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**Bhimani MD**
Department of Plant Pathology, College of Agriculture, JAU, Junagadh, Gujarat, India

**Golakiya BB**
Department of Plant Pathology, College of Agriculture, JAU, Junagadh, Gujarat, India

**Akbari LF**
Department of Plant Pathology, College of Agriculture, JAU, Junagadh, Gujarat, India

**Correspondence**
**Bhimani MD**
Department of Plant Pathology, College of Agriculture, JAU, Junagadh, Gujarat, India

# Characterization of Indian isolates of *Fusarium oxysporum* Schlecht. causing fenugreek wilt

**Bhimani MD, Golakiya BB and Akbari LF**

**Abstract**
*Fusarium oxysporum* schlecht. is a soil borne fungus that causing wilt disease in fenugreek [*Trigonella foenum-graecum* L.]. Fenugreek plant showing typical wilt symptoms were collected from fifteen different locations of Saurashtra regions of Gujarat. Isolation from diseased roots portion of wilted plant were carried out which yielded species of *Fusarium* with different cultural and morphological characters on potato dextrose agar media. Koch's postulates were performed by standard method for all fifteen isolates and they gave different response in form of varied disease incidence. On the basis of cultural, morphological, molecular characteristics and pathogenicity test, the fungus was confirmed as *Fusarium oxysporum* Schlecht. The pathogenic nature of fifteen isolates tested on fenugreek wilt susceptible cultivar Gujarat methi-2, three isolate (Thari, Balagam and Char) were found non pathogenic gave zero per cent disease incidence (PDI), while one isolate (Khadpipali) found highly pathogenic with 93.33 per cent PDI which was further used for molecular identification and screening of agro-chemicals. Study of cultural characters and conidial morphology of different isolates were carried out which showed variation in growth habit, pigmentation, sporulation, shape and size of macro and micro conidia, structure and size of chlamydospores, etc.

**Keywords:** *Fusarium oxysporum* Schlecht., fenugreek, PDI, chlamydospores

## 1. Introduction
Fenugreek (*Trigonella foenum-graecum* L.) is an important seed spice, originated in South-Eastern Europe belonging to the family Fabaceae. Fenugreek is believed to be native to the Mediterranean region (Petropoulos, 2002) [12], but now is grown as a spice in most parts of the world. It is a native of India and leading fenugreek producing country in the world. It is the third largest seed spice in India after coriander and cumin. In India, it is cultivated in about 0.9 lakh hectare and produces 1.15 lakh tone seed (Anon., 2014). Rajasthan produces the lion's share of India's production, accounting for over 80% of the nation's total fenugreek yield. Fenugreek is regarded as the oldest known medicinal plant in recorded history (Lust, 1986). Fenugreek has been referred to as a medicinal herb both in Indian Ayurvedic and traditional Chinese medicines (Tiran, 2003) [17].
There are several factors responsible for low productivity in which disease causes considerable losses. Fenugreek is suffering from various diseases like wilt (*Fusarium oxysporum* Schlecht.), powdery mildew, downey mildew, rust, leaf spot, collar Rot, root rot, stem rot, anthracnose, bacterial blight, root knot nematode and soybean mosaic virus. Fenugreek wilt is caused by *Fusarium oxysporum* Schlecht. *F. oxysporum* is an abundant and active saprophyte in soil and organic matter, with some specific forms that are plant pathogenic (Smith *et al.*, 1988). The fungus can survive either as mycelium, or as any of its three different spore types microconidia, macroconidia, and chlamydospores (Agrios, 2005) [1] and is one of the important diseases resulting in losses both in quantity and quality (Shivpuri and Bansal, 1987) [14]. Typical symptoms of vascular wilt are observed. The foliage droops down and on splitting the roots exhibit brown discolouraion. In case of severe disease incidence pre mature plant death cause heavy yield loss.

## 2. Materials and Methods
### 2.1 Sample collection, Isolation and Purification
Fenugreek plants, naturally infected and showing typical wilt were collected from different locations of Saurashtra regions which includes Gir Somnath, Jamnagar, Porbandar and Junagadh districts of Gujarat. Isolation of the fungus was made by tissue isolation technique.

The resulting fungal culture was purified by hyphal tip method. Purified culture was maintained on PDA slants by storing it under refrigeration (4°C) (Kulkarni, 2006). To maintain the culture for further studies, periodical transfers were made after every three months. The fungus was isolated, purified and sub cultured in aseptic condition with a laminar flow.

