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EVALUATING ENTOMOPATHOGENIC FUNGI FOR THE BIOCONTROL OF THE RED PALM WEEVIL, *Rhynchophorus ferrugineus* (COLEOPTERA: DRYOPHTHORIDAE)

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ABSTRACT

Rhynchophorus ferrugineus Olivier, commonly known as the Red Palm Weevil (RPW), is one of the most destructive invasive pests of palm species worldwide, causing severe economic losses in coconut and date palm plantations and posing a potential threat to oil palm plantations. Biological control using entomopathogenic fungi (EPF) is an important component of integrated pest management (IPM) and offers a more environmentally sustainable approach than relying solely on chemical insecticides. This study aimed to screen and identify indigenous virulent EPF strains against adult *R. ferrugineus* under laboratory conditions. Twenty-two fungal isolates comprising *Metarhizium anisopliae*, *Beauveria bassiana*, and *Isaria fumosorosea* known as *Cordyceps fumosorasea* were evaluated in a laboratory bioassay. Mortality was recorded daily for 21 days, and the median lethal time (LT_{50}) was estimated using probit analysis. Among the tested isolates, five strains demonstrated high virulence, with UPMC 867 (*Metarhizium anisopliae*) exhibiting the shortest LT_{50} (9.18 days) and the highest infection rate (93%). This study systematically screened a collection of indigenous Malaysian entomopathogenic fungal (EPF) isolates against adult Red Palm Weevils (*Rhynchophorus ferrugineus*) and identified UPMC 867 as a highly virulent candidate for biological control. These findings provide a scientific basis for selecting locally adapted EPF strains for future bioformulation development and sustainable management of the Red Palm Weevil.

Keywords: Patogenisiti kulat; penilaian virulensi; bioasai makmal; masa maut; isolat tempatan; pengurusan perosak bersepadu

ABSTRAK

Rhynchophorus ferrugineus Olivier yang juga dikenali sebagai Kumbang Merah Palma (RPW) adalah salah satu daripada perosak invasif spesies palma yang paling merosakkan di seluruh dunia, menyebabkan kerugian ekonomi yang teruk dalam ladang kelapa dan kurma dan mempunyai potensi ancaman kepada ladang kelapa sawit. Kawalan biologi menggunakan kulat entomopatogen (EPF) merupakan salah satu komponen penting dalam Pengurusan Perosak Bersepadu (IPM) dan menawarkan pendekatan yang lebih lestari terhadap alam sekitar berbanding hanya bergantung kepada racun serangga kimia. Kajian ini bertujuan untuk menyaring dan mengenal pasti strain EPF yang virulen terhadap *R. ferrugineus* dewasa di bawah keadaan makmal. Sebanyak dua puluh dua isolat kulat yang terdiri daripada *Metarhizium anisopliae*, *Beauveria bassiana*, dan *Isaria fumosorosea* yang juga dikenali sebagai *Cordyceps fumosorosea* telah dinilai dalam bioasai makmal. Kematian direkodkan setiap hari selama 21 hari, dan masa kematian median (LT_{50}) dianggarkan menggunakan analisis probit. Dalam kalangan isolat yang diuji, lima strain menunjukkan virulens yang tinggi, dengan UPMC 867 (*Metarhizium anisopliae*) mencatatkan nilai LT_{50} yang paling singkat (9.18 hari) serta kadar jangkitan yang tertinggi (93%). Kajian ini telah menilai secara sistematik koleksi isolat kulat entomopatogen (EPF) tempatan Malaysia terhadap Kumbang Merah Palma dewasa (*Rhynchophorus ferrugineus*) dan mengenal pasti UPMC 867 sebagai calon yang sangat virulen untuk kawalan biologi. Dapatan kajian ini menyediakan asas saintifik bagi pemilihan strain EPF tempatan yang sesuai untuk pembangunan bioformulasi pada masa hadapan serta pengurusan kumbang merah palma yang mampan.

