INFLUENCE OF MICROBIAL INOCULANT ON THE OVIPOSITION AND COMPLETION OF LIFE CYCLE OF *Chrysomya megacephala* AND *Chrysomya rufifacies* INFESTING RABBIT CARCASSES

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Dedicated to my beloved parents and siblings.

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ABSTRACT

Considering any surrounding factors as well as the cunning use of microbial inoculants to cover the smell of decomposing bodies could potentially alter (delay or speed up) the time of initial oviposition and developmental rate of insects infesting the corpses, thereby resulting in erroneous estimation of post mortem interval (PMI); such factors led to the initiation of this research. This research was conducted to assess the influence of a commercially available microbial inoculant (EM.1®) on the initial oviposition and developmental rate that lead to completion of life cycle of the two prevalent necrophagous flies (*Chrysomya megacephala* and *Chrysomya rufifacies*) in Malaysia infesting rabbit carcasses. In this research, nine rabbit carcasses were equally divided into control (C) and two treated (T1 and T2) groups. The T1 and T2 carcasses were individually sprayed with two different concentrations at 1:500 and 1:100 of the same microbial inoculant, respectively. The durations taken for each species of fly to first oviposit and complete their life cycle in treated carcasses were compared with that of control carcasses. Results revealed that single application of the microbial inoculant on both the treated carcasses did not statistically impede oviposition of both species as well as their subsequent developmental patterns (Kruskall-Wallis H: $P > 0.05$) when compared to the control carcasses. Since this is the first research that reported on the time of initial oviposition and completion of life cycle for *C. megacephala* and *C. rufifacies* in the presence of microbial inoculant, the results observed here may prove useful for estimating PMI in Malaysia particularly in cases where the use of microbial inoculant was suspected.

ABSTRAK

Mengambil kira sebarang faktor di sekeliling serta inokulan mikrob yang boleh digunakan dengan licik oleh pembunuh untuk melindungi bau mayat yang mereput berpotensi untuk mengubah (melewatkan atau mempercepat) masa permulaan pengovipositan dan kadar perkembangan serangga yang menginfestasi mayat, seterusnya mengakibatkan penganggaran sela-masa kematian (PMI) yang tidak tepat; faktor-faktor tersebut membawa kepada permulaan penyelidikan ini. Penyelidikan ini dijalankan untuk menilai pengaruh inokulan mikrob yang boleh didapati secara komersial (EM.1®) ke atas permulaan pengovipositan dan kadar perkembangan yang membawa kepada kitaran hidup lengkap bagi dua lalat nekrofagus yang lazim di Malaysia (*Chrysomya megacephala* dan *Chrysomya rufifacies*) yang menginfestasi bangkai arnab. Dalam penyelidikan ini, sembilan bangkai arnab telah dibahagikan kepada kumpulan kawalan (C) dan dua kumpulan dirawat (T1 dan T2). Bangkai-bangkai T1 dan T2 disembur dengan inokulan mikrob yang sama pada dua kepekatan berbeza iaitu 1:500 dan 1:100, masing-masing. Tempoh yang diambil oleh setiap spesies lalat untuk oviposit yang pertama dan melengkapkan kitaran hidup mereka pada bangkai dirawat dibandingkan dengan bangkai kawalan. Hasil penyelidikan menunjukkan bahawa aplikasi tunggal inokulan mikrob pada kedua-dua bangkai yang dirawat secara statistiknya tidak menjejaskan pengovipositan kedua-dua spesies serta corak perkembangan mereka (Kruskall-Wallis H: $P > 0.05$) apabila dibandingkan dengan bangkai kawalan. Oleh kerana ini adalah penyelidikan pertama yang melaporkan mengenai permulaan pengovipositan dan kitaran hidup lengkap untuk *C. megacephala* dan *C. rufifacies* dengan kehadiran inokulan mikrob, dapatan yang diperoleh di sini mungkin berguna untuk menganggarkan PMI di Malaysia terutamanya di dalam kes di mana penggunaan inokulan mikrob disyaki.

