PROTEOMIC AND BIOCHEMICAL ANALYSIS OF DETOXIFICATION ENZYME IN *Musca domestica* L. (Diptera: Muscidae) AND ITS RESPONSE UPON PROLONGED EXPOSURE OF INSECTICIDE

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by

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF PLATES

LIST OF ABBREVIATIONS AND SYMBOLS

ACh	Acetylcholine
AChE	Acetylcholinesterase
APS	Ammonium persulphate
BHC	hexachlorocyclohexane
BSA	bovine serum albumin
CCE	carboxylesterase
CDNB	1-chloro-2,4-dinitrobenzene
Cyt	Cytochrome
Cyt P450	Cytochrome P450
DDT	Dichlorodiphenyltrichloroethane
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
Est	Esterase
DVS	Department of Veterinary Services Malaysia
F1	First filial generation
GABA	γ-aminobutyric acid
GO	Gene ontology
GSH	Glutathione
GST	Glutathione s-transferase
IMR	Institute of Medical Research, Kuala Lumpur.
KEGG	Kyoto encyclopedia of genes and genomes
Kdr	Knockdown resistance
kDa	Kilodalton
LC_{50}	50% Lethal concentration
LCMS	Liquid chromatography mass spectrometry
M	Molar
mM	millimolar

Min	Minutes
Mg	Milligram
MFO	Monooxygenase
MW	Molecular weight
NA	Naphthyl acetate
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
nm	Nanometre
NSE	Non-specific esterase
OD	Optical density
PAGE	Polyacrylamide gel
PPI	Protein-protein interactions
PTU	Phenylthiourea
ROS	Reactive oxygen species
RR_{50}	50% resistance ratio
SD	Standard deviation
SDS	Sodium dodecyl sulphate
TCA	Tricarboxylic acid
TEMED	Tetramethylethylenediamine
TMBZ	3,3’,5,5’-tetramethylbenzidine
TMT	Tandem mass tag
ULV	Ultra-low application
VCRU	Vector Control Research Unit
VGSC	Voltage-gated sodium channel
VRI	Veterinary Research Institute, Ipoh
WHO	World Health Organisation
μ	Micro
α-est	α-esterase
β-est	β-esterase

LIST OF APPENDICES

ANALISIS PROTEOMIK DAN BIOKIMIA TERHADAP ENZIM DETOKSIFIKASI DALAM *Musca domestica* L. (Diptera: Muscidae) DAN TINDAK BALASNYA KE ATAS PENDEDAHAN BERPANJANGAN INSEKTISID.

ABSTRAK

Kajian ini membekalkan analisis proteomik dan biokimia enzim detoksifikasi iaitu, asetilkolinesterase (AChE), α-esterase (α-est), β-esterase (β-est), glutathione s-transferase (GST) dan sitokrom P450 (Cyt P450) dalam strain rentan (dewasa, pupa dan larva) serta strain ladang (larva) *Musca domestica*. Menerusi analisis profil protein, di dapati bahawa bilangan protein yang ditemui dalam larva adalah tertinggi (441), diikuti oleh dewasa (181) dan pupa (115) yang menyimpulkan protein khusus diperlukan untuk perkembangan wajar bagi setiap fasa perkembangan. Sementara itu, aktiviti enzim serta kandungan protein strain rentan didapati mempunyai aktiviti enzim yang berbeza-beza secara ketara merentasi peringkat perkembangan ($p<0.05$). Sebanyak, 34 peptida detoksifikasi yang disasarkan telah ditemui pada pelbagai peringkat perkembangan *M. domestica*. Analisis fungsi peptida sasaran bagi strain rentan mendedahkan bahawa sebagai tambahan kepada fungsinya dalam detoksifikasi, enzim juga didapati terlibat sebagai protein berkaitan perkembangan yang bertanggungjawab dalam pelbagai proses biologi, fungsi molekul dan laluan KEGG untuk melengkapkan metamorfosisnya. Secara keseluruhannya, peptida detoksifikasi ekspress lebih tingggi di peringkat larva, disamping diperkaya dengan proses biologi, fungsi molekul dan KEGG. Oleh itu, ini membawa kepada penyiasatan lanjut yang memfokuskan pada peringkat larva strain rentan dan ladang

