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Status and characterization of stem bark canker of pear (*Fusicoccum aesculi*) in Jammu & Kashmir

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Abstract
Pear (*Pyrus communis* L.) is one of the important pome fruits grown throughout the world. Despite the fact that the agro-climatic conditions of valley are ideally suited for its cultivation the productivity is very low compared to other parts of the world due to many biotic and abiotic factors. The major biotic factors include fungal diseases. Among these diseases canker diseases of pear are now assuming alarming status resulting in huge economic losses (Jones and Aldwinkle, 1990). The present study was therefore, undertaken to study the status and to characterize the pathogens associated with pear canker in Kashmir. A survey was carried out in the months of August and September, 2012 in major pear growing areas of Kashmir valley to record the incidence and intensity of fusicocum canker of pear. Among the different districts surveyed, the orchards in the district Anantnag exhibited the maximum average canker incidence and intensity of 18.96 and 10.10 per cent respectively, followed by orchards of district Kulgam with an average canker incidence and intensity of 14.22 and 8.11 per cent respectively. The orchards in district Baramulla exhibited least incidence and intensity of 10.45 and 5.56 per cent, respectively. Fusicoccum cankers mostly developed on small branches and twigs as small, sunken, reddish brown lesion/area with or without a fissure, which on enlargement became depressed and formed small elliptical cankers, completely girdling the affected branch. Hyphae of the fungi were branched, thick walled, septate, hyaline to light brown, 2.95-3.80 µm in width. The pycnidia were globose to conical with a papillate ostiole. The pycnidial size ranged from 142-190 × 167-240 µm wide, with an average size of 164.32 × 198.75 µm.

Keywords: status, characterization of stem bark, Jammu & Kashmir

Introduction
Pear (*Pyrus communis* L.) is one of the important pome fruits grown throughout the world. Globally pear is cultivated over an area of 1580 thousand hectares, with an annual production of 22.5 million tonnes. The major producers being China, Italy, USA, Spain, Argentina, Republic of Korea, Turkey, Germany, Japan and South Africa (Anonymous, 2009) [2]. In India its cultivation is mainly confined to temperate regions of North particularly in the states of Jammu and Kashmir, Punjab, Haryana, Himachal Pradesh, Uttar Pradesh and also in some parts of South over an area of 279 thousand hectares, with an annual production of 317 thousand metric tonnes (Anonymous, 2009a) [1]. Of this area and production the Jammu and Kashmir State alone accounts for about 4.73% (13.21 thousand hectares) area and 18.36% (58.21 thousand metric tonnes) production, respectively (Anonymous, 2012) [3].
Pear is of great economic significance in Kashmir valley, being the most important fruit crop after apple. The productivity of pear in Kashmir valley is very low compared to other parts of the world owing to many biotic and abiotic factors inflicting huge economic losses, despite the fact that the agro-climatic conditions of valley are ideally suited for its cultivation. The major biotic factors include fungal diseases like pear scab [*Venturia pirina* Aderh.], powdery mildew [*Podosphaera leucotricha* Ell. & Ev. Salmon.], pear leaf spot [*Mycosphaerella pyri* (Auersw) Boerema.], fabraea leaf and fruit spot [*Fabraea maculate* Atk.] and cankers caused by various fungal pathogens. Among these diseases canker diseases of pear are now assuming alarming status resulting in huge economic losses through girdling of branches and limbs, blightening and die back of twigs, ultimately leading to death of either whole or part of the tree (Jones and Aldwinkle, 1990) [6].

Materials and Methods
An extensive survey was carried out in the months of August and September, 2012 in major pear growing areas of Kashmir valley to record the prevalence and severity of canker disease of pear and to establish the casual pathogens. The pear orchards at 12 locations viz. Liser, Achabal, Kanilwan, Nagam, Charar-i-sharief, Chadoora, Devsar, D. H. Pora, Manzgam,

