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Prevalence of *Phomopsisvexans* (*Diaporthevexans*) causing leaf blight and fruit rot disease of Brinjal in Jharkhand

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Abstract
Brinjal (*Solanummelongena* L.) is an important vegetable crop which gives very high economic return to the growers. Fruit rot caused by *Phomopsisvexans* (Sacc. & Syd.) is one of the major constraints in its successful cultivation. During survey, the disease was prevalent in all area surveyed. The incidence of disease ranged from 18.33 to 62.00per cent in different locations. Maximum incidence (62.0 per cent) was recorded in the cultivar Pusa Purple Long at Technology Park of Birsa Agricultural University, Kanke, Ranchi whereas least disease incidence (18.33 per cent) was recorded in cultivar Pratima at Nagri village of Kanke block. It causes damping-off of seedlings in nursery, leaf blight; fruit rot and stem blight at various stages of plant growth and development. It produces two types of conidia such as alpha and beta; alpha conidia are hyaline, biguttulate, oval and infective and beta conidia are the diagnostic feature of the genus Phomopsis. Pathogenicity of the pathogen was proved by seed, soil, leaves and fruit inoculations. Maximum seedling mortality (40 %) was observed in seed and soil inoculation followed by seed inoculated in healthy soil (9 %) and healthy seed sown in inoculated soil (6 %).

Keywords: leaf blight, fruit rot disease, Brinjal

Introduction
Brinjal (*Solanummelongena* L.) is one of the most important vegetable crop cultivated in the tropics and sub-tropics, and grown extensively in China, India, Bangladesh, Pakistan and Philippines. It is also cultivated in America, Europe and other parts of Asia. The crop is susceptible to various biotic and abiotic stresses at different stages of growth and development, among them the most significant being Phomopsis blight and fruit rot disease. Many fungal pathogens especially species of *Fusarium*, *Colletotrichum*, *Phytophthora* and *Phomopsis* are associated with fruit rot disease of brinjal. But the rot incited by *Phomopsisvexans* (Sacc. and Syd. 1899) Harter is most destructive. It is second only to bacterial wilt in extent of damage and is reported to cause 10-20 per cent losses in yield in India.In recent years, the Phomopsisblight/ fruit rotdisease of brinjal has become a major constraint in its successful cultivation. It is an unsightly disease that not only harms the eggplants but makes them inedible and unmarketable. The pathogen causes over 50 per cent loss in production and productivity in various parts of the world (Akhtar *et al*., 2008) [1]. Hence, the present study was conducted to determine disease severity of *Phomopsis* in major brinjal growing regions of Jharkhand, and to characterized *P. vexans* by morpho-cultural methods.

Materials & Methods
Field survey and data collection
A field survey was conducted to gather information on the severity of Phomopsis blight / fruit rot of brinjal in farmer's field from Hochar, Hussir, Bukru, Sukurhuttu, Chutia, Itki, and Pithoria villages in the district of Ranchi as well as from brinjal growing plots of Research Farm of Birsa Agricultural University, Kanke during *Rabi*, 2016-2017 when the crop was three to four months old. The Phomopsisblight/fruit rot disease incidence was assessed from June to October by recording the number of plants showing characteristic symptoms and the total number of plants examined. In each village, five fields were selected and in each field 100 plants were examined randomly and scored for disease incidence by using following formula.

$$\text{Per cent disease incidence} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants examined}} \times 100$$

Isolation and identification of pathogen

Brinjal plants showing the characteristic symptoms of Phomopsis blight /fruit rot disease was collected from farmer's field, wrapped in polythene bag, labelled properly and brought to the laboratory for the purpose of isolation of the pathogen.The standard tissue isolation procedure was followed to isolate the pathogen. For this purpose brinjal fruit showing typical fruit rot symptoms were cut into 2 mm small bits and surface sterilized with 1:1000 mercuric chloride (Hg_2Cl) solution for 30 seconds followed by 3 rinsing in sterilized distilled water. The cut pieces were placed in between two layers of sterilized blotting sheets so as to remove moisture and transferred ascetically to PDA plates with the help of sterilized inoculation needle and incubated in the BOD incubator at temperature of 27 ± 1^0C. Identification of the fungus was made after examining of conidia under microscope (under 10X) from mature pure culture. Stage and Ocular micrometer were used to measure the length, breadth and number of septa of the conidia. To get mature pycnidial bodies the cultures were further incubated up to thirty days. Diameter of ten pycnidial bodies were recorded with help of screw gauge. Total number of pycnidia produced per cm^2 and shape of pycnidia were also recorded.

