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RESEARCH ARTICLE

# Identification and Characterization of Citrus Fungal diseases in the Wilaya of Jijel

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**ABSTRACT**

Non-compliance in the maintenance and management of citrus orchards for various reasons, causes very significant damage by pests and parasitic diseases, among others, fungal, some of which are very serious, leading to the decline of the tree. This study aimed to isolate and identify fungal strains contaminating citrus orchards in the wilaya of Jijel. We took 300 samples from different parts of the trees (root, trunk and leaf) in different sites. The isolation and purification of fungal strains were carried out on the PDA medium. The identification of isolates was done on the basis of morphological and molecular criteria. A total of 59 fungal strains were isolated; The 6 species identified are: Fusarium sp (67.80%), Phythophthora sp (6.78%), Alternaria sp (1.70%), Diplodia sp (3.39%), Mortierella (3.39%) and Trichoderma (16.94%). In short, this study showed that citrus trees in the wilaya of Jijel are contaminated by a diversity of fungal species.

## INTRODUCTION

Citrus fruits are of vital interest to a large number of countries due to their economic importance. They generate appreciable income through their marketing as fruits and as various derivatives such as juice, jam etc. (Mutin, 1977 in Biche et al. , 2012).

Microbial plant pathogens, including fungal pathogens, have the capacity to infect a small number or a large variety of plant species, causing quantitative and qualitative losses of varying magnitude in crops grown in different ecosystems.

Global losses caused by crop diseases have been estimated at between 9 and 14.2% of potential yield (Narayanasam P,2011). Subsequent loss estimates indicated that about 14.1% of production could be lost due to crop diseases, for a monetary value of $220 billion per year, with developing countries suffering more losses than developed countries (Narayanasam P, 2011).

Algeria, due to its geographical location, climate and the quality of its production, can rightly claim to occupy a prime position on the world markets. Indeed, traditionally an exporter of citrus fruits, the country was one of the major citrus producing countries in the Mediterranean basin: in 1960, citrus fruits represented 20% of the value of agricultural production (Mutin, 1977 in Biche et al., 2012). This shows that citrus growing in Algeria experienced good years after independence until the 1980s. It has enormous potential in terms of citrus production.

### The Importance of Citrus Fruits

From an economic point of view, citrus fruits represent the world's leading fruit production with 95 million tonnes produced per year. The Mediterranean basin alone represents 20% of production with a major producer: Spain.

With an annual production of 23.6 million tonnes (FAOSTAT 2021), Mediterranean citrus growing is an important sector of agricultural production in the world. It is therefore a question of protecting this primacy, not only from competition, but also from new parasites and changes in operating conditions imposed on us by climate change. (Minuto G et al, 2022)

Citrus fruits are among the basic elements of agricultural production to meet food needs due to population growth and increasing demand for their products, as well as to provide many raw materials for the food, medical and cosmetic industries, citrus fruits have nutritional value and great economic importance, and the economic importance of citrus fruits lies in the multiplicity of species and varieties to which they belong, whose fruits ripen at different stages of the year, which makes their fruits available on the market most of the time (Allaf, 2020)

Citrus fruits have great economic and commercial importance. They are many varieties and forms, and citrus fruits produce different types of fruits at different stages of the year. Citrus fruits are cultivated and their fruits are marketed in many parts of the world, such as Brazil, China, India, the United States of Mexico, the Maghreb, Egypt, Syria, Algeria, Palestine, Jordan and Lebanon (Al-atoum, 2021)

The economic importance of citrus fruits is reflected in the multiplicity of uses of their fruits and consumption methods. Where citrus fruits are consumed fresh and various types of juices, jams and sweets. The economic importance of citrus fruits increases by extracting volatile oils from their leaves, flowers and fruit peels, as these oils are used in the manufacture of perfumes and cosmetics, and the wood of citrus fruits is also used after the production stops in order to obtain a good amount of wood which is used in the heating process (Al-atoum, 2021).

In cultivation, citrus fruits are very sensitive to crypto gamic diseases which constitute a significant part of this drop in yield and cause enormous damage and influence the profitability of Algerian and partly Jijelian citrus orchards. It is in this context that we propose to isolate fungal strains from citrus trees and determine their pathogenicity.

### Citrus Pathologies

Crops are infected by one or more fungal pathogens at different growth stages. In addition, the propagules of the pathogen may be present in soil, water, air, natural vectors and other host plant species providing inoculum for newly planted crops. The incidence of previously unknown diseases may be observed from time to time. The nature of the cause of the newly observed disease should be determined immediately to minimize its further spread. (Narayanasamy, 2011)

## MATERIALS AND METHODS

The study was conducted in the orchards of the wilaya of Jijel with the aim of identifying the different fungal diseases of orchards. This study was carried out in different citrus growing areas (5 potential communes). It focuses on the citrus species cultivated by taking into consideration the following aspects: Identification and location of the species, the homogeneity of the orchard, the varieties of citrus fruits, the age of the orchards, the management of the orchards and the irrigation method used. A total of 30 orchards were surveyed.