ke atas kesan pendedahan berpanjangan insektisid. Status kerentanan strain ladang iaitu strain Sungai Lembu (SL) dan Tapah Road (TR) terhadap piretroid dan organofosfat menunjukkan rintangan sederhana hingga rintangan tinggi iaitu diantara 13.78 kali ganda kepada 48.82 kali ganda. Penyiasatan perbandingan pemprofilan enzimatik mendedahkan bahawa strain SL dan TR telah mempunyai aktiviti α-est, β-est, GST, dan Cyt P450 dengan lebih tinggi berbanding strain IMR yang menandakan penglibatan enzim detoksifikasi dalam pendedahan racun serangga berpanjangan. Aktiviti AChE bagi strain ladang didapati berkurang dan ini menolak penglibatan AChE dalam cabaran toksikologi. Siasatan proteomik mendedahkan kehadiran 16 Est, 15 isoform atau kelas GST, dan 11 isoform Cyt. Analisis kuantitatif peptida menunjukkan peningkatan peptida GST (GSTD1-1, GSTD1-3, GSTO1 dan GSTS1-2) dan Cyt (Cyt C-5) di mana peningkatan GSTO1 dan GSTS1-2 adalah paling tinggi (>2.5 kali ganda). Oleh itu, disimpulkan bahawa lalat rumah menjalani detoksifikasi metabolik melalui metabolisma langsung glutation, detoksifikasi pasif yang melibatkan konjugasi GST kepada kumpulan sulfhidril, detoksifikasi produk peroksidasi lipid, dan mekanisme pengasingan. Selain itu, didapati peptide Cyt c oxidase menyediakan pembaikan sel yang rosak dan perlindungan terhadap spesis oksigen reaktif (ROS) yang disebabkan oleh pendedahan kepada pyrethroid dan organofosfat.

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ABSTRACT

This study provides the proteomic and biochemical information of the detoxification enzymes namely acetylcholinesterase (AChE), α-esterase (α-est), β-esterase (β-est), glutathione s-transferase (GST) and cytochrome P450 (Cyt P450) in susceptible (adult, larvae and pupae) and poultry strains (larvae) *Musca domestica.* Upon whole protein analysis, number of proteins discovered in larvae was highest (441), followed by adults (181) and pupae (115) which concluded specific proteins are necessary for the proper progression of each developmental phase. Meanwhile, the enzyme activities as well as the protein content of the susceptible strain were found to have significantly varied enzymatic activity across the developmental stages ($p<0.05$). Thirthy four distinct peptides of the targeted detoxification enzyme were discovered at various developmental stages of the susceptible strain. Functional analysis of the targeted peptides of the susceptible strains exposed that in addition to their functions in detoxification, the multifunctional enzymes were also found to be developmental-related proteins that responsible in various biological process, molecular functions and KEGG pathways to complete metamorphosis. Overall, it has been observed that at the larvae stage, the targeted developmental related detoxification peptides were more expressed and were the most enriched in terms of biological process, molecular function and KEGG. Therefore, this led to further investigation focusing on the susceptible and poultry strains larvae for further study on the effect of prolonged insecticide exposure. The susceptibility status of the poultry strains namely Sungai Lembu (SL) and Tapah Road (TR) strain towards pyrethroid and organophosphate revealed moderate to high resistance in comparison to the susceptible Institut Medical Research (IMR) strain (13.780fold to 48.82-fold). Comparative investigation of differential enzymatic profiling revealed that SL and TR strains had significantly elevated α-est, β-est, GST, and Cyt P450 activities compared to susceptible IMR strains signifying the involvement of detoxification enzymes in prolonged exposure to insecticides in poultry farms. The involvement of AChE in prolonged insecticide exposure was ruled out as enzymatic activity was reduced in poultry strain. The proteomic investigation revealed the presence of 16 isoforms of Est, 15 isoforms or class of GST, and 11 isoforms of Cyt. Though, peptide abundance analysis showed upregulation of GSTs (GSTD1-1, GSTD1-3, GSTO1 and GSTS1-2) and Cyt (Cyt C-5) whereby GSTO1 and GSTS1-2 fold-change were noticeably high (>2.5-fold). Thus, it is concluded that the housefly undergoes metabolic detoxification via direct metabolism of glutathione, the passive detoxification provided by GST conjugation to sulfhydryl groups, the detoxification of lipid peroxidation products, and sequestering mechanisms were all attributed to GSTs including the involvement of Cyt c oxidase which assist in the damaged cell repair and protection against reactive oxygen species due to exposure of pyrethroid and organophosphate.

CHAPTER 1

INTRODUCTION

1.1 Overview

Musca domestica also known as housefly or domestic fly has successfully evolved synanthropic insect that has been identified as a mechanical vector for more than 100 pathogens. Housefly breeds and feeds organic material such as faeces, decaying organic material and garbage. During its contact with the feeding material, housefly tends to pick up pathogens on the external body parts as well as the internal body that may give a negative impact on both humans and animals where they transmit. It has been proved that this species transmits more than 100 pathogens that eventually affect the health of humans and animals (Abbas *et al.*, 2014; Abbas *et al.*, 2015; Ma *et al.*, 2017).

M. domestica has a complete metamorphosis with distinct eggs, larvae, pupae and adults. The adult female could lay up to 500 eggs in several batches of 75 to 159 eggs in a 3 to 4 days period. Due to the abundant and effective breeding, *M. domestica* had become a major pest in the domestic, medical, and veterinary fields (Axtell, 1986). Studies also indicated that there is evidence showing the correlation between the rise in the occurrence of diarrhoea and the population of houseflies (Levine and Levine, 1991; Nichols, 2005; Farag *et al.*, 2013; Hafiz *et al.*, 2014).

