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## Regional survey, isolation, and morphotypes diversity of *Fusarium* Wilt pathogen in chilli from Andhra Pradesh and Tamil Nadu

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### Abstract

*Fusarium* wilt, caused by various species of *Fusarium*, is a severe disease affecting chilli (*Capsicum annuum*) crops, leading to significant yield losses in major chilli-growing regions. This study aimed to assess the incidence of *Fusarium* wilt in Andhra Pradesh and Tamil Nadu during the 2021 rabi season. A systematic survey was conducted across chilli-growing districts, including Guntur, Kadapa, Cuddalore, Trichy, and Dindigul, to determine disease prevalence and severity. The highest percent disease incidence (PDI) was recorded in Peesapadu (38.96%), followed by Karambilipatti (35.57%), while lower incidence levels were observed in other locations. Isolation and identification of the wilt pathogen were performed using the tissue segment method, with morphological characterization revealing the presence of *Fusarium* sp. as the primary causal agents. The isolates exhibited significant variability in colony morphology, conidial dimensions, and growth patterns, suggesting diverse *Fusarium* populations in the surveyed regions. These findings highlight the need for continuous monitoring, precise pathogen identification, and the development of integrated management strategies, including breeding for resistance, to mitigate the impact of *Fusarium* wilt on chilli production.

**Keywords:** Chilli, yield losses, morphological characterization, spore morphology

### Introduction

Chilli (*Capsicum annuum* L.) is one of the highly significant vegetable spice and aromatic cultivated worldwide (Hami *et al.*, 2021) [8]. It is one of the key and widely cultivated spice in India, often referred to as 'the home of spices, contributes a substantial 25% of the global chilli production (Nazneen *et al.*, 2021; Manikanta Chowdary and Sutha Raj Kumar, 2021) [18, 15]. Widely cultivated in Asia on large scale (Tariq *et al.*, 2014) [36]. Chilli belonging to the family *Solanaceae* (Sahi and Khalid, 2007) [28] and is called with different names (hot pepper, sweet pepper, chili pepper, cayenne papper and bell pepper *etc.*), having superfluous nutritional and medicinal value (Hunziker, 2001; Knapp *et al.* 2004) [8, 11].

Major chilli growing countries are India, China, Indonesia, Korea, Pakistan, Turkey and Sri Lanka in Asia; Nigeria, Ghana, Tunisia and Egypt in Africa; Mexico, United States of America in North – Central America; Yugoslavia, Spain, Romania, Bulgaria, Italy and Hungary in Europe and Argentina and Peru in South America.

India being the world's largest producer, consumer and exporter of chillies, having the largest cultivated area of 7.33 lakh ha (18.11 lakh acres) constituting 42.81% of the global chilli cultivation. India leads global chilli production with 17.64 lakh tonnes surpassing the other major producers such as China (3.21 lakh tonnes), Ethiopia (2.94 lakh tonnes), Thailand (2.47 lakh tonnes) and Pakistan (1.48 lakh tonnes) in 2019-20. In India the major chilli producing states are Andhra Pradesh (6.60 lakh tonnes), Telangana (3.28 lakh tonnes), Madhya Pradesh (2.18 lakh tonnes), Karnataka (1.80 lakh tonnes) and West Bengal (1.04 lakh tonnes) accounting for 35%, 17%, 12%, 11% and 6% of the total chilli production in the country, respectively.

Chilli production frequently faces challenges due to various fungal, bacterial, and viral infections, with fungi alone being responsible for over 40 different diseases (Rangaswami, 1979, 2005) [25, 24]. Among the various disease, chilli *Fusarium* wilt is one of the most problematic in chilli production (Sanakar Reddy and Rajamohan, 2023) [31].

Yield losses in chilli production range from 10-50% globally and 10-80% in India (Thoyajakshi Bai, 2018) [38]. *Fusarium* wilt is characterized by symptoms such as yellowing and wilting of the plant, accompanied by the upward and inward rolling of leaves. The younger leaves may die in succession, leading to the wilting and eventual death of the entire plant within a few days (Sankara Reddy and Rajamohan, 2022) [30]. The high incidence and severity of *Fusarium* wilt necessitate an integrated approach to disease management, combining effective cultural practices, biological, resistant cultivars, and precise diagnostic tools.

