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Morphological and molecular characterization of *Trichoderma* species isolated from rhizosphere soils in Malaysia

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ABSTRACT

Aims: Knowledge of the *Trichoderma* taxa is important for both control efficiency and environmental conservation. Therefore, the objective of this study is to isolate and identify *Trichoderma* species from various rhizosphere soil samples using phenotypic and molecular characterization.
Methodology and results: Native *Trichoderma* spp. were isolated from agricultural fields in 17 sites from seven states of Malaysia. These isolates were characterized via morphological observation and molecular phylogenetic analysis based on the translation elongation factor-1α (tef1-α) gene. About 42 isolates were classified into eight *Trichoderma* species, which are *Trichoderma asperellum*, *T. hamatum*, *T. harzianum*, *T. koningiopsis*, *T. rodmanii*, *T. spirale*, *T. viride* and *T. virens*. Comparison of DNA sequences of tef1-α showed that the isolates were 98-100% similar to respective *Trichoderma* species from GenBank, thus confirming the fungal identity. Phylogenetic trees of maximum likelihood (ML) dataset of tef1-α inferred that the isolates were clustered according to species.
Conclusion, significance and impact of study: Findings in the present study will be beneficial for the purposes of biodiversity conservation and plant disease management using biocontrol agents.

Keywords: Filamentous fungi, morphology, translation elongation factor, *Trichoderma*, soil

INTRODUCTION

Trichoderma is a rhizocompetent filamentous fungi that free-living and can be found in all types of soil especially in agricultural soil (Samuels, 2006). They are genetically diverse and can be found on decaying wood, bark, and other plant-decomposed materials that may attribute to their diverse metabolic capability and aggressive competitive nature (Howell, 2003; Lorito *et al*., 2010). These characteristics make them significant decomposers of woody and herbaceous material and are necrotrophic against other decomposers. In addition, they are important for soil fertility (Contreras-Cornejo *et al*., 2009; Lorito *et al*., 2010). They are extremely helpful in maintaining soil function, can colonize the root and populate the rhizosphere (Ahmad *et al*., 2011). As soil fungi, *Trichoderma* can survive in various type of media such as top soil, mixed soil and some of the agro wastes where coconut fiber best promotes sporulation (Easa Hasan *et al*., 2020).

Trichoderma species are among the most studied fungal biological control agents and commercially marketed as biopesticides (Harman, 2000). *Trichoderma* can act as a secondary opportunistic invader, a fast-growing fungus, a strong spore producer, a source of cell wall degrading enzymes and important antibiotic producers (Vinale *et al*., 2008). *Trichoderma* also plays key roles in suppressing soil-borne plant diseases and promoting plant growth (Garbeva *et al*., 2004; Lorito *et al*., 2010). These diverse activities of *Trichoderma* render them a beneficial component of the soil ecosystem. Based on Suhaida and Nur Ain Izzati (2013), the application of *T. harzianum* T73s has successfully inhibited the Fusarium ear rot of maize.

Trichoderma spp. have recently received greater attention in nanotechnology such as in the synthesis of several bioactive inorganic nanoparticles (Guilger *et al*., 2017; Elamawi *et al*., 2018). On top of that, biodiversity conservation of fungi is underestimated although they are important agents influencing the biodiversity of an ecosystem. Therefore, this study was conducted to isolate *Trichoderma* species from various soil samples and to identify the isolates at species level using phenotypic and molecular characterization. The present study may provide useful isolates, which in the future can be used for disease management strategies in preventing diseases, enhance plant growth, and increasing the yields.

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MATERIALS AND METHODS

Soil sampling

Rhizosphere soils of different cultivated crops were obtained from 17 sampling locations in seven states (Kedah, Melaka, Pahang, Perak, Sabah, Selangor and Terengganu) throughout Malaysia (Table 1). The sampling locations were selected based on availability and accessibility to collect the soil samples. The soil samples (200 g) were collected in triplicate by using a sterile trowel at depth 10 cm within a radius of 0.5 m around the trunk or stem of plants. The soil samples were kept in an envelope paper and stored at 4 °C until being used.

Fungal isolation and purification

Fungal species were isolated from soil samples using dilution plating by mixing 10 g of soil with 100 mL sterile distilled water before agitating on a shaker (Infors HT) at 100 rpm for 10 min. The soil was diluted until 10^{-3} and every 1 mL of the final dilution from 10^{-1} until 10^{-3} diluted soil solution was pipetted into a Petri dish and was done in triplicate. About 9 mL of Rose Bengal agar (RBA) was poured into the Petri dish of diluted soil, swirled gently, and left to solidify. The soil plates were examined daily and fungal colonies that had been grown on RBA were subculture onto Potato Dextrose Agar (PDA). Single spore isolation was carried out on a new PDA to obtain the pure culture of *Trichoderma* isolates.

Morphological characteristics of *Trichoderma* species

The *Trichoderma* isolates were tentatively identified into the species level based on macro- and micromorphological characteristics and species confirmation by molecular analysis. For macromorphological observation, the isolates were grown on PDA. The colony feature, conidia shape and size, pigmentation and sporulation pattern were observed, and the growth rate was measured.

