Prevalence of olive tree phytopathologies of microbial origin in Fquih Ben Salah, Azilal and Beni Mellal (Morocco)

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ABSTRACT
The herein search has as an objective to determinate the prevalence of olive tree phytopathologies of microbial origin in some olive groves of Fquih Ben Salah, Azilal and Beni Mellal. To reach this, 23 orchards were investigated which permitted us to identify the presence of three diseases of microbial origin. Two of them are of fungal origin, e.g., verticillium wilt and olive scab. While the third phytopathology has a bacterial origin which is olive tuberculosis. Moreover, the geographical distribution of the identified foci varied from one disease to another. In fact, olive scab was the most prevalent in the three studied provinces, followed by verticillium wilt and bacterial olive tuberculosis. On the other hand, psyllid, olive scab and verticillium wilt were the most cited diseases by the farms responsible for olive orchards recruited in our study. Conversely, more than 50% of these farmers affirmed not using a phytosanitary treatment against pests and diseases.

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1. Introduction:
The olive tree (Olea europaea L.) represents the main fruit species in Morocco where it occupies areas of more than 50% of the national arboreal area, or 6% of the area of the Mediterranean basin [1-2]. Currently, Morocco is the world's second largest producer of canned olives and the fifth largest producer of olive oil [3-4]. Beni Mellal, Azilal and Fquih Ben Salah are three provinces which were included in Khenifra – Beni Mellal region before the territorial division of 2015. In this region, the olive tree constitutes the regional heritage of excellence of agriculture, occupying an area estimated at 65,500 ha representing 12% of national olive growing and 60% of fruit species at the level of this region [5-6]. Although Morocco has substantial genetic diversity among its olive varieties, it differs from other olive-growing countries by the dominance of a single variety called “Moroccan picholine” which has a dual use: olive oil and table olives. Locally, this variety is also called “Zeitoun Beldi” and covers 90% of the total Moroccan olive orchards [3-4,7-8]. Its importance is justified by the quality of its products and their secular uses as well as its multiple functions of erosion control and enhancement of agricultural land [7]. Despite its good adaptation, it has an average oleic yield of 18% and it presents a high index of alternation of production as well as a high sensitivity to certain diseases and pests [3]. In fact, the various studies carried out at the national level have revealed that the olive tree and its products can be damaged by the presence of numerous pest insects [9-11]. This phytosanitary problem of the olive tree is the main obstacle to the productivity of olive growing. Among the main Moroccan olive pests, the most harmful are the olive moth (Prays oleae); the olive fly (Bactrocera olea); the olive psyllid (Euphyllura olivina) and the black scale (Saissetia oleae) [7,9,10,12]. In addition to these pests, microorganisms are also involved in attacking Moroccan olive trees. The main fungi identified are Fusarium oleaginum responsible for peacock’s eye [13]. Verticillium dahliae the causative agent of verticillium wilt [12] as well as Fumago salicina and Capnodium oleaginum involved in the appearance of sooty mold [7]. Among the bacteria involved, Pseudomonas

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savastanoi pv savastanoi has been identified as responsible for tuberculosis in olive trees characterized by the presence of tubers on the branches and stems [7,10]. The symptoms of these diseases are diverse and can affect all organs of the olive tree e.g. twigs, leaves, flowers, and fruits. Damage can range from premature leaf drop to a reduction in the quantity and quality of oil. Consequently, this can lead to an economically significant damage in the absence of any health intervention [7,9,12].

In this context, the objective of this work is to locate possible foci of infections of fungal and bacterial origins in some olive groves prospected in the region of Khenifra - Beni Mellal and to estimate the infestation rates on the basis of a symptomatological diagnosis. The investigation also aims to assess the level of knowledge of farmers on the presence of pests and microbial diseases in the study area as well as to determine whether control methods are adopted.

