First report of *Helicoceras celtidis* causing foliar disease of *Celtis australis* from Jammu and Kashmir, India

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Key words: *Celtis australis*, *Sirosporium celtidis*. – Plant pathogen, leaf spot, dematiaceous hyphomycete.

Abstract: In autumn 2013, a foliar disease was observed on the important ornamental tree *Celtis australis* affecting several trees planted in different locations in Jammu and Kashmir. Symptoms appeared on lower leaf surfaces as reddish to dark brown velvety irregular spots, later becoming greyish brown on the upper surface. Spots on lower leaf surfaces were covered by mycelium, conidiophores, and conidia. Fungal isolates were recovered directly from the structures present on the lesions. Morphological characters such as mycelial septation, conidiophore attachment, conidial size, and conidial ornamentations revealed by scanning electron microscopy corresponded to the description of *Helicoceras celtidis*, which was described from Sicily (Italy) in 1815 as *Sirosporium celtidis*. It has been recorded on various *Celtis* species in Mediterranean countries, India, Japan and the USA. The present study is the first report of this disease from Jammu and Kashmir.

Zusammenfassung: Im Herbst 2013 wurde eine Blattkrankheit auf dem wichtigen Zierbaum Celtis australis beobachtet, die etliche angepflanzte Bäume an verschiedenen Orten in Jammu und Kaschmir betraf. Die Symptome traten auf der Blattunterseite als rötliche bis dunkelbraune, samtige unregelmäßige Flecken auf, später als graubraune Flecken auf der Oberseite. Die Flecken auf der Blattunterseite waren mit Myzel, Konidiophoren und Konidien bedeckt. Pilzisolate wurden direkt aus den befallenen Stellen gewonnen. Die im Rasterelektronenmikroskop beobachtetetn morphologischen Merkmale, wie Myzelseptierung, Konidienträger, Haftpunkt, Konidienform, -größe und Ornamentierung, entsprachen der Beschreibung von *Helicoceras celtidis*, die von Sizilien (Italien) im Jahre 1815 als *Sirosporium celtidis* beschrieben wurde, und auf verschiedenen Celtis-Arten festgestellt worden war. Die Art ist aus den Mittelmeerländern, Indien, Japan und den USA bekannt. Die vorliegende Studie ist der erste Bericht dieser Krankheit von Jammu und Kaschmir.

The State of Jammu and Kashmiris located in the far north of the Indian republic. It is a mountainous zone in the NW Himalayas that borders on Pakistan in the west. The Kashmir valley lies between 33º 20’ and 34º 54’ N latitude and 73º 55’ and 75º 35’ E longitude. The Kashmir valley, a fertile basin whose soil is formed of deposits laid down on the floor of the lake that once covered it, is bounded in the north by the Great Himalaya and separated from the plains of northern India by the Pir Panjal range (HUSAIN 2001).

