First report of *Diplocarpon mespili* on loquat (*Eriobotrya japonica*) in Pakistan

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Abstract: The present study reports the pathogenic fungus of loquat, *Diplocarpon mespili* (= *Entomosporum m.*), responsible for leaf spot, from Pakistan. Leaf symptoms were observed as circular, bright red spots on young leaves that later on turned to purple blotches with ash brown grey centres. The spots coalesced to form large dead areas on leaf surfaces. The fungus was identified as *D. mespili* on the basis of typical insect like conidia and morphological characters. The chlamydosporic phase of the fungus was observed when the fungal cultures were incubated for three months at 20°C. The fungus grew well on PDA at 25 °C. Pathogenicity tests conducted on healthy plants under lab conditions resulted in appearance of typical leaf spots on young leaves. This is the first report of *Diplocarpon mespili,* with chlamydosporic stage causing leaf spot of loquat from Pakistan.

Zusammenfassung: Die vorliegende Studie berichtet über den pathogenen Pilz *Diplocarpon mespili* (= *Entomosporium mespili*), der für Blattflecken der Japanischen Mispel, verantwortlich ist, erstmals aus Pakistan. Befallsymptome wurden als kreisförmige, helle rote Flecken auf jungen Blättern beobachtet, die später lila verfärbte flächige Flecken mit aschgrauem Zentrum bilden. Die Flecken verschmelzen schließlich und führen zu großen abgestorbenen Bereichen auf den Blattoberflächen. Der Pilz wurde aufgrund der typischen, Insekten-ähnlichen Konidien und weiterer morphologischer Merkmale als das anamorphe Stadium von *Diplocarpon mespili* identifiziert. Die chlamydosporenbildende Phase des Pilzes konnte beobachtet werden nach Inkubation der Pilzkulturen für drei Monate bei 20 °C. Der Pilz wuchs auch auf PDA bei 25 °C. Pathogenitäts-Tests an gesunden Pflanzen unter Laborbedingungen durchgeführt, führten zum Auftreten typischer Blattflecken an jungen Blättern. Dies ist der erste Bericht über *Diplocarpon mespili* mit blattfleckenverursachendem Chlamydosporen-Stadium von der Japanischen Mispel in Pakistan.

Loquat (*Eriobotrya japonica* LINDL.) is an important sub-tropical fruit tree of Pakistan with an annual production of 10479 tons. Punjab and Khyber Pakhtunkhwa (KPK) are the major loquat producing provinces with 98% contribution of the total production (ANONYMOUS 2008). In Punjab, it is mostly grown in the Pothohar region while in KPK it is mainly cultivated in Mardan, Peshawar and Hari Pur districts (HUSSAIN & al. 2007). Loquat leaves have great significance from medicinal and nutritional point of view. In China, loquat leaves are being used as folk medication for treatment of pulmonary tuberculosis (ZHANG & al. 2004). Loquat leaves are also used to cure various skin diseases, pain, inflammation, coughing, diabetes and liver disorders (HAMADA & al. 2004, NISHIOKA & al. 2002, SAKURAMATA & al. 2004).

Loquat is attacked by many pathogens. Fire blight is a serious bacterial disease of loquat caused by *Erwinia amylovora* (SEYMOUR 1965). Other pathogens are: *Pseudomonas eriobotryae* (canker), *Phytophthora* spp. (crown rot), *Spilocaea pyracanthae* (=*Fusicladium eriobotryae*) (leaf scab), *Lasiodiplodia theobromae* (=*Diplodia natalensis* (collar and root rot), and *Colletotrichum gloeosporioides* (anamorphic *Glomerella*) (anthracnose) (CRANE & CALDEIRA 2006). Leaf spot incited by *Diplocarpon mespili* (SORAUER) B. SUTTON, is an important disease of several genera of subfamily *Pomoideae* of the *Rosaceae*. Several species of *Photinia*, juneberry (*Amelanchier* spp.), flowering quince (*Chaenomeles* spp.), loquat (*Eriobotrya japonica*), firethorn (*Pyracantha coccinea* var. *formosana*), Indian hawthorn (*Rhaphiolepis indica*), and mountain ash (*Sorbus sitchensis*) are among the noticeable hosts of this fungus. In past years, the disease has proven itself capable of rendering an entire fruit crop unmarketable (DAVIDSON 1986). In 1990 and 1994, *D. mespili* proved costly to saskatoon industry when cumulative losses were estimated to the amount of $ 1000000 (BAINS & al. 1996). *Entomosporium mespili*, (= *E. maculatum)* is the anamorphic stage of *Diplocarpon mespili* (= *Fabraea maculata*), the rare teleomorphic stage. The present paper reports on the association of the anamorph and its chlamydospore stage with loquat from Pakistan.

