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Asian J Agric & Biol. 2019;7(4):602-609.

Original Article

First record of *in vitro* growth evaluation of wild mushroom, *Schizophyllum commune* from Pulau Kapas in Malaysia

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Received:
April 16, 2019

Accepted:
October 14, 2019

Published:
December 31, 2019

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Abstract

Schizophyllum commune Fr. is an edible mushroom which is easily recognised based on its morphology and has the potential to be commercialized in Malaysia. This study was undertaken to evaluate the relationship between abiotic factors to the growth of mycelia and fruiting bodies formation of *S. commune*. The optimum temperature for mycelial growth was obtained at 28°C. The observed variations in colony size, mycelia density and number of fruiting body formation, shows that pH 5 was the most favourable for mycelia growth of *S. commune*. Interestingly, all seven culture media tested were suitable for the vegetative growth of *S. commune*. However, in the consideration on the mycelia spread rapidly and growth phenotype of mycelia, the most favourable culture media for *S. commune* was malt extract agar supplemented with yeast extract and glucose (MYGPA). Aeration was significantly affecting the mycelia growth, mycelia density and fruiting body formation of *S. commune* ($p<0.05$). It shows that unsealed culture condition produced lower mycelia growth and moderately compact of mycelia density but could produce more fruiting body formation compared to sealed culture condition. It was concluded that the mycelium growth of *S. commune* was affected by different temperature, culture media, pH and aeration conditions which can be used as a guideline to mushroom growers.

Keywords: *Schizophyllum commune*, Culture conditions, Mycelia growth, Mycelia density, Fruiting body

How to cite this:

Rosnan ND, Chuen N and A. Ngadin A, 2019. First record of *in vitro* growth evaluation of wild mushroom, *Schizophyllum commune* from Pulau Kapas in Malaysia. Asian J. Agric. Biol. 7(4):602-609.

Introduction

Split gill mushroom (*Schizophyllum commune* Fr.) belongs to the family of Schizophyllaceae of Agaricales and is an edible white rot fungus that commonly found grows abundantly in the wet season. *Schizophyllum commune* naturally appearing on decaying logs and classified as a wood degrader (Arun et al., 2015; Alam et al., 2010; Dasanayaka and Wijeyaratne, 2017). It is one of the most commonly found and widely distributed saprophytic basidiomycete around the world (Imtiaj et al., 2008; Khatua et al., 2013; Ediriweera et al., 2015). It is easily recognized based on its morphology that has a small

size (1–5 cm wide), tiny, elastic, tough and leathery fruiting bodies with hairy wet split gill (Schmidt, 2006). The fruit body is usually wrinkled at the upper surface, fan to shell-shaped with short striped and grey-white to brown in colour (Yim et al., 2013). Fruiting can be solitary or in clusters on decaying wood (Nasreen et al., 2015). The spore is composed of a cylindrical or elliptical morphology with 3~4 × 1~1.5µ and spore print is white (Kuo, 2003).

The life cycle of *S. commune* begins when single meiospores germinate and grow to develop a haploid, monokaryotic mycelia, in which each hyphae contains a single nucleus. The dikaryotic mycelium will form during mating sexually in which reciprocal nuclei exchange occur within the haploid monokaryotic hyphae. Then, clamp connections at each septum will be developed by the hyphae of dikaryotic only because it is the main structure of *S. commune*. Under favourable environments, the dikaryotic mycelia induce fruiting bodies development and produce the haploid spores that will germinate and start a new life cycle (Nieuwenhuis, 2012).

In general, the growth of mycelia and fruiting body formation are influenced by various physical and chemical factors that comprise of temperature, pH value, nutrients, aeration and others. Mushrooms are classified according to the favourable temperatures as either tropical (24~36℃) or sub-tropical (16~26℃). Most studies reported that *S. commune* was suitable to grow under the tropical region with the range of temperature was 25~35°C. The growth rate of mushroom is also influenced by the pH of the media either favourable to acidic (less than pH 7) or alkaline (more than pH 7) (Chang and Miles, 2004). Different mushroom species may differ in their optimal pH value. For example, the optimum range for basidiomycetes is often in a slightly acid environment of pH 4 to pH 6 (Schmidt, 2006).

