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Survey and characterisation of *Fusarium oxysporum* f.sp. *cepae* causing basal rot of onion (*Allium cepa* L.) in Perambalur district of Tamil Nadu

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Abstract
Basal rot of onion, incited by *Fusarium oxysporum* f.sp. *cepae*, is one of the most destructive soil-borne diseases limiting onion productivity worldwide. A systematic survey was conducted across ten major onion-growing areas of Perambalur district, Tamil Nadu, to determine the incidence of basal rot and to isolate the causal pathogen. Disease incidence ranged from 11.26% to 60.15%, with the highest recorded at Settikulam and the lowest at Nakkasalem. Ten isolates of *F. oxysporum* f.sp. *cepae* were obtained and evaluated for cultural and morphological variability. Distinct differences were observed in colony colour, mycelial growth, conidial morphology and sporulation intensity among the isolates, with Foc3 identified as the most virulent based on growth and sporulation parameters. Sand–maize medium supported maximum fungal biomass production compared to sorghum and cumbu grains. The results confirm significant pathogenic diversity among *F. oxysporum* f.sp. *cepae* isolates in Perambalur district of Tamil Nadu and highlight the necessity for region-specific management strategies.

Keywords: Basal rot, *Fusarium oxysporum* f.sp. *cepae*, onion, pathogenic variability, Perambalur district

Introduction
Onion (*Allium cepa* L.) is one of the most important bulb crops cultivated worldwide and is often referred to as the "Queen of the kitchen" due to its indispensable culinary and nutritional value. Belonging to the family *Alliaceae*, it is rich in essential nutrients such as vitamin C, vitamin B6, folic acid and dietary fibre and possesses several pharmacological properties including antioxidant, antimicrobial and anticancer activities [26, 14]. India ranks second globally in onion cultivation, with Tamil Nadu contributing significantly through districts such as Dindigul, Tiruppur, Perambalur and Namakkal [16].

Despite its economic importance, onion productivity is severely constrained by soil-borne diseases, among which basal rot, caused by *Fusarium oxysporum* f.sp. *cepae*, is highly destructive. The pathogen infects the basal plate, leading to yellowing, wilting and eventual plant death, causing yield losses of up to 50% [4]. The survival of the fungus as chlamydospores for extended periods (8–10 years) in the soil further complicates its management [17]. Chemical control and resistant cultivars have shown limited success due to environmental concerns, cost factors and the emergence of virulent pathogen strains [9].

Although several studies have reported the occurrence of *Fusarium* wilt in onions, detailed investigations on the incidence, isolation and pathogenic variability of *F. oxysporum* f.sp. *cepae* under Tamil Nadu conditions remain scarce. Moreover, limited information exists on the occurrence of native antagonists with potential for biocontrol applications in the region. Understanding the pathogenic and cultural variability among isolates and identifying effective native biocontrol agents are crucial for developing sustainable disease management strategies.

Therefore, the present investigation was undertaken with the following objectives:

1. To conduct a survey to assess the incidence of basal rot of onion in major onion-growing areas of Tamil Nadu and to isolate the pathogen *Fusarium oxysporum* f.sp. *cepae* and native antagonists.

2. To assess the cultural, morphological and pathogenic characterisation among the isolates of *Fusarium oxysporum* f.sp. *cepae*.

Materials and Methods

Survey to Assess the Incidence of Basal Rot of Onion

A field survey was conducted across ten major onion-growing regions of Perambalur district, Tamil Nadu, to evaluate the incidence of basal rot. In each field, 100 plants were randomly selected and the number of infected plants was recorded. The mean disease incidence was calculated as a percentage using the formula of McKinney [11]. Onion plants exhibiting characteristic symptoms of Fusarium basal rot were collected along with their rhizosphere soil for pathogen isolation. Additionally, data on locality, crop variety and soil type were recorded [10].

$$\text{Disease Incidence \% (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Isolation and Identification of *Fusarium oxysporum* f. sp. *cepae*

