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Nisar Ahmad Dar
Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences & Technology, Shalimar, Srinagar, Jammu and Kashmir, India

Nisar Ahmad Khan
Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences & Technology, Shalimar, Srinagar, Jammu and Kashmir, India

Mudasir Ahmad Bhat
Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences & Technology, Shalimar, Srinagar, Jammu and Kashmir, India

Corresponding Author:
Nisar Ahmad Dar
Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences & Technology, Shalimar, Srinagar, Jammu and Kashmir, India

Status, symptomatology and partial characterization of stem bark canker disease of apple (*Malus domestica* Borkh.) in Kashmir Valley

Nisar Ahmad Dar, Nisar Ahmad Khan and Mudasir Ahmad Bhat

Abstract
Surveys were conducted to determine the occurrence and distribution of Stem bark canker affecting Apple in Kashmir valley during 2013 and 2014**.** Apple orchards were more affected in district Ganderbal with highest canker incidence (31.33%) and the least in district Shopian (15.33%). Among the villages surveyed, Watlar of district Ganderbal exhibited the highest canker incidence of 41.00 per cent. Village Batapora of district Shopian exhibited the least canker incidence of 13.00 per cent. The data over the two years further revealed that maximum canker incidence of 23.50% was observed on tree trunks followed by scaffold branches (17.70%) and least on fruiting wood (10.09%). Infected samples were collected from different localities in four districts of Kashmir valley. Stem bark symptoms mostly characterized under field conditions by the appearance of small, sunken, reddish brown lesions, which on enlargement became depressed and developed elliptical cankers with vertical and horizontal slits, partially or completely girdling the affected trunk or branch. In advanced stages, the cankered surface however, became was black and brittle which remained studded with numerous black fissures. The fungus isolated from stem bark canker produced olivaceous to violaceous black fungal colonies with dense aerial mycelium. The hyphae were smooth, thick walled, septate and dark brown in colour. The pycnidia formed only in presence of light were globose to sub-globose distributed uniformally over the culture medium. The conidiogenous cells were smooth, hyaline, sub-cylindrical, swollen at the base, producing single apical conidium. The conidia were smooth, thin walled, hyaline, unicellular, fusoid to ellipsoidal with an obtuse apex and truncate or sub-truncate to rounded base. Based on morphological characters both on host as well as in culture, symptom expression and pathogenicity tests, the fungus causing the disease was identified as *Fusicoccum aesculi* Corda.

Keywords: Apple, stem bark canker, incidence, lesions and *Fusicoccum aesculi* Corda

1. Introduction
Apple (*Malus domestica* Borkh.) is a premier table fruit of the world and has been under cultivation since time immemorial. Apple tree owes its origin in South Eastern Europe and Tien Shan Mountains of Kazakhstan in Asia (Gasteir, 2000) where vast forests of wild apple trees exist even today. The ten leading apple producing countries contributing to world's annual production of 63 million tonnes are USA, China, France, Italy, Turkey, Argentina, West Germany, Spain, Japan and erstwhile USSR (Snowdon, 1990) [24]. In India, the commercial cultivation of apple is largely confined to the states of J & K., H.P. and U.K. which together accounts for 99 per cent of the total production with productivity of 13.07 metric tonnes per hectares in J&K followed by 8.95 metric tonnes per hectares in H.P. and 3.52 metric tonnes per hectares in U.K. (Anonymous, 2011) [3].

In spite of the unique agro climatic conditions of the Kashmir valley being quite conducive for temperate fruit production, apple productivity per unit area is low owing to many biotic and abiotic stresses. The major biotic factors inflicting huge economic losses are the fungal diseases, the predominant among them being scab, powdery mildew, collar rot, Alternaria leaf blotch, Marssonina blotch, Sooty blotch and stem and branch cankers caused by various fungi (Bilgrami *et al.*, 1979, Kanwar, 1988; Sharma and Bhardwaj, 1999) [4, 9, 21]. Among these canker diseases, stem and branch cankers have assumed an alarming proportion and cause huge economic losses through girdling of branches, limbs, blighting and die-back of twigs ultimately resulting in the death of whole or part of the plant (Jones and Aldwinkle, 1990) [8]. Apart from girdling of branches, losses also occur through fruit rotting and premature defoliation (Sharma and Bhardwaj, 1999) [21]. Stem bark canker, Silver leaf, Smoky Canker, Phomopsis canker, Valsa canker and anthracnose cankers has also been reported from Jammu & Kashmir state (Malik, 1967; Chib and Andotra, 1985, Khan *et al.*, 2010, 2011 and 2011a) [14, 11].

Keeping in view the extent of damage inflicted by the canker diseases, the present study on stem bark apple canker disease of apple excluding its leaf spot and fruit rot phase, if any, were, therefore undertaken with main emphasis on status and symptomatology.