**Table No. 1: Areas and distribution of citrus fruits in the wilaya of Jijel**

| Municipalities | Total area (Ha) | Total area (%) |
|---|---|---|
| Elmilia | 208,78 | 31,78 |
| Taher | 95,05 | 14,47 |
| Kaous | 74,2 | 11,29 |
| El Ancer | 69,5 | 10,58 |
| Chekfa | 66,5 | 10,12 |
| Sidi Abdelaziz | 49,75 | 7,57 |
| Settara | 30,75 | 4,68 |
| Sidi Maarouf | 23,38 | 3,56 |
| El Aouana | 22.98 | 3,50 |
| Texenna | 16.05 | 2,44 |
| Total | 656,94 | 100,00 |

**(Source: DSA.2024)**

In the wilaya of Jijel there are 656.94 Ha of citrus growing areas. In light of Table No. 1, it appears that the different citrus producing areas, most of whose orchards are located in the municipalities of El Milia (208.78 Ha), Taher (95.05 Ha), Kaous (74.2 Ha), El Ancer (69.5 Ha) and Chekfa (66.5 Ha); Sidi Abdelaziz, Settara, Sidi Maarouf, El Aouana and Taksana. In the wilaya of Jijel, the citrus growing areas reach 656.94 ha.

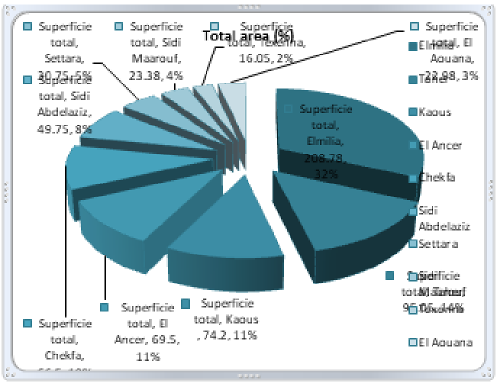


**Figure 1: Areas and distribution of citrus fruits in the wilaya of Jijel**

**Table No. 2: Area and distribution of varieties**

| Species<br>Common | Orange tree | Clementine tree | Lemon tree | Total (Ha) |
|---|---|---|---|---|
| Elmilia | 153 | 41,5 | 18 | 212,5 |
| Taher | 46,75 | 37,3 | 11 | 95,05 |
| Kaous | 38,6 | 20,25 | 15,35 | 74,2 |
| El Ancer | 42,5 | 12,5 | 14,5 | 69,5 |
| Chekfa | 30 | 23,5 | 13 | 66,5 |
| Sidi Abdelaziz | 16 | 17 | 16,75 | 49,75 |
| Settara | 19,5 | 2,25 | 9 | 30,75 |
| Sidi Maarouf | 9,61 | 3,38 | 6,67 | 19,66 |
| El Aouana | 11,1 | 0 | 11,88 | 22,98 |
| Texenna | 13 | 0 | 3,05 | 16,05 |
| Total | 380,06 | 157,68 | 119,2 | 656,94 |

**(Source: DSA, 2024)**

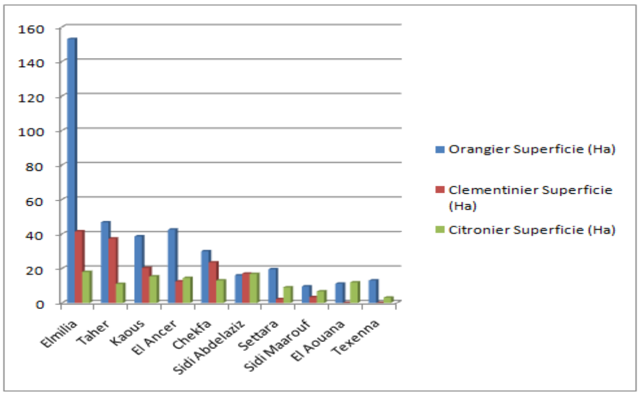


**Figure No. 2: Area and distribution of varieties**

## Comment

The citrus orchards of the wilaya of Jijel contain three species; oranges (Citrus sinensis), clementines (Citrus clementina) and lemons (Citrus limon) with a predominance of oranges (Thomson navel and Washington navel) followed by clementines and lemon. All regions contain the three species but always predominance of orange trees. These varieties are scratched on the bitter orange rootstock

**Diagnosis, Field Surveys and Epidemiological Study**

**Sampling**

This in-depth study on the incidence of citrus diseases and its severity is carried out in 5 different citrus growing regions of the wilaya of Jijel; El Milia, Taher, Chekfa, Elanser and Kaous. Successive observations are carried out during the 2017-2018, 2018-2019 and 2019-2020 seasons in the selected orchards, localities taken into consideration for each municipality. 6 orchards of 0.25 Ha to 0.5 Ha for each municipality were selected including 3 orange orchards, 2 clementine orchards and 1 lemon orchard according to the dominance of the species, density and age of the orchard (large, medium, small). 5 trees in each orchard were chosen diagonally, from these trees 15 samples were taken of the organs showing symptoms (trunks, roots and leaves).

The samples are taken according to the most frequent characteristics of the trees that appear in the orchards and that have anomalies compared to other healthy trees.

The characteristics monitored are: decline, wilting of the tree. Cankers on the trunk and branches, deformed parts of the leaves and drying of the branches especially at the ends of citrus trees.

We have taken into consideration some parameters such as the identification and location of species and varieties, the age of the orchards (trees), the management of the orchards, the nature of the cultivation operations, the equipment used and the periods of intervention, phytosanitary maintenance (products used and period of intervention), pruning, irrigation, etc. These parts are obtained from observations and surveys of farmers and sector managers.

The realization of these surveys will give an overview of the distribution of diseases as well as the presence of different fungal species in the main citrus producing municipalities in Jijel.

