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Cultural and Morphological Characterisations of Fusicoccum *sp., the Causal Agent of Rubber* (Hevea brasiliensis) *Leaf Blight in Malaysia*

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Leaf blight caused by Fusicoccum *sp. is becoming a serious canopy disease of rubber trees in Malaysia. Despite its increasing significance, information regarding the biology of the causal fungus is scarce. This study provides morphological data useful for its characterisation. Fungal isolates obtained from leaf samples collected from several rubber plantations in Selangor, Johor and Perak were compared in terms of their behaviour in various cultural media potato dextrose agar (PDA), Czapex-dox agar (CDA), corn meal agar (CMA) and malt extract agar (MEA). Furthermore, their incidence on rubber leaves was assessed. In all media and different pH tested, significant differences were observed in spore production, dimension of conidiomata and growth rates among isolates. On PDA, pH 6 supported the best growth compared to other media used. The growth rate was 16.8 mm/day on PDA and MEA, against 14.4 mm / day on CDA and 11.4 mm/day on CMA. The highest spore production (161.33 × 10^6 spores / mL) was obtained on PDA. The optimum temperature for spore germination in water agar was 24ºC - 28ºC within a period of eight hours. All fungal isolates tested were pathogenic to* H. brasiliensis *but showed variation in symptoms.*

Keywords: leaf blight; *Hevea brasiliensis*; *Fusicoccum* sp.; sporulation; disease incidence

The first report of *Fusicoccum* infection on rubber in Malaysia was by Radziah and Chee[1]. The fungus causes large lesions with concentric brownish zone and rusty brown pin head size spots on rubber leaves. Infected young leaves fall about four months later. As new clones were planted in new localities, this disease which first appeared in 1987 in Johor[1] has spread to Perak and Selangor in 2003. *Fusicoccum* leaf blight was recently classified among the five major rubber canopy diseases in Malaysia[2] and is now, reported[3] to be present in all the rubber growing areas. The disease affects several clones such as, PR 261,

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PB 260, PB 217, PB255, RRIM 600, RRIM 2023, RRIM 2024, RRIM 2025, KT 35/39 and PB 350[1,4].

The genus *Fusicoccum* is known as a cosmopolitan fungus that can cause canker, leaf blight, fruit rot, twig dieback and blossom blight on deciduous woody plants[5–7]. It has been reported as a pathogen of fully expanded leaves of *Hevea brasiliensis* in Malaysia[1]. It has been suggested as the anamorph of *Botryosphaeria* spp. such as *B. ribis*, *B. dothieda* Grossenb. and Duggar[8–10]. *Fusicoccum* sp., which belongs to the family of Botryosphaeriaceae is considered as a threat to forest species because of its role as endophyte in healthy plants and causing disease after stress[11]. Due to the large variation in morphology of this family of fungi, the aim of our study was therefore to identify isolates of *Fusicoccum* from rubber leaves in Malaysia and to determine the disease prevalence on rubber clones. This information will be valuable in the disease management of rubber clones and also for the evaluation of pathogen genetic variability.

MATERIALS AND METHODS

Collection of Disease Samples

Leaves showing symptoms of blight were collected from five rubber clones in three locations in West Malaysia from July to September 2010 (*Table 1*). Leaves were noted according to their clones and year of planting. Ten infected leaves were collected from each of ten trees per clone in each location. They were wrapped in paper, kept in labelled polythene bags and isolation procedures were conducted not later than one day after they were brought to the laboratory.

Identification of the Causal Fungus

Colony: From each diseased leaf, thin sections (5 mm in diameter) of infected tissues with some adjacent healthy tissues were cut in small pieces (*Figure 1a*). The sections were surface sterilised by dipping in 70% ethanol for 1 min, rinsed in sterile distilled water, dried on sterile blotting papers (Chmlab group, Spain) and placed on potato dextrose agar (PDA) supplemented with 1mg mL^{-1} streptomycin to suppress bacterial growth[12,13]. Plates were incubated under continuous white fluorescent light for 3 days at 25ºC. Once a full growth was obtained, the mycelia underwent repeated sub-cultures, until pure cultures were obtained.

Identification: From culture description[1], all cultures with a peripheral morphology resembling the genus *Fusicoccum*, initially white aerial and cottony mycelia appearance,

TABLE 1. ORIGIN OF FUSICOCCUM ISOLATES OBTAINED FROM MATURE RUBBER LEAVES IN THREE DIFFERENT LOCATIONS IN WEST MALAYSIA

Location State	Area	Clone	Year of planting	Code
Selangor	Sg. Buloh	RRIM 2024	2004	SB3
Johore	Penawar	RRIM 2023	2004	SJ2
Johore	Penawar	KT 39/35	2005	SJ4
Perak	Sg. Biong	RRIM 2024	2004	SK3
Perak	Sg. Biong	PB 350	2005	SK1

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with spore bearing cavity surrounded by a pycnidium, were transferred to fresh petri dishes containing PDA[14]. After three days, mycelia were scrapped under laminar flow by gently touching the edge of a clean microscope slide over the surface of the whole colony. The petri dishes were re-sealed then placed under continuous light to produce conidia. The average lengths and width of 100 conidia were measured under light microscope (Olympus Optical, Model CX31RBSF) for each representative isolate.

