experiments were conducted in isochoric and unstirred conditions. They are user-friendly and easily adaptable for scale-up. Figures 1 *a* and 2 *a* show gas uptake during hydrate formation in sI and sII respectively. Insets in these figures depict the pressure–temperature (*p*–*T*) trajectory during freezing (black) and thawing (red) cycles, along with computed equilibrium (brown) curves. At ~5.0 MPa pressure, hydrate nucleation occurs around ~274.5 ± 1.9 and 287.8 ± 1.4 K in the sI and sII systems respectively. As shown in the shaded portion, the time taken for 90% of gas uptake in the hydrate is around ~23 ± 2.4 and 62 ± 8.1 min respectively. A small dose (0.5 wt%) of amino acid is added to water to accelerate hydrate formation in sI. Similarly, a stoichiometric amount of tetrahydrofuran is added to form sII hydrates.

The next step is to assess the stability near ambient pressures. Some hydrates are in the metastable state outside their thermodynamic stability limits. For example, methane hydrates retain their stability below the ice-melting point at lower pressures. This state is popularly known as the self-preservation window. The hydrates can be stable for a longer duration at near-ambient pressure and sub-zero temperature conditions[3,4]. The hydrate dissociation rate in this window is vital for designing the storage vessel and transportation distance. The stability of sI and sII hydrates was monitored for 100 and 45 h at constant temperatures of ~270 and 283 K respectively, Figures 1 *b* and 2 *b* depict the results. The overall pressure increase (triggered by the hydrate dissociation) is about 950 kPa in sI hydrates, while it is significantly less in sII hydrates. Thus the gas in SNG form can easily be transferred for about 4000 km without measurable loss. Further, it can be stored in lighter insulated tanks as the required temperature is not very low.

Finally, gas is recovered by dissociating the hydrate at the dispensing location. The hydrates can be dissociated quickly by exposing the storage/crystallizer tank to ambient temperature. As shown in Figures 1 *b* and 2 *b*, typically, the temperature rises at 3°–5° per hour, and the hydrates dissociate rapidly.

The *p*–*T* trajectory of Figure 1 *b* (shown as (ii)) exemplifies the self-preservation of sI hydrates. A sudden surge in pressure is observed around the ice-melting temperature region. Although the precise reason for this phenomenon is unknown, a general view is that an ice layer around the hydrate grains prevents rapid dissociation. However, no such effect is seen in sII hydrates (Figure 2 *b*(ii)), and hydrate dissociation is along the thermodynamic phase boundary.

In summary, the present study demonstrates HBT for gas transport. Rapid and efficient hydrate conversion in sI offers a distinct alternative to conventional CNG or PNG modes using low-dose amino acids. More extensive storage (~143 v/v) capacity in SNG has a clear advantage in the gas supply chain for the power or fertilizer industry. On the other hand, the requirement for city-gas supply can easily be fulfiled by adopting sII-type SNG.

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Fusarium verticillioides as an acaropathogenic fungus on two-spotted red spider mite (*Tetranychus urticae* Koch) from South Gujarat, India

Two-spotted red spider mite, *Tetranychus urticae* Koch, is an important polyphagous species of family Tetranychidae, attacking several agri-horticultural crops and causing economic loss. A moderate population of the spider mite may significantly affect crop production, while heavy infestation will result in the death of plants[1]. Spider mites puncture the epidermal layer and suck the oozing sap; they damage the internal tissues surrounding the area punctured using their chelicerae[2]. The two-spotted red spider mite remains active throughout the year under polyhouse as well as in open field conditions, causing 36.8–83.2% yield loss in okra[3]. It causes serious damage in various crops 10–15% in rice, 15–20% in tea, 10–25% in sugarcane, and 13–31%, 20–25% and 27–39% in brinjal, okra and chilli respectively[4].

Most species of *Fusarium* are saprophytic and 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 to insects and their host range includes members of Coleoptera, Lepidoptera, Hymenoptera, Diptera and Hemiptera[7,8]. In South Gujarat, India, only a few farmers use biological methods for management of mites in different crops. Therefore, there is a need to identify native strains of new acaropathogenic organisms having the ability to control these mites. The aim of the present study was to identify effective means of biological control of two-spotted red spider mite on okra crops in South Gujarat.

