Available online at www.jibresearch.com; ISSN: 2348-6473 (online)

Original Research

Journal of Innovative Biology

June 2014 Vol. 1, Issue 2, P. 112-116

Leaf-spot disease of *Trianthema portulacastrum* – a new record from world

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Abstract: *Trianthema portulacastrum* L., an indigenous plant to South Africa, is a very common weed of tropical and subtropical areas throughout the world. In the years 2011-13 a series of surveys for natural enemies of horse purslane were conducted in Haryana, Punjab and Uttar Pradesh. A leaf spot disease was found on horse purslane at Kurukshetra. A species of *Fusarium* was isolated on PDA and TeDA media from infected leaves. On the basis of cultural, morphological and molecular characteristics, it was identified as *Fusarium chlamydosporum* Wollenw. & Reinking. *In vitro* inoculation on *Trianthema* leaves, the pathogen showed similar symptoms as occurred in nature. Thus, proving pathogenicity and Koch's posulates. This is the first report of occurrence of *F. chlamydosporum* causing leaf spot on horse purslane from the world.

Keywords: Horse purslane, *Fusarium chlamydosporum*, leaf spot, infestation.

Received: 02 May 2014 / Accepted: 13 May 2014 / Published Online: 20 June 2014

Trianthema portulacastrum L. (Aizoaceae), commonly called by several names such as horse purslane, hogweed, itcit or santha, is an introduced terrestrial weed in India which is an indigenous plant to South Africa. It enjoys the weed status in different parts of the world including tropical and subtropical areas (Aneja et al. 2000). It is one of the troublesome weeds in Haryana, Punjab, Rajasthan, Uttar Pradesh and Delhi infesting important agricultural crops such as mustard, maize, sorghum, sugarcane (Balyan and Bhan, 1986). Up to 60-70% infestation of this weed has been reported in pigeon pea and soybean fields and 80-90% in maize and brassica fields (Aneja, 2010). It is currently controlled mechanically and by the application of pre- and post-emergence herbicides such as acifluorfen, alachloral, atrazine, bentazon, fluchloralin, fomesafen, paraquat and pyrivate. But due to increasing global concern about pesticide residues in the biosphere and public demand for pesticide free food, exploitation of microorganisms especially plant pathogenic fungi is now emerging as an effective and eco-friendly alternative to conventional methods of weed control (Aneja, 2009; 1999; Charudattan, 1991).

Fungal pathogens namely *Cercospora trianthemae* (Chiddarwar, 1962), *Gibbago trianthemae* (Aneja and Kaushal, 1998; Simmons, 1986), *Drechslera* (*Exserohilum*) *indica* (*Bipolaris indica*) (Taber et al. 1988; Rao and Rao, 1987), *Colletotrichum gloeosporioides* (Darshika and Daniel, 1992), *Fusarium oxysporum* (Darshika and Daniel, 1992), *Fusarium semitectum* (Darshika and Daniel, 1998), *Alternaria alternata* (Bohra et al. 2005; Gupta and Mukherji, 2001) and *Phoma herbarum* (Lakshmi and Ray, 2013) have been reported on this weed around the world. Of these *G. trianthemae* have shown potential to control this weed (Aneja et al. 2013; 2010; 2000). The purpose of the present study was to search for virulent fungal pathogens which could be exploited for its development as mycoherbicide/s.

MATERIALS AND METHODS

Diseased leaves were collected in polythene bags from different places of Kurukshetra and brought to

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the laboratory for study of symptoms, isolation and pathogenicity test of the organism involved following the method of Aneja and Kaushal (1998).