Mushroom culture media is made up of some or all of the following components: source(s) of carbon, nitrogen, minerals such as sulphur, phosphorus, potassium and magnesium, vitamins and also solidifying agents. These components play an important role for mushroom growth since mushroom is heterotrophic. For example, the most common carbon source used for the growth of mushrooms in *in vitro* condition is 6-monosaccharide glucose. Carbon sources are very important to provide the structural and energy to the mushroom cell. While nitrogen is needed in the synthesis of the essential compounds including proteins, purines and pyrimidines. Besides carbon and nitrogen, minerals and vitamins also important for mushroom growth even these elements may be needed in lower concentrations. Most of the mushrooms utilize minerals and vitamins in mushroom metabolism and also as cofactor or activator for many enzymes systems (Chang and Miles, 2004).

In addition, *S. commune* has the potential to be cultivated and commercialized in a local market or also in the world market as it is not only consumed as good functional food with a unique flavour but also outstanding in its pharmacological effect and as the best degrader of lignocellulosic materials. Thus, it is needed to create a method for mycelia growth with a fast growth rate, gain thick mycelia density with more fruiting body formation in *in vitro* condition. However, there are limited studies associated with mycelia growth, mycelia density and fruiting body formation of *S. commune* in Malaysia. Therefore, this study was designed to optimize the suitable physiological culture conditions of *S. commune* based on temperature, pH, type of media used and aeration. Furthermore, the results obtained in this present study will be beneficial to mushroom growers in producing a high yield of the split gill mushroom in a short time.

Material and Methods

Pure mycelium of *Schizophyllum commune*

Samples of *S. commune* were collected on a dead branch at Pulau Kapas, Terengganu at attitude 45m (5°13'05.8"N 103°16'05.5"E). A pure culture of the *S. commune* mycelium was obtained by growing tissue of fruiting body aseptically on potato dextrose agar (PDA) medium at the Laboratory for Pest, Disease and Microbial Biotechnology (LAPDiM), School of Food Science and Technology, Universiti Malaysia Terengganu. This experiment was performed based on completely randomized design (CRD).

Temperature cultures

A 10-mm diameter agar plug of the inoculum was cut from 10 days old culture grown and placed on PDA plate. Temperature of four different tropicals at 24, 28, 32, 36°C, included the temperature of a humid sub-tropical at 16 and 28°C (as control) were evaluated to investigate the optimum temperature for the mycelia growth, mycelia density and fruiting bodies development of the *S. commune* according to standard protocol by Imtiaj et al., (2008). *S. commune* was incubated in five different temperatures on PDA

medium of pH 6. The growth of mycelial was determined by measuring the radial growth of three directions with a ruler and average value was calculated. To calculate final mean value of mycelial growth, three replication were setup. The measurement of mycelial growth was observed every two days for 10 days. The characteristics of mycelia morphology as density and fruiting body formation was also identified by visual observation as described by Guadarrama-Mendoza et al., (2014). The mycelial density can be classified as follow: compact, somewhat compact, somewhat thin and thin (Figure 1).

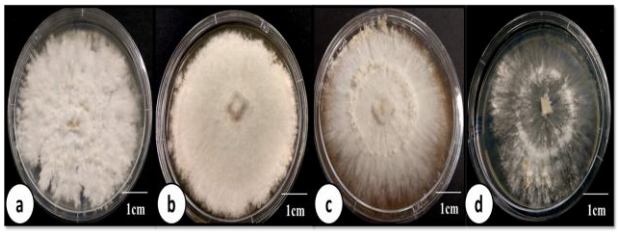

Figure-1: Mycelium morphology type for *S. commune*:
(a) Culture plate with compact of mycelia density (abbreviation as 'C') (b) Culture plate with somewhat compact of mycelia density (abbreviation as 'SC') (c) Culture plate with somewhat thin of mycelia density (abbreviation as 'ST') (d) Culture plate with thin of mycelia density (abbreviation as 'T')

pH media
The PDA medium was set to the pH 4, 5, 6, 7, and 8 by adding 0.1 M NaOH or HCl, and cultured for 10 days at 28ºC. The measurement of mycelial development and the morphology characteristics were conducted following the method mentioned previously.

