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Isolation of Microfungi from Malay Traditional Vegetables and Secondary Metabolites Produced by *Fusarium* Species

(Pemencilan Kulat Mikro daripada Sayuran Traditional Melayu dan Metabolit Sekunder dihasilkan oleh Spesies *Fusarium*)

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ABSTRACT

Microfungi isolated from Malay traditional vegetables such as Centella asiatica, Cosmos caudatus, Oenanthe javanica, Persicaria odorata *and* Psophocarpus tetragonolobus *are well diverse. A total of 40 isolates of the fungi were identified and classified into four genera such as* Aspergillus, Fusarium, Penicillium *and* Trichoderma. *Five species of* Fusarium *were morphologically identified as* F. oxysporum, F. semitectum, F. proliferatum, F. solani *and* F. konzum. *Three species of* Aspergillus *were identified as* A. niger, A. fumigatus *and* A. flavus. *The highest number of microfungi was isolated from* Cosmos caudatus *(12 isolates), followed by* Persicaria odorata *(9 isolates),* Oenanthe javanica *(8 isolates),* Centella asiatica *(6 isolates) and* Psophocarpus tetragonolobus *(5 isolates). Four isolates of* Fusarium *species were able to produce moniliformin (MON) and five isolates were able to produce fumonisin B_1 (FB_1). This is the first report on diversity of microfungi associated with some Malay traditional vegetables.*

Keywords: Aspergillus*; fumonisin B_1;* Fusarium*; Malay traditional vegetables; moniliformin;* Penicillium

ABSTRAK

Kulat mikro yang dipencilkan daripada sayuran tradisi Melayu seperti Centella asiatica, Cosmos caudatus, Oenanthe javanica, Persicaria odorata *dan* Psophocarpus tetragonolobus *adalah pelbagai. Sejumlah 40 isolat kulat telah dikenal pasti dan dikelaskan ke dalam empat genus iaitu* Aspergillus, Fusarium, Penicillium *dan* Trichoderma. *Lima spesies* Fusarium *telah dikenal pasti secara morfologi sebagai* F. oxysporum, F. semitectum, F. proliferatum, F. solani *dan* F. konzum. *Walau bagaimanapun, tiga spesies* Aspergillus *telah dikenal pasti sebagai* A. niger, A. fumigatus *dan* A. flavus. *Bilangan tertinggi kulat mikro telah dipencilkan daripada* Cosmos caudatus *(12 isolat), diikuti dengan* Persicaria odorata *(9 isolat),* Oenanthe javanica *(8 isolat),* Centella asiatica *(6 isolat) dan* Psophocarpus tetragonolobus *(5 isolat). Empat isolat spesies* Fusarium *berupaya menghasilkan moniliformin (MON) dan lima isolat berupaya menghasilkan fumonisin B_1 (FB_1). Kajian ini merupakan laporan pertama mengenai kepelbagaian kulat mikro yang berasosiasi dengan beberapa sayuran tradisional Melayu.*

Kata kunci: Aspergillus*; fumonisin B_1;* Fusarium*; moniliformin;* Penicillium*; sayuran tradisional Melayu*

Introduction

Malay traditional vegetables are plants that can be eaten fresh as the accompaniment to rice and locally known as 'ulam'. 'Ulam' group consists of 120 plant species that represent many families from herbs to trees (Mansor 1988). 'Ulam' constitutes an important part of the food intake among the local peoples especially the Malay and indigenous communities. 'Ulam' become very popular to all Malaysians and also among visiting foreigners who have acquired the 'ulam' taste. 'Ulam' are predominantly taken as salads, usually their leaves part, and sometimes they may be blanched, curried and fried (Bautista et al. 1988; Mansor 1988). Basically, 'ulam' contain high value of nutrients and served as healthy dishes.

Microfungi are free living, widely distributed and mostly acting as saprophytes, endophytes or pathogen on various plants, including 'ulam' (Liddel 1991). Microfungi belong to the genera of *Aspergillus, Fusarium, Penicillium* and *Trichoderma* are filamentous fungi group under division Ascomycota. Filamentous fungi grow as multicellular networks of filament shaped cells called as hyphae and each cell contains a number of nuclei (Hawksworth et al. 1995). Some microfungi such as *Fusarium* species that are associated with plants may produce mycotoxins as their secondary metabolites. Mycotoxins were produced by aerobic microfungi under favorable conditions of temperature and moisture, and have the ability to impair health and productivity of human and animal (D'Mello & MacDonald 1997).

