

EFFECT OF CITRONELLA AND CHLORPYRIFOS ON OVIPOSITION OF
CALLIPHORID FLIES IN DECOMPOSING RABBIT CARCASSES

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ABSTRACT

The independent influences of citronella and chlorpyrifos on oviposition and duration for completing life cycles for *Chrysomya megacephala* and *C. rufifacies* on decomposing rabbit carcasses in Johor Bahru were studied in view of its application for forensic entomological assessment of postmortem interval (PMI). Twelve male rabbit carcasses were equally divided into control, citronella-treated and chlorpyrifos-treated groups and left to decompose for 14 consecutive days. Results revealed that *C. megacephala* was the first necrophagous fly to oviposit followed by *C. rufifacies* in all the control and citronella treated carcasses. The initial oviposition of *C. megacephala* was significantly delayed by 4-6 hours in all the citronella treated carcasses ($p < 0.05$) although significant prolongation in the duration for completing its life cycle was not observed. In contrast, delay in the initial oviposition as well as prolongation in the duration of life cycle for *C. rufifacies* in citronella treated carcasses were not observed. Although delayed in oviposition was observed for the chlorpyrifos treated carcasses, the eclosion of the eggs were not successful hence rendering inability to identify the fly species that oviposited. Since forensic entomological baseline data for Johor as well as the individual influences of citronella and chlorpyrifos on oviposition and completion of life cycle for *C. megacephala* and *C. rufifacies* have not been reported, the results reported here may prove useful for estimating PMI via entomological assessment within this region, especially whenever these two chemical agents are suspected to be present.

ABSTRAK

Pengaruh serai wangi dan klorpirifos secara berasingan ke atas pengovipositan dan tempoh kitaran hidup lengkap bagi *C. megacephala* dan *C. rufifacies* pada bangkai arnab yang mereput di Johor Bahru dikaji dari sudut entomologi forensik bagi menganggar selang masa kematian (PMI). Dua belas bangkai arnab jantan telah digunakan dan dibahagikan sama rata dalam tiga kumpulan iaitu kawalan, dirawat dengan serai wangi dan dirawat dengan klorpirifos, ditinggalkan mereput selama 14 hari berturut-turut. Kajian mendapati bahawa *C. megacephala* adalah lalat nekrofagus yang pertama bertelur, diikuti oleh *C. rufifacies*, pada bangkai kawalan dan bangkai yang dirawat serai wangi. Pengovipositan terawal bagi *C. megacephala* adalah signifikan dengan kelewatan bertelur selama 4–6 jam pada bangkai arnab yang dirawat dengan serai wangi ($p < 0.05$). Namun demikian, pemanjangan tempoh dalam melengkapkan kitar hidup spesies nekrofagus terawal tersebut adalah tidak signifikan. Berbeza bagi *C. rufifacies*, pengovipositan terawal dan tempoh kitaran hidup lengkap bagi spesies tersebut tidak dilihat pada bangkai yang dirawat serai wangi. Walaupun kelewatan bertelur didapati pada bangkai yang dirawat dengan klorpirifos, telur-telur pada bangkai tersebut gagal menetas dan ini menyebabkan identifikasi spesies lalat yang bertelur tidak dapat dilaksanakan. Memandangkan, data asas untuk Johor dan juga pengaruh serai wangi dan klorpirifos secara berasingan ke atas pengovipositan dan tempoh kitaran hidup lengkap bagi *C. megacephala* dan *C. rufifacies* tidak lagi dilaporkan, hasil kajian ini dibuktikan berguna bagi menganggar PMI melalui penilaian entomologi di rantau ini, terutama apabila terdapat kehadiran bagi kedua-dua agen bahan kimia ini.

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LIST OF ABBREVIATION

AChE	-	Acetylcholinesterase
a.m.	-	<i>Ante meridiem</i>
ANOVA	-	Analysis of Variance
C	-	Control
<i>C. megacephala</i>	-	<i>Chrysomya megacephala</i>
<i>C. rufifacies</i>	-	<i>Chrysomya rufifacies</i>
cm	-	Centimetre
CPO	-	Chlorpyrifos- oxon
CPY	-	Chlorpyrifos
DC	-	Dorsal comu
<i>e.g.</i>	-	For example
g	-	Gram
G	-	Gena
GC-MS	-	Gas chromatography-mass spectrometry
<i>i.e.</i>	-	Such as
kg	-	Kilograms
KOH	-	Potassium hydroxide
L	-	Litre
mL	-	Millilitres
Mm	-	Millimetres
Oct	-	October
OPCs	-	Organophosphorus compounds
P	-	Post-gena
PE	-	Peritremes
pH	-	Potential hydrogen

p.m.	-	<i>Post meridiem</i>
PMI	-	Postmortem interval
S	-	Spiracular slits
SD	-	Standard deviation
Sept	-	September
T1	-	Citronella – treated
T2	-	Chlorpyrifos – treated
TCP	-	Trichloropyridinol
VC	-	Ventral comu

