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Fusarium virguliforme, a soybean sudden death syndrome fungus in Malaysian soil

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Abstract Numerous *Fusarium* species associated with soil and different plants have been reported from Malaysia. Until now there are no reports on *F. virguliforme* in Malaysia. *Fusarium virguliforme* is the etiological agent of soybean sudden death syndrome (SDS). Morphological studies combined with molecular analysis using sequences from the translation elongation factor 1α (TEF-1α) gene and nuclear ribosomal DNA internal transcribed spacer (ITS) regions were conducted to identify *F. virguliforme* (2.1 %) isolates from soil in Malaysia.

Keywords *Fusarium solani* species complex · Malaysia · ITS regions · TEF-1α

Fusarium solani species complex (FSSC) Clade 2 encompasses at least eight phylogenetic species including *F. phaseoli* (bean root rot pathogen), *Fusarium virguliforme* and related species that have been reported from all major plantation regions in South America (Aoki et al. 2003). Sudden death syndrome of soybean, caused by *Fusarium virguliforme*, is one of the most destructive diseases affecting soybean production in North and South America (Aoki et al. 2003). Aoki et al. (2003) extensively investigated the bean root rot pathogen and SDS pathogenic strains from North and South America based on comprehensive morphological comparisons and molecular phylogenetic analyses of multilocus DNA sequences Table 1. According to this study, they described three morphologically and phylogenetically distinct species within Clade 2. *Fusarium virguliforme* (formerly known as *F. solani* f. sp. *glycine*) and *F. phaseoli* (formerly known as *F. solani* f. sp. *phaseoli*) were described for the United States strains and *F. tucumaniae* was illustrated for Argentinian strains (Roy 1997; Rupe et al. 2001). Aoki et al. (2005, 2012), based on detailed morphological comparisons, phenotypic and molecular phylogenetic analyses of multiple loci of DNA sequences, described *F. brasiliense*, *F. cuneirostrum* and *F. crassistipitatum* as novel SDS pathogens. Until now, no attempt has been made to classify members of the FSSC in Malaysia. Therefore, the objectives of the present study were to re-identify strains of FSSC stored in the *Fusarium* Culture Collection Unit of School of Biological Sciences, Universiti Sains Malaysia by using morphological and molecular markers. In this survey, 140 strains were investigated based on morphological characteristics as shown in Table 2. All strains were purified by the single-spore isolation technique. Single germinated macroconidia were transferred onto PDA plates and colony appearance was used to select isolates for further study. *Fusarium* strains were grown on PDA and carnation leaf agar (CLA) (Fisher et al. 1982) in 9 cm plastic Petri dishes. Cultures were incubated under 12 h alternating light (black/white) at 25±2 °C for 1 week. Colony morphology and colour were based primarily on cultures grown on PDA. Cited colours are given according to Kornerup and Wanscher (1978). For comparison of mycelial growth rates, agar blocks ca 5×5 mm were cut from the margins of 1 week old cultures on CLA and transferred onto PDA and incubated at 25 °C for 1 week in the dark. Thirty randomly selected conidia of each septation class (macroconidia and microconidia), sporodochial phialides, chlamydospores and conidiophores were measured and analysed by the 2-Sample T-Test using MINITAB® 15 (Table 2). For species determination, the descriptions by Aoki et al. (2003, 2005, 2012) were adopted.

Representative FSSC strains were grown on PDA with sterile dialysis membranes (Lui et al. 2000) for 5 days (Table 3). The mycelium was harvested and ground in a sterile mortar with liquid nitrogen to a fine powder and then DNA

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Table 1 Strains of *Fusarium solani* species complex recovered from various hosts and substrates in Malaysia