Therefore, regular travelling of houseflies from the breeding sites to human and livestock habitats may lead to disease transmission between humans and animals (Khamesipour, 2018). According to WHO (1991), the housefly is the main mechanical vector for the spreading of eye infections. This synanthropic creature also has been regarded for spreading skin disease, leprosy poliomyelitis and enteric infections such as dysenteries, diarrheoa, typhoid, cholera, and helminths (Keiding, 1986).

There are also some reported human and animal cases where the housefly tends to deposit its eggs on the flesh of the host and leading to myiasis (Antunes *et al.*, 2011). With the emerging and re-emerging diseases caused by the most distributed houseflies, more drugs, antimicrobials and vaccines are developed and produced by humankind to ensure the betterment of public health, and these are directly increasing the cost of healthcare.

The housefly is considered the main pest in livestock industries especially poultry following in annoyance and oblique harm in livestock production. The poultry industry in Malaysia is vital in supplying a major source of protein to the nation. Nevertheless, farms cultivated by the farmers have been raised with issues regarding the increasing population of the housefly and the disease that they bring to the farm animals. The waste such as faeces that are produced by farms often attracts the housefly for breeding. In order to control the population of the housefly, the management tends to use chemical insecticides which are effective and economical in cost wise, however, it leads to other issues such as insecticide resistance, environmental pollution, the killing of housefly pray ecological impact and increasing the production cost. Saddening, livestock farms that are unable to manage the housefly problem in Malaysia will be subjected to the lawsuit and even compounds are also applied.

1.2 Background

Globally, the population growth of humans has increased from 1 billion in the year 1800 to 7.7 billion in the year 2019 (Roser *et al.,* 2019). This number was estimated to hike to 9.7 billion in 2050 by the United Nation (Elferink and

Schierhorn, 2016). The increasing human population leads to a rise in food demand. Thus, we are moving to a bigger scale in food chain supply especially in agriculture industries such as livestock in order to fulfil the demand. Indirectly agriculture activities are generating more waste at large which has increased the population of pests with improper management of waste. Both highly populated and unhygienic both rural and urban settlements attracted pests such as houseflies to invade and breed successfully. Houseflies often exist in the large population creating a nuisance to humans by entering the house and land, feeding on human food at open restaurants and even spotting the windows with their faeces (Gregory *et al.*, 2009).

It is well known that *Musca domestica* is responsible to be a mechanical vector for more than 100 types of pathogens which includes bacteria, virus, fungus, and parasites (Abbas *et al.*, 2015; Abbas *et al.*, 2014; Ma *et al.*, 2017). These pathogens served both human medical and veterinary health importance (Axtell, 1986). The most commonly reported diseases that are transmitted by pathogens carried by houseflies are typhoid, cholera, salmonellosis, dysentery, polio, anthrax, tuberculosis, helminths infections and eye infections such as trachoma and epidemic conjunctivitis (Isa, 2019). Thus, the impact caused by flies on public health risks is quite large. The presence of houseflies at human settlements, food stalls and farms are labelled as unhygienic, and the owners can be facing a lawsuit for improper hygienic management that attracts the housefly to breed.

The life cycle of *M. domestica* consisting of adults, eggs, larvae, and pupae takes only 7-10 days and an adult life span of 15 to 25 days could go up to 2 months. This able the houseflies to breed in large numbers and be effective in maintaining their population. Strategy to control the population of houseflies is implemented by targeting all levels of the life cycle.

Control approaches that are commonly practised for the control of *M. domestica* are cultural, biological, and chemical. Among the approaches used to control the problem of house flies is chemical-based pesticides were vitally used as it is effective and easily available on the market. However, inappropriate and prolonged use of these substances had developed resistance in housefly. According to Sawicki (1987), resistance is defined as a genetic change in response to the selection by toxicants that may impair control in the field. Besides, the misconduct and improper use of chemical insecticides are much hazardous to livestock as well as the environment. Thus, it is a serious problem for the farmers to get rid of these vectors for disease control.

Major classes of chemical insecticides that are used in controlling the *M. domestica* are pyrethroid, carbamate, and organophosphate groups (Keiding, 1986). Four main enzyme systems were identified that involved in resistance towards these chemical insecticides in insects including houseflies namely acetylcholinesterase (AChE), esterase (Est), glutathione s-transferase (GST) and cytochrome P450 (Cyt P450) are focused in this study (Hamzah *et al.*, 2022). These enzymes play a primary role in the detoxification of endogenous and xenobiotic compounds besides being involved in protection against oxidative stress.

M. domestica is selected in this study as it is interestingly holding the record for the greatest ability to develop evolutionary xenobiotic resistance in insect groups (Keiding *et al.*, 1977). Thus, much ongoing research and studies related to xenobiotic resistance conducted on this species in present. Profiling the housefly will enable us to understand the resistance mechanism that the species has developed in order to overcome this challenging pest. Additionally, in this study, the interactions between proteins in *M. domestica* species which elucidate the biological function that contributes to the insecticide resistance mechanism are also determined.

