www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(8): 1445-1454

www.thepharmajournal.com
Received: 01-05-2022
Accepted: 05-06-2022

Muthukumar G
PG Scholar, Department of Plant Pathology, Annamalai University, Chidambaram, Tamil Nadu, India

Udhayakumar R
Assistant Professor, Department of Plant Pathology, Annamalai University, Chidambaram, Tamil Nadu, India

Muthukumar A
Associate Professor, Department of Plant Pathology, Annamalai University, Chidambaram, Tamil Nadu, India

Muthukumaran N
Assistant Professor, Department of Entomology, Annamalai University, Chidambaram, Tamil Nadu, India

Ayyandurai M
Ph.D. Scholars, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author
Muthukumar G
PG Scholar, Department of Plant Pathology, Annamalai University, Chidambaram, Tamil Nadu, India

Survey on disease incidence of basal rot of onion incited by *Fusarium oxysporum* f. sp. *cepae* in major onion growing tracts of Tamil Nadu

Muthukumar G, Udhayakumar R, Muthukumar A, Muthukumaran N and Ayyandurai M

Abstract
Onion (*Allium cepa* L. var. *aggregatum* Don.) is the world's most important commercial vegetable crop. *Fusarium* basal rot (FBR) is an economically significant onion disease that causes yield losses of up to 50 per cent in the field and 30 to 40 per cent during post-harvest storage of bulbs. Survey was conducted in onion growing areas of Tiruchirappalli, Perambalur, Salem, Karur and Madurai districts of Tamil Nadu during 2021-2022 and collected the infected diseased samples, from this ten isolates of *Fusarium* were isolated under *in-vitro.* FOC pathogens multiplied with different substrates, among them sand maize medium is a best source for production of inoculum of pathogens. During the pathogenicity among the ten isolates, FOC_6 isolated from Thogaimalai village was identified as a virulent isolate which recorded 56% disease incidence. The isolates were confirmed by both morphological and cultural characters. The conidial and resting structure also seen under SEM Molecular confirmation was done by using fungal universal primer ITS1 and ITS4 and we obtained approximately 550-600 bp common fragments in FOC_6 isolate of *Fusarium.* Different solid media was used for evaluate the growth of the FOC isolates, among them potato dextrose agar medium shows vigorous mycelial growth under *in vitro* condition.

Keywords: Onion, *Fusarium* basal rot, mycelial growth, pathogenicity, *in vitro*

1. Introduction
The most important vegetable crop is onion (*Allium cepa* L. var. *aggregatum* Don). It was commonly referred to as the "queen of kitchen" because it is used as a food, salad, spice, condiment and medicine (Shilpa *et al*., 2017) [29]. Onion's nutritional value is primarily determined by its content of vitamin B6, vitamin C, dietary fiber, folic acid and carbohydrates. Fresh onion contains 88.9-92.8 percent water, 0.0-1.6 per cent protein, 0.120 mg pyridoxine, 0.027 mg riboflavin and 0.046 mg thiamin (Choudhary *et al*., 2015) [7]. Due to the presence of volatile compounds such as allyl-propyl disulphide, onions are pungent (Shamyuktha *et al.* 2020) [28]. China is the leading onion producer in world followed by India. In India, it is cultivated in Maharashtra, Karnataka, Gujarat, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Rajasthan and Haryana. In Tamil Nadu, 54,000 ha were devoted to onion cultivation, primarily in the districts of Perambalur, Namakkal, Trichy and Dindigul (www.newindianexpress.com 2021) [21]. According to the ministry's first advance estimate, onion cultivation is expected to increase from 1.62 million ha in 2021-22 to 1.91 million ha in 2022-23 (www.business-standard.com 2022). *Fusarium* basal rot (FBR) is an economically significant onion disease that causes yield losses up to 50 per cent in the field and 30 to 40 per cent during post-harvest storage of bulbs (Sintayehu *et al*., 2011) [33]. *Fusarium* basal rot (FBR) is an economically significant onion disease that causes yield losses of up to 50 per cent in the field and 30 to 40 per cent during postharvest storage of bulbs (Mishra *et al*. 2014) [19]. According to Kalman *et al.* (2020) [4], basal rot infected plant which shows the growth rate of 0.83-0.87 cm per day. Significant amount of yield losses was observed in the affected onion field ranging from 30-100% (Priya *et al.* 2016) [24]. The optimum temperature required for the successful establishment of pathogen invasion is 25-32 °C (Howard *et al.* 2007) [12]. Infection occurs at soil temperatures of 15 °C to 32 °C, with an optimum at 28 °C - 32 °C, because infection and disease development is favored by high soil temperature (Mishra *et al.* 2014) [19]. The agent of basal rot infects the onion root epidermis and then extends into the vascular tissue at the basal region areas. The characteristic symptoms of the rot disease include onions leaf curving, drooping, turning pale and discoloring of onion and then the contaminated tissue gets

brown and hydrated. As a result of these serious infections, the plant eventually dies (Idris Bektas *et al.* 2019) [13]. In this study mainly focusing on variability between the different isolates of FOC pathogens and influence the virulence of them, and also optimizes the suitable substrate for mass multiplication, nutrient providing medium.

2. Materials and methods

2.1 Survey and collection of disease samples

The survey was conducted in Tiruchirappalli, Perambalur, Salem, Karur and Madurai districts of Tamil Nadu during 2021 and 2022 in order to assess the incidence of basal rot in various onion fields. In each village, four fields were chosen at random, and four plots with an average area of ten square meters were marked in each field. From the seedling to maturity stage of the onion crop, Basal rot symptoms were collected from various locations, including Padalur, Manapparai, Chettikulam, Thalaivasal and Oddanchatram during the survey. The disease incidence was determined by counting the total number of infected plants relative to the total number of observed plants. Other information about the site, including the type of soil and onion cultivar, was also recorded. The per centage of basal rot disease incidence calculated using a formula (McKinney 1923) [18] (Plate 2).

$$\text{Per cent Disease incidence (PDI)} = \frac{\text{Number of plants infected with FOC}}{\text{Total number of plants observed}} \times 100$$

2.2 Isolation of the pathogen

The infected bulbs were isolated using the tissue segment technique described by Rangaswami (1958) [26]. Onion samples infected with basal rot were collected from ten different locations. The infected portions were then cut into small pieces (1-2cm) using a sterilized scalpel and washed in 1 per cent Sodium hypochlorite for one minute, 70 per cent ethanol, and then three times with sterile distilled water. Using sterile, dried tissue paper, excess moisture in the sample was extracted. The sample was placed in a Petri dish containing Potato Dextrose Agar (PDA) medium at equal distances. The pure culture of FOC was obtained using the single hyphal tip method, then transferred to agar slants and stored at 4 ºC for future research. Ten total isolates were obtained from distinct samples and designated FOC_1 through FOC_{10} (plate 1).

