*Original Research Article*

# Studies on cultural, morphological and pathogenic variability among the isolates of *F. verticillioides* associated with maize stalk rot in Telangana state

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if you go to india
NOTE: Telangana State - India


## ABSTRACT

Maize (*Zea mays* L.) is one of the important cereal crops of the world and world's third most leading cereal crop, after wheat and rice. Maize is affected by various biotic and abiotic stresses. Among the biotic stresses, fusarium wilt of maize caused by *Fusarium verticillioides* is most serious disease of maize. Tweleve isolates of *F. vericillioides* were studied for its cultural, morphological and pathogenic variability. Microconidia were hyaline, oval to club shaped with a flattened base and measured 5.12-7.11 µm *X* 2.04-3.18 µm (L×W). Macroconidia were sickle shaped with 3-5 septa and measured 20.01-31.12 µm *X* 2.01-3.21 µm (L×W). The radial mycelial growth of test isolates ranged from 4.32 mm to 8.65 mm at 10 days after inoculationon on PDA medium. However, maximum mycelial growth was recorded by the isolate F-ISO-5 in all the three mediums and mean maximum growth of isolates were observed in CMA. The fungal colony of *Fusarium* isolates on PDA were initially white, floccose which turned purple to dark brown after 7 days of incubation at 28 ± 2 ºC. Cultures developed pigmentation like pink, light purple, dark violet which varied with age. All the tested isolates were pathogenic on tested maize cultivar (kaveri- 50). However, the disease severity was varied among the isolates. *Fusarium* isolates F-ISO-7 was highly virulent which caused severe disease upon inoculation with disease score of 8.0 on 1-9 scale followed by F-ISO-1, F-ISO-3, F-ISO-4, F-ISO-5, F-ISO-6 and F-ISO-8.



**Keywords:** Cultural, Morphological, Pathogenic variability, *Fusarium*, Maize


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## INTRODUCTION

Maize (*Zea mays* L.) is one of the most versatile emerging crops having wider adaptability under varied agro-climatic conditions. It is cultivated in tropics, sub tropics and temperate regions under irrigated and rainfed conditions. Globally, maize is known as queen of cereals, because it has the highest genetic yield potential among the cereals. In most of the developing countries maize is consumed directly as food. Maize occupies an important place as a source of human food (26%), animal feed (11%), poultry feed (48%), industrial products (12%) and seed (3%) in India. Maize is cultivated in an area of 9380.07 thousand hectares with an annual production of 28752.8 thousand tons in India. In Telangana State, India, the crop is grown in almost all districts in an area of 630 thousand hectares with a production of 2555.64 thousand tonns and productivity of 4057 $Kg^{-1}$ hectare (INDIANSTAT, 2017-2018). The other important maize growing states in India are Karnataka, Bihar, Rajastan, Maharashtra, Madhya Pradesh, Utter Pradesh, Andhra Pradesh, and Himachal Pradesh etc. Maize is affected by various biotic and abiotic stresses. Among the biotic stresses, fungal diseases are one of the major constraints in realizing the potential yields of this crop.

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Of the fungal diseases, post flowering stalk rots poses a major threat to the productivity of maize crop. Post flowering stalk rot is complex disease which occurs at post flowering stage of the crop in both *kharif* and *Rabi* season. In India, eight fungi and three bacteria were reported to cause stalk rots (Raju and Lal, 1976). Among all, *Fusarium* stalk rot (*Fusarium verticillioides),* Charcoal rot *(Macrophomina phaseolina),* Late wilt *(Cephalosporium maydis)* are more prevalent and destructive in

India (Khokhar *et al*., 2014). Among the stalk rots, *Fusarium* stalk rot caused by *F. verticillioides* was first reported from USA by Pammel (1914) as a serious root and stalk disease. Later, Valleau (1920) reported that *F. moniliforme* was a primary cause of root and stalk rot of maize. In India, the disease was first reported from Mount Abu, Rajasthan State, India (Arya and Jain, 1964) and prevalent in most of the maize growing areas of country where water stress occurs at the flowering stage of the crop. The disease becomes apparent when crop enters senescence phase and severity increases during grain filling stage. The rotting extends from the infected roots to the stalk and causes premature drying, stalk breakage and ear dropping and thus resulting in reduction of maize yields (Colbert *et al*., 1987). The disease causes internal decay and discoloration of stalk tissues, directly reducing yield by blocking translocation of water and nutrients, thus resulting in death and lodging of the plant (Dodd, 1980).The fungus survives on crop residues in the soil or on the soil surface.