1.3 Objectives

This study focused on research objectives as listed below: -

1. To profile and analyse the protein of susceptible strain of *M. domestica* in different development stages.
2. To profile and analyse the targeted detoxification enzyme in different developmental stages of susceptible strain of *M. domestica*
3. To determine the susceptibility status of the poultry strains and the detoxification enzyme that response towards prolonged insecticide exposure of poultry strain of *M. domestica* larvae.
4. To determine the role of peptides that respond to the prolonged insecticide exposure in poultry strain *M. domestica.*

CHAPTER 2

LITERATURE REVIEW

2.1 *Musca domestica*

Musca domestica or housefly possibly originated from central-asia is the most prevalent species found on both farm and house (Sarwar, 2016). This dipteran order species was classified under the Muscidae family are distributed worldwide and considered pests in rural and urban housing, livestock farms and even in the food processing industry (Sarwar, 2016; Geden *et al.*, 2021).

2.1.1 Classification

The classification of the houseflies is as follows (Pont, 1981):

Domain	: Eukarya
Kingdom	: Animalia
Phylum	: Arthropoda
Subphylum	: Hexapoda
Class	: Insecta
Subcalss	: Pterygota
Order	: Diptera
Suborder	: Brachcera
Family	: Muscidae
Subfamily	: Muscinae
Tribe	: Muscini
Genus	: *Musca*
Species	: *Musca domestica*

2.1.2 Morphology

In the tenth edition of the Systema Naturae published in 1758, Linnaeus provided the first description of *Musca domestica* (Pont, 1981). Linnaeus utilised wings to distinguish between the several orders, and mouthparts to describe genera. The 6 to 7 mm long adult common housefly can be recognised by its crimson eyes, sponging mouth parts and has a 13–15 mm wingspan as in **Plate 2.1** (Geden *et al.*, 2021). The front of the grey thorax has four prominent longitudinal stripes that are particularly noticeable there (Borror *et al.*, 1989). At the wing margin, the fourth vein on the wing almost touches the third vein before bending sharply forward. Particularly in males, the sides of the basal half of the abdomen are yellowish buff and occasionally translucent (Hewit, 1914). The final portions are covered by a central longitudinal band that gets wider at the back. Adult houseflies have short antennae and a grey thorax with four darker longitudinal stripes, as well as a grey or yellow abdomen with a darker median line and an irregular light yellowish patch at the anterior lateral edges. Males have eight abdominal segments, whereas females have nine (Dahlem, 2003). The first five segments are seen externally in females. The last four segments are ordinarily retracted, but when the female deposits her eggs, they stretch to form the ovipositor. This enables females to bury their eggs several millimetres below the surface. Females are a little bigger than males.

The eggs of the houseflies are creamy white in colour, about 1 mm long, and often deposited singly in little batches of 75 to 150 eggs apiece. in damp substrates (**Plate 2.3**). The eggs are placed singly in tiny batches of 75 to 150 eggs each, and they are around 1.2 mm long (Azmi *et al.*, 2016).

The larva of a housefly begins as a 3 to 9 mm long, creamy-white, cylindrical, and tapers towards the end larvae as shown in **Plate 2.4** (Ceden *et al.*, 1988). It has a mouth on its head with two black hooks but lacks eyes, legs, or any other appendages. It is divided into 13 segments, 12 of which are clearly visible (West, 1951). It develops through three instars, or two moults, to form a 7 to 12 mm long maggot. It finishes developing after 4 to 13 days at ideal temperatures (23°C to 30°C) or after 14 to 30 days at unfavourable temperatures from 12°C to 17°C (West, 1951; Azmi *et al.*, 2016).

The oval pupae measure around 8 mm in length (Azmi *et al.*, 2016). The pupa's colouring varies as it ages, going from creamy-white to yellow, brown, red, and finally black (Azmi *et al.*, 2016). Its development is finished in 2 to 6 days at temperatures between 32°C and 37°C, or in 17 to 27 days at about 14°C (Azmi *et al.*, 2016).

Plate 2.1 Adult housefly, *Musca domestica* Linnaeus
View under a dissecting microscope at 1.0 x magnification

Plate 2.2 Lateral view of the head of an adult housefly, *Musca domestica* Linnaeus
View under a dissecting microscope at 1.2 x magnification

Plate 2.3 Egg of housefly, *Musca domestica* Linnaeus
View under a dissecting microscope at 4.0 x magnification

Plate 2.4 Larvae of housefly, *Musca domestica* Linnaeus
View under a dissecting microscope at 1.5 x magnification

Plate 2.5 Pupae of housefly, *Musca domestica* Linnaeus
View under a dissecting microscope at 1.2 x magnification

2.1.3 Development stages

Insects undergo significant developmental changes as they evolve from immature to adulthood. Metamorphosis refers to a series of physical, physiological, and or behavioural changes that help a species' survival, dispersion, and reproduction (Rolff *et al.*, 2019). The housefly goes through four distinct phases during its life cycle **(Figure 2.1.)**, beginning with an egg and progressing to a larva, pupa, and adult, which can be completed in 7 to 10 days in ideal conditions or up to 2 months in poor settings (Azmi *et al.*, 2016). The housefly can have more than 20 generations per year in tropical and subtropical climates, and 10 to 12 generations in temperate regions (Azmi *et al.*, 2016).

