**Alternaria alternata** Isolated From Lemons (Citrus lemon) in Libya

El-Gali, Zahra Ibrahim

Department of Plant Protection, Faculty of Agriculture, Omer Al-Mukhtar University, El-Beida Libya.

zelgali@yahoo.com

**Abstract:** Lemons (Citrus lemon Burmann) is one of the citrus fruits of Libya. Blight symptoms was observed on plants were grown in homes gardens. I isolated and examined a fungus which causes leaf lesions and blight and fruits drop on lemons by using morphological method. Based on classification keys, the pathogen was compared on basis of morphological and characteristics, the recovered isolate from the plant tissues was identified as *Alternaria alternata* (Fr.) Keissler, and identified was confirmed by pathogenicity test.

**Keywords:** Lemons, Blight, Alternaria alternata, Morphology, Pathogenicity, Libya.

---

**1. Introduction**

Citrus occupies a prominent position in fruit industry of the world. Libya is among the leading citrus growing countries of the world and earns substantial amount of foreign exchange annually. In Libya, citrus is the largest group of fruits produced over an area of 10 000 hectares with a total production of 84 Mt. annually, which is very low as compared to other citrus growing countries. Diseases are one of the major factors which impede the fruit yield and quality. Among diseases, Alternaria leaf spot caused by *Alternaria* spp. one of the most devastating and occurs throughout citrus growing countries of the world [1,2] including Libya. *Alternaria* is a ubiquitous fungal genus associated with a wide variety of substrates including seeds, plants, agricultural products, animals, soil, and the atmosphere. Most species are well-known and significant plant pathogens causing a range of diseases and post-harvest rots of numerous agronomic and ornamental plants [3]. Some species are commonly correlated with deterioration of painted wall surfaces [4], found as saprophytes in soil, and decaying plant tissues, or as endophytes in various plants [5,6]. In addition, several taxa have emerged as animal or human pathogens [7]. *Alternaria* species have been generally identified and classified based on cultural and conidial morphology [8,9,10]. *Alternaria* spp. cause several diseases of citrus, including Alternaria brown spot of tangerine, leaf and fruit spot of rough lemon and Rangpur lime, affecting Mexican lime, and black rot of fruit of several *Citrus* spp. [11,12]. Species of *Alternaria* also commonly occur as saprophytes and on all aboveground tissues of citrus trees and also may colonize citrus leaves as endophytes (T. L. Peever and L. W. Timmer, unpublished data). In addition to the diseases mycotoxins produced by *Alternaria* and specifically by *Alternaria alternata* are numerous. Among them the most widely studied are: tenuazonic acid (TA), alternariol (AOH), alternariol monomethyl ether (AME), alternenuene (ALT), alter toxin I and ten toxin [13,14].

Lemons was the most planting in homes gardens in El-Beida, Libya and the blight symptoms was observed on leaves and fruits. Therefore the objective of this study included isolation, purification and identification of pathogenic fungus causing leaf and fruit blight disease of lemons.

**2. Materials and Methods**

**2.1. Field observations and symptoms.**

Lemons plants showing infection on leaves and fruits were collected from the experimental area of the homes gardens, in El-Beida city at 25 km from the coast, with an elevation ranging from 612 ± 8.4 meters above sea level between lat 32° 45’ and 30°16’ N, and long 21°42’ and 21°37.9’ E. Maximum and minimum mean temperatures are 25°C and 10°C, respectively. Annual rainfall averages 650 mm and is most abundant during December to February. Soil of the site is a typical clay soil, composed of 11.8% sand, 35.2% silt and 50.1% clay. Digital photographs of leaf and fruit symptoms were recorded using a Sony DSC-S700 model digital camera.

**2.2. Collection and isolation of the fungus.**

Naturally infected fruit and leaves were collected and processed for isolation of causal disease in the laboratory of Department of Plant Pathology, Faculty of Agriculture. A special medium known as Potato Dextrose Agar (PDA) at 24°C was used for isolation of pathogen and observed periodically for fungal growth. The isolated fungal was
purified and multiplied which was later on identified as fungi on the basis of morphology characteristics.

2.3. Microscopic Analysis
The fungi were grown on PDA at 25°C for 7-9 days, and the conidia of the isolates were examined under a microscope comparing morphological and cultural characteristics (i.e., size of conidia, number of cells/conidia, color of conidia, and type of mycelia to those described in [10, 15, 16]).