**Isolation from fragments of roots and collars and leaves**

The roots were washed with tap water, cut into small pieces of 1 cm in length, and placed on the surface of the culture medium (Ippolito et al. 1991)

First, the organelles (roots and collars and leaves) are cut into small fragments, rinsed with water and then disinfected with sodium hypochlorite. After rinsing with sterile distilled water and drying with sterile papaya filter, the fragments are placed in Petri dishes containing PDA culture medium to minimize contamination, the latter are incubated at 25C° for one week (Davet and Rouxel, 1997)

**Purification**

For the purification of fungal isolates, the method applied is successive subculture on PDA medium until pure strains are obtained. This purity is controlled by microscopic observation of the cultures. (Samson et al., 1981; Botton et al., 1990; Guiraud J.P., 2003).

The aim of the culture is to obtain genetically homogeneous fungal material (Booth, 1971)

**Identification of Isolates**

The determination of genera and species uses morphological and cultural characteristics. After purification, the isolates are inoculated on PDA media. Preparations fixed with lactophenol are examined under an optical microscope (X 40). The color and texture of the thallus, the reverse of the culture, and the growth rate are noted. Identification is based on the mold determination keys recommended by Botton et al. (1990), Rémi (1997) and Messiaen (1991).

**Conservation of isolates**

For fungi, once the isolates are purified, they are inoculated into tubes containing the inclined PDA medium, after 72 h of development, the tubes are stored at 4 ° C (Botton et al., 1990; Davet and Rouxel, 1997). Another method of conservation is applied which consists of preserving the fungal strains in the form of spores in a solution of 30% glycerol/physiological water at -20 ° C.

## RESULTS

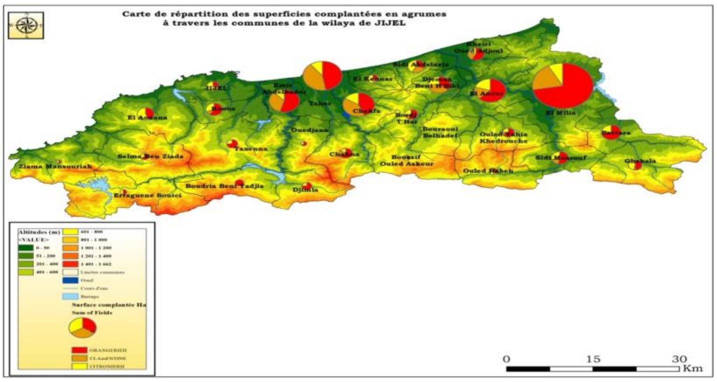


**Figure No. 3: The distribution of citrus orchards in the wilaya of Jijel**

According to the geographical map, citrus orchards are located on the side of the Djendjen, Oued Nil and Oued Lekbir valleys.

Most of the orchards are located in the plains confined between 0 and 200m above sea level. Other orchards with modest areas are distributed in the internal areas of the wilaya above 200m above sea level.

According to PINHAS, ELIEZER 1996 and POLESE, 2008, citrus fruits are native to the tropical and subtropical regions of Southeast Asia where the coolest temperatures rarely fall below 15°C. However, they have been introduced in colder regions (in the Mediterranean for example) and are now cultivated on both sides of the equator over a very wide geographical area (from 40° north to 40° south). Therefore the orchards of Jijel are located in areas that perfectly meet the climatic requirements of citrus fruits. The dominant varieties in the orchards are: • Early varieties: Clementines (24%) • Seasonal varieties: Thomson Navel, Washington Navel (57.85%) and lemon (18.14%) In addition, it appears that a certain number of varieties previously cultivated no longer exist today. The age of citrus orchards is divided as follows: • -5 years: 10% • 5 to 30 years: 50% • 30 to 50 years: 25% • +50 years: 15%

Isolation from tree fragments from potential areas of the wilaya of Jijel made it possible to obtain 59 fungal isolates. The identification of the isolates was done on the basis of morphological and molecular criteria. The species that have been discovered at laboratory level are: Fusarium sp, Phythophthora sp, Alternaria sp, Diplodia sp, Mortierella and Trichoderma.

### Frequencies

Our laboratory results are translated strictly into statistical analysis attached:

| **Statistics** | | | | | |
|---|---|---|---|---|---|
| | | **Especes** | **Organes** | **Substrats** | **Communes** |
| **N** | **Valid** | 59 | 59 | 59 | 59 |
| | **Missing** | 0 | 0 | 0 | 0 |
| **Mode** | | 1 | 1 | 1 | 5 |
| **Range** | | 5 | 2 | 2 | 4 |
| **Minimum** | | 1 | 1 | 1 | 1 |
| **Maximum** | | 6 | 3 | 3 | 5 |

### Frequency Table

**Table No. 3: Frequency of identified fungal species**

| Especes | | | | | |
|---|---|---|---|---|---|
| | | Frequency | Percent | Valid Percent | Cumulative Percent |
| Valid | Fusarium sp | 40 | 67.8 | 67.8 | 67.8 |
| | Phythophtora sp | 4 | 6.8 | 6.8 | 74.6 |
| | Alternaria sp | 1 | 1.7 | 1.7 | 76.3 |
| | Diplodia sp | 2 | 3.4 | 3.4 | 79.7 |
| | Trichoderma | 10 | 16.9 | 16.9 | 96.6 |
| | Mortierella | 2 | 3.4 | 3.4 | 100.0 |

| | | | | | |
|---|---|---|---|---|---|
| | Total | 59 | 100.0 | 100.0 | |

shows the different species identified at the laboratory level which are manifested by Fusarium sp, Phythophtora sp, Alternaria sp, Diplodia sp, Trichoderma and Mortierella, it emerges that citrus trees contain a great diversity of fungal species including the genus Fusarium sp presents a high frequency of appearance on the majority of species analyzed of the order of 40 (67.8%). It is also noted that Trichoderma is the second species with a reduction in the percentage always in relation to Fusarium sp of the order of 10 (16.9%) followed by Phythophtora sp of the order of 4 (6.8%). For the other species Alternaria sp, Diplodia sp Mortierella, their appearance is very modest.