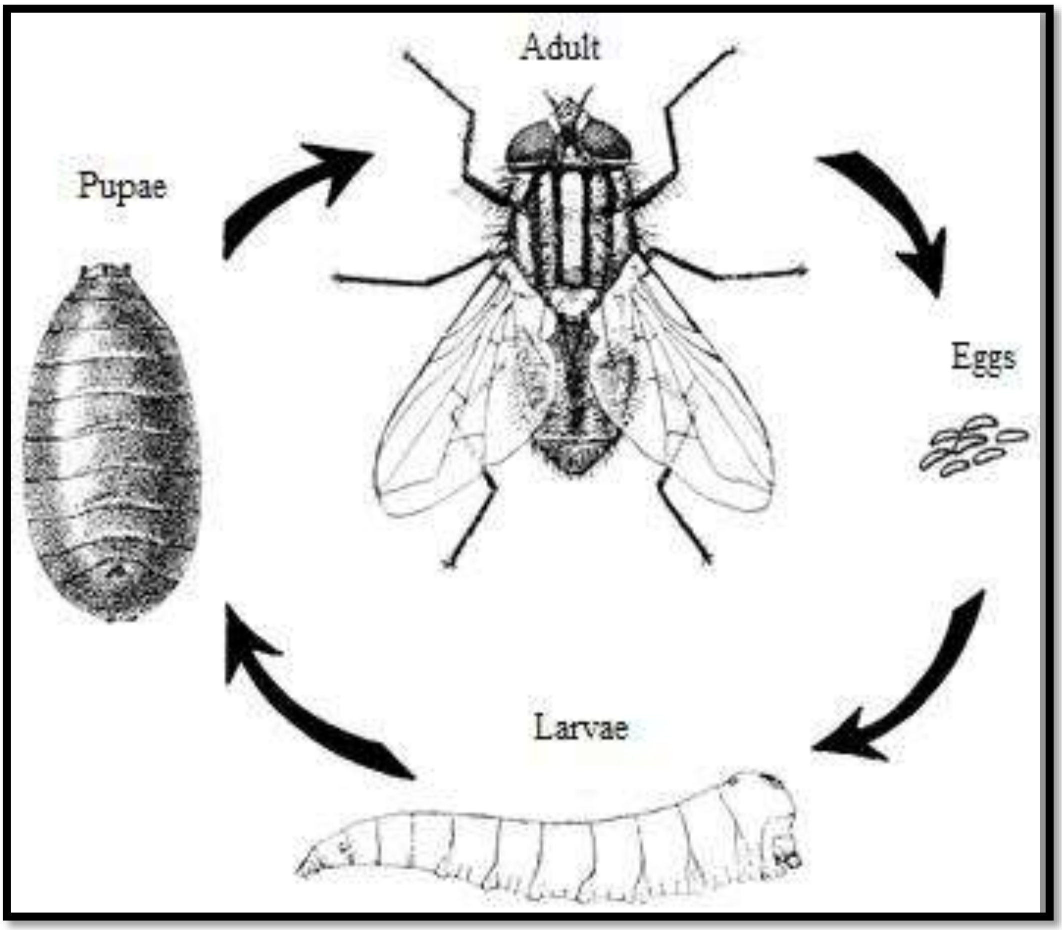

Figure 2.1 Life cycle of *Musca domestica* (WHO, 1986)

2.1.4 Feeding habit

Houseflies consume a variety of organic substances, such as faeces and other liquids as well as waste manure which has high nutritional value. As omnivorous, housefly's mouth is designed such that it can only eat liquid and semi-liquid meals. As a result, when it touches solid food, it vomits on it, turning it into a solution that it then swallows, contaminating the food (WHO, 1986). Nevertheless, in order to preserve a housefly's longevity, carbohydrates are a crucial component of its diet. While proteins are essential for the development and laying of eggs (Azmi *et al.*, 2016). The housefly's feeding mechanism, the proboscis, works like a straw for the insect (Azmi *et al.*, 2016). Limiting the fly to liquids would prevent the need to prepare solid substrates for ingestion. Typical suction and filtering of liquid food is a common method of feeding for houseflies, in which food is pulled by the suction power produced by muscular contraction. Houseflies utilise the prestomal teeth found in the labella to scrape solid food, and then moisten it with saliva or vomited food to create a solution that can be absorbed later by sucking (Azmi *et al.*, 2016). The most active feeding habit occurs during larvae stage (Hewitt, 1914). At certain temperatures, larvae will devour paper and textile materials such as wool, cotton, and sacking (Hewitt, 1914). At the pupae stage, larvae will be in a stagnant condition and unable to feed (Kelling *et al.*, 2002).

2.1.5 Mating and breeding

The female housefly releases the pheromone muscalure to find a partner and start the reproduction process (Carlson *et al.*, 1971). When a male captures a female, mating commences. It lasts for a few seconds to a few minutes and occurs on an item (Azmi *et al.*, 2016). In order to get the female to extend her ovipositor so that it could link with the male's penis, which descends from his genital atrium, the male would use his front tarsi to rub the female's head after capture. The male can ascertain the probable mate's species, sex, and status for mating by caressing the female with the fore tarsi (Dahlem, 2009).

