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SUSCEPTIBILITY OF THE POULTRY STRAIN *Musca domestica* LINNAEUS (DIPTERA: MUSCIDAE) LARVAE TOWARD PYRETHROIDS AND ORGANOPHOSPHATE INSECTICIDES

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ABSTRACT

Musca domestica Linnaeus is well-known as a pest that mechanically spreads diseases that are both medical and veterinary importance. Resistance has developed in the field as a result of the prolonged and improper application of insecticides to control the number of houseflies. The purpose of this study was to investigate the resistance status of poultry strain *M. domestica* larvae towards pyrethroid insecticides namely permethrin and deltamethrin, as well as organophosphate insecticides namely malathion and temephos. The third instar of susceptible poultry strains of *M. domestica* larvae was subjected to topical insecticide bioassay with concentrations ranging from 0.0005mg/L to 2000.0mg/L. The resistance ratio was calculated to categorize the level of resistance by dividing the LC_{50} value of the poultry strain by the susceptible strain. The susceptibility status of the poultry strains, Sungai Lembu (SL) and Tapah Road (TR) towards pyrethroid and organophosphate revealed moderate to high resistance in comparison to the susceptible IMR strain (13.78-fold to 48.82-fold). Both strains were found to be resistant and possess multiple resistance and cross-resistance towards the tested insecticide. Understanding the resistance status of targeted pests is critical for effective, economically feasible, and environmentally sustainable pest management strategies in agriculture and beyond. It contributes to food security, environmental protection, and long-term pest control efficacy. Additionally, it is advisable to implement integrated pest management strategies and utilize synergists to enhance the effectiveness of both chemical-based insecticides and biopesticides.

Keywords: *Musca domestica*, resistance, insecticide, pyrethroid, organophosphate

ABSTRAK

Musca domestica Linnaeus terkenal sebagai perosak yang secara mekanikal menyebarkan penyakit yang mempunyai kepentingan perubatan dan veterinar. Kerintangan telah berkembang di lapangan akibat penggunaan racun serangga yang berpanjangan secara tidak betul untuk mengawal lalat rumah. Tujuan kajian ini adalah untuk mengkaji status kerintangan larva *M. domestica* strain ladang terhadap racun serangga piretroid iaitu permetrin dan deltamethrin, serta organofosfat iaitu malathion dan temephos. Instar ketiga strain rentan larva *M. domestica* telah diuji melalui kaedah bioassay insektisida topikal dengan kepekatan antara 0.0005mg/L hingga 2000.0mg/L. Nisbah rintangan bagi mengkategorikan tahap rintangan diperoleh dengan membahagikan nilai LC_{50} strain ladang dengan strain rentan. Status kerentanan strain ladang (strain SL dan TR) terhadap piretroid dan organofosfat menunjukkan rintangan sederhana hingga tinggi berbanding strain rentan IMR (13.78 kali ganda kepada 48.82 kali ganda). Kedua-dua strain didapati tahan dan mempunyai corak rintangan pelbagai dan rintangan silang terhadap racun serangga yang diuji. Memahami status rintangan perosak sasaran adalah penting untuk strategi pengurusan perosak yang berkesan, boleh dilaksanakan dari segi ekonomi dan alam sekitar dalam pertanian. Ia menyumbang kepada keselamatan makanan, perlindungan alam sekitar, dan keberkesanan kawalan perosak jangka panjang. Selain itu, adalah dinasihatkan untuk melaksanakan strategi pengurusan perosak bersepadu dan penggunaan sinergi untuk meningkatkan keberkesanan kedua-dua racun serangga dan biopestisid berasaskan kimia.

Kata kunci: *Musca domestica*, kerintangan, racun serangga, piretroid, organofosfat

INTRODUCTION

Musca domestica Linnaeus also known as housefly or domestic fly have successfully evolved synanthropic insects that have been identified as mechanical vectors for more than 100 pathogens (Abbas & Shad 2015; Khamesipour et al. 2018; Ma et al. 2017). Houseflies breed and feed organic material such as feces, decaying organic material and garbage (Helena et al. 2015). During their contact with the feeding material, housefly tends to pick up pathogens on the external body parts as well as the internal body that may have a negative impact on both humans and animals where they transmit disease causing pathogens (Khamesipour et al. 2018). *Musca domestica* has a complete metamorphosis with distinct eggs, larvae, pupae and adults. The adult female might lay up to 500 eggs in numerous batches of 75 to 160 eggs in 3-4 days, allowing the housefly to adapt and spread globally, making it difficult to control (Axtell 1986).

