SHORT COMMUNICATION

First report on leaf tip blight disease of tuberose (*Polyanthes tuberosa* L.) caused by new *Phoma* sp., *Phoma mondouriensis* from West Bengal

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First report on Leaf Tip Blight disease of Tuberose *(Polyanthes tuberosa* L.*)* caused by new *Phoma* sp., *Phoma mondouriensis* from West Bengal

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During last three years, a severe leaf tip blighting of tuberose (*Polyanthes tuberosa* L., family Asparagaceae,) has been observed initially at Mondouri, the Horticultural Research Farm of Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India and later on at the farmers' field and private gardens situated at 11 districts of the State. The disease first appears as brown elongated lesions on one or both margins of leaf towards the leaf tip. Lesions coalesce to form broad patch, progress basipetally and become brown to grey or straw coloured. Numerous black dots like erumpent pycnidia are formed sub-epidermally. Pathogen appears greyish black from the top but dark brown to black at the bottom on potato dextrose medium (PDA) medium, covers 90 mm diameter of Petri plate within four days after inoculation and produces dry biomass of 0.222 - 0.268 g (av. 0.249 g) within 7 days when grown on 30 ml potato dextrose broth in 250 ml borosil conical flask. Septate, branched, hyaline to dark brown mycelia with 3.4 – 6.2 μm (av. 4.2 μm) width and 11.9 – 34.1 μm (av. 21.9 μm)septal distance were measured along with the recoding of immersed, numerous micro-sclerotia with 54.2 - 101.1 μm (av. 75.4 μm) x 47.3 – 75.2 μm (av. 60.2 μm) in size, 194.1 – 284.2 μm (av. 227.4 μm) in perimeter and 2749.1 – 5412.9 μm^2 (av. 3754.9 μm^2) in surface area. Numerous ostiolate [ostiol diameter 10.0 – 11.5 μm (av. 10.9 μm)], erumpent, solitary or confluents, glabrous, sub-globose to globose, brown to black pycnidia [148 – 173.1 μm (av. 163.1 μm) x 134.8 – 167.7 μm (av. 147.5 μm) in size] lined with pseudo-parenchymatous wall of 3 – 4 layers with 3.8 - 4.8 μm (av. 4.3 μm) thickness. Conidia are unicellular, hyaline, elongated, sub-cylindrical, slightly uneven wall, very finely echinulate/ warty, eguttulate, rounded tips with 11.8 – 25 μm (av. 19.6 μm) x 3.7 – 5.5 μm (av. 4.8 μm) in size. No pycnidia production was observed in the PDA medium. Pathogenicity test of the isolated fungus has been established in the laboratory with detached leaf and in the field with attached leaves. Host range studies indicate that the pathogen is specific to tuberose and does not infect other five plants *viz.* agave [*Agave americana* L], aloe [*Aloe vera* (L.) Burm f.], dracaena [*Dracaena fragrans* (L.) Ker Gawl], nolina (*Beaucarnea recurvata* Lem.) and sansevieria (*Sansevieria trifasciata* Prain) under the same family. This is the first report of tuberose leaf tip blight disease caused by new *Phoma* sp.,*Phoma mondouriensis* from West Bengal.

Key words: Tuberose, *Polyanthes tuberosa*, tip blight, *Phoma mondouriensis*, host range

Tuberose (*Polyanthes tuberosa* L.), belongs to the family Asparagaceae, is one of the economically and commercially important flower crops grown in West Bengal occupying third position, covering

5000 ha area under cultivation and contributing 13% of the total tuberose cultivable area and 7% of the total tuberose production of India. It is affected by eight fungal diseases reported from different corners of the world. Of those, five fungal diseases *viz.* leaf blight disease caused by *Alternaria polyanthi* Mariappa, Babu and Kandaswamy from Tamil Nadu and *Alternaria alternata* (Fr.) Keissler from Gujrat, stem/root/foot rot caused by *Sclerotium rolfsii* Sacc. from Maharashtra, West Bengal, Assam, Karnataka and Bihar, blossom blight caused by *Fusarium equiseti* (Corda) Sacc. from Assam, anthracnose caused by *Colletotrichum capsici* (Syd.) E.J. Butler and Bisby and a disease caused by *Phoma polyanthis* Died from Bihar have been reported in India (De, 2013).