The toxins can cause illnesses and economic losses (Charmley et al. 1995; D'Mello & MacDonald 1997). One of the major health risks associated with plant-based food is the consumption of mycotoxins because most of the plant-based food are commonly infected with toxigenic fungi

such as *Fusarium*, *Penicillium* or *Aspergillus* (Christensen & Kaufmann 1969). Filtenborg et al. (2000) had reported the presence of about 400 mycotoxins. *Fusarium* species has been reported to produce the mycotoxins fumonisin B_1 (FB_1) and moniliformin (MON) (Leslie & Summerell 2006).

The objectives of this study were to isolate and identify the microfungi i.e. *Aspergillus*, *Fusarium*, *Penicillium* and *Trichoderma* species associated with 'ulam' samples and to detect the presence of FB_1 and MON using thin layer chromatography (TLC) analysis.

Material and Methods

Isolation of the microfungi

All the fresh leaves samples of 'ulam' such as *Cosmos caudatus* (ulam raja), *Persicaria odorata* (kesom), *Psophocarpus tetragonolobus* (kacang botol), *Oenanthe javanica* (selom) and *Centella asiatica* (pegaga) were surface sterilized with 10% Chlorox® and cut into small pieces and cultured directly onto pentachloronitrobenze agar (PPA) and incubated for 7 days under the standard incubation conditions i.e. 12 h under light fluorescence, 12 h under UV light with 28±2ºC of room temperature. The cultures were further purified by single spore technique according to Hansen and Smith (1932) on potatoes dextrose agar (PDA). After 24 h incubation, the small colonies appeared and the new germinated conidia were tranfered onto the new PDA for allowing the development of the uniform colonies. The pigmentation and colony features of the pure cultures were recorded after 7 days, and growth rates of the cultures were measured after 72 h incubation.

Identification of microfungi

For the identification of *Fusarium*, the cultures were transferred onto the carnation leaf agar (CLA) and incubated for 7 days. The microscopic characteristics of the all *Fusarium* cultures were observed and identified into the species level according to Burgess et al. (1994) and Leslie and Summerell (2006) by using the light microscope (Zeiss, model AxioCamMR). The small section of water agar with the mycelia were cut and placed onto the slide for observing the *in situ* characteristics such as the ontogeny of microconidia. Other microscopic characteristics were observed such as the shape of macroconidia, presence or absence of microconidia, shape and mode of formation of microconidia, nature of the conidiogenous cell bearing microconidia and presence or absence of chlamydospores.

Others microscopic fungi such as *Aspergillus, Trichoderma* and *Penicillium* species were identified by observing the slide cultures. The colony features and microscopic characteristics of the *Aspergillus, Penicillium* and *Trichoderma* species such as the formation and arrangement of conidia and conidiophore types were observed and recorded. *Aspergillus* species were identified according to Raper and Fennell (1965), *Penicillium* species according to Pitt (1979) and *Trichoderma* species according to Domsch et al. (1980) and Rifai (1969).

Conidial suspension of *Fusarium* species

The conidia of 7 days old cultures of *Fusarium* isolates were harvested by pouring 10 mL of sterile distilled water onto the culture plate and conidia were gently dislodged using a hockey-shaped glass rod. The suspension was filtered by using a sterile double-layered muslin cloth in order to remove the mycelial debris. Conidia concentration was adjusted to 1×10^5 conidia/mL by using heamocytometer.

Preparation of inoculum

For detection of FB_1 and MON, 40 g of cornmeal grits were added into the conical flask with 10 mL of distilled water before autoclaving at 15 psi, 121ºC and 5 mL of sterile water after autoclaving. All the flasks were covered with cotton wool and aluminum foil and re-autoclaved for 30 minutes. Each of sterile cornmeal grits flask was inoculated with 1 mL of conidial suspension and was shaken twice daily for three days to homogenize the inoculated cornmeal. Then, all the cultures were incubated in the dark for 28 days. All the treatments were carried out in triplicates and controls were only inoculated with sterile distilled water.

Extraction and TLC analysis

MON A total of 30 g of each cornmeal culture was soaked in 120 mL of acetonitrile-H_2O (3:1; v/v). All the cornmeal solutions were milled in a blender (ELBA, model EBM-9661X). The extract was filtered through Whatman no. 1 filter paper and 40 mL of n-hexane has been used to soak the filtered solution. The supernatant was separated from n-hexane by using a separating funnel and was evaporated to dryness at 65ºC using a rotary vacum evaporator (Buchi 461, Switzerland). The residue was dissolved in methanol and 10 µL of the suspended residues was spotted onto a silica gel TLC plate (Merck, Darmstadt, Germany) (20 cm^2, 0.25 mm thick silica gel 60 F_{254}) along with a standard marker (Sigma, US) of MON. A heat gun has was used to dry the plate and developed in specific solvent system A; toluene-acetone-methanol (5:3:2, v/v/v), (Burmeister et al. 1979). The plates were sprayed with 20% of aluminum chloride ($AlCl_3$) where 20 g $AlCl_3$ were dissolved in 100 ml of ethanol-H_2O (1:1, v/v) for visualizing the MON and heated at 110ºC for 10 min. Then, the plates were sprayed again with 20% H_2SO_4 and reheated at 110ºC for 10 min. TLC plates were observed under the longwave UV light (365 nm).

