



***Botryosphaeria dothidea* ASSOCIATED WITH WHITE ROT AND STEM BARK CANKER OF APPLE IN JAMMU & KASHMIR**

Nisar Ahmad Khan, Mushtaq Ahmad and M.Y. Ghani

Division of Plant Pathology, S.K. University of Agriculture Sciences and Technology of Kashmir,
Shalimar, Srinagar (J&K) 191 121 - India
e-mail:nissar_dr2008@rediffmail.com

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ABSTRACT

Apple orchards in nine locations of Kashmir valley were surveyed in 2006 and 2007 for white rot and stem canker bark disease. Maximum disease incidence was noticed on scaffold branches and trunks of the trees. The anamorph of causal pathogen of white rot and stem canker was identified as *Fusicoccum aesculi* Corda, while the teleomorph as *Botryosphaeria dothidea* (Mung.Fr.) Ces. & De Not. *Botryosphaeria dothidea* is reported for the first time from the Jammu and Kashmir State.

Key words: Apple trees, *Botryosphaeria dothidea*, disease incidence, Jammu & Kashmir, stem bark canker, white rot

INTRODUCTION

Apple (*Malus domestica* Borkh.) is a predominant fruit crop of Jammu & Kashmir State; grown on an area of 1,07,900 ha and contributes towards 81.0% of the total fruit production of the State (Anonymous, 2005). The apple crop in J&K State is affected by many diseases, including scab, root rot, *Alternaria* leaf blight and powdery mildew. In recent years the apple trees have encountered new problems in the form of cankers. The disease has assumed epiphytotic proportions in some pockets of Kashmir valley (Nazir *et al.*, 2009), resulting in huge economic losses through girdling of limbs and branches, blightening and die-back of twigs and ultimately leading to the death of either whole or some parts of the tree (Jones and Aldwinkle, 1990). In view of the alarming damage inflicted by canker diseases on tree crops, the present study was undertaken to ascertain the status of white rot and canker disease in Jammu & Kashmir and to identify the causal agents responsible for the disease.

MATERIALS AND METHODS

In August-September, 2006 and 2007, survey of major apple growing belts in Kashmir valley was carried out to record the prevalence and severity of various canker diseases and ascertain their causal pathogen(s). The apple orchards at nine locations across the valley viz., Rafiabab, Sopore, Pattan, Beerwa, Budgam, Nowgam, Pulwama, Shopian and Keller were surveyed and disease incidence on various parts of the trees recorded. Three locations in each district (Baramulla, Budgam and Shopian) and five orchards in each of the five villages selected were taken to represent a location. Besides main trunk, three scaffold branches (limbs) and nine fruiting wood (branches/twigs) for each of the ten randomly selected trees were examined for assessing the canker incidence and intensity. The canker intensity was calculated after rating the level of disease on branches/twigs on 0–5 scale adopted by Crosse (1975) with slight modifications wherein: 0 = no incidence; 1 = 0.1-10% of branch/twig surface area diseased; 2 = 10.1-20.0% of branch/twig surface areas diseased; 3 = 20.1-30.0% of the branch/twig surface areas diseased; 4 = 30.1-40.0% of the branch/twig surface area disease or one side

of the branch/twig showing partial girdling; 5 = >40.0% of the branch/twig surface area diseases or girdling the branch/twig, the branch alone the girdled portion complete dead/died. Isolation of the associated pathogens from cankered wood/ fructification was made by adopting the procedure of Proffer and Jones (1989) and Wilkinson (1945). Single spore isolates, obtained by the transfer of germinating spores individually onto oat meal agar medium, were used for various studies. The morphological characters of isolated fungus (anamorph) and its teleomorph were compared with the descriptions given by Punithalingum and Holliday (1973), Pennycook and Samules (1985), Slippers *et al.* (2004) and Philips *et al.* (2005). The pathogenicity was established as per the method of Mikholland (1972) and Spiers (1977) on one year potted apple saplings cv. "Red Delicious". Re-isolations from the resultant cultures were compared with the original inoculants to satisfy the Koch's postulates.

RESULTS AND DISCUSSION

During orchard survey among the various canker diseases, the stem bark canker was observed in all the surveyed locations as well as on all the parts of trees with disease incidence ranging from 18 to 41% (Fig. 1). The incidence was maximum on scaffold branches (13.7-37.0%) and trunk (9.0-33.0%) and minimum on fruiting wood (6.8 to 22.7%).