CULTURAL CHARACTERISATION

Effects of Media on Mycelia Growth

Agar plugs of five mm diameter (D_a) containing mycelia from each of the five representative isolates were taken from actively growing margin of a three–day–old culture and were placed centrally on four artificial media *viz* potato dextrose agar (PDA) (Difco), Czapex-dox agar (CDA) (Difco), corn meal agar (CMA) (Difco) and malt extract agar (MEA) (Difco) to study the growth rate, size, shape and colour of the conidia, appearance and texture of the mycelium. Cultures were incubated at room temperature (26ºC ± 2ºC). The experiment was arranged in a completely randomised design with three replications for each medium and each isolate. Colony diameter of each isolate of the fungus was measured daily in two perpendicular direction (D_1 and D_2) without opening the petri dishes, until the plate was fully covered with mycelia. The daily fungal growth rate (D_a) of each isolate at each media was computed for each replication as a difference between the mean diameter and the diameter of the agar plugs and expressed as mm/day[15]. The radial growth rate (mm/day) for each media tested was calculated for each replicate from the linear regression slope by univariate analysis of variance using SPSS version 17.

Effects of pH and Media on Sporulation

Based on the results of growth rates on these four media, PDA and CDA provide ideal conditions for conidia as they support abundant production of spores. Each medium was prepared separately by pouring 15 mL of media into each petri dish (9 cm diameter); their pH levels were adjusted with either lactic acid (CH3.CHOH.COOH) or potassium hydroxide (KOH)[16] to get the required pH 5, 6, 7, and 8. The pH of the media was adjusted by using a pH meter and the media was sterilised in autoclave at 121ºC, for 20 minutes. Agar plugs of five mm diameter containing mycelium were transferred onto the middle of each plate at different pH levels. The inoculated plates were incubated and kept at 25ºC for five days. Six day old cultures were scraped, sealed back and kept under continuous light to induce sporulation. After twelve days, 10 mL of sterilised distilled water was added on each the petri dish tested, and slowly swirled for three to five minutes. The number of spores per mL was determined by placing a drop of 0.01 mL solution on a haemocytometer and examined under a microscope (Nikon Model Eclipse E200).

Spore Germination

Production of one germ tube from the conidia was assessed using modifications by Melo and Mello[17]. Spores from isolates SK3, SB3, SK1 SJ2, and SJ4 were evaluated for their germination capacity. 100 µL of homogenous conidial suspension of the test fungus containing 200 spores mL^{-1} in sterile distilled water were spread on each petri dish containing water agar (WA) and incubated at 15, 20, 25, 30, and 35ºC. Four dishes for each isolate and for each temperature were removed after 4, 8, 12, 16, 20 and 24 hours. Germination was determined by visualising 100 conidia on microscopic fields of 400X magnification

and photographs of conidia germinating were captured by light microscopy (Nikon Model Eclipse E200).

Pathogenicity of *Fusicoccum*

Pathogenicity tests were conducted on recent series clones RRIM 2000 (RRIM 2002, RRIM 2007, RRIM 2023, and RRIM 2025) developed by Nursery Perbadanan RRIMCORP Ladang 5, Rubber Research Institute of Malaysia, Sungai Buloh to score their susceptibility to *Fusicoccum*. The information generated would be useful to optimise the utilisation of these new clones with regards to damages inflicted on leaves[18]. Clones PB 260 and PB 350 were chosen for the purpose of comparison with previous clones' series[1].

Detached Leaf Assay

Following modified method by Ismail and Indran[19], detached leaflets, at limp green stage of rubber (*Hevea brasiliensis*) clones were floated on water in 15 cm diameter petri dishes. For inoculation, spore suspension of four isolates originated from different clones (SB3, SJ2, SJ4, and SK1) were prepared from 12 day old cultures of each isolate grown on PDA plates and the concentration was adjusted at 3 × 106 spores/mL using haemocytometer. The leaflets were inoculated with micro-pipettes by placing ten droplets of the spore suspension onto the abaxial side and placed in a humid condition (20ºC) with 70%–80% relative humidity under continuous fluorescent light. Four replicates of leaves were sprayed with each spore suspension and control leaves were inoculated with distilled water. Scoring of the intensity of necrotic lesion size was carried out 5, 10, 15, and 20 days after application of treatments. The severity of the infection was rated following a 5-level grading scale with 0 = leaves without lesion (no symptom), 1 = 1-25% lesions (light symptom), 2 = 25-50% lesions (moderate symptom), 3 = 50-75% lesions (severe symptom), 4 = 75-100% lesions (very severe symptom). For each isolate, the mean number of lesions per leaflets in each experimental unit (four leaflets) was computed and expressed as disease severity index (DSI) according to the following formula:

DSI= [(Number of lesions developed / number of inoculated spots)] × 100%).
The disease index (DI) for each leaf was calculated using the formula[20]:

$$\mathrm{DI} = \left(\sum_{i=0}^{4} N_i \times i\right) \Big/ \sum_{i=0}^{4} N_i \qquad \ldots 1$$

Where *i* is severity (0 to 4) and *Ni* is the number of leaves with the severity of *i*.

