

EFFECTS OF NEEM OIL, *Azadirachta indica* A. JUSS ON GROWTH AND SURVIVAL OF BAGWORM, *Metisa plana* (LEPIDOPTERA: PSYCHIDAE) LARVAE

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ABSTRACT

Bagworm, *Metisa plana* Walker (Lepidoptera: Psychidae) is a major leaf-defoliating insect pest that has gained national attention due to the severe damage and loss to oil palm crops, notably in Peninsular Malaysia. The plant-derived insecticide neem, *Azadirachta indica* A. Juss provides a sustainable alternative as a biological control tool. This study was aimed to evaluate the growth and survival of *M. plana* after exposed to different concentrations of neem oil (75, 37.50, 18.75, 9.38, 4.69, and 2.35 mg/L) under laboratory conditions with distilled water used as control treatment. The bioassays of the neem extract were assessed using a leaf-dipping technique on fourth and fifth instar larvae stages of *M. plana* collected from Tapah, Perak, Malaysia. Observations were made during three exposure intervals: 1, 2, and 3 days after treatment (DAT). Higher concentrations of the neem oil had significantly increased the bagworm larval mortality at 3 DAT (ranging from 30% to 97.67%) and inhibited larvae growth which survived to adulthood. The highest % mortality was 96.67% and 83% for the fourth and fifth instar larvae after 3 DAT at a concentration of 75 mg/L, respectively. The lethal concentration, LC₅₀ within 3 DAT indicated a value of 3.59 and 6.15 mg/L for the fourth and fifth instar larvae, respectively. The findings demonstrated that neem oil as an insect growth regulator exhibited inhibitory properties against the growth and survival of the bagworm larvae, resulting in a notable increase in mortality. Therefore, neem possesses the potential to be utilized as a substitute biopesticide for *M. plana*, which requires more field investigations.

Keywords: Insect growth regulator, biopesticide, neem, bagworm, oil palm

ABSTRAK

Ulat bungkus, *Metisa plana* Walker (Lepidoptera: Psychidae) ialah serangga utama pemakan daun yang mendapat perhatian kerana menyebabkan kerosakan yang teruk pada tanaman sawit terutamanya di Semenanjung Malaysia. Racun serangga berasaskan semambu, *Azadirachta indica* A. Juss dapat menjadi kawalan biologi alternatif secara berterusan. Kajian ini bertujuan untuk menilai pertumbuhan dan kelangsungan hidup *M. plana* selepas rawatan kepada minyak semambu di bawah keadaan makmal pada kepekatan yang berbeza (75, 37.50, 18.75, 9.38, 4.69, dan 2.35 mg/L) dan air suling sebagai kawalan. Bioasai minyak semambu telah dinilai menggunakan teknik celup daun terhadap larva instar keempat dan kelima *M. plana* yang telah dikumpulkan dari Tapah, Perak, Malaysia. Pengamatan dilakukan semasa tiga selang waktu pendedahan: 1, 2, dan 3 hari selepas rawatan (DAT). Kepekatan semambu yang lebih tinggi secara signifikan meningkatkan kematian larva ulat bungkus pada 3 DAT (dari 30% hingga 97.67%) dan menunjukkan penghalang pertumbuhan mereka yang bertahan hingga dewasa. Kematian tertinggi yang dilaporkan ialah 96.67% dan 83% untuk instar keempat dan kelima, masing-masing selepas 3 DAT pada kepekatan 75 mg/L. Kepekatan mematikan, LC₅₀ dalam 3 DAT menunjukkan nilai 3.59 dan 6.15 mg/L, masing-masing untuk instar keempat dan kelima ulat bungkus. Penemuan ini mendedahkan bahawa semambu menunjukkan sifat menghalang terhadap pertumbuhan dan kelangsungan hidup larva ulat bungkus, yang membawa kepada peningkatan ketara dalam kematian. Oleh itu, semambu mempunyai potensi untuk digunakan sebagai pengganti biopestisid untuk *M. plana* selepas penyelidikan lapangan yang lebih lanjut.