FB_1 Ten gram of cornmeal cultures were soaked in 100 mL of methanol-H_2O (3:1, v/v) and were milled in the blender (Scott et al. 1999). Filtration of the extract has been done using the Whatman no. 1 filter paper and the supernatant was evaporated to dryness at 60°C using a rotary vacuum

evaporator. The residue was dissolved in acetone and then 10 µL of the suspended residues was spotted on a silica gel TLC plate along with a standard of FB_1 (Sigma). The dryness of the plates was taken placed before developing in specific solvent systems: solvent system B; chloroform-methanol-acetic acid (6:3:1, v/v/v) (Ross et al. 1991), solvent system C; ethyl acetate-acetic acid-H_2O (6:3:1, v/v/v) (Tseng et al. 1995; Fadl Allah 1998). FB_1 was visualized following Tseng et al. (1995) and Fadl Allah (1998). The plate was sprayed with 0.5% ρ-anisaldehyde in methanol-H_2SO_4-acetic acid (90:5:5, v/v/v) after air-drying. The TLC plate was heated at 100°C for 5 min before visualizing under longwave UV light (365 nm).

The R_f value of secondary metabolites were calculated according to Fessenden et al. (2001). R_f value is known as distance of the certain compound spot and the standard moved up on the TLC plate relative to the distance moved by the solvent front.

Results

Diversity of microfungi isolated from 'ulam'

A total of 40 isolates of microfungi were obtained and identified into four genera i.e. *Aspergillus* species, *Fusarium* species, *Penicillium* species and *Trichoderma* species (Table 1). The highest number of the fungal isolates were classified into *Aspergillus* species (22 isolates), followed by *Fusarium* species (9 isolates), *Trichoderma* species (7 isolates) and *Penicillium* species (2 isolates). Five species of *Fusarium* were morphologically identified as *F. oxysporum* (4 isolates), *F. semitectum* (2 isolates),

TABLE 1. Microscopic fungi isolated from leaves of the 'ulam'

No.	Isolate no.	Sample	Locality	Genus/Species
1	A13	*Oenanthe javanica*	Semenyih, Selangor	*A. fumigatus*
2	A14	*Oenanthe javanica*	Semenyih, Selangor	*A. fumigatus*
3	A15	*Oenanthe javanica*	Semenyih, Selangor	*A. fumigatus*
4	A16	*Oenanthe javanica*	Semenyih, Selangor	*A. niger*
5	A17	*Oenanthe javanica*	Semenyih, Selangor	*A. niger*
6	A18	*Oenanthe javanica*	Semenyih, Selangor	*A. niger*
7	A19	*Oenanthe javanica*	Semenyih, Selangor	*A. fumigatus*
8	A08	*Cosmos caudatus*	Puchong, Selangor	*A. flavus*
9	A09	*Cosmos caudatus*	Puchong, Selangor	*A. flavus*
10	A10	*Cosmos caudatus*	Puchong, Selangor	*A. niger*
11	A11	*Cosmos caudatus*	Puchong, Selangor	*A. niger*
12	A12	*Cosmos caudatus*	Puchong, Selangor	*A. fumigatus*
13	A21	*Cosmos caudatus*	Cameron Highland, Pahang	*A. fumigatus*
14	A01	*Persicaria odorata*	Kajang, Selangor	*A. flavus*
15	A02	*Persicaria odorata*	Kajang, Selangor	*A. niger*
16	A20	*Persicaria odorata*	Kajang, Selangor	*A. fumigatus*
17	A22	*Persicaria odorata*	Kajang, Selangor	*A. niger*
18	A07	*Persicaria odorata*	Puchong, Selangor	*A. niger*
19	A03	*Psophocarpus tetragonolobus*	Cameron Highland, Pahang	*A. niger*
20	A04	*Psophocarpus tetragonolobus*	Cameron Highland, Pahang	*A. niger*
21	A05	*Psophocarpus tetragonolobus*	Cameron Highland, Pahang	*A. niger*
22	A06	*Psophocarpus tetragonolobus*	Cameron Highland, Pahang	*A. fumigatus*
23	F282	*Centella asiatica*	Puchong, Selangor	*F. oxysporum*
24	F283	*Centella asiatica*	Puchong, Selangor	*F. proliferatum*
25	F284	*Centella asiatica*	Puchong, Selangor	*F. semitectum*
26	F285	*Centella asiatica*	Puchong, Selangor	*F. konzum*
27	F286	*Cosmos caudatus*	Puchong, Selangor	*F. semitectum*
28	F287	*Cosmos caudatus*	Cameron Highland, Pahang	*F. oxysporum*
29	F288	*Cosmos caudatus*	Jengka, Pahang	*F. oxysporum*
30	F289	*Cosmos caudatus*	Jengka, Pahang	*F. solani*
31	F290	*Persicaria odorata*	Puchong, Selangor	*F. oxysporum*
32	T61	*Persicaria odorata*	Kajang, Selangor	*Trichoderma* sp.
33	T62	*Persicaria odorata*	Puchong, Selangor	*Trichoderma* sp.
34	T63	*Centella asiatica*	Puchong, Selangor	*Trichoderma* sp.
35	T64	*Centella asiatica*	Puchong, Selangor	*Trichoderma* sp.
36	T65	*Cosmos caudatus*	Puchong, Selangor	*Trichoderma* sp.
37	T66	*Psophocarpus tetragonolobus*	Puchong, Selangor	*Trichoderma* sp.
38	T67	*Oenanthe javanica*	Semenyih, Selangor	*Trichoderma* sp.
39	P01	*Cosmos caudatus*	Puchong, Selangor	*Penicillium* sp.
40	P02	*Persicaria odorata*	Puchong, Selangor	*Penicillium* sp.