Culturing media
Seven different nutrients media: PDA, Potato Carrot Agar (PCA), Malt Extract Agar (MEA), Malt Extract Agar supplemented with yeast extract and glucose (MYGPA), Oat Meal Agar (OMA), Sabouraud Dextrose Agar (SDA) and V8 Agar (V8A) were prepared and adjusted to pH 6 aseptically to screen favourable nutrient media of the mycelial growth and fruiting body development of the *S. commune* (Table 1). All culture plates were grew for 10 days at 28°C, were monitored every two days and recorded their mycelia growth and the characteristics of mycelia morphology like the same technique as before.

Table-1: Culture media and their composition of media used for the growth

	Media and composition(g/*l*)						
	PDA	**PCA**	**MEA**	**MYGPA**	**OMA**	**SDA**	**V8A**
Agar	15	20	20	15	20	15	20
Carrot		20					
Dextrose	20					40	
Glucose				5			
Malt Extract			20	30			
Oat Meal					30		
Peptone				5		10	
Potatoes	200	20					
'V8' Vegetable juice							200
Yeast-Extract				3			

Aeration affecting the mycelia growth and fruit bodies formation
The inoculated PDA plates of *S. commune* were prepared in two conditions, sealed with parafilm to prevent the direct oxygen while the other culture plates were unsealed and were exposed to aeration. The PDA culture plates were grew for 10 days at 28°C and measurement of mycelia growth and the characteristics of mycelia morphology were recorded as described earlier.

Statistical analysis
All the experiments were conducted in triplicates. Data were analysed in the Statistical Package for Social Sciences (SPSS) version 20 using one-way analysis of variance (ANOVA) and Tukey test to analyse the significant treatment comparison at 5% level of significance. Data with two treatments were analysed using the t-test. The results were represented as the mean ± standard deviation.

Results and Discussion

Effect of temperature
Temperature factors affecting the mycelia growth performance and their fruit bodies formation were observed in this study. The mushroom can be categorized as either temperate, semi-temperate, or tropical depending on the favourable temperature for mycelial growth performance (Lin, 2004; Dulay et al., 2012). The effect different temperatures to the mycelia growth of *S. commune* showed the most favourable is 28°C, followed by 32, 36, 24 and 16°C (Table 2).

Table-2: Effect of temperature on mycelia growth, mycelia density and fruiting body formation of *S. commune* grown on PDA with pH 6 medium

Culture Media	Mycelium Colony Diameter (cm)					*Mycelia Density	*Average No. of Fruit Bodies
	Day 2	Day 4	Day 6	Day 8	Day 10		
PDA	0.73 ± 0.14^{bc}	2.55 ± 0.37^{b}	4.40 ± 0.37^{b}	5.81 ± 0.43^{b}	7.33 ± 0.42^{a}	SC	+ +
PCA	0.44 ± 0.10^{c}	3.00 ± 0.31^{b}	5.91 ± 0.32^{a}	7.35 ± 0.21^{a}	7.84 ± 0.14^{a}	T	+
MEA	1.16 ± 0.27^{ab}	3.18 ± 0.22^{ab}	5.51 ± 0.54^{a}	7.13 ± 0.29^{a}	7.87 ± 0.17^{a}	SC	+ +
SDA	1.00 ± 0.20^{ab}	3.33 ± 0.61^{ab}	5.80 ± 0.20^{a}	7.20 ± 0.20^{a}	7.80 ± 0.20^{a}	SC	+
MYGPA	1.38 ± 0.14^{a}	3.40 ± 0.13^{ab}	5.73 ± 0.06^{a}	7.45 ± 0.20^{a}	7.91 ± 0.10^{a}	SC	+ + +
V8A	0.91 ± .10^{abc}	3.40 ± 0.13^{ab}	6.27 ± 0.37^{a}	7.71 ± 0.14^{a}	7.89 ± 0.10^{a}	ST	+ +
OMA	0.93 ± .31^{abc}	4.20 ± 0.53^{a}	6.13 ± 0.42^{a}	7.20 ± 0.20^{a}	7.73 ± 0.31^{a}	ST	+

Means ± Standard Deviation (SD) (n=3). Mean with different letters in the same column differ significantly at p <0.05.