2.3 Mass multiplication of pathogen

2.3.1 Sand maize medium (Ricker and Ricker, 1936) [27]

Maize and river sand were added proportionately @ 50g kg^{-1} of soil and thoroughly mixed, then transferred to open-mouthed bottles, closed with a sterile cotton wool plug, and the desired amount of water was added. The bottle containing the media was sterilized at 121.5 °C under 15 lbs pressure for 2 hours over two days. After sterilization, the media-containing bottle was inoculated with 9 mm mycelial discs of FOC obtained from a seven-day-old culture of *F. oxysporum* f. sp. *cepae*. The bottle was then incubated for 15 days at 28 ± 2 ºC for colonization of FOC and the colonization of FOC isolates was observed.

2.3.2 Sorghum and Cumbu grains medium (Diaraa 1996) [9]

Cumbu and Sorghum grains (19:1) were added to three-quarters of glucose bottles, which were then sealed with sterile cotton wool. The bottles were sterilized at 121.5 °C under 15 lbs pressure for two hours over two days. Each bottle was inoculated with six 9 mm-diameter discs from a FOC culture that was seven days old. The bottles were incubated at room temperature (28 ± 2 ºC) for fifteen days, and the resulting inoculums were used to inoculate the soil with the pathogen.

2.4 Effect of different media on the growth of *F. oxysporum* f. sp. *cepae*

In order to compare the growth of *F. oxysporum* f. sp. *cepae* on various solid media, the following sterilized warm media were poured into 90 mm sterile Petri plates: potato dextrose agar, corn meal agar, beetroot medium, carrot juice agar and oat meal agar at 15 ml each. The pathogen's 9 mm culture disc was placed in the center of a Petri plate that was incubated at room temperature (28±2 ºC) and three replications were maintained in each medium. Seven days after inoculation, the radial growth of the mycelium was measured. On the culture media, the colony and its growth pattern were recorded.

2.5 Pathogenicity characteristics

All *Fusarium* wilt pathogen isolates were grown in sand maize medium. For optimal multiplication, sand maize medium was uniformly inoculated with four fungal discs and incubated at room temperature (28±2 ºC) for 15 days (Ricker and Ricker, 1936) [27]. The potting soil (Red soil: sand: cow manure at a ratio of 1:1:1 by weight) was sterilized. The virulence and pathogenicity of ten isolates were evaluated by artificially inoculating a sand maize culture in potting soil at a concentration of 6.5×10^5 spores per gm of soil. The potting soil containing the fungus was placed in pots with a diameter of 30 cm. The onion bulbs were surface sterilized for 30 seconds with a 0.1% mercuric chloride solution, rinsed three times with sterile distilled water, and then planted in the pot. This study utilized a total of three plants per pot and three replications. Regularly, the symptom expression, virulence, and pathogenicity of the observed disease incidence were monitored. The incidence of the disease was determined by measuring the incidence of basal rot from the vegetative stage to maturity. From the vegetative stage onward, the observation of disease incidence was routinely tracked. From the manifestation of symptoms, virulence and pathogenicity were determined. The incidence of the disease was calculated using the following formula:

$$\text{Per cent Disease incidence (PDI)} = \frac{\text{Number of plants infected with FOC}}{\text{Total number of plants observed}} \times 100$$

2.6 Morphological characteristics of *F. oxysporum* f. sp. *cepae* isolates

In order to examine the cultural and morphological characteristics of FOC isolates, a nine mm culture disc of each isolate was transferred to Petri plates containing PDA medium and incubated for seven days. All ten *Fusarium* isolates were examined for morphological characteristics, including mycelium colour, growth pattern, micro and macro conidia, and chlamydospores. Brayford (1996) [5] Pathogen identified based on cultural and morphological characteristics (plate 9).

2.7 Cultural characteristics

In order to examine the cultural and morphological characteristics of FOC isolates, a nine mm culture disc of each isolate was transferred to Petri plates containing PDA

medium and incubated for seven days. All ten *Fusarium* isolates were examined for morphological characteristics, including mycelium colour, growth pattern, micro and macro conidia and chlamydospores. Pathogen identified based on cultural and morphological characteristics (Brayford 1996) [5]. Cultural characteristics were evaluated 15 days after the plates were prepared and subjected under the compound microscope to observe mycelial growth, sporulation, colony color etc.

2.8 Scanning Electron Microscopy (SEM)

Actively growing fungal culture was fixed at overnight for 28 ˚C in 0.05M phosphate buffer containing 4% glutaraldehyde. On the next day, fungal mat was washed three times with phosphate buffer and dehydration of the sample was done using ethanol for 15 minutes. Then, the fixed and dehydrated samples were dried with CO_2 for 5 minutes and were fixed on aluminium stubs and sputter coated with carbon polaron E-500 spotted coated and immediately observed under scanning electron microscope at 15 KV. This work was carried out in Department Physics, Annamalai University (plate 4).

2.9 Molecular characterization of *F. oxysporum* f. sp. *cepae* isolates DNA isolation and PCR amplification (Manikandan *et al.* 2018) [17]

2.9.1 DNA extraction

All the Fungal isolates were grown in 100 ml of PDA broth for 7 days at 28 ± 2 °C. The genomic DNA was extracted and purified using the CTAB buffer method modified from Nicholson *et al*. (1996). Fungal mycelium was harvested and 2g of dried mycelium were ground into fine powder in liquid nitrogen using mortar and pestle until it forms dry powder. 200 mg of powdered mycelium was transferred to 2.0 ml Eppendorf tubes and 1ml of 20 mM EDTA (pH: 8.0) and 2% CTAB, mercaptoethanol (0.1) was added, vortexed and incubated at 65 °C for 10 minutes. The mixture was transferred to clean tube and chloroform: isoamyl alcohol (24:1) was added in equal volume. The mixture was centrifuged at 10,000 rpm for 10 minutes. Equal volume of 5 M NaCl and ice-cold isopropanol was transferred to the supernatant taken in clean tube and mixed well. It was incubated at 65 °C for DNA precipitation or incubated overnight at -20 °C. The content was centrifuged at 13000 rpm for 10 minutes and the pellet was collected by discarding the supernatant. The pellet was washed with 70% ethanol twice. 50µl of TE buffer or double sterile water was used for resuspending the pellet (10mM Tris, 1mM EDTA, pH 8.0).