Katakunci: Pengawal pertumbuhan serangga, biopestisid, semambu, ulat bungkus, kelapa sawit

INTRODUCTION

Bagworm *Metisa plana* Walker (Lepidoptera: Psychidae) is a significant insect pest that leads to leaf defoliation in oil palm plantations in Peninsular Malaysia, particularly in the regions of Perak and Johor (Nur Robaatul Adhawiyah 2021; Wood & Norman 2019). This pest has garnered nationwide attention due to its ability to cause substantial damage and significant economic losses. The Malaysian government officially classified this leaf-eating pest as harmful on November 15th, 2013, under the Malaysia Act 167 (Plant Quarantine Act 1976) (MPOB 2016). It was previously reported that the oil palm industry has suffered significant financial losses of over USD 25 million a year due to the bagworm outbreak's recurrence, and immediate control measures need to be implemented (Mazmira et al. 2022). To safeguard the crop, chemical pesticides are repeatedly used against any insect pests by some planters to achieve immediate control.

Over-dependence on insecticides paved the way for bagworms to develop insecticide tolerance and resurgence, which may increase environmental pollution and cause adverse effects on the beneficial insects (especially natural enemies and pollinators) and human health. An upsurge of studies focusing on biological control methods has recently emerged, corresponding with the awareness of the Integrated Pest Management (IPM) concept. The adverse consequences of using chemical pesticides have prompted a renewed interest in using alternative insecticides, with reduced cost and little ecological repercussions. The IPM approaches in oil palm are much preferred to suppress this pest, which includes the application of biopesticides using aircraft and drones (Mazmira et al. 2022), pheromone trapping

(Noorhazwani et al. 2021), and utilization of beneficial plants and insects (Nasir et al. 2023; Siti Nurulhidayah et al. 2023), which are currently utilized to reduce damaging pest populations. However, for the biological control agents to perform efficiently, highly toxic insecticides must be avoided as much as possible when integrating both insecticides and beneficial insects in the bagworm IPM system.

As an outcome, more emphasis have been placed on the development of environmentally friendly insecticides that can aid in an effective pest management strategy. The use of plant-derived insecticides is one such option (Bartelsmeier et al. 2021; Mohd Arafah et al. 2023). Neem-based substances have demonstrated effective and environmentally favourable characteristics, such as minimal impact on non-targets, multiple modes of action, and affordability (Campos et al. 2016; Mantzoukas et al. 2020). Neem oil is derived from the neem tree, *Azadirachta indica* Juss., which belongs to the Meliaceae family. Originally from the Indian subcontinent, it is now regarded globally as the primary provider of phytochemicals for use in human health and pest management (Campos et al. 2016; Islas et al. 2020). The main component of neem is azadirachtin, which is well-known for being a key component in insecticides. Neem promotes sterility in insects and functions as an antifeedant and repellent by hindering oviposition and halting insect males' sperm production. By applying neem, the metamorphosis process of insect pests can be inhibited, particularly in their immature stages (Bartelsmeier et al. 2021; Priyono et al. 2020; Shannag et al. 2013). Despite the abundance of articles on the efficacy of neem in agricultural uses, there is less information on its suppressive effect towards the bagworms in oil palms. Therefore, this study aimed to assess the growth-regulating effects of neem oil on fourth and fifth instar larvae of *M. plana*.

MATERIALS AND METHODS

Insect Collection and Rearing

Bagworm, *M. plana* Walker samples were collected from Tapah, Perak, Malaysia at their early instar larvae and reared in aluminum mesh cages (measuring 40 cm length x 40 cm width x 40 cm height) under laboratory conditions (27±1°C, 75±1 R.H. with a 12:12 h light: dark cycle). Fresh oil palm leaf stems were provided in the rearing cages as a food source for the larvae. To maintain the freshness, oil palm leaf stems were cut and placed through a hole in the transparent plastic lid of a vial containing water. The fourth and fifth instar larvae from this rearing colony were used for bioassay and further assessment. The purpose of using these late instars is to observe the subsequent stage formation, particularly the pupae, and determine if they are affected by the neem treatment or not.

Preparation of Neem Serial Dilutions and Bioassays

Neem extract containing a 3% concentration of neem oil was supplied by a local agrochemical company. The neem serial concentrations were prepared at 75, 37.50, 18.75, 9.38, 4.69, and 2.35 mg/L with distilled water as the control. The dosage applied is approximately half of the manufacturer's recommended rate for field application.

