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# Isolation and Identification of Xanthomonas spp. of Almond Trees in Suluq District, Libya

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## Abstract

Bacterial leaf spot (*Bls*) disease caused by *Xanthomonas* spp. is one of the most economically important bacterial diseases that attack stone fruit trees and almond trees in particular. We conducted the current study to determine and identify the causative pathogen, *Xanthomonas* spp., of almond trees at the University of Benghazi farm in the Suluq district. We used biological and morphological features such as gram stain reaction and bacterial colony color on growth medium as tools for accurate identification. Results in the present study showed that *Xanthomonas* spp. was the main causative of leaf spot disease on almond trees in Suluq district.

**Keywords:** Bacterial leaf spot, *Xanthomonas*, almond trees.

## 1. Introduction

Almond (*Prunus dulcis*) is a deciduous tree that planted for its edible seeds and other medicinal usages. Almond belongs to genus *Prunus* L (Family: Rosaceae) (USDA, 2023, Gupta et al., 2020) is an important orchard tree that has been grown in a relatively cold climate. Almond fruits are extremely beneficial to human health because of their antioxidant properties, such as phenolic compounds and Omega-3 fatty acids, in which can help in preventing or reducing cardiovascular chronic diseases, for example, heart attack (Blomhoff et al, 2006). Almond wild type believed to be originated from central Asia and distributed to the other continents via human trading (Ladizinsky, 1999). Because of its bitter taste, humans cannot consume the wild type directly and they consume only the sweet type.

According to Food Agriculture Organization (FAO, 2017) statistic, the United States of America (U.S) is the largest producing country, and Spain's production ranks second with 70%, 20% worldwide, respectively. The other producing countries, include Italy, Portugal, Morocco, and Tunisia, are accounted for the rest of almond's production.

There are various factors that limit almond yields. Of these, frost in the spring, shortage of irrigation, nutrient deficiency, especially nitrogen (N), poor soil structure, lack of plant disease management, namely fungal and bacterial pathogens (Agrios, 2005). These abiotic and biotic stresses collectively decrease almond productivity substantially per hectare.

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Several bacterial diseases (e.g., leaf spot, canker, crown gall and bacterial blight), and fungal diseases (e.g., powdery mildew, brown rot, and dieback) attack almond trees, and cause significant quantitative and qualitative fruit loss (Naqvi, 2004; Schumann and DArcy, 2010). Of importance, leaf spot (Syn. shot-hole or black spot of leaf) is caused by *Xanthomonas arboricola* pv. *pruni* (phylum: proteobacteria) (Anonymous. 2006; Kado , 2010), and is considered a quarantine pathogen by all major almond-producing countries worldwide (EPPO, 2013). It is noteworthy to mention that nine pathovars of *X. arboricola* complex have been identified based upon the host they infect. Much of studies has reported that pathovars *corylina*, *juglandis* and *pruni* cause the most destructive damage on their hosts (Fischer-Le Saux, 2015). Previously, *X. arboricola* was formerly known as *X. campestris* (Vauterin et al, 1995). The word *arboricola* comes from Latin origin, which means “tree dwellers”.

The discovery of *Xanthomonas* genus dates back to 1881 by Wakker (Wakker, 1883). The causative agent of bacterial spot was first described on Japanese plum in 1903, U.S.A (Smith, 1903). The bacterium is described as a Gram negative, rod-shaped, aerobic, and motile by one flagellum.

Disease symptoms appear first on lower surface as pale green to yellow color. On the upper surface, angular, dark or brown spots are seen. With disease encroachment, theses spots gradually coalesce with each other and resulting in premature leaf drop. As a result, yield losses and fruit quality and quality decrease significantly because of photosynthesis reduction (Stefani, 2010). Recent study conducted by Rasoulnia et al., 2018 that inoculation of citrus (*Citrus aurantifolia*) with *X. citri* subsp. *citri* significantly reduced photosynthesis parameters such as chlorophyll a, b, and carotenoid pigments compared to control plants. The specific aim of this present study is to identify and isolate *Xanthomonas* spp. from orchard almond trees.

## *2*. Material and methods

### 2.1 Study Area Description:

The experiment was conducted during the year of 2017, at the University of Benghazi farm. Benghazi is the second largest city in the eastern part of Libya. The climate is relatively semi-arid with an annual precipitation below 300 mm. The farm is located between 31.76 and 20.23. The current study was conducted in late June 2017 (Fig. 1).