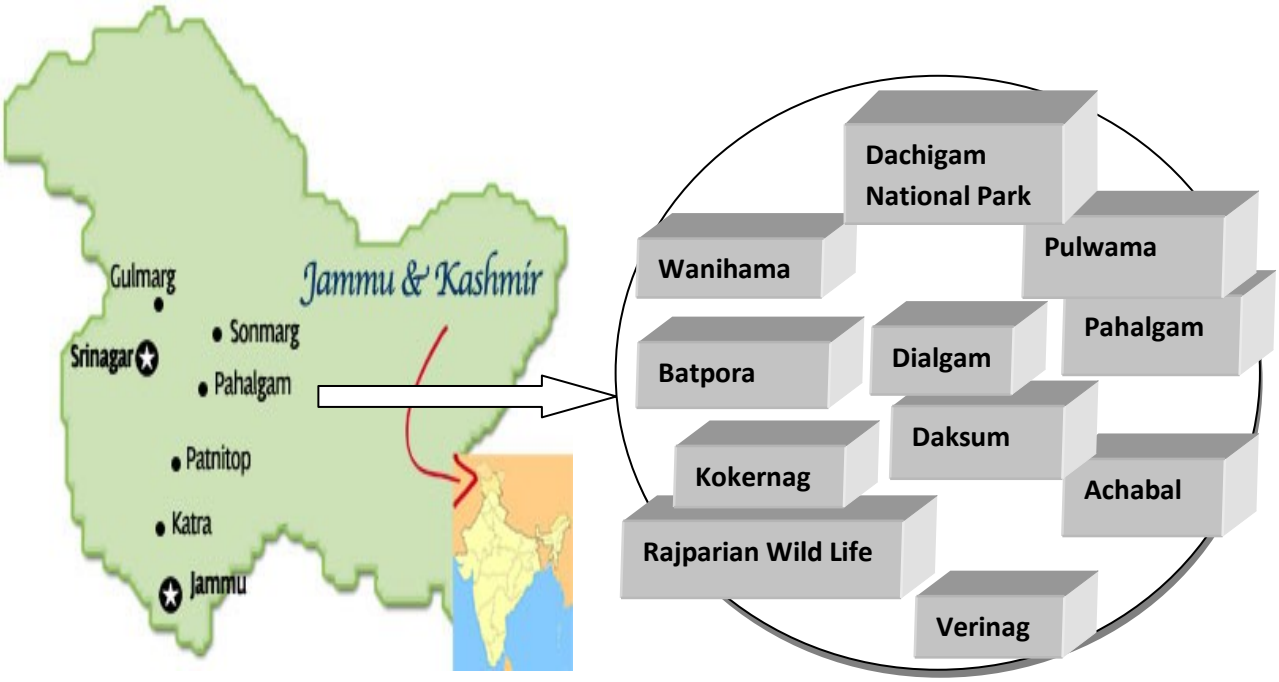


Fig. 1. Map of different sites selected and surveyed in Jammu and Kashmir forests for *Helicoceras celtidis* occurrence.

The fungal diversity scaling relationships relative to that of plants is important to understand ecosystem functioning. The forests, constituting approximately 20% of the geographical area, harbor diverse fungal species due to the wide variability in climate altitude and nature of species constituting them. Fungi are key functional components of forest ecosystems (BROWN & al. 2006) and they have received less attention than animals and plants, although they are omnipresent and highly diverse in nature (PIEPENBRING 2007). Defining the exact number of fungi on the earth has always been a point of discussion and several studies have focused simply on the enumeration of world's fungal diversity (CROUS & al. 2006). Organisms like fungi and algae have not been studied exhaustively. Information on the micro-flora of isolated regions is available for some plant orders and families only. So far 270 macrofungal species have been reported from Jammu and Kashmir state (e.g. ATRI & KAUR 2003, BEIG & al. 2008, BILGRAMI & al. 1991, DAR & al. 2009, JAMMALUDIN & al. 2004). There is urgent need to thoroughly explore these forests for foliicolous fungi and to conserve the biodiversity prevailing in the state for future use.

Celtis australis is a medium to large sized deciduous tree of subtropical to temperate climate. In India, especially of Northern Himalayan forests, it grows in association with horse chestnut, maple, bird cherry and oak in moist localities of blue pine and deodar forests, where it is commonly known as batkar, khark, khirk, roku and brimji. It is often planted as an ornamental being resistant to air pollution. Most of the areas where it grows experience frost in the winter. The bark is smooth and grey, almost elephantine (MORE & WHITE 2003). The height reaches up to 25 m and 60 cm dbh with crown spreading, bluish-grey bark, twigs smooth and greenish. The leaves are alternate, obliquely ovate to lanceolate and strongly 3 nerved. Old leaves are shed in October, November, and new ones appear in March, April simultaneously with flowers.