2. Materials and Methods:
2.1. Presentation of the studied region:
Tadla-Azilal region was one of the sixteen regions of Morocco before the territorial division of 2015. It was integrated with the provinces of Khenifra and Khouribga in the new region of Khenifra -Beni Mellal. It is located in the center of the country over an area of 7125 Km². From its location between two phosphate plateaus and the Middle Atlas, the Tadla Azilal region with an average altitude of 400 to 700 m is characterized by a very continental climate and the amount of precipitation varies between 300 and 750 mm depending on the year [14]. This region includes three provinces which are Beni Mellal, Azilal and Fquih Ben Salah. Agriculture is the dominant activity in the region, both through the jobs offered (81% of the rural working population in 2008) and through the induced effects on the regional economy. All the more so as the region, with its plains (Tadla) and its significant water resources, offers the possibility of developing modern and industrializing agriculture [15]. Regarding plantations in the region, fruit crops are mainly made up of olive orchards [6]. Indeed, this heritage covers an area of 65500 hectares in the region [5] whose olives represent around 12% of national production among fruit trees [6]. Moreover, the olive growing activity plays an indisputable socio-economic role by creating 2 million working days annually, including 6500 permanent jobs [5]. In addition, the olive oil sector is characterized by development linked to increased demand [6]. In fact, production in 2016-2017 is in the order of 150000 tons. This production is ensured by an irrigated olive-growing area which represents 80% with 5000 Ha conducted in water-saving irrigation (drip) and 1000 Ha in super intensive [5].

2.2. Prospecting and identification of diseases of microbial origin:
According to the olive-growing stations of the studied region and its provincial geographical distribution, this region was divided into three zones:
- Zone 1: Fquih Ben Salah.
- Zone 2: Azilal.
- Zone 3: Beni Mellal.

The field surveys consist in randomly choosing several olive farms spread across the region. Thus, nine municipalities and villages with significant olive growing activity were chosen and distributed as follows:
- Zone 1: Bradia, Oulad Youssef, Oulad said, Fquih Ben Salah.
- Zone 2: Afourar, Beni Ayat.
- Zone 3: Mghila, Tagzirt, Sidi Jaber.

Even plus, in the prospecting of olive orchards, routes following a diagonal model were made whose movements in the field are in zigzags according to the protocol adopted by Dieudonné (1989) [16] (Figure 1). This method aims to cover the largest area of the field and inspect as many olive trees as possible while reducing the likelihood of missing a focus of infestation. Field observations were based on a symptomatological diagnosis, indicative of the presence of the disease, using guides provided by the National Institute of Agronomic Research.

Figure 1. The zigzag inspection process carried out in the studied olive orchards.
2.3. State of knowledge of farmers on pests and microbial diseases:
To ensure a good spatial coverage of the areas studied, a questionnaire was adopted that targets farmers practicing olive cultivation in order to:
• Estimate the state of knowledge of these farmers about pests and microbial diseases.
• Determine if the means of control are practiced against these phytopathologies.

3. Results and discussion:
3.1. Prospecting and identification of diseases of microbial origin:
The surveys were performed in 23 orchards of the three designated olive growing areas. This allowed to identify the presence of three diseases of microbial origin, two of which of fungal origin, namely verticillium wilt as well as olive scab and one of bacterial origin, tuberculosis of the olive tree.

3.1.1. Olive scab:
- Significance of the disease:
Olive scab, also called peacock's eye, is caused by Fusarium oxysporum, a pathogen specific to subcutaneous development that manifests as circular lesions on the leaves of the olive tree [13,17]. The importance of this disease was around 91.30% in olive groves in the three studied areas. It is also noted that Beni Mellal was the most affected province by the disease (Figures 2 and 3).
It should be noted that the percentage of affected orchards by olive scab in each province was calculated basing on the number of contaminated fields out of the number of prospected fields of the visited villages and municipalities. While the importance of olive scab in Tadla Azilal region was calculated basing on the total number of contaminated fields out of the number of prospected fields localized in the nine visited villages and municipalities of Fqui Ben Salah, Azilal and Beni Mellal. Peacock's eye is considered to be one of the most widespread olive leaf diseases in many olive-growing regions, causing yield losses estimated at 20% [13,17]. Undoubtedly, the disease is particularly severe in nurseries and orchards where olive cultivars are heavily planted when environmental conditions are favorable for temperature and humidity for the development of the fungus [17]. In Morocco, this disease is also one of the most important diseases of the olive tree [18]. It has appeared in several regions, namely Gharb, Ouazzane [18], Beni Mellal, Marrakech [19] and Kelaa Sraghna [20].

