Applied Biological Research 21(3): 274-282; (2019)
DOI: 10.5958/0974-4517.2019.00036.3

Venturia crataegi CAUSING SCAB ON *Crataegus songarica:* MORPHO-MOLECULAR CHARCTERIZATION AND A NEW RECORD FROM INDIA

M.S. Dar[1]*, Mushtaq Ahmad[2], M.D. Shah[3], Bilal A. Padder[3], Nazir A. Bhat[4], M. Anwar Khan[5], F.A. Ahanger[1], Asha Nabi[1] and Aqleema Banoo[3]

[1]Division of Plant Pathology, [5]Division of Genetics and Plant Breeding, Faculty of Agriculture, S.K. University of Agricultural Sciences & Technology of Kashmir (SKUAST-K), Wadura, Sopore - 192 301, Jammu & Kashmir (India)
[2]Directorate of Extension, SKUAST-K, Shalimar, Srinagar - 190 025, Jammu & Kashmir (India)
[3]Plant Virology and Molecular Plant Pathology Laboratory, Division of Plant Pathology, Faculty of Horticulture, SKUAST-K, Shalimar, Srinagar - 190 025, J&K (India)
[4]Mountain Field Crop Research Centre, SKUAST-K, Khudwani, Anantnag - 192 001, J&K (India)
*e mail: *saleemdar0001@gmail.com*

(Received 12 September, 2019; accepted 4 December, 2019)

ABSTRACT

A field survey was conducted in the years 2017 to 2019 for the collection of diseased samples of various crops including *Crataegus* species, from Pulwama and Srinagar districts of Jammu and Kashmir. The leaf and fruit samples of *Crataegus songarica* showing scab symptoms were collected and brought to the laboratory. During disease monitoring it was noticed that the disease initiated as dark spots on leaves and fruits; and then often covered the entire surface. The mycelium on host was septate, immersed, subcuticular and pale olivaceous; hyphae branched and 2-4 µm wide. On MEA medium the mycelium was septate, branched, interwoven, light pale to olivaceous brown and 3-5 µm wide. The conidia were solitary, fusiform to obclavate having 0-1 septum (avg. size; 15.64 × 5.76 µm). Based on morphocultural characteristics the fungus was identified as *Venturia crataegi*. The identity was confirmed by sequencing of ITS region of the pathogen using ITS1 and ITS4 primer. The ITS sequence was submitted to Genbank of NCBI under Accession No. MK478372. For phylogenetic analysis, the sequence was compared with other sequences of *Venturia* species available at NCBI which showed close relatedness of the present sequence with *V. crataegi* (Accession No. MH855705.1). The present study forms the first report of crataegus scab caused by *Venturia crataegi* from India.

Keywords: *Crataegus sonarica*, phylogenetics, rDNA-ITS, scab, *Venturia crataegi*

INTRODUCTION

Venturia species are most widely distributed in the temperate areas of world and occur either as saprobes or parasites on a large number of host plants. Most *Venturia* species are notorious phyto-pathogens like *V. inaequalis* (Cooke) Wint. causing apple scab and *V. pyrina* Aderh. inciting pear scab (Sivanesan, 1977). *Venturia* was first described by De Notaris (1844) who identified its two species *viz*., *V. rosea* and *V. dianthi*. Subsequently, Cesati and De Notaris (1863) described two more species of this genus, *V. dickiei* and *V. eres* (Zhang *et al.*, 2016). Saccardo (1882) amended the description of *Venturia*, excluded both *V. rosea* and *V. dianthi* and retained *V. dickiei* and *V. eres*.