2. Materials and Methods

An extensive survey of different Apple (*Malus domestica* Borkh.) growing localities in four districts of Kashmir Valley was conducted during 2013 and 2014 cropping seasons (in the months of August to September) to determine the distribution and incidence of Stem bark canker in the state. In each district, three locations were selected and five orchards in each of the five villages taken to represent a location. Ten trees were randomly selected from each orchard for assessing the incidence of the canker disease. Besides the main trunk, three scaffold branches (major limbs) and nine branches/twigs (fruiting wood) from each tree were randomly selected and examined for the presence of the canker disease and recorded as per cent canker incidence.

$$\text{Per cent canker incidence} = \frac{\text{No. of cankered units}}{\text{Total No. of assessed units}} \times 100$$

The per cent canker intensity of stem bark canker occurring only on branches and twigs was calculated after rating the level of disease on 0-5 scale of Crosse (1957) [5] adopted by Khan *et al.* (2011).

Per cent canker intensity was assessed using the formula:

$$\text{Per cent canker intensity} = \frac{\Sigma(n \times v)}{N \times S} \times 100$$

Where,

n = number of branches or twigs in each category;

v = numerical value of each category;

N = number of branches or twigs examined; and

S = the maximum numerical value.

Disease samples from trunks, limbs, branches of apple trees showing distinct cankerous symptoms, collected during the course of Survey, were washed with running tap water to remove the dirt and dust. After removing the bark, the infected woody tissue was thoroughly sterilized with cotton swab dipped in absolute alcohol (95%). Small sections of 5 mm² size were cut at the transition zone between healthy and diseased tissue with a sterilized scalpel and surface sterilized in 0.1 per cent mercuric chloride solution for 30 seconds. The sections were then rinsed in distilled water to remove the traces of mercuric chloride solution, blotted dry and transferred aseptically onto acidified potato dextrose agar (PDA) medium contained in sterilized Petri plates and incubated at 25±1 ℃. The culture thus obtained was purified by hyphal tip method (Pathak, 1972) [16]. Pure cultures obtained were maintained by repeated sub-culturing at regular intervals for further studies. The stock cultures were stored in a refrigerator at 4 ℃. The composition of Potato Dextrose Agar medium was:

1. Peeled potato: 250 grams
2. Dextrose: 20 grams
3. Agar Agar: 20 grams
4. Water: 1000 ml

The pathogenicity test of the isolated pathogen(s) was performed on healthy one year old potted saplings of apple cultivar "Red Delicious" as per the technique employed by Milholland (1972) [15] and Spiers (1977). The saplings were sprayed with copper oxy-chloride 50 WP @ 0.3% 20 days before inoculations to exclude any harbouring pathogen. The pots containing the saplings were kept in diffused sunlight in polythene chambers, designed for the purpose, maintaining high humidity inside the chambers by timely irrigating the pots and intermittently spraying with distilled sterilized water. On one year old potted apple saplings 5mm vertical and horizontal incisions of "T" shape were made on the selected twigs after surface sterilizing the site with absolute alcohol and a 4 mm test mycelial plug inserted inside the "T" shaped flap. The inoculated incision was covered with moistened absorbent cotton and wrapped with paper tape. Incised inoculated twig with plain PDA medium covered with moistened cotton and wrapped with paper tape served as control.

Pathogenicity tests were closely monitored for symptom/canker development. Re-isolation of the pathogen from artificially inoculated twigs was carried out and resultant cultures compared with the original inoculant to satisfy Koch's postulates.

The morphological characteristics of the causal organism were studied taking in pathogen thallus from the host and after culturing it in the laboratory. The pathogen cultures were grown on potato dextrose agar medium and the semi-permanent slides prepared from 7 and 21 days old colonies. The important characters studied were as under:

- Mycelium, width, septation and colour
- Fruiting body structure, size and colour
- Conidia, shape, size and colour

The diseased branches and twigs were cut off along with some healthy portion, kept in a humid chamber at room temperature (20±1℃) and observed for mycelial colour, size and septation after 72 hours. Diseased branches/twigs were also observed under stereoscopic microscope for the presence of fruiting bodies and their morphological characteristics studied under compound microscope previously calibrated with the aid of stage and ocular micrometres.

Further the over-wintered branches and twigs collected at fortnightly intervals were examined under stereoscopic microscope for the presence of perfect state fruiting bodies. The morphological details of these fruiting bodies were also studied using a compound microscope. The morphological characters of the causal organism studied were compared with authentic descriptions for their identification and nomenclature.

3. Results and Discussion

3.1 Survey and Incidence

In the present investigation, an extensive survey of apple orchards in Anantnag, Kulgam, Ganderbal and Shopian districts of Kashmir valley during the months of August-September in 2013 and 2014 was under taken to record the status of stem bark canker disease. During the surveys stem bark canker disease was observed on different apple tree parts with varying degrees of incidence. The cankers were observed on all the major tree parts like trunks, scaffold branches (limbs) and fruiting wood (branches and twigs).

The data averaged for two years Table 1; Fig. 1 revealed an overall total canker incidence of 23.66 per cent throughout the areas surveyed. The average incidence was maximum on main trunk (19.64%) followed by that on scaffold branches (15.14%). The incidence on fruiting wood being the least

(9.43%). The total canker incidence recorded over the two years was highest in district Ganderbal (31.33%) followed by that in district Kulgam (27.00%). However, the incidence was least (15.33%) in district Shopian.

