Pathogenicity and infection behaviour of *Exserohilum rostratum* on wheat and associated collateral hosts.

Tulasi Korra
Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005 India
Sudhir Navathe (sudhir.agro123@gmail.com)
Agharkar Research Institute
Ramesh Chand
Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005 India

Research Article

Keywords: Fungal disease, Poaceae, Triticum aestivum, Triticeae

Posted Date: September 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2027087/v1

Abstract

Exserohilum leaf spot is a newly arising fungal disease that mostly affects monocots. Thirty-two plant species of 14 families were evaluated for pathogenicity to *Exserohilum rostratum* as a potential pathogen. The isolate collected from diseased wheat leaves produced typical dark brown lesions upon inoculation to healthy wheat plants and produced similar symptoms. The artificial inoculation of detached leaf assay symptoms appeared on major cereals like *Triticum aestivum*, *Oryza sativa*, *Echinochloa esculenta, Panicum miliaceum* and *Eleusine coracana.* The symptoms were reddish-brown in most of the genera of Poaceae. Histopathological studies revealed that conidia produce the appressoria within 24 hrs and penetrate the host through stomata or epidermal cells after germination. Study reveals that collateral hosts serve as reservoirs for the infection, allowing it to persist in the absence of its primary host. These secondary hosts aid the pathogen in continuing the infection cycle and spreading the disease.

1. Introduction

Exserohilum has been described as a pathogen on cereals and grasses (Kusai et al. 2016). Every year about 10–20% losses due to diseases are reported in wheat (Figueroa et al. 2018). Major wheat diseases are rusts, spot blotch, smut, common root rot, Fusarium head blight and several bacterial and viral diseases (Gulyaeva et al. 2020). Helminthosporium is classified as one of the influential groups under Hyphomycetes, including pathogens of plants and animals. Based on conidial ontogeny and morphology, this group is divided into three genera Exserohilum, Bipolaris, and Drechslera (Alcorn, 1988). Presently, less than 50 species are described as true *Helmithosporium* spp. Based on the conidial and protruding hilum, the Exserohilum was erected from Bipolaris by Leonard and Suggs (1974) with sexual morph *Setosphareia rostrata*.

Exserohilum pathogenic species include *E. pedicellatum, E. prolatum* producing leaf spot on maize and wheat, but *E. rostratum* infect numerious hosts, including wheat, banana, maize and grasses (Lin et al. 2011). Exserohilum leaf blight is characterized by leaf and stem lesions that spread to the entire leaf after a few days of infection (Kusai et al. 2016). A strong interaction between the pathogen and the environment is also reported (Kusai et al. 2016). Climate change has also brought behavioural changes in pathogens, and many pathogens of minor significance have become major (El-Sayed and Kamel, 2020). *E. rostratum* is a known potential pathogen whose outbreak is expected due to climate change. However, the biology and ecology of this pathogen are least known. Therefore, this study aimed to determine the host range and histological studies during infection.

2. Materials And Methods

All the investigations were carried out during 2019–2021 at Laboratory and Polyhouse, Mycology and Plant Pathology Department, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India (North Eastern Plain Zone, India, 25.26° N and 83.99°E).

2.1 Fungal isolation, identification and inoculum preparation

Wheat-infected leaves were collected from the Dharwad, Karnataka (15.3518° N, 75.1291° E). Infected samples were air dried and kept in a paper bag labelled. The pathogen was isolated on potato dextrose agar (PDA), and the spore suspension of each fungal isolate was streaked on autoclaved 4% water agar (WA). It was then incubated for 24 h at 28^0C (Laboratory, Varanasi). Pure culture of the fungus was prepared by single spore isolation, and pure culture was maintained on PDA (Chand et al.2013). For spore measurements, a minimum of 30 spores were analyzed per culture using Adobe CS5 Sofware.

For molecular identification, DNA was idolated following Al-Samarraj and Schmid, (2000). For identification fungal barcodes ITS1 + ITS4 (White et al. 1990) and *Tef1 α* (EF1-983 + 22108; Rehner and Buckley, 2005) were used. PCR purified products were commercially sequenced at Eurofins Pvt. Ltd., India following sanger sequenng. BioEDIT was used to edit the sequences for good quality and further subjected to nBLAST to check the homology in the genbank database to further confirm the species. After a preliminary check of the nucleotide sequence, it is submitted to NCBI genebank.

The identified culture was multiplied on PDA after inoculating a bit of mycelium in the plate's centre and incubating at 25 ℃ for a week. The inoculum was prepared by washing the conidia from the cultures grown in Petri plates using sterile water. Spore suspensions were prepared and adjusted to 10^4 spores/ml with a drop of Tween 20 (0.05%) as spreader. Spore suspensions were sprayed on the leaves at the Zadoks stage 45 (Zadoks et al. 1972).

Host range and inoculation studies:

Host range test was conducted on locally available and reported weeds (Table 1). Under poly house conditions, seeds of different hosts were grown in pots (20 cm diameter) filled with sterilized mixtures with equal portions (v/v) of soil, sand and clay (5 seeds/pot). All the plants were inoculated when they were 35 days old. The spore suspension was spread on each plant with hand-held automizers. For each isolate, a separate automizer was used. The inoculated plants were covered with plastic bags for 48 h to maintain high humidity. Three replicates of each host were sprayed with the isolate. Uninoculated plants serve as a negative control. Six days post-inoculation, plants were examined for the appearance of symptoms. The method described above was followed in the detached leaf assay technique with three leaves as three replications and control in Petri plates for 31 hosts (Bhattarai et al. 2020). The incubation period (IP) was recorded from the inoculation to the appearance of the first visible symptoms described by Aquino et al. (1990). The total number of lesions counted on each infected leaf one week after post-inoculation. The leaf was divided into four parts with the help of a marker pen and the number of lesions count (LN) in each part (Smith, 1996). Lesion size (LS) was determined by measuring the length and width of the lesion produced by the pathogen one week after post-inoculation (Bashyal et al. 2011). The grey value was obtained by transforming the RGB image into an 8-bit greyscale and calculating the mean grey value of the pixels making up each lesion. The greyscale ranges from 0 (black) to 255 (white) and can be used for the degree of the colour of the lesion in *E.rostratum* (Lendenmann et al. 2014).