The isolates of the pathogen were identified based on colony characters and spores morphology (Booth, 1971) [4]. Micro photographs of the *F. oxysporum* isolates were taken by using imaging microscope to describe spore morphology.

**2.2 Pathogenicity of isolates**

The fifteen isolates were screened for their pathogencity on fenugreek cultivar Gujarat Methi-2 during *rabi* season 2016-17 under net house. The inoculum of each isolates of *Fusarium oxysporum* was prepared on half boiled jowar media and incubated at $25^0$ C for 10 days. These inoculums were used for soil inoculation at 40 g $kg^{-1}$ soil in all the pots (Kala *et al.,* 2016) [7].

For each isolate, three sets of pots (15 cm width x 15 cm depth) were prepared. One set of pot constituting three pots to be filled with sterilized soil only. These pots were considered as uninoculated control. Three test tubes filled with soil were inserted at equidistance and about 6 cm deep in each pot for supplementary inoculation.

Fifteen seeds of fenugreek cultivar Gujarat Methi-2 were sown in each pot. Germination was counted after five DAS. Watering was done as and when required. The plants were observed regularly for the appearance and development of disease symptoms. Secondary inoculation done by adding inoculum prepared on potato dextrose broth. Liquid culture (30 ml/pot) along with piece of mycelial met (2 x $10^7$ cfu/ml) inoculated in hole made by removal of test tubes so that inoculum was directly leached to the root zone.

Inoculation was done in all pots except control. As the symptoms of disease appeared, the fungus was re-isolated from the roots of diseased plant and the re-isolated fungus was brought to pure culture, which was later compared with the original one. The per cent wilt incidence was calculated by following formula.

$$\text{Per cent Disease Incidence} = \frac{\text{Total number of wilted plants per pot}}{\text{Total number of plants per pot}} \text{x}100$$

**2.3 Cultural, morphological and molecular characters of different isolates of *Fusarium oxysporum* of fenugreek wilt**

**2.3.1 Cultural and morphological studies**

All fifteen isolates of *F. oxysporum* were separately grown on PDA in Petri plates and incubated at 28 ± 2ºC for seven days. Observations on cultural characters *viz.,* colony colour & type, growth and pigmentation were recorded a week after inoculation. Observations on cultural characters *viz.,* colony colour and type, growth and pigmentation were recorded a week after inoculation. Morphological characters of spores of different isolates were studied by observing in cotton blue stained slides under imaging microscope. Measurements of macro-micro conidia and chlamydospores were made with the help of imaging microscope which shows size of conidia and diameter of chlamydospores. Sporulation was recorded by microscopic examinations using following scale given by Tuite (1969) [18].

| | | |
|---|---|---|
| - | = | Absent, |
| + | = | Scanty (1-10 spore/MF), |
| ++ | = | Poor (11-20 spores/MF), |
| +++ | = | Good (21-30 spores/MF), |
| ++++ | = | Abundant (>30 spores/MF), |

Where, MF denotes Microscopic field.

**2.3.2 Molecular characterization**

**Fungal DNA isolation and sequencing**

The fungal genomic DNA was extracted from mycelia grown in 250 ml of PDB at 28 °C for 5 days. The mycelia were harvested from broth and lyophilised and stored at -20 °C for further process. The genomic DNA for PCR was extracted by using HiMedia fungi DNA isolation kit. The ITS region of fungi, including ITS2 (5'-GCTGCGTTCTTCATCGATGC-3'), ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were amplified. The amplification was performed in 30 µl reaction volume with 0.1 mM of each dNTP and 100pmol of both forward and reverse primer. Veriti PCR (Thermo fisher) was programmed for initial denaturation at 94 °C for 4 min, and 35 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. The amplification was completed with a final extension at 72°C for 5 min. Further it was sequenced by ABI 3130 capillary sequencing.

After sequencing identification of fungal sequences were analysed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

**3. Results and Discussion**

**3.1 The Pathogen**

**3.1.1 Isolation and purification of pathogen**

The wilt affected fenugreek plants were identified in the field based on key symptoms like withering, yellowing of leaves and drying of plants. Roots of wilt infected plants when split open vertically showed brown discolouration of the xylem vessels. The fungus was isolated from infected roots of fenugreek plants under aseptic conditions using tissue segment method on PDA. The fungus was further purified by single hyphal tip method on PDA. Pure culture was depicted in fig-I.