The side culture technique was used to observe the micromorphological features of *Trichoderma*. A block (1 cm^2) of PDA was placed on a sterile slide and then cultured with *Trichoderma* on all four sides of the agar block and covered with a coverslip. The culture was then incubated for 3 days (28 ± 2 °C) in a sterile glass Petri dish layer with damp filter paper. A sterile coverslip was put on the slide and then observed under a microscope. The slide culture was examined using a 40× magnification under a light microscope (Olympus CX 21, America Inc.). *Trichoderma* species were identified via microscopic observation of the morphology of conidia, conidiophore, phialides, and chlamydospore using taxonomic keys (Samuels *et al*., 2012).

Table 1: Locations of soil sampling with their respective crop.

State	City	Crop
Kedah	Langkawi	Paddy
Melaka	Telok Mas	Mango
Pahang	Maran	Rubber, oil palm
	Cameron Highland	Cabbage
Perak	Segari Bidor	Oil palm
Sabah	Kundasang	Banana
Selangor	Meru	Banana, rubber
	Tanjung Karang	Paddy
	Serdang	Banana, papaya
	Banting	Oil palm
	Hulu Selangor	
	Kajang	
	Semenyih	
	Dengkil	Oil palm, banana, jackfruit
Terengganu	Bukit Besi Ketengah	Oil palm

Translation Elongation Factor 1 Alpha (TEF-1α) sequence analysis

Isolates were grown on PDA and incubated at 28 ± 2 °C for 3 days. DNA was extracted using UltraClean® Microbial DNA Isolation Kit (MO BIO, Carlsbad, CA, USA), following the manufacturers protocol. The gDNA was stored in –20 °C. Translation Elongation Factor (tef) 1α region of genomic DNA of all *Trichoderma* isolates were amplified using a TProfessional Standard Thermocycler (Biometra Company). For tef-1α amplification, the PCR mixture was completed by using 25 µL reaction master mix that contains 5 µL of 5× PCR buffer, 1.25 µL of 0.5 µM primer, 2.5 µL of 0.2 mM deoxynucleotide triphosphate (dNTPs), 2.5 µL of 2.5 mM Magnesium chloride ($MgCl_2$), 0.125 unit of Taq Polymerase and 20 ng of the DNA template. A set of primer was used: EF1728F (5'-CATCGAGAAGTTCGAGAAGG-3') and TEF1LLErev (5'-AACTTGCAGGCAATGTGG-3') (Jaklitsch and Voglmayr, 2015). The PCR cycling for tef-1α was conducted as follows: initial denaturation at 94 °C for 85 sec, followed by 35 cycles of denaturation at 95 °C for 35 sec, annealing at 58 °C for 55 sec, extension at 72 °C for 90 sec, final extension at 72 °C for 10 min and kept at 4 °C until further use.

About 5 µL PCR products were loaded in 1.5% agarose gel with 0.1% FloroSafe DNA stain and undergone electrophoresis for 35 min at 90 V. The amplicon of tef-1α regions in size between 1.0-1.2 kb was determined based on its migration and conformation relative to the 1.0 kb molecular size marker (BIORON GmbH, Germany) and 6× Loading Dye (Thermo Fisher Scientific, Carlsbad, California). PCR products were purified using QIAGEN (QIAquick® Gel Extraction Kit) following the manufacturer's instruction. The purified PCR

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products of tef-1α were sequenced using an Applied Biosystem 3730xl DNA Analyzer (MyTACG Bioscience Enterprise, Selangor, Malaysia).

Sequence similarity searches were performed for each of the representative fungal sequences by BLAST and compared to the sequences in GenBank by using the Standard Nucleotide BLAST network services for similarities present in the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/) (Huang *et al*., 2009). ClustalW of MEGA X software was used to generate the consensus sequences to align the consensus sequence to each other and to the sequences in GenBank (Kumar *et al*., 2018). All the assembled sequences were deposited to GenBank, NCBI (http://www.ncbi.nlm.nih.gov/).

RESULTS AND DISCUSSION

A total of 42 isolates of *Trichoderma* were successfully obtained from rhizosphere soils of different crops that collected from 17 different sampling sites around Malaysia. The most frequently isolated species were *T. asperellum* (11 isolates) followed by *T. virens* (8 isolates), *T. harzianum* (7 isolates), *T. koningiopsis* (7 isolates), *T. viride* (4 isolates), *T. hamatum* (3 isolates), *T. spirale* (1 isolate) and *T. rodmanii* (1 isolate). The differences in macro- and micromorphological characteristics of eight *Trichoderma* species were summarized in Table 2. *Trichoderma* has gained immense significance since years ago which reflects to its biocontrol properties against various plant pathogens and their ability to promote plant growth. Until 2015, 256 names of *Trichoderma* species have been listed (Bissett *et al*., 2015).

Tef1 is one of the best-resolving markers used for species identification of *Trichoderma*, in categorized separation at the species level (Lorito *et al*., 2010; Jaklitsch and Voglmayr, 2015). The tef-1α region was successfully amplified and the amplicon size ranged between 1.0-1.2 kb (Figure 1). The sequences showed a value of 89-99% similarity with sequences in GenBank. The maximum-likelihood analysis resulted in the isolates of the same species that were grouped in the same cluster (Figure 2). Table 3 shows the accession numbers of *Trichoderma* isolates that have been deposited in Genbank (http://www.ncbi.nlm.nih.gov/).