## Materials and Methods

### Survey and collection of *Fusarium* spp. isolates from different regions of Andhra Pradesh and Tamil Nadu

Survey was conducted in major chilli growing districts of Andhra Pradesh (Guntur, Kadapa) and Tamil Nadu (Cuddalore, Trichy and Dindigul) during rabi season, March 2021 (Table 1). Samples are collected at maturity growth stage of the crop to know the incidence of *Fusarium* wilt from different locations of Andhra Pradesh and Tamil Nadu. Infected plants were identified based on diagnosis of typical symptoms of *Fusarium* wilt diseases such as leaf yellowing, wilting, vascular browning and death of plants (Figure 1). A random sampling has been done at the interval of 5-10 km along road side and in some interior villages and diseased samples are collected by selecting different plots from each village and assessed the disease incidence. Average percent disease incidence (PDI) was calculated by using the following formula.

$$\text{PDI} = \frac{\text{Number of plants observed}}{\text{Total number of plants observed}} \times 100$$

### Isolation and identification and purification of the pathogen

The wilt pathogenic isolates were cultured (Figure 1) in a PDA media and isolated by tissue segment method (Agrios, 2005) [1] from the infected root and stem samples. Infected roots and stems samples were washed under running tap water for 10 min and blotted (Katan, 1971) [10]. Since the pathogen is confined to vascular bundles, so brown colour vascular bundles were selected for isolation. The infected vascular bundles, along with healthy portions, were cut into small pieces (3-5 mm) and surface sterilized with 1% sodium hypochlorite (NaOCl) for 30 seconds, followed by three rinses in different variants of sterile distilled water (Dongzhen *et al.*, 2020) [7] and dried with tissue paper. 2-4 pieces were placed in a Petri plate containing solidified potato dextrose agar (PDA) media supplemented with Streptomycin sulphate to prevent bacterial contamination, the culture is incubated at 28±2 °C for 3-5 days. The culture was purified by using single spore isolation method and stored in agar slants at 25±2 °C (Leslie and Summerell, 2006) [12].

### Morphological features of *Fusarium* sp.

Twenty isolates of *Fusarium* were identified based on their colony morphology, the characteristics of macroconidia and microconidia, and the measurement of chlamydospores (Table 2). The *Fusarium* isolates were identified by comparing their morphology with the descriptions in a

reference manual (Booth, 1971) [4] and atlas of *Fusarium* (Leslie and Summerell, 2006; and Samson *et al.* 2008) [12, 29]. The pathogen is identified based on growth and morphological nature of the isolates viz., colony morphology, mycelial growth, colony colour, conidia size, shape and septation were observed, and measured (Soesanto *et al.*, 2011) [34]. The sizes of macroconidia, microconidia, and chlamydospores were measured using a trinocular microscope (Labomed-Lx-500, model LR-HD with a monitor system) equipped with a 5MP HDMI camera. Phase contrast images of the macroconidia and microconidia were captured at 400X magnification (Tsegaye and Tesfaye, 2020) [39].

## Results and Discussion

### Survey on the incidence of *Fusarium* wilt of chilli from major chilli growing areas of Andhra Pradesh and Tamil Nadu

An intensive survey was carried out in major chilli growing areas of Andhra Pradesh and Tamil Nadu reveals the endemic nature of the disease. Among the different locations surveyed Peesapadu (F1) recorded with maximum disease incidence with 38.96, followed by Karambilipatti (F13), Abburu (F7), Ponnampalle (F9), Andukuru (F4) and Phanidham (F6) with 35.57, 34.76, 32.11, 31.16 and 30.33 respectively. The other locations viz., Dugganapalli (28.98), Kottapatti (26.90), Kattamuru (25.76, 23.15) and Sivapuri (22.86) showed modest disease incidence and least wilt incidence was recorded in Athicombai (6.85). When compared to locations surveyed in Andhra Pradesh the disease incidence was much higher compared to locations in Tamil Nadu. The reason might be the disease susceptibility of the varieties grown in different locations and soil type. The previous data from the other surveyors also reveals the susceptibility of Teja varieties compared to local varieties grown over the locations and the entire data was furnished in Table 1. Birla (2014) [3] conducted survey and assessed the pervasiveness of chilli wilt caused by *Fusarium solani* f.sp. *capsici* from Nimar Valley and Malura Plateau zone and reported the maximum average disease incidence was recorded from Khargone with 34.93%. The results are in order with Manasa *et al* (2022) [14] who conducted roving survey in tomato intensive growing areas of Rayalaseema zone of Andhra Pradesh during 2021 and the disease incidence ranges from 15-60%. The maximum PDI was recorded at chinnahuithy village in Kurnool district with 59.5% followed by settivaripalle with 48% in YSR Kadapa, whereas minimum was recorded in Anantharajupeta of YSR Kadapa with 15% PDI. The variations in the disease incidence are observed and it might be due to geographical locations and difference in virulence level of the isolates.

