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# Regional and seasonal variation of *Fusarium* and Oomycetes species associated with apple seedlings decline in Tunisian nurseries

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## Abstract

Apple decline, which is responsible for seedlings root and collar rot in the nurseries, is a serious disease that causes reduction in apple plant production. The aim of this study was to evaluate the regional and seasonal distribution of the fungi and Oomycetes, which were associated with the apple decline disease in the Tunisian nurseries. In this study, surveys were conducted from autumn, 2015 to summer, 2016. Apple plants were sampled to detect and quantify the inoculum density of these pathogens. Based on the morphological characteristics; two *Fusarium* and two Pythiaceae spp. were obtained. The most dominant species were *F. oxysporum* (33.9 %); *Pythium ultimum* (33.05 %), *F. solani* (16.95 %), and *Phytopythium mercuriale* (16.1 %). Results of the seasonal variation showed that *Fusarium* spp. and Pythiaceae populations had peaked in June. The populations of *F. oxysporum* and *F. solani* were significantly and positively correlated to temperature. In relation to the soil physicochemical characteristics; the Pearson correlation showed that the population of *P. ultimum* was positively related to the nitrogen (N) content (r = 0.59); sand (r= 0.82), organic matter (r = 0.85), and organic carbon (r = 0.84). However, this species was negatively correlated with the silt content (r = -0.79); clay (r = -0.84), and electrical conductivity (r = -0.74). The *Phytopythium mercuriale* population was positively correlated with nitrogen content (r = 0.64), and negatively correlated with soil pH (r = -0.62); clay content (r = -0.47), and silt (r = -0.54).

**Keywords**: Apple decline, Climatic conditions, Nursery, *Fusarium* spp., Pythiaceae

## 1. Introduction

Apple (*Malus domestica*) is an economically important crop in Tunisia. Overall 17500 ha of apple are cultivated in the different governments of Tunisia, such as Kasserine (8570 ha); Siliana (1976 ha), Kairouan (1496 ha), and Manouba (1227 ha) (Anonymous. 2021). The apple rootstocks used in

Tunisia are MM106 that is susceptible to root and crown rot caused by *Phytophthora* spp., and MM111 that is moderately susceptible to the same diseases (Wilcox, 1992). Several species of the pathogenic fungi and Oomycetes have been reported as root pathogens of apple in the different apple-growing areas throughout the world, such as Europe (Manici *et al*., 2003); North America (Willett *et al*., 1994), Africa (Mannai *et al*., 2018; Tewoldemedhin *et al*., 2011), Asia (Xiang *et al*., 2021; Zhou *et al*., 2021; Duan *et al*., 2022), and Australia (Dullahide *et al*., 1994).

The problem of apple decline had been reported in Tunisian orchards since 2004 (Boughalleb *et al*., 2006). Several *Pythium* and *Phytophthora* spp., including *Pythium rostratifingens*; *P. undulatum*, *P. indigoferae*, *P. irregulare*, *P. sterilum*, *Phytophthora parasitica*, *Phytophthora inundata*, and *Phytophthora. cactorum*, have been reported as the major causal agents of apple decline in the orchards (Boughalleb *et al*., 2006; Souli *et al*., 2011a, b; Souli *et al*., 2014). In the nurseries, the plants can be attacked by more than one telluric pathogen (Gilbert, 1995; Mannai *et al*., 2018). In the nurseries, several *Fusarium* spp. such as *F. oxysporum*; *F. solani*, *F. equiseti*, and *F. proliferatum*, have been associated with the apple seedlings' diseases (Mannai *et al*., 2018).

After establishment of the orchard, the microbial pathogens can survive in the plants rhizosphere for one to two years, or even longer (Mazzola, 1999; Tewoldemedhin *et al*., 2011). An investigation has been conducted in the South-African apple nurseries revealed that the roots of the nursery seedlings are infected by several fungal and Oomycetes pathogens, including *Fusarium*; *Pythium*, and *Phytopythium* spp. (Moein *et al*., 2019). Association of the apple orchards decline causative agents with the nursery trees suggested that there is a relationship between the nursery management and the development of a disease in the orchards. Thus, to prevent the biotic diseases; the investigations should start from the nurseries.

The life cycle of the soil-borne pathogens is influenced by several environmental parameters, such as the temperature; rain, humidity, and frost, which can increase the multiplication and spread of these pathogens (Scortichini, 2010). As the environment is one of the important components of disease development; changes in this environment can be strongly correlated with the changes in the disease severity and its associated losses (Elad and Pertot, 2014). In addition, the physicochemical soil characteristics can affect the pathogens population (Höper *et al*., 1995; Mallett and Maynard, 1998; Rimé *et al*., 2003). Höper *et al*., (1995) revealed that soil suppressiveness to the *Fusarium* wilt of flax was correlated with several soil physicochemical characteristics, including the texture; pH, calcium (Ca) and magnesium (Mg) contents; in addition to the biological characteristics of the pathogens, such as the population densities and the fluorescent pseudomonads. The objectives of the present investigation were to (i) evaluate the regional and seasonal distribution of the fungi and Oomycetes associated with apple seedlings decline in the Tunisian nurseries; and (ii) determine the influence of some environmental and soil characteristics on the pathogen populations.

## 2. Materials and methods

### 2.1. Surveys and sampling

In this study, surveys were carried out from autumn, 2015 to the summer, 2016 at three nurseries of apple, which were located in 3 different Tunisian regions (*i.e*., Ben Arous; Monastir, and Kasserine), according to Khouja *et al*., (2008). From each apple rootstock (*i.e*., MM106 and MM111) that was found in each prospected nursery; root samples of 10 apple plants ageing from 6 to 18-months were collected. The rootstock MM106 was used in all the surveyed nurseries, whereas the rootstock MM111 was used only in the nursery that was located in the Monastir region. Samples were taken every three months (every season). The first sampling was performed in autumn, 2015; the second was in the winter, 2015, the third one was in spring, 2016, and the last sampling was in the summer, 2016.

### 2.2. Isolation of Oomycetes and *Fusarium* spp.

Isolation of the Oomycetes and *Fusarium* spp. was conducted in reference to Souli *et al*., (2014); Mannai *et al*., (2018). Roots samples were washed under running tap water and surface-sterilized using ethanol (70 %). The sterilized roots tissues of each plant sample were cut into 3 to 5 mm pieces using a sterile scalpel. Then, 10 pieces/ plant were placed aseptically onto the surface of 90 mm Petri-plates of Potato dextrose agar (PDA) medium amended with streptomycin sulfate (300 mg/ l). Moreover, a selective medium of Pimaricin + Ampicillin + Rifampicin + Pentachloronitrobenzene (PARP) [17 g of cornmeal agar (CMA) amended with 10 µg/ ml of pimaricin; 250 µg/ ml of ampicillin, 10 µg/ ml of rifampicin, and 25 mg/ ml of pentachloronitrobenzene (PCNB)] (Jeffers and Martin, 1986), was also inoculated with the root pieces. Three Petri plates were used per each sample and per each type of medium. The plates were incubated at 25 °C in the darkness, and examined for fungal growth within 2-3 d. The obtained Oomycetes-like isolates were purified by the hyphal tips method (Souli *et al*., 2014), while the *Fusarium*-like isolates were purified using a single-spore colony technique (Souli *et al*., 2014).