Onion samples collected from various growing regions of Perambalur district, Tamil Nadu, were used for pathogen isolation. The pathogen was isolated from infected onion bulbs using the tissue segment method as described by Rangaswami [19]. The infected bulb tissues were cut into small pieces with a sterile scalpel and surface sterilised with 1.0% sodium hypochlorite for 30 seconds. The tissues were then rinsed three times with sterile distilled water and transferred aseptically onto Petri plates containing sterilised Potato Dextrose Agar (PDA). Plates were incubated at room temperature (28 ± 2 °C) for 5–7 days and observed for fungal growth. The hyphal tips of emerging colonies were subcultured onto PDA slants for maintaining pure cultures. The isolated fungi were identified based on their cultural and morphological characteristics.

Morphological and Cultural Characteristics of *Fusarium oxysporum* f. sp. *cepae*

Ten isolates of *Fusarium* spp. obtained from infected onion bulbs were examined for variations in their morphological and cultural characteristics on both solid and liquid media. Ten-day-old cultures of each isolate were inoculated separately and incubated at 28 ± 2 °C for seven days. After incubation, parameters such as radial mycelial growth, microconidia and macroconidia population, colony characteristics, sporulation intensity and size of microconidia, macroconidia and chlamydospores were recorded. The conidial and chlamydospore counts and measurements were carried out following standard mycological procedures.

Mass Multiplication of the Pathogen

Sand–Maize Medium

Mass multiplication of *F. oxysporum* f. sp. *cepae* isolates was carried out using the sand–maize medium following the method of Riker and Riker (20). Sterilised sand and ground maize seeds were mixed in a 19:1 ratio and adjusted to approximately 50% moisture content. The mixture was filled into glucose bottles and autoclaved at 20 psi for 2 hours. Seven-day-old actively growing mycelial discs (9 mm diameter) were inoculated into each bottle under aseptic conditions. The bottles were incubated at room temperature (28 ± 2 °C) for 15 days. The colonised substrate was then used as inoculum for subsequent experiments.

Sorghum and Cumbu Grain Medium [6]

Sorghum and cumbu grains were filled up to three-fourths of the glucose bottles and plugged with cotton wool. The bottles were sterilised at 15 lbs pressure for 2 hours on two consecutive days. Six mycelial discs (9 mm) from 10-day-old *F. oxysporum* f. sp. *cepae* cultures grown on PDA were inoculated into each bottle. The cultures were incubated at room temperature (28 ± 2 °C) for 15 days and the resulting inoculum was used for soil inoculation.

Inoculation

Five kilograms of sterilised garden soil were filled into earthen pots (30 cm diameter). The soil was sterilised in an autoclave at 15 lbs pressure (1.04 kg cm^{-2}) for two successive days and inoculated by thoroughly mixing the freshly prepared *Fusarium* inoculum (multiplied on sand–maize medium) at a rate of 50 g kg^{-1} soil [13]. Two onion bulbs were planted in each pot, with three replications per treatment. The pots were maintained in a greenhouse under uniform and regular watering conditions and observed periodically for symptom development. The percentage disease incidence for each isolate was recorded 60 days after inoculation.

Statistical Analysis

All data collected were statistically analysed using the WASP Version 2.0 software developed by the Indian Council of Agricultural Research (ICAR), Goa, following the procedure of Gomez and Gomez [7]. Prior to analysis of variance (ANOVA), percentage data of the disease index were arcsine-transformed. ANOVA was performed at a significance level of $P<0.05$ and mean comparisons were made using Duncan's Multiple Range Test (DMRT). Pot culture, laboratory and field experiments were designed following the Randomised Block Design (RBD). Percentage values were subjected to arcsine or square root transformation as appropriate.