Leaves bearing disease symptoms were re-isolated to confirm the presence of the pathogen based on spore morphology.

Rubber Seedlings

Tests were conducted with the same four isolates (SB3, SJ2, SJ4, and SK1) in the glasshouse on plants grown in polythene bags. These seven month old seedlings of *Hevea brasiliensis* clones (RRIM 2002, RRIM 2023, RRIM 2025, RRIM 2007, PB260 and PB350) were inoculated with *Fusicoccum* on their young leaflets at brown to light green stage by spraying 50 mL of the spore suspension per plant. Inoculation was done during cool morning hours. To create favourable conditions for infection, each seedling was covered with a white moist polythene bag and dampened cotton wool was wrapped around the central internodes of the stem. The polythene bag was removed after 24 h and the inoculated seedlings were maintained for two days at the same temperature. Seedlings

were then transferred into a greenhouse at ambient temperature (24ºC to 28ºC) and 70% to 80% relative humidity was controlled by watering the seedlings. Control seedlings of un-inoculated plants which were irrigated with only distilled water were included. There were three replicates for each of the six clones tested, arranged in a completely randomised design. The blighted leaves were recorded for each isolate per clone 5, 10, 15 and 20 days after inoculation respectively following the scale (0 to 4). Seedlings were examined for symptoms four weeks after inoculation and compared with common field symptoms. Seedlings bearing disease symptoms were re-isolated to confirm the presence of the fungus.

EXPERIMENTAL DESIGN AND DATA ANALYSIS

All the experiments were performed in completely randomised design (CRD) in three replications. For the morphological study, radial growth rate (mm/day), sporulation (spores/mL) and germination data were recorded. The linear regression slope was subjected to univariate analysis of variance with statistical package SPSS version 17. For the pathogenicity test, the analysis of variance was performed on disease index (DI) data using the ANOVA procedure of SPSS. Statistical significance was tested at $p < 0.05$ level. Where analysis revealed significant difference, Tukey HSD test for separation of means was performed.

RESULTS

Disease Symptom Description

The disease was usually initiated on young leaves developing later into symptoms such as brown spots on the midrib of the leaves. Infection expands and becomes a somewhat elongated black lesion then leading to the blighting of the leaf. Leaves located in damp and moist areas are the first to be infected usually from the leaf margins (*Figure 1a*). Small spots will change from light to dark brown often separated from healthy tissue by a band of crimson colouration. These spots will coalesce to form larger necrotic areas on leaves (*Figure 1b*). The characteristic symptoms on older leaves are the appearance of undulated margins and a concentric brownish zone with embedded pycnidia on the upper surface (adaxial) of leaves. The infected leaf will defoliate with perforations observed on the surface, veins and margins to necroses of the leaves. Severely infected leaves will become desiccated, defoliated or persist abnormally on the branches.

Identification of the Causal Agent

Isolates: Five isolates were identified and considered representative of the *Fusicoccum* sp., based on colony colour and conidial morphology. In general, all the isolates from each media were morphologically similar and all media were suitable for growth of the fungus. However, there were some variations on macroscopic appearance of the four media used as shown in *Table 2*.

Mycelia. Colony edge was well defined, dark colored tuft appeared at the centre of the media (*Figure 2a*) with maximum colony growth at 5 to 8 days after incubation.

Pycnidia. Scattered black dots were observed on PDA and CDA indicating pycnidia production usually appearing after 8 days. Cultures were scraped at 5 days after incubation and exposed to continuous light. On MEA there were less dots observed and appearing only after 14 days. When the colony became slightly darker, these become more abundant. However, on CMA all isolates