During last three years, a severe leaf tip blighting of tuberose has been observed initially at the experimental field of All India Co-ordinated Floriculture Improvement Project (ICAR) at Mondouri, the Horticultural Research Farm of Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India ($22^0$43' N latitude and $88^0$34'E longitude with an elevation of 9.75 m above mean sea level) (De, 2013) and later on at the farmers' field and private gardens situated at Nadia, South and North 24 Parganas, Howrah, Hooghly, Medinipur, Bankura, Budwan, Birbhum, Murshidabad and Cooch Behar districts of West Bengal.

Tuberose leaves showing typical tip blight disease symptoms (Figure 1a, 1b and 1c) were collected from the experimental field, cut into small pieces, surface sterilized with 0.1% mercuric chloride ($HgCl^2$) solution for 45 seconds, rinsed serially 5 – 6 times with sterile distilled water and made blotted dry with sterilized blotting paper. Then the surface sterilized diseased leaf pieces were transferred on to the solidified water agar medium in sterilized Petri plates, incubated in BOD at 28 ± 1^0C and observed periodically for 1.5 – 2.0 cm growth of fungus. Then the bits of fungal growth from such area (Figure 1d) were transferred to PDA slants, incubated in BOD for few days and finally preserved the full grown slant (Figure 1e) in a refrigerator for further studies.

Pathogenicity test of isolated fungus was done in the laboratory with detached leaf (Figure 1h) and in the field with attached leaves (Figure 1i). For both the laboratory and field testing, apparently disease free leaves were selected, washed with sterile distilled and made blotted dry and inoculated with 5mm disc of 48 hours young active fungal culture near its tip; but the inoculated leaves were kept inside moist-chambered Petri plates in the former whereas in the latter these were wrapped with absorbent cotton soaked with sterile distilled water followed by wrapping with polyethylene strip and tying with a cotton thread and kept under cover for 48 hours. The procedure of inoculation as followed in case of tuberose under field condition was also followed during the inoculation of other five plants *viz.* agave [*Agave americana* L], aloe [*Aloe vera* (L.) Burm f.], dracaena [*Dracaena fragrans*(L.) Ker Gawl], nolina (*Beaucarnea recurvata* Lem.) and sansevieria (*Sansevieria trifasciata*Prain) under the same family.

Disease first appears as brown elongated lesions on one or both margins of leaf towards the leaf tip (Figure 1a). These spots coalesce with each other, later under high humid condition or during rainy season form uniform water soaked patch and progress downward (Figure 1b). The leaf tip dries up basipetally. Colour of the affected portion of leaf changes from brown to grey or straw. Numerous black dots like erumpent pycnidia are formed sub-epidermally on straw coloured dead tissues (Figure 1c).

Typical leaf tip bight symptoms develop in tuberose within 3 – 5 days after inoculation under laboratory whereas within 6 - 8 days after inoculation under field condition. Black dot like pycnidia did not developed under laboratory condition but the same developed within 15 – 20 days after inoculation only under field conditions. Neither the tip blight symptom nor any black dot like pycnidia was observed on the five inoculated hosts considered for host range studies even at one month after inoculation. Re-isolation of pathogen and collection of pycnidia from inoculated tuberose leaves was done and comparison was made with previously isolated culture and collected pycnidia for establishing firmly the pathogenicity of isolated fungus.

Colony morphology of the isolated fungal pathogen on PDA medium appears greyish black from the top but dark brown to black from the bottom of Petri plates. Mycelia of the pathogen grown on this medium covers 90 mm diameter of Anumbra glass