The disease symptoms, mostly observed on sun burnt surfaces of tree parts, appeared as small, sunken, oval, reddish brown lesions with or without a fissure occasionally with purplish margins which on enlargement became depressed forming elliptical cankers with longitudinal and transversal slits (Plate 1,i-iii). In advanced stages the cankered surfaces became black and brittle; and remained studded with numerous black fissures. The bark of affected branches easily sloughed off as thin papery filaments. Black pimple-like protuberances of pycnidia and pseudothecia formation were also observed on the cankered surfaces. Fruit rot, commonly known as white rot, initiated as small, sunken light green spot surrounded by light brown area (Pate 2,i). On subsequent enlargement the entire rotten fruit turned light brown. The affected area became depressed and developed a series of concentric rings with spongy fruit flesh. Stereo-microscopic examination of cankered surface revealed the presence of numerous dark brown to black, submerged or erumpent papillate pycnidia (Plate 2,iii) exuding creamy conidial mass. Spermatia borne on spermatophores, produced occasionally in locules of

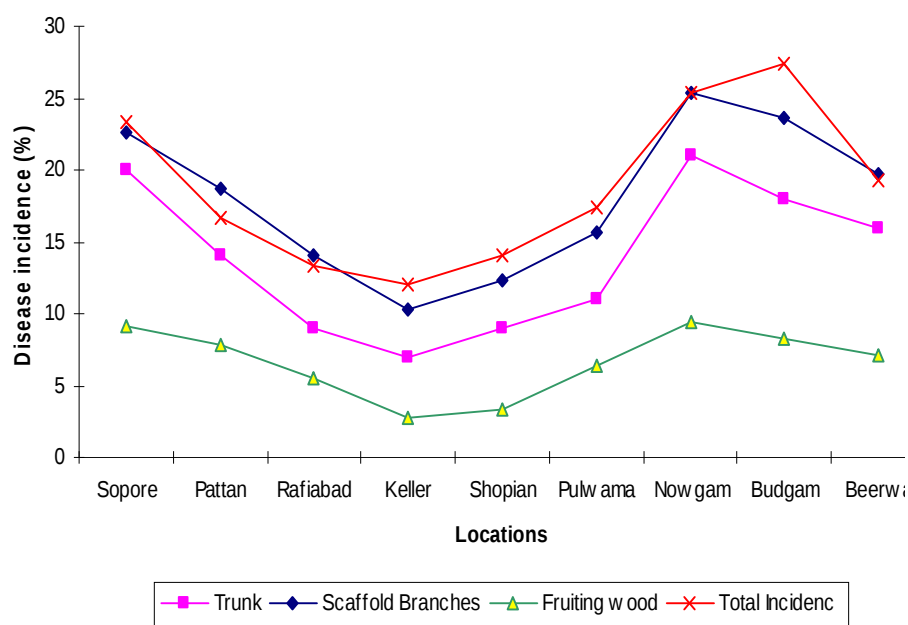


Fig. 1: Prevalence of stem bark canker on various tree parts of Kashmir valley (average of two year data, 2006-2007)



Plate 1: Disease symptoms of stem bark canker on apple (from left to right): i) reddish brown lesions with fissures, ii) reddish brown areas with purplish margin, iii) formation of transverse slits, and iv) blackened brittle canker with fissures

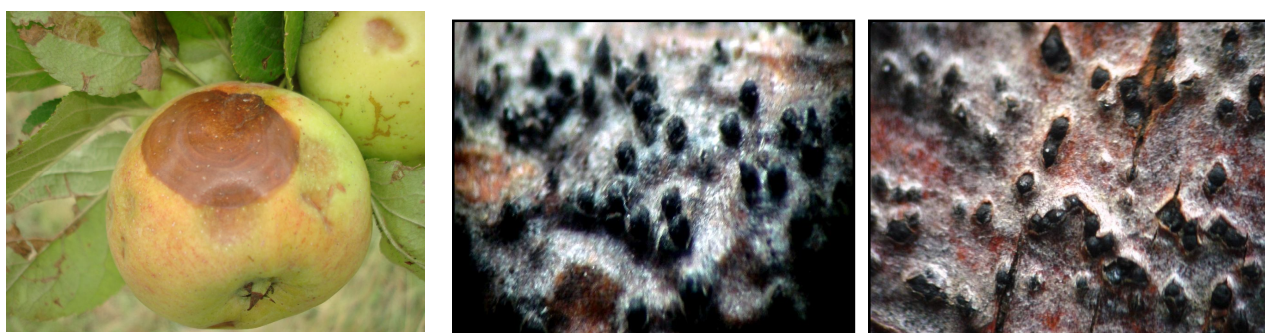


Plate 2: (from left to right) i) Disease symptoms of fruit rot, ii) pseudothecia, iii) pycnidia