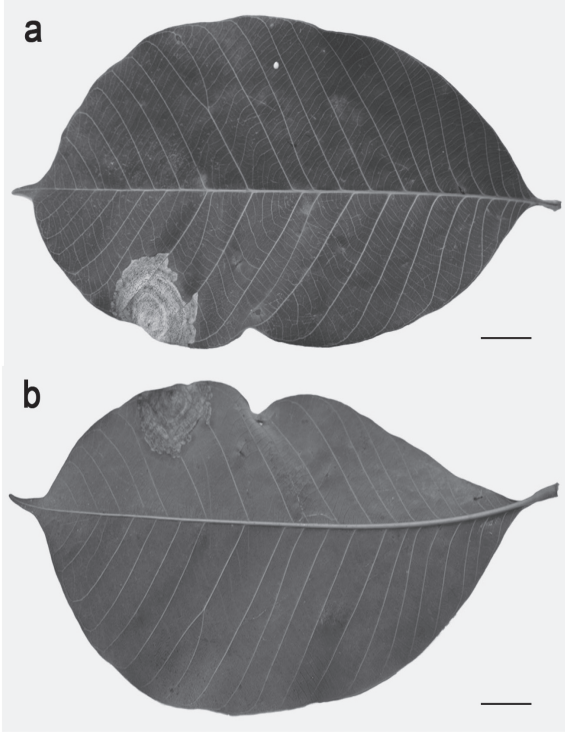

Figure1. Infected leaflet of H. brasiliensis *by* Fusicoccum *sp. (a) Adaxial of leaf showing undulate margins and a concentric brownish zone. (b) Brown developing spots visible abaxial of leaf surface. Bars = 15 mm.*

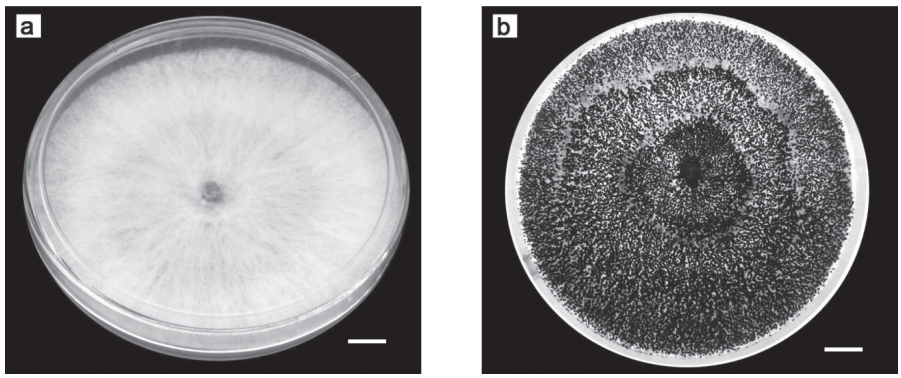

Figure 2. Fusicoccum *sp. culture on potato dextrose agar. (a) Aerial mycelium well-defined and highly branched after five days. (b) Pycnidia were produced after twelve days. Bars = 10 cm.*

TABLE 2. SUMMARY OF MACROSCOPIC APPEARANCE OF *FUSICOCCUM* ISOLATES

Parameter	Medium			
	PDA	CDA	MEA	CMA
Colour	White to grey	Faint white to slightly dark	White to olive green	Faint white
Mycelium	Aerial and cottony	Flat	Aerial	Flat and thin
	Highly branched	Highly branched	Branched	Branched
Growth (day)	5	6	6	7-8
Conidia	Very abundant	Very abundant	Abundant	Less abundant

produced pycnidia slightly later than all other media at 20 days after incubation. The best production of conidia was achieved on PDA and CDA.

The daily radial growth of fungus isolates and statistical comparisons are presented in *Figure 3*, *Table 3a* and *b*. Growth rates of the isolates on four media at 26ºC showed full coverage within 5 days on PDA, within 6 days on CDA and MEA, and 7 to 8 days on CMA. Growth rates were 16.8 mm/ day on PDA, 14.5 mm /day on CDA, 11.9 mm/ day on MEA and 16.4 mm/ day on CMA.

Conidia: Younger macroconidia of 12 to 15 days old pycnidia of isolates appeared similar on different media. There were hyaline in colour, variable in shapes (oval-shaped, elliptic, ovaloid or subglobose) and aseptate. Older macroconidia of more than 2 weeks were 1-2 septate. Conidia generally were aseptate, thin brown and globose (*Figure 4*). The variation of conidia size among isolates was observed and the ratio (length / width) of these shapes differed among isolates (*Table 4a*). Based on measurements of 100 conidia size of isolates SB3 and SK3 gave the highest length (18.0 µm) compared to isolate SJ2 which gave (14.2 µm) and highest width (5.2 µm). Meanwhile, SK1 registered the lowest (4.0 µm) (*Table 4b*).

Sporulation: *Fusicoccum* sp. sporulation on CDA and PDA occured at different pH (*Figures 6* and *7*). CDA produced the highest spore number at pH 7 and the lowest at pH 5 (*Figure 6*), while the highest spore number production was at pH 6 and the lowest was observed at pH 8 on PDA (*Figure 7*). Isolate SB3 gave the highest number of spores (91.7 $\times$ 10^6 spores/mL) and SJ4 gave the lowest number for all levels of pH tested (*Figure 6*). In comparison to CDA, isolate SJ2 gave the highest number of spores on PDA (161.3 $\times$ 10^6 spores/mL) while SJ4 gave the lowest one (85.7 $\times$ 10^6 spores/mL) at all levels of pH tested (*Figure 7*).