The 10 days old cultures of *F. oxysporum* f. sp. *cepae* were transferred into 250 ml conical flasks containing 150 ml potato dextrose broth (PDB) and incubated at room temperature for 7 days. Mycelium was harvested by filtration through sterile filter and used for DNA extraction using standard CTAB method. PCR assay was carried out using total DNA from FOC_6 isolate as templates to amplify the ribosomal DNA intergenic spacer (IGS) region in the FOC_6 genome.

Forward primer ITS 1-5′-GTAAGCCGTCCTTCGCCTCG-3′
Reverse primer ITS 4-5′-GCCATACTATTGAAT TTT GC-3′

These primers were used to amplify ribosomal DNA intergenic spacer regions of *F. oxysporum* f. sp. *cepae* isolate along the positive control obtained from Department of Plant Pathology, Annamalai University, Chidambaram, India. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer in MEDAUXIN SEQUENCING PVT, Limited, Bangalore. Consensus sequence of 18S rRNA gene was generated from forward and reverse sequence.

18S rRNA gene sequence was used to carry out BLAST with the database of NCBI GenBank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Finally acquiring accession numbers, these sequences were submitted to NCBI (National Centre for Biotechnology Information) gene bank, USA (Vignesh 2020) [36] (plate 5).

3. Results and Discussion

3.1 Survey and collection of disease samples

Maximum disease incidence was observed in Thogaimalai village of Karur district (28.45%) followed by Chettikulam village of Perambalur district recorded 24.87%. Minimum basal rot incidence (10.12%) was observed in Oddanchatram village of Dindigul district. The native isolates of *F. oxysporum* f. sp. *cepae* were isolated from respective locations and designated as FOC_1 to FOC_{10}. (Table 1).

Similar studies carried out by, Jayanta *et al.* (2018) [15] conducted a survey in four districts of Northeastern Karnataka and the wilt incidence was noticed in all locations surveyed with a range of 8.33 to 38.66 per cent attributed by specific variety. Similar findings are accord with, Shamyuktha *et al.* (2020) [28] conducted a survey in Tirunelveli, Thoothukudi and Tenkasi districts of Tamil Nadu and the wilt incidence was noticed in all locations surveyed with a range of 27.00 to 74.33 per cent attributed by local variety. Jagraj Singh *et al*. (2018) [14] also concluded that sandy soil supported highest wilt incidence in watermelon, tomato and marigold and the least incidence was found in silt clay soil.

3.2 Mass multiplication of *F. oxysporum* f. sp. *cepae* in different substrate

Mass multiplication of *F. oxysporum* f. sp. *cepae* using different substrates *viz.*, sand maize, sorghum grains and cumbu grains were tested and the result were depicted in the (Table 5). Among different substrate tested, sand maize medium found to be the best which supports the maximum mycelial growth of *F. oxysporum* f. sp. *cepae* followed by sorghum grains and the least mycelial growth was obtained in cumbu grains after 15 days of inoculation (Plate 7).

Similarly, Asish Mahato *et al*. (2017) [2] reported that mass multiplied isolate were incorporated in collar region at the rate of 20 g kg^{-1} soil (multiplied on wheat and sorghum grains) recorded the maximum incidence of collar rot of tomato. In past study carried out by, Sivakumar *et al*. (2018) [34] reported that the sand maize and sorghum grains are used in the mass multiplication of *F. oxysporum* f. sp. *lycopersici*.

3.3 Evaluation of different media on the growth of *F. oxysporum* f. sp. *cepae*

The growth of virulent isolate (FOC_6) was evaluated on different medium (Table 4) revealed variation. Potato dextrose agar medium showed maximum mycelial growth on 7^{th} day incubation (90.00mm) followed by carrot juice medium (86.78mm) and oat meal medium (73.90mm). The least growth was observed on beet root medium (53.78mm) (Plate 8).

Similarly, Singh *et al*. (2016) [30] studied effect of different

solid media and liquid media on radial growth and sporulation of *Fusarium oxysporum* f. sp. *lentis* and reported that Potato Dextrose Agar and Richard's Agar were the best medium for radial growth and sporulation of *Fusarium oxysporum* f. sp *lentis.* Maximum colony diameter of *Fusarium udum* (82.0mm) was recorded on Potato Dextrose Agar medium, followed by Richard's Agar which yielded 79.33mm of colony diameter (Desh Raj Chaudhary *et al*. 2018) [8].

3.4 Pathogenicity of *F. oxysporum* f. sp. *cepae* isolate under pot culture condition

Pathogenicity study for the soil-borne pathogen, *F. oxysporum* f. sp. *cepae* isolates showed that the symptoms were expressed after 7 days of inoculation. The symptoms were characterized as affected roots become dark brown to dark pink, and a white fungal growth is sometimes evident at the base of infected bulbs. When cut vertically, an infected bulb show brown discoloration of the stem plate tissue (Plate 6).

A pot culture experiment was conducted to test the pathogenicity of isolates collected from different villages of onion growing tracts of Tamil Nadu. Among ten isolates collected (Table 6) the isolate FOC_6 collected from Thogaimalai recorded maximum diseases incidence of 56.00 per cent under artificial inoculation and was identified as virulent culture followed by FOC_7 which was recorded 45.74 per cent from Vedasandur. The least disease incidence was observed in FOC_1 which recorded 18.86 per cent (Table 6).

