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Pathogenicity and Morphological Variabilities of *Lasiodiplodiatheobromae* Isolates in Tuberose

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ABSTRACT: Peduncle blight, hitherto an unknown disease was found to be a major limiting factor to the cultivation of tuberose, as the disease incidence was noticed up to 42.60 per cent in pockets of Madurai district. Though Lasiodiplodia theobromae is an ubiquitous pathogen, its occurrence on tuberose is a new record. Peduncle blight disease infected tuberose plants were collected from seven places of Tamil Nadu, India. Among the seven isolates screened for virulence, isolate I_1collected from Agricultural College in Madurai district recorded maximum infection under artificial inoculation. The isolate I_1 significantly recorded 570 mm lesion length in pinpricked buds and also recorded 47.00 mm mycelial growth per day. Lasiodiplodiatheobromae produced dark brown, flask-shaped, ostiolate pycnidia, 110-170 mm x 60-130 mm in size appeared in seven-day-old cultures.Conidia were initially globose to oblong, hyaline and unicellular later turning brown and septate, measuring 18.5-21.7 mm x 8.0-11.2 mm. Compact, fluffy and sparse colony types were observed among the isolates. Significant variation was observed in morphological character and virulence among the Lasiodiplodia theobromaeisolates.

Key words: Morphological character, Lasiodiplodia theobromae, Pathogenicity, Tuberose

INTRODUCTION

Tuberose (*Polianthes tuberosa* Linn.) is one of the most important ornamental plants which is extensively cultivated in many sub-tropical and tropical areas of the world (Biswas *et al.*, 2002) Total area under tuberose in India is around 3000 ha with an average yield of 6300 kg per ha. The flower production in Maharashtra was 9180 tonnes (Bhattachaaarjee *et al.*, 1994). In India the incidence of peduncle blight disease was first reported by (Durgadevi and Sankaralingam, 2012). The causal organism *Lasidiplodia theobromae* was found to be associated with this disease, producing blossom blight, peduncle blight and blighting of leaf tips.

Morphological characters are important tools inthe identification and classification of fungus. Several workers described enormous pathogenic variability among *Lasidiplodia theobromae* isolates (Patil *et al.*, 2006; Khanzada *et al.*, 2006). Hence, the present investigationwas conducted to study the morphological andpathogenic variability among isolates of *Lasidiplodia theobromae.*

ISOLATION OF PATHOGEN

Peducle blight diseas infected plants were collected from tuberose growing areas of The pathogen causing peduncle blight in tuberose was isolated from the samples by tissue segment method Infectedportions of diseased plants were cut into smallpieces using sterilized scalpel and surface sterilizedwith 0.1 per cent mercuric chloride for one minuteand washed in three changes of sterile distilledwater and then placed on Petri dish containingsolidified Potato Dextrose Agar (PDA) medium.These plates were incubated at room temperature(28 ± 2°C) for five days and observed for the growthof the fungus. The hyphal tips of fungi weretransferred aseptically to PDA slants for maintenanceof the cultureon potato dextrose agar (PDA) and the fungus was purified by single spore isolation and maintained on PDA.The causal organism was identified based on spore morphology and confirmed further (ID.NO. 6751/11) by Indian Type Culture Collection Centre (ITCC) of Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi.

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MORPHOLOGICAL VARIABILITY AMONG *LASIDIPLODIA THEOBROMAE* ISOLATES

From the five day old culture plates nine mmculture disc of the pathogen was cut by a sterilizedcork borer and placed at the center of each sterilePetri dish containing 20 ml of previously sterilizedand solidified PDA medium. The plates wereincubated at room temperature (28+2oC) for fivedays. The growth and morphological characters ofthe isolates *viz.*, colony morphology, mycelial growthrate, colony colour, pycnidiasize, shape and septationwere observed, measurement was made under themicroscope (Olympus BX41).

