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Morphology, pathogenicity and physiology of *Ceratocystis fimbriata* causing black rot disease of *Colocasia esculenta*

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Arvi (*Colocasia esculenta*) is widely cultivated sweet and starchy corms as a staple food in most of the African and Asian countries. Black rot caused by *Ceratocystis fimbriata* is the most damaging post-harvest disease of Arvi in many countries. In June, 2019, the black rot disease symptoms were noticed on edible corms of Arvi for sale in different vegetable shops in Faisalabad, Pakistan. These symptoms were comprised of small, brown to greenish, slightly sunken spots or lesions which later on enlarged as black rot on the harvested corms. A comprehensive study was initiated to explore the pathogen associated with symptomatic corms. White to grey mycelia and black perithecia with long necks were observed within the lesions. The isolated pathogen was identified as *Ceratocystis fimbriata* based on morpho-physiological characteristics. Pathogenicity was proved on inoculated corms at 26±2°C and 70% relative humidity (RH) in plant growth chamber. The fungus was further tested for growth and reproduction physiology under different growth media and environmental parameters. A significant interaction between mycelial growth and culture media was observed. Highest mycelial growth (9 cm) of *C. fimbriata* was showed by Potato Carrot Agar (PCA) and lowest growth (0%) was observed on Water Agar (WA). There was a significant correlation between Temperature, RH, and hydrogen ion (pH) concentration responsible for the growth of *C. fimbriata* at ($P < 0.01$). The optimum fungal growth (8.75, 8.96, and 8.95 cm) was measured at 25°C, 70% RH, and pH 8 respectively. The paper may act as basic resource for future research on the epidemiology of this disease.
Keywords: Arvi, post-harvest disease, morphology, growth media, physical parameters.

INTRODUCTION

Arvi or Taro (*Colocasia esculenta*) is widely cultivated sweet and starchy corms as a staple food in most of the African and Asian countries (Jianchu *et al*., 2001, Vinning, 2003 and Atiquzzaman *et al*., 2008). It has been ranked as the fifth most widely consumed root vegetable (Rao *et al*., 2010). About 145 000 tonnes per annum of world trade in Arvi is estimated (Vinning, 2003). It is a good source of industrial starch, fibres and ash. It is also a good source of carbohydrates, flavonoids, thiamine, copper, iron, manganese, phosphorus, potassium, zinc, and vitam B6 and C (Quach *et al*., 2001).

Post-harvest diseases cause 10-30% crop losses and in some perishable crops, especially in developing countries, losses up to 30% have been assessed (Kader, 2002; Agrios, 2005). The earliest report of black rot of Arvi dates back in 1939 in Japan (Shimizu, 1939). The black rot of Arvi is a significant post-harvest disease caused by *Ceratocystis fimbriata* in Brazil (Harrington *et al*., 2005) and China (Huang *et al*., 2008).

Ceratocystis fimbriata is the most destructive soil-borne pathogen globally, with a broad host range (Oliveira *et al*., 2018). It has been associated with various horticultural crops: *Alocasia* sp., *Ipomoea batatas*, *Manihot esculenta* and *C. esculenta* (CABI, 2005, Roux and Wingfield, 2009). *C. fimbriata* is well known pathogen osn pomegranate, loquat, eucalyptus, mango and shisham trees in Pakistan (Alam *et al*., 2018 and Ul Haq *et al*., 2019), but there is no literature available on this fungus causing black rot of Arvi in the country.

In June 2019, black rot disease symptoms were noticed on edible corms of Arvi for sale in different vegetable shops in Faisalabad, Pakistan. A *Certacystis* sp., later identified as *C. fimbriata*, was found sporulating on diseased corms when examined under a stereoscope. Studying fungi and how they respond to their environment to cause disease is the subject of significant concern.

The knowledge of conditions that favours fungal growth and sporulation is important in microbiology and plant pathology. Temperature, relative humidity, and Hydrogen ion

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concentration are the main players in growth, reproduction and survival of the microorganisms (Brito *et al*., 2021). Physiological studies on fungi have played an important role in agriculture, industry, medicine and industrial production of vitamins (Madan and Thind, 1998). Knowing how fungi interact with their growing environment is a key to control it (Walker and White, 2017). The more we understand physiology, the better we can utilize it and prevent its harmful activities. Like all other living organisms, fungi also require some vitamins and other growth factors to perform different life activities. They derive their nutrition from their hosts utilizing degradation of organic substance(s), symbiosis, or some others in the natural environment (Madan and Thind, 1998). However, to culture them in the laboratory, there is no universal growth media upon which all kinds of fungi can grow and reproduce well (Sonyal *et al*., 2015b: Gururaj *et al*., 2017) Physiological studies of fungi can be carried out on different growth media (Madan and Thind, 1998). Morphological characteristics of fungi vary significantly with environmental conditions (Kumara and Rawal, 2010). This kind of information provides a strong background to understand the survival ability of fungi under different environments and soil types with various pH levels. The objective of the study was to identify *Ceratocystis* sp. associated with rotted corms based on cultural morphology to study its physiology. The paper may act as basic resource for future research on the epidemiology of this disease.

