



Identification of *Cylindrosporium padi* associated with leaf spot disease of cherry in Kashmir Valley, India

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Abstract

Cherry an important stone fruit crop has been found to face a serious threat due to *Blumeriella* leaf spot disease. Survey of cherry orchards in Srinagar, Ganderbal and Baramulla districts revealed prevalence of the disease to varying extent. The disease incidence and intensity ranged from 13.00 to 52.50 per cent and 5.78 to 30.48 per cent, respectively. Maximum disease incidence of 41.02 per cent and intensity of 18.62 per cent was recorded in Ganderbal district, while minimum disease incidence of 18.10 per cent and intensity of 8.4 per cent was recorded in Baramulla district. The disease first appeared as small, circular to irregular, purple red speck on upper leaf surface. Periodical changes in colour, shape and size of the spots coupled with formation of irregular necrotic patches led to pre-mature defoliation. The fungus isolated on Potato Dextrose Agar medium produced compact and circular greyish white fungal colonies composed of hyaline, thick walled, septate and branched mycelium. Acervuli produced after 20 days of incubation at 20±1°C were dark brown to black, circular, discoid and measured 260.50 µm in diameter. Conidia (39.01 × 2.89 µm) were hyaline, bicelled, elongated, curved or flexuous with tapered apex and rounded base. The pathogenic nature of the fungus was established on potted cherry saplings of cv. *Bigarreau napoleon* (Double). Based on morphological characters, pathogenicity test and comparison with the authentic description, the pathogen causing the disease was identified as *Cylindrosporium padi* (Lib.) P. Karst. Ex Sacc. the anamorph of *Blumeriella jaapii* (Rehm) Arx.

Key words: cherry, *Blumeriella* leaf spot disease, *Cylindrosporium padi*.

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Introduction

Cherries occupy unique position among temperate fruits all over the world and are season's first tree fruit to reach the market, maturing within 60-70 days after full bloom and therefore, fetches premium price. Cherries are classified under the genus *Prunus* and belong to the family *Rosaceae*. The cultivated cherries are divided into two groups i.e. sweet cherries (*Prunus avium* L.) and sour cherries (*Prunus cerasus* L.). The primary cherry producing countries contributing to world's annual production of 2256 thousand MT are Turkey, U.S.A., Iran, Italy, Chile, Uzbekistan and China (Anonymous, 2014). In India, cherry is mainly grown in the state of Jammu and Kashmir (J&K) and to some extent in the North-Western Himalayan region of Himachal Pradesh and Uttarakhand hills, India. Exports of cherry from Jammu and Kashmir State earns substantial foreign exchange up to 150.00 crore (Anonymous, 2012). The cherry fruit is mostly consumed as fresh, besides being used in confectionery, ice-creams, bakery, Juice-making, syruping and liquors. Cherry wood and fruit are also usable in veneer industry, dying, pharmaceutical and food industries (Chinnici et al., 2015). Owing to its high content of vitamin C and other chemicals that might act as antioxidants, cherries are used to prevent cancer and other cardiovascular diseases besides treating osteoarthritis and gout (Bastos et al. 2015; Pacifico et al., 2014). The agroclimatic conditions of Kashmir valley are quite conducive for stone fruit crop cultivation and also the area under cherry is increasing marginally every year, but the cherry productivity per unit

area is low owing to many biotic and abiotic factors. Among the diseases, *Blumeriella* leaf spot (BLS) has assumed an alarming proportion in the major cherry growing countries of the world and causes huge economic losses through mid-summer pre-mature defoliation to the extent of 80-90% reported from Pennsylvania, which ultimately results in reduced fruit bud survival and fruit set in the following year (Gianessi & Williams, 2011; McManus et al., 2007). Further, the disease results in the production of soft and poorly coloured fruits with low content of soluble solids besides increasing tree mortality during severe winters (Jones, 1995). The disease has been first observed in USA by Karsten in 1884. Since then the disease has been recorded from many other countries of the world, but in India, particularly in Jammu and Kashmir State, no attempt has been done except for a report (Khan et al., 2014) and hence the present study was undertaken to characterize the disease based on morphological characters and pathogenicity test from three cherry growing districts of Kashmir valley, India.