*Fusarium* wilt freshly became more serious disease in all chilli growing zones of India (Singh *et al.*, 1998; Nishani *et al.*, 2021) [33, 19], especially in black cotton soils in Karnataka leading up to 25 percent yield loss (Madhukar and Naik, 2004; Mishra *et al.*, 2018) [13, 16], 0.0-70.0 (Ravikumara *et al.*, 2022) [26] and 15-20 percent in dry areas of Pakistan (Siddiqui *et al.*, 2007) [32]. The wilt incidence varied from 0.0-75.0 percent (Anonymous, 2005) [2] and 10-80 percent (Devika Rani., 2006) in different in different parts of India, the average recovery percentage of *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *capsici* was 53.30 percent (Mishra *et al.*, 2018) [16].

### Identification and morphological characters of the pathogen

All the isolates of *Fusarium* spp. produce white, creamy, pale brown, brownish colored fluffy, greyish, pinkish and cottony mycelial growth on potato dextrose agar medium (PDAM). The isolates F1 and F13 collected from Peesapadu and Karambilipatti recorded the maximum mycelial growth of 85.67 and 73.65. This was followed by Abburu (F7), Ponnampalle (F9), Andukuru (F4) and Phanidham (F6) with 71.16, 70.05, 68.60 and 68.10 in the decreasing order of merit after 7 days of plating. The isolates F4 and F6 are on par with each other and the lowest mycelial growth was recorded in isolate F18 collected from Athicombari village of Dindigul district in Tamil Nadu and the values are presented in Table. 2 (Figure 3). Production of microconidia, macroconidia and chlamydospores of 20 isolates are recorded and represented in table 2. The size of microconidia ranges from 13.89-26.91  $\mu\text{m}$  length to 3.45-7.32  $\mu\text{m}$  breadth, size of macroconidia ranges from 38.86-65.40  $\mu\text{m}$  length to 4.98-7.45  $\mu\text{m}$ , chlamydospores diameter ranges from 9.80-12.01  $\mu\text{m}$ , the number of septa of micro and macroconidia are 0-1 and 2-6 respectively (Table 2). All conidia are sickle to crescent shaped with blunt ends and

chlamydospore are produced terminal to intercalary with round to oval shaped (Figure 4).

All the twenty isolates of *Fusarium* sp. grown on the potato dextrose agar (PDA) medium showed various in growth pattern and colony characters white, fluffy to cream white cottony mycelium. The isolates F1, F3, F4, F8, F10, F14 produces white to pinkish brown cottony mycelium, whereas F2, F6, F11, F12, F13, F18, 19 produces pale brown to dark brown mycelium (Table 1). The pathogen is identified based on the morphological and cultural characters as described by Butler, 1910 [5]; Padwick, 1940 [21]; Booth 1971 [4] and Leslie and Summerell (2006) [12]. Oljira and Berta (2020) [20] isolated and characterised *Fusarium* wilt of pepper pathogen from Gurgeon zone, Ethiopia and identified *Fusarium oxysporum* f. sp. *capsici* responsible for the disease. Similarly, Hami *et al* (2021) [8] reported *F. equiseti* involved in causing chilli wilt from Kashmir region. *F. oxysporum* has also been reported from many parts of India and in Kashmir valley (Rajeswari and Kannabiran, 2011) [23], *F. solani* (Naik *et al.*, 2008) [17]. The results are in order with Soleha *et al* (2022) [35] who identified the etiology of acacia seedling wilt disease caused by *Fusarium oxysporum* in south Sumatra.

**Table 1:** Survey and incidence of *Fusarium* wilt of chilli from major chilli growing areas of Andhra Pradesh and Tamil Nadu