Kata kunci: Fungal pathogenicity; virulence assessment; laboratory bioassay; lethal time; indigenous isolates; integrated pest management

INTRODUCTION

The Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae), is an invasive and highly destructive pest that attacks various palm species throughout Asia, the Middle East, Africa and Europe (Faleiro 2006). Its larvae tunnel into palm trunks and feed within the tissues, making early detection difficult and frequently resulting in the death of affected palms (Giblin-Davis et al. 2013). Heavy infestations have caused substantial economic damage to both the coconut and date palm industries (Azmi et al. 2017). RPW infestation causes severe damage through larval feeding within the trunk, often requiring the removal of infested trees and the implementation of additional control measures. The absence of early infestation symptoms further facilitates the spread of this pest (Sabbahi & Hock 2023).

This invasive weevil has the potential to become one of the most destructive pests threatening Malaysia's oil palm industry. With approximately 5 million hectares of oil palm plantations contributing around RM70 billion annually to the national economy, the establishment and spread of RPW could result in substantial economic losses (Department of Agriculture Malaysia 2025). Control measures for RPW have primarily relied on chemical insecticides and pheromone-based trapping methods. Nevertheless, the overuse of insecticides can cause environmental pollution, negatively affect non-target organisms, and contribute to the development of insecticide resistance (Faleiro 2006). As a result, environmentally sustainable biological control approaches are increasingly being integrated into integrated pest management (IPM) strategies.

Entomopathogenic fungi (EPF) are naturally occurring insect pathogens that show strong potential for use as bioinsecticides. Indigenous EPF strains are generally better adapted to extreme environmental conditions and may therefore provide effective control against the RPW (Lee et al. 2017). Indigenous isolates of *B. bassiana* and *M. anisopliae* have demonstrated high virulence against a range of economically important insect pests, including stored-product beetles and other coleopteran and lepidopteran species highlighting the importance of selecting locally adapted strains to enhance the efficacy of biological control programs (Panwar & Szczepaniec 2024; Sutanto et al. 2024).Species such as *M. anisopliae*, *B. bassiana* and *I. fumosorosea* infect insects by directly penetrating the insect cuticle (Sabbahi & Hock 2023). Following attachment, the fungal conidia germinate, invade the cuticle, multiply within the hemocoel and eventually kill the host through tissue damage and toxin secretion (Vega et al. 2009). These fungi are regarded as environmentally friendly biological control agents with limited impact on non-target organisms (Lacey et al. 2015).

Earlier studies have confirmed the pathogenic effects of entomopathogenic fungi against the Red Palm Weevil, although the level of virulence varies among fungal strains (Dembilio et al. 2010; Sabbahi & Hock 2023). A study by Ment et al. (2023) found that *B. bassiana* and *M. brunneum* caused 60–88% mortality in female RPW, while *M. brunneum* reduced egg hatchability by approximately threefold and significantly suppressed palm infestation. Another research by Halim et al. (2024) found that indigenous *B. bassiana* isolates caused 100% mortality of adult RPW within 21 days, with mortality increasing significantly as conidial concentration increased. Consequently, evaluating highly virulent indigenous isolates is important for selecting suitable candidates for bioformulation development. Although numerous studies have demonstrated the potential of entomopathogenic fungi (EPF) for controlling RPW (Irshaid et al. 2024; Yang et al. 2023), most have focused on commercial products or isolates obtained from other geographical regions. Information on the virulence of indigenous Malaysian EPF isolates against adult RPW remains limited (Wakil et al. 2024). Therefore, this study systematically screened indigenous Malaysian EPF isolates against adult RPW to identify promising candidates for sustainable management.

MATERIALS AND METHODS

Insect Collection and Maintenance

Adult RPW used in this study were obtained from laboratory-reared colonies maintained by the Department of Agriculture Terengganu and the Malaysian Agricultural Research and Development Institute (MARDI), Manir, Terengganu. Only healthy and active male and female adults were selected for the bioassays. The insects were maintained under laboratory conditions prior to experimentation in ventilated 12 oz round plastic containers with lids at a controlled temperature of 27±2°C and relative humidity of 70±5%. Fresh sugarcane was provided ad libitum as a food source and replaced with fresh pieces as needed throughout the bioassay to ensure continuous availability. Maintaining the insects under standardized laboratory conditions helps minimize variability and ensures a consistent physiological status during pathogenicity screening (Lacey et al. 2015).

