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Short communication

Three fungal pathogens associated with horse purslane (*Trianthema portulacastrum*) in North India

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Weeds represent one of the major kinds of pests worldwide which reduce yield and the quality of crops through competition for essential inputs such as water, nutrients and sunlight. Of the total annual loss of agricultural products due to various pests in India, weeds roughly account for 37%, insects for 29%, diseases for 22% and other pests for 12% (Yaduraju 2006). It is widely accepted that sole dependence on herbicides for weed control is inopportune and that alternative and complementary control options should be developed. The application of plant pathogens is considered especially for parasitic weeds, difficult to control *via* chemical means, or for small-scale and specialized crops where the development of specific chemical solutions is too expensive (Auld and Morin 1995).

Trianthema portulacastrum L. (Aizoaceae), commonly known as horse purslane, santhi or santha, is one of the troublesome weed in Haryana, Punjab, Rajasthan, Uttar Pradesh and Delhi affecting important agricultural crops such as maize, sorghum, sugarcane, cotton, mungbean, potato, soybean, black gram, pearl millet, pigeon pea (Kumar and Aneja 2016). The biological control of weeds by plant pathogens has gained acceptance as a practical, environmentally safe and beneficial method. The use of fungal pathogens as biological control agents for weeds is provoking increasing interest worldwide. Some commercial products are already available in developed countries as potential mycoherbicides to control terrible weeds (Aneja *et al.* 2013). In the present study, survey to find out the natural enemy associated with the horse purslane that may be used as potential mycoherbicide agents against *T. portulacastrum* was conducted during 2012-2015,

Preparation of *Trianthema* extract dextrose agar medium (TEDA)

A specific and economic growth medium was prepared growing. This medium was found to be very suitable with the supplementation of dextrose for growing pathogens that are specific to horse purslane. The plant extract of *T. portulacastrum* was prepared by boiling and dextrose was added into the extract as a supplement and sterilized by autoclaving at 121 ^{O}C at 15 lbs pressure for 15 minutes.

Infected leaves with different types of symptoms were collected in sterilized polythene bags and brought to the laboratory for the study of symptoms, isolation, identification and pathogenicity tests of the pathogen/s involved.

Isolation and identification of the fungal pathogens

Leaf surfaces of horse purslane were washed with distilled and sterilized distilled water in order to remove epiphytic fungi and adherent soil particles**.** The infected leaves were cut into 1.0-1.5 cm fragments, surface sterilized with 70% ethanol by dipping for 1-2 minutes and then rinsed in sterile distilled water for 3 to 4 times. These fragments were transferred on to the *Trianthema* extract dextrose agar (TEDA) medium supplemented with streptomycin sulphate. Petri-plates were incubated at 25 °C for 3 to 4 days (Aneja *et al.* 2014). After appearance of fungal growth on leaf surface, fungus was sub cultured and purified on *Trianthema* extract dextrose agar (TEDA) and Potato Dextrose Agar (PDA). Isolated pathogens were morphological characterized and identification was confirmed by molecular characterization.

Pathogenicity test

The pathogenicity was determined under *in vitro* conditions. The leaves were washed with sterile distilled water and wiped with a cotton swab dipped in 70% alcohol. Some of the leaves before inoculation were injured by pricking on adaxial surface with a flamed needle. Mycelial discs of 8 mm taken from 5 days old culture were placed on injured and uninjured portions. The inoculated leaves were kept in sterilized moist chambers and incubated at 25 °C. Regular observations were made for the appearance of symptoms for 3 days of incubation.

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A total of three fungal pathogens namely, *Cochliobolus australiensis, Cochliobolus spicifer* and *Colletotrichum gloeosporioides* were reported on this weed (**Table 1**). Molecular identification of *C. australiensis* was confirmed from Macrogen Inc., Advancing through Genomics, Korea and sequence was submitted to the National Center for Biotechnology Information (NCBI) with accession number KM999998. *C. spicifer* (IMI no. 503552) and *C. gloeosporioides* (IMI no. 503551) were identified by Commonwealth Agricultural Bearux International (CABI), International Mycological Institute (IMI) Egham, England.

