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Symptomatology and morphological, cultural characterization of Entomosporium leaf and fruit spot disease of pear (*Pyrus communis* L.)

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Abstract

The disease on leaves was characterized by the appearance of small, reddish to dark brown pin-pointed spots measuring approximately 3 mm in size, with angular to irregular margins, and as the spots grew, they become more or less circular in outline, with reddish brown to black centers and were surrounded by a yellowish halo which on coalescing formed irregular necrotic patches. Severe infection resulted in premature defoliation. Small, sunken, circular, black spots, averaging 6 mm in size surrounded by reddish brown margins, embedded with dark brown to black acervuli releasing creamy white spore droplets under moist conditions. Severely infected fruits were roughened and developed cracks were the characteristic disease symptoms observed on fruit. The pathogen constantly associated with the disease was isolated. The pathogen on potato dextrose casein hydrolysate thiamine agar medium produced compact, glistening, dull white to reddish brown cottony mycelial mat after 45 days of incubation at 22±1°C. The morphological characters of the pathogen associated with the disease were studied both on host (*in vivo*) as well as on after culturing on potato dextrose agar medium incorporated with casein hydrolysate thiamine (*in vitro*). On the host the hyphae were branched, smooth, thick walled, septate, hyaline and measured 8.25-8.75 µm in width averaging 8.48 µm, the size of the conidia ranged from 14.46-20.68 × 9.42-12.46 µm. with an average size of 17.57 × 10.94. From culture on potato dextrose casein hydrolysate thiamine agar medium the fungus exhibited compact, dull, white, glabrous, glistening cottony growth. The white colour of the fungal colony finally changed to reddish brown after 30 days of incubation at 22±1°C. The hyphae were hyaline, smooth, thick walled, branched, septate, and measured 8.54 to 8.98 µm in width averaging 8.76µm, the conidial size ranged from 16.36-22.70 µm in length and 10.37-14.65 µm in width averaging (19.56×12.51 µm).

Keywords: Leaves, fruits, culture, hyphae, conidia

Introduction

Pear (*Pyrus communis* L.) is one of the important pome fruits grown throughout the world. It belongs to genus *Pyrus* which is a member of family Rosaceae. Pear is of great economic significance in Kashmir valley after apple, having good taste and flavor. Its cultivation dates backs to 35-40 centuries and seems to have originated from west Asia. Pear is mainly grown for consumption as fresh fruit in India, while in other countries it is also used for juice, wine making, canning, drying and cooking. 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) [3]. Of this area and production, Jammu and Kashmir State accounts for about 4.73% (13.21 thousand hectares) area and 18.36% (58.21 thousand metric tonnes) production, respectively (Anonymous, 2012). Among various diseases, Entomosporium leaf and fruit spot, caused by the fungus *Fabraea maculata* (Lev.) (anamorph: *Entomosporium maculatum* Lev.) has assumed an alarming proportion resulting in huge economic losses through pre-mature mid-summer defoliation besides reducing the tree vigour, fruit quality and fruit yield (Goncalves *et al.*, 2013) [6]. Infected fruits are disfigured, cracked, misshapen, and unmarketable as fresh fruit. Davidson (1989) [5] reported the occurrence of distinct, circular, and reddish to purple pin pointed spots on leaves, which on enlargement developed dark brown colour with black centers possessing minute black fruiting bodies (acervuli) of the fungus. Sivanesan and Gibson (1976) reported that conidia of the fungus were hyaline, cruciform, four celled, sometimes 3-6 celled and measured 12-20 µm × 8-14 µm. with an apical setae on each cell.

Methods and Materials

The present investigations were conducted in the Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, Srinagar.

Symptomatology

Fresh pear leaves and fruits with typical disease spots collected during the course of survey were brought to laboratory. These leaves and fruits were critically examined for symptom expression and later used for isolation of the pathogen. Some of the leaves were also preserved as dry specimen for further investigations and reference. In addition, two trees of cv. Chinese sandy pear in the orchards of Division of Fruit Science SKUAST-K, Shalimar were kept unsprayed throughout the growing season and were used for recording the disease symptoms at periodic intervals.

Morphological and Cultural Characteristics

The morphological and cultural characteristics of the causal organism were studied taking the pathogen thallus from the host (*in vivo*) and after culturing it on artificial culture medium in the laboratory. For microscopic examination the leaves and diseased fruit portion was kept in moist chamber for 24 hours to get the acervuli bulged. These bulged acervuli were lifted with the aid of a teasing needle under stereoscopic microscope and mounted on cotton blue in lacto phenol. The pathogen cultures were grown on potato dextrose agar medium incorporated with casein hydrolysate thiamine and then semi-permanent slides prepared on sterilized glass slides stained with cotton blue in lacto phenol. The important characteristics studied were as under-

Cultural characters

1. Nature of growth of colony
2. Nature of pigmentation, if any

Morphological characters

Hyphae : Septation, width and colour

Acervuli : Shape, size and colour

Conidia : Shape, size and colour

These observations were later on used as criteria for the identification of the fungus.

Results and Discussion

In the present investigations, the symptoms of Entomosporium leaf and fruit spot disease of pear developed, under natural conditions of infection in the field were studied both on leaves as well as on fruits.

