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MASTER IN

FONDAMENTAL MICRIOBIOLOGIE

Isolation, characterization of phytopathogenic fungi *Alternaria sp,* and physico-chemical study.

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Dedication

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Summary:

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Abreviations list

- **%:** Percentage.
- **° C:** degree Celsius.
- **A.:** Alternaria.
- **AME:** Alternariol monomethyl ether.
- **ATB:** Antibiotic.
- **C:** carbon.
- **Cm:** centimeter.
- **DNA:** deoxyribonucleic acid.
- **e.g:** example.
- **F:** Fahrenheit.
- **FAO:** food agriculture organization.
- **g:** gram.
- **in:** inches.
- **m:** meter
- **MEA:** Malt extract agar.
- **min:** Minutes.
- **ml:** milliliter.
- **mm:** millimeter.
- **N:** Nitrogen.
- **PCA:**Composition of Plate Count Agar.
- **PDA**: Potato dextrose agar.
- **PSA:**Composition of Peptone-Sucrose-Agar.
- **S.** : Solanum.
- **Syn:** synonym.
- **WA Government:** Western Australia Government.

Abstract:

This work consists of studying the fungal diseases of tomato and carrot, mainly caused by *Alternaria sp*. Major disease of this culture. The objective is the morphological characterization through the cultural aspects of various strains of *Alternaria sp*, with different tests: carbon source test, nitrogen source, and best culture medium, the influence of pH and temperature on growth of *Alternaria sp*.

The results of morphological and cultural characteristics showed that the best source of carbon is glucose, the best source of nitrogen is both sodium nitrate and potassium nitrate, the best culture medium for Alternaria is Czapek medium, the pH between 6 and 8 is very optimal for the growth of Alternaria, and the best temperature for this is 25 ° C.

The results of this study seem to us to be able to contribute to the knowledge of the morphological and pathogenic characteristics of Alternaria sp in order to recommend an effective control measure in the field.

Key words: tomatoes, carrot, plant, fungus, fungal disease, *Alternaria sp*, phytopathogenic fungus.

ملخص:

يتكون هذا العمل من دراسة الأمراض الفطرية للطماطم والجزر، والتي تسببها بشكل رئيسي*Alternaria sp.*

المرض الرئيسي لهذه الفئة.

الهدف هو التوصيف المورفولوجي من خلال تحديد الجوانب الزراعية لسلالات مختلفة من*Alternaria sp*،و ذلك من خلال اختبارات مختلفة: اختبار مصدر الكربون ، مصدر النيتروجين، وأفضل وسط استزراع ، وتأثير درجتي الحموضة و الحرارة على نمو *Alternaria sp.*

أظهرت نتائج الخصائص المورفولوجية و الزراعية أن أفضل مصدر للكربون هو الجلوكوز، وأفضل مصدر للنيتروجين هو كل من نترات الصوديوم ونترات البوتاسيوم، وأفضل وسط لاستزراع Alternaria هو وسط .Czapek ، تعتبر درجتا الحموضة 6 و 8 مثاليَّة جدًا لنمو Alternaria ، كما أن أمثل درجة حرارة لذلك هي 25 درجة مئوية.

نتائج هذه الدراسة قادرة على المساهمة في معرفة الخصائص المورفولوجية والممرضة لـ Alternaria sp من أجل التوصللإجراء تحكم فعال في هذا المجال.

الكلمات المفتاحية:الطماطم،الجزر،النبات،الفطريات، الأمراض الفطرية،*Alternaria sp*، الفطريات الممرضة للنبات.

Résumé :

Ce travail consiste à étudier les maladies fongiques de la tomate et de la carotte, principalement causées par *Alternaria sp*. Maladie majeure de cette culture. L'objectif est la caractérisation morphologique à travers les aspects culturels de différentes souches d'*Alternaria sp*, avec différents tests : test de source de carbone, source d'azote et meilleur milieu de culture, influence du pH et de la température sur la croissance d'*Alternaria sp.*

Les résultats des caractéristiques morphologiques et culturelles ont montré que la meilleure source de carbone est le glucose, la meilleure source d'azote est à la fois le nitrate de sodium et le nitrate de potassium, le meilleur milieu de culture pour Alternaria est le milieu Czapek, le pH entre 6 et 8 est très optimal pour la croissance d'Alternaria, et la meilleure température pour cela est de 25 ° C.

Les résultats de cette étude nous semblent pouvoir contribuer à la connaissance des caractéristiques morphologiques et pathogènes d'*Alternaria sp* afin de recommander une mesure de lutte efficace sur le terrain.

Mots clés : tomates, carotte, plante, champignon, maladie fongique, *Alternaria sp*, champignon phytopathogène.

Introduction

Introduction:

Nowadays diseases has becoming a worldwide problem and becoming highly concerning factor. Not only in human beings, animals but also in plants it is becoming a problem with various factors like fungi, bacteria, viruses, etc.,. Fungi represent the major pathogenic microorganisms that infect plants, causing numerous. They constitute the largest number of plant pathogens and are responsible for a range of serious plant diseases. Fungi are parasitic on almost all groups of eukaryotic organisms. Parasitic fungi are best known through their extensive damage to plants, especially cultivated plants which causes fungal diseases in plants. (Longdom., 2015).

Such as tomatoes (*Lycopersicum esculentum*) and carrot (*Daucus carota*), they are known as the most affected plants, they are very susceptible plants to fungal diseases. This is evident in several symptoms, including changes such as yellowing, browning, and blackening.These symptoms can appear at any level in the plant.

Alternaria is a ubiquitous fungal genus that causes pre- and post-harvest damage to agricultural products including cereal grains, fruits and vegetables. In addition to spoiling a wide variety of foods, several Alternaria species are able to produce secondary metabolites considered as both phytotoxins, which play an important role in the pathogenesis of plants, and mycotoxins, which can be harmful to humans and animals. (Patriarca et al., 2018). Alternaria may cause several diseases such as: Early blight of tomatoes, and Leaf blight of carrot

The main objective of my study is the isolation, identification characterization of Alternaria genus from tomato and carrot in the region of Mostaganem.

This work is made up of two main parts:

1- A bibliographical section made up of four chapters:

- Chapter I and II: General information on the host plants: *Lycopersicum esculentum* and *Daucus carota.*

- Chapter III and IV: General information on the parasite (*Alternaria sp*) and means of control.

2- An experimental part made up of two chapters:

- Chapter I: Materials and methods.

- Chapter II: Results and discussion.

Literature review

I. Tomatoes

1. Origin:

The tomato (Lycopersicum esculentum) is native to the Andes of South America. It was first domesticated in Mexico, then introduced to Europe in 1544. (Si Mohammed., 2014).

From there, its culture spread to South and East Asia, Africa and the Middle East.

Among the common names used to designate the tomato: tomato (French and Spanish), jitomate (Mexican Spanish), pomodoro (Italian), tomati (West Africa), tomat (Indonesian), faanke'e (Chinese).(Si Mohammed., 2014).

Etymologically, the word tomato is a deformation of the Inca word Tomalt and the word Lycopersicum which means in Latin "Peach of wolf", appellation not very enticing to which one added in the 18th century the adjective esculentum because of the gustatory properties of this vegetable- fruit (Si Mohammed., 2014).

2. The botanical description:

The tomato is a herbaceous plant belonging to the nightshade family, this family includes other species which are also well known, such as potato, tobacco, pepper and eggplant.

The tomato is generally grown as an annual plant; it can reach a height of more than two meters (Chaux et Foury, 1994).

a) **Root:**Strong taproot that grows to a depth of 50 cm or more. The main root produces a high density of lateral roots and weeds.(Naika et al., 2005).
b) **Stem:** The growth habit varies between erect and prostrate. The stem grows to a length of 2 to 4 m. The stem is full, strongly hairy and glandular.(Naika et al., 2005).
c) **Foliage:** Leaves arranged in a spiral, 15 to 50 cm long and 10 to 30 cm wide. The leaflets are ovate to oblong, covered with glandular hairs. The large leaflets are sometimes pinnate at the base. The inflorescence is a cyme formed from 6 to 12 flowers. The petiole measures between 3 and 6 cm.(Naika et al., 2005).
d) **Flowers:** Bisexual, regular and between 1.5 and 2 cm in diameter. They grow opposite to - or between the leaves. The calyx tube is short and hairy, the sepals are persistent. In general there are 6 petals which can reach a length of 1 cm, which are yellow and curved when they are ripe. There are 6 stamens and the anthers have a bright yellow color and surround the style which has an elongated sterile tip. The ovary is superior with between

2 and 9 carpels. In general the plant is autogamous, but cross-fertilization can take place. Bees and bumblebees are the main pollinators.(Naika et al., 2005).

e) **Fruit:** Fleshy berry, globular or flattened with a diameter of 2 to 15 cm. When not yet ripe, the fruit is green and hairy. The color of ripe fruit varies from yellow to red to orange. In general, the fruits are round and regular or ribbed.(Naika et al., 2005).

f) **Seeds:** Numerous, kidney-shaped or pear-shaped. They are hairy, beige, 3 to 5 mm long and 2 to 4 mm wide. The embryo is coiled in the albumen. 1000 seeds weigh approximately 2.5 to 3.5 g (Naika et al., 2005).

3. Tomato taxonomic classification

Kingdom: *Plantae – Plants*

Subkingdom: *Tracheobionta – Vascular plants*

Superdivision: *Spermatophyta – Seed plants*

Division: *Magnoliophyta – Flowering plants*

Class: *Magnoliopsida – Dicotyledons*

Subclass: *Asteridae*

Order: *Solanales*

Family: *Solanaceae – Potato family*

Genus: *Solanum*

Species: *S. lycopersicum.*(Yogesh., 2007).

4. nutritional value of tomato:

Table 1: Nutritional value of 100g of raw tomato. (Adda., 2019).

Nutrient	**Amount**
Water	94.52
Proteins	0.88
Fat (lipids)	0.20
Carbohydrates	3.89

Fibers	1,2
Calories	15
Vitamins	A, C, B3, B6, B9, E, K1

5. Tomato growing conditions:

Tomatoes are warm season plants and should only be planted after the frost has passed. Tomato temperature tolerance for extreme heat or cold snaps is of extreme importance to the development of blossoms and subsequent fruit set. Blossom drop will occur in the spring if daytime temperatures are warm but night temperatures drop below (13°C.). In the summer when temperatures soar over (32°C.) with nights over (24°C.); again, the tomato plant will suffer damage to immature fruit or loss of flowers. Additionally, when nights become too warm, the pollen grains of the tomato flower begin to burst, thwarting pollination, hence no fruit set. This is doubly true when the air is saturated with relative humidity. The growing temperature for tomato seedlings should be maintained at constant temperatures of between (14-16°C.), whether starting in the greenhouse or indoors, and then not transplanted until the last frost has passed. (Amy., 2019).

6. Harvest time for tomatoes:

Harvest time for tomatoes will occur at the end of its growing season, usually late summer, once the tomatoes are at their mature green stage. Tomatoes harvested before this, such as those, you buy at the supermarket, have often been picked before this stage so they can ripen during transport and, thus, have a lesser flavor than those left on the vine a bit longer. There is a fine line when picking tomatoes at the mature green stage. Look for the first light blush of color as an indicator of when to pick tomatoes to ensure no loss in their essence. Of course, you can also harvest tomato fruit when it is ripe; ripe fruit will sink in water. These vine ripened tomatoes may be the sweetest, but some types of tomato are too heavy to vine ripen, hence picking tomatoes at their mature green stage and allowing the ethylene gas to continue the ripening process.

Once you've harvested the tomatoes, store them indoors to continue to ripen. Green tomatoes will ripen faster if wrapped in newsprint, which will contain the ethylene gas and hasten the process. Store them at 55-70 F. (13-21° C.) — or cooler if you wish to slow the ripening and warmer to hasten it, and check routinely for ripeness. They may last from three to five weeks stored this way (Amy., 2019).