Table 1
List of hosts tested during the study

Host	Botanical name	EPPO CODE	References
Rice	*Oryza sativa*	ORYSA	Imrani et al. 2017
Ragi	*Eleusine coracana*	ELECO	Young et al. 1947
Pearl Millet	*Pennisetum glaucum*	PESGL	Misra and Singh, 1970
Maize	*Zeamays.L*	ZEAMX	
Sorghum	*Sorghum bicolor*	SORVU	Lele and Dhanraj, 1966
Sugarcane	*Saccharum officinaru*	SACOF	Ahmadpour et al. 2013
Banana	*Musa paradisiaca Linn.*	CYPRO	Lin et al. 2011
Rhapis palm	*Rhapis excels*	RPJEX	Chase 1982
Napier grass	*Pennisetum sp.*	PESSS	Thite and Chavan, 1977
Crepe Ginger	*Costus speciosus*	CQTSC	Centko et al. 2017
Ear leaf acacia	*Acacia auriculiformis*	ACAAF	Mohanan and Sharma,1988
Switch grass	*Panicum virgatum*	PANVI	Tsukiboshi, 2002
Tiger grass	*Thysanolaena maxima*	THSMA	Brunings et al. 2009
Bermuda grass	*Cynodon dactyle*	CYNDA	Pratt, 2006
Johnson grass	*Sorghum halepense*	SORHA	
Green foxtail	*Setaria viridis*	SETVI	
Broadleaf signal grass	*Brachiaria platyphylla*	BRAPP	
Clustered Lovegrass	*Eragrostis elongate*	ERAEL	Misra et al. 1970;
Buffel grass	*Cenchrus ciliaris*	PESCI	Wernham and Kirby, 1941
Cogon grass	*Imperata cylindrica*	IMPCY	
	Cymbopogon sp.	CYGSS	
Edible amaranth	*Amaranthus tricolor*	AMATR	Lin et al. 2011
Spiny amaranth	*Amaranthus spinosus*	AMASP	
African arrowroot	*Canna indica*	CNNIN	
Water spinach	*Ipomoea aquatic*	IPOAQ	
Muskmelon	*Cucumis melo*	CUMME	
Pumpkin	*Cucurbita moschata*	CUUMO	
Sponge gourd	*Luffa cylindrica*	LUFAE	
Nut grass	*Cyperus rotundus*	CYPRO	
Papaya	*Carica papaya*	CIAPA	
Fire plant	*Euphorbia heterophylla*	EPHHL	
Purging nut	*Jatropha curcas*	IATCU	
Bitter cassava	*Manihot esculenta*	MANES	
Love Grass	*Chrysopogon aciculatus*	CYSAC	
Egyptian crowfoot	*Dactyloctenium aegyptium*	DTTAE	
Mung bean	*Vigna radiata*	PHSAU	
Chinese hibiscus	*Hibiscus rosa-sinensis*	HIBRS	
White mulberry	*Morus alba*	MORAL	

2.2 Measurement of the lesion number, the colour of the lesion, mean grey value and lesion size of the infected leaves:

Photography of individual infected leaves was done by the NIKON Camera D5200 (Nikon Japan) on red background with a white scale (Poudel et al. 2019). The Photographs of symptoms were taken from randomly selected three tagged plants in each row for all the hosts after post-inoculation. These photographs were saved in jpg format. The lesions were analyzed for colour measurement on a grey scale by Adobe CS5 Software. Selected infected leaves were open in

the software and went to the analysis settings for modification; Pixel length, logical length, Logical unit (https://helpx.adobe.com/photoshop/using/color-modes.html). The number of Lesions was measured using the analysis menu's count tool. Grey values were displayed, extracted to the notepad, and finally recorded in the excel file.

2.3 Histopathology :

E. rostratum and samples inoculated to the leaves of the different hosts were collected at 0, 24 and 48 hai. Histopathology was performed using a bleaching solution (Ethanol: Glacial acetic acid: Glycerol = 3:1:1 v/v). Cut leaf segments into 1 cm× 1cm were poured in 5 ml bleaching solution into a test tube (10 ml) containing leaf segments in each test tube. The bleaching solution and leaf segments were placed in boiling water for 15–20 minutes (Sillero and Rubiales, 2002). The transparent leaf segments were used for microscopic study after treating the leaf with Trypan blue solution (Trypan blue, 0.025 mg + Ethanol, 50ml + 50 ml, Lactophenol + 50 ml Distilled water) (Sillero and Rubiales, 2002). Spore germination and infection processes were observed using the Nikon Eclipse E 200 microscope (Nikon Instruments Inc). The observations on the germination percentage (G%), germ tube length (LGT), total count formation of appressoria (NAF), total penetration count through a stoma (SP) and total count penetration(EP) through the epidermal cell were noted at 0, 24, 48 hai (Bashyal et al. 2011).

2.3 Scanning electron microscope (SEM) :

For cryo-SEM, leaf lesions were excised into small pieces size of 1mm^2 by chemical fixation of glutaraldehyde (2–4%) method (Kiernan et al.2000). The tissue bearing symptoms attached to carrier sputter coated with gold always achieved before scanning by the method of Riauet al.(2010). The coated samples were examined under a ZEISS EM 10 SEM operating at keV (Low-voltage electron microscope) at a magnification of 100 to 5000 fold.