Similar methodology was followed by Nikam *et al.* (2011) [10] collected wilt infected fenugreek plant samples from different locations and isolated *Fusarium oxysporum* on potato dextrose agar (PDA) in the laboratory.

**Fig 1:** Pure culture of *Fusarium oxysporum*

### 3.1.2 Pathogenicity of Isoaltes

Pathogenicity of fifteen isolates *Fusarium oxysporum* Schlecht were tested on fenugreek cultivar Gujarat methi-2 by “Soil inoculation method” as described under “Materials and Methods”. Pathogenicity test indicated that these isolates varied in the percentage of infection.

Among the all isolates highest disease incidence was recorded in Khadpipali isolate with 93.33 per cent disease incidence, in KGF-1 isolate and Vakiya isolate PDI were 80 per cent. Toraniya isolate showed 73.33 per cent PDI followed by EF-1 isolate (66.66 per cent PDI). Nandarkhi isolate found least pathogenic showed only 26.66 per cent disease intensity while, Thari, Balagam and Char isolates were found non pathogenic (Table 1).

**Table 1:** Variation in wilt incidence among different isolates of *Fusarium oxypsorum* wilt in fenugreek

| S. No | Isolates | Total plants/pot# | Total Wilted plant/s# | Per cent Disease Incidence# |
|---|---|---|---|---|
| 1 | Nandarkhi | 15 | 4 | 26.66 |
| 2 | Madhupur | 15 | 7 | 46.46 |
| 3 | Thari | 15 | 0 | 0 |
| 4 | Khadpipali | 15 | 14 | 93.33 |
| 5 | E.F.* | 15 | 10 | 66.66 |
| 6 | Toraniya | 15 | 11 | 73.33 |
| 7 | Parbadi | 15 | 6 | 40.00 |
| 8 | Balagam | 15 | 0 | 0 |
| 9 | Choki | 15 | 5 | 33.33 |
| 10 | K.G.F.* | 15 | 12 | 80.00 |
| 11 | V.R.F.* | 15 | 8 | 53.33 |
| 12 | Char | 15 | 0 | 0 |
| 13 | Vakiya | 15 | 12 | 80.00 |
| 14 | Dhrol | 15 | 7 | 46.46 |
| 15 | Vagudal | 15 | 9 | 60.00 |

#mean of three replications (pots),

*- E.F. - Engineering Farm J.A.U., K.G.F. - KrushiGadh Farm J.A.U., V.R.F. -Vegetable Research Farm J.A.U.

This result indicates that the isolates isolated from the infected roots may or may not be pathogenic. Hence once the isolates received in pure culture requires was tested further for their pathogenicity so the pathogenic culture to be used for remaining laboratory and pot trials.

Our results were in agreement with that of Hashmi (1988) [5] who recorded pathogenic nature of *Fusarium oxysporum* in fenugreek which also affect seed germination, vigour index, plant growth and the grain yield.

### 3.1.3 Identification of pathogen

#### 3.1.3.1 Cultural characteristics of different isolates

Observations on cultural characters of *Fusarium oxysporum* Schlecht. *viz.,* colony color, growth, pigmentation and sporulation were recorded a week after inoculation and presented in Table 2 and fig. II.

**Table 2:** The colony characters, growth habit and sporulation of *Fusarium oxysporum* causing wilt in fenugreek

| S. No | Isolates | Mycelial arrangement and color | Pigmentation | Growth habit | Sporulation* |
|---|---|---|---|---|---|
| 1 | Nandarkhi | Sparse Cottony white | Pale yellow | Slow | + |
| 2 | Madhupur | Sparse Dirty white | Brown | Moderate | +++ |
| 3 | Thari | Dense Cottony white | Light Brown | Fast | ++ |
| 4 | Khadpipali | Dense Dirty white | Pale yellow | Moderate | ++++ |
| 5 | EF-1 | Sparse Dirty white | Light Brown | Fast | ++++ |
| 6 | Toraniya | Dense Cottony white | Pale yellow | Moderate | +++ |
| 7 | Parbadi | Sparse Dirty white | Brown | Fast | +++ |
| 8 | Balagam | Dense Cottony white | Light Brown | Moderate | ++ |
| 9 | Choki | Sparse Cottony white | Pale yellow | Slow | + |
| 10 | KGF-1 | Dense Dirty white | Light Brown | Fast | +++ |
| 11 | VRF-1 | Dense Cottony white | Pale yellow | Moderate | +++ |
| 12 | Char | Dense Cottony white | Brown | Fast | ++ |
| 13 | Vakiya | Sparse Cottony white | Pale yellow | Slow | ++++ |
| 14 | Dhrol | Dense Cottony white | Light Brown | Moderate | +++ |
| 15 | Vagudal | Sparse Dirty white | Light Brown | Moderate | ++ |