Similarly, Manikandan *et al.* (2018) [17] carried out an experiment for pathogenicity in different isolates of *F. oxysporum* f. sp. *lycopersici*, all the isolates produced significant symptoms under pot culture studies and per cent disease incidence (PDI) ranged from 52.50 to 100 was noticed. According to Vignesh *et al*. (2021) [37], isolates of *F. oxysporum* f. sp. *lycopersici* collected from different tomato growing areas of Krishnagiri district, isolate from Uthangarai was found to be more virulent and recorded the maximum incidence of 65.52 per cent was recorded. Similarly, Singha *et al*. (2011) [32] carried out an experiment for virulence in different isolates of *F. oxysporum* f. sp. *lycopersici* and the result showed that 88.8 per cent disease incidence in virulent isolates. Sundaramoorthy and Balabaskar (2013) [35] artificially inoculated the *Fusarium* wilt pathogen in tomato plants under controlled environment and they observed incidence of wilt disease up to 57.73%. Similarly, Rajendran *et al*. (2018) [26] mentioned that the *F. oxysporum* f. sp. *lycopersici* isolates produced significant symptoms from 47 days after transplanting. The per cent wilt incidence ranged from 48 to 100% between the isolates.

3.5 Morphological characteristics of *F. oxysporum* f. sp. *cepae* isolates

Among the ten isolates of FOC, FOC_6 shows vigorous growth, and the mycelium is dark pink in colour with violet colour pigmentation in the centre of the mycelium and it covered the plate within 7 days. FOC_3 isolate produced milky white mycelial growth with slight shrinkage of mycelium in the ends and vigorous growth. FOC_5 isolate generates profuse dark pinkish mycelium with pink to violet pigmentation in the centre of the mycelium and fast growth. FOC_4 isolate produced slight fluffy mycelial growth and light pink color pigment in the later stage with slow growth. FOC_8 produced dull white colour thin mycelium. The isolates of FOC showed variation with respect to colony characters. The color of the isolates varied from white to pale pink and pinkish. Most of the isolates produced fluffy cottony aerial mycelium other than the isolates FOC_5 and FOC_8 produce smooth fluffy growth (Table 2 and Plate 9).

Similarly, Mandal *et al*. (2018) [16] isolated 14 isolates of FOC and they differ in colour of the mycelium (slight pink, violet and white) and also varied in growth. Similarly, Most of the FOC isolates produced fluffy to moderately fluffy cottony aerial mycelium (Vignesh *et al*. 2021) [37]. According to Patra and Biswas (2017) [23], the micro conidia of *F. oxysporum* f. sp. *cepae* were oval to kidney shaped having 0-1 septa and macro conidia were sickle shaped having 3 septa. Similarly, Mrinmoy *et al*. (2018) [20] carried out an experiment to conclude that; maximum sporulation was counted from FOC isolate (103.33 lakh ml^{-1}) in PDA medium. Both macro and micro conidia of each isolate was measured after slide culture using PDA medium.

3.6 Cultural characteristics

The isolates of *F. oxysporum* f. sp. *cepae* showed variation with respect to colony characters. The color of the isolates varied from white to pale pink and pinkish. Most of the isolates produced fluffy cottony aerial mycelium other than the isolates FOC_5 and FOC_8 produce smooth fluffy growth (Table 3).

3.6.1 Mycelial growth

Among the isolates of *F. oxysporum* f. sp. *cepae*, the maximum mycelial growth (90.00 mm) was recorded by the isolate FOC_6 followed by FOC_{10} and FOC_7 (88.84 and 87.74 mm). The minimum mycelial growth (75.45 mm) was observed by the isolate FOC_4 (Table 3).

3.6.2 Sporulation and growth type

Microscopic examination revealed that all the isolates produced two types of conidia namely micro and macro conidia. Micro conidia are small, oval shaped, hyaline and single or bi-celled. In case of macro conidia were sickle shaped hyaline and multi-celled with three to five septate. (Plate 3) Which shows the number of micro conidia was more as compared to macro conidia. In addition chlamydospores were observed terminally and intercalary. Based on sporulation, the isolates were divided into three category *viz*., poor sporulation, medium sporulation and good sporulation. Ten isolates *viz.*, FOC_6, FOC_7 had produced good sporulation while five isolates *viz.*, FOC_3, FOC_4, FOC_5, FOC_8 and FOC_9 produced sporulation at medium level. Least sporulation and growth was recorded in isolate namely FOC_1.

Similarly, Mandal *et al*. (2018) [16] isolated 14 isolates of FOC and they differ in colour of the mycelium (slight pink, violet and white) and also varied in growth. Similarly, Most of the FOC isolates produced fluffy to moderately fluffy cottony aerial mycelium (Vignesh 2021) [37]. Also, Rajendran *et al*. (2018) [26] reported that the pathogen produced different colony colors *viz*., light pink, pink, dark pink, creamy white, pale white with pink and the mycelial growth pattern showed two different pattern namely adherent smooth and fluffy growth.

3.7 Molecular characterization of *F. oxysporum* f. sp. *cepae* isolates DNA isolation and PCR amplification

PCR reaction was performed with initial denaturation step at 94 ºC for 5 min, 35 cycles of amplification (1min for denaturation at 94 ºC, 1 min for primer annealing at 60 ºC and

2 min for extension at 72 ºC) and one cycle of final extension at 72 ºC for 5 min in PCR Thermo Cycler. The amplified PCR products were run on 1.5% agarose gel and visualized on an UV-trans-illuminator and photographed in the gel documentation unit.

In this study, we selected ITS region for amplification using previously reported primer ITS1 and ITS4 and we obtained approximately 550-600 bp common fragments in FOC_6 isolate of *Fusarium.* Amplified fragments of FOC_6 sequenced through the gateway of BLAST search in NCBI website (www.blast.ncbi.nlm.nih.gov/Blast).

Finally conclude that the isolate FOC_6 as *F. chlamydosporium* and receive the accession number OM978680 (plate 5).