Pharmacologically, the extract from the tree is used to treat edema, headache and boils. The leaves and fruits are astringent, lenitive and stomachic (CHIEJ 1984, CHEVALLIER 1996). The leaves are also a rich source of flavonoid C glycosides (SPITALER & al. 2009, KALTENHAUSER & al. 2010). Decoction of both leaves and fruit is used in the treatment of amenorrhea, heavy menstrual and intermenstrual bleeding and colic (DUKE 1985; CHOPRA 1956, 1969).

Material and methods

Collection of fungal specimens

Random field visits were carried out to maintained and unmaintained (rich in biodiversity) plantations of *Celtis australis* to different locations in Jammu & Kashmir (Daksum, Dialgam, Wanihama, Rajparian wild life sanctuary, Dachigam National Park, Pahalgam, Pulwama, Achabal, Batpora and Verinag) from June 2012–November 2014 (Fig. 1., Tab. 1). Field equipments were autoclaved polybags, magnifying glass, digital camera, sterile scissors, tags and maps. An official forest guard was provided by the Chief Conservator Officer (CCO). The collected specimens were pressed and preserved using naphthalene to avoid microbial decomposition. Voucher specimens are deposited in the University mycological herbarium with Accession no. DHSGU111.

Tab. 1. Sites selected and surveyed showing different percentage of *Helciceras celtidis* occurrence in locations of Jammu & Kashmir.

Site no.	Area surveyed	No. of Plants observed	No. of plants infested	% of Fungal Occurrence
1	Daksum Kokernag	25	07	28
2	Dialgam Anantnag	15	05	33.33
3	Wanihama	10	03	30
4	Rajparian wild life sanctuary	23	06	26.08
5	Dachigam National Park	33	11	33.33
6	Pahalgam	21	05	23.8
7	Pulwama	33	12	36.7
8	Achabal	26	09	34.6
9	Verinag	16	04	25
10	Batpora	09	02	22.22
	Total	**211**	**64**	**30.33%**

Laboratory work

The collected specimens were identified and studied using a compound microscope, chemical reagents (lactophenol, 3% KOH, 1% cotton blue) and relevant literature (USDA, UNITE fungal databases). Preliminary identification was followed by Scanning Electron Microscopy (FEI Nova Nano SEM-450) using magnifications ranging between 800–6000×. Most SEM specimens have been imaged at ambient temperature in a high vacuum, having first been chemically fixed, dehydrated and sputter coated with gold (HiRes-Gold on Carbon).

Production of cultures

The fungus was isolated and inoculated using a sterile loop on three different growth media (PDA with added vitamin H, 6-BAP and MEA) and the bottom of each Petri plate was labelled. The buffer (HIMEDIA) and Streptomycin (Ranbaxy) tablets were added to media to maintain pH (4–5) and avoid bacterial contamination, respectively. The culture plates were incubated at 25–28 ^{o}C for 15–17 days.
On the 18th day, the fungal cultures were examined for CFU using hemocytometer and the spore suspension of 1×10^3 concentration were inoculated in potato dextrose broth medium. Subculturing of

heterogeneous polycultures was carried out under aseptic conditions. The cultures were incubated again at 28° C for 15 days until the homogenous cottony growth appeared (Fig. 2). Previsualization/identification was made under a compound microscope followed by SEM. Morphological characters, such as mycelial septation, conidiophore attachment, conidial size and ornamentation were considered.

Fig. 2. Images of the host, *Celtis australis*, infected with *Helicoceras celtidis* and its homogenous pure culture (PDA) incubated at 28 °C for 15 days.