MATERIALS AND METHODS

***Sample collection*:** A total of 120 black rot suspected corm samples were collected by visiting different sales in distribution centres, vegetable shops, farmer markets, and supermarkets in five Punjab districts (Bahawalpur, Faisalabad, Lahore, Multan, and Sahiwal) in 2019. Initial symptoms were small circular brown to greenish-black spot or slightly sunken lesions on the infected corms' surfaces. With disease progression these spots become enlarged, and dark brown to black. The rot was usually firm and dry that was often restricted to the cortex. White to grey mycelia was observed near the central portion of the lesion. Within those growth blackish perithecia, a diagnostic sign of black rot disease caused by *Ceratocystis* spp., were easily observed with a hand lens. In most of the infected corms (45%), superficial black rot, a few millimetres deep with pink to orange discoloration in the starchy interior, has been noticed (Fig 3). Samples were placed in plastic bags, labelled, and kept on an ice box until they could be brought to the Fungal Molecular Biology Laboratory (FMB) in Plant Pathology, University of Agriculture, Faisalabad, and stored at 4^{o}C until ready for the user.

Isolation and Purification of pathogen: Naturally infected samples were then tested for the presence of suspected pathogen(s). The samples were thoroughly washed under running tap water, surface sterilized with 70% ethanol and air-dried on sterile tissue towels in the laminar cabinet. The samples were placed in moist chambers at room temperature (25°C) for 3-4 days to induce sporulation directly on infected tissue. After 7-10 days of incubation, a fragrant banana odour was produced by naturally infected corms. Blackish perithecia, fungal fruiting bodies, were removed and directly transferred to Potato Dextrose Agar (PDA) medium supplemented with rifampicin (0.4 mg/mL) using a stereoscope. The fungus was further purified by sub-culturing using single hyphal tip technique (Narayanasamy 2001).

Identification of the Ceratocystis sp.: Isolated fungus was identified using morphological characters including colony colour, type of spores produced and size of perithecia as Engelbrecht and Harrington, 2005 and Firmino *et al*. 2012; 2013 described. Samples were microscopically examined by mounting various fungal structures on glass slides. Observations of microscopic structures were made using the Nikon SMZ 18 stereo microscope and Nikon Eclipse Ni compound microscope with differential interference contrast (DIC) illumination. All the *C. fimbriata* isolates were morphologically identical, thus one representative isolate was selected from each district for pathogenicity trials.

***Pathogenicity test*:** The pathogenicity test was proved on fresh healthy corms. These corms were surface sterilized with 70% ethanol. The representative isolate(s) were cultured on PDA for five days at 26±2°C, spores were collected by scratching the fungal mat by adding sterile water and spore suspension was adjusted at 1.0×10^5 spores/mL using a hemocytometer. Artificial wounds were made using a sterile needle and 20 µL of spore suspension was inoculated into these wounds, whereas control corms were inoculated with 20 µL of distilled water only (Paul *et al*., 2018). The inoculated corms were kept in a plastic box and incubated at 26±2°C and 70% relative humidity (RH) in the plant growth chamber to allow symptoms development. The experiment was repeated twice with three replications (Paul *et al*., 2018). The corms were monitored at various time points to develop disease symptoms such as brown to black spot or slightly sunken lesions, and fruiting bodies called perithecia.

***Physiological studies*:** For the physiological studies of *C. fimbriata* an array of agar media was used (Table 1). The respective medium was poured (20 mL) into Petri plates having a 9 cm diameter. On solidification, cultures were inoculated with 7-days old pure culture of test fungus. The experiment was arranged under Completely Randomized Design (CRD) in five replicas and the Tukey HSD test was used for pairwise comparison of different media. The plates were incubated at 26±2^{o}C and 70% RH. Four observations were recorded with three-day intervals until any of the plates were entirely covered with the fungal growth. Data regarding different physiological parameters were recorded when culture(s) were fully matured.

Table 1. The composition of growth media used.

