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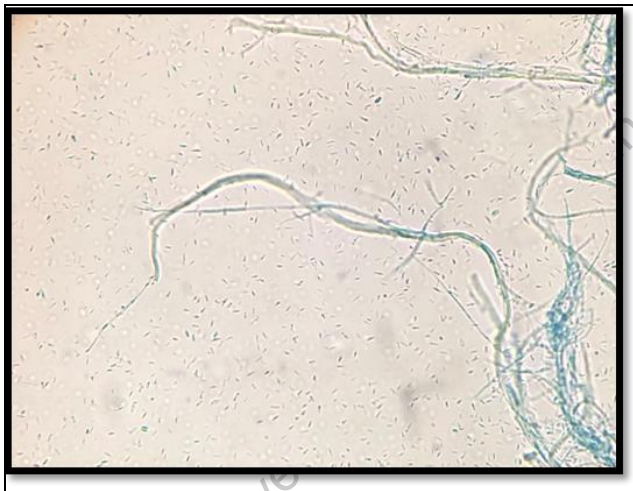
|  |  |
| **Fig. 1: Macro- and microconidia of *Fusarium verticillioides*** | **Fig. 2: *Fusarium verticillioides* on *Tetranychus urticae*** |

# New record of *Fusarium verticillioides* as acaropathogenic fungi on two spotted red spider mite (*Tetranychus urticae* Koch) from South Gujarat



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# Abstract

"The nymphs and adults of *Tetranychus urticae* Koch (two spotted red spider mite) were collected from Okra fields, infected with *Fusarium verticillioides* (Sac.) Niren. The symptoms, developed on mite's body, as white cottony mass. The fungus was isolated and pure cultures were raised on PDA. Single-celled, oval micro-conidia on monophialides and sickle-shaped macro-conidia having 3-5 septa were found developing in culture. The pathogenic nature of *F. verticillioides* has been tested on healthy individuals of *T. urticae* and confirmed. *F. verticillioides* has been recorded for the first time as an acaropathogen on two spotted red spider mites in the South Gujarat region of India."



**Key words:** Acaropathogen, *Fusarium verticillioides*, *Tetranychus urticae*, Two spotted red spider mite,




**Introduction**

Two spotted red spider mite, *Tetranychus urticae* Koch, is one of the most imperative polyphagous species of the family Tetranychidae, attacking several agri-horticultural crops and causing economic loss. Moderate population may significantly affect crop production and heavy infestation results in death of the plants [1]. Spider mites puncture the epidermal layer and suck the oozing sap, and they damage the internal tissues surrounding the area punctured by their chelicerae [2]. The two spotted red spider mite remains active throughout the year under poly house as well as in open field condition causing 36.8 to 83.2 per cent yield loss in Okra[3]. It causes serious damage in various crops like 10 to 15 per cent in rice, 15 to 20 per cent in tea, 10 to 25 per cent in sugarcane and 13 to 31, 20 to 25 and 27 to 39 per cent losses in brinjal, okra and chilli respectively [4].

Most of the species of *Fusarium* are saprophytic and are relatively abundant as soil microbiota [5], however, several *Fusarium* species are pathogenic on plants, insects and humans too [6]. More than 13 species are pathogenic on insects and have host range on the members of Coleoptera, Lepidoptera, Hymenoptera, Diptera and Hemiptera [7,8]. In South

Gujarat, only few of the farmers utilize biological means of management for mites in different crops, and therefore, there is an apt need to identify the native strains of newer acaropathogenic organisms having ability to control these mites. During the present investigations, studies have been made to identify effective means of biological control of two spotted red spider mites on Okra crops in South Gujarat.



**Materials and Methods**

The mite infested okra fields were frequently visited during the month of June-July, 2016 and the infested okra plants were minutely observed to collect diseased mites. The samples were immediately taken to the laboratory at N. M. College of Agriculture and ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari (Gujarat) where the infected mites with leaf were kept in sterile Petri dish in a rearing room under controlled conditions (25±2ºC temperature and 70-80% relative humidity).

The infected mites were surface sterilized in 0.5% sodium hypochlorite solution for 10 seconds and washed with sterile distilled water and were inoculated on sterile solidified PDA (200 g Potato + 20 g Dextrose + 30 g Agar per litre) in disposable Petri plates (90 mm in diameter), sealed with parafilm and incubated in BOD incubator (at 27ºC temperature).

