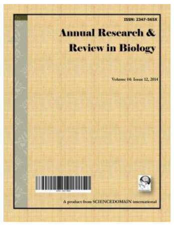

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# Influence of Variations in Climatic Factors and Some Cultural Practices on Knot Disease Development on Oleaster and Olive Tree (*Olea europaea* L,) Northwest of Morocco

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***Authors' contributions***

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The present study is conducted to know the effect of certain cultural practices and the variation of temperature and humidity (bioclimatic stage, slope exposure) on the distribution and importance of the development of tuberculosis disease of olive trees, caused by *Pseudomonas savastanoi* pv. *savastanoi* (PSS), in Northwest of Morocco. 1584 trees of oleaster and olive trees (cuttings and grafts) were observed during the period 2013-2014 in several groves in the northwest regions of Morocco. The percentage of infection was calculated by the number of olive trees showing the symptoms of tuberculosis caused by *Pseudomonas savastanoi* pv. *savastanoi*. The intensity of the disease in the oleaster and the olive tree was determined by counting the number of knots.

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The prospection was showed that olive plots are more or less attacked by tuberculosis in the northwest part region of Morocco and more apparent in olive trees in development station in Zoumi and in Boukkara (North and West oriented slopes of 68% and 28% respectively). The percentage of infection was very high in oleaster and graft plants (31% and 28% respectively) in two humid stations (Zoumi and Boukkara). The intensity of the tuberculosis disease showed that oleaster trees are most attacked. The development of olive trees is highly influenced by the symptoms in branches. The fruit size and weight were significantly higher in healthy olive trees than in those showing the symptoms of tuberculosis.

*Keywords: Tuberculosis; oleaster; olive; slopes; exposure; node; humidity; temperature; Morocco.*

## 1. INTRODUCTION

The cultivation of olive trees in the Mediterranean basin is of a great socio-economic importance, with 715 million olive trees covering an area of about 7.5 million hectares [1] that represents 9.5% of the world's olive-growing heritage.

In Morocco, the olive sector is the main fruit speculation, with an area of about 680000 hectares, almost 55% of the national arboreal orchard. It actively contributes to the establishment of the rural population by creating more than 11 million work days [2]. Moreover, olive oil production contributes actively to the balance of trade resulting in Morocco which is the second largest exporter of table olives with an annual average of nearly 70000 T. Today, the olive tree is subject to different types of phytosanitary problems that can cause serious economic losses. It is the pests, fungal and bacterial diseases [3-5] that cause most damage by attacking different organs of the olive tree [6-13].

Tuberculosis is one of the bacterial diseases of olive tree. It is caused by *Pseudomonas savastanoi* pv. *savastanoi* (PSS), and considered one of the most serious diseases affecting olive trees especially in the Mediterranean countries [14]. It was first described by the Greeks in the 4$^{th}$century BC and has been reported in Africa since the early 19$^{th}$century [15]. However, in Morocco, tuberculosis was reported for the first time in the region of Meknes 1960, since then, the disease spread to the Moroccan olive growing areas and mainly caused damage in the northern regions of the country, in Meknes, Fez and Zerhoun [14]. The economic consequences of this disease are considerable on the cultivation of the olive tree affecting both the quality and the yield of oil [16-18].

Tuberculosis infections begin timidly on branch twigs and do not spare carcasses and trunk [19]. Several factors are involved in triggering the infectious process such as injuries that can be natural like those caused by leaf fall or mechanical due to pruning and picking [20] and disease can also occur through irrigation and transport of size residues [21,22] also reported that the effect of insects is not negligible as well. In fact, a homopteran (*Ceresa* sp., Membracidae Family) deposits its eggs and causes wounds that constitute an infection site by the bacteria. The propagation of the PSS bacterium among the entire olive tree is due to its diffusion through xylem [23].

In addition, many conditions may help in this propagation like the variation in climatic factors (continentality, altitude and slope exposure) and the origin of olive plants. Thus, the aim of this study is to establish the variation effect by certain climatic factors, and the exposure effect of slopes on the development of tuberculosis disease of olive tree and oleaster caused by *Pseudomonas savastanoi* pv. *savastanoi* in the region of Ouazzane, Northwestern Morocco.