The mite-infested okra fields were visited frequently during June–July 2016 and the infested okra plants were examined to collect the diseased mites. The samples were taken immediately 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 leaves were kept in sterile petri dish in a rearing room under controlled conditions (25° ± 2°C temperature and 70–80% relative humidity).

The infected mites were surface sterilized in 0.5% sodium hypochlorite solution for 10 sec, washed with sterile distilled

water and then inoculated on sterile solidified PDA (200 g potato + 20 g dextrose + 30 g agar per litre) in disposable petri plates (90 mm diameter), sealed with parafilm and incubated in a BOD incubator (at 27°C).

Fungal mycelial disc of 5 mm from 10-day-old pure culture was inoculated in 100 ml of potato dextrose broth (200 g potato + 20 g dextrose per litre) supplemented with chloramphenicol (0.5 g) at room temperature for fungal growth. The mycelial mat, developed after 18–21 days, was crushed with liquid broth and filtered through double-layered muslin cloth. The number of spores was counted using Neubauer's haemocytometer.

Healthy mites were collected with fresh leaves from the field and placed in a sterile petri plate with wet cotton swab. Next, 2 ml liquid suspension containing 2×10^8 cfu/ml of *Fusarium verticillioides* was sprayed on healthy mites with the help of a Potter tower and calibrated at 10 psi, according to the IOBC/WPRS methodology[9] corresponding to an average deposition of 2.5 mg/cm^2 suspension droplets in a petri dish (150 mm diameter × 22 mm depth) and kept under controlled condition (25–27°C temperature and 70–80% relative humidity).

Total fungal genomic DNA was extracted and its quality was evaluated on 1.0% agarose gel. A single band of high-molecular weight DNA was observed. A fragment of the 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 ITS-1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATGC-3′) primers using the BDT v3.1 cycle sequencing kit on ABI 3730xl Genetic Analyzer.

The ITS sequence was used to carry out BLAST with the NCBI GenBank. Based on maximum identity score, the first 10 sequences were selected and aligned using multiple alignment software programs. DNA sequence homology searches were performed using the online BLAST search engine in GenBank (www.ncbi.nlm.nih.gov). Phylogenetic analysis was done using MEGA-7.

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

The evolutionary history was inferred using the neighbor-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 likelihood method[13] (in the unit of number of base substitutions per site). The analysis involved 11 nucleotide sequences. Codon positions included were first + second + third + 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 (Figure 3) in the final dataset and the phylogenetic tree (Figure 4). Evolutionary analyses were done using MEGA7 (ref. 14).

Following the pathogenicity experiment, all the mites had died after eight days and were found to be covered with a white, cottony, fluffy growth of *F. verticillioides*, thus confirming its pathogenic nature. It has been reported that *F. verticillioides* acts as an entomopathogenic on grasshoppers (*Tropidacris collaris* Stoll) in Argentina[15] as well as on short-horned grasshopper (*Oxya*