![Figure 2](image1.png)
**Figure 2.** Number of contaminated fields by olive scab in the 23 prospected orchards localized in nine municipalities and villages of Fqui Ben Salah, Azilal and Beni Mellal provinces.

![Figure 3](image2.png)
**Figure 3.** Percentage of contaminated fields by olive scab of the 23 prospected orchards localized in nine municipalities and villages of Fqui Ben Salah, Azilal and Beni Mellal provinces.
• **Symptoms of the disease in the field:**

Surveys executed in the olive groves of the three visited areas revealed mainly on the upper surface of the leaves zoned circular spots surrounded by yellow halos, hence the term of "peacock's eye". These spots vary in color from gray to brownish or dark brown to yellow-orange. These symptoms observed in olive groves are characteristic of olive scab. Indeed, when the temperature and humidity conditions are favorable for the germination of the conidia of the fungus *F. oleagineum*. This microorganism penetrates through the cuticle of the upper surface of the leaves, developing a mycelium which propagates in the internal tissues of the leaf [21] thus causing typical lesions which are often surrounded by yellow halos [17]. When the attack is severe, necrosis reaches the main veins; then the leaves turn yellow leading to their premature abscission [13]. Moreover, recurrent infections cause a branch dieback, a weakness in olive trees and even a significant decrease in production due to a low photosynthetic capacity as well as a limited vegetative growth [17,22]. The disease can also appear on fruits, fruit peduncles and young shoots leading to weakening of the whole plant [13,17].

3.1.2. **Verticillium wilt:**

• **Significance of the disease:**

Verticillium wilt is caused by a microscopic fungus present in the soil, *Verticillium dahliae*, which first affects the roots and then the vascular system of the tree, and causes damage in the aerial parts [12]. The importance of this disease was around 65.2% in olive groves. In general, and within the limits of the municipalities and douars visited in the study region, the disease was found to be well distributed and established. In fact, in the region of Fquih Ben Salah, 75% (n = 6) of olive tree fields surveyed contain foci of the disease followed by the region of Beni Mellal and Azilal with 66.7% and 55.6 %, respectively (Figures 4 and 5). In Morocco, the disease was first described in Meknes region on young and well-maintained olive groves where it caused significant damage. After that, more than 40 isolates were collected during different surveys carried out in the same region with an attack importance ranging from 3.72% to 30.3% on average. Afterwards, the disease was detected in several other regions e.g., Figuig, Beni-Mellal, and El Kelâa Sraghna. In addition, in certain localities of Haouz region, this tracheomycosis has been found to be well distributed and established with a contamination percentage of 40.9% of douars, 61.9% of cooperatives and 3.9% of 2823 visited farms. The incidence varied from 1 to 50% of affected trees depending on the olive orchards [1].

The percentage of affected orchards by verticillium wilt in each province was calculated basing on the number of contaminated fields out of the number of prospected fields of the visited villages and municipalities. While the importance of verticillium wilt in Tadla Azilal region was calculated basing on the total number of contaminated fields out of the number of prospected fields localized in the nine visited villages and municipalities of Fquih Ben Salah, Azilal and Beni Mellal.

![Figure 4](image4.png) *Figure 4.* Number of contaminated fields by verticillium wilt in the 23 prospected orchards localized in nine municipalities and villages of Fquih Ben Salah, Azilal and Beni Mellal provinces.

![Figure 5](image5.png) *Figure 5.* Percentage of contaminated fields by verticillium wilt of the 23 prospected orchards localized in nine municipalities and villages of Fquih Ben Salah, Azilal and Beni Mellal provinces.
• **Symptoms of the disease in the field:**

