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Short Communication

First Report of *Exserohilum rostratum* Inducing Leaf Spot of *Solanum melongena* in Pakistan

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Abstract

Eggplant (*Solanum melongena* L.) is an imperative solanaceous vegetable crop in sub-tropics and tropics and widely cultivated in Pakistan. Epidemic of asymmetrical necrotic leaf spots was perceived on *S. melongena* during a survey of experimental station of Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. On the basis of structural features followed by sequence analysis of ITS region of rDNA the pathogen was recognized as *Setosphaeria rostrata* (Wakker) Boedijin syn. *Exserohilum rostratum* (Drechsler) K.J. Leonard & Suggs. The pathogenic potential of *E. rostratum* was endorsed by implementation of Koch's postulates. To our awareness, the report of *E. rostratum* as leaf spot pathogen of *S. melongena* in Pakistan is novel. © 2020 Friends Science Publishers

Key words: Eggplant; Leaf spots; Pathogenicity; Sequence analysis

Introduction

Eggplant or aubergine is an edible fruit possessing nutrients, minerals, antioxidants, vitamins, nutritional fiber and body constructing elements and proteins (Matsubara *et al*. 2005). Eggplant is a popular vegetable crop of Pakistan having exclusive range of health benefits. Among various vegetables, brinjal is common and extensively grown all over the country but the brinjal production is facing a lot of threats. In Pakistan a lot of reasons are involved for its low productivity like biotic factors (insects, pests and pathogens) as well as abiotic factors (temperature and humidity) (Gangwar and Sachen 1981; Shafique *et al*. 2019). Fungal diseases affecting eggplants cause severe damage in the nursery resulting in reduced fruit yield. The common fungal diseases are wilts, blights, damping off, and leaf spots which are caused by a number of fungi. Thus to manage eggplant diseases in Pakistan the overall objective is the isolation of the most pathogenic strains of fungi that cause severe damage in brinjal plants. Numerous leaf spot diseases ensure comparable biology and consequently identical management alternatives. The work thus emphasizes the correct identification of causal agent isolated from leaf spot disease of *S. melongena*.

Materials and Methods

Collection of diseased plant material

The infected brinjal leaves were taken from the field of Institute of Agricultural Sciences, University of the Punjab, Lahore, during a survey conducted in March 2016. The outburst of irregular necrotic leaf spots was observed. These symptomatic leaves were photographed and collected in sterilized polythene bags. They were thus brought to the laboratory for disease study and refrigerated till further use for pathogen isolation.

Isolation and identification of fungal pathogen

For isolation and identification of mycological pathogen, the protocol of Sime *et al.* (2002) was used. The infected leaves of different plants with 2–3 leaf spots on a leaf were carefully chosen, cut into small pieces (3 mm) and sanitized in 1% NaOCl for 10 min and washed. Few pieces of leaf were inoculated on plates of 2% MEA followed by incubation at 25 ± 2°C for 3–4 days for growth. Hyphae from the emerging fungal colonies were sub-cultured to new medium petri plates and kept on same conditions for the purification of fungal culture. Purified colonies were further identified for their characterization. Isolated fungal strains were primarily examined on the basis of cultural and microscopic characters. For morphological study one week grown cultures were used. The colony characters and the microscopic characteristics of isolated culture were recorded and the species were identified by comparing with any authentic published literature (Ellis 1971; Simmon 2007). Microscopic structural identification was inveterated by sequencing the Internal Transcribed Spacer Sequence (ITS) of rDNA. Molecular analysis was carried out in order to

determine the genomic range of the culture. The primer pair ITS1/ITS4 successfully amplified ~650 bp DNA fragment of total genomic DNA (White *et al*. 1990). The obtained DNA sequences were scrutinized by nucleotide BLAST. The pathogen was confirmed based on maximum homology with the species sequences in the GenBank database. Nucleotide sequences were also deposited to GenBank.

Pathogenicity test

The pathogenic potential of *E. rostratum* was validated by implementation of Koch's postulates on healthy host plants using detached leaf method and pot trial method.

Detached leaf method

The separated leaves of healthy plants were arranged in Petri plates saturated with 2 mL double dist. water by dipping the petiole ends in filter paper. Then 1 mL of spore suspension of the culture was given to the healthy leaf surface separately. All the plates were incubated at 25 ± 2°C up to 7 days and monitored for symptoms development. Characteristic disease symptoms induced by the pathogen on the test plants' leaves were snapped to match the outcomes with the symptoms witnessed in the field.

