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Short Communication

ALTERNARIA ALTERNATA AND ALTERNARIA SONCHI: TWO NEW RECORDS OF FUNGAL PATHOGENS ON *SONCHUS ASPER* (ANNUAL SOW THISTLE) IN PAKISTAN

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ABSTRACT

Sonchus asper (annual sow thistle) is an annual or winter annual herbaceous plant native to Europe. It has become a very aggressive invader in many regions of the world. In February 2013, *S. asper* plants grown in and around the chickpea and tomato fields at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan were found to be severely affected by a leaf blight disease. Initially the disease symptoms began as small, circular, dark, necrotic lesions usually on the older leaves. At later stage, these lesions enlarged rapidly up to 4-12 mm in diameter and when spotting was abundant the entire leaf turned yellow. However, in April 2013, *S. asper* plants grown in and around the tomato and wheat fields at NIAB, Faisalabad, Pakistan were found with different leaf blight symptoms. The leaf spots were initially small, epiphyllous, irregular, scattered to marginal and dark-brown. Later on these spots enlarged, became orbicular to irregular but often angular, with brown to cinereous necrotic centers and usually with a narrow dark margin. On the basis of symptoms, morphological and cultural characteristics of the isolated pathogen, the causal agent was identified as *Alternaria alternata* from February infected plants while it was identified as *Alternaria sonchi* from April infected plants and Koch's postulates were fulfilled. This is the first report of *Alternaria alternata* and *Alternaria sonchi* from *S. asper* plants in Pakistan.

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Sonchus asper (annual sow thistle) is a serious annual herbaceous weed plant with spiny leaves and yellow flowers impacting crop production in many regions of the world (Hutchinson et al., 1984). The leaves of *S. asper* are bluish-green, simple, lanceolate, with wavy and sometimes lobed margins, covered in spines on both the margins and underneath. The base of the leaf surrounds the stem. The plant can reach up to 180 cm in height. The leaves and stems emit a milky sap when

cut. The flowers grow in clusters and the end of the stems. *S. asper* is native to Africa, temperate Asia (Middle East to China and Siberia), tropical Asia (India, Nepal, Pakistan), and Europe (Grin., 2000). It has been used medicinally, and as a food plant (Haughton, 1978). It is commonly found in the cool and shady places, water channels, field boundaries, in wheat and winter vegetables, gardens, orchards, lawns, ditch banks, and waste places.

In February 2013, *S. asper* plants grown in and around the chickpea and tomato fields at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan were found to be severely affected by a leaf blight disease. Initially the disease symptoms began as small, circular, dark, necrotic lesions usually on the older leaves. At later stage, these lesions enlarged rapidly up to 4-12 mm in diameter and when spotting was abundant the entire leaf turned yellow (Figure 1). However, in April 2013, *S. asper*



Figure 1. Leaf spots on *Sonchus asper* due to *A. alternata*

Symptomatic *S. asper* leaves were collected during February and April 2013. The leaves were then excised aseptically into small (5 × 5 mm) segments and surface sterilized in 0.25% sodium hypochlorite solution. Then after drying under sterile air flow, these segments were transferred into Petri plates containing Potato dextrose agar (PDA) medium and incubated in an incubator at 25±2°C under a 12-h light-dark regime (Akhtar et al., 2013; Akhtar et al., 2012). After approximately 5-7 days of incubation, a grey fluffy fungal growth resulted from the *S. asper* leaves collected during February. Observations under a light microscope (CXRII, Labomed, CA, USA) showed that the conidiophores were branched, straight, golden brown in colour, measuring 15 mm long and 2-6 µm thick. The conidia were formed in long chains, golden brown in colour, and were obclavate and muriform, often with a short conical or cylindrical, pale beak, less than one-third of the length of the conidium. The size of conidia varied from 22.75 to 63.70 µm in length and 13.65 to 18.20 µm in width with an average

plants grown on water channels around the tomato and wheat fields at NIAB, Faisalabad, Pakistan were found with different leaf blight symptoms. Spots initially were small, epiphyllous, irregular, scattered to margins and dark-brown. These spots enlarged rapidly up to 2-15 mm in diameter, became orbicular to irregular but often angular when limited by veins of the leaf, with brown to cinereous necrotic centers and usually with a narrow dark margin (Figure 2).