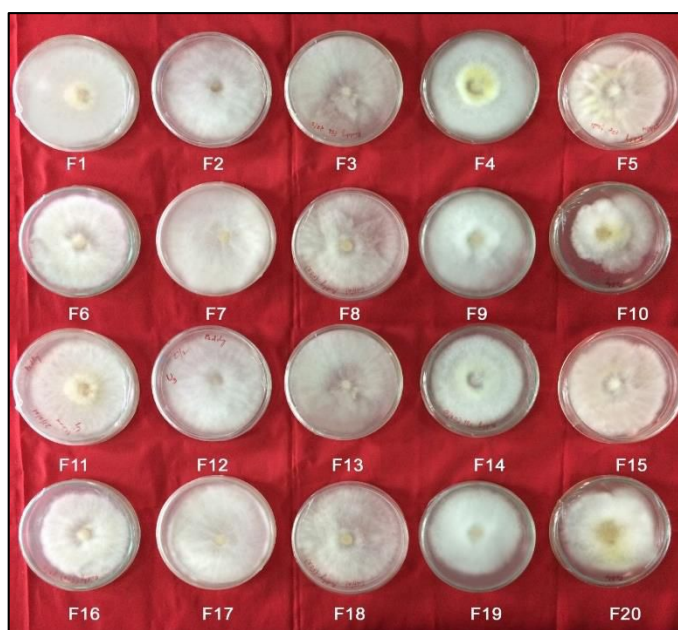
| S.NO                                                                                                                           | Isolate | Area              | District  | Variety  | Soil type  | Coordinates   |               | <i>Fusarium</i> wilt incidence (%) |
|--------------------------------------------------------------------------------------------------------------------------------|---------|-------------------|-----------|----------|------------|---------------|---------------|------------------------------------|
| 1                                                                                                                              | F1      | Peesapadu         | Guntur    | Teja     | Red soil   | 16°28'56" N   | 80°8'23" E    | 38.96 <sup>a</sup> (38.61)         |
| 2                                                                                                                              | F2      | Kattamuru         | Guntur    | Teja     | Red soil   | 16°29'31" N   | 80°8'58" E    | 23.15 <sup>a</sup> (28.75)         |
| 3                                                                                                                              | F3      | Kattamuru         | Guntur    | Teja     | Red soil   | 16°28'56" N   | 80°10'57" E   | 25.76 <sup>f</sup> (30.49)         |
| 4                                                                                                                              | F4      | Andukuru          | Guntur    | Teja     | Red soil   | 16°31'13" N   | 80°11'1" E    | 31.16 <sup>cd</sup> (33.93)        |
| 5                                                                                                                              | F5      | Gaarapadu         | Guntur    | Teja     | Red soil   | 16°30'42" N   | 80°13'11" E   | 18.65 <sup>b</sup> (25.58)         |
| 6                                                                                                                              | F6      | Phanidham         | Guntur    | Teja     | Red soil   | 16°45'85" N   | 80°17'71" E   | 30.33 <sup>de</sup> (33.41)        |
| 7                                                                                                                              | F7      | Abburu            | Guntur    | Teja     | Red soil   | 16°44'24" N   | 80°16'24" E   | 34.76 <sup>b</sup> (36.12)         |
| 8                                                                                                                              | F8      | Thorrivemala      | Kadapa    | Endo 5   | Clay loam  | 14°53'43.5" N | 78°19'14.9" E | 13.66 <sup>i</sup> (21.68)         |
| 9                                                                                                                              | F9      | Ponnampalle       | Kadapa    | Endo 5   | Clay loam  | 14°54'44.3" N | 78°18'28" E   | 32.11 <sup>c</sup> (34.51)         |
| 10                                                                                                                             | F10     | Dugganapalli      | Kadapa    | Endo 5   | Clay loam  | 14°56'5.7" N  | 78°19'42.6" E | 28.98 <sup>e</sup> (32.56)         |
| 11                                                                                                                             | F11     | Sivapuri          | Cuddalore | Local    | Red soil   | 11°22'0.1" N  | 79°42'51.0" E | 22.86 <sup>e</sup> (28.56)         |
| 12                                                                                                                             | F12     | Kuduraikuthipatty | Trichy    | K1       | Clam loam  | 10°38'24.5" N | 78°24'45.2" E | 12.10 <sup>ij</sup> (20.35)        |
| 13                                                                                                                             | F13     | Karambilipatti    | Trichy    | K1       | Red soil   | 10°40'11.4" N | 78°25'17.6" E | 35.57 <sup>b</sup> (36.61)         |
| 14                                                                                                                             | F14     | Kottapatti        | Trichy    | K1       | Red soil   | 10°39'52" N   | 78°24'57" E   | 26.90 <sup>f</sup> (31.23)         |
| 15                                                                                                                             | F15     | Aamayapuram       | Trichy    | K1       | Red loam   | 10°38'43.6" N | 78°24'36.6" E | 10.89 <sup>ijkl</sup> (19.26)      |
| 16                                                                                                                             | F16     | Nalupulikottai    | Dindigul  | K2       | Black soil | 10°28'3" N    | 77°49'49" E   | 12.89 <sup>i</sup> (21.03)         |
| 17                                                                                                                             | F17     | Puducharam        | Dindigul  | K2       | Black soil | 10°28'53.3" N | 77°47'22.4" E | 9.86 <sup>kl</sup> (18.30)         |
| 18                                                                                                                             | F18     | Athicombari       | Dindigul  | Bangaram | Black soil | 10°29'05.4" N | 77°46'31.4" E | 6.85 <sup>m</sup> (15.17)          |
| 19                                                                                                                             | F19     | Naganampatty      | Dindigul  | K2       | Black soil | 10°20'03.9" N | 77°45'34.9" E | 11.16 <sup>kl</sup> (19.51)        |
| 20                                                                                                                             | F20     | Kalanjipatty      | Dindigul  | Bangaram | Black soil | 10°30'40.8" N | 77°46'53.7" E | 9.45 <sup>l</sup> (17.90)          |
| Mean of three replications                                                                                                     |         |                   |           |          |            |               |               |                                    |
| Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range test (DMRT) |         |                   |           |          |            |               |               |                                    |