Field observations revealed the difference in virulence with in *Fusarium verticillioides* populations from different conventional maize growing areas indicating the emergence of new pathotypes. The pathogen is mainly soil borne therefore needs to know the nature of pathogen for better management practice of *Fusarium* wilt disease of maize. However the present study was investigated to cultural, morphological and pathogenic variability of *Fusarium* isolates present in the different soils of Telangana State, India.

## MATERIALS AND METHODS

Survey was carried out in the major maize growing regions of Telangana State, India, during kharif – 2019, where cereals are cultivated extensively by the farmers and experimented at Department of Maize Pathology, Maize Research Centre, ARI, Rajendranagar.

**Reagents and equipments**

Sulfuric acid (Synth, Brazil)....

UV-Vis spectrophotometer (BelPhotonics, Mod. M-51, Italy)...

**Isolation of *Fusarium verticillioides* isolates**

Maize plants showing typical symptoms were collected from different locations and used for isolation of the pathogen. These samples were first washed with tap water followed by sterile distilled water. Diseased portions with some healthy portion were cut into small bits of 3-5 mm size, surface sterilized by dipping them in sodium hypochlorite conc. (1% - *v/v*) solution for one minute and then 3-4 bits were transferred aseptically to petri plates containing potato dextrose agar (PDA) medium and were incubated at 25 ± 1 ºC in an incubator (Aneja, 2003).

**Cultural and Morphological variability**

Observations on colony colour, pigmentation, sporulation, growth pattern of each isolate were recorded 12 days after incubation at 28 ± 2 ºC.

**Colony colour and pigmentation**

Colony colour and pigmentation of all the isolates grown on different media was determined with the help of Munsell's colour chart (Soil Survey Staff, 1951). Twelve day old culture of *Fusarium verticillioides* were used to note down the colony colour and pigmentation. The pigmentation of the colony was recorded from the under surface of the *Petri* plate.

**Sporulation**

To determine sporulation in each isolate, discs of 5 mm size were cut from 10

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day old cultures. Three such discs were placed in a test tube containing 15 mL of distilled water and were vortexed to dislodge the conidia from mycelial mat. Spore load was measured by using haemocytometer (Aneja, 2003).

**Preparation of slides**

A small amount of pure culture was taken using a sterile needle and transferred onto a clean sterile slide. The culture of each isolate was taken from four positions of the culture plate. Total three culture plates of each isolate were used for the morphological studies of length, width and septa after 10 days of incubation at 28 ± 2 ºC. The culture was stained with conc. 0.1% (*m/v*) lacto phenol cotton blue and observations on different morphological characteristics were recorded by computerized inverted microscope for each of the isolate on different media *viz*., potato dextrose agar (PDA), corn meal agar (CMA) and czepec dox agar (CDA) media.

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**Pathogenicity test**

Pathogenicity of the different *F. verticillioides* isolates was tested by toothpick method (AICMIP, 1983). For this purpose, round bamboo tooth picks about 6.5 cm long were boiled three times (about 1 h each time) in tap water to remove toxic substances. After each boiling these were thoroughly washed in fresh water and dried in the sun. When these were thoroughly dried, they were loosely packed in bundles and put into the glass jars/ bottles and enough potato dextrose broth is added to thoroughly moisten the toothpicks.

Sterilized toothpicks were inoculated with the culture of the pathogen aseptically. The growth of the fungus covers the toothpicks and inoculum is ready for use in about 10 days and inoculated on 50 days old plants. The lower internode (second/third) above soil level is opened with a jabber and the toothpick was inserted into the hole. The Symptoms were recorded on 45 days after inoculation. For scoring disease severity of *Fusarium* stalk rot 1-9 disease rating scale (AICMIP, 1983) is followed.

**RESULTS AND DISCUSSION**

**Morphological variability among the isolates**

Morphological characters such as size, shape, septation and colour of conidia were studied for 12 isolates. Conidiophores were elongated and branched, each branch usually terminated with a spore bearing monophialide. The pathogen was found to produce two types of asexual spores *viz.*, microconidia and macroconidia. The resting spores called chlamydospores were also observed in age old culture.

Microconidia were hyaline, oval to club shaped with a flattened base and measured 5.12-7.11 µm *X* 2.04-3.18 µm (L×W). They were formed from monophialides and were found in long chains. Macroconidia observed were sickle shaped, hyaline with apical cell curved and tapered, and basal cell notched (Plate 1). They were typically 4-6 celled with 3-5 septa and measured 20.01-31.12 µm *X* 2.01-3.21 µm (L×W). Chlamydospores were globose, intercalary, solitary or in chains. Morphological variability of all the 12 isolates was depicted in the Table 1.