Growth medium	Abbre-viation	Composition (/litre of distilled water)
Potato Dextrose Agar	PDA	200g infusion from Potato, 20g Dextrose and 15g Agar
Potato Carrot Agar	PCA	200g infusion from Potato, 200g infusion from Carrot, and 15g Agar
Corn Meal Agar	CMA	50g infusion from Corn meal and 15g Agar
Yeast Extract Agar	YEA	5g Peptic digest of animal tissue, 3g Yeast extract and Agar 15g
Malt Extract Agar	MEA	30g Malt extract, 5g Mycological Peptone and 15g Agar
Yeast Malt Extract Agar	YMEA	20g Malt extract, 5g Peptone, 2g Yeast extract and 15g Agar
Oat Meal Agar	OMA	60g Oat meal and 12.5g Agar
Oat Dextrose Agar	ODA	20g Oat, 15g Dextrose and 15g Agar
Water Agar	WA	15g Agar

***Effect of temperature, relative humidity (RH) and hydrogen ion (pH) concentration on fungal growth*:** To evaluate mycelial growth of *C. fimbriata* at different temperature, Relative humidity, and hydrogen ion concentration levels, three different experiments were conducted using 5 treatments and 4 replicates for each factor, each replicate being composed of one Petri dish (9 cm diameter) for each level of temperature, relative humidity and hydrogen ion activity. PDA plates having 20 mL of media were inoculated with mycelial disks of 5 mm in diameter of *C. fimbriata*. The treatments for temperature, relative humidity and hydrogen ion activity were (10, 15, 20, 25 and 30ºC), (30, 40, 50, 60 and 70%), and (5, 6, 7, 8 and 9) respectively. The experiment was arranged under completely randomized design (CRD) in four replicas per treatment and incubated in the growth chamber. Five observations were made with three days interval. The data were analyzed using Statistical Software (IBM SPSS Statistics version 22).

RESULTS

***Fungal isolation*:** Isolation from symptomatic tissues yielded 15 isolates of *Ceratocystis* sp. The overall percentage of isolation of *Ceratocystis* sp. was 75%, and rest were saprophytic fungi like *Aspergillus* (19%), and *Rhizopus* (6%). The *Ceratocystis* sp. isolated from black rot infected corms was identified as *Ceratocystis fimbriata* based on cultural and conidial morphology.

***Identification of Ceratocystis sp.*:** A total of 5 isolates, one representative from each district, was selected to study morphology of the fungus. 7-10 days fresh fungal cultures were used to measure morphological parameters. The fungal mycelia were initially white and gradually turned to greenish or greyish; pure colonies were olivaceous brown, producing fruity aroma and cylindrical, single-celled conidia. Perithecia were black, globose and measured 237.30 x 233.70 µm and had a 729 µm long rostrum (neck) ending in ostiolar hyphae. Ascospores were hat-shaped (3.62 x 4.68 µm), cylindrical endoconidia were 17.80 x 3.93 µm whereas Barrel-shaped endoconidia were present in chains measuring 7.95 x 6.89 µm and aleurioconidia were brown (12.43 x 9.50 µm). Ostiolar hyphae were smooth-walled, divergent, hyaline and non-septate (Engelbrecht and Harrington, 2005 and Firmino *et al*. 2012;2013). All the *C. fimbriata* isolates were morphologically identical, thus one representative isolate was selected from each district for pathogenicity trials (Fig 3).

***Pathogenicity and symptoms development*:** The inoculated corms began to show black rot symptoms in the second week of inoculation in the form of a small circular brown to black spot followed by superficial black rot. The fungus was re-isolated from 100% corms inoculated with *C. fimbriata* isolates. All morphological characteristics expected for isolates classified as *C. fimbriata* were observed for all isolates recovered from inoculated corms. Corms inoculated with sterile water remained symptomless throughout the experiment.

***Physiological studies*:** Morpho-cultural characteristics of *C. fimbriata* on nine different substrates were studied (Table 1, 2, Fig 1). Observations revealed that the physiological behavior of the test fungus varies significantly on various substrates. Test fungus varies in physical appearance and type of spore(s) produced on different media. However, opacity and elevation of the colonies on all the test media were alike.

Table 2. Morpho-taxonomic characteristics of *C. fimbriata* on different growth media

Growth medium	Size (cm)	Form	Elevation	Margin	Color	Surface	Opacity	Sporulation		
								A	B	C
PDA	7.32	Irregular	Flat	Undulate	Greyish	Smooth	Transluscent	+	+	+
PCA	9.00	Irregular	Flat	Undulate	Greyish	Wrinkled	Transluscent	+	+	+
CMA	7.72	Irregular	Flat	Lobate	Greyish, brown centers	Smooth	Transluscent	+	+	+
YEA	6.16	Filamentous	Flat	Undulate	Light grey	Rough	Transluscent	+	+	_
MEA	8.04	Rhizoid	Flat	Undulate	Greyish	Smooth	Transluscent	+	+	+
YMEA	7.42	Irregular	Flat	Undulate	Olivaceous green	Smooth	Transluscent	+	+	+
OMA	8.76	Irregular	Flat	Undulate	Olivaceous green	Smooth	Transluscent	+	+	+
ODA	6.70	Irregular	Flat	Undulate	Olivaceous green	Smooth	Transluscent	+	+	+
WA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	_	_	_