7. Tomato fungal diseases:

a) Early Blight:

This disease is caused by the fungi *Alternaria tomatophila* and *A. solani* and is first observed on the plants as small, brown lesions mostly on the older foliage. Spots enlarge and concentric rings in a bull's-eye pattern may be seen in the center of the diseased area. Tissue surrounding the spots may turn yellow. If high temperature and humidity occur at this time, much of the foliage is killed. Lesions on the stems are similar to those on leaves and sometimes girdle the plant if they occur near the soil line (collar rot). On the fruits, lesions attain considerable size, usually involving nearly the entire fruit. Concentric rings are also present on the fruit. Infected fruit frequently drops. (Home and garden information center., 2018).

Figure 1: Early blightt (*Alternaria solani*) on tomato foliage. Joey Williamson, ©2012 HGIC, Clemson Extension.

b) Late Blight:

Late blight is a potentially serious disease of potato and tomato, caused by the fungus *Phytophthora infestans*. Late blight is especially damaging during cool, wet weather. The fungus can affect all plant parts. Young leaf lesions are small and appear as dark, water-soaked spots. These leaf spots will quickly enlarge and a white mold will appear at the margins of the affected area on the lower surface of leaves. Complete defoliation (browning and shriveling of leaves and stems) can occur within 14 days from the first symptoms. Infected tomato fruits develop shiny, dark or olive-colored lesions, which may cover large areas. Fungal spores are spread between plants and gardens by rain and wind. A combination of daytime temperatures in the upper 21°C with high humidity is ideal for infection. (Home and garden information center., 2018).

Figure 2: The aspect of tomatoes' late blight disease. (Bighhat., 2018).

c) Septoria Leaf Spot:

This destructive disease of tomato foliage, petioles and stems (fruit is not infected) is caused by the fungus *Septoria lycopersici*. Infection usually occurs on the lower leaves near the ground, after plants begin to set fruit. Numerous small, circular spots with dark borders surrounding a beige-colored center appear on the older leaves. Tiny black specks, which are spore-producing bodies, can be seen in the center of the spots. Severely spotted leaves turn yellow, die and fall off the plant. The fungus is most active when temperatures range from 20 to 25° C, the humidity is high, and rainfall or over-head irrigation wets the plants. Defoliation weakens the plant, reduces the size and quality of the fruit, and exposes the fruit to sunscald (see below). The fungus is not soil-borne, but can overwinter on crop residue from previous crops, decaying vegetation and some wild hosts related to tomato. (Home and garden information center., 2018).

Figure 3: Septoria leaf spot (*Septoria lycopersici*) on tomato. Joey Williamson., ©2013 HGIC, Clemson Extension.

d) Leaf Mold:

The fungus *Passalorafulva* causes leaf mold. It is first observed on older leaves near the soil where air movement is poor and humidity is high. The initial symptoms are pale green or yellowish spots on the upper leaf surface, which enlarge and turn a distinctive yellow.

Under humid conditions, the spots on the lower leaf surfaces become covered with a gray, velvety growth of the spores produced by the fungus. When infection is severe, the spots coalesce, and the foliage is dead. Occasionally, the fungus attacks stems, blossoms and fruits. Green and mature fruit can have a black, leathery rot on the stem end.(Home and garden information center., 2018).

The fungus survives on crop residue and in the soil. Spores are spread by rain, wind or tools. Seeds can be contaminated. The fungus is dependent on high relative humidity and high temperature for disease development. (Home and garden information center., 2018).

Figure 4: Leaf mold (*Passalora fulva*) on lower leaf surface. Joey Williamson., ©2012 HGIC, Clemson Extension.

e) Buckeye Rot:

Buckeye rot is a disease of the fruit caused by the fungus *Phytophthora parasitica*. The first fruit symptoms appear as brownish spots, often at the point of contact between the fruit and the soil. As the spots enlarge, dark, concentric rings can be seen. Lesions of buckeye rot resemble those of late blight, except that the former remain firm and smooth, whereas late blight lesions become rough and are slightly sunken at the margins. Under moist conditions, a white, cottony fungal growth appears on the buckeye rot lesions. With time, the entire fruit will rot. The fungus does not affect the foliage. The disease is most common during periods of prolonged warm, wet weather and in poorly drained soils. The fungus survives in the soil and is spread by surface water and rain. Peppers are also susceptible to this disease. (Angie., 2018).

Figure 5: Tomatoes buckeye rot caused by the fungus *Phytophthora parasitica*. (Angie., 2018).

II. The carrot :

1. Origin:

Carrot is one of the most important root vegetable plants in the world. In its wild state it is a tiny, bitter root with little appeal as a food, but years of human cultivation and domestication, with a helping hand from nature, has made it an extremely versatile vegetable, appearing in several colors, shapes, and sizes. Although cultivated for over 2000 years, and originally used only as a medicinal plant, the domestic carrot (*Daucus carota* var. *sativus*, *Apiaceae* or Umbelliferae) remains an important world crop with production expanding rapidly in Asia. Current world annual production is 27 million tons; the leading producing countries, China, Russia, and USA, produce 45% of World output (FAO, 2008). The swollen taproots are eaten both raw and cooked, in sweet and savory dishes and it is known for its high beta-carotene content, which the body converts to Vitamin A. It also forms a major ingredient in the food processing industry, a significant constituent of cosmetic products and its image has been used to symbolize healthy eating. The leaves are also consumed in salads and the seeds made into an herbal tea. (World Carrot Museum - The Encyclopedia of Carrots., 2011).

2. Botanical description :

Carrot, *Daucus carota*, is an edible, biennial herb in the family Apiaceae grown for its edible root. The carrot plant produces a rosette of 8–12 leaves above ground and a fleshy conical taproot below ground. The plant produces small (2 mm) flowers which are white, red or purple in color. The root can grow to between 5 and 50 cm (2.0–20 in) long and reach 5 cm (2.0 in) in diameter. The foliage of the plant can reach a height of 150 cm (59.1 in) when in flower. The carrot plant can be annual or biennial and may also be referred to as wild carrot. The plant is believed to have originated in Europe or the Western Mediterranean. (Lemmens., 2020).

3. Classification :

Domain : Biota

Reign : Plantae (Haeckel, 1866)

Sub-Kingdom : Viridaeplantae

Infra-Reign : Streptophyta(John, Williamson &Guiry, 2011)

Class : Equisetopsida(C. Agardh, 1825)

Clade : Tracheophyta(Sinnott ex Cavalier-Smith, 1998)

Clade : Spermatophyta

Subclass : MagnoliidaeNovák ex Takht., 1967

Super-Order : AsteranaeTakht., 1967

Order : ApialesNakai, 1930

Family : ApiaceaeLindl., 1836

Genus : Daucus L., 1753

Species :*Daucus carota* L., 1753

Subspecies :*Daucus carota* subsp. sativus (Hoffm.) Schübl. & G. Martens, 1834

Variety :*Daucus carota* var. sativus DC. (Schübl and Martens, 1834).

4. Nutritional value of carrot :

Table 2: Nutritional value of carrot. (Adda., 2015).

Nutrient	Amount
Calories	41
Water	88 %
Protein	0.9 g
Carbohydrate	9.6 g
Sugar	4.7 g
Fibers	2.8 g
Fat :	0.2 g
- Saturated	0.04 g
-Mono-unsaturated	0.01 g
-Poly-unsaturated	0.12 g
-Omega-3	0 g
-Omega-6	0.12 g
-Trans fat	~

5. Carrots growing conditions :

Carrots grow best in temperatures between 15 and 21°C. Temperatures below 10 °C will stunt the growth of the foliage. Temperatures above the mid-27 °C will produce undesirable flavors in the carrots. Carrots can tolerate a light frost but prolonged exposure should be avoided. Sandy soils, sandy loam and silted loam are traditionally consideredas the best soils for carrots. Heavy, clay soils or compacted soils may produce a warped or stunted crop. In soils that grow dense below 0.3 meters of depth, dwarf varieties of carrots may be more appropriate. Amend clayey soils should with loam builder or organic material to create a lighter, better-draining soil.Keep the soil well-watered, but not waterlogged. Irregular watering can produce split or diseased carrots. Avoid this by putting carrots on a regular watering schedule or by watering carrots with a timed irritation system. (Leslie., 2019).

6. Carrot harvesting time:

Knowing how to tell when carrots are ready to harvest is important for getting a good crop. First, consult your seed packet to see how many days it takes your chosen variety of carrots to mature. Baby carrots are usually ready to harvest 50 to 60 days from the planting date. Mature carrots need a few more weeks and are usually ready in about 75 days. Most carrots are ready to harvest when the shoulders are 1/2 to 3/4 inch in diameter, but again, there is much variation depending on the variety.(Jackie., 2020).

7. Carrot fungal diseases:

a) Cavity spot disease :

Cavity spot disease of carrots is caused by the soil-borne fungus *Pythium sulcatum*.

Cavity spots are small elliptical lesions (usually less than 10mm across) often surrounded by a yellow halo. Infection can take place anywhere along the carrot root and lesions start as pinhead-size spots. (WA Government., 2019).

In most cases visible symptoms develop in the month before harvest maturity and develop rapidly if conditions are favorable. Cavity spot reduces quality so that carrots become unacceptable for local and export fresh markets. It has resulted in severe losses and has been difficult to control. (WA Government., 2019).

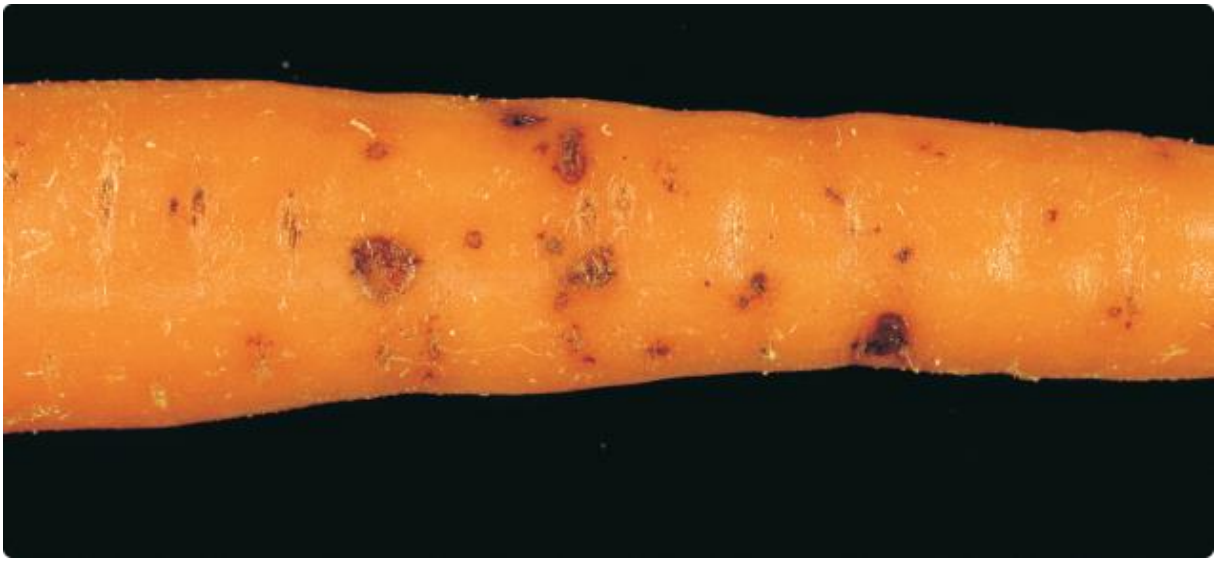

Figure 6: Carrot with cavity spot disease. (WA Government., 2019).

b) Leaf blight:

Carrot leaf blight is a disease commonly found in carrot crops in Western Australia. It is usually caused by the fungus *Alternaria dauci* and occasionally by *A. radicina*. Another fungus, *Cercosporacarotae*, causes leaf spotting of carrots. Both Alternaria and Cercospora can weaken leaves and in severe cases can defoliate crops. (WA Government., 2019).

Alternaria dauci appears on leaves as small variously-sized dark brown to black lesions. The lesions often appear on the edges or margins of the carrot leaf. In severe cases, the lesions expand, causing the leaflets to turn brown, shrivel and die. The leaf may have a scorched appearance.

The petiole or leaf stems can also become infected and develop brown irregular-shaped lesions(WA Government., 2016).