LIST OF SYMBOLS

°C	-	Degree Celcius
~	-	Approximately
%	-	Percent
>	-	Greater than
<	-	Less than

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Forensic entomology is one the most useful tool in estimating post mortem interval (PMI) especially when dealing with highly decomposed bodies found 72 hours or more after death (Greenberg and Kunich, 2002; Gennard, 2007). In general, it has been reported that *Chrysomya megacephala* (Fabricius) is the dominant and the first necrophagous species on human corpses (Lee *et al.*, 2004) and animal models (Heo *et al.*, 2008; Ahmad and Ahmad, 2009 ; Mahat *et al.*, 2009; 2014) decomposing in several parts in peninsular Malaysia namely Penang, Selangor, Kuala Lumpur and Kelantan, followed by *Chrysomya rufifacies* (Macquart). While, it is pertinent to indicate that the empirical baseline data established in one biogeoclimatic region may not be useful in estimating PMI in other regions (Wells and Stevens, 2008) and the fact that similar studies focusing on the southern part of peninsular Malaysia (Johor) remains unreported; such studies conducted within this southern region may prove to be useful.

It has been reported that many commercial formulations especially those originated from plant materials such as citronella would deter the colonisation of insects such as mosquitoes (Debbown *et al.*, 2007) and ants (Soria *et al.*, 2012). In contrast, except for a study reported by Charabidze *et al.* (2009), review of the literature reveals no other studies ever reported pertaining to the influence of citronella

preparations on necrophagous flies emphasising on its significance in forensic context. Such a scarce state of information with regards to this matter may lead to erroneous application of forensic entomology related data in assessing PMI. Interestingly, while the effect of malathion (an organophosphorous insecticide) (Mahat *et al.*, 2009; Liu *et al.*, 2009) and paraquat (quaternary ammonium herbicide) (Mahat *et al.*, 2014) on oviposition and developmental patterns of necrophagous insects have been duly reported; studies pertaining to other organophosphorous insecticides that rarely cause death, but potentially affecting such patterns *e.g.* chlorpyrifos remain unreported so far. In this context, it is pertinent to indicate here that any factors that may interrupt insect colonisation as well as developmental patterns may lead to erroneous estimation of PMI (Sharanowski *et al.*, 2008), resulting from inaccurate interpretation of forensic entomological empirical baseline data. Therefore, this present study that was specifically designed to investigate the possible influences of citronella and chlorpyrifos individually on the oviposition and developmental patterns of *C. megacephala* and *C. rufifacies* using rabbits as animal models, merits forensic consideration.

1.2 Problem Statement

Considering that crimes committed by cunning individuals are gaining notoriety, easy accessibility of natural insect repellents (*e.g.* citronella) and insecticides (*e.g.* chlorpyrifos) in the market as well as availability of scientific publications; the tendency of such criminals to capitalize on these aspects for confusing forensic investigators could not be ruled out. Hence, it was found pertinent to conduct this present research for exploring the influence of these products on oviposition and infestation patterns of the first two dominant necrophagous insects, in view of its application in estimating PMI based on entomological evidence. In addition, this research also attempted at establishing forensic entomological baseline data in Johor, considering its evidential value in forensic investigation of death cases within this part of the country.

1.3 Objectives

This present research was therefore aimed to compare the independent influences of citronella and chlorpyrifos on the initial oviposition and durations for completing life cycles for *C. megacephala* and *C. rufifacies* with that of controls.

1.4 Hypotheses

There are differences in the time of initial oviposition and completion of the life cycles for *C. megacephala* and *C. rufifacies* between rabbits treated with citronella, chlorpyrifos and controls (tap water).

1.5 Scope

This research involved 12 male domestic rabbit (*Oryctolagus cuniculus*) carcasses, weighing about 1 to 1.5 kg obtained from the local meat sellers in Johor Bahru. These carcasses were equally divided (n=3) into three groups *viz.* Controls (C), Treated 1 (T1) and Treated 2 (T2). For preparing the citronella treated solution, one part of the organic concentrated preparation of citronella was diluted in two parts of tap water. Similarly, tap water was also used for preparing the chlorpyrifos solution (0.5 g/L). While tap water alone was used for the control carcasses, the freshly prepared citronella treated and chlorpyrifos solutions, were individually homogenized and applied onto the T1 and T2 carcasses respectively. The decomposition was conducted in a sunlit area within the Universiti Teknologi Malaysia (UTM), Johor Campus, and observed until the completion of life cycles of *C. megacephala* and *C. rufifacies*. During this period, data for rainfall, ambient and carcass surface temperatures as well as the relevant entomological specimens were collected. Chemical analyses for revealing the presence of the compounds of interest in both of

the citronella and chlorpyrifos formulations used in this study were performed using Gas chromatography- Mass spectrometry (GC-MS).

1.6 Significance of Study

In addition to provide the first ever empirical evidence for forensic entomology in the southern part of peninsular Malaysia, the data reported here would also prove useful in elucidating the possible individual influences of citronella and chlorpyrifos on the prevailing necrophagous insects (*i.e.* *C. megacephala* and *C. rufifacies*) and their implications on the accuracy of the PMI estimates. Since PMI remains to be one of the pertinent issues in forensic investigations especially in proving and/or disproving alibis, the availability of these data would pave the way to the utilization of insect evidence in crime investigations within this region (Johor) as well as other locations with similar biogeoclimatical conditions.

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