Plate 3: (from left to right) i) Asci, ii) ascospores, iii) grayish fungal colony

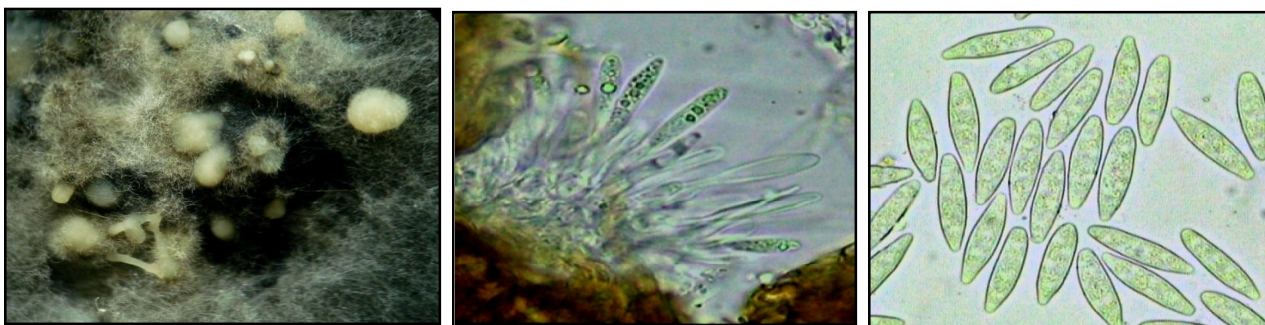


Plate 4: (from left to right) i) Creamy conidial ooze of culture, ii) conidiogenous cells, iii) conidia of the fungus

pycnidia or ascogenous stromata, were hyaline, unicellular, allantoids or straight with an average size of $2.53 \times 1.00 \mu\text{m}$. Pseudothecia (Plate 2,ii), scattered on diseased twigs, were globose to sub-globose having a papillated ostiole with average size of $169 \times 231 \mu\text{m}$. Asci (Plate 3,i), interspersed among fillform paraphysis were clavate or cylindrical, hyaline, short-stalked and bitunicate measuring $109 \times 19 \mu\text{m}$ in average size. Ascospores (Plate 3,ii) were hyaline, unicellular, ovoid or fusoid with granular contents measuring $24.4 \times 7.6 \mu\text{m}$ in average size.

For isolating the casual pathogen, several sections of diseased bark were collected from trunk, limbs, branches and twigs. The bark surface was removed with a scalpel and small pieces of 5 mm^2 were cut from the edge of diseased area and surface sterilized in 0.5% sodium hypochlorite for 1 min., rinsed in sterilized water and dried on sterilized filter paper before placement on acidified oat meal agar (OMA) medium and incubation at $25 \pm 1^\circ\text{C}$. The diseased fruit surface was sterilized by rubbing with a cotton swab dipped in 0.5% sodium hypochlorite and peeled off with a sterilized blade. Small portion of diseased pulp along with the line of healthy and rotted area were removed aseptically and transferred in acidified OMA medium. Single spore isolates, maintained by the transfer of germinating conidia individually in the medium produced after isolating the fungus from the diseased material, were used for cultural, morphological and pathogenicity studies. Under alternate cycles of light and darkness, compact, velvety, olivaceous buff to olivaceous grey coloured fungal colonies appeared after 10 to 12 days of incubation which finally turned violaceous black. Pycnidia, initially clothed with hypha-like appendages, started developing within 7 to 8 days of incubation exuding creamy conidial mass (Plate 4,i) in later stages. Conidiogenous cells (Plate 4,ii) lining inside the pycnidial wall were hyaline, sub-cylindrical, proliferating with 1 to 2 proliferations, $9.50 \times 2.58 \mu\text{m}$ in average size producing single apical conidium. Conidia (Plate 4,iii) were hyaline, unicellular, fusoid to ellipsoidal with an obtuse apex to sub-truncate base measuring $19.0 \times 6.3 \mu\text{m}$ in average size. The colour of conidia remained unaltered without developing any septa in later stages of growth.