**Pathogenicity test**

Fungal mycelial disc of five millimetres from 10 days old pure culture was inoculated in 100 ml of Potato Dextrose Broth (200g potato + 20g dextrose per litre) supplemented with chloramphenicol (0.5gm) at room temperature for fungal growth. The mycelial mat, developed after 18 to 21 days, was crushed with liquid broth and filtered through double layered muslin cloth. The number of spores was counted with the help of Neubauer's haemocytometer.

Healthy mites were collected with fresh leaves from the field and placed it in sterilized Petri plate with wet cotton swab. Two millilitre liquid suspension was sprayed containing $2\times10^8$ cfu/ml on healthy mites with the help of Potter tower and calibrated at 10

psi according to IOBC/WPRS Methodology [9] that corresponded to an average deposition of 2.5 mg/cm$^2$ suspension droplets in Petri dish (150mm diameter x 22mm depth) and kept under controlled condition (25-27ºC temperature and 70-80% relative humidity).

**Molecular identification of fungi**

Total fungal genomic DNA was extracted and its quality was evaluated on 1.0% Agarose gel, a single band of high-molecular weight DNA has been observed. Fragment of ITS gene was amplified by PCR. A single discrete PCR amplicon band was observed when resolved on Agarose gel. The PCR amplicon was purified by column purification to remove contaminants. DNA sequencing reaction of PCR amplicon was carried out with ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') primer using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.

The ITS sequence was used to carry out BLAST with the database of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software programs. DNA sequence homology searches were performed using the online BLAST search engine in GenBank (available at: www.ncbi.nlm.nih.gov). Phylogenetic analysis was done using MEGA-7.

**Results and Discussion**

The isolated fungus was morphologically identified as *F. verticillioides* having prolific asexual spores - ~~as~~ microconidia and macroconidia with 3-5 septa (Fig. 1). Infected mites showed white cottony appearance that covered the entire body (Fig. 2).

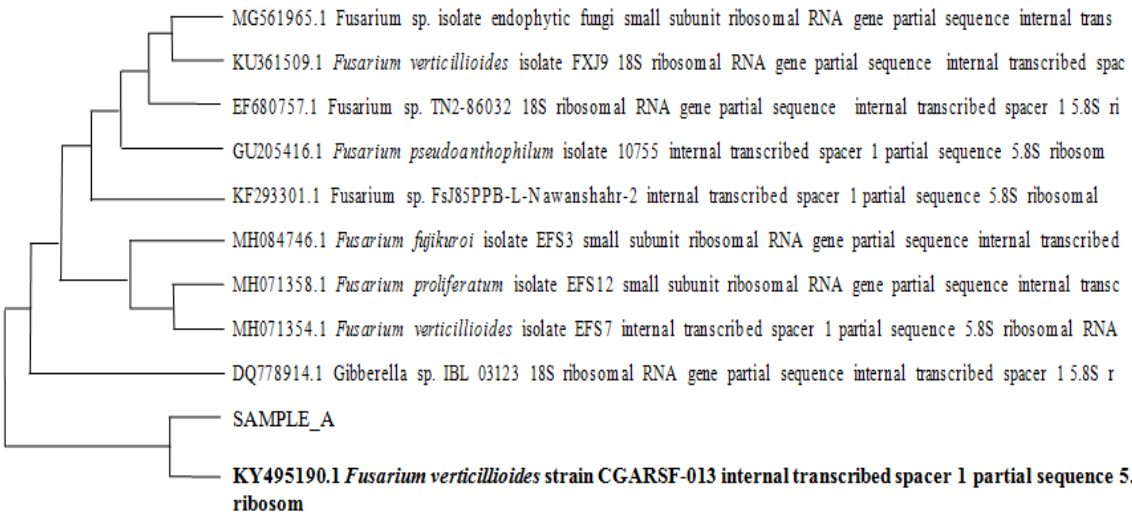
The evolutionary history was inferred using the Neighbour joining method [10]. The original tree with the sum of branch length = 0.01185892 is shown. The confidence probability (multiplied by 100) that the interior branch length ID greater than 0, as estimated using bootstrap test (500 replicate is shown next to the branches [11, 12]. The evolutionary distances were computed using the maximum composite like-hood method [13] and are in the unit of number of base substitutions per site. The analysis involved 11 nucleotide sequences.

Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions with less than 95 % site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data and ambiguous bases were allowed at any position. There were a total 471 positions (Fig. 3) in the final dataset and the phylogenetic tree (Fig. 4). Evolutionary analyses were conducted in MEGA7 [14].

**Fig. 3: Total of 471 bases sequenced**

ATGCACATACCAATTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTG
TTTCCATGTGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA
ACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGT
ATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCAGCTTGGTGTTGGGACTCGCGAGTCAAATCGCGTTC
CCCAAATCGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCG
TTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAGGCGCGGAGGAG
AGGGATCATTAACGAAGTTTACAACTCCAAACCTGGGAAACATACCCAAATGGTTCCTGCGGGGAAAAAACGCTCCGGTAA
AACGGGACCGCCGCGAAGGACCTAAACTCTGTTTTCATGTGTAACTTCTGAGTAAAAACATAAATAAATCAAAACTTTCACA
ACGGATCTCTTGGTTCTGGCATCATGAAAACGCAGCAAATGCGATAAGTAATGTGAATTGCAGAATTCGTGAATCATCAATC
TTTTGAACGCACATTTGCGCCGCCGTATTCTGGGCGGGCATGC

**Fig. 4: Phylogenetic Tree**




Following the pathogenicity experiment, all the mites were dead after eight days and were found to be covered with the white cottony fluffy growth of *F. verticillioides* and its pathogenic nature has been confirmed. It has been reported that *F. verticillioides* also act as entomopathogenic on grasshoppers, (*Tropidacris collaris* Stoll) in Argentina[15] as well as on Short-horned grasshopper (*Oxya hyla intricate* Hollis) in Philippines. [16] *F. verticillioides* also act as plant pathogen causing disease in Sweet corn at Athens [17], in Maize it shows seedling decay, stalk rot, ear rot and mycotoxin contamination at Ames [18]. Post flowering

stalk rot has also been reported in corn at Rajasthan, India [19]. It is also reported as pathogenic to sugarcane, which showed discoloration from red to brown in sugarcane stalks at Iran [20]. It also causes stalk and root rot of sorghum in Spain [21]; contamination of some cereal grains *viz*. maize, sorghum, paddy and wheat with *F. verticillioides* was reported at Karnataka, India [22]. While in case of fruit crop banana was found to be infected with rotten fruit at Mexico [23] and banana neck rot in Hungary [24], whereas, in case of pineapple it causes fruit rot and leaf spot disease at Malaysia[25]. Similarly, at Malaysia this phytopathogen causes fruit rot disease in Banana, Papaya and Guava [26].

The literature indicates that *F. verticillioides* is recorded for the first time as an acaropathogen on two spotted red spider mite (*T. urticae*) in the South Gujarat region of India. There is need to have further research work on many applied aspects including testing the efficacy of the *F. verticillioides* on different mites infesting various crops, testing this native isolate as an effective biological control tool on mites.



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**Figures:**

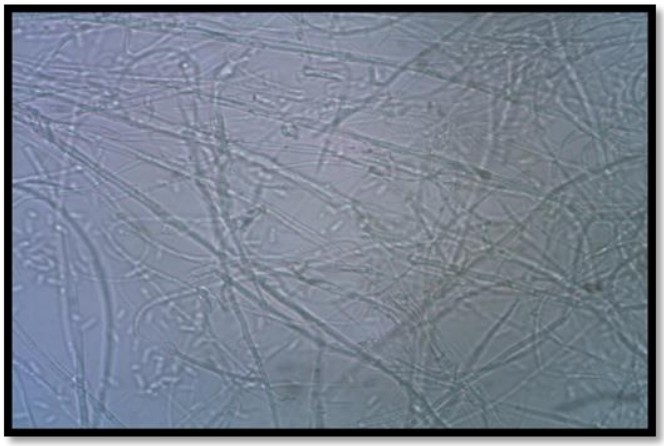

**Fig. 1: Macro- and microconidia of *Fusarium urticae***

**Fig. 2: *Fusarium verticillioides* on *Tetranychus verticillioides***