# STUDIES ON THE LIFE HISTORY OF BLOW FLIES (DIPTERA: CALLIPHORIDAE) IN CENTRAL KERALA AND ITS FORENSIC SIGNIFICANCE

Thesis submitted to the University of Calicut for the award of degree of

**DOCTOR OF PHILOSOPHY IN ZOOLOGY**

**By**

**REJECT PAUL M P**

U.O.NO: 6522/2019/Admn, Dated, 21.05.2019

Under the supervision of

**Dr. C F BINOY**

Research and Post Graduate Department of Zoology

St. Thomas' College (Autonomous), Thrissur


University of Calicut, Kerala

July 2023

Dr. C.F. Binoy,
Associate Professor &
Head of Department,
Research & Post Graduate Department of Zoology,
&Dean of Science,
St. Thomas' College (Autonomous),
Thrissur-680001, Kerala, India
Email Id: binoycf@stthomas.ac.in , drcfbinoy@gmail.com
Mobile: 8921645795

## CERTIFICATE

This is to certify that the corrections/suggestions, recommended by adjudicators of the PhD thesis, of Mr. REJECT PAUL. M. P, titled "STUDIES ON THE LIFE HISTORY OF BLOW FLIES (DIPTERA: CALLIPHORIDAE) IN CENTRAL KERALA AND ITS FORENSIC SIGNIFICANCE" have been incorporated in the thesis and that the contents in the thesis and the soft copy (CD) are one and the same.

Thrissur

04/01/2023

Dr. C.F. Binoy

Research Guide

Dr. C. F. BINOY, Ph.D.
Dean of Science
Associate Professor & Head
Research & Postgraduate Department of Zoology
St. Thomas' College, Thrissur - 680 001, Kerala
E-mail : drcfbinoy@gmail.com

## DECLARATION

I hereby declare that the thesis entitled **"STUDIES ON THE LIFE HISTORY OF BLOW FLIES (DIPTERA: CALLIPHORIDAE) IN CENTRAL KERALA AND ITS FORENSIC SIGNIFICANCE"**, submitted to the University of Calicut for the award of degree of Doctor of Philosophy in Zoology is a bonafide research work done by me under the supervision and guidance of Dr. C F Binoy, Dean of Science, Associate Professor and HOD, Research and Post Graduate Department of Zoology, St. Thomas' College (Autonomous), Thrissur.

I also declare that the findings presented in this thesis are original and do not form the basis for the award of any other degree, diploma, or other similar titles of any other University.

Reject Paul M P

Thrissur

Dr. C.F. Binoy,
Dean of Science,
Associate Professor &
Head of Department,
Research & Post Graduate Department of Zoology,
St. Thomas' College (Autonomous),
Thrissur-680001, Kerala, India
Email Id: binoycf@stthomas.ac.in , drcfbinoy@gmail.com
Mobile: 8921645795

## CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON THE LIFE HISTORY OF BLOW FLIES (DIPTERA: CALLIPHORIDAE) IN CENTRAL KERALA AND ITS FORENSIC SIGNIFICANCE",** submitted to the University of Calicut for the award of degree of Doctor of Philosophy in Zoology is a bonafide account of research work carried out by Mr. Reject Paul M P under my supervision. The work has not been submitted either partially or fully to any other University or institution for the award of any other degree, diploma, fellowship, title or recognition.

Thrissur

04/07/2023

Dr. C.F. Binoy

Research Guide

Dr. C. F. BINOY, Ph.D.
Dean of Science
Associate Professor & Head
Research & Postgraduate Department of Zoology
St. Thomas' College, Thrissur - 680 001, Kerala
E-mail : drcfbinoy@gmail.com

*ACKNOWLEDGMENTS*

*First and foremost, all praises go to God almighty and I thank Almighty God the most Gracious and the most Merciful, for blessing me with this life with good health, guiding me and for showing me the right path in my life, career and research.*

*The completion of this thesis would not have been possible without the support, motivation, encouragement and love of many people, whom I would like to thank from the bottom of my heart.*

*I would like to thank with deep sense of gratitude, Dr. C. F. Binoy, my research supervisor, for his guidance, conscientiousness, constant support and enthusiasm throughout my work. His thoughtful suggestions and sharing of his knowledge have provided me with the freedom to pursue my research interests and helped me to construct and modify my arguments while writing my thesis. The painstaking efforts put by him by taking extra hours during working days and holidays while making scrupulous corrections of my thesis is really praiseworthy and commendable.*

*I also owe a thank you to Dr. M. Nasser, external subject expert and Pro-Vice Chancellor, University of Calicut for his critical feedback and suggestions that helped improve this work. And I thank all members of research advisory committee for their valuable suggestions.*

*I am grateful and indebted to Rev. Fr. Dr. Martin Kolambrath, Principal and the management of St. Thomas' college (Autonomous), Thrissur, for providing me all the facilities for the conduct of research. I take this opportunity to convey my thanks to former principals; Dr. Joy. K. L and Dr. Ignatious Antony for their encouragement and support.*

*I record my sincere gratitude to Dr.Francy K Kakkasery (Retd), Dr. Britto Joseph K (Retd), Dr.C V David (Retd) and Dr.V M Chacko, for their encouragement and well wishes.*

*My gratitude and love go to all teaching staffs, research scholars and other staff of research and post graduate Dept. of Zoology.*

*And I wish to thank all my colleagues in teaching and research and clients in Forensic consultation who worked or acquainted with me for the last 25 years, some of whom are across different states in India and across the globe, for their motivation and support.*

*I express my heartiest thanks to Mr. Sanjoe Jose, Librarian for his unconditional support in getting me the research papers what I needed time to time.*

*I am forever indebted and thankful to Dr. Sunanda for the statistical work done by her and for having had detailed deliberations with me on the interpretations. Special thanks to Dr. Shiva Prasad for his constant support and motivation.*

*My deepest, sincere and warmest thanks go to my loving parents; Pushpa and Paul who made this endeavor possible through their never ending blessing and prayers and the life and formal education provided to me with all support and love.*

*Above all, of course, I am beyond thankful to my beloved life partner, Joshlin and my beloved children, Valentina Rose and Angelino Saul who make my life so much more beautiful and worthwhile and who have taken this journey with me with constant support, motivation, encouragement and unconditional love. Thank you for your boundless support and unwavering faith in me; for keeping me in shape, bearing with me when I was too busy while doing my research and writing thesis, and somehow knowing exactly when to clock me off for the day. My heartfelt thanks are to them who have been patiently waiting for the completion of my research work to get more time for them from me and I am sorry Joshlin, Rose and Saul for me becoming impatient while doing my research and writing of thesis. I thank you for being by my side all these years and also thank you for celebrating all the exciting moments of this Ph. D work with me and for always knowing how to cheer me up. Thank you for always supporting me with what seemed like an impossible journey and providing me with love, wisdom, and motivation.*

*Love to Diana, my pet dog who kept me company and offered her own kind of support and love although I have had compromised in caring time given for her.*

*Finally I am thankful to all my dearest students, whom I have taught from 1998 till today, wherever they are who have been always a constant true inspiration for me to study, research and excel in academics.*

*All this support, encouragement, motivation, help and love from you all have been everything I needed to buckle down in solitude to complete this feat.*

*Reject Paul M P*

*Dedicated to*

*My beloved family*

*"Insects are major players in nature's recycling effort, and in nature a corpse is simply organic matter to be recycled. Left to its own devices, nature quickly populates a corpse with a diverse community of organisms, all dedicated to reducing the body to its basic components."*

*M. Lee Goff*

*A Fly for the Prosecution*

*……though there are animals which have no attractiveness for the senses, yet for the eye of science, for the student who is naturally of a philosophic spirit and can discern the causes of things, Nature which fashioned them provides joys which can not be measured"*

*Aristotle*

*De Partibus Animalium*

## CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## ABBREVIATIONS

| | |
|---|---|
| % | percentage |
| $\beta_0, \beta_1$ | parameters of the regression model to be estimated |
| °C | degree Celsius |
| µl | microliter |
| ADD | accumulated degree hours |
| ADH | accumulated degree hours |
| ANOVA | analysis of Variance |
| BLAST | basic local alignment aearch tool |
| bp | base pair |
| *C.chani* | *Chrysomya chani* |
| *C.megacephala* | *Chrysomya megacephala* |
| *C.rufifacies* | *Chrysomya rufifacies* |
| CABI | centre for agriculture and bioscience international |
| CO I | cytochrome oxidase subunit I |
| CO II | cytochrome oxidase subunit II |
| $D_0$ | developmental threshold temperature |
| DNA | deoxyribonucleic acid |
| *E* | expectation |
| ft | feet |
| *H.ligurriens* | *Hemipyrellia ligurriens* |
| h/hr/hrs | hour/s |
| K | thermal summation constant |
| km | kilometer |
| LSD | least significance difference |
| m | meter |
| M | molar |
| mA | milliampere |
| mg | milligram |
| ml | milliliter |

| | |
|---|---|
| mm | millimeter |
| NCBI | national center for biotechnology information |
| ns | non-significant |
| ORF | open reading frame |
| PCR | polymerase chain reaction |
| PMI | post mortem interval |
| $R^2$ | coefficient of determination |
| RH/H | relative humidity/humidity |
| RNase | Ribonuclease |
| rRNA | ribosomal RNA |
| SEM | scanning electron microscopy |
| T | temperature |
| t | time |
| TBE | tris borate EDTA |
| UV | ultraviolet |
| v/v | volume/volume |
| w/v | weight/volume |
| $Y_t$ | length (mm) at duration '*t*'(hrs |

## ABSTRACT

The flies belonging to the Diptera, Calliphoridae are the first visitors to inhabit and colonize the dead body within a short period of time of cadavers found. The importance of generating location specific data of forensically important blow fly species for accurate assessment of PMI was evident from the previous works. The present investigation has recorded 17 blow fly species belonging to 4 subfamilies and 8 genera from central Kerala. In this study four forensically significant blow flies; *Chrysomya megacephala, Chrysomya rufifacies, Chrysomya chani* and *Hemipyrellia ligurriens* were identified based on their morphological and molecular characteristics. Seasonal abundance of blow flies were found to be significantly higher in monsoon. Morphology of egg, larval instars and pupa were studied. The ultra structural details of larval instars were analyzed using Scanning Electron Microscopy (SEM). Life cycle parameters like fecundity, length and weight of larval instars and duration of life of eggs, different larval instars and pupation were studied. The survival rate (%) in the different life stages were studied and it was found that higher survival rate was seen in the egg and instar I. Effect of temperature and humidity on the life cycle of blow flies was investigated and it showed that the pre-oviposition period was significantly higher in winter in all the four species. The number of eggs laid in a day and during the life span by all species were significantly higher in monsoon. The periodicity of egg laying was significantly higher in winter for all species. The time dependent growth data corresponding to the length and weight of larval instars of blow flies during different seasons were investigated and found that the length and weight was significantly higher in monsoon for *C. megacephala* and *C. rufifacies* and in summer for *C. chani* and *H. ligurriens*. The total duration taken by the fly for its development from the egg stage till

the emergence of adult fly was shorter in summer, followed by monsoon and winter. Comparative studies of laboratory rearing of the four species with the outdoor rearing results showed that the developmental duration of different stages in the outdoor rearing were higher. The survival rate of all species were found to be significantly higher in monsoon in comparison to other seasons. The major outcome of this study is that the results of outdoor rearing cannot be simulated in the laboratory. A high value of coefficient of determination ($R^2$) was obtained for all the four blow fly species for the predicted regression equation which indicated that higher percent of variability in larval length can be explained by duration. The regression equation method developed in this study emerged as the best suitable method for the estimation of PMI using life history of the blow flies. The changes observed in the rate of developmental data of the blow flies might be due to the changes in humidity, rainfall and temperature prevailing in the geographically different areas and genetic variations of blow flies. This cautions that while performing the assessment of PMI, the investigators should be very careful about the climatic conditions prevailing in the respective study area and signifies the importance of generating location specific data of forensically important species of blow flies. This is the first report on the developmental rate of these blow fly species during different seasons from South India. Present investigation on the life cycle of above mentioned blow flies and the regression equation model constructed for the PMI assessment of dead bodies has been found to be useful for application in forensic investigations in the study region. Further research can be conducted to augment the present study results and for exploring new dimensions in future.

# സംഗ്രഹം

ഡിപ്റ്റിറാ : കാലിഫോറിഡേ (Diptera : Calliphoridae) ഇനത്തിൽപ്പെട്ട ഈച്ചകൾ, മൃതശരീരം കണ്ടെത്തി ചുരുങ്ങിയ സമയത്തിനുള്ളിൽ ആദ്യം തന്നെ അതിൽ ആവാസമുറപ്പിച്ച് കോളനി രൂപീകരിക്കുന്നു. പോസ്റ്റ്മോർട്ടം ഇടവേളയുടെ കൃത്യമായ വിലയിരുത്തലിനായി ഫോറൻസിക് പ്രാധാന്യമുള്ള മണിയനീച്ചകളുടെ പ്രദേശ നിർദ്ദിഷ്ട ഡാറ്റ സൃഷ്ടിക്കുന്നതിന്റെ പ്രാധാന്യം മുൻകാല ഗവേഷണകൃതികളിൽ നിന്ന് വ്യക്തമാണ്. ഈ പഠനഗവേഷണത്തിന്റെ ഭാഗമായി മധ്യകേരളത്തിൽ 8 ജനുസ്സുകളിലും 4 ഉപകുടുംബങ്ങളിലും ഉൾപ്പെട്ട പതിനേഴ് ഇനം മണിയനീച്ചകളെ രേഖപ്പെടുത്തി. പഠനത്തോടനുബന്ധിച്ച് ക്രൈസോമിയ മെഗാസെഫാല (*Chrysomya megacephala*), ക്രൈസോമിയ റൂഫിഫേസീസ് (*Chrysomya rufifacies*), ക്രൈസോമിയ ചാനി (*Chrysomya chani*), ഹെമിപൈറെലിയ ലിഗൂറിയൻസ് (*Hemipyrellia ligurriens*) എന്നീ നാലിനം മണിയനീച്ചകളെ അവയുടെ രൂപശാസ്ത്രപരവും ജനിതകപരവുമായ സവിശേഷതകളെ അടിസ്ഥാനമാക്കി തിരിച്ചറിയാൻ കഴിഞ്ഞു. കാലാവസ്ഥാനുസൃതമായി മണിയനീച്ചകളുടെ സമൃദ്ധി കാലവർഷത്തിൽ വളരെ അധികമുള്ളതായി കണ്ടെത്തി. മുട്ട, ലാർവ, പ്യൂപ്പ എന്നിവയുടെ രൂപഘടന പഠനത്തിന് വിധേയമാക്കി. ലാർവകളുടെ സൂക്ഷ്മ ഘടനയുടെ വിശദാംശങ്ങൾ സ്കാനിംഗ് ഇലക്ട്രോൺ മൈക്രോസ്കോപ്പി (SEM) ഉപയോഗിച്ച് വിശകലനം ചെയ്തു. ജീവചക്രത്തിലെ വിവിധ ഘടകങ്ങളായ ഫീക്കണ്ടിറ്റി, ലാർവകളുടെ നീളവും ഭാരവും, മുട്ട, ലാർവ, പ്യൂപ്പ എന്നിവയുടെ ജീവചക്ര കാലയളവ് മുതലായവ പഠനത്തിന് വിധേയമാക്കി. വിവിധ ജീവചക്ര ഘട്ടങ്ങളിൽ അതിജീവനതോത് മുട്ടകളിലും ആദ്യത്തെ ഇൻസ്റ്റാറിലും ആണെന്ന് കണ്ടെത്തി. മണിയനീച്ചകളുടെ ജീവിത ചക്രത്തിൽ താപനിലയും ഈർപ്പവും ചെലുത്തുന്ന സ്വാധീനവും വിശകലനം ചെയ്തു. ഇതിൽ മുട്ടയിടുന്നതിനു മുമ്പുള്ള കാലയളവ് നാല് മണിയനീച്ചകളിലും ശീതകാലത്തിൽ ഗണ്യമായി കൂടുതലായി കാണപ്പെട്ടു. ദിവസത്തിലെയും മുഴുവൻ ജീവിതകാലയളവിലും ഇടുന്ന മുട്ടകളുടെ എണ്ണം കാലവർഷത്തിൽ കൂടുതലായി കാണപ്പെട്ടു. മുട്ടകൾ ഇടുന്ന ഇടവേളകളിൽ കൂടുതൽ ദൈർഘ്യം ശീതകാലത്തിലാണെന്ന് കണ്ടെത്തി. വ്യത്യസ്ഥ കാലാവസ്ഥകളിൽ സമയാടിസ്ഥാനത്തിലുള്ള മണിയനീച്ചകളുടെ വളർച്ചാ നിരക്ക്, ലാർവകളുടെ നീളം, ഭാരം എന്നിവയെ ആശ്രയിച്ച് നിരീക്ഷിച്ചപ്പോൾ ക്രൈസോമിയ മെഗാസെഫാല, ക്രൈസോമിയ റൂഫിഫേസീസ് എന്നിവയ്ക്ക് വർഷകാലങ്ങളിൽ നീളവും ഭാരവും താരതമ്യേന ഉയർന്നതായി കണ്ടെത്തി. ക്രൈസോമിയ ചാനി, ഹെമിപൈറെലിയ ലിഗൂറിയൻസ് എന്നിവയ്ക്ക് വേനൽക്കാലത്താണ് കൂടുതൽ നീളം ആർജ്ജിച്ചതായി കാണപ്പെട്ടത്. ഈ ഈച്ചകളുടെ മുട്ടയിൽ നിന്ന് മുതിർന്ന ഈച്ച വരെയുള്ള ജീവചക്രപരിണാമത്തിനുള്ള കാലയളവ് വേനൽക്കാലത്താണ് ഏറ്റവും കുറവ് രേഖപ്പെടുത്തിയത്. നാല് മണിയനീച്ചകളുടെ ലബോറട്ടറിയിലും സ്വാഭാവിക സാഹചര്യങ്ങളിലും നടത്തിയ വളർത്തലിൽ ലഭിച്ച കണ്ടെത്തലുകളെ താരതമ്യപഠനത്തിന് വിധേയമാക്കിയപ്പോൾ വികാസപരിണാമ കാലയളവ് സ്വാഭാവിക സാഹചര്യങ്ങളിലാണ് കൂടുതലെന്ന് തിരിച്ചറിയാൻ കഴിഞ്ഞു. നാല് മണിയനീച്ചകളുടെയും അതിജീവനതോത് കാലവർഷത്തിൽ ഗണ്യമായി കൂടുതലാണെന്ന് കണ്ടെത്തി. സ്വാഭാവിക സാഹചര്യങ്ങളെ പരീക്ഷണശാലയിൽ അതേപടി അനുകരിക്കാൻ സാധ്യമല്ലെന്ന് കണ്ടെത്തിയത് നിലവിലെ അന്വേഷണത്തിലെ ഒരു പ്രധാന കണ്ടെത്തലായി കണക്കാക്കുന്നു. മണിയനീച്ചകളുടെ

ജീവചക്രത്തെ അടിസ്ഥാനമാക്കി പോസ്റ്റ്മോർട്ടം ഇടവേള (PMI) കണക്കാക്കാനുള്ള റിഗ്രഷൻ സമവാക്യ പ്രവചനമാതൃക നിർമ്മിച്ചതിൽ ഓരോ മണിയനീച്ചകൾക്കും ഒരു ഉയർന്ന കോയിഫിഷന്റ് ഓഫ് ഡിറ്റർമിനേഷൻ ($R^2$) ലഭിക്കുകയുണ്ടായി. അത് ലാർവകളുടെ നീളത്തിലുള്ള ഉയർന്ന തോതിലുള്ള വ്യതിയാനത്തെ കാലയളവുകൊണ്ട് വിശദീകരിക്കാമെന്ന് സൂചിപ്പിച്ചു. ഈ ഗവേഷണപഠനത്തിൽ നിർമ്മിച്ച റിഗ്രഷൻ സമവാക്യ മാതൃക ഒരു മികച്ച അനുയോജ്യമായ രീതിയായി കണക്കാക്കാം. മണിയനീച്ചകളുടെ വളർച്ചാനിരക്കിൽ കണ്ടെത്തിയ വ്യത്യാസത്തിന്റെ കാരണം ഭൂമിശാസ്ത്രപരമായി വൈവിധ്യങ്ങളായ പ്രദേശങ്ങളിലെ താപനില, ഈർപ്പം, മഴയുടെ തോത് കൂടാതെ ജനിതക വ്യതിയാനങ്ങൾ എന്നീ ഘടകങ്ങളായിരിക്കും എന്ന് കരുതുന്നു. പോസ്റ്റ്മോർട്ടം ഇടവേള കണക്കാക്കുമ്പോൾ പഠനവിധേയമാക്കുന്ന പ്രദേശങ്ങളിൽ, അന്വേഷകർ നിലവിലുള്ള കാലാവസ്ഥയെപ്പറ്റി ജാഗരൂകരാകണമെന്ന് മേൽ സൂചിപ്പിച്ച കണ്ടെത്തലുകൾ മുൻകരുതൽ നൽകുന്നു. ഇത് ഫോറൻസിക് പ്രാധാന്യമുള്ള മണിയനീച്ചകളുടെ പ്രദേശനിർദ്ദിഷ്ട ഡാറ്റ വികസിപ്പിക്കുന്നതിന്റെ പ്രാധാന്യം സൂചിപ്പിക്കുന്നു. കാലാവസ്ഥാടിസ്ഥാനത്തിലുള്ള മണിയനീച്ചകളുടെ വികാസപരിണാമ നിരക്കിനെപ്പറ്റിയുള്ള ഈ പഠനം തെക്കെ ഇന്ത്യയിലെ ആദ്യപഠനമായി വിശേഷിപ്പിക്കാം. നിലവിലെ അന്വേഷണത്തിൽ നടത്തിയ മണിയനീച്ചകളുടെ ജീവചക്രപഠനവും പോസ്റ്റ്മോർട്ടം ഇടവേള കണക്കാക്കാനായി നിർമ്മിച്ച റിഗ്രഷൻ സമവാക്യ മാതൃകയും നിലവിലെ പഠനവിധേയമായ പ്രദേശങ്ങളിൽ ഫോറൻസിക് അന്വേഷണങ്ങൾക്ക് പ്രയോഗിക്കാമെന്ന് വിലയിരുത്തുന്നു. നിലവിലെ പഠനഫലങ്ങൾക്ക് അനുബന്ധമായി കൂടുതൽ ഗവേഷണങ്ങൾ നടത്തുന്നത് ഭാവിയിൽ പുതിയ മാനങ്ങൾ പരിവേഷണം ചെയ്യപ്പെടാൻ സാധിക്കും.

# CHAPTER 1

# INTRODUCTION

# CHAPTER 1

# INTRODUCTION

## 1.1. Insects as evidence for forensic investigation

Forensic entomology characterizes the implementation of certain techniques focusing on insects in forensic contexts belonging to the various classes of different arthropods (Goff, 2001). The general use of evidences from such organisms towards medico legal investigations definitely helps the scientific community to estimate the time since death that's called the PMI (Harvey et al., 2008).

The organisms of forensic importance belonging to the Phylum Arthropoda which includes the insects, crustaceans, arachnids, and scorpions. They are synanthropists and had been observed from crime scenes as well. During the past few years, the application of forensic entomology in determining the post mortem interval (PMI) has been extensively documented in Asia (Singh et al., 2022) and from other parts of the world including USA, Europe, and Australia with the help of various experimental works and numerous case studies (Campobasso & Introna, 2001).

A study by Amendt et al., (2004) discussed the various observations done by Smith, (1986) and found that there are mainly four categories of insects that can be found on the decomposing carrion: 1) Parasites and predators usually consume the necrophagous organisms: this cluster also encompasses the schizophagous species, which are previously known to consume the dead body first and in later stages, they become predaceous in nature; 2) Necrophagous organisms targeting the carrion for feed; 3) Omnivorous organisms typically consuming the carrion, wasps, some beetles and ants; 4) Organisms

including the spiders and springtails and which are primarily using the cadaver as an extension for their setting. They also reported that among the various analyzed groups, the first and second groups of the classification are found to be significant for forensic entomology. The organisms belonging to this category are primarily Coleoptera (beetles) and Diptera (flies).

As reviewed in the former part, the major species having forensic significance in this order are Calliphoridae (blow flies), Sacrophagidae (flesh flies) and Muscidae (house flies). Among them, the Calliphoridae species are typically found glossy with metallic colouring, usually with green, blue, and black thorax. They principally arrive at the cadaver immediately after the death happens (Amendt et al., 2004; Sukontason et al., 2022).

Arthropods located at a crime scene and the body of the victim or the carrion can assist several kinds of forensic aspects. Specifically, they can provide sufficient information regarding the time of particular incident, death environment, primary crime scene, concealment of the evidences following the incident and the use of drugs/toxic substances (Archer et al., 2005). It is worth introducing that such instances and experiments illustrate the potential implementation of the aforesaid perspectives in the forensic investigations not only for the determination of PMI but also for the detection of poisons involved in the crime (Marchetti et al., 2013). Notwithstanding being exceptionally informative, arthropods have the ability to strongly control and alter the crime scene along with the body of the victim. The forensic pathologists need to be aware of the impairment that arthropods can instigate along with the beneficial records, they are able to offer (Viero et al., 2019). Hence, to understand the complexity of the specific crime scene and to recognize it’s dynamics, the forensic entomological expertise is of utmost significance.

Literature review revealed that 55 insect taxa belonging to three insect orders such as Hymenoptera (sawflies, wasps, ants and bees), Diptera (flies), and Coleoptera (beetles) had been discovered to colonize human dead body (Lutz et al., 2021). The extensive species variety with 37 taxa and 13 families was found belonging to Diptera which indicated its forensic importance. Among the various investigated insect species, the blow flies (Diptera: Calliphoridae) had been the most leading insect group.

The following insect species were found to have significant roles in forensic entomology; Coleoptera; *Saprinus semistriatus* (Scriba, 1790), *Nicrophorus humator* (Gled, 1767) *Nicrophorus vespilloides* (Herbst, 1748), *Thanatophilus sinuatus* (Fabricius, 1775), *Necrodes littoralis* (Linnaeus, 1758), *Thanatophilus rugosus* (Linnaeus, 1758), *Thanatophilus* sp., Diptera: *Lucilia sericata* (meigen, 1826), *Calliphora vicina* (Robineau-desvoidy, 1830), *Lucilia ampullacea* (Villeneuve, 1922), *Protophormia terraenovae* (Robineau-desvoidy, 1830), *Calliphora vomitoria* (Linnaeus, 1758), *Chrysomya albiceps* (Wiedemann, 1819), *Phormia regina* (Meigen, 1826) *Lucilia caesar* (Linnaeus, 1758), *Lucilia illustris* (Meigen, 1826), *Lucilia silvarum* (Meigen, 1826), *Triphleba autumnalis* (Becker, 1901), *Megaselia scalaris* (Loew, 1866), *Megaselia abdita* (Schmitz, 1959), *Megaselia rufipes* (Meigen, 1804), *Conicera tibialis*(Schmitz,1925) and *Triphleba aequalis* (Schmitz, 1919) (Lutz et al., 2021).

Blow flies exhibit prominent significance in the veterinary, medical, agricultural, sanitary, and forensic researches. Primarily, they were known to have extensive ability in transmitting a plethora of bacteria, viruses, fungi, helminthes, protozoans, and other diseases causing pathogens responsible for diarrhoea, poliomyelitis, plague, bacillary dysentery, tuberculosis and cholera. The recent years have witnessed the presence of them

in the dead bodies, excreta and the carrion of various animals including the human population. This vital presence has made them a significant candidate for the future forensic research (Greenberg, 1991).

The blow flies (Diptera: Calliphoridae) are typically the prime group to initially colonize the carcass. The various developmental stages of them, the length and weight of the larval body followed by the morphological alterations at intra-puparial stages can deliver an accurate estimation of the PMI (Amendt et al., 2011). Hence, it is, in specific, vital to set up accurate primary developmental records for the blow fly species.

The precise identification of forensically vital species is encompassed as the initial step and also the most important element in the forensic investigation (Harvey et al., 2008). Identification of the insect species gives us the information regarding the developmental stages and the insect succession styles (Chen et al., 2004; Potapov et al., 2022). If the species identification is found to be inaccurate, then simultaneously the predicted PMI can also be incorrect, which justifies the significance of species identification and the choice of selection of the specific taxonomic approach in forensic entomology (Byrd & Tomberlin, 2019).

From the above-said facts, it is clear that the insect taxonomy sprawl at the coronary artery of forensic entomology. The identification of insect species were mostly based on morphological observations in the early works. Morphology of insects is a consistent strand upon which numerous dichotomous keys are based totally and has brought about the identification of over one million insect species (Chen et al., 2004; Stork, 2008). Be it though, still it is anticipated that hundreds of thousands of insect species are yet to be identified and labelled (Chen et al., 2004).

Molecular strategies are the precise techniques to discover and identify insect species (Potapov et al., 2022), supplying unique benefits compared with the conventional approach. Precise identification of insects up to species level in forensic entomology remains challenging in many instances (Hebert et al., 2003; Kjer et al., 2016).

In order to collect the blow fly specimens for forensic investigations, preference should be given to carrion. As introduced in the former sections, forensic entomology is specially implemented for estimating the minimal PMI i.e. revealing the time of insect colonization in the carcass, by means of analyzing the age of the target insect species inhabiting the dead body (Matuszewski et al., 2010, 2011). But, it can also be integrated into many more situations, e.g. cadaver repositioning or manipulation of a situation to escape from the crime. Moreover, the various developmental stages of the insects, especially the larvae and pupae have promising records for the investigation of sexual crimes including rape, particularly when the victim was discovered in a complicated stage of decomposition (Clery, 2001).

Google search for the term “forensic entomology” generated about 2.190.000 hits on 16.10.2020 which included research articles, interviews, films and newspaper articles evidencing the relevance of the research in the current scenario (Lutz et al., 2021). But notwithstanding the global positive improvement in this discipline mainly in the last 20 years, insects are nonetheless too rarely considered as significant elements in the practical implementation of strategies concerning the forensic field. Even in a developed country like Germany, it was found that, out of 41 country-wide crime investigation centers, only three of them have employed forensic entomologists (Lutz et al., 2021).

The development of such forensically important species, especially the blow flies, was precisely characterized in previous forensic entomology studies (Richards & Villet, 2009). The cosmopolitan species such as the *Phormia regina* (Meigen, 1826), *Calliphora vicina* (Robineau-Desvoidy, 1830) and *Lucilia sericata* (Meigen, 1826) were primarily used to develop the life cycle stages as a fundamental developmental record for unique provinces over the world. *Chrysomya megacephala*, usually referred to as the oriental latrine fly, formerly Pacific and Australasian in distribution, has expanded its range tremendously (Wells & Kurahashi, 1994).

The most common approach chiefly employed to calculate PMI is the thermal summation model, which is principally based on the growth of various developmental stages of immature insect species in context with the influence of temperature (Catts & Goff, 1992). This approach actually assumes a linear link between the rate of developmental stages and the surrounding environmental temperatures (Richards & Villet, 2009).

Although huge number of studies concerning the development of blowflies are available, data regarding the same aspect is found to be lacking for populations from specific geographical regions (Reibe-Pal & Madea, 2015). Also, specifically, no different is the case with the current study site of Kerala, India. Moreover, the differences in various seasons may also result in a discrepancy in the developmental time. Hence, it is of extensive significance to set up fundamental data concerning the above-said facts with special inference on the developmental stages of the flies in context with the seasonal perspective for different areas to enhance the easiness in the estimation of PMI.

Since morphological characters do not help us in confirming the identity of the species, it is imperative to choose technologies like SEM and Mitochondrial DNA (mtDNA) for the successful and precise identification of the larvae and the adult. The mitochondrial cytochrome c oxidase subunit I (COI) was known to encompass the advantageous effects of forensic entomology in species identification (Harvey et al., 2003). Molecular studies thus supplement the morphology based taxonomy internationally. However, studies focusing on the same aspect in the Indian regions, especially in the Kerala region are scanty.

According to Zajac et al., (2016), as mentioned in the former section, the COI gene analysis seemed to be more decisive for the discrimination of species than 28S rRNA for identification. Absence of overlap between the intra and interspecific genetic distances can also be noted as an additional factor that made the aforesaid gene a strong candidate for species identification.

From the above said forensic significance perspectives and the output from the study by Sontigun et al., (2018), it was perceived that the environmental and other factors linked with the seasonal fluctuation, daily activity and reproductive ability of the same are vibrant. For instance, the various environmental factors such as rainfall, relative humidity, altitude, land use types and temperature (Sontigun et al., 2018) can directly affect the abundance and the distribution of blow flies. A similar perspective in terms of seasonal fluctuations in blowfly populations was also verified by Lertthamnongtham et al., (2003). Perusal of literature reveals that the studies concerning the aforesaid aspect in Kerala is scanty. In this regard, it is essential to analyze the seasonal activity of the respective location or habitat of the blow flies for forensic entomology needs.

Among the various factors discussed, the temperature and the humidity have been recognized as significant factors in investigating the biology of blowflies, since such factors can directly affect the various developmental stages of the same including its behaviour, survival, population dynamics and the longevity ( Sukontason et al., 2008).

In addition to the above-said factors, Sontigun et al., (2018) also found that fecundity plays a significant impact on blow fly population dynamics because it truly governs the population growth potential of the same. It has been observed that larval nutrition plays a major role in life-history characteristics including body size, growth, longevity and survival followed by reproduction (Li et al., 2014).

Many investigations have witnessed that the higher temperature and lower temperature enhanced and slowed down the developmental process respectively (Queiroz, 1996). In contrast to these discussed facts, some of the studies reported that the environmental conditions and specific geographic locations may found to be major probable factors behind this situation. However, many of them have reported the direct influence of temperature on the fast developmental stages and growth. In this regard, many earlier research works have been conducted on the following blowfly species on the same aspect as mentioned in the former parts; *Chrysomya albiceps, Chrysomya megacephala, Chrysomya rufifacies, Calliphora vicina, Chrysomya erythrocephala, Calliphora vomitoria, Calliphora dubia, Protophormia terraenovae, Phormia regina, Muscina stabulans, Eucalliphora latifrons, Lucilia illustris, Ophyra capensis, Ophyra aenescens* and *Muscina assimilis* (Sukontason et al., 2008).

Besides principally identifying the species of blow flies collected from a death scene, the developmental rate of collected sample specimens is also found to be essential in

order to use as a piece of evidence in forensic investigation, specifically for the PMI estimation. As discussed by many of the researchers, it is also clear that the development of insects including the blow flies is primarily temperature dependent. For this reason, the normal metabolic rate of the same upsurges with augmented temperature, thereby making the developmental process happen at a faster rate (Anderson, 2000).

As discussed in the former parts regarding the temperature and other factors, there exists a controversy regarding the exact influence of these factors on the growth rate of blow flies. Moreover, many previous studies have documented that the pattern of influence may be changed with the geographic location. Although the facts follow these patterns, as stated in the above-said parts, some of the studies have suggested that there exists no noticeable difference between various seasons while following the same experimental conditions. A very similar range of temperature existing during their experimental conditions (summer - 23.3–32.0°C; rainy- 23.3–29.6°C: and winter-21.0–25.8°C) may be recognized as the major reason behind the aforesaid circumstance.

However, the blow flies, showed local adaptation to the different provinces over the globe, and earlier studies have reported that the various stages in the development of blow flies from different geographic locations may be found different (Grassberger & Reiter, 2002) in many of the instances and this justifies the significance of investigating the forensic entomological aspects of blow flies from Kerala, India.

In fact, a previous study recognized that an established life table of a particular species of fly is extremely beneficial in forensic entomology since it can deliver significant information concerning the survival growth as well as the fecundity of the studied fly (Abou Zied et al., 2003). The rate of development of blow flies having forensic importance

has been previously described in Thailand under natural temperature .In the same study it was observed that specific photoperiod followed by extensive development occurred in the summer season, while the rainy season was reported with relatively lower development (Hadura et al., 2018).

The concluding remarks by Shiao & Yeh, (2008) revealed that, the contributing factors in other species of *Chrysomya*, the temperature and various other factors significantly and obviously affect the larval development directly and thereby primarily impact the PMI estimations. Many of the investigators have suggested that the complexity emerged during such research in a geographic location perspective view should be clarified and rectified through an effective way.

In addition to the blow flies belonging to the *Chrysomya* genus, other species belonging to different genera like *Lucilia* may also exhibit great significance in forensic entomology. The precise and accurate identification of such specimens having forensic significance is one of the very significant elements from an applied point of sight (Catts & Goff, 1992).

In southern India, studies relating to the time since death assessment have not been conducted so far. The estimates of PMI based on the known characteristics of the infesting fauna in the natural conditions of the specific geographical location are very important.

The studies focusing on the use of insects as evidence in forensic investigations in different geographical areas are often considered to be the initial steps in developing the reference base line data (Acosta et al., 2021; Dawson et al., 2022). In the collection of ecological data with regard to the decomposing model, insects are found to be a major element in forensic investigations. The pre-colonization phase of the forensically significant insect is usually witnessed in the following phases; a) detection b) finding the

remains c) evaluating the cadaver d) colonization and e) post-colonization (Tomberlin et al., 2011).

While discussing the practical implementation of various strategies in forensic entomology it was evident that, developing baseline data using various parameters like temperature, humidity, successional pattern, and developmental time, is essential for the PMI determination. In addition to this, it is also required to consider the fact that successional sequence and developmental rate may be strongly influenced by the geographic region, various seasons, climate change and life cycle pattern (Brundage et al., 2014).

The process of natural variation in different geographical locations significantly impacts the distribution of forensically significant taxa. In addition to this, it should be remembered that seasonal variations should also be considered, as different species are showing variations in their life cycle even if the species have been found within the same locality (Cammack et al., 2016).

There are many approaches that are likely to provide support for PMI estimation in the various decomposition stages. A number of extrinsic and intrinsic factors are previously witnessed as the core of forensic entomology research. The former consists of the environmental conditions such as concealment, temperature, insect activity, clothing, moisture and various seasons while the latter encompasses the elements linked with the cause and manner including gender, body mass, injuries, and age (Amendt et al., 2011).

While focusing on the various factors discussed, the significant variables influencing the life cycle stages of insect include the temperature, humidity and seasonal variations (rainy, winter and summer seasons) (Amendt et al., 2011). For the early determination of PMI, post mortem hypostasis followed by algor mortis and rigor mortis

are recognized to be the most reliable and applicable method. The influence of various parameters including the temperature, humidity and other significant factors on the insect life cycle stages would be recognized as the most efficient method for PMI determination (Pittner et al., 2020). The lifecycle pattern and succession of flies inhabiting the cadavers usually followed a particular pattern and can be used as a reference for forensic investigations.

In order to estimate the PMI based on the developmental stages, geographical differences and seasonal variations, it is essential to accomplish the proper identification of the species (Sukontason et al., 2022).

Recent studies have validated that the knowledge concerning the carrion-inhabiting insects found at the specific geographic location is vital for determining the PMI (Amendt et al., 2011). The various developmental stages of these carrion-inhabiting insects are influenced by many factors including the geographical region, climate, temperature, humidity and seasons and may also result in diapause. The PMI determination principally relied on the information concerning the life cycle of the chosen insect and the various factors linked with it. The growth rate and the developmental pattern of insects having forensic significance might vary in several regions over the world. Hence the seasonal variations in different regions may also illustrate considerable interest in forensic investigations (Verma & Reject Paul, 2016).

In this regard, it is essential to warrant studies concerning the above-said aspect in insect species belonging to Calliphoridae, since their forensic significance have been previously reported in various provinces over the world except in Kerala. The output gathered from this investigation could be used to estimate the PMI in the Kerala region, which would be recognized as the novelty of this investigation.

## 1.2. Relevance of the Study

Among the various previously described necrophagous insects, flies belonging to Calliphoridae are known to inhabit and colonize the cadaver as the first comers within a short period of time. For this reason, studies focusing on the determination of the age of the blow fly life stages with special inference on the PMI estimation have gained extensive significance in the past few years. Many of the previous studies have discussed that among the various studied flies, blow flies are prominently found to exhibit great significance in the current scenario. Moreover, the studies from different parts of the world clearly validate that the studies concerning the influence of various parameters on the development rate, growth rate and lifecycle stages of blow flies are of great forensic significance. From this point of view, the research gap found in India, especially in Kerala were the main reasons for the present investigations in the above said perspectives in blow flies. As we all know, the data obtained from forensic entomology studies with special inference on the PMI estimation is usually allied with the geographic location. In this regard, the data obtained from the current study can be explored to determine the PMI in India especially, Kerala.

## 1.3. Objectives of the present study

The prime objectives of the current study are as follows:

1) To generate data on blow fly fauna of Central Kerala
2) To assess the seasonal differences in blow fly population in Central Kerala
3) To study the life history of selected blow flies in carrion
4) To find out the relation between the temperature and humidity on the life cycle of calliphorid flies
5) To relate the life cycle of flies and time since death assessment for forensic application

# CHAPTER - 2

# REVIEW OF LITERATURE

# CHAPTER - 2

# REVIEW OF LITERATURE

## 2.1. International

The first report, where blowflies were used for criminal investigation perspectives was in China during the 13th century, is mentioned in Sung Tzu's *The Washing Away of Wrongs*. According to the aforesaid incident, a farmer was murdered with a sharp weapon in a field and the investigators said that after the murder people placed their sickles in the field. Among the various sickles placed in the field, only one of the sickles attracted blow flies, since the sickle concerned had a trace of blood that cannot be seen by the naked eye. This extraordinary and scientific view has opened the way towards the confession of the murderer. This marked the beginning of forensic entomology.

The application of the above said perspective in front of the court, especially in the the modern world was witnessed in France during the 18th century where the data from entomological evidence was recognized as valid proof for clearing the existing inhabitants at a home.

According to Patton, (1931), the size of the larvae of blow flies differed according to the temperatures they inhabited and larvae contracted quickly just prior to the pupariation. The drawings and illustrations of *C. megacephala* with special inference to the conventional taxonomy were unveiled (James, 1947).

Zumpt, (1965) in his treatise mentioned that the rapid dissemination of *C. megacephala* all over the world was witnessed in the past few epochs. Moreover, many studies also unveiled certain significant records indicating the geographical distribution of

the same in Indian regions. The adult flies of *C. megacephala* were usually attracted to decaying cadavers and reach within a few hours of death of the animal.The literature concerning the comparative investigations that would reveal the conventional identification of *C. megacephala* using eggs was found scanty in the current scenario. Typically, the eggs of *C. megacephala* are sausage-shaped with whitish cream in colour measuring 1.5–1.6 mm.

James, (1971) was of the view that some of the previous literature found that the identification of *C. megacephala* for forensic needs exhibited some degree of difficulty since it may be markedly confused with other species which have common identification features with closely related *C. megacephala.* The *Chrysomya saffranea* is known for the aforesaid perspective all over the world. In this regard, it is essential to remember the fact that maximum care should be engaged while verifying the taxonomic information of every insect which has forensic significance including *C. megacephala.*

*C. megacephala* is recognized to have a diverse habitat, and in addition to the human inhabiting regions, they have also been reported from different ecotypes, rural and natural forested provinces, and straddling urban and peri-urban regions (Tumrasvin et al., 1979).

Kurahashi, (1979) described *Chrysomya chani* from Singapore. The information on life cycle, the influence of various factors in its development, life cycle and forensic importance followed by molecular analysis of *C. chani* were limited. According to Kurahashi, (1982), *C. megacephala* was found to be initially reported from Australasian regions while considering the most abundant blow flies.

Smith, (1986) discussed about the oviposition preferences of blow flies in open wounds and also categorized the group of invertebrates infesting on carrion. Tullis & Goff, (1987) reported that measurement of larval length, width and weight can be used for the age determination of particular instar for the PMI estimation.

Liu and Greenberg (1989) provided the key for egg and all the larval instars of flies of forensic significance. In their study they also suggested that need for identification of larvae becomes significant when larval specimens are presented. So rapid and accurate identification will be possible through the ultrastructural examination of the larvae.

Erzinclioglu, (1990) did a study on the morphology of larval instars of *Chrysominae* species and tried to compare them through the spinulation characteristics on the larval body.

A study by Goodbrod & Goff, (1990) revealed that the rate of development of insect larvae inhabiting the cadaver may depend on the environmental temperature, which suggests the role of temperature in forensic entomology studies. In addition to this, it was also evident that each stage of insect development has a specific temperature and other factors' needs. Each insect species that has forensic significance has its own specific days or degree hours to complete the various developmental stages during its development.

While reviewing the geographical distribution of *C. megacephala*, a study by Kurahashi, (1991) reported the presence of the same from the Australasian regions including New Caledonia, New Guinea and Samoa.

Medico legal cases world over have reported the forensic relevance of *C. megacephala* (Goff and Flynn, 1991, Amendt et al., 2004, Richards and Villet, 2009, Gruner et al., 2017). The age of the different species of the blow flies like *C. megacephala*

(Fabricius), can be used to estimate the PMI from the development rate linked with the environmental temperature. Since blowflies regularly find a corpse and promptly lay eggs on the same without delay (Turner & Howard, 1992), they are noted to be ideal candidates for estimating the PMI.

*C. rufifacies* was originally described in 1978 from Central America and have been reported from the various provinces like Arizona, Texas, California, Guatemala and Florida during 1986 to 1991. A silent invasion of the same in Puerto Rico was also reported in 1991 (Baumgartner, 1993).

When death happens, the cell's enzyme digestion, as well as the cell death takes place in the cadavers, thereby offering the habitat for the insect species. According to Ashworth & Wall, (1994), once the body starts to decompose, the microbial consortia living in the dead body start to destroy the tissues thereby resulting in the production of liquids as well as the following gases; carbon dioxide, hydrogen, sulphur dioxide, apneumones, methane, hydrogen sulphide and ammonia. Thus formed chemical elements and gaseous candidates escaping from the dead body may invite the insect species to the decomposing matter. Each stage of the decomposition may witness the presence of a plethora of chemical constituents and the scientific community all over the world is trying to identify such constituents.

The earlier experiments conducted by Wells & Kurahashi, (1994) at 16L: 8D light conditions and a temperature of 27°C, unveiled the pupariation of the studied species *C. megacephala* at 144 h.

The identification of *C. megacephala* species-group with special inference to their adult has been published by Wells & Kurahashi, (1996). The various drawings and

photographs of the adult *C. megacephala* along with the shape of female frons and eyes of male have also been discussed by James, (1947) and Zumpt, (1965).

Many previous investigations have already studied the developmental stages of *C.megacephala*, which include development at regular temperatures, and changing temperatures followed by outcomes of different feeding matters. The results from such studies primarily display the developmental plasticity in *C.megacephala*, and that extraordinary populations have differences in development according to the environment as well as the geographic location (Wells & Kurahashi, 1994, Ma et al., 1998) prominently depends on their surrounding temperature.

The morphological identification of insects having forensic significance, especially *C. megacephala,* has been previously reported (Wells & Kurahashi, 1996). To get the data that can be used to estimate the PMI in the future, it is essential to investigate the developmental rate of forensically significant fly species like *C. megacephala* with special inference to the various environmental factors that have been found in the death scene (Queiroz, 1996). If so, such studies can be used to compare the forensically important blow flies collected from death scenes or environments as stated in the former sections.

The developmental rate of *Chrysomya* species in context with the various natural temperatures and cyclic temperatures, results in an extensive difference ranging from 4h to 38 h (Byrd & Butler, 1997). Many investigations have been performed previously by them to analyze the pattern of the various developmental stages of *C. rufifacies* for forensic needs. They investigated the growth curve for the various developmental stages of *C. rufifacies* including the larva, and pupa under a constant temperature of 25°C and at average cyclic temperatures of 15.6°C, 21.1°C, 26.7°C, and 35.0°C.

In addition to the forensic significance shown by the *H. ligurriens,* these flies were also found to make nuisances in gardens and markets. Moreover the adult flies of *H. ligurriens* are also responsible for the transmission of a plethora of pathogens to humans as well and they were also attracted to the human excreta usually found near human-inhabited surroundings (Kurahashi, 1997).

Earlier studies have reported that in addition to the adult flies obtained from the carcass, the various developmental stages of the same, specifically the eggs, and larva flowed by puparia can also be used to determine the postmortem interval (Anderson, 1999; Smith, 1986)

A fact sheet published by CABI in 2001 revealed that the following countries reported the presence of *C. rufifacies*: Bangladesh, Fiji, Papua, China, Arizona, Nebraska, Texas, Saudi Arabia, Alabama, New South Wales, New Caledonia, Sri Lanka, Australia, Western Australia, Costa Rica, Malaysia, India (Kerala, Karnataka, West Bengal and Tamil Nadu),, Pakistan, Philippines, New Zealand, Argentina Vanuatu, Arkansas, Hawaii, Louisiana, Thailand, Queensland, New Guinea, and Mexico (CABI, 2001).

Likewise the, fact sheet by CABI, 2001 revealed that *C. chani* was reported from the following regions over the world; Saudi Arabia, Sri Lanka, Australia, Bangladesh, Fiji, Papua, New South Wales, New Caledonia, Argentina Western Australia, China, Arizona, Nebraska, Texas, Philippines, Costa Rica, Arkansas, Hawaii, Louisiana, Thailand, Malaysia, New Zealand, Alabama, Vanuatu, Queensland, New Guinea, Mexico and Pakistan.

*H. ligurriens* was known for its prominent contribution to forensic investigations and are primarily distributed in the following countries; Australia, The Philippines, India,

Malaysia, Papua New Guinea, Laos, Indonesia, Taiwan, Korea, China, Thailand, Singapore, and Sri Lanka (Tumrasvin et al., 1979, Kurahashi and Chowanadisai, 2001).

*C. rufifacies* larvae usually exhibit an unfamiliar activity. For example, they start to prey on other larvae once they have attained the second instar stage. Hence, they can alter the population composition on the cadavers, occasionally imposing other *Chrysomya* sp. to attain the wandering stage in a quick manner. In accordance with the various contributing factors, the rate of *C. rufifacies* larval weight was previously known to possess extensive statistical significance towards temperatures and time (Madeira, 2001).

According to Amorim & Ribeiro (2001), the mouth hooks of the mature larvae which can also be seen sticking inside the enclosed puparium, contribute to the identification of the same at the species level.

The prominent characteristic feature used for the identification of *C. chani* is the densely sclerotized spiracle on the posterior part, complete peritreme followed by the presence of accessory sclerite (Greenberg & Kunich, 2002).

Von Aesch et al., (2003) conducted study on the abundance and diversity of blow flies in the outdoor to relate the influence of climatic factors on the fly activity and also to check the level of attraction of the blow flies to different stages of decomposition.

Lertthamnongtham et al., (2003) have investigated the seasonal activity of *C. megacephala* from the Chiang Mai Province, the northern region of Thailand and also validated the same perspective in terms of seasonal fluctuations in blowfly populations. The seasonal fecundity of the same insect was also analyzed in their study and found that the ovarioles number statistically differed between the various seasons studied, and there

exists a significant increase of the same in the rainy season than in the summer and winter seasons.

Sukontason et al., (2003) clearly depicted the major characteristic features of the ultrastructure of the larvae of *C. megacephala* for species identification using Scanning Electron Microscopy. The authors emphasized about the significance of hairy nature of *C. rufifacies* that can be used to differentiate it from *C. megacephala*. Their study also revealed about the importance of spiracular hairs in the posterior region of the *C. rufifacies* to differentiate its various developmental stages.

The establishment of forensic entomology with sufficient scientific evidence was initially done by Yovanovich and Megnin's evaluation concerning the succession of insects on the cadavers. The presence of insect species belonging to the above said group can deliver significant information for the estimation of PMI. For this, the precise identification of the insect specimen inhabiting the cadaver could be recognized as the initial step. In order to consider the insect specimen as significant evidence for the criminal investigation, especially for the estimation of PMI, the insect larvae need to be determined in terms of their length, width, growth rate and other influencing factors (Amendt et al., 2004).

Amendt et al., (2007) discussed in detail about the good methods in forensic entomological work and stipulated certain standards and guidelines with respect to collection, preservation, and transportation of entomological evidences.

From a forensic entomology perspective and also based on the many investigations carried out, the construction of the life table for a particular species of fly like *C. rufifacie*s and *C. megacephala* could be noted as a significant study in the current scenario (Sukontason et al., 2007).

While considering the same perspective in an international concern, specifically in the Brazilian context, it was observed that there exists a constant increase in the abundance of *C. megacephala* found between the end of spring, October and February (all of summer) and attained its maximum level in December (Mello et al., 2007). The study unveiled that the fluctuation in its abundance and diversity is usually specific to its habitat and location which are noted to be altered according to various seasons including pre-monsoon, monsoon and post-monsoon.

Studies focusing the development of *C. megacepehala* has been done previously (Sukontason et al., 2008; Niederegger et al., 2010; Zhang et al., 2018). *C. megacephala* has been considered as an important fly for the determination of minimum postmortem interval (Wang et al., 2008). Many of the studies performed from various regions all over the world including Malaysia have reported the forensic significance of *C. rufifacies*. The predacious activity exhibited by *C. rufifacies* larvae is recognized to be the major reason behind the same. One of the major points while discussing the life cycle stages of *C. rufifacies* is the influence of *C. megacephala* in stimulating the oviposition activities. This essentially means that the *C. rufifacie*s consume the larvae of *C. megacephala* (Shiao & Yeh, 2008).

Due to the various complications linked with the conventional taxonomic analysis of the *C. megacephala*, a study by Stevens et al., (2008) has suggested the use of the whole mitochondrial genome of *C. megacephala* in species identification.

Wang et al., (2008) reported that the presence of three slits in the posterior spiracles and 11–13 branched anterior spiracles on the third larval instar of *C. megacephala* could be used for distinguishing it from the first and second instar larvae. According to them, as a predatory and necrophagous species, the immature stages of *C. megacephala* have been

found on almost all cadavers reported from Southern China. *C. rufifacies* are found on carcasses almost all year usually in warmer areas while they are mainly found during the warmer months in the temperate zone of China.

According to a study by Evaldo et al., (2008), there exists a lot of environmental factors such as rainfall, relative humidity, temperature and altitude including the land use types. They have a significant role in the rapid distribution and extensive abundance of flies belonging to the Calliphoridae.

Sukontason et al., (2008) reported the morphological characteristic features of the *C. rufifacies* from Thailand. They primarily compared the morphological characteristic features of the *C. rufifacies* and *C. megacephala* with special reference to the cephalopharyngeal skeleton, posterior spiracle, cuticular spines and appearance of the body.

Among the various previously studied flies, *C. megacephala* has relevance to medical entomology, forensics, and public health in various provinces over the world especially in Asia, South America, Africa and Australia (Richards & Villet, 2009). The authors also reviewed the rate of development and influence of various parameters including the environmental temperature, physiological age and surrounding environmental condition of *C. megacephala*.

Estimates of postmortem interval (PMI) based on the known characteristics of the infesting fauna in the natural conditions of the specific geographical location are very important (Sukontason et al., 2008). Niederegger et al., (2010) suggested that negligence of fluctuating temperatures in legal cases can lead to distinctly wrong estimates of the PMI.

Recent studies have validated the use of molecular strategies for the identification of blow flies (Tan et al., 2009).

According to the findings by Ahmad et al., (2010), it was evident that they principally used the light microscope for the examination of the morphological structures (anterior spiracles, posterior spiracles and cephalopharyngeal skeleton) of second and third instar larvae of *Hypopygiopsis* from Malaysia. Also the developmental data generated by the authors really helps the scientific community to estimate the PMI of corpses from the study region of Malaysia.

According to Gallagher et al., (2010), to implement the approaches targeting the insect for forensic entomology, the study concerning all the immature stages followed by the developmental rate in relation to different parameters including the temperatures and the humidity in geographically distinct populations is having great forensic significance, because such insect studies will be useful for the precise estimation of PMI in the specific region.

The morphology of various developmental stages of the *H. ligurriens* was revealed through the light microscope and SEM by Firdaus et al., (2010); Sukontason et al., (2004), justifies the use of SEM for the identification of *H. ligurriens*.

As mentioned by Mendonça et al., (2010), the identification of insect specimen using conventional approaches, especially based on the morphological features has some advantages but it has certain limitations when applied to immature stages. Moreover, when damaged specimens were obtained from the field or crime scene, DNA-barcoding, specifically the mitochondrial COI genome has great potential for the identification of insect specimens particularly Calliphoridae. Mendonça et al., (2010) also validated the

efficacy of SEM to study the morphological characteristic features of flies belonging to the Calliphoridae.

Studies involving microscopic investigation of larval instars of blow flies were reported by Ahmad et al., (2010) from Malaysia. The presence of *C. chani* in outdoor and indoor circumstances was witnessed by a study that used *Macaca fascicularis* Raffles as a candidate animal. From their observations, the adults of *C. chani* were collected from 6 to 13 days, which are generally known for the decomposition stages. While considering an indoor perspective, it was clear that the *Chrysomya chani* was sampled from 4 to 30 days, which justifies a preference for distended to extensive decay (Ahmad et al., 2011).

Sukontason et al., (2011) tried to identify the larval instars of *C. megacephala* using Scanning Electron Microscopy (SEM). According to Olea et al., (2011), the appearances of *C. megacephala* in new regions draw attention to the current scenario; also reminds us of the increased rate of the spread of the same from one region to another. They are able to travel about 2-3 km/day and recent studies indicated that the *C. megacephala* extended its habitat to a region that is 500 km far from its original habitat. Brundage et al., (2011) investigated the abundance and distribution of insects belonging to the Calliphoridae with special reference to seasons and habitats for two years.

SEM studies on ultrastructure of *C. megacephala* larvae have been reported by Mendonça et al., (2012). They claimed that the spinulations present on the body integuments and the cluster of spines having either one or two tips on the cephalic region can distinguish *C. megacephala* from various *Chrysomya* species.

Yang and Shiao, (2012) studied about the oviposition preferences of *C. rufifacies* and *C. megacephala* in context with the possibility of using them as prominent candidates

for the estimation of PMI. In addition to this, they have also validated that the adult female of *C. rufifacies* and *C. megacephala* usually arrive on corpses immediately less than an hour of their presence, and are typically found to lay about 220–325 eggs at a time. The influence of various parameters like temperature, humidity from their surroundings on rate of development was also recommended by them in forensic investigations.

. In addition to the above-reviewed species, the blow flies belonging to *Hemipyrellia* have also exhibited sturdy significance in forensic entomology. Among the various investigated blow flies, *H. ligurriens*, were found to have prominent significance in forensic entomology as stated in various studies previously from Malaysia and Thailand.

A study by Bunchu et al., (2012) revealed the geographical distribution of *H. ligurriens* over the following regions; Taiwan, Korea, China Papua New Guinea, Australia, The Philippines, India, Thailand, Singapore, Sri Lanka Malaysia, Laos and Indonesia.

*Hemipyrellia* is represented by four species in the Oriental region. In India, it is represented by two species; *H. pulchra and H. ligurriens* (Senior-White et al., 1940, Nandi, 2004., Bharti, 2011). *H. ligurriens* was reported on decomposing human cadavers in Malaysia (Rajagopal, 2013), Thailand (Moophayak et al., 2014) and other regions (Chen et al., 2004, Lee et al., 2004, Sukontason, 2007) and also has significant forensic importance. The development studies of *H.ligurriens* has been done by a few researchers (Sinha and Nandi, 2007, Sukontason et al., 2008, Sukontason et al., 2010, Bunchu et al., 2012, Yang et al., 2015).

The metallic copper green coloured adult, possesses about 12.23 mm in length and 2.85 mm in width. The head of the male and female were holoptic and dichoptic respectively (Bunchu et al., 2012). There exists a whitish coloured squama and the thorax

was noted with postsutural acrostichal setae. All the above said morphological characteristic features are reported to be the major contributing factors in *H. ligurriens* species identification.

As a comparison perspective against the findings of Sukontason et al., (2008), Bunchu et al., (2012) verified the forensic significance of *H. ligurriens* in context with the developmental rate and the morphology of all stages. In their study, they maintained natural ambient settings for their experiments and used a light microscope for identification purposes. The final findings of them clearly validated that *H. ligurriens* took about 270.71 h for completing its life cycle. Some of the immature stages of *H. ligurriens* larvae, specifically the third instar larvae have been investigated using the light microscope.

Szpila et al., (2013) have developed keys to identify the larvae up to species level from Europe and Mediterranean regions. They also reported that in the larval instars of *C. megacephala,* cuticular spines in the dorsal region between the mesothorax and the prothorax are organized close together in a single form. They also pointed that various ranges of temperatures have really affected the developmental process and growth rate *C. megacephala*.

Successional studies conducted on the in *Oryctolagus cuniculus*, the New Zealand white rabbit, illustrated that *C. chani* adults are typically active in the bloated stage of decomposition. The larvae of *C. chani* at second and third developmental stages were also collected during active decay. However, the larvae at third developmental stages were also reported from 6 to 8 days of decay, which is usually referred to as the advanced stages of decay (Silahuddin et al., 2015).

The information concerning the influence of various parameters including the temperature humidity and other factors towards the growth rate and the development of other *Chrysomya* sp. except *C. chani* were reported from many countries. Jordaens et al., (2013) appended the mitochondrial COI gene analysis of *C. megacephala* for species identification.

Moophayak et al., (2014) sampled 2,115 blow flies belonging to six genera including *H. ligurriens*, and found that there exists a prominent link between the studied seasons and the frequency of occurrence. Furthermore, they concluded that many of the insect species in their study had shown their peak during the summer seasons, as found in various literatures reported in Thailand previously.

According to the perspective of Owings et al., (2014), it was evident that the variations in the abundance and life cycle of blow flies may probably be due to the vast differences in the in population variation, study sites sampling strategies, and the sampling period.

A study by Zabala et al., (2014) sampled 28,507 adult calliphorids and analyzed for various forensic perspectives with special inference on the influence of various seasons including summer, winter and monsoon. According to their findings, it was evident that *C. vomitoria* (6530) and *C. vicina* (9883) were the most abundant species in winter season. In addition to this, *L. ampullacea* and *L. caesar* were abundant. Some of the following species reported in their study also possessed significant seasonal abundance and exhibited a maximum peak in the summer season like *C. albiceps, L. sericata* and *L. illustris.*

Studies conducted by Whitaker, (2014) proved that same species of blow flies were attracted to both pig and human cadaver and comparable results were obtained regarding

the oviposition sites, larval development which reinstated the claim for pigs as a better substitute for forensic entomological studies.

A study performed by Akbarzadeh et al., (2015) clearly defined the various morphological structures and specific pattern which can be used for the identification of *C. rufifacies.*

Yang et al., (2015) while studying the PMI estimation, suggested that it is essential to highlight the significance of implementing the various technologies and concepts to get enough knowledge concerning the biology of the various developmental stages of the native species belonging to the Calliphoridae with special inference on the diverse affinity toward the geographical regions. In a previous study, they uncovered the possibilities of constructing growth curve models for *H. ligurriens* using different constant temperature regimes.

*C. megacephala* was originally reported from Australasian regions including the New Caledonia, New Guinea and Samoa. The dramatic spread of the same has resulted in the record of the species grew from various other regions such as Mauritius, Rodriguez, and India (Akbarzadeh et al., 2015).

The larval development rates of *C. rufifacies* within the South-East Asia region were reported by Yanmanee et al., (2016) and found that the different geographic locations have a prominent role in the rate of development of *C. rufifacies*. The results clearly validated that the developmental times varied among different temperatures from egg to adult stage of *C. rufifacies*.

Yang et al., (2016) and Gruner et al., (2017) have validated the probable use of the thermal accumulation models for forensic entomology research. The aforementioned

thermal accumulation models are primarily focused on either the landmarks or developmental events, which were typically followed by maintaining the samples of live insects for forensic needs.

It has been observed that *C. megacephala* lay eggs at night in certain circumstances where their surrounding environment is found to be warm (Williams et al., 2017). Previous research has shown that *C. megacephala* is one of the first reported insects to arrive at a cadaver, and its exceedingly huge population size makes them unquestionably the dominant species on the remains (Wang et al., 2017 & Sukontason et al., 2022). The observation of Sontigun et al., (2018) validates that the ecological niches inhabited by the *C. megacephala* may influence the fecundity of the same.

Mendonça et al., (2010), used mitochondrial COI gene to identify the blow flies; *C. bezziana, C. megacephala, H. ligurriens, C. nigripes, C. chani, C. villeneuvi, C. pinguis, Lucilia porphyrina, C. rufifacies, L. papuensis, Hypopygiopsis infumata, H. tumrasvini C. thanomthini, L. sinensis, L. cuprina,* and *H. pulchra.* Many of the previous studies have primarily used the applicability of DNA-based strategies, especially the use of mitochondrial COI gene to identify blow flies (Qiu et al., 2017, Yusseff and Agnarsson ., 2017, Sukontason et al., 2008).

The influence of seasonal variations including the summer, monsoon and winter on blow flies (Dipter: Calliphoridae) was reported by Sontigun et al., (2018). According to them, the various environmental factors including the relative humidity, altitude, rainfall, land use types and temperature can influence the abundance and the distribution of insects belonging to Calliphoridae. In this regard, they sampled and investigated about 88,273 flies belonging to *Chrysomya* and found that among the various analyzed insects, *C.*

*megacephala* was found to be predominant in summer and the abundance got gradually weakened in rainy and winter seasons.

Sukontason et al., (2018) have verified that *C. rufifacies* and *C. chani* had been known to illustrate their prominent significance in forensic entomology. Based on the analysis, *C. megacephala, C. rufifacies* and *C. chani* were the predominant insect species that have prominent significance in forensic entomology and confirmed that PMI was six days. The authors examined the larval instars of *C. chani* and suggested that the elongate tubercles with innumerable small spines on body, sclerotized spine bands and heavily sclerotized peritreme of the spiracles were characteristic and could help in identifying the species. Their study also validated that the biological information was found to be limited. The authors also suggested that phylogenetic analysis using COI and COII genes can be employed to distinguish *C. chani* from closely resembling *Chrysomya pinguis* and *C. megacephala.* They described larval instars of *C. rufifacies* using the light microscopy and mentioned the presence of large tubercles on abdominal segments with spines on the tip can be used for the identification of the species.

The identification of *C. megacephala*, along with its biology and geographical distribution were reviewed by Badenhorst and Villet, (2018) from South African provinces to get an extensive background for various strategies that might be used for this fly for forensic needs.

Irish et al, (2014) and Badenhorst and Villet, (2018) studied the various morphological features of *C. megacephala* using photographs and drawings along with the ultrastructure analysis of first, second and third instar larval stages. According to them, first, second, and third instar larvae can attain 1.7–3.5 mm, 6–8 mm, and 16 mm long

respectively. They treated *Lucilia flaviceps, Musca flaviceps, Musca remuria, Pollenia basalis, Somomyia dives, Somomyia cyaneocincta, Somomya cyaneocincta, Musca bata, Musca combrea, Musca megacephala, Somomya pfefferi,* and *Somomyia cyaneocincta* as synonyms of *C. megacephala.*

A study by Sontigun et al., (2018) revealed the various findings concerned with the seasonal variation perspectives of *C. megacephala* activity with special inference on the various developmental stages of the same collected from the Chiang Mai area of Northern Thailand. They reported that the highest abundance of *C. megacephala* was found to be in summer and the abundance gradually diminished in the rainy and winter seasons.

The growth and development patterns of *C. megacephala* from Yangtze River Delta region of China was studied by Zhang et al., (2018). They have constructed three models; isomegalen diagram, thermal summation model and isomorphen diagram for PMI estimation. They found that various development stages of *C. megacephala* such as eggs, first instar second instar and third instar larvae, followed by the pupae were reported with diminishing duration of development with higher temperatures. The total developmental duration was also found to be diminished to 171.8 h at 34.0 °C from 794.8 h at 16.0 °C, justifying the significant link between the temperature and developmental duration in *C. megacephala*.

Klong-Klaew et al., (2018) studied geographical and ecological information of insects having forensic significance with special inference on the PMI estimation. They collected nearly 1298 flies belonging to Chrysomyinae and *C. chani* was predominant in their study and the maximum abundance of the fly was observed during summer with

temperatures ranging from 25–30 °C.While the rainy and winter seasons witnessed a drastic reduction in their numbers.

A study from China by Li et al., (2016) also found the similar observations as reported by Klong-Klaew et al., (2018). However, certain kinds of conflict were also found in some previous studies by Moophayak et al., (2014).

Regarding seasonality, according to Klong-Klaew et al., (2018), *H. ligurriens* species was predominantly reported during the late summer and the diversity was drastically reduced in the rainy season. In addition, the population diversity of *H. ligurriens* was reported as reduced in the winter season. There exists a positive correlation between relative humidity and increased temperature in the above-said contexts.

In agreement with the findings reported by Sukontason et al., (2018); Singh et al., (2011), validated the applicability of 2386 bp of combined nuclear carbamoylphosphate synthetase and COI gene in the molecular characterization of *C. chani*. In contradiction to their above findings, the use of 28S nuclear rRNA gene along with a size of 1000 bp placed *C. chani* into the hairy maggot blow flies group indicating the significance in choosing the particular gene for specific analysis. One of the major reasons behind the group change may be the prominent range of variations that happened in the different genes. Such movements will definitely result in the alteration of the taxa arrangements (Zajac et al., 2016).

Zhang et al., (2019) investigated the thermal summation, development duration and larval growth of *Chrysomya* sp. under a specific range of temperatures typically within the range of 16–34°C and found a prominent range of links between the developmental rate and temperature. They also suggested that the data obtained from their findings can be used

to build developmental models and that geographically isolated populations of insects belonging to *Chrysomya* genus can differ in their developmental time. This is in conformity with studies by Grassberger & Reiter, (2002).

Constant range of temperature primarily at 25°C formed the pupation times and adult emergence of *C. rufifacies* from 134 h to 162 h and 237 to 289 h respectively (Hu et al., 2019). The most preferable temperature determined for the larva of *C. rufifacies* while using the gradient system was 35.1°C. The various benefits including the slight larval length variation and extremely foreseeable developmental time followed by low cohort variation highlight *C. rufifacies* as a highly predominant candidate for PMI determinations based on forensic entomology.

Similar to *C. megacephala*, the adult and immature stages of *C. rufifacies* collected from corpses have been primarily used as forensic evidence in various investigations mainly to estimate the PMI (Herrera et al., 2021). Nigoghosian et al., (2021) recently proposed a two-step protocol for the mounting of cephalopahryngeal skeleton of calliphoridae larvae for the microscopic examination.

According Lutz et al., (2021), in instances where entomological evidence was collected and insects identified (n = 279), many of the dead bodies have been inhabited by one or two species with almost 10 unique species colonizing a carcass. Collectively, mono-colonization is found more regularly interior than outdoor environment. It cannot be forgotten that this particular pattern was based upon numerous factors along with the person who was incharge for the sample collection (medical expert, forensic entomologist, crime scene technician, or police), the sampling area (scene of death, place of autopsy) or specific pattern for sample collection.

A recent study by Acosta et al., (2021, 2022), reported the fact that humidity and controlled temperature have a direct impact on the life cycle of various flies belonging to Calliphoridae.

The early 1900s witnessed the first report of *C. rufifacies* from Hawaii as a new fly and the drastic dissemination of the same towards various regions across the world were reported since the ships and aircraft that link abroad paid them clear route for the dissemination. Due to this, in 1958, *C. rufifacies* was reported from Japan which was far about 6,611 km from Hawaii, thereby justifying the role of international travel and migration approaches in the extensive spread and diversity of *C. rufifacies* from various parts all over the world (Baumgartner, 1993; Dawson, et al., 2022). A recent study by Jeong et al., (2022) revealed the distribution of blowflies having forensic significance in terms of various seasons such as spring, summer and autumn followed by winter.

Pelletti et al., (2022) validated and concluded that SEM can be used for the morphological characteristics-based identification of *C. vomitoria.* The identification of any insect which has forensic significance needs a proper identification approach to validate its significance in forensic entomology (Abdullah et al.,, 2022; Apasrawirote et al., 2022; Greenberg & Ibrahim, 2022). The data concerning the various developmental stages and life cycles of *C. megacephala* was studied by many of the authors (Xu et al., 2022).

**2.2. National**

While considering the review of perspectives for the study area, it was evident that some of the studies (Subramanian & Mohan, 1980) have analyzed the biology of flies belonging to *Chrysomya* and the fact is that many of the studies have focused on *C.*

*rufifacies* and *C. megacephala*. But the aforesaid species were not reported with forensic significance from the current study region except in this study.

