, small unbranched mitochondria. Each mitochondrion has a smooth outer membrane and an inner membrane that extends into ridges that penetrate the matrix. The tricarboxylic acid cycle takes place in the matrix while the transport of electrons and the production of ATP take place at the ridges. **(NASRAOUI, 2015)**

I - 2 - 4 - Plasma Membrane

Fungi have a plasma membrane similar to that of other eukaryotes, and it is made up of a bilayer of phospholipids, proteins, and sterols associated with them. However, the main membrane sterol in fungi is ergosterol and not cholesterol as in animals and phytosterols like cholesterol as in plants **(GHODBANE et RAHMANI , 2020).**

I - 3- Fungi nutrition

In order to grow, fungi require nutrients that are in direct contact with the environment. The fungus can absorb nutrients that are small molecules, such as simple sugars and amino acids dissolved in the hydrophilic membrane surrounding the hyphae. On the other hand, nutrients made up of large insoluble polymers, such as cellulose, starch, and proteins must first be disassembled before they can be used. This digestion is carried out by extracellular enzymes that control hydrolysis reactions that break down large molecules into simpler components. The complete decomposition of large polymers into simple soluble molecules is a process in which various enzymes outside the cell are involved. Once the single-molecule is absorbed into the cell, it passes under the influence of intracellular enzymes **(NASRAWI, 2015).**

I - 4- Fungi ecology

Fungi are found in almost all regions and climates of the world with the ability to adapt to environments There are different types, and each type has a characteristic ; fungi are said to be saprophytic It breaks down and recycles organic matter from plant residues

mycorrhizal or Symbiotic fungi, It is also common in fungi and plants some fungi are phytopathogenic (causing hidden diseases in plants) **(ACHOUB et FRIKHA, 2011).**

The life of phytopathogenic fungi depends on the host and their share in debris, plants and soil. In the facultative parasite which grows parasitically on the host, but continues its life near the dead tissues of the host, or the other type is that the net parasites are dependent on the host plant, and the spores spread at the end of their life cycle in the soil where they die or remain inactive. Dead plants where they live or die **(ACHOUB and *al* , 2011) .**

I - 5 - Classification of fungi

Advances in analytical and molecular methods have allowed scientists to classify fungi primarily based on their biochemical properties: primary metabolites, secondary metabolites, and symantides that carry genetic information **.**

The preceding characters put the fungi into six divisions: Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota, Basidiomycota and Deuteromycota **(FIN *et al.*, 2010).**

I - 5 - 1 - Zygomycota: the filamentous thallus also called hyphae, they are non-partitioned, flagellate cells absent, reproduction, sexual by way of cystogamy (gametangial copulation), from which results a sexual spore "<zygospore" from which is produces the sporangium **(ABDICHE, 2008)**

I - 5 - 2 – Deuteromycota : (Imperfect Fungi) This division includes all species that reproduce asexually. Recent data based on electron microscopy on the one hand, and molecular biology on the other hand, make it possible to establish a close association with basal fungi. Fungi knew only their asexual phase **(STRUMIA *et al.*, 2021).**

I - 5- 3- Ascomycotin : these fungi, with divided thalli or yeasts, have a particular structure called ascus, a specific sporangium that forms during sexual reproduction. Ascos usually contain a definite number of spores or ascospores formed after the fusion of two nuclei followed by meiosis. It can be spherical, cylindrical or more or less than the collarbone **(AISSI, 2019) .**

I - 5- 4 - Basidiomycota: They are characterized by the production of sexual spores, called basal spores, which form by budding at the apex of elongated cells, the basal ones. Basal fungi have a divided thallus with "rings" at the septal level **(AISSI, 2019) .**

I - 5- 5- Chytridiomycota : is the oldest strain of the previous five, unlike other members of the kingdom, it is non-vegetative, motile and asexual (animal) spores. Along with other fungi that produce zoospores in open water in soil, they have short life cycles and drought-tolerant resting stages, making them well suited for living in terrestrial habitats. **(LONGCORE, 2006)**

I -5 - 6 – Glomeromycota: They currently comprise approximately 150 species, Glornyromycetes produce (relatively large spores of 40-800um). it is assumed that they are formed asexually since there is no evidence that glornomycetes reproduce sexually **(BOUALI, 2016)**

I - 6 - Reproduction in fungi

Reproduction is the biological process by which new organisms are produced, it is the basic characteristic of every living thing that exists in life .Fungi reproduce sexually and/or asexually. In the perfect fungi sexually and asexually, while in the imperfect fungi (by mitosis) **(PUISSANT *et al.*, 2021).**

I - 6 - 1 - Asexual reproduction

Asexual reproduction, it is more widespread, does not involve genetic transformation, and plays a major role in the spread of species. This is done either by fragmentation of the stalk or by the production of asexual spores **(BRANGER *et al.*, 2007)**

Asexual reproduction occurs without the fusion of gametes. This is a common reproductive condition for almost all fungi. Asexual reproduction in mushrooms may appear by budding, binary fission, fragmentation or spore formation (**IHSAN et *al* , 2013).**

- **Budding and binary fission :** are the simplest forms of asexual reproduction. Budding and uneven division of the cytoplasm results in a parent cell and a daughter cell, these being smaller than the parent cell. Binary fission, on the other hand, results in two identical cells **(NEH *et al.*, 2021)**

- **The fragment and the sporulation:** Fragmentation is a form of asexual reproduction where a new organism develops from a parent fragment. Sporulation is the most asexual form of reproduction in fungi. It is done through the asexual spores **(CLÉMENCE et DONGMO , 2009).**

I - 6 - 2 - Sexual reproduction

Fungi can reproduce sexually by fusing their filaments together in an interconnected network called an anastomosis. Sexual reproduction begins when the haploid filaments of fungal organisms meet and join. Although the cytoplasm of each organism fuses sexually, in which the nuclei fuse into a single nucleus**.** The cell then undergoes meiosis to form haploid spores and the cycle repeats. Some fungi do not have a diploid stage except for the sexual sporangium, while others have completely lost the ability to reproduce sexually **(PUISSANT *et al.*, 2021).**

I - 7 - Phytopathogenic soil fungi

There are several genera of soil fungi capable of infecting the roots of wild and cultivated plants and causing serious damage. These include Aspergillus, Penicillium, Fusarium, Rhizoctonia, Alternaria, Pythium, Verticillium. All these microorganisms cause diseases in various vegetable crops, cereals, plants **(HAMMANI and SAADA ; 2020) .**

Damping off is a set of diseases that are widely. They are found in forests, fields and greenhouses. The host range is also very wide. The disease affects plants of all age categories. However, the greatest damage is observed at the level of seedlings and germinating seeds **(HAMMANI and SAADA ; 2020) .**

According to Perrin, 1988, damping off is a universally known cryptogamic event. The attack corresponds to an alteration of the tissues of the collar of the seedling accompanied by a shrinkage in diameter. The seedling droops as if sheared at the base, then withers. In the open ground, the disease progresses step by step, forming spots of mortality characteristic of the damping-off **(ABDELKADER, 2012).**

The latter caused by pseudo fungi of the genus Pythium which live as saprophytes in the soil behave as parasites of seedlings or young seedlings when conditions are unfavorable. Some fungi such as Phytophthora, Rhizoctonia and Fusarium cause symptoms

similar to those caused by Pythium. These fungi can also cause rotting diseases of fruits growing on the soil surface **(ABDELKADER - 2012) .**

I - 7- 1 - The genus Alternaria : The gender of Alternaria includes more than 100 species spread everywhere on a very large scale in the soil, vegetation, air or food while some species live in the air case that can sometimes be the causes of opportunistic diseases, they are generally found on the seeds, causing the loss of emergence or Pressing. The affected small buds are an important source **(HAMMANI, SAADA , 2020)**

I - 7- 2 - The genus Fusarium : The genus includes incomplete fungi. The genus includes approximately 40 widely distributed species. Species of the genus Fusarium can attack cereals (corn, wheat, barley, and oats), vegetables, ornamentals, and many fruit trees. This type of disease also occurs in many cultivated plants, such as vegetables and tropical crops in the form of wilt and rot, which are caused by the fungus *F. oxysportum* **(N HAMMANI, S SAADA - 2020)**

I - 7 - 3 - The genus Pythium : The name Pythium is given to the genus of microorganisms classified within the uromycetes. They are fungi that cause economically important plant diseases such as wheat. Pythium is more dangerous as the same species can attack a large number of crops. Most of the places it infects are concentrated in germinating seeds, young seedlings and fresh tissues and causing them to rot. Symptoms of pythioses vary, but most of them subside. **(ABDELKADER - 2012) and (N HAMMANI, S SAADA - 2020) .**

II - Methods of combating phytopathogenic fungi

Many alternative methods of control have been studied and implemented. Used alone or in combination, these methods have been very successful, however, they require a good knowledge of the parasite to find the weak points of its biological cycle.