Strain number	Species	Origin	Host	Substrate and symptoms
[a]USM [b]FSSC-[c]P3P	*F. solani*	Penang	Potato	Tuber, dry rot
USM FSSC-P53B	*F. solani*	Penang	Bean sprout	Root, rot
USM FSSC-P54B	*F. solani*	Penang	Bean sprout	Root, rot
USM FSSC-P57G	*F. solani*	Penang	Onion	Leaf, rot
USM FSSC-P43R	*F. solani*	Penang	Rice	Seed
USM FSSC-P0112S	*F. solani*	Penang	Soil	–
USM FSSC-P104S	*F. solani*	Penang	Soil	–
USM FSSC-P1235A	*F. solani*	Penang	Asparagus	Stem, brown spot
USM FSSC-P21H	*F. solani*	Penang	Human	Finger
USM FSSC-P200S	*F. solani*	Penang	Soil	–
USM FSSC-P1361G	*F. solani*	Penang	Onion	Bulb, rot
USM FSSC-P1531S	*F. solani*	Penang	Soil	–
USM FSSC-P1532S	*F. solani*	Penang	Soil	–
USM FSSC-P1555S	*F. solani*	Penang	Soil	–
USM FSSC-P1557S	*F. solani*	Penang	Soil	–
USM FSSC-P2103S	*F. solani*	Penang	Sand	–
USM FSSC-P1194W	*F. solani*	Penang	Watermelon	Stem, blackish brown leaves
USM FSSC-P1225W	*F. solani*	Penang	Watermelon	Stem, blackish brown leaves
USM FSSC-P767M	*F. solani*	Penang	Mango	Stem
USM FSSC-P2214An	*F. solani*	Penang	Angsana	–
USM FSSC-P2142An	*F. solani*	Penang	Angsana	–
USM FSSC-P2143An	*F. solani*	Penang	Angsana	–
USM FSSC-P5004B	*F. solani*	Penang	Long bean	Leaf, spot
USM FSSC-P1517S	*F. solani*	Penang	Soil	–
USM FSSC-P1531S	*F. solani*	Penang	Soil	–
USM FSSC-P3602Gr	*F. solani*	Penang	*Digitaria setigera*	Root, rot
USM FSSC-P3598Gr	*F. solani*	Penang	*Paspalum orbiculare*	Root, rot
USM FSSC-P52B	*F. solani*	Penang	Bean sprout	Root, rot
USM FSSC-P993P	*F. solani*	Penang	Potato	Tuber, dry rot
USM FSSC-P1263S	*F. solani*	Penang	Soil	–
USM FSSC-P878V	*F. solani*	Penang	Vanda	Black, stem rot
USM FSSC-P6S	*F. solani*	Penang	Soil	–
USM FSSC-P2108S	*F. solani*	Penang	Soil	–
USM FSSC-P17E	*F. solani*	Penang	Eggplant	Stem, rot
USM FSSC-P2106S	*F. solani*	Penang	Soil	–
USM FSSC-P65S	*F. solani*	Penang	Soil	–
USM FSSC-P2109S	*F. solani*	Penang	Soil	–
USM FSSC-P76V	*F. solani*	Penang	Cassava	Leaf, spot
USM FSSC-P0111S	*F. solani*	Penang	Soil	–
USM FSSC-P86S	*F. solani*	Penang	Soil	–
USM FSSC-P88S	*F. solani*	Penang	Soil	–
USM FSSC-P1980S	*F. solani*	Penang	Soil	–
USM FSSC-P91S	*F. solani*	Penang	Soil	–
USM FSSC-P93S	*F. solani*	Penang	Soil	–
USM FSSC-P97S	*F. solani*	Penang	Soil	–
USM FSSC-P98S	*F. solani*	Penang	Soil	–
USM FSSC-P34L	*F. solani*	Penang	Oil palm	Seed

Table 1 (continued)