Sporulation: A=Endoconidia, B=Aleurioconidia, C=Perethecium production

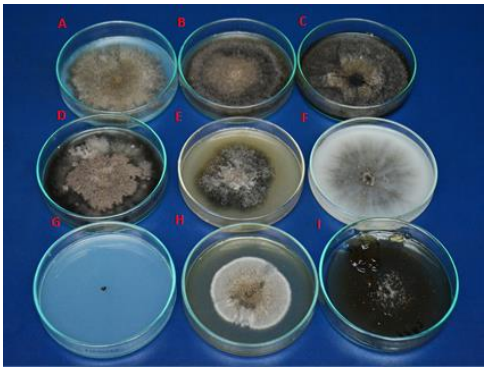

Figure 1. A) PDA, B) MEA, C) PCA, D) CMA, E) OMA, F) ODA, G) WA, H) YMEA, I) YEA

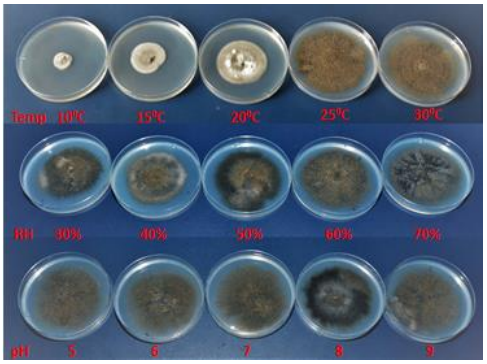

Figure 2. Effect of different treatment levels of temperature (1st row), Relative Humidity (2nd row, RH) and Hydrogen Ion (3rd row, pH)

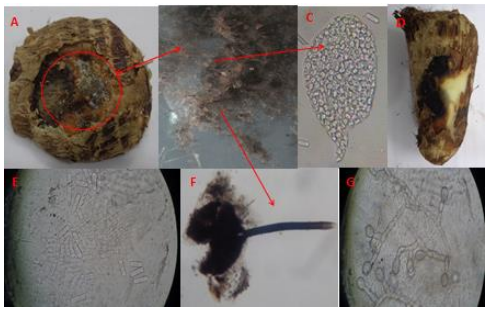

Figure 3. A) Corm of *C. esculenta* with white to gray, sporulating areas of *C. fimbriata* B) White mycelia and black perithecia as observed on the surface of a black rot lesion C) Figure 3: Sticky drops containing masses of ascospores D) A dry, black rot lesion on corms, typical of black rot, in the starchy tissue below sporulation by *C. fimbriata* E) Cylindrical conidia of *C. fimbriata* F) Perithecium of *C. fimbriata* along with long neck G) Aleuroconidiophores with aleuriconidia.

A significant interaction between mycelial growth and culture media was observed. Maximum colony size of 9 cm was obtained from PCA, followed by OMA (8.76 cm), MEA (8.04 cm), on CMA (7.72 cm), YMEA (7.42 cm) and PDA (7.32 cm) growth was moderate, ODA (6.7 cm) and YEA (6.16 cm) were least effective and no growth at all was observed on WA. The fungus produced Endoconidia, Aleurioconida and Perithecia on all the media except YEA, where it fails to produce perithecia. However, PDA, PCA, MEA, OMA and CMA produced abundant uniformly dense mycelia with numerous perithecia. Only PDA, MEA, PCA, CMA, ODA and OMA produced banana-like aroma, typical of *C. fimbriata*, while others could not produce it.

Effect of temperature, relative humidity (RH) and hydrogen ion (pH) concentration on fungal growth: The effect of different media on the growth of *C. fimbriata* significantly varies from each other. Multiple mean comparisons test revealed that WA, YEA and ODA significantly differ from all other media. PDA, YMEA and YE were not substantially different from each other. YE and MEA were also non-significant (Table 3).

Table 3. Tukey HSD test for Multiple Mean Comparison among different treatment levels of media on *C. fimbriata* growth

		Tukey HSD[a]					
Media	**N**	**Subset for alpha = 0.05**					
		1	**2**	**3**	**4**	**5**	**6**
WA	5	0.000					
YEA	5		6.160				
ODA	5			6.700			
PDA	5				7.320		
YMEA	5				7.420		
CMA	5				7.720	7.720	
MEA	5					8.040	
OMA	5						8.760
PCA	5						9.000
Sig.		1.000	1.000	1.000	0.059	0.228	0.593

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 5.000.