F. proliferatum (1 isolate), *F. solani* (1 isolate) and *F. konzum* (1 isolate). Whereas, three species of *Aspergillus* were identified as *A. niger* (11 isolates), *A. fumigatus* (8 isolates) and *A. flavus* (3 isolates).

Among the five 'ulam' samples, the highest number of microfungi was isolated from *Cosmos caudatus* with 12 isolates followed by *Persicaria odorata* (9 isolates), *Oenanthe javanica* (8 isolates), *Centella asiatica* (6 isolates) and *Psophocarpus tetragonolobus* (5 isolates) as shown in Figure 1. All the 'ulam' samples were collected from five different locations in Peninsular Malaysia. The highest number of fungal were isolated from samples obtained from Puchong, Selangor with 19 isolates, followed by; Semenyih, Selangor (8 isolates), Cameron Highland, Pahang (6 isolates), Kajang, Selangor (5 isolates) and Jengka, Pahang (2 isolates).

MORPHOLOGICAL CHARACTERISTICS OF *ASPERGILLUS* SPECIES

A. flavus *A. flavus* was green mould on PDA, and the isolates produced white pigmentation with green at the center of the plate. The aerial mycelia were white and become green yellowish with age. The colony was usually grown uniformly and the growth rate of *A. flavus* isolates were 4.33 ± 0.10 mm/day. The conidia were subglobose and formed in cluster with smooth-walled. The diameter of conidia was 4.67 – 9.30 µm. The conidiophores were smooth-walled with conidia chains at the tips, that were formed from primary sterigmata.

A. fumigatus On PDA, the pigmentation of the colony was light to dark green at the center of culture. The texture of colony was flattened and grows uniformly. The growth rate