* + Poor, ++ Moderate, +++ Profuse, ++++ Abundant

The cultural characteristics of 15 isolates of *Fusarium oxysporum* Schlecht. revealed that isolates differed in colony type and growth habit, pigmentation and sporulation. The isolates were differed in their mycelial arrangement and growth habit. On the basis of the mycelium growth pattern, the isolates could be categorized into two groups’ i.e. sparse growth and dense growth. Most of the isolates had dense or sparse growth with smooth margin, while dense growth with irregular margin was present in Thari isolate and VRF-1 isolate. Sparse growth with irregular margin was observed in Nandarkhi isolate and Parbadi isolate. (Fig. II)

Typical pale yellow pigmentation was observed in most of the isolates even after one month of incubation, whereas three isolates *viz*., Madhupur, Parbadi and Char isolates had brown pigmentation, while Thari, EF-1, Balagam, KGF-1**,** Dhrol and Vagudal isolates showed light brown pigmentation.

Madhupur, Khadpipali, EF-1, Toraniya, Parbadi, KGF-1, VRF-1 and Vakiya isolates showed good sporulation. Five isolates showed moderate sporulation were Thari, Balagam, Char, Vagudal and Dhrol isolates. Poor sporulation was observed in Nandarkhi isolate and Choki isolate.

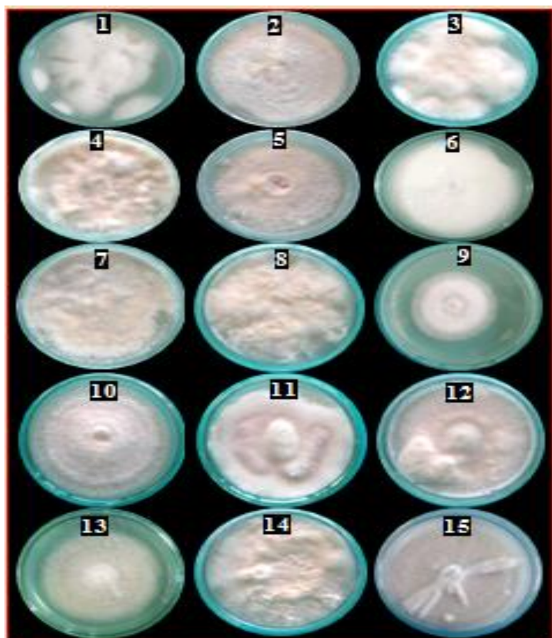


**Fig 2:** Growth characters of *Fusarium oxysporum* Schlecht. isolate on PDA (Isolates 1-15)

Based on the growth habit, the isolates were categorized into 3 groups *viz.,* fast growing, moderate growing and slow growing. Five isolates: Thari, EF-1, Parbadi, KGF-1 and Char isolates showed fast growth habit, while three isolates: Nandarkhi, Choki and Vakiya showed slow growth habit and the remaining seven isolates have moderate in growth habit.
In the present investigation, common characters for the highly virulent isolates were sparse to dense mycelial growth, mostly pale yellow pigmentation, moderate to fast growth habit and abundant sporulation.

Saxena and Singh (1987) [13] reported that *F. oxysporum* f. sp. *ciceris* is septate, profusely branched growing on potato sucrose/dextrose agar at 25˚C, initially white turning light buff or deep brown later, fluffy or submerged. The growth becomes felted or wrinkled in old cultures. Various types of pigmentation (yellow, brown, crimson) can be observed in culture.
Sonkar *et al.* (2014) [16] studied cultural and morphological characters of *Fusarium oxysporum* f. sp. *lycopersici*. On the basis of the mycelial growth pattern, Most of the isolates showed fluffy growth. Sporulation of the macroconidia, microconidia and varied highly among the isolates.