Results and Discussions

Survey and Incidence of Basal Rot of Onion in Perambalur District of Tamil Nadu

An extensive field survey was conducted in major onion-growing areas of Perambalur district, Tamil Nadu, to assess the incidence of basal rot caused by *Fusarium oxysporum* f. sp. *cepae*. The disease incidence varied between 11.25% and 60.15% across the surveyed locations (Table.1, Plate 1). Among the villages, Settikulam recorded the highest basal rot incidence, which could be attributed to the cultivation of a susceptible variety and favourable environmental conditions for pathogen development. In contrast, Nakkasalem village showed the lowest disease incidence.

Findings from the present study are supported by earlier reports [26], which documented basal rot incidence in major onion-growing regions of Tamil Nadu, with the highest (18.07%) observed in Pollachi, Coimbatore district. Similarly, in Karnataka, the incidence of *Fusarium* wilt ranged from 1.71% to 42.77%, with the highest incidence (42.77%) at Annigeri village in Dharwad district [2]. Further surveys in the districts of Tirunelveli, Thoothukudi and Tenkasi also reported basal rot incidence in all locations, with the highest (78.50%) in Vallanadu village, Thoothukudi district [22]. Soil texture was also found to influence the incidence of basal rot. In the present survey, higher disease incidence was observed in red soils compared to black or clay loam soils (Table 1). Similar findings were reported earlier [5], indicating higher *Fusarium* wilt populations in sandy soils, followed by loamy sand and loam textures.

Table 1: Survey on the incidence of onion basal rot in major Onion growing areas of Perambalur district

Sl. No.	Isolate name	Location	Soil type	Variety	Disease incidence (%)
1.	Foc_1	Siruvayalur	Clay loam	Co 5	45.76^{c} (42.57)
2.	Foc_2	Mavilangai	Sandy loam	Co 1	27.54^{g} (31.65)
3.	Foc_3	Settikulam	Clay	Co 3	60.15^{a} (50.86)
4.	Foc_4	Pommanapadi	Sandy loam	Co 1	29.88^{f} (33.14)
5.	Foc_5	Navalapuram	Sandy loam	Co 5	39.87^{d} (39.16)
6.	Foc_6	Alambadi	Clay loam	MDU 1	35.64^{e} (36.65)
7.	Foc_7	Esanai	Clay	Co 1	23. 86^{h} (29.24)
8.	Foc_8	Koneripalayam	Red soil	MDU 1	19.32^{i} (26.07)
9.	Foc_9	Alathur	Clay loam	Co 3	58.65^{b} (49.98)
10.	Foc_{10}	Nakkasalem	Sandy clay loam	Co (on) 5	11.26^{j} (19.60)

* Mean of the three replications
* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Isolation and Cultural Characteristics of Various Isolates of *Fusarium oxysporum* f. sp. *Cepae*

Isolation and Colony Characters

The pathogen *Fusarium oxysporum* f. sp. *cepae* was isolated from diseased onion bulb samples on PDA medium. The isolates exhibited considerable variation in colony colour and texture, ranging from normal white to violet, dirty white and pink. Most isolates produced fluffy to moderately fluffy aerial mycelium, except the isolate Foc10, which showed thin, flat mycelium distinct from others (Table 3).

Earlier reports [18] indicated that the pathogen exhibited diverse colony colours such as light pink, dark pink, creamy white and pale white with pink, along with growth patterns like adherent, smooth and fluffy. Similar groupings among isolates of *Fusarium oxysporum* f. sp. *cepae* were also observed [22]. Colony colours described as purplish white, whitish orange, creamy white and cottony white were reported in previous studies (2017). Comparable diversity was also noted among *F. oxysporum* f. sp. *cubense* [21] and *F. oxysporum* f. sp. *lycopersici* [23, 24]. These earlier findings are in agreement with the present observations.