**Table No. 4: the frequency of fungal agents according to the organs**

| **Organes** | | | | | |
|---|---|---|---|---|---|
| | | **Frequency** | **Percent** | **Valid Percent** | **Cumulative Percent** |
| **Valid** | **Racine** | 25 | 42.4 | 42.4 | 42.4 |
| | **Tronc** | 20 | 33.9 | 33.9 | 76.3 |
| | **Feuille** | 14 | 23.7 | 23.7 | 100.0 |
| | **Total** | 59 | 100.0 | 100.0 | |

Shows the frequency of the appearance of fungal agents according to the organs (root, trunk and leaf) which are successively 25 (42.4%), 20 (33.9%) and 14 (23.7%). According to these results we note that the roots are the most contaminated organ followed by the trunk then the branches with a small proportion.

These results are justified first by the direct contact of the roots with the soil. The latter is often the main reservoir of inoculum for a large number of species. For the trunk, it is the organ closest to the ground.

**Table No. 5: The frequency of fungal agents according to citrus species**

| **Substrats** | | | | | |
|---|---|---|---|---|---|
| | | **Frequency** | **Percent** | **Valid Percent** | **Cumulative Percent** |
| **Valid** | **Oranger** | 24 | 40.7 | 40.7 | 40.7 |
| | **Clementinir** | 13 | 22.0 | 22.0 | 62.7 |
| | **Citronnier** | 22 | 37.3 | 37.3 | 100.0 |
| | **Total** | 59 | 100.0 | 100.0 | |

From the table we can see that the citrus species are all affected and contaminated by certain fungal agents with different proportions which shows the sensitivity of each species compared to the other (Host plant).

In light of the table we notice that the orange tree and the lemon tree are the most contaminated species therefore the most sensitive to diseases then comes the clementine tree less than the others in second position. In addition, the comparison of the differences in frequency between the orange tree and the lemon tree (2) with the sampling protocol from 3 orange orchards, 2 clementine orchards and 1 lemon orchard clarifies that the lemon tree is the most sensitive and most contaminated species

**Table No. 6: The frequency of fungal agents according to the municipalities**

| **Communes** | | | | | |
|---|---|---|---|---|---|
| | | **Frequeny** | **Percent** | **Valid Percent** | **Cumulative Percent** |
| **Valid** | **Elmilia** | 11 | 18.6 | 18.6 | 18.6 |
| | **Elancer** | 9 | 15.3 | 15.3 | 33.9 |
| | **Chekfa** | 11 | 18.6 | 18.6 | 52.5 |
| | **Taher** | 8 | 13.6 | 13.6 | 66.1 |
| | **Kaous** | 20 | 33.9 | 33.9 | 100.0 |
| | **Total** | 59 | 100.0 | 100.0 | |

The table shows that fungal agents appeared in the 5 municipalities of which the Municipality of Kaous compared to the others holds the lion's share in the emergence of fungal agents. On the other hand, the others have similar frequencies.

Like all regions of the Algerian coast, the Wilaya of Jijel benefits from a temperate climate with a mild winter characteristic of Mediterranean areas and a rainfall of around 1,200 mm/year. It is among the wettest regions. So the conditions are favorable for the development of different fungal agents. We can also justify the increase in the frequency of the municipality of Kaous by the highest humidity rate which is due to the existence of two areas, several rivers plus the proximity of orchards with the forest.

### Crosstabs

| **Case Processing Summary** | | | | | | |
|---|---|---|---|---|---|---|
| | **Cases** | | | | | |
| | **Valid** | | **Missing** | | **Total** | |
| | N | Percent | N | Percent | N | Percent |
| Especes * Organes | 59 | 100.0% | 0 | 0.0% | 59 | 100.0% |

**Table No. 7: Frequency of occurrence of each fungal agent on organs**

| Species * Organs Crosstabulation | | | | | |
|---|---|---|---|---|---|
| Count | | | | | |
| | | Organes | | | Total |
| | | Racine | Tronc | Feuille | |
| Especes | Fusarium sp | 16 | 12 | 12 | 40 |
| | Phythophtora sp | 2 | 2 | 0 | 4 |
| | Alternaria sp | 0 | 0 | 1 | 1 |
| | Diplodia sp | 0 | 1 | 1 | 2 |
| | Trichoderma | 5 | 5 | 0 | 10 |
| | Mortierella | 2 | 0 | 0 | 2 |
| Total | | 25 | 20 | 14 | 59 |

The table shows the frequency of occurrence of each fungal agent on the sampled organs.

Fusarium sp: is the most common pathogen in the three organs with a pre dominance in the roots. Therefore, fusarium attacks all parts of the tree.

The occurrence of each of the fungal agents phythophtora sp, Alternaria sp and Diplodia sp is very modest compared to fusarim sp.

Phythophtora sp appears only on the trunk and roots.