The fusarium isolates F-ISO-1, F-ISO-4, F-ISO-5, F-ISO-6, F-ISO-8, F-ISO-9. F-ISO-10, F-ISO-11 and F-ISO-12 showed sickle shaped macroconidia with blunt ends and *Fusarium* isolates F-ISO-2, F-ISO-3 and F-ISO-7 showed elongated sickle shaped

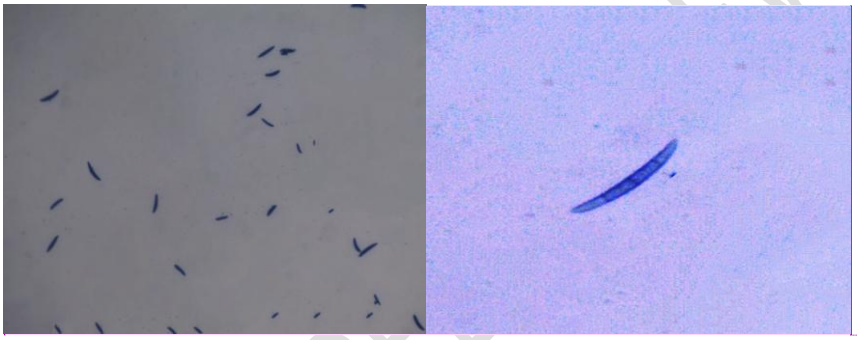
macroconidia. Pyriform to oval microconidia were observed in F-ISO-1, F-ISO-2, F-ISO-3, F-ISO-4, F-ISO-5, F-ISO-6, F-ISO-9 and F-ISO-12 where as in F-ISO-7, F-ISO-8, F-ISO-10 and F-ISO-11 round to oval microconidia were seen. More number of septa was found in F-ISO-7 with 4-6 septa and remaining isolates were showed 4-5 septa only. Thaware *et al*. (2017) also reported eight isolates of *Fusarium oxysporum* f.sp. *ciceri* with similar characteristics. Anita *et al*. (2017) found the significant variations in morphology and cultural characters of different isolates of *Fusarium verticillioides viz.,* Fv SC-01, Fv SC-02, Fv SC-03 and Fv SC-04.

**Table 1. Morphological characteristics of different isolates of *Fusarium verticillioides***

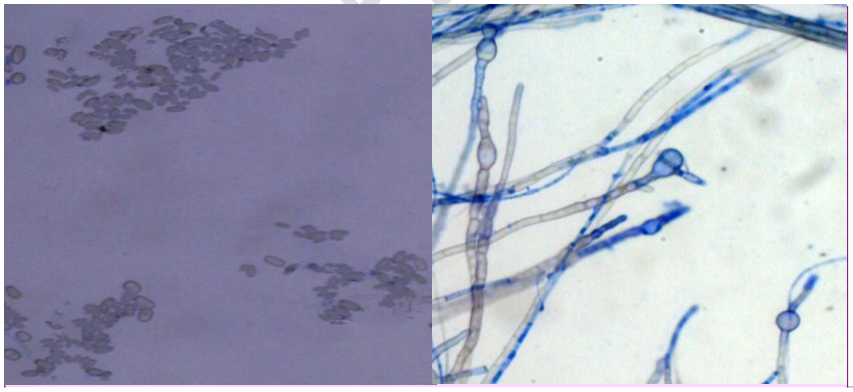
| S. No. | Isolates | Macro conidia | | | Micro conidia | | | Colour |
|---|---|---|---|---|---|---|---|---|
| | | Septation | Size ( µm) | Shape | Septation | Size ( µm) | shape | |
| 1 | F-ISO-1 | 3-5 | 25.41x2.92 | Sickle shape with blunt end | 0-1 | 5.83x2.51 | Pyriform to Oval | Hyaline |
| 2 | F-ISO-2 | 3-5 | 31.12x3.11 | Elongated sickle shape | 0-1 | 6.41x3.12 | Pyriform to Oval | Hyaline |
| 3 | F-ISO-3 | 3-5 | 30.11x2.97 | Elongated sickle shape | 0-1 | 5.71x2.76 | Pyriform to Oval | Hyaline |
| 4 | F-ISO-4 | 3-5 | 26.13x2.71 | Sickle shape with blunt end | 0-1 | 6.22x2.31 | Pyriform to Oval | Hyaline |
| 5 | F-ISO-5 | 3-5 | 29.01x3.11 | Sickle shape with blunt end | 0-1 | 5.92x2.72 | Pyriform to Oval | Hyaline |
| 6 | F-ISO-6 | 3-5 | 23.08x2.17 | Sickle shape with blunt end | 0-1 | 7.06x3.18 | Pyriform to Oval | Hyaline |
| 7 | F-ISO-7 | 4-6 | 28.36x2.42 | Elongated sickle shape | 0-1 | 7.11x2.04 | Round to Oval | Hyaline |
| 8 | F-ISO-8 | 3-5 | 25.12x3.21 | Sickle shape with blunt end | 0-1 | 5.81x2.91 | Round to Oval | Hyaline |
| 9 | F-ISO-9 | 3-5 | 23.96x2.01 | Sickle shape with blunt end | 0-1 | 6.27x2.82 | Pyriform to Oval | Hyaline |
| 10 | F-ISO-10 | 3-5 | 20.01x2.12 | Sickle shape with blunt end | 0-1 | 5.12x2.24 | Round to Oval | Hyaline |
| 11 | F-ISO-11 | 3-5 | 22.12x2.12 | Sickle shape with blunt end | 0-1 | 5.42x2.22 | Round to Oval | Hyaline |
| 12 | F-ISO-12 | 3-5 | 25.71x2.45 | Sickle shape with blunt end | 0-1 | 5.91x2.41 | Pyriform to Oval | Hyaline |