### 2.3. Morphological identification

Each fungal isolate was grown on PDA medium at 25°C in darkness to assess some colony features, such as growth rate and pigmentation. The Pythiaceae-like isolates were identified on the basis of their mycelial characteristics, including colony morphology, in addition to the sporangia, oogonia and antheridia production; morphology, and dimensions, as described by Belbahri *et al*., (2008). The isolates were inoculated on V8 agar medium (200 ml of V8 juice, 2 g Calcium carbonate ($CaCO_3$), 20 g agar, in 800 ml dist. water), and incubated at 25 °C for five d in the darkness. After incubation, 5-mm-diameter mycelial plug of each isolate was inoculated into 20 ml of sterile soil extract (100 g of soil in 900 ml of dist. water), and incubated for 2-3 d in light at room temperature. The presence or absence of oogonia; antheridia, oospore, and sporangia produced by each isolate was checked, and then 25 measurements of each structure were taken under a compound Leica microscope (DM 2500, Germany). Conversely, each *Fusarium* isolate was grown on PDA medium at 25 °C for 7 d, and the growing isolates were identified based on their morphological criteria, according to the keys of Leslie and Summerell, (2006).The percentage (%) of *Fusarium* and Pythiaceae population was calculated (%) for each sample using the following formula:

$$P(\%) = \left(\frac{n}{N}\right) \times 100$$

Where; n: mean number of *Fusarium* or Pythiaceae colonies, N: number of root pieces used in isolation

### 2.4. Analysis of the physicochemical characteristics of soil

About 200 g of each soil sample was shipped to the laboratory for soil analyses, based on the soil texture; pH, electrical conductivity, organic matter, and measurement of the total organic carbon. The soil texture was classified using the Soil Triangle Hydraulic Properties Calculator (Saxton *et al*., 1986). The soil pH and the electrical conductivity (EC) were measured with a glass electrode using a 1:5 soil (w: v) ratio (ISO. 5725-2. 1994; ISO. 11464. 1994). The total N content was determined according to ISO. 11261. 1995, which consisted of mineralization of the sample by hot concentrated $H_2SO_4$ in the presence of selenium as a catalyst. This mineralization process transformed the organic nitrogen into an ammonium ion; followed by alkalization of the reaction products and distillation of the liberated ammonia, which was neutralized with boric acid and finally titrated with 0.05 M $H_2SO_4$. The nitrogen content Kjeldahl (N-NTK) was expressed as the percentage (%) of dry matter (ISO. 11261. 1995):

$$N\text{-}NTK\ (\%) = [(V1 - V0) \times 14 \times C \times 100 \times 0.001] / m$$

Where; V1: volume of the $H_2SO_4$ solution used for the dosage of the sample (ml); V0: volume of the $H_2SO_4$ solution used for the blank test (V0 = 0.2 ml); C: concentration of the $H_2SO_4$ solution used during the titration

(mol/ l); m: weight of the tested portion (g); the atomic mass of nitrogen is 14 g/ mol.

A protocol that was modified and described by Naanaa and Susini, (1988) was followed to determine the organic carbon content. In brief, 0.5 g of each fine dry compost soil sample was placed in a beaker; 5 ml of potassium dichromate (8 %) and 10 ml of conc. $H_2SO_4$ was added. After cooling for 30 min., approximately 25 ml of $H_2O$ was added and the suspension was whiskered using a glass rod. After standing overnight in the darkness, the treated soil samples were run in a colorimetric analysis, and then using the calibration curve and the given colorimeter values; the carbon values for each sample were deduced in mg, according to the formula of Naanaa and Susini, (1988):

C content (%) = [(C (mg) ×100) / (P×1000)]

Where; P: compost weight in g (0.5 g), C: carbon content in mg

The determination of the organic matter (OM) content was based on determination of the carbon content (C %), using the formula reported by Naanaa and Susini, (1988):

OM (%) = (C % ×1.724)

Where; OM: Organic matter; C: carbon content; 1.724: is the conversion factor, which assumes that the OM contains 58 % of organic carbon

### 2.5. Weather data

To analyze the seasonal variation of the Pythiaceae and *Fusarium* populations in the Tunisian apple nurseries; the temperatures; relative humidity, and rainfall data pertaining to the survey periods from autumn, 2015 to summer, 2016 were recorded from the National Institute of Weather of Tunisia.

### 2.6. Statistical analyses

Data were subjected to a one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences software (SPSS), version 20.0. Means of the values were separated using the Student-Newman–Keuls (S-N-K) test to identify the significant differences at Alpha = 0.05. Principal Component Analyses (PCA) were done to identify the main climate variables and soil characteristics, which discriminate the variation of *Fusarium* and Pythiaceae spp. using the software XLSTAT 2018. The correlation analyses between Pythiaceae and *Fusarium* populations, and the different parameters were carried out using Pearson's correlation analysis.

## 3. Results

### 3.1. Survey and sampling

From autumn, 2015 to summer, 2016, a total of 120 apple samples were collected. The infected apple seedlings showed symptoms of drying and browning of the apical parts of the scion and/ or browning at the collar; thus, inducing a complete decline and death of the apple plants. The uprooting showed a root browning (Fig. 1). These symptoms were observed in the used apple rootstocks of MM106 and MM111.

### 3.2. Prevalence of fungi and Oomycetes

Based on morphological characterization; two *Fusarium* spp. were isolated from the apple seedling roots in the surveyed nurseries; mainly *F. solani* and *F. oxysporum*. Meanwhile, two spp. of Pythiaceae were also obtained from apple, including *P. ultimum* and *Phytopyhthium mercuriale*. Results showed that the most dominant species was *F. oxysporum* (33.9 %), followed by *F. solani* (16.95 %), *P. ultimum* (33.05 %), and *Phytopyhthium mercuriale* (16.1 %) (Figs. 2 and 3). Results of this study demonstrated that in the Monastir nursery; the average of infected roots by *Fusarium* spp. was 44.67 %, while in the nursery of the Kasserine region; the percent of roots infection by *Fusarium* spp. was 32.33 %. Finally, in the Ben Arous nursery, the percent of infection was 31.33 %. Indeed, the *F. oxysporum* and *F. solani* were recorded in all nurseries used during the different periods of the surveys.

