Mycopath (2018) 16(2): 111-115 **Research Article**

Surveillance and characterization of *Botryosphaeria obtusa* causing frogeye leaf spot of Apple in District Quetta

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Abstract

Botryosphaeria obtusa (Schwein.) Shoem is an important plant pathogen that causes black rot, frogeye leaf spot and limb canker disease of apple. It is more prevalent in cooler temperate regions and causes substantial yield losses. A survey of different orchards in eight locations of the district Quetta was conducted during 2016 and 2017 for *B. obtusa* infection. The disease was 100% prevalent in all the surveyed locations. Maximum mean disease incidence was found in Killi Muhammad Hasni (22%) followed by Qambrani (19.5%), Hanna Orak (16%), Killi Gul Muhammad (15.5%), Kuchlak (12.5%) and Lohra Nullah (12%) while minimum in Chashma Achozai (8.5%) and Chiltan (7.5%). Recovered fungal isolates exhibited dark green colonies with moderate aerial mycelium growth that changed to dark brown at maturity. Isolates produced small, single, dark brown to black coloured pycnidia. Rounded large, dark brown to black coloured, aseptate conidia (25 to 26.8 × 10.5 to 12.03 µm) were observed on these pycnidia. Based on cultural appearance and microscopic studies, recovered pathogen was identified as *Botryosphaeria obtusa* and was reconfirmed by pathogenicity tests on apple cv. Golden Delicious seedlings. This is the first report of *B. obtusa* infection on apple from Pakistan and results of the present studies can serve as basis of management strategies against this disease.

Keywords: Frogeye leaf spot, *Botryosphaeria obtusa*, disease assessment.

Introduction

Apple is an important fruit grown in all temperate regions of the world. It is a pomaceous fruit of the apple, species *Malus domestica* in the rose family Rosaceae (Potter *et al.,* 2007). In Pakistan, climatic conditions of Balochistan, Khyber Pakhtunkhwa, Azad Jammu and Kashmir and Gilgit-Baltistan are suitable for apple production. Balochistan accounts for 34% of the country's apple production (0.52 million tons) from an area of 88 thousand hectares (GOP, 2016). Apple is grown in 17 districts of Balochistan *viz*. Killa Saifullah, Zhob, Mastung, Pishin, Killah Abdullah, Ziarat, Quetta, Loralai, Khuzdar, Kalat, Barkhan, Kohlu, Musa Khail, Sherani, Harnai and Panjgur (Wahid, 2001; GOB, 2014).

Apple is highly nutritive fruit containing essential food elements such as sugar 11 %, fat 0.4 %, protein 0.3%, carbohydrates 14.9% and vitamin C, A and B in a balanced form (Amiri *et al.,* 2008). It ranks 3rd in consumption after citrus and banana and is almost available throughout the year. Compared to many other fruits and vegetables, apples contain a relatively low amount of vitamin C but are a rich source of other antioxidant compounds (Boyer and Liu, 2004). Owing to its high nutritive value, apple is prone to several biotic and abiotic diseases. Among biotic diseases, fungal pathogens especially *Botryosphaeria obtusa* is one of the prevailing issues for apple farming. It causes black rot of fruit, frogeye leaf spot and limb canker diseases on Apple (Arauz and Sutton, 1990; Phillips, 2007). Foliage infections can occur as early as silver tip.

Named for its symptoms, this pathogen can cause severe losses especially in cooler temperate regions where apples are grown. Tiny, purplish to reddish brown flecks appear on apple leaves as early infection and enlarge to circular brown lesions surrounded by purple radiance and leaves become chlorotic and abscise. Fungal reproductive structures (pycnidia) may develop in spot centers; visible as tiny black specks but may best be seen with a hand lens. Pycnidia are filled with spores that are the source of continued infections. Lesions on heavily infested leaves appear irregular in shape and dark brown to black in colour (Venkatasubbaiah *et al.,* 1991). Apple trees exposed to low-temperature injury and drought resistance are more vulnerable to be attacked by *B. obtusa* (Fathi and Tari, 2016). The fungus normally infects apple plants through pruning that is a common cultural practice to cope with fire blight disease of apple (Sutton, 1991).

A preliminary survey for reporting prevailing fungal pathogens in apple growing orchards revealed the significance of the disease however, no literature concerning disease loss assessment or pathogen characterization was found from Pakistan. The objective of this study was to report field susceptibility to frogeye leaf spot on Apple. An

extensive survey of major apple growing orchards of district Quetta was conducted for disease assessment. We've also characterized *Botryosphaeria obtusa* isolates recovered from symptomatic samples of apple leaves.

Materials and Methods

Studies on disease documentation of *Botryosphaeria obtusa* on apple were made in district Quetta which includes several orchards in locations *viz*.; Killi Muhammad Hasni, Qambrani, Lohra Nullah, Chashma Achozai, Hanna Orak, Killi Gul Muhammad, Chiltan and Kuchlak. Quetta district is situated between 30.1798°N and 66.9750°E with an elevation of 1,679 m above sea level.