For statistical analysis, the data were subjected to ANOVA (analysis of variance, CRD) and LSD (least significant differences) to determine the significant differences between means. The correction coefficients between disease and histological components were calculated by R software.

3. Results

3.1 Isolation and identification of the pathogen

E. rostratum isolated from the infected leaf samples has been deposited at CSIR- Mycrobial type culture collection(MTCC), Chandigarh, India with accession number MTCC 13118. The conidial dimensions of the isolate ranged from 14–85 µm in length and were 7–17 µm wide. The conidia have protruding hilum, conidiophores slightly curved or erect, septate and geniculate, olivaceous–brown, conidiogenous cells with circular conidial scars. A dark septum is observed on both polar ends of each conidium. The conidial dimensions of the isolates ranged from 85–114 µm in length and were 7–17 µm wide. The conidia have protruding hilum, conidiophores slightly curved or erect, 5 to 15 distoseptate and geniculate, olivaceous–brown, conidiogenous cells with circular conidial scars (Fig. 1a). A dark septum is observed on both polar ends of each conidium. Additionally the identity of the isolate was confirmed based on the phylogenetic analysis of ITS and *Tef − 1* alpha regions of *E.rostratum* (Fig. 1b). The sequences are deposited in NCBI genbank with accession MN599631 (ITS) and OM752309 (Tef1 α).

3.2 Symptomatology on different hosts and pathogenicity aspects:

The incubation period among the host was significant (p = 0.05). For *Triticum aestivum*, *Echinochloa esculenta, Eleusine coracana*, *Panicum miliaceum* and other hosts, small brown to dark brown lesions were formed and later, these lesions coalesced, leading to blight appearance. Furthermore, in *Sorghum bicolour*, *Saccharum spontaneum* and *Saccharum officinarum* where lesions coalesce to produce reddish colour symptoms. (Fig. 2 and Fig. 3). *Mangifera indica* has not shown infection.

Incubation period

The detached leaf assay and polyhouse-grown plant incubation period varied. Variation was less in grasses. In detached leaf assay, the incubation period was three days on *Cynodon dactyle*, *Megathyrsus maximus, Panicum virgatum* and *Sorghum bicolour*. The most prolonged incubation period was five days on *Oryza sativa*, *Saccharum officinarum*, *Eichhornia crassipes*, *Zea mays, Ficus religiosa* and *Ficus hispida* (Table 2). Under polyhouse, the incubation period was 4.34 days on *Zea mays L.* and the shortest *Dactyloctenium aegyptium*, *Setaria italica*, *Oryza sativa*, and *Saccharum spontaneum*, respectively (Table 3).

Table 3
Host range pathogencity and histological test against E. rostratum under Polyhouse assay

Family	Host	Common name	Incubation period	Mean gray value colour of lesion	Lesion size per (leaf/cm^2)	Lesion (count/ leaf cm^2)	Germination (%)	No. of appersoria formed (mm^2)	Length of germ tube (µm)	Appressorium Penetration based Incubation period	
										Penetrated through stoma (mm^2)	Pene throu epid cell (
Poaceae	*Echinochloa esculenta*	Banyard millet	4.00 ± .0.00	135.99 ± 3.25	2.23 ± 0.12	79.00 ± 7.08	29.29 ± 1.47	2.62 ± 0.14	1.65 ± 0.0.69	0.00 ± 0.00	0.71 0.05
	Cyandon dactyle	Bermuda grass	3.34 ± 0.58	94.87 ± 6.37	0.66 ± 0.07	107.0 ± 7.08	30.39 ± 0.50	1.32 ± 0.20	0.84 ± 0.75	0.63 ± 0.12	0.27 0.13
	Dactyloctenium aegyptium	Egyptian crowfoot grass	3.00 ± .0.00	146.64 ± 2.53	0.92 ± 0.07	31.00 ± 2.83	50.96 ± 1.05	2.83 ± 0.14	1.06 ± 0.55	0.00 ± 0.00	0.39 0.11
	Setaria italica	Fox tail millet	3.00 ± 0.00	120.0 ± 21.3	1.75 ± 0.09	58.00 ± 5.66	26.74 ± 3.34	1.44 ± 0.37	1.80 ± 0.95	0.82 ± 0.06	0.72 0.50
	Megathyrsus maximus	Guniea grass	4.00 ± 0.00	152.98 ± 19.01	1.06 ± 0.09	31.00 ± 4.25	23.66 ± 3.21	0.36 ± 0.10	0.41 ± 0.96	1.23 ± 0.47	0.26 0.67
	Zea mays.	Maize	4.34 ± 0.58	153.36 ± 12.6	1.26 ± 0.12	64.00 ± 11.32	31.67 ± 1.71	2.09 ± 0.40	1.11 ± 0.41	1.24 ± 0.45	0.46 0.15
	Panicum miliaceum	Prosomillet	4.00 ± 0.00	57.02 ± 7.07	2.54 ± 0.07	147.00 ± 4.25	32.57 ± 2.24	2.55 ± 0.38	1.41 ± 0.76	0.00 ± 0.00	0.66 0.08
	Eleusine coracana	Finger millet	4.00 ± 0.00	108.3 ± 9.66	2.74 ± 0.17	87.00 ± 4.25	22.71 ± 2.41	1.67 ± 0.04	1.47 ± 0.57	0.14 ± 0.03	0.69 0.44
	Oryza sativa	Rice	3.00 ± 0.00	131.19 ± 3.34	2.74 ± 0.14	75.50 ± 19.09	39.12 ± 3.59	3.69 ± 0.12	2.05 ± 0.39	0.00 ± 0.00	1.29 0.06
	Sorghum bicolor	Sorghum	3.34 ± 0.58	83.69 ± 7.71	1.89 ± 0.62	131.00 ± 2.83	24.69 ± 0.60	1.28 ± 0.24	2.02 ± 0.49	0.00 ± 0.00	0.89 0.27
	Saccharum officinarum	Sugarcane	4.00 ± 0.00	107.6 ± 20.05	3.65 ± 0.23	57.00 ± 7.08	63.86 ± 0.50	5.56 ± 0.35	1.17 ± 0.79	0.00 ± 0.00	0.35 0.18
	Saccharum spontaneum	Wild sugarcane	3.00 ± 0.00	61.08 ± 4.48	0.52 ± 0.04	57.50 ± 7.78	29.14 ± 0.50	5.20 ± 0.18	1.28 ± 0.63	0.19 ± 0.11	0.60 0.13
	Triticum aestivum	Wheat	3.67 ± 0.58	66.14 ± 10.05	2.66 ± 0.10	38.50 ± 9.19	39.87 ± 0.50	1.29 ± 0.145	0.54 ± 0.41	0.19 ± 0.11	0.32 0.20
Musaceae	*Musa paradisiaca*	Banana	3.34 ± 0.58	105.01 ± 14.76	4.73 ± 0.07	59.00 ± 7.08	25.39 ± 1.04	1.120 ± 0.07	1.05 ± 0.81	0.00 ± 0.00	0.52 0.09
	CV		10.30	7.88	9.37	8.07	4.38	7.67	9.27	NS	8.98
	SEM P < 0.05		0.21	4.95	0.11	3.41	1.15	0.11	0.49	-	0.14