Saccardo's description of *Venturia* was widely accepted and neotypified by *V. inaequalis* (Sivanesan, 1977). The genus *Venturia* currently contains more than 250 species with a wide range of hosts belonging to the families *Acaraceae*, *Betulaceae*, *Cornaceae*, *Oleaceae*, *Rosaceae* and *Salicaceae* (Sivanesan, 1977; Gautam, 2014). Most of the *Venturia* species are host-specific and occur only on one host or a few host species as a result of host-fungus coevolution (Schnabel *et al.*, 1999; Beck *et al.*, 2005). *Fusicladium*, *Pollaccia* or *Spilocaea* are the anamorphs of *Venturia* (Zhang *et al.*, 2011). These genera are distinguished on the basis of proliferation mode of their conidiogeous cells and conidial formation in which *Fusicladium* has sympodial proliferation, *Pollaccia* has monoblastic with determinate to percurrent conidiogenous cells having a few inconspicuous annellations, and *Spilocaea* has percurrent proliferation with numerous conspicuous annellations (Schubert *et al.*, 2003). Braun *et al.* (2002) suggested that the separation of these anamorphic genera is not tenable since conidiogenesis and structure of conidiogenous loci were uniform.

Molecular studies have shown that the *Venturia* is a monophyletic clade that could not be separated into sub-clades relevant to these anamorphic genera (Beck *et al.*, 2005), and it was proposed that the genera could be merged in one anamorphic genus. Hence, the name *Fusicladium* was conserved against other names (Braun, 2005). The asexual and sexual morphs of same genus must have one name with preference for *Venturia* over *Fusicladium* or *Pollaccia* (Rossman *et al.*, 2015), being the more widely known. The diagnostic characteristics of *Venturia* species were habitat biotrophic or hemi-biotrophic on dicotyledonous leaves; ascomata small-sized, solitary, scattered or gregarious, initially immersed, becoming erumpent, globose to subglobose, wall black, papillate, ostiolate; peridium thin, composed of a few rows of pigmented cells of *textura angularis*; hamathecium rare, usually evanescent in mature ascomata; asci 8-spored (rarely 4-spored), bitunicate, oblong to obclavate with a short, thick pedicel or pedicel lacking, with an inconspicuous ocular chamber; ascospores obliquely uniseriate and partially overlapping to biseriate, especially at the base, ellipsoidal, with broadly rounded ends, pale brown, usually 1-septate, slightly constricted at the septum, the upper cell shorter than the lower one and smooth-walled (Zhang *et al.*, 2011).

Various species of *Venturia* have been reported from Kashmir *viz*., *V. inaequalis*, *V. pyrina*, *Cladosporium humile, F. radiosum* and *V carpophila* by Nath (1935); Putto and Chaudhary (1984); Singh *et al*. (1983); Kaul *et al.* (1989) and Kacho *et al.* (2017), respectively; but most reports are available on epidemiology, disease management, development of resistant cultivars (Farooqi and Dalal., 2003), distribution of apple scab race flora and identification of resistance sources against *V. inaequalis* (Dar *et al*., 2015). No information is available on the phylogenetic studies on *Venturia crataegi* in India, although other species like *V. inaequalis* have previously been characterized (Padder *et al*., 2013). Schubert *et al.* (2003) reported that *Fusicladium crataegi* is the only cosmopolitan fungus that parasitizes *Crataegus* members. *F. crataegi* was for the first time reported from Poland on *Crataegus coccinnae* (Michalska and Połec, 2006). During survey for ascomycetous fungi in Jammu and Kashmir (India), we collected scab-infected leaves and fruit samples from hawthorn (*Crataegus songarica*). The present study was aimed to characterize the scab pathogen of hawthorn on morpho-cultural and molecular basis.

MATERIALS AND METHODS

Hawthorn (*Crataegus songarica*) samples comprising of leaves and fruits were collected during survey (2017-2019) in Pulwama and Srinagar districts of Jammu and Kashmir. The samples were dried with absorbent paper in a specimen press.