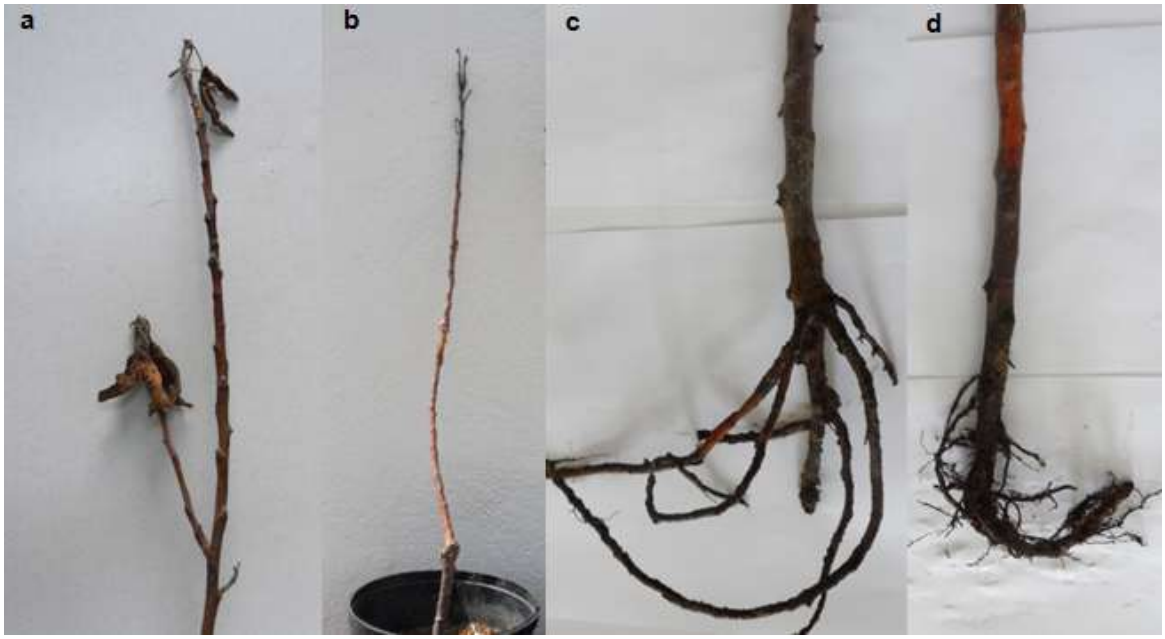



**Fig. 1**. Symptoms of browning of the apple plant apical parts (a, b) and root rot (c, d) of seedlings of apple rootstock 'MM106' cultivated in Tunisian nurseries

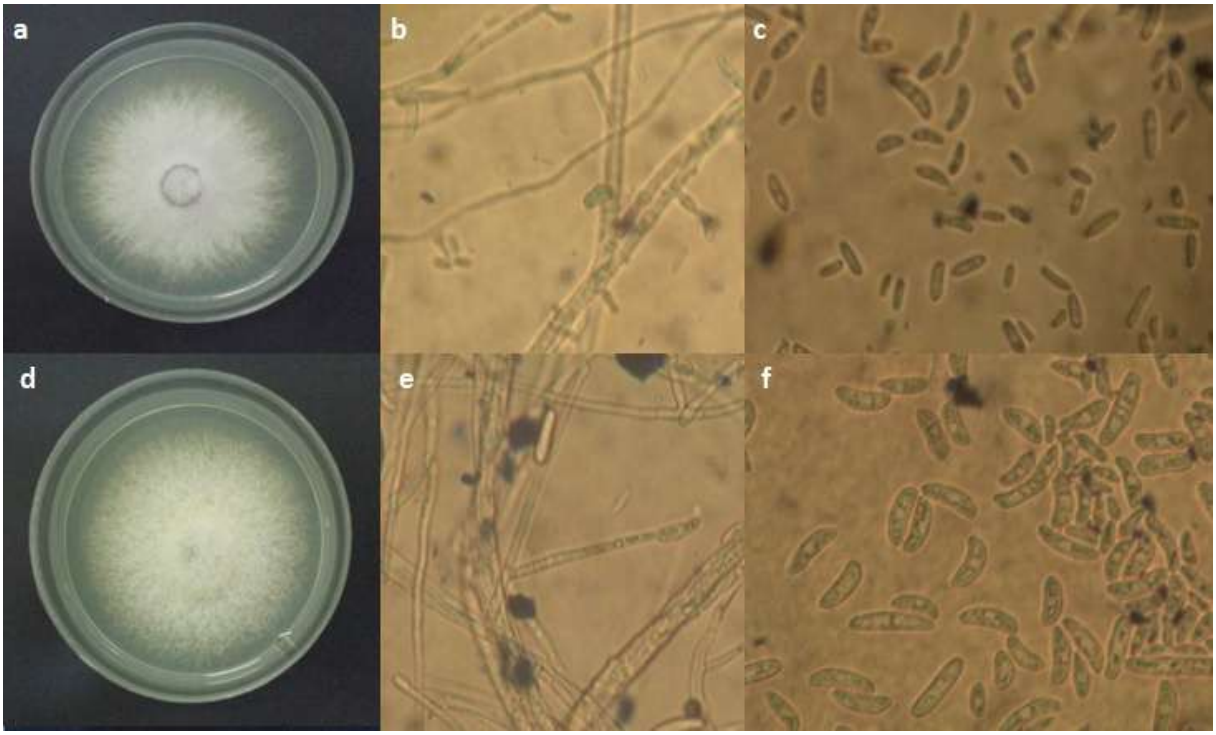



**Fig. 2**. Morphological characteristics of *F. oxysporum*: (a): mycelium growth on PDA medium, (b, c): microconidia and short phyalides, and of *Fusarium solani*: (d) mycelial growth on PDA medium, (e, f): long phyalides and microconidia