Table 2: Isolation and cultural characteristics of various isolates of *Fusarium oxysporum* f.sp. *cepae*

Sl. No.	Isolate name	Location	Cultural characteristics	Mycelial growth (mm)	Conidial population × 10^6ml
1.	Foc_1	Siruvayalur	Moderate aerial mycelium with white to pink colour	88.12^{b}	2.4^{c}
2.	Foc_2	Mavilingai	Moderate aerial cottony mycelium white to pink colour	84.12e	1.8^{g}
3.	Foc_3	Settikulam	Profuse fluffy cottony growth mycelium with white to pink colour	90.00^{a}	2.8^{a}
4.	Foc_4	Pommanapadi	Whitish fluffy growth mycelium, slightly pink colour	85.99^{d}	1.9^{f}
5.	Foc_5	Navalapuram	Aerial mycelium with white colour	87.18^{c}	2.2^{d}
6.	Foc_6	Alambadi	Moderate fluffy cottony growth mycelium, white to pale pink colour	86.66^{d}	2.0^{e}
7.	Foc_7	Esanai	Fluffy mycelium white to pink colour	83.24^{f}	1.3^{h}
8.	Foc_8	Koneripalayam	Thin flat mycelium, white to pale pink colour	78.78^{g}	0.9^{i}
9.	Foc_9	Alathur	Moderate fluffy cottony growth mycelium with slightly pink colour	89. 45^{b}	2.6^{b}
10.	Foc_{10}	Nakkasalem	Thin flatmycelium with slight pink colour	76.15^{h}	0.6^{j}

* Mean of the three replication
* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Mycelial Growth

All ten isolates of *Fusarium oxysporum* f. sp. *cepae* exhibited moderate, fluffy mycelial growth with dirty white to violet pigmentation on PDA medium. Among the isolates, Foc3 recorded the maximum mycelial growth (90 mm), followed by Foc9, Foc1, Foc5, Foc6, Foc2, Foc7 and Foc8, while Foc10 showed the minimum (78.78 mm) (Table 3). Comparable variations in mycelial growth among *F. oxysporum* f. sp. *cepae* isolates have also been reported earlier [22].

Table 3: Mycelial dry weight and conidial characters of different isolates of *Fusariumoxysporum f.sp. cepae* (Foc)

Sl. No.	Isolates	Mycelial dry weight(mg)	Micro conidia			Macroconidia			Chlamydospore size (µm)
			Size (µm)	Shape	Septation	Size (µm)	Shape	Septation	
1.	Foc_1	198.57^{c}	7.43×4.28	Oval	0	26.97×4.26	Sickle	3	7.12-7.30
2.	Foc_2	178.69^{e}	6.26×4.25	Fusiform	0	25.39×4.14	Sickle	4	6.76-6.92
3.	Foc_3	228.47^{a}	10.07×4.58	Oval round	0	29.25×4.78	Sickle	4	7.51-7.92
4.	Foc_4	167.44^{f}	6.10×4.13	Fusiform	0	23.16×3.62	Sickle	3	6.62-6.82
5.	Foc_5	199.59^{c}	7.45×4.30	Oval	0	26.99×4.31	Sickle	3	7.17-7.32
6.	Foc_6	187.48^{d}	7.14×4.80	Oval	0	23.22 ×3.50	Sickle	2	6.96-7.26
7.	Foc_7	155.24^{g}	5.84×3.68	Round	0	22.42×3.61	Sickle	2	6.52-6.78
8.	Foc_8	141.60^{h}	5.55×3.55	Oval	0	23.02×3.44	Sickle	4	6.48-6.64
9.	Foc_9	216.86^{b}	8.17× 5.25	Oval round	0	27.04×4.56	Sickle	3	7.22-7.62
10.	Foc_{10}	135.45^{i}	5.43×3.25	Fusiform	0	21.63×3.14	Sickle	2	6.23-6.52

* Mean of the three replications
* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Size of Micro- and Macroconidia