#### 3.1.3.2 Morphological characteristics of different isolates

The fungus *Fusarium oxysporum* wilt of fenugreek produce two types of conidia *viz*., microconidia (small in size) and macroconidia (large in size). The conidial width and length of 15 isolates were measured and presented in Table 3 and depicted in fig III-A.
Microscopic observation revealed that the microconidia (fig. III-A) in all isolates were small, one to two celled, hyaline with oval to reniform and oval to oblong with slightly curved shape. Its length ranged from 5.97 to 12.26 µm, while width ranged from 2.32 to 5.85 µm. The measurement of microconidia varied considerably.
Macroconidia (fig. III-B) in all these isolates were long, variable in size and shape, somewhat of uniform width except at the end, curved toward the end where they were narrow, blunt and smoothly rounded or pointed at the tip, mostly 2-3 septate and hyaline in colour. Its length ranged from 12.14 to 27.10 µm, while the width ranged from 2.78 to 7.25 µm. Toraniya, Parbadi, VRF-1, Char, and Vakiya isolates produced comparatively larger macroconidia (27.10 - 21.87 x 2.32 - 4.92µm).

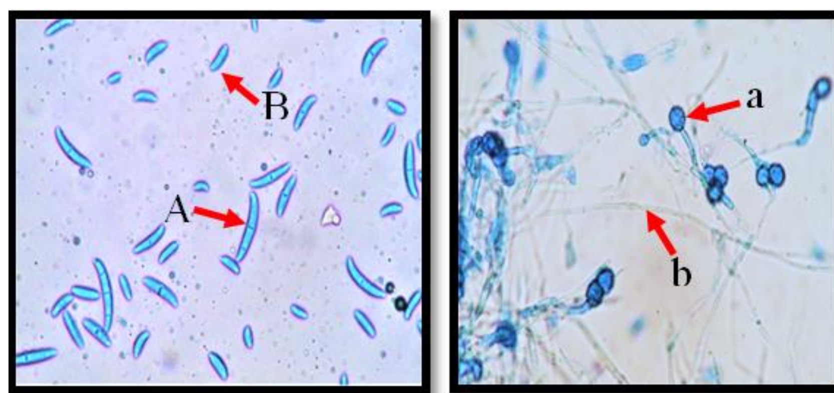


**Fig: III-A:** A) Microconidia, B) Macroconodia Fig: III-B: a) Chlamydospore, b) Mycelium

In old culture, chlamydospores were formed, which were rough or smooth walled, intercalary or terminal and may be formed singly, in chains or pairs (Plate 3-b). Variation among diameter of chlamydospore is presented in table 3. Chlamydospore of Nandarkhi isolate was found large in size measuring 12.64µm diameter while Chittal isolate having comparatively small (07.35 µm) chlamydospore. Thari, Balagam and Char isolates were not found chlamydospore.

**Table 3:** The measurement of macroconidia, microconidia and chlamydospore of *Fusarium oxysporum* causing wilt in fenugreek

| S. No | Isolates | Microconidia* | | Macroconidia* | | Chlamydospores* |
|---|---|---|---|---|---|---|
| | | Length (µm) | Width (µm) | Length (µm) | Width (µm) | Diameter (µm) |
| 1 | Nandarkhi | 8.69 | 2.43 | 14.31 | 3.78 | 12.64 |
| 2 | Madhupur | 7.13 | 3.55 | 13.60 | 2.95 | 9.81 |
| 3 | Thari | 5.97 | 3.50 | 18.56 | 4.25 | 11.91 |
| 4 | Khadpipali | 10.25 | 4.75 | 18.84 | 5.23 | 11.19 |
| 5 | EF-1 | 8.89 | 2.49 | 14.88 | 2.78 | 11.11 |
| 6 | Toraniya | 10.29 | 2.32 | 22.51 | 3.46 | 7.35 |
| 7 | Parbadi | 8.90 | 4.92 | 24.17 | 6.15 | 9.39 |

| 8 | Balagam | 8.85 | 5.27 | 12.14 | 6.40 | 10.00 |
|---|---|---|---|---|---|---|
| 9 | Choki | 11.36 | 4.10 | 19.43 | 5.86 | Absent |
| 10 | KGF-1 | 7.88 | 5.85 | 19.97 | 7.25 | 9.05 |
| 11 | VRF-1 | 11.68 | 3.55 | 24.55 | 5.25 | 9.78 |
| 12 | Char | 12.26 | 2.87 | 21.87 | 4.76 | 10.79 |
| 13 | Vakiya | 11.60 | 3.43 | 27.10 | 4.78 | 12.05 |
| 14 | Dhrol | 8.40 | 2.90 | 13.59 | 3.78 | 9.35 |
| 15 | Vagudal | 10.68 | 3.18 | 17.52 | 4.24 | Absent |