Pathogenicity

Pathogenicity test was carried out to determine the fungal association and incidence of disease of brinjal (Koch's Postulation).Pathogenicity of the isolated fungus was tested with three methods viz., (i) seeds and soil inoculation (ii) leaf inoculation (iii) fruit inoculation

Seed and Soil inoculation: For Pathogenicity test of the pathogen through seed and soil inoculation, one hundred surface sterilized seeds of variety, Pusa Purple Long (susceptible) were sown in a tray (9 dia.) containing sterilized soil and another tray containing inoculated soil. In another set, inoculated seed were sown in same manner and kept in glass house.

Treatment

1. Seed & soil inoculated
2. Healthy seed and soil inoculated
3. Seed inoculated and soil uninoculated
4. Control (seed & soil uninoculated)

Leafinoculation: Inoculation of the pathogen in leaf was made by two methods i.e. spraying with glass automizer and smearing spore suspension with the help of a camel hair brush. Prior to inoculation, leaf injury was done with the help of an entomological needle. Four pricks (1-2 mm deep) at equal distance (5 mm) were made. Inoculated area was covered with sterilized non-absorbent cotton to avoid contamination and provide moisture. Observations on disease expression were recorded upto eight days. Uninoculated control was maintained.

Fruitinoculation: A small amount (0.2 ml) of spore suspension was placed on the healthy fruit surface without injury and with injury by pin-prick method and then covered with sterilized cotton. Suitable uninoculated checks were maintained.

Results

In order to determine the prevalence of fruit rot disease, a roving survey were under taken in brinjal growing adjoining areas of Ranchi during *Rabi*, 2016-2017 cropping season. The disease was prevalent in all the ten locations surveyed and incidence of disease varied from 18.33 to 62.00 per cent. Maximum incidence (62.0 per cent) was recorded in the cultivar Pusa Purple Long at Technology Park of Birsa Agricultural University, Kanke, Ranchi whereas as least disease incidence (18.33 per cent) was recorded in cultivar Pratima at Nagri village of Kanke block (Table 1). Sharma *et al*. (2011) [6] conducted a field Survey in the brinjal growing regions of Jammu and recorded 07.0−14.7 per cent of fruit rot incidence and 03.0−08.0 per cent fruit rot intensity of Phomopsis leaf blight of brinjal during two consecutive years 2007 and 2008. Jayaramaiah *et al*. (2013) [3] recorded 5−23 per cent disease incidence of leaf blight and 30−60 per cent fruit rot incidence in different brinjal growing areas of Mysore and Mandya Districts of Karnataka.

Isolation and Morphological and cultural identification

The isolation of the pathogen was made from infected fruits of brinjal by following tissue isolation technique. The pathogen was purified by hyphal tip and by transfer of single pycnidium on the fresh medium. The colony of *P. vexans* is fluffy with wavy margins, Floccose in appearance, compact and thick**,** whitish to pale in color with distinct concentric ring. A distinct yellowish zone alternated with dark brownish zone was observed on the reverse side of the colony (Table 2). Morphologically the pathogen produced hyline, septate and branched mycelium uneven in thickness often swollen at the base of branch. It was observed that the hyphal thickness was more in the medium as compared to host (Table 3). Numerous brown, globose to irregular pycnidia were also produced after 27 days of inoculation densely distributed in mycelium and Produces alpha conidia which are hyaline, one celled, sub-cylindrical and 5-8.8 x 2-3.2µ in size (Table 4). Punithalingam and Holliday (1972) [5] isolate the pathogen from symptomatic tissues of brinjal on PDA having white floccose mycelium and black, globose to irregular pycnidia up to 290 µm in dia. Alpha conidia were one celled, hyaline, and ellipsoidal (5.5-9.05 µm long x 1.9-2.3 µm wide); beta conidia were one celled, hyaline, filiform and straight or curved (19.9-28.2 µm long x 0.95-1.32 µm wide). Similar findings was reported by Sugha *et al.* (2002) [7] that alpha and beta are the two forms of the same conidium. *Phomopsisvexans* produces only one type of conidia in its pycnidia, which are hyaline, one celled, sub-cylindrical and 5-9 x 2-2.8 µ in size during summer months, which gradually changed into the beta form. According to Mahadeva kumar *et al.* (2017) [4] the pathogen produced whitish colony with weavy margin. Conidia were single celled, bi-guttulate, and 4.6-7.4 × 1.2-2.0 µm. Pycnidia were submerged and produced all over the surface when isolated from fruit rot symptom of brinjal. On the basis of cultural and morphological characters, the fungus was tentatively identified as *Phomopsisvexans*.