## 2. MATERIALS AND METHODS

### 2.1 Design

A survey on olive tuberculosis in the Northwest of Morocco area was carried out during the period September 2013 – October 2014. Eleven sites from Souk El Arbaa to Zoumi via various stations (Kariat Ben Aouda, Foukra, Tnin Srafah, Sabt Masmoda, Ouazzane, Brikcha, Sabt Sidi Radouane, Ain Douraij, Mjaara, Zoumi and Boukara) were involved. In each site, 100 olive trees were selected at random to determine the tuberculosis infection percentage of olive trees. The effects of altitude and slopes on the severity of tuberculosis have been studied in a humid climate site of Zoumi, a 970 m high mountain located in this site has been divided into three levels according to its height: Level A (low slope), level B (mid-slope) and level V (high slope). The

North and West exposed slopes are more humid than those exposed South and East, in fact in each slope, a plot has been chosen to quantify the disease of the olive knot in each slope. At the level of each olive tree, the importance of the disease was established at the level of the sides (North side, South side), five plants were randomly chosen under the same planting conditions. In order to study the effect of tuberculosis on the yield and quality of the olives, samples of the olives were taken from diseased branches carrying nodes and others from the branches of other non-diseased plants.

### 2.2 Infection Parameters

The percentage of infection was calculated by the number of olive trees showing the symptoms of tuberculosis disease. The intensity of the disease in the oleaster and the olive tree was determined by counting the number of tumorous (nodes) on two branches from 1 to 1.5 m long each side of the tree. The quality of the olives was quantified by measuring the weight with a scale and size with caliper of the olives.

### 2.3 Separation and Identification of Isolates

Small branches containing tumors were cut with pruning shears, placed in a paper bag and transported directly to the laboratory. The tumors were disinfected using 96% ethanol-impregnated sterile filter paper [24]. The internal tissue was cut into small fragments of 1 to 2 mm and placed in 200 µl of sterile distilled water. After 30 min of maceration, 50 µl of the macerate is spread in Petri dishes containing a King B medium [25]. The dishes were subsequently incubated at 26ºC for 3 to 5 days. After incubation, purification was carried out to obtain different isolates. The isolates obtained are seeded on inclined GN (nutrient agar) and then incubated and stored at 4ºC.The identification of isolates took into account the macroscopic, morphological and biochemical characteristics. Biochemical tests called LOPAT were carried out: levan, oxidase, pectinolytic activity, presence of arginine dehydrolase and hypersensitivity on tobacco leaves.

### 2.4 Statistical Analysis

All analyses were carried out using statistica software and the Statistical Package for the Social Sciences (SPSS, version 17.0). The data were represented as mean and standard deviation (SD) for quantitative values normally distributed, and extremes (minimum and maximum). Normality of distribution was tested by the Kolmogorov-Smirnov test. Student test and one-way ANOVA for independent samples were used to test the significance. The Fischer's test was used in the case of qualitative variables. P-values $<0.05$ were considered significant.

## 3. RESULTS AND DISCUSSION

The purified isolates were characterized by macroscopic, morphological and biochemical characteristics such as *Pseudomonas savastanoi* pv. *savastanoi.*

The symptoms of tuberculosis observed on the twigs and the branches of olive trees developing in the study regions are woody growths, Sometimes very numerous and whose dimensions vary from 0.5 cm to 5 cm. These parenchymatous tumors are very irregular, green in color at the beginning and with a smooth surface, sometimes spherical in shape (Fig. 1- a, b). But the diameter of these tubers productions increase with the evolution of the attack, and become irregular, depressed at their summits by a deep cavity, cracked, and sometimes more or less divided into lobes (Fig. 2- a, b, c, d). Then, the tumors dry up and take the brown color, causing the death of the twigs that bear them (Fig. 2 f).

The humidity influences the development of tuberculosis of the olive trees. The tuberculosis in olive trees seems to have a heterogeneous distribution; the percentages of infection at the level of some sub-humid sites are zero (Ain Doraij, Srafah, Souk El Arbaa and Kariat Ben Aouda). But in others, between 1% (Mjaara, Ouazzane) and 10% (site of Sabt Masmoda). On the other hand, the percentages of infection at the sites belonging to the humid bioclimatic stage reach 28% (Boukara site) and 31% (site of Zoumi).