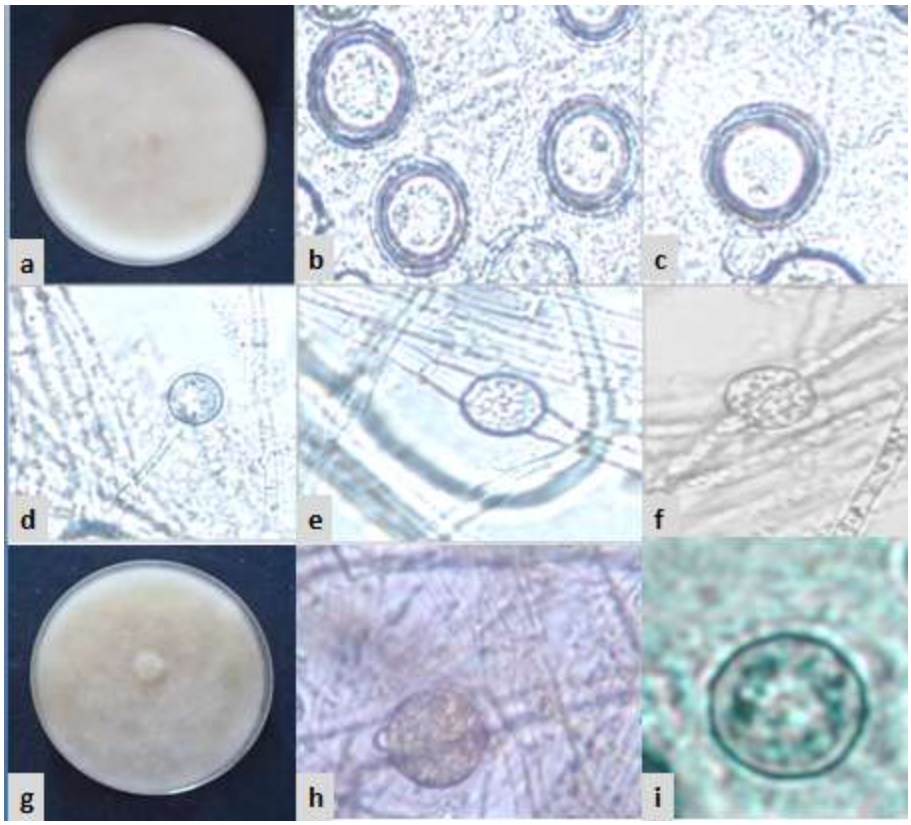
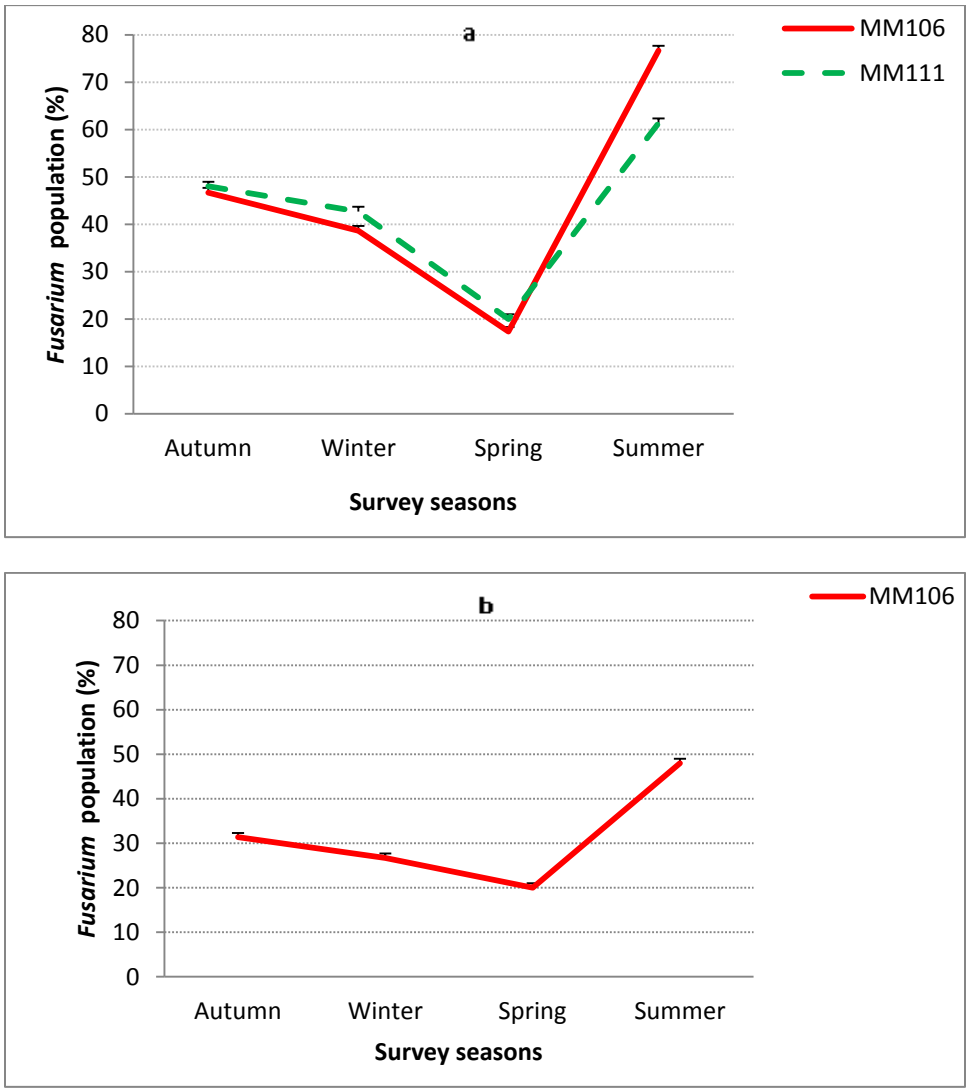



**Fig. 3**. Morphological characteristics of *Pythium ultimum*: (a): mycelial growth on PDA medium; (b): Aplerotic Oospores; (c): Oogonia with monoclinous antheridia; (d): swelling of the terminal hyphae; (e, f): swelling of the intermediate hyphae; and (g): *Phytopythium mercuriale* colony on PDA; (h): sporangia (h); (i): oogonia

### 3.3. Seasonal distribution of the causal agents of apple plants decline

From autumn, 2015 to summer, 2016, studying the seasonal variation of *Fusarium* spp. in the three nurseries in the different regions of Tunisia showed a peak of the *Fusarium* population in the summer for the two tested rootstocks; MM106 and MM111, followed by the population isolated in the autumn (Fig. 4). The seasonal variation of *Fusarium* spp. from autumn, 2015 to summer, 2016 in the Monastir nursery, showed a highly significant increase ($p \leq 0.001$) of the *Fusarium* population in the summer (76.67 %), followed by the autumn (46.67 %) for the rootstock MM106 (Fig. 4a). A significant variation was revealed for the rootstock MM111 at the same nursery (Fig. 4a). In the Ben Arous nursery, the *Fusarium* spp. population isolated from the MM106 rootstock had peaked in the summer (48 %); however, the change of the percentage from autumn to summer was not significant (Fig. 4b). In the Kasserine region, the seasonal variation of *Fusarium* spp. population at MM106 was highly significant ($p \leq 0.001$); with an isolation percentage ranging from 16 % at the spring to 60 % at the summer (Fig. 4c). Studying the seasonal variation of Pythiaceae from autumn, 2015 to summer, 2016 in the three nurseries in the different regions did not show any difference for the two tested rootstocks (Fig. 5). At the Monastir nursery, the Pythiaceae populations isolated from the two rootstocks MM106 and MM111 were comparable. In fact, the isolation percentage was between 12.67 % and 12 % in the spring for MM106 and MM11; respectively, 33.33 % in the summer for MM106 and 37.33 % in the autumn for MM111 (Fig. 5a). At Ben arous nursery, the

Pythiaceae population was about 4 % in the winter and 8.67 % in the summer (Fig. 5b). The Pythiaceae population at the Kasserine nursery varied between 17.33 % in the winter and spring and 23.33 % in the autumn (Fig. 5c). Indeed, the *P. ultimum* was observed in all nurseries in the different seasons. *Phytopyhthium mercuriale* was recorded in all nurseries and at all seasons; except for Kasserine nursery, where it was detected in the spring.