Figure 7: Infected carrot leaf with leaf blight. (WA Government., 2016).

c) Black root rot :

Black root rot of carrots is a nasty fungal disease caused by *Alternaria radicina* that plagues gardeners around the world. Once established, carrot black root rot is difficult to eradicate,

Carrots with black root rot typically display a black or brown, decayed ring at the top of the carrot, at the point where the leaves are attached. The disease results in wilting stunted growth and carrots that break off in the soil when pulled. Carrot black root rot can affect carrots at any stage of growth. It can show up on seedlings, and may appear during storage, evidenced by decay and black lesions that can spread to healthy carrots. (Mary., 2018).

Figure 8: Black root rot of carrot caused by *Alternaria radicina*. (Mary., 2018).

d) Fusarium Dry Rot:

Dry rot of carrot roots is caused by *Fusarium spp*., soil-borne fungi that occur wherever carrots are grown. Fusarium dry rot is commonly a carrot root disease, but the fungi can also be associated with seeds. Disease is severe on carrots held in fields after maturity and it can develop in storage. Fusarium spp. spores survive in soil, plant debris and crop residues and the spread of the pathogen occurs through mycelia and airborne spores. Moisture, warm temperatures and wounds caused by equipment, insects and other fungi facilitate the growth and spread of Fusarium spp. Symptoms of the disease include brown, leathery lesions, side

cankers and crown decay. In storage, rapid growth of the fungi is favored by free moisture and temperatures between 7and 21 º C and contamination of adjacent roots can occur quickly. (Catrina and Mary K., 2005).

Figure 9: Carrot dry rot disease caused by *fusarium sp*. (Davis and Raid., 2002).

III. The parasite:

1. Definition:

Alternaria is a ubiquitous fungal genus that causes pre- and post-harvest damage to agricultural products including cereal grains, fruits and vegetables. In addition to spoiling a wide variety of foods, several Alternaria species are able to produce secondary metabolites considered as both phytotoxins, which play an important role in the pathogenesis of plants, and mycotoxins, which can be harmful to humans and animals.(Patriarca et al., 2018).

2. Characteristics of Genus Alternaria:

Members of the genus Alternaria have septate conidia with transverse septaand longitudinal, the cells are multinucleated (multicellular) of dark colorgenerally piriform in size varying according to the species, they havea melanin-type pigment that protects them from the conditionsadverse environmental conditions, including resistance to microbes and enzymes hydrolytic. Alternaria fungi are Deuteromycetes(syn. Adelomycetes, fungi imperfecti). This class includes all fungiscloisonne mycelium whose reproductive form is generally unknown but havea mode of asexual multiplication. Some species of Alternaria have a reproductionsexual and their perfect form belongs to the Loculoascomycetes (genus Pleospora). (Zerigui and Mouzaoui., 2018).

3. Classification :

Kingdom: Fungi (A diverse group of eukaryotic organisms (unicellular and multicellular) that obtain their nutrition from organic matter; yeast, fungi, molds etc.).

Phylum: Ascomycota (Fungi that are largely characterized by their ascus; a sac-like structure used for reproduction purposes).

Class: Euascomycetes (class/sub-class largely associated with the development of Asci and a fruiting body).

Order: Pleosporales (Tend to form lichens and they are differentiated from other pyrenomycetes by their large pseudo-paraphyses and bitunicate asci).

Family: Pleosporaceae (sac fungi)

Genus: Alternaria (Thomma., 2003).

4. Infectious cycle:

Alternariosis is favored by a similar infectious cycle for all the species of Alternaria, responsible for this disease. This cycle is devised in several stages: conservation, penetration and invasion, sporulation then dissemination.(Zerigui and Mouzaoui., 2018).

a) Conservation, source of inoculum:

Alternaria can be kept in crop residues, contaminated soil and infected tubers for several years. Chlamydospores can also serve as a survival structure. It would also be able to maintain itself from one season to the next on other nightshades like tomato, carrot, eggplant, and pepper.(Zerigui and Mouzaoui., 2018).

b) Penetration and invasion:

Once the Alternaria spores are in contact with plant cells, they are able to germinate and produce one or more germ tubes, penetration into the tissueseither directly through the stomata or the wounds, or by enzymatic penetration, this strategy is most evident in the Alternaria. Colonization of the host is facilitated by enzymes (cellulase, methyl pectin galacturonase). The fungus quickly invades leaf tissue, lesionsbecome visible 2-3 days after infection, spore production occurs 3-5 days later.(Zerigui and Mouzaoui., 2018).

c) Sporulation and dissemination:

Conidia and conidiophores are produced in temperature ranges between 8 and 28C °, in the presence of a relative humidity of 96 to 100%.

Spores are disseminated by wind, rain and insects; the conidia produced ensure secondary contamination and thereafter several parasitic cycles can take place in the culture (Zerigui and Mouzaoui., 2018).

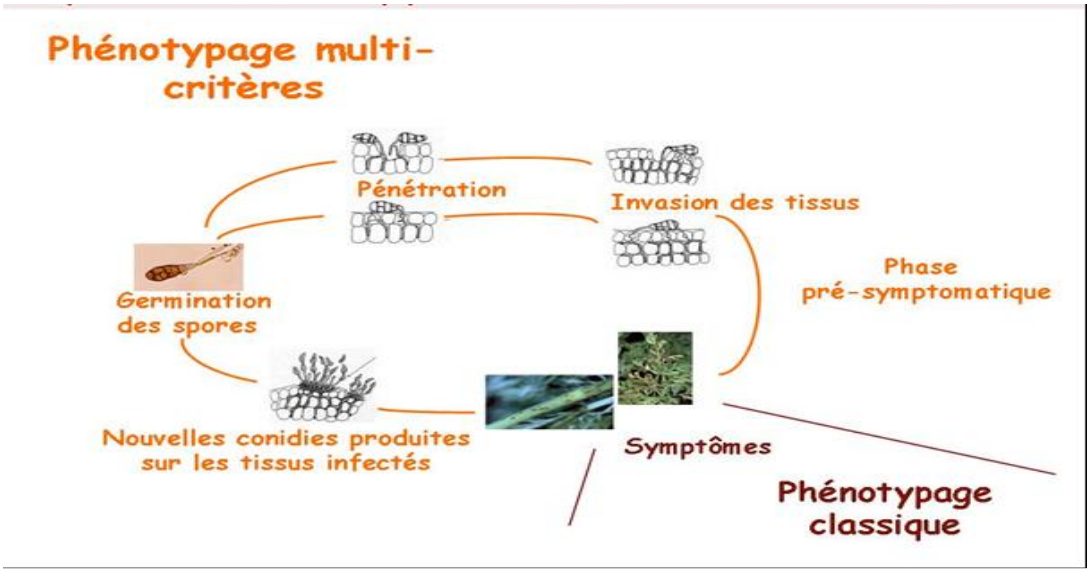

Figure 10: Infection cycle of *Alternaria sp*. (Clerc et al., 2016).

5. **Major species of Alternaria:**

a) Alternaria solani:

Alternaria solani is a fungal pathogen that produces a disease in tomato and potato plants called early blight. The pathogen produces distinctive "bullseye" patterned leaf spots and can also cause stem lesions and fruit rot on tomato and tuber blight on potato. Despite the name "early," foliar symptoms usually occur on older leaves. If uncontrolled, early blight can cause significant yield reductions(Varma., 2017).

Infection cycle of Alternaria solani :

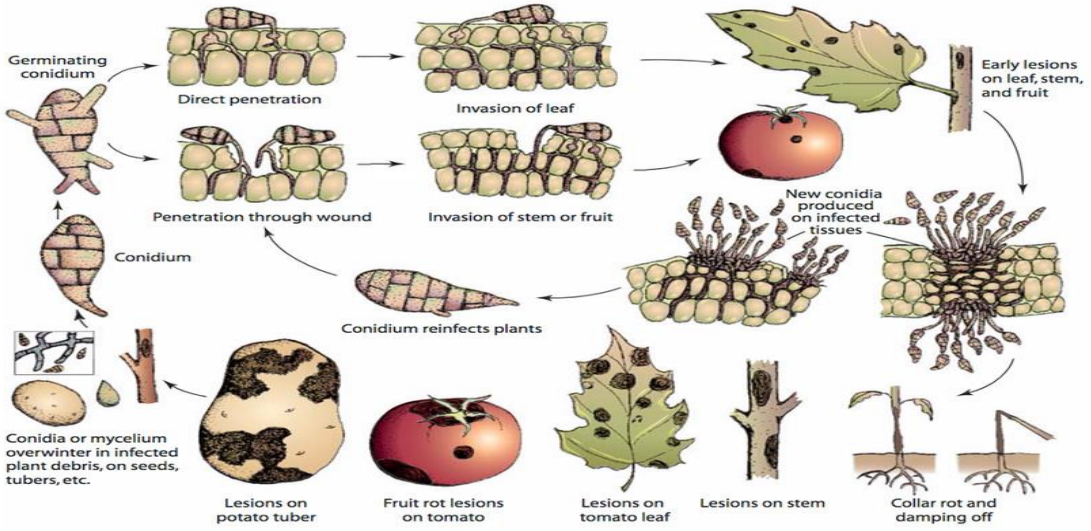

Figure 11: Disease cycle of early blight caused by *Alternaria solani.* (Agrios., 2005).

b) Alternaria alternata:

Alternaria alternata is a yeast from the soil that is found very frequently in the atmosphere and in homes. It develops optimally in heat and humidity. The presence of plants can favor its multiplication. It belongs to the class of Deuteromycetes and to the family of Pleosporaceae.

Alternaria alternata is a toxic and pathogenic species. It can cause epidermal affections in humans, respiratory allergies, asthma, leukopenia (due to mycotoxins), mycoses of the skin and rhinitis.

In plants, it presents itself as a phytopatogenic fungus causing various symptoms, black spots, rot, rust, etc. on the different organs of the plant. (Laurence., 2012).

c) Alternaria dauci:

*Alternaria dauci*is most well known for its characteristic dark lesions on the leaves of carrots. These lesions are most often found on mature leaves, where full necrosis often follows. Younger leaves remain, for the most part, relatively unharmed. Immediately after the lesions form on the leaves, chlorosis begins to occur. One phytotoxin in particular, all toxin, has been shown to both reduce chlorophyll production in leaves as well as cause stunting (Dugdale et al., 2020).

*Alternaria dauci*can be kept on crop debris and especially on seeds. It can also be perpetuated on weed host plants, especially wild carrots which are an important source of contamination.

Alternaria dauci's favorite conditions are high humidity and high temperatures between 15 and 30 ° C with an optimum at 25 ° C. Spores are spread by wind, runoff and splashes. Symptoms appear at most 2 weeks after a contaminating period, with temperatures> 18 ° c and high humidity. (e-phytia., 2019).

6. Mycotoxins:

The term mycotoxin is derived from the Greek word 'mycos' meaning mold, and the Latin word 'toxicum', which means poison. Mycotoxins are relatively low-molecular weight secondary metabolites (after the growth phase) of fungal origin that are capable molecules, at lowconcentrations, to induce a toxic effect. These are metabolites side effects produced by the fungus (Boudih., 2013).

The genus Alternaria includes both plant-pathogenic and saprophytic species, which may affect crops in the field or cause harvest and postharvest decay of plant products.Species of Alternaria

are known to produce many metabolites, mostly phytotoxins, which play an important role in the pathogenesis of plants.

The major problems associated with Alternaria mycotoxin contamination of agricultural products are illustrated by focusing on various crops and their relevant diseases, e.g. black rot of tomato, olive, and carrots; black and grey rot of citrus fruits; black point of small-grain cereals; and Alternaria diseases of apples.(Logrieco et al., 2009).

a) Alternariol :

Alternariol is a benzochromenone, It is the most important mycotoxin produced by the black mold Alternaria species, which are the most common mycoflora infecting plants worldwide.(PubChem., 2005).