The colonies of *Trichoderma* species proliferated on PDA with growth rate ranging from 2.00 to 2.80 cm/day and many isolates produced concentric rings which grow outwards from the center of the colonies. All of the fungi initially produced a pure white mycelium, which gradually turned to green, or yellow-green in colour except for *T. koningiopsis*, where all the isolates remain white, however, after being incubated more than 14 days the mycelia colour gradually turned to green. The pigmentation and the concentration of the phialospores gave rise to the green colour of the colony. The conidiophore branching structure and the conidial shape were variables between species (Table 2).

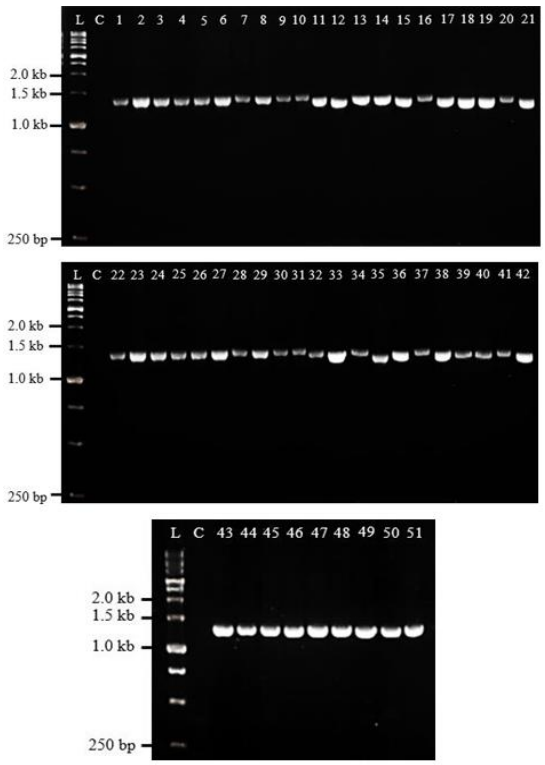

Figure 1: The banding pattern of *TEF*-1α gene amplification. Expected band size ranging from 1.0-1.2 kb. Lane 1-42: isolate A190s, A237s, B8s, B99s, B101s, B108s, B129s, B142s, B304s, B1581, B1584, B1881, B1890, B1895, B1896, B1902, B1952, B2115, B2230, B2235, C261s, C1665, C1667, C1932, K1968, K1970, M1891, T2005, T2007, T2014, T2018, T2023, T2031, T2034, T2037, T2040, T2045, T2052, T2073, S1972, S1984 and S1987. Lane L: Marker 1.0kb, Lane C: Control.

Macromorphology of *Trichoderma asperellum* in the PDA plate is sparse cottony from whitish mycelia to whitish green and then dull green in colour (Figure 3A-B). It also formed 1-2 concentric rings with green conidial production. The conidia production was denser in the center than towards the margins of the PDA plate. It has many green spores. *T. asperellum* was also a rapid growth colony on PDA that ranged between 20.0-25.0 mm/day and covered the full plate within four days. As shown in Figure 1, *T. asperellum* formed in repeatedly paired branches conidiophores along the main axis (Figure 3C-D). The phialospores of *T. asperellum* were subglobose to ovoid with smooth-walled (Figure 3E). *T. asperellum* also produced terminal or apical subglobose and granulated chlamydospores (Figure 3F). The phialides formed cylindrical shapes, which enlarged at the opposite side of the phialospores position (Figure 3G). The phialospores formed were cluster accumulated at the tips of phialides and formed a globose conidial head. Morphological of *T. asperellum* reported in this study agrees with a previous study by Wijesinghe *et al.* (2010).

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Table 2: Macro- and micromorphological characteristics of *Trichoderma* species.

Species	Phialospore		Phialides	Conidiophores	Chlamydospores	Colony colour on PDA
	Size (µm)	Shape				
T. asperellum	3.25-3.50 × 2.80-3.00	Subglobose to short ovoid with smooth walled	Cylindrical shape; enlarged on opposite side of the phialospores position	Repeated paired branches along the main axis	Apical subglobose and granulate	Whitish to dull green
T. hamatum	3.40-5.00 × 2.70-3.95	Ellipsoidal	Short swollen bottle-like pear shaped	Long and thick with short and thick side branches	Terminal and intercalary in globose with granulate	Whitish to yellowish green
T. harzianum	2.48-3.25 × 2.21-2.85	Subglobose to short obovoid	Cylindrical shape which swollen-like at the middle	Paired branches along the main mycelia axis	Terminal and intercalary in globose or oval	Whitish to dull almost dark green
T. koningiopsis	3.00-4.00 × 2.00-3.00	Ellipsoidal with smooth-walled	Long cylindrical shape	Formed in long branches	Terminal and intercalary in globose	Cottony tufted whitish and turned green
T. rodmanii	4.00-5.50 × 2.80-4.50	Elliptical-subcylindrical	Long cylindrical shape swollen near the tips	Paired branches on the tips	Terminal and intercalary in globose and oval with granulated	Whitish to slight dark green
T. spirale	3.40-4.50 × 2.30-2.55	Ellipsoidal	Cylindrical long shape	Repeated paired branches along the main axis	Terminal and intercalary in globose in shape with granulated	Whitish to slight dull green
T. virens	2.00-2.25 × 2.21-2.55	Globose in shape	Cylindrical shaped with enlarged at the uneven paired branched body	Uneven number paired branched of phialides	Terminal and intercalary in globose and oval in shape with granulated	Whitish to dull green
T. viride	3.30-3.50 × 2.50-3.05	Subglobose or obovoid	Cylindrical swollen and some were bend at the tips	Uneven paired phialides like whorled shaped	Terminal and intercalary in globose or oval in shape	Whitish to green yellow