Strain number	Species	Origin	Host	Substrate and symptoms
USM FSSC-P156R	*F. solani*	Penang	Rice	Seed
USM FSSC-P178R	*F. solani*	Penang	Rice	Seed
USM FSSC-P183S	*F. solani*	Penang	Soil	–
USM FSSC-P1282S	*F. solani*	Penang	Soil	–
USM FSSC-P383N	*F. solani*	Penang	Banana	Fruit, rot
USM FSSC-P391N	*F. solani*	Penang	Banana	Fruit, rot
USM FSSC-P458N	*F. solani*	Penang	Banana	Fruit, rot
USM FSSC-B565S	*F. solani*	Selangor	Soil	–
USM FSSC-B636S	*F. solani*	Selangor	Soil	–
USM FSSC-B1409M	*F. solani*	Selangor	Mango	Fruit, spot
USM FSSC-B1479G	*F. solani*	Selangor	Ginger	Root, vascular wilt
USM FSSC-B1481G	*F. solani*	Selangor	Ginger	Root, vascular wilt
USM FSSC-B1765S	*F. solani*	Selangor	Soil	–
USM FSSC-B1766S	*F. solani*	Selangor	Soil	–
USM FSSC-B1767S	*F. solani*	Selangor	Soil	–
USM FSSC-B1769S	*F. solani*	Selangor	Soil	–
USM FSSC-B1770S	*F. solani*	Selangor	Soil	–
USM FSSC-B2285L	*F. solani*	Selangor	Oil palm	Crown, rot
USM FSSC-B2466N	*F. solani*	Selangor	Banana	Root, vascular wilt
USM FSSC-B2982$	*F. solani*	Selangor	Coffee	Stem, dieback
USM FSSC-B2983$	*F. solani*	Selangor	Coffee	Stem, dieback
USM FSSC-B2989Br	*F. solani*	Selangor	Brinjals	Stem
USM FSSC-B3401An	*F. solani*	Selangor	Angsana	–
USM FSSC-B3819Gr	*F. solani*	Selangor	*Ischaemum magnum*	Leaf, rot
USM FSSC-B3823Gr	*F. solani*	Selangor	*Eragrostis amabilis*	Stem, rot
USM FSSC-B3824Gr	*F. solani*	Selangor	*Eragrostis amabilis*	Leaf, rot
USM FSSC-B3827Gr	*F. solani*	Selangor	*Dactyloctenium aegyptium*	Root
USM FSSC-B3829Gr	*F. solani*	Selangor	*Dactyloctenium aegyptium*	Root
USM FSSC-B3830Gr	*F. solani*	Selangor	*Dactyloctenium aegyptium*	Root
USM FSSC-B3832Gr	*F. solani*	Selangor	*Digitaria ciliaris*	Root
USM FSSC-B3834Gr	*F. solani*	Selangor	*Pennisetum purpureum*	Root
USM FSSC-B3846Gr	*F. solani*	Selangor	*Echinochloa colona*	Root
USM FSSC-B3822Gr	*F. solani*	Selangor	*Eragrostis amabilis*	Root
USM FSSC-B3024	*F. solani*	Selangor	UPM 31-P1	–
USM FSSC-A3034L	*F. solani*	Perak	Oil palm	Crown, rot
USM FSSC-A1966S	*F. solani*	Perak	Soil	–
USM FSSC-A491S	*F. solani*	Perak	Soil	–
USM FSSC-A998W	*F. solani*	Perak	Watermelon	Root, rot
USM FSSC-A1444S	*F. solani*	Perak	Soil	–
USM FSSC-A1449S	*F. solani*	Perak	Banana	Root, vascular wilt
USM FSSC-A1450N	*F. solani*	Perak	Banana	Root, vascular wilt
USM FSSC-A1463N	*F. solani*	Perak	Banana	Root, vascular wilt
USM FSSC-A1863W	*F. solani*	Perak	Watermelon	Seed
USM FSSC-A1881W	*F. solani*	Perak	Watermelon	Fruit, vascular wilt
USM FSSC-A1882W	*F. solani*	Perak	Watermelon	Fruit, vascular wilt
USM FSSC-A1883W	*F. solani*	Perak	Watermelon	Stem, vascular wilt
USM FSSC-A2333Gr	*F. solani*	Perak	Jojoba	Root, wilt
USM FSSC-A263R	*F. solani*	Perak	Roselle	Root, rot
USM FSSC-A1901S	*F. solani*	Perak	Soil	–

Table 1 (continued)