**Plate 1. Morphological characters of *Fusarium verticillioides* (F-ISO-7)**

**(a)** **(b)**



**(c)** **(d)**



**(e)** **(f)**

**Note: (a) Mycelialgrowth o PDA (b) mycroscopic view of mycelia, conidia, conidiophores, monophialides (c) & (d) macroconidia (e) microconidia in groups (f) Chlamydospores of *Fusarium verticillioides*(F-ISO-7)**

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**Cultural variability among *Fusarium* isolates**

All the twelve isolates were cultured on three different media *viz.*, potato dextrose agar (PDA), czepek dox agar and corn meal agar and grown at 28 ± 2 ºC, were studied separately for their cultural characters and mentioned in Table 2, 3 and 4 respectively. Observations on colony colour, mycelial growth pattern and growth rate were recorded at 12 days after inoculation. The fungal colony of *Fusarium* isolates on PDA were initially white, floccose, compact and dense which turned to dark brown after 7 days of incubation at 28 ± 2 ºC. Cultures developed pigmentation like pink, light purple, dark violet which varied with age (Plate 2, 3 and 4 respectively). Mahsane (2013) studied the cultural characteristics and reported that three isolates were produced light pink, five were creamy, one were light brown, six were light yellow, three were light to dark coloured pigmentation. Spongy, compact and dense growth of colonies was observed on PDA and Czepek dox agar medium, sparse and fast growth was observed on CMA.

The radial mycelial growth of test isolates ranged from 4.32 to 8.65 on PDA. However, maximum mycelial growth was recorded by the isolate F-ISO-5 in all the three mediums and mean maximum growth of isolates were observed in CMA. These findings are in agreement with the Nurbaya *et al.* (2014) findings who reported that fungi isolated from soil, or from substrates in the soil, i.e., plant debris, grow well on CMA, a relatively weak medium compared to PDA. These results of the present study are in consonance with the previous findings of many workers (Ahmed, 2010; Islam *et al.*, 2011; Nagare, 2011 and Ansar and Srivastva, 2013).

**Table 2. Cultural characters of different isolates of *F. verticillioides* on PDA medium**

Comment [RV12]: Complete name

| S.No | Isolates | Colony diameter (cm)* | Growth rate (mm day)* | Colony type | Colony colour on the reverse side of the plate |
|---|---|---|---|---|---|
| 1 | F-ISO-1 | 5.17 | 5.26 | spongy and White | Orange |
| 2 | F-ISO-2 | 7.87 | 6.82 | Sparse and orange | Light yellow |
| 3 | F-ISO-3 | 8.28 | 9.17 | Dense and violet | Brown |
| 4 | F-ISO-4 | 5.60 | 6.13 | Dense and white to violet | Brown to white |
| 5 | F-ISO-5 | 8.65 | 9.40 | Dense and violet | Dark brown |
| 6 | F-ISO-6 | 8.38 | 10.19 | Dense and light violet | Light brown |
| 7 | F-ISO-7 | 8.37 | 9.31 | Dense and light violet | White |
| 8 | F-ISO-8 | 4.95 | 5.22 | Spongy and orange | Light yellow |
| 9 | F-ISO-9 | 5.03 | 5.27 | Dense and light brown | White |
| 10 | F-ISO-10 | 4.32 | 4.46 | Spongy and orange | Orange |
| 11 | F-ISO-11 | 4.71 | 4.94 | Spongy and white | Light yellow |
| 12 | F-ISO-12 | 4.92 | 5.14 | Dense and light brown | Brown |
| CD (P=0.05) | | 0.089 | 0.075 | - | - |

