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RESEARCH COMMUNICATION

First report of *Septoria silybi* associated with leaf blotch of *Silybum marianum* from Iran

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Abstract

During March to April 2013, in the course of routine sample collection, a leaf spot disease was observed on *Silybum marianum* in different areas of Kermanshah province, Iran. Initial symptoms of the disease were pale brown, necrotic lesions, mostly 8-10 mm long on leaves. On the surface of the infected leaves conidiomata were observed, which were pycnidial, amphigenous, scattered, dark brown to blackish, globose, immersed in host tissue, becoming partly erumpent, unilocular, 90-150 µm in diameter, with an ostiole of 18-24 µm in diameter. Conidiogenesis was enteroblastic. Conidia were hyaline, filiform, sub-straight to mildly flexuous, truncate at the base, 20-48 × 1.2-2.8 µm, 2-5-septate, with indistinct septa. On the basis of symptoms, fungal morphology and completion of Koch's postulate, the fungal isolates from the leaf spots were identified as *Septoria silybi*. This is the first report of *S. silybi* on leaves of *S. marianum* in Iran.

Keywords: *Septoria silybi*; *Silybum marianum*; leaf blotch; Iran mycobiota

Introduction

Milk thistle [*Silybum marianum* (L.) Gaertn.] is an annual or biannual plant native to the Mediterranean region and belongs to the *Asteraceae* family. *S. marianum* is widespread in the East, South and West of Iran, where it invades land disturbed by grazing, machinery, or natural erosion. Milk thistle is an ancient medicinal plant used to purify and protect the liver as early as 23-79 AD. Milk thistle positively affects all forms of liver disease. The active chemical component of milk thistle is silymarin, which is a combination of three flavonoids. The seeds contain the highest amount of silymarin, but the whole plant is used medicinally. Silybin (part of the chemical structure of silymarin) is an antioxidant; it also alters the membrane structure of the liver cells, blocking the absorption of penetrating toxins into the cell (Morazzoni and Bombardelli, 1995). Silybin stimulates the production of new liver cells to replace damaged cells (kcweb.com/herb/milkt.htm). Silicristin inhibits the enzymes peroxidase and lipoxygenase (Francis, 1981). The genus *Septoria* Sacc. belongs to sympodial Blastopycnidiineae of Coelomycetes, Fungi Imperfecti (Sutton, 1980).

The genus *Septoria* has reported to include more than 1000 (Kirk *et al.*, 2001) to 2000 (Markevicius, 1996) species distributed all over the World. All species of this genus are plant pathogens causing a range of disease symptoms including leaf and fruit spots in agricultural crops, as well as horticultural and native plants (Moscow and Lindow, 1989). These damages affect the vitality and biological productivity of the attacked plants. In a survey conducted during March to April 2013 in Kermanshah province, symptoms of *Septoria* leaf blotch were observed on milk thistle plants. Initial symptoms of the disease were pale brown necrotic lesions, mostly 8-10 mm long on leaves. Therefore, present investigation was carried out to study the morphology and cause of leaf blotch infection on *S. marianum*.

Materials and Methods

The infected leaf samples were collected in different localities and natural habitats in Kermanshah province, taken to the laboratory; air dried and examined using standard light microscopy. The fungus was isolated from pycnidia (pycniospores) on leaves of *S. marianum*

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Fig. 1. Leaf spots on the leaves of *Silybum marianum* infected with *Septoria silybi*. Bar = 1cm

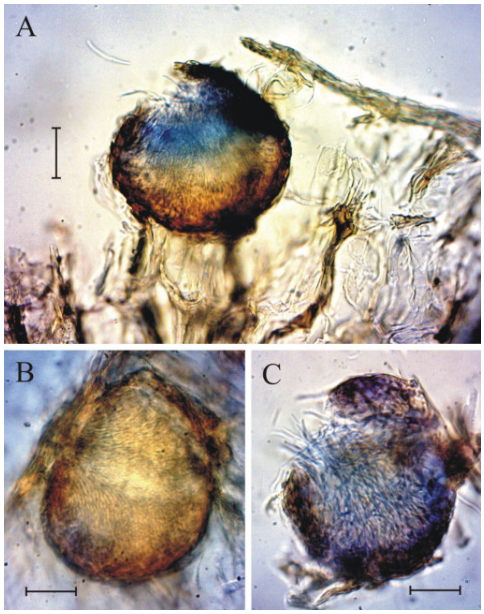

Fig. 2. A, B, Pycnidium immersed in host tissue and C, on the leaf lesion. Bars = 25 µm