Similar to the present investigation Nirmaladevi *et al*. (2016) [22] reported that using primers ITS 1 and ITS 4, the amplified region specifically ranged from 334 bp to 509 bp fragment from all *Fusarium* species isolates. Gao *et al*. 2016 [10] reported that *F. proliferatum* causing tomato leaf spot in China. Sundaramoorthy and Balabaskar, (2013) [35] identified that the *Fusarium* isolates *viz*., ATF04, ATF05 and ATF19 as *F. fusarioides* casing wilt complex disease in tomato through BLAST search of ITS1 and ITS4 in NCBI. Molecular profiling using ITS region sequencing is an indispensable method for identification studies as studied by Singh *et al*. (2016) [30]. Molecular identification based on Internal Transcribed Spacer (ITS) region and TEF-1α gene sequencing some species of *Fusarium* causing tomato wilt pathogen identified are *F. incarnatum-equiseti*, *F. graminearum*, *F. acuminatum* and *F. solani* (Asma Akbar *et al*. 2018). The isolate of *F. oxysporum* was sequenced and submitted in NCBI database and it's based on the closest match of BLAST analysis, it showed 100% homology with *F. oxysporum* (Amutha Kuppusamy *et al*. 2018) [1].

Plate 1: Axenic culture

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

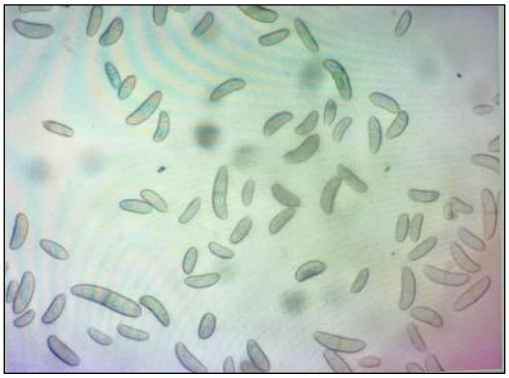
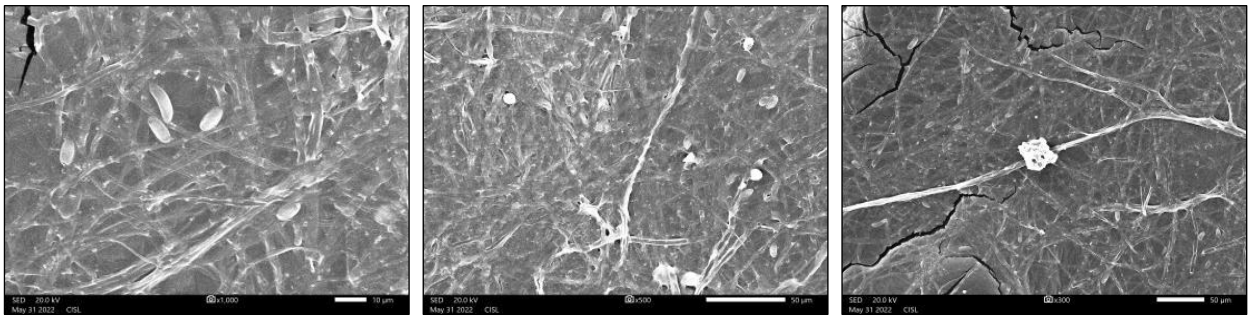


Plate 3: Microscopic view of *F. oxysporum* f. sp. *cepae* (Micro conidia)



Micro conidia Mycelium Chlamydospore

Plate 4: Scanning Electron Microscope view of *F. oxysporum* f. sp. *cepae* (SEM)

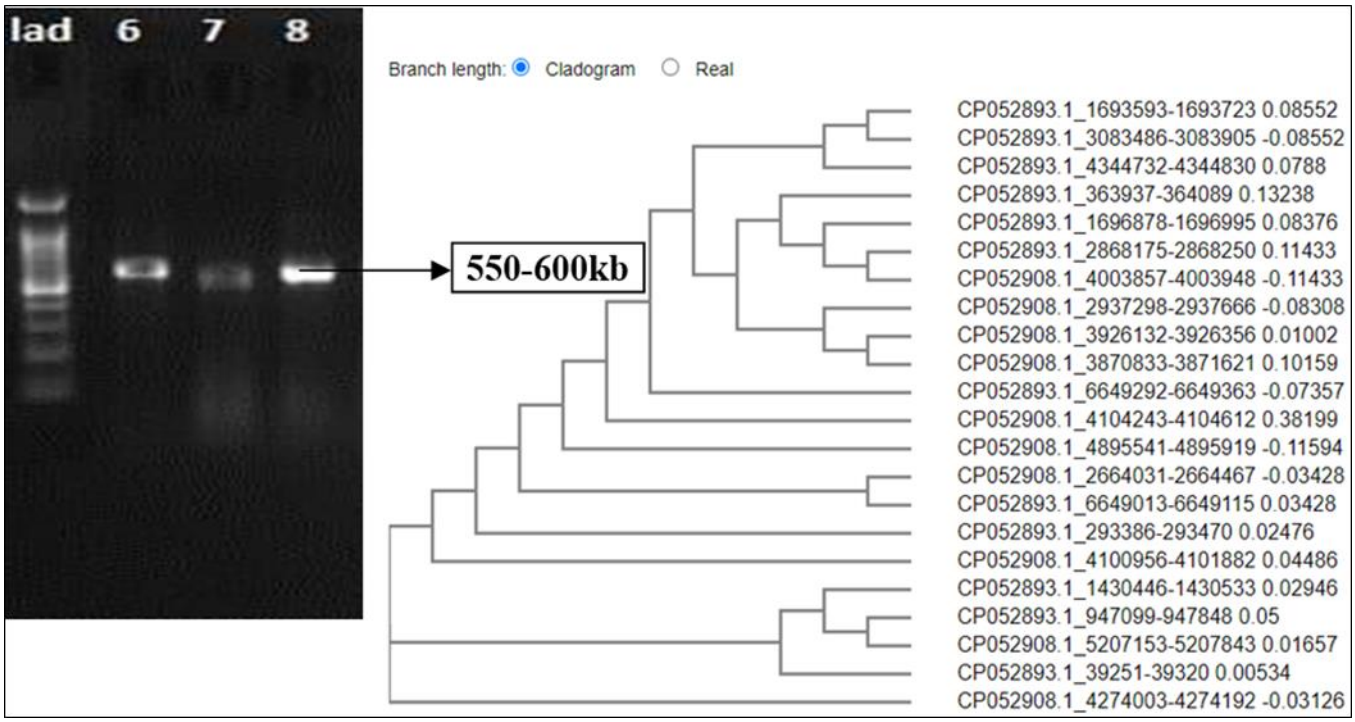


Plate 5: Gel documentation and cladogram of *F. oxysporum* f. sp. *cepae*

Plate 6: Pathogenicity test of *F. oxysporum* f. sp. *cepae*

Plate 7: Mass multiplication of *F. oxysporum* f. sp. *cepae*

Plate 8: Growth of *F. oxysporum* f. sp. *cepae* on different solid media

A. Control, B. Rose Bengal agar, C. Corn meal agar, D. Beet root agar, E. Carrot juice agar, F. Czabek's Dox agar, G. Potato Dextrose Agar medium.