Surveys carried out in the region's olive groves have revealed diebacks. Thus, we noticed the defoliation of the ends of the branches which, however, retain a few parched leaves at their base. We had also observed the presence of young shoots completely defoliated and others almost dry, but which can sometimes retain a few brown leaves at their end. In other olive trees, we noticed on the young twigs the presence of leaves having a light brown color, rolled up lengthwise, withered and brittle. Sometimes these leaves are very fragile and drop at the slightest touch. Similar symptoms of verticillium wilt in olive trees were observed according to the study performed by Sedra (2002) [1] by leading surveys in 2823 farms in Haouz area of Morocco. Effectively, when temperature and humidity conditions are favorable, sclerotia, a form of preservation of the fungus, produce spores which germinate and penetrate into the root system and at the base of the trunk [1,23]. The fungus infestation is favored by the presence of wounds [23]. The mycelium grows in the xylem tissues, which carry the raw sap to the leaves [1]. Thus, once all the xylem is colonized, the sap becomes blocked [23]. The leaves of leafy organs such as twigs and branches lose their greenish color to light brown, which causes them to dry out especially in young olive trees. Moreover, on other olive trees, the wilted leaves become brittle with a tendency to fall [24].

### 3.1.3. Olive tree tuberculosis:

• **Significance of the disease:**

Fake tree tuberculosis is the main bacterial disease of olive trees caused by *Pseudomonas savastanoi* [12,25]. This bacterium settles on wounds and causes aerial tumors on different parts of the plant [25]. The importance of this bacteriosis in olive groves was about 13%. Out of six examined orchards in the visited douars and municipalities of Beni Mellal province, two (or 33.3%) were affected by the disease. While no signs of the phytopathology were seen in the province of Azilal (Figures 6 and 7).

The percentage of affected orchards by olive tree tuberculosis in each province was calculated basing on the number of contaminated fields out of the number of prospecteds fields of the visited villages and municipalities. While the importance of olive tree tuberculosis in Tadla Azilal region was calculated basing on the total number of contaminated fields out of the number of prospecteds fields of the nine visited villages and municipalities in Fquih Ben Salah, Azilal and Beni Mellal. Olive tree tuberculosis is widespread throughout the Mediterranean basin and California [25]. In Morocco, this phytopathology is considered to be the main bacterial disease of the olive tree [26]. In fact, it was reported for the first time in the region of Meknes in 1960. Since then, it has spread since in all the olive growing areas of the country namely Beni Mellal, El Kalaa, Marrakech, Kenitra, Sidi Kacem, Oujda, Hoceima, Taza, Fez, Taounate and Ouazzane [25,27,30]. In addition, field surveys performed between 2012 and 2013 in several Moroccan regions revealed that the incidence of the disease is variable from one region to another and even from one municipality to another ranging from 1% up to 100%. However, the progression of the disease in the central and northern regions of the country is favored by climatic conditions such as humidity and temperature [30].

**Figure 6.** Number of contaminated fields by olive tree tuberculosis in the 23 prospected orchards localized in nine municipalities and villages of Fquih Ben Salah, Azilal and Beni Mellal provinces.

**Figure 7.** Percentage of contaminated fields by olive tree tuberculosis of the 23 prospected orchards localized in nine municipalities and villages of Fquih Ben Salah, Azilal and Beni Mellal provinces.
Symptoms of the disease in the field

Surveys led in Tagzirt and Bradia olive groves revealed tumor growths of wood, 2 to 10 cm in diameter, occurring on young twigs, branches and stems of the tree. These symptoms showed that the olive trees in these regions are affected by tuberculosis or bacterial canker of the olive tree. Indeed, at the beginning, the olive knot manifests itself by parenchymal tumors with irregular shape and a smooth surface of green color on twigs, main trunk branches, roots, damaged leaves, stems, fruits and young shoots [27]. After few months, the hyperplastic growths acquire a spongy and irregular appearance, becoming hard and brown on the small shoots [27,28]. It seems that the intensity of damage caused by olive tuberculosis is strongly related to the number of tumors per tree. In fact, as the number of tumors increases, the growth of the twig is retarded and the number of fruit clusters is affected [25]. In addition, the disease presents a significant problem for olive crops due to its effect on vegetative growth (decline of branches and shoots, death of small branches and twigs), olive yield, and even possibly on the olive oil quality due to inferior organoleptic characteristics such as an unpleasant odor as well as a bitter and rancid taste [26]. In addition, infection of the plant by *Pseudomonas savastanoi* requires the presence of openings provided by leaf scars, pruning wounds, cracks due to frost and hail or harvesting operations [27,28]. The abscission points of leaves, flowers and fruits are also gateways for the bacteria [27]. Tumors are developed in response to phytohormones produced by the bacterium; including indole-3-acetic acid (IAA) and cytokinins [29,30]. This pathogenicity is induced by a "Ti" plasmid (Ti for tumor inducing) which integrates into the genome of the host plant cell. The new DNA formed triggers autonomous production of IAA which plays a role in cell enlargement and cytokinins that promote cell division [29].