*C; compact, SC; somewhat compact, ST; somewhat thin, T; thin (Refer to figure 1 for mycelia density indicator)

*(-); no fruiting body, (+); 1-10 of fruiting body, (+ +); 10-20 of fruiting body, (+ + +); ≥20 of fruiting body

This result was supported with the data studied by Hoa and Wang (2015), as the mycelia of *Pleurotus ostreatus* and *Pleurotus cystidiosus* shows the higher growth rate in the temperature of 28°C. Adejoye et al. (2007) reported that *S. commune* was favourable grow at 25°C and was inhibited at low temperature (0, 10 and 15°C) as well as at high temperature (45 and 50°C). According to Imtiaj et al. (2008) and Alam et al. (2010), the suitable temperature for the mycelia growth of *S. commune* was obtained at range 30~35°C and 30°C respectively. Both findings also mentioned that the lowest mycelial growth was obtained at 15°C. These findings were quite similar to the present studies where favourable temperature for the mycelia growth of *S. commune* was recorded at the range 28~36°C and the mycelial growth rate will be reflected at the low temperature. The mycelia density of mushroom *S. commune* was somewhat compact when exposing to all temperatures tested except at 32°C, which was shown very compact of mycelia density (Figure 1). The fruiting body was formed on culture media with a temperature of 16 and 28°C. Even though the fruiting body was able to form in low temperature (16°C), the fruiting body produced was small in size compared to control temperature (28°C). These observations could be due to the protein structure modification and inactivation of enzymes activity (Jayasinghe et al., 2008; Magday et al., 2014). Therefore, this optimum temperature result showed that *S. commune* was favourable to grow in tropical climate and this is a good opportunity for the mushroom industry in Asia (Hoa and Wang, 2015). It has been shown that the appropriate temperature and fully controlled for the cultivation of mushrooms is an important environmental factor of mycelium growth and formation of fruiting bodies (Choi et al., 2003).

Effect of pH

The pH range of 4~8 was evaluated on the performance of mycelia development, mycelia density and fruiting bodies development of *S. commune*. The results of mycelia growth were generally favourable in the pH level of PDA range 4~7 (Table 3). The optimum mycelia development was found 8.00 ± 0.00 cm at pH 4 and pH 5 whereas, the lowest level of mycelia growth was found 5.36 ± 0.80 at pH 8. This result indicates that species prefer acidic conditions. This present finding was supported by previous studies by Imtiaj et al. (2008) and Alam et al. (2010), which pH 5 is the most favourable for the mycelia growth of *S. commune*. Adejoye et al. (2007) also reported that the significant mycelia development for the *S. commune* was detected at pH 5.5. The highest mycelia growth of *Cystoderma amianthinum* was showed at the same pH 5 (Shim et al. 2005). Other species of *Grifola umbellate* reported by Shim et al. (1997) shows the most suitable and unsuitable pH at 4 and 9, respectively.

Table-3: Effect of pH on the mycelia growth, mycelia density and fruiting body formation of *S. commune* grown on PDA with pH 6 medium

pH	Mycelium Colony Diameter (cm)					*Mycelia Density	*Average No. of Fruit Bodies
	Day 2	Day 4	Day 6	Day 8	Day 10		
4	0.93 ± 0.07^{a}	2.62 ± 0.10^{ab}	5.44 ± 0.44^{a}	6.84 ± 0.20^{a}	8.00 ± 0.00^{a}	SC	+ +
5	0.71 ± 0.32^{ab}	2.73 ± 0.18^{a}	4.73 ± 0.27^{ab}	7.04 ± 0.21^{a}	8.00 ± 0.00^{a}	SC	+ + +
6	0.73 ± 0.07^{ab}	2.69 ± 0.10^{a}	4.62 ± 0.14^{bc}	6.53 ± 0.07^{a}	7.69 ± 0.10^{ab}	SC	+ + +
7	0.55 ± 0.10^{ab}	2.22 ± 0.14^{bc}	3.93 ± 0.13^{cd}	5.69 ± 0.17^{b}	7.00 ± 0.07^{b}	SC	+ + +
8	0.45 ± 0.10^{b}	2.16 ± 0.25^{c}	3.33 ± 0.35^{d}	4.27 ± 0.47^{c}	5.36 ± 0.80^{c}	SC	+ +

Means ± Standard Deviation (SD) (n=3). Mean with different letters in the same column differ significantly at $p < 0.05$.