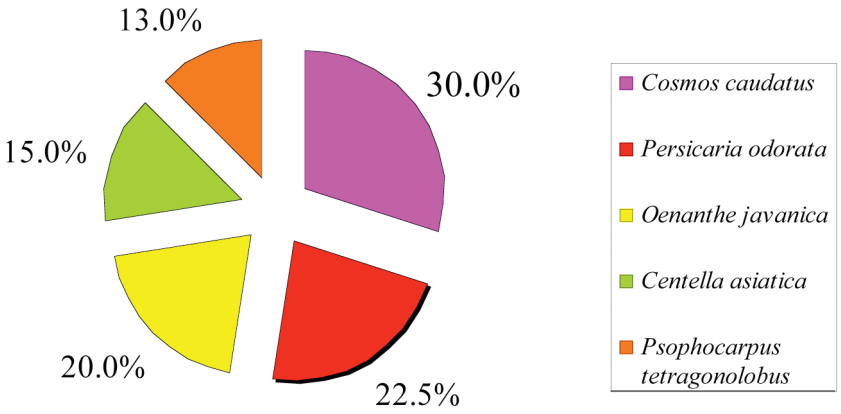

FIGURE 1. Percentage of microfungi based on different 'ulam' samples

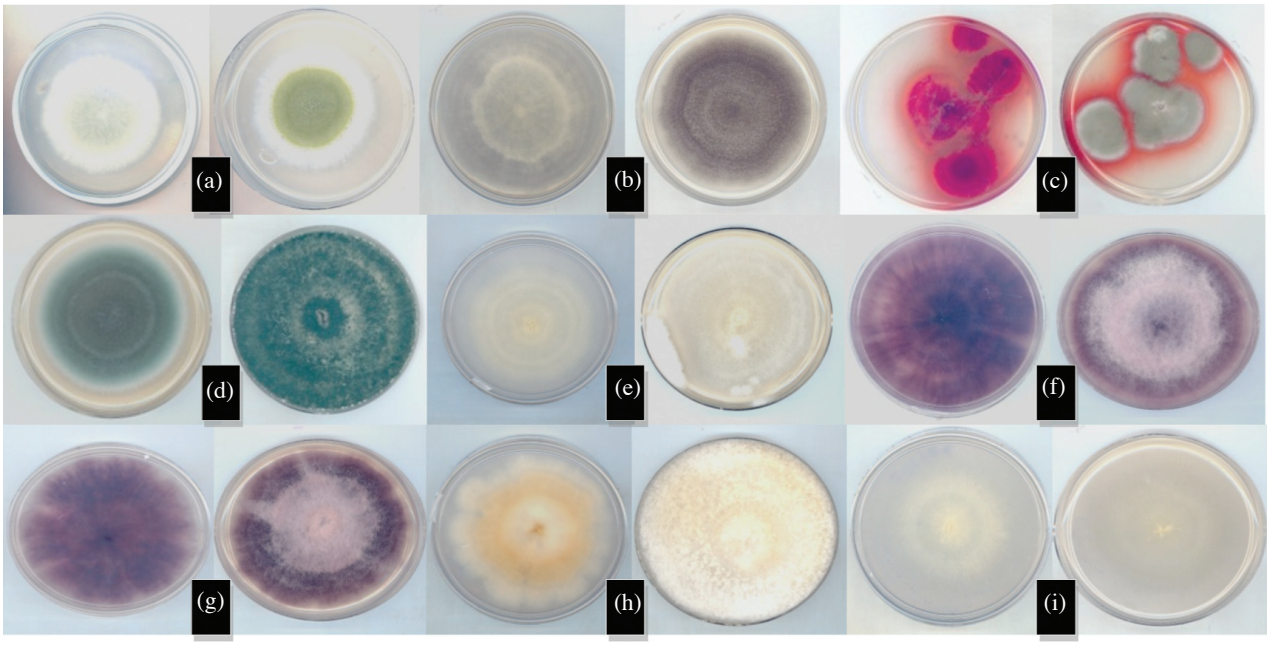

FIGURE 2. Colony features and pigmentations of microfungi isolated from 'ulam'. a) *A. flavus,* b) *A. niger,* c) *Penicillium* species, d) *Trichoderma* species, e) *F. konzum*, f) *F. oxysporum*, g) *F. proliferatum*, h) *F. semitectum*, i) *F. solani*.

of *A. fumigatus* isolates were 6.37 ± 0.40 mm/d. Conidia at the tips of primary sterigmata were globose with smooth-walled in cluster. The diameter of conidia was between 1.79 – 5.36 µm. Conidiophores were long with smooth-walled and slightly roughened.

A. niger On PDA, the pigmentation of the colony was white to cream in the center of the agar and become colorless at the edges of medium. The colonies were grown uniformly and produced abundant aerial mycelia with brown to black in colour. Therefore, they were also known as black mould fungi. The growth rate of *A. niger* isolates were 6.27 ± 0.20 mm/d. The conidia shape was globose with smooth-walled and found in clusters. The diameter of conidia was 1.43– 3.93 µm. The conidial head were consisted of globose vesicle with conidia grow from this structure. Conidiophores were smoothed-walled and long.

MORPHOLOGICAL CHARACTERISTICS OF *FUSARIUM* SPECIES

F. konzum On PDA, the pigmentation was initially pigmentless and become cream and light yellowish color with age. Colonies have floccose mycelia that initially white and become cream and light orange in color with age. The mycelia were abundantly and grown flat on plate where it may become cottony with age. The growth rate of *F. konzum* was 5.35 ± 0.8 mm/d. On CLA, macroconidia were thin walled, slender, straight and slightly curved with usually 3-septate. Apical cell was slightly curved whereas basal cell was foot-shaped. The size of macroconidia was 5.71 – 8.57 µm × 44.57 - 64.57 µm. Microconidia are oval and 0 to 1-septate and borne in false heads. Mesoconidia with 2-septate can also be found. The size of microconidia is 2.86 – 8.57 µm × 8.05 – 32.0 µm. Conidiogenous cells were monophialides and simple polyphialides.