3.2. State of knowledge of farmers about pests and microbial diseases:

Faced with the phytopathologies identified in Tadla-Azilal region, we assessed the level of knowledge of farmers regarding pests and diseases of microbial origin that affect olive trees. On the other hand, we wanted to know if any methods of struggle were adopted. Thus, among 103 farmers responsible for olive orchards recruited in our study, 68 farmers, or 66%, who responded to the questionnaire. 82.8% of these farmers knew about the presence of olive pests and microbial diseases, of which peacock's eye, psyllid and verticillium wilt were the most mentioned phytopathologies (figure 8). Nevertheless, only 48.5% of these cooperative farmers declared the adoption of a phytosanitary treatment against 51.5% who did not use any treatment. In addition, no farmer practiced biological control of olive pests, nor uses plant extracts and / or microorganisms which are less toxic and non-polluting to the environment in comparison with the pesticides used in the phytosanitary treatment to deal with microbial phytopathologies [31].

![Figure 8. Percentage of farmers with knowledge of olive pests or diseases in their olive orchards.](image)

Some farmers mentioned that they are adopting methods for disease monitoring and control through continuous observations of fruits, leaves, twigs and branches, especially during wet periods. Others employed pruning of olive trees accompanied by incineration of pruning wood and followed by chemical treatment. These precautions are the same as those indicated by Benjama (2003) [25], taken against bacteria in olive trees. Removing knots from the twigs was also fulfilled carefully during the dry season. Otherwise other farmers eliminated vector plants as means of prevention and prophylaxis against verticillium wilt. Undoubtedly, *Verticillium dahliae* is able to resist several years in the soil and to perpetuate itself through vector plants [23]. On the other hand, for good phytosanitary management, it is important to mention that farmers must be trained to have a professional qualification or to have qualified technical staff who can conduct all production operations in good conditions. They must also have the necessary equipment for the maintenance and protection of the plants [7].

4. Conclusion:

The olive activity in Fquih Ben Salah, Azilal and Beni Mellal occupies an important place in terms of fruit plantations. During the period of our study, we identified that the olive orchards in these provinces harbored two diseases of fungal origin and one disease of bacterial origin. These phytopathogenic species can cause significant damage, both quantitatively and qualitatively, thus presenting a serious potential threat to the olive groves in the region. According to their degree of importance, olive scab (91.3%) was the most prevalent in the region, followed by verticillium wilt (65.2%) and bacterial
olive tuberculosis (13%). The geographical distribution of the identified foci varied from one disease to another and from one region to another. In addition, the assessment of the knowledge of agriculture in the region vis-à-vis diseases of microbial origin and control methods revealed that almost the majority of these farmers (82.8%) had knowledge about pests and microbial diseases and confirms the identification of these agents in their olive orchards. Moreover, 48.5% of farmers declared the adoption of a phytosanitary treatment against 51.5% didn’t treat against pests and diseases. However, these phytopathologies must be monitored, treated and controlled by limiting the spread of causal agents in other olive groves still unharmed by all farmers in collaboration with several actors including the Ministry of Agriculture and national agricultural research institutes. This with the objective of preserving, on the one hand, the olive tree as a regional heritage of excellence and, on the other hand, to avoid unacceptable imperfections in production which would risk Morocco losing its place as the world's second largest producer of canned olives and the fifth largest producer of olive oil. Thus, as perspectives of this preliminary study, this work may lead to other lines of investigations, namely:

- The isolation and molecular identification of the microbial species responsible for olive scab, verticillium wilt and bacterial olive tuberculosis;
- Evaluation of the antimicrobial effect in vitro and in vivo of several extracts (aqueous and organic) and essential oils of Moroccan medicinal and aromatic plants against the various isolated causal agents;
- Purification of the active substances of plants of interest in order to develop non-toxic bio-pesticides that don’t pollute the environment.

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