Colour of lesions (Mean grey value)

The colour of the lesion indicates a grey value. In the laboratory, the mean grey value was least in *Alstonia scholaris* (15.75) and the highest grey value at114.00 (*Sorghum bicolour*), followed by *Eleusine coracana* (104.50) (Table 2). The grey value for polyhouse grown, *Megathyrsus maximus*(152.98) and *Zea mays L*. (153.35) with no significant difference in grey value, followed by *Dactyloctenium aegyptium* (146.64) and lowest in *Panicum miliaceum* (57.05) and *Mangifera indica* with no infection. (Table 3).

Lesion count(cm^2): For lesion count on detached leaf assay presented in Table 2. Maximum number (93.50cm^2) recorded *Eichhornia crassipes*. *Solms* followed by *Sorghum bicolor* (66.0 cm^2) and minimum number (8.50cm^2)in *Vigna radiata*. In the greenhouse, the maximum lesion number (147cm^2)was in *Panicum miliaceum*, followed by (131cm^2) in *Sorghum bicolor*. In *Triticum aestivum*, the lesions were (38.5cm^2) (Table 3).

Lesion area(cm^2)

The highest lesion was recorded in *Musa paradisiacal* (4.73 cm^2), followed by *Saccharum officinarum* (3.65 cm^2) and the lowest in *Saccharum spontaneum*(0.52 cm^2). (Table 3).

3.3 Infection behaviour on different hosts

The histological assessment for 31 hosts in detached leaf assay and polyhouse (14 hosts) were done at 0 h (control), 24h, and 48h of hours after inoculation (hai) (Fig. S1). Scanning electron microscope (SEM) images showed sporulation on *Triticum aestivum, Zea mays L*. and *Panicum virgatum* (Fig. 4). Significant variation was observed in conidial germination percentage. On *Ficus hispida*, germination was 86.22% and lowest (19.42%) in *Alstonia scholaris*.

Significant variation was observed for the germ tube length on different hosts. The largest (5.98 µm) germtubewas produced in *Zea mays*, followed by *Triticum aestivum* (4.38 µm). The smallest germ tube was produced on *Eichhornia crassipes* (1.78 µm) (Table 2). The maximum count of appressoria was recorded in *Thysanolaena latifolia* (5.56 mm^2), followed by *Ficus religiosa* (4.94 mm^2), and the minor count in *Triticum aestivum* (0.59 mm^2). The appressorium penetration through stoma was maximum in *Ficus religiosa* (2.53 mm^2) and through epidermal cells, highest in *Argyreia Nervosa* (1.91 mm^2).In polyhouse assay (Table 3, Fig S1, S2), *Saccharum spontaneum* germination was 63.86% and the lowest (22.71%) in *Eleusine coracana.* The largest (5.56 µm) germ tube was produced in *Saccharum officinarum*, followed by *Saccharum spontaneum* (5.21 µm). The smallest germ tube was produced on *Megathyrsus maximus* (0.368 µm) (Table 4). The maximum count of appressoria was recorded in *Oryza sativa* (2.05 mm^2), followed by *Sorghum bicolor* (2.02 mm^2), and the minor count in *Megathyrsus maximus*(0.41 mm^2). The appressorium penetration through stoma was maximum in *Zea mays.* (1.24 mm^2) and through the epidermal cell, highest in *Oryza sativa* (1.29 mm^2) and lowest in *Megathyrsus maximus* (0.26 mm^2). The germination behaviour was bipolar (Fig. S1).