Materials and methods

Survey, disease incidence and intensity: Survey of cherry growing areas in three districts viz., Srinagar, Ganderbal and Baramulla of Kashmir valley, India was done during late August (peak period of the disease) in the year 2014 to record incidence and intensity of *Blumeriella* leaf spot in cherry. Random selection of cherry orchards irrespective of cultivars from three villages per district, five orchards per village and ten trees per orchard was

done. The disease incidence was recorded by counting the total number of leaves and the number of diseased leaves

on randomly selected four branches in four directions of each tree, using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves assessed}} \times 100$$

The disease intensity was recorded by visual observations using 0-4 scale (Schuster & Tobutt, 2004). Per cent disease intensity (PDI) was calculated as per the formula:

$$\text{PDI} = \frac{\sum(n \times v)}{N \times G} \times 100$$

Where, n= No. of diseased leaves in each category, v= Numerical value of each category, N= No. of leaves examined, G= Maximum numerical value.

Symptomatological studies: Cherry leaves of cultivar Bigarreau Napoleon (Double) from four randomly selected trees were used for symptomatological studies in the orchard of Division of Fruit Sciences, SKUAST-K, Shalimar. The trees were kept unsprayed throughout the growing season to study the symptoms of *Blumeriella* leaf spot under natural epiphytotic conditions. Leaves were examined daily for the disease appearance. Periodic observations with respect to petiole infection, leaf curling and pre-mature defoliation besides size, shape and colour of the lesions on leaves was recorded.

Isolation of the pathogen: Cherry leaves exhibiting typical disease symptoms, collected during the course of survey, were repeatedly used for isolation of the pathogen. The diseased leaves were first examined for associated fungus by teasing the diseased portion with the aid

of a teasing needle and observed under microscope. For isolation of the fungus, tissue bit technique (Joshua & Mmbaga, 2014) was used and incubated at $20 \pm 1^\circ\text{C}$ for 21 days in sterilized Potato Dextrose Agar (PDA) media Petri plates.

Isolation, purification and maintenance of the pathogen:

Isolations were made from diseased leaves showing typical symptoms. After 72 hours of incubation at $20 \pm 1^\circ\text{C}$ whitish mycelial growth started emerging from the diseased leaf tissues, inoculated on Potato Dextrose Agar medium. The culture was purified by single spore isolation (Johnston & Booth, 1983). The pure culture thus, obtained was maintained by repeated subculturing at an interval of 30 days for further studies. The stock culture in PDA slants was stored at 4°C in a refrigerator. To retain the vigour of the fungus, it was isolated repeatedly from naturally infected leaves and purified by the method described.

Pathogenicity test: One year old apparently healthy, budded plants of cultivar Bigarreau Napoleon (Double), obtained from Division of Fruit Sciences, SKUAST-K, Shalimar were planted in 40 cm diameter plastic pots containing sterilized soil. The potted plants were kept in polythene chambers, especially designed for the purpose. High humidity inside the chamber was maintained by timely irrigation of the pots and intermittent spraying with distilled

sterilized water. The plants were sprayed with copper oxy-chloride 50 WP @ 0.3% to exclude any infection. Also, the plants were constantly observed for 10 days to rule out any latent infection. Prior to inoculations, the leaves were sprayed with distilled sterilized water. One set of the plants was given injuries with carborandom powder, while another set was kept uninjured. Inoculation were made by spraying spore suspension (1×10^4 spores/ml) from young and vigorous culture of *Cylindrosporium padi* on the abaxial surface of both injured and uninjured leaves with the help of an atomizer. Plants with injured and uninjured leaves sprayed with distilled sterilized water served as check. The inoculated and un-inoculated plants were closely monitored for symptom development. Re-isolations of pathogen from artificially inoculated leaves were carried out and resultant cultures compared with original inoculant to prove Koch's postulates.

Morphological and cultural characteristics of the pathogen: The morphological characters of the causal pathogen were studied both on host (*in vivo*) as well as on artificial culture in the laboratory (*in vitro*). Monoconidial cultures were first grown on PDA medium and then semi-permanent slides were prepared from 21 days old culture, stained with cotton blue in lacto phenol. The important morphological characteristics studied were nature of colony, hyphae, conidiophore, conidia and conidiomata. The morphological characters of the causal organism studied were compared with authentic descriptions for the identification of the fungus.

Results

Disease incidence and intensity: disease incidence and intensity recorded during survey indicate that *Blumeriella* leaf spot disease of cherry was prevalent in all the three districts with varied degrees Figure 1. Statistical analysis of the surveyed data revealed that the limits for average disease incidence and intensity fluctuated between 26.45 to 46.57 and 09.10 to 24.24 per cent, respectively for Srinagar district and 34.95 to 47.09 and 14.50 to 22.74 per cent, respectively for Ganderbal district and 15.18 to 21.02 and 06.97 to 09.97 per cent respectively for Baramulla district, indicates highest for Ganderbal. However, the average statistical limits in all the three districts at all the locations in incidence and intensity fluctuated between 27.09 to 36.65 and 11.59 to 17.57 per cent, respectively.