Tunisian authors [26]. explained the emergence of the disease in the central and south-eastern regions of the country (ElKarma and Bouzouita) being favored by climatic conditions such as hail fall causing injuries to the twigs, wind and rainfall, on the other hand, the discovery of the disease in the South region of Matmata (Djbel Toujen) is due rather to micro-wounds appearing after the fall of the leaves at the time of the picking. Sand winds may also play a role in the appearance of the disease. Thus, they can cause injuries to the branches, allowing the bacteria to settle on the plant and initiate the infectious process [26]. *PSS* can infect olive trees at any time of the year but the triggering of the nodes occurs when the conditions are favorable [27]. The size of the PSS population in the stems and on the surface of the leaves is positively correlated to precipitation and at a temperature ranging between 19-25ºC and a relative humidity [28], which explains the absence of the disease in some stations. On the other hand, it is omnipresent in other stations, because at the level of stations with a sub-humid climate in fresh winter the disease of tuberculosis is absent. This is the case of the city of Souk el Arbaa and Kariat Ben Ouada where the disease was absent (0%). But as the continentality increases the humidity takes its amplitude with the increase of the altitude. This process favors the appearance of tuberculosis disease, as in the case of Brikcha and Sabt Masmoda stations where infection percentages were 7% and 10% respectively. In addition to the bacterial species *PSS* finds their prosperity in the stations with humid climate; case of two stations Zoumi and Boukkarra with 31% and 28% respectively as infection percentage. Previous studies experimentally confirmed that the rain is responsible for the spread of the disease and wind-driven rain responsible for the lateral distribution of microorganisms [27]. Temperatures in the range observed during most winter and spring seasons do not appear to be a limiting factor [29]. In addition, Ercolani [30] for three consecutive years 1971, 1972 and 1973 showed that the population of bacteria is high during spring and autumn which witnessed an alternation of humidity and temperature [30,31]. In Spain, Quesada et al [28] reported that the size of the population becomes important in the rainy months. These last two stations Zoumi and Boukkarra know a condensation of the plants which constitutes a main agent in the increase of the relative humidity; it is consequently the infection in the presence of the injuries.

Concerning the tuberculosis severity in study sites, the results showed that Oleaster are more susceptible to tuberculosis than olive trees. Indeed, the average number of nodes per foot of oleaster is about 160 protuberances (galls). This number is in the order of 100 per olive tree carried in oleaster graft and 10 in olive trees derived from ligneous cuttings. In this sense, Sanhaji [13] reported to Ain Taoujtat that tuberculosis has wreaked in Ain Taoujtat in natural olive plantations and in olive trees whose graft is an oleaster. This author noted that the susceptibility of oleaster to tuberculosis is favored by the presence of certain plant species very sensitive to the infection by the bacterium PSV (case of pink laurel, ash, privet, forsythia, jasmine...) that grow inside the natural populations of oleasters. A wide spread of oleander plants at the borders of rivers can constitute a source of inoculums. Indeed, the severity of the disease increases when increasing inoculum concentration and the number of scars and injuries [32].


**Fig. 1. Production of tumors on olive stems (cv. Arbequina) after 60 days of inoculation with the isolates *Pseudomonas savastanoi*, 2064-10 (A) and 2064-8 (B)**


**Fig. 2. Symptoms of tuberculosis of the olive tree: (a) gall at the beginning of its development in the branches of the olive tree; (b) cracked and smooth-surfaced gall; (c) developing gall with a rough surface and a hollow apex; (d) black galls with a rough surface; (f) drying of the attacked twigs**

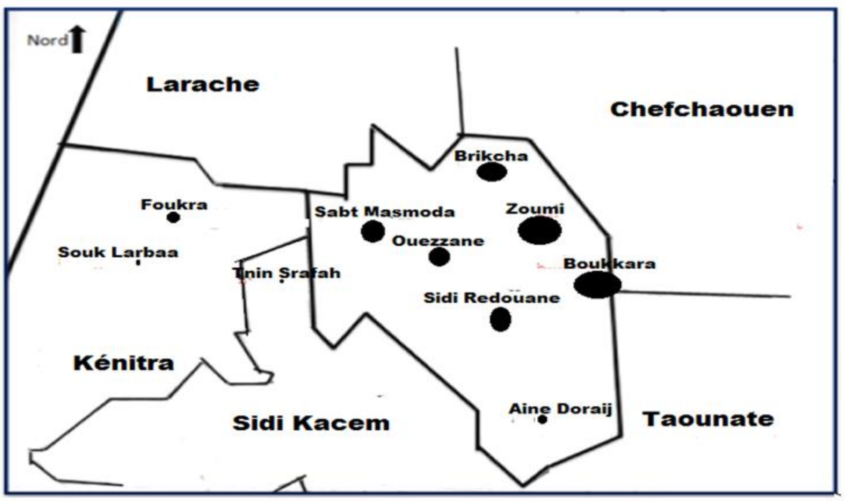


**Fig. 3. Prospective sites mapping the tuberculosis disease of the olive tree in Northwest Morocco**

**Table 1. Variation in the percentage of infection of olive trees in function to bioclimatic stage in olive orchards in Northwest of Morocco**