Alternaria sp appears only on the leaves

Diplodia sp appears only on the trunk and leaves

For the fungal agent Trichoderma and Mortierella, both appear in the roots and in the trunk for trichoderma. These two are considered biological control agents.

| **Chi-Square Tests** | | | |
|---|---|---|---|
| | **Value** | **df** | **Asymptotic Significance (2-sided)** |
| **Pearson Chi-Square** | 12.997[a] | 10 | .224 |
| **Likelihood Ratio** | 17.190 | 10 | .070 |
| **Linear-by-Linear Association** | 2.191 | 1 | .139 |
| **N of Valid Cases** | 59 | | |

a. 15 cells (83.3%) have expected count less than 5. The minimum expected count is .24.

| **Symmetric Measures** | | | | | |
|---|---|---|---|---|---|
| | | **Value** | **Asymptotic Standard Error[a]** | **Approximate T[b]** | **Approximate Significance** |
| **Interval by Interval** | **Pearson's R** | -.194 | .106 | -1.496 | .140[c] |
| **Ordinal by Ordinal** | **Spearman Correlation** | -.179 | .118 | -1.370 | .176[c] |
| N of Valid Cases | | 59 | | | |

| |
|---|
| a. Not assuming the null hypothesis. |
| b. Using the asymptotic standard error assuming the null hypothesis. |
| c. Based on normal approximation. |
| c. Based on normal approximation. |

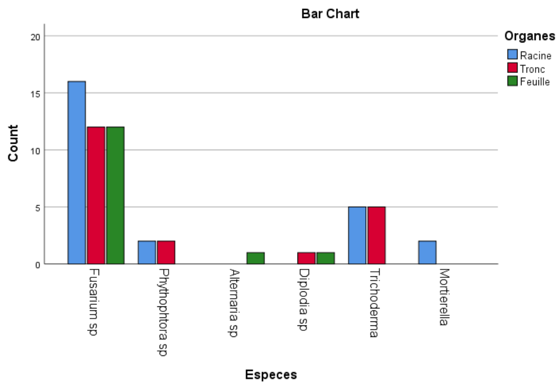


**Figure N° 4: La fréquence d'apparition de chaque agent fongique sur les organes**

## Crosstabs

| Case Processing Summary | | | | | | |
|---|---|---|---|---|---|---|
| | Cases | | | | | |
| | Valid | | Missing | | Total | |
| | N | Percent | N | Percent | N | Percent |
| **Especes * Substrats** | 59 | 100.0% | 0 | 0.0% | 59 | 100.0% |

**Table No. 8: The frequency of appearance of each fungal agent on citrus species**

| Especes * Substrats Crosstabulation | | | | | |
|---|---|---|---|---|---|
| **Count** | | | | | |
| | | **Substrats** | | | **Total** |
| | | **Oranger** | **Clementinir** | **Citronnir** | |
| **Especes** | **Fusarium sp** | 14 | 12 | 14 | 40 |
| | **Phythophtor sp** | 2 | 0 | 2 | 4 |
| | **Alternaria sp** | 1 | 0 | 0 | 1 |
| | **Diplodia sp** | 2 | 0 | 0 | 2 |
| | **Trichoderma** | 5 | 1 | 4 | 10 |
| | **Mortierella** | 0 | 0 | 2 | 2 |
| **Total** | | 24 | 13 | 22 | 59 |

Fusarium sp is a cosmopolitan species that is frequently found in all species (Orange, Clementine and Lemon) so Fusarium sp attacks all varieties of citrus fruits.

The appearance of Phythophtora sp is linked to the Orange and Lemon species. For the two fungal agents Alternaria sp and Diplodia sp their appearance is linked to the Orange species.

For the control agents Trichoderma occurs in all species on the other hand Mortierella occurs on the Lemon tree

| Chi-Square Tests | | | |
|---|---|---|---|
| | **Value** | **df** | **Asymptotic Significance (2-sided)** |
| **Pearson Chi-Square** | 11.295[a] | 10 | .335 |
| **Likelihood Ratio** | 13.811 | 10 | .182 |
| **Linear-by-Linear Association** | .000 | 1 | .993 |
| **N of Valid Cases** | 59 | | |

a. 15 cells (83.3%) have expected count less than 5. The minimum expected count is .22.

| Symmetric Measures | | | | | |
|---|---|---|---|---|---|
| | | Value | Asymptotic Standard Error[a] | Approximate T[b] | Approximate Significance |
| **Interval by Interval** | **Pearson's R** | .001 | .138 | .009 | .993[c] |
| **Ordinal by Ordinal** | **Spearman Correlation** | -.022 | .140 | -.165 | .869[c] |
| **N of Valid Cases** | | **59** | | | |
| a. Not assuming the null hypothesis. | | | | | |
| b. Using the asymptotic standard error assuming the null hypothesis. | | | | | |
| c. Based on normal approximation. | | | | | |

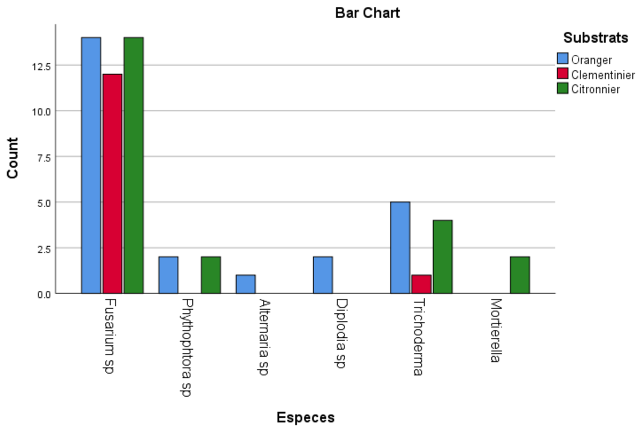


**Figure No. 5: The frequency of appearance of each fungal agent on citrus species**

## NPar Tests

## Kruskal-Wallis Tes

| Ranks | | | |
|---|---|---|---|
| | **Communes** | **N** | **Mean Rank** |
| **Especes** | Elmilia | 11 | 26.32 |
| | Elancer | 9 | 27.61 |
| | Chekfa | 11 | 33.23 |
| | Taher | 8 | 24.50 |
| | Kaous | 20 | 33.53 |
| | Total | 59 | |

| Test Statistics[a,b] | |
|---|---|
| | **Especes** |
| **Kruskal-WallisH** | 3.996 |
| **Df** | 4 |
| **Asymp. Sig.** | .407 |

| |
|---|
| a. Kruskal Wallis Test |
| b. Grouping Variable: Communes |

At the end of this work, it appears that citrus trees contain a great diversity of fungal species, including the genus Fusarium with a high frequency of appearance on the majority of the samples analyzed in the order (67.80%), followed by the genus