Conidia Germination

Most of the macroconidia of all the isolates germinated on water agar (WA) between 15ºC and 35ºC (*Figure 8*). At 25ºC, the percentage of germinated spores was more than 50% for all the isolates. There was statistical significance ($p < 0.001$) difference between isolates and temperatures and between isolates and incubation periods (*Figure 8*, *Table 5*). The germination of conidia began 4 h after incubation at all the temperatures, and reached a peak at 12 h incubation for isolates SB3, SJ2, and SK1, and after 16 h for isolates SJ4 and SK3 (*Table 5*). Isolate SJ2 recorded the highest percentage of germination (72.6%) followed by isolate SJ4 (53%). In general, the highest percentage of germinated spores was observed between 24ºC and 28ºC. Some of these macroconidia were aseptate while others had one septum. One to two germ

TABLE 3a. RADIAL GROWTH RATES (MM / DAY) OF ISOLATES OF *FUSICOCCUM* ON CULTURE MEDIA

Isolate	Radial growth rate *(mm / day)			
	PDA	CDA	CMA	MEA
	Mean ± s.e	Mean ± s.e	Mean ± s.e	Mean± s.e
SB3	16.7 ±2.32 b	14.5 ±2.70 ab	12.5 ±0.77 a	16.2 ±2,62 b
SJ2	17.1 ±2.11 c	14.5 ±2.82 b	12.0 ± 1.11a	16.3 ±2.52 bc
SJ4	16.9 ±2.53 b	13.7 ±5.08 ab	11.7 ±1.73 a	16.1 ±1.97 b
SK1	16.7 ±2.49 b	14.6 ±2.38 b	11.5 ±1.39 a	16.5 ±2.12 b
SK3	16.8 ±2.46 b	14.9 ±2.71 b	11.8 ±1.35 a	16.9 b±2.52 b

*Means followed by the same letters within each column are not significantly different at P<0.05

TABLE 3b. SUMMARY OF ONE WAY ANOVA FOR RADIAL MYCELIA GROWTH (MM /DAY) WITH MEDIA AND ISOLATE AS SOURCE OF VARIATION.

Source of variation	df	MS	VR	Probabilities
Media	3	308.469	44.477 ***	0.0001
Isolate	4	1.70	0.225 ns	0.912
Media x Isolate	12	1.26	0182 ns	0.999
Replication	2	0.762	0.110 ns	0.896

ns not significant, *** significant at p < 0.001, df = degree of freedom, MS = mean square, VR = variance ratio.

TABLE 4a. MEANS AND RANGES OF DIMENSIONS IN LENGTHS AND WIDTHS OF 10 TO 15 DAYS OLD CULTURE OF *FUSICOCCUM* SP. CONIDIA (n =100)

Isolates	Length (µm)		Width (µm)		Ratio
	Mean ± s.e	Range	Mean ± s.e	Range	Length/Width
SB3	18.04±2.96c	14.40-24.00	5.25±1.09c	3.92 - 8.10	3.49
SJ2	14.24±1.31a	9.00 - 17.70	4.53±0.70b	2.94 - 5.50	3.23
SK3	18.06±2.92c	13.40 - 23.06	5.27±0.97c	4.20 - 8.10	3.46
SJ4	17.02±4.14bc	6.00 - 24.00	5.10±0.99c	3.92 - 8.10	3.34
SK1	16.25±2.75b	10.00 - 21.20	4.09±0.86a	1.96 - 6.00	4.08

Means with same letter (s) within the column are not significantly different at p=0.05, according to Tukey HSD test.

TABLE4b. SUMMARY OF ONE WAY ANOVA MEANS AND RANGES OF DIMENSIONS IN LENGTHS AND WIDTHS OF *FUSICOCCUM* SP. CONIDIA

Dimension	Source of variation	df	MS	VR	Probabilities
Length	Isolates	4	250.26	25.586 ***	0.0001
Width	Isolates	4	2 7.11	31.234***	0.0001