Morpho-cultural characterization and pathogenicity of causal organism

The collected materials (infected fruits and leaves) were examined for morphology of associated fungi. Single conidia were isolated on 2% malt extract agar (MEA) medium. Colonies were sub-

cultured on fresh MEA and potato-dextrose agar (PDA) media; and incubated at 20±2°C for two months. The fungus was scrapped, placed in a drop of distilled water with lactic acid on a microscope slide and examined under oil immersion by standard light microscopy. Mycelial growth including the shape, size and colour of fungus was recorded and compared with the available literature (Schubert *et al*., 2003). The pathogenicity of fungus was established by detach leaf technique. For this, young succulent actively growing leaves from *C. songarica* were collected and washed with distilled sterilized water. Leaf surfaces were air-dried and leaves kept in a moist in 200 mm dia. Petri-plates containing moist Whatman filter paper. Two to three leaves per plate, in triplicate, were inoculated with uniform drops of inoculum (1×10^7 spores mL^{-1}) on leaf surface. The control plates containing leaves were inoculated with distilled water only. The plates were incubated at 20 ± 1°C in a growth chamber with 16 h photoperiod. The plates were monitored daily for disease symptoms, if any, for 6 weeks after inoculation (Liebhard *et al*., 2003; Dar *et al.*, 2015).

DNA extraction, PCR amplification and sequencing

DNA was extracted from pathogen mycelium grown on MEA plates with CTAB (cetyltrimethyl ammonium bromide) method (Murray and Thompson, 1980). The internal transcribed spacer (ITS) region was amplified with PCR based primers ITS-1 and ITS-4 was amplified and custom sequenced [White *et al*., 1990]. PCR amplification was performed in 0.2 mL PCR tubes in a T-gradient Whatman Biometra thermal cycler using 40 ng genomic DNA in a final volume of 25 µL per reaction containing 1X buffer (Fermentas), 1.5 mM $MgCl_2$, 0.2 mM dNTP mix (Fermentas), 1 U Taq DNA polymerase (Fermentas), 40-50 ng DNA template, 0.4 pmol primers and 14.8 µL sterilized distilled water. The reaction mixture was vortexed in microfuge (Thermo Scientific, Thermo Electron Corporation) and the tubes placed in 96 wells of thermal cycler. PCR amplifications were performed in thermal cycler programmed for initial denaturation at 94ºC for 5 min, followed by 35 cycles with denaturation at 94ºC for 1 min, annealing at 58ºC for 1 min, extension at 72ºC for 2 min and a final extension at 72ºC for 10 min. After PCR amplification, 5 µL PCR product of each isolate was electrophoresed to ensure successful amplification, and the remaining 20 µL PCR product sent for custom sequencing under cooled conditions to Xcelaris India, Pvt. Ltd, Ahmadabad (India). The sequence was retrieved from chromatogram, trimmed and assembled manually using Bioedit ver.7.09 (Hall, 1999) to remove ambiguous base and primer sequences, and the consensus sequences were reconfirmed by comparing with the original data output. Comparison to other available DNA sequences in databases was conducted by BLASTn (*http://www.ncbi.nlm.nih.gov/BLAST*) analysis. The present sequence of the pathogen was submitted to GenBank (NCBI) for accession number.

Sequence alignment and phylogenetic analysis

The sequence of the fungus was analyzed and compared with other sequences from GenBank (NCBI). A BLAST query was done to find the possible sister groups of sequenced taxon; and closely related sister groups of other species included in the phylogeny were chosen (Zhang *et al*., 2011). A multiple alignment was conducted in MEGA v. 6.02 (Tamura *et al*., 2013) and analysis was performed to construct a dendrogram.

RESULTS AND DISCUSSION

Symptomatology, morpho-cultural studies and pathogenicity

The scab disease on leaves and fruits of *Crataegus songarica* initiated as dark spots which were scattered, small, crustaceous, subcircular or irregular usually 2-4 mm wide and often covered the entire surface of host (Fig. 2A and B). On host, the fungal growth was observed on both the sides of leaves as well as on fruits as dark olivaceous brown spots with margins dark brown to blackish in colour, sometimes confluent. Mycelium was septate, immersed, subcuticular, pale olivaceous, hyphae