Strain number	Species	Origin	Host	Substrate and symptoms
USM FSSC-A1909W	*F. solani*	Perak	Watermelon	Root, lesion
USM FSSC-A1910W	*F. solani*	Perak	Watermelon	Root, lesion
USM FSSC-A1936W	*F. solani*	Perak	Watermelon	Soil
USM FSSC-A1937W	*F. solani*	Perak	Watermelon	Stem, vascular wilt
USM FSSC-A1968W	*F. solani*	Perak	Watermelon	Root
USM FSSC-A1969W	*F. solani*	Perak	Watermelon	Root
USM FSSC-A1970W	*F. solani*	Perak	Watermelon	Stem, vascular wilt
USM FSSC-A1973W	*F. solani*	Perak	Watermelon	Root
USM FSSC-A1974W	*F. solani*	Perak	Watermelon	Root
USM FSSC-A1977W	*F. solani*	Perak	Watermelon	Stem, vascular wilt
USM FSSC-A2072W	*F. solani*	Perak	Watermelon	Root
USM FSSC-A2073S	*F. solani*	Perak	Watermelon	Soil, vascular wilt
USM FSSC-Q724Q	*F. solani*	Sarawak	Sorghum	Stalk, rot
USM FSSC-Q725Co	*F. solani*	Sarawak	Cocoa	–
USM FSSC-Q726D	*F. solani*	Sarawak	Black pepper	–
USM FSSC-Q4992D	*F. solani*	Sarawak	–	Flower
USM FSSC-Q728	*F. solani*	Sarawak	–	Flower
USM FSSC-Q729D	*F. solani*	Sarawak	Black pepper	–
USM FSSC-Q737	*F. solani*	Sarawak	–	Flower
USM FSSC-Q1014Q	*F. solani*	Sarawak	Sorghum	Fruit
USM FSSC-Q1015Q	*F. solani*	Sarawak	Sorghum	Fruit
USM FSSC-Q1016Q	*F. solani*	Sarawak	Sorghum	Fruit
USM FSSC-Q1017Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1018Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1021Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1022Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1023Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1024Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1025Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1026Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1027Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1033Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1034Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1035Q	*F. solani*	Sarawak	Sorghum	Fruit
USM FSSC-Q1036Q	*F. solani*	Sarawak	Sorghum	Root
USM FSSC-Q1037Q	*F. solani*	Sarawak	Sorghum	Fruit
USM FSSC-Q1038Q	*F. solani*	Sarawak	Sorghum	Fruit
USM FSSC-Q1039Q	*F. solani*	Sarawak	Sorghum	Fruit
USM FSSC-Q1165Gr	*F. solani*	Sarawak	Grass	–
USM FSSC-Q1162Fe	*F. solani*	Sarawak	Feces	Feces
USM FSSC-D992S	*F. virguliforme*	Kelantan	Soil	–
USM FSSC-T531S	*F. virguliforme*	Terengganu	Soil	–
USM FSSC-J969S	*F. virguliforme*	Johor	Soil	–

[a] *USM* Universiti Sains Malaysia

[b] *FSSC Fusarium solani* species complex

[c] *P3P* The first letter denotes a particular state in Malaysia (A- Perak, B- Selangor, D- Kelantan, J- Johor, P- Penang, Q- Sarawak, and S- Sabah) and the P symbol represents the host i.e. potato

Table 2 Comparison of morphological characteristics of representative strains within the *Fusarium solani* species complex

Strain number	Species	Shape of microconidia	Shape of basal cell and apical cell	Length × width of macroconidia (μm)[a]	
				3-septate	5-septate
USM FSSC-B1409M	*F. solani*	Oval, clavate, reniform	Barely notched and papillate curved	41.5±1.5×5.4±0.2	45.5±2.5×5.8±0.2
USM FSSC-Q1371W	*F. solani*	Oval, clavate, reniform	Barely notched and papillate curved	41.5±1.5×5.5±0.2	45.5±2.5×5.9±0.2
USM FSSC-Q1165Gr	*F. solani*	Oval, clavate, reniform	Barely notched and papillate curved	40.5±1.5×5.4±0.2	45.5±2.5×5.8±0.2
USM FSSC-T531S	*F. virguliforme*	Comma-shaped, elongated oval	Distinctly notched and tapered, curved	54.5±2.5×5.6±0.5	59.5±2.5×5.7±0.5
USM FSSC-J969S	*F. virguliforme*	Comma-shaped, elongated oval	Distinctly notched and tapered, curved	54.5±2.5×5.8±0.5	57.5±2.5×5.6±0.5
USM FSSC-D992S	*F. virguliforme*	Comma-shaped, elongated oval	Distinctly notched and tapered, curved	55.5±1.5×5.8±0.5	61.0±2.5×5.8±0.5

[a] Mean values of 30 random conidia ± standard deviation

was extracted using a DNeasy® Plant Mini Kit (Qiagen) according to the manufacturer's instruction. Amplification of the translation elongation factor-1α (*tef1*) gene, and internal transcribed spacer (ITS) regions was conducted utilising the primer pair ef1 and ef2 for *tef1* (O'Donnell et al. 1998), and ITS1 and ITS4 for ITS region (White et al. 1990). PCRs were performed in a Peltier Thermal Cycler, PTC-100® (MJ Research, Inc. USA) in a total volume of 25 μl. The PCR mixture contained 4 μl 5× buffer (Promega, Madison, WI, USA), 4 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphate (dNTP) (Promega), 0.8 μM of each primer, 0.75 units of Taq DNA polymerase (Promega®, USA), and 6 ng of template DNA. To prevent evaporation, the reactions were overlaid with 25 μl of sterilised mineral oil. PCR products were purified using Qiagen columns according to the manufacturer's instructions and stored at −20 °C. The purified PCR products were sent to First BASE Laboratories Sdn. Bhd. for sequencing of *tef1* gene and ITS regions in both directions (forward and reverse) using ABI 3730x1 model of sequencer. Forward and reversed sequences of *tef1* gene and ITS regions were edited and aligned using BioEdit version 7.0.5 (Hall 1999). Consensus sequences were used as query to search for similarities using two sources: I. BLAST network services at the National Centre for Biotechnology Information (NCBI); II. FUSARIUM-ID v.1.0 database (http://fusarium.cbio.psu.edu) (Geiser et al. 2004).