Table 4. Tukey HSD test for Multiple Mean Comparison among different treatment levels of temperature on *C. fimbriata* growth.

		Tukey HSD[a]		
Temperature (ºC)	**N**	**Subset for alpha = 0.05**		
		1	**2**	**3**
10	4	1.950		
15	4	2.300		
20	4		4.450	
30	4			8.275
25	4			8.750
Sig.		0.614	1.000	0.334

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 4.000.

Table 5. Tukey HSD test for Multiple Mean Comparison among different treatment levels of Relative Humidity (RH) on *C. fimbriata* growth.

Tukey HSD[a]					
RH (%)	**N**	**Subset for alpha = 0.05**			
		1	**2**	**3**	**4**
30	4	4.850			
40	4		6.600		
50	4			8.275	
60	4			8.575	
70	4				8.975
Sig.		1.000	1.000	0.103	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 4.000.

Table 6. Tukey HSD test for Multiple Mean Comparison among different treatment levels of Hydrogen Ion (pH) on *C. fimbriata* growth.

Tukey HSD[a]			
PH	**N**	**Subset for alpha = 0.05**	
		1	**2**
5	4	8.575	
6	4	8.650	8.650
9	4	8.850	8.850
7	4		8.925
8	4		8.950
Sig.		0.081	0.051

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 4.000.

Table 7. Correlation among different treatment levels of pH, temperature and RH on *C. fimbriata* growth (N=20).

		PH	**Temp.**	**RH**
PH	Pearson Correlation	1.000	1.000**	1.000**
	Sig. (2-tailed)		0.000	0.000
Temperature	Pearson Correlation	1.000**	1.000	1.000**
	Sig. (2-tailed)	0.000		0.000
RH	Pearson Correlation	1.000**	1.000**	1.000
	Sig. (2-tailed)	0.000	0.000	

**. Correlation is significant at the 0.01 level (2-tailed).

There was a significant difference between varying levels of temperature (Table 4, Fig 2), relative humidity (Table 5, Fig 2), and hydrogen ion (pH) concentration (Table 6, Fig 2). Temperature is a significant factor concerning fungal growth. The fungus tends to grow at all temperatures that were evaluated. However, its growth was very poor at 10 and 15°C, moderate at 20°C and Optimum at 25 and 30°C. Similarly, fungus grew well at all levels of relative humidity. However, low fungal growth was noticed at 30 and 40%, moderate at 50% and optimum at 60 to 70% relative humidity. Mycelial production was low at pH level 5, moderate to high at levels 6 and 9 and optimum at levels 7 and 8. There was a significant correlation between Temperature, relative humidity, and pH responsible for the growth of *C. fimbriata* (Table 7).

DISCUSSION

Results of field observations, pathogenicity assay, and morphology of the isolates have shown that *Ceratocystis fimbriata* is associated with black rot disease of Arvi in Pakistan. *C. fimbriata* grew and sporulated over a wide range of growth medium, temperature, relative humidity and hydrogen ion concentration (pH). The highest mycelial growth, 9 cm, was measured on potato carrot agar. No growth or sporulation, 0%, was observed on water agar. Fungal sporulation (Endoconidia, Aleurioconida and Perithecia) was observed on all the media except YEA, where it could not produce perithecia. The optimum fungal growth, 8.75 cm, occurred at 25°C and lowest growth, 1.95 cm, was observed at 10°C. Mycelial production was optimum (8.96 cm) at 70%, and lowest (4.85 cm) at 30% relative humidity. Growth and sporulation of *C. fimbriata* also affected by the pH of the medium. However, a very small difference in pH was observed for mycelial growth. The Optimum and lowest growth was 8.58 and 8.95 cm at pH 8 and 5 respectively. The research showed that there was a significant correlation between temperature, relative humidity, and pH responsible for the growth of *C. fimbriata* at ($P < 0.01$).

In our efforts to fulfil Koch's postulates and to identify the causal agent associated with rotted corms, we collected naturally infected corms from various sales in distribution centres, vegetable shops, farmers markets, and supermarkets of five districts of Punjab. Our findings revealed that black rot is present in most of the locations visited. We analysed morphological characteristics of the fungus to confirm its identification. The combined data analysis confirmed the species identification as *C. fimbriata*, and are consistent with Harrington *et al*., 2005; Firmino *et al*., 2012; Oliveira *et al*., 2015; Li *et al*., 2019, who reported similar morphology of *C. fimbriata* causing black rot disease on different hosts. For the confirmation of the associated fungus, *C. fimbriata*, Koch's postulates were completed on healthy corms by inoculating 20 µL of spore suspension into the wounded area. Our study confirms the association of *C. fimbriata* with black rot of Arvi in Pakistan. Similar results have been reported on different hosts of the family Araceae by other workers (Harrington et al., 2005; Thorpe et al., 2005; Huang et al., 2008; Paul et al., 2018).