Malaysia's poultry sector is growing as a result of rising demand for chicken meat and eggs. With an annual per capita consumption of 46.8 kg, Malaysia is one of the nations with the greatest consumption of chicken meat in the world (DVS 2022). An increase in the demand for chicken and egg eventually generated more waste especially manures which are ideal for the housefly to feed and breed. Houseflies are attracted to the waste produced, causing unhygienic conditions in poultry farms, potentially leading to lawsuits and compounds. Pathogens carried by the housefly served both medical and veterinary health importance. Some pathogenic strains of microorganisms that are carried by houseflies such as Newcastle disease and highly pathogenic avian influenza, H5N1 economically affect the livestock industry, especially poultry farms.

Malaysia poultry units heavily rely on chemical insecticides, particularly conventional chemicals in the control of house flies (Ong et al. 2015). When a pesticide is prolonged and misused, insecticide resistance can develop (Kaufman et al. 2001; Sawicki 1987; WHO 1986). This results in selection pressure on the insect population, whereby those that are vulnerable to the insecticide are eliminated and those are tolerant would live and reproduce. Resistance has been built up over time, and pesticide effectiveness has decreased (Pathak et al. 2022).

A previous study, which examined the susceptibility status of houseflies from Balik Pulau, Pinang, was conducted by Bong and Zairi back in 2010. The susceptibility status is crucial since it will define the house fly's level of resistance to insecticides and, consequently, the effectiveness of the insecticides. The most recent research on the resistance status of *M. domestica* in Malaysia was carried out by Ong et al. (2015). The study concentrated on the efficacy of insecticides that can be purchased commercially, specifically thiamethoxam (Agita 10WG), imidacloprid (Toxilat 10WP), cyfluthrin (Responsar WP), lambda-cyhalothrin (Icon 2.8EC), and fipronil (Regent 50SC). Nevertheless, before this present investigation, there has been little information provided regarding the susceptibility status of *M. domestica* and the efficiency of temephos, malathion, deltamethrin, and permethrin in controlling the housefly population.

MATERIALS AND METHODS

Sources of *Musca domestica*

Susceptible strain of M. domestica

The susceptible strain (F21) of *Musca domestica* was acquired from the Kuala Lumpur Institute of Medical Research (IMR) Setia Alam, Selangor, Malaysia. The strain was cultivated and raised in a standard lab setting without the introduction of any chemicals, insecticides, or biological control agents, and served as a control in this study (Farooq & Freed 2016).

Field strain of M. domestica

Field strains of housefly populations were gathered from two distinct commercial poultry farms in Sg. Lembu Bukit Mertajam, Pulau Pinang, and Tapah Road Tapah, Perak which used chemical based insecticides to control the housefly population. These farms are identified as SL strain and TR strain respectively. Using a metal scoop, the larvae were removed from the excrement and placed into a plastic container. It was considered that these strains were introduced to exposure to insecticides and chemicals. Then, these houseflies were nurtured in a standard lab setting as the susceptible strain of *Musca domestica.*

Musca domestica cultivation

The field strains of *Musca domestica* namely Sungai Lembu (SL), Tapah Road (TR), and susceptible strains of *M. domestica*, Institute of Medical Research (IMR) were all cultivated in a lab setting at the Veterinary Research Institute (VRI) in Ipoh, Malaysia, with a photoperiod of 12 hours of light and 12 hours of darkness (Farooq & Freed 2016). The field strains of larvae were raised in a medium made of soaked hamster pellets (Figure 1) in distilled water at a ratio of 1:15 (w/v) for 24 hours or until all the water was absorbed. The larvae were allowed to pupate for about four to five days. When the housefly emerged after 4-5 days, the pupae were then moved to a different cage made of metal that was reshaped into cubes and had perforated cloth, as shown in Figure 2 with a supply of water, milk, and sugar for feeding. Wet cotton was used to provide 10% glucose for the adults. A bed medium was put into a plastic container and set up in a cage so that the female housefly could lay her eggs for four hours. The F_1 of third instar larvae were then harvested after 3–4 days after the eggs hatched and were used in the topical insecticide bioassay.