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a.m.	-	Ante meridiem
ANOVA	-	Analysis of Variance
C	-	Control
C. megacephala	-	*Chrysomya megacephala*
C. rufifacies	-	*Chrysomya rufifacies*
DC	-	Dorsal cornu
EM	-	Effective microorganisms
G	-	Gena
KOH	-	Potassium hydroxide
mPMI	-	Minimum Postmortem interval
PG	-	Postgena
PE	-	Peritreme
p.m.	-	Post meridiem
PMI	-	Postmortem interval
S	-	Spiracular slits
SD	-	Standard deviation
T1	-	Treated 1 (1:500)
T2	-	Treated 2 (1:100)
VC	-	Ventral cornu
VOC	-	Volatile organic compound
Viz.	-	Videlicet (namely)

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Application of entomology in death investigations involves interpretation of the insects' developmental data, particularly for providing accurate estimation of minimum post mortem interval (mPMI) (Mahat *et al*., 2014). In addition, entomological evidence is also useful for revealing the possibility of postmortem relocation of bodies, suggesting the possible cause of death as well as identification of the deceased (Gennard, 2007; Goff, 2009; Mahat and Jayaprakash, 2013). Forensic entomological evidence becomes particularly important in cases which the time since death is more than 72 hours, whereby during this period pathological changes (e.g. rigor mortis, algor mortis, and livor mortis) become less reliable for estimating PMI (Gennard, 2007). This can be done by examining the necrophagous insects infesting the corpse, observing their current life cycle stage, calculating the age of their immatures (e.g. instar larvae), and subsequently estimate the time when female flies first arrived and laid eggs (Rajagopal *et al*., 2008). Pertinently, the accuracy of mPMI estimation is based on the ability to accurately identify the oldest necrophagous insect species (particularly Calliphoridae), prior to utilizing the appropriate growth data.

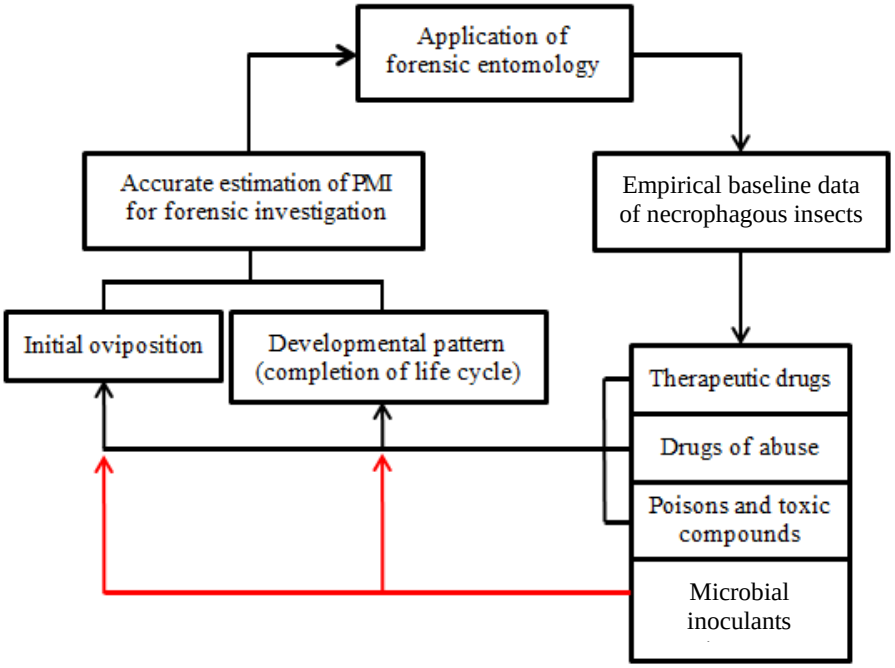
In Malaysia, *Chrysomya megacephala* (Fabricius) has been identified as the first and dominant necrophagous species infesting corpses and carcasses, followed by *Chrysomya rufifacies* (Macquart) (Mahat *et. al*., 2016). There are many factors that can affect initial oviposition, development and succession of species that include the biogeoclamatic factors (e.g. rain) (Mahat *et al*., 2009), presence of therapeutic drugs and toxic substances (e.g. paracetamol) (O'brien and Turner, 2004), as well as physical barriers such as covering or wrapping (Goff, 1992). Review of literature reveals that while chemicals such as malathion may delay initial oviposition and prolong the development of blowfly species (Mahat *et al*., 2009), faster development of necrophagous insects has been associated with cocaine (Goff *et al*., 1989) and diazepam (Carvalho *et al*., 2001). Besides, there are also chemicals (e.g. bleach) that do not affect the development of necrophagous insects (Aubernon *et al*., 2015). In this context, it is pertinent to indicate here that any factors that alter oviposition and development pattern of necrophagous insects would subsequently result in erroneous estimation of PMI. Hence, exploring the different chemicals and/or formulations that are commonly available in the market, in view of their potential influences on the oviposition and duration for completing life cycle among necrophagous insects, merits forensic consideration.