*** (p < 0.001), df = degree of freedom, MS = mean square, VR = variance ratio.

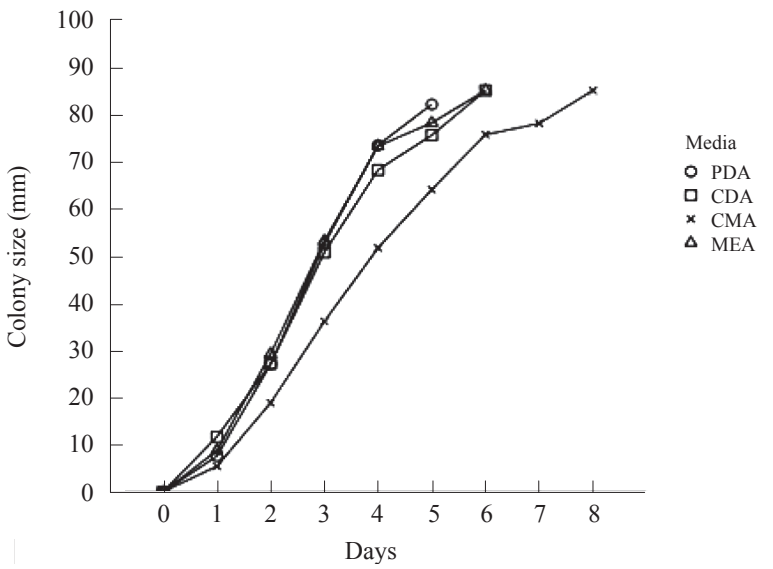

Figure 3. Effects of media on radial growth rates of Fusicoccum *isolates.*

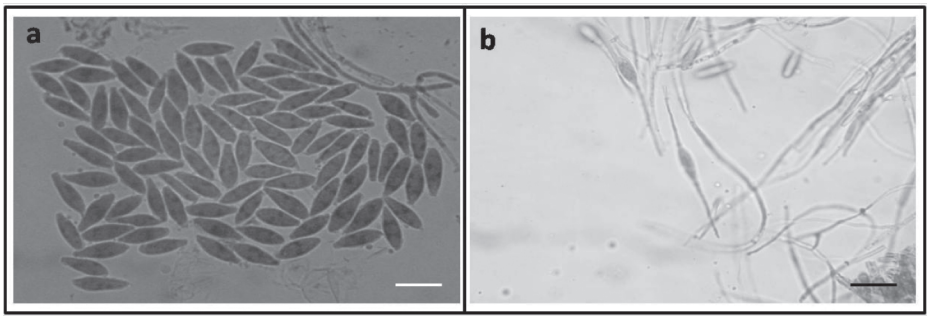

Figure 4. Conidia of Fusicoccum *sp. observed under the light microscope. (a) Macroconidia of* Fusicoccum *sp. (b) Germinated macroconidia of* Fusicoccum *sp. on water agar. Bars = 10 µm.*

tubes were formed on each macroconidium at 25ºC. Microconidia did not germinate at all temperatures.

Pathogenicity of *Fusicoccum*

The result of the pathogenicity tests confirmed all the fungal isolates were pathogenic to *Hevea brasiliensis*.

By the detached leaf test, all isolates were capable of infecting leaves causing variable degrees of lesions. Symptoms on the leaves appeared as small light spots five days after inoculation. They became slightly brown ten days later. By day 15, undulated margins with pycnidia on the leaf surface appeared, which turned completely brown by day 20. Isolates and clones showed a highly significant difference ($P < 0.001$) (*Table 6a*) as shown by

TABLE 5. MEAN GERMINATION OF FIVE ISOLATES AT DIFFERENT INCUBATION PERIODS AT 25ºC.

Isolate	Incubation period						
	0	4	8	12	16	20	24
SB3	0.00a	60.00a	60.00a	75.33a	75.00a	80.00 a	85.00a
SJ2	0.00a	70.76b	81.00b	81.33b	85.33b	95.33b	95.00a
SJ4	0.00a	49.67c	54.33c	55.00a	65.33c	74.33c	78.33a
SK1	0.00a	54.33 b	65.00d	77.67d	78.33d	80.00 a	80.00a
SK3	0.000	59.60 e	65.13d	75.33a	80.00e	80.00 a	75.00a

For each isolate, means with same letter (s) in the same column are not significantly different at p=0.05, according to Tukey HSD test.

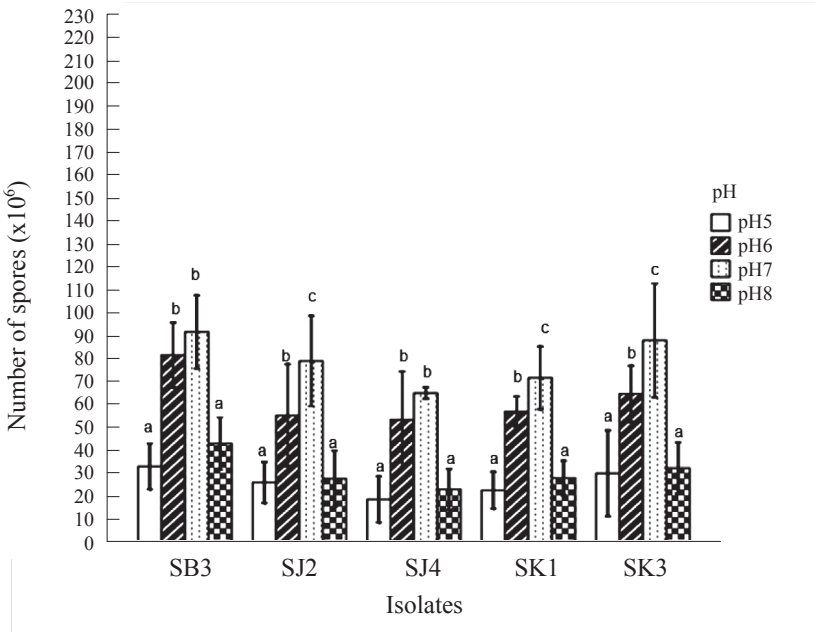

Figure 6. Sporulation of five isolates of Fusicoccum *sp. on CDA at different pH after 12 days of incubation.*

the result where isolate SJ2 gave the largest lesions compared to three other isolates SJ4, SK1 and SB3 (*Table 6c*).