C. fimbriata causes wilt and cankers on woody hosts and rots diseases of storage roots and corms of economically important plants worldwide (Thorpe *et al*., 2005). It has been reported in Japan (Shimizu, 1939), Brazil (Harrington *et al*., 2005) and China (Huang *et al*., 2008) causing the black rot of

Taro. Thorpe *et al*., 2005 found that *C. fimbriata* is pathogenic to Arvi and causes black rot disease. Other important horticultural crops affected by black rot disease include *Alocasia sp*., *Arracacia xanthorrhiza, C. esculenta, Cucumis sativus, Ipomoea batatas, Manihot esculenta* and *Passiflora edulis* (CABI, 2005; Firmino *et al*., 2013; Melo *et al*., 2016; Paul *et al*., 2018 and Li *et al*., 2019).

Morphological features and the physiological behavior of culture-able fungus may vary under changing culture media and environmental conditions (Papagianni, 2004). The knowledge of conditions that favors fungal growth and sporulation is considered essential in Plant Pathology (Brito *et al*., 2021). Like all other living organisms, fungi also require some vitamins and other growth factors to perform different life activities. In general, fungi can use any carbon source as food, but to study the physiology of fungi, it is necessary to cultivate it on growth media. Hence, a comprehensive investigation was carried out on morphological variations, growth and sporulation physiology of *C. fimbriata* on different growth media and physical environmental factors. The experiment revealed that PCA, OMA, CMA, MEA, YMEA and PDA are suitable for growth and sporulation of this fungus. *C. fimbriata* produced sufficient mycelial growth on OMA and PDA medium (Sonyal *et al*., 2015b). Greater mycelial growth of the same fungus was recorded on ODA and MEA medium (Oliveira *et al*., 2015). MEA and YMEA produced good mycelial growth and maximum sporulation, while PDA's effect on mycelial growth and sporulation was moderate (Oliveira *et al*., 2016). Abundant production of endoconidia, aleurioconida and perithecia was found on OMA, MEA, PCA and PDA medium (Sonyal *et al*., 2015b and Gururaj *et al*., 2017). ODA and YEA were not suitable for mycelial growth and studying physiological parameters. The fungus did not grow at all on WA medium, this could be attributed to the un-availability of carbon source, glucose as the sole carbon source. Similar result has been obtained by Gururaj *et al*. (2017). Olutiola and Cole, 1977, working on *Ceratocystis paradoxa*, reported that glucose, sucrose and fructose increased fungal growth and sporulation. Flat type growth of *C. fimbriata* was obtained on MA, OMA, PCA and PDA. The physical characteristics of *C. fimbriata* vary significantly in different media (Gururaj *et al*., 2017). Among physical environmental factors that influence growth and sporulation in fungi, the temperature is a significant contributor that affects almost every fungus function, including sporulation (Madan and Thind, 1998). The growth of *C. fimbriata* increased with an increase in temperature and RH and vice versa. In the present work, maximum fungal growth was harvested at 25ºC. The optimum temperature for the growth of *C. fimbriata* is ranged from 24 to 26ºC (Kile, 1993 and Oliveira *et al*., 2015). According to Brito *et al*., 2021, the optimum average temperature mycelia growth and sporulation of *C. fimbriata* is 22.4 to 22.5ºC. The test fungus showed maximum growth at 70% RH and minimum at 30% RH. The growth of test fungus was not much different from various pH levels, indicating that the fungus can survive in all kinds of soils with varying pH concentrations. However, maximum mycelial production was observed at pH ranging from 7 to 8 (Sonyal *et al*., 2015a). Significant factors contributing to the growth and proliferation of *C. paradoxa* include pH, RH, and soil moistures (Yadahalli *et al*., 2010).

***Conclusion*:** The results of surveys, isolations, cultural morphology, and pathogenicity assay illustrate clearly that black rot of Arvi corms, caused by *C. fimbriata* is emerging post-harvest disease in Pakistan. By testing the effect of growth medium and physical environment on *C. fimbriata*, this study established that the hydrogen ion concentration, temperature, and relative humidity do indeed have a significant effect on fungal sporulation and physiology. Its presence in Pakistan is particularly alarming because the pathogen may pose serious health and food security risks. The paper may act as basic resource for use in host-pathogen interaction studies, and for future research on the epidemiology of this disease.

