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Research Article

Unravelling emerging pathogens: The pathogenic potency of *Fusarium luffae* and *Fusarium chlamydosporum* in watermelon wilt in Tamil Nadu

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ABSTRACT: In Tamil Nadu, Watermelon (*Citrullus lanatus* L.) is a fruit crop that is popular during the Rabi season. In December 2020, a disease resembling *Fusarium* wilt was observed in the villages of Soonambedu in Villupuram district and Marungulam in Thanjavur district. The incidence of the disease in those villages was 43.15% and 38.75%, respectively. Typical symptoms, such as wilting of individual runners, yellowing of foliage, and damping off of seedlings, were observed in these villages. The vascular tissue was observed to have a brown discolouration when it was cut longitudinally. The isolates obtained from Soonambedu and Marungulam villages were designated as Fus01 and Fus02, respectively. These isolates showed a cottony white aerial profuse mycelial growth in the Potato Dextrose Agar (PDA) medium. The identity of this fungus was confirmed by amplifying and sequencing the internal transcribed spacer and 5.8S rRNA regions of Fus01/Fus02. The GenBank accession numbers of Fus01 and Fus02 isolates were MW845763 (*Fusarium luffae*) and MW845764 (*Fusarium chlamydosporum*). The pathogenicity tests for both isolates showed positive results that were similar to those of natural symptoms. For the first time, watermelon wilt has been reported to be caused by different *Fusarium* species.

KEYWORDS: *Fusarium chlamydosporum*, *Fusarium luffae, Fusarium* wilt, Watermelon

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INTRODUCTION

Watermelon (*Citrullus lanatus* L) is a fruit crop that is considered one of the most crucial and profitable in the Cucurbitaceae family and is grown all over the world. According to the Food and Agriculture Organisation of the United Nations (FAO, 2023), watermelon is the highest-ranked fresh fruit consumed worldwide, with annual global output estimated at around 100 million tons. This fruit crop is a significant source of income for small-scale farmers and is considered to be the most consumed and economically significant fruit crop (Levi *et al.*, 2017). The sweet flesh of this fruit contains sucrose, glucose, fructose, vitamin A and C, minerals such as K, Fe, and Ca, and is also rich in citrulline and lycopene (Reetu & Tomar, 2017). Due to its attractive colour, sweet taste, and high moisture content, watermelon is a refreshing drink that can help alleviate thirst during the summer (Romdhane *et al.*, 2017).

India is the second-largest producer of watermelon fruit in Asia, with a production of 2.48 million tonnes from 1.01 lakh hectares, with the productivity of 24.58 t per ha (HSD, 2017). The main states that are involved with

the production of watermelon are Tamil Nadu, Karnataka, Maharashtra, Uttar Pradesh, Madhya Pradesh, and Bihar. In Tamil Nadu, the major districts producing watermelon are Villupuram, Chengalpattu, Karur, Thanjavur, Thirunelveli, Trichy, Cuddalore, and Thiruvannamalai. The varieties that are most commonly grown in Tamil Nadu include NS 295, Dragon King, Maharaja, and Apoorva (Naveenkumar *et al.*, 2017). Tamil Nadu has an average productivity of 23.52 t per ha and produces 1.63 lakh tonnes (DES, 2017).

Pests and diseases are the primary impediment to watermelon production. Watermelon can suffer from a variety of diseases, resulting from different agents like fungi, bacteria, viruses, nematodes and abiotic factors. *Fusarium* wilt, which is caused by *Fusarium spp.*, is recognised as a major limiting factor for watermelon production among the various diseases. Monocropping with watermelons was easily prone to *Fusarium* wilt disease (Zhang *et al.*, 2022a). It is crucial to investigate the causal pathogens and regulate the loss of watermelon crops due to their economic importance as a fruit and the substantial crop loss. To combat the disease, the current work has focused on identifying the primary fungal pathogens and understanding their mode of infection.

MATERIALS AND METHODS

Survey on the incidence of *Fusarium* Wilt on watermelon

Using feedback from farmers and traders, a field survey was conducted in the year 2021 (January-May) to evaluate the prevalence of *Fusarium* wilt in major watermelon villages of Tamil Nadu. Three fields were chosen for each village. Ten plots of each 1 m^2 area were randomly selected in each field. To determine the incidence of wilt, the number of affected plants within each area was counted against the total plant count.