Fungal Isolates and Culture

Twenty-two entomopathogenic fungal (EPF) isolates comprising *M. anisopliae*, *B. bassiana*, and *I. fumosorosea* were cultured on Potato Dextrose Agar (PDA) using standard fungal cultivation procedures. All isolates were obtained from FRIM 7 strains (6 *M. anisopliae* and 1 *B. bassiana*), from IBS 9 strains (6 *M. anisopliae*, 2 *I. fumosorosea* and 1 *B. bassiana* and

from Department of Plant Protection UPM Toxicology Lab 6 strains (5 *M. anisopliae*, 1 *I. fumosorosea*). The isolates were revived and activated through passage in mealworms (*Tenebrio molitor*) larvae to restore virulence prior to testing. Subsequently, isolates were subcultured for two to three generations to obtain pure single-spore cultures and ensure genetic stability and pathogenic consistency (Vega et al. 2009).

Preparation of Conidial Suspension

Conidia were harvested from 14-day-old fungal cultures grown on potato dextrose agar (PDA) by adding sterile distilled water containing 0.05% (v/v) Tween 80 onto the sporulated culture surface. The conidia were gently dislodged by scraping the colony surface with a sterile glass spreader (or sterile inoculating loop/spatula), allowing the conidia to disperse into the suspension. The suspension was transferred to a sterile tube and vortexed thoroughly for 1–2 min to obtain a homogeneous conidial suspension and minimize conidia clumping. The conidial concentration was then adjusted to 1×10^8 conidia mL^{-1} using serial dilution and hemocytometer counting following standard procedures. Tween 80 was included to improve conidial dispersion and reduce aggregation (Vivekanandhan et al. 2023).

Bioassay Procedure

A dipping bioassay method adapted from Dembilio et al. (2010) was employed. Each adult RPW were immersed individually in conidial suspension (1×10^8 conidia mL^{-1}) for 60 seconds to ensure uniform contact between fungal propagules and insect cuticle. Treated RPW were air-dried on sterile filter paper and transferred to clean rearing containers. The experiment was arranged in a Completely Randomized Design (CRD) with three replications per treatment and five RPW per replicate. Cypermethrin served as a positive control, while sterile distilled water with 0.05% Tween 80 was used as a negative control. The dipping method ensures direct conidial exposure and is widely used to simulate spray application under laboratory conditions (Inglis et al. 2012).

Data Collection and Statistical Analysis

Mortality was recorded daily for 21 days. Dead RPW adults were individually placed in sterile Petri dishes lined with moistened sterile filter paper and observed for the development of mycosis. Cadavers that showed no visible fungal growth within 24 h were surface sterilized with a 2% sodium hypochlorite (NaOCl) solution, followed by three rinses with sterile distilled water. The sterilized cadavers were then transferred to fresh sterile Petri dishes lined with moistened sterile filter paper and incubated at 27±1°C to promote fungal growth and sporulation. Cadavers were examined daily, and mycosis was confirmed based on the presence of external fungal growth on the cadavers (Alwaneen et al. 2024). Mortality data were analyzed using one-way ANOVA to detect significant differences among treatments. Median lethal time (LT_{50}) values were estimated using probit analysis following Finney (1971). When necessary, mortality data were corrected using Abbott's formula to account for control mortality (Abbott 1925). Statistical analyses were conducted at a significance level of $P<0.05$ using SAS software (Version 9.3).

RESULTS AND DISCUSSION

Screening of Entomopathogenic Fungal Isolates Against *Rhynchophorus ferrugineus*

A total of 22 entomopathogenic fungal (EPF) isolates were successfully revived and screened against adult RPW, demonstrating variable levels of pathogenicity (Table 1). The results showed clear differences in infection performance among the isolates, indicating strong strain-dependent variability in virulence, which has been widely reported in EPF studies

(Vega et al. 2009; Zimmermann 2007). Among the tested isolates, *Metarhizium anisopliae* dominated the screening results, with several isolates (UPMC 867, UPMC 870, FRIM 866, and UPMC 869) demonstrating comparatively higher infection levels than the other isolates. Notably, UPMC 867 and UPMC 870 each infected 14 individuals, indicating strong pathogenic potential under laboratory conditions. The high effectiveness of these isolates may be associated with improved conidial attachment, faster germination, and successful penetration of the insect cuticle through enzymatic activities involving proteases, chitinases, and lipases (Vega et al. 2009). Furthermore, *M. anisopliae* synthesizes secondary metabolites such as destruxins, which contribute to insect mortality by interfering with the host's immune system and physiological functions (Zimmermann 2007).