II - 1 -Chemical control

Chemical struggle: Chemical treatments are widely used to combat fungal diseases and bacteria. To combat plant pathogens, the most used products are fungicides (non-systemic, contact or systemic, penetrating) **(LEPOIVRE, 2003).**

II - 2 - Biological control

Biological control has an important role in phytopathological control than the use of chemical fungicides (an alternative way to the use of chemical products which constitutes a danger for humans and is more interesting on the environmental and economic level). It is a method that consists in using the biological capacities of a living organism (a control auxiliary, which can be predatory or parasitic such as insects, nematodes, etc.) in order to limit, stop or inhibit the development of "another living organism without using pesticides with several control mechanisms: antibiosis, competition, parasitism **(JOURDHEUIL *et al.*, 1991).**

II - 3 - physical struggle

It is with soil disinfection with steam which consists of passing a stream of water vapor over the ground, The steam comes from a fuel generator and is distributed in the ground by perforated pipes, on the surface under a tarpaulin or a bell box. The duration of exposure varies according to the parasites targeted, the depth to be disinfected, and the type of soil or solarization of the soil obtained by raising the temperature of the soil thanks to solar energy, covering the soil with a transparent plastic that captures the energy and accumulates heat (solarization can modify the physical and chemical characteristics of the soil, indirectly disfavoring certain pathogens **(CHRISTIAN *et al.*, 2007).**

II - 4 - Cultural control methods

These techniques reduce the risk of diseases before or during implantation by eliminating plant regrowth, eliminating weeds

- **s AlternariaGenu**

Alternaria is a common fungus in our environment. It can affect field crops or plant products during harvest and post-harvest **(LOGRIECO et al., 2009).** The success of infection is related to the aging of leaves and plants, in addition to some climatic conditions. The disease is easily recognizable by the close concentric circles that form inside the spots. Hot and humid weather exacerbates diseases that can lead to death **(MUNRO, 1975)**. It is a fungal disease that can infect leaves, stems, and, in severe cases, fruits. **(BATISTA *et al.*, 2006).**

Alternaria is usually controlled by the use of fungicides that fight mildew **(UNECE, 2014**). Fungicides are commonly used to control leaf blight and consist of protective products **(BLANCARD *et al.*, 2012).** Work was carried out on Bacillus spp. In addition to pathogen-fighting agents in harvested and stored products, bacillus-based products are commonly used in agriculture as bio-pesticides, fungicides, and plant growth stimulants for storage and harvesting **(SHARMA *et al.*, 2009).**

Causative agents Alternariaspp. This disease is ubiquitous and can affect all parts of the plant, including leaves, stems, crowns, fruits, and even seeds. It can appear at different stages of crop growth (seedlings and adult plants) **(FERRAG and GUERIOUNE, 2018).**

- **Genus Rhizoctonia**

Rhizoctonia is an ubiquitous fungus that infects many cultures and organs. These foliar symptoms mainly appear when watering is intensified during periods of extreme heat his disease also appears mainly on organs that come into during the months of the year. T contact with moist soil and occurs when the foliage is dense and the plants are not **DAUGHTREY, 2006)** sufficiently spaced **; ،DREISTADT2001).**

f blade. Sometimes there i Basal leaves are affected first and appear red in the lea the formation of a fungal web that takes the form of a spider web. Sometimes they are .rotten and covered with brown mycelium To control it, we must ensure that aerial roots too dense, ensuring regular watering, reduce air circulation by avoiding seedlings that are **DAUGHTREY,)** groups of plants that are too wide and narrow, as well as excess leaves **2006 ; ،DREISTADT2001).**

- **Genus Botrytis**

Identification of botrytis blight often involves brown spots on leaves and buds or darker spots on flower petals. As the disease progresses, flowers and fruits rot, and spots begin to show fuzzy gray mold. One such fungal infection where you eventually see the causative fungus, not just the symptoms of the underlying disease, Masses of gray, dust-like fungal spores develop on dead and dying plant tissues where they can be easily seen.

How to control it Once botrytis blight becomes active, it becomes more difficult to control. Immediate action is critical. This disease often enters plants through small wounds and moves quickly once it has started. Prophylactic application of a fungicide early in the

season helps prevent Botrytis blight infections and stops their spread. Treat them at the first hint of symptoms. GardenTech's highly effective Daconil fungicides provide 3-way protection to stop, control, and prevent Botrytis blight and more than 65 fungal diseases. Last, these products can process tomatoes until harvest day or process beans up to seven days before harvest time. This ready-to-use pesticide provides the perfect choice for treating containers, individual plants, or small garden spaces. Shake the sprayer container and it is ready. Spray all the top and bottom surfaces of the plant until completely wet. **(ANONYME,02.2022)**

Chapter II: Anti-fungal

I - Anti-fungal definition

A fungicide is a natural product whose role is to kill, limit, or prevent the development of plant-parasitic fungi. Organic fungicides, however, prove to be better in a preventive role, which must be essential, and their curative capacity is limited to recently and slightly affected areas. In addition, curative treatments based on sulfur or copper remain toxic elements whose use has an impact on the balance of the soil, which must therefore be avoided as much as possible **(CORENTIN, 2010**). Some fungicides only inhibit fungi. (fungistats) rather than killing them (fungicide). Fungicides must be applied continuously throughout the life of the plant to suppress disease development. For example, mefenoxam will not prevent zoospores from entering roots but will only inhibit established Phytophthora infection **(PSCHEIDT, 2021)** .There are three main reasons why fungicides are used:

(a) Disease control during crop development and establishment.

(b) Increases the productivity of a crop and reduces defects

(c) Increase the shelf life and quality of harvested plants and products.

II - Types of fungicides

II - 1 - Systemic fungicide

Systemic fungicides are absorbed through the plant cuticle and underlying tissues and can work by killing spores and hyphae as well as incipient infections where the fungus has penetrated the plant surface. When they stop infections and prevent symptoms from developing, they are called "healing."

However, the symptoms already present will not be "cured" by the fungicide in question. After symptoms appear, some fungicides can reduce or inhibit fungal sporulation; they are called "anti-sporulate". The term "eradicates" is often used for products like lime sulfur, which kills overwintering fungal structures in woody plant tissues when applied as a dormant spray. However, eradicators rarely remove all of the overwintering inoculums. Sometimes people use the term "eradication" for highly effective fungicides (e.g., Ridomil) that prevent current season infections to the point that the disease appears to have been eradicated. The term translaminar refers to the movement of a fungicide from one side of

the leaf to the other, providing disease control on both sides of the leaf **(Annemiek Schilde, 2010).**

II - 2 - Protective fungicides

Protectant fungicides are fungicides that are applied to protect plants from potential infection. This means that the plants are healthy when the fungicide is applied and that the application is done to prevent potential infection. This is usually done when a certain plant disease is known to attack the plants at a certain time of the year, and the correct timing of the fungicide will then prevent that fungus from attacking the plants. As with humans, prevention is better than cure, and in general, this works well when fungicides are applied preventively **(POTTER, 2021).**

II - 3 - Treatment fungicides

Treatment fungicides, which are generally applied after the first symptoms of plant disease have been noticed, are used to eradicate the fungus from the plant. Because the plant is already infected, it is sometimes more difficult to eliminate diseases with treatment fungicides, especially when disease symptoms are advanced **(POTTER, 2021).**

II - 4 - Fungicides used in contact

contact fungicides and also better activity because they can move from where they settle on the upper surface of the leaves, where pathogens often grow best **(MCGRATH, 2020).**

III - Resistance to fungicides

Fungicide resistance is a form of selection that describes the ability of a fungus to survive and reproduce in the presence of a fungicide **(BECKERMANN, 2013).**

- There are several ways to avoid a build-up of resistance by a fungus or reduce the risk.
- Use of fungicides in conjunction with non-chemical methods to reduce disease risk.
- Avoid growing large areas of highly susceptible varieties in areas where disease incidence is generally high.

- Whenever possible, use fungicides with different modes of action. (i.e., from different groups) when several are to be used on the same crop.
- Use approved tank mixes of fungicides with different modes of action rather than always relying on single fungicides.
- Apply fungicides only when necessary; use disease predictions and thresholds to avoid unnecessary treatment **(FINCH *et al.*, 2002).**

IV- Characteristics of fungicides

The important particularity is the very high specific activity with respect to its target:

1. Preventive: inhibits the growth of germ tubes of the fungus, which can no longer penetrate the sheet.
2. The Curative is the encapsulation of the haustoria by the plant itself. The haustoria lose their function of nourishing the fungus on the surface of the leaf.

IV- 1 - Fungicide Chemical Families or Groups

Carbamates: they can be subdivided into derivatives of carbamic acid and dithiocarbamic acid. The first are systemic fungicides, essentially grouping together benzimidazoles, and the second are contact fungicides.

IV - 2 - Carbamic Acid Derivatives

IV - 2 - 1 - Benzimidazoles: Azoles such as propiconazoles, cyproconazoles, and flusilazoles.

IV - 2 - 2 - Carbendazims: They are fed by the green organs but also by the roots of the plants and are conveyed by the current of the raw sap.

IV - 2 - 3 - Thiophanate-methyl: It breaks down into carbendazim if stored too long, with a very similar mode of action. It is a very important group that is derived from dithiocarbamic acid and has a mode of action that makes it very phytotoxic **(SIMON *et al.*, 1994; LEROUX 2003)**

IV - 3 - Behavior of fungicides at the plant level(Plant-level fungicide behavior)

Fungicides can be divided into three main categories depending on their behavior at the plant level: contact, penetrating, or systemic:

- ✓ **Contact or surface products:** they have antifungal activity bound exclusively to the part at the level of the external barriers of plants (the cuticle of the aerial parts) and are not damaged. As for the internal transfers, they cannot cross the epidermal barrier remaining on the surface of the plant.
- ✓ **Permeable:** After a limited translocation of plants (without displacement of the xylem or bark), these products are likely to inactivate a parasite present in the tissues of the plant, and this property is the origin of their therapeutic activity against fungal parasites. They penetrate inside the plant without further transportation.
- ✓ **Systemic products:** after transfer to the vascular system through the xylem and/or phloem, they can inhibit the parasite present in the treated area. Similar to penetrating leaf uptake, a passive diffusion phenomenon is defined as the movement of chemicals from the leaf surface through the epidermis into the plant interior **(COUVREUR, 2002).**

V - Mode of action

The mode of action, indicating the physiological process of the fungus that is inactivated by the fungicide, is indicated by a letter. The group describes the target site of a fungicide in a fungus. Fungicides may have the same mode of action but different target sites**(ANONYME ; 03 . 2022) .**

Table 01 : Anti-fungal mechanisms of action (LAGROUH, 2017)

Inhibition of cell wall formation	The fungal cell wall primarily consists of b-glucans and chitin. If the synthesis of these compounds is inhibited, the cell wall integrity will disrupt .
Cell membrane disruption	The ergosterols are essential for the cell membrane. If these sterols are bound by anti-fungal drugs, or the synthesis of them are inhibited by ergosterol biosynthesis inhibitors, the cell membrane’s integrity will disrupt. Thereby the membrane becomes leaky

Dysfunction of the fungal mitochondria	Inhibition of the mitochondrial electron transport will result in reduction in mitochondrial membrane potential. The inhibition can occur via inhibition of the proton pumps in the respiratory chain, leading to reduction in ATP production and subsequent cell death
Inhibition of cell division	Inhibition of cell division can happen via inhibition of microtubule polymerization, and thereby inhibiting the formation of the mitotic spindle
Inhibition of RNA/DNA synthesis or protein synthesis	If the antifungal agent enters the cell, for instance via active transport on ATPases, and interferes with the RNA, it can cause faulty RNA synthesis and inhibition of DNA transcription. Inhibition of protein synthesis is also a known antifungal target
Inhibition of efflux pumps	Efflux pumps are presentin all living cells and their function is to transport toxic substances out of the cell . This transport often includes transport of accumulated drug out of the fungal cell. Over expression of efflux pumps can lead to drug-resistance. By inhibiting the efflux pumps it is believe d that drug resistance can be reduced

Chapter I: Material & Methods

I - Study type and location

This is a 4-month pilot study, from February 10 to April 24, 2022, conducted inside the equipped toxicology lab of the Faculty of Natural and Biological Sciences at the University of El-Chahid Hamma Lakhdar El-OUED University.