Figure 1. Medium prepared for larvae cultivation

Figure 2. Modified cube shaped metal cage with perforated cloth for adult housefly cultivation

Preparation of Insecticide

Insecticide stock solution at 10g/L was diluted with acetone using the $M_1V_1=M_2V_2$ dilution equation value to create a wide range of insecticide concentrations ranging from 0.0005mg/L to

2000.0mg/L. M_1, V_1, M_2, and V_2 stand for the initial concentration, initial volume, final concentration, and final volume, respectively. The technical grade of insecticide was provided by the Vector Control Research Unit (VCRU), USM.

Topical Insecticide Bioassay

Insecticide response bioassays were performed on 3rd instar larvae susceptible (F_{21}) and poultry strain (F_1) houseflies using technical grade insecticides from the pyrethroid (permethrin and deltamethrin) and organophosphate (temephos and malathion) groups that were diluted with analytical grade acetone (WHO 2005). All of the larval strains were examined in a lab setting with a 25°C ambient temperature, a humidity level kept between 60% and 80%, and a photoperiod of 12 hours of light and 12 hours of darkness. Acetone solution was used to dilute the insecticides, while the acetone solution alone served as the control. First generation (F_1) of 3rd instar larvae (F_1) were used (n=30). They were first placed in a petri dish and then transferred to plastic cups containing 10g of moist larvae medium after receiving a topically applied insecticide treatment with a range of concentrations from 0.0005 mg/L to 2000.0 mg/L (Burgess et al. 2020). Then, each test was repeated three times, with three of those repetitions being treated with acetone alone, which served as the study's control. Larvae mortality was tracked every 24 hours for a total of 72 hours.