* Mean of five replications

**Table 3. Cultural characters of different isolates of *F. verticillioides* on czepek dox agar medium**

Comment [RV13]: Complete name

| S. No | Isolates | Colony diameter (cm)* | Growth rate (mm day)* | Colony type | Colony colour on the reverse side of the plate |
|---|---|---|---|---|---|
| 1 | F-ISO-1 | 4.95 | 5.27 | Spongy and white | Dark yellow |
| 2 | F-ISO-2 | 8.56 | 9.14 | Sparse and light orange | White |
| 3 | F-ISO-3 | 6.45 | 7.44 | Dense and light violet | White |
| 4 | F-ISO-4 | 8.20 | 8.09 | Dense and light violet | Light yellow |
| 5 | F-ISO-5 | 8.91 | 9.15 | Dense and violet | Dark brown |
| 6 | F-ISO-6 | 8.42 | 8.53 | Dense and light orange | Light yellow |
| 7 | F-ISO-7 | 8.23 | 9.33 | Dense and light violet | White |
| 8 | F-ISO-8 | 4.83 | 5.64 | Spongy and orange | Light yellow |
| 9 | F-ISO-9 | 5.34 | 3.21 | Sparse and brown | White |
| 10 | F-ISO-10 | 4.35 | 4.94 | Spongy and white | Light yellow |
| 11 | F-ISO-11 | 4.26 | 4.54 | Spongy and white | White |
| 12 | F-ISO-12 | 7.63 | 7.20 | Sparse and violet | Violet |
| CD(P=0.05) | | 0.035 | 0.308 | - | - |

*Mean of three replications

**Table 4. cultural characteristics of different iolates of *Fusarium verticillioides* on corn meal agar**

| S.No | Isolates | Colony diameter (cm)* | Growth rate (mm day)* | Colony type | Colony colour on the reverse side of the plate |
|---|---|---|---|---|---|
| 1 | F-ISO-1 | 3.20 | 4.22 | Spongy and white | White |
| 2 | F-ISO-2 | 8.72 | 9.11 | Very sparse and light orange | White |
| 3 | F-ISO-3 | 8.67 | 9.40 | Very sparse and light violet | Violet |
| 4 | F-ISO-4 | 8.82 | 10.38 | Very sparse and light violet | Violet |
| 5 | F-ISO-5 | 8.93 | 9.62 | Very sparse and violet | Violet |
| 6 | F-ISO-6 | 8.85 | 10.31 | Very sparse and light violet | Violet |
| 7 | F-ISO-7 | 8.13 | 8.75 | dense and creamish | Light yellow |
| 8 | F-ISO-8 | 6.29 | 7.17 | Spongy and orange | Light yellow |
| 9 | F-ISO-9 | 5.46 | 6.32 | Dense and brown | Brown |
| 10 | F-ISO-10 | 4.42 | 4.94 | Spongy and light yellow | Yellow |
| 11 | F-ISO-11 | 4.07 | 4.28 | Spongy and white | White |
| 12 | F-ISO-12 | 5.93 | 6.45 | Dense and creamish | White |
| CD(P=0.05) | | 0.045 | 0.054 | - | - |