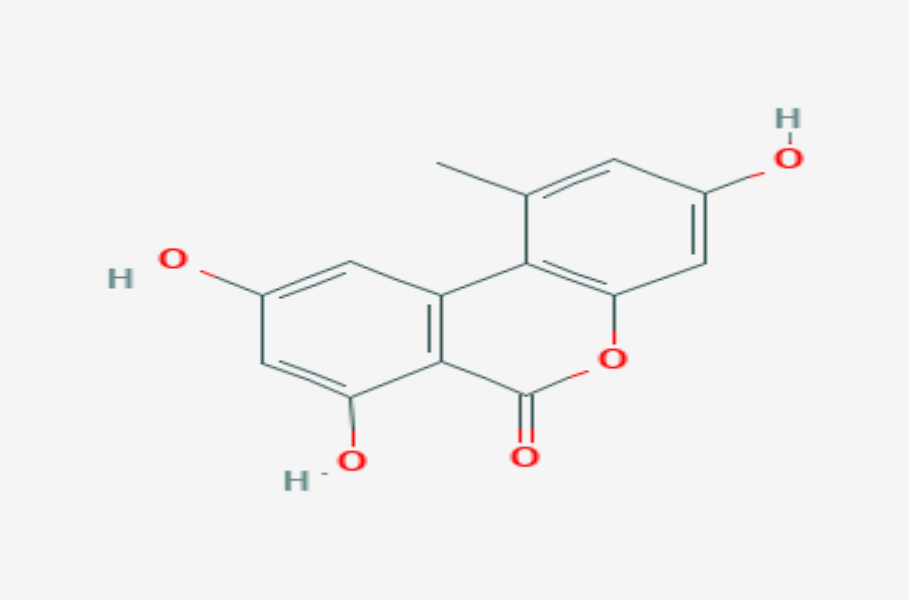

Figure 12: Chemical structure depiction of alternariol mycotoxin. (PubChem., 2005).

b) Alternariol monomethyl ether:

Alternariol monomethyl ether (AME) is a mycotoxin originally isolated from *Alternaria brassicae* extracts. AME is a common contaminant in cereal grains such as wheat, barley, and sorghum that is cytotoxic to bacterial and mammalian cells. It also provokes DNA damage that induces mitochondrial permeability transition pore-mediated activation of apoptosis.2. (Pero et al., 2020).

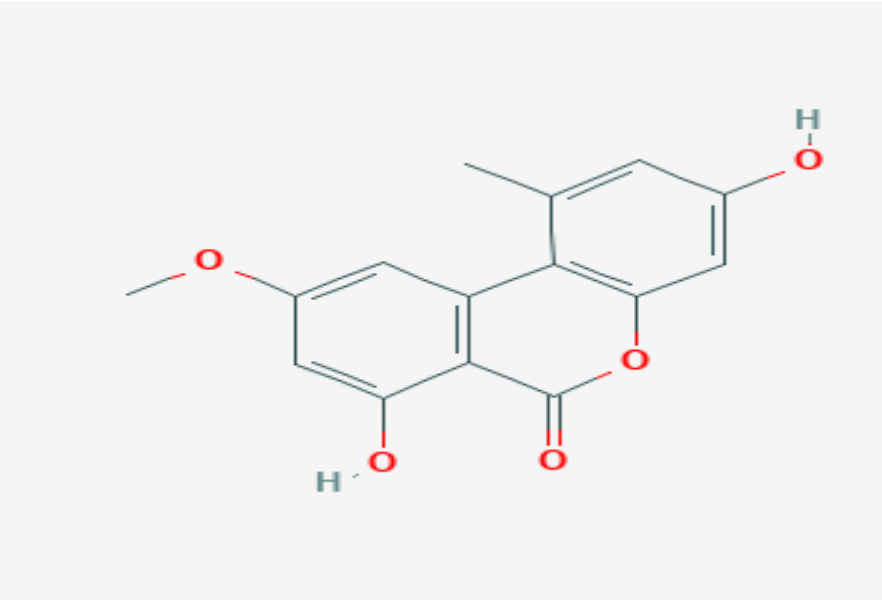

Figure 13: Chemical structure depiction of alternariol monomethyl ether. (PubChem., 2005).

IV. Means of control:

1. Chemical control:

- To mainly use an antifungal treatment. This fungicide is administered at a fixed rate. An anti-mildew contact fungicide is used on the current culture.
- To treat the alternariose of the tomato when the disease is declared but better still to prevent it thanks to a preventive treatment, because its development is fast. The epidemic can indeed spread over the entire tomato crop.
- Apply preventive treatment at a sustained rate, especially in hot and humid regions where the risk of tomato Alternaria is high. This action prevents the disease from spreading. To protect the following crops, a contact fungicide is used to treat seeds. It is also essential to disinfect the soil (Ooreka., 2020).

2. Biological control of Alternaria:

a) Bacteria:

Bacillus subtilis is used for prevention on many vegetable crops as well as in orchards. It constitutes a physical barrier preventing the spores of *Alternaria spp*. to settle on treated plants. In addition, this bacterium secretes molecules that inhibit the germination of spores and all the development of the fungus. It also plays a role in strengthening the resistance of plants.

b) Fungi:

Aureobasidium pullulans is a fungicide tested against *A. alternata*, working by "nutritive and spatial competition (Gamm vert., 2018).

3. Genetic control:

This method consists in introducing resistance genes at the level of plants called trans-genetic plants. These genes are responsible for the synthesis of proteins capable of eliminating the parasite. (Si Mohammed., 2014).

4. Plant extracts:

The use of plant extracts and natural products is highly encouraged, as these products are safe for health and do not cause pollution. Several laboratory studies carried out on different plant tissues, such as roots, leaves, seeds and flowers have shown that plant extracts have inhibitory properties against bacteria, fungi and insects. These unconventional products have been tested with more or less success in order to induce resistance in tomatoes, in particular to Alternaria. In the same register, various plant extracts, vegetable oils (*Acacia concinna* (climbing shrub),

garlic, *Azadirachta indica* (neem tree) ...etc.) would limit the development of this parasite. (Bessadat., 2013).

Material and methods

Material and Methods:

I. Sampling process:

First, we had to do a **sampling** process, which my friend did from Sidi Lakhder (district number 10) and Achaacha (district number 01) from the Wilaya of MOSTAGANEM, which is a coastal Algerian state, located in the northwest of the country. The capital is Mostaganem.

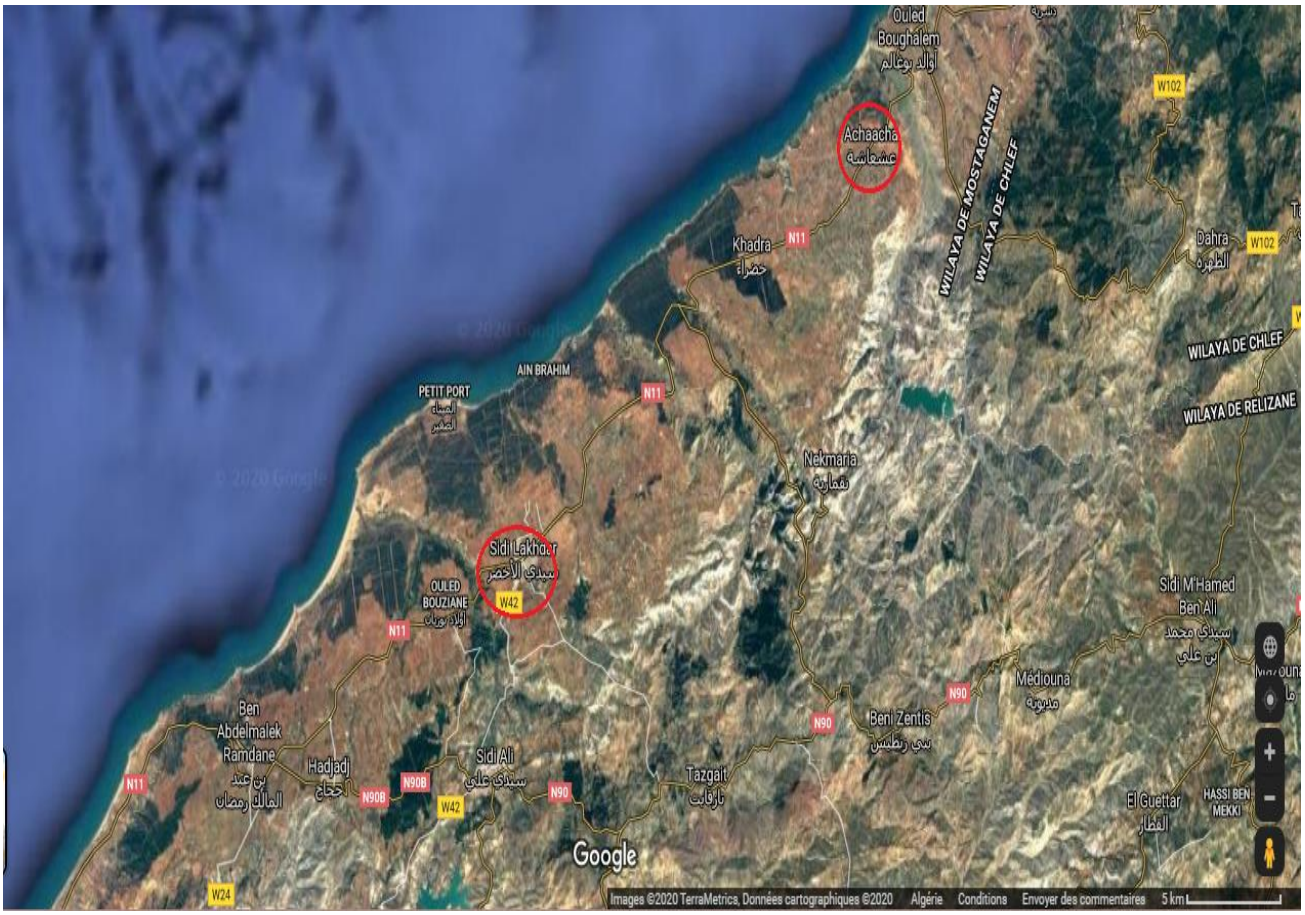

Figure 14: Sampling location: Achaacha and Sidi Lakhder. (Google Maps., 2020).

II. Isolation of the parasite:

we isolatedthe fungi from the stems , leaves, roots, and fruit of host plants (carrots, tomatoes) that showed changes such as yellowing, browning, and blackening (figure(15,16,17)),. By cutting, the infected areas using a sterile scalpel blade into a small fragment (1 to 2 cm), after that we emerged the fragments in bleach for 3 minutes to remove the saprophytic microflora. The next step is rinsing them 3 times with sterile distilled water (3 minutes each), then drying them on a sterile blotting paper.

The last step was the deposit of the fragments on the Petri dishes that contains a culture medium (MEA or PDA) and incubate them for 4 to 7 days at 27°C (figure (18, 19)).

Figure 15: Tomatoes collected from Achaacha.

Figure 16: Carrots collected from Achaacha.

Figure 17: Tomatoes collected from Sidi Lakhder.

Root Leaves Fruit

Figure 18: Isolated samples from carrot plant.

stem Leaves Fruit

Figure 19: Isolated samples from tomatoes plant.

III. Successive transplanting process:

After a week of incubation, mycelial filaments appear around the small fragments of the sample (stems, leaves, and roots). From there, we then proceed to the search for Alternaria. After a primary identification, we carry out successive subcultures in new Petri dishes containing PDA ATB medium (Gentamicin) to obtain pure cultures.

With a sterile handle (anse), cut a small square from the previous culture medium (which contains a culture that looks identical) and put it in a reverse way on a new medium that contains an antibiotic, in order to obtain more pure colonies after another week of incubation at 27°C.