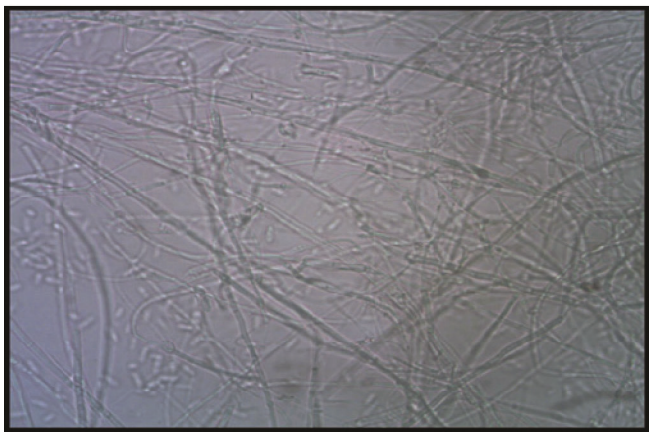

Figure 1. Macro- and microconidia of *Fusarium urticae.*

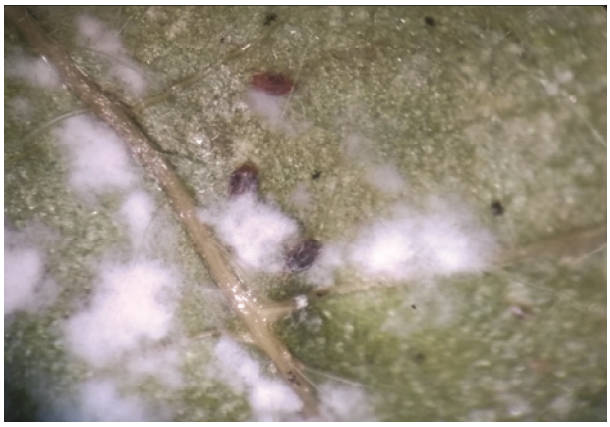

Figure 2. *Fusarium verticillioides* on *Tetranychus verticillioides*

ATGCACATACCAATTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTG
TTTCCATGTGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA
ACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGT
ATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCAGCTTGGTGTTGGGACTCGCGAGTCAAATCGCGTTC
CCCAAATCGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCG
TTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAGGCGCGGAGGAG
AGGGATCATTAACGAAGTTTACAACTCCAAACCTGGGAAACATACCCAAATGGTTCCTGCGGGGAAAAAACGCTCCGGTAA
AACGGGACCGCCGCGAAGGACCTAAACTCTGTTTTCATGTGTAACTTCTGAGTAAAAACATAAATAAATCAAAACTTTCACA
ACGGATCTCTTGGTTCTGGCATCATGAAAACGCAGCAAATGCGATAAGTAATGTGAATTGCAGAATTCGTGAATCATCAATC
TTTTGAACGCACATTTGCGCCGCCGTATTCTGGGCGGGCATGC

Figure 3. Total of 471 bases sequenced.

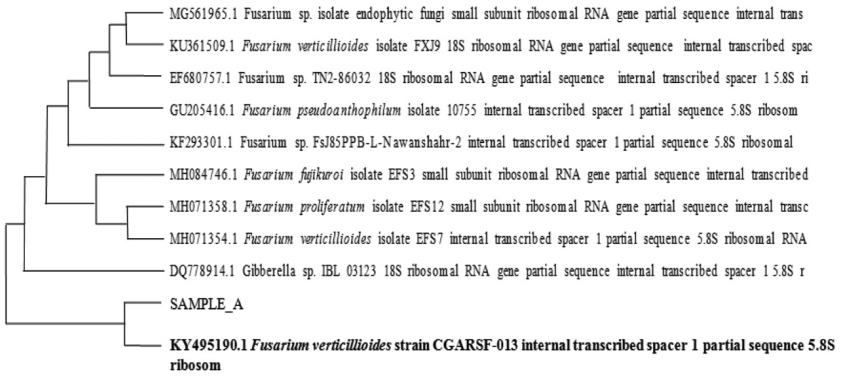

Figure 4. Phylogenetic tree.

hyla intricate Hollis) in the Philippines[16]. It also acts as a plant pathogen causing disease in sweet corn in Watkinsville, Georgia, USA[17]. In maize it shows seedling decay, stalk rot, ear rot and mycotoxin contamination in Ames, Iowa, USA[18]. Post flowering stalk rot has been reported in corn at Rajasthan, India[19]. *F. verticillioides* is also reported as pathogenic to sugarcane, showing discoloration from red to brown in sugarcane stalks in Iran[20]. It 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 in Karnataka, India[22]. In the case of fruit crops, banana was found to be infected with rotten fruits in Mexico[23] and banana neck rot in Hungary[24], whereas in the case of pineapple it caused fruit rot and leaf spot disease in Malaysia[25]. This phytopathogen also caused fruit rot disease in banana, papaya and guava in Malaysia[26].

The literature indicates that *F. verticillioides* has not been recorded earlier as an acaropathogen on two-spotted red spider mite (*T. urticae*) in the South Gujarat region. Further research is needed on various aspects like testing the efficacy of *F. verticillioides* on different mites infesting various crops, and also testing the native isolate as an effective biological control agent on mites.

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