The isolates produced both micro- and macroconidia, ranging from 0.6 to 2.8 × 10^6 conidia ml^{-1}. Among them (Plate 4 & 5), Foc3 recorded the largest conidial size, with microconidia measuring 10.07 × 4.58 µm and macroconidia 29.25 × 4.78 µm, whereas the smallest conidia were observed in Foc10, with microconidia measuring 5.43 × 3.25 µm and macroconidia 21.63 × 3.14 µm. All isolates produced aseptate microconidia and 1–4 septate macroconidia (Table 3). Earlier studies [2] reported that *F. oxysporum* f. sp. *cepae* produces white, cottony mycelium with abundant hyaline, ovoid to ovate microconidia (3.5–8.0 × 2.5–3.5 µm) and sparse 3–5 septate macroconidia (19.5–29.5 × 3–5 µm). Comparable observations on conidial and chlamydospore variability among *Fusarium* spp. were also documented [27, 8, 21, 23]. Further, microconidia ranging from 4.3–12.5 × 2.4–3.1 µm and macroconidia 15.5–31.5 × 3.0–4.0 µm were recorded in *F. chlamydosporum* [12].

Mass Multiplication of *Fusarium oxysporum* f. sp. *cepae*

Mass multiplication of *Fusarium oxysporum* f. sp. *cepae* was

carried out using different substrates sand–maize medium, sorghum grains and cumbu grains (Table 4). Among these, maximum fungal growth was recorded in the sand–maize medium, followed by sorghum grains, while the least growth was observed in cumbu grains (Plate 3). Earlier studies [13] reported the use of sand–maize and sorghum grains for the mass multiplication of *F. oxysporum* f. sp. *cepae*. Similarly, sand–maize and sorghum media were used for the multiplication of *F. oxysporum* f. sp. *lycopersici* [24]. Sorghum grains were also identified as a suitable substrate for inoculum multiplication of *F. oxysporum* f. sp. *ciceris* [1]. In addition, pathogen isolates multiplied on wheat and sorghum grains and incorporated into the collar region at 20 g kg^{-1} soil were found to cause maximum collar rot incidence in tomato [3].

Table 4: Mass multiplication of *F.oxysporum* f. sp*cepae* in different substrates

SI. No.	Isolates	Mycelial growth in different substrates		
		Sand maize	Sorghum grains	Cumbu grains
1.	Foc_1	Profuse	Moderate	Profuse
2.	Foc_2	Profuse	Moderate	Moderate
3.	Foc_3	Profuse	Profuse	Profuse
4.	Foc_4	Moderate	Profuse	Moderate
5.	Foc_5	Moderate	Profuse	Moderate
6.	Foc_6	Moderate	Profuse	Moderate
7.	Foc_7	Moderate	Poor	Moderate
8.	Foc_8	Moderate	Moderate	Poor
9.	Foc_9	Moderate	Moderate	Profuse
10.	Foc_{10}	Poor	Moderate	Moderate

Plate 1: Basal rot symptom of onion incited by *Fusarium oxysporum* f.sp. *cepae*