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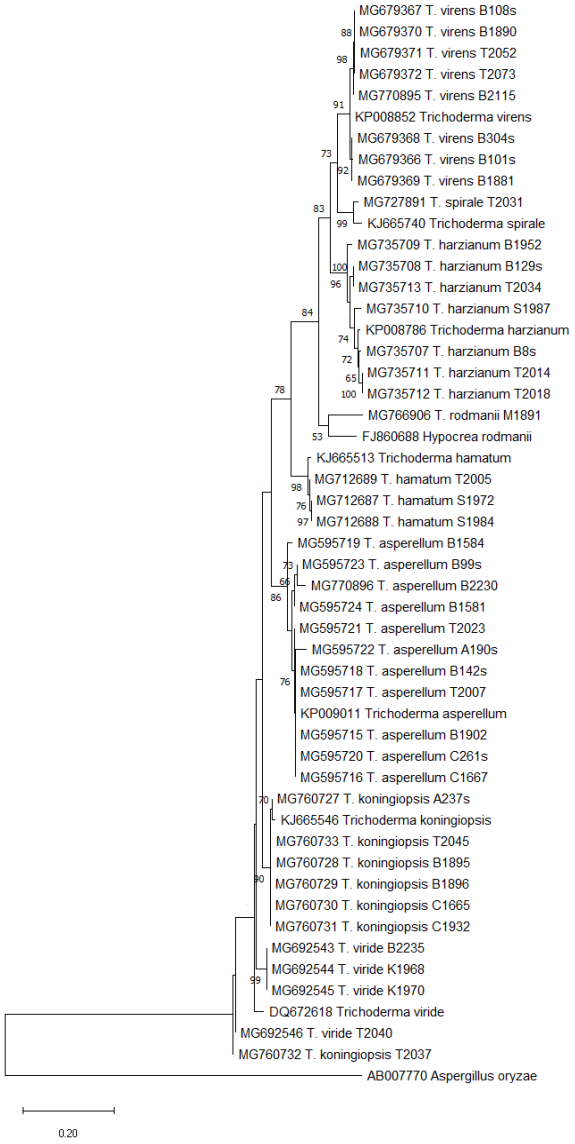

Figure 2: Phylogenetic tree generated from the Maximum Likelihood method based on the translation elongation factor 1-alpha sequences. The tree generated using Tamura-Nei model with bootstrap values of 1000 replications involved 42 sequences from *Trichoderma* isolates and an outgroup sequence of *Aspergillus oryzae*. All positions with less than 50% are not shown in the tree.

Macromorphology of *T. hamatum* in PDA plate is floccose sparse cottony from whitish at first then turned to yellowish-green in colour after more than 7 to 14 days (Figure 4A-B). The conidia production was denser in centre then towards the margins of PDA plate. *T. hamatum* growth rate on PDA ranged between 22.3-25.5 mm/day and cover the full plate within four days. The spores were produced gradually from yellowish to light green in colour at maturity. Conidiophores of *T. hamatum* were formed in long and thick with short and thick side branches (Figure 4C). The phialides were short swollen bottle-like pear-shaped (Figure 4D). The phialospores were ellipsoidal-shaped (Figure 4E). The conidia were oblong or ellipsoid, often with parallel sides, green, smooth shape. The chlamydospores present were at the terminal and intercalary in globose in shape with granulated (Figure 4F). For *T. hamatum*, in comparison with the research done by Jaklitsch and Voglmayr (2015), the characteristics of *T. hamatum* almost the same as the colony growth in yellow-brown or dull orange in colour on

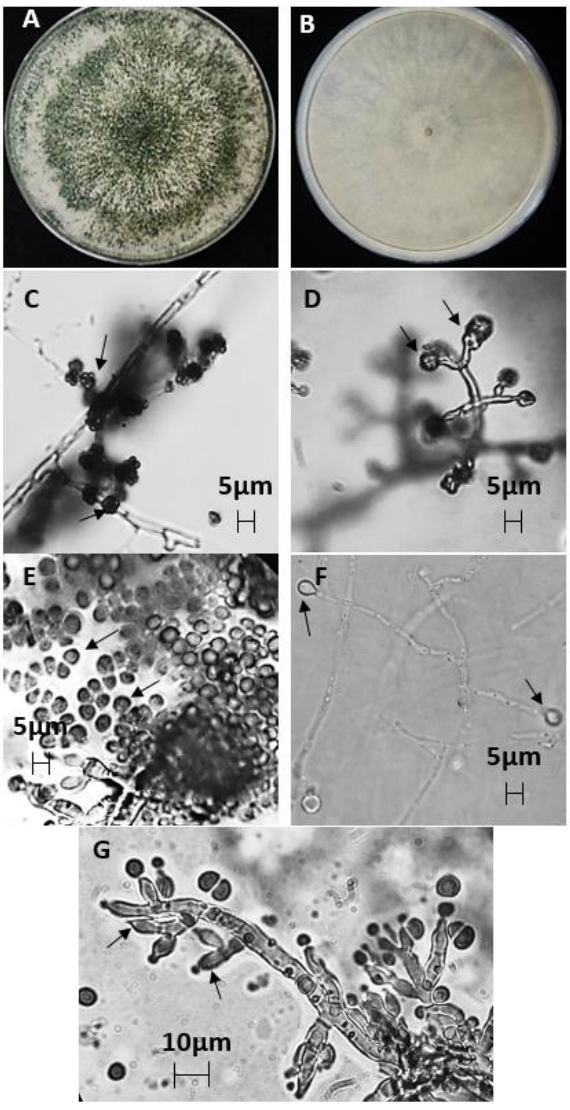

Figure 3: Morphological characteristics of *T. asperellum.* A-B: Colony features on PDA; C-D: Branches of conidiophores with spore masses; E: Phialospores (arrows); F: Chlamydospores (arrows); G: Phialides (arrow).