Dellina, Rafiabad and Sopore were surveyed and canker incidence and intensity was recorded. In each district (Anantnag, Kulgam, Budgam and Baramulla) three locations were selected and five orchards in each of the five villages were taken to represent a location. Ten trees were randomly selected from each orchard for assessing the incidence of stem bark cankers. Besides the main trunk, three scaffold branches (major limbs) and nine branches/twigs from each tree were randomly selected and examined for the presence of canker and recorded as per cent canker incidence. The percent canker intensity was calculated after rating the level of disease on 0-5 scale of Crosse (1957) [5] with slight modifications as adopted by Khan *et al*., 2011 [7] as described below:

Category	Numerical value	Criterion
I	0	No disease
II	1	0.1-10.0% of branch/twig surface area diseased
III	2	10.1-20.0% of branch/twig surface area diseased
IV	3	20.1-30.0% of branch/twig surface area diseased
V	4	30.1-40.0% of branch/twig surface area diseased or one side of the branch/twig showing partial girdling
VI	5	More than 40% of branch/twig surface area diseased or complete girdling of the branch/twig. The branch above the girdled portion completely dried

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

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 and twigs of pear trees showing distinct cankerous symptoms, collected during the course of survey, were repeatedly used for isolation of pathogen. The isolations were made as per the procedure adopted by Proffer and Jones (1989) [11]. The samples from the diseased trunk /limbs/ branches/ twigs collected during the 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 twice in distilled water to remove the traces of mercuric chloride solution, blotted dry and transferred aseptically in acidified potato dextrose agar (PDA) medium, in sterilized Petri plates and incubated at 25±1°C. The culture(s) thus obtained were purified by transferring the germinating spores individually into culture medium as employed by Schreiner (1930) [12] and Christensen (1939) [4].

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°C. Cankered branches and twigs bearing fungal fructifications were cut and incubated in moist chambers for 48-72 hours at 20±1°C. The conidial mass oozing out of the fruiting bodies was transferred to Petri plates containing PDA medium and incubated at 25±1°C under diffused light for the development of fungal colonies and the production of fruiting bodies. The conidial suspension in sterile water obtained from such fruiting bodies was plated on water agar in petri plates and incubated at 25±1°C for 24-48 hours. Single spore isolates were obtained by transferring 5-10 germinating conidia individually to PDA plates. Pure cultures thus obtained were maintained by repeated sub-culturing as described in. Single spore isolates obtained by transferring the germinating spores individually into culture medium as employed by Schreiner (1930) [12] and Christensen (1939) [4] were used for various studies. The pathogenicity of the isolated pathogens were performed on healthy one year old potted saplings of pear cultivar "Willium-bartlet" as per the technique employed by Milholland (1972) and Spiers (1977) [14]. The saplings were sprayed with copper oxy-chloride 50 WP @ 0.3% to exclude any harbouring pathogen(s) and were kept in diffused sunlight in polythene chambers, especially 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 pear 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. The morphological characters of the causal organism(s) studied were compared with authentic descriptions for their identification and nomenclature. Pathogenicity tests were closely monitored for symptom/canker development. Re-isolations of the pathogen(s) from artificially inoculated twigs were carried out and resultant cultures compared with the original inoculated fungus to satisfy Koch's postulates.

The morphological characteristics of the causal organism(s) 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°C) 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 triocular compound microscope previously calibrated with the aid of stage and ocular micrometers.

Results and Discussion

Incidence and intensity

The data presented in table 1 revealed that the Fusicoccum canker was also present in varying degrees of incidence and intensities at all the locations surveyed. However, the trunks and major limbs were found free from the disease. The canker incidence and intensity ranged between 8.21 to 23.32 and 4.15 to 12.05 per cent, respectively with an average incidence and

intensity of 13.70 and 7.63 per cent respectively. Among the different districts surveyed, the orchards in the district Anantnag exhibited the maximum average canker incidence and intensity of 18.96 and 10.10 per cent respectively, followed by orchards of district Kulgam with an average canker incidence and intensity of 14.22 and 8.11 per cent respectively. The orchards in district Baramulla exhibited least incidence and intensity of 10.45 and 5.56 per cent, respectively. Among the different locations surveyed, the maximum canker incidence and intensity of 23.32 and 12.05 per cent, respectively was observed at Achabal, while, the average minimum incidence and intensity of 8.21 and 4.15 per cent, respectively were recorded at Chadoora in district Budgam.