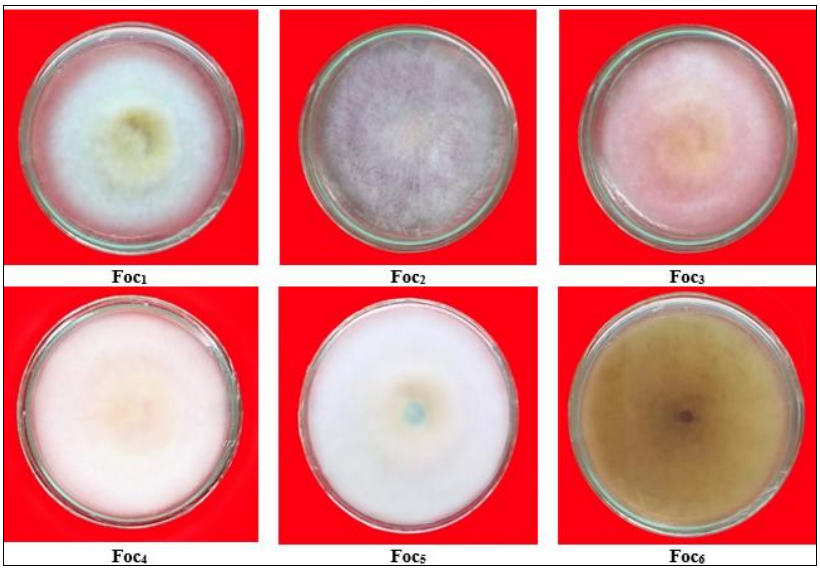

Plate 2: Virulent isolates of *F.oxysporum* f.sp. *cepae*

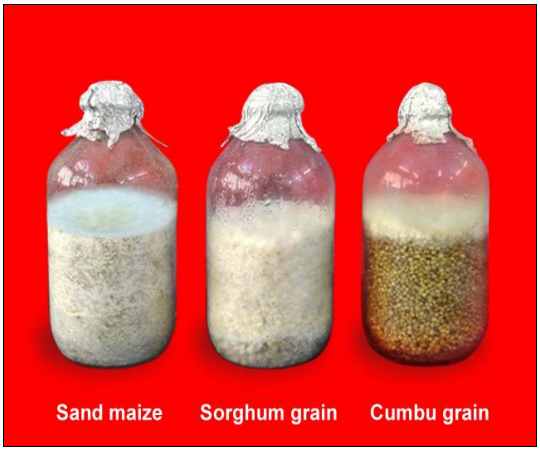

Plate 3: Mass multiplication of *Fusarium oxysporum* f.sp. *cepae*

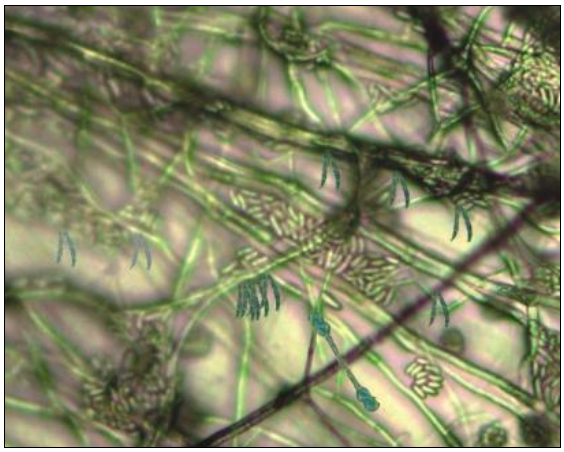

Plate 4: Macroconidia of *F. oxysporum* f.sp. *cepae*

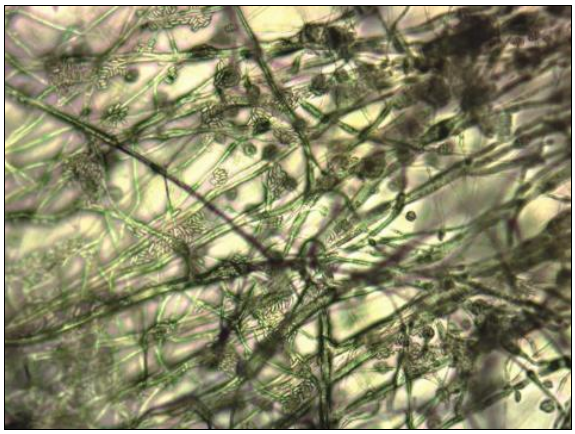

Plate 5: Microconidia of *F. oxysporum* f.sp. *cepae*

Conclusion

The present study revealed wide variability in the incidence and morphology of *Fusarium oxysporum* f.sp. *cepae* isolates from Perambalur district of Tamil Nadu. Sand–maize medium proved most effective for pathogen multiplication under laboratory conditions. Settikulam recorded the highest disease incidence, indicating the possible influence of environmental and varietal factors on pathogen development. The observed cultural and conidial diversity among the isolates suggests the presence of multiple virulent strains in the region. These findings provide a basis for future screening of resistant onion genotypes and the formulation of integrated management strategies tailored for Perambalur district and similar onion-growing regions.

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