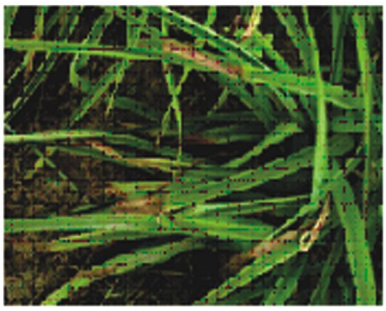

Fig. 1a : Basipetal leaf tip drying

Fig. 1b : Starting of pycnidia formation on the lesion

Fig. 1c : Pycnidia on dried straw coloured leaf tip

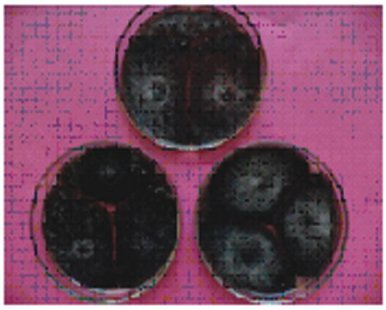

Fig. 1d : Isolation of pathogen from diseased leaf

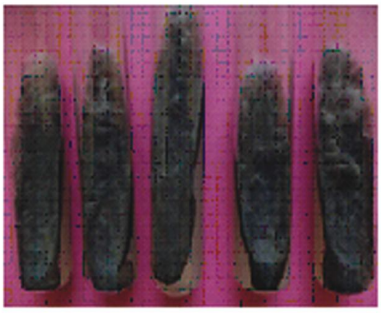

Fig. 1e : Purified culture of pathogen

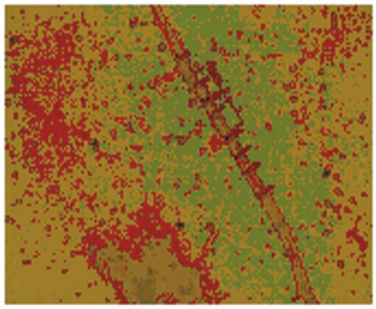

Fig. 1f : Brown septate hypha of pathogen

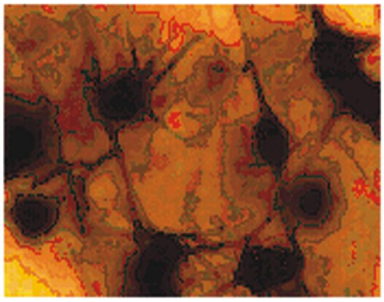

Fig. 1g : Micro-sclerotia of pathogen

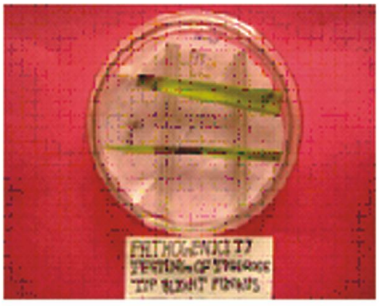

Fig. 1h : Pathogenicity testing under lab. condition

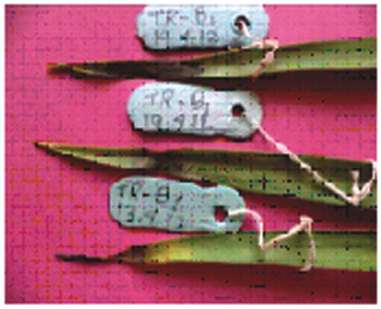

Fig. 1i : Pathogenicity testing under field condition

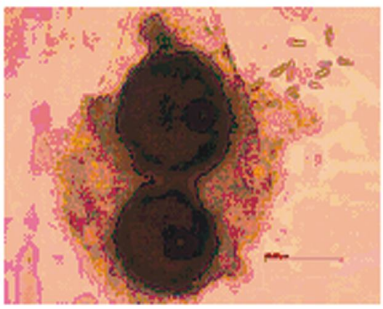

Fig. 1j : Pycnidia bearing ostiole and pycnidiospores

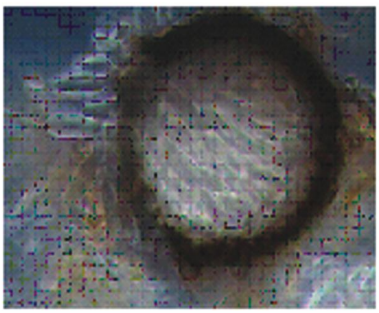

Fig. 1k : Cavity of pycnidium packed with pycnidiospores

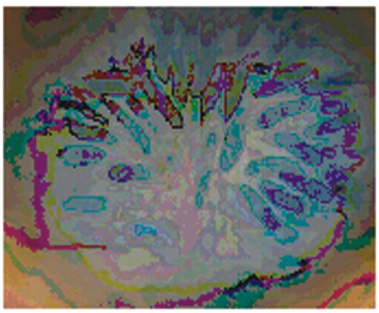

Fig. 1l : Enlarged view of pycnidiospores

Fig. 1 : Disease symptoms caused by *Phoma mondouriensis* , its isolation, purification, pathogenicity testing and its hyphae, micro-sclerotia, pycnidia and pycnidiospores

Petri plate within four days after inoculation but produce dry biomass of 0.222 - 0.268 g (av. 0.249 g) within 7 days when grown on 30 ml potato dextrose broth in 250 ml borosil conical flask. Septate and branched mycelia are hyaline when young but dark brown when matured**.** Width of the hyphae varies from 3.4 – 6.2 μm (av. 4.2 μm) and the distance between two septa ranges from 11.9 – 34.1μm (av. 21.9 μm) (Figure 1f). Very minute, immersed, numerous micro-sclerotia are produced with sizes ranged from 54.2 - 101.1 μm (av. 75.4 μm) in length x 47.3 – 75.2 μm (av. 60.2 μm) in breadth, perimeter ranged from 194.1 – 284.2 μm (av. 227.4 μm) and surface area ranged from 2749.1 – 5412.9 μm^2 (av. 3754.9 μm^2) (Figure 1g). No pycnidia production was observed in the PDA medium.