Cochliobolus australiensis

Effuse velvety, grey to blackish-brown growth with pale dark brown, smooth, septate hyphae. Conidiophores appear as single, flexuous, geniculate, septate, smooth, cylindrical, reddish-brown, up to 150 µm long, 3-7 µm wide. Conidiogenous cells are polytretic, integrated, terminal and intercalary, sympodial, cicatrized, bearing verruculose conidiogenous nodes. Conidia are straight, ellipsoidal or oblong, rounded at the ends, pale brown to mid reddish-brown, usually 3- rarely 4- or 5-distoseptate, 14-40 × 6-11 µm (**Figure 1**).

Cochliobolus spicifer

It produced olive green to dark brown coloured colonies (**Figure 2. A**). Conidiophores appear solitary or in small groups, flexuous, mid to dark brown, up to 300 µm long, 4-9 µm thick. Conidiogenous cells are polytretic, integrated, terminal, sympodial, cylindrical, and prominently cicatrized. Conidia are straight, oblong or cylindrical, round at ends, golden brown when mature except for a small area just above the scar which remains hyaline, smooth, constantly three-pseudoseptate, 20-40 x 9-14 µm (mostly 30-36 x 11-13 µm); hilum 2-3 µm wide (**Figure 2**).

Colletotrichum gloeosporioides

Symptoms are dark blackish brown coloured start at the margins and enlarge over the leaf. Fungus produces orange white colored colonies with white margins on the potato dextrose agar medium. The fungus produces hyaline, one-celled, ovoid to oblong, slightly curved or dumbbell shaped conidia, 10-15 µm in length and 5-7 µm in width (**Figure 3**). The waxy acervuli that are produced in infected tissue are sub-epidermal, typically with setae, and simple, short, erect conidiophores.

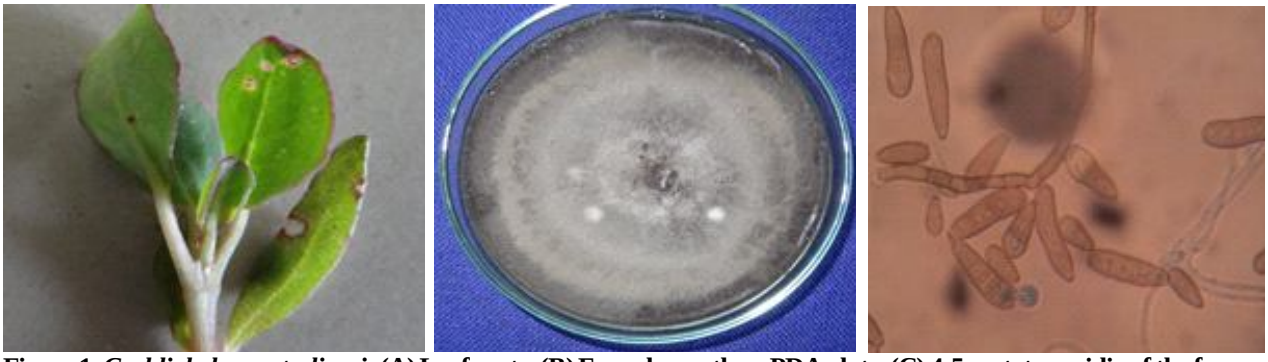

Figure 1. *Cochliobolus australiensis* (A) Leaf spots; (B) Fungal growth on PDA plate, (C) 4-5 septate conidia of the fungus

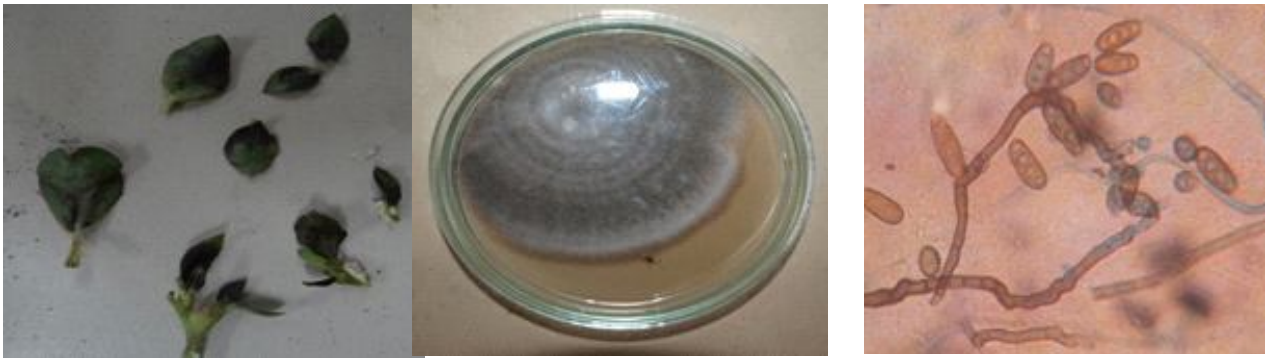

Figure 2. *Cochliobolus spicifer* (A) Leaf spots, (B) Growth on PDA plate, (C) 2-3 septate golden brown conidia of the fungus