*C; compact, SC; somewhat compact, ST; somewhat thin, T; thin (Refer to figure 1 for mycelia density indicator)

*(-); no fruiting body, (+); 1-10 of fruiting body, (+ +); 10-20 of fruiting body, (+ + +); ≥20 of fruiting body

Mycelia density was observed to be moderately compact in all pH tested (Figure 1). Interestingly, all pH tested (pH 4~8) were suitable to induce the formation of the fruiting body. The fruiting bodies appear with brown in colour, fan-shaped and grow in solitary and also in clusters on culture media. However, it is observed that culture in pH 5 produces a higher number of the fruiting body compared to pH 4. Therefore, it is suggested that pH 5 was the most favourable for mycelia growth and fruiting body formation of *S. commune.*

Effect of favourable culture media

The mycelial growth of seven different culture media was observed in the range 7.33 ± 0.42 ~ 7.91 ± 0.10 cm at 10 days after inoculation (Table 4). These seven nutrient media are favourable and not significantly different for the vegetative growth of *S. commune*. However, mycelia density was observed to be very thin in PCA, moderately thin in V8A and OMA, and moderately compact in MEA, MYGPA, SDA and PDA. High formation of fruiting bodies was obtained in MYGPA, a moderate formation of the fruiting body in V8A, MEA, and PDA, and formation of the fruiting body was poor in PCA, SDA and OMA. Thus, considering the mycelia spread rapidly and mycelia growth phenotype that included the mycelia density and number of fruiting body formation, the most suitable media culture for *S. commune* was Malt Extract Agar supplemented with yeast extract and glucose (MYGPA). In the previous study, the mycelia growth of *S. commune* were suitable on several media of Hamada, Hennerberg, PDA, Yeast Malt Extract and glucose peptone (Imtiaj et al., 2008; Alam et al., 2010). In other studies, the PDA and Yeast Malt Extract also are favourable culture media to *Cystoderma amianthinum* from Basidiomycota (Shim et al. 2005). These results were quite similar to the present study.

Effect of aeration

The influence of aeration on the mycelial growth of *S. commune* was also examined in this study. Oxygen and carbon dioxide are an important component for the fungi growth. Most fungi require good aeration for mycelia development and formation of the fruiting body (Chang and Miles, 2004). Table 4 presents the mycelia colony diameter, mycelia density and the average number of fruiting body formation in sealed and unsealed culture plates. The result showed that the mycelia colony diameter sealing with parafilm was significantly different from the unsealed culture plate ($p<0.05$). A similar result was reported by Magday et al. (2014) that *Ganoderma lucidum* on sealed condition produced higher radial mycelia growth than the unsealed condition. However, the results contradict the report on the mycelia growth response of *Lentinus tigrinus*, *Lentinus sajor-caju* and *Pleurotus djamor* where the aeration is not a main physical aspect to effect the mycelia development (Dulay et al., 2012; De Leon et al., 2017b; Bumanlag et al., 2018).

Table-4: Effect of culture media on the mycelia growth, mycelia density and fruiting body formation of *S. commune*

Temp. (°C)	Mycelium Colony Diameter (cm)					*Mycelia Density	*Average No. of Fruit Bodies
	Day 2	Day 4	Day 6	Day 8	Day 10		
Tropical Temperature							
24	0.44 ± 0.10^{c}	2.05 ± 0.10^{b}	3.20 ± 0.41^{c}	5.27 ± 0.13^{a}	6.89 ± 0.25^{b}	SC	-
28	0.71 ± 0.10^{b}	2.49 ± 0.10^{a}	4.22 ± 0.33^{b}	5.82 ± 0.44^{a}	7.62 ± 0.20^{a}	SC	+ +
32	0.91 ± 0.10^{ab}	2.58 ± 0.10^{a}	5.40 ± 0.35^{a}	6.36 ± 0.66^{a}	7.42 ± 0.25^{ab}	C	-
36	1.07 ± 0.07^{a}	2.07 ± 0.07^{b}	3.89 ± 0.23^{bc}	5.84 ± 0.39^{a}	7.09 ± 0.27^{ab}	SC	-
Sub-Tropical Temperature							
16	0.17 ± 0.04^{d}	1.53 ± 0.37^{c}	2.93 ± 0.18^{d}	4.42 ± 0.17^{b}	5.47 ± 0.18^{c}	SC	+ + +

Means ± Standard Deviation (SD) (n=3). Mean with different letters in the same column differ significantly at p <0.05.

