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Incidences of Fungal Leaf Diseases on Mastic Shrubs in Libya

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Abstract: *Mastic, an important evergreen and medicinal plant grown in the forest of Al-Jabal Al-Akhdar, Libya has suffered heavy losses due to a leaf disease. Various kinds of leaf spots and blight were found on plant leaves. The objective of this work was to study fungi based on morphological characterize and to identify those that are pathogenic. Disease incidence were quite variable between seasons. Rate of diseased leaves was recorded about 58% in spring while 24.3% in winter season. Four fungal species were frequently isolated from the disease symptoms. Alternaria alternata was the most predominant fungus associated with the brown leaf spot on mastic, followed by Pestalotiopsis fici was commonly isolated from leaf blight and P. guepinii and P. palmarum were isolated from leaf tip death and silvery gray leaf spots respectively. Koch´s postulate was verified by inoculating healthy mastic leaves. The literature indicates that this is the first report of a leaf disease of mastic in Libya.*

Keywords: *Mastic, leaf disease, fungi, Libya.*

1. INTRODUCTION

Mastic is a small evergreen tree or shrub, up to 4 min height, and distributed in the Mediterranean region up to 886 m above sea level. It is an important medicinal plant grown in several regions geographical in Al-Jabal Al-Akhdar province at 45' - 59' 32° N and long. 44' - 30' 21° E in northeastern of Libya (Fig. 1).The native flora is exposed to different abiotic and biotic environmental stresses.

The genus *Pistacia* belongs to the family *Anacardiaceae* that comprise about 70 genera and over 600 species. A mix of *Pistacialentiscus* L., *Juniperusphoenica* L. and different flora occupied around 9000 km2 of the reserve and they form around 1 % of the plant cover of the reserve (Final Report, 2005). Bozorgi*et al*., (2013) reviewed wide phytochemical and pharmacological properties from various parts of *Pistacia* species since they utilized by people widely for different nutritional and medicinal proposes. *P. lentiscus* leaves extracts showed antioxidant, antimicrobial activities and anti diabetic (Benhammou*et al*., 2008; Abdeljelil et al., 2014; Mohd et al., 2014).

In recent years, afforestation of this species in the northeastern of Libya have been severely affected by dieback and tree mortality. Four different symptoms, brown spot, leaf blight, leaf tip death and silvery gray leaf spots were observed on mastic leaves. The aim of the present work was to investigate the etiology of the leaf spot and blight observed in season 2015-2016 on mastic shrubs and to identify those that are pathogenic based on morphological characters.

Figure1. *Map of northeastern of Libya and location of Al-Jabal Al-Akhdar*

2. MATERIALS AND METHODS

2.1. Survey of Incidences of Leaf Diseases

Incidences of leaves diseases were investigated four times from spring 15 to winter 15 on 250 leaves of randomly selected 10 shrubs during the 2015-2016 growing season.

2.2. Isolation and identification of fungi

Plant samples were collected from Al-Jabal Al-Akhdar forest in the northeastern of Libya, they were transported to the laboratory for isolating pathogenic fungi. After sterilization with 1% sodium hypochlorite for 2 min., diseased leaf tissues cut into 5mm length were placed on potato sucrose agar (PSA) plates, and incubated at 23°C for 7 days to promote fungal growth and sporulation. The growth fungi was isolated, and pure culture were maintained on potato sucrose agar medium in plates. The morphological and culture characteristic of the isolated organism were studied also conidia and conidiophores details were considered under light microscope.

2.3. Pathogenicity

Pathogenicity test was confirm on detached healthy leaves of *P. lentiscus* removed from the shrubs. Before inoculation, leaves were surface disinfested by immersion in 1% sodium hypochlorite for 2 min, rinsed in sterile distilled water, and then air-dried in a laminar flow hood and maintained in Petri dish containing small glass beads and sterile distilled water. A mycelial plug (5 mm in diameter) of fungus culture was placed over the intact leaf. Others Leaves were inoculated by placing a PSA plug without fungal mycelia on upper surfaces of the leaves served as control. The Petri dishes were covered and placed under light of a 12 h photoperiod at 25°C and 95% relative humidity for 7 days. The pathogenicity test was repeated three times. The symptoms were observed and compared with the original symptoms. The fungus was isolated again from artificially inoculated mastic leaves and compared with original culture isolate

3. RESULTS

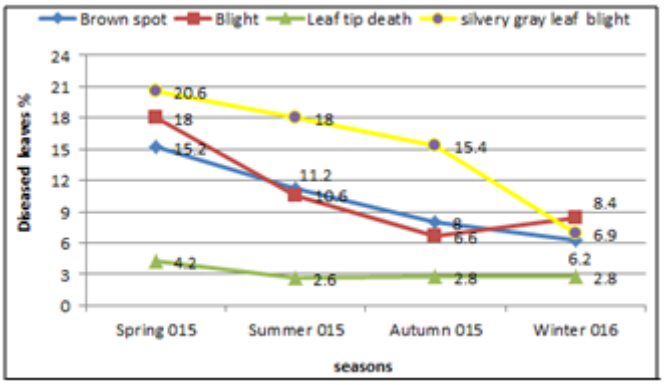

Figure2. *Incidences of leaf diseases on mastic shrubs during 2015-2016 growing season*

3.1. Incidences of Leaves Diseases

All the four diseases were recorded in all seasons during the yearly survey. Silvery grey leaf blight was the most diseases outbreak then leaf blight, followed by brown spot and leaf tip death. The highest level of all disease incidence (58%) in spring season and the lower level of disease incidence (24.3%) in winter season (Fig. 2).