3.6 Correlations between histological and disease components

The estimates of linear correlation coefficient among the nine components in detached leaf assay *viz*., germination (%), number of appressoria formed, length of the germ tube, number of appressoria formed, penetrated stoma count, penetrated epidermal cell count. The incubation period (days),mean grey value, lesion count (cm^2) and lesion area (cm^2), respectively,are presented in Table 2. Germination (%) was positively correlated with a number of appressoria formed($r = 0.43, P < 0.01$).Penetration by stoma positively correlated with the length of the germ tube ($r = 0.82, P < 0.001$), and penetration by epidermal cell positively correlated with a number of appressoria formed ($r = 0.41, P < 0.05$). The mean grey value was positively correlated with Penetrated stoma count ($r = 0.42, P < 0.05$). Further, Incubation period was positively correlated with Penetrated epidermal cell count ($r = 0.38, P < 0.05$), Penetrated stoma count ($r = 0.45, P < 0.05$),Length of germ tube($r = 0.50, P < 0.01$) and number of appressoria formed ($r = 0.37, P < 0.05$), Lesion size was positively correlated with Incubation period (r = 0.38), number of appressoria formed ($r = 0.43, P < 0.05$) with significant P = 0.05 and penetrated epidermal cell ($r = 0.47, P < 0.01$). The lesion number significantly positive correlated with lesion size($r = 0.50$), germination percentage G% ($r = 0.47$) with significant P = 0.05, Incubation period ($0.44, P < 0.01$), Length of germ tube ($0.38, P < 0.01$) and number of appressoria formed (NAF)($0.77, P < 0.001$) (Fig. 5b). In polyhouse assay (Table. 3). germination (%) was positively correlated with the length of the germ tube ($r = 0.75, P < 0.01$). Penetration by epidermal cells positively correlated with a number of appressoria formed ($r = 0.94, P < 0.001$). Lesion size was positively correlated with a number of appressoria formed ($r = 0.96$) and penetrated epidermal cells ($r = 0.97$) with a significant P = 0.001. The lesion number significantly positive correlated with lesion size ($r = 0.97$), number of appressoria formed (NAF) ($r = 0.95$) and penetrated epidermal cell ($r = 0.94$) with significant P = 0.001, respectively (Fig. 5a).The incubation period (IP), mean grey value (MGV), and stomatal penetration (SP) did not show a significant difference in the polyhouse assay method.

4. Discussion

Exserohilum leaf spot is a new emerging fungal disease in the present scenario. In this study, the fungus was isolated from diseased leaves of wheat exhibiting leaf blight symptoms. The fungus was identified based on conidia morphology and molecular characterization of ITS and *Tef 1* alpha sequences which showed the same homology (95–100%) as other isolates of *E. rostratum* in Genbank. (Cardona and Gonzalez 2007; Lin et al. 2011). Kalekar (1973) reported *E. rostratum* on wheat and paddy (Kusai et al. 2016). The primary symptoms appeared dark to purplish spots and became chlorotic to necrotic areas. (Farag 2020, Brunings 2009). This pathogen was symptomatic seven days after inoculation in bean plants and turned into reddish borders (Cardonaand González, 2007). Lesions of *E. rostratum* on *Thysanolaena latifolia* were elliptical between leaf veins and sometimes appeared as yellow halos, ranging from 0.2 cm to 1.2 cm long (Brunings, 2009). The higher densities of inoculums lead to disease progression, coalescing lesions resulting in widespread necrosis and death of leaves (Brunings, 2009). Additionally, it infects ornamental plants. Plant pathogens shift to new hosts for their survival and host expansion in nature (Zhong et al. 2016). *E. rostratum* was isolated from the diseased leaf of wheat and more infectious to the Poaceae family members than dicots. *E. rostratum* would establish a compatible interaction with graminaceous hosts, resulting in divergence. Using grey values extracted from 8-bit greyscale images, we obtained a degree of the lesion's colour based on the assumption that a dark colour lesion represents more sporulation and disease spread on different host leaves. The fungal, conidial germination takes place by specific stimuli from plant surfaces. (Ijadpanahsaravi et al. 2021). The globose appressorium penetrated the leaf epidermal or stomata cells to colonize on host cells. The chemicals such as calcium, potassium ions, sucrose, phenols, and plant extract may suppress or stimulate the appressoria formation by a fungal pathogen (Lee and Bostock, 2006). These mechanisms stimulate or inhibit a maximum number of appressoria in different hosts (Visai and Vanoli, 1997, Podila et al. 1993). Similarly, observations on bulbous appressorium development in tiger grass were reported by Lin et al. (2012). However, appressorium penetration through stomata and epidermal cells and breaches on or inside the mesophyll cells in *E. rostratum* in rice, wild sugarcane, and maize ear rot (Lin et al. 2012, Sun et al. 2022). This investigation yielded similar results in wheat infected with *E. rostratum*. A significant positive correlation between disease and histological components was observed. Similar findings lesion number was positively and significantly correlated with lesion area and sporulating lesions reported by Poudel et al. (2019) and Yusuf et al. (2016) in *B. sorokinana* infections in wheat. These findings indicate that long-term monitoring for fungus is still needed.

This study reveals that *E. rostratum* was mainly pathogenic to the members of the Poaceae family. Reddish colour lesions were evident in Sugarcane, Sorghum and Wild sugarcane, while remaining hosts exhibited light to dark brown lesions in both detached leaf assay and poly house assay. A maximum number of appressoria was observed at 24 hai. The maximum number of appressoria positively correlated with lesion size and lesion number. Collateral hosts act as reservoirs for the pathogen to survive without their primary host. These collateral hosts help the pathogen continue the infection chain and spread the disease. Although, there is a need to use approaches like cultural, biological and chemical management to reduce the virulent strain of the pathogen.

Declarations

Acknowledgements: This work was partly supported by the National Fellowship for the Scheduled Tribes (Grant No. 201718-NFST-AND-00071 to TK.

Author's contribution: SN and RC contributed to conceptualization and supervision. TK contributed to research work and writing.

Conflict of interest statement: The authors declare no conflict of interest.