Oil palm stems were cut into 20 cm segments and subsequently immersed in the serially diluted neem solutions in a 100 ml measuring cylinder for 10 seconds with gentle agitation. Afterward, the leaflets were allowed to dry in the air for two hours on a foldable aluminium foil rack. The surface-dried leaflet was placed in a transparent cylinder container, with dimensions of 12.5 cm in diameter and 23.5 cm in height, and equipped with a lid that allows

ventilation. Using forceps, ten active *M. plana* larvae were randomly selected from their colonies and placed onto the oil palm leaf. The experiment was replicated six times for each treatment (Figure 1).



Figure 1. Bioassay layout in the laboratory (left) and bagworm feeding on neem-treated leaf (right)

Assessment on Growth and Survival of Bagworm, *Metisa plana*

To address possible insect growth regulator effects, the mortality, survival, and growth of survivors of *M. plana* larvae were assessed. The larvae were checked every 24 hours for 1, 2, and 3 days after treatment (DAT), and the developmental stage was recorded. Larvae that were unable to exhibit coordinated movement in response to a gentle stimulus applied to the posterior body segment using a seeking pin or fine-pointed brush are classified as dead (Kok et al. 2012; Siti Nurulhidayah et al. 2023). Upon completion of the experiment, the larvae were taken out from the bags to conduct further morphological observations using a stereo microscope (Model: Olympus SZ61, Japan) (Shawal et al. 2023). The remaining alive larvae were retained until they reached pupation or adulthood.

Statistical Analysis

The results obtained were analysed using a one-way analysis of variance (ANOVA) using SigmaPlot software Version 12.5. Differences among the results were statistically significant when the P value was <0.05. The bioassay data on the bagworm larvae were subjected to Finney's Probit Analysis in Microsoft Excel 2019 to determine the LC₅₀ and 95% confidence intervals of the upper and lower confidence limits (Mekapogu 2021). All mortalities were corrected utilizing Abbott's (1925) formula if required.

RESULTS AND DISCUSSION

Results of the laboratory experiments have shown that the effectiveness of neem oil at different concentrations influences the growth and survival of *M. plana*. The study showed that higher neem concentrations resulted in increased mortality of *M. plana* larvae for both larval instars across all time intervals (ranging from 0% to 96.67%) and showed growth inhibition of those

surviving to adulthood. The highest recorded mortality was 96.67% and 83% for the fourth and fifth instar larvae after 3 DAT at a concentration of 75 mg/L, respectively. The bar graph in Figure 2 shows the percentage mortality of both instars of *M. plana*. Fourth instar larvae were more susceptible to neem exposure with higher mortality recorded at all time intervals than the fifth instar larvae. Different susceptibilities to neem by different instar stages could be linked to neem's mode of action as an insect growth regulator (Bartelsmeier et al. 2021; de Oliveira Lima et al. 2024; Stark & Rangus 1994). The lethal concentration (LC₅₀) of neem, which caused a 50% mortality rate, varied for the fourth and fifth instar larvae of *M. plana*. For the fourth instar group, the wide range of LC₅₀ values was 906.75 mg/L at one day after treatment (1 DAT) to 3.59 mg/L at 3 DAT. On similar intervals, higher LC₅₀ values for the fifth instar group ranged from 3,890.19 to 6.15 mg/L, demonstrating that the fourth instar group was comparatively more sensitive to neem in comparison (Table 1). This observation may be explained by the lesser size and weight of the larval instar. In this study, it was demonstrated that both the treated larval instar's development stage and the exposure duration influenced the growth-regulating effects of neem.

Table 1. Lethal concentrations of neem on fourth and fifth *Metisa plana* instar larvae on 1, 2 and 3 days after treatment (DAT) were calculated using Probit analysis

Development Stage	Exposure Interval	n	LC ₅₀	95% CI	Slope±SE	R ²
Fourth instar	1 DAT	60	906.75	n.a	n.a	n.a
	2 DAT	60	34.43	14.58–81.28	0.90±0.19	0.979
	3 DAT	60	3.59	1.75–7.36	1.15±0.16	0.806
Fifth instar	1 DAT	60	3890.19	n.a	n.a	n.a
	2 DAT	60	33.65	14.96–75.70	0.96±0.18	0.946
	3 DAT	60	6.15	2.73–13.86	0.95±0.18	0.967

Note: n.a: data not available, n: number of test insects, LC: Lethal concentration, CI: confidence interval, SE: standard error, R²: linear regression