Phythophthora sp (6.78%), Alternaria sp (1.70%), Diplodia sp (3.39%), Mortierella (3.39%) and Trichoderma (16.94%)

Fusarium sp, Phythophthora sp, Alternaria sp, Diplodia sp are identified by macroscopic and microscopic criteria. On the other hand, Mortierella and Trichoderma are identified by PCR.

## Pathogens

### Fusarium

In the laboratory, colony morphology on PDA varies considerably. Mycelia may be flocculent, sparse, or abundant, and range in color from white to pale purple. Abundant pale orange or pale purple macroconidia are produced in a central spore mass in some isolates. Some isolates produce no pigment at all.

Most isolates produce macroconidia and microconidia that differ in morphology, length, and number of septa (Figure 6)

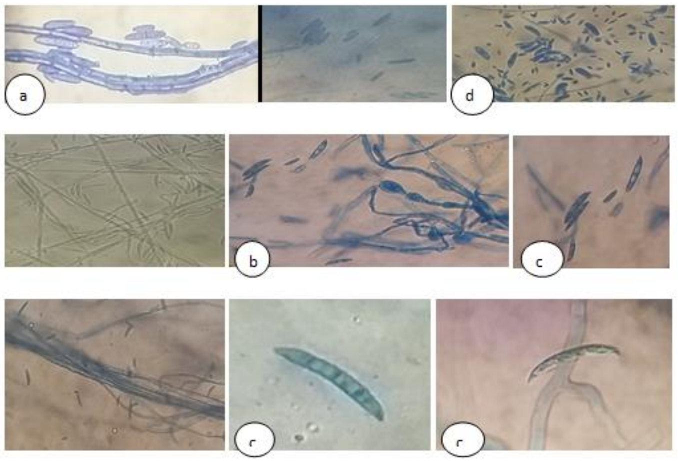

**Figure No. 6: a-Septate mycelium, b-Chlamydospores, c-Macroconidia, d-Microconidia, of Fusarium sp.**

Fusarium is a genus of ascomycete fungi first described by Link in 1809 as Fusisporium. Members of the genus are numerous and can be recovered from plants and soils worldwide as pathogens, endophytes and saprophytes (Brown and Proctor, 2013). Many phytopathogenic Fusarium species produce a number of secondary metabolites (J. Cheikowski, 1989; Brown and Proctor, 2013) upon infection of host plants that trigger physiological and pharmacological responses in plants e.g. plant phytotoxicoses (J. Cheikowski, 1989).

**Table No.: Fusarium species regularly recovered from various parts of diseased plants as saprophytes. (Leslie and Summere, 2006)**

| | |
|---|---|
| **Roots & stem bases** | F. acuminatum, F. avenaceum, F. compactum, F. equiseti, F. proliferatum, F. oxysporum, F. solani |
| **Leaves & aerial parts** | F. proliferatum, F. semitectum |
| **Flowers** | F. semitectum |
| **Seed & grain** | F. chlamydosporum, F. equiseti, F. poae, F. semitectum |

### Phytophthora sp

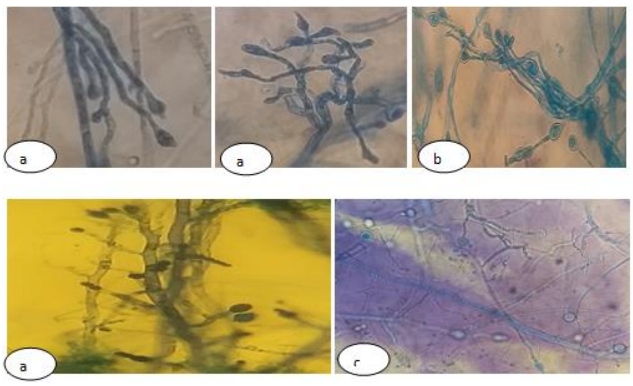

**Figure No. 7: Phytophthora sp, , viewed under a microscope (*40): (a) three sporangiophores; a number of lemon-shaped sporangia; (b) sporangia; (c) contents of sporangia dividing to form zoospores.**

Phytophthora is a cosmopolitan oomycete of warm temperate, subtropical and tropical environments. It has a wide host range. Phytophthora spp. cause the most serious and economically important soil-borne diseases of citrus crops.

Production losses of trees and crops occur due to damping-off in the seedbed, root and collar rot in nurseries, butt and fibrous root rot and brown rot of fruits in orchards.

The most important species are Phytophthora parasitica Dastur (P. nicotianae), P. citrophthora and P. palmivora. P. parasitica (Savita Gurdeep Singh Virk and Avinash Nagpal, 2012).