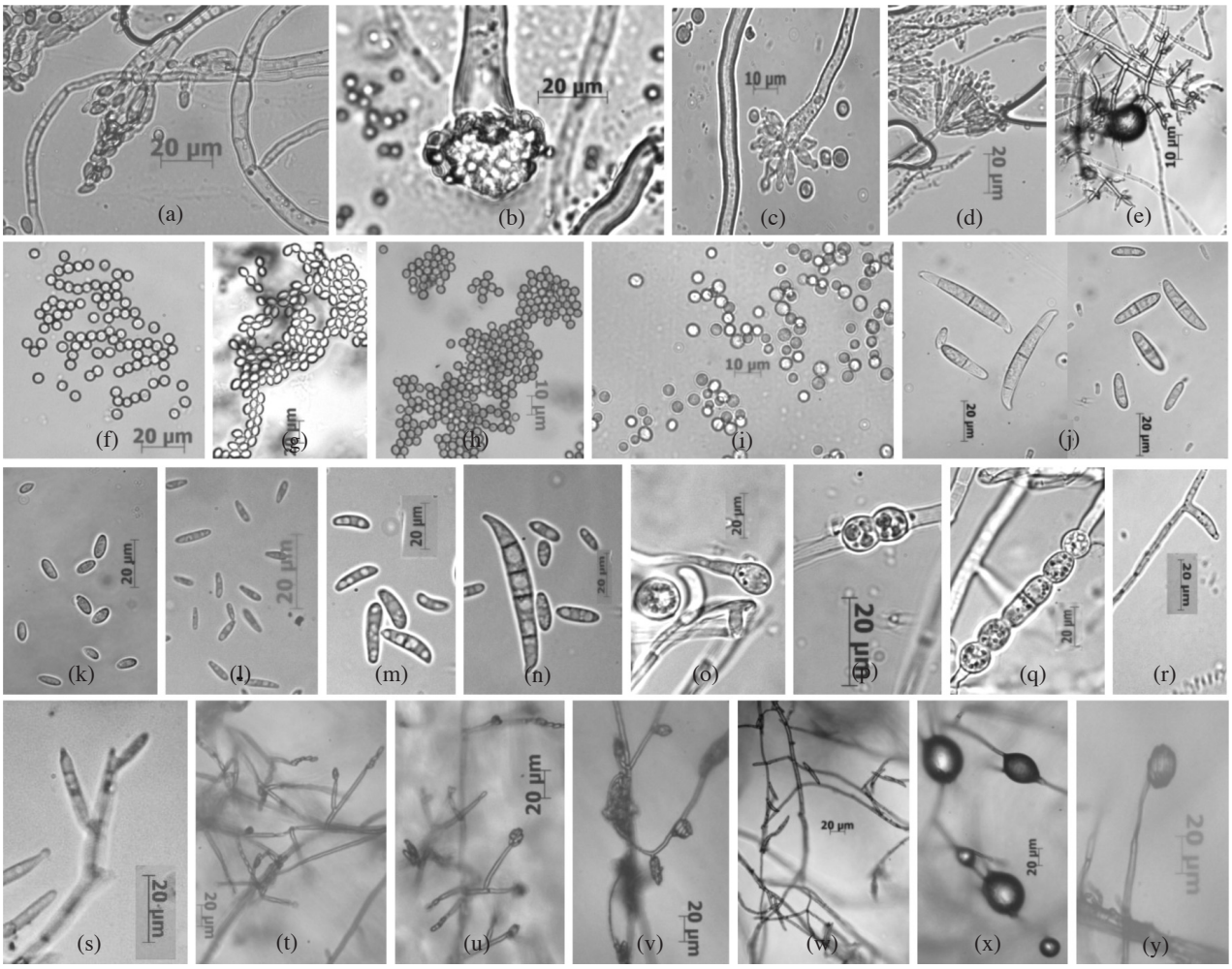

FIGURE 3. Microscopic characteristics of microfungi isolated from 'ulam'. Conidiophores of *A. flavus* (a), *A. niger* (b), *A. fumigatus* (c), *Penicillium* (d), *Trichoderma* (e); Conidia of *A. niger* (f), *Penicillium* (g), *Trichoderma* (h), *A. fumigatus* (i), *F. konzum* (j), *F. solani* (k), *F. proliferatum* (l), *F. oxysporum* (m), *F. solani* (n); Chlamydospores arrangement of *F. oxysporum*, terminal (o), pair intercalary (p), in chain (q); Monophialide of *F. oxysporum* (r), polyphialides of *F. proliferatum* (s); Conidia in chain (t) and false heads of *F. proliferatum* (u), *F. oxysporum* (v), *F. semitectum* (w), *F. solani* (x) and *F. konzum* (y)