Among the different locations surveyed, highest canker incidence was recorded at Watlar (41.00%) followed by Lar (34.00%) and Devsar (29.00%) while, it was least 13.00 and 14.00 per cent recorded at Batapora and Kapran respectively. The data presented in Table 3 further reveals that the number of cankers per tree varied from 1.00 to 2.65 at different locations surveyed with an average number of 1.75 cankers per tree. The maximum number of cankers per tree (2.65) was recorded at Lar in district Ganderbal, while, minimum number (1.00) was recorded at Wakoora in district Ganderbal.

Table 1: Incidence of stem bark canker on various tree parts of apple at different locations of Kashmir during the year 2013 and 2014

District	Location	Canker incidence (%)				Number of cankers per tree
		Trunk*	Scaffold branches*	Fruiting wood*	Total incidence**	
Anantnag	Achabal	15.56	12.15	8.76	19.00	1.85
	Kellar	18.39	14.45	8.89	21.00	1.84
	Kanilwan	21.59	17.30	8.67	23.00	1.81
	Mean	18.51	14.63	8.77	21.00	1.83
Kulgam	Devsar	20.14	13.73	9.95	29.00	1.93
	D.H.pora	20.71	13.81	10.95	24.00	1.41
	Sopat	20.84	14.24	8.19	28.00	1.84
	Mean	20.56	13.92	9.70	27.00	1.72
Ganderbal	Wakoora	25.84	11.93	10.84	19.00	1.00
	Lar	20.49	22.54	12.47	34.00	2.65
	Watlar	24.16	18.65	6.97	41.00	2.11
	Mean	23.49	17.70	10.09	31.33	1.92
Shopian	Kapran	21.11	22.96	10.61	14.00	1.55
	Batapora	11.25	11.25	8.75	13.00	1.57
	Shirmal	15.63	8.70	8.09	19.00	1.52
	Mean	16.00	14.30	9.15	15.33	1.55
Grand Mean ± SD		19.64±3.47	15.14±2.71	9.43±1.56	23.66±6.28	1.75

*Figures are the per cent of cankered trees out of the total number of trees examined

**Observations based on means of fifty trees recorded in August-September

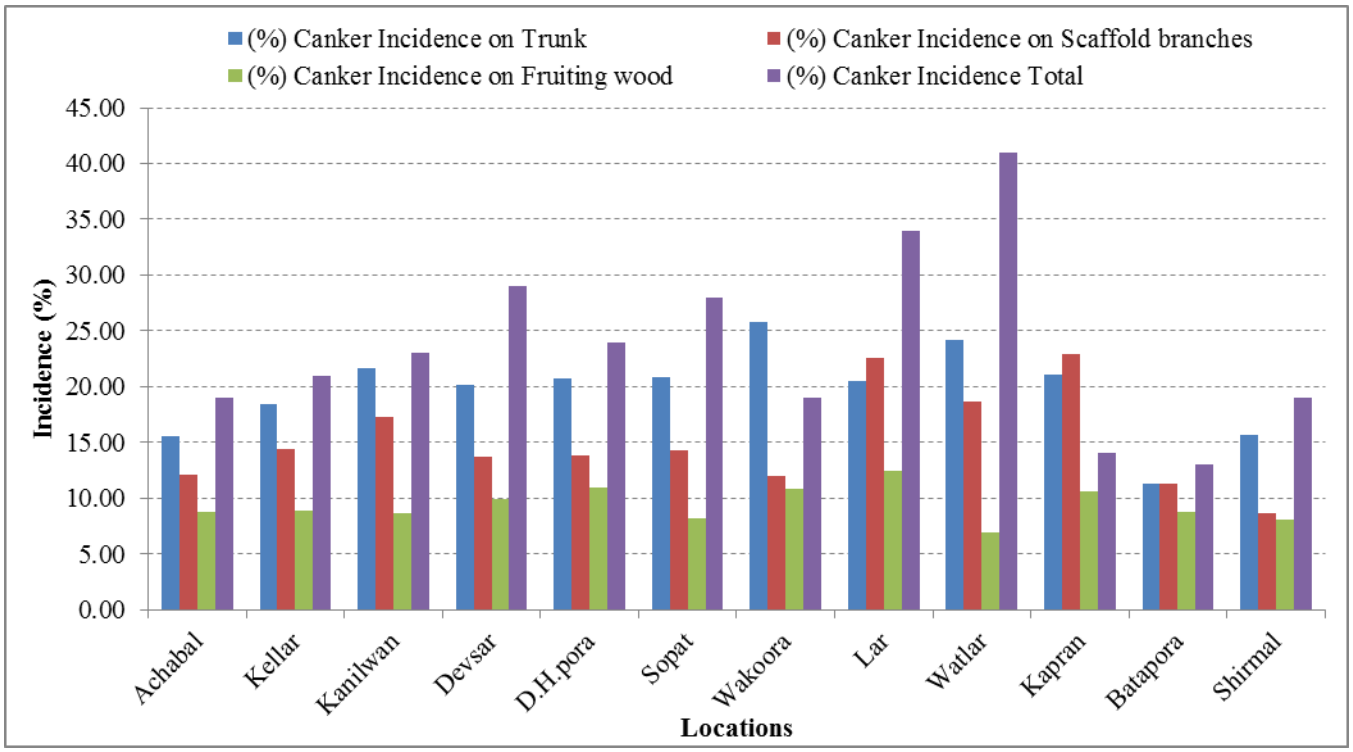


Fig 1: Incidence of stem bark canker on various tree parts of apple at different locations of Kashmir during the year 2013 & 2014