Other Phytophthora spp. namely P. boehmeriae, P. cactorum, P. cinnamon, P. citricola, P. citrophthora, P. dreschleri, P. hibernalis, P. megasperma, P. palmivora and P. syngiae have been reported as pathogens on citrus from different citrus growing areas of the world, P. citropthora and P. parasitica are widely distributed in tropical and subtropical citrus areas causing citrus stem rot, root rot, gummosis and brown rot (Savita Gurdeep Singh Virk and Avinash Nagpal, 2012)

Phytophthora pathogens can cause many different diseases and disease symptoms over a wide range of plant species.

• Root rot in general, seedlings of many plants are very susceptible to root rot and damping-off caused by Phytophthora.

• Crown rot: Crown rot often develops at or just below the soil line. The infection moves upward from the roots, rotting the lower bark tissue and discoloring the lower stem.

• Tree canker: Many species of Phytophthora can form cankers on the stems of host plants. These cankers have different names, including: band canker (cinnamon), spot canker (durian), or trunk canker.

• Leaf blight: A number of Phytophthora species cause leaf blight. These include P. palmivora on many species. (Drenth and Sendall,2001)

The identification of Phytophthora is based on the taxonomic keys of Waterhouse (1963) and Stamps et al. (1990). Characters used to classify Phytophthora species include: morphology of the sporangium; morphology of sexual structures such as antheridia, oogonia and oospores; presence or absence of chlamydospores and morphology of hyphae. (Drenth and Sendall,2001)

## Alternaria sp

Alternaria is distinguished by the formation of conidial chains. Branching of the chains occurs sympodially by the elongation of secondary conidiophores from the distal terminal conidial cells and the subsequent formation of conidia. Conidia are oval in shape, divided by transverse and vertical walls, with minimal development of apical extensions. Hyphae and conidiophores are light brown and septate

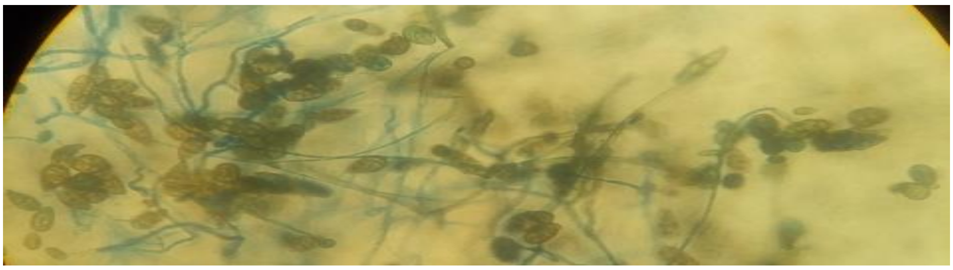

**Figure No. 8: The septate appearance of conidia. Presence of conidia chains**

The pathogen Alternaria mainly causes two diseases on citrus fruits: brown spot disease and black rot. Both diseases begin with dark brown to black necrotic spots on the fruit and manifest as black necrotic spots on the leaves that can follow the veins. They can also occur on the twigs in the form of black lesions (ACHETBI et al,2021)

Black rot of citrus fruits is a major post-harvest problem that can appear even in the field. The disease most commonly occurs on oranges (Navel) (C. sinensis in the field and on mandarins and their hybrids in storage (ACHETBI et al, 2021)

**Alternaria Diseases of Citrus Represent Interesting Pathosystems**

Alternaria sp is a common saprophyte on citrus leaves in the orchard, but these isolates are capable of causing black rot of fruits after harvest. (LAVERN et al, 2003)

**Diplodia SP**

Isolates identified as diplodia sp have a moderate, fluffy aerial mycelium with rapid growth on PDA culture medium. Indeed, it is able to fill the entire petri dish, after 72 hours of incubation. They are initially white in color which quickly becomes pale gray. Conidia are initially hyaline and aseptate, ellipsoid to ovoid in shape, with a rounded apex and base.

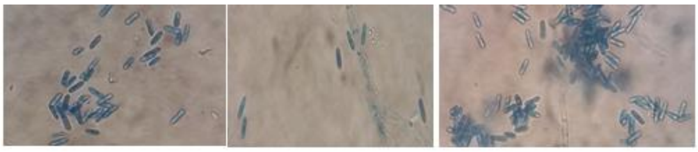

**Figure No. 9: conidia of Diplodia sp**

Lasiodiplodia species, better known as diplodia, like other members of the Botryo sphaeriaceae family, are pathogens distributed worldwide in tropical and subtropical regions and cause different types of diseases in many woody plants, including fruit and tree crops such as mango, avocado, citrus, apple, peach, pear, Eucalyptus spp., Azadirachta, and pine. (Wei Zhao,2014)

Species of Diplodia, like other members of the family Botryosphaeriaceae, are known to be pathogens, endophytes and saprophytes on a wide range of mainly woody hosts (Phillips and al, 2012)

In citrus, there are reports that diplodia have been associated with "citrus gummosis," resulting in discoloration of the tree bark and gum exudation (Wei Zhao,2014)

In citrus, invasion of fruit tissues by diplodia can cause stem tip rot, which is usually a postharvest disease (Wei Zhao, 2014)

**Biological agents: PCR identification**

**Trichoderma SP**

The genus Trichoderma is characterized by a rapid growth rate and abundant sporulation on culture medium. It is easily recognizable in culture thanks to the generally green color of its spores. This genus is characterized by conidiophores that are highly branched in a pyramidal structure and end with one or more phialides. These phialides can be cylindrical or subglobose, grouped in masses or solitary. The conidia are hyaline, ellipsoid and smooth in most species, globose conidia are rare. Some species can produce globose chlamydospores, which are intercalary or terminal.