Table 1: Incidence and intensity of Fusicoccum canker on pear (*Pyrus communis* L.) tree branches and twigs at different locations of Kashmir during the year 2012

District	Location	Canker Incidence (%)*	Canker Intensity (%)*
Anantnag	Liser	18.00	10.10
	Achabal	23.32	12.05
	Kanilwan	15.55	7.98
	Mean	18.96	10.04
Budgam	Nagam	10.22	7.20
	Chrar-i-sharief	15.11	9.13
	Chadoora	8.21	4.15
	Mean	11.18	6.83
Kulgam	Devsar	18.22	10.03
	D. H. Pora	14.22	8.20
	Manzgam	10.22	6.10
	Mean	14.22	8.11
Baramulla	Dellina	8.68	4.81
	Rafiabad	10.22	5.76
	Sopore	12.44	6.10
	Mean	10.45	5.56
	G.M. ± S.D.	13.70 ± 3.87	7.63 ± 1.91

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

G. M. = Grand mean

S. D. = Standard deviation

Symptomatology

Fusicoccum cankers mostly developed on small branches and twigs as small, sunken, reddish brown lesion/area with or without a fissure, which on enlargement became depressed and formed small elliptical cankers, completely girdling the affected branch (Plate 1). Occasionally, subsequent callus formation on one side of cankered surface along its entire length was also observed which restricted the canker proliferation. The wood below the cankered areas was necrotic and stained dark brown. Numerous black pimple like elevations developed on the cankered surface as a result of pycnidial formation beneath it. The canker also developed wart like protuberances on the branch/twig surface, which were surrounded by dark brown lesions/areas causing withering of the twig. Die-back type symptoms characterized by browning of the bark with simultaneous yellowing of the leaves were also observed on current season growth.

Morphology of the pathogen

The morphological characters of the fungus associated with Fusicoccum 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 2. Hyphae were branched, thick walled, septate, hyaline to light brown. 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. Conidiogenous cells were hyaline, sub-cylindrical, producing 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.

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 to olivaceous grey and finally to black. The hyphae were smooth, thick walled, septate and dark brown. 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 uniformally over the medium surface. However no pycnidial production was observed under complete darkness. The conidiogenous cells were smooth, hyaline, sub-cylindrical, swollen at the base, 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 (Plate 2).

Pathogenecity of the test fungus was performed on one year old potted pear saplings of cv. Willium bartlet. The initial 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 canker development was observed in control plants. Reisolation from the infected twigs yielded original inoculants thus satisfied Koch‟s postulates. Based on morphological characteristics, pathogenecity test and comparison with the authentic descriptions given by Pennycook and Samuels (1985), Slippers *et al*. (2004) and Phillip *et al*. (2005) [10] the fungus was identified as *Fusicoccum aesculi* Corda. the anamorph of *Botryosphaeria dothidea* (Moug. Fr.) Ces. & Not.

Table 2: Morphological characters of *Fusicoccum aesculi* Corda. causing Fusicoccum canker of pear (*Pyrus communis* L.)

Thallus part	Shape and character	Colour	Size	Septation
On Host				
Mycelium	Hyphae smooth, thick walled, branched	Hyaline to light brown	2.95-3.80 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	142-190 ×167-240 µm (Av. 164.32 × 198.75 µm)	Septate

Conidiogenous cell	Smooth, sub-cylindrical, slightly swollen at base	Hyaline	6.36-14.65 × 2.13-4.10 µm (Av. 11.25 × 3.21 µm)	-
Conidia	Smooth, thin walled, fusoid to ellipsoidal, somewhat clavate, apex obtuse, base truncate to round, slightly wider in the middle region	Hyaline	18.35-29.70 × 3.89-7.90 µm(Av. 25.32 × 5.96µm)	Aseptate
In Culture				
Colony	Cottony and floccose, aerial mycelium cincr-eous, becoming compact and velvety, slightly appressed along the margins with raised center	White turning olive-ceous grey and final-ly olivaceous black	-	-
Mycelium	Hyphae smooth, thick walled, branched	Hyaline to dark brown	3.35-4.13 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	156-210 × 172-254 µm (Av. 175.13 × 223.40 µm)	-
Conidiogenous cell	Smooth, sub-cylindrical, slightly swollen at base	Hyaline	6.43-16.35 × 2.10-4.15 µm (Av.11.76 × 3.45 µm)	-
Conidia	Smooth, thin walled, fusoid to ellipsoidal, apex obtuse, base truncate to round	Hyaline	17.22-30.41 × 4.00-8.31 µm(Av.26.47 × 6.12 µm)	Aseptate