Kulshrestha & Chandra (1987) detailed 25 case studies with inferences drawn on the PMI estimation based on the data of developmental stages of blow flies obtained through rearing in the prevailing conditions.

Kashyap and Pillay (1989) checked the efficacy of entomological method over other conventional medico legal approaches for the estimation of PMI by studying 16 insect infested dead bodies. In their study they found that the entomological method was superior to other methods.

Senior-White et al., (1940), Nandi, (2004), Singh & Sidhu, (2004) and Bharti, (2011) recorded 30 genera and 119 species of Callophoridae from India. Among the various genera found in the Indian region, *Chrysomya* was noted to be the most predominant genus in Calliphoridae with forensic, medical and veterinary significance (Bharti, 2011). Bharti & Kurahashi, (2009), revealed that the feral derived form (fdf) of *C. megacephala* was reported from the Himalayan forests in India.

The various blow files belonging to Calliphoridae with forensic significance were enlisted by Singh & Bharti, (2001). While considering the environmental factors, especially the influence of various seasons, Aggarwal, (2005) performed an investigation in Punjab in the context of the autumn, rainy, summer, spring, and winter seasons.

Study by Wall et al., (2001) discussed that the abundance of *C. megacephala* is directly and positively linked with the relative humidity. However, there exists a negative pattern in the abundance of *C. megacephala* while rainfall and temperature are considered as parameters. Their findings revealed that the trimodal peaks of *C. megacephala* supported

the above said inferences in which the highest abundance was reported in January, followed by September and June. According to their findings, the population rate of *C. megacephala* was extensively augmented in the rainy season and the dry hot season was reported with relatively low population rate.

Bharti et al., (2003) conducted an insect succession study on the decaying rabbit carcass in Punjab and found that among the insect species recorded, Calliphoridae were the first to arrive on the carcass in all seasons.

A study by Sinha & Nandi, (2004) reported that the synanthropic form of the aforementioned species is usually found common in the human-inhabited regions which allow direct interaction with the human population. The occurrence of *C. megacephala* from fruits, carcasses, dead fish, human excrement and sweet was previously reported by the authors which justifies the significance of *C. megacephala* in forensic entomology in Indian scenario.

Singh and Sidhu, (2004) prepared the check list of blowflies of North-West India and reported the presence of *Idiella mandarina* (Wiedemann, 1830) and *Chrysomya pinguis* (Walker, 1858).

One of the most predominant contributions to the blow flies checklist was done by the Occasional Paper NO. 231, Zoological Survey of India (Nandi, 2004). According to the list, it was evident that the following species were primarily reported from Kerala; *Bengalia jejuna* (Fabricius, 1787) (Cochin, Trivandrum and Valayar), *Bengalia surcoufi* Senior-White 1924 (Cochin, Kerala), *Lucilia ampullacea* (Malabar Coast)), *Lucilia papuensis* (Malabar Coast)), *I. mantlarina* (Wiedemann), 1830 (Trivandrum), *Rilinia unicolor* (Townsend), 1917 (Cochin), *Cosmina bicolor* (Walker, 1856) (Kerala), *Cosmina*

*simplex* (Walker), 1858), *Strongylura strongylura* (Van Hasselt, 1823) (Chalakudy, Kerala) and *Borbororhinia bivittata* (Walker, 1856) (Cochin, Kerala). In addition to this, many of the studies have reported the presence of blow flies from the study region and such data has been provided below with special inference to the source and distribution of the acquired data. The data concerning the sources have also been added in the reference part.

Studies focusing the development of *C. megacepehala* has been previously studied in India by few authors (Subramanian and Mohan, 1980; Bharti et al., 2007; Verma and Reject Paul, 2013; Bala and Singh, 2015).

Age grading studies on immature stages of *C. megacephala* at different temperatures in the laboratory has been done at Punjab, India where the fly took 6.3 days for development from egg to adult stage at 30°C (Bharti et al., 2007; Bala and Singh, 2015).

An investigation by Bharti and Singh, (2007) reported the fact that the humidity and controlled temperatures have a direct impact on the life cycle of various flies belonging to Calliphoridae.The pattern of various stages in the lifecycle of the blowfly *C. megacephala* was analyzed (Bharti et al., 2007) with the following constant temperatures; 22 °C, 25 °C, 28 °C, and 30 °C. The findings from their study revealed that the range of temperature was inversely related to the development periods (63 days to 155 days).

The presence of *C. megacephala* has been reported in some regions in India and only three following studies had discussed its predominant significance in forensic entomology; (Bharti & Kurahashi, 2009; Roy & Dasgupta, 1975; Wall et al., 2001). According to Bharti & Kurahashi, (2009), the Indian form (fdf) of the blow fly *C. megacephala* may usually be recognized as a prominent form and they were typically

found all over the year in the warmer conditions. However, the abundance may drastically be diminished in winter seasons.

The Indian scenario has witnessed the presence of about 119 species belonging to 30 genera for the Calliphoridae cosmopolitan group (Bharti, 2011). Radhakrishnan et al., (2012) reported the presence of maggots of *C. albiceps* from the cadaver of sambar deer from Kerala. *C. albiceps* was previously reported from North Africa, Madagascar, Rodriguez, Africa, Cape Verde, Socotra, United States, Afro-tropical Islands of Aldabra, South America, Palearctic region, and north-west India, which justified the significance of the same in an international perspective.

A study by Verma and Reject Paul, (2013) verified that the rate of insect development especially the various developmental processes in *Chrysomya* are strongly influenced by various environmental factors including temperature and humidity.Among the various studied flies, *Chrysomya* was noted to be one of the most predominant genera under Calliphoridae having veterinary, forensic and medical significances (Ramaraj et al., 2014).

According to Ramaraj et al., (2014), the presence of synanthropic form of *C. megacephala* and its diversity analysis in the various regions of South Indian region especially Tamil Nadu indicate the significance and scope of forensic entomology studies in the South Indian region. The authors validated for the first time that the aforesaid region witnessed an extensive abundance of the blow fly *C. megacephala.* The output gathered from their study can be used to identify the same species in their region for forensic entomology needs, especially for PMI estimation. For the identification of the blow fly *C.*

*megacephala*, they have used the following studies: Sinha & Nandi, (2004); Siriwattanarungsee et al., (2005) and Sukontason et al., (2008).

While considering the above-said perspectives for *C. chani*, a few international articles were found, and of which many of them cited that *C. chani* was reported from India. In addition, certain studies from India, Bharti et al., (2014) and Kurahashi, (1997) have revealed the habitat preferences of *C. chani* with special reference to the tropical and secondary forests. However, based on the previous literatures from an Indian perspective, it is clear that none of the studies had reported the developmental rate and life cycle studies of *C. chani*, and this justifies the significance of investigating the same for the current study.

While considering the geographic distribution of *C. chani*, it was reported that the following regions were found to be endemic for the aforesaid species that include Bangladesh (Chittagong), China (Hainan, Guangdong), India, Indonesia (Kalimantan), Malaysia (Perak, Selangor, Kuala Lumpur, Pahang), Philippines, Nepal (Mindanao, Samar), Sri Lanka, Vietnam Singapore, and Thailand. The natural forested areas were predominantly known to possess their extensive diversity in northern Thailand while adults have also been reported from the secondary forest in Indian regions (Bharti, 2014).

Abd Al Galil and Zambare, (2015) investigated the morphological parameters along with the life cycle duration of *C. rufifacies* and observed that the fly took longer duration for completion of its life cycle during winter in comparison to rainy and summer seasons.

Bharti and Singh, (2017), while reviewing the Forensic entomology perspective in the Indian context, one of the major things that has been found from the literature is that reliable and specific keys for the identification of various immature stages of flies using

morphological characteristics are found to be either incomplete or unavailable in the current scenario. The authors have enlisted the *Chrysmoya* species along with their locations from Kerala, India and are as follows; *C. rufifacies* (Calicut, Kerala; Patiala, Punjab), and *C. megacephala* (Calicut, Kerala; Patiala, Punjab; Paonta Sahib, Himachal Pradesh;. *C. nigripes* (Calicut, Kerala); *C. chani* (Calicut, Kerala). Based on the aforesaid studies and various other literatures from the international perspective, *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* have prominent significance in the Indian forensic scenario.

Siddiki and Zambare, (2017) studied the life cycle of *C. megacephala* and *C. rufifacies* and observed a shorter life cycle duration in summer when compared to winter season. Gruner et al., (2017), in their study mentioned that the age of larvae would be helpful for the determination of minimum postmortem intervals.

Bharti and Singh, (2017) have used the mitochondrial COI gene to identify blow flies from India. The presence of *C. megacephala* in the Indian region was also reported by Badenhorst & Villet, (2018). Bharti, (2019) provided a revised key for the identification of known Indian *Chrysomya* species.

None have piloted the study in South India concerning the forensic implications on *H. ligurriens* and *C. megacephala* with special inference on the development rate and life cycle during different seasons except few recent studies (Reject Paul & Binoy, 2021 and 2022). Another study concerning the ultra structural details of larval instar using SEM on *H. ligurriens* with special inference on forensic significance was reported by Reject Paul and Binoy, (2021) for the first time from India.

# CHAPTER - 3

# METHODOLOGY

# METHODOLOGY

### 3.1. Blow fly fauna of Central Kerala

Studies were conducted for three years in different sites of Central Kerala to collect the blow flies. The data was generated by attracting blow flies to the decomposing carrion, by using sweep nets and also based on the literature survey. Blow fly species trapped were collected and identified. Blow fly sampling was done during different seasons; summer (April/May), monsoon (July/August) and winter (December/January) during 2019-2021.

The sites chosen were in three different districts: Thrissur. (10° 35′ 34.87″ N, 76° 11′ 22.6″ E), Palakkad (10° 46′ 12″ N, 76° 22′ 48″ E) and Ernakulam (10° 7′ 58.8″ N, 76° 28′ 58.8″ E) of Kerala, India (Fig. 3.1)

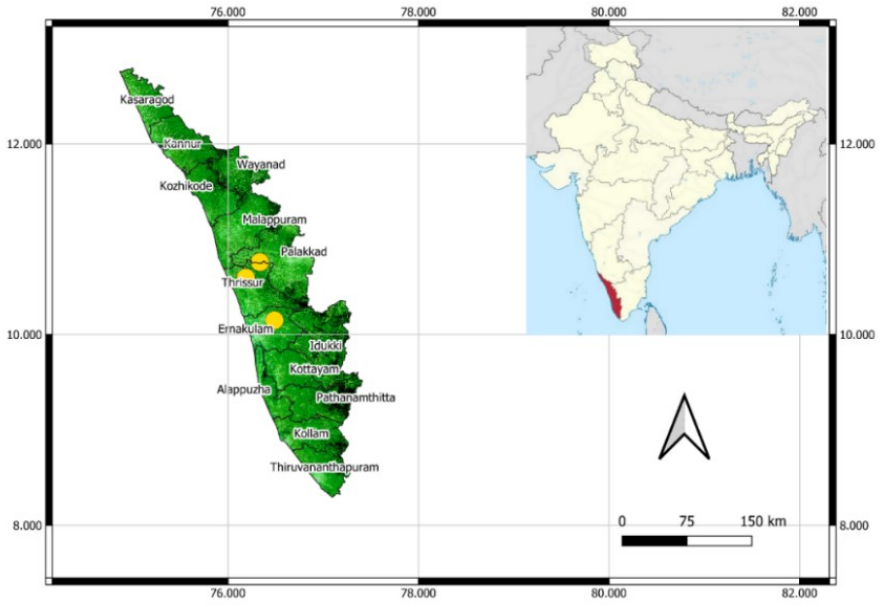



**Fig. 3.1. Blow fly collection sites in Thrissur, Palakkad and Ernakulam districts**

In addition to the collected and identified species of blow flies of forensic significance, data generation was also done based on published works, case study reports, articles, books and catalogues (Nandi, 2004, Bharti, 2011 and 2017).

### 3.1.1. Collection and Identification of blow flies

**Materials:** Aerial/sweep insect nets, collection vials, wide mouth jars with screw caps, eye dropper pippets, pork (collected from meat outlet of Kerala Veterinary and Animal Sciences University), vermiculite/sand, rearing cabinets (size: 2ft ×1ft ×1ft).

The adult blow flies were collected from the sampling sites after trapping with decomposing pork as bait kept in the portable rearing cabinets (size: 2ft ×1ft ×1ft) positioned in the outdoor. Adult flies were collected using fly collecting sweeping net (Fig. 3.2. A-F). Sample specimens were killed by keeping the flies in closed plastic bags having cotton soaked in ethyl acetate and pinned as dry specimens for morphological identification and a few were also preserved in 70% of ethanol for molecular identification.

**Preservation**

**Materials**: Insect pins, Insect net, insect boxes, naphthalene balls, needle, pointed watch makers forceps, medium/fine point dissecting curved forceps, paint brush, plastic container, plastic specimen cups, polythene bags, paper labels, marking pencils, paper towels/tissue paper, disposable surgical/polyethylene gloves, funnel, beaker, measuring jar, hand lens, ethyl acetate, ethanol (100%).

For morphological identification, preservation of the adult blow flies were done after killing the collected flies using the liquid killing agent, ethyl acetate. The killed flies


**Fig. 3.2. Collection methods of blow flies are shown. A. Bait kept in the portable rearing cabinets. B. Blow flies attracted to the decomposing bait. C. Trapped blow flies in the cabinet. D. Arrangement for collection of trapped blow flies. E. Collection of blow flies in the insect net. F. Segregation and sexing of blow flies**

were pinned using stainless steel entomological pin (size no: 2). Pinning was done through thorax between or little behind the base of the forewings and to the right of the midline so that no characters of the body were obscured. Legs were pushed down and away from thorax and wings were turned upwards or sideways from the body. Wings were straightened by using a paint brush dipped in 70% alcohol. The pinned flies were fixed in the standard insect box with Naphthalene balls to prevent the entry of any predatory insects. The killed flies were kept for drying under a table lamp with 60W bulb to remove any moisture. For molecular work, the killed flies were preserved in absolute alcohol (100%).

**Morphological identification**

**Materials:** Dissection Microscope, Compound microscope, Camera-Canon and Hand loop (40X). Greenough stereo microscope, Leica S8 APO Stereo Microscope -LEICA-S8APO with digital camera assembly (Leica MC170 HD) and Stereo microscope.

Identification of adult blow flies were done based on keys and characters described in standard literature (Senior-White et al., 1940; Bharti, 2019). Microscopic examinations were done using LEICA-S8APO stereomicroscope with camera attachment.

**Molecular characterization**

Molecular identification of the species was done using mitochondrial Cytochrome oxidase Subunit I (COI) gene. The DNA sequencing was done at Regional Facility for DNA Fingerprinting (RFDF), Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram, Kerala, India.

Isolation of mitochondrial DNA from the tissues was done using NucleoSpin® Tissue Kit (Macherey-Nagel). Agarose gel electrophoresis was used for checking the quality of the isolated DNA. Gel documentation system (Bio-Rad) was used for capturing the image under UV light after visualizing the gel in a UV transilluminator (Genei).

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol.

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al., 2010). Sequence similarity was searched using NCBI BLAST. BIOEDIT Software version 7.2.5 was used to edit and assemble the sequences. After making sure that the sequence has no CDS error or frame shift problems, the sequence was submitted in GenBank by mentioning the coding region or position in the slot provided. After submission of the sequence in GenBank, NCBI, GenBank accession numbers were received.

**Isolation of genomic DNA**

**Materials:**NucleoSpin® Tissue Kit (Macherey-Nagel), T1 buffer, proteinase K, water bath, 1.5 ml micro centrifuge tube, RNase A, B3 buffer, 100% ethanol, NucleoSpin® Tissue column, 2 ml collection tube, Ultracentrifuge, BW buffer, B5 buffer and BE buffer.

Isolation of genomic DNA was done using the NucleoSpin® Tissue Kit (Macherey-Nagel).The tissues separated out from the blow fly specimen was introduced into a 1.5 ml micro centrifuge tube. 25 µl of proteinase K and 180 µl of T1 buffer were added to the

same and were incubated at 56ºC in water bath. It was made sure that the tissue had undergone complete lysis. This was followed by the addition of 5 µl of RNase A (100 mg/ml) and the mixture was incubated at the room temperature for 5 minutes. After adding 200 µl of B3 buffer, further incubation was performed at 70ºC for 10 minutes. Addition of 210 µl of 100% ethanol was done and the mixture was vortexed. The solution mixture was pippeted into the NucleoSpin® Tissue column which was kept in 2 ml collection tube. It was centrifuged at 11000 x g for 1 minute. After transferring the NucleoSpin® Tissue column into fresh 2 ml tube, 500 µl of BW buffer was used for washing. 600 µl of B5 buffer was used for repeated washing. NucleoSpin® Tissue column was replaced in a fresh 1.5 ml tube. 50 µl of BE buffer was used for eluting the DNA.

**DNA quality check by Agarose Gel Electrophoresis**

**Materials:** Agarose, 0.25% bromophenol blue, 30% sucrose, 6X TE buffer (Tris – EDTA), 0.5X TBE buffer (Tris-Borate-EDTA), Ethidium bromide, bromophenol dye, UV transilluminator (Genei), Gel documentation system ( Bio-Rad) and Electrophoretic Unit.

Agarose gel electrophoresis was used for quality check of the isolated DNA. 5µl of DNA was added with 1µl of 6X loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0). 0.8% Agarose gel was made in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. The DNA samples were loaded to the gel. 0.5X TBE as electrophoresis buffer was employed as the tank buffer. Electrophoresis was done at 75 V till the movement of the bromophenol dye to the anodic end of the gel. Visualisation of the DNA bands was done using UV transilluminator (Genei). UV light was used for imaging using Gel documentation system (Bio-Rad).

**COI gene PCR amplification using universal primers of CO1**

**Materials:** Primers; LCO (Forward) (GGTCAACAAATCATAAAGATATTGG) & HCO (Reverse) TAAACTTCAGGGTGACCAAAAAATC, PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems), 2X Phire Master Mix, distilled water, isolated DNA.

For the PCR analysis, 5µL of 2X Phire Master Mix, 4µL of distilled water, 0.25µL of forward primer, 0.25µL of reverse primer and 1 µL of isolated DNA were used.

The COI target primers used were;

LCO (Forward) (GGTCAACAAATCATAAAGATATTGG) &

HCO (Reverse) TAAACTTCAGGGTGACCAAAAAATC;

both in 5' → 3' sequence direction. PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) was used for the PCR amplification.

PCR amplification profile is as given below:

**COX1**

| | | | |
|---|---|---|---|
| 98° C | - | 30 sec | |
| 98° C | - | 5 sec | } |
| 45° C | - | 10 sec | } 10 cycles |
| 72° C | - | 15 sec | |
| 98° C | - | 5 sec | } |
| 50° C | - | 10 sec | } 30 cycles |
| 72° C | - | 15 sec | |
| 72° C | - | 60 sec | |
| 4° C | - | ∞ | |

**Quality check of the PCR products by Agarose Gel Electrophoresis:**

**Materials:** Agarose, 0.25% bromophenol blue, 30% sucrose, 6X TE buffer, 0.5X TBE buffer, ethidium bromide, bromophenol dye, 2-log DNA ladder (NEB) as the molecular standard, UV transilluminator (Genei), Gel documentation system (Bio-Rad) and Electrophoretic Unit.

1.2% agarose gels were prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 4 µl of PCR product was mixed with 1 µl of 6X bromophenol blue and was loaded in the gel. Using 0.5X TBE buffer, electrophoresis was performed for 1-2 hours at 75V till the bromophenol front reached till the end of the gel bottom. 2-log DNA ladder (NEB) was used as the molecular standard. UV transilluminator (Genei) was used for the visualisation. Gel documentation system (Bio-Rad) was used for capturing the image under UV light.

**ExoSAP-IT Treatment**

**Materials:** Exonuclease I and Shrimp Alkaline Phosphatase (SAP) (ExoSAP-IT -GE Healthcare)

0.5µl of ExoSAP-IT consisting of the hydrolytic enzymes; exonuclease I and Shrimp Alkaline Phosphatase (SAP) was mixed with 5µl of PCR products and incubated at 37°C for 15 minutes followed by enzyme inactivation at 85ºC for 5 minutes for the removal of unwanted primers and dNTPs from PCR product mixture to avoid interference in downstream applications.

**Sequencing**

**Materials:** Sequencing PCR mix: distilled water, 5X sequencing buffer, forward Primer, reverse primer, sequencing mix and Exosap treated PCR product.

PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems)**,** BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA), ABI 3500 DNA Analyzer (Applied Biosystems), Sequence Scanner Software v1 (Applied Biosystems), Geneious Pro v5.1 (Drummond et al., 2010).

PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) was used for the sequencing.

For the PCR analysis, 6.6 µl of distilled water, 1.9µL of 5X sequencing buffer, 0.3µl of forward primer, 0.3µl of reverse primer, 0.2 µl of sequencing mix and 1 µl of Exosap treated PCR product were used.

**PCR amplification profile for the sequencing is as given below:**

| | | | |
|---|---|---|---|
| 96° C | - | 2min | |
| 96° C | - | 30sec | } 30 cycles |
| 50° C | - | 40sec | |
| 60° C | - | 4min | |
| 4° C | - | ∞ | |

**Clean up of post sequencing PCR**

**Materials:** Distilled water, 3M Sodium acetate, EDTA and 100% ethanol, 70% ethanol, Sequencing plate, Vortex (Mixmate) and Ultracentrifuge.

5 µl of distilled water, 1 µl of 3M sodium acetate, 0.1 µl of EDTA and 44 µl of 100% ethanol were properly mixed. Each well of the sequencing plate having the PCR product was added to 50 µl of the prepared mix. The sequencing plate was vortexed by Mixmate vortex for 30 minutes at room temperature. The mixture was centrifuged at 3700 rpm for 30 minutes. The supernatant was decanted. 50 µl of 70% ethanol was added to each well for washing. The mix was again centrifuged at 3700 rpm for 20 minutes and the previous step was repeated with 70% ethanol. The supernatant was decanted and the pellet was air dried.

The air dried cleaned up product was sequenced in ABI 3500 DNA Analyser (Applied Biosystems).

**Analysis of the sequence**

Sequence Scanner Software v1 (Applied Biosystems) was used for checking the quality of the sequence. Alignment of sequence and basic editing of the obtained sequences were done using Geneious Pro v5.1 (Drummond et al., 2010).

**Sequence similarity check using NCBI BLAST and submission of sequence in GenBank, NCBI**

Sequence similarity was searched using NCBI BLAST. To begin with, BIOEDIT software was used to open both the forward and reverse fasta file through open sequence set. Nucleotide positions at the beginning and at the end that showed irregular blue bars were noted. AB1 file was used for BIOEDIT for both forward and reverse sequences. The narrow peak formations with clear peaks were checked. Non-performing peaks were noted which were broad. After noting down the nucleotide positions, extract option for trimming was used to give the nucleotide positions in range. This was done for both forward and reverse extracted sequences.

'VecScreen' of NCBI (https://www.ncbi.nlm.nih.gov/tools/vecscreen/) was used for checking the presence of any vector nucleotides used in the PCR amplification of the COI gene. To check for any similarity of sequence found, the sequence was taken to bio-edit again and those portions were trimmed. Reverse compliment was prepared using the software in the bioinformatics site (https://www.bioinformatics.org/sms/rev_comp.html).

Single Contig file sequence (positive strain 5' to 3') was made using CAP3 Sequence Assembly Programme (http://doua.prabi.fr/software/cap3). ORF (Open Reading Frame/coding region) finder of NCBI (https://www.ncbi.nlm.nih.gov/orffinder/ ) was used to find the largest ORF.

Blast of the sequence with query range from start and end codon was performed using Blastn of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi? PAGE_TYPE= Blast Search). The alignment and frame shift (presence of red letter at the beginning and end of the sequence) was noted. Bioedit was used to remove the nucleotides till the first red letter

and nucleotide sequences from the last red letters. Blast was conducted again on the obtained sequence.

After making sure that the sequence was with no CDS error or frame shift problems, the sequence were submitted in GenBank by mentioning the coding region or position in the slot provided. After submission of the sequence in GenBank, NCBI, GenBank accession numbers were received.

**Molecular phylogeny**

**Evolutionary molecular phylogenetic analysis of sequences by Maximum Likelihood method**

The evolutionary history analysis was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree was shown. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and were in the units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

### 3.2. Seasonal abundance in blow fly population in Central Kerala

#### 3.2.1. Monitoring of climatic conditions

Humidity and temperature were monitored in different seasons; summer, monsoon and winter in the months of April-May (2019-2021), June-September (2019-2021), December (2019-2021) and January (2020-2022). Temperature and humidity were monitored using hygrometer (HTC-1, Model No: AP-IS11A056FBA).

### 3.2.2. Study on seasonal abundance of blow flies

Studies on the influence of seasonal differences on the population abundance were conducted in three seasons of all three years; summer (April-May (2019-2021), monsoon (June-September (2019-2021) and winter in the months of December (2019-2021) and January (2020-2022). As a part of this, preliminary observations on blow flies were conducted from dawn to dusk to find out the peak activity hours. The study revealed two peak periods of activities; one in the morning, starting from 10:30 a. m. to 12:30 p. m. and another in the evening starting from 4:00 p. m. to 5:30 p. m. during the third day of keeping the bait in the cabinet.

### 3.2.3. Study sites

Blow fly sampling was done from different sites in Central Kerala during different seasons; summer (April/May), monsoon (July/August) and winter (December/January) during the years from 2019-2021. The sites chosen were in three different districts; Thrissur (Kolangattukara; 10°34'29.4"N 76°11'01.8"E, Choolissery; 10°35'45.0"N 76°11'18.3"E, and Thangaloor; 10°37'35.5"N 76°11'15.3"E), Palakkad (Vaniamkulam-II; 10°45'32.1"N 76°20'04.8"E, Palappuram; 10°45'48.3"N 76°24'54.1"E, Varode; 10°48'50.0"N 76°22'47.2"E) and Ernakulam (Aimuri; 10°08'52.8"N 76°29'18.5"E, Kanjirakkad; 10°07'37.8"N 76°28'03.0"E, Kuruppampady; 10°07'01.8"N 76°30'12.6"E) of Kerala, India. (Fig. 3.3, 3.4, 3.5).

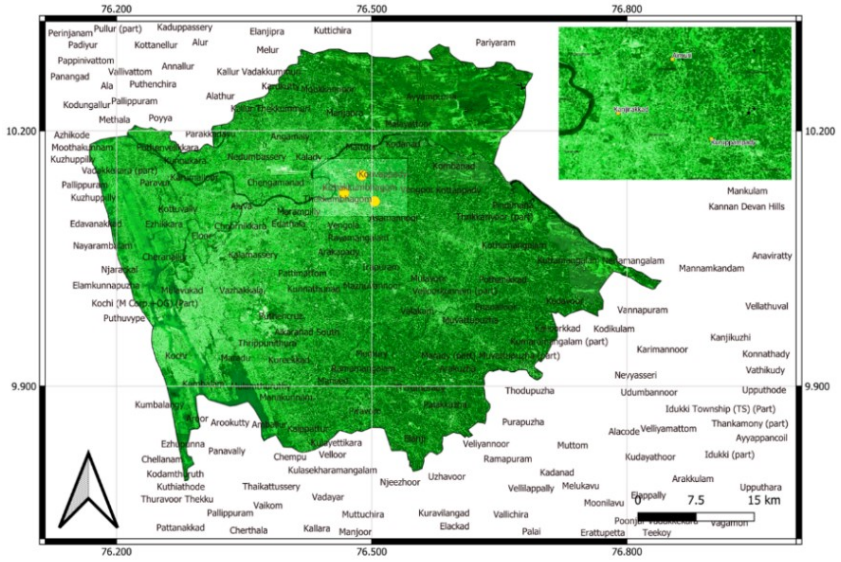



**Fig. 3.3. Blow fly collection sites in Ernakulam (Aimuri, Kanjirakkad, and Kuruppampady)**

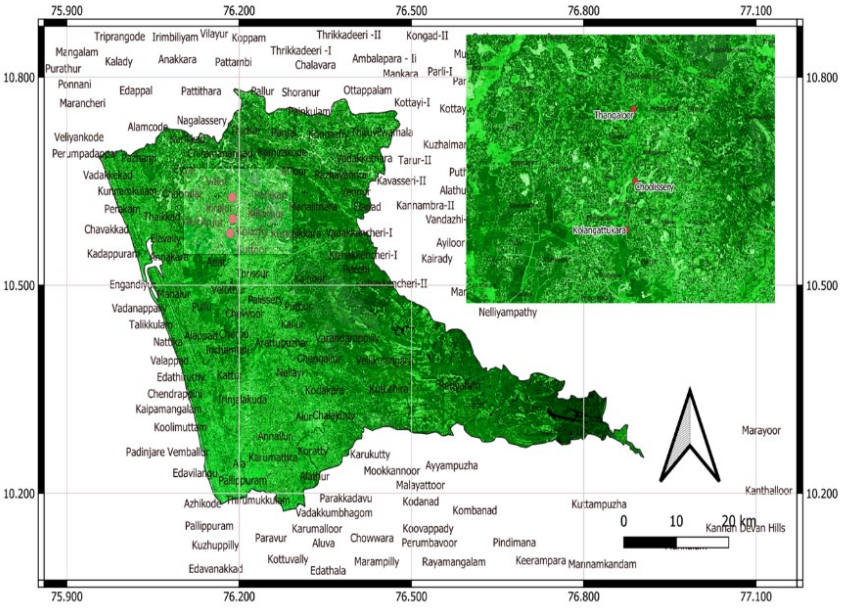



**Fig. 3.4. Blow fly collection sites in Thrissur (Kolangattukara, Choolissery, and Thangaloor)**

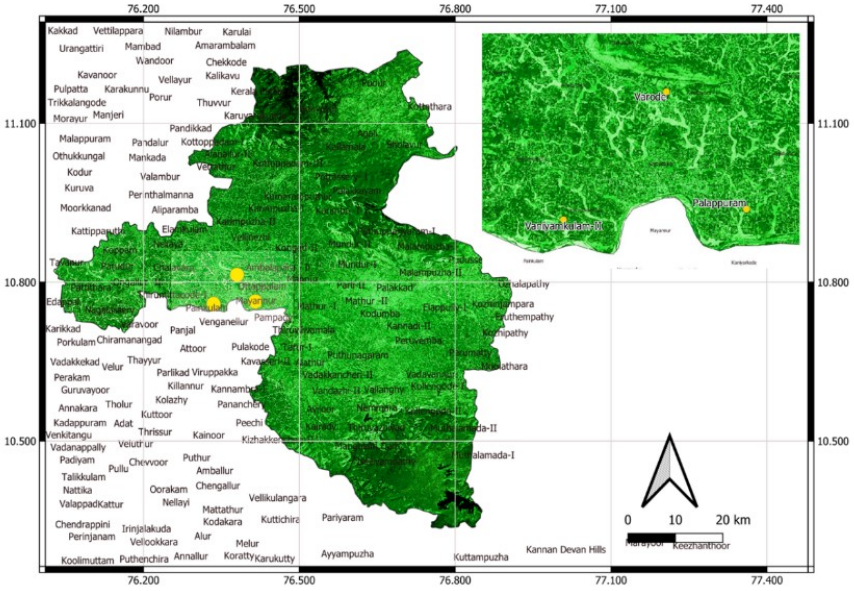



**Fig. 3.5. Blow fly collection sites in Palakkad (Vaniamkulam-II, Palappuram and Varode)**

Temperature and humidity was monitored using hygrometer (HTC-1, Model No: AP-IS11A056FBA). The insects were trapped in the rearing cabinets (Size: 2ft ×1ft ×1ft) having decomposing pork (*Sus scrofa*) as bait positioned in the outdoor facility (Fig. 3.7. A - B). The collection was done in triplicate in different cabinets. Adult blow fly abundance was monitored by keeping fly net covered over the cabinet. The flies entered into the net during the peak hours as mentioned before were trapped with insect net. Species determination and sexing were done for all the flies trapped in the study. The total number of adult flies were counted and were later segregated into species and then to male and female flies by studying their morphology under Stereo microscope. Studies were done in all seasons to find out the changes in seasonal abundance in the blow fly population.

### 3.3. Life history of blow flies in carrion

#### 3.3.1. Outdoor rearing of blow flies

The rearing of adult flies were done in the outdoor rearing facility in Thrissur (Kolangattukara; 10°34'29.4"N 76°11'01.8"E and Thangaloor; 10°37'35.5"N 76°11'15.3"E)

A

B

C

D


E

F

G

H

**Fig. 3.6. Outdoor rearing facility: A-D. Rearing cabinets with bait and vermiculite E. Hygrometer hung in the facility, F. Round rearing plastic containers with pork and vermiculite, G. 10%Sugar Solution, 1.5%(v/v) multivitamin syrup solution in plastic containers with wicks as diet for blow fly, H. Glass made rearing cabinet with rearing containers, diet and vermiculite**

A

B

C

D

E

**Fig. 3.7. Rearing methods I: A-B. Blow fly attracted to carrion C-D. Identified female blow fly of F1 generation released into the fresh bait, E.1st instars in the decomposing bait**

A

B

C

D

E

**Fig. 3.8. Rearing methods II: A. 2nd Instars on the bait, B. 3rd Instars on the bait, C. Migration of the larva for pupation D. Slow moving adult blow flies just emerged from pupae E. Fast moving emerged adult flies**

during summer (April-May, 2019-2021), monsoon (June-September, 2019-2021) and winter (December, 2019-2021 and January, 2020-2022) seasons. Temperature and humidity were monitored using hygrometer.

**Materials:** Milk powder, 10% Sugar Solution, and 1.5 % (v/v) multivitamin syrup solution. Bait: Pork meat (collected from the meat outlet of Kerala Veterinary and Animal Sciences University), Vermiculite/Sand, Rearing Cabinets (Glass) of size: 2ft ×1ft ×1ft with steel mesh framed with Aluminum, Hygrometer (HTC-1, Model No: AP-IS11A056FBA) and Exhaust fan.

Pure culture of the insects was accomplished after rearing for two generations followed by identification and the next generation was considered F1 generation in the study. Rearing was performed in the rearing cabinets in the outdoor facility having decomposing minced pork (*Sus scrofa*) (Byrd and Tomberlin, 2019, Wang et.al, 2017, Bernhardt .et.al 2017, Whitaker, 2014, Catts and Goff, 1992) as bait (Fig.3.6.A-H). To facilitate oviposition, round plastic containers (500 ml) with 10cm diameter opening were used for keeping 100 gm. of pork over a layer of vermiculite kept in polythene pouch. The F1 generation male and female flies were released into the containers (Fig.3.7.C-D). The containers were covered with muslin cloth tightened with rubber bands. The adult insects were provided with milk powder, 10 % (w/v) sugar solution, 1.5 % (v/v) multivitamin syrup solution and water *ad libitum* using cotton wicks kept through the perforated lid of plastic containers. The decomposing pork was served as a reflex stimulus for the adult female fly to lay eggs and also served as a food source for the larvae (Fig. 3.8).

### 3.3.2. Preservation

**Materials:** Low tension larval forceps, needle, pointed watch makers forceps, medium/fine point dissecting curved forceps, paint brush, plastic container, plastic vials, plastic specimen cups, paper labels, marking pencils, paper towels/tissue paper, disposable surgical/polyethylene gloves, funnel, beaker, measuring jar, hand lens, 95% ethanol, ethanol (100%), formaldehyde, glacial acetic acid, and Kahle's Solution (30 ml of 95% ethanol: 12 ml of formaldehyde: 4ml of glacial acetic acid: 60 ml of distilled water)

Eggs laid on the bait were collected using a fine brush and transferred to watch glass having 70% ethanol. It was kept for 10 minutes to promote the dissolution of egg mass. Different larval instars were collected and boiled for two to three minutes at 80°C to prevent the shrinkage of larvae and to kill the microbes present in the gut of larvae and thus preventing darkening of the specimens in alcohol (Tantawi and Greenberg 1993, Adams and Hall, 2003). The specimens were preserved in 70% ethanol to facilitate the measurement of length and weight. For long term preservation, the boiled larval specimen were stored in Kahle's Solution.

### 3.3.3. Morphological identification of eggs and different stages of larval instars

**Materials:** Microscopic slides, cover slips, sharp blade, test tubes, spirit lamp, DPX mountant, compound microscope, Westwox Optik-Model: JXL No: 77126, with camera attachment (Keowa CE-500X), 10% (w/v) of KOH, alcohol grades: 50%, 70%, 80%, 95% and absolute ethanol (99%), 1% glacial acetic acid, Xylene and distilled water.

Morphological examination of 1$^{st}$, 2nd and 3$^{rd}$ Instars to study cephalopharyngeal skeleton and posterior spiracle was done using potassium hydroxide enabled clearing. The alcohol preserved larvae were cut at the middle of second thoracic segment and at 11$^{th}$

segment using a sharp blade under LEICA-S8APO stereomicroscope to study the cephalopharyngeal skeleton and posterior spiracle by digesting the anterior and posterior portions as per the methods stated by Sukontason et al., (2004). The cut anterior and posterior portions were treated with 10% (w/v) of KOH solution in a watch glass for 48 hours and were cleaned 4 times using distilled water. This was followed by treating the specimen in a mixture of 1% glacial acetic acid and 35% ethyl alcohol in a watch glass for 30 minutes. Serial dehydration of the specimens was done using alcohol gradient (50%, 70%, 80% and 95%) and absolute ethanol by placing the specimen in each for 30 minutes. The specimens were treated in xylene for 60 seconds and mounted on a microscopic slide with a coverslip using DPX mountant. The cephalopharyngeal skeleton, anterior and posterior spiracles were observed and photographed using compound microscope and Westwox Optik-Model: JXL No: 77126, with camera attachment (Keowa-CE-500X).

### 3.3.4. Ultrastructure study of larvae using Scanning Electron Microscopy

**Materials:** Distilled water, alcohol grades; ethanol-70 & 99.5%, carbon double tapes, aluminium stubs, ultra sonicator, sputter unit (JFC 1600, Japan) and Scanning Electron Microscope (JEOL Model JSM-6390 LV, JEOL Ltd. Japan).

The first, second and third instar larvae were collected and washed many times in distilled water. To kill the larvae and to prevent deformation changes, the larvae were kept in boiling water (80ºC) for two to three minutes and finally preserved in 70% alcohol (Tantawi and Greenberg 1993, Adams and Hall, 2003). Sample preparation for SEM included dehydration using 99.5% alcohol, followed by ultra sonification and air drying. After being dried at room temperature, larval specimens were gently placed onto stubs fixed with double tape. The specimen was coated with gold using gold sputtering for 10

seconds with 10mA current in the sputter unit (JFC 1600, Japan). Images were taken under JEOL Model JSM-6390 LV, SEM, JEOL Ltd. Japan, in Sophisticated Analytical Instrumentation Facility (SAIF), Cochin University of Science and Technology, Kochi, Kerala. Larval terminology follows Courtney et al. (2000) with a few additional terminologies prepared by Szpila and Villet (2011).

### 3.3.5. Life cycle

**Developmental time of eggs, larvae and pupae**

Once the eggs were found, the bait with the eggs was transferred into the new larval rearing round plastic containers (500ml). After eclosion occurred, in total, 1050 larvae were considered per season in which three equal replicates of 350 larvae were transferred separately on 400g of fresh pork kept in the silver foil pack. Each of the pack was kept in transparent round new plastic containers (500 ml) with 10cm diameter opening. The container was kept in the rearing cabinets with steel mesh framed with Aluminum. 200 grams of fresh pork was put into the container during different stages frequently as larval feed. This was continued until the instars reached the non-feeding stage and started pupal migration. Fresh pupae were collected from the vermiculite in the bottom of the rearing cabinet and were transferred to a new rearing round plastic container with moist vermiculite at the bottom and it was kept inside the rearing cabinet for the emergence of the adult fly (Fig. 3.8. D-E). Different larval instars were collected for studying their morphology and length/weight parameters and the developmental time.

**Study on preoviposition time, fecundity and incubation time**

Fecundity studies were done in all seasons of three consecutive years (2019-2021). In each trial, preoviposition period in days, maximum number of eggs laid by the adult fly

in a day, periodicity for egg laying in days, number of eggs laid by the fly in its life span and incubation period of eggs in hours were observed. Three such trials were conducted for each species for every season of the year.

For obtaining the eggs for the fecundity studies, 50 gm. of fresh pork kept in aluminum foil was placed into round plastic containers (500 ml) with 10cm diameter opening. Female fly of the respective species was released into the container. The flies were provided with milk powder, 10 % (w/v) sugar solution, 1.5 % (v/v) multivitamin syrup solution and water *ad libitum* using cotton wicks kept through the perforated lid of small plastic containers. The containers were covered with muslin cloth tightened with rubber bands. The containers were kept in the rearing cabinet and observed for the next 24 hour period every 30 minutes. The time of laying of eggs were monitored through close observation. Thus, preoviposition period was determined for every replicate per species per season in all three consecutive years.

Egg masses laid by the blow flies on the bait were collected within 24 hours, the meat was removed and examined for eggs laid for fecundity determination. Eggs laid on the bait were collected using a fine brush and transferred to a cavity block having 70% ethanol. It was kept for 10 minutes to promote the dissolution of egg mass. Then ethanol was drained off for egg counting. Dissolution was performed on egg masses by transferring the egg mass to a black fine mesh cloth for a better background contrast. A fine small tissue paper moistened with distilled water was kept over the black cloth having egg mass and was kept for 5 minutes. The eggs were physically separated by gentle manipulation with a 10 mm synthetic paint brush. The eggs were counted using Labomed-CSM2 Stereo zoom microscope.

**Study on survival of different stages of blow fly species**

Survival studies in triplicate were undertaken in all seasons of three consecutive years. In each trial, survival rate in percentage was calculated for each life stage; egg, 1st Instar, 2nd Instar, 3rd Instar, post feeding stage and pupa till the emergence of adult flies.

For conducting this experiment, three groups of 110-130 eggs were considered in each trial, per season for all three years. The eggs were transferred from the outdoor rearing cabinets and placed in the new larval rearing round plastic containers (500ml). After eclosion occurred, live larvae were counted at each stage. The larvae were then transferred to 200 gm. of fresh pork kept in the silver foil pack. Each of the pack was kept in transparent round new plastic containers (500 ml) with 10cm diameter opening. The container was kept in the glass made rearing cabinets. 200 grams of fresh pork was put into the container as larval feed. This process was continued until the instars reached the non-feeding stage and started pupal migration. Fresh pupae were collected from the vermiculite in the bottom of the rearing cabinet and were transferred to a new rearing round plastic container with moist vermiculite at the bottom and were kept inside the rearing cabinet for the emergence of the adult fly. During rearing, the number of live 1st instar, 2nd Instar, 3rd Instar and pupae which have undergone eclosion were recorded.

### 3.4. Effect of temperature and humidity on the life cycle of calliphorid flies

#### 3.4.1. Assessment of development rate

**Materials:** 70% Ethanol, hand loop (40X), dissection microscope, Greenough stereo microscope ( Leica S8 APO Stereo Microscope -LEICA-S8APO) with digital camera assembly (Leica MC170 HD), Stereo microscope, Vernier caliper, hot air oven, electronic Balance- Schimadzu - ELB 300.

Seasonal study on the fecundity measures during summer, monsoon and winter seasons during the life cycle of the fly was conducted from the time of mating till ovipositioning. Different parameters like pre-oviposition period, maximum number of eggs laid by adult fly in a day**,** egg laying time interval between successive batches (days) and the total number of eggs laid by the fly in its life span were considered for data analysis. In the rearing cabinet, the observations were made during the period between the mating and the time of oviposition in triplicate trials.

Preoviposition time was noted in each trial**.** Incubation time in all replicates were observed. The phase from the time of oviposition till the emergence of adult fly from pupae was considered for the study of developmental rate. After eclosion occurred, in total 1050 larvae were considered per season in which three equal replicates of 350 larvae were transferred separately on 400g of fresh pork kept in the silver foil pack. Each of the pack was kept in transparent round new plastic containers (500 ml) with 10cm diameter opening. 200 grams of fresh pork were put into the container during different stages frequently as larval feed.

The time taken by the eggs for hatching was noted. The freshly hatched larvae were transferred to the new larval rearing chamber and 100 grams of fresh pork was provided as food. Six larvae were collected every six hours and also at different stages of ecdysis for the length meassurements.They were boiled for two to three minutes at 80°C to prevent the shrinkage of larvae, to coagulate the protein and to kill the microbes present in the gut of larvae; and preserved in 70 % alcohol. Larval measurements were made as per the protocol suggested by Adams and Hall (2003) and Donovan et al., (2006).

Seasonal study encompassing the life cycle of the fly with special emphasis on the length and weight of larval instars during summer, monsoon and winter was conducted from the time of egg hatching till the pupation. In the rearing cabinet, the observations were made during the period between the egg hatching and the commencement of pupation on the decomposing meat in triplicate trials.

Time dependent data was recorded in accordance with the length and weight of the instars of blow flies during their developmental stages with special inference on the summer, winter, and monsoon seasons. Length of all instar stages were done using stereomicroscopy with scale *in situ* for the first instar and vernier caliper and dissection microscopy especially for the $2^{nd}$ and $3^{rd}$ instars. This was followed by the weight measurement of larvae. The time spent by the species in each life stage was recorded. Larvae of the same batch and phase were boiled for two minutes at 80°C and preserved in 70 % alcohol (Tantawi and Greenberg 1993, Adams and Hall, 2003). The larvae were dried in hot air oven at 50°C for 36 hours. The dried larvae were weighed on an electronic weighing balance. Based on these observations, growth curves were plotted. The development rate assessment was done according to the protocol of Greenberg and Wells (1998). The effect of temperature and relative humidity on preoviposition period, incubation time of eggs and larval period were noted. Larval development especially the length and weight parameters were also studied in detail.

Seasonal study on the survival rate measures during summer, monsoon and winter during the life cycle of the fly was conducted from the time of ovipositioning till the emergence of adult fly. Comparison of survival rate of different stages between seasons and also between years were considered for data analysis.

### 3.4.2. Laboratory rearing of blow flies under controlled conditions and validation

**Materials:** 10% sugar solution, 1.5% (v/v) multivitamin syrup solution, pork meat, Vermiculite / Sand, Incubator, 70% ethanol, hand loop (40X), dissection microscope, Greenough stereo microscope (Leica S8 APO Stereo Microscope -LEICA-S8APO) with digital camera assembly (Leica MC170 HD), stereo microscope, Vernier caliper, hot air oven, electronic balance- Schimadzu - ELB 300.

Laboratory rearing was done to validate the outdoor rearing studies conducted on blow flies. The average temperature (T) and relative humidity (RH) recorded in the three seasons were considered for conducting laboratory rearing. The average values of temperature and relative humidity used for laboratory studies were the following. For summer: T-32.28°C, RH-68.23%; for monsoon: T-26.17°C, RH-97.44% and for winter: T-25.19°C, RH-46.32% (Group I -III). A photo period of 12:12 (L: D) h was maintained in the incubator.

Trials were conducted in triplicate per species for a particular constant temperature and relative humidity in incubator (Fig. 3.9. A-D). A total of 375 eggs were considered per species with 125 each in three replicates. 50 gm. of fresh pork kept in aluminum foil were placed into round plastic transparent containers (500 ml) with 10cm diameter opening. Adult male and female fly of the respective species was released into the container. The flies were provided with milk powder, 10 % (w/v) sugar solution, 1.5 % (v/v) multivitamin syrup solution and water *ad libitum* using cotton wicks kept through the perforated lid of plastic containers. The containers were covered with muslin cloth tightened with rubber bands. The decomposing pork was used which served as a reflex stimulus for the adult female fly to lay eggs and also served as a food source for the larvae.

Once the eggs were found, the bait with the eggs was transferred into the larval rearing rectangular plastic containers (Size: 166×124×60mm). The containers were covered with a wet muslin cloth. Wet vermiculite was kept as the bottom layer in the cabinet to assist migration of third instars for pupation and to maintain humidity. 100 grams of fresh pork was put into the container as larval feed. This was continued until the instars reached the non-feeding stage and started pupal migration. Six individuals of different larval instars and pupae were randomly collected every six hours from all the three replicate per species. Fresh pupae were collected and transferred to a new rearing jar with moist vermiculite at the bottom and the jar was kept inside the rearing cabinet for the emergence of the adult fly. Six larvae were collected every six hours and boiled for two minutes at 80°C and preserved in 70 % alcohol for the assessment of length (Tantawi and Greenberg 1993, Adams and Hall, 2003). The time spent by the species in each life stage was recorded. Dry weight was also recorded using precision analytical balance. Based on these observations, growth curves were plotted.

The validation of the outdoor rearing results was done by comparing the data of laboratory rearing under controlled conditions and outdoor rearing data to check the presence of any differences of significance pertaining to length, weight and life cycle duration data for all species.

### 3.5. Relation between life cycle and time since death assessment for forensic application

#### 3.5.1. Estimation of Post Mortem Interval (PMI)

Time dependent growth functions / equations are used to represent the biological growth of the larvae and this describes the variations experienced in length in relation to age. The growth in terms of length increases upto the post feeding stage and thereafter, a

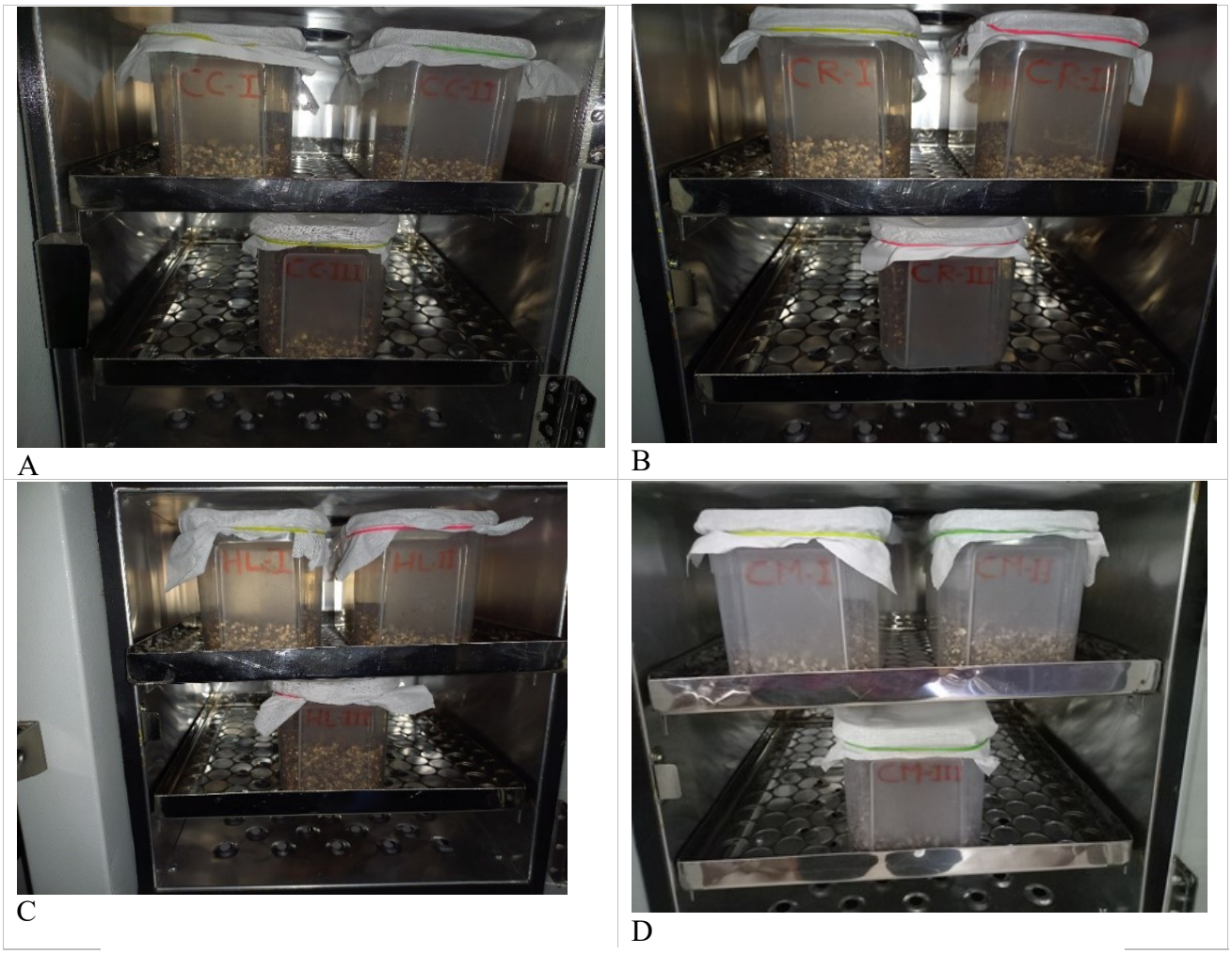



**Fig. 3.9. Laboratory rearing showing brood chamber with culture replicates of blow flies**

slight decrease was observed. A sigmoid curve was used to represent the evolution of size over time. The pattern of the growth curve (sigmoid) obtained was similar irrespective of the species or seasonal climatic conditions. In this study, the growth models best fitting to the data were used (Kiviste et al., 2002).

For the estimation of PMI, growth biology was represented by a graph plotted between time and length. PMI was estimated in the current study through the development rate with respect to the length of larval instars (Wells and LaMotte, 2017). The construction of growth curves were done based on the age-specific length parameters of larvae versus time taken for the development of each stage of the blow fly species. Regression method was used for constructing a model for the estimation of PMI.

### 3.6. Statistical analysis of data

Entire statistical analysis was performed using the software SPSS version 21.0. The analysis of variation in length, weight and percentage of survival between years, between seasons, between larval stages and all its interactions were tested by using three-way ANOVA with one factor as year, the second factor as season and the third factor as stage. If the interaction between these factors was found to be significant, pairwise comparison was done between the levels of one factor with different levels of other factor using Least Significant Difference (LSD) test by using least square means.

In the case of fecundity parameters and seasonal abundance, two-way ANVOA with one factor as year and the second factor as season was done to compare between years, between seasons and also to estimate the interaction between years and seasons. Pair wise comparison was done using LSD test by using least square means. The laboratory

rearing data of length and weight of larval instars were subjected to two factor ANOVA to compare between stage and temperature / humidity and also to test their interaction.

The duration of different life cycle stages compared between seasons and years was done using two-way ANOVA. The pairwise comparison between seasons and also between years was done by using LSD test.

For the estimation of PMI, regression equations were constructed as models. (Das and Giri 1979, Draper and Smith, 1988). The regression equations were fitted with length as the dependent variable and duration as the independent variable. Among the different models tested using curve estimation procedure in SPSS, S(sigmoid) function of the form, $E(Yt)=\exp(\beta 0+\beta 1/t)$ was used for predicting length in terms of duration, where $E$ represents expectation, $Y_t$ is the length (mm) at duration '$t$'(hrs.) and $\beta_0$ and $\beta_1$ are parameters of the regression model to be estimated. The above model was fitted by logarithmic linearization. Then the model became

$\ln(Y_t) = \beta_0 + \beta_1/t$ where, ln represents natural logarithm.

The best model was selected with the help of coefficient of determination ($R^2$) and root mean square error. The model having higher $R^2$ and lesser root mean square error was selected as the best model. To obtain an estimate of development time (Post mortem interval) from these models, reverse prediction procedure was used where the data for the response variable, length, was entered in the equation to obtain duration in hours (t).

# CHAPTER – 4

# RESULTS

# CHAPTER – 4

# RESULTS

## 4.1. Blow fly fauna

The family Calliphoridae is encompassed of a group of flies having veterinary, ecological, medical and forensic significance with worldwide distribution. Currently, 1500 species of blow flies were reported from all over the world. In India, the family is represented by 9 subfamilies, 30 genera and 119 species. The subfamilies included are; Melanomyinae, Calliphorinae, Bengaliinae, Luciliinae, Rhiniinae, Helicoboscinae, Chrysomyinae, Ameniinae and Polleniinae. The blow flies in Kerala are represented by 4 subfamilies 8 genera and 17 species (Subramanian & Mohan, 1980, Nandi, 2004, Bharti, 2011, Radhakrishnan et al., 2012, Bharti and Singh 2017, Reject Paul and Binoy, 2021 and 2022). The description and distribution of the seventeen species found in Kerala are discussed below;

**Subfamily: Bengaliinae**

**Genus: *Bengalia* Robineau- Desvoidy, 1830**

***Bengalia jejuna* Fabricius, 1794**

Distribution: Kochi, Walayar, Thiruvananthapuram

Remarks/Source: (Bharti, 2011)

Distinguishing features: Absence of concavity on the posterior margin of eye, upper part of anepimeron with 9-11 black setulae, broad cercus narrowing down to pointed tip, 3$^{rd}$ and 4$^{th}$ tergite with marginal bands, bacilliform sclerite with an oblique distal margin, distiphallus with a constriction at the middle of dorsal wall, broad distal lip process with broad wing like membranes.

### *Bengalia surcoufi* Senior-White, 1924

Distribution: Kochi

Remarks/Source: (Nandi, 2004)

Distinguishing features: Brownish grey parafrontalia, first and second antennal segments reddish brown, pale palpi with black bristles, all segments of abdomen black banded, pale yellowish squamae, tarsi tips darkened.

## Subfamily: Luciliinae

### Genus: *Hemipyrellia,* Townsend, 1917

### *Hemipyrellia ligurriens* Wiedemann, *1830*

Distribution: Foot of Nilgiri hills, Thrissur, Palakkad and Ernalulam, Kerala

Remarks/Source: (Nandi, 2004), collected from Thrissur, Palakkad and Ernakulam in Kerala and identified in the current study (Reject Paul and Binoy, 2021)

Distinguishing features: Genae and parafrontalia silver white, antennae tawny yellow to brownish, orange palpi, upper squama with creamish white short cilia and lower squama with light brown cilia, short hairs on the edges of tergites and first visible sternite, bare stem vein, 1st longitudinal vein without any setulae, 3rd longitudinal vein with short setulae on dorsal and ventral aspects.

### Genus: *Lucilia* Robineau-Desvoidy, 1830

### *Lucilia ampullacea* Villeneuve, 1922

Distribution: Malabar Coast (Kerala)

Remarks/Source: (Nandi, 2004)

Distinguishing features: Third to fifth tergites without marginal band, basicostal scale brownish black, subcostal sclerite with upstanding hairs, post sutural acrosctichal 2, alar squama white with tuft of hair on the lower margin, and lower squama infuscated, tibiae black.

### *Lucilia papuensis* Macquart, 1843

Distribution: Malabar Coast (Kerala)

Remarks/Source: (Nandi, 2004)

Distinguishing features: Frons broader than inter post ocelli distance, parafacialia broader than the third antennal segment, occiput with more than two irregular rows of black post ocular setae, posterior surface of post gena with black hairs, anterior pair of post sutural acrostichals present posterior to the second pair of post sutural dorsocentrals, alar and thoracic squama infuscated with a tuft of blackish brown hairs at the lower margin.

### *Lucilia sericata* Meigen, 1826

Distribution: Calicut (Kerala), throughout India

Remarks/Source: (Priya and Sebastian, 2015, Nandi, 2004)

Distinguishing features: Parafrontalia with short decumbent bristles, cerebrale with 8-9 occipital bristles on either side, metallic green abdomen, non arched abdomen, abdomen metallic golden green with sparse pruniosity, absence of tuft of long hairs on sternites, hypopygium inconspicuous.

## Subfamily: Chrysomyinae

### Genus: *Chrysomya* Robineau-Desvoidy, 1830

### *Chrysomya megacephala* Fabricius, 1794

Distribution: Throughout India, Thrissur, Palakkad and Ernakulam in Kerala

Remarks/Source: Calicut (Bharti and Singh, 2017), collected from Thrissur, Palakkad and Ernakulam in Kerala and identified in the current study (Reject Paul and Binoy, 2022)

Distinguishing features: parafrontalia slightly narrower than the breadth of frons, covered with golden tomentum. Antennae, arista and palpi orange, Parafacialia and genae completely orange, Anterior spiracles dark brown, Subcostal sclerite covered with brown

felted pubescence and small erect hairs. A row of setulae on the upper posterior side on the stem vein. Upper calypter was with ventral hairs on the opaque white basal part.

### *Chrysomya chani* Kurahashi, 1979

Distribution: Western Ghats, Thottilpalam, Calicut, Thrissur, Palakkad and

Ernakulam, Kerala

Remarks/Source: Western Ghats (Bharti, 2014), Calicut (Bharti and Singh, 2017),

Thrissur, Palakkad and Ernakulam in Kerala and identified in the current study (Reject Paul and Binoy, 2021)

Distinguishing features: Fuscous to black coloured genae and parafacialia, setulae and hairs on parafacialia and parafrontalia were black in colour, brown to fuscous 1$^{st}$ , 2$^{nd}$ and 3$^{rd}$ antennal segments, black hairs on the venter of tergite V, prothoracic spiracle fuscous black in colour, black coloured epaulet and basicosta, dense basal tuft of black hairs on the subcostal sclerite, black setae on the upper margin of 3$^{rd}$ longitudinal vein, base of alar squamae white and ventrally bare except for fringe.

### *Chrysomya nigripes* Aubertin, 1932

Distribution: Calicut, Kerala, India

Remarks/Source: ( Bharti and Singh, 2017)

Distinguishing features: Parafrontalia and parafacialia with grey tomentum, genae grey, antennae dark brown, anterior spiracle white, only one katepisternal setae developed, all hairs on the tergite V black, prothoracic stigma white, hind margins of second and third segments of abdomen dark banded, basicostal scale dark brown, sub costal sclerite with pale hairs, squama white.

### *Chrysomya rufifacies* Macquart, 1842

Distribution: Throughout India, Calicut, Thrissur, Palakkad and Ernakulam in Kerala

Remarks/Source: Nandi, 2004, Calicut (Bharti and Singh, 2017) Thrissur, Palakkad and Ernakulam in Kerala and identified in the current study (Reject Paul and Binoy, 2021)

Distinguishing features: Third antennal segment brownish red on the inner surface. Parafrontalia narrowed with a black colour in the upper half, lower half covered with silver tomentum with upstanding white hairs, parafacialia and genae light yellowish and covered with white hairs, anterior spiracle white, few white hairs present on the tergite V, and upper squama white. The lower squama was slightly fuscous with white hairs.

### *Chrysomya albiceps* Wiedemann, 1819

Distribution: Periyar Lake, in the Periyar Tiger Reserve, Thekkady, Kerala,

Remarks/Source: (Radhakrishnan et al., 2012)

Distinguishing features: Third antennal segment blackish brown, proepimeral seta absent, two katepiternal setae, dorsal part of thorax glossy with a little dusting, anterior spiracle white, few white hairs on the posterior edge of tergite V with incision, black transverse narrow marginal abdominal bands on the 3$^{rd}$ and 4$^{th}$ segments.

## Subfamily: Rhiniinae

### Genus: *Idiella* Braeuer and Berensteamn, 1889

### *Idiella euidielloides* Senior-White, 1922

Distribution: Cardamom Estate (Kerala)

Remarks/Source: (Arce et al., 2020)

Distinguishing features: Basicosta black, sternopleuron and mesopleuron with distinct piliferous spots, first and second tergite with few black lateral bristles, posteroventral surface of hind tibia with longer hairs, tibial hairs not exceeding the width of tibia.

### *Idiella mandarina* Wiedemann, 1830

Distribution: Thiruvananthapuram, Kerala

Remarks/Source: (Nandi, 2004)

Distinguishing features: Frontal stripe brownish black, white parafrontalia with black spots, genae shining black, antennae brown, black palpi, lower half of the occiput with dense hairs, pleurae with dense golden hairs, tibiae and first tarsal joint brown and rest of tarsi black.

### Genus: *Stomorhina* Rondani, 1861

### *Stomorhina discolor* Fabricius, 1794

Distribution: Cardamom Estate (Kerala)

Remarks/Source: : (Nandi, 2004)

Distinguishing features: Frontal stripe dark brown, parafacialia and parafrontalia white with shining black spots, epistome and genae shining black, antennae and palpi brown, green thorax densely grey dusted with small black spots, anterior lower meso pleuron and anterior sternopleuron glossy black, abdominal segments with black hind margins with a black median stripe, hind femur yellowish at base, tibiae and tarsi brownish yellow.

**Genus: *Cosmina* Robineau-Desvoidy, 1830**

***Cosmina bicolor* Walker, 1856**

Distribution: Nilgiris (Kerala)

Remarks/Source: (Nandi, 2004)

Distinguishing features: Parafrontalia greyish with black spots, parafacialia silvery white, antennae yellowish brown, palpi black, propleuron hairy, mesopleuron metallic green, sub median mesonotal stripes broad, abdominal segments with a median stripe, strong bristles close to the apex of fifth sternite, hypopygium without strong spines, epaulet reddish brown.

***Cosmina simplex* Walker, 1858**

Distribution: Kochi, Kerala

Remarks/Source: (Nandi, 2004)

Distinguishing features: Parafacialia silvery white with black spots, parafrontalia greyish with shining black spots, genae shining black, antennae yellowish brown, thorax copper green with black spots, long bristles on the entire surface of fifth visible sternite, hypopygium with curved laterally directed spines.

**Genus: *Strongyloneura* Bigot, 1886**

***Strongyloneura prolata* Walker, 1860**

Distribution: Chalakudy, Kerala

Remarks/Source: (Nandi, 2004)

Distinguishing features: Mesopleuron without bristle on its upper part, third sternite without tuft of hair, fourth sternite with tuft of hair, fifth sternite and hypopygium are well developed, last sternite projected posteriorly and widely uncovered by corresponding tergites, bend of vein $M_{1+2}$ gently curved.

### 4.1.1. Identification

***Chrysomya megacephala***

**Morphological identification**

**Adult fly**

Blow fly samples collected during the current study were identified up to the level of species using existing literature and taxonomic keys. Morphological features of head, thorax, abdomen and wings of *C. megacephala* are discussed below.

Head

Eyes were holoptic in males and dichoptic in females. Facets of upper two-thirds in the male eyes were enlarged and was clearly demarcated from the smaller facets below. In females, the facets were uniformly small. In males, the parafrontalia was reduced to a fine line covered with golden tomentum. The right and left paprafrontalia slightly narrower than the breadth of the frons. Antennae, arista and palpi orange in colour. Outer vertical bristles were absent in males. Parafacialia and genae completely orange in colour. Hairs and setulae on the parafacialia yellowish. In females, the frontal stripe is broader at the middle of frons than in male fly (Fig. 4.1 B-C).

Thorax

Thorax was bluish green with anterior spiracles dark brown. Femora was not swollen in both sexes (Fig. 4.1. D).

Abdomen

Bluish green. First segment bluish black and second and third segments were banded posteriorly. Absence of median incision in tergite V of females. Ventral aspect of tergite V

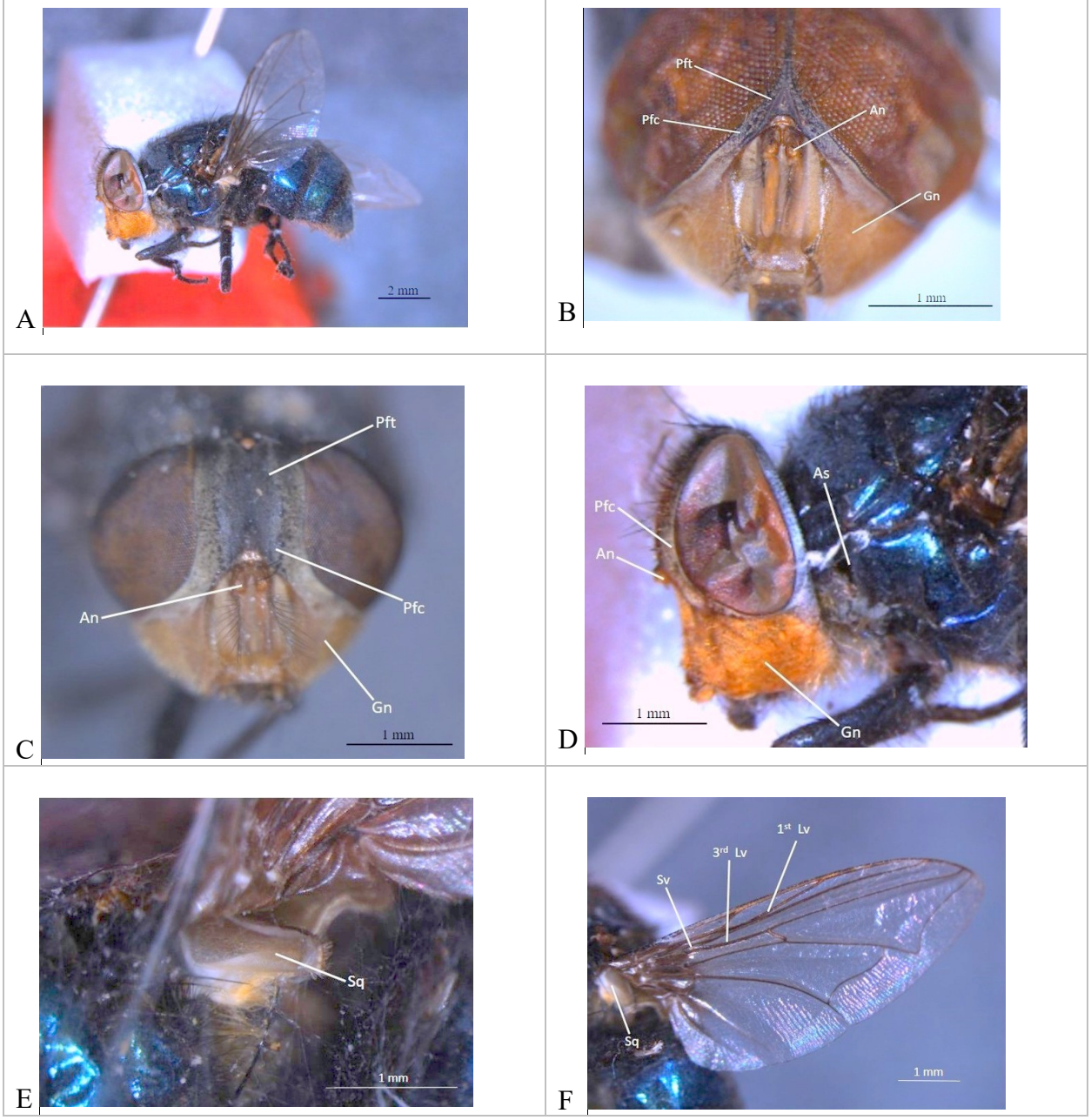



**Fig. 4.1. Characteristics of *C. megacephala* A. Adult fly B. Frons of the male showing holoptic eyes C. Frons of the female showing dichoptic eyes D. Lateral view of head and thorax E.Wing base showing calypter F. Wing venation with setulae**

**An- Antenna, As - Anterior spiracle, Gn - Gena, Lv - Longitudinal vein, Pfc- Parafacialia, Pft- parafrontalia , Sq- Squama, Sv - Stem vein**

was intermixed with yellow hairs. Golden hairs were seen on the sternites and tergites (Fig. 4.1. D-E).

Wings

Darkened at the base and hyaline in nature. Basicostal scale dark brown to black. Sub costal sclerite covered with brown felted pubescence and also with small erect hairs. A row of setulae were seen on the upper posterior margin on the stem vein. Squama showing upper calypter with ventral hairs on the opaque white basal part. Except the pale base, upper and lower calypters were brown (Fig. 4.1. F).

**Molecular characterisation**

**Molecular sequencing of CO I gene**

Molecular diagnosis was done by amplifying the partial coding sequence of mitochondrial COI gene of *C. megacephala* using the primers; LCO (forward) and HCO (reverse). The DNA sequence interpret (Fig. 4.2), conceptual translational product (Fig.4.3), Agarose Gel Electrophoresis of PCR products (Fig.4.4), and the electropherogram (Fig. 4.5 a & b) are given below.