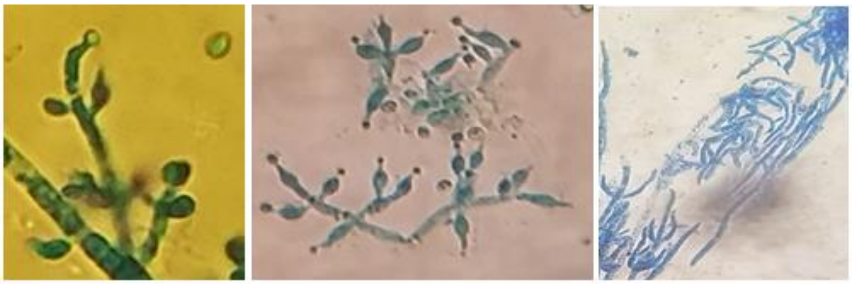

**Figure N°10: Conidiophores, phialides et Conidies of Trichoderma sp**

Trichoderma is effective when allowed to settle before the arrival of pathogenic fungi. Its action is therefore preventive. At the root level, it creates a protective sleeve around them and thus prevents pathogens from entering the roots. The same effect is observed when it is used in aerial spraying. Once established, Trichoderma can have a stimulating effect on the plant in the absence of pathogenic fungi. (Johanne Caron, 2002)

The fungus Trichoderma is considered an essential factor in biological control since most of these species are involved in the control of harmful microorganisms, especially fungi such as Phytophthora infestants. The genus Trichoderma is often found in soil or on plant constituents, and their growth is rapid (Harman and Kubicek, 1998).

**Mortierella SP**

In the laboratory -Mortierella species are characterized by white cottony colonies with a rosette appearance, and a garlic odor is often present

The main characteristic of representatives of this genus is the production of sporangiophores with a swollen base and the absence of columellae

The positive contribution of these fungi in agricultural soils as well as in plant tissues should lead to the discovery of optimal cultivation conditions and sporulation media for analyzing complex interactions between plants and microorganisms. It should be emphasized that pathogenic species are very rare in this genus, therefore Mortierella species are very promising sources of plant growth-promoting inoculants for agriculture (Ozimek and Hanaka, 2020)

Mortierella sp. effectively dissolved rock phosphate decreasing the pH of the medium from 7.7 to 3.0. The low molecular weight of organic acids, such as oxalic, malic, acetic, formic, gluconic, citric, lactic, 2-ketogluconic, tartaric and citric acids secreted by filamentous fungi is considered as one of the main mechanisms of inorganic phosphate solubilization by Mortierella sp. (Ozimek and Hanaka, 2020)

The branching patterns of sporangiophores are the main characteristic to differentiate Mortierella sections (Melo Gonçalves et al, 2020)

## CONCLUSION

Citrus trees are subject to attacks by many diseases, some of which are very serious, since they can lead to the death of the tree, some are difficult to control

As we mentioned previously, the samples are taken according to the most frequent characteristics of the trees that appear in the orchards and which have anomalies compared to other healthy trees. The characteristics monitored are: decline, wilting of the tree. Cankers on trunk and twigs, deformed parts of leaves and drying of twigs especially at the tips of citrus trees

The observations made in the field and supplemented by laboratory work have confirmed the presence of a wide variety of fungal species (pathogen and biological agent) including the Fusarium genus which has a high frequency of occurrence on the majority of the samples analyzed.

The disease is more prevalent in citrus production areas with a Mediterranean climate characterized by cool, wet winters and hot, dry summers than in humid areas. Climate change, which tends to be wetter and warmer, is expected to cause an increase in the intensity of diseases caused by various fungal pathogens.

Climate change affects citrus production in terms of quantity as well as quality. In terms of plant diseases, fungal pathogens can spread to a new area that was previously unsuitable for them. Warmer and more humid climatic conditions can encourage certain fungi to emerge as more virulent pathogens which, in turn, can cause an epidemic of diseases

Despite the fact that the bitter orange tree gives citrus fruits good vigor, significant development, adapted to most types of soils, normal longevity, suitable fruit quality and resistance to phytophthora and root rot, the orchards of the wilaya of Jijel are still faced with several problems, including dieback, which is one of the most frequent and widespread constraints in orchards. In recent years, it has struck our orchards and affected the majority of orchards in production, often leading to the death of trees. Plants over 4 years old would be particularly affected.

Visits to several orchards have noted that on average 80% of orchards are affected by diseases. The phenomenon is manifested by a stunting of the tree, a general weakening and then its death. Added to this are the failures in good cultural and hygiene practices

Our citrus orchards are at a productivity level below the normative economic profitability threshold in the Jijel area of the order of 10 to 20 T / Ha. According to the results obtained, we note a diversity of fungal species that manifests itself in the study area, thus causing serious diseases on citrus orchards. In short, promising solutions are to be identified to obtain healthy plants free of contamination for better improvement and stabilization of production at the regional level.

The use of integrated orchard management methods remains the best practice to minimize the harmful effects of pesticides on the environment.

. Tree health protection involves a set of techniques, all of which are essential: - health selection of plant material (healthy variety and rootstock), - choice of variety and rootstock (not very susceptible to diseases), - chemical control: it is only used as a last resort.

In the case of preventive control, the best results will be obtained by the following approach: - correctly identify the disease to be combated, - estimate the importance of the attack (notion of tolerance threshold) to know whether it is necessary to intervene or not,

. The need to organize the fight on a genetic level, by opposing the parasite with plant material selected for its resistance, has become essential. This method requires that we have in-depth knowledge of the parasite and in particular, its diversification potential, which is linked to the structure of its population dependent on a particular ecosystem and its behavior in the face of this ecosystem.

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