*C; compact, SC; somewhat compact, ST; somewhat thin, T; thin (Refer to figure 1 for mycelia density indicator)

*(-); no fruiting body, (+); 1-10 of fruiting body, (+ +); 10-20 of fruiting body, (+ + +); ≥20 of fruiting body

On the other hand, there is also a difference between aerated and unaerated conditions in mycelia density and number of fruiting body formation. The sealed culture plates were observed more compact mycelia density with the absence of fruiting body formation while in the unsealed culture plates condition were showed compact mycelia density with more fruiting body formation after 10 days of the incubation period (Figure 1). This response of *S. commune* to aeration conforms to the observation by De Leon et al. (2017a) that *Lentinus squarrosulus* cultured in sealed condition produced a thick mycelia density compared to the unsealed condition. Therefore, it can be concluded that aeration was affecting the mycelia growth, the mycelia density and fruiting body formation of *S. commune.*

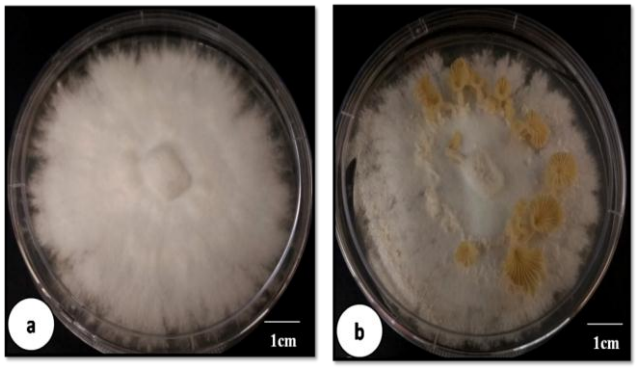

Figure-2: Mycellium growth of *S. commune* grown for 10 days after inoculation on PDA with pH 6 at 28°C. (a) Culture plate sealed with parafilm (b) Culture plate unsealed with parafilm

Table-5: Effect of aeration on the mycelia growth, mycelia density and fruiting body formation of *S. commune* grown on PDA with pH 6 medium

Aeration	Mycelium Colony Diameter (cm)					*Mycelia Density	*Average No. of Fruit Bodies
	Day 2	Day 4	Day 6	Day 8	Day 10		
Sealed	0.92 ± 0.07	3.00 ± 0.07	5.31 ± 0.10	6.99 ± 0.09	8.00 ± 0.00	C	-
Unsealed	0.73 ± 0.07	2.70 ± 0.10	4.67 ± 0.17	6.53 ± 0.07	7.69 ± 0.10	SC	+ + +

Means ± Standard Deviation (SD) (n=3).

*C; compact, SC; somewhat compact, ST; somewhat thin, T; thin

*(-); no fruiting body, (+); 1-10 of fruiting body, (+ +); 10-20 of fruiting body, (+ + +); ≥20 of fruiting body

Conclusion

In vitro evaluation of the wild mushroom, *S. commune* collected from Pulau Kapas, Terengganu is a very important study for mushroom growers in the tropical region, especially in Malaysia. The mycelium growth of *S. commune* were effected by different temperature, culture media, pH and aeration conditions. Maximum mycelium growth of *S. commune* was observed at 28℃ and is suitable for producing mushroom culture and grain spawn to increase the production of mushrooms in Malaysia. Further studies on spawn production, *S. commune* cultivation, nutrition values and bio-demineralisation can be investigated due to the huge potential to be commercialized in Malaysia and this can reduce the vast harvest of wild mushrooms to sustain the sustainability of this species in Malaysia's forests.

Acknowledgement

This work was funded by Fundamental Research Grant Scheme (FRGS – Vot. number 59463) from the Ministry of Higher Education (MOHE).

Disclaimer: None
Conflict of Interest: None
Source of Funding: Fundamental Research Grant Scheme (FRGS – Vot. number 59463) from the Ministry of Higher Education (MOHE), Malaysia.

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Contribution of Authors

Rosnan ND: Conducted the study, analysed the data and wrote the manuscript with support from other authors

Ngadin A: Supervised the study, read and approved the final draft of manuscript

Ng LC: Helped supervise the study, read and revised the manuscript