Materials and methods

During surveys of loquat orchards in 2013, spots were observed on leaves. The spotted leaves were brought to the laboratory of Plant Pathology for identification of the disease symptoms and the associated pathogen. The colour and shape of the spots were observed. For the isolation of the pathogen, the spots were cut, surface sterilized in ethanol and plated on autoclaved Potato Dextrose Agar (PDA) in 9 cm Petri plates. The plates were then incubated at 25 °C for the growth of the fungus. The fungus was purified from a single conidium and identified on the basis of morphological characters (NAG RAJ 1993). For isolation of conidia of *D. mespili* a modified protocol suggested by PARK & al. (2011) was followed. For production of chlamydospores, the culture was kept at 20 °C for 3 months and then observed.

For determination of optimum temperature for the growth of the fungus, the purified fungus was grown on PDA and Malt Extract Agar and incubated at different temperatures (15, 20, 25 and 30 °C) with a 12 h photoperiod. Ten mm scoops of the purified fungus were inoculated in the centre of the two media individually. Each treatment was replicated ten times. The growths on the two media at different temperatures were recorded after 7 days of incubation.

For pathogenicity test, the conidial suspension was prepared from the first generation of the pure culture on PDA and quantified with a haemocytometer. The conidial suspension was sprayed on leaves of one year old loquat plants and placed in green house and symptoms were recorded.

Results and discussion

Leaf symptoms were observed as circular, bright red spots on young leaves that later on turned to purple blotches with ashen brown grey centres. The spots coalesced to form large dead areas on leaf surfaces (Fig.1 a, b). The single spore culture grew as plane white hyaline immersed mycelia in the beginning and later turned denser with profuse sporulation and yellow to reddish brown coloration (Fig. 1 c). Conidia varied in size from 16–18 µm in length and 10–12 µm in width. The presence of characteristic ashy brown raised acervuli in the centre of spots (Fig. 1 d) and the insect like conidia separated by a septum served as a confirmation of *D. mespili* (Fig. 1 e). Dark coloured structures were developed that appeared as black spots embedded in media and were