In seedlings, there were different responses of rubber clones inoculated with fungal isolates compared to control (*Table 6b*). Isolates from rubber clones RRIM 2023, RRIM 2025, PB 350, PB 260 gave higher percentage infections on seedlings while isolates RRIM 2002 and RRIM 2007 gave lower percentage infections. The lesions developed fully after one month of inoculation extending upwards and downwards from the point of inoculation. Wavy concentric brownish zones with black pycnidia embedded were produced within

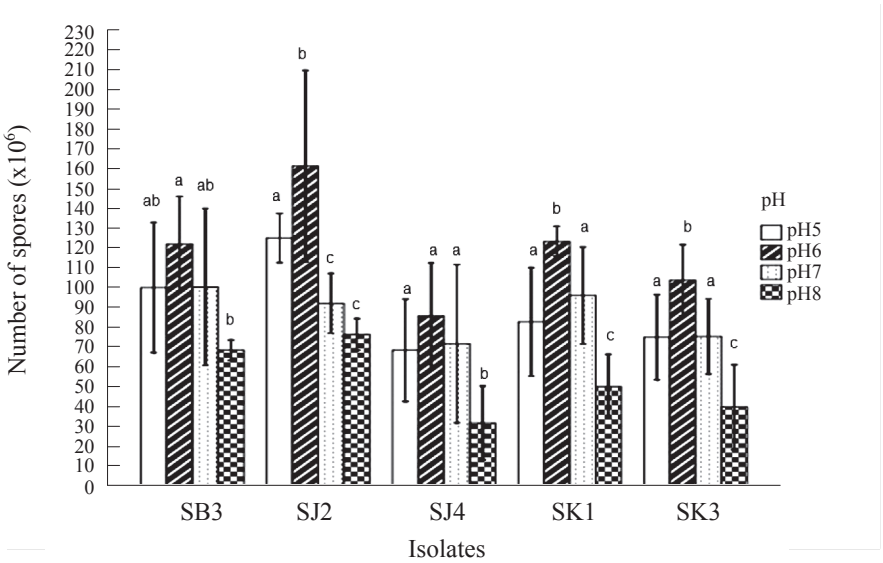

Figure7. Sporulation of five isolates of Fusicoccum *sp. on PDA at different pH after 12 days of incubation.*

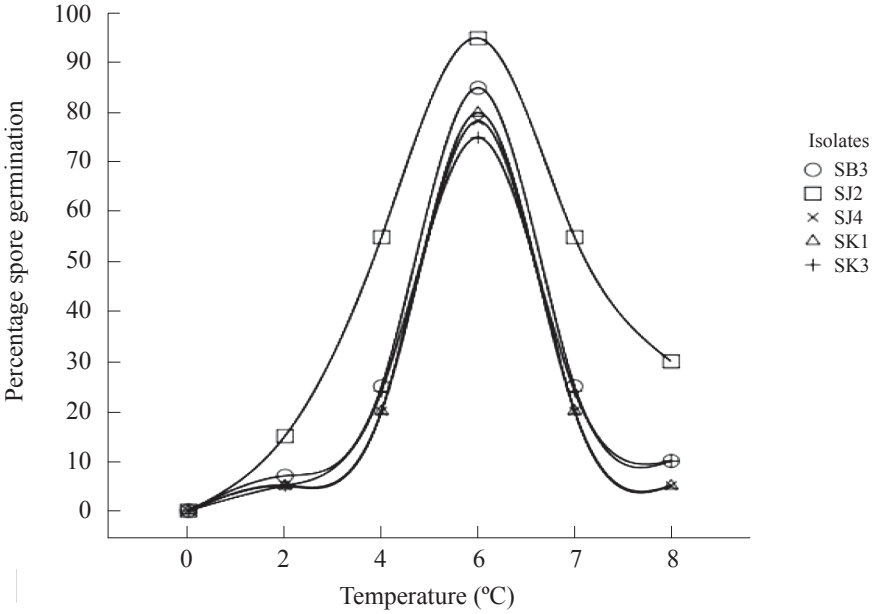

Figure 8. Percentage germination of five isolates on water agar (WA) at six different temperatures after 24 hours incubation.

TABLE 6a. MEAN NUMBER OF LESIONS PER LEAFLET EXPRESSED BY *FUSICOCCUM* SP. ISOLATE

Isolates	Days after inoculation			
	5	10	15	20
Control	0.0000a	0.0000a	0.0000a	0.0000a
SJ4	0.2056b	0.8333b	1.8278b	2.8722b
SB3	0.2222bc	1.0056bc	2.0556bc	2.8889b
SK1	0.3167c	1.1167cd	2.1444c	2.9444b
SJ2	0.4278d	1.2278d	2.2167c	2.9611b

For each isolate, means with same letter (s) in the same column are not significantly different at p=0.05, according to Tukey HSD test.