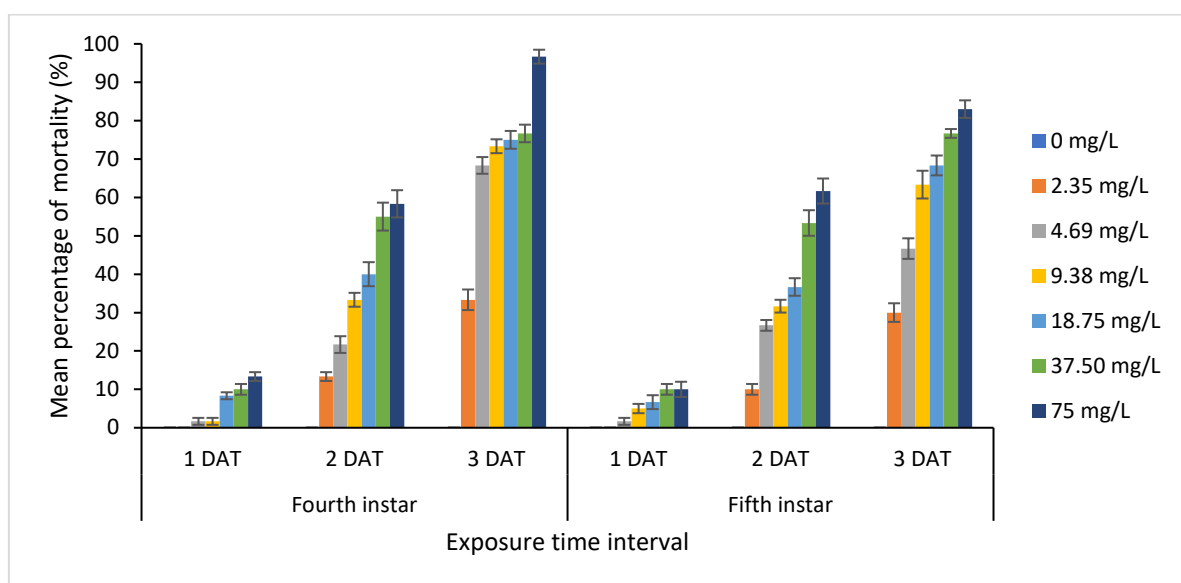


Figure 2. Mean percentage of mortality at different neem concentrations (in mg/L) on fourth and fifth *Metisa plana* instar larvae on 1, 2 and 3 days after treatment (DAT)

When administered at greater dosages, mortality increased and the effects became apparent following treatment, as observed on both bagworm instars. It was shown that the neem concentration of 9.38 mg/L treatment caused above 50% mortality on 3 DAT for both instars. At lower concentrations, the occurrence of moulting happened and led to disrupted growth and the development of deformities during the moulting process. Most of the observed samples died after being inactive for a few days or undergoing a prolonged moulting period.

Figure 3 illustrates the larvae survival rates after 1, 2, and 3 DAT. Data from Figure 2 can be related to the data in Figure 3. As the concentration increases, the survival rate of larvae decreases. During the 3 DAT observation period, it was found that only 16.67% and 3.33% of the fourth and fifth instar survived, respectively. This finding was similar to data recorded by Rawani et al. (2010), Oke et al. (2014), and Bartelsmeier et al. (2021), who discovered that lethal concentration values gradually reduced with exposure time, showing that the mortality rate is positively correlated with concentration. Neem, although less harmful than other pesticides, induces significant morphological alterations and reduces the survival of bagworm larvae, leading to a substantial mortality rate. After two weeks, it can be seen that the effect of neem as an insect growth regulator ultimately causes high *M. plana* larvae mortality and might delay the development time to the pupal stage for larvae survivors before they eventually die. The continuous observation of up to two weeks provides evidence that the survival of the fourth and fifth instar to the pupal stage decreased to 11% and 18.3%, respectively. None of the bagworm survivors reached adulthood, although the fifth instar had a chance to develop to the pupal stage. Similarly, the survivors of the fourth instar only had the ability to undergo metamorphosis into the fifth stage, but with certain abnormalities. Apart from the external abnormalities on the survived *M. plana* larvae, the visual inspection revealed the length and weight were found to decrease if compared to the control. The outer cuticle shrunk, with underdeveloped head capsules and abdominal segments observed (Figure 4).