I - 1 - The purpose of the study

- ✓ Knowing the Proper Fungicide Concentration.
- ✓ Know the effectiveness of the fungicide on fungi.
- ✓ Possibility of having fungicide resistance.

II - Plant material

Samples were taken from different places in El-Oued State. This sample is taken from different cities; we take it from the market, these plants.

Figure 02 : Samples (original photo, 2022)

II - 2 - Laboratory equipment

Table 02 : Materials used in our study

Laboratory equipment and glassware*	Culture media and reagents	Plant material
Benzene spout ; analytical balance pressure furnace; sterile cotton test tube 500ml; conical flask steam room; Laminar flow hood blade and lamella. Micropipette 5 to 50 µL, 1000 µL; optical microscope; filter paper; clamp. stove; cooling; spatula; Tube rack cups. petri dishes; Graduated test tube rinse reservoir.	PDA medium (Agar Dextrose Potatoes); Middle Sabouraud; Sterile distilled water; methyl blue .	potatoes, zucchini, tomatoes, peas, carrots, salad, and beans.

II - 3 - Study Methods

II - 2 - 1 - Preparation of the implant culture

Many media can be used for growing fungi, some are very specific to a group of species others allow the cultivation of very many species which presents a problem of adaptability of the strains on the cultural media, since some strains have preferences for one or another specific medium.

- **PDA (Potato-Dextrose-Agar) medium**

Composition : Potato 200g; Glucose 20g; Agar 20 g and Distilled water complete up to 1000 ml **(LABIOD and CHAIBRAS, 2015)**

- **Sabouraud-agar medium**

It is a commonly used medium for the enumeration and cultivation of fungi.

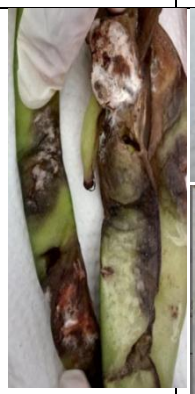
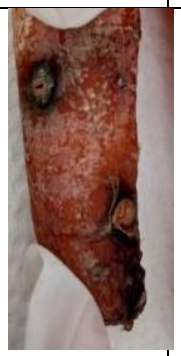
Composition : Glucose 30g; Peptone 10g; Agar 20g and Water q.s.p 1000ml **(BOUKHNISSA *et al.*, 2011)**

We brought 06 host vials, put them in autoclave for 45 minutes, then put them in a five-minute open pack. We pour a huge 05cm of implants into the petri dish and show you to dry for 20 minutes until it is Agar, then we close the Petri dishes and put them in the refrigerator for 24 hours.

Figure 03 : Implant culture preparation (original photo, 2022)

II - 2 - 2 - Sow the infected specimens in a growing medium

We got seven different samples: potatoes, zucchini, tomatoes, peas, carrots, salad, and beans, as shown in the following **table 03** is :

The plants	Green Bean	Fennel *Anisosciadium*	Carrots	Salad	Tomatoes	Potato	Zucchini *Cucurbita pepo*
The location of the fungal disease and planting medium	Fruit PDA	Stem and root PDA Sabouraud	Fruit PDA Sabouraud	Leaves PDA	Fruit PDA	Fruit ; every tuber PDA Sabouraud	Fruit PDA
Plant picture							

II - 2 - 3 - Isolation of fungal strains

We sterilize Bunsen burner and start by taking 03 pieces and transplanting them into petri dishes using a PDA and Sabouraud medium in a triangle shape. We close it and put it in the incubator for 5 days at a temperature (27 ° C - 30 ° C) until we obtain pure fungal strains. **(LAOUID and NEFTIA, 2007)**

Figure 04 :Fungal isolation (original photo, 2022)

Seeding is always done in the sterile zone of the Bunsen burner. On the inoculated dishes, the culture medium and the name of the diseases are indicated **(BOUGHACHICHE *et al.*, 2005).**

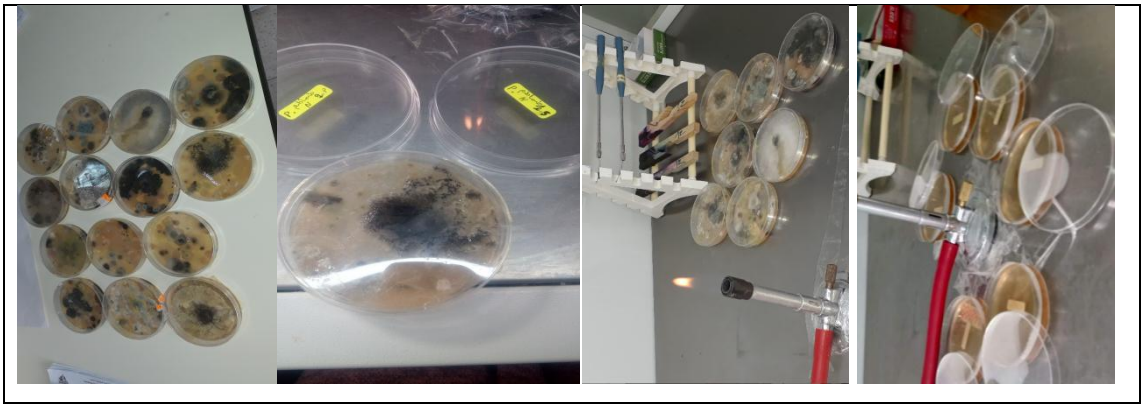


Figure 05 : Seeding technique adopted (original photo, 2022)

The inoculated boxes are incubated in an oven at a temperature of 27°c to 30°c for 05 days until the appearance of good development of the fungi.

II - 2 - 4 - Macroscopic and microscopic observation

We took the Petri dishes from the incubator and read them with the naked eye in the host around the Bunsen burner, identifying the Petri dishes where the mushrooms appeared most clearly. Both samples contained a Petri dish containing PDA and Sabouraud implants.

A portion of the infected specimen (from each PDA petri dish for all specimens, as well as Sabouraud for all specimens) was removed by sterile forceps and a knife in an autoclave according to the fungi to be taken **(BOURGEOIS et LEVEAU, 1980).**

II - 2 - 5 - Transplanting

The method used to evaluate the antifungal activity of the extracts is the method of dilution in an agar medium. The extract to be tested is incorporated into the agar medium and then an actively growing mycelial disc is placed in the center of the Petri dish **(WILKINSON, 2006).**

After the good development of the colony, subcultures are performed for each colony to purify the fungi and reduce the risk of contamination, until a single colony of a specific fungus is isolated in each Petri dish.

This piece is placed in the center of a new box in which the date of transplantation and the coordinates of the collection box are indicated. The transplant is performed aseptically near the Bunsen burner.

Where the transplant process is carried out by bringing a knife and sterile forceps in an Autoclave, by removing the colony to be transplanted by cutting the middle around it and carrying it with sterile forceps, and planting it in the center of the center so that the fungi touch the surface of the medium in which the transplant is done. then closed the Petri dishes and placed them in the incubator for 04 days at 27°C-30°C **(GUIRAUD, 1998).**

II - 2 - 6 - Preservation of stains

To promote viability, and limit the possibilities of variation of the isolated molds, conservation is carried out on slanted agar.

After purification and obtaining pure cultures, the isolates obtained were sub-cultured into test tubes containing the inclined PDA medium. Cultures were stored at 28°C for one week and then stored at 4°C. Sub-culturing and storage of isolates are repeated every three months **(BOTTON, 1990).**

II - 3 - Study of effect of fungicide doses

II - 3 - 1 - Disk method

Sterile 5 mm Whatman paper is infused into the supernatant for each fungicide used. Then, the discs are placed on the agar surface of a Petri dish containing cultured fungi **(HWANHLEM et al., 2011)**.