**Plate 2. Growth of isolates *F. verticillioides* on PDA medium**

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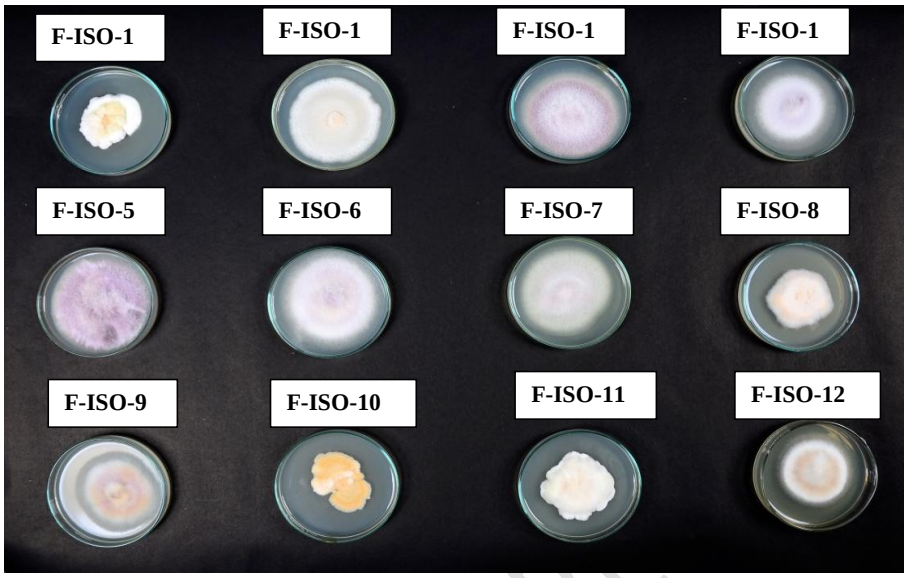
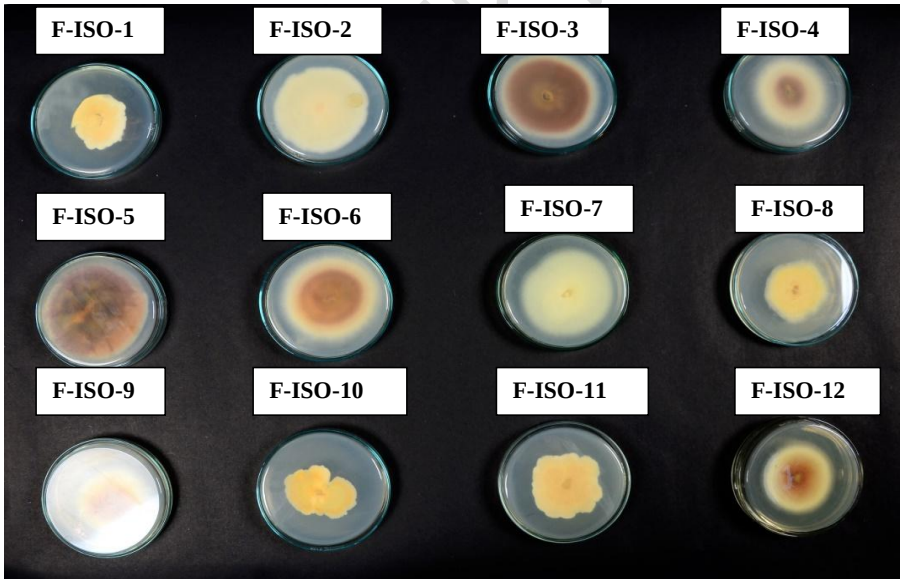



**a) Aerial colony growth**




**b) Colony growth on reverse side of *Petri* plate**

**Plate 3. Growth of isolates *F. verticillioides* on czepek dox agar medium**

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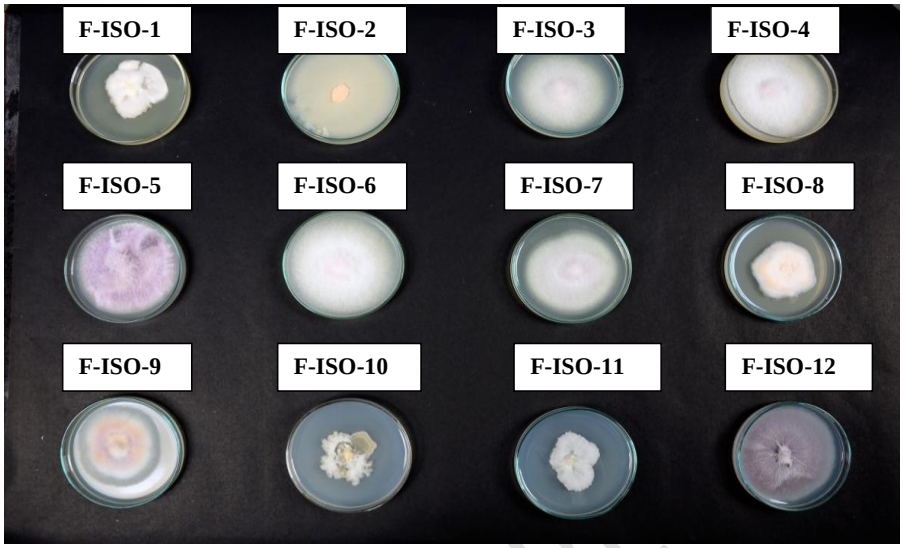
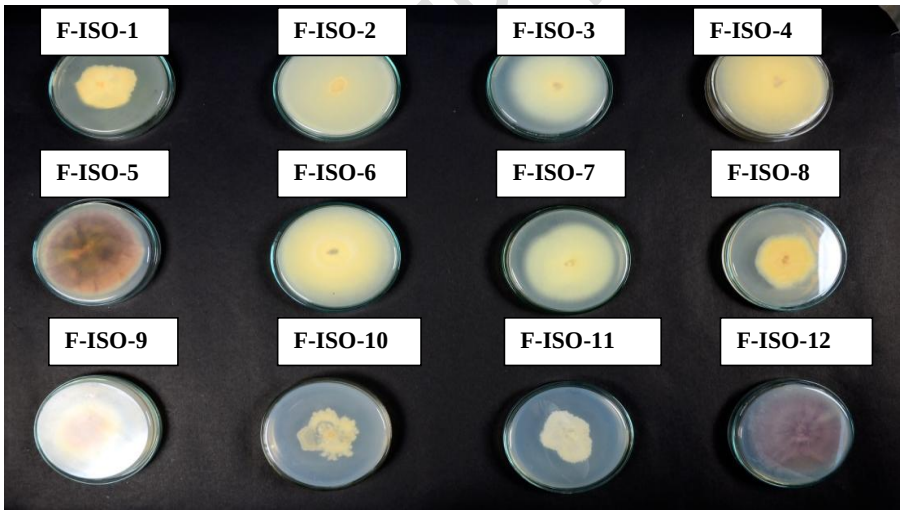