PATHOGENIC VARIABILITY AMONG *LASIDIPLODIA THEOBROMAE* ISOLATES

Detached Flower Bud Technique

A five- mm culture disc of *L. theobromae* was placed closer to the calyx of healthy detached flower bud and kept in 150-mm-dia Petri dish over a layer of moistened filter paper. An empty five- mm disc of PDA served as control.

Three replications were maintained and the plates were incubated at room temperature (28 ± 2° c). The formation of lesion on flower bud was closely monitored and the lesion length was recorded at regular intervals.

Pathogenicity in Glasshouse

The pathogenicity of the fungus was confirmed by Koch's postulates using five numbers of four-month-old healthy plants. Plants were inoculated by making a vertical cut (3 mm) in the peduncle region below the calyx using a sterilized needle and placing a fungal disc over the wound. The inoculated area was covered with moist cotton and wrapped with parafilm. The plants were covered with polythene bags to maintain humidity and monitored for symptom expression. Proper controls were maintained with PDA plugs.

RESULTS AND DISCUSSION

The growths and colony characters of seven isolates of *L. theobromae* were assessed on PDA. Of the seven isolates studied for their cultural characters, five isolates, namely I_1, I_2, I_3, I_4 and I_5, showed colony growth rate of 1.63 to 1.88 mm/h indicating their fast growing nature (Table 1, Figure 1a, 1b). The isolate I2attained full nine-cm-dia growth at 48 h after inoculation. On the same day growth of the other isolates I_2 (88.30mm), I_3 (87.00mm) and I_4 (86.60mm) were on par with I_1. The lowest growth was observed with the isolate I_6 (64.30 mm). The colony colour of the isolates were dull white (I_1, I_7), cottony white changing to black (I_4, I_6) and greyish white (I_2, I_5). The isolates I_1, I_4 and I_6 showed dense, fluffy growth. I_3 was partial fluffy. However, I_2 and I_4 were sparse in growth. The growth of I_5 was appressed. The mycelial were fast spreading, branched, septate and pycnidia were brown coloured. Conidia were initially globose to oblong, hyaline and unicellular turning brown and septate later. This is in accordance with the descriptions of *L. theobromae* reported earlier by Punithalingam (1979). The results indicated that difference in morphological character was positively correlated with its virulence.

Peduncles of tuberose showing typical symptoms of blight were collected from seven locations (Table 2). The age of the crop varied from 9 to 28 months and cultivars with single flower were common than double flower types. The disease incidence ranged from 12.00 to 42.60 per cent.In the current survey, blighting of flower buds of tuberose followed by dieback of peduncle from tip downward are the major

Table 1
Morphological Characters and growth of Different Isolates of *L. theobromae* on Potato Dextrose Agar

Sl. No.	*Isolatecode*	*Colony type*	*Colour*	*Mycelial growth (mm)*	*Mycelial growth rate(mm/h)*
				48 h	
1	I_1	Coarse, fluffy	Dull white	90.00	1.88
2	I_2	Sparse	Greyish white	88.30	1.84
3	I_3	Partial fluffy	Dull white	87.00	1.81
4	I_4	Coarse	Black	86.60	1.80
5	I_5	Flat	Greyish white	78.30	1.63
6	I_6	Dense, Fluffy	Black	64.30	1.34
7	I_7	Sparse	Dull white	77.00	1.60
		CD		6.44	