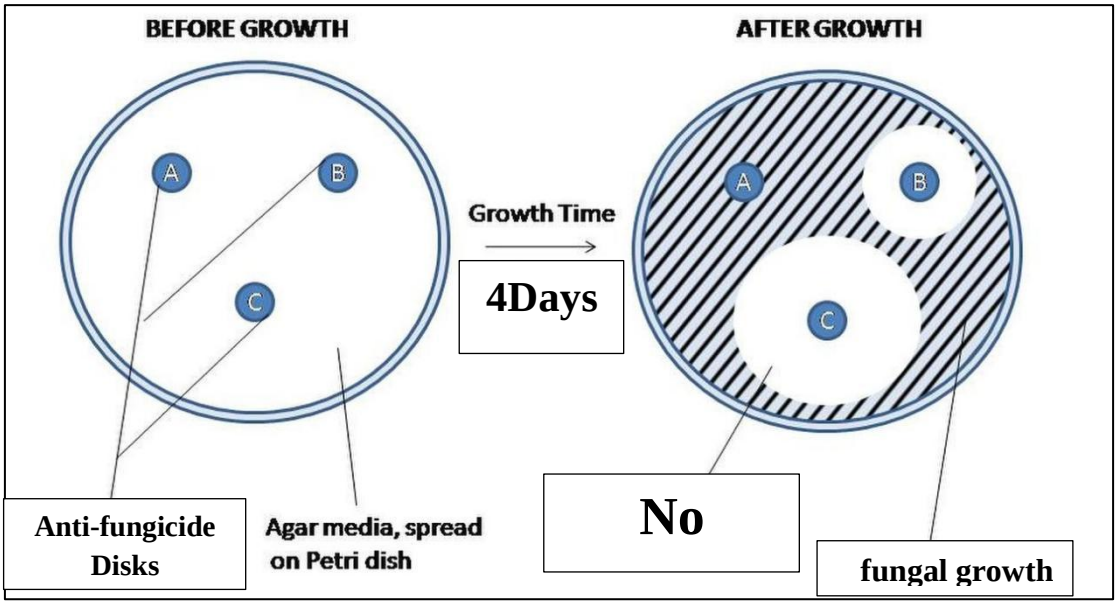


Figure 06 : Method for studying targeted anti-fungal activity by disc method**(Anonyme 04 , 2022).**

II - 3 - 2 - Diffusion methods

We cultured fungal strains at different concentrations (D1, D2, D3) of fungicide such that it was D1 > D2 > D3.

We started the cultivation process on Monday, April 22, 2022. We tracked the resistance stage of the fungus and its ability to multiply and grow in the treated medium for 7 consecutive days at a temperature of (30-32°C) degrees Celsius.

So that :

D1=> V: 4g / 10 ml

=> T: 1 ml / 100 ml

=> C: 5 ml (V) + 5 ml (T)

D2=> V: 4 g / 20 ml

=> T: 1 ml / 200 ml

=> C: 10 ml (V) + 10 ml (T)

D3=> V: 4g / 40 ml

=> T: 0.5 ml / 200 ml

=> C: 10 ml (V) + 10 ml (T)

V : V sign up for an anti-fungal VALETTE .

T : T sign up for an anti-fungal TRACHIGAZOLE .

C : C sign up for an anti-fungal that is a mixture of TRACHIGAZOLE + VALETTE.

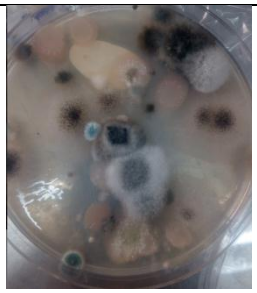
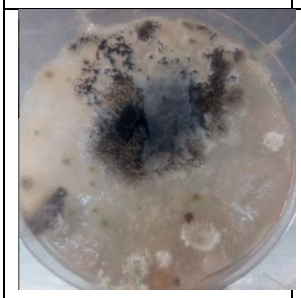
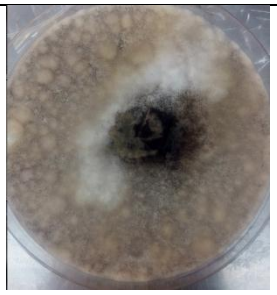
Chapter II : Results & Discussion

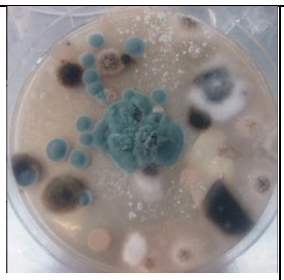
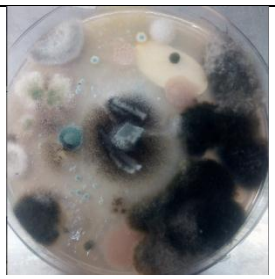
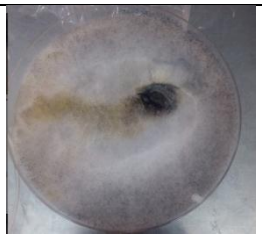
I - Pre identification of fungal strains

I - A - Macroscopic observation

The result of the macroscopic observation are presented in the following table

Table 04 : Identification of selected strains ;

The Plants	Planting medium	Shape of the samples	Sample color	Noticing
Salad	PDA		Black	Large black spots, the presence of green spots, and spores.
Tomatoes	PDA		Green	Black spots, dark green, and light green spots with spores.
			Black	Many black spots with little green spots with spores.
Potato	Sabouraud		Black	Black spots with spores spread all over.

	PDA		Green	Green spots in the center and black spots and germs around.
Carrots	PDA		Green	Some spots of dark green color with spores on each other with a large number of black dots.
	Sabouraud		Black	Black spots and spores are spread over them.

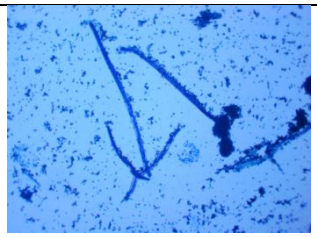
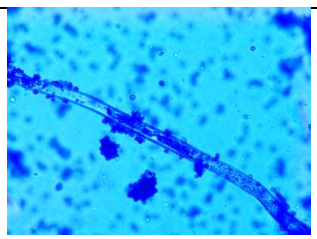
- **For Salad:** The emergence of large black spots in most of the center, which begins to spread from the center due to cultivation in the center (the box) with green spots interspersed on one side with spores.
- **For Tomatoes :** The appearance of black spots is distributed in the center with the presence of spores on some of them. The appearance of dark green and light green spots with spores around them. Black fungi is : Many black spots appear in the center with a few green spots with spores in the middle.
- **For Potato :** The appearance of black spots in the center due to planting in the center, with spores around it distributed throughout the center. Noticeably green spots appear in the middle of planting. There are black and white spots, with only germs around the black spots.
- **For Carrots :** Black spots appear in the center with spores on them scattered all over the center. The appearance of some spots of dark green color with the presence of spores on some green spots. A large number of black dots appear in the middle.

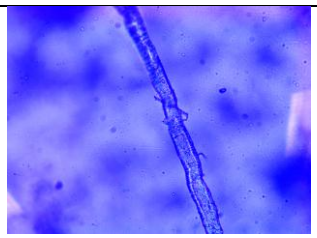
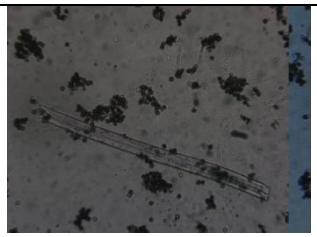
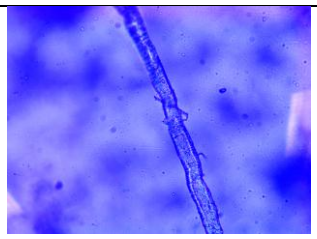
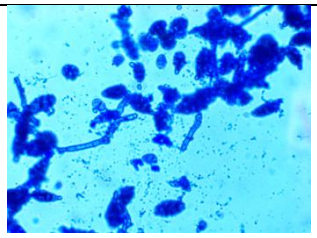
I - B - Microscopic observation

Fungi identification requires observation under a light microscope based on microscopic identification criteria and when they can be identified. For this, using a sterile platinum ring, we superficially take a portion of the culture placed on a slide. The swab prepared in this way is then smeared with lactophenol blue or lactic acid. The blade is then covered with a cap, which is then observed under a light microscope at magnification G X40.This is what **BOUTON (1990)** watch to be specified:

1. **Hyphae:** divided or not
2. **Conidiophores:** absent, simple, branched
3. **Cone cells:** annular, phialide...
4. **Conidia:** unicellular or multicellular, solitary, in groups or in chains, shape (round, oval, in mass) .

Table 05: microscopic observations under 40X;

The Plants	Sample color	Microscopic note 40X	Noticing
Carrots	Black	X	**We don't notice anything on this zoom.**
	Green		**Thick-walled bacilli with parallel filaments.**
Potato	Black		**Inside the wall is cytoplasm with a thick, parallel, stick-shaped wall.**

	Green		**Parallel wall sticks, filaments shape.**
Tomatoes	**Black**		**Thin threads inside the thick wall in the form of sticks.**
	Green		**Parallel surface wall in sticks, threads with the letter.**
Salad	**Black**		**Several shapes, including bacilli, spherical and semi-oval. They have extended tree-like filaments.**

- **For Carrots :** We notice elongated close to each other, and each elongated form contains two parallel thick walls interspersed with threads extending along with the bacilli.
- **For Tomatoes :** We note a solid and robust parallelepiped wall in the form of a stick, interspersed with threads formed with the letter (X) with sticks. We note the shape of sticks surrounded by a parallel wall interspersed with thin threads it.
- **For potato:** We note a solid and robust parallelepiped wall in the form of a stick, interspersed with threads formed with the letter (X) with sticks. We notice two thick parallel walls, inside these bacilli.
- **For Salad:** We notice several shapes. There are elongated as well as globules in shape and semi-oval. The shape is interspersed with strings extending along its length, transverse and longitudinal, resembling a tree.

Through the macroscopic and microscopic study of the isolated strains, they can be listed in the following genera :

- ✓ Black fungi of Salad: Alternania.
- ✓ Black fungi of Potato : Rhizoctonia .
- ✓ Green fungi of Potato : Rhizoctonia .
- ✓ Black fungi of Tomatoes : Botrytis .
- ✓ Green fungi of Tomatoes : Botrytis .
- ✓ Black fungi of Carrots : Rhizoctonia .
- ✓ Green fungi of Carrots : Rhizoctonia .