*mean of 10 spores from 2 microscopic fields

The comparison between size and septation in micro and macro conidia of pathogenic and non-pathogenic did not gave clear picture; hence it is clearly observed in the present study that conidial measurement has no relevance with its virulence. In two isolates namely choki and vagudal chlamydospore were not found. This has been supported by following findings by Anjaneyareddy and Muhammad, (2006) [2] reported that the size of macroconidia varied from 13.03 x 3.6 µm (Bangalore isolate) to 20.6 x 2.1 µm (ICRISAT isolate), while the size of microconidia varied between 5.26 x 1.78 µm (ICRISAT isolate) to 9.09 x 1.94 µm (Gulbarga isolate) in *Fusarium udum*.

Sonkar *et al.* (2014) [16] reported that the length × breadth of the macroconidia varied between 15-37.5µ x 2.5-4µ and that of the microconidia was around 2.5-15µ x 2-3µ among the isolates in *Fusarium oxysporum* f. sp. *lycopersici*. Patra and Biswas (2016) noticed the size of macroconidia was ranged from 13-15 x 2-3 µm to 15-19 x 3-4 µm, in microconidia was from 3-4 x 1-2 µm to 5-6 x 2-3 µm.

#### 3.1.3.3 Molecular identification of pathogen

Among fifteen isolates Khadpipali isolate, KGF-1 isolate and Vakiya isolate were selected for molecular identification based on their virulence proved during pathogenicity test. Sequencing was done by following procedure as described in section 3.4.1. At the end of the procedure sequence was found for both of the isolates which was BLAST online in NCBI data base and concluded that the pathogen associated with wilt of fenugreek was *Fusarium oxysporum* (Table 4). Khadpipali isolate was further utilized for evaluate agro chemicals *in vitro* as well as in pots.