F. oxysporum The pigment produced by this species was dark violet from the center of colony with grayish violet at the edges of the PDA plate. White orange pigmentation can also be observed in some cultures. Mycelia were floccose and violet sclerotia were produced abundantly. The growth rate of *F. oxysporum* was 6.33 ± 1.40 mm/d. On CLA, the isolates produced 1-septate mesoconidia, and macroconidia which were generally short, straight to slightly curved, relatively slender, and thin walled. Apical cell was tapered and curved with usually 3-septate. The size of macroconidia was 7.50 – 8.75 µm × 43.75 – 83.13 µm. Microconidia have oval and allantoids shapes with 0-1 septate. The size of microconidia was 4.38 – 6.25 µm × 14.38 – 29.38 µm. Chlamydospores produced in singly, terminal and pair intercalary. Microconidia were formed in false heads on short monophialides.

F. proliferatum The pigmentation of the colonies on PDA was grayish violet and dark violet in some isolates. The aerial mycelium initially was white but become violet in age. The aerial mycelium grows uniformly on plate and the colony is slightly cottony with growth rate at 7.45 ± 0.80 mm/d. On CLA, macroconidia were slender, thin-walled and slightly straight whereas apical cell was slightly curved with usually 3-5 septate. The size of macroconidia was 4.32 – 3.24 µm × 26.49 – 45.41 µm. Microconidia were obovoid with truncate base without septate in size 2.16 – 3.78 µm × 5.95 – 15.68 µm. The conidiophores were monophialides, branches monophialides or simple polyphialides. Microconidia produced in chains and false heads on branched-monophialides or polyphialides.

F. semitectum On PDA, white orange pigment can be observed and the cultures grow rapidly with abundant dense aerial mycelium that initially white and become beige and brown with age. The growth rate of *F. semitectum* was 7.80 ± 1.10 mm/day. On CLA, macroconidia were slender with curved dorsal surface and straighter ventral surface where commonly referred as spindle-shaped macroconidia with 3 to 5 septate. The size of macroconidia was 4.0 – 5.10 µm × 26.55 – 63.64 µm. Most of the isolates produced mesoconidia with usually 1-3 septate with 4.0 – 4.36 µm × 24.36 – 33.45 µm that often have the appearance of 'rabbit ears' on CLA. The conidiophores were monophialides.

F. solani On PDA, the cultures produced cream and yellowish pigments but sometimes no pigmentation produced. Colonies were white to cream with sparse mycelia. The aerial mycelia were slimy growing on the plate with growth rate was 3.98 ± 0.20 mm/day. Macroconidia were relatively wide, straight, stout and robust. Apical cell was slightly curved and rounded end whereas basal cell was foot-shaped with 3 to 5 septate. The size of macroconidia was 4.58 – 7.08 µm × 31.25 – 55.42 µm. Microconidia were oval with 0-1 septate with size 2.08 – 3.75 µm × 4.17 – 7.92 µm. Long monophialides and single intercalary chlamydospore can also be observed.

MORPHOLOGICAL CHARACTERISTICS OF *PENICILLIUM* SPECIES

On PDA, reddish pigmentation can be observed and the cultures was flat, green with slow growth rate at 4.15 ± 3.60 mm/day. The shape of conidia of *Penicillium* species was globose and found in clusters with diameter 3.13 µm – 5.63 µm. The phialides were produced from branch metulae that giving a brush-like appearance known as a penicillus where bearing conidia at their tips. Conidiophores were hyaline and smooth-walled.

MORPHOLOGICAL CHARACTERISTICS OF *TRICHODERMA* SPECIES

On PDA, the colony of *Trichoderma* species showed a dark green color in matured cultures. The *Trichoderma* cultures were rapidly growing with growth rate at 9.0 ± 0.9 mm/day. Conidia of *Trichoderma* species have globose shape with smooth-walled and formed globular clusters at the ends of the conidiophores and also can be found in clusters. The diameter of conidia was 2.14 – 3.57 µm. Single chlamydospore can also be observed and the conidiophores were smooth-walled with regularly verticillate and more irregular of branches patterns.

SECONDARY METABOLITES PROFILES OF *FUSARIUM* SPECIES ISOLATED FROM 'ULAM'

MON R_f value for standard marker on MON was ranged between 0.55 – 0.65 with yellow greenish florescence when developed in solvent system A (Table 2). Only *F. oxysporum* isolates F287, F288 and *F. proliferatum* isolate F283, *F. semitectum* isolate F286 produced MON.