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Table 3: GenBank accession number of TEF 1-alpha *Trichoderma* isolates.

No.	Isolates	Scientific name	Origin	Crop	GenBank accession no.
1	A190s	*T. asperellum*	Bidor	oil palm	MG595722
2	A237s	*T. koningiopsis*	Segari	oil palm	MG760727
3	B8s	*T. harzianum*	Semenyih	oil palm	MG735707
4	B99s	*T. asperellum*	Banting	oil palm	MG595723
5	B101s	*T. virens*	Meru	rubber	MG679366
6	B108s	*T. virens*	Meru	rubber	MG679367
7	B129s	*T. harzianum*	Kajang	oil palm	MG735708
8	B142s	*T. asperellum*	Kajang	oil palm	MG595718
9	B304s	*T. virens*	Hulu Selangor	oil palm	MG679368
10	B1581	*T. asperellum*	Tanjung Karang	paddy	MG595724
11	B1584	*T. asperellum*	Tanjung Karang	paddy	MG595719
12	B1881	*T. virens*	Dengkil	oil palm	MG679369
13	B1890	*T. virens*	Dengkil	oil palm	MG679370
14	B1895	*T. koningiopsis*	Dengkil	banana	MG760728
15	B1896	*T. koningiopsis*	Dengkil	banana	MG760729
16	B1902	*T. asperellum*	Dengkil	banana	MG595715
17	B1952	*T. harzianum*	Meru	banana	MG735709
18	B2115	*T. virens*	Dengkil	jackfruit	MG770895
19	B2230	*T. asperellum*	Serdang	banana	MG770896
20	B2235	*T. viride*	Serdang	banana	MG692543
21	C261s	*T. asperellum*	Cameron Highland	cabbage	MG595720
22	C1665	*T. koningiopsis*	Maran	oil palm	MG760730
23	C1667	*T. asperellum*	Maran	oil palm	MG595716
24	C1932	*T. koningiopsis*	Maran	oil palm	MG760731
25	K1968	*T. viride*	Langkawi	paddy	MG692544
26	K1970	*T. viride*	Langkawi	paddy	MG692545
27	M1891	*T. rodmanii*	Telok Mas	mango	MG766906
28	S1972	*T. hamatum*	Kundasang	banana	MG712687
29	S1984	*T. hamatum*	Kundasang	banana	MG712688
30	S1987	*T. harzianum*	Kundasang	banana	MG735710
31	T2005	*T. hamatum*	Bukit Besi	oil palm	MG712689
32	T2007	*T. asperellum*	Bukit Besi	oil palm	MG595717
33	T2014	*T. harzianum*	Bukit Besi	oil palm	MG735711
34	T2018	*T. harzianum*	Bukit Besi	oil palm	MG735712
35	T2023	*T. asperellum*	Bukit Besi	oil palm	MG595721
36	T2031	*T. spirale*	Bukit Besi	oil palm	MG727891
37	T2034	*T. harzianum*	Bukit Besi	oil palm	MG735713
38	T2037	*T. koningiopsis*	Bukit Besi	oil palm	MG760732
39	T2040	*T. viride*	Bukit Besi	oil palm	MG692546
40	T2045	*T. koningiopsis*	Ketengah	oil palm	MG760733
41	T2052	*T. virens*	Ketengah	oil palm	MG679371
42	T2073	*T. virens*	Ketengah	oil palm	MG679372

PDA. The microscopic observation of *T. hamatum* was typical for pachybasium-type conidiophores with ampulliform phialides.

Trichoderma harzianum on PDA plate rather lose or compact cottony tufts from whitish at first mycelia growth then to dull that dark green in color when increasing time. It formed 1-2 concentric rings with dark green conidial production (Figure 5A-B). The conidia production was denser in centre then towards the margins of PDA plate. Its produced no distinguishes odour however when being incubated in more than 14 days, some isolates will emit some pungent odour like 'coconut'. *T. harzianum* is rapid growth colonies on PDA that ranged between 21.0-25.0 mm/day and covers the full plate within four days. Conidiophores of *T. harzianum* were formed in paired branches along the main mycelia axis (Figure 5C). The phialides naturally bend towards the apex and formed cylindrical shape which swollen-like at the middle (Figure 5D). The phialospores formed were abundant and accumulated at the tips of phialides and formed globose conidial head. The phialospores of *T. harzianum* were subglobose and short obovoid in shape (Figure 5E). The chlamydospores were at the terminal and intercalary in globose or oval (Figure 5F). For microscopic observation of *T. harzianum*, almost the same with Suhaida and Nur Ain Izzati (2013). Their mycelium, initially of a white color, acquired green, yellow shades, or remained white, due to