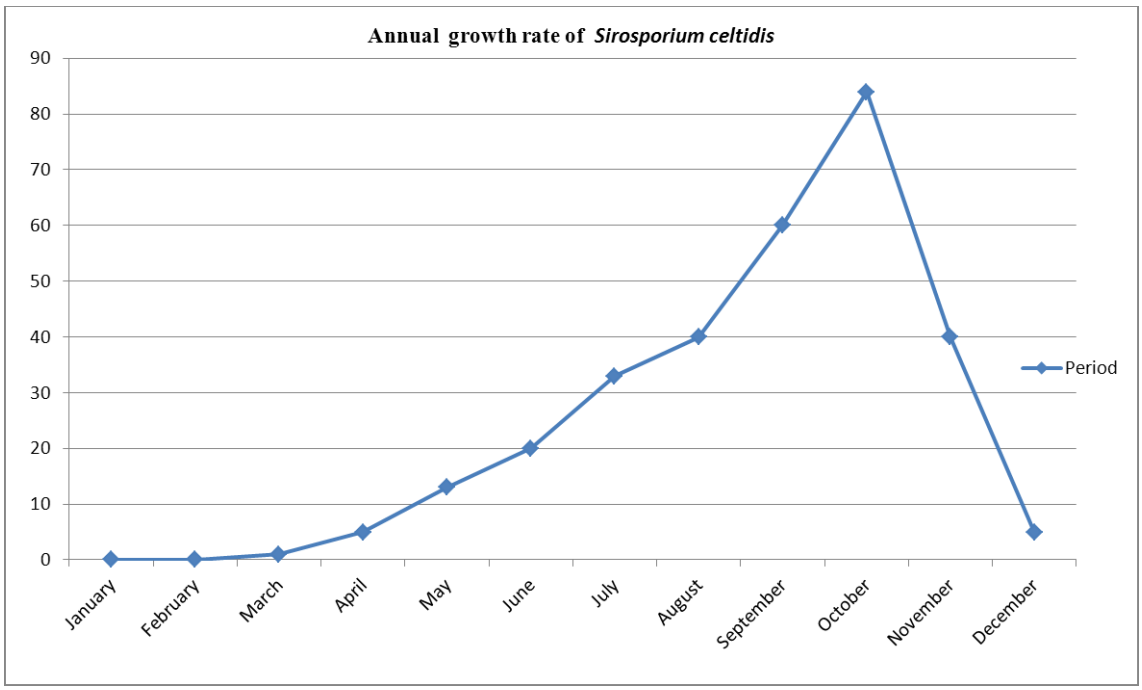


Fig. 3. Growth rate of *Helicoceras celtidis* under different environmental conditions; y-axis: percentage of plants infested per site against no. of plants observed per site