3.2. Brown Spot

The symptoms observed were small circular to oval dark brown necrotic sunken spots located on the leaf margin (Fig. 3). The spots were characterized by having grey centers with brown margins. Circular, necrotic and sunken dark brown spots on both side of the leaf (Fig. 3a and 2b). In a later stage of infection, the affected leaves discolored to yellowing and finally dried up. The causal agent of this disease was successfully isolated on potato sucrose agar (PSA) from the diseased leaves.

The fungus produced flat, downy to woolly and grey colonies with olive green peripheries. The *Alternaria* grew well on PSA and formed an olivaceous black colony with dark olive margins, reverse black at 25±1 °C after 10 days. The surface was greyish white at the beginning which later darkens and became greenish black or olive brown (Fig. 3c).

The conidiophores were branched, straight, Pale brown to olive brown in colour, measuring 25-60 µm long and 3-3.5 µm thick. The conidia were pale brown to light brown in colour and produced in long branched chains, obclavate to obpyri form or ellipsoid in shape with short conical beak at the tip, or beakless. The size of conidia varied from 18.2-26 µmin length and 10-15.6µm in width. Conidia had two to three transverse septa and usually several longitudinal septa (Fig. 3d,e). Based on the symptoms, mycelial and conidial characters, the fungus was identified as *Alternariaalternata* (Ellis 1971).

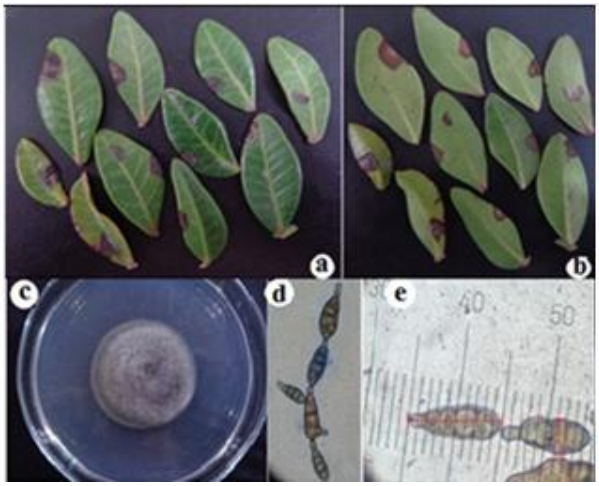

Figure3. *Necrotic spot on the leaf (a) upper surface, (b) lower surface, (c) culture of A. alternata on PSA medium and (d,e) morphological characteristics of conidia*

3.3. Leaf Blight

During a disease survey of several shrubs, performed in 2015-2016, leaf blight was found causing defoliation in shrubs of Mediterranean forest. Symptoms on large blight that were located mainly, exclusively, along the leaf margin (Fig. 4a,b). The margins blight were bordered by a red halo. The fungus consistently isolated from symptoms identified as *Pestalotiopsisfici* based on morphological characters.

Colonies on PSA attaining 7 cm diam. after 7 days at 25°C, with edge undulate, whitish, aerial mycelium on surface (Fig. 4c), fruiting bodies black, concentric; reverse of culture yellow to pale brown. Conidia 19-26 × 5-7 µm, setulae 2-3 and 30-32 µm(Fig. 4d), fusi form, straight to slightly curved, 4-septate, smooth, greyish brown; basal cell conical, hyaline, with three median cells, dark brown, concolorous, septa and periclinal walls darker than the rest of the cell, apical cell hyaline, conic to subcylindrical, with 2 tubular apical appendages without knobs, arising from the apex of the apical cell, 19-24µm long basal appendage filiform, short 9-16 µm. Acervuli are immersed within epidermal cell layer, and then break through it mature (Sutton, 1981).