Like canker incidence, the canker intensity on fruiting wood (branches and twigs) also varied at all the locations surveyed. The data presented in Table 2; Fig 2 revealed that the canker intensity ranged between 4.81 to 9.73 and 5.76 to 12.04 per cent during the years 2013 and 2014 respectively and was highest in 2014 with 7.41 per cent compared to that 5.56 per cent in 2013, with an overall canker intensity of 6.49 per cent. Among the different districts surveyed, district Ganderbal exhibited the maximum average canker intensity (9.09%) followed by Kulgam with an average canker intensity (8.44%). While, minimum canker intensity of 6.49 per cent was recorded in district Shopian. Among various locations surveyed the maximum canker intensity of 10.89 per cent was recorded at Lar of district Ganderbal, while the minimum canker intensity of 5.24 per cent respectively were recorded at Achabal of district Anantnag.

Table 2: Intensity of stem bark canker on fruiting wood (branches & twigs) of apple at different locations of Kashmir during the year 2013 & 2014

District	Location	Canker intensity (%)		
		2014	2015	Mean
Anantnag	Achabal	4.15	6.33	5.24
	Kellar	9.13	10.03	9.58
	Kanilwan	7.20	8.79	8.00
	Mean	6.83	8.38	7.61
Kulgam	Devsar	8.20	10.07	9.14
	D.H.pora	8.78	11.72	10.25
	Sopat	6.10	5.76	5.93
	Mean	7.69	9.18	8.44
Ganderbal	Wakoora	8.89	10.11	9.50
	Lar	9.73	12.04	10.89
	Watlar	4.81	8.97	6.89
	Mean	7.81	10.37	9.09
Shopian	Kapran	4.81	6.43	5.62
	Batapora	5.76	7.37	6.57
	Shirmal	6.1	8.43	7.27
	Mean	5.56	7.41	6.49
Average ± SD		6.97 ± 1.72	8.83 ± 1.85	7.91 ± 1.72

*Observations based on means of fifty trees recorded in August-September

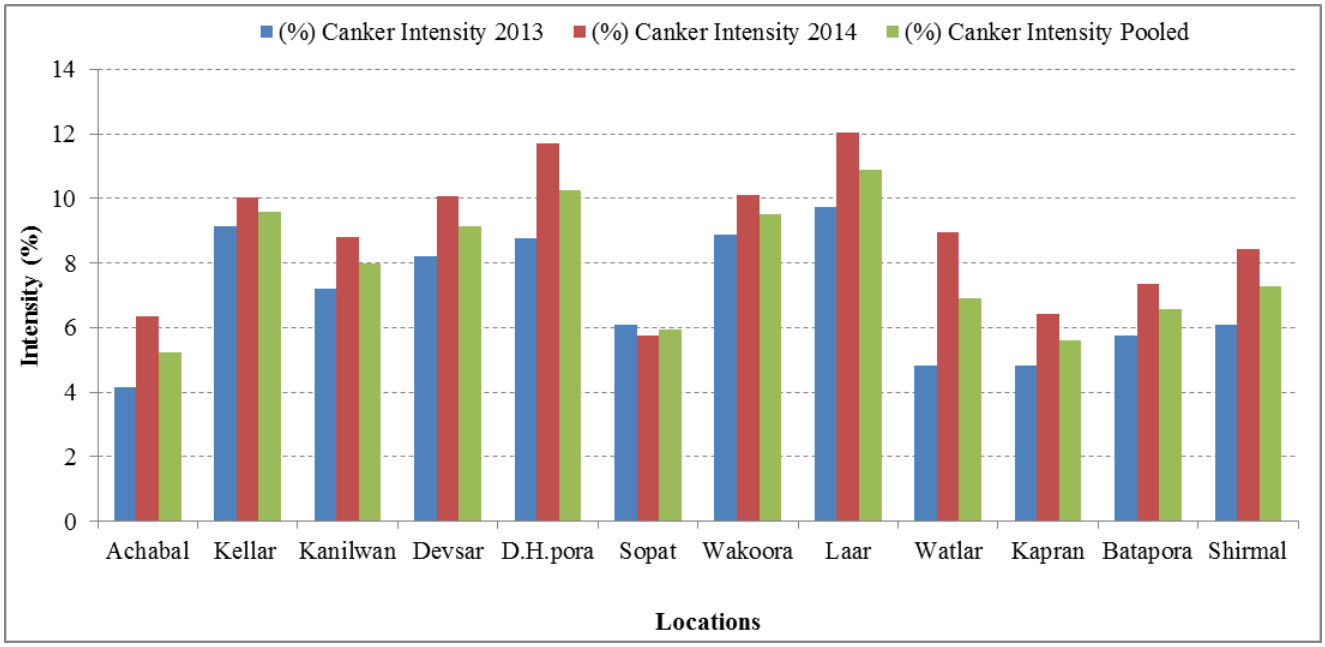


Fig. 2: Intensity of Stem bark canker on fruiting wood (branches & twigs) of apple at different locations of Kashmir during the year 2013 & 2014