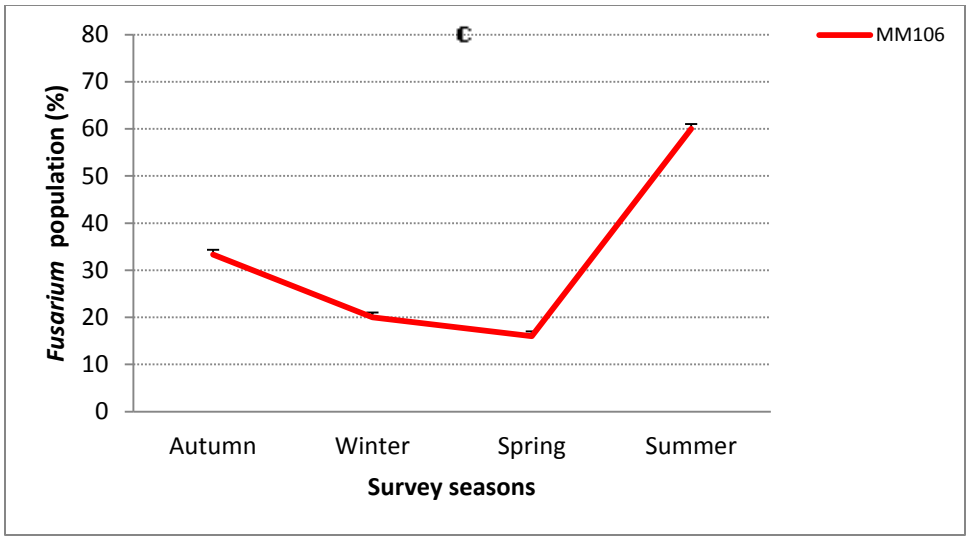
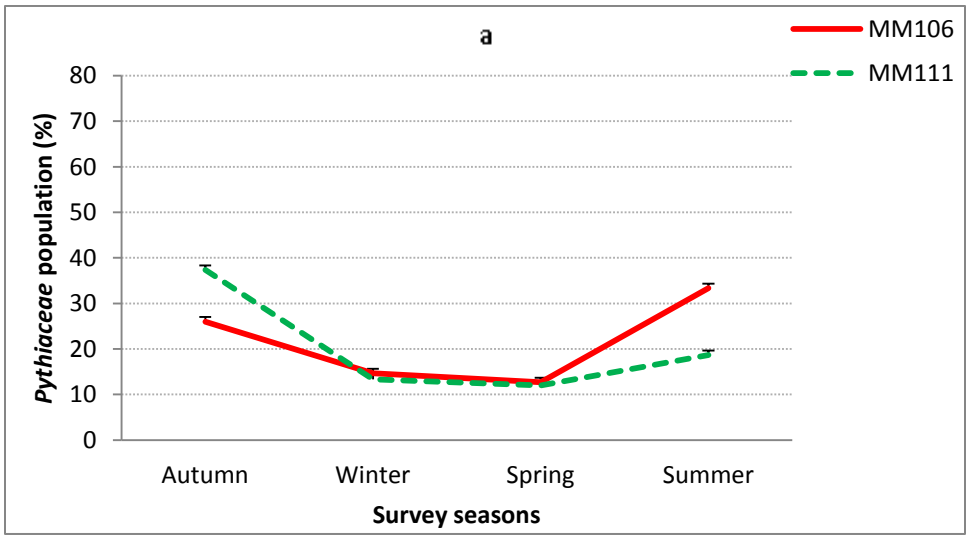



**Fig. 4**. Seasonal variation of the *Fusarium* spp. population from the rhizosphere of the rootstock MM106 in (a): Monastir; (b): Ben arous; (c): Kasserine nurseries; and (a): the rootstock MM111 in the Monastir nursery. The error bar represents the standard deviation (SD)



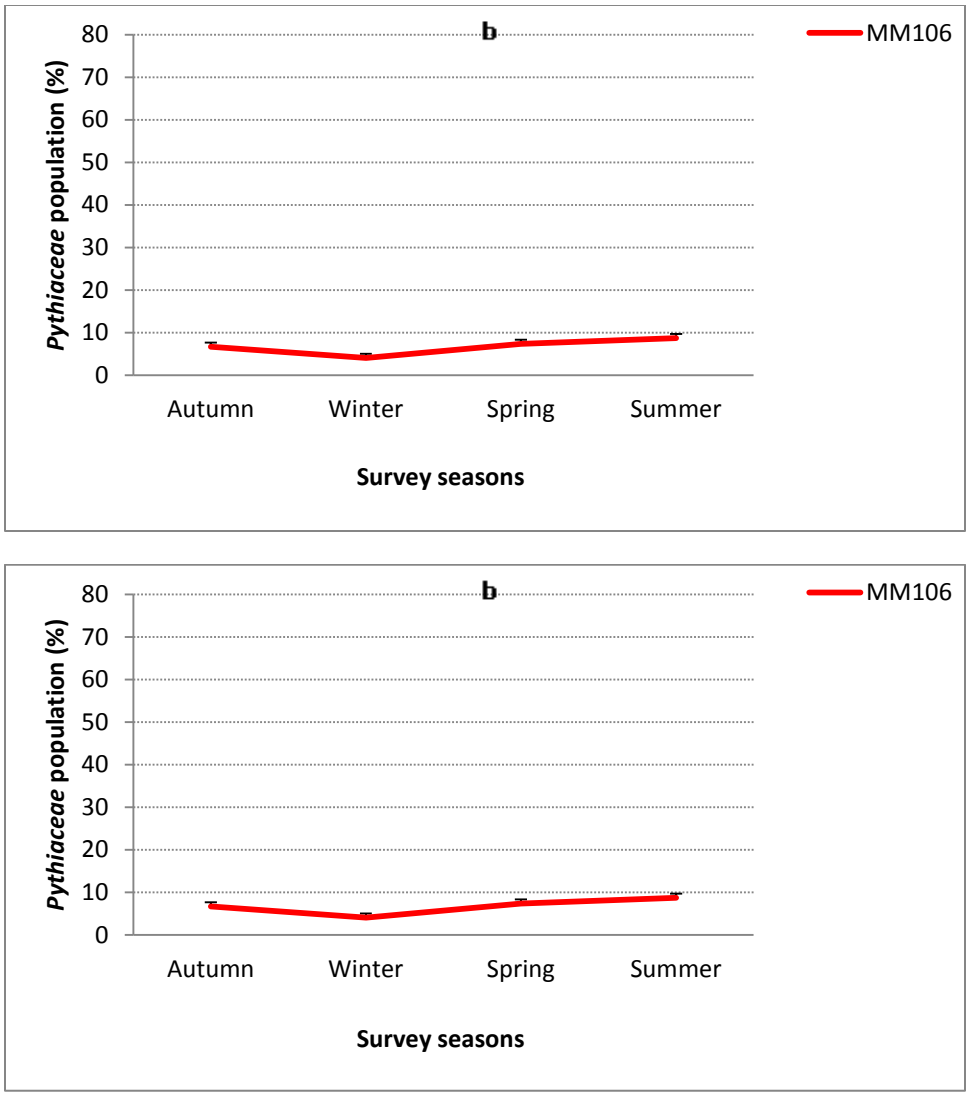



**Fig. 5**. Seasonal variation of the Pythiaceae population from the rhizosphere of the rootstock MM106 in (a): Monastir; (b): Ben arous; (c): Kasserine nurseries, and (a): the rootstock MM111 in the Monastir nursery. The error bar represents the standard deviation (SD)

### 3.4. Correlations between *Fusarium* spp. and Pythiaceae populations and the prevailing environmental conditions

The PCA showed that the populations of *Fusarium* spp.; *F. oxysporum*, F. solani, and temperatures had the highest contributions in the construction of the first axis with respective values of 18.09; 26.77, 22.70, and 16.64 %; respectively, while axis 2 was composed mainly of the populations of *P. ultimum* (40.15 %), *Phytopyhthium mercuriale* (19.31 %), the Pythiaceae population (11.03 %), relative humidity (11.40 %), and the *Fusarium* population (10.01 %) (Table 1).