Female house flies normally mate just once, however, males frequently procreate with a variety of mates (Gowathy, 2012). A total of five to six batches of 75 to 100 eggs, which hatch in 12 to 24 hours, may be laid by the female (Azmi *et al.,* 2016; Sarwar, 2016). The number of eggs each female produce is proportionate to her size, therefore the bigger the female, the more eggs she produces. A female housefly can often deposit up to 500 eggs over the course of three to four days (Azmi *et al.,* 2016). Most of the time, numerous flies will deposit their eggs near to one another, producing numerous fly larvae and pupae at the location (Azmi *et al.,* 2016). At poultry farms, housefly deposit it eggs in the manure which is damp and nutritious whereby accumulation of larvae frequently observed **(Plate 2.6)**.

Plate 2.6 Housefly larvae accumulated at the faeces of the chicken at a poultry farm

2.2 Medical and veterinary health impacts

The housefly acts its role as a mechanical vector for the transmission of disease pathogens which includes parasites, bacteria, viruses and fungus (Khamesipour *et al*, 2018). These pathogens that are carried by the housefly may have medical and veterinary health impacts that cause disease to organisms wherever they land. Houseflies on the whole feed and reproduce in feces, animal carcasses and other decaying organic material, and consequently, houseflies live in close association with various microorganisms (Khamesipour *et al.,* 2018). There is also the possible tendency for the transmission of antimicrobial and multi-drug resistant pathogens from houseflies that are captured in hospital environments and livestock farms where antibiotics are used extensively as disease control and growth promoters (Davari, 2010; Nassiri, 2015; Nazari, 2017).

Houseflies picked up pathogens through external body parts such as body hair and tarsi (Keiding, 1986). The pathogen that sticks to the houseflies lasted a few hours but those ingested may survive in the guts for a few days (Axtell, 1985; Williams *et al.,* 1985). Well known for causing nuisance and annoyance to human and animals (Iqbal *et al.,* 2014) housefly also carries a negative psychological impact as their existence is considered an indication of unhygienic condition (Keiding, 1986).

Studies also indicated that there are evidence showing the correlation between the rise in occurrence of diarrhea and the population of houseflies (Aha, 2014; Levine, 1991). Therefore, regular travelling of houseflies from the breeding sites to human and livestock habitats may lead to disease transmission between humans and animals (Khamesipour, 2018). According to Keiding, (1986), housefly is the main mechanical vector for the spreading of eye infections. This synanthropic creature also has been regarded for spreading skin disease, leprosy poliomyelitis and enteric infections such as dysenteries, diarrhea, typhoid, cholera and helminths (Keiding, 1986).

There are also some reported human and animal cases where the housefly tends to deposit the eggs on the flesh of the host and leading to myiasis (Antunes *et al.,* 2011). With the emerging and re-emerging diseases caused by the most distributed houseflies, more drugs, antimicrobials and vaccines are developed and produced by humankind to ensure the betterment of public health, and these are directly increasing the cost of healthcare.

The housefly is considered as the main pest in livestock industries especially poultry ensuing in annoyance and oblique harm in livestock production. The poultry industry in Malaysia is vital in supplying a major source of protein to the nation. Nevertheless, farms cultivated by the farmers have been raised with issues regarding the population of houseflies and the disease that they bring to the farm animals. The waste such as faeces that are produced by farms often attracts the housefly for breeding. In order to control the housefly population, the management tends to use chemical insecticides which are effective and economical cost-wise, however, it leads to other issues such as insecticide resistance, environmental pollution, the killing of houseflies pray ecological impact and increasing the production cost. Saddening, livestock farms that are unable to manage the housefly problem in Malaysia will be subjected to lawsuits and even compounds are also applied.

2.3 Control measures

The most pervasive synanthropic pest in the world is the housefly (Hinkle and Hogsette, 2021). Houseflies infiltrate the housing settlements and buildings, but rarely reproduce there (Hinkle and Hogsette, 2021). Due to the restricted capacity of urban pest management professionals to identify and manage larval habitats, the majority of housefly management in urban settings concentrates on adult fly reduction. Whereas in the agricultural field, the management of housefly populations was concentrated at all development stages since they reproduce massively there. According to WHO (1986), the control measurement of the houseflies can be categorized into cultural control, biological control and chemical control.

Local municipal councils in Malaysia are given the authority in controlling the pest including disease causing vectors such as the housefly and mosquitoes. This includes imposing compounds and legal actions to house owners, food premises, food industries and agricultural premises that failed to compile the rules and regulations that have been set up in order to control the pest. The local municipals are also responsible for educating and creating awareness among the public through campaign mandatory training as well as certification and permit for relevant premises.

2.3.1 Cultural control

Cultural control refers to the manipulation of the production system and environment in order to minimize and eventually eliminate the population of pests and the most common practice that adheres to cultural control is avoidance (James, 2018). Avoidance the housefly population to invade the poultry farm by having proper sanitation, razing possible breeding places, minimizing light, implementing biosecurity measurements and scheduled monitoring of housefly population at the premises.