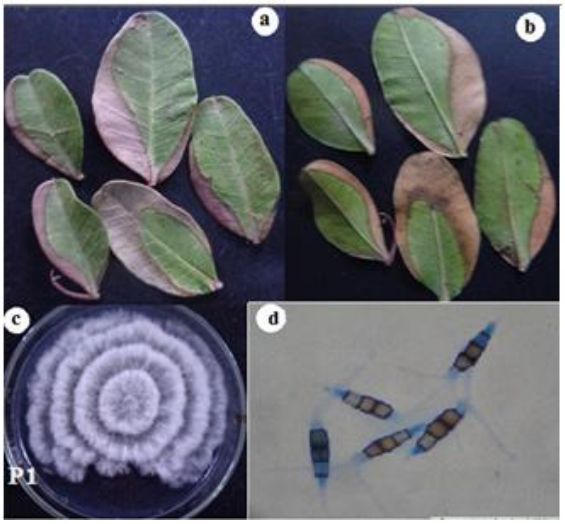

Figure4. *Symptoms of blight on leaf, (a) upper surface (b) lower surface, (c) culture of P. fici on PSA medium and (d) conidia*

3.4. Leaf Tip Death

Leaf tip death and leaf chlorosis with a dark brown border, was observed on leaves mastic shrubs (fig. 5a,b).

Mycelial growth on potato sucrose agar (PSA) was cottony white (fig. 6c) and conidia were produced in ink-like fruiting bodies. After 2 weeks, acervular conidiomata (up to 200 in number) developed on the infected leaf incubated at 100%relative humidity and 20–24°C, and in cultures grown on PSA at 24°C. All isolates had 5-celled smooth conidia. Apical and basal cells were hyaline, cylindrical to conic (Fig. 5d). The three intermediate cells were dark brown, with the two upper ones sometimes darker. Conidia were 22.8–29.1 µm.5.3–8.9 µm. There were typically three apical appendages 17.3–28.7 µmlong and a basal appendage 4.3–13µmlong.

Based on morphological characters described above, the fungus was identifiedas *P. guepinii* (Sutton, 1981).

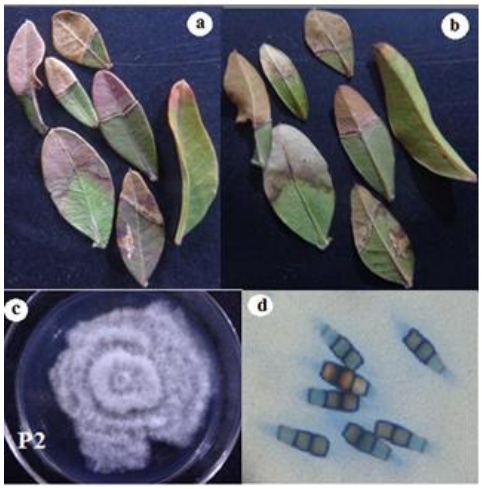

Figure5. *Symptoms of leaf tip death (a) upper surface, (b) lower surface, (c) culture of P. guepinii on PSA medium and (d) conidia*

3.5. Silvery Gray Leaf Spots

Grey leaf spot, and leaf tip blight of mastic shrubs. The symptoms observed were small circular to oval dark brown necrotic sunken spots located mostly on the leaf tip (Fig. 6a,b). Older leaves can be severely blighted.

P. palmurum formed white aerial mycelial mats with circular shape on PSA plates after incubation at 25°C over 7 days (Fig. 6c). after 12 days of incubation at 20°C, dark colored acervuli formed all over the mycelial mat. Conidia were fusiform, 5-celled, 17–26 × 4.5–7.5 µm (Fig. 6d), fusiform to ellipsoid, mainly straight, 4-septate; three median cells, concolorous, olivaceous, lower cell of 3 sometimes paler, apical and basal cells hyaline; with three appendages, 5–29 µm long, arising from the apex of the apical cell; filiform basal appendage, 2–14µm long(Sutton, 1981).*Pestalotiopsis* spp. without identify has previously been reported as a affected leaf pathogen of mastic shrubs (El-Gali, 2016).

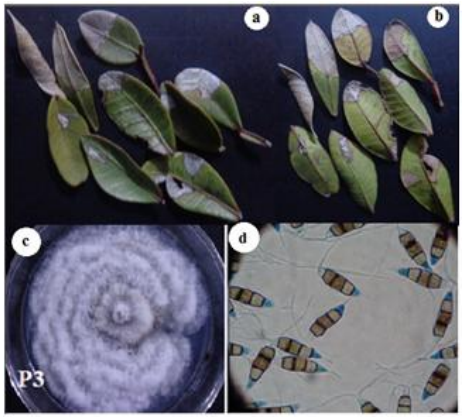

Figure6. *Symptoms of silvery gray spot on leaf, (a) upper surface, (b) lower surface, (c) culture of P. palmarum on PSA medium, (d) conidia*

4. CONCLUSION

A alternata, Pestalotiopsisfici, P. guepinii and *P. palmurum*. are causal agents of mastic shrubs leaf spot and blight. Colony diameter is an important character for species differentiation of *Pestalotiopsis* spp.; however, it is important to evaluate the most possible characters of conidia, as number of cells, color of medium cells, conidia dimensions, and position and length of apical appendage. According to our studying, this is the first report of leaf spot and blight disease of mastic shrubs in Libya. The cultures of all isolates fungihas been deposited in the culture collection of Department of Plant Pathology, Faculty of Agricultural, Omer Al-Mukhtar University, Libya.

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