Based on the first major component (F1); the relative humidity and rainfall were negatively correlated with the populations of Pythiaceae and *Fusarium* spp. The temperature was positively correlated with populations of all the pathogenic species except for *P. ultimum* (Fig. 6).

**Table 1**. Contribution percentages of the different variables (pathogens population and climatic conditions) in the edification of the axes 1 and 2 of the PCA

| | Axis1 | Axis 2 |
|---|---|---|
| Pythiaceae Population | 0.777 % | 11.032 % |
| *Fusarium* Population | 18.091 % | 10.013 % |
| Temperature | 16.639 % | 0.273 % |
| Rainfall | 2.690 % | 5.261 % |
| Relative humidity | 6.213 % | 11.401 % |
| *F. oxysporum* | 26.769 % | 2.038 % |
| *F. solani* | 22.695 % | 0.521 % |
| *P. ultimum* | 0.022 % | 40.147 % |
| *P. mercuriale* | 6.104 % | 19.314 % |

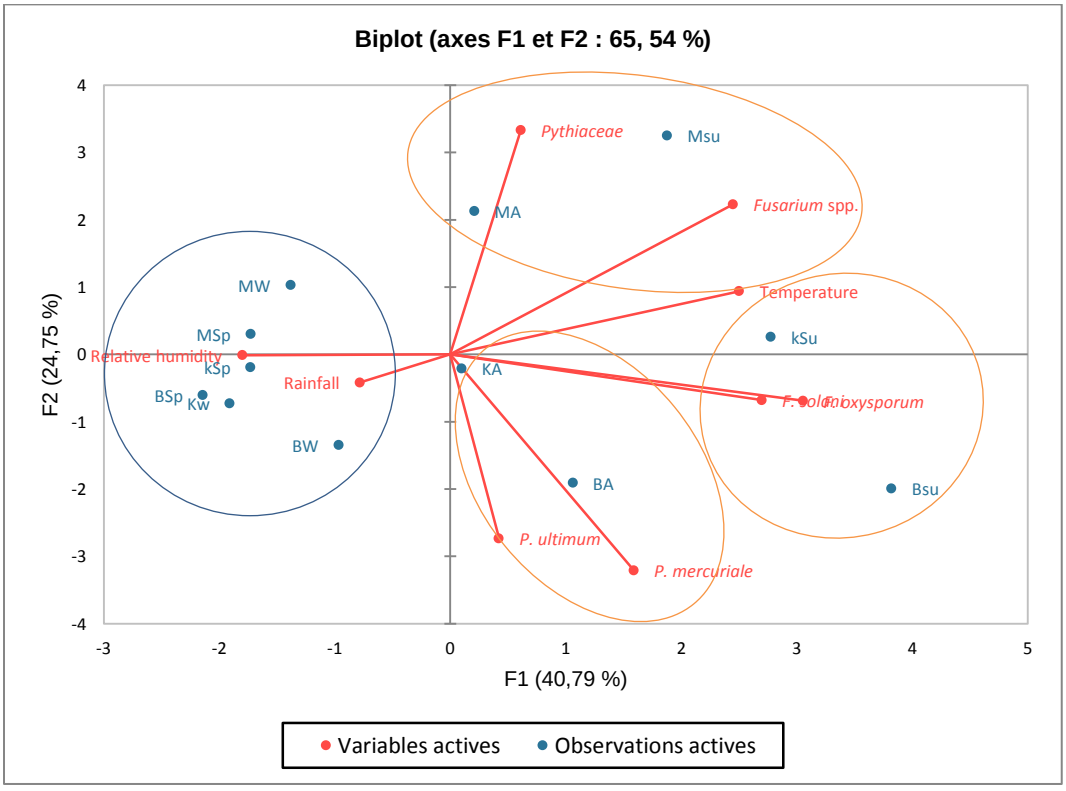



**Fig. 6**. Projection of the climatic characteristics and the populations of the Pythiaceae and *Fusarium* spp., and their dispersion with the region and seasons of samplings in the plan generated by axes 1 (F1) and 2 (F 2). Where; BA: Ben Arous in autumn; BW: Ben Arous in the winter; BSp: Ben Arous in the spring; BSu: Ben Arous in the summer; KA: Kasserine in autumn; KW: Kasserine in the winter; KSp: Kasserine in the spring; KSu: Kasserine in the summer; MA: Monastir in autumn; MW: Monastir in the winter; MSp: Monastir in the spring; MSu: Monastir in the summer

Based on the seasonal distribution, the populations of *Fusarium* spp. associated with apple seedlings in the nurseries were higher in the summer, whereas the Pythiaceae populations were very high in the autumn (Fig. 6). Pearson's correlation demonstrated that the populations of *F. oxysporum* and *F. solani* were positively and significantly correlated with the temperature (r = 0.72 and r = 0.51, respectively); however, they were negatively correlated and non-significant with the rainfall (r = -0.21 and r = -0.32, respectively). Negative correlations were observed between these populations and the relative humidity. These correlations were significant only for the population of *F. oxysporum* (r = 0.57) (Table 2 and Fig. 6). For Pythiaceae, the *Phytopyhthium mercuriale* population was positively and non-significantly correlated with the temperature (r = 0.28). Meanwhile, the population of *P. ultimum* was negatively and non-significantly correlated with the temperature (r = -0.06) and the relative humidity (r = -0.38) (Table 2 and Fig. 6).

**Table 2**. Correlations between *Fusarium* spp. and Pythiaceae populations under different climatic conditions

| | **Temperature** | **Rainfall** | **Relative humidity** |
|---|---|---|---|
| *F. oxysporum* | 0.72** | -0.21 | -0.57** |
| *F. solani* | 0.51** | -0.32 | -0.14 |
| *P. ultimum* | -0.06 | 0.06 | -0.38 |
| *Phytopyhthium mercuriale* | 0.28 | 0.04 | -0.12 |

Where; **: Significant correlation at 0.01, *: Significant correlation at 0.05

### 3.5. Relationships among the physicochemical soil characteristics, Pythiaceae, and *Fusarium* populations

The soils collected from the surveyed nurseries appeared to have different characteristics. In fact, the soil texture was sandy clay in Ben Arous and Kasserine nurseries, while it was silty clay in the Monastir nursery. The electric conductivity was high in Monastir and Ben Arous nurseries with values of 2210 and 1849, respectively. For the organic matter and the nitrogen content, the Monastir nursery showed the lowest values (Table 3).

The PCA revealed that the soil physicochemical characteristics (*i.e*., pH; electrical conductivity, nitrogen content, clay, sand, and silt) and the populations of *P. ultimum* and *Phytopyhthium mercuriale*, had the highest composition of the first axis, while axis 2 consisted mainly of the populations of *F. oxysporum* (20.26 %), *F. solani* (27.70 %), and *Phytopyhthium mercuriale* (10.34 %) (Table 4).