The percentage of disease incidence was calculated by using phytopathometry (Mayee & Datar, 1986). Additionally, the plants that were infected and exhibited the typical symptoms of *Fusarium* wilt were gathered to isolate the pathogen. In the respective survey fields, data were recorded on the soil type used to grow the crop, the farming situation, and the variety of watermelon cultivated. The formula below was used to calculate the percentage of disease incidence.

PDI = (Number of infected plants/Total number of plants observed) X 100

Isolation of pathogen

Infected plants showing symptoms like damping off, dead runners, and dead plants were collected from the fields of watermelon in Villupuram and Thanjavur districts of Tamil Nadu, India. Vascular discolouration was visible on the stem portions of watermelon plants that were infected. The infected regions were separated into tiny pieces approximately 5 mm long, sterilised with 0.1% mercuric chloride solution for 30 s, and rinsed with sterile distilled water. The surface-sterilised samples were transferred under sterile conditions to Petri plates containing Potato Dextrose Agar medium for incubation at 28±2°C over a period of 7 days. The purified fungal mycelia were obtained from a culture that had been 8 days old and kept at 28 ± 2°C for further studies. Two fungal isolates, named Fus01 and Fus02, were collected from Soonambedu and Marungulam villages, respectively.

Microscopic observation

The Leica Application Suite V4.4 microscope was used for visualising the fungal isolate (Fus01/Fus02). Observations were made on Fus01/Fus02 isolate culture plates after 9 days of incubation. The morphological characteristics of fungi, which involve hyphal septation, type, and shape of spores, were observed and recorded according to Amadi *et al.* (2014).

Mass multiplication of Fus01 and Fus02 isolates

Separately, the sand maize medium was used to multiply the isolates of Fus01 and Fus02. In a conical flask, the mixture of sand and ground maize seeds was mixed and moistened to a 50% level, then filled with the mixture and autoclaved at 21 psi for two hours. In each flask, four actively growing mycelial discs (9 mm) of Fus01 and Fus02 were inoculated in an aseptic environment, and the flasks were placed in an incubator for 15 days at room temperature (28 ± 2°C). The inoculum thus obtained was used for further experiments (Riker & Riker, 1936).

Assessing the virulence of Fus01 and Fus02 isolates

A 1:1:1:1 ratio of clay loam, soil, sand, and farm yard manure was used to prepare the potting mixture in a thorough way. Fus01 and Fus02 fungal suspension 50 ml was made and poured into the Rhizosphere region. After sterilising the watermelon seeds with 0.1% $HgCl_2$ solution for 30 seconds, they were washed in sterile water twice and sown at a rate of 4 seeds per pot. Each treatment was treated with three replications. The pots were cared for in a glasshouse and watered on a regular, judicious and uniform

basis. The wilt incidence occurred after 25 and 36 days after sowing for Fus01 and Fus02 isolates, respectively.

Cultural characteristics of the isolates

Sterile Petri dishes were filled with fifteen ml of sterile PDA medium and allowed to solidify. Aseptically, a 9 mm culture disc of isolates such as Fus01 and Fus02 was removed from an actively growing region in the middle of the dish and incubated. The mycelium's radial growth and colour were measured in millimetres seven days after inoculation.

Isolation of fungal DNA

The *Fusarium* isolates (*Fus01* and *Fus02*) were cultured in 100 mL Potato Dextrose Broth (PDB) at 28 ± 2°C for one week. To extract genomic DNA, a CTAB-based protocol with minor refinements was employed (Nicholson *et al.*, 1996). Mycelia were harvested, frozen with liquid nitrogen, and finely ground using a chilled mortar and pestle. About 200 mg of finely ground tissue was transferred into 2 mL tubes containing 1 mL extraction buffer (20 mM EDTA, pH 8.0; 2% CTAB; 0.1% β-mercaptoethanol). Samples were vortexed thoroughly and then incubated at 65°C for 10 min. Equal volumes of chloroform: isoamyl alcohol (24:1) were added to each sample, mixed well, and centrifuged at 10,000 rpm for 10 minutes. The aqueous layer was collected, and DNA was precipitated using 5 M NaCl and ice-cold isopropanol, either after incubation at 65°C or following overnight storage at –20°C. The pellet was obtained by centrifugation at 13,000 rpm for 10 min, washed twice with 70% ethanol, air-dried, and dissolved in 50 µL of TE buffer or nuclease-free water (Vignesh *et al.*, 2023).