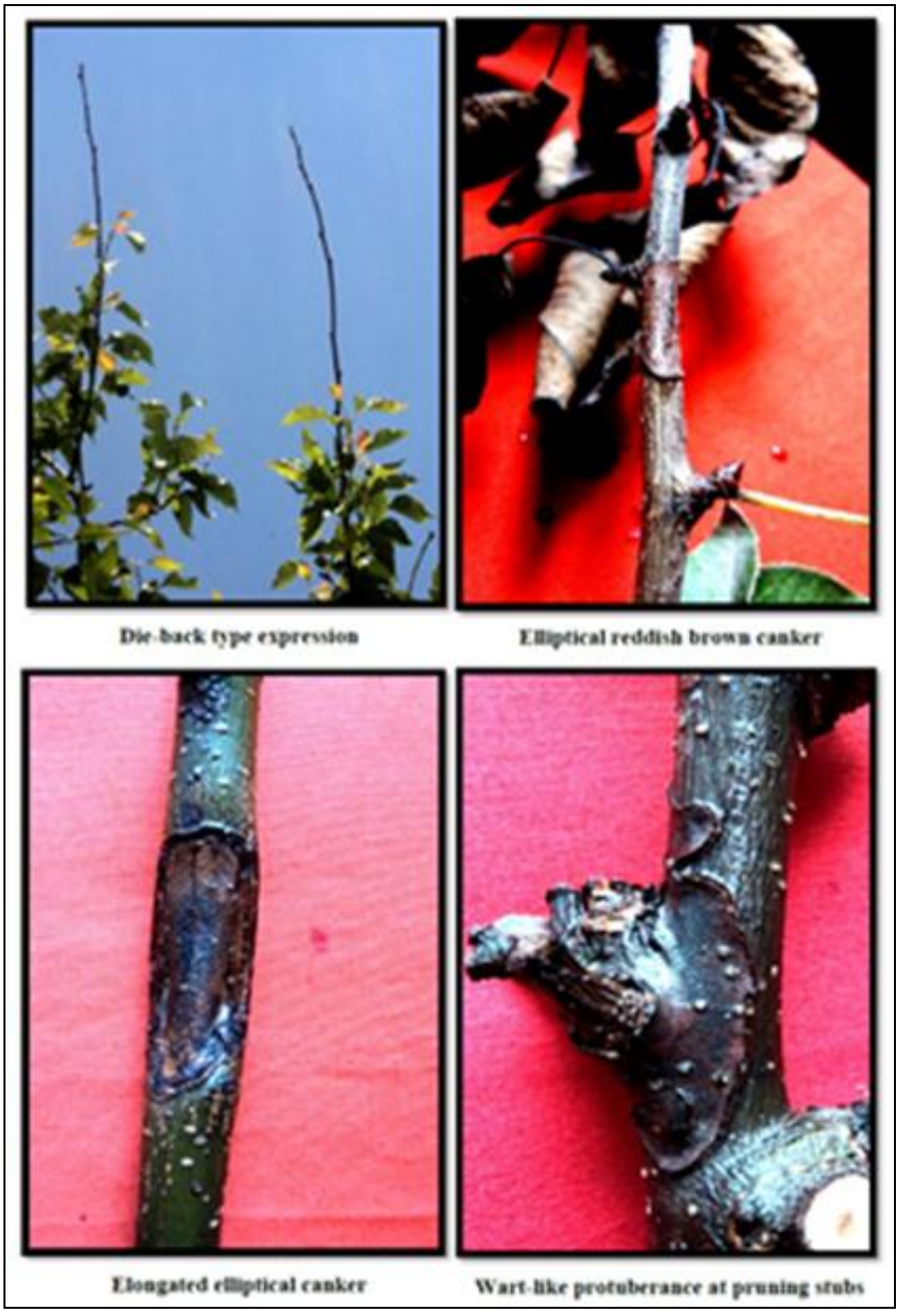

Plate 1: Symptoms expressed by Fusicoccum canker under field conditions

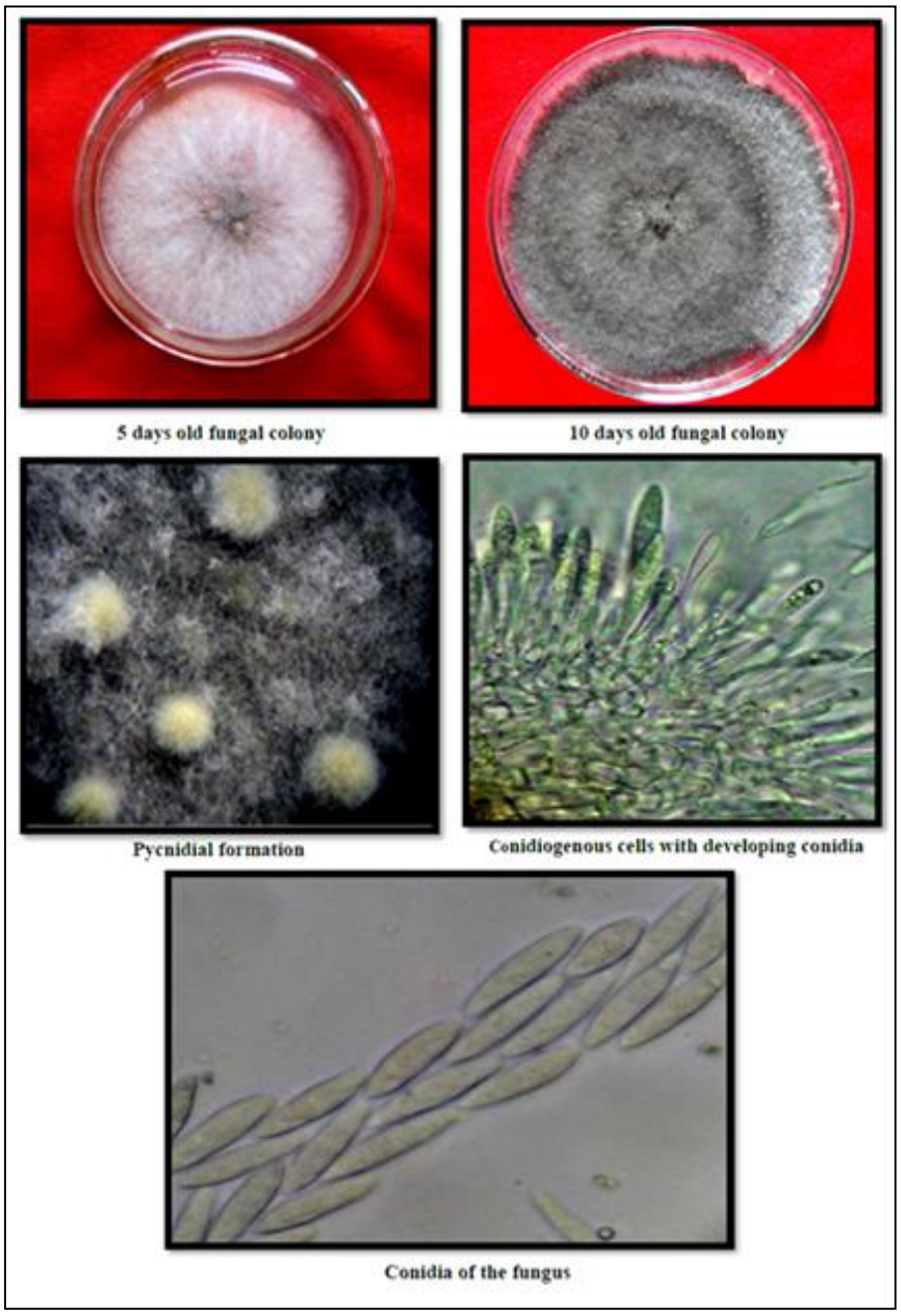

Plate 2: Cultural and morphological characters of *Fusicoccum aesculi*

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