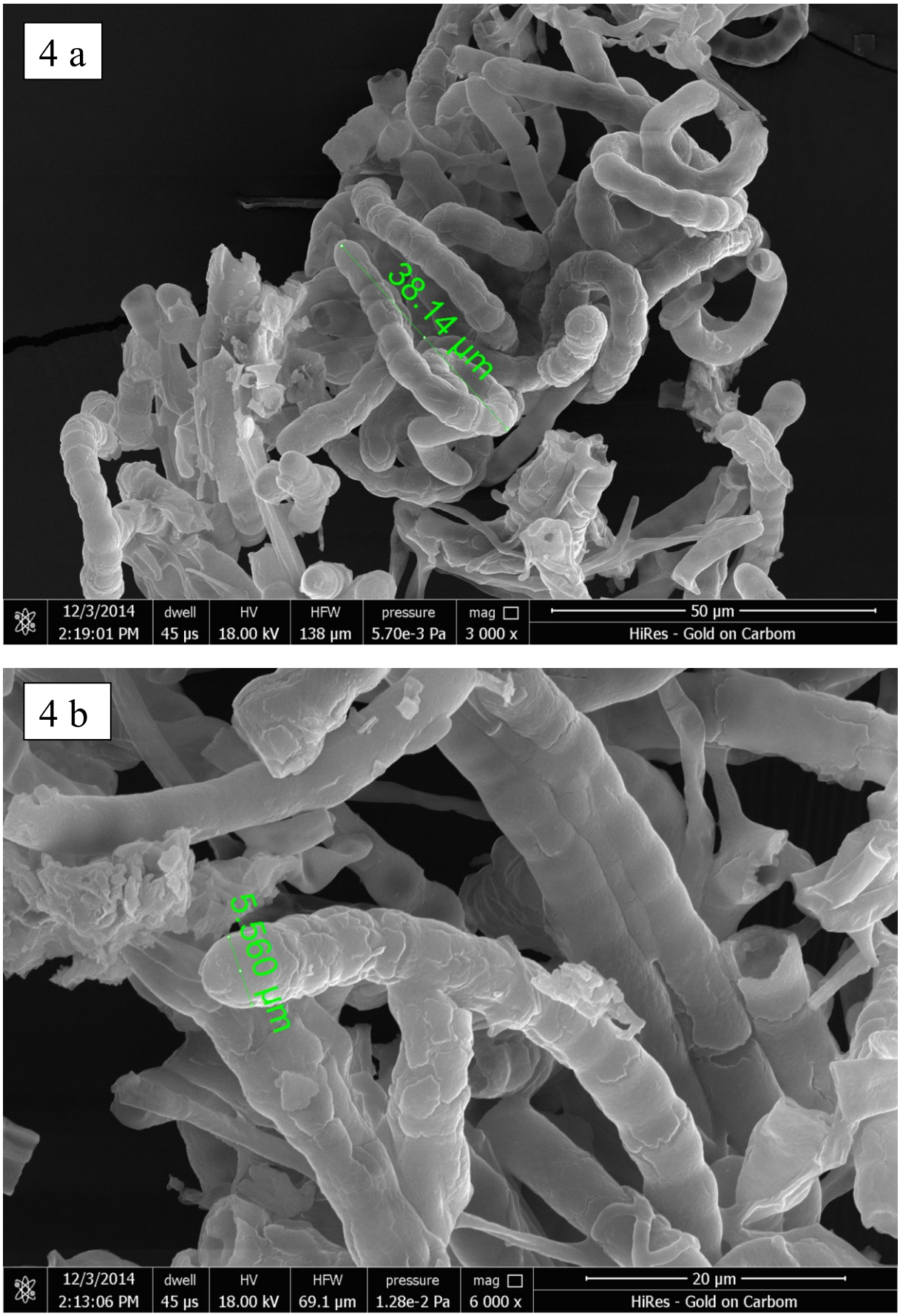