The Neighbor Joining method allowed us to identify the species at the molecular level with precision and accuracy (Fig. 4.6). The isolated sequence was submitted in GenBank, NCBI with accession No: MW 522614 (Appendix II).

```
CGGAGCTTGA TCCGGAATAG TAGGAACTTC ATTAAGTATT TTAATTCGAG CTGAATTAGG
ACACCCTGGA GCATTAATTG GAGACGACCA AATTTATAAT GTAATTGTAA CAGCTCACGC
TTTTATTATA ATTTTCTTTA TAGTAATGCC AATTATAATT GGAGGATTTG GAAATTGACT
AGTTCCTTTA ATGTTAGGAG CTCCAGATAT AGCTTTCCCA CGAATAAATA ATATAAGTTT
CTGACTTTTA CCTCCTGCAT TAACTTTATT ATTAGTAAGT AGTATAGTAG AAAATGGGGC
TGGAACAGGA TGAACTGTTT ACCCACCTTT ATCTTCTAAT ATTGCTCATG GAGGAGCATC
AGTTGATTTA GCTATTTTCT CCTTACACTT AGCAGGAATT TCTTCAATTT TAGGAGCTGT
AAATTTTATT ACAACTGTAA TTAATATACG ATCTACAGGA ATTACATTTG ATCGAATACC
TTTATTTGTA TGATCTGTAG TTATTACTGC TCTATTATTA TTATTATCTT TACCAGTATT
AGCTGGAGCT ATTACTATAT TATTAACTGA CCGAAATCTA AATACTTCAT TCTTTGATCC
AGCAGGAGGA GGAGATCCTA TTTTATATCA ACATTTAA
```

**Fig. 4.2. Partial coding sequence of mitochondrial COI gene of *C.megacephala.***

(>MW522614.1 *C. megacephala* isolate CMRP02 cytochrome c oxidase subunit I (CO I) gene, partial cds; mitochondrial (638 bp)

```
GAWSGMVGTSLSILIRAELGHPGALIGDDQIYNVIVTAHAFIMI

FFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPALTLLLVSSMVENGAGT

GWTVYPPLSSNIAHGGASVDLAIFSLHLAGISSILGAVNFITTVINMRSTGITFDRMP

LFVWSVVITALLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHL
```

**Fig. 4.3. Translational product of mitochondrial COI gene of *C.megacephala***

>QQX23394.1 cytochrome c oxidase subunit I, partial Amino acid sequences

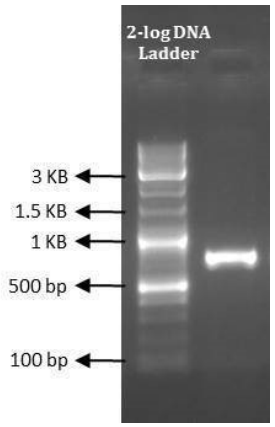



**Fig. 4.4. Agarose Gel Electrophoresis of the PCR product**

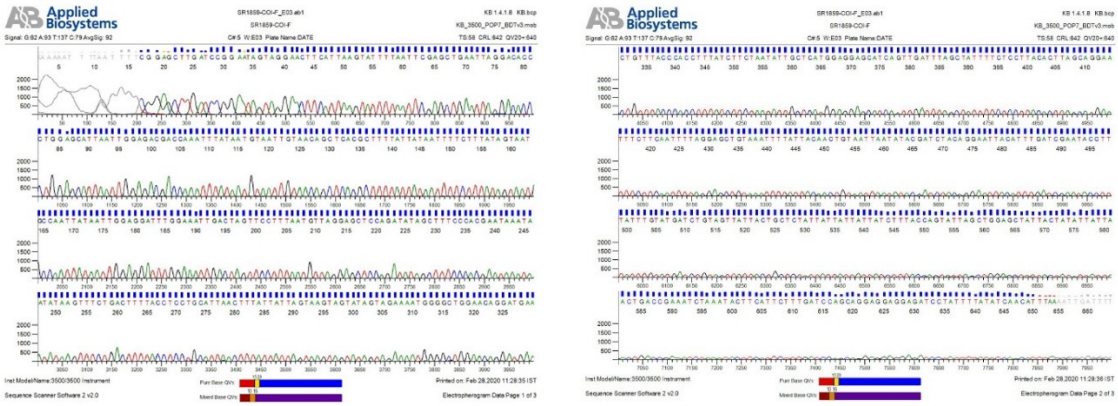


**Fig. 4.5 a. Electropherogram of mitochondrial COI gene of *C.megacephala* using forward primer**

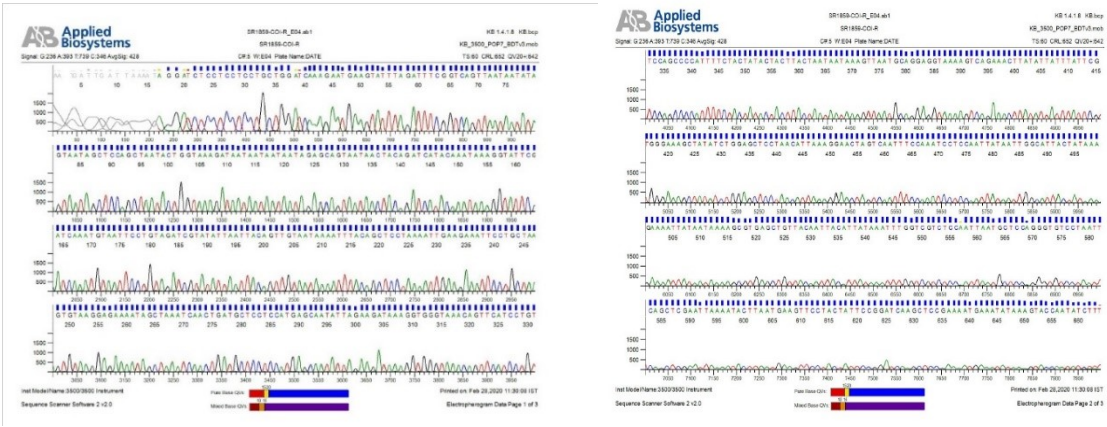


**Fig. 4.5. b. Electropherogram of mitochondrial COI gene of *C.megacephala* using reverse primer**

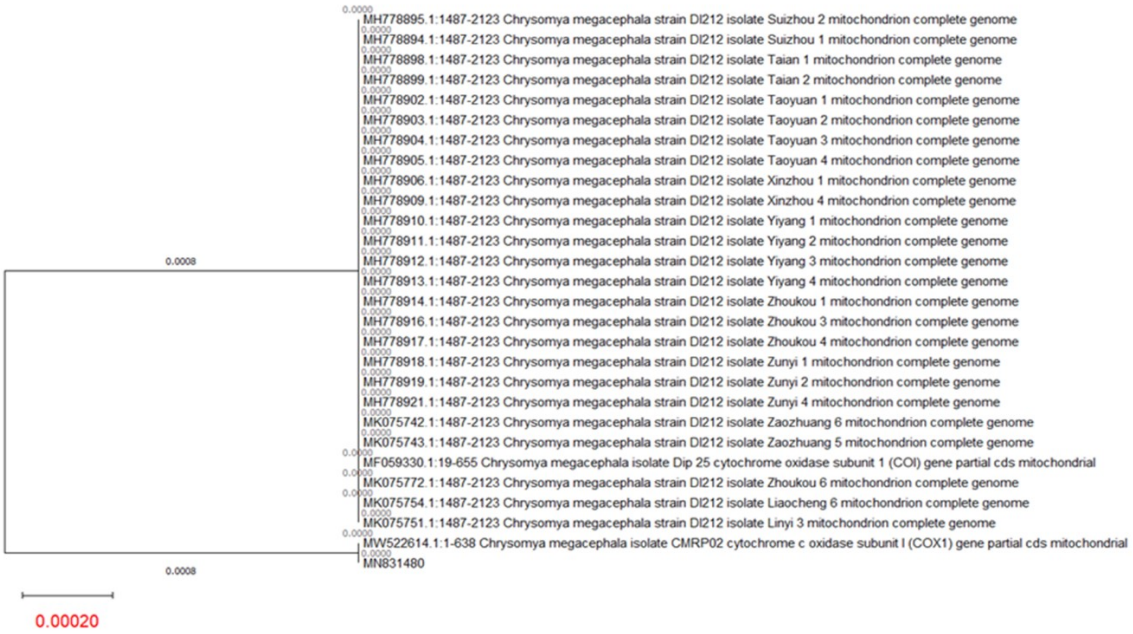



**Fig. 4.6. Phylogenetic tree based on COI sequence, SR1859-COI-F_E03 of *C.megacephala* by Maximum Likelihood method**

This analysis involved 28 nucleotide sequences and the codon positions included were 1st+2nd+3rd+Noncoding. There were 638 positions in the final dataset. The phylogenetic analysis confirmed the species identity of *C.megacephala* with strong boot strap support.

***Chrysomya rufifacies***

**Morphological identification**

**Adult fly**

**Head**

Facets of eyes in both sexes were small. Third antennal segment brownish red on the inner surface. Parafrontalia narrow with a black colour in the upper half. The lower half covered with silver tomentum with white erect hairs. Frons greyish in colour. Parafacialia

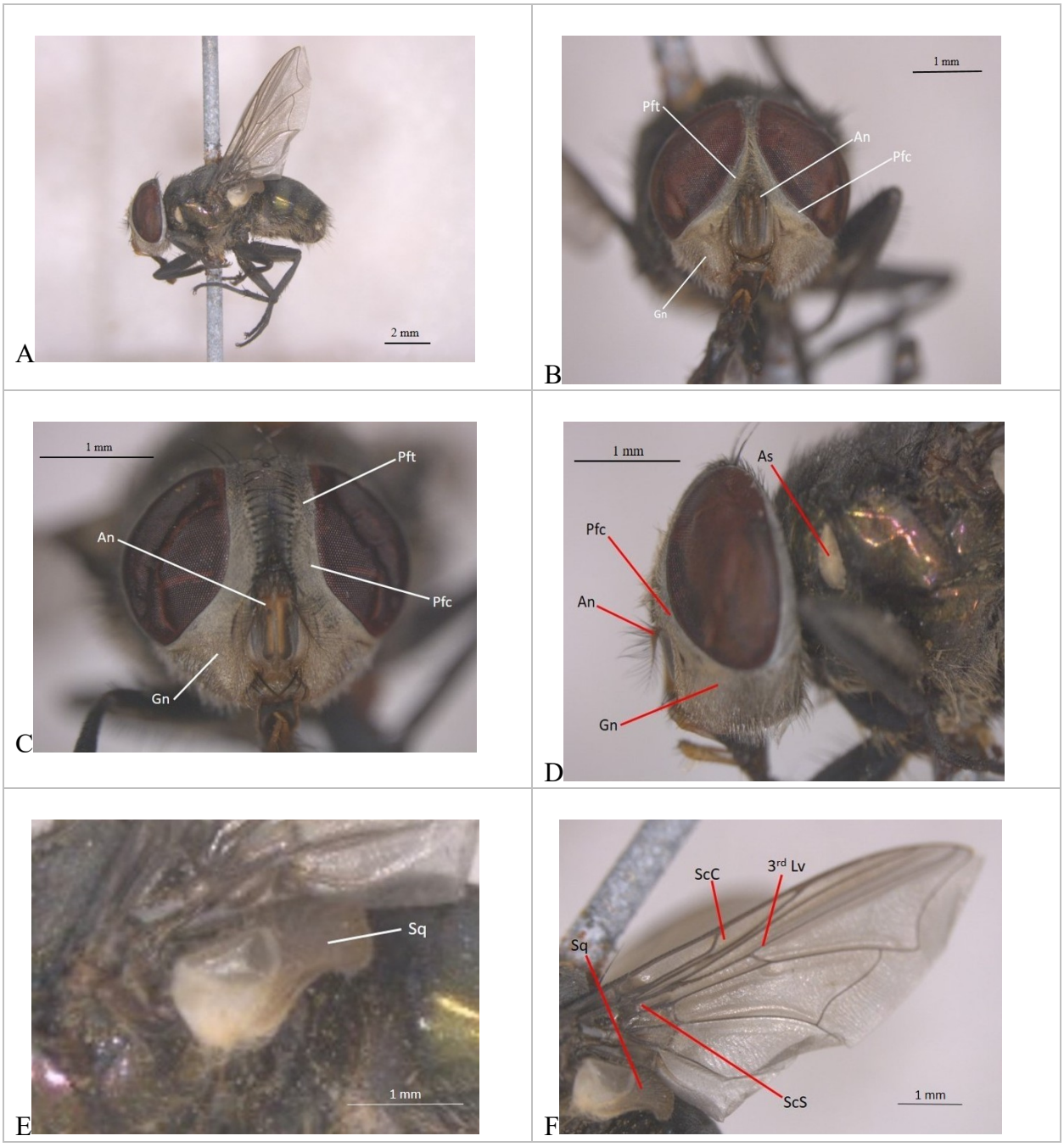



**Fig. 4.7. Characteristics of *C. rufifacies* A. Adult fly B. Frons of the male showing holoptic eyes C. Frons of the female showing dichoptic eyes D. Lateral view of head and thorax E.Wing base showing calypter F. Wing venation with setulae**

**An- Antenna, As - Anterior spiracle, Gn - Gena, Lv - Longitudinal vein, Pfc- Parafacialia, Pft- parafrontalia , ScC - Subcostal cell, ScS - subcostal sclerite, Sq- Squama, Sv - Stem vein**

and genae light yellowish in colour and covered with white hairs. Antennae brown in colour. The palpi and epistome orange in colour (Fig. 4.7 B-C).

**Thorax**

Dorsum of the thorax shiny and greenish blue colour with no dusting. Anterior spiracle white. First quarter of 3$^{rd}$ and one sixth of the 4$^{th}$ abdominal segments very narrow. Proepimeral setae present, two setae present on the katepisternum. Median incision present on the posterior edge of tergite V of females. Few white hairs present on the tergite V (Fig. 4.7 D-E).

**Abdomen**

Second and third segments dark banded on the posterior margins. Fine golden hairs were present on the venter.

**Wings**

Hyaline in nature and infuscated at the base of the subcostal cell. Basicostal scale was brownish black. Subcostal sclerite with no hairs. Upper squama was white in colour. The lower squama was slightly fuscous in colour with white hairs. (Fig. 4.7 F).

The length of the fly was 9-10mm.

**Molecular characterisation**

**Molecular sequencing of CO I gene**

Molecular diagnosis was done by amplifying the partial coding sequence of mitochondrial COI gene of *C. rufifacies* using the primers; LCO (forward) & HCO (reverse). The DNA sequence interpret (Fig. 4.8), conceptual translational product (Fig.4.9), Agarose Gel Electrophoresis of PCR product (Fig.4.10), and the electropherogram (Fig. 4.11 a & b), are given below.

The Neighbor Joining method allowed us to identify the species at the molecular level with precision and accuracy (Fig. 4.12). The isolated sequence was submitted in GenBank, NCBI with accession No: OM019083.1 (Appendix II).

```
GGAACTTCTT TAAGAATCCT AATTCGAGCT GAATTAGGAC ATCCTGGAGC ACTAATTGGG
GATGACCAAA TTTATAATGT AATTGTAACA GCTCATGCTT TTATTATAAT TTTCTTTATA
GTAATACCAA TTATAATTGG AGGATTTGGA AATTGACTAG TCCCTCTAAT ACTAGGAGCC
CCAGATATAG CTTTCCCACG AATAAATAAT ATAAGTTTTT GACTTTTACC TCCTGCATTA
ACTTTACTAT TAGTAAGTAG TATAGTAGAA AATGGAGCTG GAACAGGATG AACTGTTTAT
CCACCTTTAT CATCTAATAT TGCACATGGT GGAGCATCAG TTGATTTAGC TATTTTTTCT
TTACACTTAG CTGGAATTTC ATCAATTTTA GGAGCCGTAA ATTTTATTAC AACTGTTATT
AATATACGAT CTACAGGAAT TACATTTGAT CGAATACCTT TATTTGTATG ATCTGTAGTT
ATTACTGCTC TTCTTTTATT ATTATCATTA CCAGTATTAG CAGGTGCAAT TACTATATTA
TTAACTGATC GAAATTTAAA TACTT
```

**Fig. 4.8. Partial coding sequence of mitochondrial COI gene of *C.rufifacies***
(> OM019083.1 *C. rufifacies* isolate CRRP04 cytochrome c oxidase subunit I (CO I) gene, partial cds; mitochondrial (565 bp))

```
GTSLSILIRAELGHPGALIGDDQIYNVIVTAHAFIMIFFMVMPI

MIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPALTLLLVSSMVENGAGTGWTVYPP

LSSNIAHGGASVDLAIFSLHLAGISSILGAVNFITTVINMRSTGITFDRMPLFVWSVV

ITALLLLLSLPVLAGAITMLLTDRNLNT
```

**Fig. 4.9. Translational product of mitochondrial COI gene of C. *rufifacies***
(> UHQ29699.1 cytochrome c oxidase subunit I, partial Amino acid sequences)

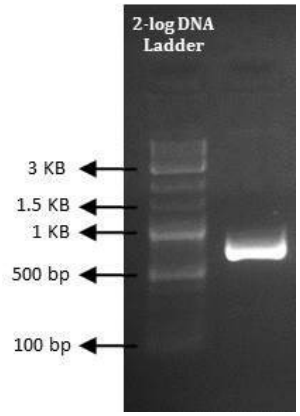



**Fig. 4.10. Agarose Gel Electrophoresis of the PCR product**

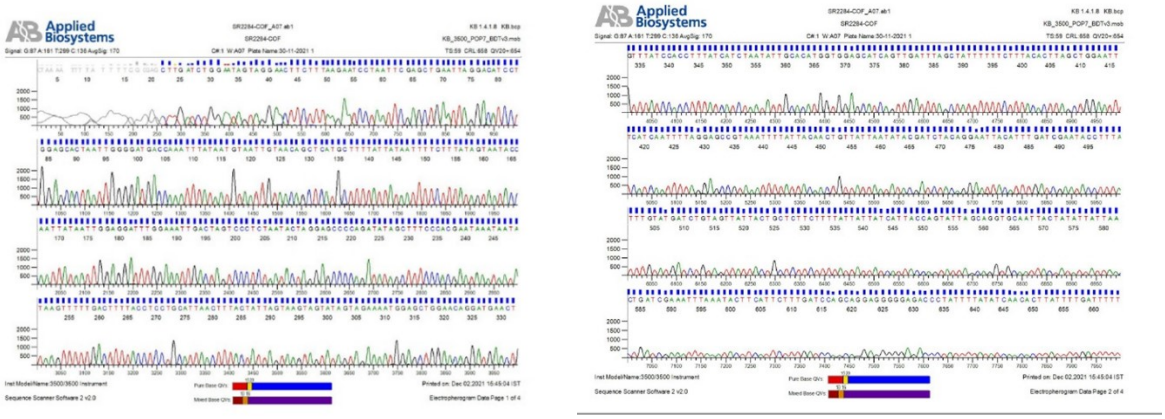


**Fig. 4.11 a. Electropherogram of the mitochondrial COI gene of *C.rufifacies* using forward primer**

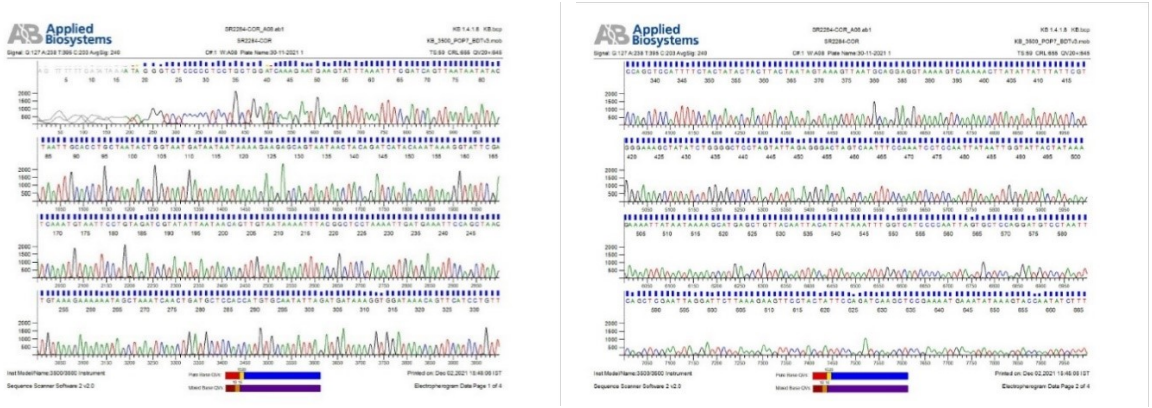


**Fig. 4.11 b. Electropherogram of the mitochondrial COI gene of *C.rufifacies* using reverse primer**

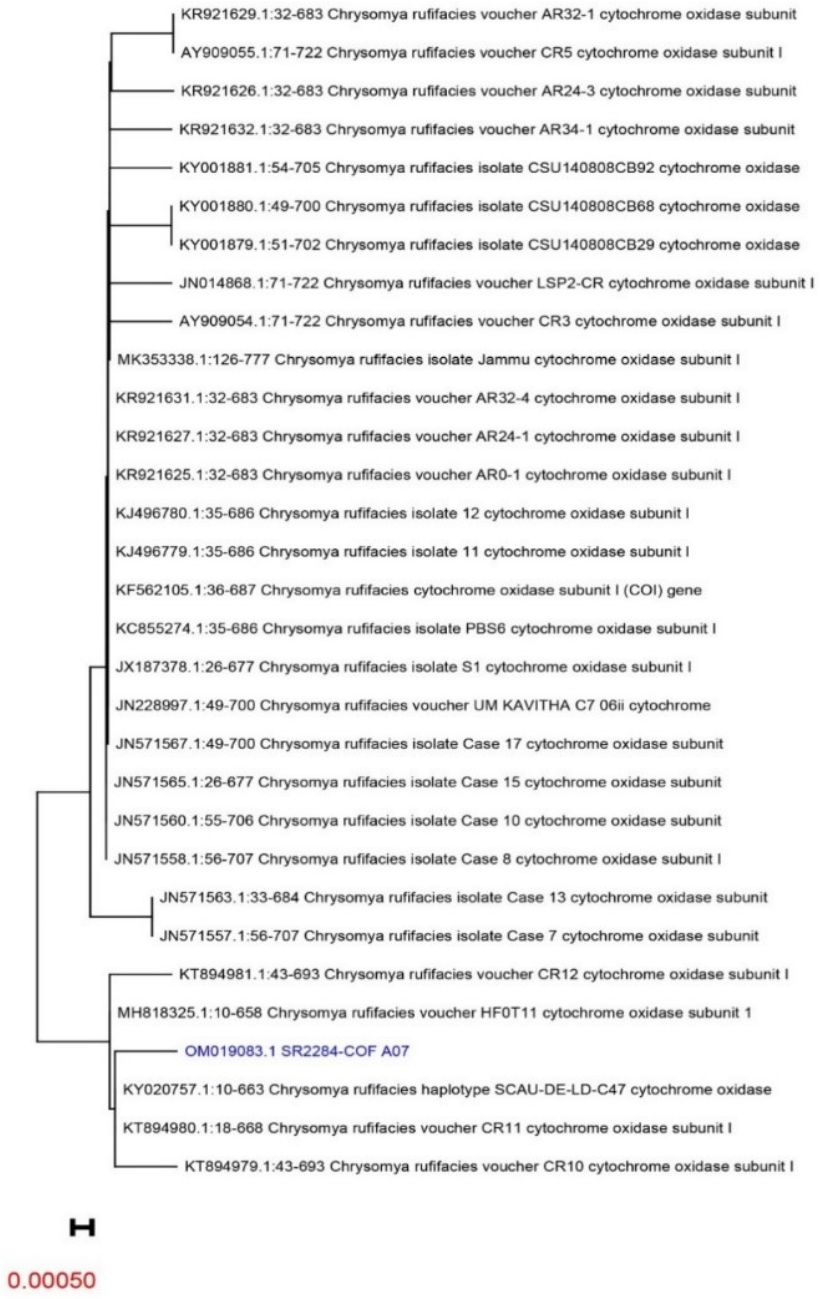



**Fig. 4.12. Phylogenetic tree based on COI sequence *SR2284-COF_A07* of *C. rufifacies* by Maximum Likelihood method**

This analysis involved 31 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were 655 positions in the final dataset. The phylogenetic analysis confirmed the species identity of *C. rufifacies* with strong bootstrap support.

*Chrysomya chani*

**Morphological identification**

**Adult fly**

**Head**

Eyes holoptic in males and dichoptic in females. The upper facets of the eye were enlarged and clearly demarcated from the small facets. Facial ridge was present. Genae and parafacialia were fuscous to black in colour. Setulae and hairs on parafacialia and parafrontalia were blackish in colour. 1st and 2nd antennal segments were brown to fuscous in colour. 3rd segment was fuscous in colour (Fig. 4.13. B-C).

**Thorax**

Thorax was bluish green coloured with anterior spiracle fuscous black in colour. Black hairs were present on the pleura. Median cleft was present on the female tergite V with black hairs on the venter (Fig. 4.13. D-E).

**Wings**

Wings hyaline. Epaulet and basicosta are black coloured. Dense basal tuft of black hairs present on the subcostal sclerite. Black setae present on the upper margin of 3rd longitudinal vein. Base of alar squamae is white in colour and ventrally it is bare except for fringe (Fig. 4.13. F).

**Molecular characterisation**

**Molecular sequencing of CO I gene**

Molecular diagnosis was done by amplifying the partial coding sequence of mitochondrial COI gene of *C. chani* using the primers; LCO (forward) & HCO (reverse).

The DNA sequence interpret (Fig. 4.14), conceptual translational product (Fig. 4.15), Agarose Gel Electrophoresis of PCR product (Fig. 4.16), and the electropherogram (Fig. 4.17 a-b), are given below.

The Neighbor Joining method allowed us to identify the species at the molecular level with precision and accuracy (Fig. 4.18).The isolated sequence was submitted in GenBank, NCBI with accession no: MW600494.1 (Appendix II).

```
TTCGGAGCTT GATCCGGAAT AGTAGGAACT TCATTAAGAA TTTTAATTCG AGCTGAATTA
GGACACCCTG GAGCATTAAT TGGAGATGAC CAAATTTATA ATGTAATTGT AACAGCTCAC
GCTTTTATTA TAATTTTTTT TATAGTAATA CCAATTATAA TTGGAGGATT TGGAAATTGA
TTAGTTCCTT TAATACTAGG AGCCCCAGAT ATAGCTTTCC CACGAATAAA TAATATAAGT
TTCTGACTTT TACCTCCTGC ATTAACTTTA TTATTAGTAA GTAGTATAGT AGAAAATGGA
GCTGGAACAG GATGAACTGT TTATCCACCT TTATCTTCTA ATATTGCTCA TGGAGGAGCA
TCAGTTGATT TAGCTATTTT TTCTTTACAT TTAGCAGGAA TTTCTTCAAT TTTAGGAGCT
GTAAATTTTA TTACTACAGT AATTAATATA CGATCTACAG GAATTACATT TGATCGAATA
CCTCTATTTG TTTGATCAGT AGTTATTACT GCTTTATTAT TATTATTATC TTTACCAGTA
TTAGCAGGAG CTATTACTAT ATTATTAACT GATCGAAATT TAAATACTTC ATTCTTTGAT
CCAGCA
```

**Fig: 4.14. Partial coding sequence of mitochondrial COI gene of *C. chani***

> MW600494.1 *C. chani* isolate CCRP03 cytochrome c oxidase subunit I (CO I) gene, partial cds; mitochondrial (606 bp)

```
FGAWSGMVGTSLSILIRAELGHPGALIGDDQIYNVIVTAHAFIM

IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPALTLLLVSSMVENGAG

TGWTVYPPLSSNIAHGGASVDLAIFSLHLAGISSILGAVNFITTVINMRSTGITFDRM

PLFVWSVVITALLLLLSLPVLAGAITMLLTDRNLNTSFFDPA
```

**Fig. 4.15. Translational product of mitochondrial COI gene of *C. chani***

>QRQ47096.1 cytochrome c oxidase subunit I, partial Amino acid sequences

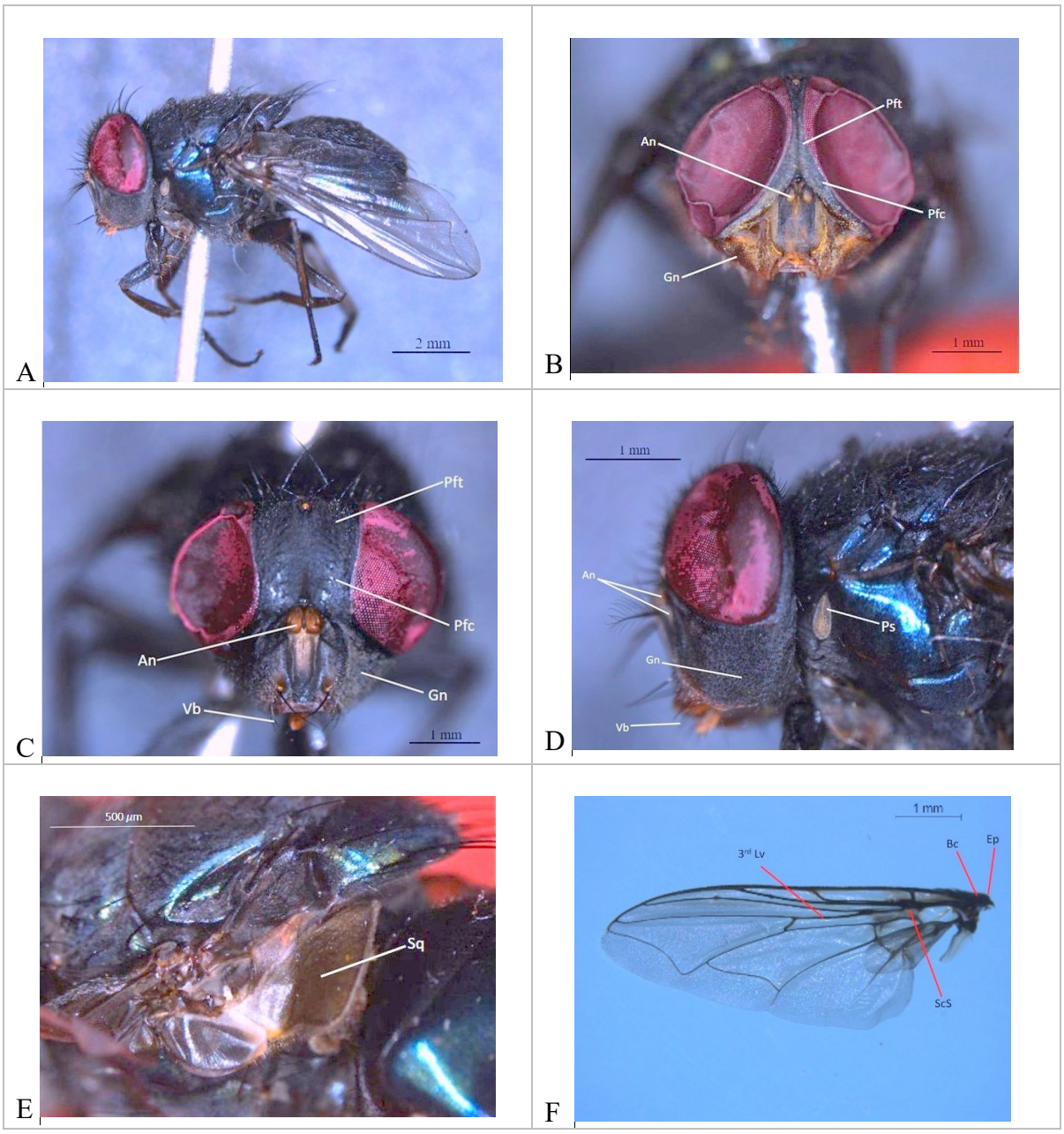



**Fig. 4.13. Characteristics of *C. chani* A. Adult fly B. Frons of the male showing holoptic eyes C. Frons of the female showing dichoptic eyes D. Lateral view of head and thorax E. Wing base showing calypter F. Wing venation with setulae**

**An- Antenna, As - Anterior spiracle, Bc – Basicosta, Ep – Epaulet, Gn - Gena, Lv - Longitudinal vein, Pfc- Parafacialia, Pft- parafrontalia , ScC - Subcostal cell, ScS - subcostal sclerite, Sq- Squama, Sv - Stem vein, Vb - Vibrissae**

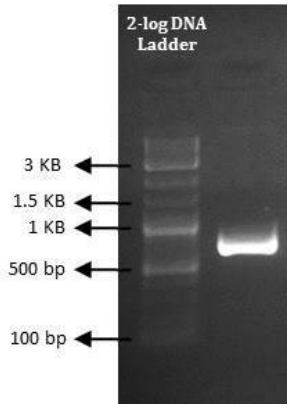



**Fig. 4.16. Agarose Gel Electrophoresis of the PCR product**

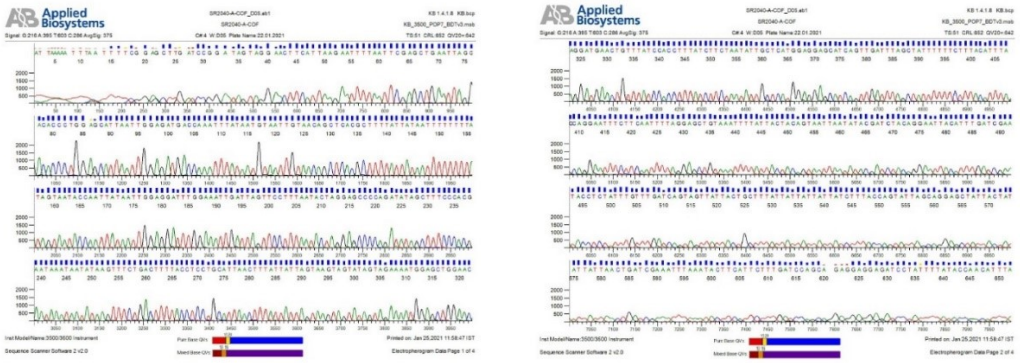


**Fig. 4.17 a. Electropherogram of the mitochondrial COI gene of *C. chani* using forward primer.**

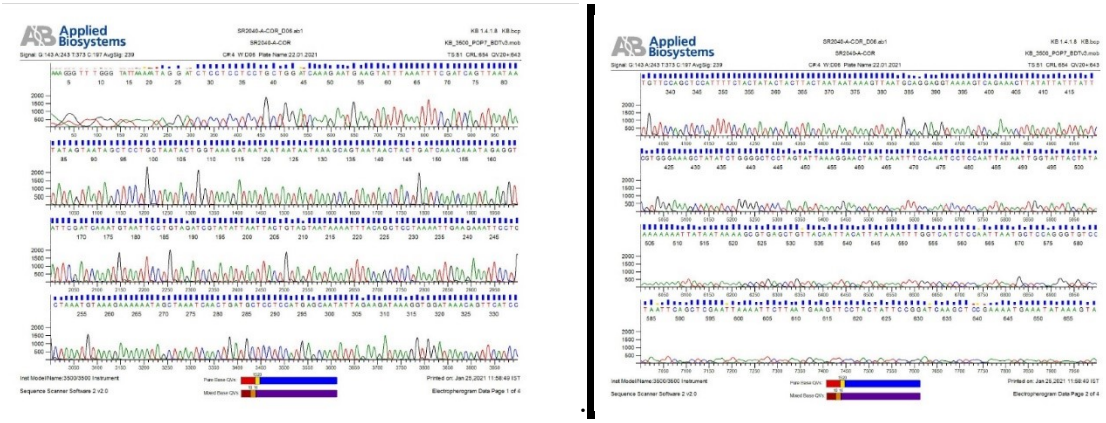


**Fig. 4.17 b. Electropherogram of the mitochondrial COI gene of *C. chani* using reverse primer**

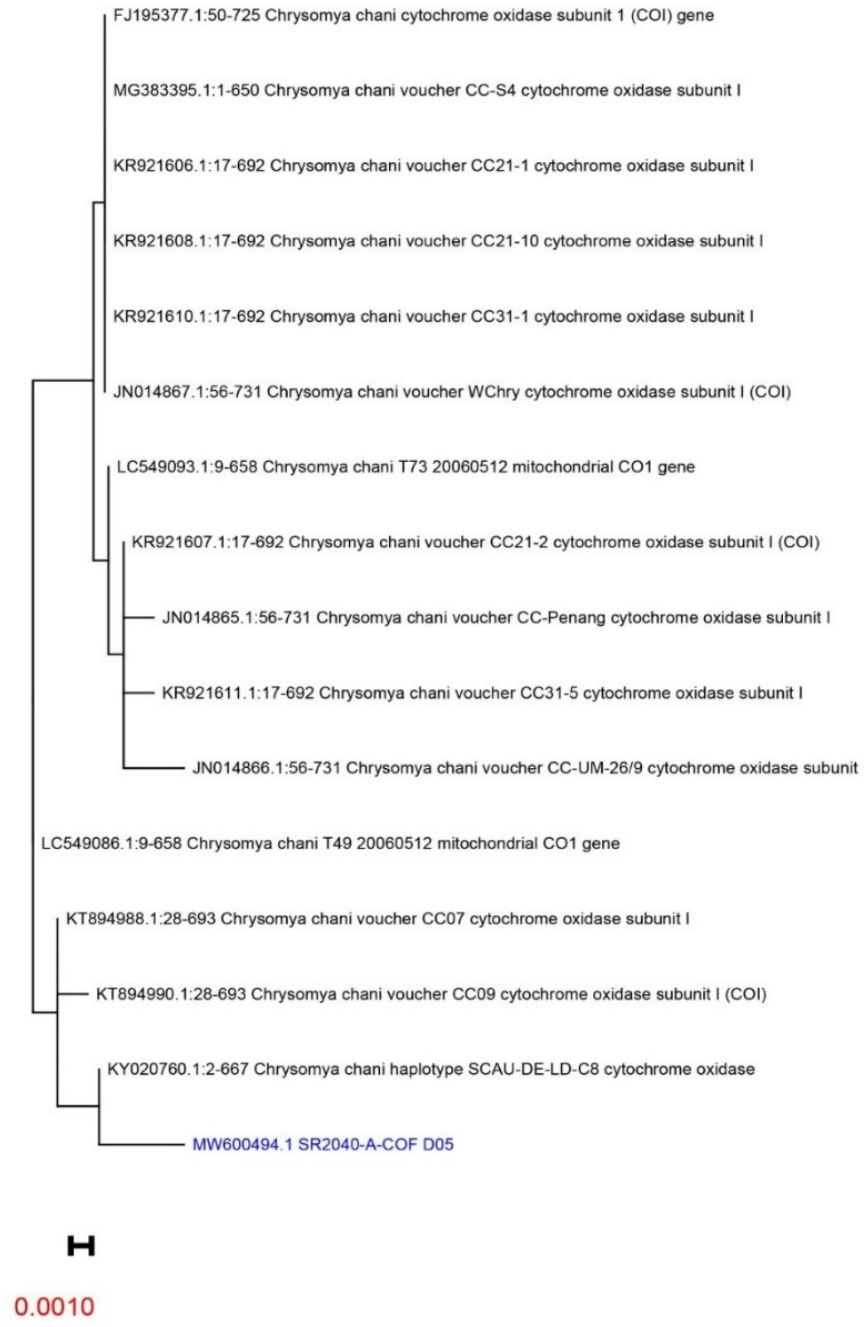



**Fig. 4.18. Phylogenetic tree based on COI sequence, *SR2040-A-COF_D05* of *C. chani* by Maximum Likelihood method**

This analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were 685 positions in the final dataset. The phylogenetic analysis confirmed the species identity of *C. chani* with strong boot strap support.

***Hemipyrellia ligurriens***

**Morphological identification**

**Adult fly**

**Head**

The length of the third antennal segment is shorter than the distance between the eyes in males. Parafrontalia is narrower. Genae and parafrontalia silver white. Antennae tawny yellow to brownish. Palpi orange in colour. Parafrontalia and frons are with similar width (Fig. 4.19. B-C).

**Thorax**

Greenish purple shining present on the thorax. Anterior part of the thorax and lower part of hypopleuron heavily dusted. The squama whitish. The upper squama creamish white with short cilia and lower squama with light brown cilia (Fig. 4.19. D-E).

**Abdomen**

Greenish purple shining is present on the abdomen. Posterior margins of the abdomen dark. In males, sparse short hairs are seen on the edges of tergites and first visible sternite.

**Wings**

Wings hyaline. Setulae are not present on the stem vein and 1$^{st}$ longitudinal vein, but short setulae are present on the dorsal and ventral aspects of 3$^{rd}$ longitudinal vein (Fig. 4.19. F).

Length of the adult fly is 9-10 mm

**Molecular characterisation**

**Molecular sequencing of CO I gene**

Molecular diagnosis was done by amplifying the partial coding sequence of mitochondrial COI gene of *H. ligurriens* using the primers; LCO (forward) and HCO (reverse). The DNA sequence interpret (Fig. Fig. 4.20), conceptual translational product (Fig.4.21), Agarose Gel Electrophoresis of PCR product (Fig.4.22), and the electropherogram (Fig. 4.23 a & b), are given below.

The Neighbor Joining method allowed us to identify the species at the molecular level with precision and accuracy (Fig. 4.24). The isolated sequence was submitted in GenBank, NCBI with accession no: MN831480.1 (Appendix II).

```
CAGGAATAAT TGGAACTTCA TTAAGAATTC TAATTCGAGC TGAATTGGGA CACCCTGGAG
CTTTAATTGG AGATGACCAA ATCTATAATG TAATTGTAAC AGCTCATGCT TTTATTATAA
TTTTTTTTAT AGTAATACCA ATTATAATTG GAGGATTTGG AAATTGATTA GTTCCTTTAA
TATTAGGAGC CCCAGATATA GCATTCCCTC GAATAAATAA TATAAGTTTT TGACTTTTAC
CTCCTGCATT AACTTTATTA TTAGTAAGCA GTATAGTAGA AAACGGAGCT GGAACAGGAT
GAACAGTTTA CCCTCCTTTA TCATCTAATA TTGCCCATGG AGGAGCTTCT GTAGATCTAG
CTATTTTCTC TTTACATTTA GCAGGAATTT CATCAATTTT AGGAGCTGTA AATTTCATTA
CAACAGTAAT TAATATACGA TCAACAGGTA TTACTTTTGA TCGAATACCT TTATTTGTTT
GATCTGTAGT AATTACAGCT TTATTACTTT TATTATCATT ACCAGTATTA GCAGGAGCTA
TTACTATACT TTTAACAGAC CGAAATCTAA ACACTTCATT CTTTGATCCA GCTGGAGGAG
GAGATCCAAT TTTATATCAA CATTTATTTT GATTTTTTGG TCACCAGA
```

**Fig. 4.20. Partial coding sequence of mitochondrial COI gene of *H. ligurriens***

(>MN831480 *H. ligurriens isolate* HLRP01 cytochrome c oxidase subunit I (CO I) gene, partial cds; mitochondrial (648 bp)

```
GMIGTSLSILIRAELGHPGALIGDDQIYNVIVTAHAFIMIFFMV

MPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPALTLLLVSSMVENGAGTGWTV

YPPLSSNIAHGGASVDLAIFSLHLAGISSILGAVNFITTVINMRSTGITFDRMPLFVW

SVVITALLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLFWFFGHQ
```

**Fig. 4.21. Translational product of mitochondrial COI gene of *H. ligurriens***

(> QNG41879.1 cytochrome c oxidase subunit I, partial Amino acid sequences)

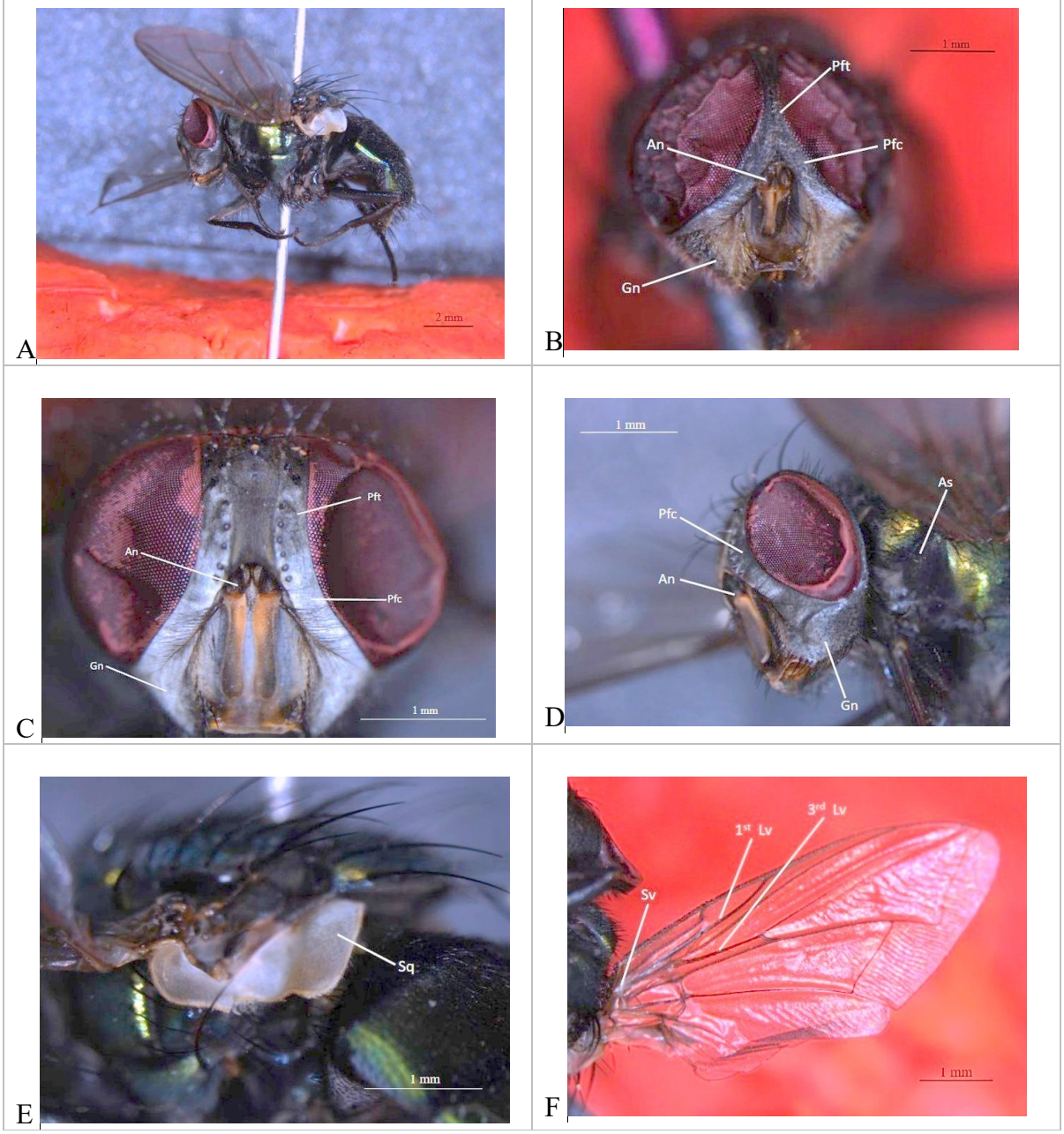



**Fig. 4.19. Characteristics of *H. ligurriens* A. Adult fly B. Frons of the male showing holoptic eyes C. Frons of the female showing dichoptic eyes D. Lateral view of head and thorax E. Wing base showing calypter F. Wing venation with setulae**

**An- Antenna, As - Anterior spiracle, Gn - Gena, Lv - Longitudinal vein, Pfc- Parafacialia, Pft- parafrontalia , Sq- Squama, Sv - Stem vein**

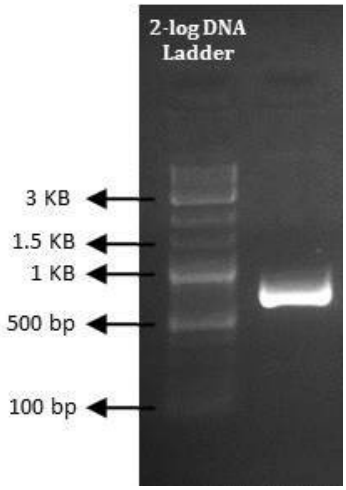



**Fig. 4.22 Agarose Gel Electrophoresis of the PCR product**

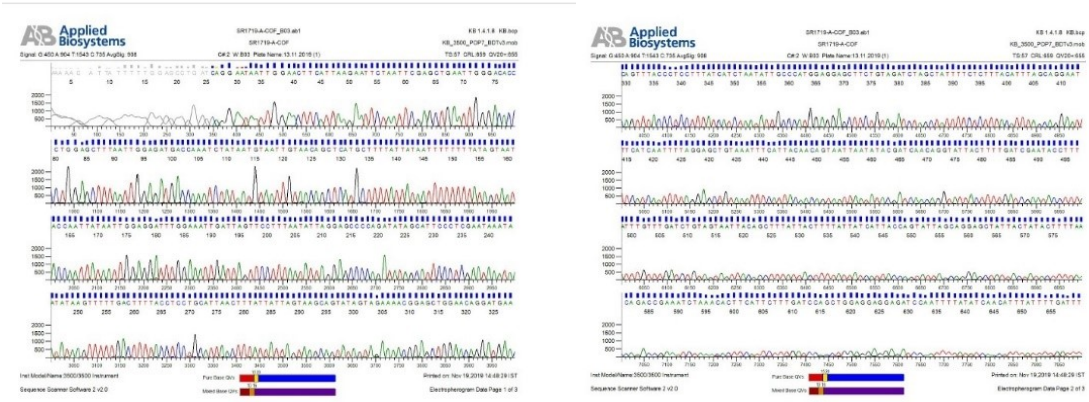


**Fig. 4.23 a. Electropherogram of the mitochondrial COI gene of *H. ligurriens* using forward primer**

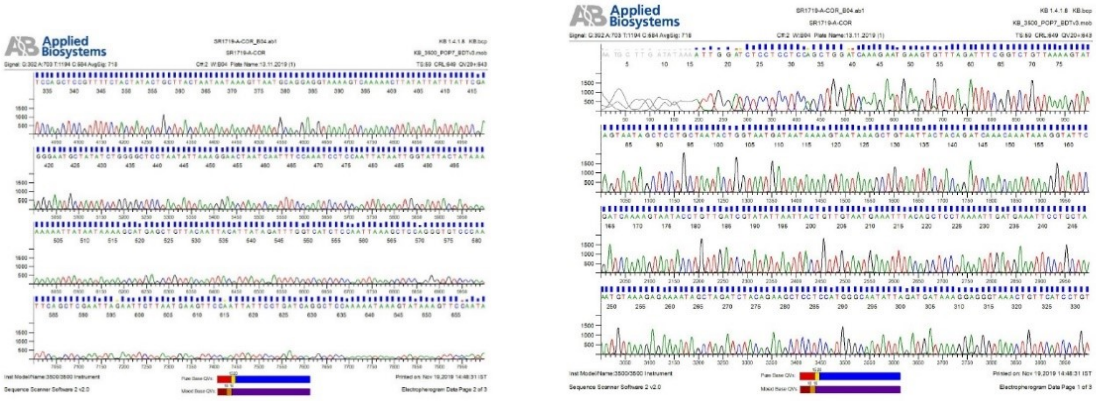


**Fig. 4.23. b. Electropherogram of the mitochondrial COI gene of *H. ligurriens* using reverse primer**

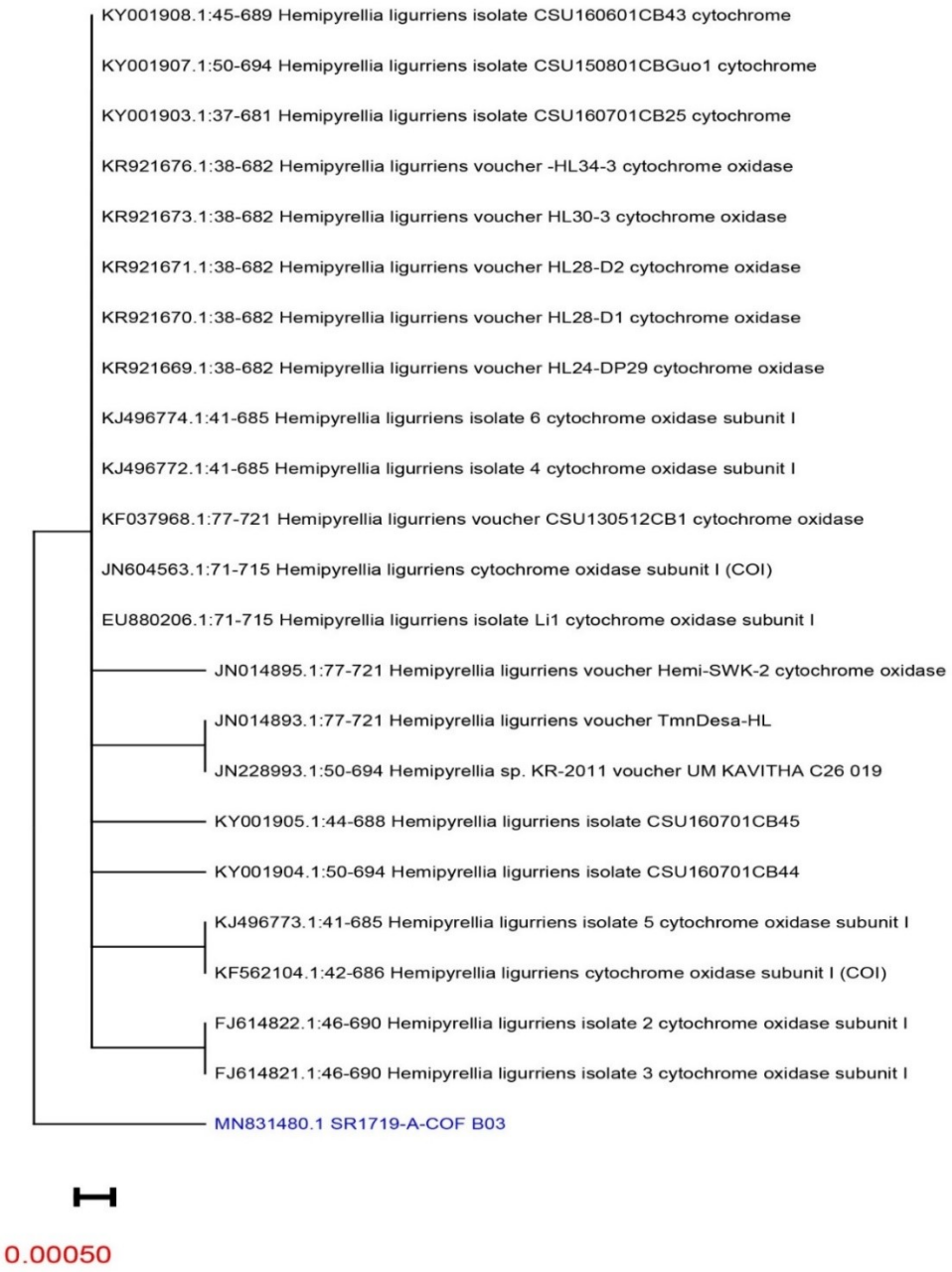



**Fig.4.24. Phylogenetic tree based on COI sequence, *SR1719-A-COF_B03* of *H. ligurriens* by Maximum Likelihood method**

This analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were 648 positions in the final dataset. The phylogenetic analysis confirmed the species identity of *H.ligurriens* with strong boot strap support.

## 4.2. Seasonal differences in blow fly population in Central Kerala

### Abundance

Seasonal differences in the number of adult blow flies, the number of male and female flies and the difference in abundance between seasons and also between years was considered for data analysis.

#### 4.2.1. *Chrysomya megacephala:*

Effect of season on the abundance of *C. megacephala* was found to be significant (F = 52.773; P = 0.01). The abundance of *C. megacephala* was significantly higher in monsoon (70.37 ± 9.52) in comparison with summer (65.33 ± 9.23) and winter (45.56 ± 7.88) (Table 4.1).The interaction between years and seasons were found to be non-significant (F = 1.567; P= 0.419) indicating that seasonal variations were same in all years.

**Table 4.1. Abundance (%) of *C. megacephala* between seasons and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 75.44 ± 8.56 | 65.44 ± 9.68 | 45.56 ± 7.88 | 62.15 ± 9.68 |
| 2020 | 67.67 ± 6.44 | 66.78 ± 8.51 | 42.00 ± 10.15 | 58.81 ± 8.51 |
| 2021 | 68.00 ± 11.67 | 63.78 ± 10.28 | 50.11 ± 9.03 | 60.63 ± 10.28 |
| Overall Season | 70.37 ± 9.52$^{a}$ | 65.33 ± 9.23$^{b}$ | 45.89 ± 9.35$^{c}$ | 60.53 ± 9.23 |
| Between year F-value = 0.879$^{ns}$; (P-value = 0.419)<br>Between season F-value = 52.773**; (P-value < 0.001))<br>Interaction between season and year F-value = 1.567$^{ns}$; (P-value = 0.192) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*

Effect of season on the abundance of *C. megacephala* male flies was found to be significant (F = 6.357; P = 0.003). The abundance of male flies was significantly lower in monsoon (23.90 ± 6.82) in comparison with summer (28.13 ± 5.74) and winter (30.30 ± 7.37) The interaction between years and seasons were found to be non-significant (F =0.557; P= 0.694) indicating that seasonal variations were same in all years (Table 4.2).

**Table 4.2. Abundance (%) of *C. megacephala* male flies between seasons and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 24.56 ± 9.74 | 29.55 ± 5.32 | 29.61 ± 6.78 | 27.91 ± 5.32 |
| 2020 | 21.66 ± 5.03 | 27.39 ± 5.50 | 27.90 ± 6.87 | 25.65 ± 5.50 |
| 2021 | 25.49 ± 4.69 | 27.46 ± 6.71 | 33.39 ± 8.09 | 28.78 ± 6.71 |
| Overall Season | 23.90 ± 6.82$^{b}$ | 28.13 ± 5.74$^{a}$ | 30.30 ± 7.37$^{a}$ | 27.45 ± 5.74 |
| Between year F-value = 1.574$^{ns}$; (P-value = 0.214)<br>Between season F-value = 6.357**; (P-value = 0.003))<br>Interaction between season and year F-value = 0.557$^{ns}$; (P-value = 0.694) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*

Effect of season on the abundance of *C. megacephala* female flies was found to be significant (F = 6.357; P = 0.003). The abundance of female flies was significantly higher in monsoon (76.50 ± 6.18) in comparison with summer (71.21 ± 6.55) and winter (67.94 ± 10.29). The interaction between years and seasons were found to be non-significant (F = 0.557; P = 0.694) indicating that seasonal variations were same in all years. (Table 4.3)

**Table 4.3. Abundance (%) of *C. megacephala* female flies between seasons and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 76.99 ± 8.38 | 68.47 ± 7.17 | 68.60 ± 10.55 | 71.35 ± 7.17 |
| 2020 | 78.02 ± 4.94 | 72.61 ± 5.50 | 71.53 ± 8.18 | 74.06 ± 5.50 |
| 2021 | 74.51 ± 4.69 | 72.54 ± 6.71 | 63.68 ± 11.43 | 70.24 ± 6.71 |
| Overall Season | 76.50 ± 6.18$^{a}$ | 71.21 ± 6.55$^{b}$ | 67.94 ± 10.29$^{b}$ | 71.88 ± 6.55 |
| Between year F-value = 1.574$^{ns}$; (P-value = 0.214)<br>Between season F-value = 6.357**; (P-value = 0.003))<br>Interaction between season and year F-value = 0.557$^{ns}$; (P-value = 0.694) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*

### 4.2.2. *Chrysomya rufifacies:*

Effect of season (F = 4.006; P = 0.022) and year (F = 3.935; P = 0.024) on the abundance of *C. rufifacies* was found to be significant. The abundance of *C. rufifacies* was significantly lower in winter (38.44 ± 8.99) in comparison with monsoon (44.22 ± 7.59)

and summer (43.56 ± 9.38). The abundance was significantly higher in 2021 (45.56 ± 7.91) compared to 2019 (39.48 ± 8.73) and 2020 (41.19 ± 6.46). The interaction between years and seasons were found to be non-significant (F = 1.567; P = 0.419) indicating that season wise variations were same in all years (Table. 4.4).

**Table 4.4. Abundance (%) of *C. rufifacies* between seasons and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 45.44 ± 9.10 | 37.78 ± 8.73 | 35.22 ± 6.83 | $39.48 \pm 8.73^{B}$ |
| 2020 | 42.33 ± 8.03 | 41.78 ± 6.46 | 39.44 ± 9.10 | $41.19 \pm 6.46^{AB}$ |
| 2021 | 44.89 ± 5.82 | 51.11 ± 7.91 | 40.67 ± 10.72 | $45.56 \pm 7.91^{A}$ |
| Overall Season | $44.22 \pm 7.59^{a}$ | $43.56 \pm 9.38^{a}$ | $38.44 \pm 8.99^{b}$ | 42.07 ± 9.38 |
| Between year F-value = 3.935*; (P-value = 0.024)<br>Between season F-value = 4.006*; (P-value = 0.022)<br>Interaction between season and year F-value =$1.890^{ns}$; (P-value = 0.122) | | | | |

* Significant at 0.05 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of season (F = 1.654; P = 0.198), effect of year (F = 2.921; P = 0.060), and the interaction between year and season (F = 0.902; P = 0.468) were found to be non-significant on the abundance of *C. rufifacies* male files. The above results showed that there were no significant variations in the abundance of male flies between seasons and between years (Table 4.5).

**Table 4.5. Abundance (%) of *C. rufifacies* male flies between seasons and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 30.18 ± 6.01 | 27.21 ± 7.17 | 30.35 ± 10.02 | 29.24 ± 7.17 |
| 2020 | 32.81 ± 10.32 | 34.74 ± 8.97 | 34.4 ± 8.9 | 33.98 ± 8.97 |
| 2021 | 28.65 ± 3.74 | 26.42 ± 6.76 | 34.83 ± 5.35 | 29.96 ± 6.76 |
| Overall Season | 30.55 ± 7.16 | 29.45 ± 8.32 | 33.19 ± 8.26 | 31.06 ± 8.32 |
| Between year F-value = $2.921^{ns}$; (P-value = 0.060)<br>Between season F-value = $1.654^{ns}$; (P-value = 0.198)<br>Interaction between season and year F-value = $0.902^{ns}$; (P-value = 0.468) | | | | |

*ns non-significant*

Effect of season (F = 1.654; P = 0.198), effect of year (F = 2.921; P = 0.060), and the interaction between year and season (F = 0.902; P = 0.468) were found to be non-significant on the abundance of *C. rufifacies* female files. The above results showed that there were no significant variations in the abundance of female flies between seasons and between years (Table 4.6).

**Table 4.6. Abundance (%) of *C. rufifacies* female flies between seasons and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 70.11 ± 6.31 | 72.5 ± 6.94 | 72.24 ± 10.98 | 71.61 ± 6.94 |
| 2020 | 67.19 ± 10.32 | 65.85 ± 9.53 | 63.69 ± 7.29 | 65.58 ± 9.53 |
| 2021 | 71.35 ± 3.74 | 69.58 ± 8.68 | 64.97 ± 5.30 | 68.63 ± 8.68 |
| Overall Season | 69.55 ± 7.24 | 69.31 ± 8.58 | 66.96 ± 8.76 | 68.61 ± 8.58 |
| Between year F-value = $2.921^{ns}$; (P-value = 0.060)<br>Between season F-value = $1.654^{ns}$; (P-value = 0.198)<br>Interaction between season and year F-value = $0.902^{ns}$; (P-value = 0.468) | | | | |

*ns non-significant*

### 4.2.3. *Chrysomya chani:*

Effect of season on the abundance of *C. chani* was found to be significant (F = 33.586; P = < 0.001). The abundance of *C. chani* was significantly higher in monsoon (30.19 ± 5.00) in comparison with summer (22.22 ± 5.18) and winter (19.78 ± 4.85) (Table 4.7). The interaction between years and seasons were found to be non-significant (F = 1.814; P = 0.135) indicating that season wise variations were same in all years.

**Table 4.7. Abundance (%) of *C. chani* between seasons and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 33.78 ± 2.99 | 22.78 ± 4.92 | 19.33 ± 4.66 | 25.30 ± 4.92 |
| 2020 | 28.22 ± 5.91 | 22.56 ± 6.27 | 18.22 ± 5.14 | 23.00 ± 6.27 |
| 2021 | 28.56 ± 3.94 | 21.33 ± 4.74 | 21.78 ± 4.55 | 23.89 ± 4.74 |
| Overall Season | $30.19 \pm 5.00^{a}$ | $22.22 \pm 5.18^{b}$ | $19.78 \pm 4.85^{b}$ | 24.06 ± 5.18 |
| Between year F-value = $1.520^{ns}$; (P-value = 0.226)<br>Between season F-value = 33.586**; (P-value < 0.001))<br>Interaction between season and year F-value =$1.814^{ns}$; (P-value = 0.135) | | | | |

** Significant at 0.01 level; *ns non-significant*

*Means having different small letter as superscript differ significantly within a row*

Effect of season (F = 1.446; P = 0.242) and effect of year (F = 1.162; P-value = 0.319), were found to be non-significant on the abundance of *C.chani* male files. The interaction between year and season was found to be significant (F = 2.832; P = 0.031). In the year 2021, abundance of male flies was significantly lower in monsoon (26.99 ± 6.70) compared to summer (36.11 ± 4.86) and winter (36.01 ± 6.29). During winter, the abundance of male flies was significantly higher in the year 2021(36.01 ± 6.29%) in comparison to 2019 (27.00 ± 4.56) and 2020 (28.46 ± 7.61) (Table 4.8).

**Table 4.8. Abundance (%) of *C. chani* male flies between seasons and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 32.06 ± 5.04 | 31.28 ± 7.27 | $27.00 \pm 4.56^{B}$ | 30.11 ± 7.27 |
| 2020 | 32.35 ± 10.84 | 32.64 ± 8.76 | $28.46 \pm 7.61^{B}$ | 31.15 ± 8.76 |
| 2021 | $26.99 \pm 6.70^{b}$ | $36.11 \pm 4.86^{a}$ | $36.01 \pm 6.29^{aA}$ | 33.04 ± 4.86 |
| Overall Season | 30.47 ± 8.01 | 33.34 ± 7.17 | 30.49 ± 7.25 | 31.43 ± 7.17 |
| Between year F-value = $1.162^{ns}$; (P-value = 0.319)<br>Between season F-value = $1.446^{ns}$; (P-value = 0.242)<br>Interaction between season and year F-value =$2.832^{*}$; (P-value = 0.031) | | | | |

* Significant at 0.05 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of season (F = 1.446; P = 0.242) and effect of year (F = 1.162; P = 0.319), were found to be non-significant on the abundance of *C. chani* female files. The interaction between year and season was found to be significant (F = 2.832; P = 0.031). In the year 2021, abundance of female flies was significantly higher in monsoon (73.01 ± 6.70) compared to summer (63.89 ± 4.86) and winter (63.99 ± 6.29). During winter, the abundance of female flies was significantly lower in the year 2021(63.99 ± 6.29%) in comparison to 2019 (73.00 ± 4.56) and 2020 (71.54 ± 7.61) (Table 4.9).

**Table 4.9. Abundance (%) of *C. chani* female flies (%) between seasons and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 67.94 ± 5.04 | 68.72 ± 7.27 | 73.00 ± 4.56$^{A}$ | 69.89 ± 7.27 |
| 2020 | 67.65 ± 10.84 | 67.36 ± 8.76 | 71.54 ± 7.61$^{A}$ | 68.85 ± 8.76 |
| 2021 | 73.01 ± 6.70$^{a}$ | 63.89 ± 4.86$^{b}$ | 63.99 ± 6.29$^{bB}$ | 66.96 ± 4.86 |
| Overall Season | 69.53 ± 8.01 | 66.66 ± 7.17 | 69.51 ± 7.25 | 68.57 ± 7.17 |
| Between year F-value = 1.162$^{ns}$; (P-value = 0.319)<br>Between season F-value = 1.446$^{ns}$; (P-value = 0.242)<br>Interaction between season and year F-value =2.832$^{*}$; (P-value = 0.031) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

### 4.2.4. *Hemipyrellia ligurriens:*

Effect of season on the abundance of *H. ligurriens* was found to be significant (F = 47.470; P = < 0.001). The abundance of *H. ligurriens* was significantly higher in monsoon (30.85 ± 7.42) in comparison with summer (18.56 ± 5.01) and winter (17.63 ± 4.58). The interaction between years and seasons were found to be non-significant (F =2.180; P=0.080) indicating that season wise variations were same in all years (Table 4.10).

**Table 4.10. Abundance (%) of *H. ligurriens* between seasons and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 30.89 ± 5.82 | 15.33 ± 3.08 | 15.89 ± 4.40 | 20.7 ± 3.08 |
| 2020 | 34.11 ± 8.72 | 19.22 ± 4.99 | 17.89 ± 4.86 | 23.74 ± 4.99 |
| 2021 | 27.56 ± 6.67 | 21.11 ± 5.26 | 19.11 ± 4.4 | 22.59 ± 5.26 |
| Overall Season | 30.85 ± 7.42$^{a}$ | 18.56 ± 5.01$^{b}$ | 17.63 ± 4.58$^{b}$ | 22.35 ± 5.01 |
| Between year F-value = 2.049$^{ns}$; (P-value = 0.136)<br>Between season F-value = 47.470**; (P-value < 0.001)<br>Interaction between season and year F-value = 2.180$^{ns}$; (P-value = 0.080) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*

Effect of season (F = 2.251; P = 0.113), effect of year (F = 0.587; P = 0.559), and the interaction between year and season (F = 1.421; P = 0.236) were found to be non-significant on the abundance of *H. ligurriens* male files. The above results showed that

there were no significant variations in the abundance of *H. ligurriens* male flies between seasons and between years (Table 4.11).

**Table 4.11. Abundance (%) of *H. ligurriens* male flies between season and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 36.95 ± 8.45 | 31.21 ± 8.23 | 26.18 ± 7.9 | 31.44 ± 8.23 |
| 2020 | 33.35 ± 7.08 | 35.17 ± 5.13 | 33.04 ± 10.6 | 33.85 ± 5.13 |
| 2021 | 35.44 ± 7.16 | 29.98 ± 11.11 | 32.64 ± 6.04 | 32.69 ± 11.11 |
| Overall Season | 35.25 ± 7.44 | 32.12 ± 8.49 | 30.62 ± 8.67 | 32.66 ± 8.49 |
| Between year F-value = $0.587^{ns}$; (P-value = 0.559)<br>Between season F-value = $2.251^{ns}$; (P-value = 0.113)<br>Interaction between season and year F-value = $1.421^{ns}$; (P-value = 0.236) | | | | |

*ns non-significant*

Effect of season (F = 2.251; P = 0.113), effect of year (F = 0.587; P = 0.559), and the interaction between year and season (F = 1.421; P = 0.236) were found to be non-significant on the abundance of *H.ligurriens* female files. The above results showed that there were no significant variations in the abundance of *H.ligurriens* female flies between seasons and between years (Table 4.12).

**Table 4.12. Abundance (%) of *H. ligurriens* female flies between season and between years**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 66.23 ± 7.66 | 68.79 ± 8.23 | 73.82 ± 7.9 | 69.61 ± 8.23 |
| 2020 | 66.65 ± 7.08 | 66.28 ± 7.79 | 66.96 ± 10.6 | 66.63 ± 7.79 |
| 2021 | 64.56 ± 7.16 | 70.02 ± 11.11 | 67.36 ± 6.04 | 67.31 ± 11.11 |
| Overall Season | 65.81 ± 7.08 | 68.36 ± 8.94 | 69.38 ± 8.67 | 67.85 ± 8.94 |
| Between year F-value = $0.587^{ns}$; (P-value = 0.559)<br>Between season F-value = $2.251^{ns}$; (P-value = 0.113)<br>Interaction between season and year F-value = $1.421^{ns}$; (P-value = 0.236) | | | | |

*ns non-significant*

### 4.3. Life history of Blow flies

#### 4.3.1. *Chrysomya megacephala*

**Morphology of eggs and larval instars**

**Egg**

Creamy white.The caudal end slightly wider than the anterior end and oblong (Fig. 4.25. A).

**Larvae**

In total, there are three larval instars including a post feeding stage. All instars have a clearly defined anterior cephalopharyngeal sclerite, three thoracic segments and eight abdominal segments.

**First instar**

Larvae whitish cream. Backwardly directed acuminate spines with dark pigmentation at the tips were present on the anterior and posterior margins of the ventral and lateral surfaces of all the three thoracic segments. Anterior spinous bands are 4-5 in number and posterior spinous bands are narrow and 2-3 in number (Fig. 4.25. B).

Cephalopharyngeal skeleton is incompletely developed with no uniform sclerotisation. Dense pigmentation is present on the dorsal cornua and it is long, pointed and slightly curved (Fig. 4.26. A).