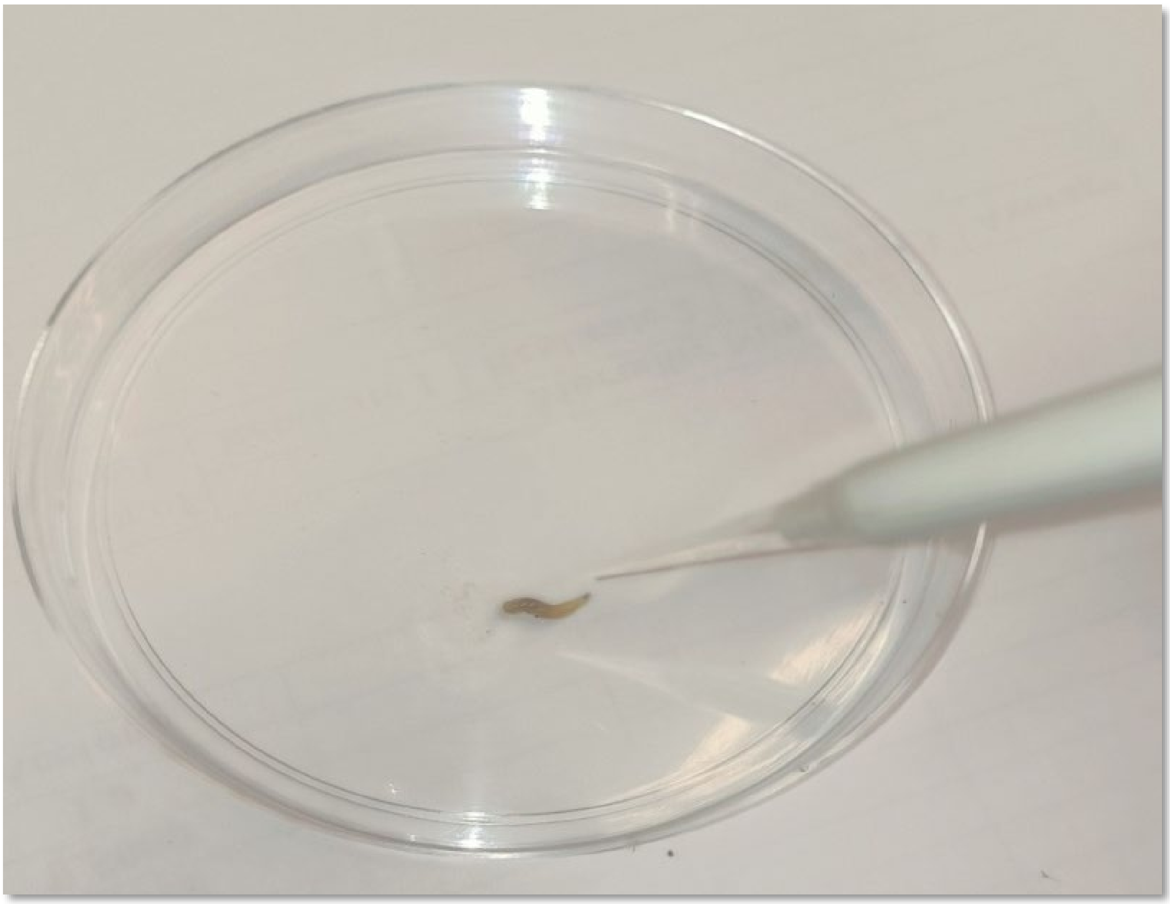

Figure 3. Topical application of insecticide on 3rd instar larvae using micro pipettor

Data Analysis

Probit analysis in SPSS Version 28 was used to examine the bioassay data of housefly mortality based on pesticide concentration to compute the LC_{50}, and a value of 0.05 is regarded as statistically significant. When the control mortality is between 5 and 20%, the Abbott formula is employed to modify (Abbott 1925). When the pupation rate exceeded 10%, the test was rejected (WHO 2005). The resistance ratio (RR_{50}) was computed by dividing the field strain's LC_{50} value by the susceptible strain's LC_{50} value. The level of resistance was assessed using the classification proposed by Khan. (2019) and developed by WHO 1980, as shown in Table 1.

Table 1. Resistance levels are classified based on the resistance ratio at sublethal concentration (RR_{50}) proposed by Khan (2019)

RR_{50}	**Resistance Level**
Less than 1	Susceptible
1 to 10	Low resistance
11 to 30	Moderate resistance
31 to 100	High resistance
More than 100	Very high resistance

RESULTS

By statistical probit analysis using the SPSS software Version 28, the sub-lethal dosage (LC_{50}) of permethrin, malathion, deltamethrin and temephos against *M. domestica* larvae were obtained at 934.81 mg/L, 451.77 mg/L, 485.00 mg/L, and 254.52 mg/L respectively for SL strain, 943.32 mg/L, 976.96 mg/L, 440.67 mg/L and 901.66 mg/L (Table 2). The two poultry strains SL and TR had probit lines with slopes <4.37, indicating that their responses to different insecticides (permethrin, malathion, deltamethrin and temephos) varied widely. According to the WHO classification of resistance level, SL strains were highly resistant towards permethrin (36.37-fold), moderately resistant towards malathion (30.81-fold), deltamethrin (21.45-fold) and temephos (13.78-fold). Surprisingly, the TR strain was categorised as highly resistant to permethrin, deltamethrin and temephos at 36.71-fold, 46.53-fold, and 48.82-fold respectively. Nevertheless, the TR strain was also found to be moderately resistant to malathion at 28.00-fold.

Table 2. Toxicity of insecticides to adult houseflies of poultry strain compared to the susceptible population

Insecticides	**Strains**	**LC_{50}(mg/L)**	**Slope±SE**	**X^2**	**RR**
Permethrin	**SL**	934.81	2.07	2.90	36.37
	TR	943.32	2.52	0.89	36.71
	IMR (susceptible)	25.70	0.583	3.53	-
Malathion	**SL**	485.00	1.33	1.65	30.81
	TR	440.67	1.43	4.15	28.00
	IMR (susceptible)	15.74	0.72	8.94	-
Deltamethrin	**SL**	451.77	1.40	4.44	21.45
	TR	979.96	1.12	21.46	46.53
	IMR (susceptible)	21.06	0.80	8.457	-
Temephos	**SL**	254.52	0.93	1.68	13.78
	TR	901.66	4.37	0.34	48.82
	IMR (susceptible)	18.47	0.48	7.13	-

LC_{50}: Lethal concentration that causes 50% mortality; SE: Standard error; X^2: Chi-square value; RR: Resistance ratio. SL: Sungai Lembu strain; TR: Tapah Road strain: IMR (susceptible): Institute of Medical Research Strain