References

1. Ahmadpour, A., Karami, S., Heidarian, Z., and Javan-Nikkhah, M. (2013). *Exserohilum rostratum* causing sugarcane leaf spot in Iran. Australasian Plant Disease Notes, *8*(1), 97–99.
2. Alcorn, J. L. (1988). The taxonomy of Helminthosporium species. Annual Review of Phytopathology, *26*, 37–56.
3. Al-Samarrai, T. H., and Schmid, J. (2000). A simple method for extraction of fungal genomic DNA. Letters in applied microbiology, *30*(1), 53–56.
4. Aquino, V. M., Shokes, F. M., Gorbet, D. W., and Nutter, F. W. (1995). Late leaf spot progression on peanuts as affected by components of partial resistance. Plant Disease, *79*(1), 74–78.
5. Bashyal, B., Chand, R., Prasad, L., and Joshi, A. (2011). Partial resistance components for the management of spot blotch pathogen *Bipolaris sorokiniana* of barley (Hordeum vulgare L.). Acta Phytopathologica et Entomologica Hungarica, *46*(1), 49–57.
6. Bhattarai, G., Feng, C., Dhillon, B., Shi, A., Villarroel-Zeballos, M., Klosterman, S. J., and Correll, J. C. (2020). Detached leaf inoculation assay for evaluating resistance to the spinach downy mildew pathogen. European Journal of Plant Pathology, *158*(2), 511–520.
7. Brunings, A. M., Datnoff, L. E., Palmateer, A. J., Locke, J. C., and Krause, C. R. (2009). Exserohilum leaf spot on tiger grass. Plant Health Progress, *10*(1), 1.
8. Calderan-Rodrigues, M. J., Guimarães Fonseca, J., de Moraes, F. E., Vaz Setem, L., Carmanhanis Begossi, A., and Labate, C. A. (2019). Plant cell wall proteomics: A focus on monocot species, *Brachypodium distachyon*, *Saccharum* spp. and *Oryza sativa*. *International journal of molecular sciences*, *20*(8), 1975.
9. Cardona, R., andGonzález, M. S. (2007). First Report of *Exserohilum rostratum* associated with rice seed in Venezuela. Plant Disease, *91*(2), 226–226.
10. Centko, R. M., Ratnaweera, P. B., Tysoe, C., Withers, S. G., de Silva, E. D., and Andersen, R. J. (2017). Alpha-glucosidase and alpha-amylase inhibiting thiodiketopiperazines from the endophytic fungus *Setosphaeria rostrata* isolated from the medicinal plant *Costus speciosus* in Sri Lanka. Phytochemistry letters, *22*, 76–80.
11. Chand, R. A. M. E. S. H., Kumar, P. R. A. B. H. A. T., Singh, V. I. N. E. E. T. A., and Pal, C. H. H. A. T. T. A. R. (2013). Technique for spore production in *Cercospora canescens*. *Indian Phytopathology*, *66*, 159–163.
12. Chase, A. R. (1982). Dematiaceous leaf spot of *Chrysalidocarpus lutescens* and other palms in Florida. Plant Disease. *66*, 697–699.
13. Eisa, M., Chand, R., and Joshi, A. K. (2013). Biochemical and histochemical traits: a promising way to screen resistance against spot blotch (*Bipolaris sorokiniana*) of wheat. European journal of plant pathology, *137*(4), 805–820.
14. El-Sayed, A., and Kamel, M. (2020). Climatic changes and their role in emergence and re-emergence of diseases. Environmental Science and Pollution Research, *27*(18), 22336–22352.
15. FAO. 2022. Food Outlook – Biannual Report on Global Food Markets. Rome. https://doi.org/10.4060/cb9427en.
16. Farag, M. F. (2020). A first record of *Exserohilum rostratum* as a new pathogen causing bean blight in Egypt. Plant Pathology.*1*(4), 1–4.
17. Figueroa, M., Hammond-Kosack, K. E., and Solomon, P. S. (2018). A review of wheat diseases—a field perspective. Molecular plant pathology, *19*(6), 1523–1536.
18. Frank, B. Uebereinigeneue und wenigerbekanntePflanzenkrankheiten. Ber. Deut. Bot. Ges. *1883*(*1*), 29–34.
19. Gulyaeva, M., Huettmann, F., Shestopalov, A., Okamatsu, M., Matsuno, K., Chu, D. H., and Bortz, E. (2020). Data mining and model-predicting a global disease reservoir for low-pathogenic Avian Influenza (AI) in the wider pacific rim using big data sets. Scientific reports, *10*(1), 1–11.
20. Ijadpanahsaravi, M., Punt, M., Wösten, H. A., and Teertstra, W. R. (2021). Minimal nutrient requirements for induction of germination of *Aspergillus niger* conidia. Fungal biology, *125*(3), 231–238.
21. Imrani, N., Boudoudou, H., Mouria, A., Touati, J., Touhami, O. A., Benkirane, R., and Douira, A. (2017). Pathogenicity of *Helminthosporium rostrata* on rice varieties widely grown in Morocco. IJEAB, *2*, 1003–6.
22. Kalekar, A. R. (1973). Varietal resistance in wheat to *Bipolaria tetramera* and *Helminthosporium rostratum* in Maharashtra State. Indian Journal of Mycology and Plant Pathology.71–75pp.
23. Kiernan, J. A. (2000). Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: what they are and what they do. Microscopy today, *8*(1), 8–13.
24. Kusai, N. A., Azmi, M. M. Z., Zainudin, N. A. I. M., Yusof, M. T., and Razak, A. A. (2016). Morphological and molecular characterization, sexual reproduction, and pathogenicity of *Setosphaeria rostrata* isolate from rice leaf spot. Mycologia, *108*(5), 905–914.
25. Lee, M. H., and Bostock, R. M. (2006). Induction, regulation, and role in pathogenesis of appressoria in *Monilinia fructicola*. Phytopathology, *96*(10), 1072–1080.
26. Lele, V. C., and Dhanraj, K. S. (1966). A note on *Helminthosporium rostratum* Drechs. on Ragi (*Eleusine coracana* (L.) Gaertn.). Current Science, *35*(17), 446–447.
27. Lendenmann, M. H., Croll, D., Stewart, E. L., and McDonald, B. A. (2014). Quantitative trait locus mapping of melanization in the plant pathogenic fungus *Zymoseptoria tritici*. *G3: Genes, Genomes, Genetics*, *4*(12), 2519–2533.
28. Leonard, K. J., andSuggs, E. G. (1974). *Setosphaeria prolata*, the ascigerous state of *Exserohilum prolatum*. Mycologia, *66*(2), 281–297.
29. Lin, S. H., Huang, C. H., Deng, Z. Y., Yan, M. X., Huang, W. H., Wei, J. J., and Qin, Z. Q. (2012). First report of leaf spot disease on sugarcane caused by *Bipolaris spicifera* in China. Australasian Plant Disease Notes, *7*(1), 51–53.