**Fig 1:** Symptoms of *Fusarium* wilt of chilli caused by *Fusarium* sp.

**Table 2:** Morphological variability of *Fusarium* sp. from different localities of Andhra Pradesh and Tamil Nadu

| Isolate | Colony characters                      | Mycelial growth (7 days)    | Micro conidia |            |                   | Macro conidia |            |                   | Chlamydospore |
|---------|----------------------------------------|-----------------------------|---------------|------------|-------------------|---------------|------------|-------------------|---------------|
|         |                                        |                             | Length (µm)   | Width (µm) | No. of septations | Length (µm)   | Width (µm) | No. of septations | Diameter (µm) |
| F1      | White fluffy raised cottony mycelium   | 85.67 <sup>a</sup> (67.74)  | 18.85         | 5.15       | 0-1               | 44.03         | 6.03       | 4-6               | 11.85         |
| F2      | Cream white mycelium                   | 57.32 <sup>f</sup> (49.20)  | 13.67         | 3.67       | 0-1               | 45.65         | 6.03       | 2-3               | 10.45         |
| F3      | White cottony mycelium                 | 60.44 <sup>e</sup> (51.02)  | 18.15         | 3.45       | 0                 | 50.56         | 5.89       | 2-4               | 10.05         |
| F4      | White cottony mycelium                 | 68.60 <sup>c</sup> (55.91)  | 26.91         | 6.32       | 0                 | 40.60         | 7.50       | 3-5               | 10.00         |
| F5      | White cottony mycelium                 | 51.67 <sup>h</sup> (45.95)  | 15.25         | 4.54       | 0-1               | 48.68         | 7.45       | 2-4               | 11.5          |
| F6      | Cream white submerged cotton mycelium  | 68.10 <sup>c</sup> (55.60)  | 22.21         | 7.32       | 0                 | 45.47         | 7.07       | 3-5               | 10.5          |
| F7      | Cream white fluffy mycelium            | 71.16 <sup>b</sup> (57.52)  | 20.86         | 5.86       | 0-1               | 65.40         | 6.82       | 3-5               | 12.01         |
| F8      | White fluffy cottony mycelium          | 44.06 <sup>i</sup> (41.58)  | 15.67         | 4.97       | 0-1               | 59.97         | 6.05       | 2-4               | 9.5           |
| F9      | White cottony mycelium                 | 70.05 <sup>bc</sup> (56.81) | 18.34         | 4.44       | 0                 | 52.13         | 8.05       | 3-5               | 9.95          |
| F10     | White fluffy cottony mycelium          | 64.15 <sup>d</sup> (53.21)  | 19.56         | 4.23       | 0-1               | 54.76         | 6.88       | 2-5               | 10.8          |
| F11     | Pale brown to white cottony mycelium   | 55.01 <sup>s</sup> (47.86)  | 18.56         | 4.01       | 0                 | 56.46         | 6.67       | 2-4               | 10.4          |
| F12     | Cream white mycelium                   | 42.11 <sup>i</sup> (40.45)  | 17.25         | 4.67       | 0-1               | 54.26         | 6.06       | 2-5               | 10.35         |
| F13     | Cream white to pinkish mycelium        | 73.65 <sup>a</sup> (69.10)  | 24.87         | 5.79       | 0                 | 40.09         | 6.29       | 2-5               | 10.86         |
| F14     | White creamy to white greyish mycelium | 62.66 <sup>d</sup> (52.32)  | 14.67         | 4.37       | 0                 | 44.87         | 5.88       | 2-5               | 10.56         |
| F15     | Pale brown to dark brown zonation      | 36.89 <sup>j</sup> (37.39)  | 16.67         | 4.09       | 0-1               | 47.98         | 6.62       | 2-4               | 11.08         |
| F16     | Whitish brown fluffy mycelium          | 43.63 <sup>i</sup> (41.33)  | 17.54         | 4.56       | 0                 | 56.39         | 5.77       | 2-4               | 9.85          |
| F17     | Milkfish white cottony mycelium        | 32.23 <sup>k</sup> (34.58)  | 16.67         | 3.67       | 0-1               | 39.84         | 5.57       | 2-3               | 10.5          |
| F18     | Pale brown cottony mycelium            | 23.67 <sup>m</sup> (29.11)  | 13.89         | 3.76       | 0                 | 38.86         | 5.51       | 2-3               | 9.80          |
| F19     | Brownish mycelium                      | 38.66 <sup>j</sup> (38.44)  | 15.56         | 3.98       | 0                 | 43.67         | 6.18       | 2-3               | 10.15         |
| F20     | Creamy white fluffy mycelium           | 29.56 <sup>l</sup> (32.92)  | 16.91         | 4.03       | 0                 | 42.10         | 4.98       | 2-3               | 9.85          |