Authors' Contributions statement: Muhammad Zunair Latif devised the project, the main conceptual ideas and proof outline. MZL and Kaleem Sarwar conducted the survey, recorded data, and collected the material for write up. MZL took the lead in writing the manuscript with input from all authors. Imran ul Haq and Siddra Ijaz provided critical feedback and helped shape the research, analysis and manuscript.

Conflict of interest: All authors declared that they have no conflict of interest.

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REFERENCES

Agrios, G. N. 2005. Plant Pathology, Academic Press, New York.

Alam, M. W., A. Rehman, M. S. Haider, M. L. Gleason, H. Rosli, M. Saira, S. M. Khan, S. Aslam, M. A. Khan, A. Hameed and S. Sarfraz. 2018. First Report of *Ceratocystis fimbriata* Causing Wilt of Loquat in Pakistan. Plant disease 102:2034.

Atiquzzanmam, M., M. M. Ali, M. A. Mondal, M. Z. F. A. Begum and Q. Y. Akhter. 2008. Effect of spacing on the

growth and yield of 'Mukhikachu'. J. Agrofor. Enviro. 2:1-6.

Brito, N. M. D., H. D. S. S. Duarte, C. D. B. Buhner, C. G. Auer and A. F. D. Santos. 2021. Morphophysiological characterization of *Ceratocystis fimbriata* isolates from yerba mate. Ciencia Rural 51:..??

CAB International. 2005. *Ceratocystis fimbriata* (original text prepared by T.C. Harrington). In: Crop Protection Compendium. Wallingford, UK: CAB International. [http://www.cabi.org/cpc]. Accessed 05 April 2019.

Shimizu, M. 1939. Black rot of dasheen. Pusan Customs, Plant Inspection Rep. No.1., Japan.

Engelbrecht, C. J. B. and T. C. Harrington. 2005. Intersterility, morphology and taxonomy of *Ceratocystits fimbriata* from sweet Potato, cacao and sycamore. Mycologia 97:57-69.

Firmino, A. C., Q. S. D. Novaes, H. J. Tozze Jr, G. R. Sobrinho, A. Santos, J. L. Bezerra and E. L. Furtado. 2013. First report of *Ceratocystis fimbriata* causing fruit-rot of *Passiflora edulis* in Brazil. New Disease Reports 27:2044-0588.

Firmino, A. C., H. J. Tozze Jr, and E. L. Furtado. 2012. First report of *Ceratocystis fimbriata* causing wilt in *Tectona grandis* in Brazil. New Disease Reports 25:2044-0588.

Gururaj, S., Y. S. Amaresh, S. T. Yenjerappa, A. Amaregouda and A. G. Shreenivas. 2017. Cultural characteristics of *Ceratocystis fimbriata* Ell. and Halst. on different solid media causing wilt in pomegranate. Plant Archives 17:51-54.

Halsted B. D. 1890. Some fungous diseases of the sweet Potato. The black rot. New Jersey Agriculture Experiment Station Bulletin 76:7-14.

Harrington, T. C., D. J. Thorpe, V. L. A. Marinho and E. L. Furtado. 2005. First report of black rot of *Colocasia esculenta* caused by *Ceratocystis fimbriata* in Brazil. Fitopatologia Brasileira 30:88-89.

Huang, Q., Y. Y. Wang, Y. Y. Zhao, Y. X. Jiao, X. F. Li, H. R. Chen and Y. Y. Zhu. 2008. First report of taro black rot caused by *Ceratocystis fimbriata* in China. Plant Pathology 57:

Jianchu, X., Y. Yongping, P. Yingdong, W. G. Ayad and P. B. Eyzaguirre. 2001. Genetic diversity in Taro (*Colocasia esculenta* Schott, Araceae) in China: An ethnobotanical and genetic approach. Economic Botany 55:14-31.

Kader, A. A. 2002. Post-harvest technology of horticultural crops (Vol. 3311). University of California Agriculture and Natural Resources.

Kile, G. A. 1993. Plant diseases caused by species of *Ceratocystis* sensu stricto and Chalara. In: *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology and Pathogenicity. Ed. by Wingfield, M. J.; Seifert, K. A.; Webber, J. A. St. Paul, MN: APS Press, pp. 173–183.

Kumara, K. L. and R. D. Rawal. 2010. Influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya (*Carrica papaya* L). Tropical Agricultural Research and Extension 11:7-12

Li, X., K. C. Xu, Y. Zhang, H. Song, M. S. Xie, Y. X. Sun and Q. Huang. 2019. First Report of *Ceratocystis fimbriata* Causing Postharvest Fruit Rot of Cucumber in China. Plant Disease 103:1432-1432.

Madan, M. and K. S. Thind. 1998. Physiology of fungi. APH Publishing.