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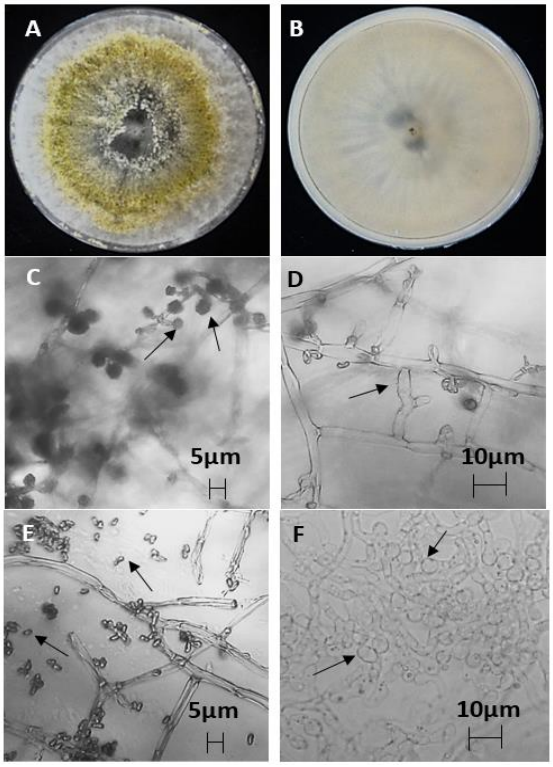

Figure 4: Morphological characteristics of *T. hamatum*. A-B: Colony features on PDA; C: Branches of conidiophores with spore masses; D: Phialide (arrow); E: Phialospores (arrows); F: Chlamydospores (arrows).

the abundant production of conidia, which presents subglobous to ellipsoid conidia, ampuliform phialides.

Macromorphology of *T. koningiopsis* in PDA plate is cottony tufted whitish in colour (Figure 6A-B); sometimes when incubated more than 14 days if formed slightly green in colour indicates green conidia. *T. koningiopsis* colonies growth on PDA was ranged between 20.5–24.7 mm/day and covers the full plate within four days. Conidiophores of *T. koningiopsis* were formed in long branches (Figure 6C). The phialides were long than *T. harzianum* and *T. asperellum* (Figure 6D). The phialospores were ellipsoidal with smooth-walled (Figure 6E). The chlamydospores present were at the terminal and intercalary in globose in shape with granulated (Figure 6F). Based on Qian *et al.* (2013), *T. koningiopsis* (strain F13V-2) was firstly reported as pathogen of leaf blight disease of *Curcuma wenyujin* in China, the growth of *T. koningiopsis* on the PDA were the same, which is white mycelium. However, the colonies grew up to about 54 mm in diameter within 33 hours and turned light green after being incubated for 72 hours different with obtained *T. koningiopsis* which can only turned to green after incubation more than 14 days. Conidia were green, smooth, ellipsoid, 3-4 × 2-3 µm in size (Qian *et al*., 2013).

In PDA plate, *T. rodmanii* culture is floccose tufted from whitish to slight dark green in colour, sometimes when incubate more than 14 days if formed powdery green conidia. The conidia production was denser outside then toward the margins of PDA plate (Figure 7A-B). *T. rodmanii* colonies growth on PDA was ranged between 21.5-25.5 mm/day and covers the full plate within four days. Conidiophores of *T. rodmanii* were in paired branches on the tips (Figure 7C). The phialides were longer than *T. harzianum* and *T. asperellum* (Figure 7D). The phialospores were elliptical-subcylindrical (Figure 7E). The chlamydospores present were at the terminal and intercalary in globose and oval with granulated (Figure 7F). In comparison with previous study done by Degenkolb *et al*. (2008), *T. rodmanii* has slower rate of growth, but the strains obtained having smaller globose conidia and phialides and partially sterile conidiophores to distinguish this species.

Trichoderma spirale shown macromorphology in PDA plate is cottony floccose tufted from whitish to slight dull green in colour at the centre, when incubated more than 14 days the dull green was expand from the centre to the margins. It formed 1-2 concentric rings with green conidial production at the centre (Figure 8A-B). *T. spirale* colonies growth on PDA was ranged between 21.5-25.5 mm/day and covers the full plate within four days. Conidiophores of *T. spirale* were formed in long and

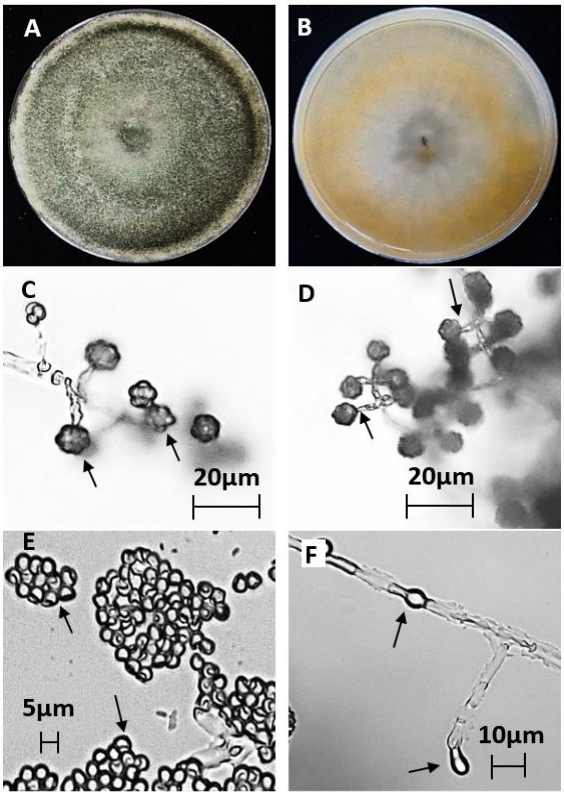

Figure 5: Morphological characteristics of *T. harzianum*. A-B: Colony features on PDA; C: Branches of conidiophores with spore masses; D: Phialides (arrows); E: Phialospores (arrows); F: Chlamydospores (arrows).