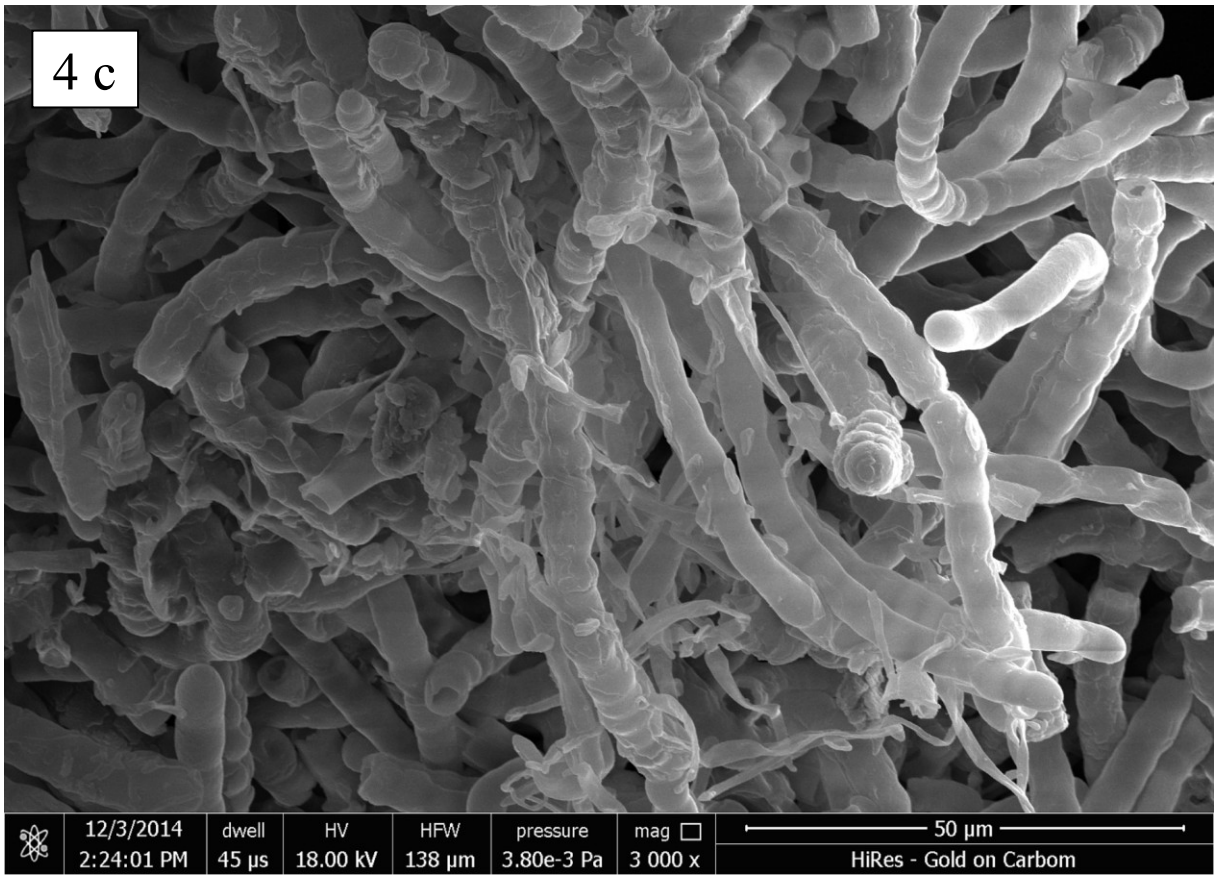