Leaves

The disease symptoms developed, under natural conditions in the field on pear leaves, were first noticed on upper surface of young leaves as small, irregular, reddish to purple pin pointed specks of size 1-2 mm in diameter. As the spots enlarged, the color of the spots changed from reddish to dark brown, became more or less circular in shape with an average size of 1-3 mm in dia. coalescing of numerous disease spots resulted in the formation of irregular dark brown to black necrotic

patches. Necrotic lesions were also observed on the leaf petioles. Dark brown to black dot like specks (acervuli) were seen scattered on upper leaf surface in the centre of the mature spots. Severe disease infection led to yellowing and pre-mature leaf defoliation.

Fruits

On fruits the disease initially appeared as small, sunken irregular black spot surrounded by reddish brown margins. Periodical changes in the shape, size and colour of the spot ultimately led to formation of sunken, circular, spot averaging 6 mm in diameter. The spots were usually studded with numerous black pin head like structures (acervuli) releasing creamy white spore droplets under moist conditions. Severely infected fruits were roughened and developed cracks.

Morphological characters of the pathogen

The morphological characters of the pathogen associated with the disease were studied both on host (*in vivo*) as well as on after culturing on potato dextrose agar medium incorporated with casein hydrolysate thiamine (*in vitro*) and are presented in Table 1.

Host (*in vivo*)

The hyphae were branched, smooth, thick walled, septate, hyaline and measured 8.25-8.75 μm in width averaging 8.48 μm . The stereoscopic examination of the diseased spots revealed the presence of acervuli scattered over the spot which were black, solitary, discoid, immersed in the host tissue, erumpent at maturity and measured 110-200 μm in diameter with an average diameter of 154.62 μm . Under moist chamber conditions acervuli oozed large number of conidia in the form of a mucilaginous droplet. The conidia ornamented with deposits of droplet like structures were hyaline, cruciform, four celled, with one large upper and one basal cell and two small lateral cells. The basal and the two lateral cells possessed slender bristle like appendages. The size of the conidia ranged from 14.46-20.68 \times 9.42-12.46 μm . with an average size of 17.57 \times 10.94 μm (Table 1).

From culture

On potato dextrose casein hydrolysate thiamine agar medium the fungus exhibited compact, dull, white, glabrous, glistening cottony growth. The white colour of the fungal colony finally changed to reddish brown after 30 days of incubation at 22 \pm 1 $^{\circ}\text{C}$. The hyphae were hyaline, smooth, thick walled, branched, septate, and measured 8.54 to 8.98 μm in width averaging 8.76 μm . The acervuli produced after 40 days of incubation, were immersed in culture medium, became partially erumpent, were discoid, solitary, grayish black in colour and measured 130-240 μm in diameter, averaging 167.48 μm . The conidia ornamented with deposits of droplet like structures were hyaline, cruciform, four celled, with one large upper cell, one basal cell and two lateral cells. The basal and two lateral cells showed the presence of bristle like appendages. The conidial size ranged from 16.36-22.70 μm in length and 10.37-14.65 μm in width averaging (19.56 \times 12.51 μm). The pathogen was very slow growing with 18-21.5 mm radial growth as recorded after 45 days of incubation.

Table 1: Morpho-cultural characteristics of *Entomosporium maculatum* Lev. causing leaf and fruit spot disease of pear

Thallus part	Shape and character	Colour	Size	Septation
On host				
Acervulus	Discoid, Solitary, embedded in host tissue, partially becoming erumpent at maturity.	Black	110-200µm (154.62µm)	-
Conidia	Cruciform, Four celled, with one large apical and basal cell and two lateral cells, possessing bristle like appendages at basal and at two lateral cells.	Hyaline	14.46-20.68 × 9.42-12.46µm (Av. 17.57 × 10.94)	Septate
Mycelium	Hyphae branched, smooth, thick walled	Hyaline	8.25-8.75 µm Av. 8.48µm	Septate
In culture				
Colony	Initially compact with tufts of aerial mycelium, becoming glistering and glabrous	Initially whitish changing to Reddish brown	18-21.5mm in dia.	
Mycelium	Hyphae branched, smooth, thick walled	Hyaline	8.54-8.98 µm Av. 8.76 µm (width)	Septate
Acervulus	Discoid, Solitary, partially embedded in culture medium.	Grayish black	130-240µm (Av 167.48µm)	-
Conidia	Cruciform, four celled, Ornamented with droplet like structures, with large basal and upper cell and two small lateral cells, appendages at two lateral cells and at basal cell	Hyaline	16.36-22.76 × 10.37-14.65µm (Av. 19.56X 12.51µm)	Septate

**Fig 1:** Bulged acervuli on disease spots**Fig 2:** Small, irregular lesions on fruit**Fig 3:** Conidia of the fungus**Fig 4:** Leaf blighting

The characteristic symptoms of the disease as observed under natural conditions were identical with those described by Anderson (1956) [1] and Mendoza and Ortiz (1984) [8]. The characteristic features of the isolated fungus were compared with the authentic descriptions given by Stathis and Plakidas (1959) [10]; Horie and Hobayashi (1979) and Sutton (1980) [11] with which these characters fully corroborated and the fungus was identified as *Entomosporium maculatum* Lev. Since the perfect state of the fungus neither developed in culture nor was it observed on host during the period of studies indicating

that under existing environmental conditions the fungus reproduces asexually, however, the fungus has been found elsewhere to reproduce sexually and the perfect state identified as *Fabraea maculata* Lev. (Atk.).

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