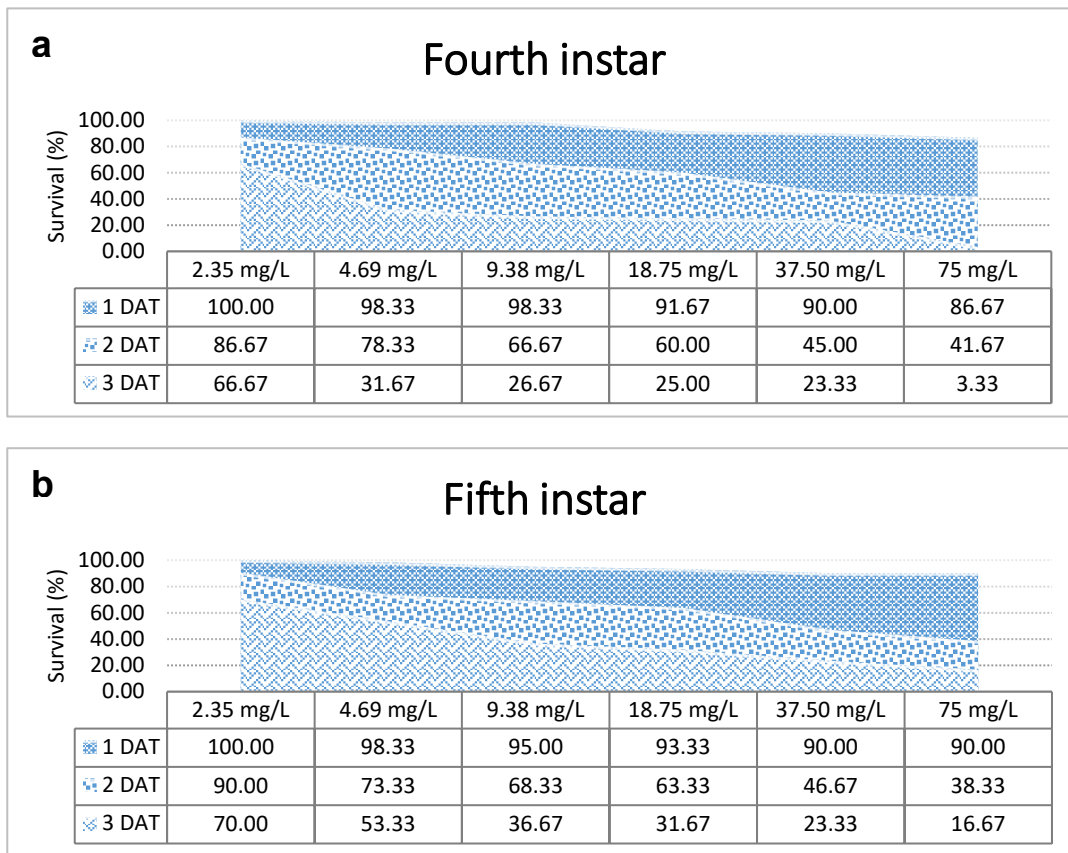


Figure 3. Percentage of survival for 1, 2, and 3 DAT for *Metisa plana* fourth instar larvae (a) and fifth instar larvae (b) at several neem concentrations



Figure 4. Dissected neem-treated fifth instar bagworm at its pupal stage after 3 DAT (right) and control (left)

Upon further examination, it was discovered that both larval instars might experience apolysis, a process characterized by swelling, in preparation for ecdysis. However, in the current study, this condition is caused by either the moulting process being unsuccessful or the larvae being unable to detach themselves from their old exoskeleton. Furthermore, bagworms feeding on neem-treated leaves might have experienced two outcomes: the complete detachment from the body without a ruptured exoskeleton, or incomplete expulsion but with a ruptured exoskeleton as stated by Lowery et al. (1994) and Ur Rahman et al. (2024). These consequences can occur as the larvae attempt to moult into the subsequent stages of their life cycle. This demonstrated that neem is responsible for the morphological changes and this condition is supported by the finding of Najla Mohamed et al. (2015) on the effects of diuron on the morphology of brine shrimp, *Artemia salina* Linn. In addition to the moulting interruptions, a disturbance in feeding was also noticed, specifically at the higher neem concentrations (37.5 and 75 mg/L), where there was little or no signs of leaf consumption. This also supported the finding of Kamaraj et al. (2018), whereby they observed an antifeedant effect on the feeding behavior of the cotton ballworm, *Helicoverpa armigera* Hub. and cotton leafworm, *Spodoptera litura* Fab. larvae when treated with neem. More importantly, the larvae that consumed neem-treated leaves were shown to be smaller in size compared to those that consumed untreated leaves.