Table 1: Survey on the incidence of onion basal rot caused by *F. oxysporum* f. sp. *cepae* (FOC) in major onion growing tracts of Tamil Nadu

S. No.	Isolates	Village	District	Variety	Soil type	DI*
1.	FOC_1	Padalur	Perambalur	Local	Black soil	12.34^{i} (20.56)
2.	FOC_2	Chettikulam		Local	Red loamy	24.87^{b} (29.91)
3.	FOC_3	Siruvachur	Salem	Local	Red soil	23.67^{c} (29.11)
4.	FOC_4	Thalaivasal		Local	Loamy soil	16.54^{g} (23.99)
5.	FOC_5	Musiri	Karur	Local	Sandy loam	20.64^{e} (27.02)
6.	FOC_6	Thogaimalai		Local	Red soil	28.45^{a} (32.23)
7.	FOC_7	Vedasandur	Dindigul	Local	Black soil	22.98^{d} (28.59)
8.	FOC_8	Oddanchatram		Local	Red loam	10.12^{j} (18.54)
9.	FOC_9	Thuraiyur	Trichy	Local	Red soil	12.34^{h} (20.56)
10.	FOC_{10}	Manapparai		Local	Red soil	18.56^{f} (25.51)

*DI-Disease Incidence.
*Mean of three replication.
*If same letter repeated means non-significant at 5% level by Duncan's Multiple Range Test (DMRT)

Table 2: Morphological variation of different isolates of *F. oxysporum* f. sp. *cepae* on potato dextrose agar medium

S. No.	Isolate	Morphological characters	Shape of conidia			Radial mycelial growth (mm)	Days to cover an entire plate
			Micro conidia	Macro conidia	Chlamydospore		
1.	FOC_1	Light pink	Oval to kidney	Falcate	One celled	85.88^{ab}	9
2.	FOC_2	Light pink	Oval to kidney	Falcate	One to two celled	86.43^{ab}	8
3.	FOC_3	Milky white	Oval to kidney	Falcate	-	78.44^{b}	12
4.	FOC_4	Pink	Oval to kidney	Falcate	-	75.45^{e}	13
5.	FOC_5	Light pink	Oval to kidney	Cylindrical	-	85.88^{ab}	9
6.	FOC_6	Dark pink	Oval shaped	Falcate	One to two celled	90.00^{a}	7
7.	FOC_7	Milky white	Round shape	Sickle shaped	Two celled	87.74^{ab}	8
8.	FOC_8	Creamy white	Oval to kidney	Falcate	-	83.83^{bc}	11
9.	FOC_9	Pinkish white	Oval to kidney	Falcate	-	80.85^{cd}	13
10.	FOC_{10}	Pure white	Oval shaped	Sickle shaped	One to two celled	88.84^{a}	8

*Mean of three replication; Means in a column followed by same superscript are not significantly different by Duncan's Multiple Range Test P=0.05

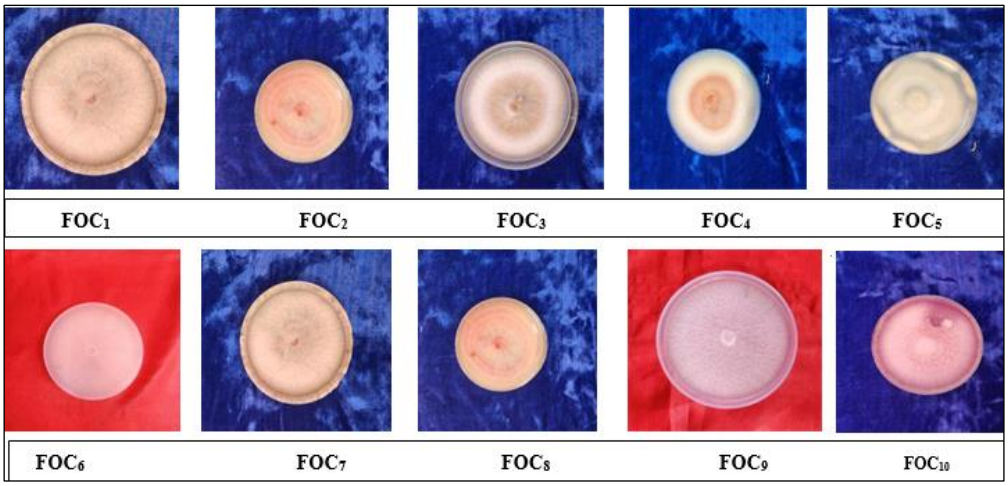


Plate 9: Morphological characteristics of different isolates of *F. oxysporum* f. sp. *cepae*

Table 3. Cultural characteristics of different isolates of *F. oxysporum* f. sp. *cepae*

S. No.	Isolate	Sporulation	Growth type	Mycelial growth pattern	Micro conidia (µm)*	Macro conidia (µm)*
1.	FOC_1	+	Slow	Adherent smooth	09×3.0	37×3.7
2.	FOC_2	+	Slow	Adherent smooth	09×3.3	42×3.6
3.	FOC_3	++	Medium	Fluffy growth	09×3.2	30×2.9
4.	FOC_4	++	Medium	Fluffy growth	08×3.0	42×3.5
5.	FOC_5	++	Medium	Smooth fluffy growth	08×3.7	28×3.4
6.	FOC_6	+++	Fast	Fluffy growth	09×3.6	41×3.7
7.	FOC_7	+++	Fast	Adherent smooth	10×3.3	37×3.7
8.	FOC_8	++	Medium	Fluffy growth	09×3.5	40×3.4
9.	FOC_9	++	Medium	Fluffy growth	09×3.8	37×3.3
10.	FOC_{10}	+	Slow	Adherent smooth	08×3.1	38×3.5