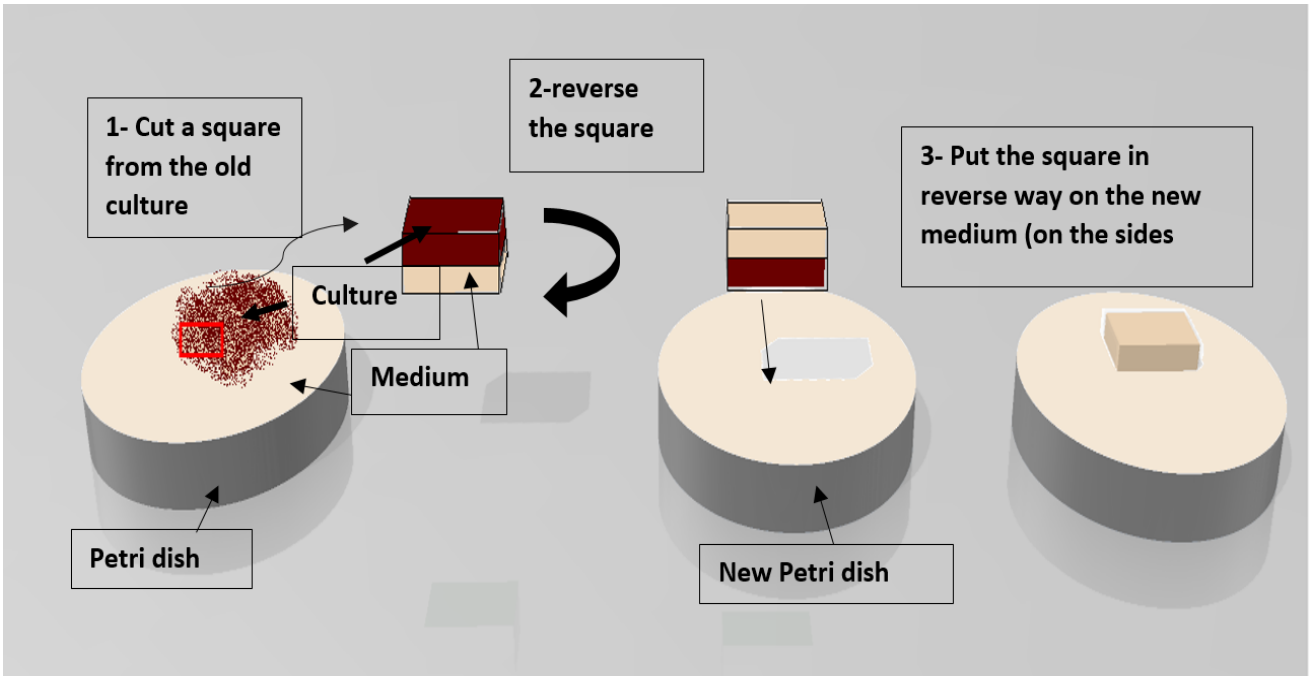

Figure 20: Transplanting process.

However, the crops obtained risk of being contaminated by bacteria and by fungi, which are sometimes harmful, in order to avoid this risk. The simplest and safest process remains that of monosporic culture.(Unfortunately, we were not able to complete the work in the laboratory due to the Coronavirus pandemic,and we had to do the work from home).

IV. Monosporic culture:

The technique of monosporic culture allows obtaining a pure culture from fungal spores by spreading on GN.

First, transplant the strain to monosporic in a Petri dish containing PDA medium and allow it to develop on the entire surface of the dish for 5 to 6 days.

Take an explant from the side of the dish and introduce it into a tube containing 9 ml of sterile distilled water after shaking, a sporal suspension is obtained.(Si Mohammed., 2014).

Dilutions to the tenth are made from the sporal suspension, which is introduced into a tube containing 9ml of sterile distilled water and then stirred.

Repeat the operation as many times until the desired dilution.

From the last dilution 10^{-3} and 10^{-4}, we take 1 ml, which is spread using sterile bile on a medium (usually GN).

After 24 h of incubation at 28°C using a binocular magnifier, we proceed to the identification and elimination of germinating spores, we take 3 to 4 conidia which are placed in a Petri dish containing the PDA or PSA medium. (Si Mohammed., 2014).

In order to identify it, first with the macroscopic observation using the bare eye to describe the morphology by noting the aspect color texture... etc.

V. Evaluation ofmacroscopic morphological characters

Observations are carried out 5 to 7 days on PSA. The macroscopic examination including the morphological characteristics of the colony such as the nature of the mycelium (color and appearance of the thallus) are carried out in order to identify some specific characteristics. Thus the mycelium can be short, undeveloped, velvety in appearance, not very fluffy or airy to cottony and brown in color, olive to light or dark gray depending on the species and the morphotypes. Even if the macroscopic characters of the colonies seem to be distinct, it is difficult to identify these pathogens on these criteria alone. In addition, it is difficult to separate pathogenic species from saprophytic species. (Bessadat., 2013).

VI. Microscopic observations:

The microscopic observation (using an optical microscope) will help to refine the diagnosis, revealing the characteristics of the spores

The most used methods are:

1. The direct observation using an adhesive tape on the surface:

- Place a piece of an adhesive tape on the sample, scrape lightly and gently remove the tape;

- On a microscope slide, place the piece of tape containing the structures taken;

- Observe the slide under the microscope (Kaba, 2018).

2. The microscopic examinations of the fungus were carried out by directly observing the mycelium (taken from a culture) between slide and coverslip in a drop of cotton blue and which is brought to microscopic observation at different magnifications in general (x10 and x40). (Belalia and Ramdani., 2019).

After comparison with Identification key whichis a printed or computer-aided device that aids the identification of biological entities, taking into considerationthe host plant, we can say that the fungi we have is Alternaria.

VII. Characterization tests:

Last but not least, we were supposed to perform the following tests:

Temperature test, pH test, different medium test, source of carbon test, source of nitrogen test, by changing the rate of each one of them, in order to know their effect on the growth of the fungi.

1. Effect of different culture media:

The biometric characterization is carried out in order to assess the best conditions for the growth of the strains.

The effect of six different solid culture media is studied on isolates from the Alternaria and group. The environments; PCA, Malt Agar, PSA, Czapek dox, Mathur and Sabouraud, are poured at a rate of 15ml per petri dish. After solidification, the dishes are inoculated with a 5

mm mycelial disc from the periphery of cultures aged seven days from each isolate and incubated at 25 ± 1 ° C. The appearance and pigmentation of the colonies are observed by visual observation after 7 days for the study of cultural characteristics. (Bessadat., 2013).

2. Effect of different sources of carbon and nitrogen:

The behavior in vitro in the presence of several sources of carbon and nitrogen on the mycelial growth and the sporulation of the different groups of Alternaria more or less reflects their natural nutritional differences. The production of enzymes (amylase, cellulase ...) allows certain species to degrade nutrient sources into assimilable compounds.(Bessadat., 2013).

In this work, several sources of carbon and nitrogen are tested in vitro on the growth and the mycelial density of species responsible for the anomalies. The basic medium used is that of Czapek dox modifying the source of nitrogen (N) or carbon (C). The contents of C and N are kept constant: they are 3% (v / v) of C and 0.2% (v / v) of N present in the basic medium. The carbonaceous and nitrogenous solutions are added to the medium before autoclaving. Eleven carbon sources are tested including: monosaccharides (glucose and fructose), disaccharides (sucrose, lactose and maltose), polysaccharides (soluble starch and cellulose), alcohols (glycerol and mannitol) and an organic acid (citric acid). Eight sources of nitrogen are also tested, inorganic nitrogen [KNO, NANO, (NH,), SO] and organic nitrogen including: amino acids (asparagine, arginine, valine and leucine) and proteins (peptone). The carbonaceous and nitrogenous compounds are added and the pH adjusted to 6.5. Then sterilized in an autoclave for 30 min at 120°C. The dishes containing 15ml of each medium are inoculated in the center by a 5mm mycelial disc taken from a culture aged seven days and then incubated in the dark and at 25 ± 1°C (Bessadat., 2013).

3. Effect of temperature and pH:

The most important environmental factors that govern the growth and sporulation of fungi are temperature and the concentration of hydrogen ions (pH). A slight variation of these factors can induce marked differences in their morphological characters, growth and sporulation.

The behavior of the fungus as a function of different temperatures is studied, for this purpose; petri dishes containing the PSA medium are inoculated with the isolates. The dishes are incubated at different temperatures: 5, 10, 20, 25, 30 and 35 ° C. Tolerance to different pH values differs considerably in some Alternaria species, their growth can be completely inhibited in media that are too acidic or too alkaline. In order to test the influence of the latter on the growth and sporulation of the different groups of isolates, PSA media are initially adjusted to a pH range from 4.0 to 10.0 using hydrochloric acid (HC1 0.5N) or an alkaline solution of sodium hydroxide (NaOH 0.5N), the inoculated petri dishes are incubated at 25 ± 1°C (Bessadat., 2013).

Results and Discussion

Results and Discussion

I. Isolation results:

The results of isolation after one week of incubation:

Figure 21: Petri dish that contains a sample of the upper part of a carrot.

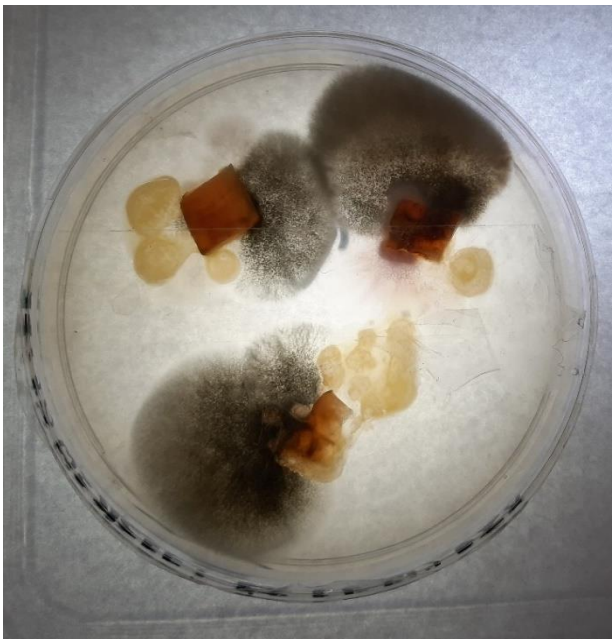

Figure 22: Petri dish that contains a sample of the center part of a carrot.

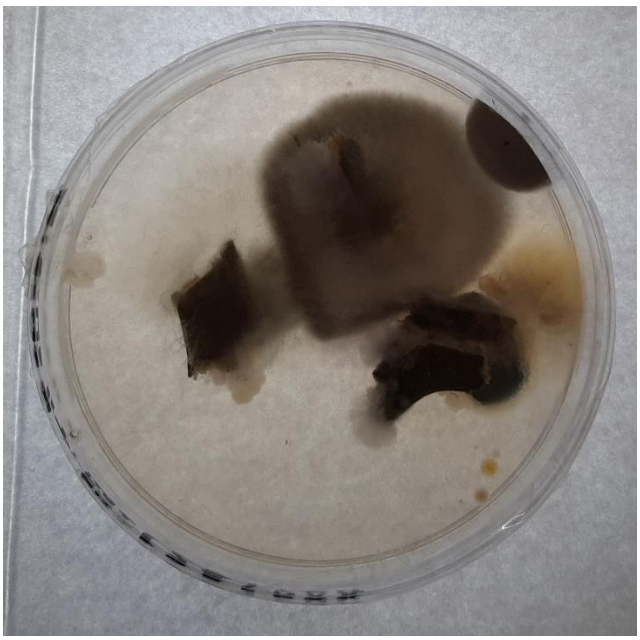

Figure 23: Petri dish that contains a sample of tomatoes' stem.

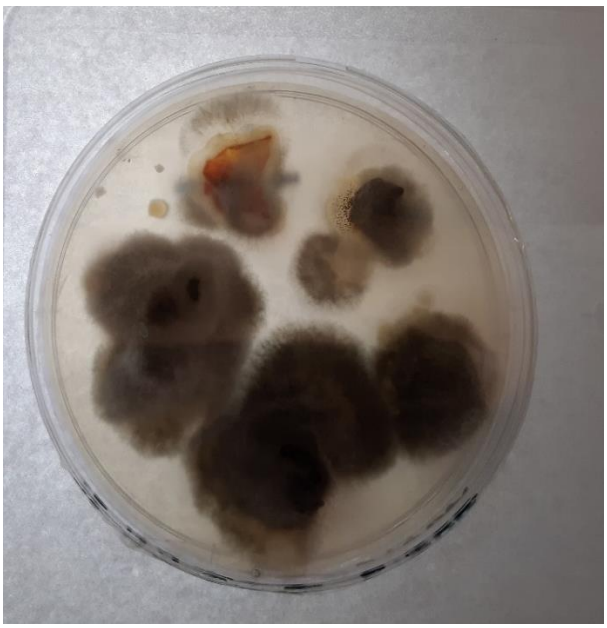

Figure 24: Petri dish that contains a sample of tomatoes' fruit.