The primary factor responsible for both deterring feeding and causing toxicity in insects is azadirachtin, a complex limonoid derived from the neem plant (Mordue (Luntz) & Nisbet 2000). Azadirachtin might possess similarities in structure with the insect hormones that cause insect metamorphosis. Chemical sensors in insects, such as taste receptors in the mouthparts, are essential for controlling an insect's feeding behaviour. As investigated by Mordue (Luntz) and Nisbet (2000), insect cells and tissues are directly affected by azadirachtin, which inhibits cell proliferation and protein synthesis upon uptake into cells. Flaccid paralysis of the muscles, necrosis of the midgut cells, loss of the gut's regenerative cells, and an inability to produce midgut enzymes are all symptoms of these effects. Compared to the antifeedant impacts, the cumulative physiological effects of azadirachtin are constant across species.

As observed in this study, the growth-regulating effect of neem was influenced by the concentration level and the larval instar stage treated as discussed by Bartelsmeier et al. (2021). Neem's insect growth regulator action has been seen in various insect species, with the effective dose (ED₅₀) causing a 50% reduction in feeding. These species include Lepidoptera, Coleoptera, Hemiptera, Hymenoptera, and Orthoptera. According to Mordue (Luntz) and Nisbet (2000), Lepidoptera has a high sensitivity to azadirachtin, which demonstrates potent antifeedant properties at concentrations ranging from 1 to 50 parts per million (ppm), depending on the species. This study showed that neem hindered the normal development and growth of bagworm larvae, thereby resulting in their larval death before adulthood. To our knowledge, the bagworm's bag or case did not influence the efficacy of neem, which is widely recognized for its capability as a feeding deterrent, particularly when administered orally. The relevance of this response is supported by the previous findings that stated the anti-feeding effect and the upsurge in larval mortality (Bartelsmeier et al. 2021; Kamaraj et al. 2018; Michereff-Filho et al. 2008; Tavares et al. 2010). Moreover, a study conducted by Prijono et al. (2020) provided evidence that neem oil suppressed the reproduction of *Spodoptera litura*. Moreover, it significantly reduces egg production by emerging females, with a reduction rate of up to 98.3%. Recently, Mawi et al. (2022) discovered similar outcomes in reducing

Nilaparvata lugens egg production by utilizing neem in combination with *Piper sarmentosum* as formulations.

Despite these important findings, it is necessary to undertake thorough follow-up investigations to examine additional parameters and possible factors that contribute to the growth regulator effects and other biological activities of neem involved in bagworm growth and reproductive systems regulation.

CONCLUSION

The findings provide an initial framework for suppressing bagworms in larval stages using neem oil as an insect growth regulator. High mortality of up to 97% was attained with some inhibitory effects on the fourth and fifth instars of *M. plana* revealed at a laboratory scale. Future research should prioritize the assessment of neem through field trials, specifically emphasizing population-level outcomes within Integrated Pest Management (IPM) programs. In its entirety, to suggest utilizing neem-based insecticide as an environmentally friendly substitute for developing more advanced and safer approaches for controlling bagworms and other oil palm pests. However, further studies should be done before better control measures can be proposed to curb the bagworm infestation and to determine whether neem has the potential as an alternative for biopesticides and subsequently to be effectively combined with the current IPM program.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Declarations

No ethical issue required for this research.

Data Availability Statement

My manuscript has no associated data.

Authors' Contributions

Siti Nurulhidayah Ahmad (SNA), Noorhazwani Kamarudin (NK), Saharul Abillah Mohamad (SAM), Norhayu Asib (NA), and MMohamed Mazmira Mohd Masri (MMM) conceived this

research, SNA, NK, and NA designed the experiments; SNA and NK did the research and collected the samples from the field; SNA, SAM, Mohamad Rosman Sulaiman (MRS), and Muhammad Nurul Yaqin Syarif (MNYS) participated in the interpretation of the data; SNA, NK, SAM, MRS, and MNYS wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript.

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