branched and 2-4 µm wide. On MEA medium, the conidia germinated within 2-3 days. The fungal growth was extremely slow, upto 1.0 mm dia. recorded after 15 days of incubation at 20±2°C in dark, 10.0 mm after 4 weeks and reached 39.0 mm in dia. after 3 months (Fig. 2C). Colony was first erumpent spreading with abundant aerial mycelium with feathery to smooth margins. Upper surface of the culture was grey-olivaceous; whereas reverse side was dark olivaceous producing septate, branched interwoven and light pale to olivaceous brown mycelium with hyphal width of 3.0-5.0 µm. The conidia were solitary, light yellow to olivaceous brown, fusiform to obclavate having 0-1 septum with slightly constricted at septum and measured 11. 47-19.18 × 4.71-6.90 µm in size [avg. size 15. 64 × 5.76 µm] (Fig. 2D and E). The conidiogenous structures were not observed. Based on morpho-cultural characteristic, the fungus was identified as *Venturia crataegi* Aderh., Ber. Deutsch. Bot. Ges. 20: 200 (1902), Anamorph: *Fusicladium crataegi*. The identity of fungus was confirmed by molecular characterization using ITS sequencing.

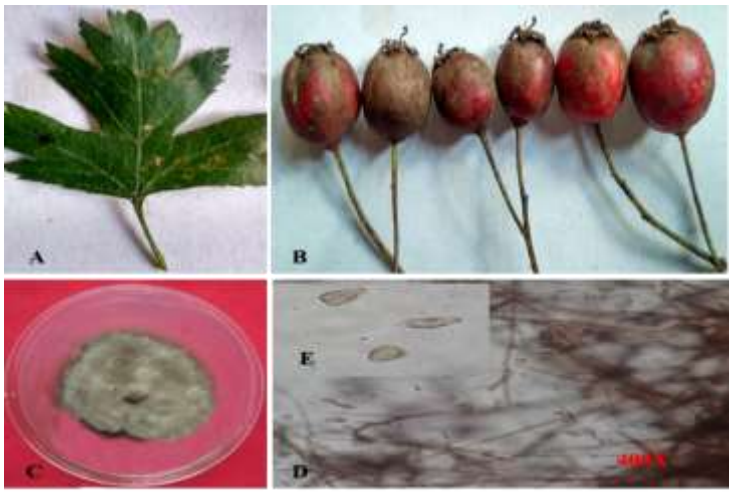

Fig. 1: Symptoms of *Venturia crataegi* on A) leaves, and B) fruits of *Crataegus songarica*, C) colony on malt extract agar, D) mycelium, and E) spores in culture

The fungus grew abundantly on the surface of inoculated leaves of *C. songarica*. Pathogenicity was established following the Koch's postulates. Visible symptoms were observed as initial chlorosis upto 3-4 weeks post-inoculation, then followed by necrosis after 5-6 weeks. Similar results have been reported in *Venturia inaequalis* which produced 5 mm circular leaf punches of detached leaf assay, successful infections and sporulation in apple cv. Golden Delicious (Liebhard *et al.*, 2003).

Fig. 2: PCR amplification of *Venturia crataegi* with S = ITS1 and ITS4 (650 bp); L = 100 bp ladder

Molecular characterization and phylogenetic analysis

PCR based amplification of DNA, obtained from *V. crataegi* causing scab on *Crataegus songarica*, was carried in thermal cycler (T-gradient Whatman Biometra) using ITS1 and ITS4 primers (Fig. 2). The amplified product was custom sequenced. The sequence was retrieved from the chromatogram of each forward and reverse primers and subjected to quality check using Phred score above 30 indicating high quality sequences (Fig. 3). The information on sequence of ITS gene of fungus was analyzed through BLASTn programme of NCBI to compare them with the available sequences of database at NCBI (*http://www.nlm.nih.gov/nuccore*). The study showed that the sequence had maximum similarity with the sequence of *Venturia crataegi* with Accession No. MH855705.1 available at NCBI (National Centre of Biotechnology Information). The sequence of pathogen was submitted to GenBank NCBI under Accession No. MK478372. The present study forms the first report of *Venturia crataegi*, causing scab on *Crataegus sonagrica* from India. To elucidate the taxonomic relationship of *Venturia crataegi* with other species, forty-four sequence of different *Venturia* species