II. Transplanting results :

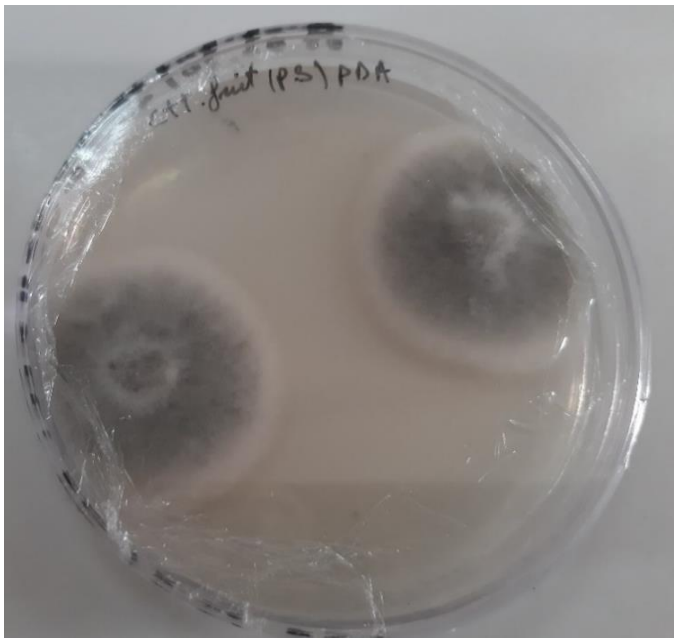

Figure 25: Front view of a petri dish that contains fragments of a previous culture of a carrot (upper part).

Figure 26: Back view of a petri dish that contains fragments of a previous culture of a carrot (upper part).

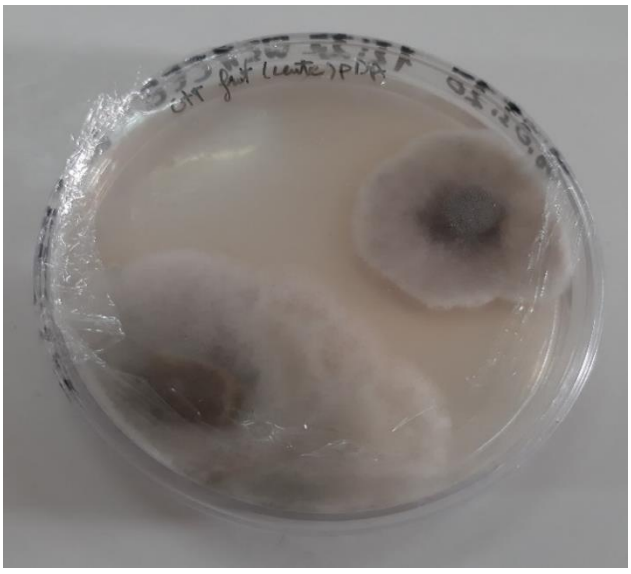

Figure 27: Front view of a petri dish that contains fragments of a previous culture of a carrot (center part).

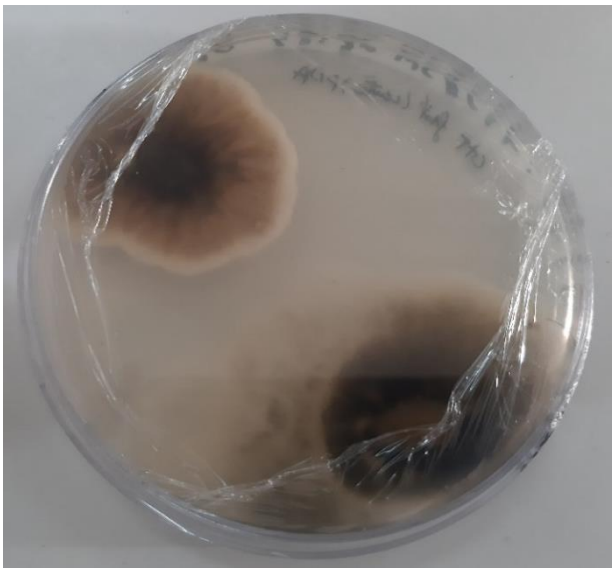

Figure 28: Back view of a petri dish that contains fragments of a previous culture of a carrot (center part).

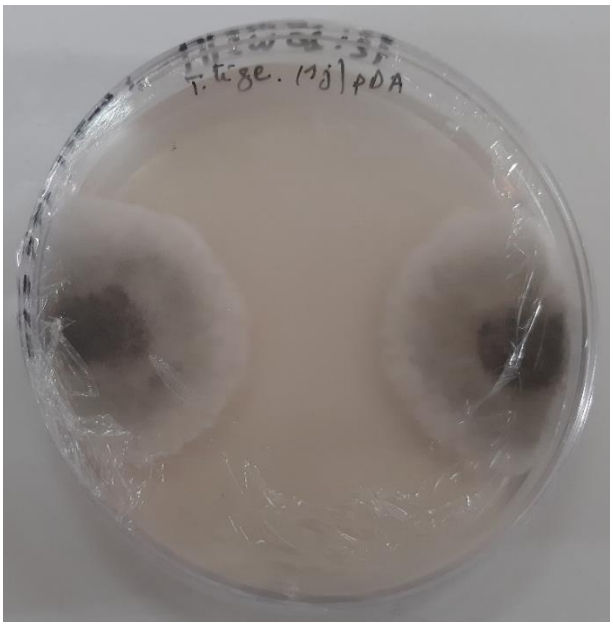

Figure 29: Front view of a petri dish that contains fragments of a previous culture of a tomatoes' stem.

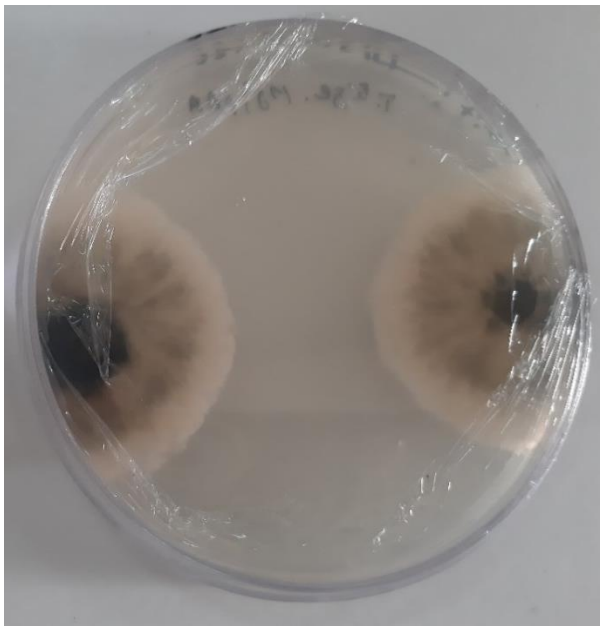

Figure 30: Back view of a petri dish that contains fragments of a previous culture of a tomatoes' stem.

Figure 31: Front view of petri dish that contains fragments of a previous culture of a tomatoes' fruit.

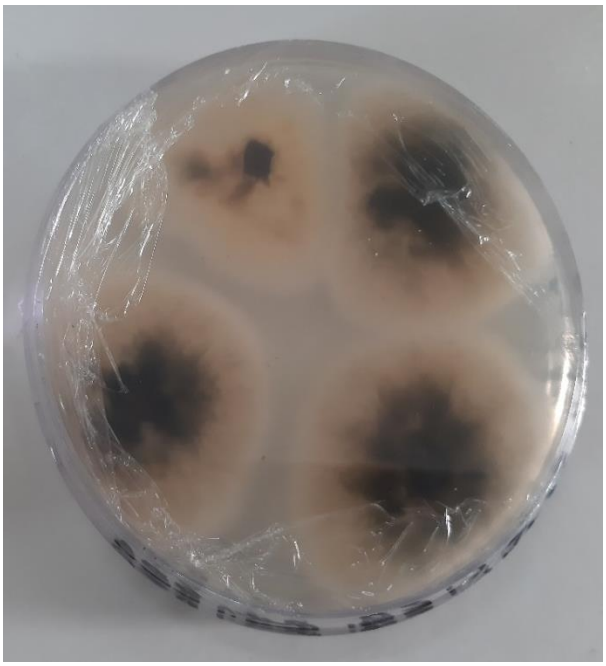

Figure 32: Back view of a petri dish that contains fragments of a previous culture of a tomatoes' fruit.

III. Macroscopic aspect study: The macroscopic study of several isolates purified after 8 days of incubation on PDA medium, made it possible to present a considerable variation between the morphological characters, the color of the colony varies from light to dark to an olive-green and greyish tint.

The majority of colonies have a fluffy or cottony appearance, with variations in mycelial growth and regular and irregular borders (Belalia and Ramdani., 2019).

IV. Microscopic examination of conidia:

Identification is based on morphological characteristics of hyphae and asexual reproductive organs. The conidia vary frompyriform to oval or oblique, yellowish to dark brown in color, the mycelium is septate of light green color.(Belalia and Ramdani., 2019).

They are shown in the following figure:

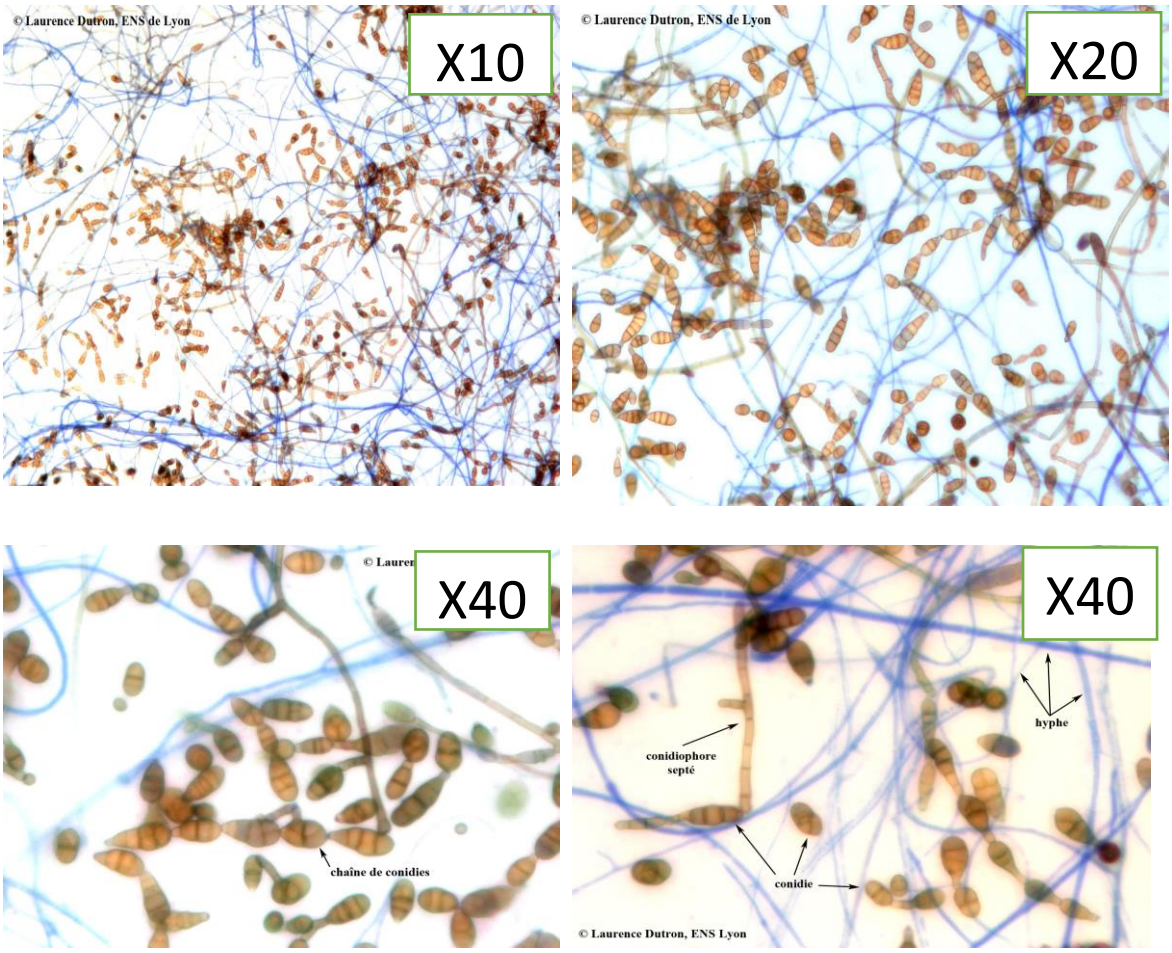

Figure 33: Microscopic observation of *Alternaria sp* with different magnification (Laurence., 2012).