| Sites | Multiplication method | Climat | Infection, % |
|---|---|---|---|
| Souk larbaa | Cutting | Sub-humid | 0.0[a] |
| Kariat ben aouda | Cutting | Sub-humid | 0.0[a] |
| Foukra | Cutting | Sub-humid | 9.0[b] |
| Srafah | Cutting | Sub-humid | 0.0[a] |
| Sabt masmoda | Cutting and graft | Sub-humid | 10.0[b] |
| Ouazzane | Cutting and graft | Sub-humid | 1.0[c] |
| Sebt sidi redouane | Cutting | Sub-humid | 4.0[d] |
| Zoumi | Cutting and graft | humid | 31.0[e] |
| Boukara | Cutting | humid | 28.0[e] |
| Brikcha | Cutting and graft | Humid | 7.0[bf] |
| Ain doraij | Cutting | Sub-humid | 0.0[a] |
| Mjaara | Cutting | Sub-humid | 1.0[c] |


**Fig. 4. Severity of the disease towards the oleaster and the olive tree**

The plots orientation influences the development of tuberculosis of olive trees. The infection percentages of olive trees by tuberculosis are functions of the orchards orientation. They were 68% and 28% for the North and West oriented plots respectively. In South and East oriented plots, the percentages of infection were 20% and 4%, respectively. The nodes number in olive trees from the exposed North and West plots was 21.8 and 17.5 respectively. This number of tumors remains low in olive trees oriented south and east 9.3 and 7.5. Similarly, olive trees on the high and middle slope show a large number of nodes 62.0 and at the bottom pouring the disease is less intense 14.4. These results are consistent with those reported by Fayard [33] in California, which showed that the disease is very important in Northern California, a humid region that receives more precipitation.

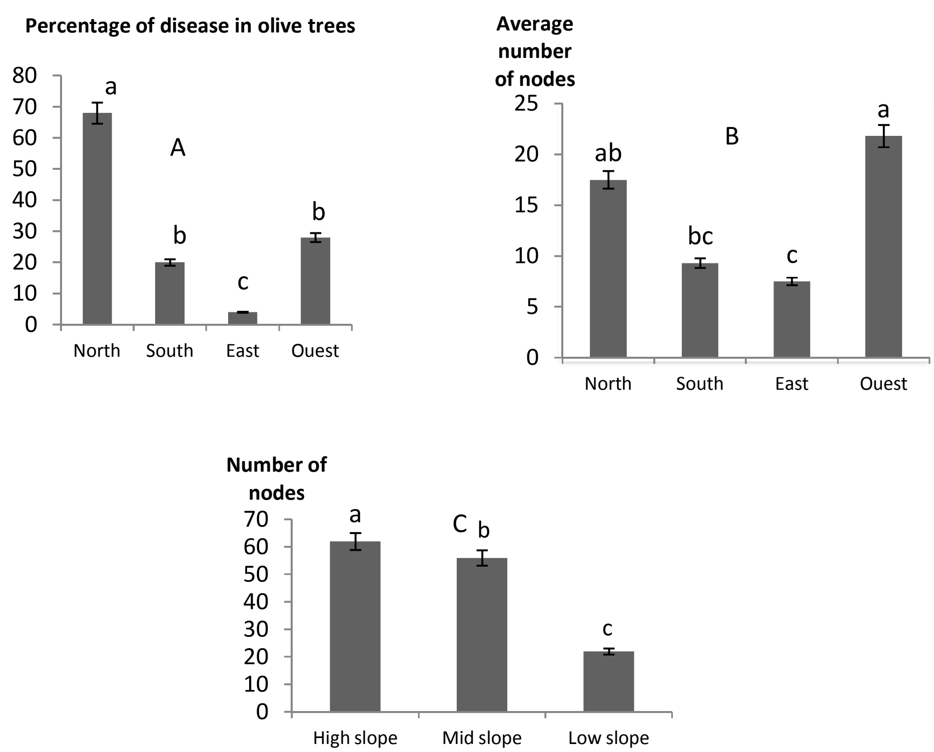


**Fig. 5. Effect of orientation (A) and elevation of plots on the presence and severity (B, C) of tuberculosis (Number of nodes)**

In addition, olive trees groves on the Southern slope benefit more from the sun, which limits the severity of the disease than tuberculosis in contrast to the North Slope which receives less sunlight. Youg [34] reported that natural plantations of Oleaceae are a source of contamination and generally grow between the high slope and the mid slope, the drafts in these two levels causing injury to the olive branches and consequently the penetration of bacterial inoculum and the outbreak of tuberculosis disease.