Symptomatology: During the periodic observation of marked trees, the initial disease symptoms were noticed in the first week of June which reached to its peak by the last week of August. The symptoms appeared in the form of small, irregular, purple red, non sporulating specks measuring 0.5 to 1.0 mm with an average size of 0.8 mm Figure 2a. The spot progression though initially slow showed a curvilinear behaviour with a maximum spot size of 3.80 mm recorded in the fourth week of June, beyond which no further enlargement in size was observed. The colour of the spot changed from purple red in the first week of June to purple-brown at the end of the month. The acervular formation on abaxial leaf surface just corresponding to spot as a whitish felt like growth was recorded in

the fourth week of June, yielding hyaline, bicelled macroconidia Figure 2b. Beyond the third week of July major portion of the leaf got covered with diseased spots. The petiole infection though less frequent was first recorded in the third week of July as brown elliptical lesions with a greyish center. Also, microconidia were formed within the same stromata in the second week of August. The shape of the spots was irregular to circular upto third week of June beyond which due to coalescing of spots large irregular necrotic patches were formed Figure 2c. Chlorosis and/or inward curling of chlorotic and/or achlorotic severely infected leaves Figure 2d along the margins was observed in second week of August which ultimately leads to

initiation of pre-mature defoliation Figure 2e by the fourth week of August.

Pathogenicity test: The pathogenic nature of the isolated fungus was proved on one year old potted cherry saplings cv. Bigarreau Napoleon (Double). Observations regarding the pathogenicity of the test fungus revealed the initiation of typical disease symptoms 10 days after inoculation on injured leaves of the potted plants. However, in case of uninjured leaves, the disease symptoms appeared 16 days after inoculation. No lesion development was observed on control plants. Re-isolations from infected leaves yielded typical cultures of the fungus, thus proved the Koch's postulates.

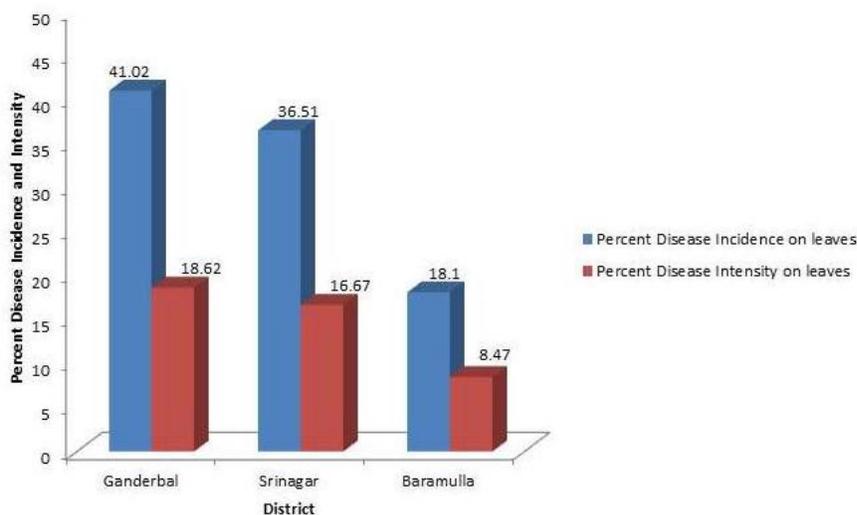


Figure 1: Incidence and intensity of BLS of cherry in three districts of Kashmir valley during year 2014