Sanitation is the primary defence measure against pests in poultry farms. Possible breeding mediums of houseflies such as manure, feed spillage, broken eggs and mortalities need to be disposed immediately is necessary to improve the monitoring and regulation of chicken manure management (Hongshun *et al.,* 2014). The manure that stays on the farm should be handled to keep it dry and friable. Wet manure can be reduced by controlling the height of drinking systems and monitoring water flow rates. In addition, leaks in drinking water systems should be monitored on a regular basis.

Housefly as well as another common agricultural pest is phototaxis naturally (Urban and Broce, 2000). Therefore, to reduce the population of barns, the use of light should be at a minimum. However, this behaviour of attraction to the light makes by a fly trap especially ultraviolet light is effective.

The installation of extraction blowers that direct airflow outward is discovered to be beneficial for halting the housefly infestation in the barns (Mathis *et al.*, 1970). Stainless steel, coated steel, polyvinyl chloride or vinyl, and aluminium are common materials for mesh screening and to successfully exclude flies, the screen hole size should be between 0.88mm and 1.22mm (Busvine, 1965).

If enough traps are utilized, positioned correctly, and employed both indoors and outdoors, fly traps may be beneficial in some fly control programmes (Hinkle and Hogsette, 2021). Houseflies are drawn to white surfaces and odour-producing sticky baits **(Plate 2.7)**. Indoors, ultraviolet light traps capture flies in an inverted cone or electrocute them with a grid. Inside structures, one trap should be put every 30 feet of wall, but not over or within five feet of food processing facilities. Near building entrances, passageways, beneath trees, and around animal sleeping areas and dung dumps are also good places to put them outside. Building openings should be thoroughly covered with regular window screens to prevent insects from entering.

Traps are commonly used in conjunction with a mechanism that traps the attracted flies and can be baited with molasses, sugar, fruit, or meat (Hinkle and Hogsette, 2021). The sex pheromone (Z)-9-tricosene, often known as muscalure, is also an aggregation pheromone (Carlson *et al.*, 1971). Muscalure is commercially available fly bait that is prepared with sugar for local population suppression and population monitoring (Carlson and Beroza, 1973).

Ultraviolet light traps are a non-chemical control approach that can be utilised indoors in both agricultural and non-agricultural settings (Hinkle and Hogsette, 2021). Electrocuting flies that enter the trap is how they usually work, while those used in restaurants and farms usually feature a sticky screen, tapes, ribbons **(Plate 2.8)** or tubes (Kaufman, *et al.*, 2005). Because flies do not orient to traps from a considerable distance, multiple traps are usually required to be effective. To take advantage of fly flight behaviour, placement should be within 4 to 8 metres of entryways and 1.5 metres of the floor. They should be used all the time but are most effective when the room lights are turned out.

Systematic fly population monitoring aids in determining when and where insecticides should be used. It can also serve as a legal document in the event of public health or nuisance complaints involving flies from the farm. Simple observation of adult flies gives a less accurate reference point for fly counts than a constant and dependable fly surveillance strategy. The fly index tool is recommended by the Department of Veterinary Services in Malaysia to be used as a guide to control house flies at poultry farms. This calculation is made using the scudder grill **(Plate 2.9)** method where the device is placed in an area where there are many flies such as under the coop (WHO, 1986). Flies that land on the grill within 30 seconds will be counted. If the number of flies in a place exceeds the set index, then the number or population of flies in that place is counted a lot and needs to be controlled. According to the state poultry plantation enactment in Malaysia, the fly index is to be kept below the level of 10 for commercial poultry farms (DVS, 2019).

Plate 2.7 Sticky bait used in farm to reduce the population of houseflies

Plate 2.8 Ribbons dipped in sticky glue-like substance used to control the housefly in farm and chicken manures that were cleaned from the floor and packed

Plate 2.9 Scudder grill used as tool to calculate the fly index at farms

2.3.2 Biological control

Biological control in managing the housefly involves plant extracts, fungal pathogens, bacteria, parasitoids, and predators (Anushree *et al.*, 2007).

Plant derived extracts and oils have been significantly recognized as natural sources of pesticides (Isman, 2006; Shaalan *et al.*, 2005). Botanical pesticides are more targeted than chemical pesticides and do not harm non-target organisms and may be advantageous from an economic and ecological standpoint. Tests reveal that only insects, not mammals, are affected by the synergistic increase in toxicity of plant pesticides (Wilikins and Mercalfe, 1993). Botanical pesticides can be used as larvicidal, pupicidal, and adulticidal while some of them can also be employed as insect growth regulators, antifeedants, ovipositional deterrents, and repellents for houseflies. A comparative study of the efficacy of neem leaf extract and chemical insecticide using Dichlorvos (DDVP) has been, conducted by Khan and Ahmed in the year 2000. It has been concluded that the neem leaf extract diluted with acetone was effective in controlling the housefly when used topically as adulticide whereby 30% of mortality was observed in the adult at 2.5 μg/fly and 85% of mortality at 80 μg/fly while for the DDVP were at 0.44, and 3.58 μg/fly respectively. Neem leaves are readily available, and the resulting product is environmentally friendly, thus their commercial feasibility should not be discounted. In another study on the insecticidal properties of 31 essential botanical oil for repellent against pulse beetle (*Callosobruchus chinensis)* and housefly, *Ocimum gratissimum, Thymus serpyllum* L., *Illicium verum* Hooks.f., *Myristica fragrans* Houtt., *Curcuma amada* Roxb. showed 100% repellency (Singh and Mehta, 2000). To date, the study field application of plant derived material is still lacking and it is crucial to carry out the botanical pesticide toxicology assay tests before advising on their field application.