Anterior spiracles not developed completely. Two slits were present on the posterior spiracles and were brown in colour (Fig. 4.26. B).

**Second instar**

The second instar larvae were muscoid, vermiform, pointed anteriorly and blunt posteriorly. Anal papillae are prominent with a broad conical base especially in the outer

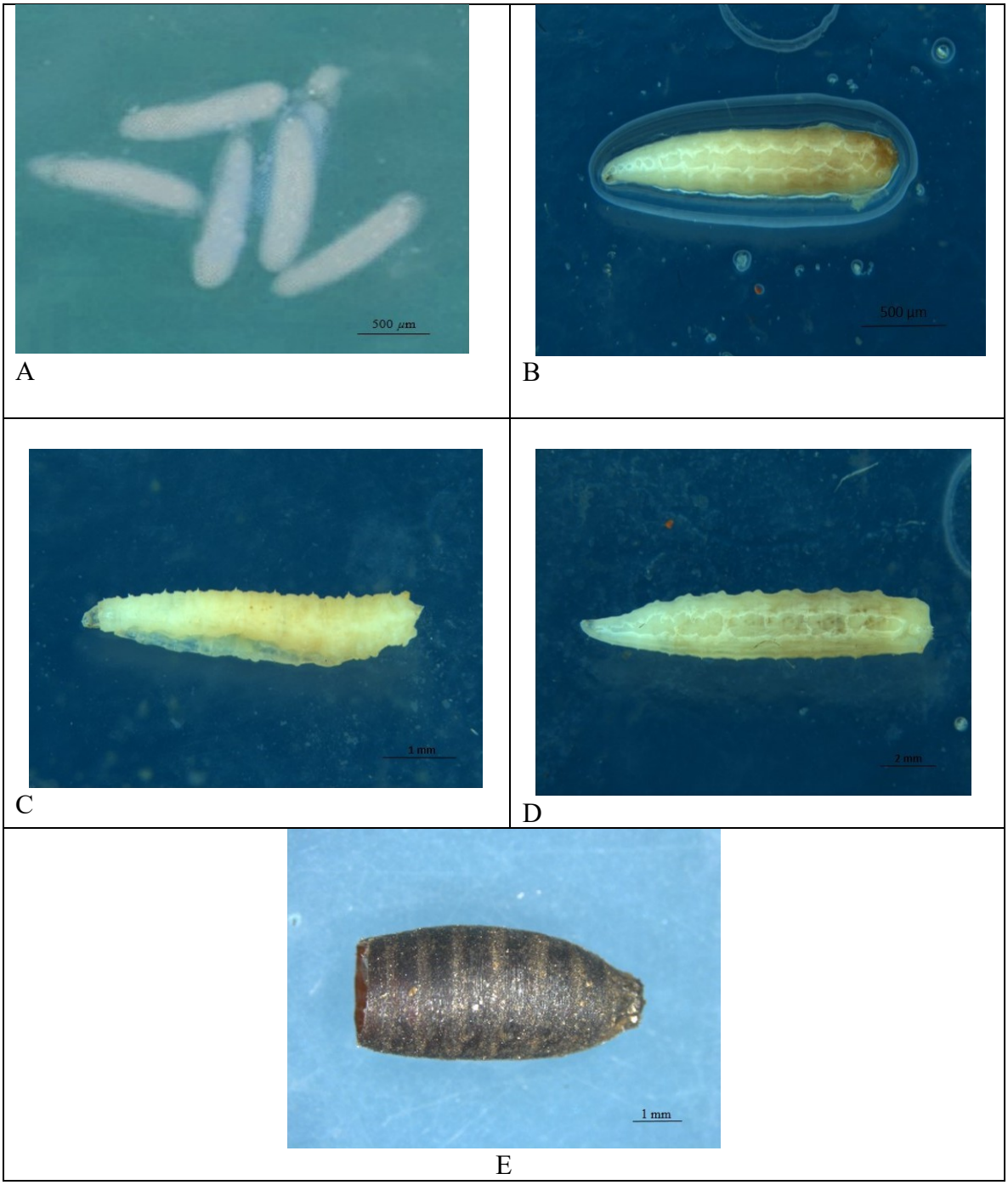



**Fig. 4.25. Eggs, different larval instars and pupal case of *C. megacephala***

**(A) Eggs (B) First Instar (C) Second Instar (D) Third Instar (E) Pupal case**

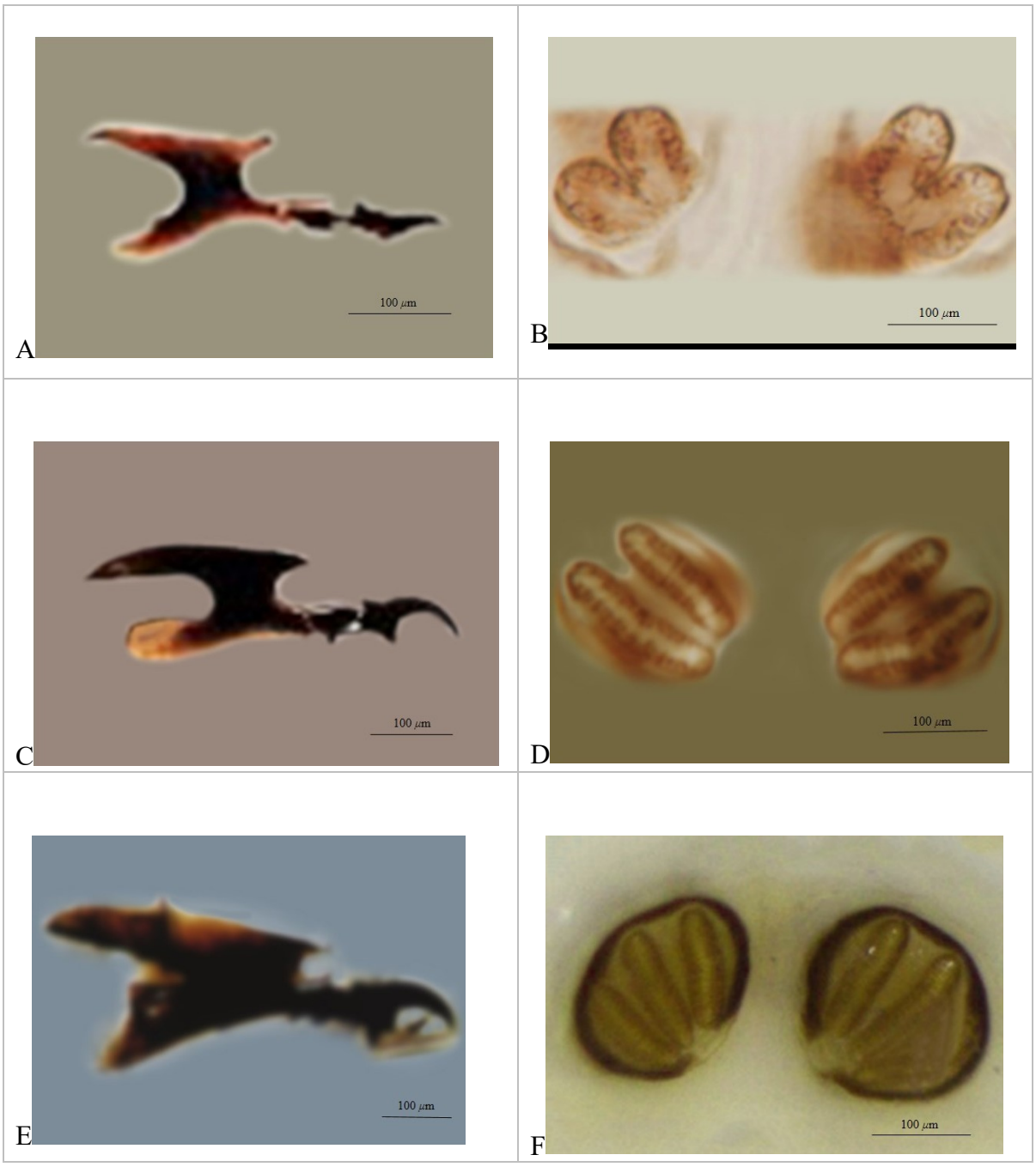



**Fig. 4.26. Cephalopharyngeal skeleton and posterior spiracle of *C. megacephala* larvae (A, B) 1st instar (C, D) 2nd instar (E, F) 3rd instar**

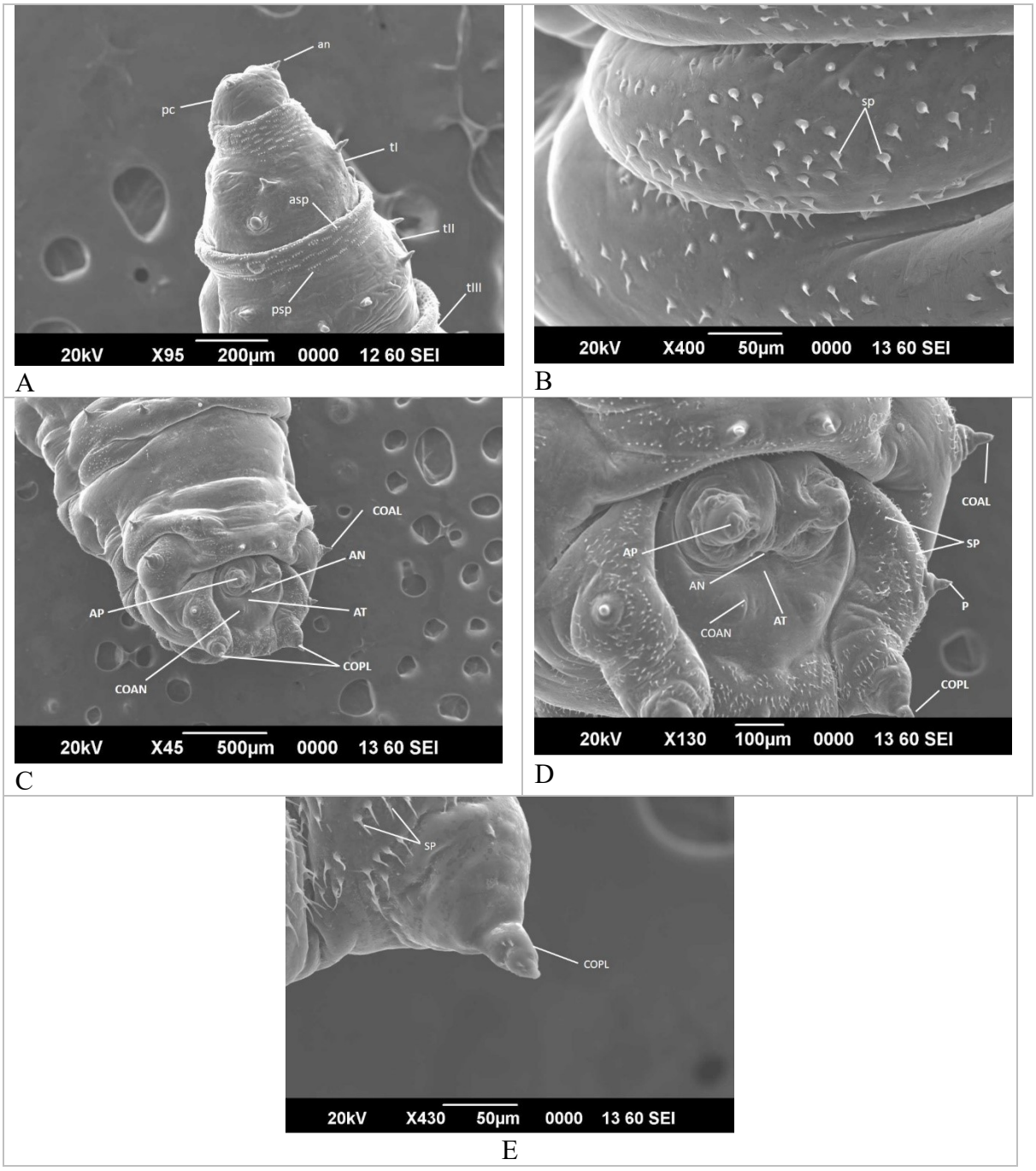



**Fig. 4.27. SEM micrographs of first instar larvae of *C. megacephala***

**A) pseudocephalon showing antennal complex (an), and anterior spinous process (asp) of second thoracic segment, thoracic segments (t I - t III) B) spines on thorax (sp), C) & D) anal segment displaying anterolateral cones (coal), postero lateral cones (copl), anus (an), anal cone (coan), anal process (ap), anal tubercle (at), spinous process (sp), E) anal segment displaying posterolateral cones (copl) and spinous process (sp)**

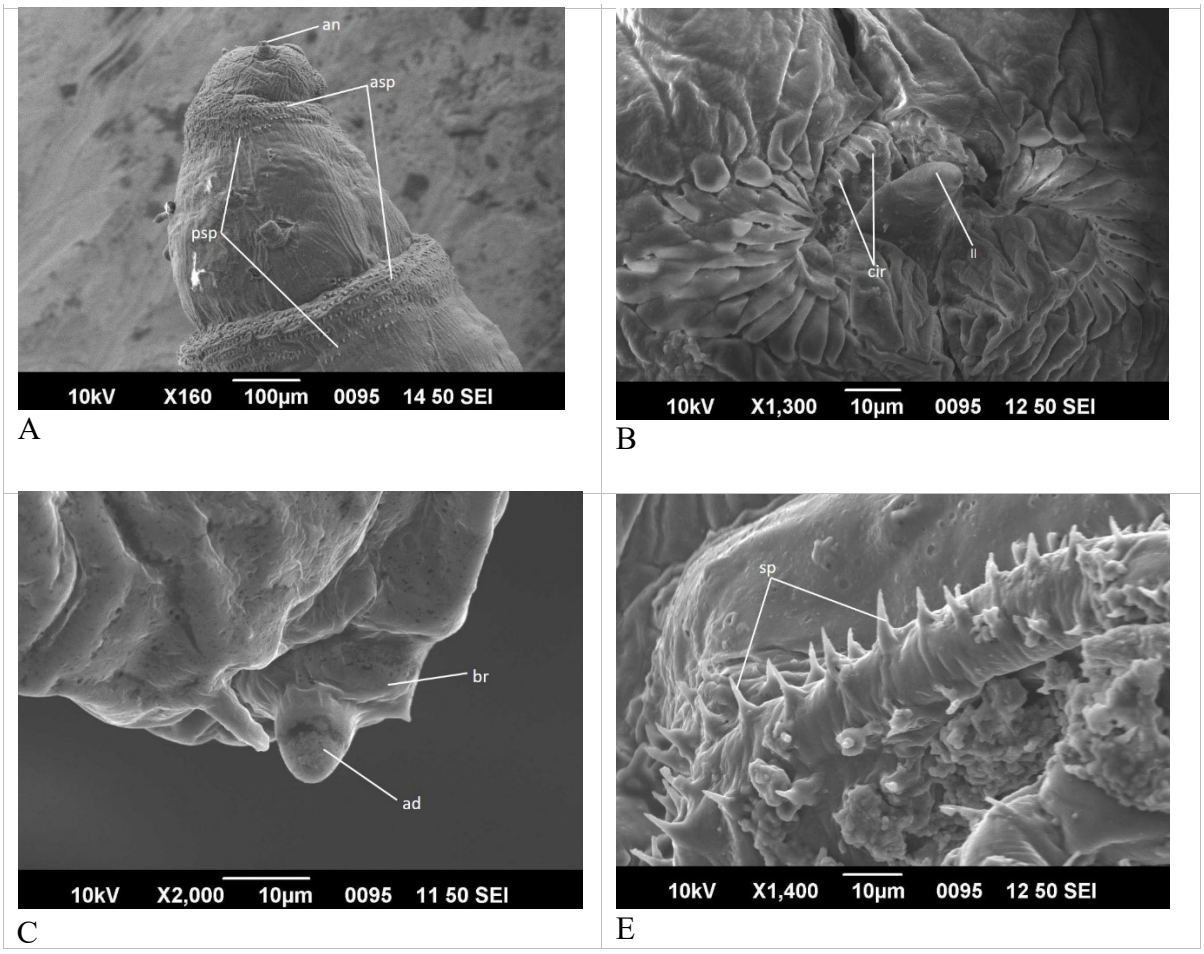



**Fig .4.28. SEM micrographs of second instar larvae of *C. megacephala***

**A) pseudocephalon showing antennal complex (an), anterior and posterior spinous process (asp & psp) B) pseudocephalon showing cirri (cr) and labial lobe (ll) C) pseudocephalon showing antennal complex having antennae (an) and basal ring (br) D) spines with flat base and sharp tips between first an d second thoracic segments (sp)**

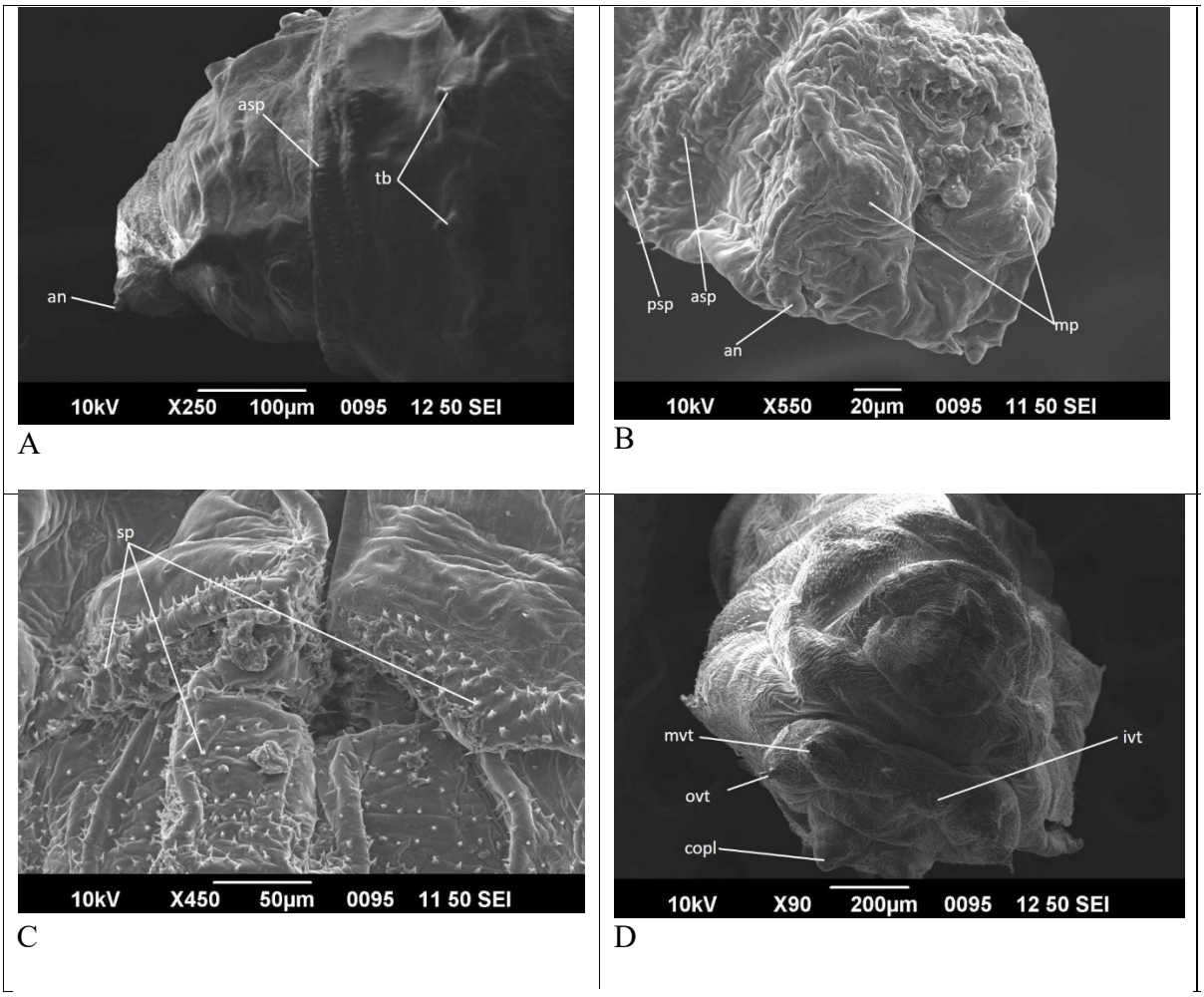
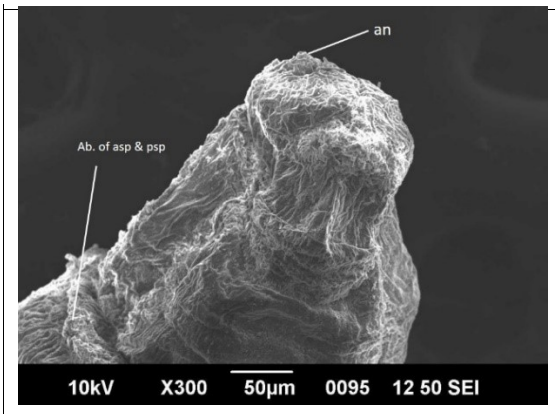
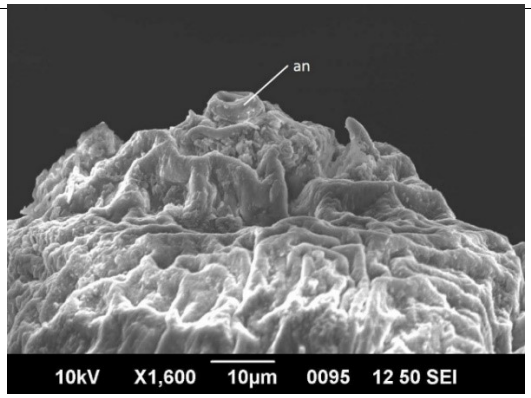



**Fig.4.29. SEM micrographs of third instar larvae of *C. megacephala***

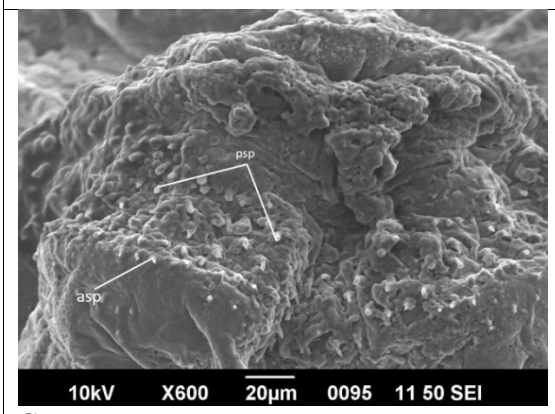
**A) pseudocephalon showing antennal complex (an), anterior spinous process (asp) and tubercles (tb) B) pseudocephalon showing antennal complex (an), maxillary palpus (mp), anterior and posterior spinous process (asp & psp) of the first and second thoracic segment C) spines with flat broad base and sharp tips on first thoracic segment (sp) D) anal segment displaying inner, medial and outer ventral tubercles (ivt, mvt & ovt), postero lateral cones(copl)**



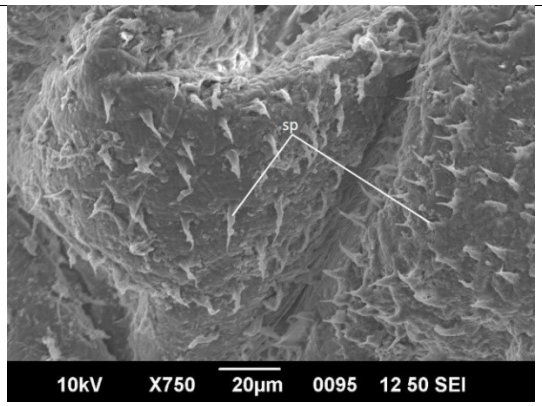

A



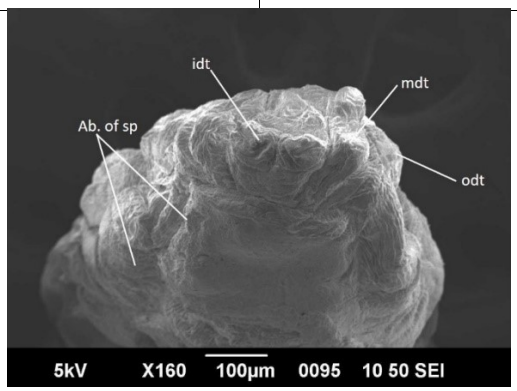

B




C




D




E

**Fig.4.30. SEM micrographs of post feeding stage of *C. megacephala***

**A) pseudocephalon showing antennal complex (an), rudimentary anterior and posterior spinous process (asp & psp) B) pseudocephalon showing rudimentary antennal complex (an), C) rudimentary anterior and posterior spines (asp & psp) with flat broad base and sharp tips on first thoracic segment D) third and fouth thoracic segments showing slender filiform spines (sp) E) anal segment displaying inner, middle and outer dorsal tubercles (idt, mdt & odt), rudimentary spines (sp)**

dorsal and outer ventral papillae. Papillae were surrounded by numerous microtrichia. Spinous pattern on thorax is similar as that of the first instar. (Fig. 4.25.C)

Cephalopharyngeal skeleton pigmentation is uniform. Postero dorsal process projected upwards. Dorsal cornua is pointed and long and is structurally similar to the first instar larva but with larger size. The ventral cornua is shorter than the dorsal cornua (Fig. 4.26. C).

Anterior spiracles are yellow while the posterior spiracles are deep brown in colour with two spiracular slits (Fig. 4.26. D).

**Third instar**

Cream to light yellow in colour. All segments from 2-11 are with spinous bands. The spines acuminate and were arranged singly in rows and have dark points on the tips. The spinous bands were found to be restricted to the lateral and ventral surfaces. The middle dorsal tubercles were moderately sized in comparison to the inner and outer tubercles. The lateral, ventral and dorsal tubercles were large and found to be fully developed (Fig. 4.25. D).

Cephalopharyngeal skeleton pigmentation is darker. Dorsal sclerite comma shaped and prominent. Dorsal cornua reduced in length and with uniform width and longer than the ventral cornua. Parastomal and accessory sclerites and anterodorsal process were present (Fig. 4.26. E).

Posterior spiracles were clearly seen with three spiracular slits. A dark pigmented incomplete peritreme was seen surrounding the three slits with a bent in the middle slit (Fig. 4.26. F).

Puparium is brown in colour (Fig. 4.25.E)

**Morphology of larval instars using SEM**

**First Instars**

Shape was muscoid having 12 segments. Dorsal organs and terminal organs were present in pairs. Mouth hooks branched with three to four rows of curved sharp tipped spines. Posterior spiracular discs were present in the caudal segment with two slits. The spiracular regions surrounded with fine spiracular hairs (Fig. 4.27).

**Second instar**

The ultra structural details were mostly similar to that of the first instar. The antennal dome and maxillary palpii were similar to the first instar. The labium and mouth hooks were well developed. Three lobes were present in the labium. The sensillae in the terminal organs were seen as two separate groups. The caudal segment have post spiracular discs with two spiracular slits. The slits were surrounded by fine spiracular hairs (Fig. 4.28).

**Third instar**

Body size relatively large. The ultra structural details were almost similar to the second instar. The posterior spiracular discs were positioned in a depression with three slits (Fig. 4.29).

**Post feeding stage**

Stage showed a rudimentary antennal complex, maxillary process, anterior and posterior spinous process (asp & psp) of the second thoracic segment, short spines with flat broad base and sharp tips on the first thoracic segment and rudimentary spines on the anal segment (Fig. 4.30).

**Life cycle**

Seasonal life cycle data is provided in Appendix III.

**Mating**

In the pure culture studies conducted, it was found that the adult flies started mating from the 3rd day to the 8th day of emergence. The duration of mating was seen as 10 ± 3 minutes.

**Fecundity**

The mature female fly laid an average of 345.48 ± 26.09 eggs in a day on the decomposing meat. The sites chosen to lay eggs on the meat were small foldings, gaps / crevices on the meat. The preoviposition period of the female flies was found to be 9.37 ± 1.15 days after mating. The next batches of eggs were laid after an interval of 4.39 ± 0.38 days. An average of 2485.44 ± 257.9 eggs were laid by the fly during its life span. The fly stopped laying eggs by 52nd day. The egg took an average of 18 hrs for hatching (Table 4. 13 – 4. 16).

**Development of Larvae and Pupae**

**First Instar**

The average length of the first instar was 1.5 ± 0.1 mm and the average dry weight was 1.47 ± 0.12 mg. The first ecdysis was completed after 17 hrs of growth. The cuticle was found to be loosening approximately one to two hours before the ecdysis. (Table 4.17, 4.22 and 4.28).

**Second Instar**

The beginning of the ecdysis was seen approximately 3 to 4 hours before the actual process. The average length of the second instar was 5.14 ± 1.32 mm and the

average dry weight was 10.19 ± 0.50 mg. The second moulting was completed after 22 hours (Table 4.18, 4.23 and 4.28).

**Third Instar**

This stage took 40 hours to enter into the post feeding stage. Till then, third instars were found to be feeding on the meat. But even after attaining the maximum length, the larval instars were found to be present on the meat. The average length of the third instar was 10.91 ± 1.02 mm and the average dry weight was 30.71 ± 1.09 mg (Table 4.19, 4.24 and 4.28).

**Post feeding stage**

This non-feeding stage is characterized by shortening of body length. Larva spent 30 hours in this stage before pupation. The average length of the post feeding stage was 10.60 ± 0.44 and the average dry weight was 29.63 ± 0.78 mg (Table 4.20, 4.25 and 4.28).

**Pupa**

The average period of pupation was 99 hours. The colour of the pupae was greyish black. The average length and width of the pupa was 6.8 mm and 3 mm respectively. The anterior end of the pupae were found to be split and through this slit the adult fly emerges (Table 4.28).

**Adult fly**

The flies were found to be emerging from the pupae during day time slowly with folded wings with dull white colour over the thorax and wings. The female flies live for about 69 days where as the males have lesser longevity and live for only 16 days. The average length of the fly was 9-10mm.

**Total life cycle period**

The total life cycle period from egg till the emergence of adult fly was found to be 227 ± 59 hours (Table 4.28)

**Survival**

The survival distribution was studied for all life stages of the fly from the egg stage till the emergence of the adult fly. The stage specific survival rates were 86.32 ± 6.50%, 84.22 ± 7.27%, 75.98 ± 8.03%, 69.26 ± 4.82% and 69.4 ± 5.38% for egg, first instar, second instar, third instar, and pupa respectively. Average survival rate of *C. megacephala* was 77.04 ± 9.65% (Table 4. 29 – 4. 31).

**4.3.2. *Chrysomya rufifacies***

**Morphology of eggs and larval instars**

**Egg**

Creamish white in colour.The caudal end was slightly wider than the anterior end and oblong (Fig. 4.31 A).

**Larvae**

In total there are three larval instars including a post feeding stage. All instars have a clearly defined anterior cephalopharyngeal sclerite, three thoracic segments and eight abdominal segments.

**First instar**

Larvae whitish cream in colour. Backwardly directed acuminate spines with dark pigmentation at the tips were present on the anterior and posterior margins of the ventral

and lateral surfaces of all the three thoracic segments. Anterior spinous bands are 4-5 in number and posterior spinous bands are narrow and 2-3 in number (Fig. 4.31. B).

Cephalopharyngeal skeleton incompletely developed and with no uniform sclerotisation. Dense pigmentation is present on the dorsal cornua and it is long, pointed and slightly curved (Fig. 4.32. A).

Anterior spiracles are not developed completely. Two slits were present on the posterior spiracles and were brown in colour (Fig. 4.32. B).

**Second instar**

The second instar larvae were muscoid, vermiform, pointed anteriorly and blunt posteriorly. Anal papillae are prominent with a broad conical base especially in the outer dorsal and outer ventral papillae. These papillae were surrounded by numerous microtrichia. Spinous pattern on thorax is similar as that of the first instar. (Fig. 4.31. C).

Cephalopharyngeal skeleton pigmentation is uniform. Postero dorsal process projected upwards. Dorsal cornua is pointed and long and is structurally similar to the first instar larva but with larger size. The ventral cornua is shorter than the dorsal cornua (Fig. 4.32. C).

Anterior spiracles are yellow while the posterior spiracles are deep brown in colour with two spiracular slits (Fig. 4.32. D).

**Third instar**

Creamish yellow in colour and muscoid in shape. All segments from 2-11 are with spinous bands. The body was hairy with long and stout tubercles on all segments. The tubercles had a broad base and tapered with pointed spines at the tip. The spines were

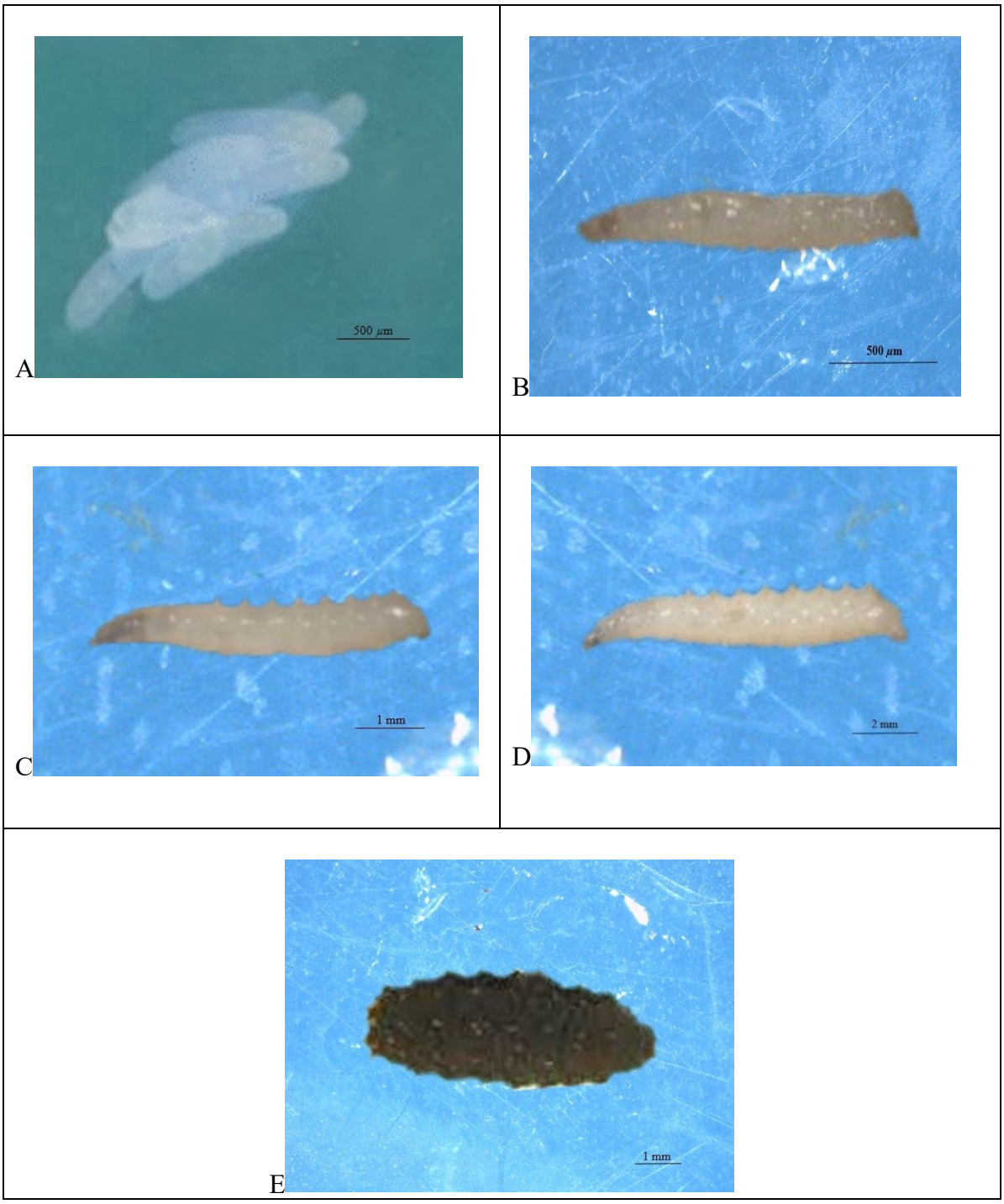



**Fig. 4.31. Eggs, different larval instars and pupa of *C. rufifacies***

**(A) Eggs (B) First Instar (C) Second Instar (D) Third Instar (E) Pupa**

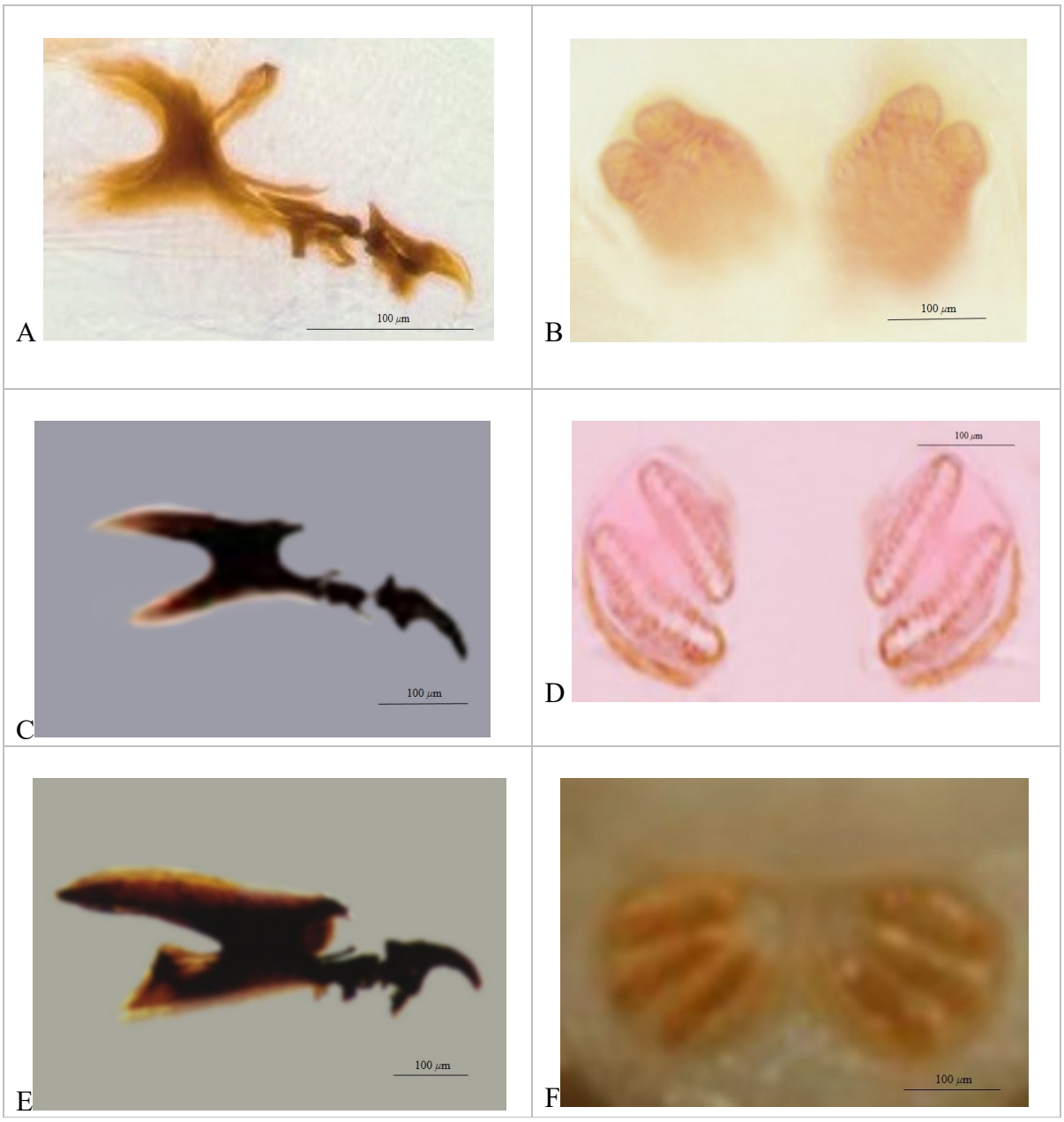
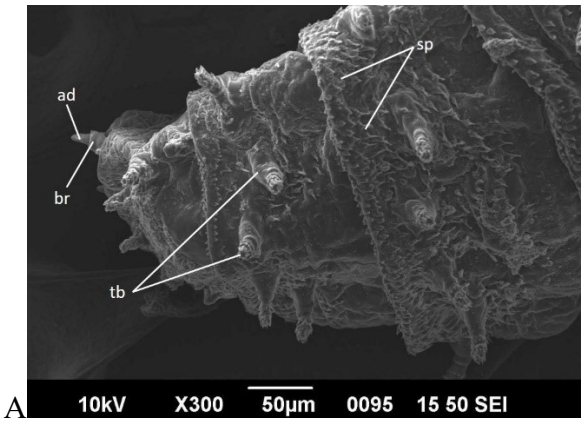
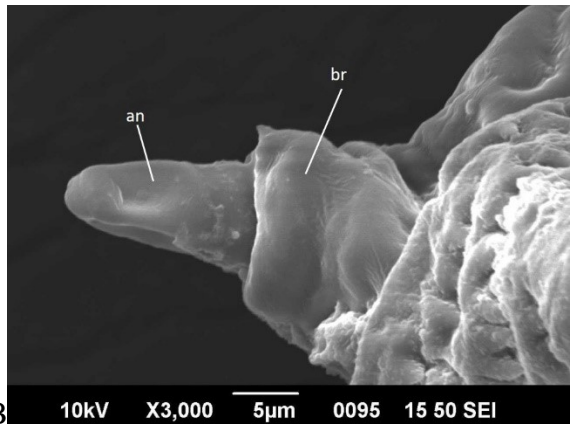



**Fig. 4.32. Cephalopharyngeal skeleton and posterior spiracle of *C. rufifacies* larvae**

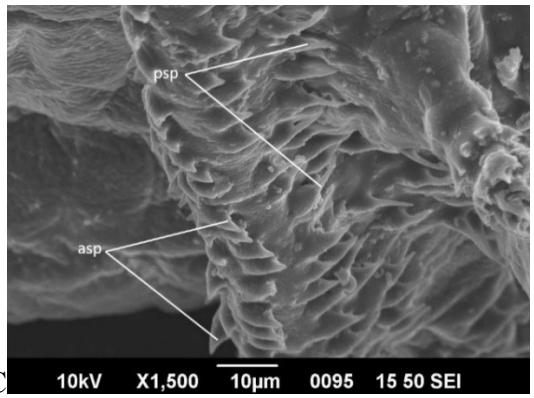
**(A, B) 1st instar (C, D) 2nd instar (E, F) 3rd instar**



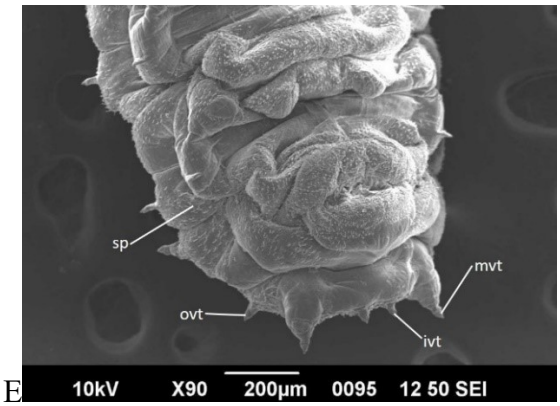

A




B




C




E

**Fig. 4.33. SEM micrographs of first instar of *C. rufifacies***

**A) pseudocephalon showing antennal complex (an), spinous process of second thoracic segment (sp), long tubercles on body (tb) B) antennal complex showing antenna (an), basal ring (br) C) spines with flat broad triangular with less sharp tips on first thoracic segment (sp) D) anal segment displaying inner, medial and outer ventral tubercles (ivt, mvt & ovt), spinous process (sp)**

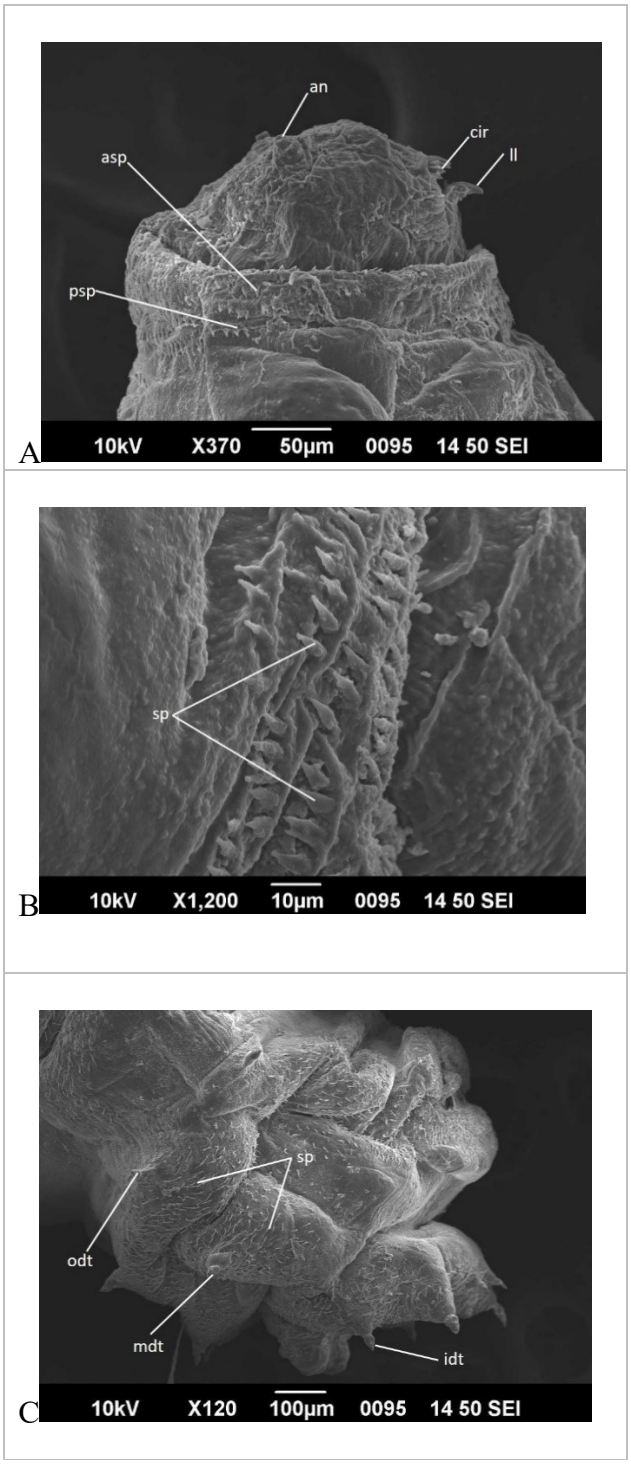
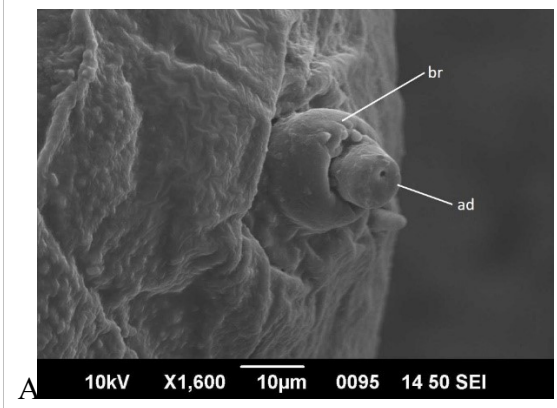
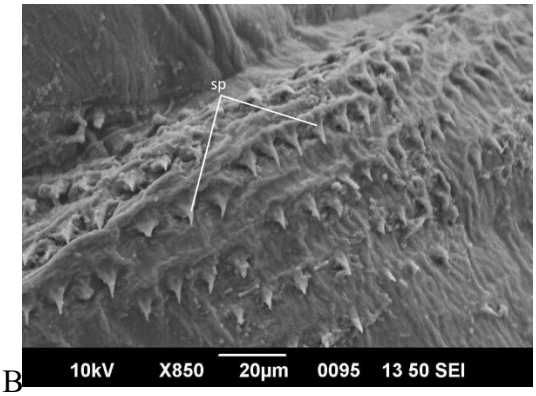



**Fig. 4.34. SEM micrographs of second instar of *C. rufifacies***

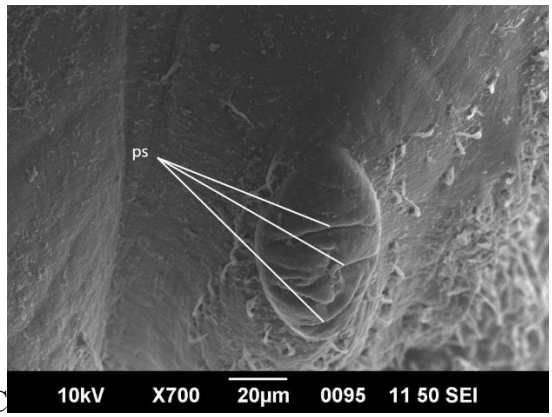
**A) pseudocephalon showing antennal complex (an), cirri (cr) and labial lobe (ll), anterior and posterior spinous process (asp & psp) of the first and second thoracic segment B) spines with flat broad triangular with less sharp tips on second thoracic segment (sp) C) anal segment displaying inner, medial and outer ventral tubercles (ivt, mvt & ovt), spinous process (sp)**



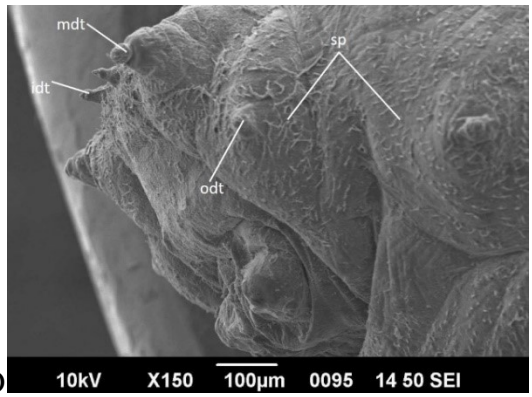

A




B




C




D

**Fig. 4.35. SEM micrographs of third instar of *C. rufifacies***

**A) pseudocephalon showing antennal complex showing antenna (an), basal ring (br) B) flat broad triangular spines with less sharp tips on second thoracic segment (sp) C) anal segment showing posterior spiracles (ps) D) anal segment displaying inner, medial and outer dorsal tubercles (idt, mdt & odt), spinous process (sp)**

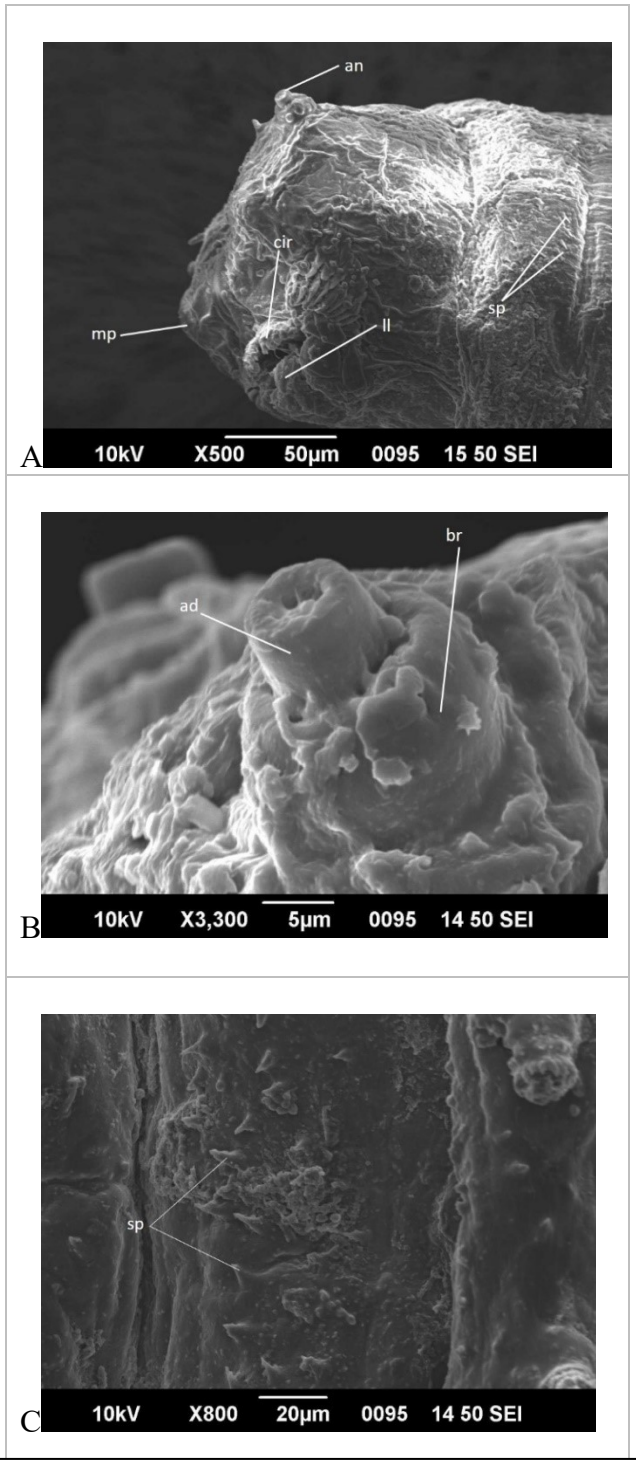



**Fig. 4.36. SEM micrographs of post feeding instar of *C. rufifacies***

**A) pseudocephalon showing, antennal dome (an), maxillary process (mp), rudimentary cirri (cir), rudimentary labial lobe (ll), spinous process (sp) of the second thoracic segment B) pseudocephalon showing rudimentary antennal complex antenna (an), basal ring (br) C)rudimentary spines second thoracic segment (sp)**

arranged singly in rows and were having dark points on the tips. Spines were present on the anterior and posterior margins on the ventral and lateral surfaces of all the three thoracic segments. The middle, inner and outer dorsal tubercles, middle, inner and outer ventral tubercles were found to be fully developed. Outer dorsal tubercles were prominent (Fig. 4.31. D).

Cephalopharyngeal skeleton pigmentation is darker. Dorsal sclerite comma shaped and prominent. Dorsal cornua reduced in length and with uniform width. The ventral and dorsal cornua were smaller in size. Parastomal and accessory sclerites were absent. Anterodorsal process was present (Fig. 4.32. E).

Posterior spiracles were clearly seen with three spiracular slits. Densely dark pigmented incomplete peritreme was seen surrounding the three slits with a medial bent in the middle slit (Fig. 4.32. F).

Puparium is brown in colour (Fig. 4.31. E).

**Morphology of larval instars using SEM**

**First instar**

Shape was muscoid. Dorsal organs were represented by a pair of antennal domes. Terminal organs were represented by a pair of maxillay palpii. Papillae were present as two separate groups. Oral cirri were curved spines and present in rows. Six to eight rows of pointed spinous process were seen on the demarcating areas between prothorax and metathorax. Six pairs of long tubercles with slender spines were present on the anal segment (Fig. 4.33).

**Second instar**

Shape was muscoid and similar to first instar. Antennal dome and maxillary palpii were well developed. Labium was trilobed. Pointed spines were present on the junction between the neighbouring body segments. Thick long tubercles were present on body segments with fine spines at the tip. Six pairs of well-developed tubercles were present on the anal segment (Fig. 4.34).

**Third instar**

Shape was vermiform and muscoid similar to second instar. Labium was not trilobed.Tubercles were elongated and present all over the body except on the pseudocephalon and anal segments. Tubercles were thicker at the bases and terminated to a narrow end with many sharp tipped fine spines. Antennal dome and terminal organs were well developed. The dorsal organs were seen present on the dorsolateral aspect of the terminal organ. The sensillae in the terminal organ were similar to the second instar. Three to four rows of anterior and posterior spines were present on the inter segmental junctions. Six pairs of well-developed marginal tubercles were present on the anal segment. Broad posterior spiracular hairs were present (Fig. 4.35).

**Post feeding stage**

Spines on the thorax, antennal dome, terminal organs, oral cirri and tubercles were rudimentary in nature. (Fig. 4.36).

**Life Cycle**

Seasonal life cycle data is provided in Appendix III.

**Mating**

In the pure culture studies conducted, it was found that the adult flies started mating from the 3rd day to 7th day of emergence. The duration of mating was seen as 9 ± 3 minutes.

**Fecundity**

The mature female fly laid average of 247.74 ± 28.43 eggs in a day on the decomposing meat. The sites chosen to lay eggs on the meat were small folding, gaps / crevices on the meat. The preoviposition period of the female flies was found to be 8.15 ± 0.99 days after mating. The next batches of eggs were laid after an interval of 4.44 ± 0.38 days. An average of 1842.26 ± 97.99 eggs were laid by the fly during its life span. The fly stopped laying eggs by 39th day. The egg took an average of 16 hrs for hatching (Table 4. 33 - 4. 36).

**Development of Larvae and Pupae**

**First Instar**

The average length of the first instar was 1.66 ± 0.20 mm and the average dry weight was 1.53 ± 0.07 mg. The first ecdysis was completed after 19 hours of growth. The cuticle was found to be loosening approximately one to two hours before the ecdysis (Table 4. 37, 4. 42 and 4. 48).

**Second Instar**

The beginning of the ecdysis was seen approximately 3 to 4 hours before the actual process. The average length of the second instar was 5.05 ± 1mm and the average

dry weight was 10.22 ± 0.84 mg. The second moulting was completed after 23 hours (Table 4. 38, 4. 43 and 4. 48).

**Third Instar**

This stage took 37 hours to enter into the post feeding stage. Till then, the third instars were found to be feeding on the meat. But even after attaining the maximum length, the larval instars were found to be present on the meat. The average length of the third instar was 10.61 ± 0.51 mm and the average dry weight was 31.49 ± 1.29 mg (Table 4. 39, 4. 44 and 4. 48).

**Post feeding stage**

This non feeding stage is characterized by shortening of body length. Larva spent 27 hours in this stage before pupation. The average length of the post feeding stage was 10.61 ± 0.43 mm and the average dry weight was 29.96 ± 1.01 mg (Table 4. 40, 4. 45 and 4. 48).

**Pupa**

The average period of pupation was 91 hours. The colour of the pupae was greyish black. The average length and width of the pupa were 6.7 mm & 3 mm respectively. The anterior end of the pupae were found to be split and through this slit the adult fly emerges (Table 4. 48,).

**Adult fly**

The flies were found to be emerging from the pupae during day time slowly with folded wings with dull white colour over the thorax and wings. The female flies live for about 71 days where as the males have lesser longevity and live for only 18 days. The average length of the fly was 10-11 mm.

**Total life cycle period**

The total life cycle period from egg till the emergence of adult fly was found to be 212.78 ± 8.98 hours (Table 4. 48).

**Survival**

The survival distribution was studied for all life stages of the fly from the egg stage till the emergence of the adult fly. The stage specific survival rates were 82.47 ± 5.45 %, 81.90 ± 6.16 %, 76.03 ± 4.66 % , 72.27 ± 5.92% and 72.33 ± 6.14 % for egg, first instar, second instar, third instar, and pupa respectively. Average survival rate of *C. rufifacies* was 77.00 ± 7.17% (Table 4.49 – 4.51).

### 4.3.3. *Chrysomya chani*

**Morphology of eggs and larval instars**

**Egg**

Creamish white in colour. The caudal end was slightly wider than the anterior end and generally it was oblong (Fig. 4.37. A).

**Larvae**

In total, there are three larval instars including a post feeding stage. All instars have a clearly defined anterior cephalopharyngeal sclerite, three thoracic segments and eight abdominal segments.

**First instar**

Larvae whitish cream in colour.Backwardly directed acuminate spines with dark pigmentation at the tips were present on the anterior and posterior margins of the ventral

and lateral surfaces of all the three thoracic segments. Anterior spinous bands are 4-5 in number and posterior spinous bands are narrow and 2-3 in number (Fig. 4.37. B).

Cephalopharyngeal skeleton incompletely developed with no uniform sclerotisation. Dense pigmentation present on the dorsal cornua and it is long, pointed and slightly curved. (Fig. 4.38. A).

Anterior spiracles not developed completely. Two slits present on the posterior spiracles and brown in colour (Fig. 4.38. B).

**Second instar**

The second instar larvae muscoid, vermiform, pointed anteriorly and blunt posteriorly. Anal papillae prominent with a broad conical base especially in the outer dorsal and outer ventral papillae. Papillae surrounded by numerous microtrichia. Spinous pattern on thorax is similar as that of the first instar. (Fig. 4.37. C).

Cephalopharyngeal skeleton pigmentation is darker. Dorsal sclerite comma shaped and prominent. Dorsal cornua reduced in length and with uniform width. Dorsal cornua is longer than the ventral cornua. Parastomal and accessory sclerites are present. Anterodorsal process is present (Fig. 4.38. C).

Anterior spiracles are yellow and posterior spiracles were brown in colour with two slits (Fig. 4.38. D).

**Third instar**

Cream to light yellow in colour. All segments from 2-11 were with spinous bands. Characteristic acuminate spines present on the first and second thoracic segments were clearly visible. The spinous bands were found to be restricted to the lateral and ventral

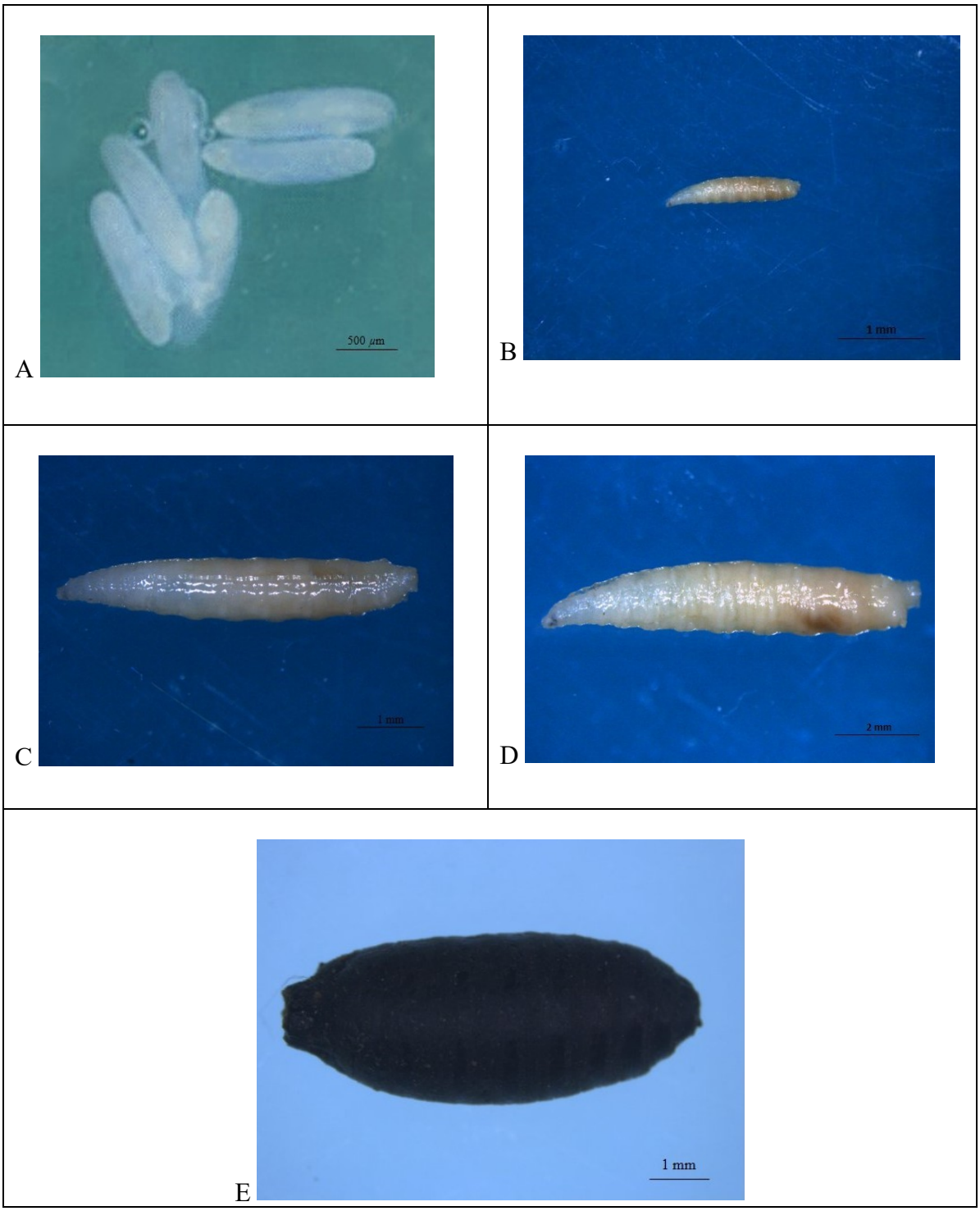



**Fig. 4.37. Eggs, different larval instars and pupa of *C. chani***

**(A) Eggs (B) First Instar (C) Second Instar (D) Third Instar (E) Pupa**

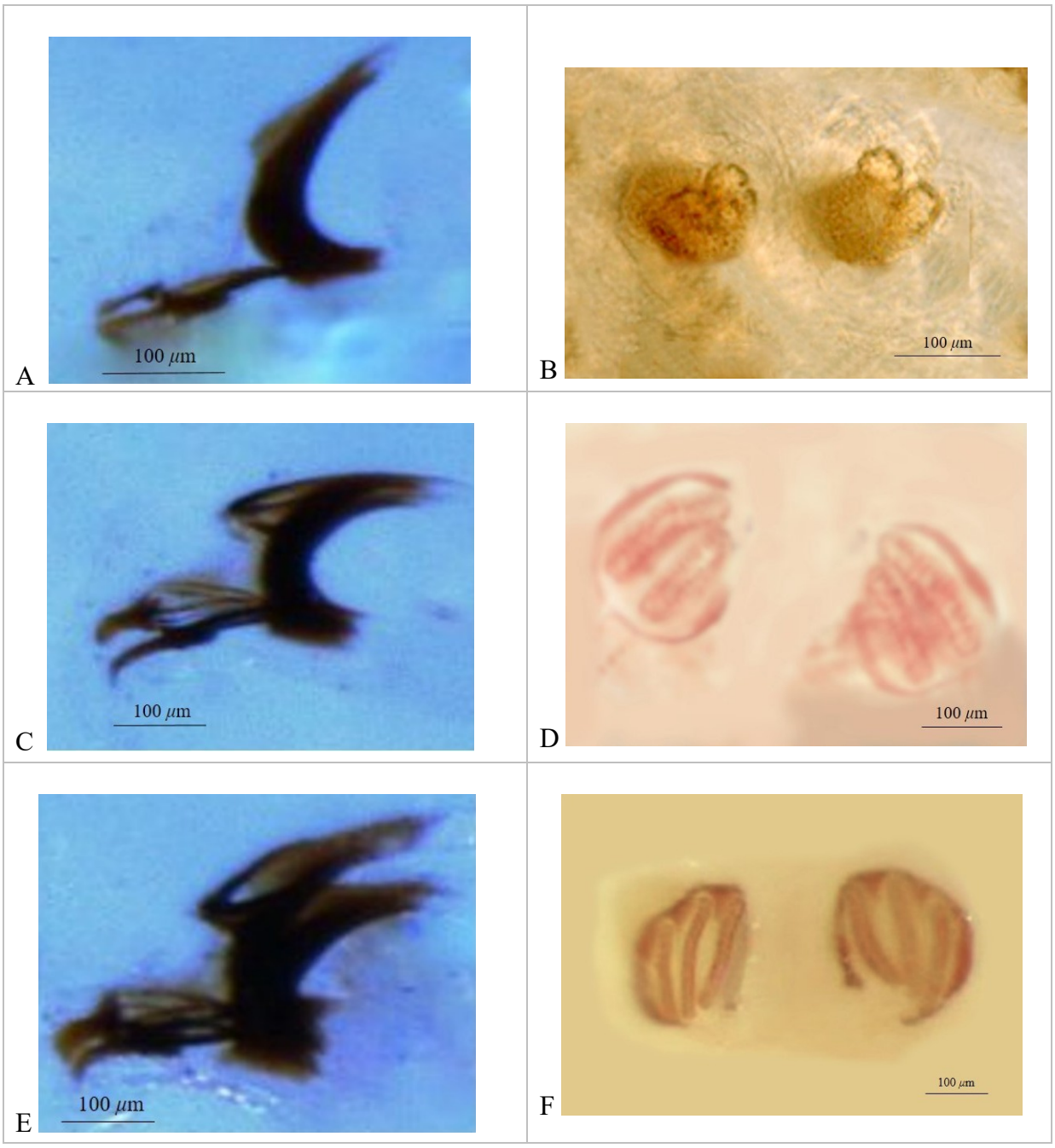



**Fig. 4.38 Cephalopharyngeal skeleton and posterior spiracle of *C.chani* larvae**

**(A, B) 1st instar (C, D) 2nd instar (E, F) 3rd instar**

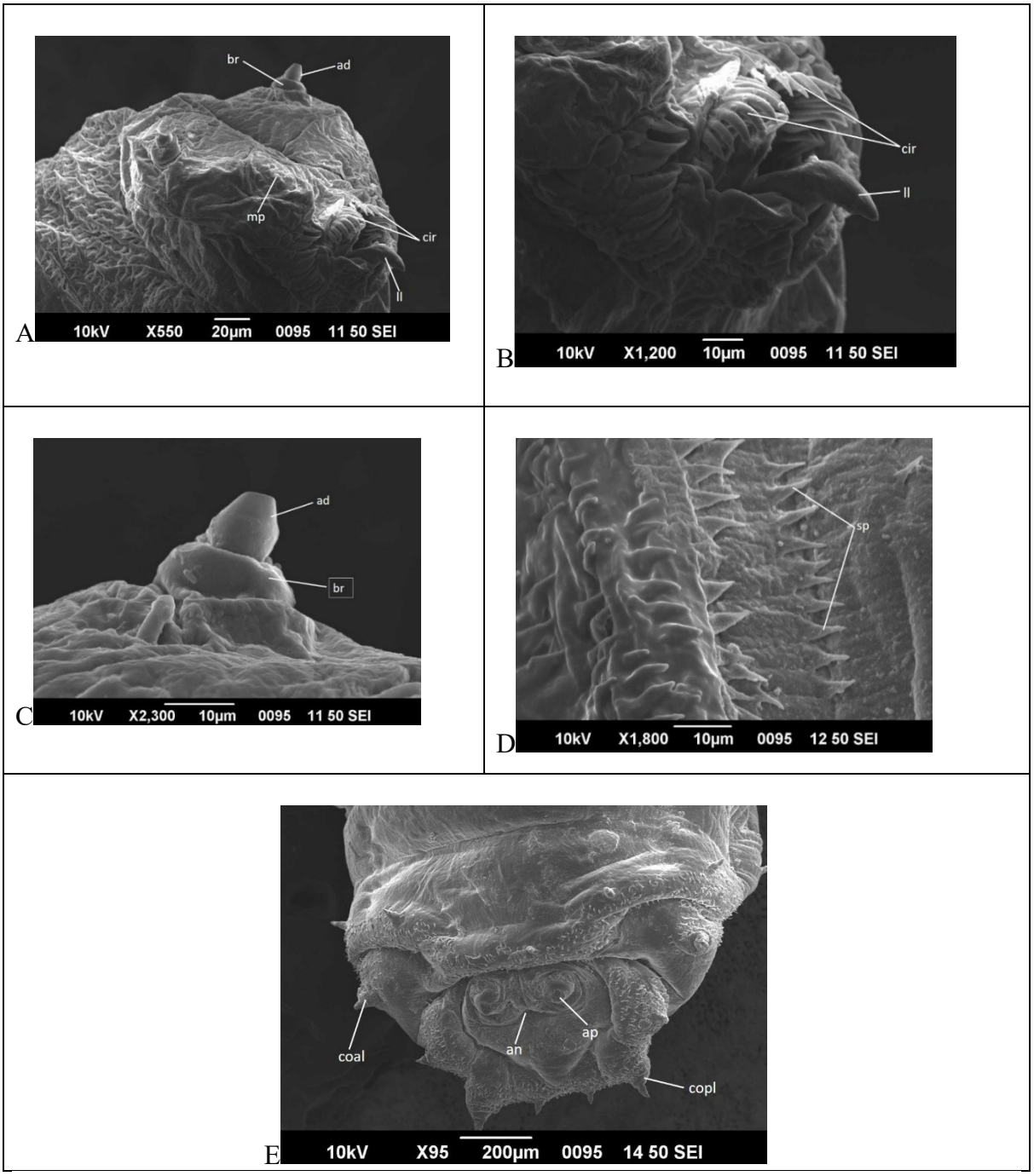



**Fig. 4.39. SEM micrographs of first instar larvae of *C. chani***

**A) pseudocephalon showing antennal complex (an), maxillary palpus (mp), cirri (cr), labial lobe (ll) B) cirri (cr), labial lobe (ll) C) antennae (an) and basal ring (br) D) spines between first and second thoracic segments (sp) E) anal segment displaying anterolateral cones (coal), postero lateral cones (copl), anus (an), anal process (ap)**