30. Lin, S. H., Huang, S. L., Li, Q. Q., Hu, C. J., Fu, G., Qin, L. P., and Yan, W. H. (2011). Characterization of *Exserohilum rostratum*, a new causal agent of banana leaf spot disease in China. Australasian Plant Pathology, *40*(3), 246–259.
31. Misra, A. P., andSingh, T. B. (1970). A new leaf spot disease of *Pennisetum typhoides* caused by *H. rostratum* in India. Indian Phytopathology, *23*(4), 701–702.
32. Misra, A. P., Prakash, O., and Singh, R. N. (1970). New records of *H. rostratum* Drechsler on graminicolous hosts. Journal of Applied Sciences, *2*(1), 60–63.
33. Mohanan, C., and Sharma, J. K. (1988). Diseases of exotic acacias in India. Journal of Tropical Forestry, *4*(4), 357–361.
34. Podila, G. K., Rogers, L. M., and Kolattukudy, P. E. (1993). Chemical signals from avocado surface wax trigger germination and appressorium formation in *Colletotrichum gloeosporioides*. Plant Physiology, *103*(1), 267–272.
35. Poudel, A., Navathe, S., Chand, R., Mishra, V. K., Singh, P. K., and Joshi, A. K. (2019). Hydrogen peroxide prompted lignification affects pathogenicity of hemi-biotrophic pathogen *Bipolarissorokiniana* to wheat. The plant pathology journal, *35*(4), 287.
36. Pratt, R. G. (2006). Johnsongrass, yellow foxtail, and broadleaf signal grass as new hosts for six species of Bipolaris, Curvularia, and Exserohilum pathogenic to bermudagrass. Plant Disease, *90*(4), 528–528.
37. Rehner, S. A., and Buckley, E. (2005). *A Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologia, *97*(1), 84–98.
38. Riau, A. K., Beuerman, R. W., Lim, L. S., and Mehta, J. S. (2010). Preservation, sterilization and de-epithelialization of human amniotic membrane for ocular surface reconstruction. Biomaterials, *31*(2), 216–225.
39. Sillero, J. C., and Rubiales, D. (2002). Histological characterization of resistance to *Uromyces viciae-fabae* in faba bean. Phytopathology, *92*(3), 294–299.
40. Smith, G. A., and Campbell, L. G. (1996). Association between resistance to Cercospora and yield in commercial sugarbeet hybrids. Plant Breeding, *115*(1), 28–32.
41. Stewart, E. L., Hagerty, C. H., Mikaberidze, A., Mundt, C. C., Zhong, Z., and McDonald, B. A. (2016). An improved method for measuring quantitative resistance to the wheat pathogen *Zymoseptoria tritici* using high-throughput automated image analysis. Phytopathology, *106*(7), 782–788.
42. Sun, H., Guo, N., Ma, H., Liu, S., and Shi, J. (2022). First Report of Maize Ear Rot Caused by *Exserohilum rostratum* in Hainan Province in Southern China. Plant Disease, *106*(1), 314.
43. Thite, A. N., and Chavan, P. D. (1977). A new leaf spot disease of *Pennisetum* spp. caused by *Helminthosporiumrostratum*. Maharashtra Vidnyan Mandir Patrika, *12*, 63–65.
44. Tsukiboshi, T., (2002). NIAES, Microbial Systemtics Lab. http://www.naro.affrc.go.jp/archive/niaes/inventory/microorg/eng/z7e-Ex-rost.html.61.
45. Van Bockhaven, J., Spíchal, L., Novák, O., Strnad, M., Asano, T., Kikuchi, Sand De Vleesschauwer, D. (2015). Silicon induces resistance to the brown spot fungus *Cochliobolus miyabeanus* by preventing the pathogen from hijacking the rice ethylene pathway. New Phytologist, *206*(2), 761–773.
46. Visai, C., and Vanoli, M. (1997). Volatile compound production during growth and ripening of peaches and nectarines. Scientia Horticulturae, *70*(1), 15–24.
47. Wernham, C. C., and Kirby, R. S. (1941). A new turf disease. Phytopathology, *31*, 24.
48. White, T. J., Bruns, T., Lee, S. J. W. T., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications, *18*(1), 315–322.
49. Young, G. Y., Lefebvre, C. L., and Johnson, A. G. (1947). *Helminthosporium rostratum* on corn, sorghum, and pearl millet. Phytopathology, *37*(3), 180–183.
50. Yusuf, C. S., Chand, R., Mishra, V. K., and Joshi, A. K. (2016). The association between leaf malondialdehyde and lignin content and resistance to spot blotch in wheat. Journal of Phytopathology, *164*(11–12), 896–903.
51. Zadoks, J.C., Chang, T.T. and Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed research, *14*(6), pp.415–421.
52. Zhong, Z., Norvienyeku, J., Chen, M., Bao, J., Lin, L., Chen, Land Wang, Z. (2016). Directional selection from host plants is a major force driving host specificity in Magnaporthe species. Scientific reports, *6*(1), 1–13.