Isolation of the pathogen

Leaves collected from different regions with leaf spots symptoms were washed in running tap water to remove soil particles and keep for some time to remove water. The infected portions of the leaves were cut into small fragments with small portion of healthy leaves. Leaves fragments are surface disinfected in 70% ethyl alcohol for 1-2 minutes and then rinsed in sterile distilled water two to three times. These fragments were transferred to Potato dextrose agar (PDA) and *Trianthema* extract dextrose agar (TeDA) plates supplemented with streptomycin sulphate (10mg/L) and were incubated at 25±2°C (Aneja et al. 2000). TeDA medium (Fresh horse purslane leaves 200.0 gm; Dextrose 15.0 gm; Agar-agar 20.0 gm and Distilled water 1000.0 ml, pH-5.6) was prepared as follows: Fresh horse purslane leaves (200 g) were washed in running tap water and then in sterile distilled water. These were boiled for 20-25 minutes in 500 ml distilled water and filtered through cheese cloth for the collection of extract. *Trianthema* extract was mixed with the other constituents as in the preparation of PDA medium (Aneja and Singh, 1989). Isolated fungi had been identified by consulting the literature, books on fungal taxonomy and monographs (Ellis, 1976; 1971).

Pathogenicity test

Pathogenicity of the chosen isolate was determined in *in vitro*. Healthy leaves of horse purslane were washed with sterile distilled water and wiped with a cotton swab dipped in 70% alcohol. Some of the leaves before inoculation were injured on adaxial surface by pricking with a flamed needle. Mycelial discs of 8 mm taken from 5 days old colony of *F. Chlamydosporum* and placed on injured and uninjured portions and covered with sterile moist cotton. The inoculated leaves were kept in sterilized moist chambers and incubated at 25±2°C. Regular observations for the appearance of symptoms were made after 3 days of incubation (Aneja et al. 2000; Aneja and Singh, 1989).

RESULTS AND DISCUSSION

During the extensive surveys conducted in the various districts of Haryana, Punjab and Uttar Pradesh in 2011-2013, infestation of horse purslane was recorded in various crops. A horse purslane population in a crop was found affected by leaf spot diseases at Kurukshetra. It was observed that small brown spots on leaves start from the margins which gradually spread towards the centre and become irregular shaped structures (Fig. 1A). Another important observation made in the present study is that young leaves showed less infection than mature leaves in the field indicating that young leaves are comparatively more resistant. Under severe conditions, the older diseased leaves are shed from the plant. The spot on PDA (Fig. 1B) and TeDA yielded a fungus pathogen which was identified as *Fusarium chlamydosporum* Wollenw. & Reinking (Fig. 1) on the basis of microscopic and molecular characteristics. Typical disease symptoms were produced on both injured and uninjured leaves in *in-vitro* and the inoculated pathogen was re-isolated and found similar to the original isolate in cultural characteristics thus confirming the pathogenicity of *F. chlamydosporum* to *Trianthema portulacastrum* and completing the Koch's postulates. This fungus has been reported to cause various leaf and fruits diseases in Aleppo pine, chilli, gauva and sorghum (Lazreg et al. 2013; Kumar et al. 2013; Gupta and Misra, 2012; Onyike and Nelson, 1992).

Identification based on microscopic characteristics

The morphological, cultural and formation of, sporodochia, two types of conidia; macroconidia and microconidia, and chlamydospores by *F. chlamydosporum* (growth on PDA) were the principle characters to identify *F. chlamydosporum* (Fig. 1B). Hyphae are septate and hyaline. Conidiophores are simple or branched monophialides and polyphialides (phialides with more than one opening not delimited by a septum). Microconidia are abundant, spindle-shaped, 0-3 septate (never globose), measuring 6-26 x 2-4 µm (Fig. 1C, D). Macroconidia are rare, 3-5 septate, sickle-shaped, measuring 30-38 x 3-4.5 µm (Fig. 1 F). The species epithet is derived from the profuse formation of brown, rough, thick-walled chlamydoconidia that occur in pairs, chains, or clumps at maturity (Fig. 1 G-I) (Gupta and Misra, 2012; Booth, 1971).