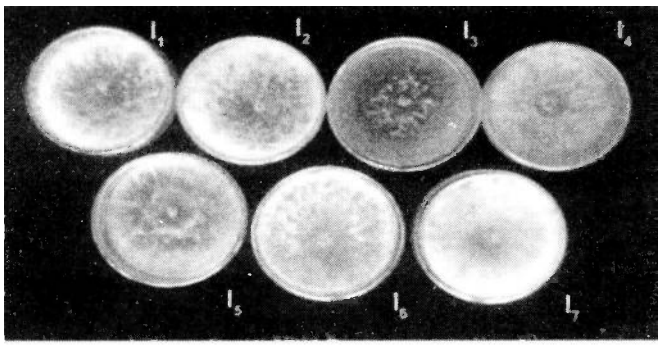
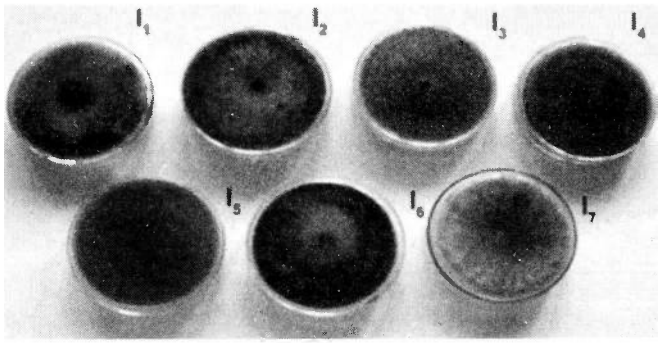


Figure 1a: Isolates of *Lasiodiplodia theobramae* on PDF Growth at 3 Days after inoculation



1b: View through the bottom of the culture plates

symptoms observed in the field. Tests of pathogenicity by detached flower technique (Figure 2) as well as those conducted at greenhouse yielded symptoms as observed in the field. The fungus also caused symptoms on flower buds without wound after an incubation period of seven days. However, wounding was found to enhance the symptom expression.

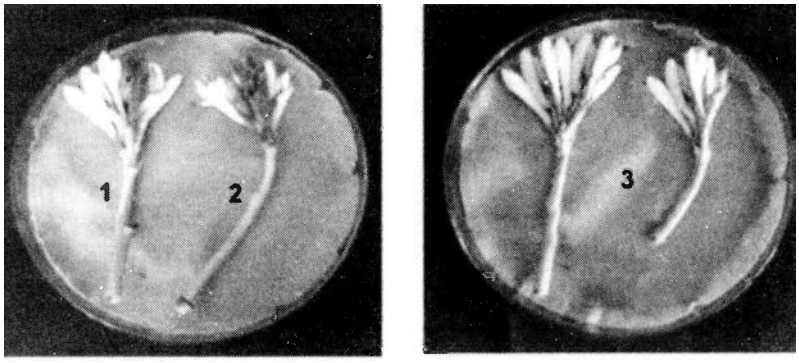
The fungus *Lasiodiplodia theobromae* has a wide host range infecting monocot and dicot plants causing an array of symptoms including shoot bight and die back

(Arx, 1987). In cashew it causes drying of petals and other flower parts followed by die back of peduncles leading to inflorescence blight (Olunloyo, 1979). The fungus causes necrosis and die back of shoots in mango (Burhan, 1987; Khanzada *et al.*, 2004) and grapevine (Wood and Wood, 2005).

The present investigation revealed that the isolates of *Lasiodiplodia theobromae* exhibited high variability in morphological characterand pathogenicity which could be used for further development of race specific resistant varieties of onion for the control of disease.

Table 2
Occurrence of Peduncle Blight in Tuberose Growing Areas

Sl. No.	*Isolate code*	*Location*	*Variety*	*Age of the crop (months)*	*Disease Incidence (%)*
1	I_1	AC and RI (Madurai)	Suvasini (Double)	24	42.60
2	I_2	Vilampatti (Dindugaldt)	Prajwal (Single)	9	19.45
3	I_3	Kodairoad (Dindugaldt)	Prajwal (Single)	14	34.33
4	I_4	Kannanur (Madurai dt)	Prajwal (Single)	8	12.00
5	I_5	Kannanur (Madurai dt)	Prajwal (Single)	12	24.65
6	I_6	Sekanoorani (Madurai dt)	Prajwal (Single)	10	17.11
7	I_7	Chellampatti (Madurai dt)	Prajwal (Single)	28	25.33



1. Pinpricked flower
2. Without pricking
3. Control

Figure 2: Pathogenicity on detached flower bud

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