Fig. 4. SEM images of *Helicoceras celtidis*, ***a*** conidia at 3000×, ***b*** conidia, conidial cell width of 5.5 µm and bursting of conidia, 6000×, ***c*** mycelium clustering, 3000×, ***d***, ***e*** attachment to the host at 800× and 1600×, respectively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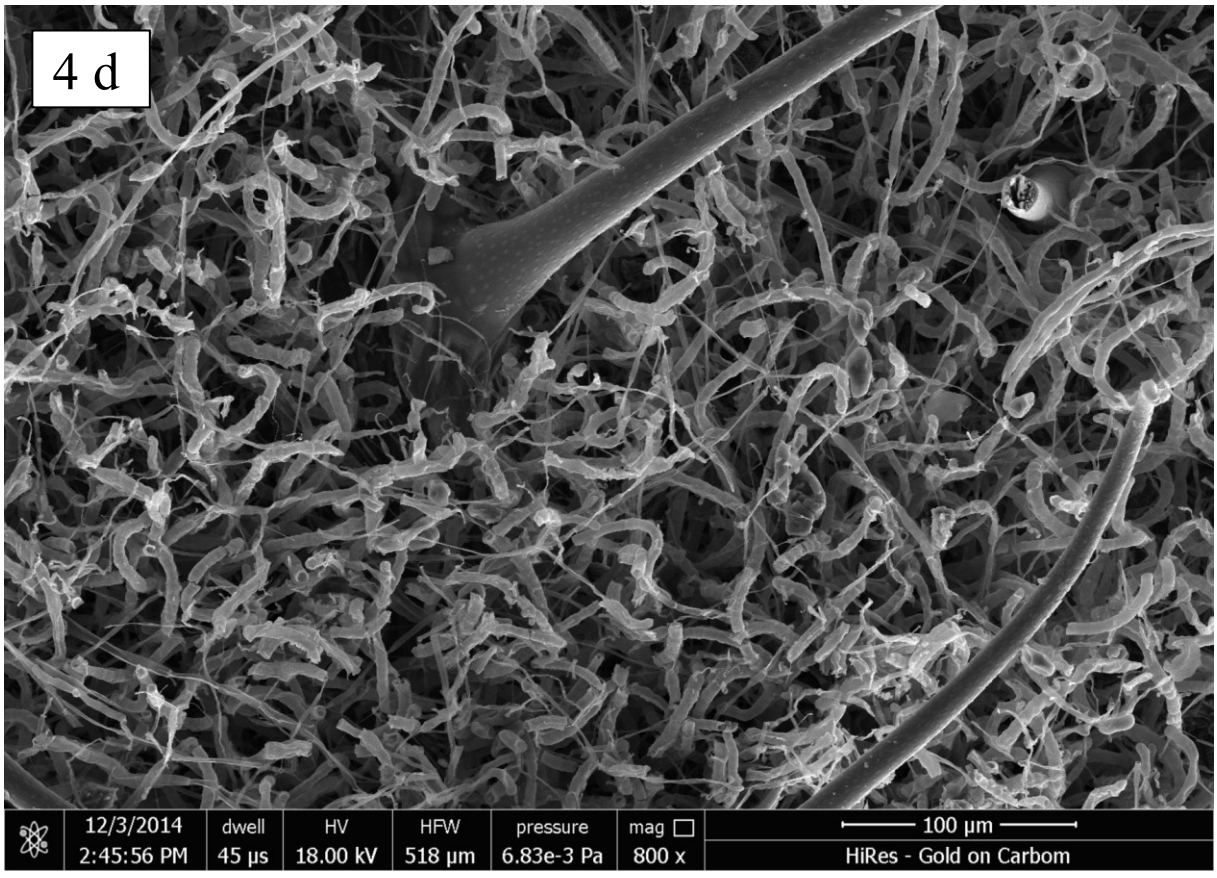
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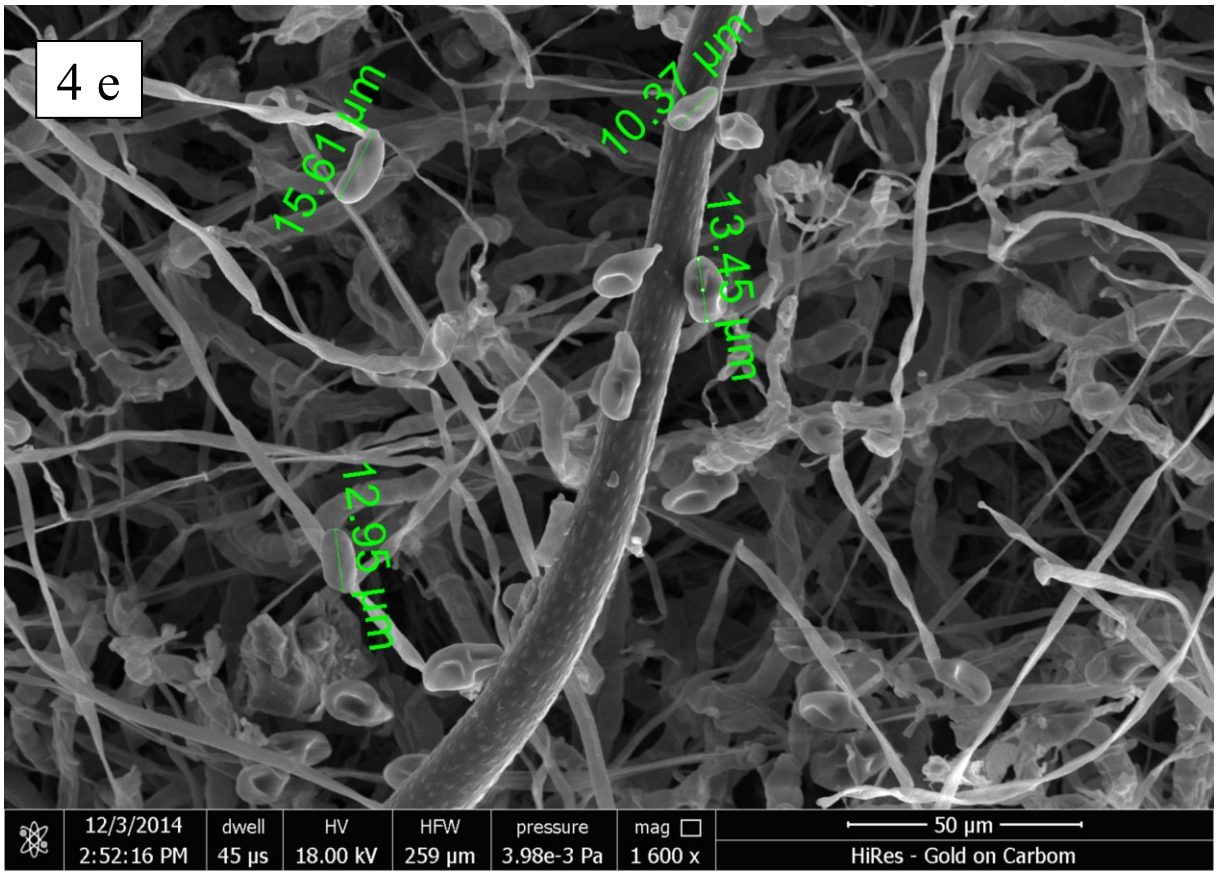