**a) Aerial colony growth**




**b) Colony growth on reverse side of *Petri* plate**

**Plate 4. Growth of isolates *F. verticillioides* on corn meal agar medium**

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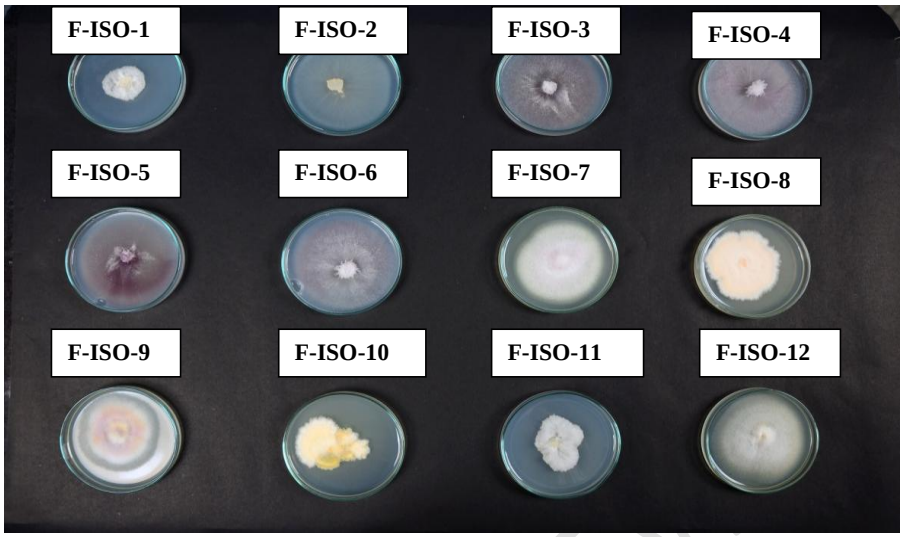
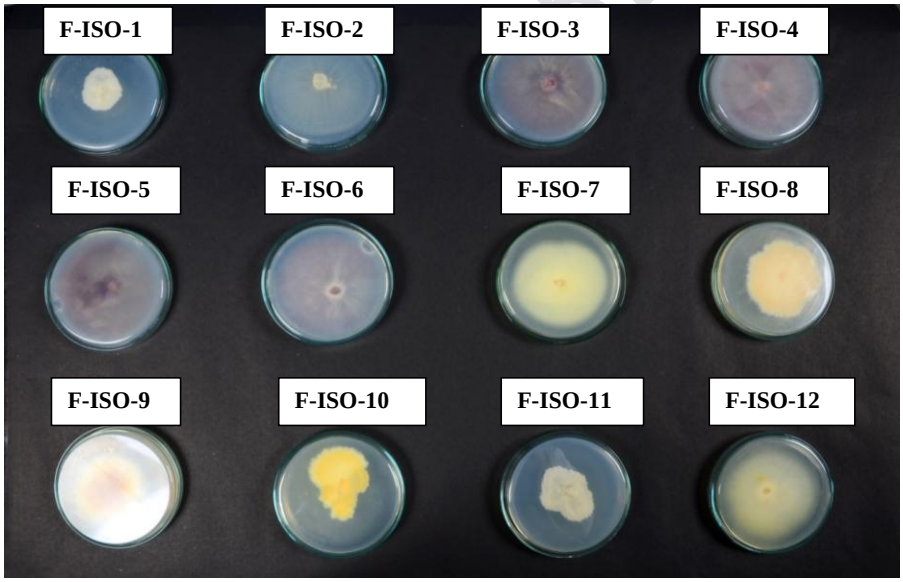