II - Study of effect of fungicide doses

II - 1 - Disk method

In this method, we observe the area of colony growth and how the fungi interacts with the antifungals during monitoring for seven continuous days. We got the following result:

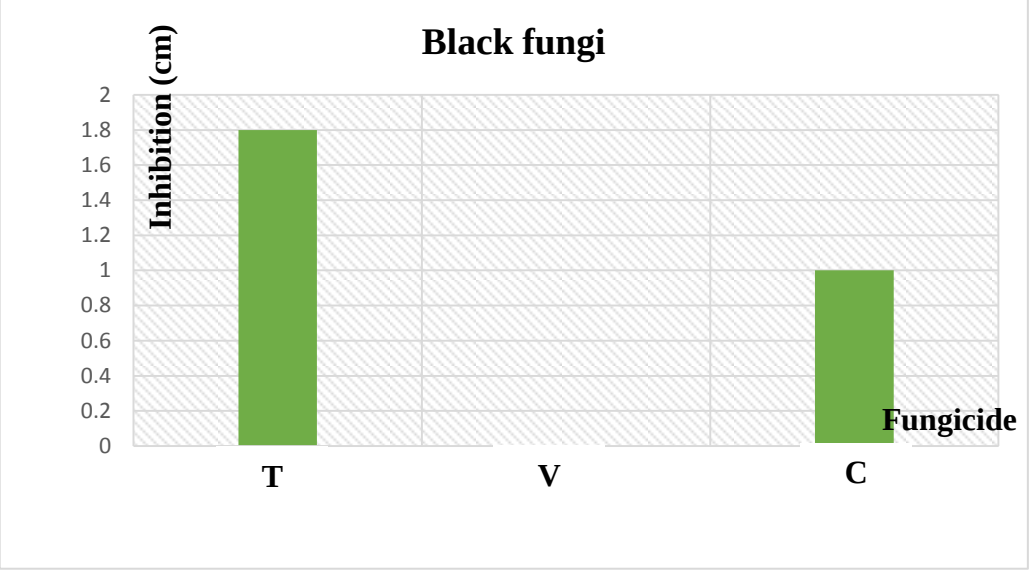


Figure 07 : Efficiency of fungicides on target fungi (C :VALETTE + TACHIGAZOLE ;V : VALETTE and T : TACHIGAZOLE) for Salad

We note that the fungus did not grow in an area of 1.8 cm around the anti-fungal T, in contrast to the anti-fungal V, we notice the growth of colonies around it. As for the anti-fungal C, the area of no colonies growth is 1 cm, during all days of the week.

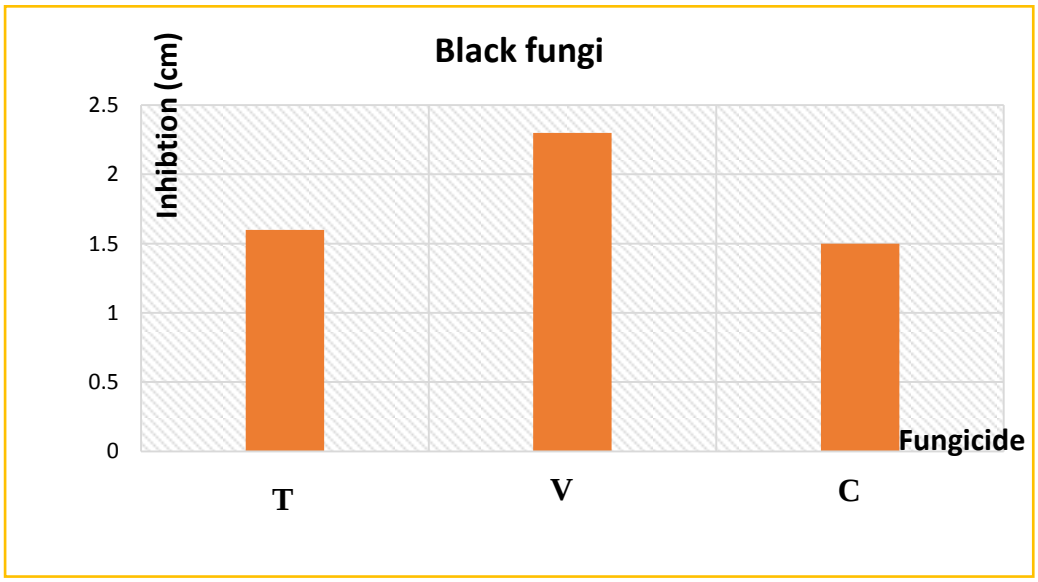


Figure 08 : Efficiency of fungicides on target fungi (C :VALETTE + TACHIGAZOLE ;V : VALETTE and T : TACHIGAZOLE) for Tomatoes .

We note that in an area of 1.6 cm there is no fungi growth around antifungal T, and we observe no growth. Colonies with an area of 2.3 cm around antifungal V. As for antifungal C, the area of no fungal growth around it is 1.2 cm. in a week.

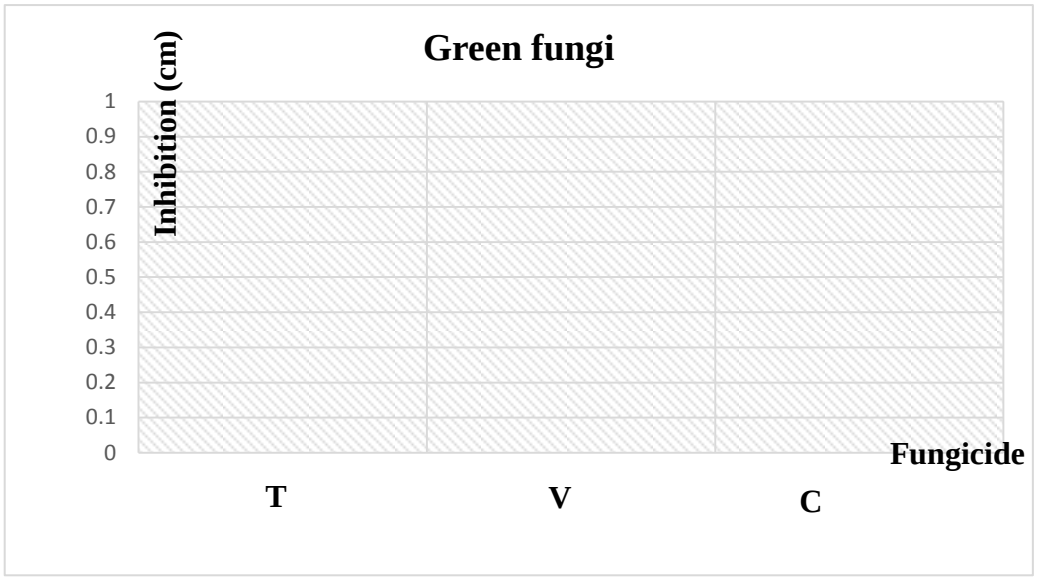


Figure 09 : Efficiency of fungicides on target fungi (C :VALETTE + TACHIGAZOLE ;V : VALETTE and T : TACHIGAZOLE)for Tomatoes .

We note the growth of colonies and their invasion of all places, even where antifungals V, T and even C are present.

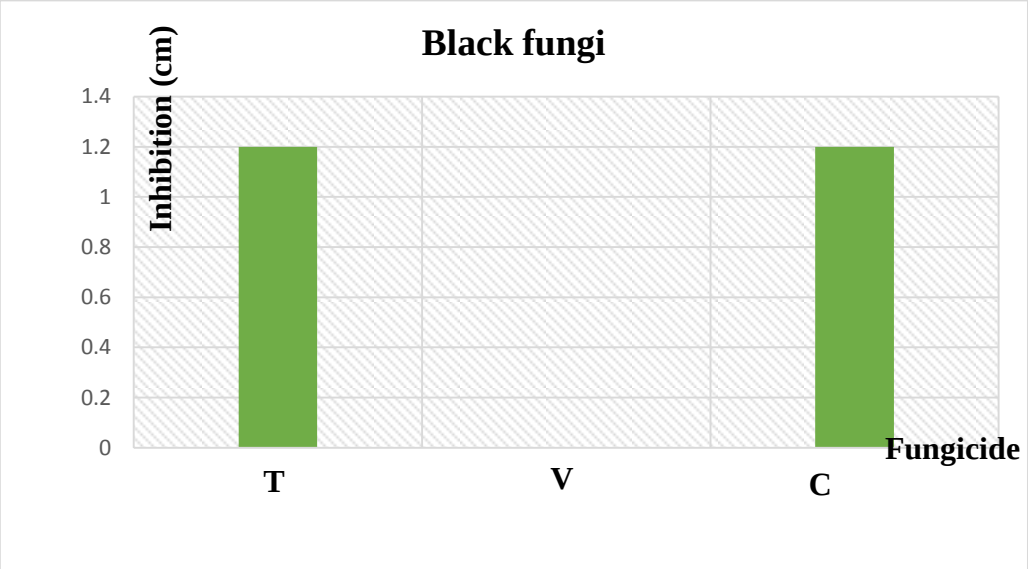


Figure 10 : Efficiency of fungicides on target fungi (C :VALETTE + TACHIGAZOLE ;V : VALETTE and T : TACHIGAZOLE)for Carrots .

We note that the area of non-growth of colonies is 1.2 cm around T and C antifungals, while . We observe the invasion of fungal colonies to the area of presence of antifungal V.

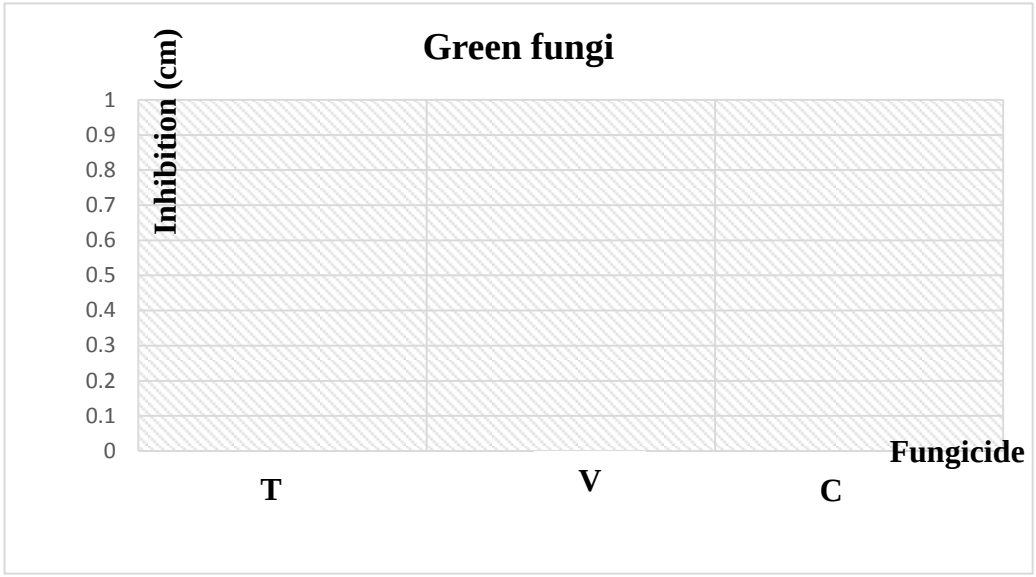


Figure 11 : Efficiency of fungicides on target fungi (C :VALETTE + TACHIGAZOLE ;V : VALETTE and T : TACHIGAZOLE)for Carrots .