Using biopesticides based on entomopathogenic, the fungus can be an alternative solution to control the housefly populations. Infections with fungi are highly prevalent in houseflies. By breaking through the host cuticle or the stomach wall of sucking insects, fungi can infect insects (Hajek and Leger, 1994). It is well known that the fungus pathogen *Beauveria bassiana* can be used to manage agricultural pests and is found naturally occur in hundreds of insect species including houseflies (Fargues and Remaudiere, 1977; Shelton *et al.,* 1998). *Beauveria bassiana* was initially discovered naturally in houseflies by Steinkraus *et al.* (1990). Since then, numerous investigations on the utilisation of *Beauveria bassiana* against houseflies in lab and field trials have been carried out. A similar study conducted by Naworaj *et al.* (2014), found two species of fungi pathogens, *Beauveria bassiana* and *Metarhizium anisopliae* have the potential to control the housefly population. A further benefit of *Beauveria bassiana* is that it may be easily cultivated on synthetic media. As a result, it is simple and affordable to manufacture this fungus, and it also gets along with the environmental conditions seen in poultry farms.

Bacteria species are also potentially recognized for their ability to control houseflies in agricultural fields. A study on the toxicity of *Bacillus thuringiensis* against houseflies resulted in 50% of the larvae population being killed by Crystal δ-endotoxins at a concentration of 10.2 μg/ml (Hodgman *et al.,* 1993). In poultry farms, *Bacillus thuringiensis* mixed with chicken feed may significantly lower house fly populations (Anushree *et al.,* 2007). Fed chicken faeces were found to be highly poisonous to breeding maggots. The maximum larvicidal activity could be reached on the fourth day of post-feeding faeces and lasts until the sixth day.

Legner (1995), has studied the biological control using parasites against the housefly population in temperate areas. Two parasites were identified to be able to control the housefly at larvae and adult stages which are nematodes and hymenoptera parasitoids. Nematode parasite possesses the entomopathogenic character of its lethal toxin and the symbiotic bacterium that it carries (Nickel, 1984; Kondo and Ishibashi, 1988). The infectious juvenile infects the insect host by directly penetrating the cuticle, anus, spiracles, or mouth and is capable of causing death in 24 to 72 hours. In a comparative field study of commercial bait (methomyl) and nematode bait using *S. feltiae* and *Heterorhabditis megidis* against housefly populations at a pig farm, the number of flies was found lower at nematode baited house (Renn, 1998). Although some nations forbid the introduction of non-indigenous species, nematodes are non-polluting and hence environmentally safe and acceptable (Anushree *et al.*, 2007). Meanwhile, Hymenoptera parasitoids species (*Pteromalidae* and *Ichneumonidae*) were observed to infect the houseflies' pupae (Skovgard and Jespersen, 1999).

Another biological method to minimise the density of house flies is predation (Anushree *et al.*, 2007). Investigations on the predaceous arthropods in manure have been carried out in the United States and it was found that, predators found in poultry manure were the histerid beetle, *Carcinops pumilio* (Erichson), and the mite, *Macrocheles muscaedomesticae* (Scopoli) (Legner and Olton 1970; Legner *et al.*, 1975; Axtell 1986; Axtell and Arends 1990; Wills *et al.*, 1990). Hulley and Pfleiderer (1988) found that *C. troglodytes* (Payle) were plentiful in poultry manure in South Africa. Similarly, the common parasitoids that attacked the housefly pupae were, the pteromalid wasps *Muscidifurax* and *Spalangia* species, correspondingly were also encountered by Hulley (1983) and Matanmi and Giliomee

(2002) in South Africa. These biological predators invade and feed on the larvae and pupae that are accumulated in the manure at farms (Achiano and Giliomee, 2006).

2.3.3 Chemical control

A class of chemicals known as pesticides are designed to eradicate and kill pest species (Naqvi *et al.,* 2016). Insecticides, herbicides, fungicides, and other pest management agents make up the pesticides. These have severe negative health effects on non-target creatures and are exceedingly poisonous. Pesticides are classified based on their origin, targeted species and function as in **Figure 2.2** (Abubakar *et al.,* 2000). Four major groups of synthetic classes of insecticide are organochlorine, pyrethroid, organophosphate and carbamates (Abubakar *et al.,* 2020). The primary pest affecting public health in Malaysia is the house fly, which is commonly controlled by insecticides applications in the agricultural field (Nazni *et al.,* 1999).