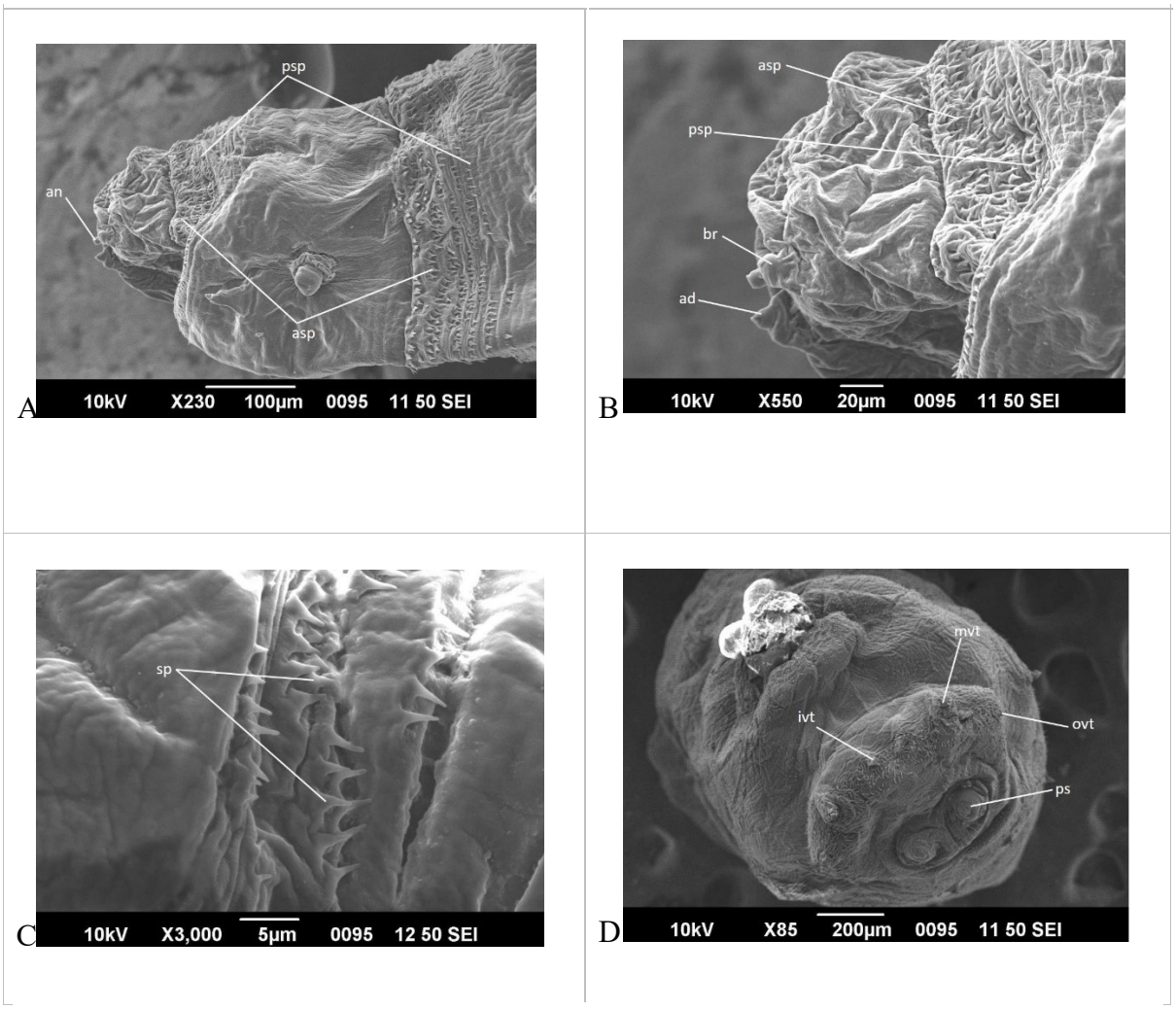



**Fig. 4.40. SEM micrographs of second instar larvae of *C. chani***

**A) pseudocephalon showing antennal complex (an), anterior and posterior spinous process (asp) B) antennae (an) and basal ring (br), anterior and posterior spinous process (asp & psp) C) spines on first and second thoracic segment (sp) D) anal segment displaying inner, middle and outer ventral tubercles (ivt, mvt & ovt), posterior spiracles (ps)**

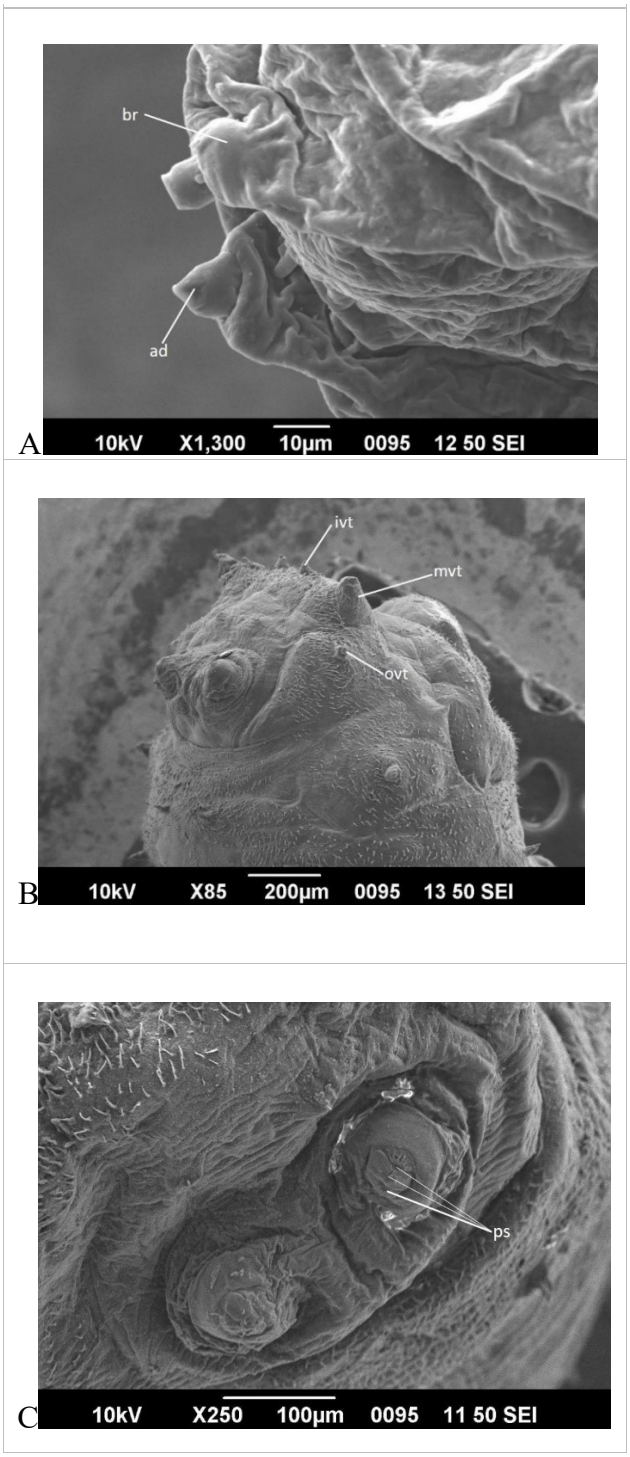



**Fig. 4.41. SEM micrographs of third instar larvae of *C.chani***

**A) pseudocephalon showing antennal complex with antennae (an) and basal ring (br) B) anal segment displaying inner, middle and outer ventral tubercles (ivt, mvt & ovt) C) posterior spiracles (ps)**

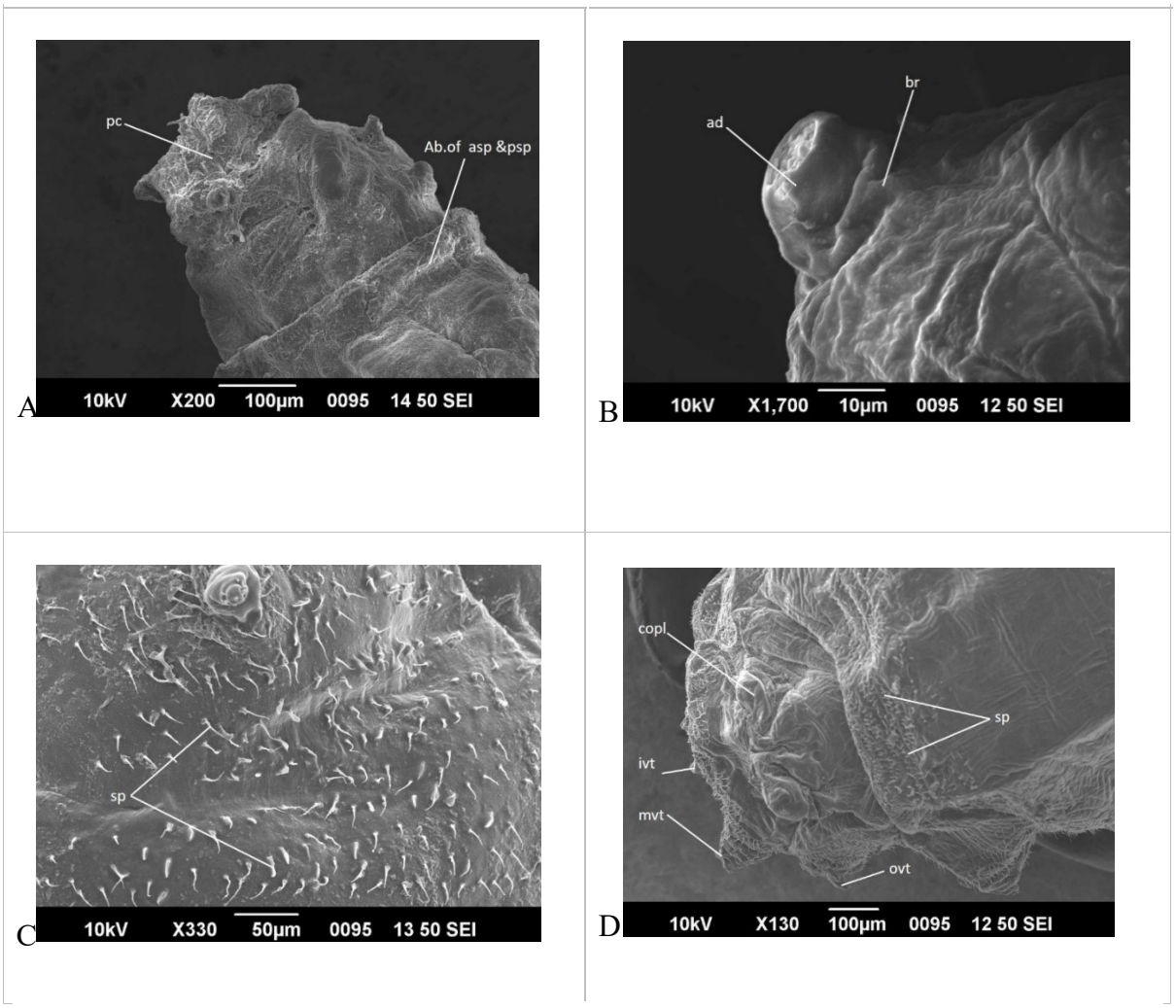



**Fig. 4.42. SEM micrographs of post feeding instar larvae of *C.chani***

**A) rudimentary anterior and posterior spinous process (asp & psp) B) pseudocephalon showing rudimentary antennal complex (an) C) rudimentary spines on first thoracic segment (sp) D) anal segment displaying inner, medial and outer dorsal tubercles (idt, mdt & odt), postero lateral cones (copl) ,rudimentary spines (sp)**

surfaces. Many tubercles present on all the abdominal segments except the caudal segment. The margin of the caudal segment are with pairs of middle, inner and outer dorsal tubercles, middle, inner and outer ventral tubercles and were found to be fully developed in almost equal sizes. (Fig. 4.37. D).

Cephalopharyngeal skeleton is with sclerotized and curved mouth hooks directed downwards. Base of the mouth hooks is broader especially at the posterior aspect. Heavily sclerotized accessory sclerites are seen near the base of the mouth hooks. Base of the mouth hooks are linked to the backwardly directed dental sclerite. Characteristic intermediate sclerite is present. An upwardly curved thin parastomal bar is present. Anterior margin of the parastomal bridge is with the same length as that of dorsal bridge. The dorsal bridge is thin and directed downwards. Intense sclerotization is present on the dorsal and ventral cornua. Ventral cornua is smaller than the dorsal cornua and the pigmentation is uniform. Dorsal cornua is pointed and long and is structurally to the first instar larva but with larger size.Postero dorsal process is projected upwards. (Fig. 4.38. E).

Posterior spiracles with three spiracular slits. A dark pigmented thick sclerotized peritremes were complete in form. Peritreme was seen surrounding the three slits. The button was indistinct (Fig. 4.38. F).

Puparium was ash black in colour (Fig. 4.37. E).

**Morphology of larval instars using SEM**

**First Instars**

Shape was vermiform and muscoid. Dorsal organs and terminal organs were present in pairs. Dorsal organ represented by a dome shaped antenna with a flat surface. Height of

the antenna was almost equal to the basal ring in the antennal dome. Sensillae basiconium was inconspicuous and was present adjacent to the sensilla coeloconicum. Mouth hooks were branched represented by two to three rows of curved sharp tipped spines. The oral cirri were arranged bilaterally with ten numbers each. The first and second antero lateral rows of spines present near the functional mouth opening were elongated and curved with broad bases (Fig. 4.39).

**Second instar**

Shape of second instars were similar to that of the first instar. The antennal dome and maxillary palpii were present with no marked differences from the first instar. The labium and mouth hooks were well developed. The labium was trilobed. The sensillae in the terminal organs were present in two separate groups. Ventral organs were curved. Posterior spiracular discs with two spiracular slits were present. The spines present on the abdomen and thorax were similar in nature. The spines present on the anal segment were filiform type. Thoracic spines were with flat triangular base and fine tips (Fig. 4.40).

**Third instar**

Body size was relatively large. The ultra structural details were similar to that of second instar. The posterior spiracular discs with three slits were positioned in a depression. Three pairs each of dorsal and venral tubercles were present on the anal segment. (Fig. 4.41).

**Post feeding stage**

Pseudocephalon showed rudimentary antennal complex (an) and rudimentary anterior and posterior spinous process (asp & psp) of the second thoracic segment

.Rudimentary spines with tapering tips were present on the first thoracic segment. Anal segment displayed inner, middle and outer dorsal tubercles (idt, mdt & odt) and postero lateral cones (copl). Rudimentary spines (sp) were present on the anal segment (Fig. 4.42).

**Life cycle**

Seasonal life cycle data is provided in Appendix III.

**Mating**

In the pure culture studies conducted, it was found that the adult flies started mating from the 3rd day to the 7th day of emergence. The duration of mating was seen as 10 ± 4 minutes.

**Fecundity**

The mature female fly laid an average of 240.15 ± 19.6 eggs in a day on the decomposing meat. The sites chosen to lay eggs on the meat were small folding, gaps / crevices on the meat. The preoviposition period of the female flies was found to be 8.74 ± 1.26 days after mating. The next batches of eggs were laid after an interval of 4.59 ± 0.31 days. An average of 1667.52 ± 49.78 eggs were laid by the fly during its life span. The fly stopped laying eggs by 41st day. The egg took an average of 21 hrs for hatching (Table 4. 53 - 4. 56).

**Development of Larvae and Pupae**

**First Instar**

The average length of the first instar was 2.54 ± 0.30 mm and the average dry weight was 1.49 ± 0.12 mg.The first ecdysis was completed after 18 hours. The cuticle was found to be loosening approximately one to two hours before the ecdysis (Table 4. 57, 4. 62 and 4. 68).

**Second Instar**

The beginning of the ecdysis was seen approximately 3 to 4 hours before the actual process. The average length of the second instar was 6.08 ± 0.33 mm and the average dry weight was 9.82 ± 0.41 mg. The second moulting was completed after 23 hours (Table 4. 58, 4. 63 and 4. 68).

**Third Instar**

This stage took 36 hours to enter into the post feeding stage. Till then, third instars were found to be feeding on the meat. But even after attaining the maximum length, the larval instars were found to be present on the meat. The average length of the third instar was 10.75 ± 0.28 mm and the average dry weight was 30.97 ± 1.09 mg (Table 4. 59, 4. 64 and 4. 68).

**Post feeding stage**

This non-feeding stage is characterized by shortening of body length. Larva spent 36 hours in this stage before pupation. The average length of the post feeding stage was 10.46 ± 0.35 mm and the average dry weight was 29.90 ± 1.13 mg (Table 4. 60, 4. 65 and 4. 68).

**Pupa**

The average period of pupation was 119 hours. The colour of the pupae was greyish black. The average length and width of the pupa were 6.7 mm & 3 mm respectively. The anterior end of the pupae were found to be split and through this slit the adult fly emerges (Table 4. 68).

**Adult fly**

The flies were found to be emerging from the pupae during day time slowly with folded wings with dull white colour over the thorax and wings. The female flies live for about 73 days where as the males have lesser longevity and live for only 20 days. The average length of the fly was 8-9 mm.

**Total life cycle period**

The total life cycle period from egg till the emergence of adult fly was found to be 252.89 ± 17.16 hours (Table 4. 68).

**Survival**

The survival distribution was studied for all life stages of the fly from the egg stage till the emergence of the adult fly. The stage specific survival rates were 75.84 ± 5.53 %, 76.04 ± 5.25 %, 76.45 ± 4.50 %, 68.49 ± 5.19% and 66.69 ± 3.81 % for egg, first instar, second instar, third instar, and pupa respectively. Average survival rate of *C. chani* was 72.70 ± 6.42 % (Table 4. 69 – 4. 71)

### 4.3.4. *Hemipyrellia ligurriens*

**Morphology of eggs and larval instars**

**Egg**

Creamish white in colour. The caudal end was slightly wider than the anterior end and generally it was oblong (Fig. 4.43. A).

**Larvae**

In total, there are three larval instars including a post feeding stage. All instars have a clearly defined anterior cephalopharyngeal sclerite, three thoracic segments and eight abdominal segments.

**First instar**

Larvae whitish cream in colour. Backwardly directed acuminate spines with dark pigmentation at the tips were present on the anterior and posterior margins of the ventral and lateral surfaces of all the three thoracic segments. Anterior spinous bands are 4-5 in number and posterior spinous bands are narrow and 2-3 in number (Fig. 4.43. B).

Cephalopharyngeal skeleton is incompletely developed and with no uniform sclerotisation. Dense pigmentation is present on the dorsal cornua and it is long, pointed and slightly curved (Fig. 4.44. A).

Anterior spiracles are not developed completely. Two slits were present on the posterior spiracles and were brown in colour (Fig. 4.44. B).

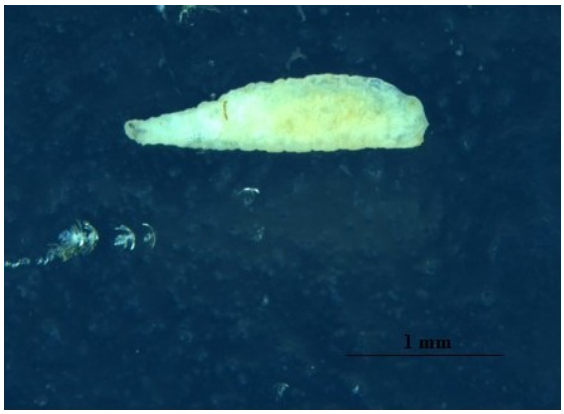
**Second instar**

The second instar larvae were muscoid, vermiform, pointed anteriorly and blunt posteriorly. Anal papillae are prominent with a broad conical base especially in the outer dorsal and outer ventral papillae. Papillae were surrounded by numerous microtrichia. Spinous pattern on thorax is similar as that of the first instar (Fig. 4.43. C).

Cephalopharyngeal skeleton pigmentation is uniform. Postero dorsal process projected upwards. Dorsal cornua is pointed and long and is structurally similar to the first instar larva but with larger size. The ventral cornua is shorter than the dorsal cornua. (Fig. 4.44. C).

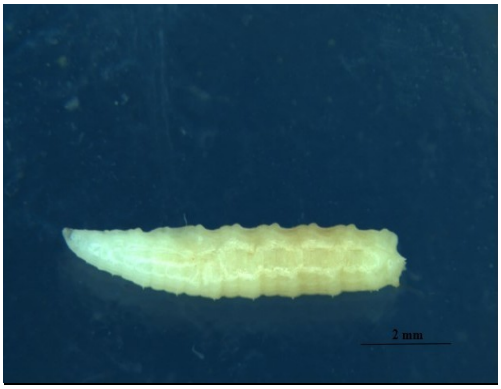
Anterior spiracles are yellow while the posterior spiracles are deep brown in colour with two spiracular slits (Fig. 4.44. D).


A




B


C




D


E

**Fig. 4.43. Eggs, different larval instars and pupal case of *H. ligurriens***

**(A) Eggs (B) First Instar (C) Second Instar (D) Third Instar (E) Pupal case`**

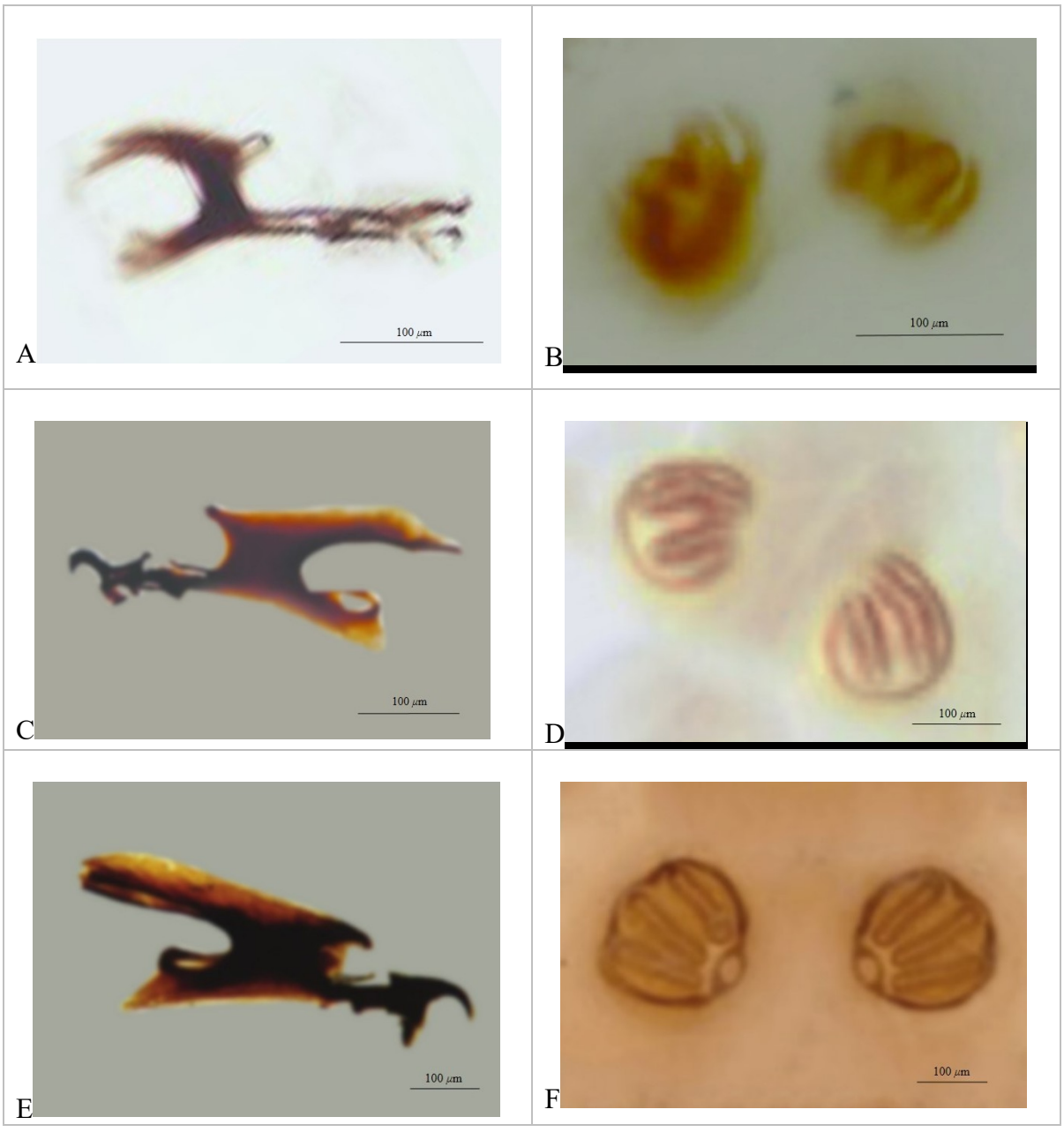



**Fig. 4.44. Cephalopharyngeal skeleton and posterior spiracle of *H. ligurriens* larvae**

**(A, B) 1st instar (C, D) 2nd instar (E, F) 3rd instar**

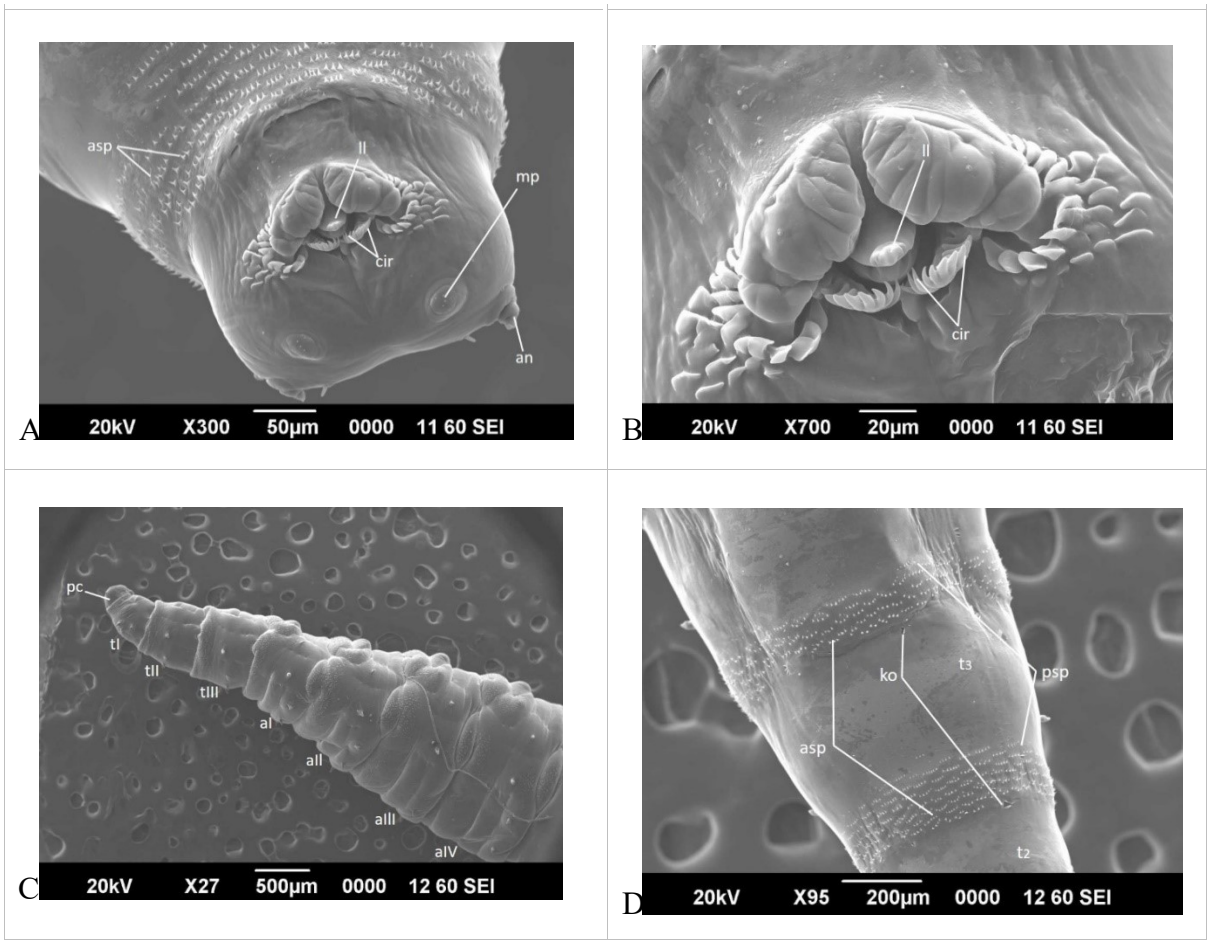



**Fig. 4.45. SEM micrographs of first instar of *H. ligurriens.***

**A) pseudocephalon showing antennal complex (an), maxillary palpus (mp), cirri (cr), labial lobe (ll) and anterior spinous process of the first thoracic segment (asp) B) cirri (cr), labial lobe C) body segments till fourth abdominal segment (t I - t III & a I a IV) D) second and third thoracic segments showing anterior spinous processes (asp), posterior spinous process (psp), Keilin's organ (ko)**

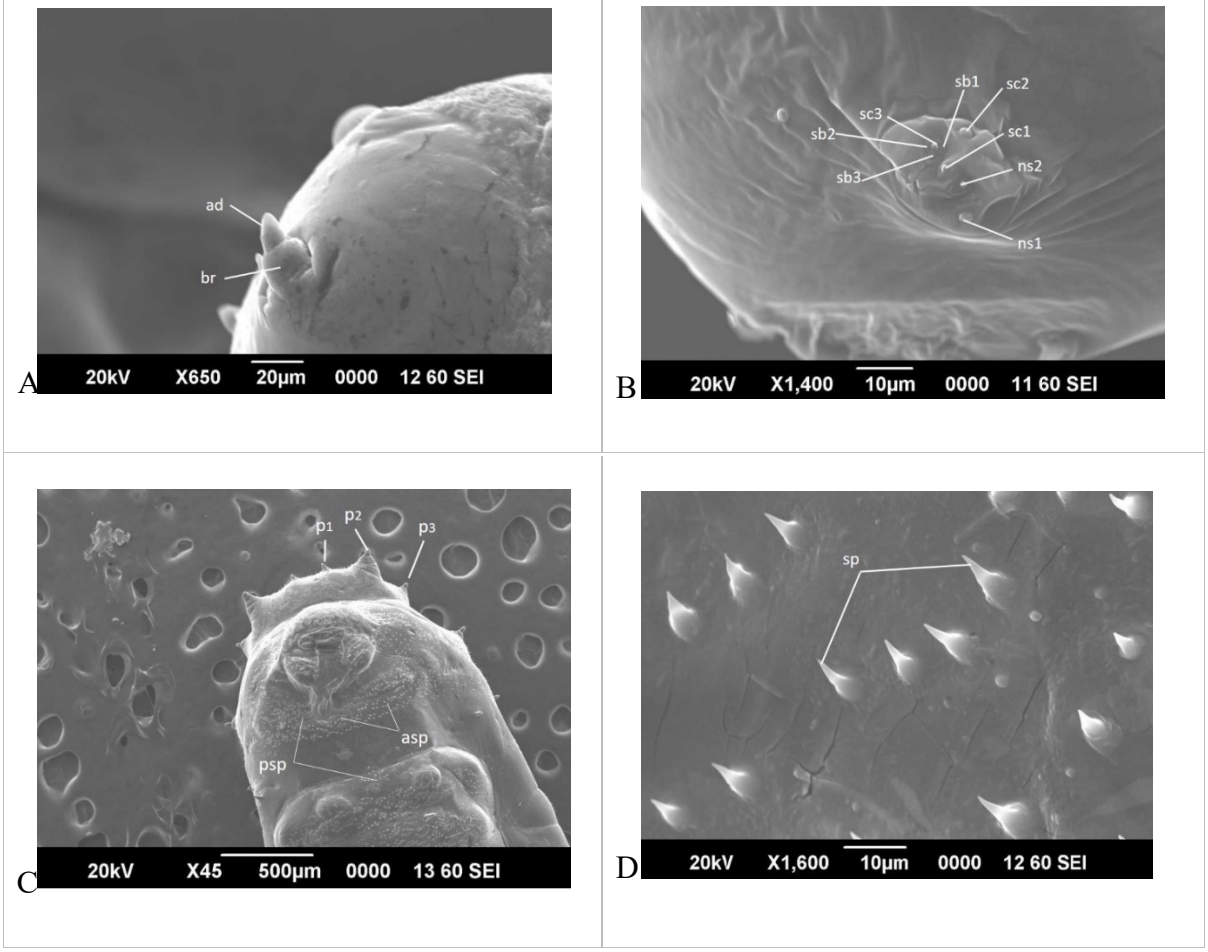



**Fig. 4.46. SEM micrographs of second instar of *H. ligurriens.***

**A) pseudocephalon showing antennal dome (ad) (B) Maxillary palpus showing eight sensillae (sc1-3; sb1-3; ns1-2) C) anal segment displaying dorsal papillae (p1 p3), D) spines with bulbous base and sharp tips between first and second thoracic segments (sp).**

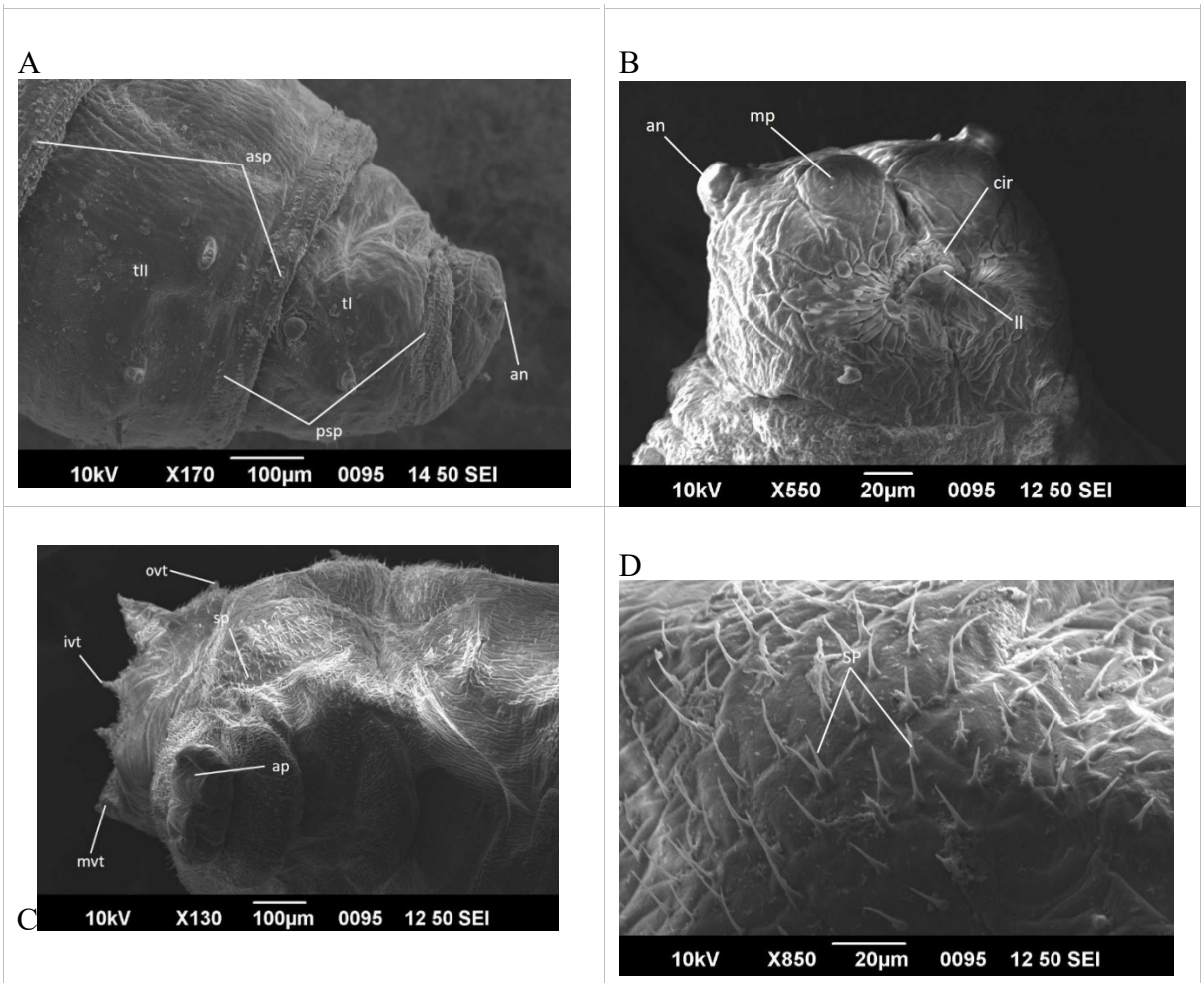



**Fig. 4.47. SEM micrographs of third instar of *H. ligurriens.***

**A) pseudocephalon showing antennal complex (an), anterior and posterior spinous process (asp & psp) of the first and second thoracic segments B) pseudocephalon showing antennal complex (an), maxillary palpus (mp), cirri (cr), labial lobe (ll). C) anal segment displaying inner, medial and outer ventral tubercles (ivt, mvt & ovt) D) slender spines with flat base and sharp tips between first and second thoracic segments (sp)**

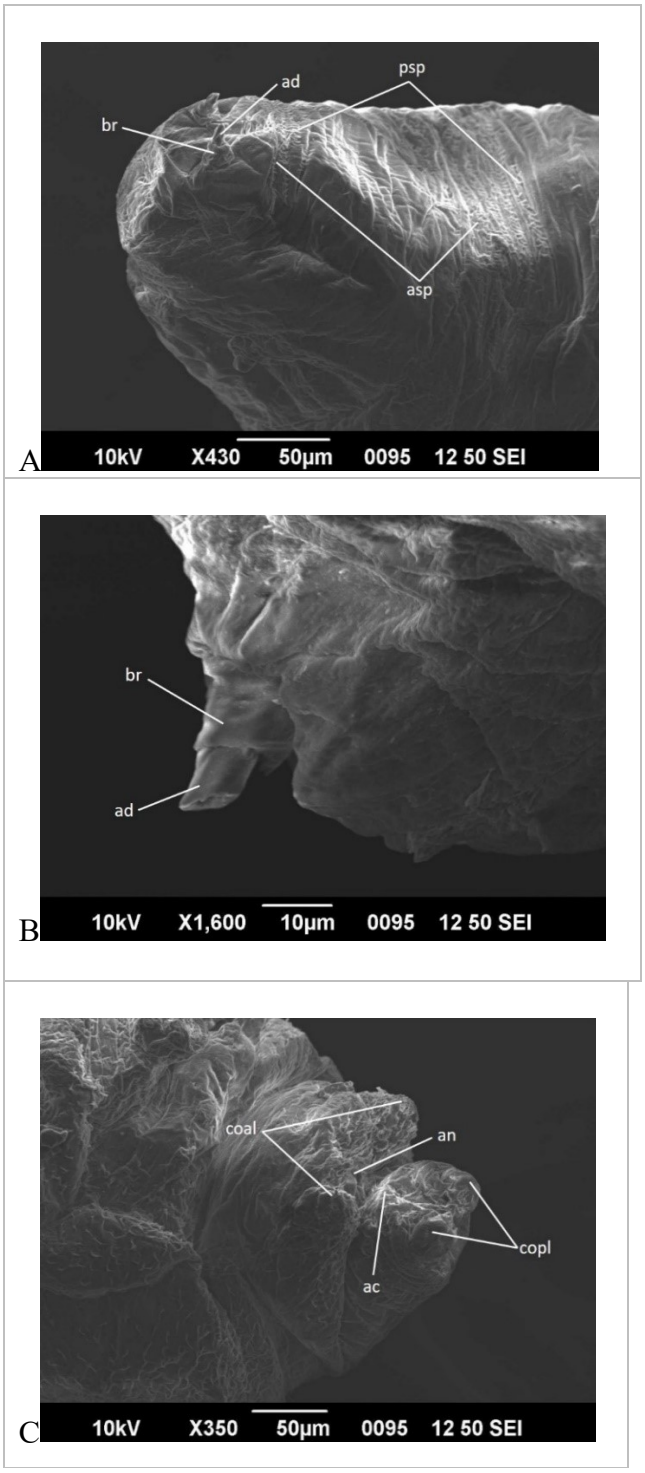



**Fig. 4.48. SEM micrographs of post feeding instar stage of *H. ligurriens.***

**A) pseudocephalon showing antennal complex having antennae (an) and basal ring (br), anterior and posterior spinous process (asp & psp) of the first and second thoracic segments B) antennal complex having antennae (an) and basal ring (br) C) anal segment displaying anterolateral cones (coal), postero lateral cones (copl), anus, and anal cone (ac)**

**Third instar**

Cream to light yellow in colour. All segments from 2-11 are with spinous bands. The spines acuminate and were arranged singly in rows and have dark points on the tips. The spinous bands were found to be restricted to the lateral and ventral surfaces. The middle dorsal tubercles were moderately sized in comparison to the inner and outer tubercles. The lateral, ventral and dorsal tubercles were large and found to be fully developed (Fig. 4.43. D).

Cephalopharyngeal skeleton pigmentation is darker. Dorsal sclerite comma shaped and prominent. Dorsal cornua got reduced in length and is with uniform width. Ventral cornua is equal in length to dorsal cornua (Fig. 4.44. E).

Anterior spiracles were yellowish and the lobes were arranged in a circle. Posterior spiracles were dark brown and size was bigger than that of the second instar. Peritreme is complete. Button is present (Fig. 4.44. F).

Puparium is brown in colour (Fig. 4.43. E).

**Morphology of larval instars using SEM**

**First instar**

Shape was muscoid and vermiform. Cephalic region were having terminal organs and dorsal organs in pairs. Mouth parts in the ventral regions were conspicuous with oral cirri and labium. The cirri were in the shape of curved spines. The antero-dorsal side of both pairs of pseudocephalon is occupied by an antenna in the shape of a dome which has a superior cleft and is placed on a ring like base and a circular disc shaped maxillary palpus. The height of dome is shorter than that of the height of basal ring. The diameter of the

maxillary palpus is more than the antennal length. Groups of many sensilla were present in the maxillary palpus.

Anterior and posterior spines were present on thorax in 3-4 rows. Characteristic acuminate spines were present on the anterior and posterior margins of the ventral and lateral surfaces of all the three thoracic segments. Backwardly directed spines have a bulbous base and slender sharp tip. Keilin's organs which are sensitive to humidity were present on the ventral side of all thoracic segments.

The shape of spines in all abdominal segments was similar to thoracic segments. Filiform spines were present on anal segment Spines were present on the ventral and lateral surfaces of all segments. Anterior spinous bands were 4-5 in number and posterior spinous bands were narrow and 2-3 in number. The spinulation pattern was similar to that of the thoracic segments (Fig. 4.45).

**Second Instar**

Shape was muscoid and similar to first instar.The antero-dorsal side of both pairs of pseudocephalon is occupied by an antenna in the shape of a dome which has a superior cleft and is placed on a ring like base and a circular disc shaped maxillary palpus. The height of dome is shorter than that of the height of the basal ring. Groups of many sensilla were present in the maxillary palpus. Sensilla coeloconica are three in number and are arranged in a single row with some space in between them. Sensillae basiconicum are also three in number, highly reduced and not visible prominently and are positioned adjacent to the sensilla coeloconicum. Two more sensillae known as 'first and second additional sensillum coeloconicum' are seen dorsal to sensilla coeloconicum and basiconicum cluster.

Facial mask is very prominent on the ventral aspect of the pseudocephalon. Numerous well-structured cirri are dominating in the facial mask and are ten in number. They are characteristically arranged bilaterally on each side of the mouth opening and are gently curved medially. Three rows of spine clusters were present dorso-medial to the functional mouth opening. The first and second anterolateral rows of spines were with shapes of elongated pyramids of different sizes having broad bases and flat blunt ends. Third postero-medial row of spines were with broad bases and thin concavo-convex apex. Oral ridges were not prominent. Labial lobe was well developed with fleshy lateral lobes constituting a very distinctively demarcated ventral arch area and a medial small lobe. Rounded sensory structures were seen on lateral lobes.

Backwardly directed acuminate spines with bulbous base and slender sharp tips were present on the anterior and posterior margins of the ventral and lateral surfaces of all the three thoracic segments. Keilin's organs which are sensitive to humidity are present on the ventral side of all thoracic segments.

In all the abdominal segments, spines were present on the ventral and lateral surfaces. The shape of spines in all abdominal segments was similar to thoracic segments except the last anal segment which has filiform. Anal papillae are prominent with a broad conical base especially in outer dorsal and outer ventral papillae. These papillae were surrounded by microtrichia. Anterior spinous bands are 4-5 in number and posterior spinous bands are narrow and 2-3 in number. The spinulation pattern was similar to that of the thoracic segments (Fig. 4.46).

**Third Instar**

The shape was vermiform and muscoid. Well developed antennal dome and terminal organs were present. The dorsal organs were present on the dorsolateral aspect of the terminal organ. The sensillae in the third instar were similar to the second instar. Three to four rows of anterior and posterior spines were seen on the thoracic segments at the inter segmental junctions. Middle ventral, inner ventral, outer ventral, outer dorsal, middle dorsal, and inner dorsal tubercles were present in pairs on the anal segment. (Fig. 4.47).

**Post feeding stage**

Spines on the thorax, oral cirri, antennal dome and the terminal organs were rudimentary (Fig. 4.48).

**Life cycle**

Seasonal life cycle data is provided in Appendix III.

**Mating**

In the pure culture studies conducted, it was found that the adult flies started mating from the 3$^{rd}$ day to the 6th day of emergence. The duration of mating was seen as 10 ± 04 minutes.

**Fecundity**

The mature female fly laid an average of 215.74 ± 22.29 eggs in a day on the decomposing meat. The sites chosen to lay eggs on the meat were small folding, gaps / crevices on the meat. The preoviposition period of the female flies was found to be 9.59 ± 0.89 days after mating. The next batches of eggs were laid after an interval of 3.67 ± 0.37 days. An average of 1451.26 ± 83.71 eggs were laid by the fly during its life span. The fly stopped laying eggs by the 44$^{th}$ day. The eggs took an average of 27 hrs for hatching (Table 4.73 - 4.76).

**Development of Larvae and Pupae**

**First Instar**

The average length of the first instar was 2.11 ± 0.23 mm and the average dry weight was 0.87 ± 0.34 mg. The first ecdysis was completed after 17 hours. The cuticle was found to be loosening approximately one to two hours before the ecdysis (Table 4. 77, 4. 82, 4. 88)

**Second Instar**

The beginning of the ecdysis was seen approximately 3 to 4 hours before the actual process. The average length of the second instar was 4.53 ± 0.47mm and the average dry weight was 7.52 ± 1.59 mg. The moulting was completed after 25 hours (Table 4. 78, 4. 83, 4. 88).

**Third Instar**

This stage took 59 hours to enter into the post feeding stage. Till then third instars were found to be feeding on the meat. But even after attaining the maximum length, the larval instars were found to be present on the meat. The average length of the third instar was 7.91 ± 0.37 mm and the average dry weight was 23.35 ± 2mg (Table 4. 79, 4. 84, 4. 88).

**Post feeding stage**

This non-feeding stage is characterized by shortening of body length. Larva spent 115 hours in this stage before pupation. The average length of the post feeding stage was 7.75 ± 0.55 mm and the average dry weight was 26.35 ± 0.99 mg (Table 4. 80, 4. 85, 4. 88).

**Pupa**

The average period of pupation was 153 hours. The colour of the pupae was greyish black. The average length and width of the pupa were 6.7 mm & 3 mm

respectively. The anterior end of the pupae were found to be split and through this slit the adult fly emerges (Table 4. 88).

**Adult fly**

The flies were found to be emerging from the pupae during day time slowly with folded wings with dull white colour over the thorax and wings. The female flies live for about 71 days where as the males have lesser longevity and live for only 19 days. The average length of the fly was 10-11 mm

**Total life cycle period**

The total life cycle period from egg till the emergence of adult fly was found to be 395.88 ± 35.82 hours (Table 4. 88).

**Survival**

The survival distribution was studied for all life stages of the fly from the egg stage till the emergence of the adult fly. The stage specific survival rate were 72.45 ±5.94 %,72.57 ± 5.68 % , 70.78 ± 5.81 % , 69.78 ± 6.69 % , 69.77 ± 6.95 % for egg, first instar, second instar, third instar, and pupa respectively. Average survival rate of *H. ligurriens* was 71.07 ± 6.26% (Table 4.89 – 4. 91).

## 4.4. Effect of temperature and humidity on the life cycle

### 4.4.1. *C. megacephala*

**Effect on fecundity**

Effect of season on the pre-oviposition period were found to be significant (F = 19.73, P = < 0.001). Effect of year was also found to be significant (F = 11.545, P = < 0.001). However seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 0.909, P = 0.480). The pre-oviposition period in *C.*

*megacephala* was significantly higher in winter (10.33 ± 0.71) in comparison to monsoon (9.33 ± 0.58) and summer 8.44 ± 0.88) (Table 4.13).

**Table 4.13. Pre-oviposition period (days) of *C.megacephala***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 10.33 ± 0.58 | 9.33 ± 0.58 | 10.67 ± 0.58 | $10.11 \pm 0.78^{A}$ |
| 2020 | 9.33 ± 0.58 | 8.33 ± 0.58 | 10.33 ± 0.58 | $9.33 \pm 1.00^{B}$ |
| 2021 | 8.33 ± 0.58 | 7.67 ± 0.58 | 10.00 ± 1.00 | $8.67 \pm 1.23^{C}$ |
| Overall Season | $9.33 \pm 1.00^{b}$ | $8.44 \pm 0.88^{c}$ | $10.33 \pm 0.71^{a}$ | 9.37 ± 1.15 |
| Between year F-value = 11.545**; (P-value = 0.001)<br>Between season F-value = 19.73**; (P-value= < 0.001))<br>Interaction between season and year F-value =$0.909^{ns}$; (P-value = 0.480) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of season on the number of eggs laid in day was found to be significant (F = 74.306, P = < 0.001). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 0.738, P = 0.578). The number of eggs laid by *C.megacephala* was significantly higher in monsoon in comparison to other seasons (Table 4.14).

**Table 4.14. Eggs laid by the *C. megacephala* in a day**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 381.00 ± 5.57 | 356.00 ± 8.19 | 319.67 ± 6.81 | 352.22 ± 27.38 |
| 2020 | 374.67 ± 7.10 | 338.00 ± 14.80 | 312.33 ± 10.02 | 341.67 ± 28.78 |
| 2021 | 368.33 ± 9.45 | 340.33 ± 17.16 | 319.00 ± 3.00 | 342.56 ± 23.61 |
| Overall Season | $374.67 \pm 8.53^{a}$ | $344.78 \pm 14.73^{b}$ | $317.00 \pm 7.16^{c}$ | 345.48 ± 26.09 |
| Between year F-value = $3.062^{ns}$; (P-value = 0.072)<br>Between season F-value = 74.306**; (P-value < 0.001))<br>Interaction between season and year F-value =$0.738^{ns}$; (P-value = 0.578) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*

Effect of season on the periodicity of egg laying was found to be significant (F = 6.300, P = < 0.008). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 1.65, P = 0.205). The periodicity of egg

laying in *C. megacephala* was significantly higher in winter (4.67 ± 0.35) in comparison to monsoon (4.33 ± 0.35) and summer (4.17 ± 0.25) (Table 4.15).

**Table 4.15. Periodicity of egg laying (days) by *C. megacephala***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 4.17 ± 0.29 | 4.33 ± 0.29 | 4.33 ± 0.29 | 4.28 ± 0.26 |
| 2020 | 4.50 ± 0.50 | 4.17 ± 0.29 | 4.83 ± 0.29 | 4.50 ± 0.43 |
| 2021 | 4.33 ± 0.29 | 4.00 ± 0.00 | 4.83 ± 0.29 | 4.39 ± 0.42 |
| Overall Season | $4.33 \pm 0.35^{b}$ | $4.17 \pm 0.25^{b}$ | $4.67 \pm 0.35^{a}$ | 4.39 ± 0.38 |
| Between year F-value = $1.200^{ns}$; (P-value = 0.324)<br>Between season F-value = 6.300**; (P-value = 0.008))<br>Interaction between season and year F-value = $1.65^{ns}$; (P-value = 0.205) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*

Effect of season on the total number of eggs laid during the life span was found to be significant (F = 323.32, P = < 0.001). Effect of year on the number of eggs laid was also found to be significant (F= 29.859; P = < 0.001). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 1.130, P = 0.373). The number of eggs laid in *C. megacephala* was significantly higher in monsoon (2796.33 ± 114.39) in comparison to summer (2442.89 ± 80.12) and winter (2217.11 ± 69.91) (Table 4.16).

**Table 4.16. Eggs laid by the *C. megacephala* during in its life span**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 2919.00 ± 57.72 | 2533.33 ± 10.97 | 2285.67 ± 61.27 | $2579.33 \pm 279.64^{A}$ |
| 2020 | 2791.33 ± 38.28 | 2426.33 ± 47.9 | 2204.33 ± 33.31 | $2474 \pm 259.04^{B}$ |
| 2021 | 2678.67 ± 64.53 | 2369.00 ± 48.88 | 2161.33 ± 52.21 | $2403 \pm 230.55^{C}$ |
| Overall Season | $2796.33 \pm 114.39^{a}$ | $2442.89 \pm 80.12^{b}$ | $2217.11 \pm 69.91^{c}$ | 2485.44 ± 257.9 |
| Between year F-value = 29.859**; (P-value < 0.001)<br>Between season F-value = 323.32**; (P-value < 0.001)<br>Interaction between season and year F-value = $1.130^{ns}$ ; (P-value = 0.373) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Effect on length of larvae**

**Instar I**

Effect of season, year and stage on the length of I$^{st}$ instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.21). The instar length in *C. megacephala* was significantly higher (1.60 ± 0.05) in monsoon in comparison to summer (1.44 ± 0.09) and winter (1.47 ± 0.05) (Table 4.17).

**Table 4.17. Seasonal changes in length (mm) of I$^{st}$ instar larvae of *C. megacephala***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $1.65 \pm 0.02^{a}$ | $1.41 \pm 0.12^{b}$ | $1.44 \pm 0.0004^{b}$ | $1.50 \pm 0.13^{AB}$ |
| 2020 | $1.54 \pm 0.04^{a}$ | $1.39 \pm 0.03^{b}$ | $1.45 \pm 0.05^{ab}$ | $1.46 \pm 0.07^{B}$ |
| 2021 | 1.61 ± 0.02 | 1.51 ± 0.08 | 1.53 ± 0.04 | $1.55 \pm 0.06^{A}$ |
| Overall Season | $1.60 \pm 0.05^{a}$ | $1.44 \pm 0.09^{b}$ | $1.47 \pm 0.05^{b}$ | 1.5 ± 0.1 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Instar II**

Effect of season, year and stage on the length of II$^{nd}$ instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.21). The instar length in *C. megacephala* was significantly higher in monsoon (6.13 ± 0.65) in comparison to summer (5.65 ± 0.96) and winter (3.65 ± 0.65) (Table 4.18).

**Table 4.18. Seasonal changes in length (mm) of II$^{nd}$ instar larvae of *C. megacephala***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $5.74 \pm 0.02^{aB}$ | $4.39 \pm 0.09^{bC}$ | $4.47 \pm 0.002^{bA}$ | $4.87 \pm 0.66^{C}$ |
| 2020 | $6.99 \pm 0.05^{aA}$ | $6.14 \pm 0.18^{bB}$ | $3.00 \pm 0.08^{cC}$ | $5.38 \pm 1.83^{A}$ |
| 2021 | $5.66 \pm 0.1^{bB}$ | $6.41 \pm 0.09^{aA}$ | $3.49 \pm 0.02^{cB}$ | $5.19 \pm 1.32^{B}$ |
| Overall Season | $6.13 \pm 0.65^{a}$ | $5.65 \pm 0.96^{b}$ | $3.65 \pm 0.65^{c}$ | $5.14 \pm 1.32$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Instar III**

Effect of season, year and stage on the length of III$^{rd}$ instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.21). The instar length in *C. megacephala* was significantly higher in summer (11.87 ± 0.35) in comparison to monsoon (11.17 ± 0.59) and winter (9.70 ± 0.40) (Table 4.19).

**Table 4.19. Seasonal changes in length (mm) of III$^{rd}$ instar larvae of *C. megacephala***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $10.41 \pm 0.07^{aC}$ | $11.72 \pm 0.14^{bB}$ | $10.21 \pm 0.02^{cA}$ | $10.78 \pm 0.72^{B}$ |
| 2020 | $11.38 \pm 0.09^{bB}$ | $11.57 \pm 0.04^{aC}$ | $9.55 \pm 0.08^{cB}$ | $10.83 \pm 0.97^{B}$ |
| 2021 | $11.73 \pm 0.01^{bA}$ | $12.31 \pm 0.05^{aA}$ | $9.33 \pm 0.04^{cC}$ | $11.12 \pm 1.37^{A}$ |
| Overall Season | $11.17 \pm 0.59^{b}$ | $11.87 \pm 0.35^{a}$ | $9.70 \pm 0.40^{c}$ | $10.91 \pm 1.02$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Post feeding stage**

Effect of season, year and stage on the length of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.21). The length of post feeding stage in *C. megacephala* was significantly higher in summer (10.98 ± 0.32) in comparison to monsoon (10.62 ± 0.38) and winter (10.2 ± 0.17) (Table 4.20).

**Table 4.20. Seasonal changes in length (mm) of post feeding stage of *C. megacephala***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $10.23 \pm 0.07^{bC}$ | $10.68 \pm 0.17^{aC}$ | $10.11 \pm 0.03^{bB}$ | $10.34 \pm 0.28^{C}$ |
| 2020 | $10.56 \pm 0.05^{bB}$ | $10.92 \pm 0.07^{aB}$ | $10.09 \pm 0.10^{cB}$ | $10.52 \pm 0.37^{B}$ |
| 2021 | $11.08 \pm 0.07^{bA}$ | $11.34 \pm 0.24^{aA}$ | $10.39 \pm 0.14^{cA}$ | $10.94 \pm 0.45^{A}$ |
| Overall Season | $10.62 \pm 0.38^{b}$ | $10.98 \pm 0.32^{a}$ | $10.2 \pm 0.17^{c}$ | $10.60 \pm 0.44$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

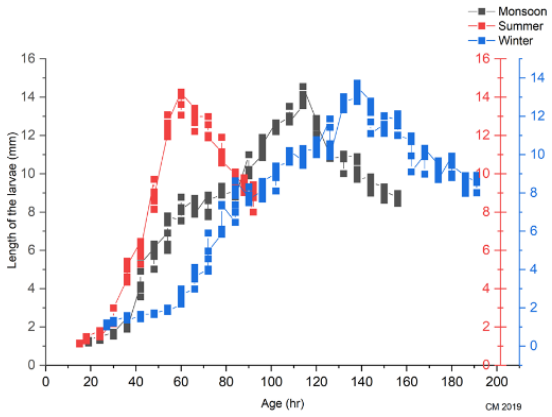
Interaction studies on year, season and stage was found to be significant (F = 115.12; P = < 0.001) indicating that seasonal variations in length of each larval instar were different in different years (Table 4.21).

**Table 4.21. ANOVA for comparing the length of larval instars of *C. megacephala***

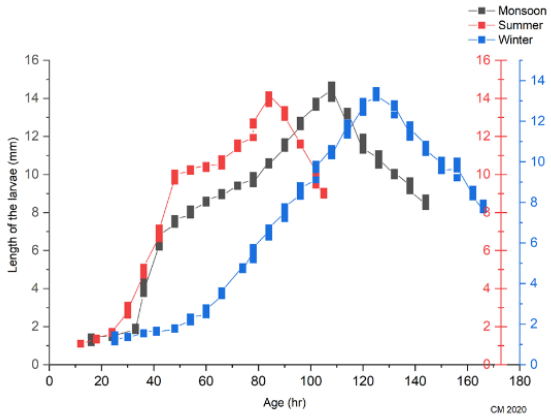
| Source | df | Sum of Squares | Mean Square | F-Value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 1.95 | 0.98 | 126.08** | <0.001 |
| Season | 2 | 33.36 | 16.68 | 2155.47** | <0.001 |
| Stage | 3 | 1672.43 | 557.48 | 72030.16** | <0.001 |
| Year * Season | 4 | 6.70 | 1.68 | 216.48** | <0.001 |
| Year * stage | 6 | 1.59 | 0.27 | 34.21** | <0.001 |
| Season * stage | 6 | 22.72 | 3.79 | 489.15** | <0.001 |
| Year * Season * Stage | 12 | 10.69 | 0.89 | 115.12** | <0.001 |
| Error | 72 | 0.56 | 0.01 | | |
| Total | 107 | 1750.00 | | | |

** Significant at 0.01 level

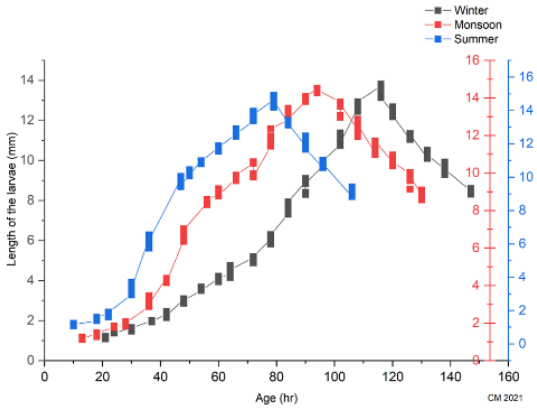
The growth curves representing the developmental rate (Length (mm) Vs. Age (hr) of *C. megacephala* from hatching until pupation during different seasons and years were prepared (Fig. 4.49 and Fig. 4.50).




2019

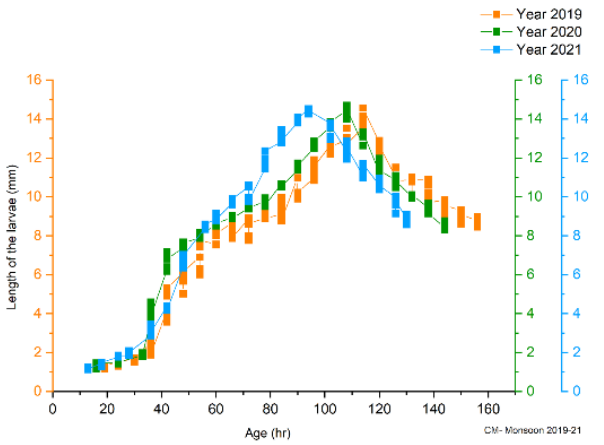



2020

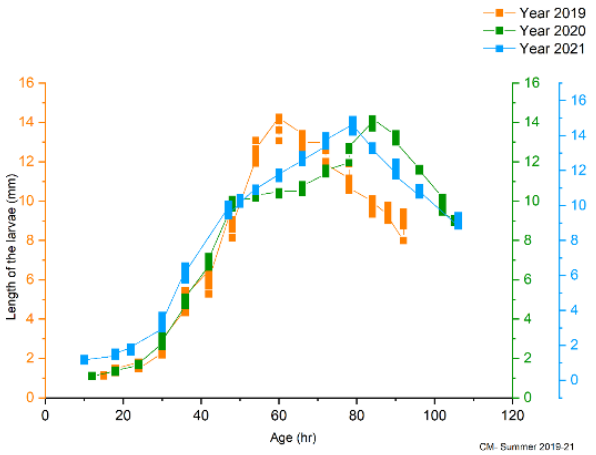



2021

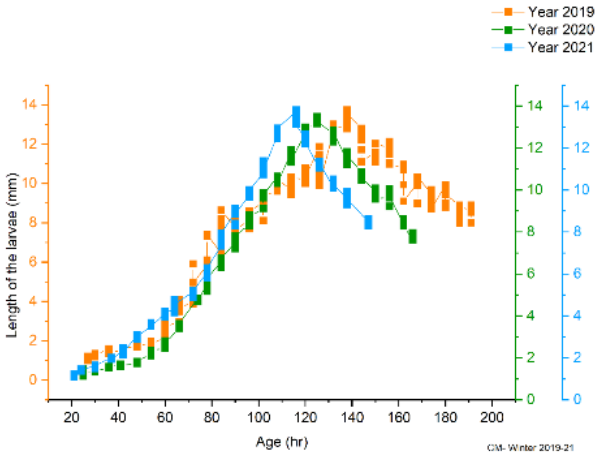
**Fig. 4.49. Seasonal developmental rate (Length (mm) Vs. Age (hr) of *C. megacephala* from hatching upto pupation**




Monsoon




Summer




Winter

**Fig. 4.50. Developmental rate (Length (mm) Vs. Age (hr) of *C. megacephala* from hatching upto pupation during the study period**

**Instar I**

Effect of season and year on the weight of I$^{st}$ instar larvae was found to be non significant. The pattern of differences in the weight was similar in all the years and seasons (Table 4. 22).

**Table 4. 22. Seasonal changes in weight (mg) of I$^{st}$ instar larvae of *C. megacephala***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 1.40 ± 0.05 | 1.28 ± 0.07 | 1.50 ± 0.06 | 1.39 ± 0.11 |
| 2020 | 1.42 ± 0.07 | 1.44 ± 0.06 | 1.70 ± 0.08 | 1.52 ± 0.15 |
| 2021 | 1.46 ± 0.10 | 1.55 ± 0.06 | 1.46 ± 0.05 | 1.49 ± 0.08 |
| Overall Season | 1.43 ± 0.07 | 1.42 ± 0.13 | 1.55 ± 0.12 | 1.47 ± 0.12 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Instar II**

Effect of season, year and stage on the II$^{nd}$ instar weight was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.26). The instar weight in *C. megacephala* was significantly higher in monsoon (10.37 ± 0.42) in comparison to summer (10.33 ± 0.40) and winter (9.88 ± 0.54) (Table 4.23).

**Table 4.23. Seasonal changes in weight (mg) of II$^{nd}$ instar larvae of *C. megacephala***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $10.66 \pm 0.30^{aA}$ | $10.60 \pm 0.44^{aA}$ | $9.26 \pm 0.27^{bB}$ | 10.17 ± 0.75 |
| 2020 | $10.54 \pm 0.12^{aA}$ | $10.05 \pm 0.33^{bB}$ | $10.24 \pm 0.29^{abA}$ | 10.27 ± 0.31 |
| 2021 | $9.93 \pm 0.38^{B}$ | $10.35 \pm 0.34^{AB}$ | $10.13 \pm 0.40^{A}$ | 10.13 ± 0.37 |
| Overall Season | $10.37 \pm 0.42^{a}$ | $10.33 \pm 0.40^{a}$ | $9.88 \pm 0.54^{b}$ | 10.19 ± 0.50 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Instar III**

Effect of season, year and stage on the weight of III$^{rd}$ instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.26). The instar weight in *C. megacephala* was significantly higher in summer (31.75 ± 1.10) in comparison to monsoon (30.61 ± 0.54) and winter (29.77 ± 0.40) (Table 4.24).

**Table 4.24. Seasonal changes in weight (mg) of III$^{rd}$ instar larvae of *C. megacephala* during the study period**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $30.46 \pm 0.18^{bB}$ | $33.05 \pm 0.41^{aA}$ | $29.58 \pm 0.21^{cB}$ | $31.03 \pm 1.58^{A}$ |
| 2020 | $31.24 \pm 0.30^{aA}$ | $31.62 \pm 0.09^{aB}$ | $29.46 \pm 0.18^{bB}$ | $30.78 \pm 1.01^{B}$ |
| 2021 | $30.13 \pm 0.28^{bB}$ | $30.58 \pm 0.12^{aC}$ | $30.25 \pm 0.14^{abA}$ | $30.32 \pm 0.26^{C}$ |
| Overall Season | $30.61 \pm 0.54^{b}$ | $31.75 \pm 1.10^{a}$ | $29.77 \pm 0.40^{c}$ | 30.71 ± 1.09 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Post feeding stage**

Effect of season, year and stage on the weight of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.26). The weight of post feeding stage in *C. megacephala* was significantly higher in monsoon (30.03 ± 0.56) in comparison to summer (29.98 ± 0.64) and winter (28.89 ± 0.59) (29.42 ± 0.93) (Table 4.25).

**Table 4.25. Seasonal changes in weight (mg) of post feeding stage of *C. megacephala***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $30.34 \pm 0.14^{aA}$ | $30.72 \pm 0.48^{aA}$ | $28.94 \pm 0.34^{bB}$ | $30.00 \pm 0.87^{A}$ |
| 2020 | $29.34 \pm 0.33^{B}$ | $29.71 \pm 0.10^{B}$ | $29.39 \pm 0.58^{A}$ | $29.48 \pm 0.38^{B}$ |
| 2021 | $30.40 \pm 0.26^{aA}$ | $29.51 \pm 0.32^{bB}$ | $28.35 \pm 0.34^{cC}$ | $29.42 \pm 0.93^{B}$ |
| Overall Season | $30.03 \pm 0.56^{a}$ | $29.98 \pm 0.64^{a}$ | $28.89 \pm 0.59^{b}$ | 29.63 ± 0.78 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

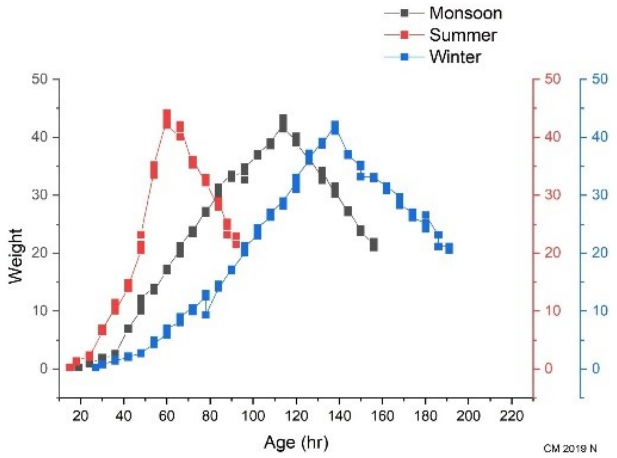
Interaction studies on year, season and stage was found to be significant (F = 14.365; P = < 0.001) indicating that seasonal variations in weight of each stage are different in different years (Table 4.26).

**Table 4.26. ANOVA for comparing the weight of larval instars of *C. megacephala***

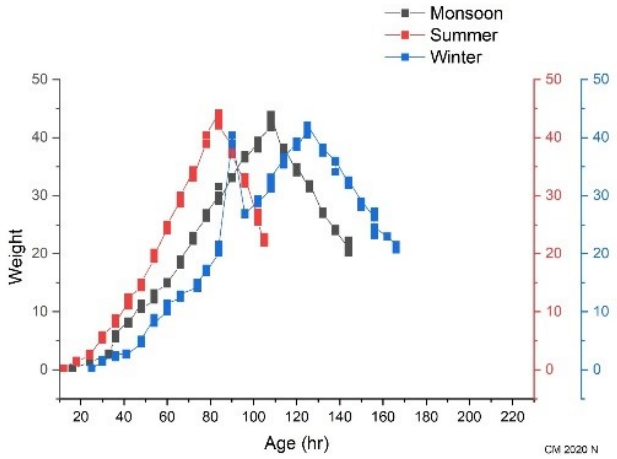
| Source | df | Sum of Squares | Mean Square | F-Value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 1.70 | 0.851 | 11.718** | < 0.001 |
| Season | 2 | 13.60 | 6.799 | 93.618** | < 0.001 |
| Stage | 3 | 17041.12 | 5680.372 | 78210.86** | < 0.001 |
| Year * Season | 4 | 5.08 | 1.269 | 17.473** | < 0.001 |
| Year * stage | 6 | 2.64 | 0.44 | 6.061** | < 0.001 |
| Season * stage | 6 | 13.13 | 2.189 | 30.135** | < 0.001 |
| Year * Season * Stage | 12 | 12.52 | 1.043 | 14.365** | < 0.001 |
| Error | 72 | 5.23 | 0.073 | | |
| Total | 107 | 17095.02 | | | |

** Significant at 0.01 level; ns non-Significant

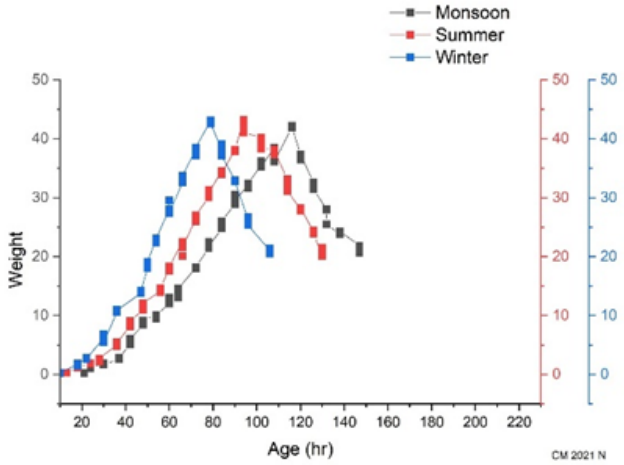
The growth curve representing the developmental rate (Weight (mg) Vs. Age (hr) of *C. megacephala* from hatching until pupation during different seasons and years were prepared (Fig. 4.51 and Fig. 4.52).