Table 2

Table 2 is available in the Supplementary Files section.

Table 4

Table 4 is not available with this version.

Figures

Figure 1

aColony of *E. rostratum* (MTCC 13118) on potato dextrose agar (PDA) and typical conidia with hilum.

bPhylogenetic tree inferred from a maximum likelihood analysis (MEGA v11) based on concatenated alignments of ITS and *Tef-1α* from *E.rostratum*. The tree was rooted to *Cercospora eucommiae* as out group. The information on the sequences used in phylogenetic analysis is detailed in Table S1.

Figure 2

Host range pathogenicity assay for*E. rostratum* infections under polyhouse conditions.

Figure 3

Host range and pathogencity for *E. rostratum* infections by detached leaf assay

Figure 4

Scanning electron microscope (SEM) insights a) Appressorium formation 12h b) Initiation of spore development 48h c,d) Conidiophores emerged from the abaxial surface stomal region on sonailika variety of wheat at 3 dai e) sporulation on Nut grass f) sporulation on maize leaf against *E. rostratum* (Scale bar=1, 10, 20 µm).

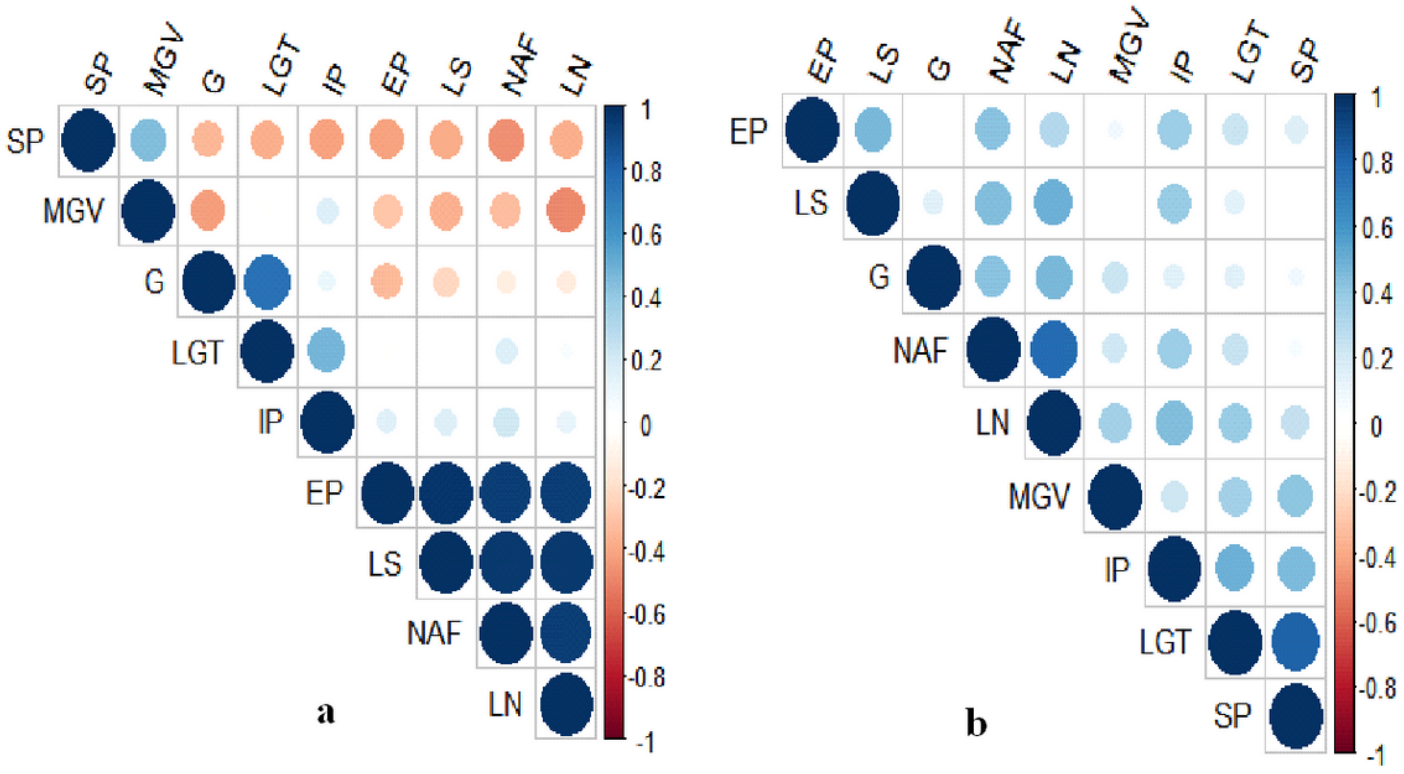

Figure 5

a) Polyhouse assay with nine components with 14 hosts b) Detached leaf assay with nine components with 31 hosts. It indicates IP(Incubation period), MGV(Mean grey value), LN(lesion size), germination percentage (G%), germ tube length (LGT), total count formation of appressoria (NAF), total penetration count through a stoma (SP) and total count penetration (EP) through epidermal cell. Correlation coefficient among the disease components were indicated by different colours as more than medium size blue ($P<0.001$) medium size blue colour ($P<0.01$) followed by small size blue colour ($P<0.05$), and non-significant difference (NS) indicated by very small size blue colour, light to dark brown colour.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2.docx
- FigS1.pptx
- TableS1.docx