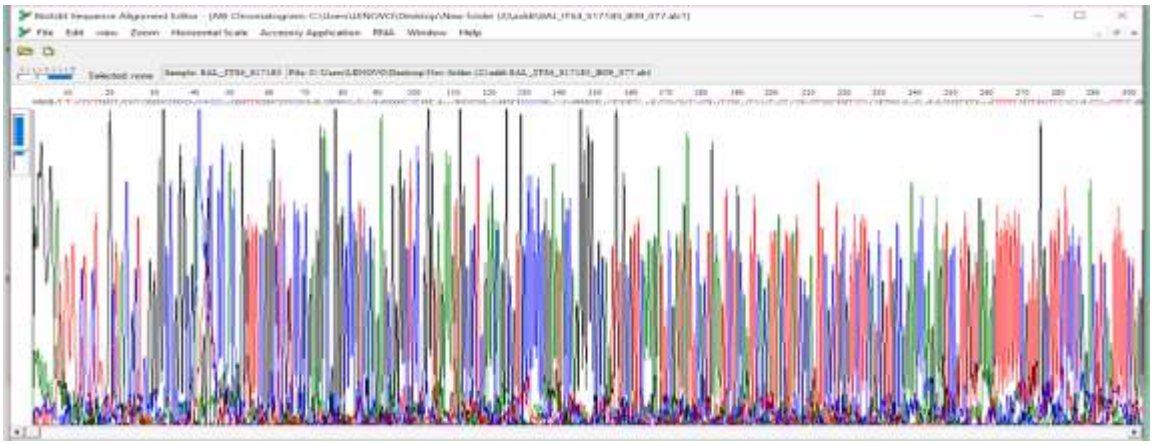

Fig. 3: Chromatogram based on ITS gene sequence of *Venturia crataegi* isolate

Fig. 4: Sequence alignment of *Venturia* species isolates through CLUSTAL W multiple alignment programme using software MEGA

were retrieved from GenBank at NCBI such as *Cladosporium caryigenum*, *Fusicladium eriobotryae*, *F. pyracanthae*, *F. caryigenum*, *F. effusium*, *Venturia inaequalis*, *V. crataegi*, *F. mandshuricum*, *V. saliciperda*, *V. populina*, *V. martianoffiana*, *V. carpophila*, *V. asperata*, *V. nashicola* and *V. pyrina* for present phylogenetic analysis. Sequences were aligned through ClustalW multiple alignment programme (Fig. 4) and phylogenetic analysis carried out on ITS1-50.8S rDNA-ITS2 sequences by Neighbor-joining method and cladogram generated. Best-fit model of nucleotide evolution (T92+G) was selected by Akaike information criterion [AIC] (Posada and Buckley, 2004) in MEGA v. 6.02 (Tamura *et al*., 2013). Bootstrap analysis with 1000 replicates was used to test the statistical support of the branches and dendrogram generated. The test isolate of *V. crataegi* formed a well robust clade with another *V. crataegi* isolate (MH855705.1) available at NCBI with well-supported bootstrap value of 93% (Fig. 5). The analysis provides the first molecular results for *V. crataegi* and the corresponding probable phylogenetic relationships. The cladogram displayed seven well supported major clades and relationships among the isolates of same species were topologically well defined. Clade I encompassed all isolates of *C. caryigenum.* Clade II was a paraphyletic group of *F. eriobotryae*, *F. pyracanthae* and *V. inaequalis* with less-supported bootstrap value (BS = 34). Clade III accommodated the sequences of *V. crataegi* isolates both custom sequenced and the available at