PCR amplification and sequencing

DNA concentration and purity were measured with a NanoDrop spectrophotometer, while integrity was confirmed by 2% agarose gel electrophoresis, which showed intact high-molecular-weight DNA. PCR amplicons were purified and subjected to bidirectional sequencing using the Big Dye Terminator v3.1 kit (Applied Biosystems) on an ABI 3730xl Genetic Analyser at MEDAUXIN SEQUENCING PVT. Ltd., Bangalore. Forward and reverse chromatograms were assembled to obtain consensus sequences. BLAST searches against the NCBI database identified homologous sequences. The top matches were aligned using ClustalW, and evolutionary relationships were inferred using MEGA v7. Accession numbers were obtained after sequence submission to GenBank (Figures 1, 2).

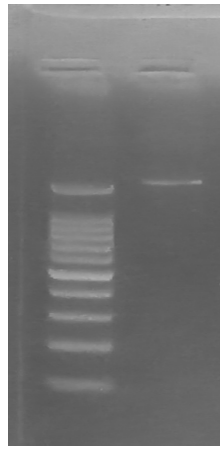


Figure 1. Gel photo of *Fusarium luffae*.

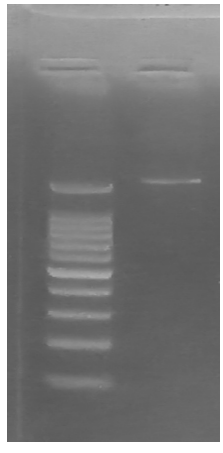


Figure 2. Gel photo of *Fusarium chlamydosporum*.

Phylogenetic analysis

Consensus sequences were compared with related fungal taxa through BLAST searches. Multiple sequence alignments were generated using ClustalW implemented in MEGA v7.0 (Kumar *et al.*, 2016). Phylogenetic trees were constructed using the Neighbour-Joining method (Saitou & Nei, 1987). Evolutionary distances were computed using the Kimura 2-parameter (K2P) model, enabling clear separation of the isolates from closely related species.

RESULTS

Survey of disease incidence

Field assessments revealed the endemic occurrence of *Fusarium* wilt of watermelon in four districts (Table 1). In Soonambedu village (Villupuram district), the mean incidence was 43.15%, while Marungulam village (Thanjavur district) showed 38.75%. Symptoms were most evident during the seedling and vining stages. In

Table 1. Survey on the incidence of *Fusarium* wilt disease in major watermelon growing areas of Tamil Nadu

S No.	Village	District	Soil type	Variety	Stage of the crop	Disease Incidence (%)
1	Soonambedu	Villupuram	Sandy loam	NS 295	Seedling	43.15 (39.95)
2	Marakkanam	Villupuram	Red loamy	SW 2208	Seedling	33.59
3	Pattanam	Villupuram	Sandy loam	Sweety	Vining	6.21
4	Sivapuri	Cuddalore	Sandy loam	Maharaja	Vining	13.48
5	Ramapuram	Cuddalore	Clay loam	SW 2208	Seedling	32.53
6	Bhuvanagiri	Cuddalore	Sandy loam	Spic	Vining	7.92
7	Mondipatti	Trichy	Clay loam	Dragon king	Seedling	28.48
8	Vellakal	Trichy	Red loamy	NOVA 46	Vining	9.23
9	Vannankovil	Trichy	Sandy loam	Sweety	Vining	7.96
10	Elupatti	Thanjavur	Sandy loam	SW 2208	Seedling	35.17
11	Marungulam	Thanjavur	Sandy loam	Maharaja	Vining	38.75
12	Eachangkottai	Thanjavur	Red loamy	Senthura	Vining	7.52

Fus01-infected fields, damping-off was observed as early as 18 days after sowing, indicating high aggressiveness at the seedling stage. In Fus02-infected fields, whole-plant mortality and runner wilt were evident around 32 days after sowing, corresponding to the vining stage. A consistent diagnostic feature was vascular browning, observed in longitudinally cut stems. The disease was most severe in sandy loam soils, which favoured pathogen proliferation (Figure 3).