Melo, M. P., L. Pimenta, L. S. Oliveira and M. A. Ferreira. 2016. First report of *Ceratocystis fimbriata* causing black rot on *Arracacia xanthorrhiza* in Brazil. Plant Disease 100:652.

Narayanasamy, P. 2001. Plant pathogen detection and disease diagnosis. CRC press.

Oliveira, L. S. S., L. M. S. Guimaraes, M. A. Ferreira, A. S. Nunes, L. V. A. Pimenta and A. C. Alfenas. 2015. Aggressiveness, cultural characteristics and genetic variation of *Ceratocystis fimbriata* on Eucalyptus spp. Forest Pathology 45:505-514.

Oliveira, L. S. S., T. C. Harrington, M. A. Ferreira, R. G. Freitas and A. C. Alfenas. 2018. Populations of *Ceratocystis fimbriata* on *Colocasia esculenta* and other hosts in the Mata Atlantica region in Brazil. Plant pathology 67:97-106.

Oliveira, L. S., M. B. Damacena, L. M. Guimaraes, D. L. Siqueira and A. C. Alfenas. 2016. *Ceratocystis fimbriata* isolates on *Mangifera indica* have different levels of aggressiveness. European Journal of Plant Pathology 145:847-856.

Olutiola, P. O., and O. O. Cole. 1977. Some environmental and nutritional factors affecting growth and sporulation of *Ceratocystis paradoxa*. Mycologia 69(3): 524-532.

Papagianni, M. 2004. Fungal morphology and metabolite production in submerged mycelial processes. Biotechnology advances 22:189-259.

Paul, N. C., S. S. Nam, A. Kachroo, Y. H. Kim and J. W. Yang. 2018. Characterization and pathogenicity of sweet Potato (*Ipomoea batatas*) black rot caused by *Ceratocystis fimbriata* in Korea. European journal of plant pathology 152:833-840.

Quach, M. L., L. D. Melton, P. J. Harris, J. N. Burdon and B. G. Smith. 2001. Cell wall compositions of raw and cooked corms of taro (*Colocasia esculenta*). Journal of the Science of Food and Agriculture 81:311-318.

Gururaj, S., Y. S. Amaresh, S. T. Yenjerappa, A. Amaregouda and A. G. Shreenivas. 2017. Cultural characteristics of *Ceratocystis fimbriata* Ell. and Halst. on different solid media causing wilt in pomegranate. Plant Archives 17:51-54.

Rao, V. R., D. Hunter, P. B. Eyzaguirre and P. J. Matthews. 2010. Ethnobotany and global diversity of Taro. In: Rao, R. V., P. J. Matthews, P. B. Eyzaguirre and D. Hunter, eds., The Global Diversity of Taro: Ethnobotany and Conservation. Biodiversity International, Rome, Italy 1-5.

Roux, J., and M. J. Wingfield. 2009. *Ceratocystis* species: Emerging pathogens of non-native plantation Eucalyptus and Acacia species. Southern Forests: A Journal of Forest Science 71:115-120.

Sonyal, S., A. Pappachan, K. B. Palanna, H. S. Mahesha, S. H. Manjunath and S. G. Madhu. 2015(a). Survival ability of *Ceratocystis fimbriata* causing Pomegranate Wilt in Different Temperature and Hydrogen Ion Concentration (pH). International Journal of Pure and Applied Biosciences 3:49-53.

Sonyal, S., S. G. Madhu, H. S. Mahesha, K. B. Palanna, S. H. Manjunath and A. Papachan. 2015(b). Cultural characteristics of *Ceratocystis fimbriata* Ell. and Halst. causing wilt in pomegranate. International Journal of Pure and Applied Biosciences 3:23-27.

Thorpe, D. J., T. C. Harrington and J. Y. Uchida. 2005. Pathogenicity, internal transcribed spacer-rDNA variation, and human dispersal of *Ceratocystis fimbriata* on the family Araceae. Phytopathology 95:316-323.

Ul Haq, I., Ijaz, S., and M. Z Latif. 2019. Multilocus sequence typing (MLST) based genetic variation analysis of Shisham dieback associated strains of Ceratocystis fimbriata sensu lato species complex in Pakistan. Applied ecology and environmental research 17:12573-12582

Vinning, G. 2003. Select markets for Taro, sweet Potato and yam. Rural Industries Research and Development Corporation, Publication No. 03/052, Canberra, Australia.

Walker, G. M. and N. A. White. 2017. Introduction to fungal physiology. Fungi: biology and applications, 1-35.

Yadahalli, K. B., S. S. Adiver and S. Kulkarni. 2010. Effect of pH, temperature and relative humidity on growth and development of *Ceratocystis paradoxa*–a causal organism of pineapple disease of sugarcane. Karnataka Journal of Agricultural Sciences 20:1.