We note the growth of colonies and their invasion of all places, even where antifungals V, T and even C are present.

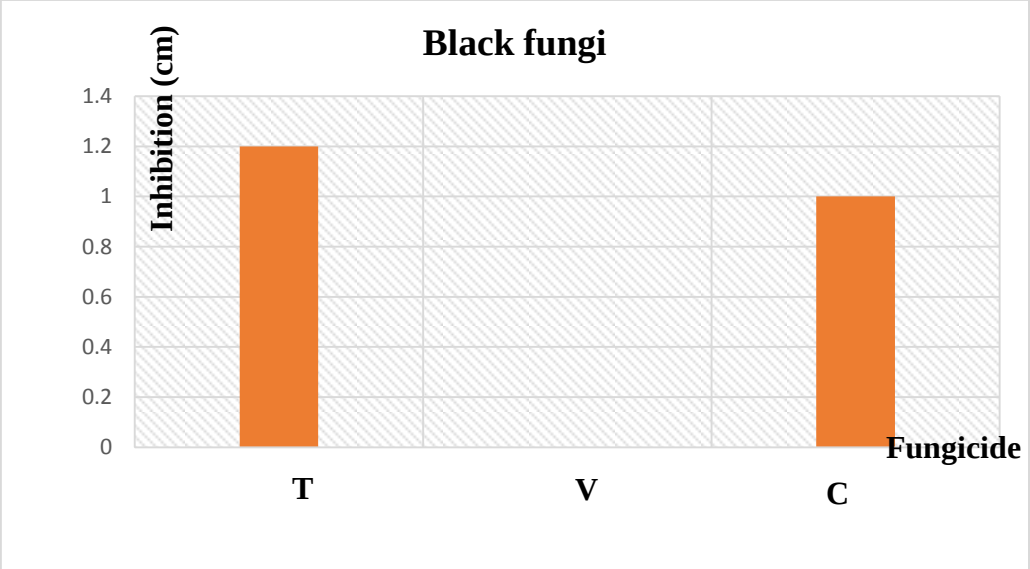


Figure 12 : Efficiency of fungicides on target fungi (C :VALETTE + TACHIGAZOLE ;V : VALETTE and T : TACHIGAZOLE)for Potato .

Around the antifungal T region, we noticed that the fungi did not grow with an area of 1.2 cm and around the antifungal C area was 1 cm, while the colonies invaded the antifungal region V.

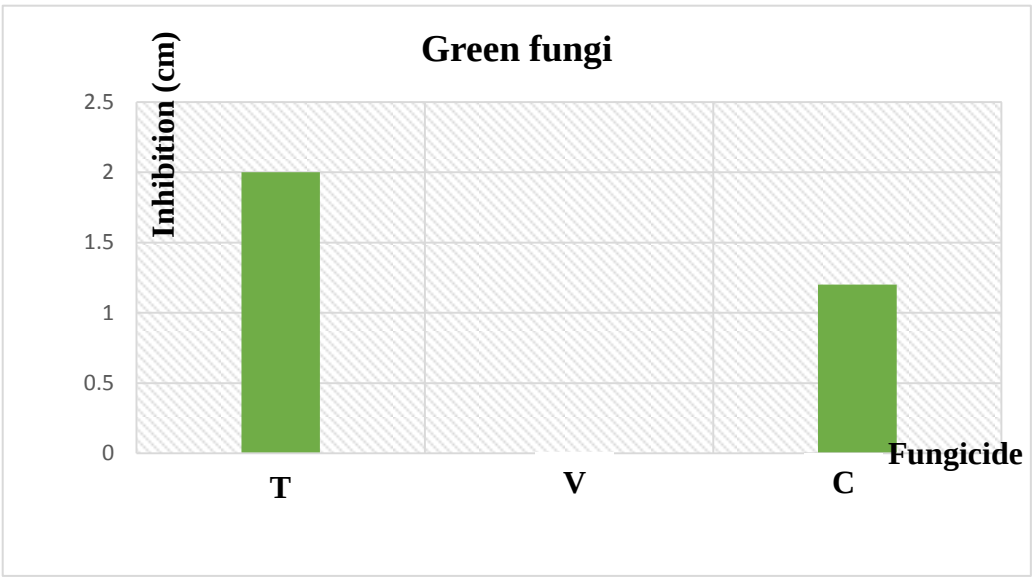


Figure 13 : Efficiency of fungicides on target fungi (C :VALETTE + TACHIGAZOLE ;V : VALETTE and T : TACHIGAZOLE) for Potato .

We note that the fungi do not grow around the anti-fungal T region of 2 cm, while in the anti-fungal region C, we note that the fungi did not grow by 1 cm but in the anti-fungal region V we observe the proportion of fungal colonies of the region.

For Salad

- **Black fungi:** the non-growth of fungi around anti-fungal T with an area of 1.8 cm, and around anti-fungal C with an area of 1 cm, proof of preventing the growth of fungi in these areas, and its growth around anti-fungal V is evidence of its resistance to it. Since the T resistance zone is higher than the C resistance zone of the fungal colonies, we conclude that T is very effective against this type of fungus.

For tomatoes

- **Black fungi:** No growth of fungi around anti-fungal T with an area of 1.6 cm, around anti-fungal V with an area of 2.3 cm, and around anti-fungal C with an area of 2.3 cm. a surface of 1.2 cm, proof of their prevention of their reproduction. Since the resistance zone V is greater than T and greater than C for fungal colonies, we conclude that V is more effective against this type of fungi.

- **Green fungi:** the growth of fungal colonies around all areas of antifungals V, T and C, and this is evidence that the fungi have been resistant to these antifungals, and we conclude that these antifungals are ineffective for this type of fungi .

For carrots:

- **Black fungus:** the growth of fungal colonies around the antifungals T and C with an area of 1.2 cm, and this is the proof of preventing their reproduction in these areas, and the growth of fungal colonies around the antifungals V is the evidence of fungal resistance to this antifungal. Since the T and C resistance areas of the colonies are equal, we conclude that the activity of antifungal T against this type of fungi is weak.

- **Green fungi:** the growth of fungal colonies in all areas of the T, V and C antifungals, evidence that the fungi were resistant to these antifungals, and we conclude that these antifungals have no efficacy against this type of fungi.

For Potato

- **Black fungus:** Absence of fungal growth around anti-fungal T with an area of 1.2 cm, and around anti-fungal C with an area of 1 cm, evidence of the prevention of fungal proliferation in these areas. As for the growth of fungi around anti-fungal V, evidence

of resistance against this anti-fungal, and from this we conclude that anti-fungal T has a resistance rate for this type of fungus.

- **Green fungi:** There is no growth of fungi around anti-fungal T with an area of 2 cm and around anti-fungal C with an area of 1 centimeter, indicating that its growth is inhibited. As for its growth around anti-fungal zone V, proof of its resistance against it. Since the resistance surface T is greater than the resistance surface C of the fungal colonies, we conclude that the anti-fungal T is effective against this type of fungus.

II - 2 - Diffusion methods

It represents the value of the growth area of the fungi in the middle of different concentrations according to the incubation period, so we put the values of the anti-fungal on which we observed the growth of colonies, which is (T). As for the rest of the antifungals (V) and (C). We got the following results: representing the value of the area of growth of fungi in the medium of different concentrations according to the incubation period .

For Salad, the black fungi, we note that colonies did not grow for 7 days within the three concentrations mentioned earlier for antifungals.

For Tomatoes, the black and the green fungi, we note that colonies did not grow for 7 days within the three concentrations mentioned earlier for antifungals.

For Potato, the black and the green fungi,we note that colonies did not grow for 7 days within the three concentrations mentioned earlier for antifungals.

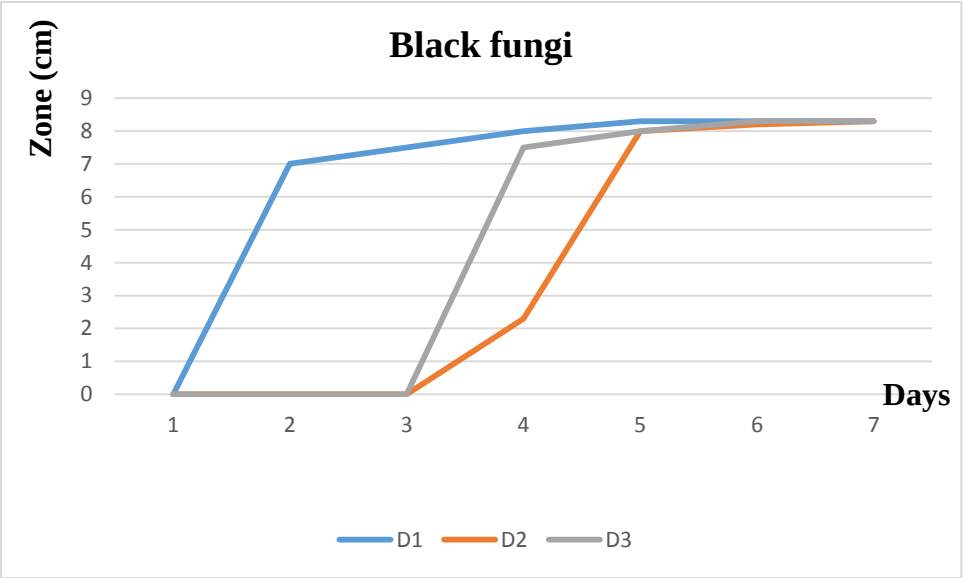


Figure 14 : The area(cm) of green fungi growth in different media in TACHIGAZOLE of anti-fungal , concentration(D1 ; D2 and D3) for Carrot.