The high performance of indigenous *M. anisopliae* isolates observed in this study is consistent with previous findings demonstrating the strong pathogenicity of this species against coleopteran pests, including RPW (Dembilio et al. 2010). The predominance of *M. anisopliae* among the top-performing isolates suggests that this species may be better adapted to local environmental conditions and host populations than other EPF species. Indigenous isolates are generally considered more suitable for field application because they are ecologically adapted and may demonstrate greater survival under local environmental conditions (Lacey et al. 2015). In contrast, *I. fumosorosea* isolates exhibited moderate to low infection levels, with UPMC 873 infecting eight individuals, whereas UPMC 876 showed no infection. Similarly, *Beauveria bassiana* isolates demonstrated relatively lower pathogenicity than *M. anisopliae*. These results highlight the importance of strain selection, as virulence is often influenced more by isolate-specific traits than by species identity alone (Vega et al. 2009). Differences in virulence among isolates may also reflect variation in conidial viability, host specificity, or tolerance to experimental conditions.

The origin of the fungal isolates appeared to influence their pathogenic performance. Several of the most virulent isolates originated from insect cadavers or forest ecosystems (e.g., Gunung Jerai and Hutan Melintang), suggesting that natural selection within insect-rich environments may enhance their infectivity and adaptability. Previous studies have reported that EPF isolated from insect hosts often exhibit greater pathogenicity due to prior host adaptation (Goettel & Inglis 1997). Interestingly, some isolates that were successfully revived did not exhibit high pathogenicity in the screening assays. This suggests that successful revival does not necessarily correlate with virulence, emphasizing the need for systematic bioassay-based screening rather than relying solely on culture viability.

Overall, the findings confirm substantial variability among EPF isolates and reinforce the importance of screening local strains to identify highly virulent candidates for biological control programmes. While laboratory results provide valuable baseline data, environmental factors such as temperature, humidity, and ultraviolet radiation can significantly affect EPF performance under field conditions (Lacey et al. 2015). Therefore, further studies involving semi-field and field validation, formulation development, and assessment of compatibility with integrated pest management (IPM) strategies are necessary before practical implementation.

Table 1. Screening results of entomopathogenic fungal isolates against adult *Rhynchophorus ferrugineus* under laboratory conditions

No	Strains Code	Species	Revived	Screening (RPW Bioassay)	Location	Infected Individuals (%)	Remarks
1	**A1**	*M. anisopliae*	√	√	RPW Cadaver	47	
2	**B1**	*M. anisopliae*	√	√	RPW Cadaver	20	
3	**C1**	*M. anisopliae*	√	√	RPW Cadaver	13	
4	**D1**	*M. anisopliae*	√	√	RPW Cadaver	40	RPW (S)
5	**UPMC 866**	*M. anisopliae*	√	√	Insects- Hutan Melintang	67	RPW (S)
***6**	**UPMC 867**	*M. anisopliae*	√	√	Insects- Gunung Jerai	93	RPW (A) Encapsulated
7	**UPMC 868**	*M. anisopliae*	√	√	Insects- Gunung Tahan	47	RPW (S)
8	**UPMC 869**	*M. anisopliae*	√	√	Insects- Hutan Simpan Air Hutan	80	NEW SUB
9	**UPMC 870**	*M. anisopliae*	√	√	Insects-Pantai Remis	93	
10	**UPMC 871**	*M. anisopliae*	√	√	Insects-Fraser Hills	27	
11	**FRIM 861**	*M. anisopliae*	√	√		53	
12	**FRIM 866 (HSAH P6-6)**	*M. anisopliae*	√	√		87	
13	**FRIM 871**	*M. anisopliae*	√	√		27	
14	**FRIM 872**	*M. anisopliae*	√			87	
15	**FRIM 881 (KOMP 15-15)**	*M. anisopliae*	√	√		13	
16	**FRIM 1150**	*M. anisopliae*	√	√	Insect cadaver (Kepinding	13	
17	**A(i)3**	*M. anisopliae*	√	√		7	
18	**UPMC 873**	*I. fumosorosea*	√	√	Insects- Hutan Melintang	53	RPW (S)
19	**UPMC 876**	*I. fumosorosea*	√	√	Insects- Hutan Melintang	0	RPW (A)
20	**ISARIA UPM**	*I. fumosorosea*	√	√		40	RPW (S)
21	**UPMC 878**	*B. bassiana*	√		Insects	20	
22	**FRIM 1297**	*B. bassiana*	√	√		7	RPW (A)