Table 4: Evaluation of different media on the growth of *F. oxysporum* f. sp. *cepae*

S. No.	Medium	Mycelial growth (mm)		
		3rd DAI	5th DAI	7th DAI
1.	Potato dextrose Agar	44.87^a	70.02^a	90.00^a
2.	Corn meal agar	35.05^c	49.56^c	65.89^c
3.	Beetroot medium	25.73^d	51.87^c	53.78^d
4.	Carrot juice agar	40.07^b	59.78^b	86.78^a
5.	Oat meal medium	38.09^b	60.89^b	73.90^b

*Mean of three replication. *Values in the column followed by common letters do not differs significantly at 5% level by Duncan's Multiple Range Test (DMRT)

Table 5: Mass multiplication of *F. oxysporum* f. sp. *cepae* in different substrates

S. No.	Isolates	Mycelial growth in different substrates (15 DAI)		
		Sand maize	Sorghum	Cumbu grains
1.	FOC_1	++	++	++
2.	FOC_2	++	+++	+
3.	FOC_3	+++	++	+
4.	FOC_4	++	+++	++
5.	FOC_5	++	++	+
6.	FOC_6	+++	++	++
7.	FOC_7	+++	++	+
8.	FOC_8	++	++	++
9.	FOC_9	+++	++	+
10.	FOC_{10}	++	+++	++

+++: Profuse mycelial growth on the grains.
++: Moderate mycelial growth on the grains.
+: Poor mycelial growth on the grains.

Table 6: Pathogenicity of *F. oxysporum* f. sp. *cepae* isolate under pot culture condition

S. No.	Isolate	Basal rot incidence (%)			Mean
		30 DAS*	45 DAS*	60 DAS*	
1.	FOC_1	12.48^i (20.68)	17.45^h (24.69)	26.65^i (31.08)	18.86
2.	FOC_2	15.15^h (22.90)	18.46^h (25.44)	28.48^h (32.25)	20.69
3.	FOC_3	18.36^g (25.37)	21.86^g (27.87)	30.18^g (33.32)	23.46
4.	FOC_4	25.24^e (30.15)	28.44^f (32.22)	35.46^f (36.54)	29.71
5.	FOC_5	28.15^d (32.04)	45.15^b (42.21)	38.36^e (38.36)	37.22
6.	FOC_6	46.75^a (43.13)	60.08^a (50.81)	61.18^a (51.46)	56.00
7.	FOC_7	45.66^a (42.51)	43.18^c (41.08)	48.40^b (44.08)	45.74
8.	FOC_8	30.18^c (33.32)	40.15^d (39.31)	45.15^c (42.21)	38.49
9.	FOC_9	35.15^b (36.36)	38.15^e (38.14)	42.18^d (40.50)	38.49
10.	FOC_{10}	19.88^f (26.47)	28.18^f (32.06)	38.22^e (38.18)	28.76

*Mean of three replication.
*Values in the column followed by common letters do not differ significantly at 5% level by Duncan's Multiple Range Test (DMRT).

Conclusion

From this study, the higher disease incidence recorded in Thogaimalai village (56.00%). These isolate recorded vigorous growth and abundant conidial production under invitro condition and also which multiplied fast manner under sand maize medium. Which suggested that positive relationship between disease incidence and morphological growth characteristics. Those isolates were forwarded to molecular determination will give the key features about the *Fusarium oxysporum* f. sp. *cepae.* Among the ten isolates of *Fusarium* basal rot pathogen (*F. oxysporum* f. sp. *cepae*), Thogaimalai isolate (FOC_6) was identified as a virulent isolate.