NCBI GenBank. Clade IV was a paraphyletic group of *F. mandshuricum*, *V. saliciperda*, *V. populina* and *V. martianoffiana.* Clade V contained all the sequence of *F. caryigenum*, *F. effusium* and *C. caryigenum*. Clade VI was a paraphyletic group containing *V. carpophila* and *V. asperata.* Clade VII contained all sequence of *V. nashicola* and *V. pyrina*. Overall, *Venturia* species proved to be the monophyletic group supported by bootstrap values and these findings are in agreement with those of Schnabel *et al.* (1999), Beck *et al.* (2005), Zhao *et al.* (2012) and Padder *et al.* (2013) who studied rDNA-ITS data of *Venturia* species in a more comprehensive way and could not differentiate them on the basis of ITS region because of considerable homogeneity between them. This supports the earlier taxonomic separation; however, clear host-specific differentiation has not been observed.

According to Schubert *et al.* (2003), *Venturia crataegi* forms conidia which are solitary, fusiform, obclavate, 10-25 × 4-6 µm in size, pale olivaceous, 0-1-septate, rarely 2-septate, more or less constricted at septum. Colonies on living leaves and fruits in present study were brown to black in colour, crustaceous and small forming subcircular spots of 1-2 mm width. These morphological characteristics fit well for *Venturia crataegi.* The identification is strongly supported by DNA sequences data (Fig. 5). Most *Venturia* species are host-specific and occur only in one or a few host species (Schnabel *et al.*, 1999; Beck *et al.*, 2005). In our study, the phylogeny of *V. crataegi* based on ITS region was in consistence with the phylogeny of other *Venturia* species based on ITS region. These observations are in conformity with others (Braun *et al*., 2003; Schubert *et al*., 2003, Beck *et al*., 2005), who put rDNA ITS data of *Venturia* anamorphs in a more inclusive context of *Cladosporium*-like fungi and concluded that these are true members of Venturiaceae. Since *Cladosporium* are taxonomically related to *Fusicladium* and could be confirmed by molecular examinations (Braun *et al*., 2003). In present study, the sequences of *Venturia* species were grouped together despite having different geographical and host origin. These results are in line with Schnabel *et al.* (1999), Beck *et al.* (2005) and

Fig. 5: Phylogenetic tree from the ITS1-5.8S rDNA-ITS4 sequences: the tree was constructed using Neighbour-joining method. Bootstrap values (1000 replicates) are indicated above the nodes. Branch lengths are proportional to the genetic distance between the taxa

Zhao *et al*., 2012) who also demonstrated partial close coevolution between *Venturia* species and their respective hosts based on ITS phylogenies and reported that the species boundaries within a host genus are less distinct (Bowen *et al.*, 2011). The switching over of *Venturia* species between the genera was interesting to note which indicated their limited ability to colonise the new host plants. These results are in agreement with Beck *et al.* (2005) who also reported the switching over between *V. saliciperda* on Salix host with *Fusicladium* species on poplar host. Although several species of genus *Fusicladium* including *F. pomi, F. pyrorum,* and *F. carpophillum* have so far been reported from Jammu & Kashmir state, but most of them are not adequately described. This is valuable information for studying the interaction between *Venturia* species and its hosts since previous information known from species of *Venturia* genus can be applied. Therefore, in mycology phylogenetic classification exclusively based on morphology is conflicting, which makes Deuteromycetes difficult to compare with sexual and asexual morphs (Bruns *et al*., 1991). The controversy can be overcome with the use of molecular markers. At present, the most reliable sequence used for such purpose is DNA encoding for small ribosomal subunit i.e. rDNA-ITS (Valente *et al*., 1999). Based on the available literature on *Venturi*a species there is no evidence of any reports from India on *V. crataegi* on *Crataegus* species (host); therefore, the present study forms the first report of *Venturia crataegi* on *Crataegus songarica* from India.

Acknowledgement: The authors are highly thankful to the Professor and Head, Division of Plant Pathology, Faculty of Horticulture, SKUAST-K, Shalimar, Srinagar (J&K) for providing necessary facilities during the study.