N = 15 adult RPW per treatment

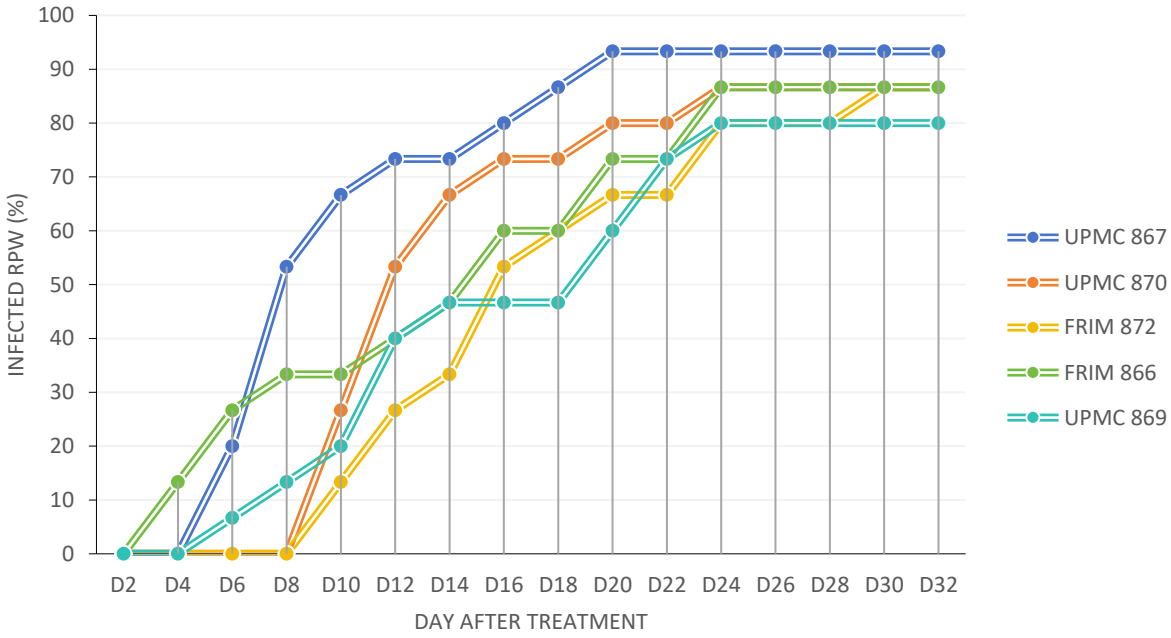

Figure 1. Percentage of infected RPW for selected top 5 strains

Based on Table 2, UPMC 867 exhibited the highest virulence with the shortest LT_{50} (9.18 days), indicating faster host mortality compared to other isolates. The results demonstrated significant variability in virulence among EPF isolates, consistent with previous findings that pathogenicity is strain-dependent (Vega et al. 2009; Zimmermann 2007). The variability in virulence among isolates suggests that pathogenicity is strain-dependent rather than species-dependent. All top-performing isolates belonged to *M. anisopliae*, supporting reports that this species is highly effective against coleopteran pests confirming its strong potential as a biological control agent against *R. ferrugineus* (Sewify et al. 2009).

Table 2. Lethal time (LT_{50}) of infected RPW

Strain Code	**Species**	**Infected Individuals**	**LT_{50} (days)**
UPMC 867	*M. anisopliae*	14	9.176
UPMC 870	*M. anisopliae*	14	13.881
FRIM 866	*M. anisopliae*	13	12.434
FRIM 872	*M. anisopliae*	13	17.560
UPMC 869	*M. anisopliae*	12	16.347

N = 15 adult RPW per treatment

The superior performance of UPMC 867 may be attributed to higher conidial adhesion efficiency, rapid germination, enhanced cuticle penetration, or greater production of secondary metabolites such as destruxins. The shorter LT_{50} observed for UPMC 867 indicates faster infection dynamics and host mortality, which are advantageous for suppressing RPW populations in biological control programmes (Dembilio et al. 2010). Rapid mortality may be

associated with enhanced conidial adhesion, increased production of cuticle-degrading enzymes and toxin production, including destruxins (Vega et al. 2009).