**Table 4:** Sequence data of three isolates of pathogen

| Isolates | Sequence (Accession No.) | Identification |
|---|---|---|
| Khadpipali | GCCCTTCTTTTGTGACAGTCAGAGGGATCATTACCGAGTTATACAACTCATCAACCCTGTGAACATA<br>CCTATAACGTTGCCTCGGCGGGAACAGACGGCCCCGTAACACGGGCCGCCCCCGCCAGAGGACCCCC<br>TAACTCTGTTTCTATAATGTTTCTTCTGAGTAAACAAGCAAATAAATTAAAACTTTCAACAACGGATC<br>TCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGA<br>GAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTC<br>ATTACAACCCTCAGGCCCCCGGGCCTGGCGTTGGGGATCGGCCACATCTGGTGCTTACACCACTCCCC<br>CCCCTTTATAGGTGGCCCGCCGGACTCAATTTCGCGTTGAATAGTAACATCACCCCACAGATGGGGGG<br>GGGGGCGCTGGCCGTTAAAAAAAACCCAACTTATGCAGGAAGACGGCCAATTCCGTTAGGAATACCC<br>ACTTACGTTTTAGTTTTTTTCAAGGCGGGAGGCCAAAAAAAAACCAACGGGGGGGAGGGTCCCCTTT<br>CTGGTGGGGTGGGGGGGGGGCCCCCGGCACACCTTTTTTAAAACCGGGGTTTCGGGGGGCCCCAGCC<br>TGGGGAAGTTGGTGGGAGGCGAGGGTTTTTGGGGGGCGGGGTGGGGTTCCCCGAGCGAGGCCGGGG<br>GATGGTGAGGGACCGTTTCAACGGGGGGTTGGATCGCCCGGTGCCGGGGAATGGGCAGACCAGAGG<br>ACCTCCTGTCCGGACGGGGGTCTCTCTCCGAAACGGAGAGGAAACGGTTTGTGTTTTGTGGAGCATTT<br>GGTTAGTCTTTCTCACTCTAAAA ( KU671029.1) | *Fusarium oxysporum* strain (99% identity) |
| KGF-1 | GAATCTTTGGATGAAGTCGAGAGGGATCATTACCGAGTTATACAACTCATCAACCCTGTGAACATACC<br>TATAACGTTGCCTCGGCGGGAACAGACGGCCCCGTAACACGGGCCGCCCCCGCCAGAGGACCCCCTA<br>ACTCTGTTTCTATAATGTTTCTTCTGAGTAAACAAGCAAATAAATTAAAACTTTCAACAACGGATCTC<br>TTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAAAATTCAGTGA<br>ATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGAATGCCTGTTCAAGCGTCAT<br>TACAACCCTCAGGCCCCCGGGCCTGGCGTTGGGGATCGGCGTCTTTTTGACTGCGCAACAACCCCCCT<br>CCCCAAAATACGGGGGCCCCCCCCGACGCATCTTCGATTGAAAATTAACTCACCCCAACAGAACGGG<br>GGGGGGGCCCGGCCCCAAAAAAAACCCCACTTTCTGAAGGTGTATTCTCCAAACTCAGGTTCGGAAA<br>AACCAGAGTGTGGATTTTTGTTTTTTTATCAATAGGCGGGGGAGGCCACGAAAGACCCCAAGGGGGG<br>TTGGGGCCCCCGTTTTCGGTTGAGTATGAAATAGCGCCCGCACAGCAAACAAATTTTTTGAAAACACG<br>GGGATTTTCGGGGGGGCCCCCAGCCCTGGGAAGTTTTGGGAGGAACGCAGAGGGTTTTTTTTGGGGG<br>GGCAGGGGTGGGGGTTTCCCCGAGGCGTTAGCGCTGGGGGGAATGGGTGAAGGGGTCCCGTTTTAAA<br>GAGCGTATGAAAACGCCCCGGTTCTTGGAGATAGGTGAGCGAACCCAGAGGATCTCTCCCGTGTCGA<br>GACGGGGGTTCCCTCTATCCCAAACCGGGAGGATCAGCCGCGTTTTGTGGTTTTTGGGGGGACATATG<br>TTGTGTATGCACTCATTCCTCACCAATCACTCGA (KU097280.1) | *Fusarium oxysporum* strain (99% identity) |
| Vakiya | CAACGCATTGAGACAGTCGAGAGGGATCATTACCGAGTTATACAACTCATCAACCCTGTGAACATAC<br>CTATAACGTTGCCTCGGCGGGAACAGACGGCCCCGTAACACGGGCCGCCCCCGCCAGAGGACCCCCT<br>AACTCTGTTTCTATAATGTTTCTTCTGAGTAAACAAGCAAATAAATTAAAACTTTCAACAACGGATCT<br>CTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG<br>AATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCA<br>TTACAACCCTCAGGCCCCCGGGCCTGGCGTTGGGGATCGGCGGAAGCCCCCTGCGGGCACAACGCCG<br>TCCCCCAAATACAGTGGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAGCTAACACCTCGCAACTGGAG<br>AGCGGCGCGGCCACGCCGTAAAACACCCAACTTGTGAATGTTGACCTCGAATCAGGTAGGAATACCC<br>GCTGATTTTAAGCATATCAATAAGCCCACGAACAGAAACCGACAGGGATTGCCCCAGTAACGGAAGA<br>GGGGAGGGGGCAACAGCTCAAATTTGAAATCTGGGTCTCGGTCCCGAGTTGTAAGATGGTGGATTAT<br>GGTGTAGTGTGATTCTCGCCGTCGGAGGGGTCGGGGAGGGCCGTTCACGGGGGAGGGGCGCAGATCT | *Fusarium* spp. strain (97% identity) |

| | | |
|---|---|---|
| | GTATGGACGATCGAATACCCCTGCCGAGGGTCTTTCCAACGGGACAAAGTTTGTTTGTGGAATTGCAG ACTTCCACCCTC (EU750688.1) | |

Hussain *et al.* (2012) [6] isolated the wilt causing fungi from roots and stems of recently wilted guava plants. Among 16 isolates eleven isolates were confirmed as *F. oxysporum* through polymerase chain reaction (PCR) using species specific primers designed from the conserved regions of 18S rRNA gene.

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