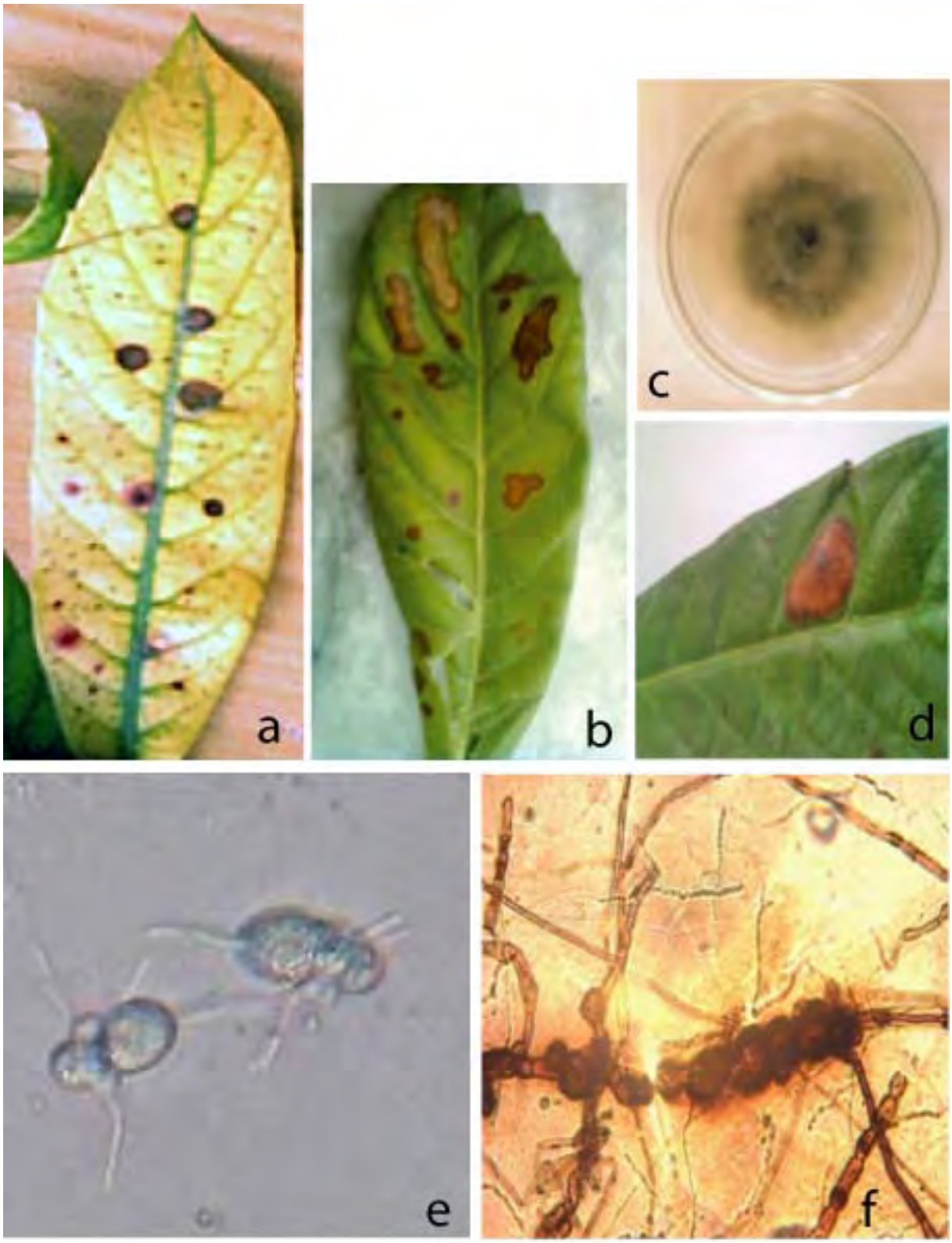

Fig. 1. *Diplocarpon mespili. a* Earlier symptoms found on younger leaves, *b* mature symptoms on loquat leaves, *c* single spore culture, *d* acervuli as dark area in the centre of the spot on loquat leaf, *e* insect like conidia, *f* chlamydospore stage observed under 40× of compound microscope

seen clearly with naked eye in light. Under microscope, they appeared as typical asexual, intercalary thick-walled, pigmented, slightly swollen spherical chlamydospores, formed between and at the ends of conidial chains (Fig.1 f). The results of present

study were found to be similar to those obtained by PIEHL & HILDEBRAND (1936) and STOWELL & BACKUS (1966) who reported the same characteristics associated with *D. mespili.* The maximum development of the fungus was observed at 25 °C temperature on PDA. The fungus did not grow well on MEA.

The pathogenicity test confirmed the association of the fungus with the disease. The fungus produced the characteristic symptoms of leaf spots which have been observed during identification. As VAN DER ZWET & STROO (1985) have already stated that after two to three transfers in vitro, *D. mespili* loses its pathogenicity, although conidia are still produced, but it becomes avirulent. So it was suggested that inocula must be obtained from diseased leaves or a conidial suspension from first generation of pathogen on artificial media, for successful inoculation and virulence.

Based on symptoms, morphological characters and pathogenicity tests, the fungus identified was confirmed as *Diplocarpon mespili*. To the best of our knowledge, this is the first report of it, with chlamydosporic stage causing leaf spot of loquat in Pakistan.

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