Although *B. bassiana* and *I. fumosorosea* are widely reported as effective entomopathogenic fungi, their performance in this study was comparatively lower than that of *M. anisopliae*. This finding highlights the importance of screening indigenous isolates to identify strains that are better adapted to local environmental conditions and target hosts. The dipping method ensured direct conidial contact with the insect cuticle, simulating exposure during field spray applications. However, environmental factors such as temperature, humidity, and ultraviolet radiation can influence EPF efficacy under field conditions (Lacey et al. 2015). Therefore, further studies involving formulation optimization, semi-field evaluation, and field validation are required before practical application and commercialization.

Variability in Virulence Among EPF Isolates

The present study demonstrated considerable variability in pathogenicity among EPF isolates against adult RPW. Such variation has been widely reported and reflects differences in isolate-specific traits, including conidial adhesion, germination rate, enzyme production, and toxin secretion (Vega et al. 2009). These findings highlight the importance of screening indigenous isolates to identify highly virulent candidates adapted to local environmental conditions and target hosts.

Mechanisms Underlying the High Virulence of *Metarhizium anisopliae*

All top-performing isolates belonged to *M. anisopliae*, confirming its strong pathogenic potential against coleopteran pests. The superior performance of UPMC 867 may be attributed to enhanced production of cuticle-degrading enzymes, including proteases, chitinases and lipases, together with secondary metabolites such as destruxins, which disrupt the host immune response (Vega et al. 2009). The shorter LT_{50} observed for UPMC 867 indicates rapid host colonization and efficient infection dynamics, characteristics that are desirable for effective pest suppression.

Implications for Integrated Pest Management (IPM)

The dipping method used in this study ensured uniform fungal exposure and provided a reliable laboratory screening approach for evaluating EPF pathogenicity. However, environmental factors such as ultraviolet radiation, temperature, and humidity can significantly influence EPF efficacy under field conditions (Lacey et al. 2015). Therefore, integration of highly virulent indigenous EPF isolates with other IPM strategies, such as pheromone trapping and cultural control, may enhance the effectiveness and sustainability of RPW management. Although the laboratory results demonstrate the high virulence of selected indigenous EPF isolates against adult *Rhynchophorus ferrugineus*, further semi-field and field evaluations are required to validate their efficacy under natural environmental conditions. In addition, formulation development is essential to improve fungal viability, persistence, and delivery, thereby facilitating practical application, commercialization, and integration into sustainable RPW management programmes.

CONCLUSION

This study successfully identified virulent indigenous entomopathogenic fungal (EPF) isolates against adult RPW. Among the tested strains, UPMC 867 (*Metarhizium anisopliae*) exhibited the highest virulence, with the shortest LT_{50} and the highest infection rate. These

findings demonstrate the potential of indigenous Malaysian EPF isolates as promising candidates for biological control and future bioformulation development against RPW. However, this study was conducted under controlled laboratory conditions and evaluated only the adult stage of RPW. Therefore, the efficacy of the selected isolates under field conditions and against other developmental stages of the pest remains to be determined. Future research should focus on developing and optimizing stable bioformulations, evaluating their efficacy and persistence through field trials and assessing their compatibility with other integrated pest management (IPM) strategies. These efforts will be essential to validate the practical application of the selected EPF isolates and support their potential commercialization for sustainable RPW management.

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AUTHOR CONTRIBUTION

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Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
No ethical issue is required for this research.

Data Availability Statement
This manuscript has no associated data.

Authors' Contributions
Masdah Mawi (MM) and Norhayu Asib (NA) developed the scope and outline of the review. MM, Muhammad Firdaus Ahmad (MFA) and Insyirah Ishak (II) performed the literature search, analysis and drafted the manuscript. Wan Muhammad Azrul Wan Azhar (WMAWA) and NA contributed to the interpretation of the results and reviewed the manuscript. All authors reviewed and edited the manuscript and approved the final version.

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