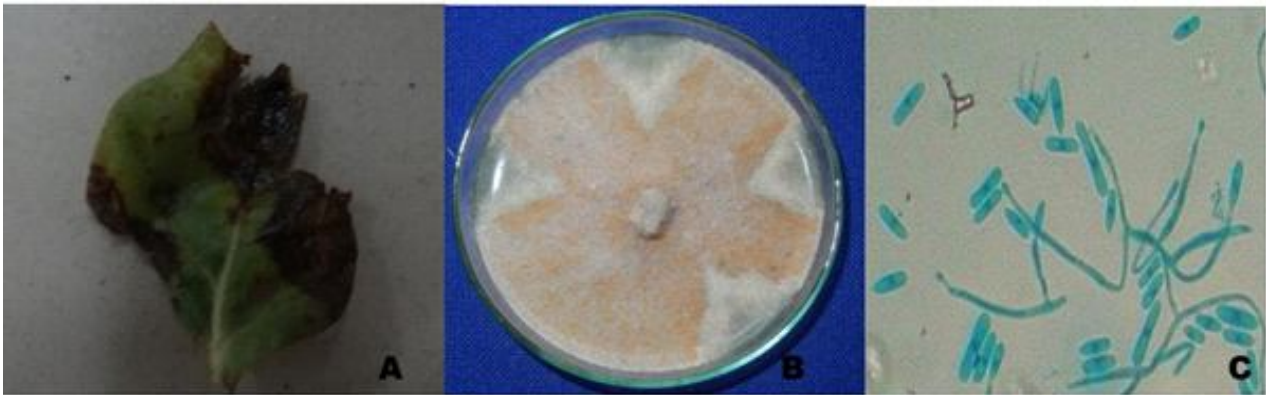

Figure 3. ***Colletotrichum gloeosporioides*** **(A) Leaf spots, (B) Growth on PDA plate, (C) Single cell conidia of the fungus**

Table 1. Total fungal pathogens isolated from ***Trianthema portulacastrum***

Fungus	Symptoms	Samples collected from	IMI number	Accession number	Gene Bank submitted name	Isolates
Cochliobolus australiensis	Leaf spot	Jyotisar	-	KM999998	*Cochliobolus australiensis* strain VKR	D-TP
Cochliobolus spicifer	Leaf spot	Kurukshetra	503552	-	-	DT-1
Colletotrichum gloeosporioides species complex	Leaf spot	Kurukshetra	503551	-	-	CT-1

Pathogenicity test

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 all the phytopathogens to *T. portulacastrum* and completing the Koch's postulates.

Molecular characterization of fungal pathogens

PCR amplification of 18S rRNA gene with universal primers for the fungal plant pathogen, *viz. Cochliobolus* sp. (D-TP) produced an amplification product of approximately 557 bp. The sequence similarity search of the sequenced products obtained were analysed through BLAST that confirmed the identification of these isolates namely, *Cochliobolus australiensis.* The alignment of retrieved sequences from NCBI database with 18S rRNA of the fungal isolate D-TP showed maximum homology with *Cochliobolus australiensis*. The gene sequences of the fungal pathogen have been submitted to NCBI under the accession number KM999998.

Out of three isolated fungal pathogens, two has been reported first time from *T. portulacastrum*, namely *Cochliobolus australiensis* and *Cochliobolus spicifer. Colletotrichum gloeosporioides* was reported previously by Darshika and Daniel (1992).

SUMMARY

A number of phytopathogenic fungi are known to be associated with horse purslane. Three new fungal pathogens have been found to be associated with horse purslane, which may be used for the preparation of mycoherbicide. There may be possibility to prepare cultural blends with suitable adjuvants. One of the fungal pathogen, *Gibbago trianthema*, has shown the potential to be used as successful biological control agent. New phytopathogenic fungal genera reported during this study, can be further exploited for the biological control of horse purslane.

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