Results and discussion

The field visits were done to explore the occurrence of the foliicolous fungus, *Helicoceras celtidis* (BIV.) LINDER [=*Sirosporium celtidis* (BIV.) M. B. ELLIS], and to trace its symptomatology and possible remedy. In autumn a foliar disease was observed affecting several *Celtis australis* trees planted in different locations as shown in Tab. 1. The symptoms appeared on lower leaf surfaces as reddish to dark brown velvety irregular spots, later becoming grayish brown on the upper surface. In the pathogenicity test, the spores of *Helicoceras celtidis* sprayed on the fresh leaves of *Celtis australis* caused the same symptoms in six weeks after the artificial inoculation, which is similar to observations in the field. Most of the infected trees were prematurely defoliated. During these explorations it was observed that the magnitude of occurrence and extent of its infection changes parabolically with the fluctuating environmental conditions. During the cold season, especially the snow period (December to March) the fungal occurrence was almost zero but with the onset of favorable conditions (May to October) it grew linearly especially in the month of October, which may be considered its peak growth season. Thereafter the fungal growth progression declined again (Fig. 3).

Description of *Helicoceras celtidis* macromorphology

Spots on the lower leaf surfaces were covered by mycelium, conidiophores, and conidia. Fungal isolates were recovered directly from the structures present on the lesions. Colonies were hypophyllous, effuse, reddish brown to dark blackish brown, velvety. Conidiophores were erect or ascending, smooth and pale brown near base, often verrucose and darker at the apex.

SEM visualization and micromorphology

Cultures as well as the host-attached-fungus on infected leaves were examined under SEM to show host-fungus attachment, conidial dimensions and hyphae length. SEM revealed that the conidia corresponded best to the description of *Helicoceras celtidis* given by ELLIS (1971, as *Sirosporium celtidis*): "conidia cylindrical or sometimes obclavate, often curved or coiled, smooth, rugulose or verrucose, subhyaline to golden or reddish brown with 1–32 transverse and occasionally 1–2 longitudinal or oblique septa, slightly constricted at the septa, 23–160(70 µm) long, 5–10(6.8) µm thick in the boradest part, 2.5–5 µm wiede at the base which bears a rather inconspicuous scar." (Fig. 4). Most of the conidia of our material falls in the range of 16–145 µm. *Helicoceras celtidis* is already recorded from India (ELLIS 1971). The present record is the first report of this disease from Jammu & Kashmir.

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