2019

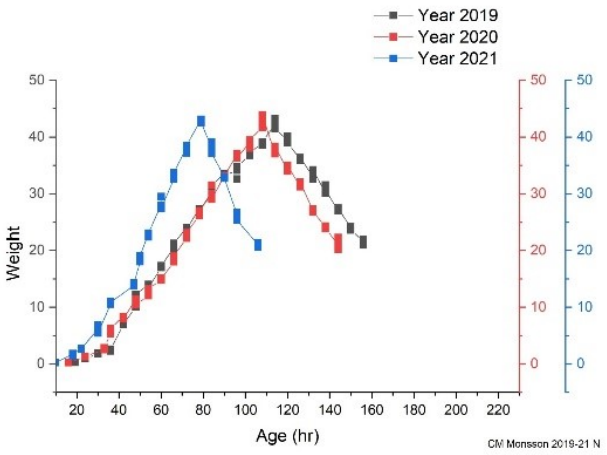



2020

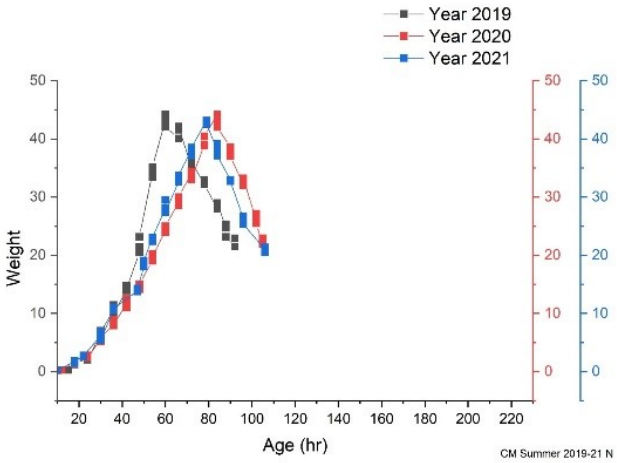



2021

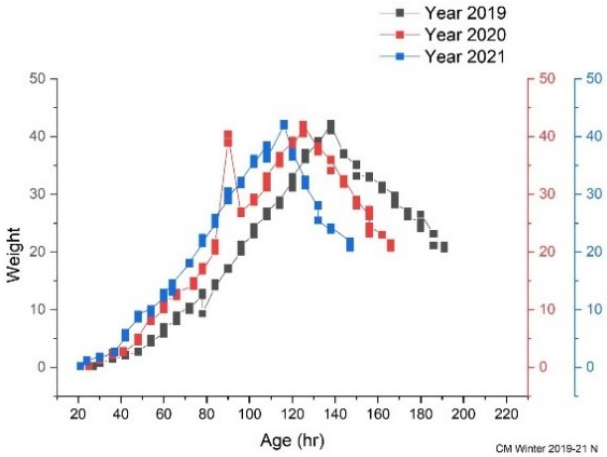
**Fig. 4.51. Seasonal developmental rate (Weight (mg) Vs. Age (hr) of *C. megacephala* from hatching upto pupation**




Monsoon




Summer




Winter

**Fig. 4.52. Developmental rate (Weight (mg) Vs Age (hr) of *C. megacephala* from hatching upto pupation during the study period**

**Effect on life cycle duration**

Significantly higher duration (hr) was observed during winter for *C. megacephala* for incubation (24.00 ± 2.65) (F = 408.40; P = <0.001), duration of II$^{nd}$ larval instar (28.00 ± 5.29) (F = 8.555; P = 0.036), post feeding stage (36.33 ± 10.69) (F = 8.704; P = 0.035), pupation (125.67 ± 7.37) (F = 480.82; P = <0.001), and total life cycle (286.00 ± 23.26) (F = 50.49; P = <0.001) (Table 4.27).

**Table 4.27. Seasonal changes in duration (hrs) of life cycle of *C. megacephala***

| Stages | Monsoon | Summer | Winter | F-value (P-value) |
|---|---|---|---|---|
| Incubation | 15.67 ± 2.52$^{b}$ | 12.00 ± 2.00$^{c}$ | 24.00 ± 2.65$^{a}$ | 408.40** (<0.001) |
| Instar I | 16.67 ± 1.53 | 12.00 ± 0 | 23.00 ± 6.08 | 6.802$^{ns}$ (0.052) |
| Instar II | 22.33 ± 2.08$^{ab}$ | 17.00 ± 1.00$^{b}$ | 28.00 ± 5.29$^{a}$ | 8.555* (0.036) |
| Instar III | 45.33 ± 13.32 | 27.00 ± 4.36 | 49.00 ± 6.25 | 5.417$^{ns}$ (0.073) |
| Post feeding stage | 32.00 ± 5.29$^{ab}$ | 23.00 ± 4.58$^{b}$ | 36.33 ± 10.69$^{a}$ | 8.704* (0.035) |
| Pupation | 95.00 ± 3.61$^{b}$ | 77.00 ± 5.57$^{c}$ | 125.67 ± 7.37$^{a}$ | 480.82** (<0.001) |
| Total time taken from egg stage till emergence | 227.00 ± 22.52$^{b}$ | 168.00 ± 5.29$^{c}$ | 286.00 ± 23.26$^{a}$ | 50.49** (<0.001) |

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*
*Means having different letter as super script differ significantly between seasons (within a row)*

Significantly higher duration (hrs) was observed during 2019 for *C. megacephala* for incubation (19.33 ± 6.11) (F = 60.40; P = < 0.001) and post feeding stage (38.00 ± 10.00) (F = 8.244; P =0.038). The duration of pupation stage was significantly higher in 2020 (105.33 ± 26.08) (F =24.029; P =0.006) (Table 4.28).

**Table 4.28. Changes in duration (hrs) of life cycle of *C. megacephala***

| Stages | 2019 | 2020 | 2021 | F-value (P-value) |
|---|---|---|---|---|
| Incubation | $19.33 \pm 6.11^{a}$ | $17.67 \pm 6.66^{b}$ | $14.67 \pm 5.69^{c}$ | 60.40** (< 0.001) |
| Instar I | $19.00 \pm 7.55$ | $15.00 \pm 2.65$ | $17.67 \pm 7.37$ | $0.926^{ns}$ (0.467) |
| Instar II | $24.33 \pm 9.07$ | $22.67 \pm 4.16$ | $20.33 \pm 3.51$ | $1.141^{ns}$ (0.405) |
| Instar III | $46.00 \pm 20.88$ | $39.67 \pm 8.74$ | $35.67 \pm 7.64$ | $1.058^{ns}$ (0.428) |
| Post feeding stage | $38.00 \pm 10.00^{a}$ | $27.67 \pm 7.77^{b}$ | $25.67 \pm 3.22^{b}$ | 8.244* (0.038) |
| Pupation | $94.67 \pm 24.11^{b}$ | $105.33 \pm 26.08^{a}$ | $97.67 \pm 23.71^{b}$ | 24.029** (0.006) |
| Total time taken from egg stage till emergence | $241.33 \pm 73.8$ | $228.00 \pm 54$ | $211.67 \pm 49.94$ | $3.202^{ns}$ (0.148) |

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*
*Means having different letter as super script differ significantly between years (within a row)*

**Effect on survival rate**

Effect of season on the survival rate (%) of *C. megacephala* was found to be significant (Table 4.32).The survival rate (%) in *C. megacephala* was significantly higher in monsoon ($80.73 \pm 9.94$) in comparison to summer ($77.9 \pm 10.38$) and winter ($72.48 \pm 6.39$) and significantly higher in 2021 ($78.06 \pm 7.08$) compared to 2019 and 2020 (Table 4.29).

**Table 4.29. Seasonal changes in survival rate (%) of *C. megacephala***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $81.33 \pm 11.64$ | $77.41 \pm 13.11$ | $72.05 \pm 8.30$ | $76.93 \pm 11.59^{AB}$ |
| 2020 | $79.87 \pm 10.57$ | $77.25 \pm 10.81$ | $71.23 \pm 5.82$ | $76.12 \pm 9.84^{B}$ |
| 2021 | $80.98 \pm 7.89$ | $79.03 \pm 6.91$ | $74.16 \pm 4.57$ | $78.06 \pm 7.08^{A}$ |
| Overall Season | $80.73 \pm 9.94^{a}$ | $77.9 \pm 10.38^{b}$ | $72.48 \pm 6.39^{c}$ | $77.04 \pm 9.65$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of year and stage on the survival rate was found to be significant (Table 4.32) The survival rate of egg was significantly higher in 2019 (90.76 ± 4.94) compared to 2020 (86.68 ± 5.97) and 2021 (81.53 ± 5.39). However, the survival rate in 2nd Instar (77.96 ± 7.05) and 3rd Instar (72.74 ± 4.14) was significantly higher in 2021. Similarly the survival rate of pupation was also significantly higher in 2021 (72.74 ± 4.12) (Table 4.30).

**Table 4.30. Survival rate (%) of life cycle stages of *C.megacephala***

| Stage | 2019 | 2020 | 2021 | Overall stage |
|---|---|---|---|---|
| Egg | $90.76 \pm 4.94^{aA}$ | $86.68 \pm 5.97^{bA}$ | $81.53 \pm 5.39^{cB}$ | $86.32 \pm 6.50^{A}$ |
| I$^{st}$ Instar | $84.70 \pm 8.58^{B}$ | $82.64 \pm 8.25^{B}$ | $85.32 \pm 5.08^{A}$ | $84.22 \pm 7.27^{B}$ |
| II$^{nd}$ Instar | $74.84 \pm 9.11^{bC}$ | $75.15 \pm 8.37^{bC}$ | $77.96 \pm 7.05^{aC}$ | $75.98 \pm 8.03^{C}$ |
| III$^{rd}$ Instar | $67.54 \pm 4.68^{bD}$ | $67.49 \pm 4.01^{bD}$ | $72.74 \pm 4.14^{aD}$ | $69.26 \pm 4.82^{D}$ |
| Pupa | $66.81 \pm 5.86^{bD}$ | $68.64 \pm 4.71^{bD}$ | $72.74 \pm 4.12^{aD}$ | $69.4 \pm 5.38^{D}$ |
| Overall year | $76.93 \pm 11.59^{ab}$ | $76.12 \pm 9.84^{b}$ | $78.06 \pm 7.08^{a}$ | $77.04 \pm 9.65$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of season and stage on the survival rate was found to be significant (Table 4.32). The survival of egg (89.70 ± 4.78), I$^{st}$ Instar (89.29 ± 2.61), II$^{nd}$ Instar (84.65 ± 2.72), III$^{rd}$ Instar larvae (73.83 ± 3.69) was significantly higher in monsoon compared to other seasons. Survival of pupa of *C. megacephala* was significantly higher in winter (73.85 ± 3.07) compared to monsoon (66.18 ± 3.31) and summer (68.17 ± 6.17) (Table 4.31).

**Table 4.31. Seasonal changes in survival rate (%) of life cycle stages of *C. megacephala***

| Stage | Monsoon | Summer | Winter | Overall stage |
|---|---|---|---|---|
| Egg | $89.70 \pm 4.78^{aA}$ | $89.39 \pm 4.12^{aA}$ | $79.88 \pm 5.23^{bA}$ | $86.32 \pm 6.50^{A}$ |
| I$^{st}$ Instar | $89.29 \pm 2.61^{aA}$ | $88.33 \pm 2.39^{aA}$ | $75.04 \pm 4.09^{bB}$ | $84.22 \pm 7.27^{B}$ |
| II$^{nd}$ Instar | $84.65 \pm 2.72^{aB}$ | $76.52 \pm 2.23^{bB}$ | $66.77 \pm 4.09^{cD}$ | $75.98 \pm 8.03^{C}$ |
| III$^{rd}$ Instar | $73.83 \pm 3.69^{aC}$ | $67.09 \pm 3.93^{bC}$ | $66.85 \pm 3.32^{bD}$ | $69.26 \pm 4.82^{D}$ |
| Pupa | $66.18 \pm 3.31^{bD}$ | $68.17 \pm 6.17^{bC}$ | $73.85 \pm 3.07^{aC}$ | $69.40 \pm 5.38^{D}$ |
| Overall Season | $80.73 \pm 9.94^{a}$ | $77.9 \pm 10.38^{b}$ | $72.48 \pm 6.39^{c}$ | $77.04 \pm 9.65$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Three way ANOVA for studying the interaction between year, season and developmental stages indicated interactions between year and stage (F=10.06; P=<0.001) as well as season and stage F = 29.87; P = <0.001) (Table 4.32).

**Table 4.32. ANOVA for comparing the survival rate of *C. megacephala***

| Source | df | Sum of Squares | Mean Square | F-value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 85.34 | 42.67 | 4.79* | 0.011 |
| Season | 2 | 1581.27 | 790.64 | 88.68** | <0.001 |
| Stage | 4 | 6963.08 | 1740.77 | 195.25** | <0.001 |
| Year * season | 4 | 29.40 | 7.35 | $0.82^{ns}$ | 0.513 |
| Year * Stage | 8 | 717.24 | 89.66 | 10.06** | <0.001 |
| season * Stage | 8 | 2130.54 | 266.32 | 29.87** | <0.001 |
| Year * season * Stage | 16 | 159.34 | 9.96 | $1.12^{ns}$ | 0.352 |
| Error | 90 | 802.40 | 8.92 | | |
| Corrected Total | 134 | 12468.60 | | | |

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

### 4.4.2. *C. rufifacies*

**Effect on fecundity**

Effect of season on the pre-oviposition period were found to be significant (F = 23.444, P = < 0.001). Effect of year on the pre-oviposition period were found to be significant (F = 2.111, P = 0.150) However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 1.778, P = 0.177). The pre-oviposition period (days) in *C.rufifacies was* significantly higher in winter (9.22 ± 0.67) in comparison to monsoon (7.56 ± 0.53) and summer (7.67 ± 0.71) (Table 4.33).

**Table 4.33. Pre-oviposition period (days) of *C.rufifacies***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 7.33 ± 0.58 | 8.33 ± 0.58 | 9.67 ± 0.58 | 8.44 ± 1.13 |
| 2020 | 7.67 ± 0.58 | 7.33 ± 0.58 | 9.33 ± 0.58 | 8.11 ± 1.05 |
| 2021 | 7.67 ± 0.58 | 7.33 ± 0.58 | 8.67 ± 0.58 | 7.89 ± 0.78 |
| Overall Season | $7.56 \pm 0.53^{b}$ | $7.67 \pm 0.71^{b}$ | $9.22 \pm 0.67^{a}$ | 8.15 ± 0.99 |
| Between year F-value = $2.111^{ns}$; (P-value = 0.150)<br>Between season F-value = 23.444**; (P-value < 0.001))<br>Interaction between season and year F-value =$1.778^{ns}$; (P-value = 0.177) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*

Effect of season on the number of eggs laid in a day was found to be significant (F = 223.63, P = < 0.001). Effect of year on the oviposition was also found to be significant (F = 3.67, P = 0.046). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 2.91, P = 0.051). The number of eggs laid by *C. rufifacies* was significantly higher in monsoon (281.00 ± 5.45) in comparison to summer (246.89 ± 11.68) and winter (215.33 ± 5.87) (Table 4.34).

**Table 4.34. Eggs laid by the *C. rufifacies* in a day**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 019 | 278.33 ± 2.08 | 254.67 ± 9.07 | 217.00 ± 8.19 | $250.00 \pm 27.5^{A}$ |
| 2020 | 282.67 ± 4.16 | 252.00 ± 4.58 | 216.33 ± 4.73 | $250.33 \pm 29.01^{A}$ |
| 2021 | 282.00 ± 9.00 | 234.00 ± 7.94 | 212.67 ± 5.69 | $242.89 \pm 31.46^{B}$ |
| Overall Season | $281.00 \pm 5.45^{a}$ | $246.89 \pm 11.68^{b}$ | $215.33 \pm 5.87^{c}$ | 247.74 ± 28.43 |
| Between year F-value = 3.67*; (P-value = 0.046)<br>Between season F-value = 223.63**; (P-value < 0.001))<br>Interaction between season and year F-value =$2.91^{ns}$; (P-value = 0.051) | | | | |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of season on the periodicity of egg laying was found to be significant (F = 5.727, P = 0.012). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 1.091; P = 0.391). The periodicity of egg laying in

*C. rufifacies* was significantly higher in winter (4.67 ± 0.35) in comparison to monsoon (4.50 ± 0.35) and summer (4.17 ± 0.25) (Table 4.35).

**Table 4.35. Periodicity of egg laying (days) by *C. rufifacies***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 4.83 ± 0.29 | 4.17 ± 0.29 | 4.67 ± 0.29 | 4.56 ± 0.39 |
| 2020 | 4.33 ± 0.29 | 4.17 ± 0.29 | 4.50 ± 0.50 | 4.33 ± 0.35 |
| 2021 | 4.33 ± 0.29 | 4.17 ± 0.29 | 4.83 ± 0.29 | 4.44 ± 0.39 |
| Overall Season | $4.50 \pm 0.35^{a}$ | $4.17 \pm 0.25^{b}$ | $4.67 \pm 0.35^{a}$ | 4.44 ± 0.38 |
| Between year F-value = $1.091^{ns}$; (P-value = 0.357)<br>Between season F-value = 5.727*; (P-value = 0.012))<br>Interaction between season and year F-value =$1.091^{ns}$; (P-value = 0.391) | | | | |

*Means having different small letter as superscript differ significantly within a row*

Effect of season on the total number of eggs laid during the life span was found to be significant (F = 835.8, P =< 0.001). However, seasonal variations were not same in all years and also the yearly variations were not same in all seasons (F = 3.28, P = 0.035). The number of eggs laid by *C. rufifacies* was significantly higher in monsoon (1953.89 ± 22.70) in comparison to summer (1849.11 ± 26.05) and winter (1723.78 ± 12.18) (Table 4.36).

**Table 4.36. Eggs laid by the *C. rufifacies* during in its life span**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $1975.67 \pm 14.19^{aA}$ | $1877.67 \pm 11.59^{bA}$ | $1734.00 \pm 11.14^{c}$ | $1862.44 \pm 105.81^{A}$ |
| 2020 | $1953.67 \pm 10.26^{aB}$ | $1848.33 \pm 13.32^{bB}$ | $1714.33 \pm 8.33^{c}$ | $1838.78 \pm 104.31^{B}$ |
| 2021 | $1932.33 \pm 18.58^{aC}$ | $1821.33 \pm 4.51^{bC}$ | $1723.00 \pm 10.44^{c}$ | $1825.56 \pm 91.35^{C}$ |
| Overall Season | $1953.89 \pm 22.70^{a}$ | $1849.11 \pm 26.05^{b}$ | $1723.78 \pm 12.18^{c}$ | 1842.26 ± 97.99 |
| Between year F-value = 22.00; (P-value < 0.001)<br>Between season F-value = 835.8**; (P-value < 0.001))<br>Interaction between season and year F-value =3.28*; (P-value = 0.035) | | | | |

** Significant at 0.01 level; * *significant at 0.05 level*
*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Effect on length of larvae**

**Instar I**

Effect of season, year and stage on the length of I$^{st}$ instar was found to be significant. Season wise variations were not same in all years and also the year wise variations were not same in all seasons (Table 4.41). The instar length in C. *rufifacies* was significantly higher in monsoon (1.79 ± 0.16) in comparison to summer (1.64 ± 0.26) and winter (1.56 ± 0.02). It was significantly higher (1.84 ± 0.22) in 2021 compared to 2019 (1.54 ± 0.10) and 2020 (1.61 ± 0.10) (Table 4.37).

**Table 4.37. Seasonal changes in length (mm) of I$^{st}$ instar larvae of *C. rufifacies***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $1.64 \pm 0.01^{aB}$ | $1.41 \pm 0.02^{bB}$ | $1.56 \pm 0.01^{a}$ | $1.54 \pm 0.10^{C}$ |
| 2020 | $1.74 \pm 0.02^{aB}$ | $1.53 \pm 0.05^{bB}$ | $1.56 \pm 0.01^{b}$ | $1.61 \pm 0.10^{B}$ |
| 2021 | $1.99 \pm 0.06^{aA}$ | $1.97 \pm 0.02^{aA}$ | $1.55 \pm 0.05^{b}$ | $1.84 \pm 0.22^{A}$ |
| Overall Season | $1.79 \pm 0.16^{a}$ | $1.64 \pm 0.26^{b}$ | $1.56 \pm 0.02^{c}$ | 1.66 ± 0.20 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Instar II**

Effect of season, year and stage on the length of II$^{nd}$ instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.41). The instar length in *C. rufifacies* was significantly higher in monsoon (5.83 ± 0.07) in comparison to summer (5.63 ± 0.08) and winter (4.76 ± 0.41). It was significantly higher (5.56 ± 0.28) in 2021 compared to 2019 (4.50 ± 1.51) and 2020 (5.08 ± 0.53) (Table 4.38).

**Table 4.38. Seasonal changes in length (mm) of IInd instar larvae of *C. rufifacies***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $6.34 \pm 0.12^{aA}$ | $2.88 \pm 0.02^{cC}$ | $4.29 \pm 0.10^{bC}$ | $4.50 \pm 1.51^{C}$ |
| 2020 | $5.77 \pm 0.09^{aB}$ | $4.69 \pm 0.18^{bB}$ | $4.77 \pm 0.07^{bB}$ | $5.08 \pm 0.53^{B}$ |
| 2021 | $5.83 \pm 0.07^{aB}$ | $5.63 \pm 0.08^{bA}$ | $5.22 \pm 0.07^{cA}$ | $5.56 \pm 0.28^{A}$ |
| Overall Season | $5.98 \pm 0.28^{a}$ | $4.40 \pm 1.22^{c}$ | $4.76 \pm 0.41^{b}$ | $5.05 \pm 1$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Instar III**

Effect of season, year and stage on the length of IIIrd instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.41). The instar length in *C. rufifacies* was significantly higher in monsoon (10.94 ± 0.10) in comparison to summer (10.29 ± 0.77) and winter (10.61 ± 0.13). It was significantly higher (10.87 ± 0.29) in 2021 compared to 2019 (10.26 ± 0.71) and 2020 (10.71 ± 0.19) (Table 4.39).

**Table 4.39. Seasonal changes in length (mm) of IIIrd instar larvae of *C. rufifacies***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $10.88 \pm 0.08^{aB}$ | $9.33 \pm 0.1^{cC}$ | $10.58 \pm 0.07^{bB}$ | $10.26 \pm 0.71^{C}$ |
| 2020 | $10.87 \pm 0.04^{aB}$ | $10.49 \pm 0.17^{bB}$ | $10.76 \pm 0.04^{aA}$ | $10.71 \pm 0.19^{B}$ |
| 2021 | $11.06 \pm 0.04^{aA}$ | $11.06 \pm 0.08^{aA}$ | $10.5 \pm 0.04^{bB}$ | $10.87 \pm 0.29^{A}$ |
| Overall Season | $10.94 \pm 0.10^{a}$ | $10.29 \pm 0.77^{c}$ | $10.61 \pm 0.13^{b}$ | $10.61 \pm 0.51$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Post feeding stage**

Effect of season, year and stage on the length of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.41). The length of post feeding stage in *C. rufifacies* was significantly higher in monsoon (10.81 ± 0.52) in comparison to summer (10.37 ±

0.22) and winter (10.66 ± 0.41). It was significantly higher (10.80 ± 0.46) in 2021 compared to 2019 (10.31 ± 0.25) and 2020 (10.73 ± 0.40) (Table 4.40).

**Table 4.40. Seasonal changes in length (mm) of post feeding stage of *C. rufifacies***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $10.15 \pm 0.05^{bB}$ | $10.65 \pm 0.05^{aA}$ | $10.14 \pm 0.02^{bC}$ | $10.31 \pm 0.25^{B}$ |
| 2020 | $10.98 \pm 0.07^{aA}$ | $10.21 \pm 0.08^{bB}$ | $11.00 \pm 0.14^{aA}$ | $10.73 \pm 0.40^{A}$ |
| 2021 | $11.30 \pm 0.09^{aA}$ | $10.26 \pm 0.09^{cB}$ | $10.85 \pm 0.09^{bB}$ | $10.80 \pm 0.46^{A}$ |
| Overall Season | $10.81 \pm 0.52^{a}$ | $10.37 \pm 0.22^{c}$ | $10.66 \pm 0.41^{b}$ | $10.61 \pm 0.43$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

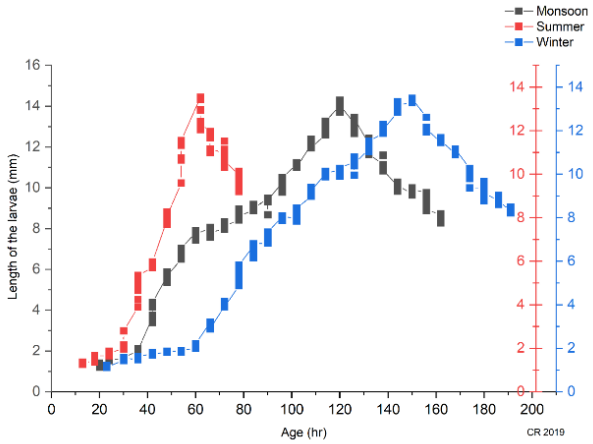
Interaction studies on year, season and stage was found to be significant (F = 0.95; P = < 0.001) indicating that seasonal variations in length of each larval instar stage were different in different years (Table 4.41).

**Table 4.41. ANOVA for comparing length of larval instars of *C. rufifacies***

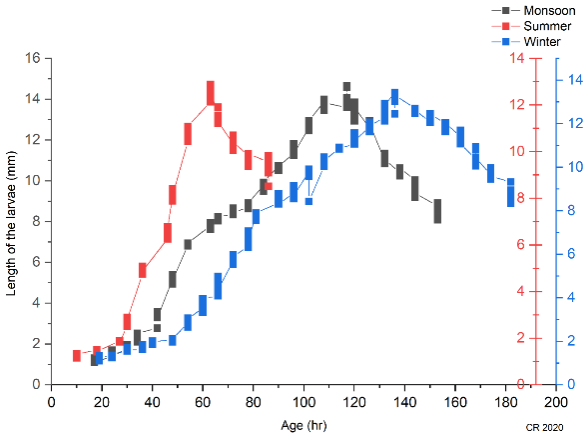
| Source | df | Sum of Squares | Mean Square | F-Value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 6.91 | 3.45 | 571.01** | <0.001 |
| Season | 2 | 9.31 | 4.66 | 769.84** | <0.001 |
| Stage | 3 | 1577.29 | 525.76 | 86910.80** | <0.001 |
| Year * Season | 4 | 2.97 | 0.74 | 122.62** | <0.001 |
| Year * stage | 6 | 1.62 | 0.27 | 44.65** | <0.001 |
| Season * stage | 6 | 6.06 | 1.01 | 167.04** | <0.001 |
| Year * Season * Stage | 12 | 11.39 | 0.95 | 156.91** | <0.001 |
| Error | 72 | 0.44 | 0.01 | | |
| Total | 107 | 1615.99 | | | |

** Significant at 0.01 level

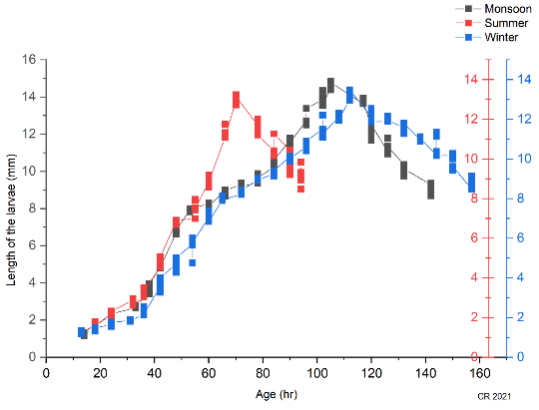
The growth curves representing the developmental rate (Length (mm) Vs. Age (hr) of *C. rufifacies* from hatching until pupation during different seasons and years were prepared (Fig. 4.53 & Fig. 4.54).




2019

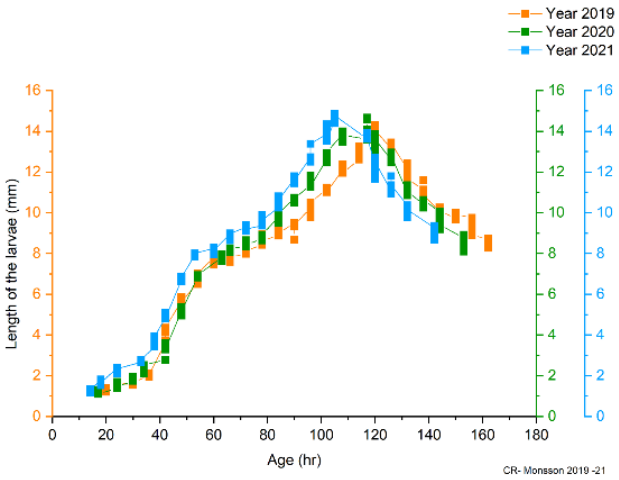



2020

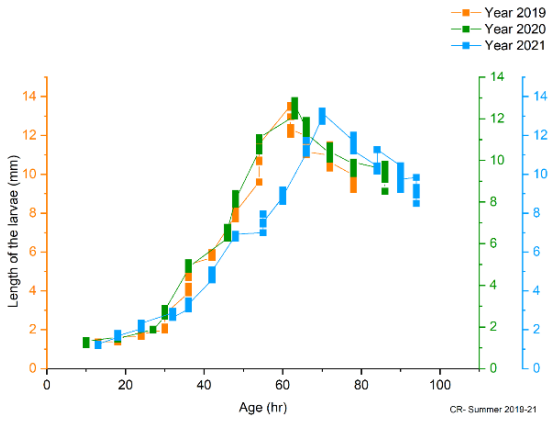



2021

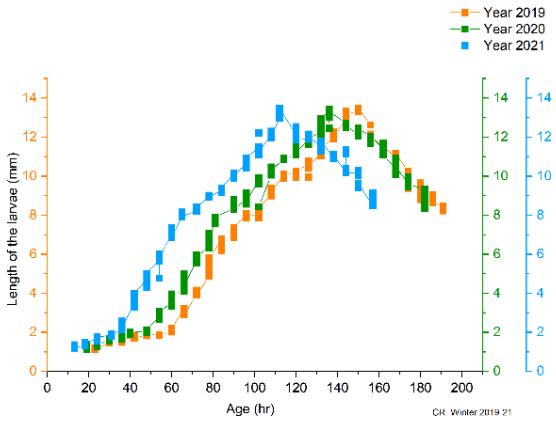
**Fig. 4.53. Seasonal developmental rate (Length (mm) Vs. Age (hr) of *C. rufifacies* from hatching upto pupation**




Monsoon




Summer




Winter

**Fig. 4.54. Developmental rate (Length (mm) Vs. Age (hr.) of *C. rufifacies* from hatching up to pupation during the study period**

**Effect on weight of larvae**

**Instar I**

Effect of season and year on the weight of I$^{st}$ instar was found to be not significant. The pattern of differences in the weight was similar in all the years and seasons (Table 4.42).

**Table 4.42. Seasonal changes in weight (mg) of I$^{st}$ instar larvae of *C. rufifacies***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 1.54 ± 0.04 | 1.54 ± 0.07 | 1.52 ± 0.04 | 1.53 ± 0.05 |
| 2020 | 1.53 ± 0.04 | 1.47 ± 0.07 | 1.61 ± 0.05 | 1.54 ± 0.08 |
| 2021 | 1.54 ± 0.04 | 1.43 ± 0.06 | 1.58 ± 0.04 | 1.52 ± 0.08 |
| Overall Season | 1.53 ± 0.03 | 1.48 ± 0.08 | 1.57 ± 0.06 | 1.53 ± 0.07 |

**Instar II**

Effect of season, year and stage on the weight of II$^{nd}$ instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table. 4.46). The instar weight in *C. rufifacies* was significantly higher in monsoon (10.59 ± 1.06) in comparison to summer (10.55 ± 0.57) and winter (9.53 ± 0.21) (Table 4.43).

**Table 4.43. Seasonal changes in weight (mg) of II$^{nd}$ instar larvae of *C. rufifacies***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $9.25 \pm 0.35^{bB}$ | $10.70 \pm 0.23^{aA}$ | $9.45 \pm 0.22^{b}$ | $9.80 \pm 0.72^{C}$ |
| 2020 | $11.25 \pm 0.28^{aA}$ | $11.08 \pm 0.29^{aA}$ | $9.61 \pm 0.29^{b}$ | $10.65 \pm 0.82^{A}$ |
| 2021 | $11.28 \pm 0.48^{aA}$ | $9.88 \pm 0.08^{bB}$ | $9.52 \pm 0.18^{b}$ | $10.23 \pm 0.84^{B}$ |
| Overall Season | $10.59 \pm 1.06^{a}$ | $10.55 \pm 0.57^{a}$ | $9.53 \pm 0.21^{b}$ | 10.22 ± 0.84 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Instar III**

Effect of season, year and stage on the weight of III$^{rd}$ instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table. 4.46). The instar weight in *C. rufifacies* was significantly higher in summer (32.47 ± 1.34) in comparison to monsoon (31.86 ± 0.31) and winter (30.13 ± 0.46) (Table 4.44).

**Table 4.44. Seasonal changes in weight (mg) of III$^{rd}$ instar larvae of *C. rufifacies***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $32.19 \pm 0.27^{a}$ | $30.72 \pm 0.28^{bB}$ | $30.60 \pm 0.13^{bA}$ | $31.17 \pm 0.79^{B}$ |
| 2020 | $31.71 \pm 0.15^{b}$ | $33.56 \pm 0.19^{aA}$ | $29.88 \pm 0.34^{cB}$ | $31.72 \pm 1.61^{A}$ |
| 2021 | $31.69 \pm 0.22^{b}$ | $33.12 \pm 0.29^{aA}$ | $29.91 \pm 0.43^{cB}$ | $31.57 \pm 1.42^{A}$ |
| Overall Season | $31.86 \pm 0.31^{b}$ | $32.47 \pm 1.34^{a}$ | $30.13 \pm 0.46^{c}$ | $31.49 \pm 1.29$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Post feeding stage**

Effect of season, year and stage on the weight of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table. 4.46). The weight of post feeding stage in *C. rufifacies* was significantly higher in monsoon (30.93 ± 0.96) in comparison to summer (29.16 ± 0.69) and winter (29.79 ± 0.36) (Table 4.45).

**Table 4.45. Seasonal changes in weight (mg) post feeding stage of *C. rufifacies***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $30.64 \pm 0.37^{aB}$ | $28.53 \pm 0.29^{bB}$ | $30.17 \pm 0.29^{a}$ | $29.78 \pm 1.00$ |
| 2020 | $30.85 \pm 0.32^{aAB}$ | $29.90 \pm 0.59^{bA}$ | $29.61 \pm 0.09^{b}$ | $30.12 \pm 0.66$ |
| 2021 | $31.31 \pm 1.75^{aA}$ | $29.04 \pm 0.13^{bB}$ | $29.59 \pm 0.32^{b}$ | $29.98 \pm 1.36$ |
| Overall Season | $30.93 \pm 0.96^{a}$ | $29.16 \pm 0.69^{c}$ | $29.79 \pm 0.36^{b}$ | $29.96 \pm 1.01$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

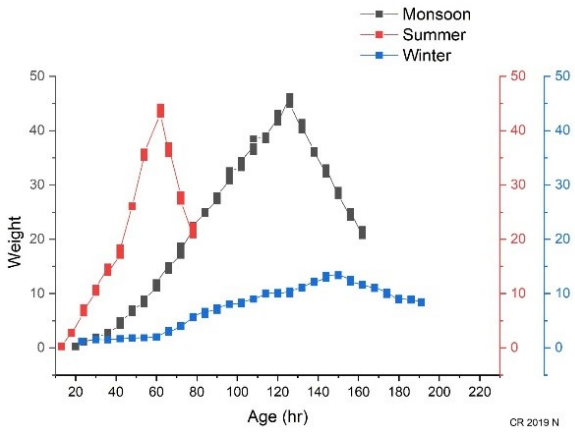
Interaction studies on year, season and stage was found to be significant (F-value = 10.095; P-value <0.001) indicating that seasonal variations in weight of each stage are different in different years (Table 4.46).

**Table 4.46. ANOVA for comparing the weight of larval instars of *C. rufifacies***

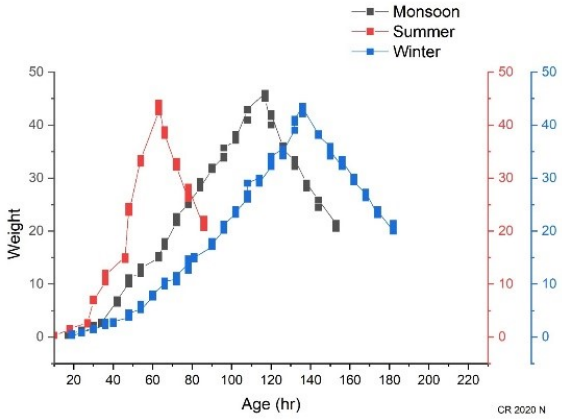
| Source | df | Sum of Squares | Mean Square | F-Value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 3.43 | 1.71 | 11.425** | < 0.001 |
| Season | 2 | 17.87 | 8.94 | 59.586** | < 0.001 |
| Stage | 3 | 17721.43 | 5907.14 | 39388.42** | < 0.001 |
| Year * Season | 4 | 6.86 | 1.72 | 11.439** | < 0.001 |
| Year * stage | 6 | 1.77 | 0.30 | 1.97** | < 0.001 |
| Season * stage | 6 | 29.77 | 4.96 | 33.081** | < 0.001 |
| Year * Season * Stage | 12 | 18.17 | 1.51 | 10.095** | < 0.001 |
| Error | 72 | 10.80 | 0.15 | | |
| Total | 107 | 17810.1 | | | |

** Significant at 0.01 level

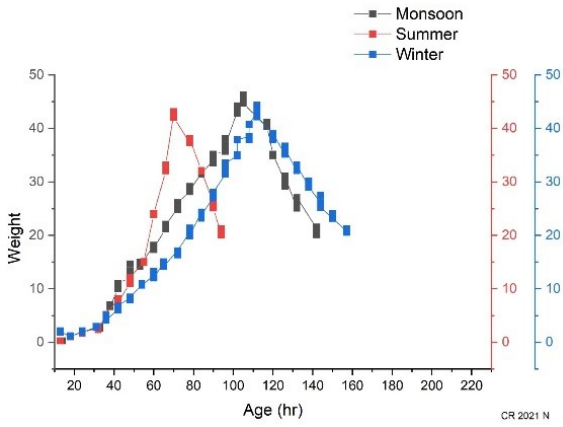
The growth curve representing the developmental rate (Weight (mg) Vs. Age (hr) of *C. rufifacies* from hatching until pupation during different seasons and years were prepared (Fig. 4.55 & Fig. 4.56).




2019

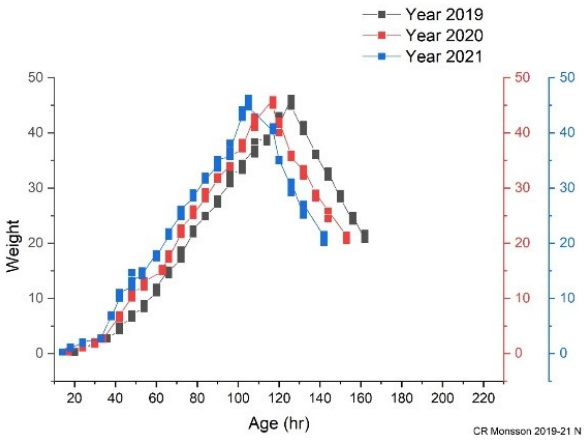



2020

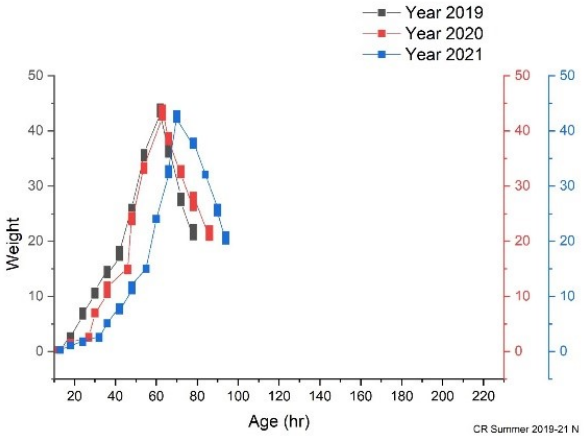



2021

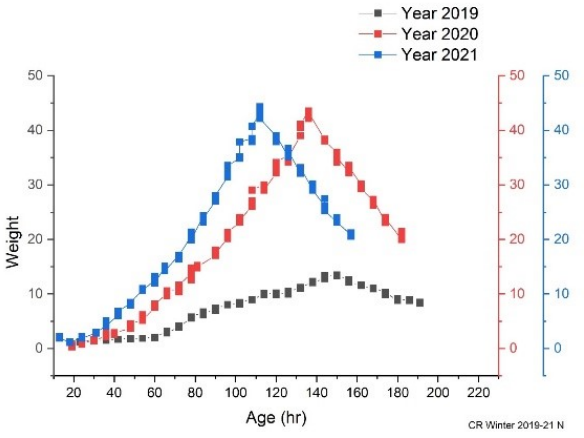
**Fig. 4.55. Seasonal developmental rate (Weight (mg) Vs. Age (hr) of *C. rufifacies* from hatching upto pupation**




Monsoon




Summer




Winter

**Fig. 4.56. Developmental rate (Weight (mg) Vs. Age (hr) of *C.rufifacies* from hatching upto pupation during the study period**

**Effect on life cycle duration**

Significantly higher duration (hr) was observed for *C.rufifacies* during winter for IInd larval instar (32.67 ± 3.51) (F = 12.250; P = 0.020), post feeding stage (36.67 ± 1.53) (F = 45.953; P = 0.002), pupation (111.33 ± 3.79) (F = 115.71; P = <0.001), and total life cycle (267.00 ± 18.68) (F = 65.588; P = < 0.001). The duration of III$^{rd}$ Instar larvae was significantly higher in monsoon (50.00 ± 4.58) (F = 491.46; P = <0.001) (Table 4.47).

**Table 4.47. Seasonal changes in duration (hrs) of life cycle of *C.rufifacies***

| Stages | Monsoon | Summer | Winter | F-value (P-value) |
|---|---|---|---|---|
| Incubation | 17.00 ± 3.00 | 12.00 ± 1.73 | 18.33 ± 5.03 | 4.300$^{ns}$ (0.101) |
| Instar I | 17.67 ± 2.08 | 16.67 ± 2.52 | 21.33 ± 3.51 | 1.606$^{ns}$ (0.308) |
| Instar II | 20.00 ± 4.58$^{b}$ | 15.67 ± 3.51$^{b}$ | 32.67 ± 3.51$^{a}$ | 12.250* (0.020) |
| Instar III | 50.00 ± 4.58$^{a}$ | 15.00 ± 5.00$^{b}$ | 46.67 ± 7.02$^{a}$ | 491.46** (<0.001) |
| Post feeding stage | 29.33 ± 4.04$^{b}$ | 16.00 ± 4.00$^{c}$ | 36.67 ± 1.53$^{a}$ | 45.953** (0.002) |
| Pupation | 89.00 ± 1.00$^{b}$ | 73.00 ± 2.65$^{c}$ | 111.33 ± 3.79$^{a}$ | 115.71** (<0.001) |
| Total time taken from egg stage till emergence | 223.00 ± 13.45$^{b}$ | 148.33 ± 6.43$^{c}$ | 267.00 ± 18.68$^{a}$ | 65.588** (0.001) |

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*
*Means having different letter as super script differ significantly between seasons (within a row)*

Significantly higher duration (hrs) was observed during 2019 for *C.rufifacies* for III$^{rd}$ larval instar (42.67 ± 19.63) compared to 2020 (37.33 ± 19.5) and 2021 (31.67 ± 18.93) (F = 39.854; P = 0.002) (Table 4.48)**.**

**Table 4.48. Changes in duration (hrs) of life cycle of *C. rufifacies***

| Stages | 2019 | 2020 | 2021 | F-value (P-value) |
|---|---|---|---|---|
| Incubation | 18.67 ± 5.13 | 15.33 ± 4.73 | 13.33 ± 0.58 | 2.800 (0.174) |
| Instar I | 18.33 ± 5.86 | 18.33 ± 2.31 | 19.00 ± 1.00 | 0.039$^{ns}$ (0.962) |
| Instar II | 24.00 ± 12.00 | 23.33 ± 8.74 | 21.00 ± 7.21 | 0.390$^{ns}$ (0.701) |
| Instar III | 42.67 ± 19.63$^{a}$ | 37.33 ± 19.5$^{b}$ | 31.67 ± 18.93$^{c}$ | 39.854** (0.002) |
| Post feeding stage | 25.67 ± 12.10 | 30.33 ± 9.29 | 26.00 ± 10.54 | 2.837$^{ns}$ (0.171) |
| Pupation | 91.33 ± 22.03 | 90.00 ± 16.09 | 92.00 ± 19.67 | 0.324$^{ns}$ (0.741) |
| Total time taken from egg stage till emergence | 220.67 ± 73.91 | 214.67 ± 56.52 | 203.00 ± 49.69 | 1.471$^{ns}$ (0.332) |

*** Significant at 0.01 level; ns non-significant*
*Means having different letter as super script differ significantly between years (within a row)*

**Effect on survival rate**

Effect of season on the survival rate (%) of *C. rufifacies* was found to be significant (Table 4.52). The survival rate (%) in *C. rufifacies* was significantly higher in monsoon (79.09 ± 7.56) in comparison to summer (76.70 ± 7.51) and winter (75.22 ± 5.93) (Table 4.49).

**Table 4.49. Seasonal changes in survival rate (%) of *C. rufifacies***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 81.34 ± 8.52 | 76.07 ± 9.66 | 73.22 ± 4.89 | 76.88 ± 8.48 |
| 2020 | 77.14 ± 8.43 | 76.90 ± 8.21 | 75.50 ± 5.94 | 76.51 ± 7.47 |
| 2021 | 78.79 ± 5.14 | 77.12 ± 3.99 | 76.94 ± 6.61 | 77.61 ± 5.30 |
| Overall Season | 79.09 ± 7.56$^{a}$ | 76.70 ± 7.51$^{b}$ | 75.22 ± 5.93$^{b}$ | 77.00 ± 7.17 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of year and stage on the survival rate of developmental stages was found to be significant (Table 4.52). The survival rate of I$^{st}$ larvar instar was significantly higher (84.76 ± 7.64) in 2019 compared to 2020 (80.66 ± 6.21) and 2021 (80.27 ± 3.54). However, the survival rate in pupation stage was significantly higher in 2021 (76.74 ± 4.37) compared to 2019 (69.96 ± 4.47) and 2020 (70.30 ± 7.12) (Table 4.50).

**Table 4.50. Survival rate (%) of life cycle of *C. rufifacies* during the study period**

| Stage | 2019 | 2020 | 2021 | Overall stage |
|---|---|---|---|---|
| Egg | 82.60 ± 6.69 | 84.39 ± 4.42 | 80.43 ± 4.84 | 82.47 ± 5.45$^{A}$ |
| I$^{st}$ Instar | 84.76 ± 7.64$^{a}$ | 80.66 ± 6.21$^{ab}$ | 80.27 ± 3.54$^{b}$ | 81.90 ± 6.16$^{A}$ |
| II$^{nd}$ Instar | 76.64 ± 5.70 | 74.70 ± 3.57 | 76.75 ± 4.72 | 76.03 ± 4.66$^{B}$ |
| III$^{rd}$ Instar | 70.42 ± 5.74 | 72.50 ± 5.57 | 73.90 ± 6.55 | 72.27 ± 5.92$^{C}$ |
| Pupa | 69.96 ± 4.47$^{b}$ | 70.30 ± 7.12$^{b}$ | 76.74 ± 4.37$^{a}$ | 72.33 ± 6.14$^{C}$ |
| Overall year | 76.88 ± 8.48 | 76.51 ± 7.47 | 77.61 ± 5.30 | 77.00 ± 7.17 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of season and stage on the survival rate of developmental stages was found to be significant (Table 4.52). The survival rate of developmental stages in *C. rufifacies* was significantly higher in monsoon (79.09 ± 7.56) in comparison to summer (76.70 ± 7.51) and winter (75.22 ± 5.93) (Table 4.51).

**Table 4.51. Seasonal changes in survival rate (%) of life cycle stages of *C. rufifacies***

| Stage | Monsoon | Summer | Winter | Overall stage |
|---|---|---|---|---|
| Egg | 84.23 ± 6.46$^{A}$ | 83.31 ± 3.52$^{A}$ | 79.89 ± 5.54$^{A}$ | 82.47 ± 5.45$^{A}$ |
| I$^{st}$ Instar | 85.29 ± 5.15$^{A}$ | 82.79 ± 6.72$^{A}$ | 77.61 ± 4.14$^{A}$ | 81.90 ± 6.16$^{A}$ |
| II$^{nd}$ Instar | 78.95 ± 5.29$^{B}$ | 75.49 ± 3.42$^{B}$ | 73.65 ± 3.81$^{AB}$ | 76.03 ± 4.66$^{B}$ |
| III$^{rd}$ Instar | 76.76 ± 4.63$^{C}$ | 72.11 ± 6.07$^{B}$ | 67.96 ± 3.44$^{B}$ | 72.27 ± 5.92$^{C}$ |
| Pupa | 70.22 ± 5.41$^{C}$ | 69.81 ± 5.87$^{C}$ | 76.98 ± 4.73$^{AB}$ | 72.33 ± 6.14$^{C}$ |
| Overall Season | 79.09 ± 7.56$^{a}$ | 76.70 ± 7.51$^{b}$ | 75.22 ± 5.93$^{b}$ | 77.00 ± 7.17 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Three way ANOVA for studying the interaction between year, season and developmental stages indicated interactions between season and stage (F = 4.965; P = <

0.001), year and stage (F = 3.082; P = 0.004), as well as year and season (F = 2.751; P = 0.033) (Table 4.52).

**Table 4.52. ANOVA for comparing the survival rate of *C. rufifacies***

| Source | df | Sum of Squares | Mean Square | F-value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 28.40 | 14.20 | $0.707^{ns}$ | 0.496 |
| Season | 2 | 343.43 | 171.71 | 8.553** | < 0.001 |
| Stage | 4 | 2672.32 | 668.08 | 33.275** | < 0.001 |
| Year * season | 4 | 220.97 | 55.24 | 2.751* | 0.033 |
| Year * Stage | 8 | 494.95 | 61.87 | 3.082** | 0.004 |
| season * Stage | 8 | 797.50 | 99.69 | 4.965** | < 0.001 |
| Year * season * Stage | 16 | 521.86 | 32.62 | $1.625^{ns}$ | 0.078 |
| Error | 90 | 1806.98 | 20.08 | | |
| Corrected Total | 134 | 6886.414 | | | |

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

### 4.4.3. *C. chani*

**Effect on fecundity**

Effect of season on the pre-oviposition were found to be significant (F = 40.111, P = < 0.001). Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (F = 4.778, P = 0.008). The pre-oviposition period in *C chani* was significantly higher in winter (10.11 ± 0.60) in comparison to monsoon (7.78 ± 0.67) and summer (8.33 ± 1.00) (Table 4.53).

**Table 4.53. Pre-oviposition period (days) of *C. chani***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $7.67 \pm 0.58^{cAB}$ | $9.33 \pm 0.58^{bA}$ | $10.33 \pm 0.58^{a}$ | 9.11 ± 1.27 |
| 2020 | $7.33 \pm 0.58^{cB}$ | $8.33 \pm 0.58^{bB}$ | $9.67 \pm 0.58^{a}$ | 8.44 ± 1.13 |
| 2021 | $8.33 \pm 0.58^{bA}$ | $7.33 \pm 0.58^{cC}$ | $10.33 \pm 0.58^{a}$ | 8.67 ± 1.41 |
| Overall Season | $7.78 \pm 0.67^{b}$ | $8.33 \pm 1.00^{b}$ | $10.11 \pm 0.60^{a}$ | 8.74 ± 1.26 |
| Between year F-value = $3.111^{ns}$; (P-value = 0.069)<br>Between season F-value = 40.111**; (P-value < 0.001))<br>Interaction between season and year F-value =$4.778^{**}$; (P-value = 0.008) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of season on the number of eggs laid in a day was found to be significant (F = 133.56, P = < 0.001). However, seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 0.91, P = 0.477). The number of eggs laid by *C chani* was significantly higher (265.00 ± 6.27) in monsoon in comparison to summer (234.44 ± 5.75) and winter (221.00 ± 5.72) (Table 4.54).

**Table 4.54. Eggs laid by the *C. chani* in a day**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 263.33 ± 7.51 | 240.00 ± 5.57 | 224.33 ± 7.02 | 242.56 ± 17.97 |
| 2020 | 264.33 ± 7.57 | 230.67 ± 2.52 | 218.67 ± 6.81 | 237.89 ± 21.16 |
| 2021 | 267.33 ± 5.51 | 232.67 ± 4.73 | 220.00 ± 3.00 | 240.00 ± 21.58 |
| Overall Season | 265.00 ± 6.27[a] | 234.44 ± 5.75[b] | 221.00 ± 5.72[c] | 240.15 ± 19.6 |
| Between year F-value = 1.44[ns]; (P-value = 0.264)<br>Between season F-value = 133.56**; (P-value < 0.001))<br>Interaction between season and year F-value =0.91[ns]; (P-value = 0.477) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*

Effect of season (F = 3.111; P = 0.069), effect of year (F = 0.111; P-value = 0.895), and the interaction between year and season (F = 1.444; P-value = 0.260) were found to be non-significant on the periodicity of egg laying in *C. chani*. The above results showed that there were no significant variations in the periodicity of egg laying between seasons and between years (Table 4.55).

**Table 4.55. Periodicity of egg laying (Days) by *C. chani***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 4.67 ± 0.29 | 4.33 ± 0.29 | 4.83 ± 0.29 | 4.61 ± 0.33 |
| 2020 | 4.33 ± 0.29 | 4.67 ± 0.29 | 4.83 ± 0.29 | 4.61 ± 0.33 |
| 2021 | 4.67 ± 0.29 | 4.33 ± 0.29 | 4.67 ± 0.29 | 4.56 ± 0.33 |
| Overall Season | 4.56 ± 0.33 | 4.44 ± 0.33 | 4.78 ± 0.26 | 4.59 ± 0.31 |
| Between year F-value = 0.111[ns]; (P-value = 0.895)<br>Between season F-value = 3.111[ns]; (P-value = 0.069)<br>Interaction between season and year F-value = 1.444[ns]; (P-value = 0.260) | | | | |

*ns – non significant*

Effect of season on the total number of eggs laid during the life span was found to be significant (F = 901.52, P = < 0.001). Effect of year on the number of eggs laid was also found to be significant (F = 12.54, P = < 0.001). Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (F = 3.65, P = 0.024). The number of eggs laid in *C.chani* was significantly higher in monsoon (1735.11 ± 12.62) in comparison to summer (1640.22 ± 5.40) and winter (1627.22 ± 7.82) (Table 4.56).

**Table 4.56. Eggs laid by the *C. chani* during in its life span**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $1740.67 \pm 5.13^{aA}$ | $1638.67 \pm 4.51^{b}$ | $1632.67 \pm 10.02^{b}$ | $1670.67 \pm 52.91^{A}$ |
| 2020 | $1719.67 \pm 4.16^{aB}$ | $1637.33 \pm 5.51^{b}$ | $1621.67 \pm 6.66^{c}$ | $1659.56 \pm 45.84^{B}$ |
| 2021 | $1745.00 \pm 6.56^{aA}$ | $1644.67 \pm 4.51^{b}$ | $1627.33 \pm 3.06^{c}$ | $1672.33 \pm 55.18^{A}$ |
| Overall Season | $1735.11 \pm 12.62^{a}$ | $1640.22 \pm 5.40^{b}$ | $1627.22 \pm 7.82^{c}$ | $1667.52 \pm 49.78$ |
| Between year F-value = 12.54**; (P-value < 0.001)<br>Between season F-value = 901.52**; (P-value < 0.001)<br>Interaction between season and year F-value = 3.65*; (P-value = 0.024) | | | | |

** Significant at 0.01 level; * *significant at 0.05 level*
*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Effect on length of larvae**

**Instar I**

Effect of season, year and stage on the length of $I^{st}$ instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.61). The instar length in *C. Chani* was significantly lower in winter (2.65 ± 0.11) in comparison to monsoon (2.48 ± 0.17) and summer (2.49 ± 0.48) (Table. 4.57).

**Table 4.57. Seasonal changes in length (mm) of I$^{st}$ instar larvae of *C. Chani***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $2.59 \pm 0.01^{aA}$ | $1.88 \pm 0.02^{bC}$ | $2.68 \pm 0.03^{aAB}$ | $2.38 \pm 0.38^{B}$ |
| 2020 | $2.59 \pm 0.03^{A}$ | $2.66 \pm 0.12^{B}$ | $2.57 \pm 0.05^{B}$ | $2.61 \pm 0.08^{A}$ |
| 2021 | $2.25 \pm 0.04^{cB}$ | $2.94 \pm 0.10^{aA}$ | $2.71 \pm 0.18^{bA}$ | $2.64 \pm 0.32^{A}$ |
| Overall Season | $2.48 \pm 0.17^{b}$ | $2.49 \pm 0.48^{b}$ | $2.65 \pm 0.11^{a}$ | $2.54 \pm 0.30$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Instar II**

Effect of season, year and stage on the length of II$^{nd}$ instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.61). The instar length in *C. chani* was significantly higher in summer ($6.40 \pm 0.22$) in comparison to monsoon ($5.79 \pm 0.23$) and winter ($6.04 \pm 0.19$) (Table 4.58).

**Table 4.58. Seasonal changes in length (mm) of II$^{nd}$ instar larvae of *C. chani***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $5.63 \pm 0.12^{cB}$ | $6.66 \pm 0.03^{aA}$ | $6.05 \pm 0.06^{bB}$ | $6.11 \pm 0.46^{A}$ |
| 2020 | $6.08 \pm 0.12^{bA}$ | $6.39 \pm 0.07^{aB}$ | $5.81 \pm 0.01^{cC}$ | $6.09 \pm 0.26^{AB}$ |
| 2021 | $5.67 \pm 0.04^{bB}$ | $6.16 \pm 0.05^{aC}$ | $6.25 \pm 0.03^{aA}$ | $6.03 \pm 0.27^{B}$ |
| Overall Season | $5.79 \pm 0.23^{c}$ | $6.40 \pm 0.22^{a}$ | $6.04 \pm 0.19^{b}$ | $6.08 \pm 0.33$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Instar III**

Effect of season, year and stage on the length III$^{rd}$ instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.61). The instar length in *C. chani* was significantly

higher in summer (11.04 ± 0.23) in comparison to monsoon (10.72 ± 0.06) and winter (10.49 ± 0.19). (Table 4.59).

**Table 4.59. Seasonal changes in length (mm) of III$^{rd}$ instar larvae of *C. chani***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $10.74 \pm 0.04^{b}$ | $11.32 \pm 0.03^{aA}$ | $10.32 \pm 0.05^{cB}$ | $10.79 \pm 0.43^{A}$ |
| 2020 | $10.71 \pm 0.09^{b}$ | $10.94 \pm 0.07^{aB}$ | $10.43 \pm 0.07^{cB}$ | $10.69 \pm 0.23^{B}$ |
| 2021 | $10.7 \pm 0.07^{b}$ | $10.85 \pm 0.13^{aB}$ | $10.71 \pm 0.12^{abA}$ | $10.76 \pm 0.12^{AB}$ |
| Overall Season | $10.72 \pm 0.06^{b}$ | $11.04 \pm 0.23^{a}$ | $10.49 \pm 0.19^{c}$ | $10.75 \pm 0.28$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Post feeding stage**

Effect of season, year and stage on the length of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.61). The length of post feeding stage in *C. chani* was significantly higher in summer (10.61 ± 0.30) in comparison to monsoon (10.50 ± 0.19) and winter (10.27 ± 0.46) (Table 4.60).

**Table 4.60. Seasonal changes in length (mm) of post feeding stage of *C. chani***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $10.31 \pm 0.03^{bC}$ | $10.89 \pm 0.04^{aA}$ | $9.68 \pm 0.03^{cC}$ | $10.29 \pm 0.52^{C}$ |
| 2020 | $10.70 \pm 0.08^{bA}$ | $10.27 \pm 0.16^{aC}$ | $10.46 \pm 0.07^{cB}$ | $10.48 \pm 0.21^{B}$ |
| 2021 | $10.50 \pm 0.15^{bB}$ | $10.66 \pm 0.19^{aB}$ | $10.66 \pm 0.15^{aA}$ | $10.61 \pm 0.16^{A}$ |
| Overall Season | $10.50 \pm 0.19^{b}$ | $10.61 \pm 0.30^{a}$ | $10.27 \pm 0.46^{c}$ | $10.46 \pm 0.35$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

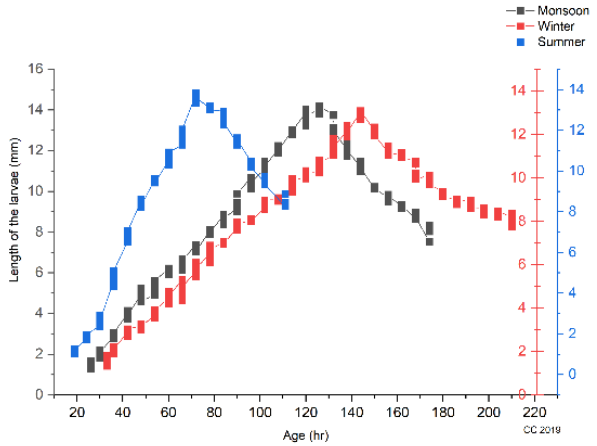
Interaction studies on year, season and stage was found to be significant (F = 0.335; P = <0.001) indicating that seasonal variations in length of each stage are different in different years (Table 4.61).

**Table 4.61. ANOVA for comparing length of larval instars of *C. Chani***

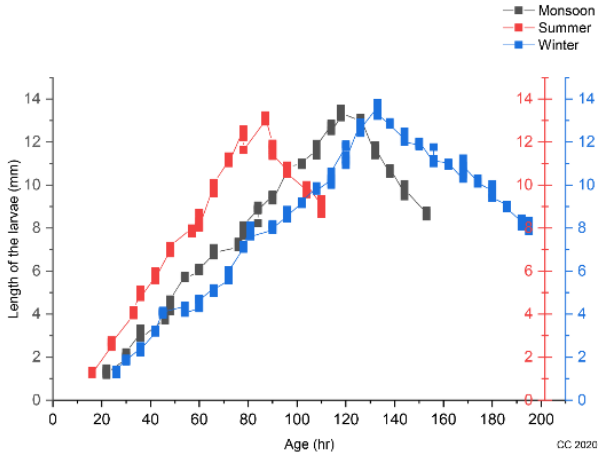
| Source | Df | Sum of Squares | Mean Square | F-value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 0.223 | 0.111 | 14.249** | <0.001 |
| Season | 2 | 1.722 | 0.861 | 110.150** | <0.001 |
| Stage | 3 | 1239.390 | 413.130 | 52853.036** | <0.001 |
| Year * Season | 4 | 1.287 | 0.322 | 41.157** | <0.001 |
| Year * stage | 6 | 0.647 | 0.108 | 13.788** | <0.001 |
| Season * stage | 6 | 2.057 | 0.343 | 43.862** | <0.001 |
| Year * Season * Stage | 12 | 4.018 | 0.335 | 42.832** | <0.001 |
| Error | 72 | 0.563 | 0.008 | | |
| Total | 107 | 1249.906 | | | |

** Significant at 0.01 level

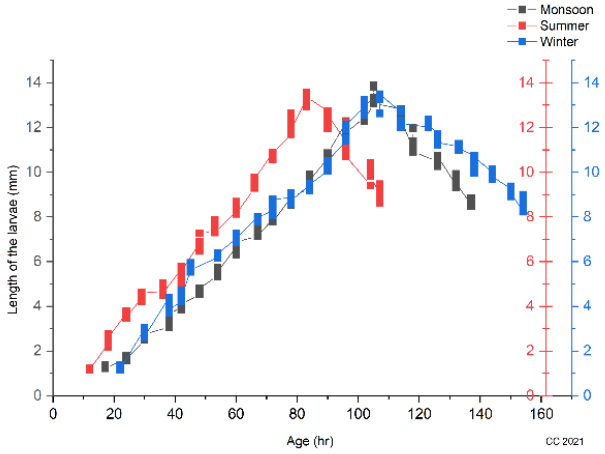
The growth curves representing the developmental rate (Length (mm) Vs. Age (hr) of *C. Chani* from hatching until pupation during different seasons and years were prepared (Fig. 4.57 and Fig. 4.58).




2019

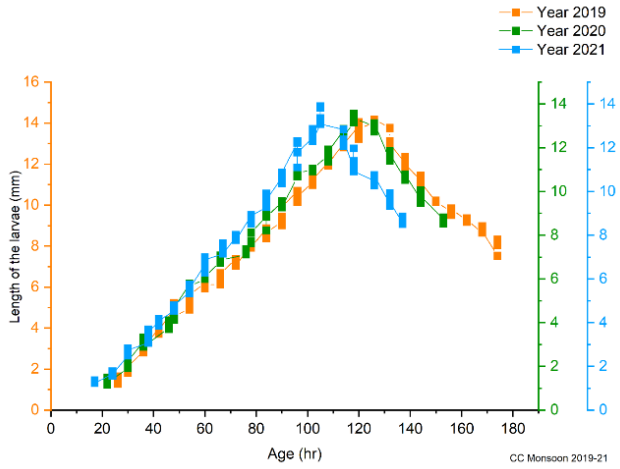



2020

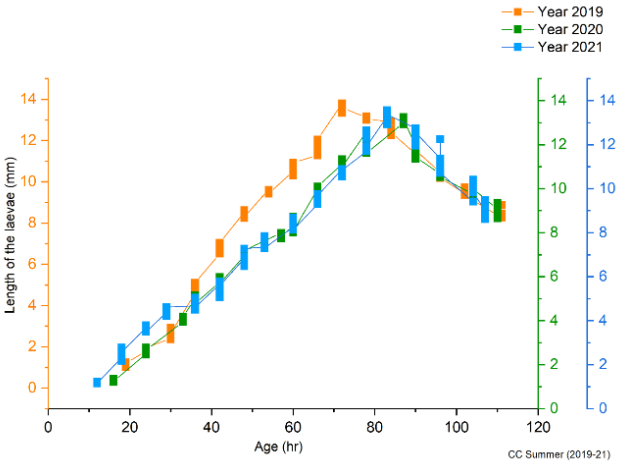



2021

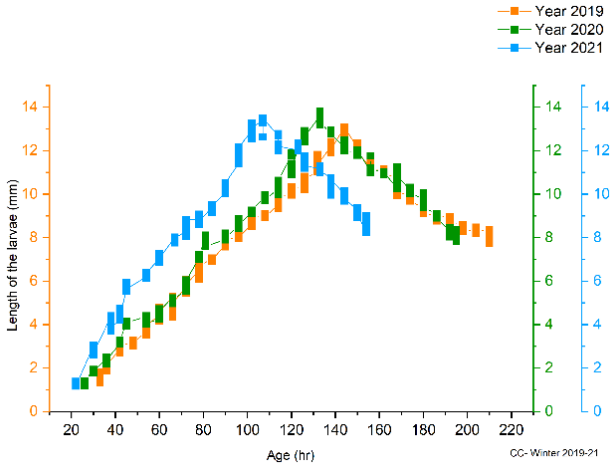
**Fig. 4.57. Seasonal developmental rate (Length (mm) Vs. Age (hr) of *C. chani* from hatching upto pupation**




Monsoon




Summer




Winter

**Fig. 4.58. Developmental rate (Length (mm) Vs. Age (hr) of *C. chani* from hatching upto pupation during the study period**

**Effect on weight of larvae**

**Instar I**

Effect of season and year on the I$^{st}$ instar weight was found to be not significant. The pattern of differences in the weight was similar in all the years and seasons (Table 4.62).

**Table 4.62. Seasonal changes in weight (mg) of I$^{st}$ instar larvae of *C. chani***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 1.41 ± 0.07 | 1.45 ± 0.12 | 1.60 ± 0.08 | 1.48 ± 0.12 |
| 2020 | 1.39 ± 0.06 | 1.40 ± 0.13 | 1.62 ± 0.10 | 1.47 ± 0.14 |
| 2021 | 1.40 ± 0.06 | 1.60 ± 0.03 | 1.55 ± 0.03 | 1.52 ± 0.1 |
| Overall Season | 1.40 ± 0.06 | 1.49 ± 0.13 | 1.59 ± 0.07 | 1.49 ± 0.12 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Instar II**

Effect of season, year and stage on the weight of II$^{nd}$ instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.66). The instar weight in *C. chani* was significantly higher in summer (10.08 ± 0.33) in comparison to monsoon (9.87 ± 0.39) and winter (9.52 ± 0.33) (Table 4.63).

**Table 4.63. Seasonal changes in weight (mg) of II$^{nd}$ instar larvae of *C. chani***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $9.98 \pm 0.40^{bA}$ | $10.5 \pm 0.12^{aA}$ | $9.49 \pm 0.32^{c}$ | $9.99 \pm 0.51^{A}$ |
| 2020 | $10.16 \pm 0.13^{aA}$ | $9.91 \pm 0.05^{abB}$ | $9.56 \pm 0.32^{b}$ | $9.87 \pm 0.31^{A}$ |
| 2021 | $9.46 \pm 0.22^{B}$ | $9.84 \pm 0.03^{B}$ | 9.52 ± 0.49 | $9.61 \pm 0.32^{B}$ |
| Overall Season | $9.87 \pm 0.39^{a}$ | $10.08 \pm 0.33^{a}$ | $9.52 \pm 0.33^{b}$ | 9.82 ± 0.41 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Instar III**

Effect of season, year and stage on the weight of III$^{rd}$ instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.66). The instar weight in *C. chani* was significantly higher in summer (32.26 ± 0.70) in comparison to monsoon (30.49 ± 0.47) and winter (30.16 ± 0.53) (Table.4.64).

**Table 4.64. Seasonal changes in weight (mg) of III$^{rd}$ instar larvae of *C. chani***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $30.23 \pm 0.27^{bB}$ | $32.87 \pm 0.20^{aA}$ | $29.66 \pm 0.38^{cB}$ | 30.92 ± 1.51 |
| 2020 | $31.05 \pm 0.23^{bA}$ | $31.53 \pm 0.62^{aC}$ | $30.60 \pm 0.22^{cA}$ | 31.06 ± 0.53 |
| 2021 | $30.2 \pm 0.20^{bB}$ | $32.38 \pm 0.36^{aB}$ | $30.21 \pm 0.51^{bA}$ | 30.93 ± 1.14 |
| Overall Season | $30.49 \pm 0.47^{b}$ | $32.26 \pm 0.70^{a}$ | $30.16 \pm 0.53^{c}$ | 30.97 ± 1.09 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Post feeding stage**

Effect of season, year and stage on the weight of post feeding stage was found to be significant. Seasonal variations were not same in all years and also the year wise variations were not same in all seasons (Table 4.66). The weight of post feeding stage in *C. chani* was significantly higher in summer (30.14 ± 0.82) in comparison to monsoon (29.77 ± 1.35) and winter (29.80 ± 1.25) (Table 4.65).