Table 1: Survey for natural occurrence of Phomopsis blight/ fruit rot disease of brinjal

Locations	Name of cultivar/ Variety	*Disease incidence (%)
Technology park	Pusa Purple Long	62.00(52.06)
Chutia	Pusa Kranti	52.00(46.56)
Bukru	Arka sheel	24.06(29.58)
Boreya	Pusa Ankur	30.00.(32.25)
Nagri	Pratima	18.33(24.98)
Sukurhutu	Kusuma	22.00(27.64)
Pithoria	Swarna Shyamli	45.33(42.30)
Huseer	Pusa Barsati	43.33(41.12)
Hochar	Pusa Purple Cluster	41.66 (40.14)
Itki	Pusa Navneet	30.66 (33.43)
S.E.m ±		2.13
C.D at 5%		6.38
CV		9.88

*Mean of three replication

Table 2: Cultural characterization of *Phomopsisvexans* in PDA

Parameter	Particular
Growth characteristics	Fast and fluffy with wavy margins
Texture	compact and thick
Color of colony (Top view)	White to pale
Color of colony (reverse view)	yellowish with dark brownish zone
Colony Diameter	88 mm
Appearance of colony	Floccose
Concentric ring	Present

Table 3: Measurement of mycelium on host surface and PDA

Particulars	On Host(µ)		PDA
	Leaf	Fruit	
Mycelium	1.29-3 .1	1·3-2.9	3.73-5 .28

Table 4: Pycnidial charactersof *Phomopsisvexans* in PDA

Parameter	Particular
Development of pycnidia	27 days
Shape of pycnidia	globose to irregular
Distribution of pycnidia	dense
No of pycnidia	90-120
Color of pycnidia	black
Size of pycnidia	
Liberation of pycniospore	Alpha conidia
Size of alpha conidia	5-8.8 x 2-3.2 µ

Pathogenecity of the pathogen was proved by three methods i.e. seed, soil, leaves and fruit inoculations. In seed and soil inoculation test by *P. vexans*there was reduction in seed germination and least germination (40%) was recorded, when both seed & soil were inoculated as compare to 78 per cent in the control. However, maximum seedling mortality (40 %) was observed in seed and soil inoculation followed by seed inoculated in healthy soil (9 %) and healthy seed sown in inoculated soil (6 %).

Pathogenecity test on leaves is done by inoculating the spore suspension of pathogen by two methods i.e. Spraying with glass automizer and smearing spore suspension with the help of a camel hair brush. Prior toinoculation, leaf injury was done with the help of an entomological needle. Typical *Phomopsis*blight symptoms developed on both injured & uninjured leaves.

Brinjal fruits inoculated with *P. vexans* following pin-prick method showed small, roughly circular, soft, light yellow lesion (5 x 7.9 min) surrounded by light brown ring after 8-10 days of inoculation whereas in uninjured fruits the lesion area (5 x 5 mm) exhibited slight discoloration only. The increase in lesion area was faster in injured as compared to uninjured. Chowdhary and Hasija (1979) [2] inoculated leaf, stem, petiole and fruits of eggplant with spore suspension of *P. vexans* for proving the pathogenecity of the pathogen. Thippeswamy *et al*. (2006) [8] conducted pathogenicity test of *Phomopsisvexans* and *Alternariasolani* on Round Green variety of brinjal by spraying conidial suspension ($1x10^4$) and observed the symptom after 2 to 3 days of inoculation. Jayaramaiah *et al*. (2013) [3] conducted Koch's postulates by sowing seeds of brinjal collected from the healthy fruits of farmer's field in tray containing soil: sand: compost (2:1:1) and spraying the conidial suspension of *P.vexans* ($1x10^5$ conidia/ml) on 30 day's old seedlings. Appearance of leaf blight symptoms was assessed after 15 days of post inoculation.

This is the first comprehensive study on the quantification of disease incidence of *Phomopsis* leaf blight and fruit rot disease on brinjal from Jharkhand (India). As the disease severity increased year after year in major brinjal growing regions, there is a need for screening and identifying disease resistant cultivars against leaf blight and fruit rot disease. This will be the long term strategy to prevent crop losses due to *D. vexans* infection.

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