3.2 Symptomatology

The stem bark canker mostly developed on the sun burnt tree parts viz., trunks, limbs and branches of young trees as small, sunken, oval, reddish brown lesion/areas with or without a fissure occasionally with purplish margins, which on enlargement became depressed and formed elliptical cankers with longitudinal and transverse slits causing loosening of the bark and completely girdling the affected limb or branch in severe cases. In advanced stages, the cankered surface become jet black, brittle and remained studded with numerous black fissures (Plate 1 and Plate 2). Occasionally, subsequent callus formation on one side of the cankered surface along its entire length was also observed which restricted the canker proliferation. The wood below the cankered areas was usually necrotic and stained dark brown. Numerous black pimple like elevations developed on the cankered surface mostly on branches and twigs as a result of pycnidial formation underneath it.

Initial Lesion on Trunk

Horizontal Canker Elongation

Formation of transverse slits

Development of fissures/loosening of bark

Plate 1: Symptom expression on apple tree trunks by stem bark canker under field conditions

Canker on Scaffold Branch

Development of Transverse Slits/Fissures

Advancement of Fissures on Scaffold Branch Cracking and Loosening of Bark

Plate 2: Symptom expression on apple scaffold branches by stem bark canker under field conditions

3.3 Isolation, purification and maintenance of fungal cultures

The isolations of the fungi associated with stem bark canker observed during the survey was made from cankered wood and also from fungal fructifications separately on potato dextrose agar (PDA) medium using standard technique. Purity and virulence of the isolated fungus was maintained by repeated sub-culturing and frequent isolations from the cankered tissues for morphological and other *in vitro* studies. The morphological characters of the fungus associated with stem bark canker were studied both on host and after culturing on potato dextrose agar medium to identify the associated pathogen. Morphological and cultural characters of the pathogen are presented in Table 3.

Table 3: Morpho-cultural characters of *Fusicoccum aesculi* Corda. causing Stem bark canker of apple

Thallus part	Shape and character	Colour	Size	Septation
On host				
Mycelium	Hyphae smooth, thick walled, branched	Hyaline to light brown	2.35-3.20 µm (width)	Septate
Pycnidium	Immersed in host tissue, or partially erumpent, solitary or botryose, globose to conical, with papillate ostiole, oozing creamy white conidial mass from mature pycnidium under moist conditions	Dark brown to black	138-186 ×157-230 µm (Av. 159.12 × 192.05 µm)	-
Conidiogenous cell	Smooth, sub-cylindrical, slightly swollen at base	Hyaline	6.24-14.35 × 2.03-3.90 µm (Av. 11.25 × 3.21 µm)	Septate
Conidia	Smooth, thin walled, fusoid to ellipsoidal, somewhat clavate, apex obtuse, base truncate to round, slightly wider in the middle region	Hyaline	17.85-28.68 × 2.79-7.46 µm (Av. 24.72 × 5.39µm)	Aseptate
In culture				
Colony	Cottony and floccose, aerial mycelium cincreous, becoming compact and velvety, slightly appressed along the margins with raised centre	White turning olivaceous grey and finally olivaceous black	-	-
Mycelium	Hyphae smooth, thick walled, branched	Hyaline to dark brown	2.95-3.88 µm (Width)	Septate
Pycnidium	Partially embedded in the culture medium, solitary, globose to sub-globose, formed only when culture exposed to diffused light, initially covered with hyphae like appendages, conidial mass oozes from mature pycnidium	Black	152-200 × 169-261 µm (Av. 169.13 × 209.62 µm)	-
Conidiogenous cell	Smooth, sub-cylindrical, slightly swollen at base	Hyaline	6.13-15.55 × 1.98-4.00 µm (Av.12.04 × 3.19 µm)	Septate
Conidia	Smooth, thin walled, fusoid to ellipsoidal, apex obtuse, base truncate to round	Hyaline	18.32-32.11 × 3.97-8.00 µm (Av.27.07 × 5.62 µm)	Aseptate

Inference: *Fusicoccum aesculi* Corda *Means of fifty observations

3.3.1 *In vivo* morphology

The hyphae were branched, smooth, thick walled, septate, hyaline to light brown in colour measuring 2.35-3.20 µm in width. Stereoscope microscopic examination of the thallus revealed the presence of numerous dark brown to black, submerged or erumpent pycnidia over cankered branch/twig surface. These were globose to conical with a papillate ostiole exuding creamy conidial droplet under moist conditions. The pycnidial size ranged from 138-186 × 157-230 µm averaging 159.12 × 192.05 µm. The conidiophore reduced to conidiogenous cells were hyaline, sub-cylindrical, measuring 6.24-14.35 × 2.03-3.90 µm, with an average size of 11.25 × 3.21 µm, producing a single apical conidium. The conidia were hyaline, uni-cellular, smooth, ornamented with granular contents, fusoid to ellipsoidal, somewhat clavate with an obtuse apex and truncate to rounded base and measured 17.85-28.68 × 2.79-7.46 µm, with an average size of 24.72 × 5.39 µm.