Identification based on molecular characteristics

The isolated pathogen has been identified by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from European Bio-informatics Institute (EBI). 18S rDNA gene and internal transcribed spacer region (ITS) comprising the ITS1-5.8S-ITS2 rDNA gene cluster was amplified and sequenced by International Mycological Institute (IMI), CABI Bioscience UK (IMI Number 503550). Sequence of *F. Chlamydosporum* was compared for the genetic position in *Fusarium* spp. evolutionary phylogenic tree (Fig. 2). The evolutionary history was inferred using the Neighbor-Joining method. The purified PCR products of approximately 1,400 bp were sequenced by using 2 Universal primers ITS 1 and ITS 4 (Table 1). These two primers amplified the non-coding spacer regions ITS 1 and 2 and the conserved 5.8S; included as well are the partial conserved coding regions of the 18S and 28S genes which are interspersed between the ITS 1 and 2 spacer regions(Siddiquee et al. 2010; Satou et al. 2001).

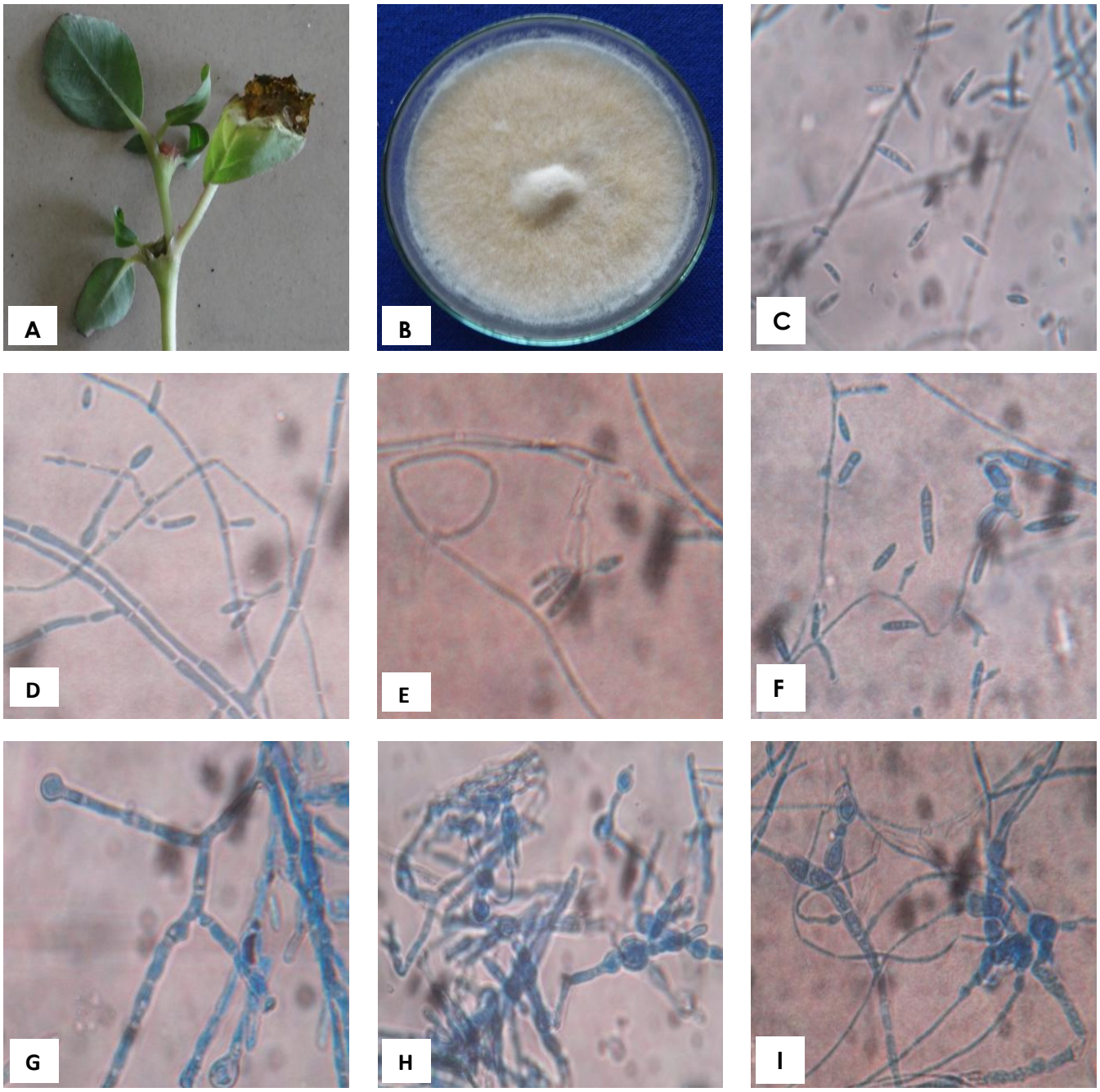