TABLE 6b. PATHOGENICITY OF FUSICOCCUM SP. ISOLATES ON SEVEN MONTHS OLD SEEDLINGS OF SIX RUBBER CLONES

Isolates	Seedlings					
	RRIM 2007	RRIM 2002	RRIM 2023	RRIM 2025	PB 350	PB 260
Control	-	-	-	-	-	-
SJ4	+	+	++	++	+++	+
SB3	+	+	++	++	+++	+
SK1	+	++	+++	++	+++	+
SJ2	+	++	++	+++	+++	+

- = no symptom
+ = slight infection
++ = moderate infection
+++ = severe infection
++++ = Very severe infection

the lesion of inoculated seedlings appearing 20 to 30 days after inoculation. Cross section of infected leaf showed black pycnidia within the lesions and *Fusicoccum* was successfully re-isolated on PDA.

DISCUSSION

In our study appearance of russet red spots, brown and spots necrotic on the leaves are often associated as disease symptoms described by several authors on other host plants[21–23]. Morphological features of five *Fusicoccum* isolates showed the same growth pattern, colour changes over time and conidia shapes on all media tested indicating their similarities. These observations concur with those described by other workers[1,4,24]. The production and germination of conidia were also similar with previous reports[1,24]. However, growth rates, spore production and percentage of germination showed some variations among isolates in terms of size (length and width of conidia). The effect of the media tested revealed highly significant meaning that the nature of the media affects the growth of mycelia in isolates screened. These observations are consistent with the variation of the medium as an experimental variable in

TABLE 6c. SUMMARY OF ONE WAY ANOVA FOR NUMBER OF LESIONS PER LEAFLETS EXPRESSED BY *FUSICOCCUM* ISOLATES.

Days after inoculation	Source of variation	df	MS	VR	Probablities
5	Isolates	4	0.450	37.188***	0.0001
	Clones	5	0.917	75.686***	0.0001
	Isolates * Clones	20	0.161	13.260***	0.0001
	Replication	2	0.012	0.24 ns	0.781
10	Isolates	4	3.856	89.707***	0.0001
	Clones	5	2.878	66.942***	0.0001
	Isolates * Clones	20	0.316	7.358 ***	0.0001
	Replication	2	0.75	0.658 ns	0.521
15	Isolates	4	15.679	264.248***	0.0001
	Clones	5	5.962	100.488***	0.0001
	Isolates * Clones	20	0.494	8.325 ***	0.0001
	Replication	2	0.014	0.013 ns	0.987
20	Isolates	4	30.650	510.829***	0.0001
	Clones	5	9.903	165.049***	0.0001
	Isolates * Clones	20	0.750	12.505***	0.0001
	Replication	2	0.057	0.29 ns	0.971

ns not significant, *** significant at $p < 0.001$, df = degree of freedom, MS = mean square, VR = variance ratio.

the realisation of sporulation[25]. *Fusicoccum* sp. conidia in our study were similar to those of *Fusicoccum aesculi*[26] and were smaller than those on pistachio[24]. Mycelia colours were observed in this study to change from white to grey and concurred with the isolate on MEA media observed for *Neofusicoccum*[11] and *Fusicoccum* colony was observed to be white to light yellowish green[24]. These variations could be due to the media used and the volume of hyphae submerged on these media. Growth rates on PDA, CDA, CMA, and MEA of isolates were variable and higher than previously observed[1]. This could be attributed to the difference in nutrient medium or the origin of plants host involved. The results of pH-growth relationships observed showed that *Fusicoccum* sp. grew optimally on acidic to neutral pH and can tolerate basic pH. Further physiological and biochemical characterisation studies are recommended to provide clearer similarity or difference among these isolates. There was no observation of teleomorph stage on artificial media used. This result supported previous observations[26,27] supporting identification of *Fusicoccum* sp. based on mycelial and pycnidiophore characteristics.

Nutritional rich media[25,28] were suggested to be favourable to vegetative growth and spore formation. Although PDA showed faster growth, higher production of *Fusicoccum* sp. conidia was observed on CDA media as well as for both media at different pH. Spore formation was therefore influenced by both media and pH. PDA was found to be suitable for culturing and sporulation of *Fusicoccum* sp.

Although little germination of conidia occurred in distilled water after eight hours

of incubation, higher germination (70%) was observed on WA. This media was previously recommended for germination of fungi[24] and therefore recommended for *Fusicoccum* sp. The optimum temperature for spore germination was 25ºC while high and low temperatures (above 35ºC and below 15ºC) generally inhibited the germination of spores. This study also found that different isolates varied in terms of spore production where SJ2 showed the highest germination rate followed by SK1 and SK3 at all incubation temperatures tested.

Typical lesions observed on rubber leaves were as described previously[1]. This may be probably due to the origin of the host[30] or to the seasonal and environmental factors during disease development[31] prevailing in Malaysia. Although this fungus was considered as a tropical plant endophyte[11,29], this study has proven that *Fusicoccum* was pathogenic to rubber.

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