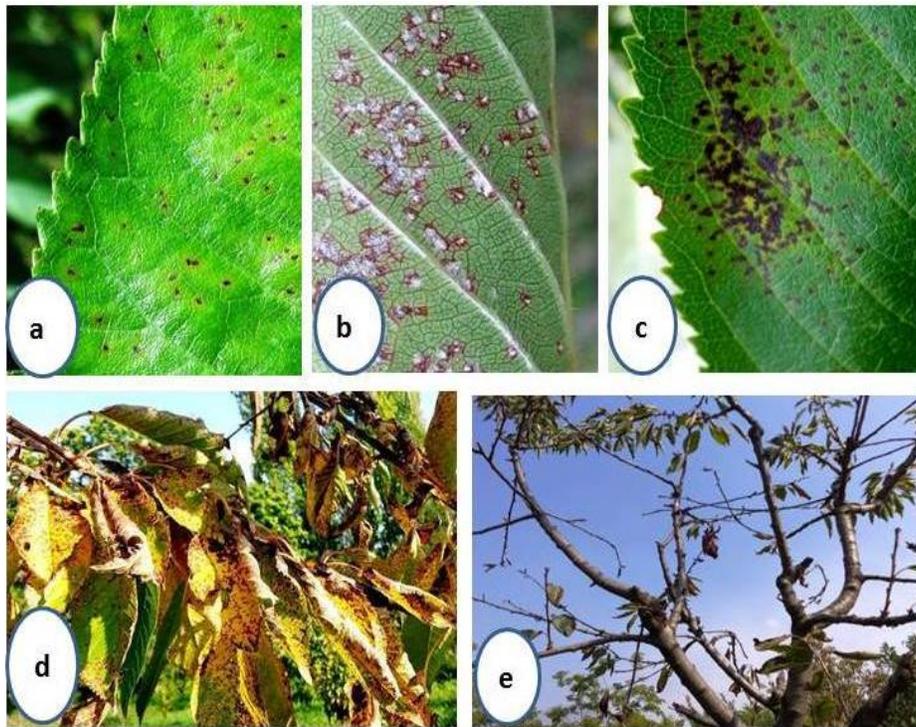


Figure 2: Symptom development of BLS disease: a) Numerous specks on leaf, b) Initiation and development of whitish felt like patches on abaxial leaf surface containing conidiomata, c) Coalescing and formation of irregular necrotic patches, d) Curling of chlorotic and achlorotic leaves, e) Premature defoliation.

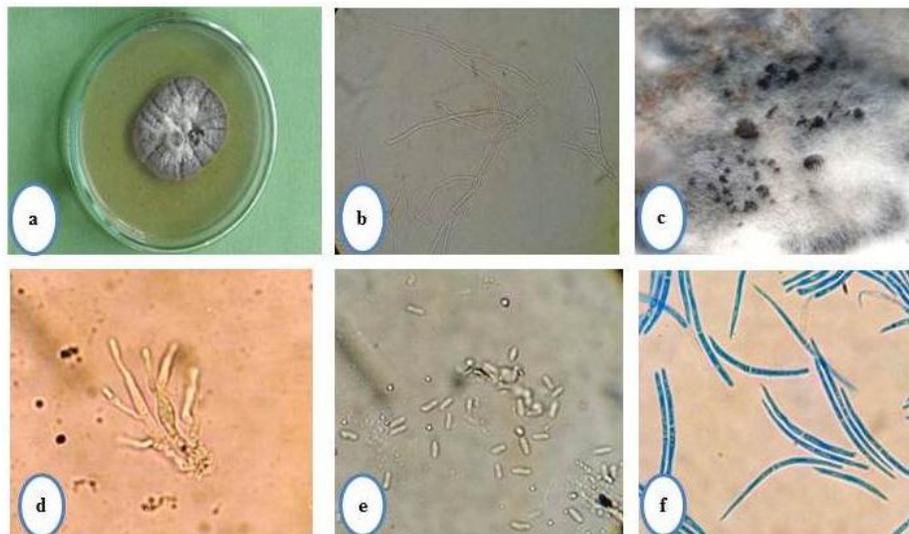


Figure 3: Morpho-cultural characteristics of *Cyindrosporium padi* (Lib.) P. Karst. Ex Sacc. a) 25 days old fungal colony, b) Young and hyaline mycelium, c) Acervuli formation in culture, d) Conidiophore, e) Microconidia f) Macroconidia.

Morphological characters: The morphological characters of the pathogen studied both on host (*in vivo*) as well as from culture (*in vitro*) are presented in Table 1. Figure 3a-f.

Identification of the pathogen: Based on the morphological characters, pathogenicity test and comparison with

authentic descriptions given by Karsten (1884), Higgins (1914) and Williamson and Bernard (1988) the fungus was identified as *Cylindrosporium padi* (Lib.) P. Karst. Ex Sacc. the anamorph of *Blumeriella jaapii* (Rehm) Arx. (Stojanovic and Boric 1973; Jones 1995). No perfect state of the fungus was observed during the investigation either on host or culture.

Table 1: Morpho-cultural characteristics of *Cylindrosporium padi* (Lib.) P. karst Ex Sacc., causing BLS disease of cherry (*Prunus* spp. L.).