Apple orchards are found on scattered locations in the district. Details on different locations surveyed from the district for is given in Table 1. A comprehensive two-years survey (2016 and 2017) was conducted in an x-plus manner at leaf emerging (March-April) and fruit maturity stage (July-September). Disease prevalence and incidence percentage were calculated in 40 different orchards using formula:

$$Disease\ prevalance\ (\%) = \frac{Locations\ exhibiting\ disease}{Total\ locations\ examined} \times 100$$

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

Symptomatic leaves were collected in paper bags, properly labelled and brought to Fungal Plant Pathology Lab. Department of Plant Pathology, PMAS Arid Agriculture University Rawalpindi for pathogen isolation and identification.

Infected portions of the leaves were cut into 5 mm segments, surface sterilized with 0.5% sodium hypochlorite and 10% ethanol, rinsed thrice with sterile distilled water, and allowed to dry on sterilized filter paper in the laminar-flow chamber. These surface disinfected pieces of infected leaves were plated on potato dextrose agar (PDA) medium in 9 cm Petri dishes and were kept in incubation chamber (25 °C) for two weeks. Pure cultures were maintained using a single spore culture technique on half strength PDA medium and placed under fluorescent light (12 h day^{-1}). Sporulation was induced by maintaining the isolates on 2% water agar medium incubated at 25 °C in a 12 h light and dark cycle. Cultural characteristics and microscopic studies of the recovered pathogen were made on seven days old pure culture of the fungus.

For the pathogenicity, freshly growing culture of *B. obtusa* was scrapped to a bottle containing 10 mL of sterile distilled water, shaken by hand to suspend the conidia. Hemocytometer was used to adjust the concentration of the conidial suspension to 1×10^5 conidia mL^{-1}. Young seedlings of apple (cv. Golden Delicious) having 8–10 fully unfolded leaves were artificially inoculated with the conidial suspension (1×10^5 conidia mL^{-1}). A set of seedlings inoculated with sterile distilled water were used as control. All seedlings were sealed in polythene bags containing a moist cotton plug to maintain humidity and were placed in controlled conditions (24 ±2 °C). Two days after inoculation, plants were unbagged and were maintained in the greenhouse. Two weeks after inoculation plant leaves were observed for disease development using disease rating scale; 1=0-3% of leaf surface lesions, 2 = 4-6%, 3 = 7-12%, 4 = 13-25% and 5 = 26-50% (Barratt & Horsfall, 1945).

Results and Discussion

Disease leaves showed circular, purple brown flecks with tan or brown centers giving frog eye appearance (Fig. 1). The disease was 100% prevalent to all the locations surveyed however showed variations in disease incidence. In the year 2016, maximum disease incidence was recorded in all 8 orchards of Killi Muhammad Hasni (23%) followed by Qambrani (19%) Hanna Orak (17%), Killi Gul Muhammad (15%), Kuchlak (13%) and Lohra Nullah (11%) while minimum in Chasma Achozai and Chiltan (7 and 9% respectively). In the year 2017, maximum disease incidence was recorded in all 8 orchards of Killi Muhammad Hasni (21%) followed by Qambrani (20%) Hanna Orak (15%), Killi Gul Muhammad (16%), Lohra Nullah (13%), Kuchlak (12%) while minimum in Chasma Achozai (8%) and Chiltan (7.5%). Overall, maximum mean disease incidence was found in Killi Muhammad Hasni (22%) followed by Qambrani (19.5%) while minimum in Chiltan (7.5%) as shown in Table 2.

Fungal isolates recovered from symptomatic leaf portions on PDA medium were morphologically characterized according to the descriptions (Taylor *et al.,* 2005; Úrbez-Torres *et al.,* 2006). Isolates purified using single spore culture technique on fresh PDA medium at temperature 25 °C showed hyphal growth on the second day of inoculation. Dark green colonies with moderate aerial mycelium growth were observed which changed to dark brown in colour at maturity. All isolates produced small, single, dark brown to black coloured pycnidia. These pycnidia produced rounded large, dark brown to black coloured, aseptate conidia. The size of these conidia ranged from 25 to 26.8 × 10.5 to 12.03 µm. Based on colony morphology and conidial characteristics, these isolates were identified as *Botryosphaeria obtusa.*

Recovered isolates of *B. obtusa* were pathogenic to all Golden Delicious seedlings used in this study except control. Symptoms appeared as brown necrotic lesions on leaves 2 days after inoculation. The culture filtrated induced necrosis on apple leaves. The pathogen was reisolated from these leaf infections and was confirmed as *B. obtusa* based on its morphology compared with the reference isolates of the original infections in the field.