Figure 2. Leaf spots on *Sonchus asper* due to *A. sonchi*

beak length of 7.73 µm. Conidia had 2-3 transverse septa with several longitudinal or oblique septa (Fig. 3). Based on these morphological and cultural characters the isolated fungus was identified as *Alternaria alternata* (Ellis, 1971).

Isolations made from the *S. asper* infected samples collected in April resulted in dark gray velvety mold growth, on which conidiophores and conidia of the pathogen appeared after approximately 5 days. Microscopic investigations revealed that conidiophores are hypophyllous/epiphyllous, effused, straight or slightly geniculate, cylindrical, obtuse, dilute brown/dirty-brown, apical portion nearly hyaline, 18-55 × 7-8µm; rarely in groups of 2-4 scarred at apex, non-constricted at septa, generally emerge through rupturing the diseased tissue (Figure 4a). Conidia were clavate to obclavate with obtuse apex, 5-8-septate, second, third, or first cells, one or all, occasionally divided by a vertical or oblique septum, 80-110 × 18-20 µm, borne singly or in chains of 2 or 3 spores and matched with *Alternaria*

sonchi (Figure 4b-d) as previously reported by Elliott (1916). On the basis of these morphological and cultural characteristics, the isolated pathogen was identified as *A. sonchi*.

To confirm the pathogenicity, both the isolated fungi *A. alternata* and *A. sonchi* were multiplied on PDA. After 10 days, conidial suspensions of each fungus was made using 15 ml (per plate) of sterilized distilled water and adjusted to 10^5 conidia per ml using a haemocytometer after sieving through a muslin cloth. The conidial suspension was sprayed on leaves of 45 days old 10 glasshouse

grown healthy *S. asper* plants separately for each fungus species using an atomizer until runoff. After inoculation, plants were covered with polyethylene bags for 72 hours to maintain high humidity and then kept in a glasshouse at 25-30°C. Three days after inoculation, numerous spots were developed on the leaves of inoculated plants. After 7-9 days these spots expanded and became similar to those examined under field condition for each test fungus. The fungi *A. alternata* and *A. sonchi* with similar cultural and morphological characteristics were re-isolated consistently from these leaves.



Figure 3. Spores of *Alternaria alternata*

A. alternata has previously been reported to cause leaf blight of cabbage, carrot, chilies, chrysanthemum, citrus, cumin, date palm, gladiolus, okra, potato, radish, rice, rubber, wheat, tomatoes and aloe vera, etc. in Pakistan (Akhtar et al., 2004; Mukhtar and Mushtaq, 2010; Shakir et al., 1997). However, *A. sonchi* was described for the first time from North America. It was also recorded from Great Britain, Uganda, Kenya, Sudan and Cyprus (Deighton, 1959). This specie was found to be parasitic on a number of composites including lettuce and chicory (Joly, 1964).

In Pakistan *A. sonchi* was earlier reported from Kasni leaves (Shakir et al., 1997). To our knowledge, this is the first report of *A. alternata* and *A. sonchi* from *S. asper* plants in Pakistan. These fungi may have potential as a useful biological control agent of *S. asper*. However, intensive studies are needed on the environmental impact and application technology on the efficiency of *A.*

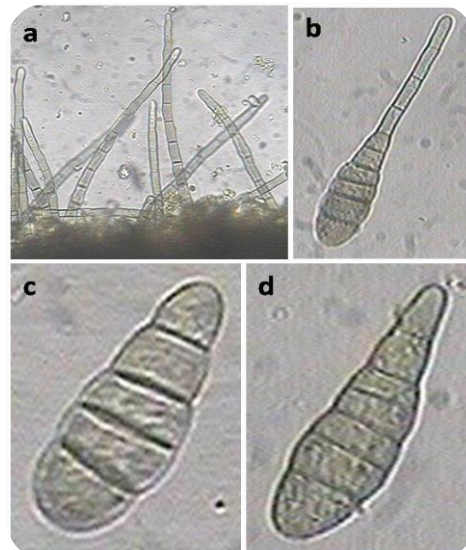


Figure 4. a) Conidiophores of *A. sonchi*; b-d: Spore of *A. sonchi*

alternata and *A. sonchi* as mycoherbicide against *S. asper*.

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