Microbial inoculants have long been widely used in farming and other agricultural activities for promoting livestock's growth, increasing nutrient level in soil, as well as improving waste management and sanitation (Kyan *et al*., 1999). Often, livestock rearing farms emanate offensive odor from the waste or manure that can adversely affect the environment that humans live in, creating inconveniences. To overcome this issue, the use of microbial inoculants that contain effective microorganisms (EM) that are proven to be effective odor reducing agents for the environment has been suggested (Sakawi and Ismail, 2015). Some of the selected species of microorganisms included in microbial inoculants that are widely sold in the market are lactic acid bacteria (*Lactobacillus casei*), photosynthetic bacteria (*Rhodopseudomonas palustris*) and yeast (Higa and Parr, 1994). This has attracted a specific attention to forensic entomologists since alteration in odor may indirectly halter the activities of flies in the surrounding areas. Therefore, when applied in forensic entomology context, it means that microbial inoculants can be one of the

contributing factors that may affect oviposition of necrophagous flies on corpse found in the area where these agents are applied in its surrounding. Moreover, the fact that microbial inoculants can also be cunningly used by the murderer to reduce the odor of a decaying body to confuse forensic entomological assessments of mPMI cannot be excluded.

1.2 Problem Statement

The accuracy of estimating PMI relies largely on understanding the intrinsic and extrinsic factors that can alter the oviposition and developmental pattern of necrophagous insects. Because scientific information has already become a public domain, and since criminals nowadays are getting better-informed, the possibility that these cunning criminals may resort to the use of various chemicals/ formulations for delaying necrophagous insect activity cannot be ruled out. Leaving alone its routine application for various purposes such as controlling bad odor in animal farming, microbial inoculants that consist of various EM can be further utilized by murderers to confuse forensic entomologists at estimating mPMI. This can subsequently result in the miscarriage of justice. The fact that such agents are readily accessible in the market and since review of literature does not reveal any specific study focusing on its influence on oviposition and development pattern of necrophagous insects, this specific research that was designed to elucidate such aspect on *C. megacephala* and *C. rufifacies* appears forensically relevant. Based on the current information available, it is expected that the initial oviposition and completion of life cycle for *C. megacephala* and *C. rufifacies* may be prolonged in carcasses sprayed with EM.1® Microbial Inoculant. The conceptual framework depicting the gap of knowledge in the body of literature examined in this present research is presented in Figure 1.1.



Note: Black line represents the established forensic entomology empirical baseline data in Peninsular Malaysia, while the red one indicates the gap of knowledge addressed by this present research.

Figure 1.1: Research conceptual framework.

1.3 Objectives and Hypotheses

The aim of this research was to investigate the influence of commercially available microbial inoculant on initial oviposition and development of *C. megacephala* and *C. rufifacies* using rabbit carcasses. Specifically, the objective of this research was to determine and compare the initial oviposition and duration for completing life cycles for *C. megacephala* and *C. rufifacies* infesting rabbit carcasses sprayed with two different concentrations of a microbial inoculant with that of control carcasses. It was hypothesized that significant differences in time of initial oviposition and completion of life cycle for *C. megacephala* and *C. rufifacies* shall be observed among the treated and control groups.

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