In the first concentration, the colony developed swiftly and stabilized at a height of 8.3cm, whereas in the second concentration, we saw an increased growth in the last two days and a height of 8.3 cm. The third concentration shows little change during the first three days, then the colony invasion and stability at 8.3 cm. And that's just for antifungals.

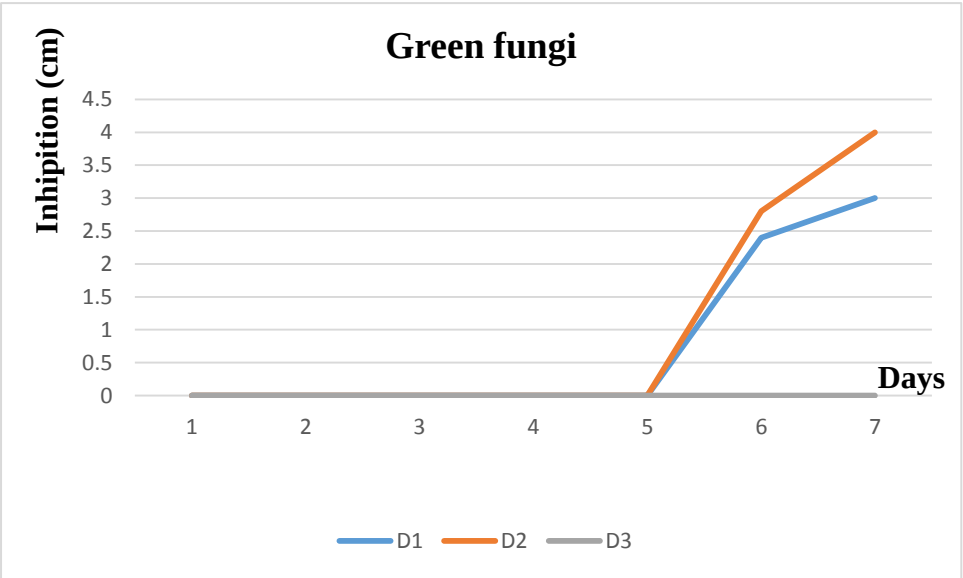


Figure 15 : The area(cm) of green fungi growth in different media in TACHIGAZOLE of anti-fungal , concentration(D1 ; D2 and D3) for Carrot .

We note that no colonies grow in the three concentrations throughout the first days of observation, but in the last few days, we notice that the green carrots begin to grow in the second concentration with a value of 2.4 cm and prove at a value of 3 cm. In the first

concentration, we notice an increase in the last day by a value of 3 cm, and it is installed in it. We do not notice any growth in the third focus.

For Salad Tomato Potato: Antifungals in the three media (V;C and T) are highly effective, because in these media the fungi did not multiply.

And from it we conclude that both are the appropriate treatment to combat these type of fungi.

For Carrots :

Black fungus: Fungi did not multiply in the environment of antifungals, evidence of their control of these fungi. And from it we conclude that these antifungals are effective against these types of fungi. As for the middle at 1 ; 2 and 3 the colonies developed rapidly until they settled at 8.3 cm during all days of the week, evidence of fungal resistance to these therapeutic doses. And from it we conclude that these doses are not suitable for treating this type of fungus.

Green fungi: fungi did not multiply in the environment of antifungals, evidence of their control of these fungi.

And from it we conclude that these antifungals are effective against these type of fungi. As for the middle no fungi growth in the three doses during the first days of observation, evidence that these doses are effective against it.

At 1 the growth of the fungi started from the fifth day until it settled at 4 cm, while at 2 it stabilized at 3 cm on the last day, this is evidence of the resistance of the fungi to these doses, but in varying proportions. 3 no fungi grow during weekdays, this is evidence of the inability of the fungi to resist these doses.

Hence, we conclude that the appropriate dose to treat this type of fungus she D3.

- Through this experiment, we noticed that there is a resistance of fungi to the antifungals used. Generally, two types of fungal resistance are distinguished:

1-Primary resistance: Natural resistance is found in certain fungal species which are insensitive to a given antifungal, or at least for which the minimum inhibitory concentrations (MIC) of antifungals are higher than the concentrations usable in therapy **(Accoceberry and Nol, 2006)** .

This type of resistance is a species characteristic expressed by all the individuals constituting the species.

2-Secondary resistance: Secondary resistance, or acquired resistance, develops in fungi that belong to a species that is a priori sensitive. This resistance is the consequence of an event that took place before or during the antifungal treatment. It is a strain trait, affecting only rare individuals within the species and giving them a selective advantage when exposed to the antifungal.

This resistance usually results from mutational events or deregulation of the expression of certain genes.

Resistance can come from: a lack of transport or penetration of the antifungal inside the fungal cell; a defect in the transformation of the antifungal into a toxic active form; overproduction of the cellular target of the antifungal; or a modification of the target, which leads to a reduction in its affinity for the antifungal; the disappearance of the target and its replacement by recruitment or diversion of another metabolite; active efflux of the antifungal . These different mechanisms are not equivalent in frequency and effectiveness, and some of them are specific to certain antifungals **(Accoceberry and Nol, 2006)**.

Conclusion

Conclusion

Fungicides are a type of pesticide used to get rid of diseases caused by fungi, either by killing the fungus that causes the disease or by discouraging its growth. sometimes it does not work due to the Controlling the disease may succeed, but .presence of resistant varieties to these fungicides

The aim of this study isfirstly to isolate some types of fungi that affect the agricultural productsin the state of El-Oued. Secondly to d etermine the optimal and .effective concentration to get rid of the fungi and be less harmful to us later

For these purpose, different products (potatoes, tomatoes, beans, carrots, zucchini, peas, salad are chosen) ;we isolated the fungi from the infected places and them in the appropriate medium for their growth (PDA, Sabouraud), then then planted we saw them under the light microscope using methyl blue to determine their type. We chose two types offungicide to treat the selected fungi using two methods: the .diffusion method disk method and the

The results of the isolation and identification of associated fungi from four types of selected plants out of seven (tomato, potato, salad, and carrot) show that they contain a variety of fungal species, withfungi riain particular:Alterna, Rh izoctonia and Botrytis.

The results of chemical control with fungicides (TACHIGAZOLE, VALLETE, and the mixture between them) against the isolated fungi proved the was effectiveness of the two fungicides, and the mixture between them that was tested effective in inhibiting the fungal growth of the three tested pathogenic fungi. The reduction in fungal growth ranges from 50 to 100 % .

:Finally, we offer some tips that we can take care of

- ✓ methods that they Educating farmers and being familiar with all the preventive must use water in moderation and consult experts and specialists when any symptoms of fungal, bacterial or other diseases appear. that infect agricultural .crops

- ✓ il We have to analyze the soil and know the compounds and nutrients that the so lacks for the plants to grow well. Identify if it is infected with any diseases and .treat it before transplanting again
- ✓ Fertilizing the soil and applying natural fertilizers before planting so that the .tead of artificial ertilizersfplants take nutrients for their natural growth ins
- ✓ Searching for natural alternatives to eliminate fungi instead of industrial and chemical pesticides, such as natural milk bacteria, as well as reducing pathogenic .bacteria using natural insects

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Annex

Annex :

- Preparation of culture medium:

Mushroom culture medium: PDA (Potato Dextrose Agar)

Potatoes ..... 200 grams

- Dextrose (glucose) ... 20 g

- Agar …….20 grams

- Distilled water …… 1 liter

To prepare the extract, wash and cut 200 grams of small cubes of unpeeled potatoes, preferably old ones (so that they are full of starch and preferably white potatoes), meter in 1 liter of water and boil for an hour, crush, drain and add to one liter.

Medium: potato extract: 1000 ml; glucose. Agar: 20 grams

Dissolve the hot agar in the extract and add the glucose. Complete in 1 L, and sterilized at 100 °C for 30 min. If depositing, move the medium before distributing . (**PARKINSON 1970) : PARKINSON. D. 1970.** Methods for the quantitative study of heterotrophic soil microorganisms, Méthodes d'étude de l'écologie du sol. Actes du colloque de Paris. UNESCO. PP: 101-105.

- **Table of fungicides that we use :**

Treatment	**Dose**	**Suitable for all types of treat.**	**The plant**	**Fungal agent**
VALETTE We do simultaneous treatment with this drug (to get rid of the poisoning, we	400g/L 10D-10D-10D-10D OU 7D-7D-7D-7D.		**Tomatoes**	Botrytis
			Salad	Alternania

must treat 4 days and then stop 10 days + mix the fungicide with water and spray it on the plant and the soil to be Treated) .				
TACHIGAZOLE We do simultaneous treatment with this drug (treatment stopped after 5 days + it is applied after the planting of after registration so that the liquide is mixed with water and applied by spraying .	100 ml / L 10D-10D-10D-10D OU 7D-7D-7D-7D.		**Potato** **Carrots**	Rhizyctonia

- Table representing the value of the area of growth of fungi in the medium of different concentrations according to the incubation period at In Disk method:

	Concentrations 1	**Concentrations 2**	**Concentrations 3**
Days 1	**0 cm**	**0 cm**	**0 cm**
Days 2	**0 cm**	**0 cm**	**0 cm**
Days 3	**6 cm**	**2 cm**	**7 cm**

Days 4	**7 cm**	**4 cm**	**7.5 cm**
Days 5	**7.5 cm**	**4.5 cm**	**7.8 cm**
Days 6	**8 cm**	**8 cm**	**8 cm**
Days 7	**8.3 cm**	**8.3 cm**	**8.3 cm**

- Table representing the area of the spread of fungi around the anti-fungal at Diffusion methods :

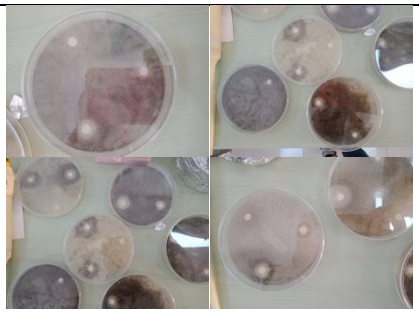
Measurements (Bio-metrics)		
C	**V**	**T**
1 cm	X	1.8 cm
1.5 cm	2.3 cm	1.6 cm
X	X	X
1.2 cm	X	2 cm
1 cm	X	1.2 cm
1.2 cm	X	1.2 cm
X	X	X

X : LACK OF AREA = 0 cm ; **C :** VAPCOTOP + TACHIGAZOLE .