DISCUSSIONS

In Malaysia, insecticides are still vitally used in pest management programmes in the agricultural area (Ong et al. 2015; Wasi et al. 2003). Although insecticides are necessary for vector management, their overuse and poor application have led to the development of insecticide resistance in houseflies. Houseflies are exposed to many xenobiotics in their natural habitat, which allows them to adapt to the environment for survival by acquiring resistance through various mechanisms. The larval stage of *M. domestica* was chosen for topical insecticide bioassay primarily because insecticide administration in Malaysian farms is frequently focused on chicken manure, where the larvae are mostly fed and reproduce. In this study, the poultry farm strains were discovered to be resistant strains with resistance ratio values ranging from 13.78 to 48.82-fold. It was also discovered that the prolonged and extensive use of chemical-based insecticide was the primary cause of *M. domestica* poultry strain (SL and TR strains) developing resistance to both pyrethroid (permethrin and deltamethrin) and organophosphate (malathion and temephos) group of insecticide (13.78 to 48.82-fold). The RR_{50} of the SL strain (13.78-fold) towards temephos was considerably lower when compared to other insecticides utilized (permethrin, malathion, and deltamethrin), indicating that this strain is more susceptible to temephos. However, as compared to other insecticides (permethrin, deltamethrin, and temephos), the RR_{50} of TR strain (21.45-fold) towards malathion was relatively lower, indicating that this strain has a higher level of susceptibility to malathion. The two poultry strains in this investigation were likewise found to be multi-resistant strains. This is because several types of insecticides have been met with resistance. The fact that these poultry strains have evolved cross-resistance is further supported by a comparison between low susceptibility to the pesticide employed in this investigation and their increased susceptibility to the IMR strain. The cross-resistance in the SL and TR strains has been attributed to the use of insecticides with a similar mode of action even though a different class.

Insecticides belonging to the pyrethroid and organophosphate classes were widely employed in Malaysia throughout the 1960s to manage the pest population (Rahim et al. 2017; Yap et al. 2000). Wang et al. (2019) proposed that pyrethroid resistance may be primarily caused by decreased cuticular penetration, decreased target site sensitivity, and metabolic detoxification. Pyrethroids damage the central nervous systems of pests, both target and non-target. Its primary method of action is by interaction with voltage-gated sodium channels in neurons (Tano 2011), which ultimately causes muscle paralysis and death in the targeted organism. Khambay & Jewess (2004) states that pyrethroids are well-known for their ability to quickly eliminate pests. But it usually takes a while to kill them. In sublethal dosage (LD_{50}) at higher concentrations, insects usually recover completely over the following couple of days (Bloomquist & Miller 1985). Secondary mechanisms like predation and desiccation are typically responsible for the targeted pest's mortality or the efficacy of pyrethroids. The present study's findings that the poultry farm strains, both SL and TR, exhibited medium resistance to deltamethrin are corroborated by a survey investigation carried out at an urban rubbish dump site in China which resulted in RR_{50} values 13–250 times greater than susceptible laboratory strain's resistance to deltamethrin (Cao et al. 2006). Remarkably, neither poultry nor the housefly control program employed deltamethrin. These strains, however, are probably less sensitive to deltamethrin, indicating the possibility of a cross-resistance scenario.

On the other hand, the insecticide's organophosphate group inhibits acetylcholinesterase, which is in charge of causing toxicity by breaking down the excitatory neurotransmitter acetylcholine and preventing nerve impulses from being transmitted at cholinergic connections (Trang & Khandhar 2022). At first, the organophosphate insecticides were effective due to their high toxicity, rapid environmental degradation, and strong biological selectivity (Eto & Zweig

2018). However, reports on the resistance of the organophosphate group of insecticides indicate that the RR_{50} ranges for diazinon and fenitrothion, respectively, were 62.47 to 309.78 and 53.08 to 261.24, placing them in the extremely resistant classification (Abobakr et al. 2022). The organophosphate group of insecticides should be discontinued right away and replaced with a novel insecticide that works through a different mechanism, according to the same study. However, earlier research conducted in Malaysia housefly strains from two chicken farms in the state of Penang (Balik Pulau and Juru) had an RR_{50} value of 7.83 for malathion (organophosphate), falling into the low resistance category, according to research done by Bong and Zairi (2010). There hasn't been a resistance study on temephos specifically on *M. domestica* published anywhere in the world or Malaysia as of yet. As a larvicide, temephos was utilized extensively and proved to be successful in reducing the Aedes mosquito population that transmits dengue fever in Malaysia (Cheah et al. 2014). Remarkably, the information in this investigation supports the cross-resistance to temephos in the *M. domestica* poultry strain. As a result, the length of time that chemical-based pesticides are used to control houseflies at poultry should be carefully considered and regulated. The poultry strains of *M. domestica* have shown a high degree of resistance to both pyrethroid and organophosphate group insecticides, therefore they are no longer an efficient chemical-based pesticide to control houseflies in the studied poultry farm. Furthermore, if these insecticide groups are used indefinitely, the genes encoding these systems will be passed down to the housefly population's progeny, creating pest populations that will be difficult to control in the future (Chong et al. 2003; WHO 1986).