**Table 3**. Soil characteristics of the surveyed nurseries

| Nurseries | Soil texture | pH | EC | TOC (%) | OM (%) | N (µg/ l) |
|---|---|---|---|---|---|---|
| Ben Arous | Sandy clay | 7.12 | 1849 | 1.26 | 2.17 | 69.81 |
| Monastir | Silty clay | 7.9 | 2210 | 0.63 | 1.08 | 48.33 |
| Kasserine | Sandy clay | 7.87 | 508 | 1.36 | 2.34 | 59.07 |

Where; EC: electric conductivity, N: nitrogen content, TOC: total organic carbon, OM: organic matter

**Table 4**. Contribution (%) of several variables (pathogens populations and soil characteristics) in the edification of the axis 1 and 2 of the PCA

| | Axis1 | Axis2 |
|---|---|---|
| Pythiaceae Population | 1.564 % | 2.053 % |
| *Fusarium* Population | 0.865 % | 13.644 % |
| *F. oxysporum* | 0.678 % | 20.262 % |
| *F. solani* | 0.047 % | 27.699 % |
| *P. ultimum* | 10.786 % | 2.418 % |
| *Phytopyhthium mercuriale* | 5.448 % | 10.340 % |
| pH | 7.693 % | 3.555 % |
| EC | 9.655 % | 4.176 % |
| N | 10.518 % | 3.135 % |
| Sand | 10.993 % | 0.293 % |
| Clay | 10.389 % | 1.997 % |
| Silt | 10.558 % | 2.632 % |
| TOC | 10.405 % | 3.839 % |
| OM | 10.400 % | 3.957 % |

Where; EC: electric conductivity, N: nitrogen content, TOC: total organic carbon, OM: organic matter

Based on the spatial distribution, the population of *P. ultimum* was higher in the nursery located in the Kasserine region. The population of *Phytopyhthium mercuriale* associated with apple seedlings (MM106) was higher in the two nurseries located in the Monastir and Kasserine regions (Fig. 7). The Pearson bilateral correlation showed that the population of *P. ultimum* was positively and significantly related to the nitrogen content (r = 0.59); sand (r = 0.82), organic matter (r = 0.85), and organic carbon (r = 0.84). This species was negatively and significantly correlated with the silt content (r = -0.79); clay (r = -0.84), and electrical conductivity (r = -0.74). *Phytopyhthium mercuriale* population was positively and significantly correlated with the nitrogen content (r = 0.64), and negatively significant with soil pH (r = -0.62); clay content (r = -0.47), and silt (r = -0.54) (Table 5). Regarding the populations of *Fusarium* spp., no significant correlation was recorded with the physicochemical characteristics of the soil; except for the electrical conductivity (Table 5 and Fig. 7). On the other hand, studying the correlation between the different *Fusarium* and Pythiaceae spp. revealed significant positive relationships of the *F. oxysporum* with the *F. solani* and *Phytopyhthium mercuriale* populations ($p \leq 0.001$; r= 0.77, and r= 0.58, respectively). Similarly, *F. solani* was related positively and significantly with *Phytopyhthium mercuriale* ($p \leq 0.001$, r=0.6).

Concerning the *P. ultimum* population, its correlation with the other species was not significant. However, it was related positively to *Phytopyhthium mercuriale* (r= 0.36) and *F. oxysporum* (r= 0.26), while being negatively correlated with *F. solani* (r= -0.06) (Table 6).

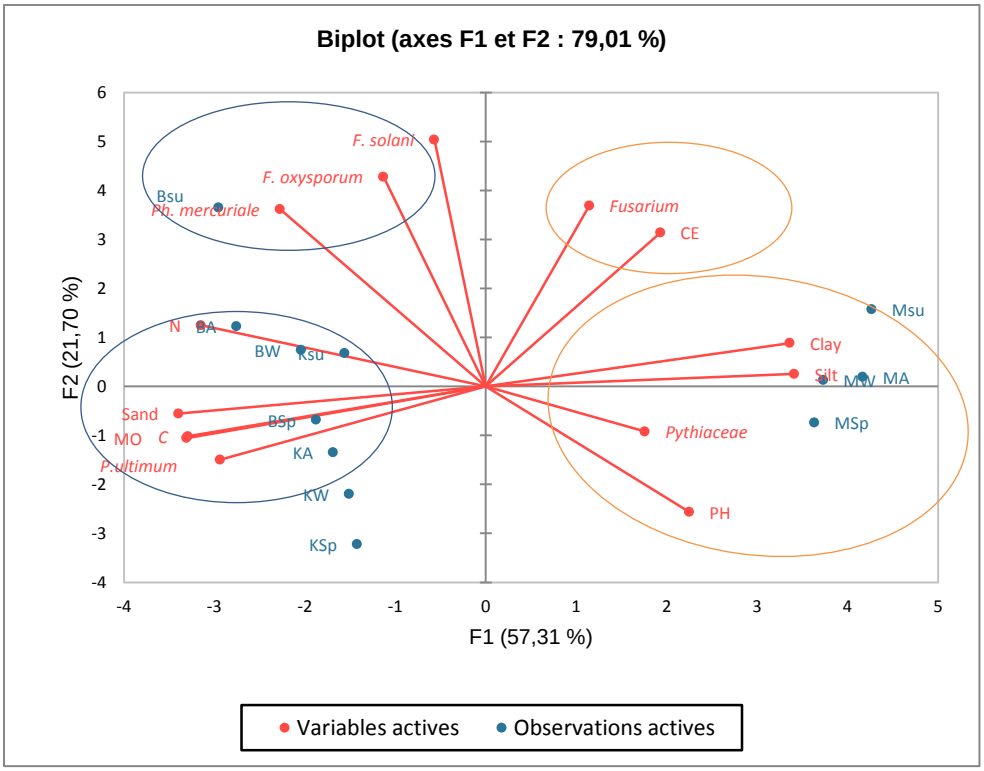



**Fig. 7**. Projection of the soil properties and Pythiaceae and *Fusarium* spp. populations, and their dispersion in the nurseries in the plan generated by axes 1 (F1) and 2 (F 2). OM: total organic matter; C: organic carbon; N: nitrogen content; EC: Electric Conductivity; BA: Ben Arous in autumn; BW: Ben Arous in the winter; BSp: Ben Arous in the spring; BSu: Ben Arous in the summer; KA: Kasserine in autumn; KW: Kasserine in the winter; KSp: Kasserine in the spring; KSu: Kasserine in the summer; MA: Monastir in autumn; MW: Monastir in the winter; MSp: Monastir in the spring; MSu: Monastir in the summer

**Table 5**. Correlations between *Fusarium* spp. and Pythiaceae populations with the soil physicochemical characteristics

| | ***F. oxysporum*** | ***F. solani*** | ***P. ultimum*** | ***P. mercuriale*** |
|---|---|---|---|---|
| pH | -0.27 | -0.33 | -0.26 | -0.62** |
| EC | -0.02 | 0.22 | -0.74** | 0.01 |
| N | 0.30 | 0.26 | 0.59** | 0.64** |
| Sand | 0.25 | 0.10 | 0.82** | 0.49 |
| Clay | -0.23 | -0.06 | -0.84** | -0.47 |
| silt | -0.26 | -0.12 | -0.79** | -0.54** |
| TOC | 0.22 | 0.05 | 0.84** | 0.42 |
| OM | 0.22 | 0.05 | 0.85** | 0.43 |