Ethical statement and Conflict of interest: This research did not involve any human and/or animal participants. The authors declare that they don't have any conflict of interests.

REFERENCES

Beck, A., Ritschel, A., Schubert, K., Braun, U. and Triebel, D. 2005. Phylogenetic relationships of the anamorphic genus *Fusicladium* s. lat. as inferred by ITS nrDNA data. *Mycological Progress*, **4**(2): 111-116.

Bowen, J.K., Mesarich, C.H., Bus, V.G.M., Beresford, R.M., Plummer, K.I.M.M. and Templeton, M.D. 2011. *Venturia inaequalis*: The causal agent of apple scab. *Molecular Plant Pathology*, **12**: 105-122.

Braun, U. 2005. Proposal to conserve *Fusicladium* against *Cycloconium* (Hyphomycetes). *Taxon*, **54**: 538.

Braun, U., Crous, P.W., Dugan, F., Groenewald, J.Z. and de Hoog, G.S. 2003. Phylogeny and taxonomy of *Cladosporium*-like hyphomycetes, including *Davidiella* gen. nov., the teleomorph of *Cladosporium* s. str. *Mycological Progress*, **2**: 3-18.

Braun, U., Ritschel A. and Schubert, K. 2002. Proposal to conserve the generic name *Fusicladium* against *Spilocaea* (Hyphomycetes). *Taxon*, **51**: 557.

Bruns, T.D., White, T.J. and Taylor, J.W. 1991. Fungal molecular systematics. *Annual Review of Ecological Systematics*, **22**: 525-564.

Cesati, V. and De Notaris, G. 1863. Schema di classificazione deglisferiacei italici aschigeri. *Commentario della Societa Crittog Italliano*, **1**: 177-240.

Dar, M.S., Irtefa, M., Sofi, T.A., Ahanger, F.A., Shah, M.D., Ahmad, M., Hamid, A., Mir, A.A., Nabi, A. and Padder, B.A. 2015. Distribution of apple scab race flora and identification of resistant sources against *Venturia inaequalis* in Kashmir. *Plant Pathology Journal*, **14**(4): 196-201.

De Notaris, G. 1844. Cenno sulla tribu de'pirenomiceti sferiacei edescrizione di alcuni nuovi generi. *Giornale. Botanico Italliano*, **1**: 322-335.

Faroouqi, K.D. and Dalal, M.A. 2003. Breeding apples for resistance to scab - An ecofriendly strategy. *Acta Horticulture,* **696**: 33-37.

Gautam, A.K. 2014. *Fusicladium ahmadii* on *Pyrus pashia*: A new record for Indian mycobiota from Himachal Pradesh. *Plant Pathology & Quarantine,* **4**(2): 86-89**.**

Kacho, N.F., Ahmed, K., Hussain, M., Bhat, M.A., Banday, S. and Nisar, Q. 2017. First record of scab disease of almond caused by *Cladosporium carpophilum* in India. *Indian Phytopathology,* **70**: 403-404.

Kaul, A.K., Ghani, M.Y. and Kaul, R.N. 1989. A new scab disease of poplar from India. *Plant Disease Research,* **4**(2): 187-188.

Liebhard, R., Koller, B., Patocchi, A., Kellerhals, M., Pfammatter, W., Jermini, M. and Gessler, C. 2003. Mapping quantitative field resistance against apple scab in a 'Fiesta' × 'Discovery' progeny, *Genetics and Resistance*, **93**: 493-501.

Michalska, M.R. and Polec, E. 2006. The genus *Fusicladium* (Hyphomycetes) in Poland. *Acta Mycologica,* **41**: 285-298.

Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research,* **8**: 4321-4325.

Nath, P. 1935. Studies in the diseases of apples in Northern India II. A short note on apple scab due to *Fusicladium dendritium* Fuckel. *Journal of Indian Botanical Society,* **14**: 121-124.