Figure 3. Damping-off symptom observed in infected field.

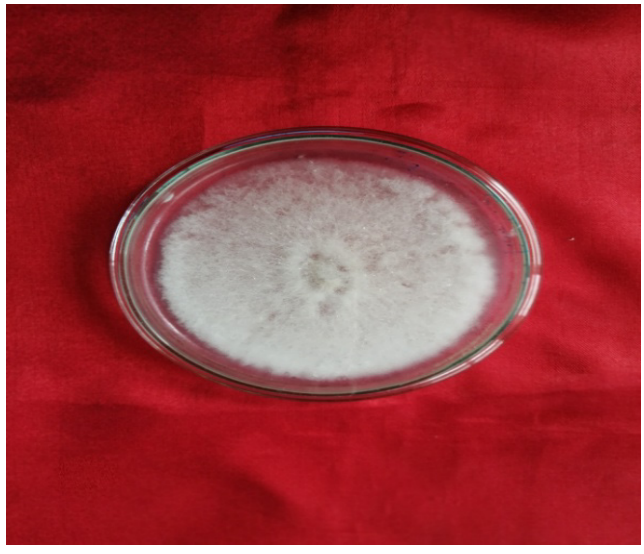


Figure 4. Pure culture of *Fusarium luffae*.



Figure 5. Pure culture of *Fusarium chlamydosporum*.



Figure 6. Microscopic view of microconidia.

Table 2. Cultural and morphological characters of Fus01 and Fus02 isolates

S. No.	Particulars		Isolate Fus01	Isolate Fus02
1	Place of collection		Soonambedu	Marungulam
2	Latitude and longitude		12.23° N and 79.88° E	10.67° N and 79.13° E
3	District name		Villupuram	Thanjavur
4	Soil type		Sandy loam	Sandy loam
5	Variety of the crop		NS 295	Maharaja
6	Stage of the crop		Seedling	Vining
7	Disease incidence %		43.15	38.75
8	Cultural characteristics		Cottony white	Cottony white
9	Mycelial growth (mm)*		90.00	89.16
10	Mycelial dry weight (mg)*		239.19	233.12
11	Micro conidia	Size (Length x Breadth µm)	11.81 × 4.33	10.82 × 3.58
		Shape	Oval	Kidney
		Septation	1	0
12	Macro conidia	Size (Length x Breadth µm)	45.13 × 5.17	41.15 × 5.22
		Shape	Sickle	Sickle
		Septation	7	5
13	Symptom expression at DAS (pathogenicity test)		18	31
14	Name of the pathogen according to NCBI		*Fusarium luffae*	*Fusarium chlamydosporum*



Figure 7. Microscopic view of macroconidia.

Morphological characterisation

Both isolates (Fus01 and Fus02) produced cottony white colonies on PDA within 7 days (Figures 4, 5). After 8 days, Fus01 achieved a radial growth of 90.00 mm, while Fus02 reached 89.16 mm. Both isolates produced macroconidia and microconidia with distinct features. Fus01 formed oval microconidia (11.81 × 4.33 µm), whereas Fus02 produced kidney-shaped microconidia (10.82 × 3.58 µm) (Figure 6). Macroconidia were sickle-shaped in both isolates; Fus01 produced 7-septate spores (45.13 × 5.17 µm), while Fus02 produced 5-septate spores (41.15 × 5.22 µm) (Figure 7). Dry mycelial weight was greater in Fus01 (239.19 mg) compared to Fus02 (233.12 mg) (Table 2). These characteristics matched earlier descriptions of *Fusarium luffae* (Namisy *et al.*, 2024) for Fus01 and *F. chlamydosporum* (Lazarotto *et al.*, 2014) for Fus02.

Pathogenicity test (Koch's postulates)

Pathogenicity assays confirmed both isolates as causal agents of wilt. Fus01 produced symptoms at 18 days after sowing, while Fus02 caused disease expression at 32 days. Control plants remained symptom-free for 45 days. Re-isolation from diseased plants yielded fungi morphologically identical to the inoculated isolates, thereby fulfilling Koch's postulates.