3.3.2 *In vitro* morphology

On potato dextrose agar medium, the fungus initially exhibited cottony and floccose growth with luxurious aerial mycelium. The colony subsequently became compact, velvety and slightly appressed along the margins. Initially white, the colony colour changed to olivaceous buff and finally to olivaceous grey after 21 days of incubation at 25±1ºC and finally to black. The hyphae were smooth, thick walled, septate, dark brown and measured 2.95 - 3.88 µm in width.

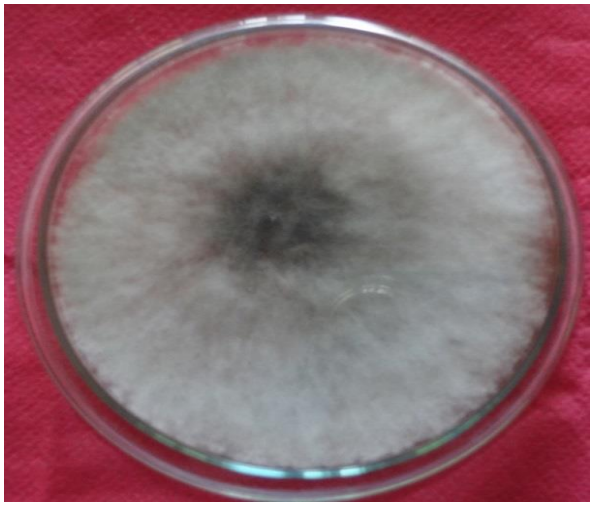
The pycnidia embedded in the culture medium, initially clothed with hyphae like appendages were formed after 10 days of incubation under alternate cycles of light and darkness. They were globose to sub-globose, distributed uniformly over the medium surface and measured 152-200 × 169-261 µm, with an average size of 169.13 × 209.62 µm. However no pycnidial production was observed under complete darkness. The conidiogenous cells were smooth, hyaline, sub-cylindrical, swollen at the base, measuring 6.13-15.55 × 1.98-4.00 µm, with an average size of 12.04 × 3.19 µm, producing single apical conidium. The conidia exuding as creamy droplets from the mature pycnidia were smooth, thin walled, hyaline, uni-cellular, fusoid to ellipsoidal with an obtuse apex and truncate or sub-truncate to rounded base and measured 18.32-32.11 × 3.97-8.00 µm, with an average size of 27.07 × 5.62 µm (Plate 3).

3.4 Pathogenicity test

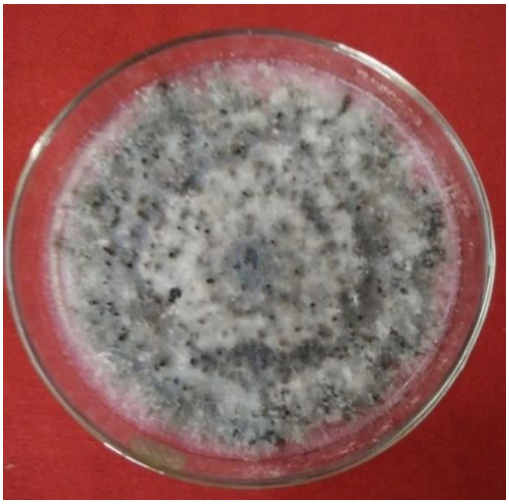
Pathogenicity of the test fungus was performed on one year old potted apple saplings cv. "Red Delicious". The initial disease symptoms of the disease appeared within 9-11 weeks of inoculation. The lesion showed upward and downward extension from the incision points involving the entire branch/stem length, which ultimately led to the death of branch. However, pycnidial production was observed after 13 weeks of inoculation. The symptoms produced were identical with those observed in the field. No lesion development however, was observed on control plants. Re-isolation from the infected twigs yielded original inoculant thus satisfied Koch's postulates.