FB_1 In solvent system B, the R_f value of FB_1 for standard marker was ranged between 0.84 – 0.88 whereas in solvent system C was 0.81 – 0.85 with red purple florescence in color as an indicator spot (Table 2). *F. konzum* isolate F285, *F. proliferatum* isolate F283 and *F. oxysporum* isolates F287, F288 and F290 produced FB_1, whereas isolate F282 did not produced FB_1.

DISCUSSION

The microfungi isolated from five 'ulam' in various locations in Peninsular Malaysia were diverse, where *Aspergillus* species were the highest number of microfungi isolated with 22 isolates (55.0%), *Fusarium* species (9 isolates, 22.5%), *Trichoderma* species (7 isolates, 17.5%) and *Penicillium* species (2 isolates, 5.0%).

Several species of *Fusarium* produce airborne conidia and are common colonizers of leaves, stems and flowers (Burgess 1981), for example *F. oxysporum*, which is one of the most variable species that can be pathogenic on plants and cause vascular wilt diseases (Beckman 1987), root, crown, tuber, corn and bulb rots (Nelson et al. 1981). *F. oxysporum* is also acting as soil saprophytes and the saprophytic members of *F. oxysporum* are usually colonise necrotic roots as secondary invaders and often mistakenly

TABLE 2. R_f values of FB_1 and MON developed in solvent systems A, B and C

Fusarium spp.	Isolates	R_f value		FB_1 Production	R_f value Solvent C	MON Production
		Solvent A	Solvent B			
F. oxysporum	F282	-	-	-	-	-
F. proliferatum	F283	0.63	0.84	+	0.60	+
F. semitectum	F284	-	-	-	-	-
F. konzum	F285	0.67	0.83	+	-	-
F. semitectum	F286	-	-	-	0.58	+
F. oxysporum	F287	0.83	0.82	+	0.60	+
F. oxysporum	F288	0.83	0.83	+	0.60	+
F. solani	F289	-	-	-	-	-
F. oxysporum	F290	0.62	0.84	+	0.79	-

Solvent system A = chloroform-methanol-acetic acid (6:3:1; v/v/v) and B = ethyl acetate-acetic acid-H_2O (6:3:1; v/v/v); Solvent system C = toluene-acetone-methanol (5:3:2), v/v/v

assumed to be the primary cause of necrosis (Burgess et al. 1994). Some species can also cause opportunistic infections of human and other animals (Rebell 1981). A few isolates of *F. oxysporum* have been reported to produce mycotoxins such as zearalenone and trichothecene (Marasas et al. 1984). There have also been reported that this species are able to produce beauvericin (Longrieco et al. 1998; Moretti et al. 2002) and fusaric acid (Amalfitano et al. 2002). Two isolates of *Trichoderma* species obtained from *Persicaria odorata* and *Centella asiatica* whereas a single isolate from *Cosmos caudatus*, *Psophocarpus tetragonolobus* and *Oenanthe javanica*.

From the present results, 2 isolates of *F. oxysporum* (isolates F287 and F288) obtained from *Cosmos caudatus* were able to produce MON. On the other hand, *F. oxysporum* (isolates F282 and F290) obtained from *Centella asiatica* and *Persicaria odorata*, respectively were not able to produce MON. This inability to produce MON may due to different types of 'ulam'. The same phenomenon was also observed in *F. semitectum* isolate F286 obtained from *Cosmos caudatus* was able to produce MON but isolate F284 that obtained from *Centella asiatica* was not able to produce MON. From these result, we can conclude that *Cosmos caudatus* was the suitable host for *F. oxysporum* and *F. semitectum* in order to produce MON.

F. oxysporum isolate F290 obtained from *Persicaria odorata* was able to produce FB_1, whereas isolate F282 originated from *Centella asiatica* was not able to produce FB_1. The variation in the R_f value may caused by many factors that occurred during the TLC analysis. These may be due to some external factors such as temperature, time as well as long-term storage of solvent systems. These factors may influence the R_f value during running of TLC analysis. Other factors will affect the value including differences of sample sources and also geographical areas of the *Fusarium* isolates. These factors may distribute to the variation of R_f value in the same solvent system. The productions of mycotoxin by *Fusarium* species isolated from 'ulam' have potentially bad effect on our vegetables sources and many dishes. Thus, the 'ulam' must be thoroughly washed in order to minimize and prevent the mycotoxins problem to animal and human.

The production of MON by *F. oxysporum*, *F. proliferatum* and *F. semitectum* isolates act as a natural contaminant in our important grains. In addition, the production of FB_1 by *F. konzum*, *F. oxysporum* and *F. proliferatum* has also huge effect on production of rice and corn. These can cause contamination on natural or processed maize that have been used as human and animal food.

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