Molecular identification

PCR amplification of the ITS region using ITS1/ITS4 primers produced ~1500 bp fragments, confirming the isolates' placement within *Fusarium*. BLAST analysis in the NCBI database verified these identities. The validated sequences were submitted to GenBank and assigned accession numbers MW845763 (Fus01) and MW845764 (Fus02).

DISCUSSION

The pathogen virulence and prevalence were based on disease incidence and severity. The incidence of *Fusarium* wilt in watermelon varied across locations. Among the surveyed sites, Veppurchekkadi village in Thiruvannamalai district registered the maximum disease incidence (40.21%), and Lalapet village in Karur District showed the least incidence (8.00%) (Naveenkumar *et al.*, 2017). This pathogen disseminates through spores, which germinate to form hyphae capable of invading vascular tissues via wounds, resulting in the production of microconidia that assist in reproduction. The disease cycle is perpetuated by the pathogen being released back into the soil as the plant dies and decomposes (Rahman *et al.*, 2021). Monocropping watermelon leads to the high vulnerability of the plant to *Fusarium* wilt disease, which can cause significant seedling deaths, stunting and growth decline, reduced vigour, wilting, plant death, and decreases in both yield and quality (Zhang *et al.*, 2022a).

Fusarium luffae caused fruit rot in muskmelon, and it produces macro conidia measuring 20 to 40 × 3.5 to 4.5 µm with 3 to 5 septate on Carnation Leaf Agar (CLA) medium (Zhang *et al.*, 2022b). The shape of microconidia ranged from oval to kidney-shaped with 0 to 1 septation, while macro conidia were sickle-shaped with 3-5 septations. The size of microconidia in *Fusarium oxysporum* f.sp. *lycopersici* varies between 6.14 and 9.75 µm, while macro conidia vary between 18-7 and 48.9 µm(Sandra *et al.*, 2020). The colony colour of pathogenic *Fusarium* species was diverse, with a variety of shades from purplish white to cottony white (Nath *et al.*, 2017).

Soybean wilt in South Dakota was reported to be caused by the isolate SLSDF2, which was identified as *Fusarium luffae* through NCBI BLAST and Fusarioid polyphasic identification databases, showing 99.40% similarity to *F. luffae* strain NRRL31167 (Sharma *et al.*, 2024). *F. luffae* was first described as the causal agent of fruit rot in muskmelon in China by Zhang *et al.* (2022b). Similarly, fruit rot of *Luffa cylindrica* was attributed to *Fusarium incarnatum* and *Fusarium chlamydosporum*, with the corresponding sequences deposited under accession numbers MN480497 and MN480498, respectively (Abdel-Kader *et al.*, 2024).

In addition, *F. chlamydosporum* has been documented as the causal agent of watermelon fruit rot disease in Nigeria (Oyedeji *et al.*, 2022).

CONCLUSION

In this study, two different *Fusarium* species were identified as causal agents of watermelon wilt. The identification of these pathogens was achieved through a combination of morphological characterisation and molecular techniques, ensuring accuracy and reliability. Their pathogenicity was further verified by fulfilling Koch's postulates, establishing a direct link between the pathogens and the observed disease symptoms. This investigation demonstrates the pathogenic potential of these *Fusarium* species, expanding the known range of causal agents responsible for *Fusarium* wilt in watermelon, which was traditionally attributed primarily to *Fusarium oxysporum* f.sp. *niveum*.

The symptoms caused by these two *Fusarium* species closely resemble those of *F. oxysporum*, including wilting, chlorosis, and vascular browning, making field diagnosis challenging. This first report of these pathogens in watermelon underscores the importance of further research on their epidemiology, host interactions, and effective management strategies. Understanding the behaviour and impact of these newly identified *Fusarium* species will be vital for developing effective and sustainable management approaches to minimise quality and potential yield losses in watermelon cultivation.

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AUTHOR CONTRIBUTION

B. Tamilselvan- carried out the experiment and wrote the manuscript, S. Sudhasha - took the lead in writing the manuscript, K.Vignesh - performed the calculations,S. Vennila - helped shape the research, analysis and manuscript, S.M. Sureshkumar - contributed to the design and implementation of the research, A.P. Srinivasaperumal - contributed to the interpretation of the results and G. Kumaresan - contributed to the interpretation of the results. All authors have read and agreed to the published version of the manuscript.