2.4. Pathogenicity test:
Lemon leaves were washed in running water and surface disinfected for 1 min in 1% Sodium hypochlorite. Then washed with sterile distilled water and dried with sterile filter paper. Twenty leaves were inoculated with a mycelial plug (5 mm in diameter) of A. alternata culture placed over the intact skin. Twenty other non-inoculated leaves served as control. The leaves were placed in humid chamber at room temperature.

3. Results and Discussion
3.1. Description of Symptoms
Alternaria attacks young fruit, leaves and twigs, producing brown-to-black lesions surrounded by a yellow halo. On young leaves, the disease produces minute brown to black spots. On the other hand Symptoms was appear in as a die back after infection. Lesions usually continue to expand and large areas of the leaf was killed (Fig. 1) even without tissue colonization. These changes caused by toxins that plays a role in disease development [17,18].

Figure 1. Spot and blight symptoms or die back on leaf

Leaf lesions are generally circular but will often have a tail, following the leaf vein which gives the lesions an eye-spot appearance (Fig. 2). The necrosis extends along the veins as the toxin spreads in vascular tissues. Lesions enlarge as leaves mature and can vary in size from 1-10 mm (0.04-0.4 inches) and will be larger if the infection occurred earlier in the season. If Alternaria blight is severe, the leaves may drop.

Figure 2. Circular lesions on leaf

Severe fruit infections, especially shortly after petal fall, result in the drop of young fruitlets (Fig. 3-a). Remaining fruit were appeared surrounded by brown lesions at the fruit base. (Fig. 3-b). On occasion, A. alternata is able to penetrate the citrus rind and cause localized necrosis, but this is relatively rare. Fruit are susceptible to Alternaria blight for 4 months after petal fall. Even when the fruit are no longer susceptible, some fruit may fall as the result of earlier infections, especially if the lesions are near the fruit stem. Severely affected fruit abscise reducing yield, and blemishes on the remaining fruit greatly diminishing marketability.

Figure 3. Symptoms on twigs (a) and young fruits (b).

3.2. Description of Species
3.2.1. Cultural characteristics.
Typical cultural and morphological characteristics are shown in Figure 4. On PDA plates colonies were velvety. The isolates first developed grayish with a very thin white margin, and cottony texture (Fig. 4-a). Colonies later developed color, the colors became dark olive as fungal age increased (Fig. 4-b).

Figure 4. a. Alternaria alternata isolated from leaf and young fruits (at narrow) and b. colony morphology on PDA medium.

3.2.2. Morphology of fungus
The isolated fungus corresponded in morphology to Alternaria alternata described by Mycelium is septate (Fig. 5-a) and the conidia are variable in size and shape, but most often short and ellipsoid to oval, tapering in the lower half.
Alternaria is easily identified by the shape of its conidia, which are large, ovoid to obclavate, dark-colored (melanized), multicellular with longitudinal and transverse septations (phaeodictyospores). Conidia are produced in single or branched chains of short conidiophores (Fig 5-b) and showed 3-8 transverse and longitudinal septa (Fig. 5-c). Conidia of the isolates were catenated in long and sometimes branched chains of 8 to 12 spores. The spores were obclavate, dark olive, with 4 to 8 trans-verse and 0 to 2 longitudinal or oblique septa (Figure 5b and c). The conidia were on average measured (22 to 38) × (5.8 to 11.2) μm. Conidia are short ellipsoid to oval, tapering in the lower half into a narrow tail extension. The upper part which was materialized by a very short beak well rounded ending abruptly appears allowing the formation of new spores, thus furnishing evidence of catenulation. Primary conidiophores arise directly from hyphae at the PDA surface; they can be simple or branched. The asexual spores of the fungus are thick-walled, multicellular, and pigmented and thus tolerate adverse conditions like dry weather. These results confirmed the isolated pathogen as Alternaria alternata.

Pathogenicity test:
To confirm pathogenicity, mycelial plugs containing conidia were used as inoculum. Lemon leaves were inoculated in the laboratory with A. alternata and maintained in a moist chamber for 2 to 5 days. Brown, corky lesions resembling symptoms that occurred in the field were observed surrounding the inoculation sites beginning around day 1 accompanied by blight symptoms or die back (Fig. 6). Symptoms were not observed on control leaves not inoculated with fungus. Symptoms was appear in as little as 24 h after infection. Lesions usually continue to expand and large areas of the leaf may be killed by the host-selective ACT-toxin even without tissue colonization This result agree with [19]. During disease development the pathogen secretes a toxin which exhibits high host specificity, plays a major role in pathogenesis and is responsible for leaf necrosis [17,18]. The fungi were reisolated from the lesions of the diseased leaves and were identical to the original isolates.

References