Padder, B.A., Sofi, T.A., Ahmad, M., Shah, M.D., Hamid, A., Saleem, S. and Ahanger, F.A. 2013. Virulence and molecular diversity of *Venturia inaequalis* in commercial apple growing regions in Kashmir. *Journal of Phytopathology,* **161**: 271-279.

Posada, D. and Buckley, T.R. 2004. Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology,* **53**: 793-808.

Putto, B.L. and Chaudhary, B.K.C. 1984. A study of pear scab in Kashmir - Symptomatology and morphology of the pear pathogen. *Indian Phytopathology,* **37**: 699-701.

Rossman, A.Y., Crous, P.W., Hyde, K.D., Hawksworth D.L., Aptroot, A., Bezerra, J.L., Bhat, J.D., Boehm, E., Braun, U., Boonmees, S., Camporesi, E., Chomnunti, P., Dia, D.Q., D-souza, M.J., Dissanayake, A., Gareth-jones, E.B., Groenewald, J.Z., Harnandez-Restrepo, M., Hongsanan, S., Jaklitsch, W.M., Jayawardena, R., Jing, L.W., Krik, P.M., Lewery, J.D., Mopook, A., McKenzie, E.H., Monkai, J., Phillips, A.J., Phookamsak, R., Raja, H.A., Seifert, K.A., Senanayake, I., Slippers, B., Suetrong, S., Taylor, J.E., Thambugala, K.M., Tian, Q., Tibpromma, S., Wanasinghe, D.N., Wijayawardene, N.N., Wikee, S., Woundenberg, J.H., Wu, H.X., Yan, J., Yang, T. and Zhang, Y. 2015. Recommended names for pleomorphic genera in Dothideomycetes. *IMA Fungus,* **6**: 507-523.

Saccardo, P.A. 1882. Fungi Veneti novi vel critici vel Mycologiae Venetae addenti. Series VIII. *Michelia,* **1**: 239-273.

Schnabel, G., Schnabel, E.L. and Jones, A.L. 1999.Characterization of ribosomal DNA from *Venturia inaequalis* and its phylogenetic relationship to rDNA from other tree-fruit *Venturia s*pecies. *Phytopathology*, **89**: 100-108.

Schubert, K., Ritschel, A. and Braun, U. 2003. A monograph of *Fusicladium* s. lat. (Hyphomycetes). *Schlechtendalia,* **9**: 1-132.

Singh, S., Khan, S.N. and Mishra, B.M. 1983. Some new and noteworthy disease of poplar in India. *Indian Forester,* **109**: 636-666.

Sivanesan, A. 1977. The taxonomy and pathology of *Venturia* species. *Bibliotheca Mycologica,* **59**: 1-139.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2013. MEGA v. 6.02: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Molecular Biology Evolution,* **28**: 2731-2739.

Valente, P., Ramos, J.P. and Leoncini, O. 1999. Sequencing as a tool in yeast molecular taxonomy. *Canadian Journal of Microbiology*, **45**: 949-958.

White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, pp. 315-322. **In**: *PCR Protocol: A Guide to Methods and Applications* (eds. M.A. Innis, D.H. Gelf, J.J. Sninsky and T.J. White). Academic Press, New York, USA.

Zhang, J., Zhou, Y., Dou. Z., He, W. and Zhang, Y. 2016. Type studies of six *Venturia* species. *Nova Hedwigia*, **102**: 173-184.

Zhang, Y., Crous, P.W., Schoch, C.L., Bahkar, A.H., Guo L.D. and Hyde. K.D. 2011. A molecular, morphological and ecological re-appraisal of *Venturiales* - A new order of Dothideomycetes. *Fungal Diversity*, **51**: 249-277.

Zhao, P., Kakishima, M., Uzuhashi, S. and Ishii, H. 2012. Multigene phylogenetic analysis of inter- and intra-specific relationships in *Venturia nashicola* and *Venturia pyrina*. *European Journal of Plant Pathology*, **132**: 245-258.