All FSSC strains were successfully investigated based on macroscopic and microscopic characteristics. One-hundred and thirty seven strains (97.9 %) were identified as *F. solani* and three strains (2.1 %) belonged to *F. virguliforme* (Fig. 1). All three strains produced pink to bluish-gray mycelium. Aerial conidiophores, unbranched or sparsely branched, up to 250 μm long and 2.5–7.0 μm wide at base, abundantly formed on CLA at 25 °C (Fig. 1a). Aerial phialides were simple, cylindrical or subcylindrical. Sporodochial conidiophores were subcylindrical or ampulliform with distinctive collarette at the tip, 20–28 μm long, 2.5–3.0 μm wide at base (Fig. 1b). Macroconidia arising from sporodochia were falcate and sometimes widest at middle, with 3-5-septa and mostly 3-septate, with a tapered and curved apical cell and distinctly notched basal cell (Fig. 1c–d). Microconidia were comma-

Table 3 Strain number, geographical origin, and GenBank accession numbers of representative strains within the *Fusarium solani* species complex

Strain number	Species	[a]*tef1*	[a]ITS	Substrate	Origin
USM FSSC-D992S	*F.virguliforme*	JX970965	JX982562	Soil	Kelantan
USM FSSC-T531S	*F.virguliforme*	JX970966	JX982563	Soil	Banana field, Terengganu
USM FSSC-J969S	*F.virguliforme*	JX970967	JX982564	Soil	Banana field, Johor
USM FSSC-B1409M	*F.solani*	KF836690	KF836675	Mango	Selangor
USM FSSC-Q1371W	*F.solani*	KF836691	KF836676	Watermelon	Sarawak
USM FSSC-Q1165Gr	*F.solani*	KF836693	KF836678	Grass	Sarawak

[a] GenBank numbers for translation elongation factor 1-alpha (*tef1*) partial sequences, and the ITS rDNA region

shaped, elongated oval to sometimes short-clavate and ellipsoidal shaped with a swollen apex often rounded and mostly 0-2-septate (Fig. 1f). The size of conidia; 0–1(−2)-septate = (11–) 15.6–26 (−33.1) × (3.8–) 5–6.4 (−7.4) μm; 3-septate= (30–) 47–56 (−65) × (4.2–) 4.6–5.4 (−6) μm; 4-septate (45–) 53–58 (−65) × (4.7–) 5.2–5.4 (−6) μm; 5-septate 56–65 × 5.3–6.1 μm. Two types of chlamydospores formed, relatively abundant in mycelium, smooth and rough-walled, 5–14 μm in diameter, may occasionally be found within macroconidia. Chlamydospores formed mostly singly, and in pairs, rarely in chains, mostly subglobose, terminal or intercalar (Fig. 1e).

A list of species names and culture collection numbers, geographical origins, original substrates, and GenBank accession numbers of the individual strains used in this study are in Table 3. Single bands of DNA fragments approximately 550-bp and 700-bp were successfully amplified for ITS region and *tef1* gene from all three strains, respectively. From similarities searched at NCBI and FUSARIUM-ID database, all three strains were similar to *F. virguliforme* with the percentage of maximum identity (100 %). Based on morphological features combined with molecular analysis using *tef1* gene and ITS regions sequences, *F. virguliforme* was reported for the first time in Malaysia.