Figure 1 *Fusarium chlamydosporum* **(A)** Leaf spot; **(B)** Colony of the fungi after 7 days at 25°C on PDA; **(C)** Macro- and Micro-conidia; **(D)** Abundant oval to obvate single microconidia with 0 - 1 septa; **(E)** Slightly larger mesoconidia with 1 - 2 septa; **(F)** Straight, rare and falcate macroconidia with 2-3 septa per conidium; **(G-I)** Chlamydospores, single, in chain at the tips and intercalary positions.

Table 1 Universal primers used during amplification

Universal Primer	Sequence	Bases
ITS1	TCCGTAGGTGAACCTGCGG	19
ITS 4	TCCTCCGCTTATTGATATGC	20

A total of eight fungal pathogens have been reported on *Trianthema portulacastrum* weed from various parts of the globe. A literature search reveals that this is the first report of the occurrence of *F. chlamydosporum* causing leaf spot on horse purslane from the world. Further studies on its evaluation for biocontrol efficacy, host-specificity, phytotoxin production are in progress in our laboratory.

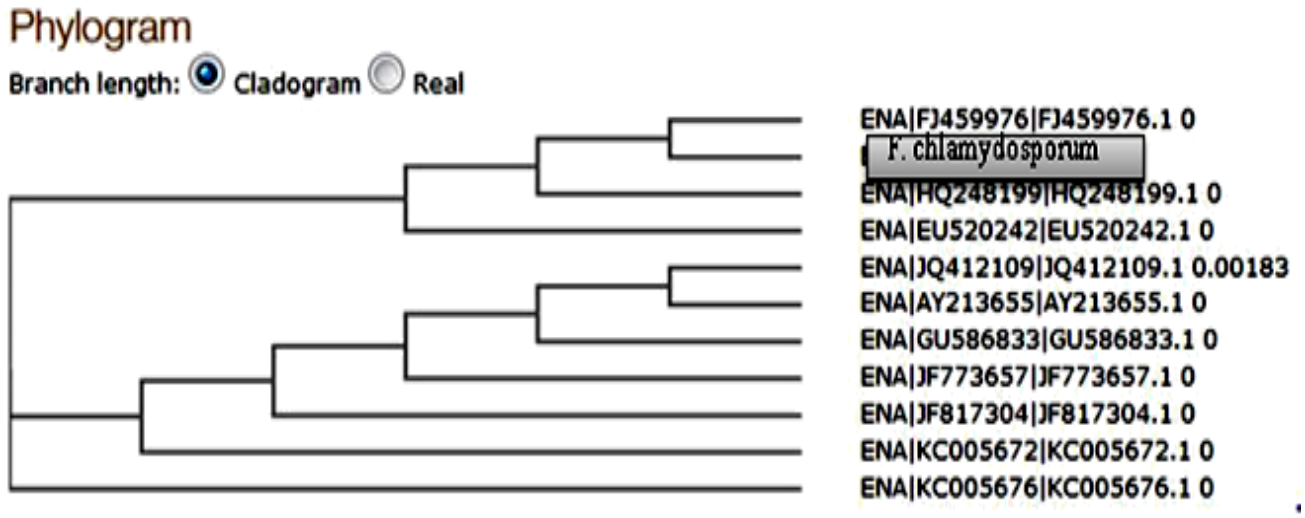

Figure 2 Phylogenetic tree using ITS sequences shows closest known relatives of *Fusarium Chlamydosporum*

Acknowledgements
The authors are thankful to the Director, CABI, International Mycological Institute, Egham, England for confirming the identification of the isolate.

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