The nature effect of plots on tuberculosis development determined according to our results showed that oleasters are more attacked by tuberculosis than olive trees (Fig 6). While, the number of nodes in oleaster plants developing in the plain is 16.6 nodes / tree and 21.7 in the mountains. The number of nodes in the olive trees of the plain is very low (0.33), but this number is important in the olive trees developing in the mountains.

### 3.1 The Tuberculosis Effect on the Size and Weight of Olives

The size of the olives of the branches with tuberculosis tumors 1.8 is lower than those carried by healthy branches 2.3 of olive tree (Fig. 6). This decrease in olive size affects weighing respectively 26.4 and 42.85 in the olives of diseased and healthy branches. These results are in agreement with those reported by Benjama et al. [35] and Cayo et al. [36] where a perfect correlation between the number of tumors and the intensity of the damage was caused by the disease.

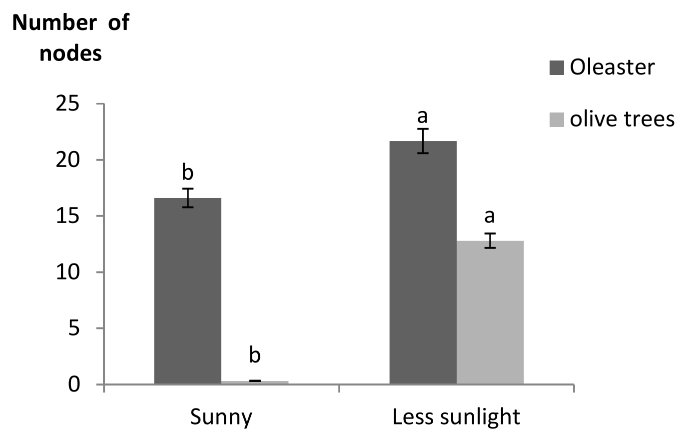


**Fig.6. Nature effect of the plot on the tuberculosis development**

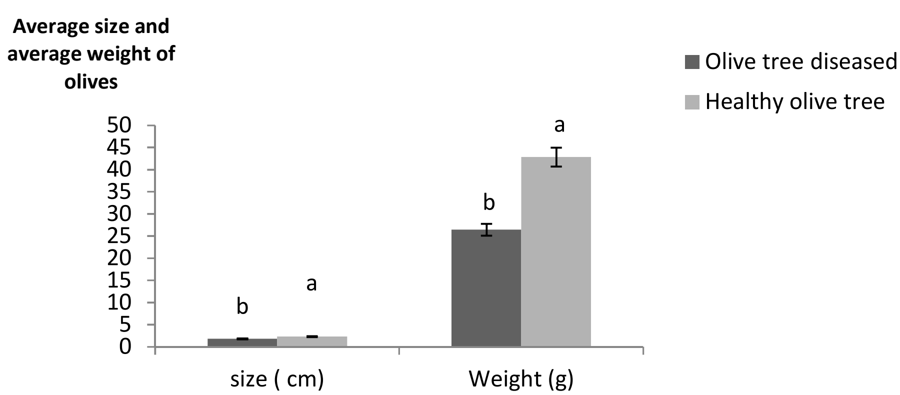


**Fig. 7. Effect of tuberculosis on the size and weight of olives**

## 4. CONCLUSION

The tuberculosis of the olive tree is omnipresent in Northwest part of Morocco. The variation in climatic factors and unhealthy cultural practices help to develop tuberculosis disease in oleaster and olive tree. Oleasters are more infected than olive trees, and they are the sources of contamination for olive trees. Moisture and lack of sunlight promote humidity agent favorable of the development of the *Pseudomonas savastanoi* pv. *savastanoi* and the manifestation of the disease, expressed by the number of tumors. The disease also affects the weight of the olives.

However, consideration of all these factors and the integration of healthy practices may contribute in reducing the disease. An urgent plan to identify plants at risk is highly recommended.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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