V. Results of using different culture media :

The growth and sporulation of the selected isolates are evaluated on different culture media (figure (34)). A difference in the growth rate is observed in each species and each isolate. Relatively high growth rates are recorded indicating that Alternaria species are able to use a wide range of sources of carbon and other nutrients. Czapek's Dox medium supported maximum growth (mean: 7.888 ± 0.723 mm / day) in the majority of isolates, but rather poor sporulation (2.166 ± 0.982 $.10^5$ spores / mm^2) which may be due to the presence of 'chloride ions. The growth rate on PCA medium is almost similar with an average of 7.763 ± 0.425 mm / day and a minimum fungal sporulation rate (average: 0.450 ± 0.207. 10^5 spores / mm^2). On the other hand, a poor mycelial growth rate is obtained on malt extract medium (average: 3.902 ± 1.141 mm / day) which, on the other hand, supported maximum sporulation (average: 16.721 ± 6.915. 10^5 spores / mm^2), with variations between isolates. A poor sporulation rate (mean: 1.334 ± 0.581.10^5 spores / mm^2) and moderate mycelial growth (mean: 6.212 ± 1.072 mm / day) are observed on the Mathur medium. Nutrient-rich environments promote mycelial growth with the ultimate loss of sporulation. The Sabouraud and PSA circles supported both moderate mycelial growth (average: 5.501 ± 1.072 mm / day and 5.639 ± 0.766 mm / day, respectively) and sporulation is favorable for isolates with small spores (6.365 ± 3.618 $.10^5$ spores / mm^2 and 4.348 ± 3.064 10^5 spores / mm^2, respectively) (Bessadat., 2013). Similarly, (Zghair et al., 2015) reported that Czpek and PSA medium showed significant differences among others of growth 8.5 cm for colony diameter and decreased rate growth in other types of media Lower growth rate of 4.0 cm for colony diameter for Sabourd agar.

In conclusion, the influence of culture media revealed a negative correlation between the growth rate and the sporulation of the isolates. The composition of the medium therefore constitutes an important physiological parameter, which significantly affects mycelial growth and the production of conidia in isolates (Bessadat., 2013).

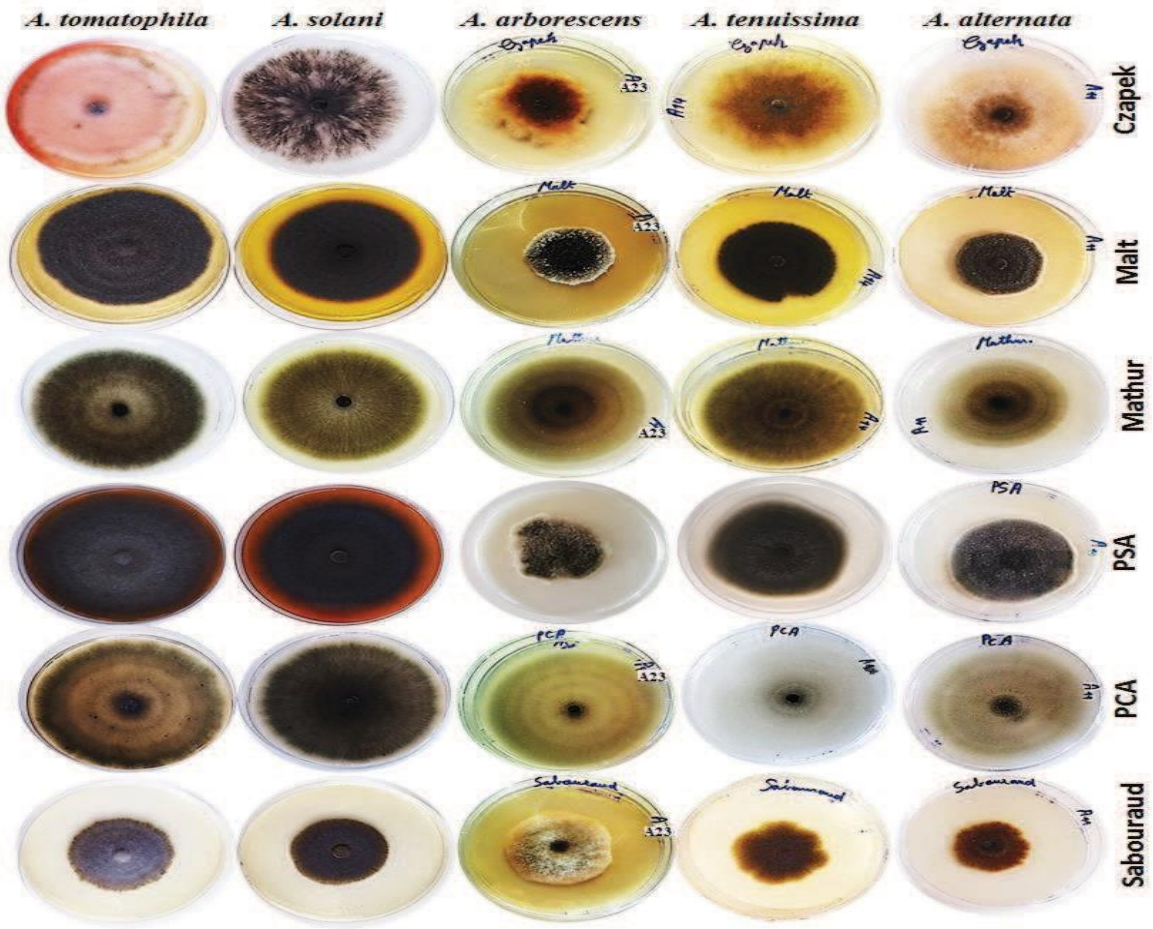

Figure 34: Appearance of *Alternaria sp* grown on different culture media. (Bessadat., 2013).

VI. Effect of carbon and nitrogen sources:

In this study, the effect of ten carbon sources (figure (35)) and eight nitrogen sources (Figure (36))is tested in vitro on modified Czapek base medium. Although the type of carbon source has few effect on the recorded growth rates, sporulation was much more affected by this parameter, and varied from $0.382 \pm 0.244.10^5$ spores / mm^2 on citric acid and $2.575 \pm 2.88.10^5$ spores / mm^2 on lactose, this results were mentioned by Bessadat (2013). Also, her results have shown that sugars are the best source of carbon, and glucose is the first one, followed by maltose secondly, while starch is third.

For alcohol, mannitol is the best source.

And for acids (less preferred), because they have not witnessed significant growth, such as that of citric acid.

Those results are similar tothe results of Taware et al., (2014) who found that The carbon sources exhibited varied radial mycelial growth and sporulation of the test pathogen. However,

highest radial mycelial growth (86.00 mm) and excellent sporulation was recorded on glucose, followed by on maltose (82.83 mm) and starch (80.33 mm) with excellent sporulation.

Contrary to what is recorded for carbon sources, a higher effect of the nitrogen source on the growth parameters is observed. The mycelial growth of isolates on different sources of nitrogen was found to be higher on two inorganic sources; sodium nitrate (7.92 ± 0.721 mm / day) and potassium nitrate (7.768 ± 0.813 mm / day) with moderate sporulation (1.58 ± 1.298 $.10^5$ spores / mm^2 and 2.041 ± 1.558 $.10^5$ spores / mm^2, respectively). Contrary to the results presented by (Gholve et al., 2015), they found that the results revealed that of the six nitrogen sources tested (table (03)), Potassium nitrate was found most suitable and encouraged maximum radial mycelial growth (82.55 mm). The second best nitrogen source found was Peptone (75.83 mm) and this was followed by Sodium nitrate (70.16 mm), Ammonium nitrate (44.83 mm) and Thiourea (26.16 mm). Urea was found least suitable which recorded minimum radial mycelial growth (19.00 mm) over the control (65.33 mm) of the test pathogen.

Table 3: Effect of different nitrogen sources on mycelial growth and sporulation of *Alternaria sp*. (Gholve et al., 2015).

Treatments	**Mean Colony Diameter(mm*)**	**Sporulation**
urea	19.00	Poor
Potassium nitrate	82.55	Excellent
Peptone	75.83	Good
Sodium nitrate	70.16	Good
Ammonium nitrate	44.83	Moderate
Thiourea	26.16	Moderate

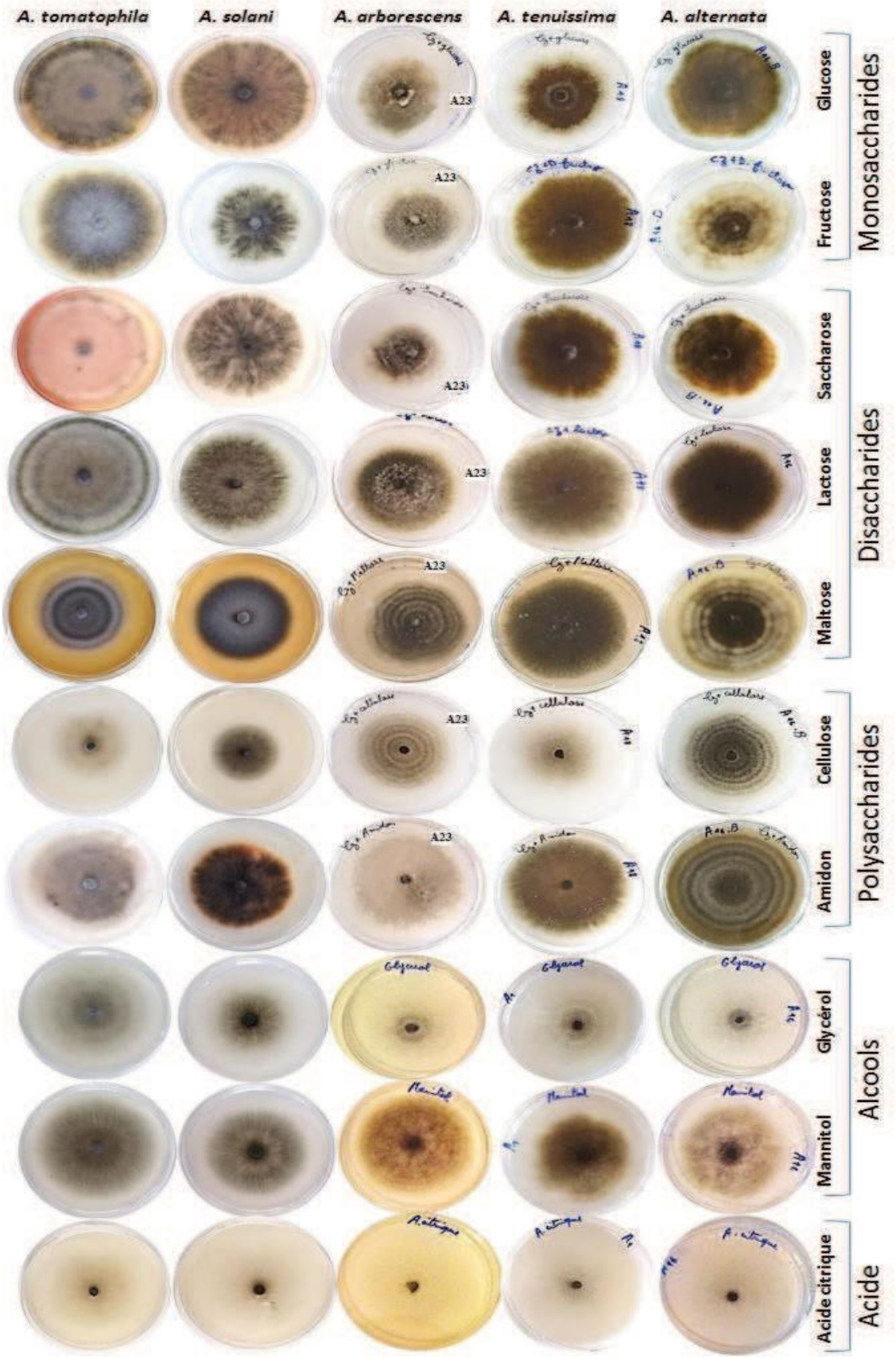

Figure 35: Appearance of colonies of *Alternaria sp* grown on ten carbon sources on modified Czapek medium. (Bessadat., 2013).