3.5 Identification

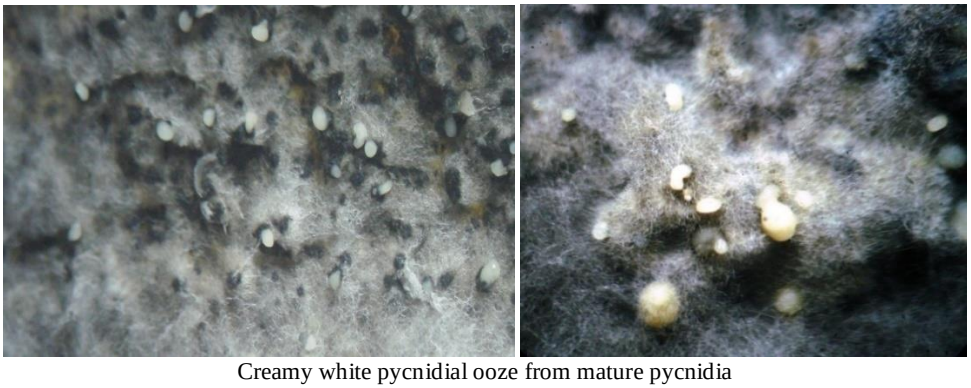
Based on morphological characteristics, pathogenicity test and comparison with the authentic descriptions given by Pennycook and Samuels (1985) [17], Slippers *et al.* (2004) [23] and Phillip *et al.* (2005) [18] as its ability to cause the disease on apple saplings, the fungus was identified as *Fusicoccum aesculi* Corda.



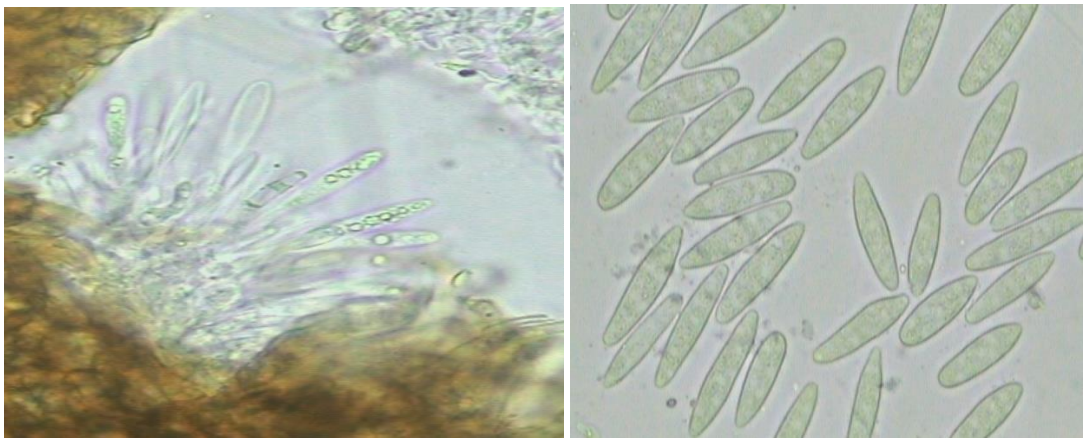
7 Days old fungal colony grown on PDA



Pycnidia formation after 15 days



Creamy white pycnidial ooze from mature pycnidia



Conidiogenous cells with developing conidia Conidia of the fungus on host

Plate 3: Morpho-cultural characters of *Fusicoccum aesculi* Corda. the causal agent of stem bark canker

4. Discussion

In the present investigation, survey of apple orchards in four districts of Kashmir valley was conducted to record the incidence and intensity of stem bark canker disease. During the survey stem bark canker disease was observed on different parts of apple trees with varying degrees of incidence and intensity. Among the different districts surveyed on an average the stem bark canker was most prevalent in district Ganderbal with 31.33 per cent disease incidence followed by district Kulgam and Anantnag with 27.00 and 21.00 per cent incidence whereas, district Shopian recorded least incidence of 15.33 per cent. The higher incidence of stem bark canker observed in district Ganderbal could probably be attributed to the fact most of the orchards in district Ganderbal and Kulgam at higher elevations with water scare situation with lesser tree densities exposing the tree trunks and limbs to intense sunlight thus favouring the development of stem bark canker. Like canker incidence, the canker intensity on fruiting wood (branches and twigs) ranged between 4.81 to 9.13 and 5.76 to 12.04 per cent during the years 2013 and 2014 respectively and was highest in 2014 (7.41%), compared to that (5.56%) in 2013 with an overall canker intensity of 6.49 per cent. Orchards of Ganderbal district exhibited the maximum average canker intensity (9.09%) followed by Kulgam orchards with an average canker intensity (8.44%). The orchards of Shopian showed least average canker intensity of 6.49%. Among various locations surveyed the maximum canker intensity of 9.37 and 12.04 per cent respectively over the years were recorded at Lar in district Ganderbal, while the minimum canker intensity of 4.15 and 6.33 per cent respectively were recorded at Achabal in district Anantnag. Malik (1967) [14] and Shandiyal *et al.* (1973) [20] recorded the varied incidence of stem bark canker caused *by B. ribis* and *B. dothidea* in different apple growing areas and its ability to cause the death of trees. Thus unusual harsh winter and summers, faulty pruning and training practices coupled with cultivation of fruit trees in water scarce uplands and rocky foot hills deficient in moisture retensive capacities are all believed to predispose the trees to the onslaught of various canker diseases.