V : VAPCOTOP ; **T :** TACHIGAZOLE .

Annex

- Table of disk methods :

 Potato of black fungi	 Carrots of green fungi
 Carrots black fungi	 Salad of black fungi
 Tomatoes green fungi	 Tomatoes black fungi
 Potatoes green fungi	 All results of disk methods

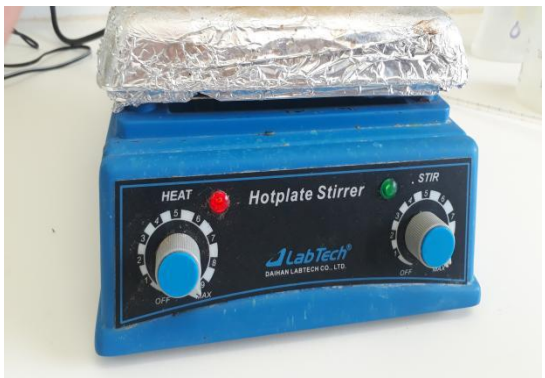
- **Diffusion methods**

 Black fungi of carrots	
	
All the results that we see in diffusion methods of carrots ; black and green fungi .	

- **Figure of some materiel that we use :**

Fungicide VALETTE

Fungicide TACHIGAZOLE



Hot plate and Stirrer + autoclave + analytical balance + host type BOF




Microscope + cave of microbial + distilled water + Labtech type oven


Swab (Ecouvillon in french) + 6 mm discs (Wattman paper) .

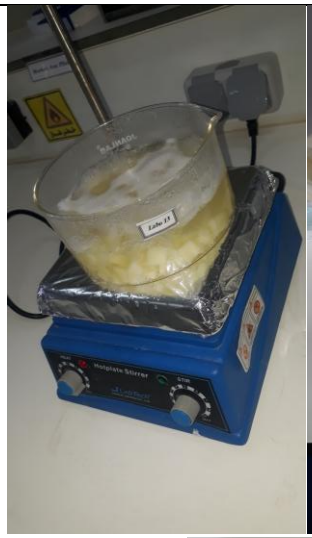
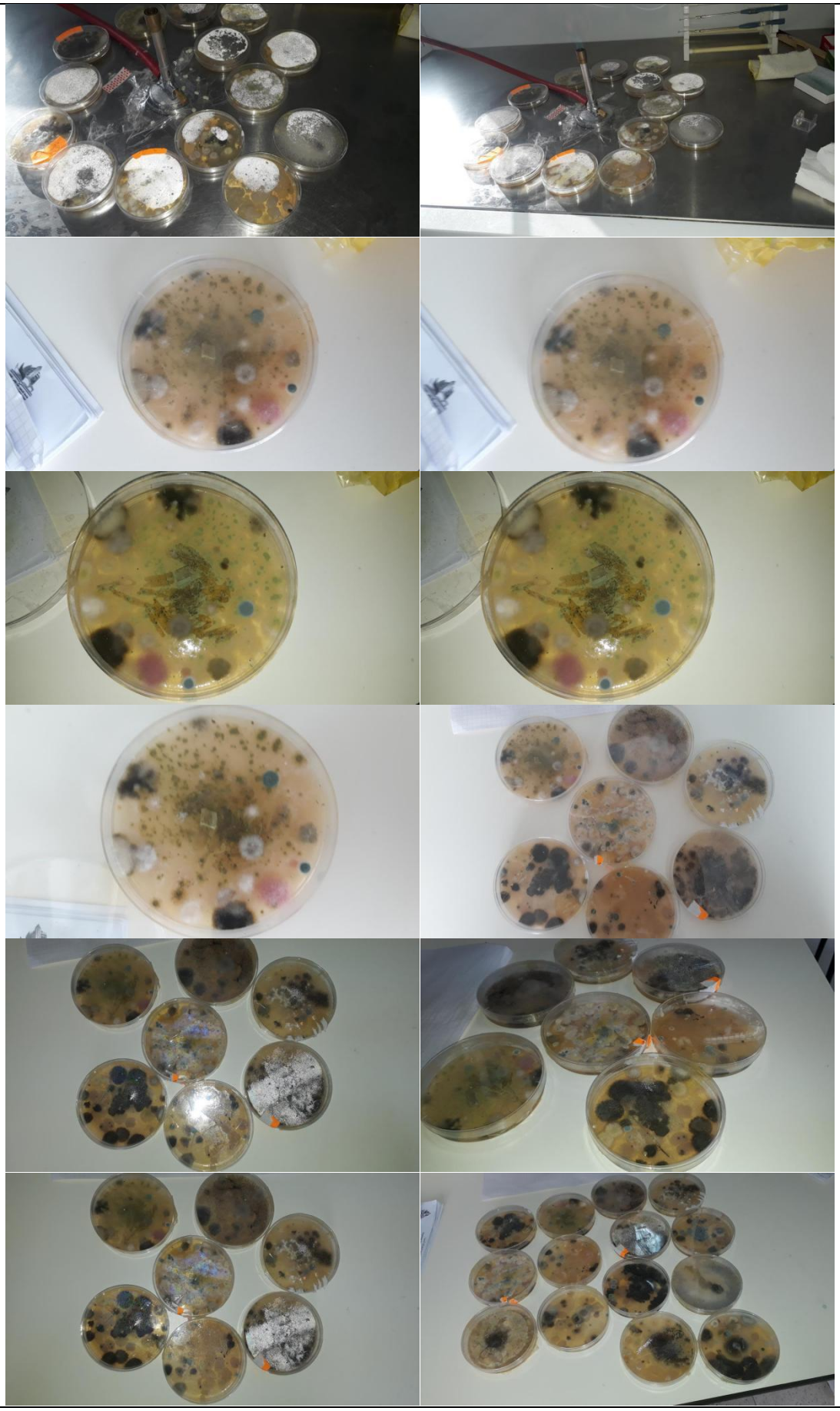



Figure of preparation the medium of PDA

Annex



Annex

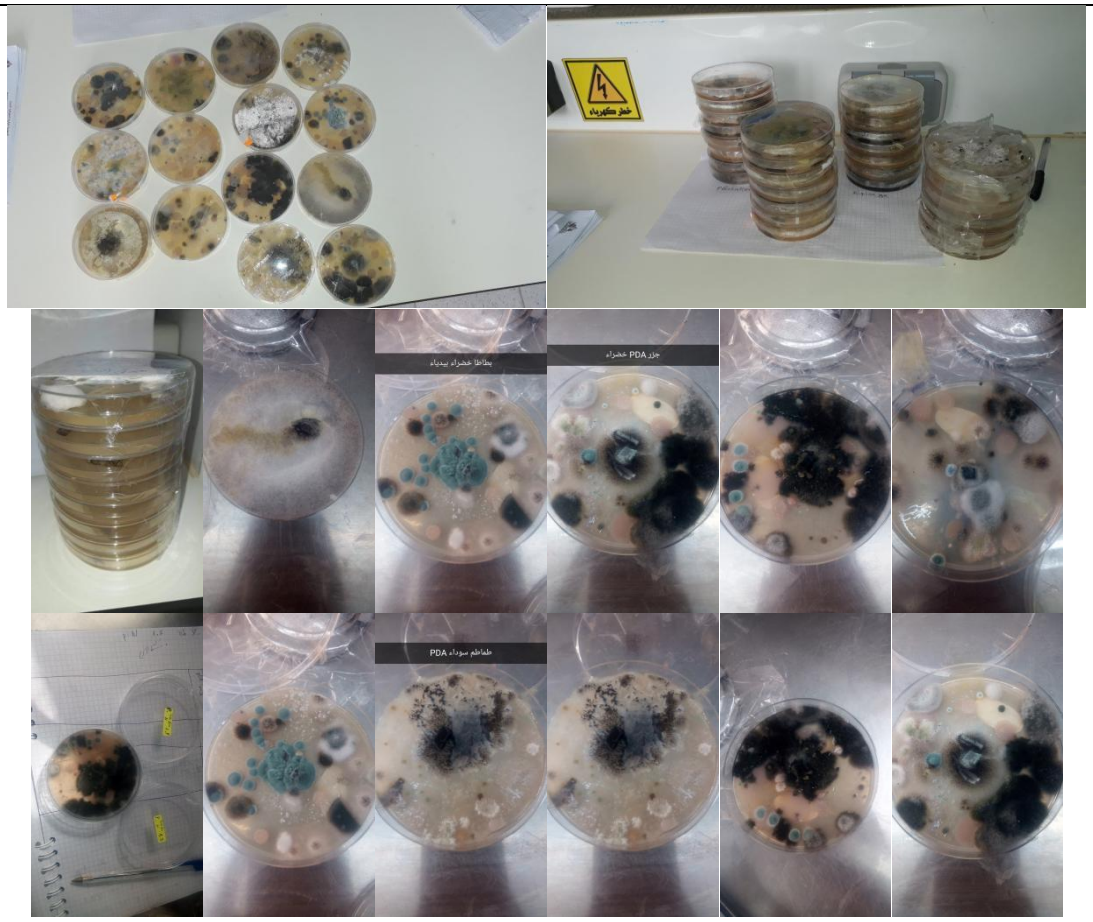


Figure of fungi that we use in all our recherche