### *2. 2* Isolation of the bacterium:

Three symptomatic trees were selected. Symptomatic leaves were cut and transferred in plastic bags to the university laboratory for further analyses. Leaves were rinsed thoroughly with tap water to remove dust and soil particles. Leaves were cut into small pieces (1-3 cm) long with sterile scissors. Leaves were soaked in 10% bleach for one minute. Leaves were transferred and immersed in sterilized deionized water for two minutes to remove the bleach. Nutrient agar medium (Fluka Chemie, Switzerland) was poured into Petri dish (13 mm), and left until solidifying. Three excised leaves were

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placed inversely on the Petri dish, sealed with Parafilm, and incubated at 27 °C for five days. Each experiment contained twenty Petri dish, and the experiment was repeated twice.

Streaking method was applied to obtain a pure culture of the bacterium for carrying out Gram staining test.


*Fig. 1 Geographic study site location on map*

As clearly illustrated in Fig 2, a colony with a yellow-color was observed in all plates. It has been demonstrated in previous studies (Palacio-Biella et al, 2010; Nagia, and Saad, 2018) that *Xanthomonas* spp produces this pigment on general bacterial media (e.g., nutrient agar). It has been reported that this yellow pigment (Xanthomonadins) is a special chemotaxonomic marker for *Xanthomonas* spp. (Chun, 2000), therefore, we used this fact as a helping tool for identification. Although few fungal plant pathogens such as *Wilsonomycets carpophilus* and *Venturia carpophila* can cause leaf spot symptoms similar to *Xanthomonas* spp, they cannot *in vitro* produce yellow-color pigment on nutrient agar medium (Fahy, and Persley, 1983). Disease symptoms were recorded in the autumn season, and *Pseudomonas* spp. typically attacks in the spring season; thus, *Pseudomonas* was excluded as the causal agent.

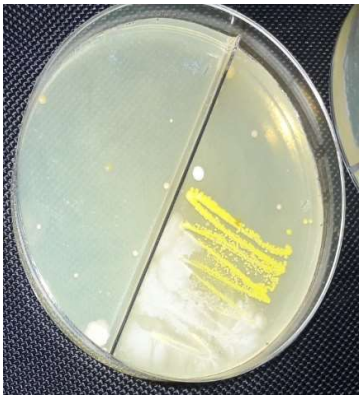

*Fig. 2 Bacterial growth on Nutrient agar medium*

In complete agreement with several studies (Boudon, 2005; Burokiene, and Pulawska, 2012; Nagia, and Saad, 2018; Holeva et al., 2023) the bacterium was a gram negative,

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with a distinctive rod-shaped under compound microscope. Symptomatic almond leaves were also used in this study to support our identification (Fig 4).

**Fig. 4** Leaf spot caused by *Xanthomonas* spp.

## Conclusion

To the best of our knowledge, this is the first report of bacterial leaf spot caused by (*Xanthomonas* spp.) of almond trees grown in Suluq district, Libya. Our results focused on morphological (e.g., the yellow pigment) and biochemical properties (e.g., Gram staining result) of *Xanthomonas* for the purpose of initial identifications and characterization. Future research should use and apply more precise and accurate methods that are based on molecular approach to detect and to characterize this bacterium to species and pathovar level.

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# عزل وتعرف بكتيريا .*Xanthomonas* spp في أشجار اللوزيات في منطقة سلوق، ليبيا

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## الملخص :

مرض التبقع البكتيري من الأمراض التي تسبب خسائر اقتصادية في أشجار الفاكهة ذات النواة الحجرية بشكل عام و في أشجار اللوز بشكل خاص. أشجار اللوز لا تحظي باهتمام كبير سواء كان ذلك من الدولة أو المزارعين مقارنة بأشجار الزيتون و الحمضيات. ولعل السبب في ذلك هو اعتماد السوق المحلي الشبه الكامل علي الاستيراد . تم استخدام الصفات البيولوجية والمورفولوجي للبكتيريا مثل صبغة جرام و لون المستعمرة كدليل تشخيصي علي أن البكتيريا المسببة لهذا المرض هي *Xanthomonas*. أيضا تم استخدام الأعراض المميزة و النوع النباتي المصاب كدليل أخر مساعد في التشخيص. تعتبر هذه الدراسة مبدئية و من الضروري استخدام وسائل تشخيص متطورة و دقيقة مثل Polymerase chain reaction (PCR) للتشخيص النهائي.

**الكلمات المفتاحية:** تبقع الأوراق البكتيرية، الزانثوموناس، أشجار اللوز.