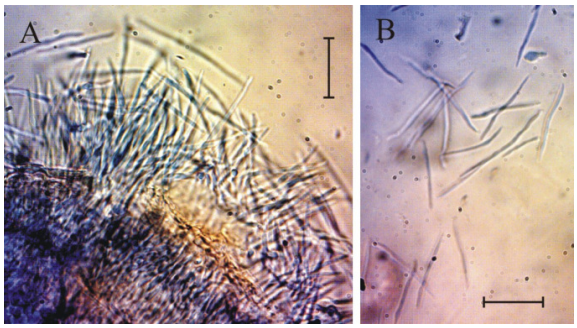

Fig. 3. A, B, Filiform conidia. Bars = 25 µm

and cultivated *in vitro*. Pycniospores were placed under aseptic conditions on 2% potato dextrose agar (PDA) plates supplemented with 20 µg/ml of streptomycin sulphate. Plates were incubated at 25-27ºC in dark for 14 days to allow the fungi to grow. For fungal identification, during the incubation period, plates were observed daily for the appearance of fungal colonies. Morphological characteristics of the fungus such as size, shape, septation of conidia, conidiomatal type, conidiogenesis and conidiogenous cells were recorded. Fifty measurements of each type of structure were made using BioloMICSMeasure software. The identification was carried out comparing the information registered with those published in the specialized literature (Teterevnikova-Babayan, 1987; Vanev *et al.*, 1997; Kirk *et al.*, 2001; Priest, 2006). The voucher specimens are housed at Razi University (SeSi-1) and a conidial isolate is kept in the Iranian Research Institute of Plant Protection. The pathogenicity tests were done with a suspension of 1×10^6 conidia per ml homogenized in sterile water. Conidial suspension was sprayed on 4 to 5, fully expanded, healthy leaves of five potted *S. marianum* plants. For the control, three *S. marianum* plants were sprayed with sterilized, distilled water and were placed 100 m away from the inoculated plants in a green house. Plants were covered with plastic sheets to maintain humidity and kept in a greenhouse for 48 hours and removed after 48 hours. Three non-inoculated plants served as a control.

Results and Discussion

In natural habitats, leaf lesions were round, irregular or angular, often delimited by veins, 2-5 mm in diameter, sometimes up to 10 mm or more when coalesced, pale brown to grayish with darker border zone and white in the center (Fig. 1A). On the surface of the infected leaves conidiomata were observed, which were pycnidial, amphigenous, but mostly epigenous, scattered, dark brown to blackish, globose, immersed in host tissue, becoming partly erumpent, separate, unilocular, 90-150 µm in diameter, with an ostiole of 18-24 µm in diameter (Fig. 2A, B, C). Conidiogenesis was enteroblastic. Conidia were hyaline, filiform, sub-straight to mildly flexuous, truncate at the base, 20-48 × 1.2-2.8 µm (n=50), 2-5-septate, with indistinct septa (Fig. 3A, B). No teleomorphic state was observed. Based on the morphological characteristics the pathogen was identified as *S. silybi* (Teterevnikova-Babayan, 1987; Vanev *et al.*, 1997; Priest, 2006). Mycelium of isolates of *S. silybi were* slow growing on potato dextrose agar and the upper side of colony was dark colored. Pycnidial conidiomata with conidia were formed in cultures after at least two weeks of incubation.

Pathogenicity of the fungal isolate was confirmed by inoculating *S. marianum* with a conidial suspension of *S. silybi*. After 14 days, leaf spots, similar to those

observed on naturally infected plants, started to develop on the leaves of inoculated plants. *S. silybi* was the only fungus consistently isolated from the leaves of experimentally infected plants, which demonstrated that it was the cause of blotch spot of *S. marianum*. The fungal sporulation was examined and found morphologically the same as the originally described pathogen. No *Septoria* was isolated from any of the control plants. In a recent book Fungi of Iran, 86 species of *septoria* collected from Iran were listed (Ershad, 2009). In Iran, *Golovinomyces cichoracearum*, *Leveilula taurica* and *Sclerotinia sclerotiorum* were reported from *S. marianum* (Yazdani and Abbasi, 1998; Aghajani *et al.*, 2008), and there have been no previous records of *Septoria* or *Septoria*-like fungi on *S. marianum*. This is the first report of *S. silybi* as well as its host plant (*S. marianum*) in Iran. The infected plants were observed in plant populations on all parts of studied territory. Up to 30 percent of plant populations infected with the fungus. *S. silybi* has been previously recorded on *S. marianum* in central California, Bulgaria and other countries (Moscow and Lindow, 1989). *S. silybi* was reported as the only *Septoria* pathogen of milk thistle and give *S. marianum* as the sole host known for *S. silybi* (Saccardo, 1884; Oudemans, 1923).

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