References

1. Amutha Kuppusamy, Godavari Amar, Nedumurath Pallikkara Roshn. Molecular characterization of *Fusarium oxysporum* by PCR amplification of 18S rRNA gene region. Indian J Exp Biol. 2018;56:842-846.
2. Asish Mahato, Mohan Kumar Biswas. Cultural, Morphological and Pathogenic Variability of Different Isolates of *Sclerotium rolfsii* Obtained from Rice-Tomato-Rice Cropping System of Undulating Red and Lateritic Zone of West Bengal, India. Int J Curr. Microbiol Appl Sci. 2017;6(3):1843-1851
3. Asma Akbar, Musharaf Ahmed, Azra Neelam, Sana Zeb Khan, Zahoor Ahmed. Characterization of the causal organism of soft rot of tomatoes and other vegetables and Evaluation of Its Most Aggressive Isolates. Amer J Plant Sci. 2018;6:511-517.
4. Ben Kalman, Dekel Abraham, Shaul Graph, Rafael Perl-Treves, Yael Meller Harel, Ofir Degani. Isolation and Identification of *Fusarium* spp., the Causal Agents of Onion (*Allium cepa*) Basal Rot in Northeastern Israel. Biology. 2020;9(4):69.
5. Brayford D. *Fusarium oxysporum* f. sp. *cepae.* Mycopathologia. 1996;133:39-40.
6. Businessstandard Onion production pegged up 16.81% to 31.12 MT in (2022-23), 2022, 2023. https://www.business-standard.com/article/economy-policy/onion-production-pegged-up-16-81-to-31-12-mt-in-2022-23-agri-ministry-1220328014221.html
7. Choudhary DR, Gora JS. Onion: Production Constraints and Their Management. Pop Kheti. 2015;3(3):40-47.
8. Desh Raj Choudhary. Scientific Cultivation of Onion (*Allium cepa* L.). Phytochemistry of Fruits and Vegetables, 2018, 239-260.
9. Diaraa C. Development of *Fusarium oxysporum* as a bioherbicide for *Striga hermaonthica* (Del.) Benth. M.Sc. thesis, McGill University, Montreal, 1996.
10. Gao, Mei-Ling, Luan, Yushi Yu, Hai-Ning Bao, Yong-Ming. First report of tomato leaf spot caused by *Fusarium proliferatum* in China. Canadian J Plant Pathol. 2016;38:1-5.
11. Gupta R, Gupta R. SAARC. J of Agri. 2014;11(2):49.
12. Howard F. Onion *Fusarium* Basal Plate Rot Identification and Life Cycle, 2007, 15.
13. Idris Bektas, Mustafa Kusek. Phylogenetic and Morphological characterization of *F. oxysporum* f. sp. *cepae* the causal agent of Basal rot on onion isolated from Turkey. Fresenius Environmental Bulletin. 2019;28(3):1733-1742.
14. Jagraj Singh, Vipul Kumar, Seweta Srivastava, Adesh Kumar. *In-vitro* evaluation of *Trichoderma* sp against *Fusarium oxysporum* f. sp. *lycopersici* causing Tomato wilt. J Plant Pathol. 2018;17(2):59-64.
15. Jayanta IN, Mallesh SB, Zaheer AB, Amaresh YA, Sreedevi SC, Ramesh G. Survey for the incidence of *Fusarium* wilt and root knot nematode complex of tomato in North Eastern Karnataka, India. Int J Cur. Microbiol Appl Sci. 2018;7(9):2060-2066.
16. Mandal Mrinmoy, Debashis Rana, Dilip Kumar Misra. Studies on cultural characteristics of *Fusarium* isolates causing panama wilt of banana under West Bengal. Journal of Pharmacognosy and Phytochemistry. 2018;7(3):833-840.
17. Manikandan R, Harish S, Karthikeyan G, Raguchander T. Comparative Proteomic Analysis of Different Isolates of *Fusarium oxysporum* f. sp. *lycopersici* to Exploit the Differentially Expressed Proteins Responsible for Virulence on Tomato Plants. Front Microbiol. 2018;9:420.
18. McKinney HH. A new system of grading plant diseases. J Agric Res. 1923;26(2):195-218.
19. Mishra RK, Jaiswal RK, Kumar D, Saabale PR, Singh A. Management of major diseases and insect pests of onion and garlic: a comprehensive review. Journal of Plant Breeding and Crop Science. 2014;6:160-170.
20. Mrinmoy M, Debashis R, Dilip Kumar M. Studies on cultural characteristics of *Fusarium* isolates causing panama wilt of banana under West Bengal. Journal of Pharmacognosy. 2018;7(3):833-840.
21. New Indian express. Area under cultivation improves in Tamil Nadu's Namakkal, 2021. Retrieved on Nov 2021, https://www.newindianexpress.com/states/tamil-nadu/2021/nov/26/area-under-onion-cultivation-improves-in-tamil-nadus-namakkal-2388172
22. Nirmaladevi D, Venkataramana M, Rakesh Srivastava K, Uppalapati SR, Vijai Kumar Gupta, Yli-Mattila T, *et al*. Molecular phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. sp. *lycopersici*. Scientific Reports. 2016;6:213-67.
23. Patra Suman, Mohan Kumar Biswas. Studies on cultural, morphological and pathogenic variability among the isolates of *Fusarium oxysporum* f. sp. *ciceri* causing wilt of chickpea. International Journal of Plant Animal and Environmental Sciences. 2017;7:11-17.
24. Priya RU, Arun Sataraddi, Darshan S. Survey of purple blotch of onion (*Alternaria porri* (Ellis) Cif.) in northern parts of Karnataka. International Journal of Agriculture, Environment and Biotechnology. 2016;9(3):367-373.
25. Rajendran M, Sankarasubramanian H, Gandhi K, Thiruvengadam R. Comparative Proteomic Analysis of Different Isolates of *Fusarium oxysporum* f. sp. *lycopersici* to Exploit the Differentially Expressed Proteins Responsible for Virulence on Tomato Plants. Frontiers in Microbiology. 2018;9:420.
26. Rangaswami G. An agar block technique for isolating soil microorganism with special reference to *Phythiacous fungi*. Science and Culture. 1958;24:85.
27. Ricker AJ, Ricker RS. Introduction to Research on Plant Diseases. John S. Swift company, Incorporated, Plant diseases, 1936, 117.
28. Shamyuktha JJ, Sheela J, Rajinimala N, Jeberlinprabina B, Ravindran C. Survey on Onion Basal Rot Disease Incidence and Evaluation of Aggregatum Onion (*Allium cepa* L. Var. *Aggregatum* Don.) Genotypes against *Fusarium oxysporum* f. sp. cepae. Int J Curr. Microbiol

App Sci. 2020;9(07):529-536.
29. Shilpa R, Koppad SB, Babaleshwar PR, Dharmatti, Shripad Kulkarni. Survey on Prevalence and Symptomatology of Major Diseases of Onion (*Allium cepa* L.) in Northern Parts of Karnataka, India. Int J Curr. Microbiol App Sci. 2017;6(9):2603-2607.
30. Singh R, Laxmikant, Singh M. Management of root rot of pea (*Pisum sativum* L.) through commercially available bioagent formulation and fungicide. 6th international Conference Plant, Pathogens and People, New Delhi, India, 2016.
31. Singh SK, Singh B, Singh V, Reena B. Morphological, cultural and pathogenic variability among the isolates of *Fusarium oxysporum* f. sp. *ciceri* causing wilt of chick pea. Ann Plant Protect Sci. 2011;19:155-158.
32. Singha IM, Kakoty Y, Unni BG, Jayshree D, Kalita MC. Identification and characterization of *Fusarium* sp. using ITS and RAPD causing *Fusarium* wilt of tomato isolated from Assam, Northeast India. Genet Eng. Biotechnol. J. 2016;14:99.
33. Sintayehu A, Sakhuja PK, Fininsa C, Ahmed S. Management of *Fusarium* basal rot *Fusarium oxysporum* f. sp. *cepae* on shallot through fungicidal bulb treatment. Crop Protection. 2011;30:560-565.
34. Sivakumar T, Balabaskar P, Sanjeevkumar K. Variability in *Fusarium oxysporum* f. sp. *lycopersici* causing wilt of tomato. Int J Chemical Studies. 2018;6(2):3655-3659.
35. Sundaramoorthy S, Balabaskar P. Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. Appl Biol Biotech. 2013;1:36-40.
36. Vignesh K. Combinational effect of AM fungi and PGPR for the management of wilt disease in tomato. Thesis, 2020.
37. Vignesh K. Survey on the incidence of *Fusarium* wilt of tomato incited by *Fusarium oxysporum* f. sp. *lycopersici* (Fol) in major tomato growing areas of krishnagiri district. Plant Archives. 2021;21:2369-2376.