Fusarium virguliforme is one of the most important causal agents of sudden death syndrome (SDS) in soybean and has been reported from all major growing regions in North

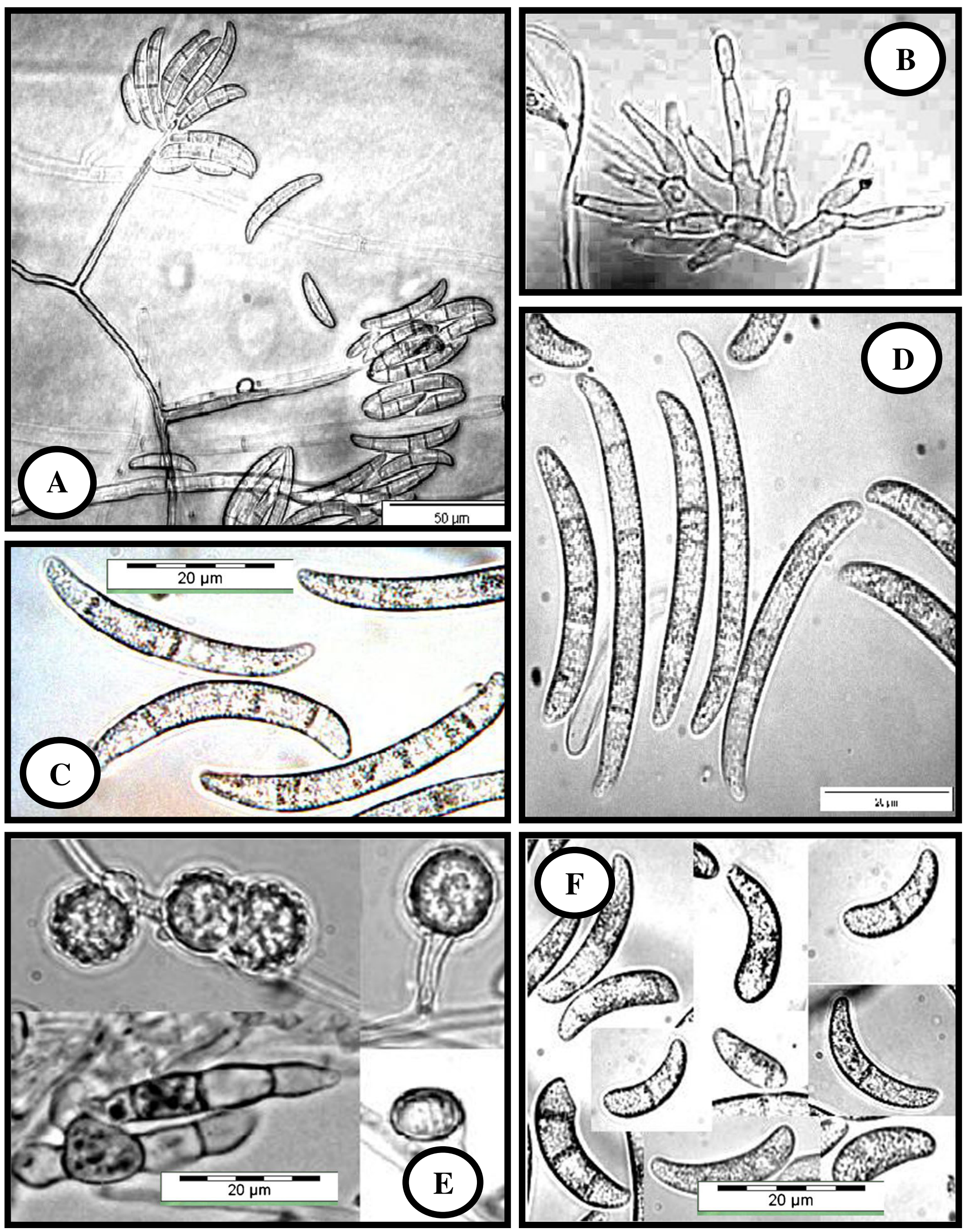

Fig. 1 *Fusarium virguliforme* grown on CLA, 2 weeks, 25 °C, cultured in the dark. **a** Slender, unbranched aerial conidiophores. **b** Branched sporodochial conidiophores forming falcate to curved cylindrical conidia. **c–d** Falcate and comma-shaped macroconidia produced in sporodochial. **e** Terminal and intercalary chlamydospores, and produced chlamydospores in conidia. **f** Comma-shaped microconidia were formed on conidiophores in hyphae. Scale bars: **a**= 50 μm, **b**–**f** = 20 μm

America (Aoki et al. 2003, 2005, 2012). Kolander et al. (2012) showed that the diversity and number of hosts for *F. virguliforme* are greater than previously reported by Aoki et al. (2005, 2012) and indicated that agricultural crops other than soybean can be damaged by *F. virguliforme* and increase pathogen inoculum in soil. Therefore, further information regarding this well-known plant pathogen within the region is needed. To the best of our knowledge this is the first report on occurrence of this species in agricultural soil in Malaysia.

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