Thallus part	Shape and Character	Colour	Size(μm)	Septation
On host				
Mycelium	Hyphae branched, hyphal segments smooth, short, thick walled	Hyaline	2.30-6.90 (4.40)*	Septate without any constriction
Conidiomata (Acervuli)	Circular, embedded in whitish felt like patch on under surface of leaf	Dark brown-black	175-250 (209.30)*	--
Conidiophore	Branched, slightly cylindrical	Hyaline	5-12 \times 2-3.5 (9.5 \times 3)	--
Macroconidia	Elongated, curved or flexous; round at base and tapered at apex	Hyaline	41.40 - 55.20 \times 2.30 - 4.60 (47.76 \times 4.46 μm)*	One
Microconidia	Straight to allantoid	Hyaline	4.60 - 6.50 \times 2.30 - 2.80 (5.75 \times 2.50)*	Aseptate
In culture				
Colony	Initially cottony, becoming furrowed, aerial mycelium sparse and submerged	Initially, whitish, turning to greyish white	--	--
Mycelium	Hyphae branched, hyphal segments smooth, short, thick walled	Hyaline	3.45-7.36 (4.87)*	Septate without any constriction
Conidiomata (Acervuli)	Discoid, bulged, globose, oozing pinkish conidial mass	Dark brown-black	220-310 (260.50)*	--
Conidiophore	Branched, slightly cylindrical	Hyaline	4-10 \times 2-2.5 (8.5 \times 2.2)	--
Macroconidia	Bicelled, elongated, curved or flexous, rounded at base and tapered at apex	Hyaline	35.50 - 43.60 \times 2.30 - 3.50 (39.01 \times 2.89)*	One
Microconidia	Not formed in culture	--	--	--

*Figures in parenthesis are average of 50 observations on Potato Dextrose Agar medium. No perfect stage was observed, either on host or in culture.

Discussion

Amongst the various cherry diseases, *Blumeriella* leaf spot causes huge economic losses through premature mid-summer defoliation which ultimately culminates in reduced fruit bud survival and fruit set in the following year, besides unevenly ripened fruit with poor taste, and increased tree mortality during severe winters (McManus et al., 2007). Highest disease incidence and intensity could probably be attributed to higher plant density, mixed cropping in cherry orchards ecosystem with maize, beans, and fodders leading to high relative humidity in the microclimate of the orchard, besides non disposal of fallen diseased leaves which serve as source of primary inoculum. Occasional or rather neglected spray programme followed in these areas to combat other fungal diseases of cherry seem to have favoured in building the highest disease incidence. The least disease incidence and intensity of (13.00 & 05.78%) and (18.50 & 08.73%) was recorded in the villages of Reram and Druoo of district Baramulla, which could be attributed to lesser plant density and better orchard management practices. Variations in incidence and intensity of *Blumeriella* leaf spot disease in various locations have also been reported by Eisensmith and Jones (1981), Ellis (2008), Wilcox (1993), Kiraly and Szentpeteri (2006), Babadoost (1995), Holb (2009) and Joshua and Mmbaga (2014). Our symptomatological findings are more or less supported by the observations made by Holb (2009), reported formation of irregular necrotic patches with change in colour and shape with the passage of time. Petiole infection though less frequent was first

observed in third week of July. Chlorosis and/or inward curling along the margins of chlorotic or a-chlorotic severely infected leaves was observed in the second week of August which resulted in pre-mature defoliation by last week of August. The characteristic symptoms of the disease as observed under natural conditions of inoculations were identical and agreed with those observed by Khan et al. (2014), Ellis (2008), and Taut et al. (2010). The morphological characters of the fungus observed on host as well as in culture were compared with the authentic description given by Karsten (1884), Higgins (1914), Williamson and Bernard (1988) and Vov Arx (1961) with which these characters closely corroborate; the anamorph was thus identified as *Cylindrosporium padi* (Lib.) P. Karst. Ex Sacc. The typical symptoms were produced by the pathogen 10 days after inoculation on injured leaves and 16 days after artificial inoculation on un-injured leaves. Reisolations from the diseased leaves yielded original inoculant repeatedly, thus satisfied Koch's postulates. Khan et al. (2014) also obtained symptoms within 10-15 days after inoculating the cherry leaves with spore suspension of *Cylindrosporium padi*. On the basis of morphological characters, pathogenicity test and comparison with the authentic descriptions (Karsten, (1884), Higgins (1914), Williamson and Bernard (1988) the pathogen was identified as *Cylindrosporium padi* (Lib.) P. Karst. Ex Sacc. Since the perfect state of the fungus neither developed in culture nor was observed on host during the period of study; however, the fungus has been reportedly found to reproduce sexually and the perfect state identified as

Blumeriella jaapii (Rehm) v. Arx. So based on morphological characters, pathogenicity test and comparison with the authentic descriptions the pathogen was identified as *Cylindrosporium padi* (Lib.) P. Karst. Ex Sacc. and no perfect state of the fungus was either developed in culture or observed on host during the period of study; however, the fungus has been reportedly found to reproduce sexually and the perfect state identified as *Blumeriella jaapii* (Rehm) v. Arx.

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