Where; **: Significant correlation at 0.01, *: Significant correlation at 0.05, EC: electric conductivity, N: nitrogen content, TOC: total organic carbon, OM: organic matter

**Table 6**. Correlation between *Fusarium* and Pythiaceae spp.

| | ***F. oxysporum*** | ***F. solani*** | ***P. ultimum*** |
|---|---|---|---|
| *F. solani* | 0.77** | - | - |
| *P. ultimum* | 0.26 | -0.06 | - |
| *Phytopyhthium mercuriale* | 0.58** | 0.60** | 0.36 |

Where; * Values are different from 0 to a level of significance alpha = 0.05; ** Values are different from 0 to one level of significance alpha = 0.001

## 4. Discussion

The present study proved the presence of the same pathogenic fungal and Oomycetes spp., which were isolated and characterized in 2013 from different Tunisian nurseries (Mannai *et al*., 2018). The results showed the diversity and seasonal variations of the causative agents of apple decline in the Tunisian nurseries; from autumn, 2015 to summer, 2016. A peak of the *Fusarium* spp. and Pythiaceae populations was observed in the summer. Also, the seasonal variation of the *Fusarium* population was significantly and positively related to the temperature data. Similar results were observed with *Phytophthora* and *Fusarium* spp. infected citrus plants (Ippolito *et al*., 1992; Khouja *et al*., 2008); with high inocula densities during the warm months and low propagules during the winter months. Besides, a recent study conducted by Delgado-Baquerizo *et al*., (2020) showed that the warm temperatures increased the relative abundances of the soil-borne potential fungal plant pathogens. Moreover, the future distribution projections under the different climate changes and land uses scenarios have proved an overall increase in the relative abundances of the potential plant pathogens worldwide (Delgado-Baquerizo *et al*., 2020). In contrast, the current study showed that in the surveyed nurseries; the seasonal variation of the Pythiaceae population was not significant for the apple rootstocks MM106 and MM111. This variation was not also related to the temperature data. This may be attributed to the difference in the dependence of Pythiaceae spp. on the climatic conditions (Khouja *et al*., 2008). In fact, the influence of temperature on *Pythium* spp. varied according to the species. In the same sense, Plaats-Niterink, (1981) highlighted that some species were more virulent at high temperatures and others at low temperatures. However, Allen *et al*., (2005) reported a

negative correlation between the population of *Pythium* spp. and the temperature of the soil and ambient air. Furthermore, these authors realized that the population of *Pythium* spp. is high in the winter and low in the spring. Our results are also in agreement with Davelos *et al*., (2004), who suggested that due to the variation in the interactions among the microbial species; it is difficult to simulate the effects of climate change on the disease suppressive soils. Accordingly, the modified chemical and biological control measures need to be implemented against the microbial diseases under the changing climatic scenario (Nazir *et al*., 2018).

In the present study, the sand content was positively correlated with all the pathogenic species. This correlation was not significant except for *P. ultimum*. Similar results were reported by Höper *et al*., (1995), who demonstrated a significant negative correlation between the soil sand content and its suppression of the *Fusarium* populations. Our findings showed a negative correlation of all species of *Fusarium* and Pythiaceae with the clay and silt contents of the soil. Several previous studies have shown no relationship between the soil texture and suppression of the root rot pathogen of asparagus that is incited by *Fusarium* (Hamel *et al*., 2005), and/ or the apple replant disease (Manici *et al*., 2003). However, Toussoun, (1975) had shown that *Fusarium* wilt was less severe in the clay soils. The increase in the Pythiaceae and *Fusarium* populations in the Monastir nursery may also be attributed to high salinity of the soil (7.73 g/ g). In this study, it had also been noted that the salinity of the substrate soil used in the Ben Arous nursery was high (6.47 g/ g). Furthermore, the Pearson's correlation analysis did not demonstrate any positive significant correlation between the electric conductivity and the different species of *Fusarium* and Pythiaceae. In contrast, there was a negative significant relationship between the electric conductivity and the *P. ultimum* population. Thus, it can be concluded that the salinity does not interfere with the observed fungal populations. Similar conclusions had been reported by Manici *et al*., (2003). The previous study conducted by Magarey, (1999) revealed that the soil biological activity was stimulated on addition of a carbon source. In addition, a variety of beneficial microorganisms existing in the soils increased their suppressiveness of disease development (Lazarovits, 2001). Our study revealed no significant correlation between the pathogen's populations neither with the organic matter nor with the total organic carbon. This study revealed that the pH of the soil samples that were collected from the different nurseries was close to neutrality (pH = 7). However, the PCA showed a negative correlation of pH with the Pythiaceae and *Fusarium* spp. These relationships were not significant except for *Phytopyhthium mercuriale*. Our findings are in agreement with several previous studies reported by Lacey and Wilson, (2001); Rimé *et al*., (2003). In fact, Höper *et al*., (1995) found a positive correlation between soil suppression and the pH; with the high pH soils being the most suppressive of *Fusarium* wilt. Lacey and Wilson, (2001) revealed the same relationship between a more acidic pH and a lower incidence of potato scab. However, Manici *et al*., (2003) reported that there was no correlation between soil pH and the disease incidence.

In the three surveyed nurseries, high populations of *Fusarium* and Pythiaceae spp. were observed in the apple seedling roots. This may be attributed to the sensitivity of both apple rootstocks MM106 and MM111 to these fungal pathogens. In the same sense, Wilcox, (1992) reported that apple trees resistance depends on the rootstock, and that MM106 was susceptible to the apple root and crown rot incited by *Phytophthora* spp., while the rootstock MM111 was moderately susceptible to the same disease. On the other hand, there are other apple rootstocks that are resistant to *Phytophthora* root and crown rot, such as M9; M2, M4, and CK1 (Wilcox, 1992); however, they were not currently recorded in the surveyed nurseries. Rotation is one of the cultural control methods used in the nurseries, which was considered in this investigation. This method is more effective for those pathogens that require the presence of their host plants

to survive and/ or pathogens that have the ability to live as saprophytes. However, this method is less effective against the polyphagous pathogens and/or the resistant forms of conservation (Umaerus *et al*., 1989).

Considering that the pathogens can establish symptomless infections in the seedlings of fruit trees (Nemec *et al*., 1978); however after the orchard establishment, these pathogens can survive for one or two years in the rhizosphere (Mazzola, 1999; Tewoldemedhin *et al*., 2011). So, further studies should start in the nurseries at the early stages of plant growth, to prevent the occurrence of the biotic diseases.

## Conclusion

The present study advances understanding of the distribution of the potential apple decline pathogens, in addition to their sensitivity to the various climatic changes and soil characteristics. This is fundamental to reduce the disease incidence and its impact on the fruit plant production in the nurseries, and accordingly to avoid the orchards' infections.

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## Conflict of interest

The authors declare that there is no conflict of interests related to this article.

## Ethical approval

Non-applicable.

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