**Table 4.65. Seasonal changes in weight (mg) of post feeding stage of *C. chani***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $30.83 \pm 0.29^{aA}$ | $31.15 \pm 0.12^{abA}$ | $30.48 \pm 0.25^{bA}$ | $30.82 \pm 0.35^{A}$ |
| 2020 | $30.44 \pm 0.25^{aA}$ | $29.46 \pm 0.14^{bB}$ | $30.76 \pm 0.29^{aA}$ | $30.22 \pm 0.62^{A}$ |
| 2021 | $28.03 \pm 0.49^{bB}$ | $29.80 \pm 0.49^{aB}$ | $28.16 \pm 0.12^{bB}$ | $28.67 \pm 0.93^{B}$ |
| Overall Season | $29.77 \pm 1.35^{b}$ | $30.14 \pm 0.82^{a}$ | $29.80 \pm 1.25^{b}$ | 29.90 ± 1.13 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

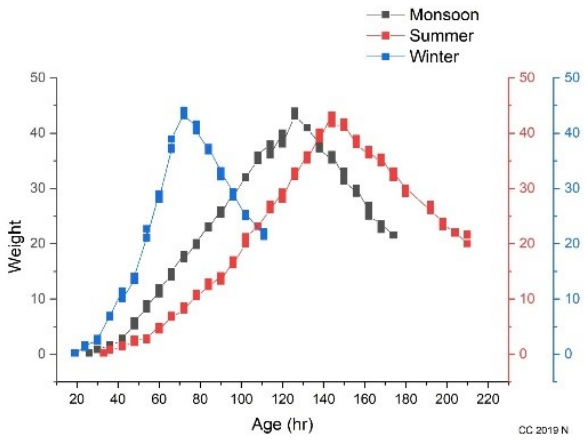
Interaction studies on year, season and stage was found to be significant (F = 14.365; P = <0.001) indicating that seasonal variations in weight of each stage are different in different years (Table 4.66).

**Table 4.66 ANOVA for comparing weight of larval instars of *C. chani***

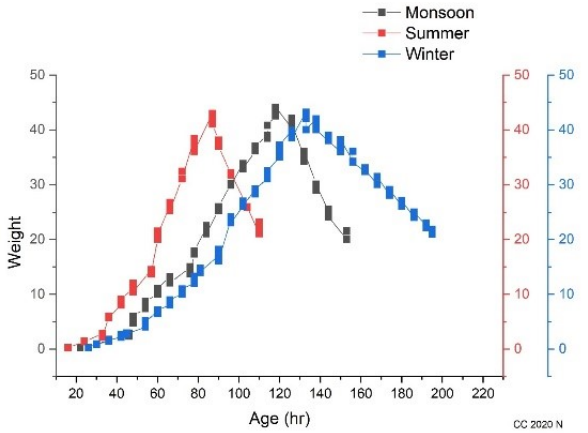
| Source | df | Sum of Squares | Mean Square | F-Value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 7.62 | 3.81 | 50.271** | < 0.001 |
| Season | 2 | 10.92 | 5.46 | 71.999** | < 0.001 |
| Stage | 3 | 17528.77 | 5842.92 | 77063.61** | < 0.001 |
| Year * Season | 4 | 7.95 | 1.99 | 26.22** | < 0.001 |
| Year * stage | 6 | 15.40 | 2.57 | 33.858** | < 0.001 |
| Season * stage | 6 | 14.44 | 2.41 | 31.739** | < 0.001 |
| Year * Season * Stage | 12 | 6.97 | 0.58 | 7.656** | < 0.001 |
| Error | 72 | 5.46 | 0.08 | | |
| Total | 107 | 17597.53 | | | |

** Significant at 0.01 level; ns non-Significant

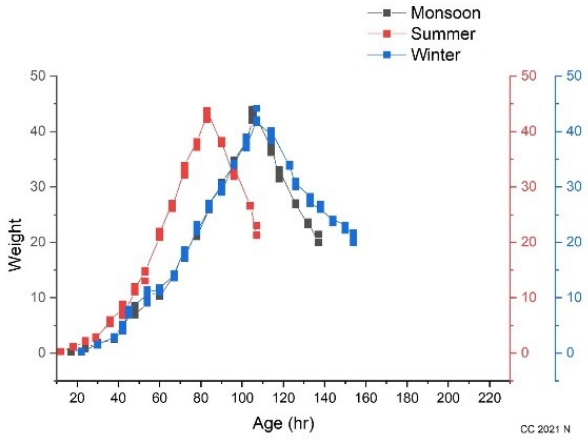
The growth curve representing the developmental rate (Weight (mg) Vs. Age (hr) of *C. chani* from hatching until pupation during different seasons and years were prepared (Fig. 4.59 and Fig.4.60).




2019

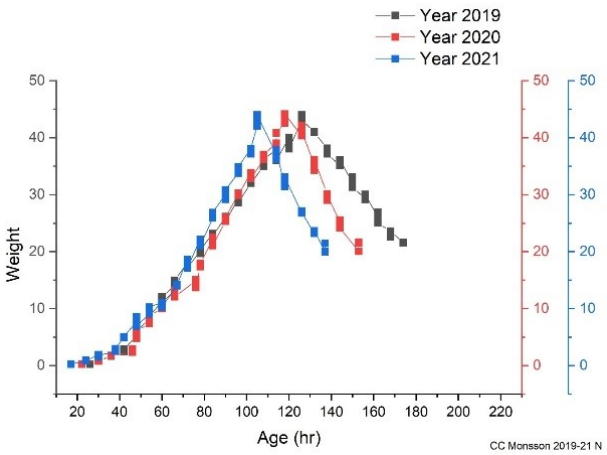



2020

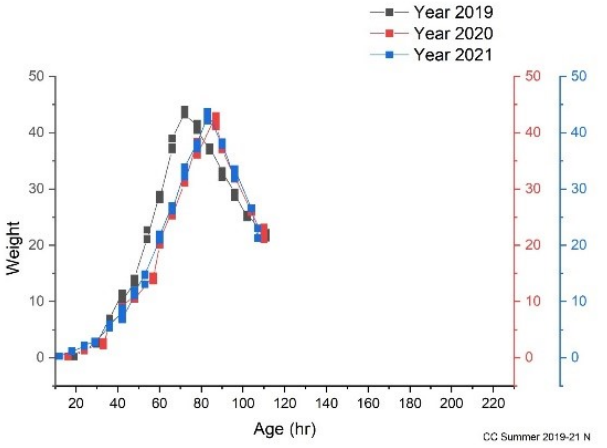



2021

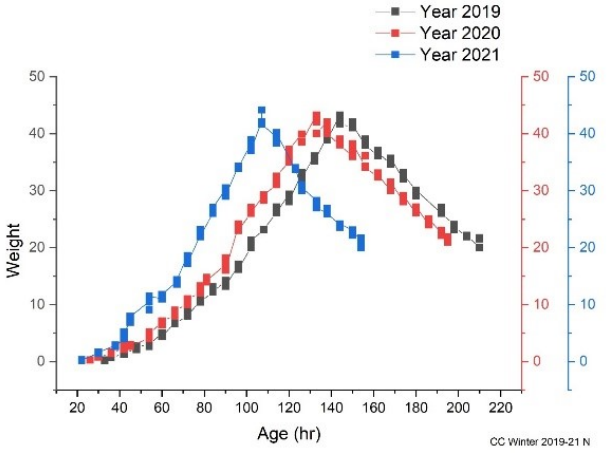
**Fig. 4.59. Seasonal developmental rate (Weight (mg) Vs. Age (hr) of *C. chani* from hatching upto pupation**




Monsoon




Summer




Winter

**Fig. 4.60. Developmental rate (Weight (mg) Vs. Age (hr) of *C. chani* from hatching upto pupation during the study period**

**Effect on the life cycle duration**

Significantly higher duration (hr) was observed for *C. chani* during winter for incubation (27.00 ± 5.57) (F = 66.77; P = 0.001), II$^{nd}$ larval instar (27.33 ± 2.52) (F = 5.59 ; P = 0.070), post feeding stage (52.00 ± 10.58) (F =29.726 ; P =0.004 ), pupation (153.33 ± 5.51) (F = 367.82; P = <0.001), and total life cycle (320.33 ± 24.03) (F = 152.50; P = <0.001) (Table 4.67).

**Table 4.67. Seasonal changes in duration (hrs) of life cycle of *C. chani***

| Stages | Monsoon | Summer | Winter | F-value (P-value) |
|---|---|---|---|---|
| Incubation | 21.67 ± 4.51$^{b}$ | 15.67 ± 3.51$^{c}$ | 27.00 ± 5.57$^{a}$ | 66.77** (0.001) |
| Instar I | 20.33 ± 4.04 | 15.33 ± 2.89 | 18.67 ± 2.52 | 1.862$^{ns}$ (0.268) |
| Instar II | 23.67 ± 5.13$^{ab}$ | 16.67 ± 4.51$^{b}$ | 27.33 ± 2.52$^{a}$ | 5.59* (0.070) |
| Instar III | 42.00 ± 11.14 | 22.67 ± 4.51 | 42.00 ± 6.56 | 6.824$^{ns}$ (0.051) |
| Post feeding stage | 30.33 ± 10.21$^{b}$ | 25.33 ± 11.93$^{b}$ | 52.00 ± 10.58$^{a}$ | 29.726** (0.004) |
| Pupation | 108.00 ± 3.61$^{b}$ | 96.67 ± 1.53$^{c}$ | 153.33 ± 5.51$^{a}$ | 367.82** (<0.001) |
| Total time taken from egg stage till emergence | 246 ± 20.08$^{b}$ | 192.33 ± 8.15$^{c}$ | 320.33 ± 24.03$^{a}$ | 152.50** (<0.001) |

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*
*Means having different letter as super script differ significantly between seasons (within a row)*

Significantly higher duration (hr) was observed for *C. chani* during 2019 for incubation (26.00 ± 7.00) (F = 42.08; P = 0.002) and post feeding stage (47.00 ± 11.36) (F = 15.69; P =0.013). However total life cycle duration was significantly higher (253.00 ± 61.99) in 2020 (F = 10.88; P = 0.024) compared to other years (Table 4.68).

**Table 4.68. Changes in duration (hrs) of life cycle of *C. chani***

| Stages | 2019 | 2020 | 2021 | F-value (P-value) |
|---|---|---|---|---|
| Incubation | $26.00 \pm 7.00^{a}$ | $21.33 \pm 5.03^{b}$ | $17.00 \pm 5.00^{c}$ | 42.08** (0.002) |
| Instar I | $16.33 \pm 4.51$ | $20.00 \pm 3.61$ | $18.00 \pm 2.65$ | $0.968^{ns}$ (0.454) |
| Instar II | $20.00 \pm 9.17$ | $25.33 \pm 3.79$ | $22.33 \pm 4.62$ | $1.359^{ns}$ (0.354) |
| Instar III | $40.00 \pm 19.29$ | $36.67 \pm 8.51$ | $30.00 \pm 6.25$ | $1.420^{ns}$ (0.342) |
| Post feeding stage | $47.00 \pm 11.36^{a}$ | $34.00 \pm 19.29^{b}$ | $26.67 \pm 11.93^{b}$ | 15.69* (0.013) |
| Pupation | $120.67 \pm 28.75$ | $115.67 \pm 28.36$ | $121.67 \pm 32.88$ | $4.227^{ns}$ (0.103) |
| Total time taken from egg stage till emergence | $270.00 \pm 73.55^{a}$ | $253.00 \pm 61.99^{ab}$ | $235.67 \pm 57.49^{b}$ | 10.88* (0.024) |

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*
*Means having different letter as super script differ significantly between years (within a row)*

**Effect on survival rate**

Effect of season on the survival rate (%) of *C. chani* was found to be significant (Table 4.72). During 2019, the survival rate (%) in *C. chani* was significantly higher in monsoon (73.51 ± 4.18) in comparison to summer (71.66 ± 3.39) and winter (69.75 ± 3.94). However, during 2021, the survival rate was significantly higher in winter (75.45 ± 9.72) compared to monsoon (73.00 ± 8.59) and summer (72.21 ± 6.95) (Table 4.69).

**Table 4.69. Seasonal changes in survival rate (%) of *C. chani***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $73.51 \pm 4.18^{a}$ | $71.66 \pm 3.39^{ab}$ | $69.75 \pm 3.94^{bC}$ | $71.64 \pm 4.07^{B}$ |
| 2020 | $73.57 \pm 6.19$ | $72.73 \pm 5.40$ | $72.44 \pm 6.69^{B}$ | $72.92 \pm 6.00^{AB}$ |
| 2021 | $73.00 \pm 8.59^{ab}$ | $72.21 \pm 6.95^{b}$ | $75.45 \pm 9.72^{aA}$ | $73.55 \pm 8.42^{A}$ |
| Overall Season | $73.36 \pm 6.43$ | $72.20 \pm 5.34$ | $72.55 \pm 7.40$ | $72.70 \pm 6.42$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of year and stage on the survival rate of developmental stages of *C. chani* was found to be significant (Table 4.72). The survival rate of developmental stages in *C. C.chani* was significantly higher in 2021 (73.55 ± 8.42) in comparison to 2019 (71.64 ± 4.07) and 2020 (72.92 ± 6) (Table 4.70).

**Table 4.70. Survival rate (%) of life cycle of *C. chani* during the study period**

| Stage | 2019 | 2020 | 2021 | Overall stage |
|---|---|---|---|---|
| Egg | $72.27 \pm 3.7^{bAB}$ | $76.36 \pm 4.45^{aA}$ | $78.90 \pm 6.38^{aA}$ | $75.84 \pm 5.53^{A}$ |
| I$^{st}$ Instar | $73.14 \pm 4.4^{bA}$ | $76.77 \pm 4.51^{aA}$ | $78.22 \pm 5.88^{aA}$ | $76.04 \pm 5.25^{A}$ |
| II$^{nd}$ Instar | $74.09 \pm 3.54^{bA}$ | $75.28 \pm 4.44^{bA}$ | $79.97 \pm 3.41^{aA}$ | $76.45 \pm 4.50^{A}$ |
| III$^{rd}$ Instar | $69.45 \pm 4.17^{B}$ | $68.65 \pm 5.73^{B}$ | $67.37 \pm 5.90^{B}$ | $68.49 \pm 5.19^{B}$ |
| Pupa | $69.25 \pm 2.57^{aB}$ | $67.51 \pm 3.92^{aB}$ | $63.31 \pm 2.05^{bC}$ | $66.69 \pm 3.81^{B}$ |
| Overall year | $71.64 \pm 4.07^{b}$ | $72.92 \pm 6^{ab}$ | $73.55 \pm 8.42^{a}$ | $72.70 \pm 6.42$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of season and stage on the survival rate of developmental stages of *C.chani* was found to be significant (Table 4.72). The survival rate of egg (78.12 ± 3.95) and I$^{st}$ Instar larvae (78.67 ± 4.02) were significantly higher in monsoon compared to other seasons. Survival rate of III$^{rd}$ Instar larvae was significantly higher in summer (73.30 ± 4.11) compared to monsoon (66.69 ± 4.46) and winter (65.48 ± 3.30) (Table 4.71).

**Table 4.71. Seasonal changes in survival rate (%) of life cycle stages of *C. chani***

| Stage | Monsoon | Summer | Winter | Overall stage |
|---|---|---|---|---|
| Egg | $78.12 \pm 3.95^{aA}$ | $72.06 \pm 3.02^{bB}$ | $77.35 \pm 7.08^{aA}$ | $75.84 \pm 5.53^{A}$ |
| $I^{st}$ Instar | $78.67 \pm 4.02^{aA}$ | $72.12 \pm 2.66^{bB}$ | $77.35 \pm 6.28^{aA}$ | $76.04 \pm 5.25^{A}$ |
| $II^{nd}$ Instar | $75.41 \pm 4.05^{A}$ | $77.66 \pm 4.74^{A}$ | $76.26 \pm 4.89^{A}$ | $76.45 \pm 4.50^{A}$ |
| $III^{rd}$ Instar | $66.69 \pm 4.46^{bB}$ | $73.30 \pm 4.11^{aB}$ | $65.48 \pm 3.30^{bB}$ | $68.49 \pm 5.19^{B}$ |
| Pupa | $67.91 \pm 3.73^{B}$ | $65.87 \pm 4.60^{C}$ | $66.30 \pm 3.07^{B}$ | $66.69 \pm 3.81^{B}$ |
| Overall Season | 73.36 ± 6.43 | 72.20 ± 5.34 | 72.55 ± 7.4 | 72.70 ± 6.42 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Three way ANOVA for studying the interaction between year, season and developmental stages indicated interactions between season and stage (F = 6.93; P= <0.001), year and stage (F = 5.59; P = <0.001), as well as year and season (F = 3.17; P = 0.018) (Table 4.72).

**Table 4.72. ANOVA for comparing survival of *C. chani***

| Source | df | Sum of Squares | Mean Square | F-value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 85.41 | 42.70 | 3.19* | 0.046 |
| Season | 2 | 31.77 | 15.88 | $1.19^{ns}$ | 0.311 |
| Stage | 4 | 2400.67 | 600.17 | 44.76** | < 0.001 |
| Year * season | 4 | 169.79 | 42.45 | 3.17* | 0.018 |
| Year * Stage | 8 | 600.00 | 75.00 | 5.59** | < 0.001 |
| season * Stage | 8 | 743.48 | 92.94 | 6.93** | < 0.001 |
| Year * season * Stage | 16 | 277.33 | 17.33 | $1.29^{ns}$ | 0.219 |
| Error | 90 | 1206.81 | 13.41 | | |
| Corrected Total | 134 | 5515.25 | | | |

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

### 4.4.4. *Hemipyrellia ligurriens*

**Effect on fecundity**

Effect of season on the pre-oviposition period were found to be significant (F = 13.727, P = < 0.001). Seasonal variations were same in all years and also the yearly variations were same in all seasons (F = 0.363, P = 0.831). The pre-oviposition period (days) in *H.ligurriens* was significantly higher in winter (10.44 ± 0.53) in comparison to monsoon (9.44 ± 0.53) and summer (8.89 ± 0.78) (Table 4.73).

**Table 4.73. Pre-oviposition period (days) of *H. ligurriens***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 9.67 ± 0.58 | 9.33 ± 0.58 | 10.67 ± 0.58 | 9.89 ± 0.78 |
| 2020 | 9.33 ± 0.58 | 8.33 ± 0.58 | 10.33 ± 0.58 | 9.33 ± 1.00 |
| 2021 | 9.33 ± 0.58 | 9.00 ± 1.00 | 10.33 ± 0.58 | 9.56 ± 0.88 |
| Overall Season | $9.44 \pm 0.53^{b}$ | $8.89 \pm 0.78^{b}$ | $10.44 \pm 0.53^{a}$ | 9.59 ± 0.89 |
| Between year F-value = $1.727^{ns}$; (P-value = 0206)<br>Between season F-value = 13.727**; (P-value < 0.001))<br>Interaction between season and year F-value =$0.363^{ns}$; (P-value = 0.831) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*

Effect of season on the number of eggs laid in a day was found to be significant (F = 417.585, P = < 0.001). Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (F = 3.562, P = 0.026). The number of eggs laid by *H. ligurriens was* significantly higher in monsoon (238.78 ± 3.96) in comparison to summer (221.33 ± 4.98) and winter (187.11 ± 4.46) (Table 4.74).

**Table 4.74. Eggs laid by the *H. ligurriens* in a day**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $237.00 \pm 2.00^{a}$ | $221.00 \pm 1.00^{bAB}$ | $190.00 \pm 3.00^{c}$ | 216.00 ± 20.78 |
| 2020 | $237.67 \pm 4.16^{a}$ | $226.00 \pm 3.00^{bA}$ | $184.00 \pm 3.61^{c}$ | 215.89 ± 24.65 |
| 2021 | $241.67 \pm 4.73^{a}$ | $217.00 \pm 5.29^{bB}$ | $187.33 \pm 5.51^{c}$ | 215.33 ± 23.98 |
| Overall Season | $238.78 \pm 3.96^{a}$ | $221.33 \pm 4.98^{b}$ | $187.11 \pm 4.46^{c}$ | 215.74 ± 22.29 |
| Between year F-value = $0.077^{ns}$; (P-value = 0.926)<br>Between season F-value = 417.585**; (P-value < 0.001))<br>Interaction between season and year F-value = 3.562*; (P-value = 0.026) | | | | |

** Significant at 0.01 level; * Significant at 0.05 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of season (F=0.750; P =0.487), effect of year (F =0.563; P-value =0.579), and the interaction between year and season (F = 0.750; P-value = 0.571) were found to be non-significant on the on the periodicity of egg laying in *H. ligurriens*. There were no significant variations in the periodicity of egg laying between seasons and between years (Table 4.75).

**Table 4.75. Periodicity of egg laying (days) by *H. ligurriens***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 3.50 ± 0.50 | 3.83 ± 0.58 | 3.50 ± 0.50 | 3.61 ± 0.49 |
| 2020 | 3.83 ± 0.29 | 3.67 ± 0.29 | 3.33 ± 0.29 | 3.61 ± 0.33 |
| 2021 | 3.67 ± 0.29 | 3.83 ± 0.29 | 3.83 ± 0.29 | 3.78 ± 0.26 |
| Overall Season | 3.67 ± 0.35 | 3.78 ± 0.36 | 3.56 ± 0.39 | 3.67 ± 0.37 |
| Between year F-value = $0.563^{ns}$; (P-value = 0.579)<br>Between season F-value = $0.750^{ns}$; (P-value = 0.487))<br>Interaction between season and year F-value = $0.750^{ns}$; (P-value = 0.571) | | | | |

*ns non-significant*

Effect of season on the total number of eggs laid during the life span was found to be significant (F = 576.86, P = < 0.001). Effect of year on the number of eggs laid was also found to be significant (F = 5.199, P = 0.017). However, seasonal variations were same in all years and also the year wise variations were same in all seasons (F = 1.192, P = 0.150). The number of eggs laid by *H. ligurriens* was significantly higher in monsoon (1531.56 ± 16.01) in comparison to summer (1481.67 ± 9.99) and winter (1340.56 ± 18.3). (Table 4.76).

**Table 4.76. Eggs laid by the *H. ligurriens* during in its life span**

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | 1539.00 ± 7.94 | 1487.67 ± 3.06 | 1354.33 ± 9.45 | $1460.33 \pm 82.79^{A}$ |
| 2020 | 1522.67 ± 7.37 | 1486.67 ± 7.23 | 1346.33 ± 4.16 | $1451.89 \pm 80.88^{AB}$ |
| 2021 | 1533.00 ± 26.51 | 1470.67 ± 8.02 | 1321.00 ± 18.03 | $1441.56 \pm 95.80^{B}$ |
| Overall Season | $1531.56 \pm 16.01^{a}$ | $1481.67 \pm 9.99^{b}$ | $1340.56 \pm 18.3^{c}$ | 1451.26 ± 83.71 |
| Between year F-value = 5.199*; (P-value = 0.017)<br>Between season F-value = 576.86**; (P-value < 0.001)<br>Interaction between season and year F-value = $1.192^{ns}$; (P-value = 0.150) | | | | |

** Significant at 0.01 level; *ns non-significant*
*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

**Effect on length of larvae**

**Instar I**

Effect of season, year and stage on the length of I$^{st}$ instar larvae was found to be significant. Seasonal variations were same in all years and also the yearly variations were same in all seasons (Table. 4.81). The instar length in *H. ligurriens* was significantly higher in winter (2.28 ± 0.09) in comparison to monsoon (2.00 ± 0.28) and summer (2.06 ± 0.19). (Table 4.77).

**Table 4.77. Seasonal changes in length (mm) of I$^{st}$ instar larvae of *H. ligurriens***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $2.15 \pm 0.03^{A}$ | $2.15 \pm 0.01$ | $2.21 \pm 0.04$ | $2.17 \pm 0.04^{AB}$ |
| 2020 | $2.21 \pm 0.02^{A}$ | $2.21 \pm 0.02$ | $2.28 \pm 0.09$ | $2.23 \pm 0.06^{A}$ |
| 2021 | $1.63 \pm 0.03^{bB}$ | $1.81 \pm 0.02^{b}$ | $2.36 \pm 0.07^{a}$ | $1.93 \pm 0.33^{B}$ |
| Overall Season | $2.00 \pm 0.28^{b}$ | $2.06 \pm 0.19^{ab}$ | $2.28 \pm 0.09^{a}$ | $2.11 \pm 0.23$ |

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

**Instar II**

Effect of season, year and stage on the length of II$^{nd}$ instar larvae was found to be significant. Seasonal variations were same in all years and also the yearly variations were same in all seasons (Table. 4.81). The instar length in *H. ligurriens* was significantly higher in winter (4.79 ± 0.27) in comparison to monsoon (4.55 ± 0.44) and summer (4.25 ± 0.53). (Table 4.78).

**Table 4.78. Seasonal changes in length (mm) of IInd instar larvae of *H. ligurriens***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $4.70 \pm 0.02^{A}$ | $4.6 \pm 0.04^{A}$ | $4.69 \pm 0.03^{AB}$ | $4.66 \pm 0.06^{A}$ |
| 2020 | $4.95 \pm 0.02^{abA}$ | $4.61 \pm 0.03^{bA}$ | $5.13 \pm 0.04^{aA}$ | $4.90 \pm 0.23^{A}$ |
| 2021 | $3.99 \pm 0.09^{bB}$ | $3.54 \pm 0.11^{bB}$ | $4.54 \pm 0.01^{aB}$ | $4.02 \pm 0.44^{B}$ |
| Overall Season | $4.55 \pm 0.44^{a}$ | $4.25 \pm 0.53^{b}$ | $4.79 \pm 0.27^{a}$ | $4.53 \pm 0.47$ |

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

**Instar III**

Effect of season, year and stage on the length of IIIrd instar larvae was found to be significant. Seasonal variations were same in all years and also the year wise variations were same in all seasons (Table. 4.81). The instar length in *H. ligurriens* was significantly higher in winter (8.18 ± 0.33) in comparison to monsoon (8.06 ± 0.20) and summer (7.51 ± 0.08). (Table 4.79).

**Table 4.79. Seasonal changes in length (mm) of IIIrd instar larvae of *H. ligurriens***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $7.85 \pm 0.03$ | $7.55 \pm 0.02$ | $7.81 \pm 0.03^{B}$ | $7.74 \pm 0.14^{B}$ |
| 2020 | $8.30 \pm 0.02^{a}$ | $7.58 \pm 0.01^{b}$ | $8.56 \pm 0.003^{aA}$ | $8.15 \pm 0.44^{A}$ |
| 2021 | $8.02 \pm 0.02^{a}$ | $7.41 \pm 0.04^{b}$ | $8.16 \pm 0.01^{aAB}$ | $7.86 \pm 0.35^{B}$ |
| Overall Season | $8.06 \pm 0.20^{b}$ | $7.51 \pm 0.08^{b}$ | $8.18 \pm 0.33^{a}$ | $7.91 \pm 0.37$ |

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

**Post feeding stage**

Effect of season, year and stage on the length of post feeding stage was found to be significant. Seasonal variations were same in all years and also the yearly variations were same in all seasons (Table. 4.81). The length of post feeding stage in *H. ligurriens* was significantly higher in summer (7.97 ± 0.05) in comparison to monsoon (7.80 ± 0.89) and winter (7.48 ± 0.19) (Table 4.80).

**Table 4.80. Seasonal changes in length (mm) of post feeding stage of *H. ligurriens***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $7.36 \pm 0.01^{bB}$ | $8.02 \pm 0.03^{a}$ | $7.26 \pm 0.01^{b}$ | $7.55 \pm 0.36^{B}$ |
| 2020 | $8.20 \pm 1.62^{aA}$ | $7.95 \pm 0.03^{a}$ | $7.46 \pm 0.02^{b}$ | $7.87 \pm 0.87^{A}$ |
| 2021 | $7.83 \pm 0.03^{aA}$ | $7.94 \pm 0.03^{a}$ | $7.70 \pm 0.02^{b}$ | $7.82 \pm 0.11^{A}$ |
| Overall Season | $7.80 \pm 0.89^{a}$ | $7.97 \pm 0.05^{a}$ | $7.48 \pm 0.19^{b}$ | $7.75 \pm 0.55$ |

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

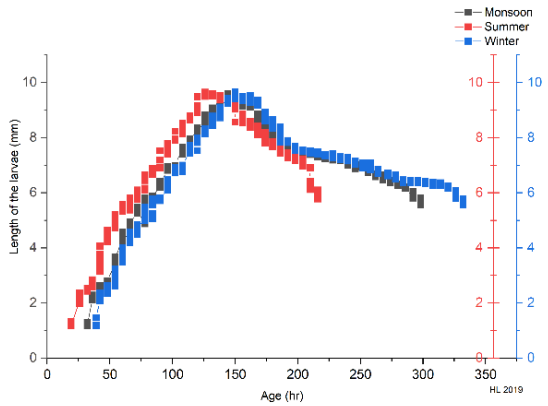
Three way ANOVA indicated that interaction between year and season (F = 4.60, P = 0.002), year and stage (F = 6.32, P = < 0.001) and season and stage (F = 9.15, P < 0.001) were found to be significant (Table 4.81).

**Table 4.81 ANOVA for comparing length of larval instars of *H. ligurriens***

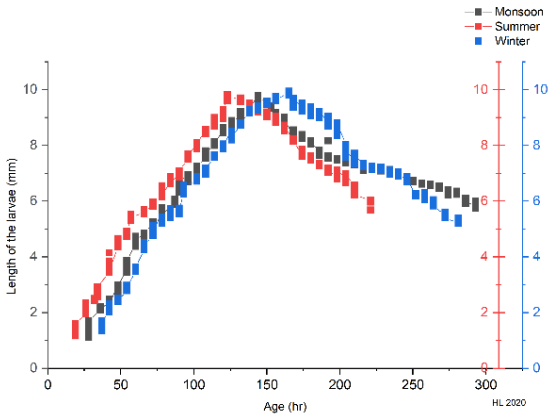
| Source | df | Sum of Squares | Mean Square | F-Value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 2.69 | 1.34 | 18.05** | < 0.001 |
| Season | 2 | 1.01 | 0.51 | 6.79** | 0.002 |
| Stage | 3 | 628.61 | 209.54 | 2813.60** | < 0.001 |
| Year * Season | 4 | 1.40 | 0.35 | 4.69** | 0.002 |
| Year * stage | 6 | 2.82 | 0.47 | 6.32** | < 0.001 |
| Season * stage | 6 | 4.09 | 0.68 | 9.15** | < 0.001 |
| Year * Season * Stage | 12 | 0.93 | 0.08 | $1.04^{ns}$ | 0.422 |
| Error | 72 | 5.36 | 0.07 | | |
| Total | 107 | 646.91 | | | |

** Significant at 0.01 level; ns non-Sgnificant

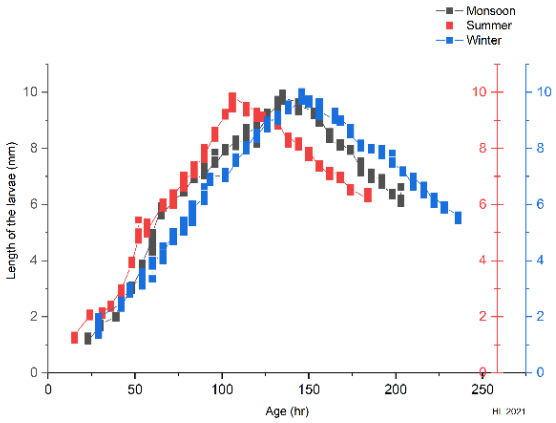
The growth curves representing the developmental rate (Length (mm) Vs. Age (hr) of *H. ligurriens* from hatching until pupation during different seasons and years were prepared (Fig. 4.61 & Fig. 4.62).




2019

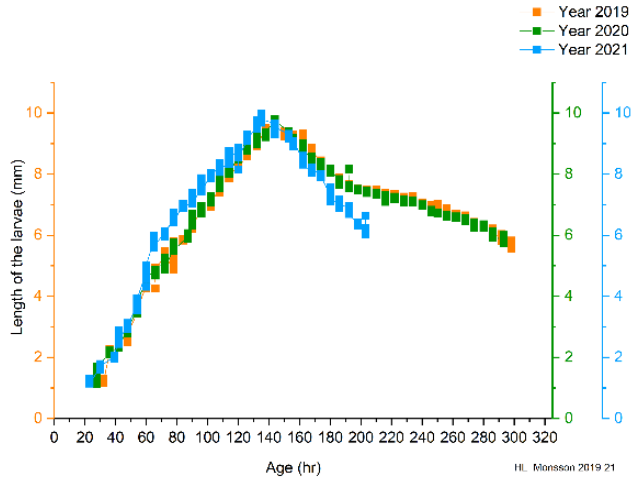



2020

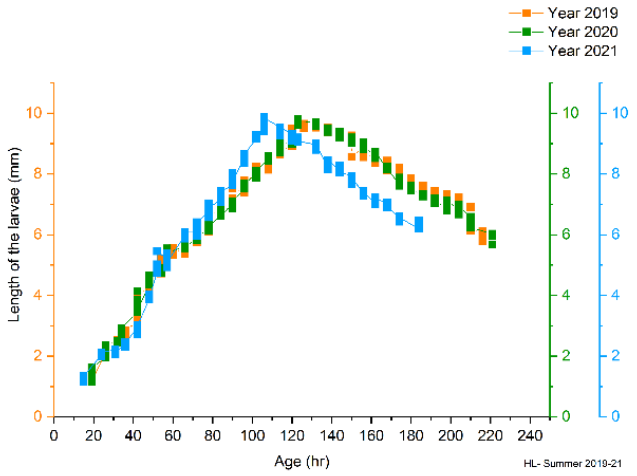



2021

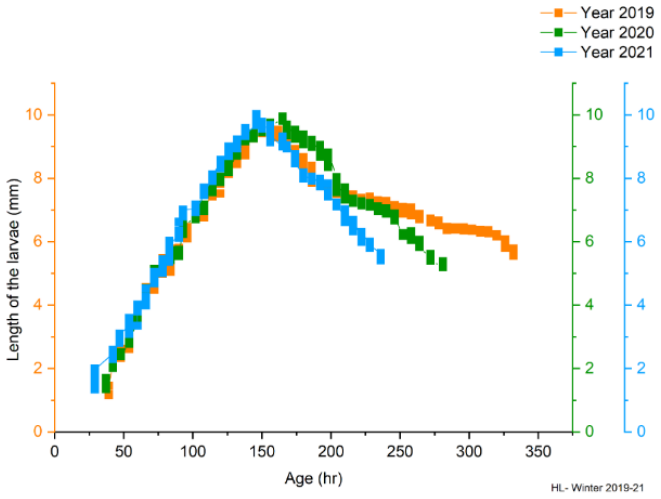
**Fig. 4.61. Seasonal developmental rate (Length (mm) Vs. Age (hr) of *H. ligurriens* from hatching upto pupation**




Monsoon




Summer




Winter

**Fig. 4.62. Developmental rate (Length (mm) Vs. Age (hr) of *H. ligurriens* from hatching upto pupation during the study period**

**Effect on weight of larvae**

**Instar I**

Effect of season, year and stage on the weight of I$^{st}$ instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.86). The instar weight in *H. ligurriens* was significantly higher in summer (1.06 ± 0.52) in comparison to monsoon (0.84 ± 0.17) and winter (0.71 ± 0.11 (Table 4.82).

**Table 4.82. Seasonal changes in weight (mg) of I$^{st}$ instar larvae of *H. ligurriens***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $0.65 \pm 0.14^{b}$ | $1.34 \pm 0.03^{aA}$ | $0.64 \pm 0.03^{b}$ | $0.88 \pm 0.36^{B}$ |
| 2020 | $0.94 \pm 0.04^{b}$ | $1.47 \pm 0.06^{aA}$ | $0.65 \pm 0.01^{b}$ | $1.02 \pm 0.36^{AB}$ |
| 2021 | $0.94 \pm 0.04^{a}$ | $0.38 \pm 0.005^{bB}$ | $0.85 \pm 0.07^{a}$ | $0.72 \pm 0.26^{B}$ |
| Overall Season | $0.84 \pm 0.17^{b}$ | $1.06 \pm 0.52^{a}$ | $0.71 \pm 0.11^{b}$ | $0.87 \pm 0.34$ |

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

**Instar II**

Effect of season, year and stage on the weight of II$^{nd}$ instar was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.86). The instar weight in *H. ligurriens* was significantly higher in monsoon (7.99 ± 0.43) in comparison to summer (7.28 ± 2.72) and winter (7.29 ± 0.47) (Table 4.83).

**Table 4.83. Seasonal changes in weight (mg) of II$^{nd}$ instar larvae of *H. ligurriens***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $7.72 \pm 0.37^{bB}$ | $9.23 \pm 0.20^{aA}$ | $7.82 \pm 0.13^{bA}$ | $8.26 \pm 0.76^{A}$ |
| 2020 | $8.49 \pm 0.15^{bA}$ | $8.93 \pm 0.15^{aA}$ | $6.74 \pm 0.03^{cC}$ | $8.05 \pm 1.01^{A}$ |
| 2021 | $7.77 \pm 0.2^{aB}$ | $3.67 \pm 0.30^{cB}$ | $7.30 \pm 0.02^{bB}$ | $6.25 \pm 1.95^{B}$ |
| Overall Season | $7.99 \pm 0.43^{a}$ | $7.28 \pm 2.72^{b}$ | $7.29 \pm 0.47^{b}$ | $7.52 \pm 1.59$ |

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

**Instar III**

Effect of season, year and stage on the weight of III$^{rd}$ instar larvae was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.86). The instar weight in *H. ligurriens* was significantly higher in summer ($24.06 \pm 0.3$) in comparison to monsoon ($23.23 \pm 2.7$) and winter ($22.77 \pm 2.16$) (Table 4.84).

**Table 4.84. Seasonal changes in weight (mg) of III$^{rd}$ instar larvae of *H. ligurriens***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $19.65 \pm 0.46^{bB}$ | $23.99 \pm 0.10^{a}$ | $19.89 \pm 0.08^{bB}$ | $21.18 \pm 2.13^{B}$ |
| 2020 | $24.98 \pm 0.08^{aA}$ | $23.92 \pm 0.46^{b}$ | $24.08 \pm 0.05^{bA}$ | $24.33 \pm 0.55^{A}$ |
| 2021 | $25.07 \pm 0.04^{aA}$ | $24.27 \pm 0.20^{b}$ | $24.34 \pm 0.18^{bA}$ | $24.56 \pm 0.41^{A}$ |
| Overall Season | $23.23 \pm 2.7^{b}$ | $24.06 \pm 0.3^{a}$ | $22.77 \pm 2.16^{a}$ | $23.35 \pm 2$ |

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

**Post feeding stage**

Effect of season, year and stage on the post feeding stage was found to be significant. Seasonal variations were not same in all years and also the yearly variations were not same in all seasons (Table 4.86). The weight of post feeding stage in *H. ligurriens* was significantly higher in winter ($27.19 \pm 0.58$) in comparison to monsoon ($25.62 \pm 1.14$) and summer ($26.24 \pm 0.35$) (Table 4.85).

**Table 4.85. Seasonal changes in weight (mg) of post feeding stage of *H. ligurriens***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $24.33 \pm 0.85^{cB}$ | $26.66 \pm 0.13^{bA}$ | $27.22 \pm 0.05^{aB}$ | $26.07 \pm 1.40^{B}$ |
| 2020 | $25.77 \pm 0.01^{cA}$ | $26.15 \pm 0.17^{bA}$ | $27.82 \pm 0.14^{aA}$ | $26.58 \pm 0.95^{A}$ |
| 2021 | $26.76 \pm 0.06^{aA}$ | $25.93 \pm 0.11^{bB}$ | $26.54 \pm 0.34^{aC}$ | $26.41 \pm 0.42^{A}$ |
| Overall Season | $25.62 \pm 1.14^{c}$ | $26.24 \pm 0.35^{b}$ | $27.19 \pm 0.58^{a}$ | $26.35 \pm 0.99$ |

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

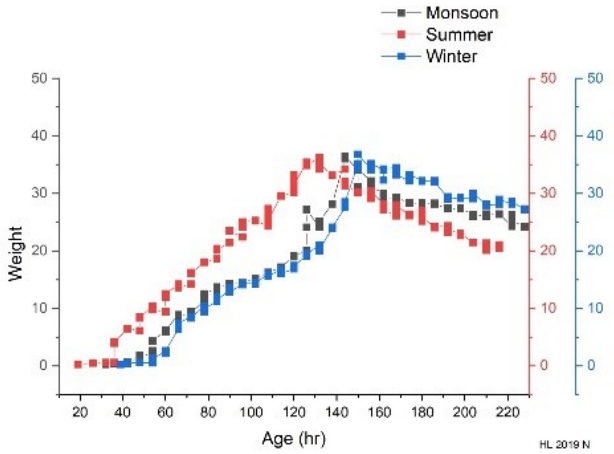
Interaction studies on year, season and stage was found to be significant (F = 14.365; P = < 0.001) indicating that seasonal variations in length of each stage were different in different years (Table 4.86).

**Table 4.86. ANOVA for comparing weight of larval instars of *H. ligurriens***

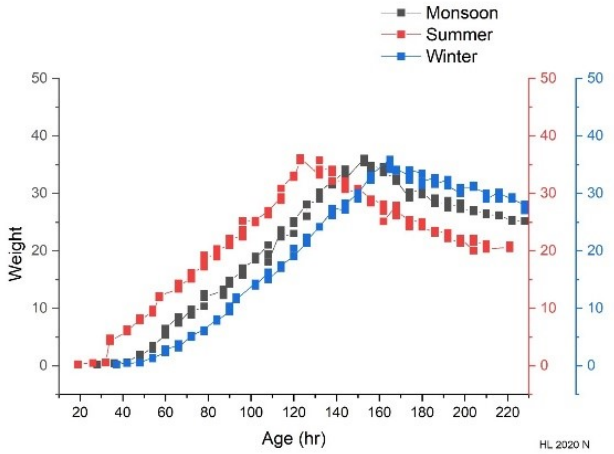
| Source | df | Sum of Squares | Mean Square | F-Value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 14.69 | 7.35 | 142.786** | < 0.001 |
| Season | 2 | 1.10 | 0.55 | 10.679** | < 0.001 |
| Stage | 3 | 12238.69 | 4079.56 | 79292.09** | < 0.001 |
| Year * Season | 4 | 46.02 | 11.51 | 223.612** | < 0.001 |
| Year * stage | 6 | 73.29 | 12.22 | 237.413** | < 0.001 |
| Season * stage | 6 | 21.52 | 3.59 | 69.713** | < 0.001 |
| Year * Season * Stage | 12 | 37.74 | 3.15 | 61.121** | < 0.001 |
| Error | 72 | 3.70 | 0.05 | | |
| Total | 107 | 12436.75 | | | |

** Significant at 0.01 level; ns non-Significant

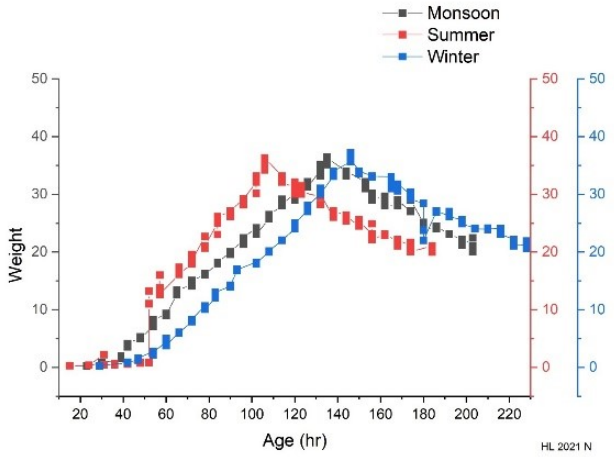
The growth curves representing the developmental rate (Length (mm) Vs. Age (hr) of *H. ligurriens* from hatching until pupation during different seasons and years were prepared (Fig. 4.63 & Fig. 4.64).




2019

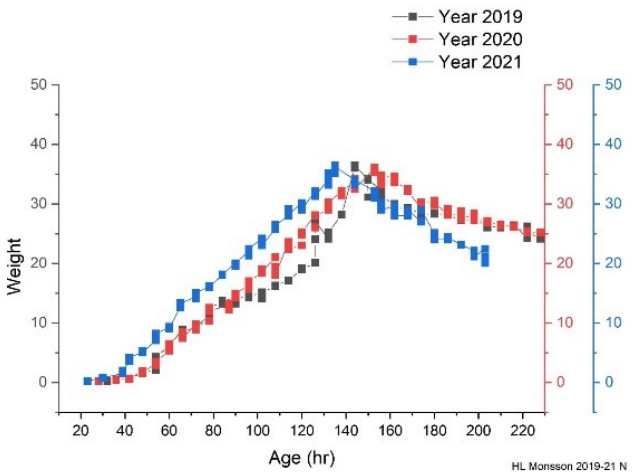



2020

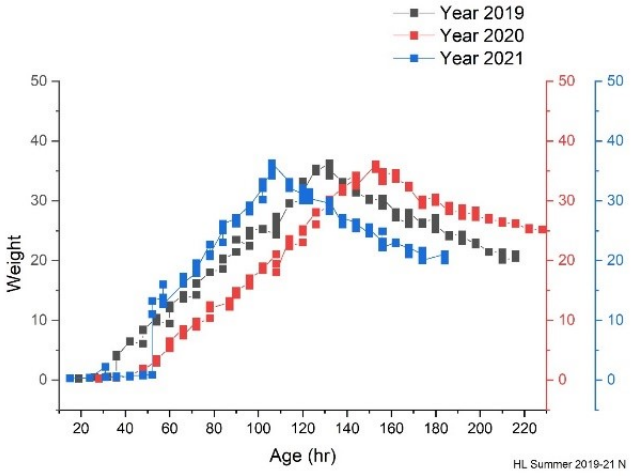



2021

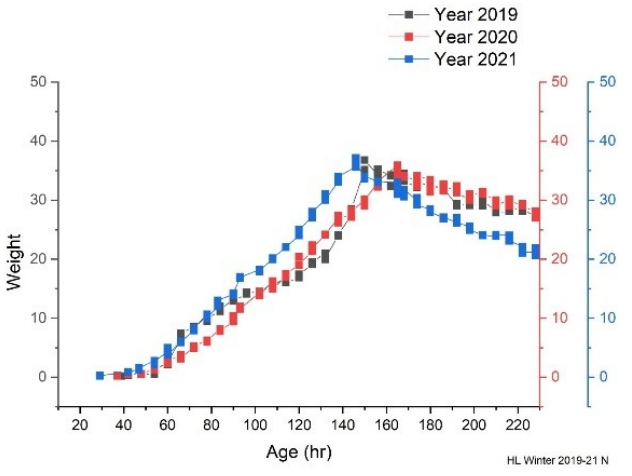
**Fig. 4.63. Seasonal developmental rate (Weight (mg) Vs. Age (hr) of *H. ligurriens* from hatching upto pupation**




Monsoon




Summer




Winter

**Fig. 4.64. Developmental rate (Weight (mg) Vs. Age (hr) of *H. ligurriens* from hatching upto pupation during the study period**

**Effect on life cycle duration**

Significantly higher duration (hr) was observed for *H. ligurriens* during winter for incubation (35.00 ± 5.29) (F = 84.88; P = < 0.001), pupation (192.33 ± 9.61) (F = 128.58; P = < 0.001) and total life cycle (457.33 ± 54.31) (F = 56.409; P = < 0.001). However significantly higher duration was observed during monsoon for II$^{nd}$ larval instar (29.33 ± 5.51) (F = 8.269; P = 0.038) (Table 4.87).

**Table 4.87. Seasonal changes in duration (hrs) of life cycle of *H. ligurriens***

| Stages | Monsoon | Summer | Winter | F-value (P-value) |
|---|---|---|---|---|
| Incubation | 27.33 ± 4.04$^{b}$ | 17.67 ± 2.31$^{c}$ | 35.00 ± 5.29$^{a}$ | 84.88** (<0.001) |
| Instar I | 18.00 ± 2.00 | 16.00 ± 1.00 | 16.67 ± 1.53 | 0.903$^{ns}$ (0.475) |
| Instar II | 29.33 ± 5.51$^{a}$ | 16.33 ± 1.53$^{b}$ | 28.67 ± 4.51$^{a}$ | 8.269* (0.038) |
| Instar III | 62.00 ± 1.73 | 55.33 ± 5.69 | 59.67 ± 3.51 | 2.575$^{ns}$ (0.191) |
| Post feeding stage | 139.33 ± 14.64 | 81.00 ± 9.85 | 125 ± 46.18 | 5.774$^{ns}$ (0.066) |
| Pupation | 154.33 ± 10.6$^{b}$ | 113.67 ± 8.15$^{c}$ | 192.33 ± 9.61$^{a}$ | 128.58** (<0.001) |
| Total time taken from egg stage till emergence | 430.33 ± 33.5$^{a}$ | 300.00 ± 23.07$^{b}$ | 457.33 ± 54.31$^{a}$ | 56.409** (0.001) |

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

*Means having different letter as super script differ significantly between seasons (within a row)*

Significantly higher duration (hr) was observed for *H. ligurriens* during 2019 for incubation (29.67 ± 10.07) (F = 16.63; P =0.012) and total life cycle (425.33 ± 104.41) (F = 10.229; P = 0.027) (Table 4.88).

**Table 4.88. Changes in duration (hrs) of life cycle of *H. ligurriens***

| Stages | 2019 | 2020 | 2021 | F-value (P-value) |
|---|---|---|---|---|
| Incubation | $29.67 \pm 10.07^{a}$ | $28.00 \pm 9.00^{a}$ | $22.33 \pm 7.02^{b}$ | 16.63* (0.012) |
| Instar I | $16.67 \pm 1.53$ | $17.33 \pm 2.52$ | $16.67 \pm 1.16$ | $0.129^{ns}$ (0.882) |
| Instar II | $24.67 \pm 7.02$ | $27.00 \pm 10.39$ | $22.67 \pm 6.51$ | $0.726^{ns}$ (0.538) |
| Instar III | $60.00 \pm 0.00$ | $61.00 \pm 3.46$ | $56.00 \pm 7.00$ | $1.575^{ns}$ (0.313) |
| Post feeding stage | $138.33 \pm 48.21$ | $113.00 \pm 24.00$ | $94.00 \pm 28.84$ | $3.091^{ns}$ (0.154) |
| Pupation | $156.00 \pm 42.57$ | $160.00 \pm 39.15$ | $144.33 \pm 37.02$ | $5.505^{ns}$ (0.071) |
| Total time taken from egg stage till emergence | $425.33 \pm 104.41^{a}$ | $406.33 \pm 77.68^{a}$ | $356.00 \pm 71.08^{b}$ | 10.229* (0.027) |

** Significant at 0.05 level; ns non-significant*
*Means having different letter as super script differ significantly between Years (within a row)*

**Effect on survival rate**

Effect of season on the survival rate (%) of *H. ligurriens* was found to be significant (Table 4.92). The survival rate (%) in *H. ligurriens* was significantly higher in monsoon ($75.08 \pm 5.18$) in comparison to summer ($73.20 \pm 3.93$) and winter ($64.94 \pm 4.21$). (Table 4.89).

**Table 4.89. Seasonal changes in the survival rate (%) of *H. ligurriens***

| Year | Monsoon | Summer | Winter | Overall year |
|---|---|---|---|---|
| 2019 | $78.30 \pm 2.94^{aA}$ | $74.50 \pm 3.19^{bA}$ | $65.32 \pm 2.50^{c}$ | $72.71 \pm 6.19^{A}$ |
| 2020 | $77.33 \pm 3.80^{aA}$ | $74.62 \pm 4.21^{bA}$ | $65.10 \pm 3.46^{c}$ | $72.35 \pm 6.49^{A}$ |
| 2021 | $69.61 \pm 3.55^{aB}$ | $70.49 \pm 2.95^{aB}$ | $64.39 \pm 6.09^{b}$ | $68.16 \pm 5.09^{B}$ |
| Overall Season | $75.08 \pm 5.18^{a}$ | $73.20 \pm 3.93^{b}$ | $64.94 \pm 4.21^{c}$ | $71.07 \pm 6.26$ |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of year and stage on the survival rate of developmental stages of *H. ligurriens* was found to be significant (Table 4.92). The survival rate of $I^{st}$ larvar instar

larvae was significantly higher in 2020 (75.97 ± 6.6) compared to other years. However, the survival rate for II$^{nd}$ (68.99 ± 4.41) and III$^{rd}$ instar larvae (64.71 ± 4.56) was significantly lower in 2021. Survival rate for pupation was significantly higher in 2019 (73.00 ±6.68) compared to 2020 (70.88 ± 6.58) and 2021 (65.43 ± 5.90) (Table 4.90).

**Table 4.90. Survival rate (%) of life cycle of *H. ligurriens***

| Stage | 2019 | 2020 | 2021 | Overall stage |
|---|---|---|---|---|
| Egg | 72.76 ± 7.13 | 72.33 ± 6.63 | 72.27 ± 4.42 | 72.45 ± 5.94$^{A}$ |
| I$^{st}$ Instar | 72.34 ± 5.69$^{b}$ | 75.97 ± 6.6$^{a}$ | 69.41 ± 2.18$^{b}$ | 72.57 ± 5.68$^{A}$ |
| II$^{nd}$ Instar | 72.91 ± 7.03$^{a}$ | 70.45 ± 5.64$^{ab}$ | 68.99 ± 4.41$^{b}$ | 70.78 ± 5.81$^{AB}$ |
| III$^{rd}$ Instar | 72.52 ± 5.75$^{a}$ | 72.12 ±6.93$^{a}$ | 64.71 ± 4.56$^{b}$ | 69.78 ± 6.69$^{B}$ |
| Pupa | 73.00 ±6.68$^{a}$ | 70.88 ± 6.58$^{a}$ | 65.43 ± 5.90$^{b}$ | 69.77 ± 6.95$^{B}$ |
| Overall year | 72.71 ±6.19$^{a}$ | 72.35 ±6.49$^{a}$ | 68.16 ± 5.09$^{b}$ | 71.07 ± 6.26 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Effect of season and stage on the survival rate of developmental stages was found to be significant (Table 4.92).Survival rate of developmental stages of *H. ligurriens* was significantly higher in monsoon (75.08 + 5.18) compared to summer (73.20 + 3.93) and winter (64.94 + 4.21) (Table 4.91).

**Table 4.91. Seasonal changes in survival rate (%) of life cycle stages of *H. ligurriens***

| Stage | Monsoon | Summer | Winter | Overall stage |
|---|---|---|---|---|
| Egg | 76.55 + 3.77 | 74.15 + 3.12 | 66.66 + 5.54 | 72.45 + 5.94$^{A}$ |
| II$^{st}$ Instar | 76.17 + 6.30 | 73.30 + 4.65 | 68.24 + 2.71 | 72.57 + 5.68$^{A}$ |
| II$^{nd}$ Instar | 75.04 + 4.43 | 73.27 + 2.80 | 64.04 + 1.89 | 70.78 + 5.81$^{AB}$ |
| III$^{rd}$ Instar | 73.25 + 5.63 | 72.55 + 5.72 | 63.55 + 3.89 | 69.78 + 6.69$^{B}$ |
| Pupa | 74.38 + 5.83 | 72.73 + 3.33 | 62.20 + 3.71 | 69.77 + 6.95$^{B}$ |
| Overall Season | 75.08 + 5.18$^{a}$ | 73.20 + 3.93$^{b}$ | 64.94 + 4.21$^{c}$ | 71.07 + 6.26 |

*Means having different small letter as superscript differ significantly within a row*
*Means having different capital letter as superscript differ significantly within a column*

Three way ANOVA for studying the interaction between year, season and developmental stages indicated interactions between year and stage (F = 3.30; P = < 0.002), as well as year and season (F = 5.85; P = < 0.001) (Table 4.92).

**Table 4.92. ANOVA for comparing survival of *H. ligurriens***

| Source | df | Sum of Squares | Mean Square | F-value | P-value |
|---|---|---|---|---|---|
| Year | 2 | 574.49 | 287.24 | 24.12** | < 0.001 |
| Season | 2 | 2619.17 | 1309.59 | 109.97** | < 0.001 |
| Stage | 4 | 205.09 | 51.27 | 4.31** | 0.003 |
| Year * season | 4 | 278.62 | 69.66 | 5.85** | < 0.001 |
| Year * Stage | 8 | 314.34 | 39.29 | 3.30** | 0.002 |
| Season * Stage | 8 | 91.02 | 11.38 | $0.955^{ns}$ | 0.476 |
| Year * season * Stage | 16 | 103.04 | 6.44 | $0.541^{ns}$ | 0.918 |
| Error | 90 | 1071.79 | 11.91 | | |
| Corrected Total | 134 | 5257.55 | | | |

*** Significant at 0.01 level; ns non-significant*

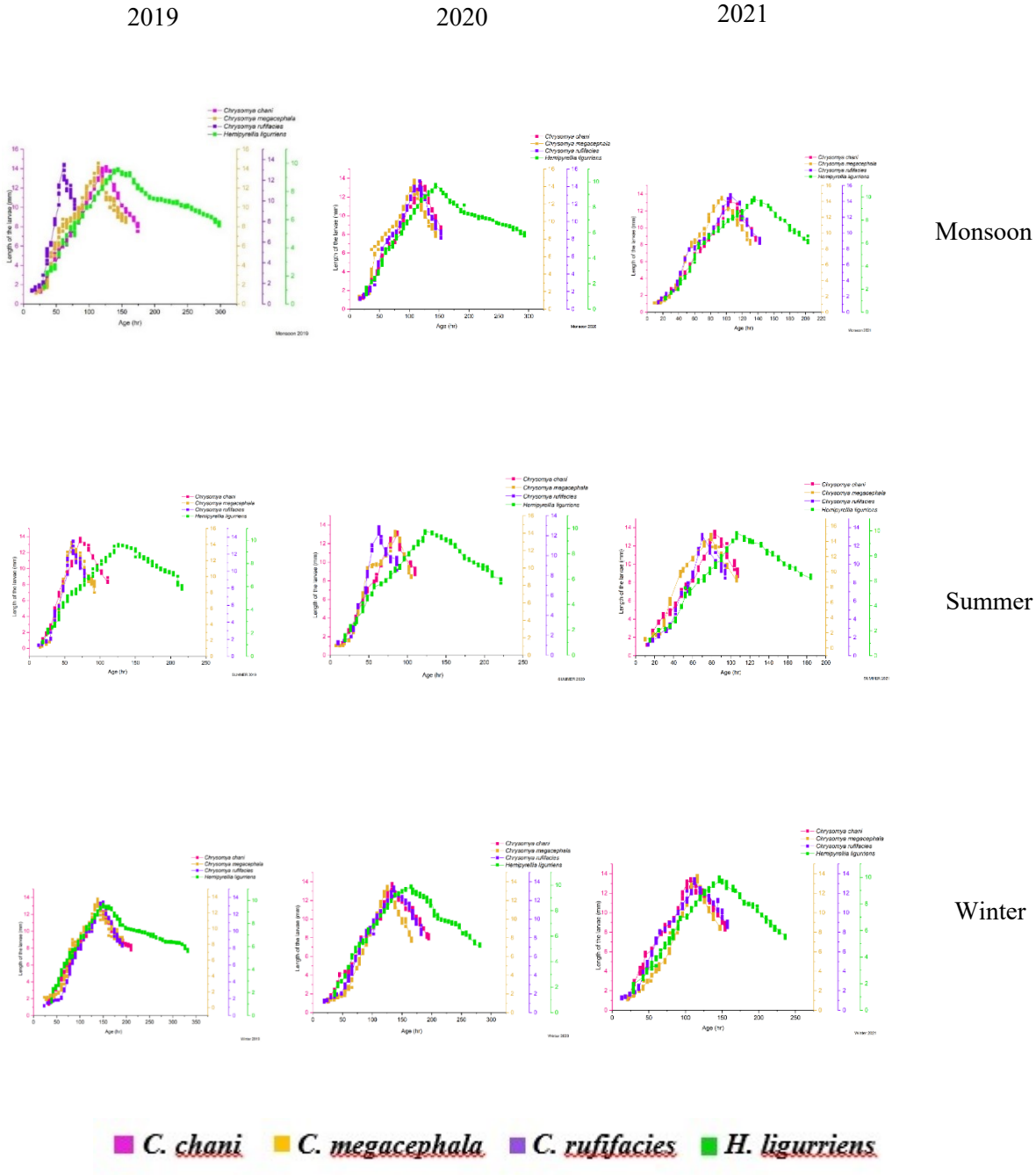



**Fig.4.65. Developmental rate (Length (mm) Vs. Age (hr) *C. chani, C. megacephala*, *C. rufifacies* and *H. ligurriens* from hatching upto pupation**

### 4.4.5 Laboratory rearing

***C. megacephala***

**Length**

Effect of temperature and humidity in controlled conditions on the length of larval instars was found to be significant (F = 56.98; P = < 0.001). The length of Instar III was found to be significantly higher (11.69 ± 0.04) in Group II while the length of Instar II was significantly lower (6.23 ± 0.04) in Group II compared to other groups. The length of post feeding stage was significantly higher in Group III (11.12 ± 0.02) compared to Group I (10.66 ± 0.06) and Group II (10.44 ± 0.01) (Table 4.93) (Fig. 4.66 and Fig.4.74).

**Table 4.93. Length (mm) of larval instars of *C. megacephala* under laboratory conditions**

| Stage | Group | | | Overall stage |
|---|---|---|---|---|
| | I. | II. | III. | |
| Instar I | $2.54 \pm 0.02^{D}$ | $2.53 \pm 0.02^{D}$ | $2.51 \pm 0.08^{D}$ | $2.53 \pm 0.04^{D}$ |
| Instar II | $6.41 \pm 0.04^{bC}$ | $6.23 \pm 0.04^{aC}$ | $6.46 \pm 0.08^{bC}$ | $6.37 \pm 0.12^{C}$ |
| Instar III | $11.45 \pm 0.02^{bA}$ | $11.69 \pm 0.04^{aA}$ | $11.51 \pm 0.03^{bA}$ | $11.55 \pm 0.11^{A}$ |
| Post feeding stage | $10.66 \pm 0.06^{bB}$ | $10.44 \pm 0.01^{cB}$ | $11.12 \pm 0.02^{aB}$ | $10.74 \pm 0.3^{B}$ |
| Between stage F-value = 78852.68**; (P-value < 0.001)<br>Between group F-value = 51.30**; (P-value < 0.001)<br>Interaction between stage and group F-value =$56.98^{**}$; (P-value < 0.001) | | | | |

** Significant at 0.01 level

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

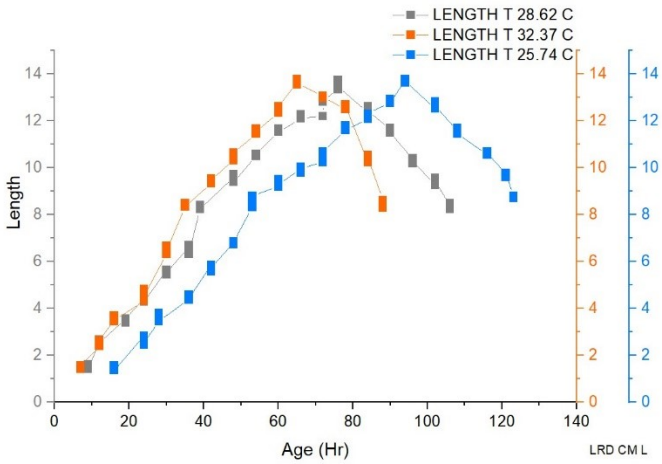



**Fig. 4.66. Developmental rate (Length (mm) Vs. Age (hrs.) of *C. megacephala* from hatching upto pupation under laboratory conditions**

**Weight**

Effect of temperature and humidity in laboratory conditions on the weight of larval instars was found to be significant (F = 11.83; P = < 0.001). The weight of instar II (11.68 ± 0.25), instar III (33.70 ± 0.47) and post feeding stage (30.21 ± 0.5) was significantly higher in Group III compared to other groups (Table 4.94) (Fig. 4.67 and Fig.4.75).

**Table 4.94. Weight (mg) of larval instars of *C. megacephala* under laboratory conditions**

| Stage | Group | | | Overall stage |
|---|---|---|---|---|
| | I. | II. | III. | |
| Instar I | $1.56 \pm 0.19^{C}$ | $1.64 \pm 0.16^{D}$ | $1.77 \pm 0.04^{D}$ | $1.66 \pm 0.16^{D}$ |
| Instar II | $10.02 \pm 0.34^{bB}$ | $10.25 \pm 0.11^{bC}$ | $11.68 \pm 0.25^{aC}$ | $10.65 \pm 0.81^{C}$ |
| Instar III | $29.50 \pm 0.83^{cA}$ | $30.37 \pm 0.86^{bA}$ | $33.70 \pm 0.47^{aA}$ | $31.19 \pm 2.02^{A}$ |
| Post feeding stage | $29.53 \pm 0.24^{aA}$ | $28.28 \pm 0.82^{bB}$ | $30.21 \pm 0.5^{aB}$ | $29.34 \pm 0.98^{B}$ |
| Between stage F-value = 7790.18**; (P-value < 0.001)<br>Between group F-value = 47.91**; (P-value < 0.001)<br>Interaction between stage and group F-value = $11.83^{**}$; (P-value < 0.001) | | | | |

** Significant at 0.01 level

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

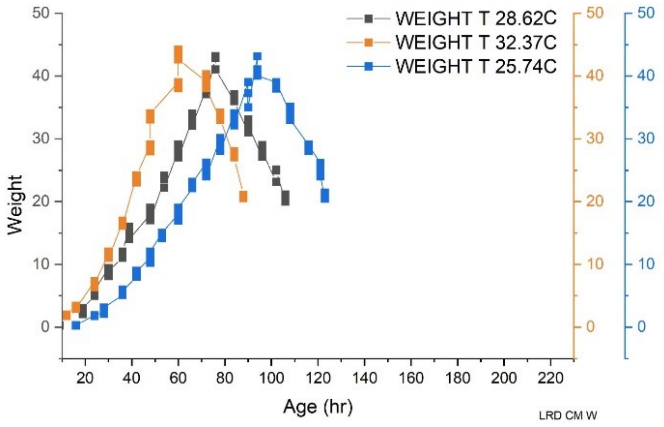



**Fig. 4.67. Developmental rate (Weight (mg) Vs. Age (hrs) of *C. megacephala* from hatching upto pupation under laboratory conditions**

**Developmental duration**

In group I conditions, it was observed that the developmental duration (hrs) for incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation were 16 ± 1.73, 12 ± 2.69, 17 ± 3.75, 34 ± 4.18, 21 ± 3.29, 112 ± 11.03 respectively. The total duration (hrs) for the completion of the life cycle was 212 ± 28.43h.

In group II, the developmental duration (hrs) for incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation were, 8 ± 2..93, 10 ± 3.36, 15 ± 2.71, 28 ± 3.90, 22 ± 4.17, 85 ± 7.92 respectively. The total duration (hrs) for the completion of the life cycle was 168 ± 17.

In group III, the developmental duration (hrs) for incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation were, 6 ± 3.66, 9 ± 2.78, 11 ± 3.19, 23 ± 3.63, 16 ± 3.89, 66 ± 8.03 respectively. The total duration (hrs) for the completion of the life cycle was 131 ± 13.03 (Table 4.95).

**Table 4.95. Duration (hrs) of life cycle of *C. megacephala* under laboratory conditions**

| Group | Duration of different life cycle stages(hrs) | | | | | | Total time taken from egg stage till emergence |
|---|---|---|---|---|---|---|---|
| | Incubation period of eggs | I instar | II instar | III instar | Post feeding Stage | Pupation stage | |
| I | 16 ± 1.73 | 12 ± 2.69 | 17 ± 3.75 | 34 ± 4.18 | 21 ± 3.29 | 112 ± 11.03 | 212 ± 28.43 |
| II | 8 ± 2..93 | 10 ± 3.36 | 15 ± 2.71 | 28 ± 3.90 | 22 ± 4.17 | 85 ± 7.92 | 168 ± 17.55 |
| III | 6 ± 3.66 | 9 ± 2.78 | 11 ± 3.19 | 23 ± 3.63 | 16 ± 3.89 | 66 ± 8.03 | 131 ± 13.03 |

The laboratory rearing data was compared with the outdoor rearing data for validation. In the case of length and weight of different larval instars, no significant differences were observed between outdoor and laboratory rearing groups. Results of laboratory rearing showed that the total duration (hrs) for the completion of the life cycle of *C. megacephala* was 212 ± 28.43 (Group-I), 168 ± 17.55 (Group-II) and 131 ± 13.03h (Group-III) respectively **(**Table 4.95).This was significantly shorter than the outdoor rearing results obtained for winter (286.00 ± 23.26), monsoon (227.00 ± 22.52) and summer (168.00 ± 5.29) respectively.

***C. rufifacies***

**Length**

Effect of temperature and humidity in controlled conditions on the length of larval instars was found to be significant (F=109.56; P=< 0.001).The length of instar I (1.75 ± 0.03) was found to be significantly higher in Group III, while the length of instar III (11.32 ± 0.03) was significantly higher in Group II compared to other groups. The length of post feeding stage (10.71 ± 0.03) was found to be significantly higher in Group I compared to other groups (Table.4.96) (Fig. 4.68 and Fig.4.74).

**Table 4.96. Length (mm) of larval instars of *C. rufifacies* under laboratory conditions**

| Stage | Group | | | Overall stage |
|---|---|---|---|---|
| | I. | II. | III. | |
| Instar I | $1.59 \pm 0.03^{cD}$ | $2.32 \pm 0.04^{aD}$ | $1.75 \pm 0.03^{bD}$ | $1.89 \pm 0.33^{D}$ |
| Instar II | $5.37 \pm 0.001^{C}$ | $5.36 \pm 0.04^{C}$ | $5.36 \pm 0.02^{C}$ | $5.37 \pm 0.02^{C}$ |
| Instar III | $10.85 \pm 0.01^{aA}$ | $11.32 \pm 0.03^{cA}$ | $10.77 \pm 0.03^{bA}$ | $10.98 \pm 0.26^{A}$ |
| Post feeding stage | $10.71 \pm 0.03^{aB}$ | $10.53 \pm 0.09^{bB}$ | $10.20 \pm 0.05^{cB}$ | $10.48 \pm 0.23^{B}$ |
| Between stage F-value = 113693.27**; (P-value < 0.001)<br>Between group F-value = 282.34**; (P-value < 0.001)<br>Interaction between stage and group F-value = $109.56^{**}$; (P-value < 0.001) | | | | |

** Significant at 0.01 level

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

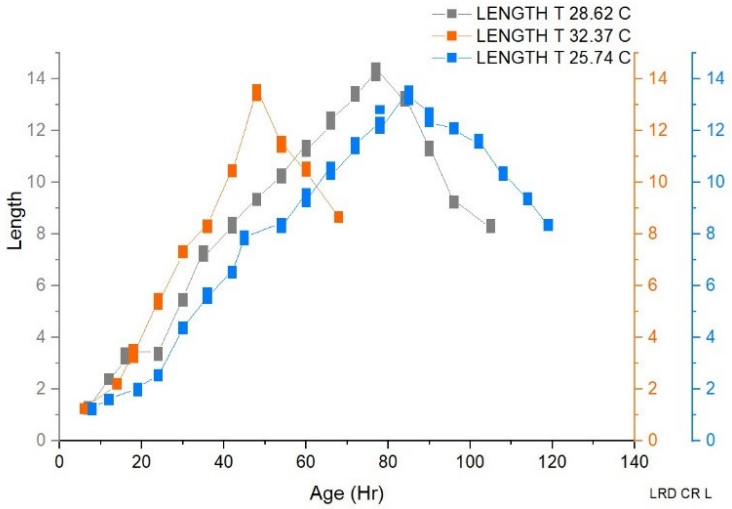



**Fig. 4.68. Developmental rate (Length (mm) Vs. Age (hrs) of *C. rufifacies* from hatching upto pupation under laboratory conditions**

**Weight**

Effect of temperature and humidity in laboratory conditions on the weight of larval instars was found to be significant (F = 16.31; P = < 0.001). The weight of instar II (11.81 ± 0.56) and instar III was significantly higher (33.48 ± 0.36) in Group III compared to other groups, while the weight of post feeding stage (29.25 ± 0.41) was significantly higher in Group I compared to Group II (28.91 ± 0.36) and Group III (27.45 ± 0.6) (Table 4.97) (Fig. 4.69 and Fig.4.75).