Pot trial method

In pot trials, experiment was designed to evaluate the nature of infection and severity induced by the pathogen on the host plant. The pathogenicity test was executed by spraying spore inoculum 5.0×10^5 conidia/mL for each replicate plant. Same quantity of dist. water was added in control. For the maintenance of appropriate moisture for better sprouting and development of disease every plant was enclosed using polythene bags separately for 2 days. The pots were watered regularly when required. Each treatment was replicated thrice. Plants were observed for disease appearance on regular basis. Disease rating scale was constructed by observing disease incidence and severity and disease index was calculated using following formula:

$$\text{Disease Severity} = \frac{\text{Area of plant part affected} \times 100}{\text{Total Area}}$$

$$\text{Disease Index} = \frac{\text{Number of plants in particular category} \times 100}{\text{Total Number of Plants}}$$

Results

Disease symptoms

About 50% portion of the leaf showed the disease with dark margins along with yellowish brown necrotic lesions. The preliminary indications were minor spherical or elliptical chlorotic spots (about 6–8 mm) on leaves which developed dim to dusky brown midpoints followed by concentric zones with the expansion of lesions (Fig. 1). In severe infections the leaves were dried and curled and eventually dropped. The infected brinjal leaves were collected from the field and brought to laboratory for disease study, pathogen isolation and identification.

Fig. 1: Leaf spots of *S. melongena*

Identification and characterization of pathogen

Results revealed that seven days old colony, grown on malt extract agar medium; was black, effuse and reaching 5–6 cm in diameter, floccose with regular and smooth margins (Fig. 2a). Conidiophores were straight, geniculate, and brown, about 500 μm elongated and 4–7 µm in width (Fig. 2b–c). Conidia were brown, slightly curved, obclavate (at maturity), pseudo-septate, end cells hyaline, and 40–90 × 8–12 μm in size (Fig. 2b–c). On the basis of these characters, pathogen was identified as *Exserohilum rostratum* (syn. *Setosphaeria rostrata* (Wakker) Boedijin) (Kusai *et al*. 2016). Cultural plates of pathogen were submitted to First Fungal Culture Bank of Pakistan with an accession number FCBP1498.

BLAST analysis of nucleotide sequence of amplicon (KY933453) exhibited 99% similarity with many other strains including strain JL-29 (JX867230) and strain CLER09 (GQ478867) of *E. rostratum* in GenBank (Fig. 3). Phylogenetic tree of known entity and meticulously correlated species was acquired (Fig. 4) from analysis by the maximum likelihood method using MEGA 6 program. The analysis involved 11 nucleotide sequences.

Pathogenicity analysis

The results obtained from detached leaf trials revealed that the symptoms from the artificial inoculation corresponded to the wild type symptoms of disease. Infection and characteristic visible symptoms were very evident on inoculated leaves after few days. However, control leaves remained asymptomatic. Initially minute spotting was noticed on leaves within 2–3 days of inoculation. With the progress of time after 10 days' disease progress was very sharp on the leaves in petri plates (Fig. 5).

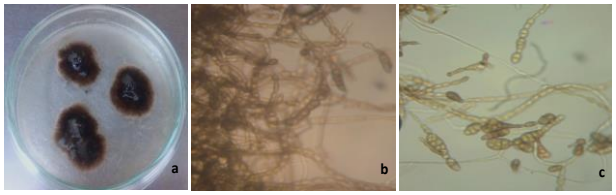

Fig. 2: *E. rostratum.* Colony on MEA (**a**) Mycelium and spore attachment (**b**) and Conidia (**c**)

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Query  10   aaaaaTGAGGGTGTGGTTTGCTGGC-ACAGCGTCCGCCCCAAGTATTTTTCACCCATGTC  68
            ||| |||||||||||||||||||| ||||||||||||||||||||||||||||||||||
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AL-33       TTAAGCATATCAATAAGGCGGAGGAA  577
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Fig. 3: ITS sequence alignment of *E. rostratum* (FBL.At01)

After 3 weeks of inoculation in pot trials; the symptoms were observed which led to about 50% of infected leaf area, while control plants remained asymptomatic. Symptoms of leaf spots observed on brinjal plants were perceived to be brownish spots and burned margins along with necrotic lesions. Firstly, yellowing was shown on the leaves which later led to chlorosis and necrosis, and then wilting occurred and eventually it lead to the death of complete plant. The rigorously infested plants exhibited diminutive growth. After 10–35 days, with a very sharp gradient of disease progress curve