The pathogenicity of fungus was proved on potted one year old apple saplings cv. 'Red Delicious' by inserting a 4 mm mycelial disc into 5 mm 'T-shaped' flap on each of the 5 saplings wrapped with paper tape. On fruit, 3 mm mycelial discs were inserted into 5 mm long and 2-3 mm deep cuts on each of the 5 surface sterilized fruits. Three non-inoculated twigs and fruits served as control. Re-isolation from inoculated diseased twigs and fruit consistently yielded the original organism, thus fulfilling the Koch's postulates. Control twigs and fruits remained healthy. The morphological characters of the isolated fungus (anamorph) and its teleomorph were compared with the descriptions given by Punithalingum and Holliday (1973), Weaver (1974), Pennycook and Samules (1985), Slippers *et al.* (2004) and Philips *et al.* (2005). The anamorph was identified as *Fusicoccum aesculi* Corda, while the teleomorph as *Botryosphaeria dothidea* (Mung.Fr.) Ces. & De Not. The identity of anamorph was confirmed by ITCC, IARI, New Delhi, India and the culture deposited vide accession No. 6505. *Botryosphaeria dothidea* has been reported to incite fruit rot and stem canker in several plants including apple and pear in temperate and tropical regions of the world (Fenner, 1925; Fulkerson, 1960; Brown and Britton, 1986), including Himachal Pradesh from India (Gupta and Agarwal, 1973). Thus this is the first report of *Botryosphaeria dothidea* (Mung.Fr.) Ces. & De Not as the causal pathogen of white rot and stem canker on apple from the Jammu and Kashmir State.

REFERENCES

- Anonymous, 2005. *Area and Production under Major Horticulture Crops in Jammu and Kashmir*. Statistical Section, Department of Horticulture, Government of Jammu and Kashmir, Srinagar, J&K. pp. 3.
- Brown, B.A. and Britton, K.A. 1986. *Botryosphaeria* diseases of apple and peach in the South Eastern United States. *Plant Disease*, **70**: 480-484.
- Crosse, J.E. 1957. Bacterial canker of stone fruits. *Annals of Applied Biology*, **45**: 19-35.
- Fenner, E.A. 1925. A rot of apple caused by *Botryosphaeria ribs*. *Phytopathology*, **15**: 230-234.
- Fulkerson, J.F. 1960. *Botryosphaeria ribs* and its relation to apple rot. *Phytopathology*, **50**: 394-398.

- Gupta, G.K. and Agarwal, R.K. 1973. Canker diseases of apple trees in Himachal Pradesh. *Indian Journal of Mycology and Plant Pathology*, **3**: 189-192.
- Jones, A.L. and Aldwinkle, H.S. 1990. White rot. pp. 100-102. In: *Compendium of Apple and Pear Diseases*. American Phytopathological Society/APS Press, St. Paul, Minnesota, USA.
- Mikholland, R.D. 1972. Histopathology and pathogenicity of *Botryosphaeria dothidea* on blueberry systems. *Phytopathology*, **62**: 654-660.
- Nazir, N.A., Maheshwari, S.K., Masoodi, S.D., Shah, T.A. and Wani, T.A. 2009. Present management strategy for diseases of apple and pear in Kashmir – Shortcomings and some suggestions for improvement. pp. 127-130. In: *State Level Seminar on Fruit Industry of Kashmir - Problems and Prospects*. Faculty of Agriculture, SKUAST-K Sopore, J&K.
- Pennycook, S.R. and Samuels, G.J. 1985. *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (kiwi fruit) in New Zealand. *Mycotaxon*, **24**: 455-458.
- Phillips, A.J.L., Rumbos, I.C., Alves, A. and Correia, A. 2005. Morphology and phylogeny of *Botryosphaeria dothidea* causing fruit rot of olives. *Mycopathologia*, **159**: 433-439.
- Proffer, T.J. and Jones, A.C. 1990. A new canker disease of apple caused by *Leucostoma cincta* and other fungi associated with cankers of apple in Michigan. *Plant Disease*, **73**: 508-515.
- Punithalingam, E. and Holliday, P. 1973. *Botryosphaeria* ribs. *Description of Pathogenic Fungi and Bacteria*. No.395. Commonwealth Mycological Institute, Kew, Surrey, England.
- Slippers, B., Corus, P.W., Denman, S., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. 2004. Combined multiple gene genologies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia*, **96**: 83-101.
- Spiers, A.G. 1977. *Botryosphaeria dothidea* infection of *Salix* sp. in New Zealand. *Plant Disease Reporter*, **61**: 664-667.
- Weaver, J.D. 1974. A gummosis disease of peach trees caused by *Botryosphaeria dothidea*. *Phytopathology*, **64**: 1429-1432.
- Wilkinson, E.H. 1945. Observation on Perennial canker fungus *Gleosporium perennans* Zeller and Childs. *Transaction of British Mycological Society*, **28**: 77-85.