In order to lessen the need for chemical pesticides and preserve the effectiveness of those that are currently in use, it is crucial to employ non-chemical approaches. Cultural strategies include clearing manure (DVS 2019), which acts as a nesting habitat for the housefly should be an ideal first step. The main cause of the excess moisture in the manure is the water leak in the chicken watering system, which should also be kept an eye on (Axtell 1986). Biopesticides are another efficient and environmentally responsible method of insect management as they break down quickly and are often effective in very little concentration and prevent the environmental issues caused by insecticides with chemicals (Essiedu et al. 2020). In addition, chemical compounds derived from plants and plant extracts have been thoroughly researched and shown to help control the housefly population (Christopher 2012). It has been discovered that plant extracts, such as neem extract, contain insecticidal qualities that can be utilized to manage blowflies and houseflies (Siriwattanarungsee et al. 2008). Recently, in a study conducted, it was suggested plant essential oils studied, *Citrus sinensis* (L.) Osbeck and *Mentha pulegium* Linnaeus essential oils have a significant effect on the larvae and pupae of the housefly (Mahyar et al. 2023). When managing the housefly population, chemical-based insecticides ought to be the last and least used option in consideration of environmental sustainability. It would be preferable to use insecticides with distinct modes of action that target the midgut, respiration, and growth and development. Apart from the results, certain research findings indicated the use of synergists such as piperonyl butoxide (PBO), which increases the insecticidal action of pyrethroid-based insecticide (Wan et al. 2008). Research on the insecticidal effects of nanoparticles in pest control has gained attention recently. Houseflies at different embryonic stages had morphological anomalies as a result of recent research on the application of magnetite nanoparticles (Fe_3O_4 NPs), with nearly no environmental damage (Toto et al. 2022). It is also recommended future studies should be more focused on investigating the genetic and metabolic mechanisms causing resistance in vector populations, as well as establishing a statewide network for routine resistance monitoring and the housefly population's reaction in agricultural settings.

CONCLUSION

The susceptibility of poultry strains (SL and TR strains) to pyrethroids (permethrin and deltamethrin) and organophosphates (temephos and malathion) revealed that these strains pose moderate to high resistance status when compared to the susceptible IMR strain. The findings of this investigation also showed multiple resistance and cross-resistance to the tested pesticide. As a result, the findings serve as a warning about the significance of current information regarding the current level of resistance in the populations of houseflies harboring the poultry strain. Consequently, for a fly control programme at a poultry farm, it is important to first analyse the necessity of using chemical-based insecticides before choosing the appropriate insecticides. This study also pointed up the significance of using IPM techniques in addition to biopesticides and essential oils that have insecticidal properties such as repellant properties, and are easily biodegradable for sustainable farming. The best solution would be to use synergists such as piperonyl butoxide to increase the effectiveness of chemical insecticides as well as chemical insecticides with a different mode of action that targets growth and development, respiration, and the midgut. Regular resistance monitoring should be done in order to preserve the efficacy of the pesticide used in the field for a longer period. As recommendations, it is advisable to focus more on investigating the biochemical and genetic mechanisms causing resistance in vector populations.

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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

This is a Master of Science Project and the data are also available in MSc. thesis of Kalaavathi A/P Manoharan (2024).

Authors' Contributions

Kalaavathi Manoharan (KM), Siti Nasuha Hamzah (SNH) and Nurulhusna Abdul Hamid (NAH) idealized and designed the research experiments; KM conducted the experiments, interpreted the data and wrote the paper under SNH supervision, SNH and NAH participated in revising the paper. All authors read and approved the final manuscript.

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