**Fig 2:** Axenic culture of *Fusarium incarnatum***Fig 3:** Mycelial growth of twenty different isolates of *Fusarium* sp.

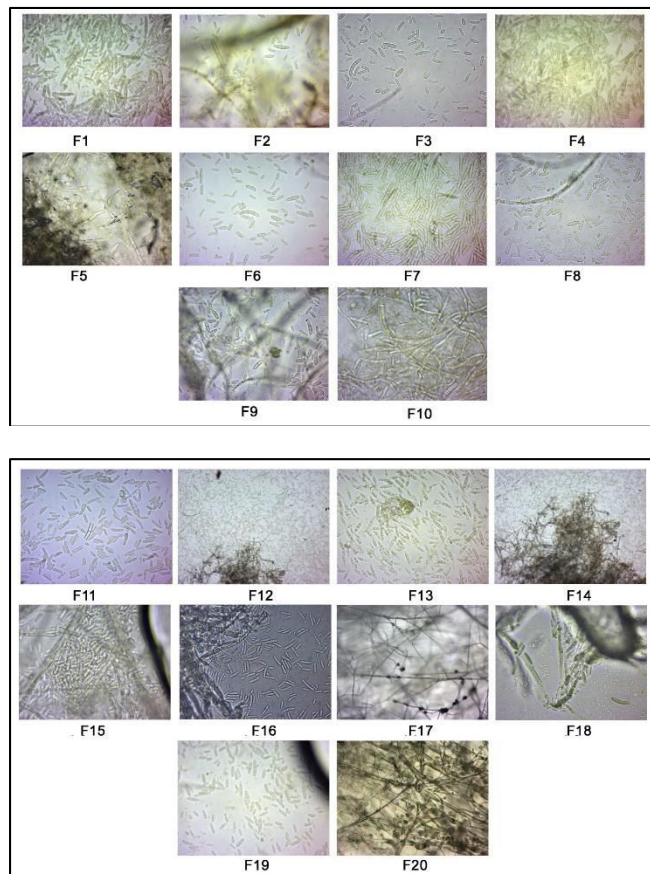


**Fig 4:** Microscopic view of conidia of twenty different isolates of *Fusarium* sp.

## Conclusion

The present study provides a comprehensive assessment of *Fusarium* wilt incidence in chilli crops across major growing regions of Andhra Pradesh and Tamil Nadu. The survey revealed that *Fusarium* wilt is a widespread and significant disease affecting chilli cultivation, with varying degrees of severity observed across different locations. Peesapadu (F1) recorded the highest disease incidence, followed by Karambilipatti (F13) and other locations. The differences in disease incidence may be attributed to the susceptibility of the varieties grown, soil types, and virulence levels of the isolates. Isolation and identification of *Fusarium* spp. were performed using tissue segment methods, and morphological characterization confirmed the presence of different *Fusarium* species. The isolates showed variability in colony morphology, mycelial growth, and conidia characteristics. This study highlights the importance of continuous monitoring and identification of *Fusarium* species to develop effective management strategies. The results underscore the need for further research on resistant varieties and integrated disease management practices to mitigate yield losses due to *Fusarium* wilt in chilli crops.

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