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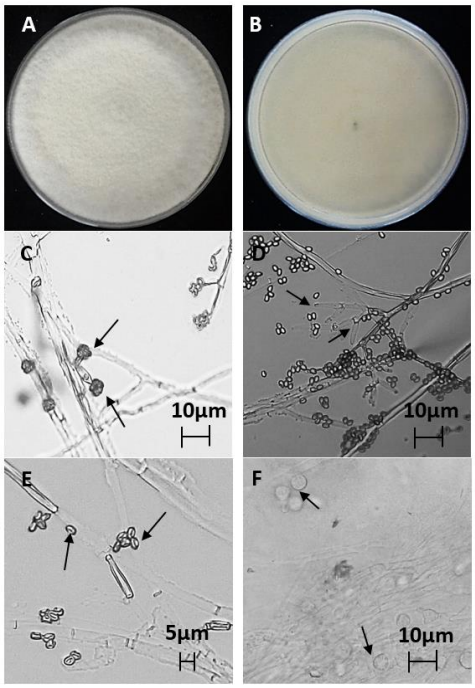

Figure 6: Morphological characteristics of *T. koningiopsis*. A-B: Colony features on PDA; C: Branches of conidiophores with spore masses; D: Phialides (arrows); E: Phialospores (arrows); F: Chlamydospores (arrows).

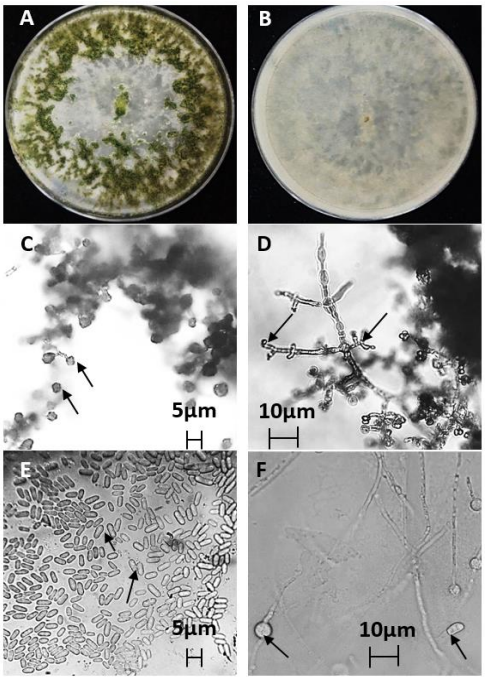

Figure 7: Morphological characteristics of *T. rodmanii*. A-B: Colony features on PDA; C: Branches of conidiophores with spore masses; D: Phialides (arrows); E: Phialospores (arrows); F: Chlamydospores (arrows).

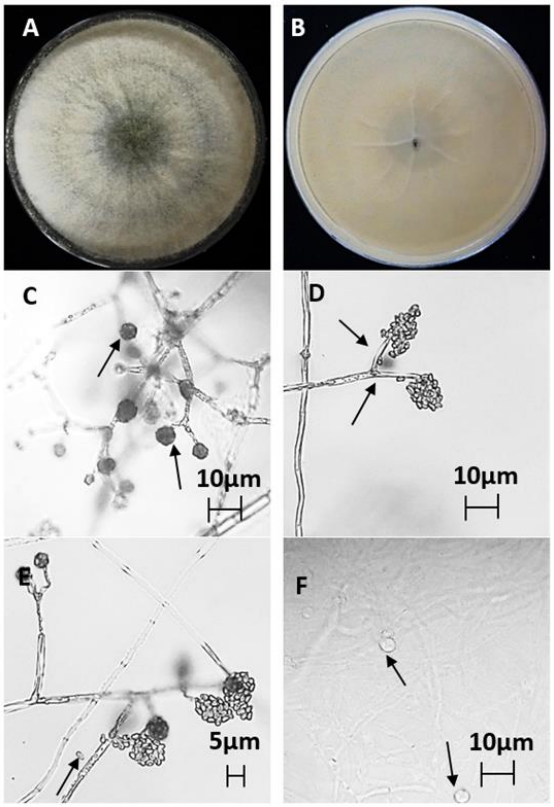

Figure 8: Morphological characteristics of *T. spirale*. A-B: Colony features on PDA; C: Branches of conidiophores with spore masses; D: Phialides (arrows); E: Phialospores (arrow); F: Chlamydospores (arrows).

repeatedly paired branches along the main axis (Figure 8C). The phialides were similar structured as *T. koningiopsis*, which are long and paired in the tip of branches (Figure 8D). The phialospores were ellipsoidal (Figure 8E). The chlamydospores present were at the terminal and intercalary in globose in shape with granulated (Figure 8F). Jang *et al.* (2017) reported *T. spirale* strains obtained from the studied showed greyish green to dark greyish green and some strains with olive yellow pigment and abundant of aerial mycelium on PDA. The conidial production forming in broad concentric rings. The conidia are smooth, oblong to ellipsoidal in size of 4.1-5.1 × 2.5-2.8 µm. The chlamydospores were not observed. The conidiophores are broad fertile branches arising from the base. The phialides arising in dense clusters, nearly doliiform, short and wide at the base.