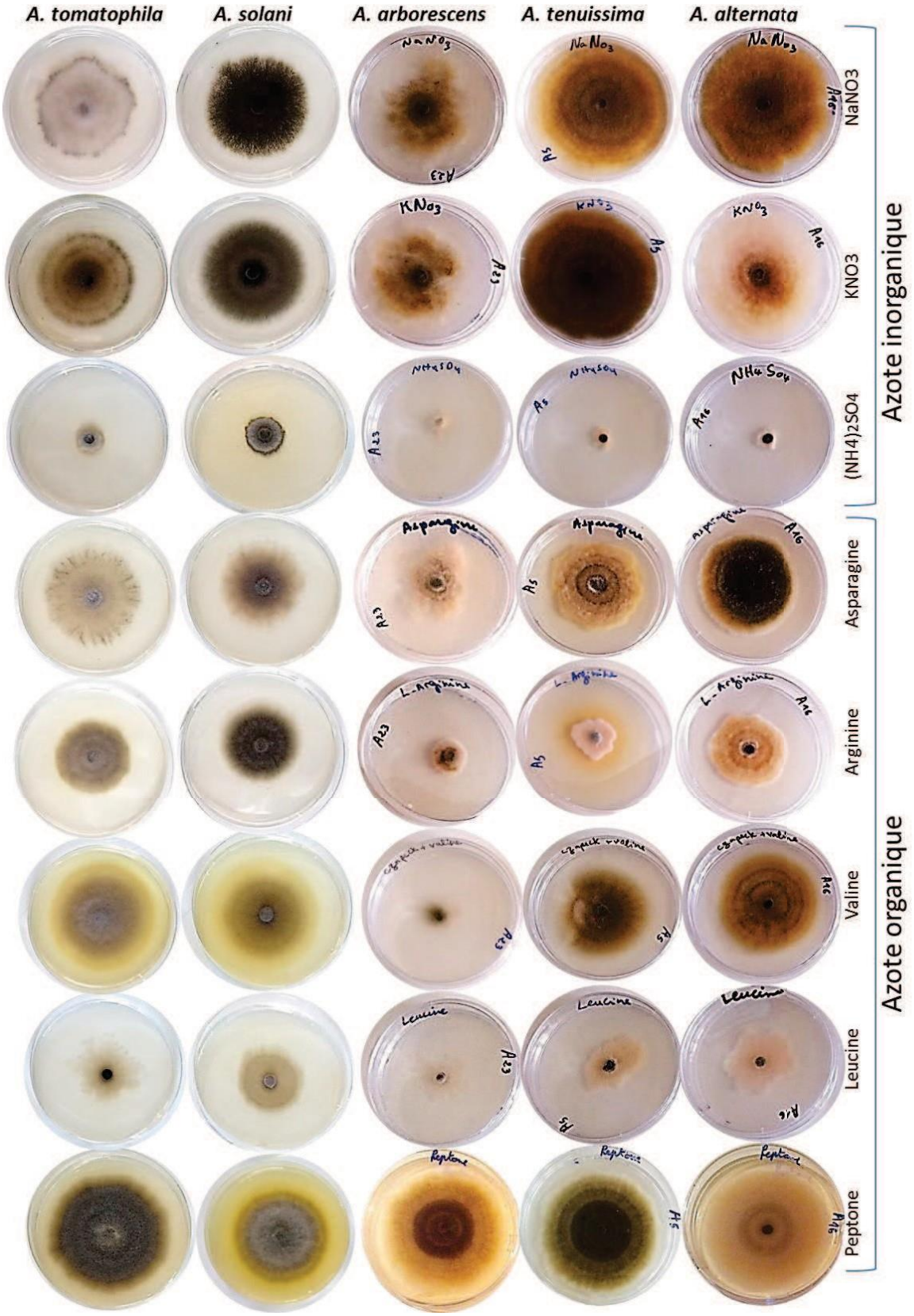

Figure 36: Appearance of colonies of *Alternaria sp* cultivated on eight nitrogen sources on modified Czapek medium. (Bessadat., 2013).

VII. Effect of pH and temperature:

The impact of the medium's pH on mycelial growth (Figures (37)), and sporulation of the isolates is studied in a pH range of 4 to 10. The results show that, generally, the fungal growth is favorable at a neutral or slightly acidic pH, the optimal pH for the growth of isolates is between 6 and 8 (approximately 6.6 mm / day). PH values below 6 and above 9 have led to slower mycelial growth, a minimum growth rate is recorded at pH 10 (5.971 ± 1.183 mm / day). (Bessadat., 2013). Similar observations have been reported by several authors: (Rajendra Kumar et al., 2019) who found that the maximum colony diameter, dry weight were observed at 6.5 pH level followed by 6.0 pH level, while minimum colony diameter and dry weight were observed at 8.5 pH. Excellent sporulation was observed at pH level of 6.0 to 7.0, while poor sporulation was recorded at 4.0, 8.0 and 8.5 pH level.

Even (Zghair et al., 2015) found the same results also, they mentioned that pH 6 giving 8.5 cm for colony diameter and a difference was significant at the pH≤ 0.05 for other pH when the pH was 8 less than the growth rate 4.3 cm for colony diameter.

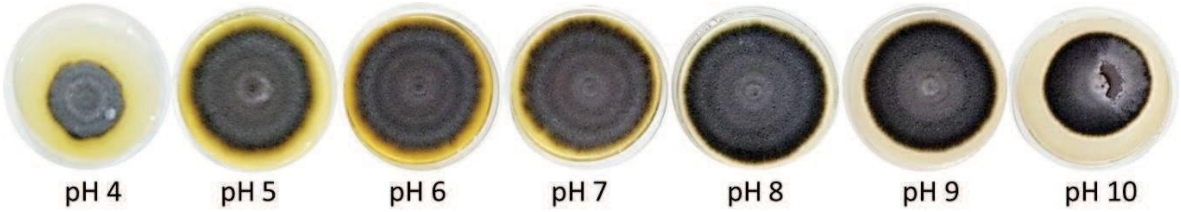

Figure 37: Effect of different pH on the mycelial growth of *Alternaria sp* after eight days of incubation. (Bessadat., 2013).

Temperature is often considered the most important physical environmental factor for regulating the growth and reproduction of fungi. The majority of isolates developed well at a temperature of 30 ° C (7.561 ± 0.692mm / day), followed by 25 ° C (6.999 ± 0.835mm / day). The lowest growth is observed at 35 ° C (1.227 ± 0.738 mm / day). In this study, it is clear that temperatures ranging from 25 to 30 °C are optimal for the growth of species in the alternata section. . (Bessadat., 2013).

Contrary to what (Jaggal et al., 2013) found, they found that Maximum mycelial growth (37.47mm) and sporulation (13.80 x 10^6spores/mL) was observed at 25ºC. A sudden fall in mycelial growth and sporulation was observed at 30ºC and 35ºC. However, 20ºC and 30ºC also favored good growth and sporulation of *Alternaria solani* but differ significantly from growth at 25ºC. It can be concluded that 25ºC is the optimum temperature for mycelial growth and sporulation of Alternaria sp, in the present study, result showed that maximum growth and

sporulation of fungus were observed at 25°C followed by 30, 20, 35 and 15ºC. These results are in agreement with the publication of (Rajendra Kumar et al., 2019), they witness that maximum colony growth was recorded at 25°C followed by 30oC and minimum colony growth was recorded at 40ºC. Excellent sporulation was noticed at 20°C and 25°C temperature. However, no sporulation was found at 40°C temperature.

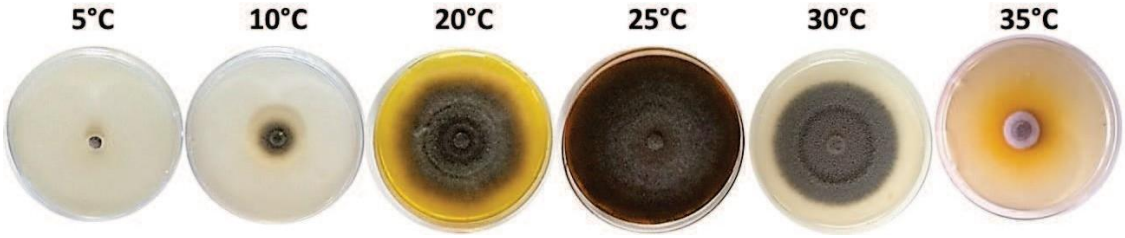

Figure 38: Effect of different temperatures on the mycelial growth of *Alternaria sp* after eight days of incubation. (Bessadat., 2013).

Conclusion

Conclusion:

This study consisted of characterizing isolates of the phytopathogenic fungus of the species *Alternaria sp.* Causative agent of various diseases of tomatoes and carrots.

This characterization was based on the morphological and cultural aspects of *Alternaria sp.*

Cultural aspects are based on colony color, mycelium type, mycelial growth and sporulation;the morphological aspects are based on the shape of the conidia.

Morphological and cultural characterization showed a broad spectrum in colony color and mycelium appearance. Both Sporulation and mycelial growth exhibited variability depending on the used culture media; sources of carbon and nitrogen, and according to different degrees of pH and temperature.

This study revealed results that may help to reduce the spread of *Alternaria sp* and protecting crops and fields from its infection and damages, by knowing and understanding the necessary conditions for its proliferation to contribute and avoid them as much as possible, through the creation of an unfavorable environment.

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Annexes

Annex 01: Composition of Czapek's Agar (CZA)

Ingredients	**g/litre**
Sucrose	30.000
Sodium nitrate	2.000
Dipotassium phosphate	1.000
Magnesium sulphate	0.500
Potassium chloride	0.500
Ferrous sulphate	0.010
Agar	15.000

Final pH (at 25°C) 7.3±0.2. (Sagar., 2019).

Annex 02: Composition of Potato Dextrose Agar (PDA):

Ingredients	**g/L**
Potatoes, infusion from	200.0
Dextrose	20.0
Agar	15.0

Final pH (at 25°C) 5.6±0.2. (Sagar., 2018).

Annex 03: Composition of Malt Extract Agar (MEA):

Ingredients	**g/L**
Malt Extract	30.0
Mycological Peptone	5.0
Agar	15.0

Final pH 5.5 +/- 0.3 at 25ºC.(HiMedia laboratoires., 2019).

Annex 04: Composition of Plate Count Agar (PCA):

Ingredients	**g/L**
Enzymatic Digest of Casein/tryptone	5.0
Yeast Extract	2.5
Glucose	1.0
Agar	15.0

Final pH 7.0 ± 0.2 at 25°C. (Sagar., 2019).

Annex 05: Composition of Peptone-Sucrose-Agar (PSA):

Ingredients	**g/L**
Ca (NO3)	0.35
FeSO4	0.35
Na2HPO4	1.4
Peptone	3.5
Sucrose	14.0
Agar	1.5
Distilled water	700

Final pH 6.8. (Mehta et al., 2005)

Annex 06: composition of Sabouraud medium:

Ingredients	**g/L**
Mycological peptone (enzymatic digest of casein and animal tissues)	10
Dextrose	40
Agar	15

pH adjusted to 5.6 at 25°C. (Rijal., 2015).

Annex 07: Reagents of Lactophenol Cotton Blue (LPCB) Staining:

- A preparation of 50ml Lactophenol cotton Blue staining solution is made up of:
- Distilled water 50ml
- Cotton Blue (Aniline Blue) 0.125g
- Phenol Crystals ($C_6H_5O_4$) 50g
- Glycerol 100ml
- Lactic acid ($CH_3CHOH\ COOH$) 50ml
- 70% ethanol. (Mokobi., 2020)