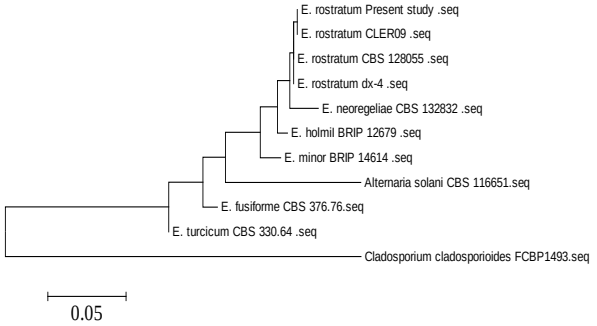

Fig. 4: Molecular Phylogenetic analysis of different species of *Exserohilum* based on sequences of Internal Transcribed Spacer region of rDNA (ITS). Evolutionary history was inferred by Maximum Likelihood method based on the Tamura-Nei model. The analysis involved 11 nucleotide sequences. Evolutionary analyses were conducted in MEGA6

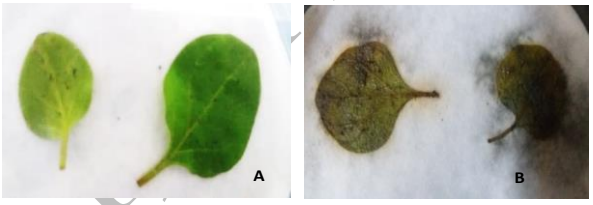

Fig. 5: Symptoms caused by *E. rostratum* in detached leaf assay. **A**: Healthy plant leaf, **B**: Infected plant leaf

100% plant death was observed by all the strains (Table 1). After 2 weeks of Symptoms development the diseased portion was re-inoculated on the medium plates to ratify the pathogen. To our awareness, it is a novel documentation of *E. rostratum* causing leaf spot of *S. melongena* in Pakistan. As *S. melongena* is an important food crop therefore there is an urgent need to control and manage this emerging pathogen.

Discussion

S. melongena is economically important edible fruit. It is severely encountered by bacterial and fungal wilt, phomopsis blight, mosaic, damping off, leaf spots. Leaf spots on brinjal by fungal pathogens are characterized by small circular spots, chlorotic abrasions, mostly hazy to brown and pointed/uneven in form. Rigorous infection led to premature dropping of leaves consequently reduction of fruit yield. Precise identification of the pathogens inducing spot diseases on a particular host plant is imperative. Numerous leaf spot infections ensure analogous biology and consequently identical management alternatives. The current research therefore underlines the correct identification as well as management of causative organism of leaf spot disease of *S. melongena*. Microscopic features and development of conidia/conidiophores is very essential for pathogen identification as physical configuration is still deliberated as the utmost persistent system to classify the

Table 1: Pictorial representation of disease rating scale on the basis of symptoms caused by *E. rostratum*

Key Scale	Disease Symptoms		Disease Severity (%)
0	No symptoms		0
1	Yellowing started on the leaves		10
2	Wilting along margins with spots		30
3	Spots increase on leaf and reduced growth		50
4	Whole plant is infected with spots		70
5	Complete death of plant		100

organism, but errors in identifications are reported (Anderson *et al*. 2006). Subsequently, numerous molecular tactics were recognized to classify the organisms up to specific level *viz*., scrutiny of ribosomal DNA (rDNA) composition to find out molecular phylogenetic relationships among different arrays of fungi (Mirhendi *et al*. 2007) or with the help of mitochondrial small subunit (SSU) rDNA sequence method (Kretzer *et al*. 1996). At present, *E. rostratum* was identified as a cause of leaf spot of brinjal by analyzing morphological microscopic features followed by genetic characterization from nucleotide sequencing of amplified ITS1-5.8S-ITS4 region of rDNA. Similarly, in a study *Phyllosticta aristolochiicola* was reported as a leaf spot pathogen of *Sonchus oleraceus* by Akhter *et al*. (2016).

In the auxiliary research, pathogenicity test was carried out for evaluation of pathogenicity of the isolated pathogen causing leaf spot on brinjal. The pathogen exhibited comparatively same symptoms on the corresponding plant. These experimental designs and their outcomes were in line with the research of Shafique *et al.* (2019) who testified similar inclination of disease progression in brinjal by *Cladosporium cladosporioides.* In another study conducted by Akhter and coworkers (2016), the same procedure of applying Koch's postulates using pot trials was adopted to evaluate the pathogenic potential of *Phyllosticta aristolochiicola* on *Sonchus oleraceus*. Similar disease symptoms on leaves started to appear after 10 days of infection as were evident on infected sample plants whereas control plants remained healthy. Re-isolation of the same pathogen was confirmed by cultural characteristics.

Conclusion

The current research reports an innovative isolation of leaf spot causing pathogen from brinjal plant. The severity of this pathogen was very high at sampling sites; therefore, there is a need of proper management of this pathogen that might have a wider host range in Pakistan.

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