Stem bark canker disease mostly observed on sun burnt surfaces of tree trunks, branches and twigs manifested as small, sunken, reddish brown lesion/areas, which on enlargement became depressed and developed elliptical cankers with vertical and horizontal slits, partially or completely girdled the affected trunk or branch. Occasionally, subsequent callus formation along the entire cankered surface was also observed which restricted the canker proliferation. Numerous black pimple like elevations developed under as a result of pycnidial formation. In advanced stages, the cankered surface was black and brittle with numerous black fissures. These observations are in close conformity with the findings of Anderson (1956) [2], Malik (1967) [14], Gupta and Agarwal (1973) [7], Kato (1973) [10], Sutton (1990b) [25] and Khan (2010) [11].

In the present investigation, the pathogen associated with the Stem bark canker disease was identified on the basis of morphological and cultural characters and compared with the authentic descriptions from the literature. The fungus isolated from Stem bark canker disease produced olivaceous to violaceous black fungal colonies with dense aerial mycelium. The hyphae were smooth, thick walled, septate, dark brown and measured 2.95 - 3.88 µm in width. The pycnidia embedded in the culture medium, initially clothed with hyphae like appendages were formed after 10 days of incubation under alternate cycles of light and darkness. They were globose to sub-globose, distributed uniformly over the medium surface and measured 152-261 µm. No pycnidial production was observed under complete darkness. The conidiogenous cells were smooth, hyaline, sub-cylindrical, swollen at the base, measuring 6.13-15.55 × 1.98-4.00 µm, with an average size of 12.04 × 3.19 µm, producing single apical conidium. The conidia exuding as creamy droplets from the mature pycnidia were smooth, thin walled, hyaline, uni-cellular, fusoid to ellipsoidal with an obtuse apex and truncate or sub-truncate to rounded base and measured 18.32-32.11 × 3.97-8.00 µm, with an average size of 27.07 × 5.62 µm. The morphological characters of the isolated fungus were compared with the authentic descriptions given by Weaver (1974) [26], Sutton (1990b) [25], Pennycook and Samules (1985) [17], Slippers *et al.* (2004) [23], Phillips *et al.* (2005) [18] and Khan *et al.* (2010) [12] with which these characters fully corroborated. Based on morphological characters both on host as well as in culture, the anamorph of the fungus causing stem bark canker disease in apple was identified as *Fusicoccum aesculi* Corda. The fungus has been found to reproduce sexually and the perfect state identified as *Botryosphaeria dothidia* Ces and De Not. In the present studies the perfect state of the fungus was neither observed on host nor does it developed in culture.

The pathogenic behaviour of the isolated pathogens has been established following Koch's postulates on one year old potted apple plants cv. "Red Delicious". Typical symptoms were produced by the pathogens 4-5 weeks after inoculations. Re-isolations from the diseased twigs yielded original inoculant repeatedly, thus satisfying Koch's postulates.

Pathogenic nature of canker fungi isolated from apple trees was proved by various workers (Shandilya, 1971 and Singh, 1985) [19, 22] both in laboratory as an excised twig method as well as under pot culture and field conditions on injured and uninjured twigs of young and grown up apple trees by inoculating culture bits from two weeks old pathogen culture and covering with moist cotton pads to provide suitable conditions for growth of the pathogen.

5. Summary and Conclusion

The survey of apple orchards in four districts of Kashmir valley viz; Ganderbal, Kulgam, Anantnag and Shopian revealed the prevalence of the disease in all the surveyed districts with an overall canker incidence of 12.67 to 26.00 per cent, during the year 2013 and 18.00 to 36.67 per cent during 2014. The highest canker incidence (31.33%) was observed in district Ganderbal and the least in district Shopian (15.33%). Among the villages surveyed, Watlar of district Ganderbal exhibited the highest canker incidence of 41.00 per cent. Village Batapora of district Shopian exhibited the least canker incidence of 13.00 per cent. The data over the two years further revealed that maximum canker incidence of 23.50% was observed on tree trunks followed by scaffold branches (17.70%) and least on fruiting wood (10.09%).

Stem bark symptoms mostly observed on sun burnt surfaces of trees was characterized by the appearance of small, sunken, reddish brown lesions, which on enlargement became depressed and developed elliptical cankers with vertical and horizontal slits, partially or completely girdling the affected trunk or branch. In advanced stages, the cankered surface however, became was black and brittle which remained studded with numerous black fissures.

The fungus isolated from stem bark canker produced olivaceous to violaceous black fungal colonies with dense aerial mycelium. The hyphae were smooth, thick walled, septate and dark brown in colour. The pycnidia formed only in presence of light were globose to sub-globose distributed uniformally over the culture medium. The conidiogenous cells were smooth, hyaline, sub-cylindrical, swollen at the base, producing single apical conidium. The conidia were smooth, thin walled, hyaline, unicellular, fusoid to ellipsoidal with an obtuse apex and truncate or sub-truncate to rounded base. Based on morphological characters both on host as well as in culture, symptom expression and pathogenicity tests, the fungus causing the disease was identified as *Fusicoccum aesculi* Corda.

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