**a) Aerial colony growth**




**(b) Colony growth on reverse side of *Petri* plate**

**Pathogenicity test**

The pathogenicity tests conducted for all 12 isolates by tooth pick method of inoculation in to the second internode of susceptible maize hybrid Kaveri-50 at 50 DAS. The typical PFSR symptoms were observed in inoculated plants. Drying of the lower leaves, lower internodes turned into grey-green color and wilt of entire plant prematurely, and stalks are hollow and weak leading to the lodging of the plant. No such symptoms were observed in controls. All the tested isolates were pathogenic on tested maize cultivar (kaveri- 50). However, the disease severity was varied among the isolates. *Fusarium* isolates F-ISO-7 was highly virulent which caused severe disease upon inoculation with disease score of 8.0 on 1-9 scale followed by F-ISO-1, F-ISO-3, F-ISO-4, F-ISO-5, F-ISO-6 and F-ISO-8. The results of pathogenicity test and disease severity score for each isolate is presented in Table 5. The fungal isolates after inoculation and upon development of characteristic symptoms were consistently re-isolated and their identity was confirmed. Koch's postulates were fulfilled thus confirming the association of stalk rots of Maize. Similar results were reported by Dharanendra Swamy *et al*., (2019) in which PFSR isolates PFSRFv_88 and PFSRFv_118 were highly virulent which caused severe infection upon challenge inoculation with disease score 8. Schoeman *et al*., (2018) reported the virulene of 15 *F. verticillioides* isolates on the maize cultivar CRN 3505 with mean ear rot symptoms not greater than 3 per cent. And no significant differences were found among the isolates at $P<0.05$ and $P<0.1$. Olowe *et al*., (2018) also reported that out of 32 *F. verticillioides* strains screened, 9.4 per cent were classified as highly virulent, 12.5 per cent as virulent, 37.5 per cent as moderately virulent, 21.8 per cent as slightly virulent, and 18.8 per cent as non-virulent.

**Table 5. Virulence of different isolates of *F. verticillioides* on maize plants at MRC – Rajendranagar State, India**

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| S. No. | Place of collection | Isolate | Disease score (1-9 scale) |
|---|---|---|---|
| 1 | Arepally | F-ISO-1 | 5.6 |
| 2 | Oglapur | F-ISO-2 | 5.0 |
| 3 | Rajendranagar | F-ISO-3 | 5.3 |
| 4 | Jammikunta | F-ISO-4 | 7.0 |
| 5 | Veenavanka | F-ISO-5 | 6.3 |
| 6 | Huzurabad | F-ISO-6 | 6.0 |
| 7 | Thimmapur | F-ISO-7 | 8.6 |
| 8 | Gundlapalli | F-ISO-8 | 7.0 |
| 9 | Allipuram | F-ISO-9 | 3.2 |
| 10 | Tanikella | F-ISO-10 | 4.0 |
| 11 | Narasimhapuram | F-ISO-11 | 3.0 |
| 12 | Chintakani | F-ISO-12 | 4.0 |

## CONCLUSION

In the present study cultural, morphological and pathogenic variability of 12 isolates causing wilt of maize in different maize growing areas of Telangana State, India showed that the radial growth of colony diameter was different for different isolates. Cultural and morphological variations among the twelve isolates of *Fusarium verticillioides*, was studied on different solid media i.e., Corn meal agar, Potato dextrose agar and czpek dox agar. Initially the colour of all isolates was white which changed gradually with different pigments like pink, pale yellow, light yellow etc. Among the isolates tested, maximum mycelial growth was recorded by the isolate F-ISO-5 in all the three Medias and mean maximum growth of isolates were observed in CMA.

Although all the isolates produced micro and macro conidia, these isolates varied in size (length and width) of the conidia, septation in macro conidia, colony colour and growth rate. All the isolates exhibited slight variation with respect to cultural and morphological characteristics. All isolates show pathogenic causing wilt disease of maize. These results provide baseline information on morphological, cultural and pathogenic variability of *F. verticillioides* which constitute an important input for further investigation of *Fusarium verticillioides* biology in order to define its evolutionary potential.

**Comment [RV19]:** Paragraph!

## REFERANCES

Comment [RV20]: See Journal Rules for References, this is not standard.


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