In the present study, 40 orchards of apple in Quetta district were surveyed for *Botryosphaeria*

obtusa infection. Apple orchards are found at the scattered location in this district with varying level of infections. Maximum mean disease incidence was found in Killi Muhammad Hasni (22%) while minimum in Chiltan (7.5%). The optimum temperature for *B. obtusa* leaf infections is around 26.6 °C with 4.5 hours of leaf wetness. The risk of infections is low when temperatures are below 10 °C (more than 24 hours of leaf wetness required), and none occur below 8 °C (Arauz and Sutton, 1990). Quetta district falls in a temperate region and features a continental arid climate with a significant variation between summer and winter temperatures maximum 42 °C and minimum −18.3 °C. Quetta usually receives snow in December and January and also affected by drought spell resulted from very low rainfall especially in the winter season (USAID, 2009)**.** These conditions also favour *B. obtusa* leaf infections. Fire blight caused by bacterium *Erwinia amylovora* is also one of the prevalent diseases of apple in this district. This also favours the infection of *B. obtusa*. This is also well accepted that farmers are not progressive and rely on traditional cultural practices. *B. obtusa* may also infect apple plants through pruning (Sutton, 1991). The virulence of *B. obtusa* in our study suggests that it is a capable pathogen of apple in Quetta and can be a serious problem for apple production. These findings are consistent with previous reports suggesting physiological stresses including low temperature, drought and predispose trees to canker favours the infection of *Botryosphaeria* spp. (Schoeneweiss, 1975, 1978). All fungal isolates recovered on PDA medium exhibited the typical symptoms of *Botryosphaeria obtusa*. Colony morphology appeared as dark green which changed to dark brown at maturity. A moderate aerial mycelium growth was observed. Sporulation was observed by growing isolates on water agar medium. Dark brown to black, aseptate conidia (25 to 26.8 × 10.5 to 12.03 µm) were observed on small, single, dark brown to black pycnidia. These descriptions were in line with the findings of Phillips *et al.* (2007), Swart and Botes (1995), and Venkatasubbaiah *et al.* (1991). Conidia are the most infective unit of *B. obtusa.* These conidia are produced throughout the year and are present on dead bark of the apple tree. Under optimum temperature and moisture conditions, these conidia become infective and cause foliage infections (Smith and Hendrix, 1984). To best of our knowledge, this is the first report of *Botryosphaeria obtusa* causing frogeye leaf spot of apple in Quetta and may be a potential threat to apple production.

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Table 1: Locations of Apple orchards surveyed for forgeye leaf spot in district Quetta.

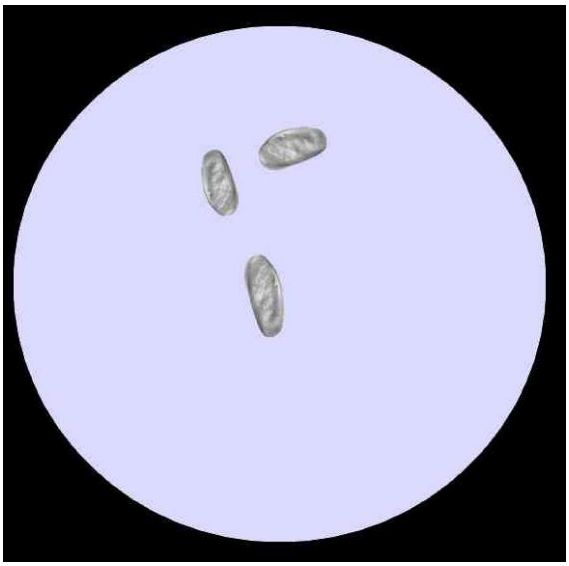
S. No.	Locations	Coordinates	Number of Orchards
1	Killi Muhammad Hasni	30°01'01.1"N 66°57'46.9"E	8
2	Qambrani	30°08'01.2"N 66°58'06.3"E	3
3	Lohra Nullah	30°09'08.2"N 66°57'50.2"E	4
4	Chiltan	30°15'55.4"N 66°58'41.2"E	3
5	Chashma Achozai	30°16'15.7"N 66°58'28.7"E	5
6	Hanna Orak	30°14'46.5"N 67°07'03.4"E	8
7	Killi Gul Muhammad	30°15'01.0"N 66°59'12.9"E	4
8	Kuchlak	30°22'44.9"N 66°58'13.4"E	5

Table 2: Disease prevalence and mean disease incidence of frogeye leaf spot of apple in various locations of district Quetta.

S. No.	Locations	Disease Prevalence (%)		Disease Incidence (%)		
		2016	2017	2016	2017	Mean
1	Killi Muhammad Hasni	100	100	23	21	22
2	Qambrani	100	100	19	20	19.5
3	Lohra Nullah	100	100	11	13	12
4	Chiltan	100	100	7	8	7.5
5	Chashma Achozai	100	100	9	8	8.5
6	Hanna Orak	100	100	17	15	16
7	Killi Gul Muhammad	100	100	15	16	15.5
8	Kuchlak	100	100	13	12	12.5

Fig. 1: Apple leaf showing symptoms of frogeye leaf spot.

Fig. 2: *Botryosphaeria obtusa* spores.



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