Macromorphology of *T. virens* in PDA plate is fluffy cottony tufted from whitish to dull green in colour. It also formed 1-2 concentric ring(s) with dull green conidial production. The conidia production was denser at the concentric ring at the centre and towards the margins of PDA plate (Figure 9A-B). *Trichoderma virens* is rapid growth colonies on PDA that ranged between 20.5-24.5 mm/day and covers the full plate within four days.

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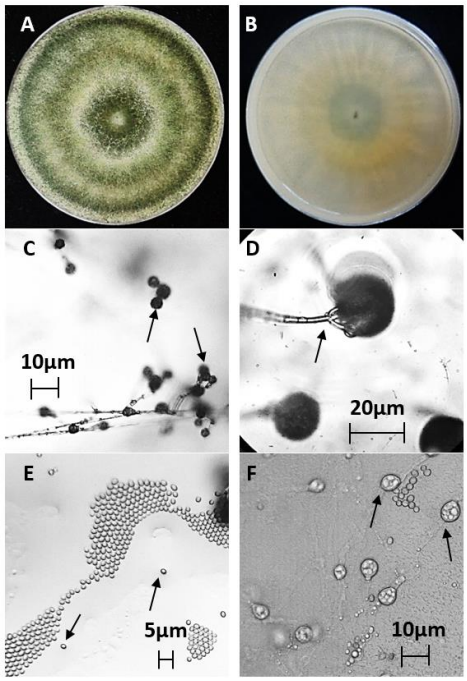

Figure 9: Morphological characteristics of *T. virens*. A-B: Colony features on PDA; C: Branches of conidiophores with spore masses; D: Phialide (arrow); E: Phialospores (arrows); F: Chlamydospores (arrows).

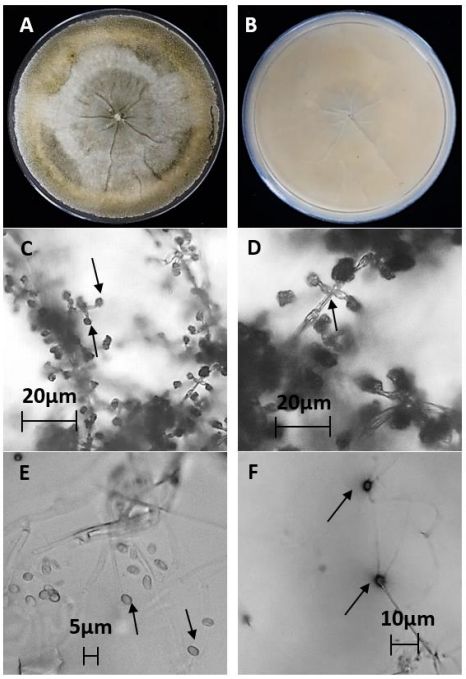

Figure 10: Morphological characteristics of *T. viride*. A-B: Colony features on PDA; C: Branches of conidiophores with spore masses; D: Phialide (arrow); E: Phialospores (arrows); F: Chlamydospores (arrows).

Conidiophores of *T. virens* were formed uneven paired branched of phialides (Figure 9C). The phialides were cylindrical shaped with enlarged at the branched body (Figure 9D). The phialospores were cluster accumulated at the tips of phialides in globose (Figure 9E). The chlamydospores presented were at the terminal and intercalary in globose and oval with granulated (Figure 9F). In comparison with Odeniyi *et al*. (2012), the conidia of *T. virens* appear dry but in some strains, they may be held in drops of clear green or yellow liquid. Typically, conidia of most strains were globose and smooth.

Trichoderma viride macromorphology in PDA plate is loose floccose cottony tufted from whitish to green-yellow in colour. It formed 1-3 concentric rings with green conidial production (Figure 10A-B). The conidia production was denser at the concentric ring whether at the centre and at the margins of PDA plate. *T. viride* growth colonies on PDA ranged between 21.0-25.0 mm/day and cover the full plate within four days. Conidiophores of *T. viride* were formed in uneven paired phialides like whorled shaped (Figure 10C). The phialides were cylindrical swollen near the tips it almost exactly like *T. koningii* but some of the *T. viride* phialides were bend at the tips (Figure 10D). The phialospores of the *T. viride* were subglobose or obovoid (Figure 10E). The chlamydospores were at the terminal and intercalary in globose or oval (Figure 10F). Based on research done by Shah *et al.* (2012), in comparison, *T. viride* appeared a bit granular with green conidia distributed, an irregular yellow zone without conidia was present and white pustules were found on the green conidia. For microscopic characterization, the conidia of *T. viride* were globose with size of 3.0 × 2.8 µm.

CONCLUSION

According to the results obtained in this work, *Trichoderma* isolates in the agricultural soil in Malaysian ecosystems are diverse. The studies of those *Trichoderma* isolates on their potential antagonistic interaction can be explored in the future for improving environmental health.

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