Numerous ostiolate [ostiol diameter 10.0 – 11.5 μm (av. 10.9 μm)], erumpent pycnidia are formed sub-epidermally on straw coloured dead tissues of the leaf tips (Figure 1c)**.** They may be solitary or confluents, glabrous, sub-globose to globose, brown to black with pseudo-parenchymatous wall, 3 – 4 wall layers with composite wall thickness 3.8 - 4.8 μm (av. 4.3 μm), length - 148 – 173.1 μm (av. 163.1 μm), breadth – 134.8 – 167.7 μm (av. 147.5 μm) (Figure 1j). Conidiophores are not clearly visible. Perhaps, conidia develop from inner basal cells of the pycnidium. Conidia are unicellular, hyaline, elongated, sub-cylindrical, slightly uneven wall, very finely echinulate/ warty, eguttulate, rounded tips with 11.8 – 25 μm (av. 19.6 μm) x 3.7 – 5.5 μm (av. 4.8 μm) in size (Figure 1k, 1l). Studying the conidial and pycnidial characteristics and comparing those with the standard key on *Phoma* (Boerema *et al*, 2004), the genus of the pathogen has been identified as *Phoma.* Pycnidial stage of the fungus obtained from tuberose was first described by Sydow and Butler (1916). Description of the pycnidial stage given therein was *Pycniidis gregariis*, tectis epidermidem poro perforantibus, applanato-globosis,atro-brunneis, parenchymaticis, 100 - 150 μ dia.; sporophoris non visis; sporulis ellisoideis, utrinque rotundatis vel etiam attenuatis, continuis, hyalines, plerumque, 1 – 2 guttulatis (an tandem 1 septatis?), 5 – 7.5 x 2.5 – 4 μ. – *Phoma polyanthis* Died. nov.spec. [Hab. in foliis caulibusque emortuis, *Polyanthis tuberosae*, Pusa, 31.12.1906, leg. Inayat (E. J. Butler no. 1680). The pycnidia and conidia of *Phoma* obtained here during present investigation differ in many respects with the description of pycnidia and conidia of *Phoma polyanthis* given by Sydow and Butler. Pycnidial size mentioned by them was 100 - 150 ìm diameter whereas the same has 148 – 173.1 μm (av. 163.1 μm) length, 134.8 – 167.7 μm (av. 147.5 μm) in breadth with 3 – 4 wall layers with composite wall thickness 3.8 - 4.8 μm (av. 4.3 μm). Besides, the pycnidia bear ostioles having diameter 10.0 – 11.5 μm (av. 10.9 μm). Conidial shape and size mentioned by Sydow and Butler was ellipsoidal, both ends rounded, 1- 2 guttulate, may or may not possess 1 septum, sizes varied from 5 – 7.5 μm in length x 2.5 – 4 μm in breadth. But the present description of conidia of *Phoma* reveals that conidia are unicellular without any septation, elongated, sub-cylindrical, slightly uneven wall, very finely echinulate/ warty, eguttulate, rounded tips with size 11.8 - 25 μm (av. 19.6 μm) in length x 3.7 – 5.5 μm (av. 4.8 μm) in breadth. So, it can be emphasized that the morphological description of present *Phoma* sp. obtained from tuberose leaf tip blight disease grossly differs from *Phoma polyanthis* described by Sydow and Butler. So, the causal agent of tuberose leaf tip blight disease can be considered here as a new species of *Phoma* and it can be designated as *Phoma mondouriensis.* Species name of the pathogen has been chosen as *mondouriensis* based on the name, Mondouri, the place of disease specimen collection. The disease specimen has been deposited at the *Herbarium Cryptogamae Indiae Orientalis* (HCIO) of Indian Agricultural research Institute, New Delhi, India (HCIO No. 51809).

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