**Table 4.97. Weight (mg) of larval instars of *C. rufifacies* under laboratory conditions**

| Stage | Group | | | Overall stage |
|---|---|---|---|---|
| | I. | II. | III. | |
| Instar I | $1.58 \pm 0.02^{C}$ | $1.5 \pm 0.05^{D}$ | $1.54 \pm 0.14^{D}$ | $1.54 \pm 0.08^{D}$ |
| Instar II | $10.49 \pm 0.46^{bB}$ | $11.75 \pm 0.29^{aC}$ | $11.81 \pm 0.56^{aC}$ | $11.35 \pm 0.76^{C}$ |
| Instar III | $31.34 \pm 0.34^{bA}$ | $32.96 \pm 0.39^{aA}$ | $33.48 \pm 0.36^{aA}$ | $32.59 \pm 1.02^{A}$ |
| Post feeding stage | $29.25 \pm 0.41^{aA}$ | $28.91 \pm 0.36^{aB}$ | $27.45 \pm 0.6^{bB}$ | $28.54 \pm 0.92^{B}$ |
| Between stage F-value = 13439.64**; (P-value < 0.001)<br>Between group F-value = 8.32**; (P-value < 0.001)<br>Interaction between stage and group F-value = $16.31^{**}$; (P-value < 0.001) | | | | |

** Significant at 0.01 level

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

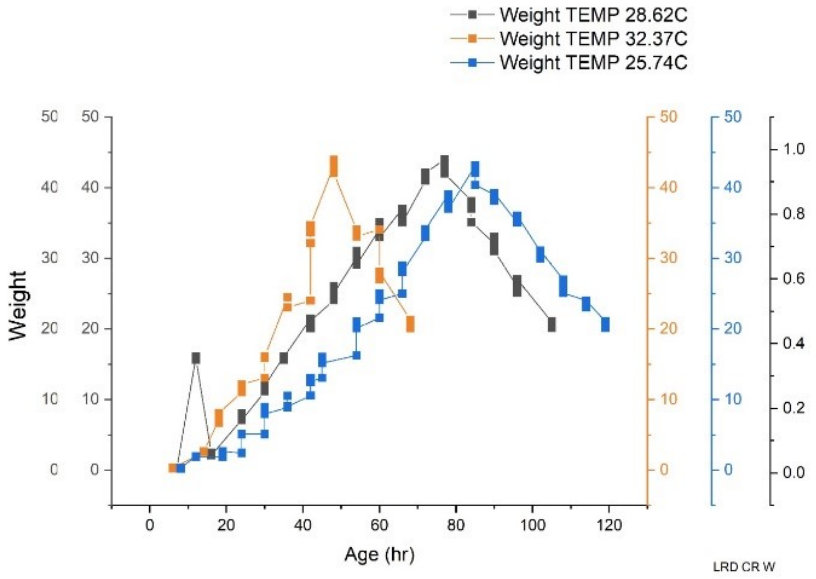



**Fig. 4.69. Developmental rate (Weight (mg) Vs. Age (hrs) of *C. rufifacies* from hatching upto pupation under laboratory conditions**

**Developmental duration**

In group I conditions, it was observed that the developmental duration (hrs) for incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation in *C.*

*rufifacies* were, 7± 2.81, 11 ± 3.11, 21 ± 4.16, 31 ± 3.83, 29 ± 4.41 and 98 ± 7.72 respectively. The total duration (hrs) for the completion of the life cycle was 197 ± 18.18 h.

In group II, the developmental duration (hrs) for incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation were, 6 ± 3.72, 9 ± 1.93, 11 ± 3.81, 35 ± 4.73, 21 ± 3.18, 78 ± 9.18 respectively. The total duration (hrs) for the completion of the life cycle was 160 ± 21.03.

In group III, the developmental duration (hrs) for incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation were, 5 ± 1.13, 8 ± 3.03, 12 ± 2.17, 29 ± 3.14, 14 ± 4.14, 62 ± 5.47 respectively. The total duration (hrs) for the completion of the life cycle was130 ± 26.77 (Table 4.98).

**Table 4.98. Duration (hrs) of life cycle of *C. rufifacies* under laboratory conditions**

| Group | Duration of different life cycle stages(hrs) | | | | | | Total time taken from egg stage till emergence |
|---|---|---|---|---|---|---|---|
| | Incubation period of eggs | I instar | II instar | III instar | Post feeding Stage | Pupation stage | |
| I | 7± 2.81 | 11 ± 3.11 | 21 ± 4.16 | 31 ± 3.83 | 29 ± 4.41 | 98 ± 7.72 | 197 ± 18.18 |
| II | 6 ± 3.72 | 9 ± 1.93 | 11 ± 3.81 | 35 ± 4.73 | 21 ± 3.18 | 78 ± 9.18 | 160 ± 21.03 |
| III | 5 ± 1.13 | 8 ± 3.03 | 12 ± 2.17 | 29 ± 3.14 | 14 ± 4.14 | 62 ± 5.47 | 130 ± 26.77 |

The laboratory rearing data was compared with the outdoor rearing data for validation .In the case of length and weight of different larval instars, no significant differences were observed between outdoor and laboratory rearing. Results of laboratory rearing showed that the total duration (hrs) for the completion of the life cycle of *C. rufifacies* was 197 ± 18.18 (Group-I), 160 ± 21.03 (Group-II) and 130 ± 26.77 (Group-III) respectively (Table 4.98). . This was significantly shorter than the outdoor rearing results

obtained for winter (267.00 ± 18.68), monsoon (223.00 ± 13.45) and summer (148.33 ± 6.43) respectively.

***C. chani***

**Length**

Effect of temperature and humidity in controlled conditions on the length of larval instars was found to be significant (F=92.660; P = < 0.001). The length of instar I (2.81 ± 0.07) was significantly higher in Group III, while that of instar II was significantly lower (5.56 ± 0.03) in Group II compared to other groups. The length of instar III (11.22 ± 0.01) and post feeding stage (10.98 ± 0.06) was significantly higher in Group I compared to other groups. (Table.4.99) (Fig. 4.70 and Fig.4.74).

**Table 4.99. Length (mm) of larval instars of *C. chani* under laboratory conditions**

| Stage | Group | | | Overall stage |
|---|---|---|---|---|
| | I. | II. | III. | |
| Instar I | $2.59 \pm 0.03^{bD}$ | $2.35 \pm 0.01^{cC}$ | $2.81 \pm 0.07^{aD}$ | $2.58 \pm 0.20^{D}$ |
| Instar II | $6.54 \pm 0.06^{aC}$ | $5.56 \pm 0.03^{bB}$ | $6.49 \pm 0.06^{aC}$ | $6.2 \pm 0.48^{C}$ |
| Instar III | $11.22 \pm 0.01^{aA}$ | $10.44 \pm 0.01^{cA}$ | $10.86 \pm 0.05^{bA}$ | $10.84 \pm 0.34^{A}$ |
| Post feeding stage | $10.98 \pm 0.06^{aB}$ | $10.44 \pm 0.01^{bA}$ | $10.47 \pm 0.03^{bB}$ | $10.63 \pm 0.27^{B}$ |
| Between stage F-value = 76347.37**; (P-value < 0.001)<br>Between group F-value = 701.69**; (P-value < 0.001)<br>Interaction between stage and group F-value =$92.660^{**}$; (P-value < 0.001) | | | | |

** Significant at 0.01 level

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

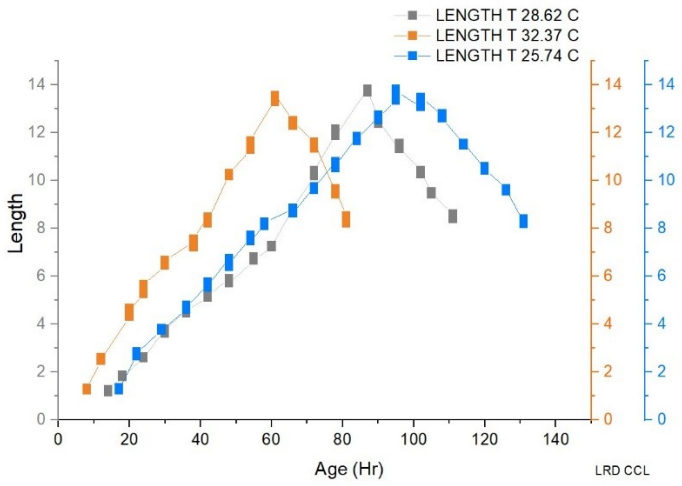



**Fig.4.70. Developmental rate (Length (mm) Vs. Age (hrs) of *C. chani* from hatching upto pupation under laboratory controlled conditions**

**Weight**

Effect of temperature and humidity in laboratory conditions on the weight of larval instars was found to be significant (F = 6.621; P = < 0.001). The weight of instar II (11.83 ± 0.71) and instar III (31.95 ± 0.52) was significantly higher in Group III compared to other groups, while the weight of post feeding stage (28.29 ± 0.62) was significantly lower in Group I compared to other groups (Table 4.99) (Fig. 4.71 and Fig.4.75).

**Table 4.99. Weight (mg) of larval instars of *C. chani* under laboratory conditions**

| Stage | Group | | | |
|---|---|---|---|---|
| | I. | II. | III. | Overall stage |
| Instar I | $1.54 \pm 0.13^{C}$ | $1.4 \pm 0.10^{D}$ | $1.36 \pm 0.18^{D}$ | $1.43 \pm 0.15^{D}$ |
| Instar II | $10.32 \pm 0.24^{bB}$ | $10.31 \pm 0.8^{bC}$ | $11.83 \pm 0.71^{aC}$ | $10.82 \pm 0.93^{C}$ |
| Instar III | $28.39 \pm 0.89^{bA}$ | $31.30 \pm 0.48^{aA}$ | $31.95 \pm 0.52^{aA}$ | $30.55 \pm 1.74^{A}$ |
| Post feeding stage | $28.29 \pm 0.62^{bA}$ | $28.79 \pm 1.06^{abB}$ | $29.50 \pm 0.53^{aB}$ | $28.86 \pm 0.85^{B}$ |
| Between stage F-value = 4975.52**; (P-value < 0.001)<br>Between group F-value = 19.291**; (P-value < 0.001)<br>Interaction between stage and group F-value = $6.621^{**}$; (P-value < 0.001) | | | | |

** Significant at 0.01 level

*Means having different small letter as superscript differ significantly within a row*

*Means having different capital letter as superscript differ significantly within a column*

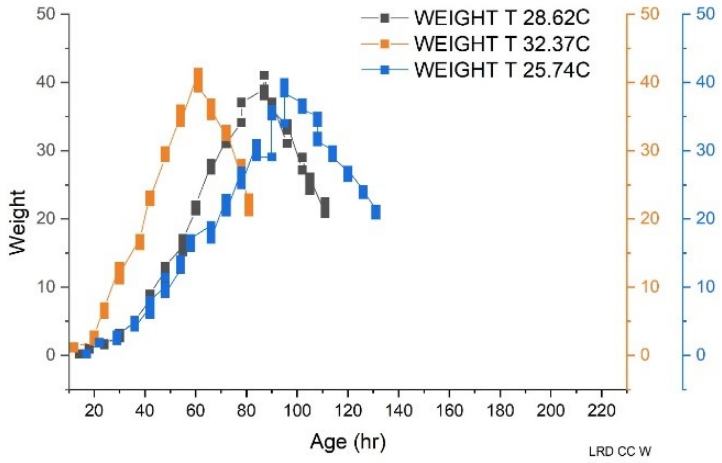



**Fig. 4.71. Developmental rate (Weight (mg) Vs. Age (hrs) of *C. chani* from hatching upto pupation under laboratory conditions**

**Developmental duration**

In group I conditions, it was observed that the developmental duration (hrs) for incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation were 16 ± 2.06, 12 ± 2.67, 28 ± 4.04, 29 ± 2.63, 29 ±4.06, 118 ± 6.81 respectively. The total duration (hrs) for the completion of the life cycle was 232 ± 33.18.

In group II, the developmental duration (hrs) for incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation were, 13 ± 2.18, 10 ± 1.18, 19±2.58, 27 ± 4.08, 21 ± 3.81, 89 ± 6.82 respectively. The total duration (hrs) for the completion of the life cycle was 179 ± 28.92.

In group III, the developmental duration (hrs) for incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation were, 7 ± 3.04, 9 ± 2.17, 14 ± 3.11, 19 ± 3.77, 15 ± 4.46, 76 ± 7.91 respectively. The total duration (hrs) for the completion of the life cycle was 140 ± 22.18 (Table 4.100).

**Table 4.100. Duration (hrs) of life cycle of *C. chani* under laboratory conditions**

| Group | Duration of different life cycle stages(hrs) | | | | | | Total time taken from egg stage till emergence |
|---|---|---|---|---|---|---|---|
| | Incubation period of eggs | I instar | II instar | III instar | Post feeding Stage | Pupation stage | |
| I | 16 ± 2.06 | 12 ± 2.67 | 28 ± 4.04 | 29 ± 2.63 | 29 ±4.06 | 118 ± 6.81 | 232 ± 33.18 |
| II | 13 ± 2.18 | 10 ± 1.18 | 19±2.58 | 27 ± 4.08 | 21 ± 3.81 | 89 ± 6.82 | 179 ± 28.92 |
| III | 7 ± 3.04 | 9 ± 2.17 | 14 ± 3.11 | 19 ± 3.77 | 15 ± 4.46 | 76 ± 7.91 | 140 ± 22.18 |

The laboratory rearing data was compared with the outdoor rearing data for validation. In the case of length and weight of different larval instars, no significant differences were observed between outdoor and laboratory rearing. Results of laboratory rearing showed that the total duration (hrs) for the completion of the life cycle of *C. chani* was 232 ± 33.18 (Group-I), 179 ± 28.92 (Group-II) and 140 ± 22.18 (Group-III) respectively. This was significantly shorter than the outdoor rearing results obtained for winter (320.33± 24.03), monsoon (246±20.08) and summer (192.33±8.15) respectively.

***Hemipyrellia ligurriens***

**Length**

Effect of temperature and humidity in controlled conditions on the length of larval instars was found to be significant (F=342.90; P = < 0.001). The length of instar I (2.41 ± 0.05) and instar II (4.53 ± 0.01) was significantly higher in Group I compared to other groups, while the length Instar III (8.22 ± 0.02) was significantly higher in Group II compared to other groups. The length of post feeding stage (7.98 ± 0.01) was significantly higher in Group III compared to Group I (7.75 ± 0.02) and Group II (7.95 ± 0.02) (Table.4.101) (Fig. 4.72 and Fig.4.74).

**Table 4.101. Length (mm) of larval instars of *H. ligurriens* under laboratory conditions**

| Stage | Group | | | |
|---|---|---|---|---|
| | I. | II. | III. | Overall stage |
| Instar I | $2.41 \pm 0.05^{aD}$ | $1.81 \pm 0.01^{bD}$ | $1.76 \pm 0.02^{bD}$ | $1.99 \pm 0.32^{D}$ |
| Instar II | $4.53 \pm 0.01^{aC}$ | $4.33 \pm 0.02^{bC}$ | $3.77 \pm 0.05^{cC}$ | $4.21 \pm 0.34^{C}$ |
| Instar III | $7.98 \pm 0.09^{bA}$ | $8.22 \pm 0.02^{aA}$ | $6.73 \pm 0.04^{cB}$ | $7.64 \pm 0.70^{B}$ |
| Post feeding stage | $7.75 \pm 0.02^{bB}$ | $7.95 \pm 0.02^{aB}$ | $7.98 \pm 0.01^{aA}$ | $7.90 \pm 0.11^{A}$ |
| Between stage F-value = 48772.16**; (P-value < 0.001)<br>Between group F-value = 874.59**; (P-value < 0.001)<br>Interaction between stage and group F-value = $342.90^{**}$; (P-value < 0.001) | | | | |

** Significant at 0.01 level

*Means having different small letters as superscripts differ significantly within a row*

*Means having different capital letters as superscripts differ significantly within a column*

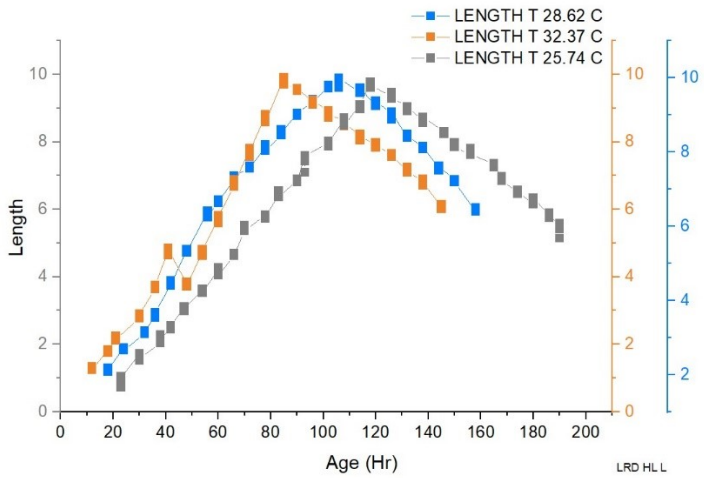



**Fig. 4.72. Developmental rate (Length (mm) Vs. Age (hrs) of *H. ligurriens* from hatching upto pupation under laboratory conditions**

**Weight**

Effect of temperature and humidity in laboratory conditions on the weight of larval instars was found to be significant (F=7.86; P=< 0.001). The weight of instar II (7.59 ± 0.54) and instar III (25.67 ± 0.53) was significantly higher in Group I compared to other groups (Table.4.102) (Fig. 4.73 and Fig.4.75).

**Table 4.102. Weight (mg) of larval instars of *H. ligurriens* under laboratory conditions**

| Stage | Group | | | Overall stage |
|---|---|---|---|---|
| | I. | II. | III. | |
| Instar I | $0.95 \pm 0.16^{D}$ | $0.79 \pm 0.04^{D}$ | $0.83 \pm 0.02^{D}$ | $0.86 \pm 0.11^{D}$ |
| Instar II | $7.59 \pm 0.54^{aC}$ | $5.24 \pm 0.13^{bC}$ | $5.63 \pm 0.13^{bC}$ | $6.16 \pm 1.13^{C}$ |
| Instar III | $25.67 \pm 0.53^{aB}$ | $24.15 \pm 0.37^{bB}$ | $24.75 \pm 0.46^{bB}$ | $24.86 \pm 0.77^{B}$ |
| Post feeding stage | $26.68 \pm 0.41^{A}$ | $26.71 \pm 0.67^{A}$ | $27.31 \pm 0.61^{A}$ | $26.9 \pm 0.59^{A}$ |
| Between stage F-value = 9404.27**; (P-value < 0.001)<br>Between group F-value = 18.39**; (P-value < 0.001)<br>Interaction between stage and group F-value = 7.86**; (P-value < 0.001) | | | | |

** Significant at 0.01 level

*Means having different small letters as superscripts differ significantly within a row*

*Means having different capital letters as superscripts differ significantly within a column*

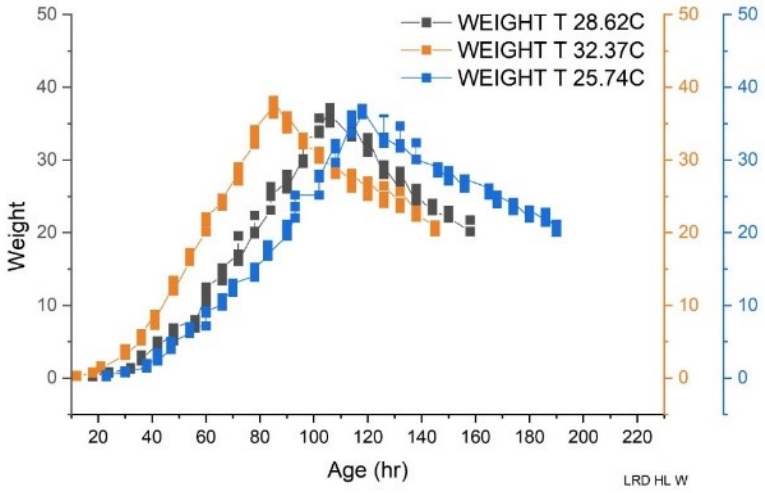



**Fig. 4.73. Developmental rate (Weight (mg) Vs. Age (hrs) of *H. ligurriens* from hatching upto pupation under laboratory conditions**

**Developmental duration**

In group I conditions, it was observed that the developmental duration (hrs) for incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation were 22 ± 3.15, 15 ± 2.33, 18 ± 2.59, 40 ± 4.77, 64 ± 5.16, 131 ± 9.03 respectively. The total duration (hrs) for the completion of the life cycle was 290 ± 29.17.

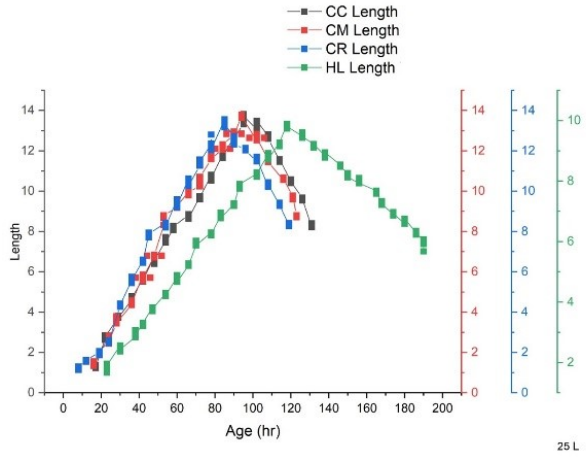
In group II, the developmental duration (hrs) of incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation were, 17 ± 4.02, 14 ± 3.04, 20 ± 3.02, 20 ± 3.02, 46 ± 5.23, 44 ± 4.20, 117 ± 11.68 respectively. The total duration (hrs) for the completion of the life cycle was 258 ± 16.38.

In group III, the developmental duration (hrs) of incubation of eggs, I$^{st}$ instar, II$^{nd}$ instar, III$^{rd}$ instar, post feeding stage and pupation were, 11 ± 2.84, 9 ± 2.17, 11 ± 2.85, 37 ± 4.99, 55 ± 3.12, 96 ± 8.16, respectively. The total duration (hrs) for the completion of the life cycle was 219 ± 21.11 (Table 4.103).

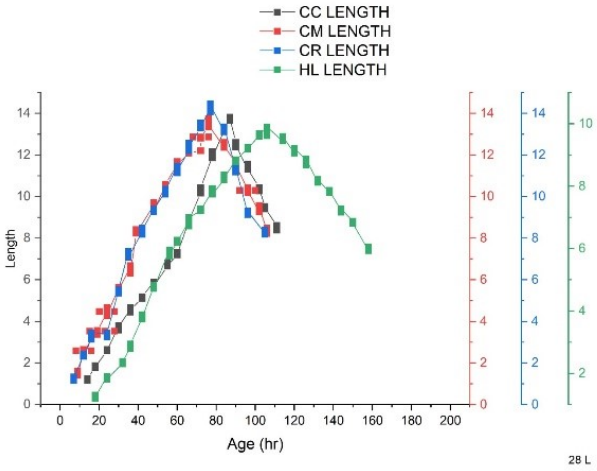
**Table 4.103. Duration (hrs) of life cycle of *H. ligurriens* under laboratory conditions**

| Group | Duration of different life cycle stages(hrs) | | | | | | Total time taken from egg stage till emergence |
|---|---|---|---|---|---|---|---|
| | Incubation period of eggs | I instar | II instar | III instar | Post feeding Stage | Pupation stage | |
| I | 22 ± 3.15 | 15 ± 2.33 | 18 ± 2.59 | 40 ± 4.77 | 64 ± 5.16 | 131 ± 9.03 | 290 ± 29.17 |
| II | 17 ± 4.02 | 14 ± 3.04 | 20 ± 3.02 | 46 ± 5.23 | 44 ± 4.20 | 117 ± 11.68 | 258 ± 16.38 |
| III | 11 ± 2.84 | 9 ± 2.17 | 11 ± 2.85 | 37 ± 4.99 | 55 ± 3.12 | 96 ± 8.16 | 219 ± 21.11 |

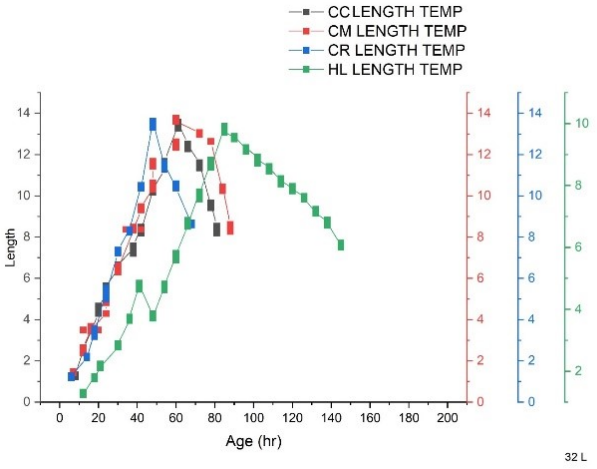
The laboratory rearing data was compared with the outdoor rearing data for validation. In the case of length and weight of different larval instars, no significant differences were observed between outdoor and laboratory rearing. Results of laboratory rearing showed that the total duration (hrs) for the completion of the life cycle of *H .ligurriens* was 290 ± 29.17 (Group-I), 258 ± 16.38 (Group-II) and 219 ± 21.11 (Group-III) respectively (Table 4.103).This was significantly shorter than the outdoor rearing results obtained for winter (457.33 ± 54.31), monsoon (430.33 ± 33.5) and summer (300.00 ± 23.07) respectively.




**Group I**

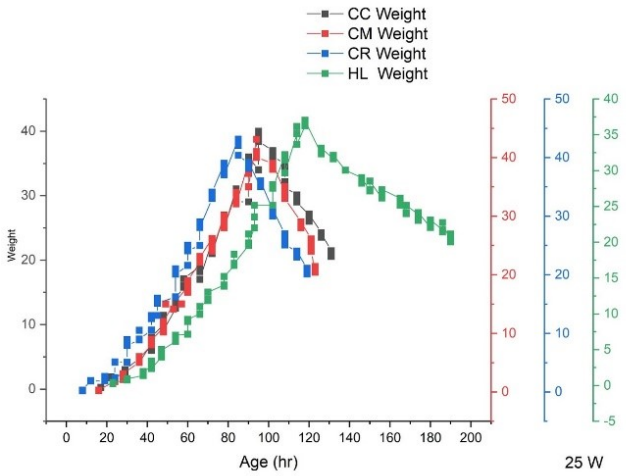



**Group II**

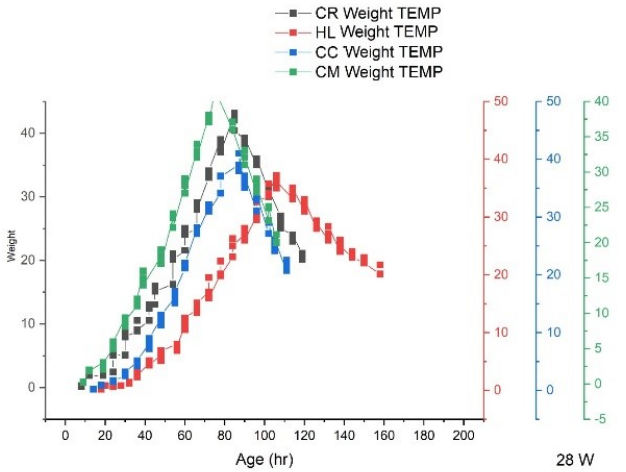



**Group III**

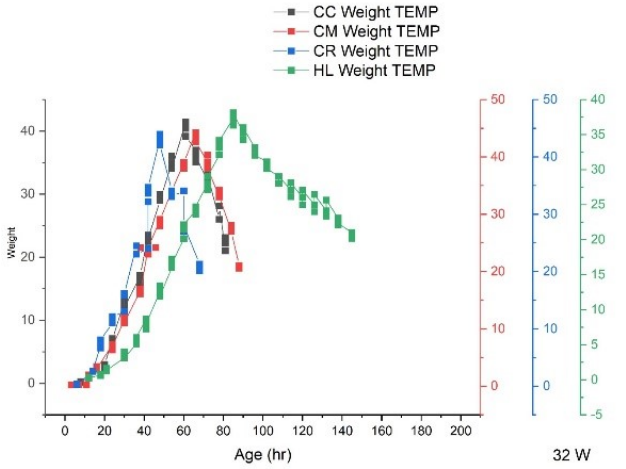
**Fig. 4.74. Developmental rate (Length (mm) Vs. Age (hrs) of *C. chani, C. megacephala*, *C. rufifacies* and *H. ligurriens* from hatching upto pupation under laboratory conditions**




**Group I**




**Group II**




**Group III**

**Fig. 4.75. Developmental rate (Weight (mg) Vs. Age (hrs) of *C. chani, C. megacephala*, *C. rufifacies* and *H. ligurriens* from hatching upto pupation under laboratory conditions**

## 4.5. Relation between life cycle of flies and time since death assessment

### **4.5.1.** Estimation of Post Mortem Interval (PMI)

The fitted equation (best fit model) for predicting duration in hours in terms of length of larvae *C. chani, C. megacephala, C. rufifacies* and *H. ligurriens* are given in table 4.104. This equation was useful for estimating the PMI.

In the case of *C. chani*, coefficient of determination ($R^2$) for the predicted equation is almost equal to 0.94 which indicates that about 94 percent of variability in length can be explained by duration and R2 for the predicted equation in the case of *C. megacephala, C. rufifacies* and *H. ligurriens* is above 0.894, 0.771, and 0.855 respectively. Pattern of variation of the observed and predicted values of the three different seasons for four different species are given in Fig. 4.76 - 4.79.

**Table 4.104. Regression equation model for the estimation of PMI**

| Species | Season | Model Fitted | $R^2$ |
|---|---|---|---|
| *Chrysomya chani* | Winter | $\ln(length) = 2.834 - 42.998/t$ | 0.940 |
| | Monsoon | $\ln(length) = 2.723 - 38.353/t$ | 0.935 |
| | Summer | $\ln(length) = 4.429 - 19.850/t$ | 0.945 |
| *Chrysomya megacephala* | Winter | $\ln(length) = 2.862 - 42.245/t$ | 0.933 |
| | Monsoon | $\ln(length) = 2.633 - 21.249/t$ | 0.911 |
| | Summer | $\ln(length) = 2.621 - 17.648/t$ | 0.894 |
| *Chrysomya rufifacies* | Winter | $\ln(length) = 2.535 - 23.123/t$ | 0.835 |
| | Monsoon | $\ln(length) = 2.576 - 18.595/t$ | 0.868 |
| | Summer | $\ln(length) = 4.162 - 15.321/t$ | 0.771 |
| *Hemipyrellia ligurriens* | Winter | $\ln(length) = 2.370 - 43.547/t$ | 0.855 |
| | Monsoon | $\ln(length) = 2.494 - 43.829/t$ | 0.902 |
| | Summer | $\ln(length) = 2.284 - 28.378/t$ | 0.869 |

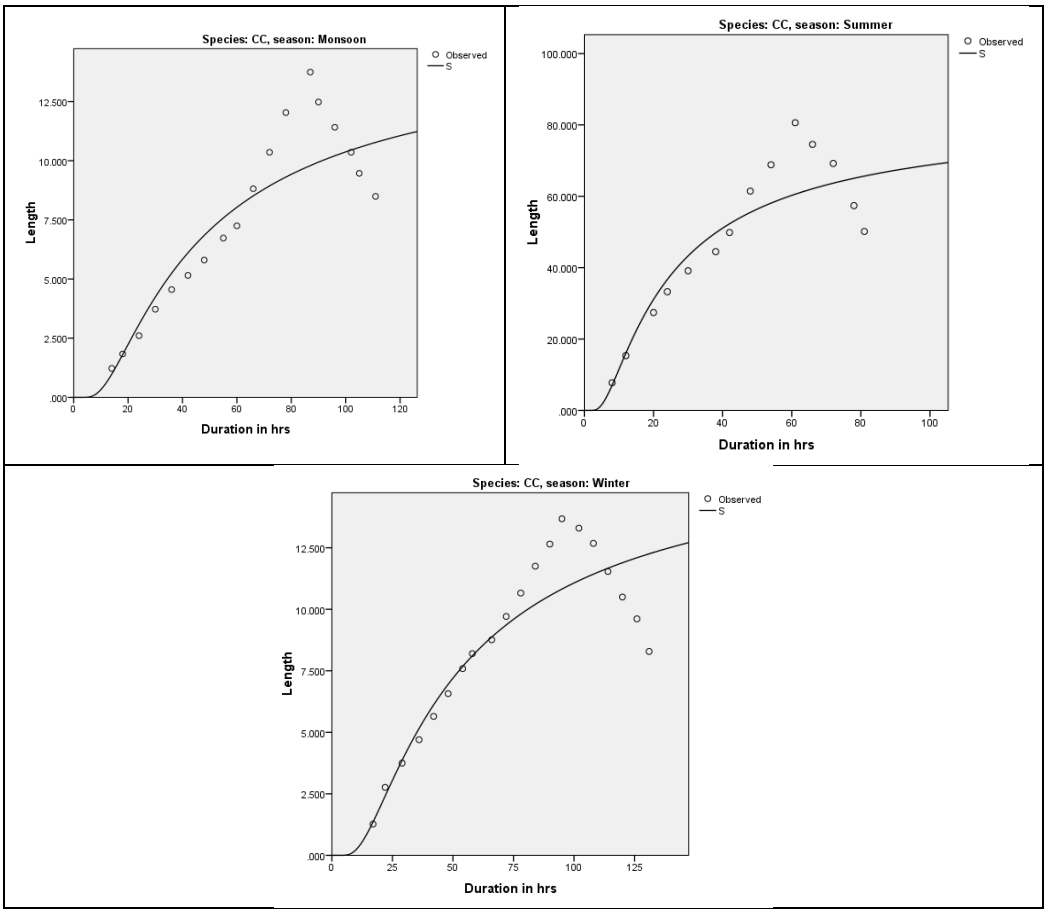



**Fig. 4.76. Growth curves of *C. chani* for the estimation of the PMI**

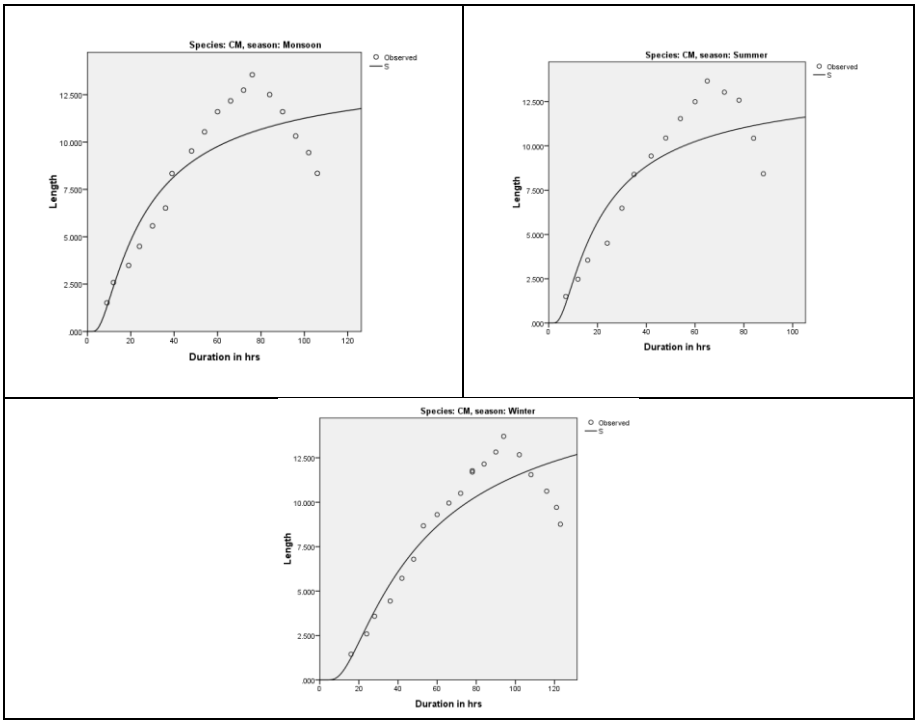



**Fig. 4.77. Growth curves of *C. megacephala* for the estimation of the PMI**

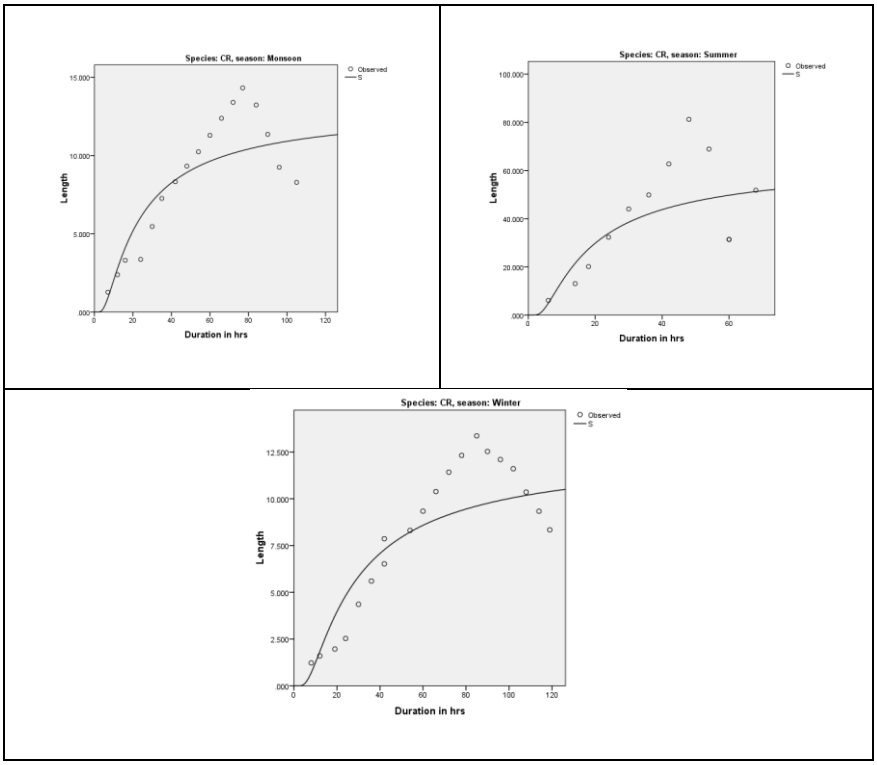



**Fig. 4.78. Growth curves of *C. rufifacies* for the estimation of the PMI**

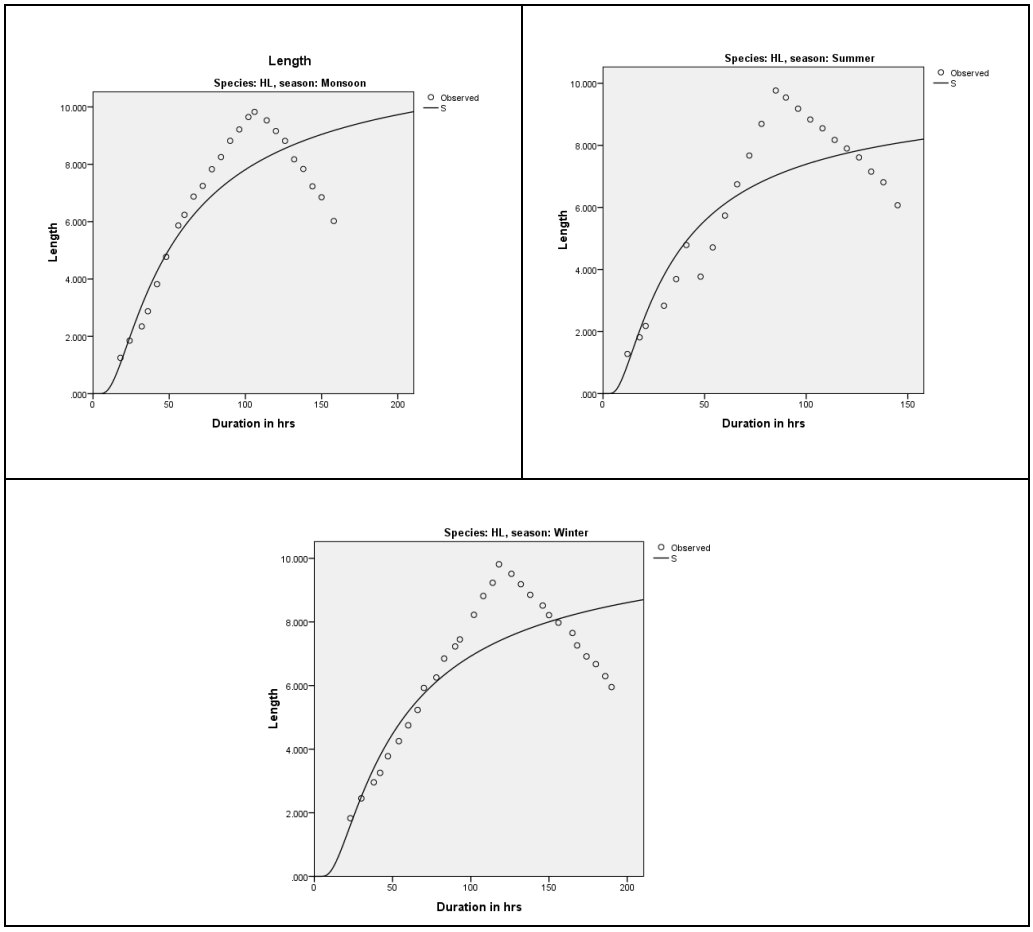



**Fig. 4.79. Growth curves of *H. ligurriens* for the estimation of the PMI**

# CHAPTER – 5

# DISCUSSION

# CHAPTER - 5

# DISCUSSION

Calliphorids are regarded as a prominent entomological evidence for estimating the PMI and ante mortem trauma. Hence, the identification of blow fly specimens are considered significant because of the pertinent evidences they offer for forensic research (Catts and Goff, 1992; Sukontason et al., 2007). The findings of the present study provide significant contribution towards the forensic science research by identifying four blow fly species; *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* based on the morphological and molecular characteristics with special inference on their life cycle.

### 5.1. Blow fly fauna of Central Kerala

Seventeen species belonging to 4 subfamily and 8 genera were recorded during this study. The genera present in Kerala are *Bengalia* (*B. jejuna* Fabricius, 1794, *B. surcoufi* Senior-White,1924), *Hemipyrellia* (*H. ligurriens* Wiedemann, 1830), *Lucilia* (*L. amplullacea* Villeneuve, 1922, *L. papuensis* Macquart, 1843, *L. sericata* Meigen, 1826 ), *Chrysomya* (*C. megacephala* Fabricius, 1794, *C. chani* Kurahashi, 1979, *C. nigripes* Aubertin, 1932, *C. rufifacies* Macquart, 1842, *C. albiceps* Wiedemann, 1819), *Idiella* (*I. euidielloides* Senior-White, 1922, *I. mandarina* Wiedemann, 1830), *Stomorhina* (*S. discolor* Fabricius, 1794), *Cosmina (C. simplex* Walker 1858, *C. bicolor* Walker, 1856) and *Strongyloneura* (*S. prolata* Walker, 1860).

Bharti, (2011) enlisted the various blow files of India. Bharti and Singh, (2017) reported the presence of *C. megacephala* Fabricius (1794), *C. rufifacies* Macquart (1842), *C. nigripes* Aubertin, (1932), and *C. chani* Kurahashi, (1979) from Calicut, Kerala, India. Radhakrishnan et al., (2012) have reported *C. albiceps* from Kerala. In addition to this, the

presence of various blow flies belonging to the central Kerala region were reported by previous workers (Subramanian & Mohan, 1980, Nandi, 2004, and Arce et al., 2020, Reject Paul and Binoy, 2021 & 2022).

### 5.1.1. Identification of blow flies

The examination of various morphological structures including the features of head, thorax, wings, egg, and the various developmental stages revealed the prominent taxonomic characters of blow flies.

*C. megacephala* has orange coloured antennae, arista and palpi. Outer vertical bristles were absent in males. Parafacialia and genae were also completely orange in colour. Hairs and setulae on the parafacialia were yellowish and anterior spiracles were dark brown in colour. Sub costal sclerite covered with brown felted pubescence and also with small erect hairs. A row of setulae were seen on the upper posterior margin on the stem vein. Upper calypter was with ventral hairs on the opaque white basal part. In addition to this, the colour of the thorax and abdomen was found to be bluish green .These observation were validated by previous works (Bharti et al., 2007, Bharti & Kurahashi, 2009, Sukontason et al., 2008, Sukontason et al., 2011, Ramaraj et al., 2014, Bharti, 2014, Bala and Singh 2015, Claver and Yaqub, 2015, Abd Al Galil & Zambare, 2015 , Siddiki & Zambare, 2017).

In the present investigation, adult flies of *C. rufifacies* were identified with the following characteristics. Parafrontalia was narrow with a black colour in the upper half. The lower half was covered with silver tomentum with upstanding white hairs. Frons was greyish in colour. Parafacialia and genae were light yellowish in colour and covered with

white hairs. Anterior spiracle was white in colour. The lower squama was slightly fuscous with white hairs. These obervations has been validated with the earlier reports. (Sukontason et al., 2008, Bharti, 2014, Bala and Singh, 2015, Abd Al Galil & Zambare, 2015, Siddiki & Zambare, 2017).

*C. chani* was having fuscous to black coloured genae and parafacialia .Seulae and hairs on parafacialia and parafrontalia were blackish in colour. 1st and 2nd antennal segments were brown to fuscous in colour. Thorax was bluish green coloured with anterior spiracle fuscous black in colour. Black setae were present on the upper margin of 3rd longitudinal vein. Base of alar squamae is white in colour. These observations were similar to the observation made earlier by Bharti et al., (2014) and Kurahashi (1997).

In the case of *H. ligurriens,* genae and parafrontalia was silver white in colour. Antennae were tawny yellow to brownish in colour. The upper squama was creamish white in colour with short cilia and lower squama has light brown cilia. Short setulae were present on the dorsal and ventral aspects of 3rd longitudinal vein. Previous works by Tumrasvin et al., (1979), Kurahashi and Chowanadisai (2001), Sinha and Nandi (2007) and Bunchu et al., (2012), have validated these observations.

Molecular characterization of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* were done. The molecular analysis of the sequences such as SR1859-COI-F_E03, SR2284-COF_A07, SR2040-A-COF_D05, and SR1719-A-COF_B03 showed strong boot strap support towards the corresponding nucletotide sequences representing *C. megacephala, C. rufifacies, C.chani,* and *H. ligurriens* respectively. Many of the previous studies have substantiated the applicability of DNA-based strategies, especially the use of mitochondrial COI gene to identify blow flies (Sukontason et al., 2008, Mendonça et al.,

2010, Bharti & Singh, 2017, Qiu et al., 2017, Yusseff and Agnarsson., 2017, Abd Al Galil & Zambare, 2017).

**5.2. Seasonal differences in blow fly population in Central Kerala**

The present investigation has analyzed the seasonal variation in the abundance of blow flies with special inference to summer, monsoon and winter seasons as such data is totally lacking in Southern India.

Effect of season on the abundance was found to be significant for *C. megacephala* (F = 52.773; P = < 0.001) (Table 4.1), *C. rufifacies* (F = 3.935; 0.024; P = 0.022) (Table 4.4), *C chani* (F = 33.586; P =< 0.001) (Table 4.7) and *H. ligurriens* (F = 47.470; P = < 0.001) (Table 4.10). Wall et al., (2001) analyzed the seasonal abundance of C. *megacephala* in Kerala and their results showed peak population of the species during monsoon which was consistent with the present study.

Seasonal influence on the abundance of blow flies were studied in earlier works and their results showed pronounced seasonal fluctuation in the population of blow flies in response to climatic conditions (Evaldo et al., 2008, Brundage et al., 2011, Zabala et al., 2014, Sontigun et al., 2018, Jeong et al., 2022).

**5.3. Life history of blow flies in carrion**

Developmental stages of *C. megacephala* was studied in the current investigation in which the eggs were oblong, creamish white in colour and the caudal end was slightly wider than the anterior end. The cephalopharyngeal skeleton was present with prominent comma shaped dorsal sclerite. Dorsal cornua has uniform width and longer than the ventral cornua. Posterior spiracles were clearly seen with three spiracular slits. A dark pigmented

incomplete peritreme was seen surrounding the three slits with a bent in the middle slit. The spinous bands were found to be restricted to the lateral and ventral surfaces. These observations were consistent with the earlier reported works (Ishijima, 1967, Omar., 2002, Sukontason et al., 2007, Sukontason et al., 2008, Ramaraj et al., 2014 ).

Present studies on *C. rufifacies* revealed that the larval body was hairy with long tubercles with broad base and tapered with pointed spines at the tip. Spines were present on the anterior and posterior margins on the ventral and lateral surfaces of all the three thoracic segments. Cephalopharyngeal skeleton was with a shorter dorsal cornua. Parastomal and accessory sclerites were absent. Posterior spiracles were clearly seen with three spiracular slits with densly dark pigmented incomplete peritreme surrounding the three slits with a medial bent in the middle slit. The results obtained were in consonance with the previously reported works (Sukontason et al., 2006, Yanmanee et al., 2016, Abd Al Galil & Zambare, 2017).

Studies on the developmental stages of *C. chani* revealed eggs as creamish white in colour. Many tubercles were present on all the abdominal segments except the caudal segment of larval body. Cephalopharngeal skeleton has heavily sclerotized accessory sclerite, upwardly curved thin parastomal bar and intermediate sclerite. Posterior spiracles were having dark pigmented thick sclerotized complete peritremes. The button was indistinct. The features described were consistent with the earlier reports (Sukontason et al., (2018).

Developmental stages of *H. ligurriens* were studied in detail which showed moderately sized middle dorsal tubercles on the larval body in comparison to the inner and outer tubercles. Pigmentation of cephalopharyngeal skeleton was darker. Well-developed

comma shaped dorsal sclerite was present. Ventral cornua was seen equal in length to dorsal cornua. Posterior spiracles were dark brown with complete peritreme and button. The characteristics observed in the present study were consistent with the previous reports (Sinha and Nandi, 2007, Bunchu et al., 2012, Eliza and Zuha, 2018).

The present investigation has used SEM for the morphology-based identification of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens*. SEM examination of larval instars in the present study revealed characterisitc ultra structural details ; three lobed labium, semicircle shaped ventral organ and post spiracular discs with spiracular slits surrounded by fine spiracular hairs in *C. megacephala*, elongated tubercles with many sharp tipped fine spines on larval body and broad posterior spiracular hairs in *C. rufifacies*, branched mouth hooks with two to three rows of curved sharp tipped spines, bilaterally arranged oral cirri, filiform spines on the anal segment and fine tipped thoracic spines with flat triangular base in *C. chani* and conspicuous oral cirri in the shape of curved spines, trilobed labium with well-developed fleshy lateral lobes, three rows of spine clusters present dorso-medial to the functional mouth opening, slender tipped acuminate spines with bulbous base on the anterior and posterior margins of the ventral and lateral surfaces of thoracic segments and filiform spines of last anal segment in *H. ligurriens.*

The results were consistent with the earlier reported works (Sukontason et al., 2008, Klongklaew et al., 2012; Szpila et al., 2013, Sanit et al., 2012). Characteristic bilateral arrangement of medially curved spinulous oral cirri was not reported in *H. ligurriens* in earlier studies (Sukontason et al., 2008). Spines on the thoracic and last anal segment of *H. ligurriens* were different from the observations made by Sukontason et al., (2008), where

thoracic spines were acuminate with flat broad bases and anal spines were verrucate and echinate.

The average mating time (hrs), pre oviposition period (days), periodicity of egg laying (days), number of eggs laid in a day and during the life span in *C. megacephala, C. rufifacies*, *C.chani* and *H. ligurriens* were studied. It was 10 ± 04, 9.59 ± 0.89, 3.67 ± 0.37, 215.74 ± 22.29 and 1451.26 ± 83.71 in *C. megacephala,* 9 ± 3*,* 8.15 ± 0.99, 4.44 ± 0.38, 247.74 ± 28.43 and 1842.26 ± 97.99 in *C. rufifacies,* 10 ± 4, 8.74 ± 1.26 ,4.59 ± 0.31, 240.15 ± 19.6 and 1667.52 ± 49.78 in *C. chani* and 10 ± 04 , 9.59 ± 0.89, 3.67 ± 0.37, 215.74 ± 22.29 and 1451.26 ± 83.71 in *H. ligurriens* respectively. The results obtained were consistent with the earlier reported works ( Rosatti et al., 2015, Lertthamnongtham et al., 2003, Yang & Shiao, 2012, Subramanian & Mohan, 1980).

Life cycle related parameters of larval instars like length, weight, life duration and pupation were studied for all the four species of blow flies. It was observed that the average length (mm) and weight (mg) of first, second, third and post feeding stage of instars for *C. megacephala* (Tables. 4.17 - 4.20; 4.22 - 4.25), *C. rufifacies* (Tables. 4.37 – 4.40; Tables. 4.42 – 4.45), *C. chani* (Tables. 4.57 – 4.60; 4.62 – 4.65) and *H. ligurriens* (Tables. 4.77 – 4.80; 4.82 - 4.85) were in consonance with the earlier reports (Gabre et al., 2005, Bharti et al., 2007, Sinha and Nandi., 2007, Sukontason et al., 2008, Bala and Singh, 2015, Bansode et al., 2016, Chakraborty et al., 2016, Siddiki & Zambare, 2017, Badenhorst and Villet, 2018, Zhang et al., 2019).

The life cycle duration (hrs) of all developmental stages such as egg, first, second and third instars, post feeding stage and pupa and the total life cycle from egg till the emergence of adult fly were studied. These were 18, 17, 22, 40, 30, 99 and 227 ± 59 for *C.*

*megacephala,* 16, 19, 23, 37, 27, 91 and 212.78 ± 8.98 for *C. rufifacies,* 21, 18, 23, 36, 36, 119 and 252.89 ± 17.16 for *C. chani* and 27, 17, 25, 59, 115, 153 and 395.88 ± 35.82 for *H. ligurriens.* The results were showing similarities with earlier works (Subramanian & Mohan, 1980, Bharti et al., 2007, Bala and Singh, 2015, Siddiki & Zambare, 2017). However, higher life cycle duration in all the stages were also reported in some previous works (Sukontason et al., 2008, Bunchu et al., 2012, Zhang et al., 2019).

The survival rate (%) of all the developmental stages for all the four species were studied. It was found that the survival rate in egg, first, second, third instars and pupae were 86.32 ± 6.50, 84.22 ± 7.27, 75.98 ± 8.03, 69.26 ± 4.82 and 69.4 ± 5.38 for *C. megacephala,* 82.47 ± 5.45, 81.90 ± 6.16, 76.03 ± 4.66, 72.27 ± 5.92 and 72.33 ± 6.14 for *C. rufifacies,* 75.84 ± 5.53, 76.04 ± 5.25, 76.45 ± 4.50, 68.49 ± 5.19 and 66.69 ± 3.81 for *C. chani* and 72.45 ±5.94, 72.57 ± 5.68, 70.78 ± 5.81, 69.78 ± 6.69, 69.77 ± 6.95 for *H. ligurriens.* The results of the study were in consonance with the earlier reports by Pitts et al., (2005), Mądra et al., (2017) and Zhang et al., (2019).

**5.4. Effect of temperature and humidity on the life cycle of Calliphorid flies**

Pre-oviposition period, eggs laid in a day, periodicity of egg laying (days) and the total number of eggs laid by four species of blow flies in its life span have been investigated.

Effect of season on the pre-oviposition period was found to be significant for *C .megacephala* (F = 19.73; P = < 0.001) (Table. 4.13), *C. rufifacies* (F = 23.444; P = < 0.001) (Table. 4.33), *C. chani* (F = 40.111, P = < 0.001) (Table. 4.53) and *H. ligurriens* (F = 13.727; P = < 0.001) (Table. 4.73). The pre-oviposition period was significantly higher in

winter in all the four species. Effect of year was found to be significant for *C. megacephala* (F = 11.545; P = < 0.001) (Table. 4.13). The interaction between years and seasons in *C. chani* were found to be significant (F = 4.778; P = 0.008) (Table. 4.53).

Effect of season on the number of eggs laid per day was found to be significant for *C. megacephala* (F = 74.306; P = < 0.001) (Table. 4.14), *C. rufifacies* (F = 223.63; P = < 0.001) (Table. 4.34), *C.chani* (F = 133.56; P = < 0.001) (Table. 4.54) and *H. ligurriens* (F = 417.585; P = < 0.001) (Table. 4.74). The number of eggs laid per day by all species were significantly higher in monsoon. Effect of year on egg laying was found to be significant for *C. rufifacies* (F = 3.67; P = 0.046) (Table. 4.34). The interaction between years and seasons were found to be significant for *H. ligurriens* (F = 3.562; P = 0.026) (Table. 4.74).

Effect of season on the periodicity of egg laying was found to be significant in *C. megacephala* (F = 6.300; P = < 0.008) (Table. 4.15), *C. rufifacies* (F = 5.727; P = 0.012) (Table. 4.35), *C. chani* (F = 901.52; P = < 0.001) (Table. 4.55) and *H. ligurriens* (F = 576.86; P = < 0.001) (Table. 4.75).

The periodicity of egg laying was significantly higher during winter in all species. Effect of year on the number of eggs laid was found to be significant in *C. megacephala* (F = 29.859; P = < 0.001) (Table. 4.15), *C. chani* (F=12.54; P =< 0.001) (Table. 4.55) and *H. ligurriens* (F=5.199; P =0.017) (Table. 4.75). The interaction between years and seasons were found to be non-significant in *C. rufifacies* (F = 3.28; P = 0.035) (Table. 4.35) and *C. chani* (F =3.65; P =0.024) (Table. 4.55).

Effect of season on the total number of eggs laid during its life span was found to be significant in *C. megacephala* (F = 323.32; P = < 0.001) (Table. 4.16), *C. rufifacies* (F =

835.8; P = < 0.001) (Table. 4.36), *C. chani* (F = 901.52; P = < 0.001) (Table. 4.56), and *H. ligurriens* (F = 576.86; P = < 0.001) (Table. 4.76). The number of eggs laid in all species were significantly higher in monsoon. Significant yearly fluctuations in egg laying were also noted in *C. chani* (F = 12.54; P = < 0.001) (Table. 4.56) and *H. ligurriens* ((F = 5.199; P = 0.017) (Table. 4.76). The interaction between years and seasons were found to be non-significant in *C. rufifacies* (F = 3.28; P = 0.035) (Table. 4.36) and *C .chani* (F = 3.65; P = 0.024) (Table. 4.56).

The time dependent data corresponding to the length and weight of developmental stages of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* during different seasons were investigated in this study. The seasonal data on the length and weight of larval instars were showing statistically significant differences due to significant interactions between years, seasons and larval stages in all the four species.

Interaction studies on year, season and stage was found to be significant for length in *C. megacephala* (F = 115.12; P = <0.001) (Table. 4.21), *C. rufifacies* (F = 0.95; P = < 0.001) (Table. 4.41) and *C. chani* (F = 0.335; P = <0.001) (Table. 4.61). However only the interaction between season and stage was significant for *H. ligurriens* (F = 9.15, P < 0.001) (Table. 4.81).

This indicated that seasonal variations in length of each stage were different in different years. The length of $I^{st}$, $II^{nd}$ and $III^{rd}$ instar were significantly higher in monsoon in *C. megacephala and C. rufifacies,* higher in summer for *C. chani* and in winter for *H. ligurriens.* The earlier studies (Singh et al., 1999, Bala and Singh, 2015) justified the strong relation between the temperature and humidity on the length and weight of larval instars.

Interaction studies on year, season and stage was found to be significant for the weight of *C. megacephala* (F = 14.365; P <0.001) (Table. 4.26), *C. rufifacies* (F = 10.095; P = < 0.001) (Table. 4.46), *C. chani* (F = 7.656; P = < 0.001) (Table. 4.66) and *H. ligurriens* (F = 61.121; P < 0.001) (Table. 4.86) indicating that seasonal variations in weight of each stage are different in different years.

The weight was significantly higher in monsoon for II$^{nd}$ and III$^{rd}$ instar and post feeding stage in *C. megacephala,* II$^{nd}$ and post feeding stage in *C. rufifacies.* The weight was significantly higher in summer for II$^{nd}$ and III$^{rd}$ instar and post feeding stage of *C. chani*, II$^{nd}$ instar and post feeding stage in *H. ligurriens.*

Recent studies also confirmed the effect of various environmental parameters including temperature and humidity on the life cycle of Calliphorid flies (Salimi et al., 2018, Reject Paul and Binoy, 2021& 2022). In a study conducted in Thailand, the length of the *C. megacephala* and *C. rufifacies* increased during the summer season which may be due to the changes in the biogeoclimatic conditions (Sukontason et al., 2008).

Length and weight of the larval instars were found to be increasing in all the four species in the current study. However, the length and weight got reduced during the post feeding stage in all species. These results were consistent with the previous reports from India and from other countries (Acosta et al., 2021, Yang et al., 2016, Bala and Singh, 2015, Siddiki & Zambare, 2017).

Rearing data on duration (hrs) of life stages related to *C. megacephala* revealed that there was significant interaction between seasons and incubation (F = 408.40; P < 0.001), second instar stage (F = 8.555; P < 0.036), post feeding stage (F = 8.704; P < 0.035) and

pupation stage (F = 480.82; P < 0.001) (Table. 4.27). These results were significantly different from the observations made by Bala and Singh (2015) as it took 10, 40, 60 and 5 at 32°C, 10, 40, 60 and 5 at 29°C and 25, 55, 85, and 5 at 25°C.

Present study revealed that total duration taken by the fly from egg stage till the emergence of adult fly was 168.00 ± 5.29 in summer, 227.00 ± 22.52 in monsoon and 286.00 ± 23.26 in winter which were significantly different from 211 in summer, 239 in monsoon and 263 in winter in Maharashtra (Siddiki & Zambare, 2017). The duration of 241.33±1.15 in monsoon (Nordin et.al., 2020) and 224 at 25.6°C (Subramanian and Mohan, 1980) were similar to the current observation.

Rearing data on duration (hrs) of life stages related to *C. rufifacies* revealed that there was significant interaction between seasons and the second instar stage (F = 12.250; P < 0.020), third instar stage (F = 491.46; P < 0.001), post feeding stage (F = 45.953; P < 0.002) and pupation stage (F = 115.71; P < 0.001) (Table. 4.47). These results were significantly different from the observations made by Bala and Singh (2015) as it took 5, 25, 45 and 5 at 32°C, 10, 55, 35 and 5 at 29°C and 15, 40, 75 and 5 at 25°C.

Present study revealed that total duration taken by the fly from the egg stage till the emergence of adult fly was 148.33 ± 6.43 in summer, 223.00 ± 13.45 in monsoon and 286.00 ± 23.26 in winter which were significantly different from the duration of 216 in summer, 239 in monsoon in Maharashtra (Siddiki & Zambare, 2017). But the duration of 286 in winter reported by the same authors were similar to the present results. Life cycle duration during monsoon was significantly lesser than that reported by Nordin et al., (2020) in Malaysia which was 266. Duration of 193 reported by Subramanian and Mohan (1980) at 25.6°C was significantly shorter than the present result.

The seasonal life cycle data on *C. chani* were found to be extremely scanty and the data obtained in this investigation could be recognized as the pioneering study. The data on duration (hrs) of life stages on rearing of *C. chani* revealed that there was significant interaction between seasons and incubation period ($F = 42.08$; $P < 0.002$) and post feeding stage ($F = 15.69$; $P= 0.013$) (Table. 4.67). Total duration taken by the fly from the egg stage till the emergence of adult fly was 192.33 ± 8.15 in summer, 246 ± 20.08 in monsoon and 320.33 ± 24.03 in winter.

The data on duration (hrs) of life stages on rearing of *H. ligurriens* revealed that there were significant interactions between seasons and incubation period ($F = 84.88$; $P<0.001$), second instar stage ($F = 8.269$; $P < 0.038$) and pupation stage ($F = 128.58$; $P < 0.001$) (Table. 4.87). The durations observed during winter season was found to be significantly different from the earlier observations made by Sinha and Nandi, (2007) in which the egg, first instar, second instar, third instar and pupal stages took 15, 6, 14, 104, and 186 respectively for completing the developmental process.

The total duration taken by the fly from the egg stage till the emergence of adult fly was 300.00 ± 23.07 in summer, 430.33 ± 33.5 in monsoon and 457.33 ± 54.31 in winter. The total life cycle duration observed in the summer was found to be significantly higher when compared to the duration of 288.4 (Yang et.al, 2015) and the duration in monsoon was significantly lesser, when compared with 532 reported by Nordin et al, (2020). The total duration observed in the winter season was significantly higher than 325 (Sinha and Nandi, 2007) and 321.9 (Yang et al., 2015).

Laboratory rearing under controlled conditions has been done to validate the outdoor results. It was observed that there existed a significant difference in the total life

cycle duration (hrs) observed in outdoor rearing and laboratory rearing in *C. megacephala* (Table. 4.95), *C. rufifacies* (Table. 4.98), *C. chani* (Table 4.100) and *H. ligurriens* (Table. 4.103). Similar observations were also reported by earlier workers (Acosta et al., 2022, Chen et al., 2019).

The variations observed in the life cycle duration in the current study and previous works may be explained by the changing weather patterns and environmental conditions and also due to the nature of the decomposing tissue used for rearing. Other reasons could be the intrinsic and extrinsic factors which may be explored in future research.

The survival rate of different developmental stages during the summer, monsoon and winter seasons was analysed and found that there exists a significant interaction between various seasons and years in *C. megacephala* (Table. 4.31), *C. rufifacies* (Table. 4.51), *C. chani* (Table. 4.71) and *H. ligurriens* (Table. 4.91). The survival rate of all species was significantly higher in monsoon in comparison to other seasons.

The results of the study were in consonance with the earlier reports by Pitts et al., (2005) who investigated the survival rate of various developmental stages of blow flies and found that seasons imposed significant contribution towards their survival. The present results were also consistent with the previous works by Mądra et al., (2017) and Zhang et al., (2019).

The major outcome of this study is that the results of outdoor rearing cannot be simulated in the laboratory (Acosta et al., 2021, 2022). Therefore a methodology was conceptualized to conduct outdoor rearing in different seasons in the study area. The results of the present study differed from the earlier works where varying seasonal climatic

variables were simulated in the laboratory using different temperature and humidity (Faris et al., 2020, Niederegger et al., 2010). Badenhorst et al., (2018) suggested to compare the laboratory rearing data with the outdoor results as it can provide sufficient information for the practical implementation of observed data for forensic science research.

Speight et al., (2008) indicated that microclimatic conditions may influence the biology of insects. The present investigation has analyzed the above perspectives for C. *megacephala, C. rufifacies, C. chani* and *H. ligurriens* and found that the data obtained in this investigation could be used for forensic research purposes in future as a reference data for Kerala, South India.

**5.5. Estimation of PMI**

The insect specimens, specifically the egg, larvae and pupae found in cadavers at death scenes have been previously used as strong evidence to determine the PMI (Catts and Goff, 1992). Accurate estimation of PMI demands the assessment to the level of minimum one hour duration for any stage of insect development.

In the current investigation, regression equation method using curve estimation was used for all larval instars. The results showed that in the case of *C. chani*, coefficient of determination ($R^2$) for the predicted equation is almost equal to 0.94 which indicates that about 94 percent of variability in length can be explained by duration and $R^2$ for the predicted equation in the case of *C. megacephala, H. ligurriens* and *C. rufifacies* were above 0.894, 0.855 and 0.771 respectively.

Another advantage of this method is that instars of any length could be applied in the equation without the requirement of the length of the largest instar. The regression

equation was found to give accurate estimate of developmental duration to the level of specific hour corresponding to the length of any particular instar.

The regression equation method developed in the current study is found to be really important as it emerged as the best suitable method for the estimation of PMI using the life history of the blow flies. This is in agreement with the earlier observations of Yang et al., (2014) on the PMI estimation using native species of blow flies in that particular geographical region.

The limitation of the use of isomegalen and isomorphen diagram method and thermal summation method for the estimation of PMI from the length of post feeding larval instars were proved by Yang et al., (2015). The study also suggested the necessity of applying the length of the largest instar in these models for determining the PMI. The simple additive methods proposed by Siddiki and Zambare (2017) could estimate the postmortem interval only up to stage specific development time of blow flies.

Similarly, the estimation of accumulated degree hours (ADH) of *C. megacephala* and *C. rufifacies* at different constant temperatures could reveal only the stage specific duration (Bala and Singh, 2015) which is inadequate for the accurate estimation of PMI in forensic investigation. ADH and ADD (Accumulated degree days) methods for PMI estimation have been criticized for not having any consideration for the post feeding stage (Arnott and Turner, 2008).

In recent studies, forensic entomologists use the data of developmental stages of insects to get precise information concerning the minimum PMI for forensic needs. For this purpose, it is essential to find a reference database of the respective species. Acosta et al.,

(2021, 2022) constructed growth models followed by isomegalen diagrams for the specific variables of the body weight and length of the larvae belonging to *Lucilia* genus.

Tachibana et al, (2006) revealed that the blowflies belonging to the *Chrysomya* genus usually have a preference for certain specific regions as well as seasons. For instance in Japan, blowflies belonging to the *Chrysomya* genus were found in mountainous zones during summer and lowlands during autumn.

This indicates that the variations in temperature have strong influence on the life cycle of blow flies and region specific studies are needed to reveal such inferences for different geographical areas and such findings have significant value for estimating the PMI for forensic research purpose (Muskan et al., 2022). In this study, such data was collected for C. *megacephala, C. rufifacies, C. chani* and *H. ligurriens* in Kerala region, India.

The differences in the developmental data of the blow fly species when compared to the previous works might be due to the changes in humidity, rainfall and temperature prevailing in these geographically different areas. The differences in developmental rate under constant temperatures are probably due to genetic variations (Tourle et al., 2009).

The changes in the developmental rate of species during different seasons cautions that while performing the assessment of PMI, the investigators should be very careful about the climatic conditions prevailing in the respective study area (Gallagher et al., 2010). This signifies the importance of generating location specific seasonal data of forensically important species for accurate assessment of postmortem interval.

In this regard, the data generated on the life cycle of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* and the regression equation model constructed for the estimation of PMI has been found to be useful for the forensic application in medicolegal investigations and for future forensic research in the study region. As this is the first report on the developmental rate of the above mentioned blow fly species, these findings can be used as forensic reference data for Kerala in future.

# CHAPTER – 6

# SUMMARY AND CONCLUSION

# CHAPTER - 6

## SUMMARY AND CONCLUSION

The flies belonging to the Diptera, Calliphoridae are the first visitors to inhabit and colonize the dead body within a short period of time of cadavers found. The importance of generating location specific data of forensically important blow fly species for accurate assessment of PMI was evident from the previous works. The present investigation has recorded 17 blow fly species belonging to 4 subfamily and 8 genera from central Kerala. Of these, four forensically significant blow fly species; *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* were prominently found to get attracted to carrion in summer, monsoon and winter seasons consistently.

Morphological identification of forensically significant blow flies; *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* were done based on the taxonomic characteristics of antennae, arista and palpi, bristles on the head, colour of parafrontalia, parafacialia, genae, colour and type of hairs and setulae on the parafacialia, colour of anterior spiracles, characteristics of sub costal sclerite, presence or absence of setulae on the stem vein, colour of suqama and the presence or absence of hairs, colour of the thorax and abdomen. Molecular diagnosis were done by amplifying the partial coding sequence of mitochondrial COI gene. The molecular analysis of the sequences showed strong boot strap support towards the corresponding nucletotide sequences representing *C. megacephala, C. rufifacies, C. chani,* and *H. ligurriens*.

Seasonal variation in the abundance of blow flies with special inference to summer, monsoon and winter seasons were done as such data is totally lacking in Southern India. The abundance of all the four species of blow flies were found to be significantly higher in monsoon. Developmental stages of *C. megacephala, C. rufifacies, C. chani, and H. ligurriens* were identified by studying the morphological features of eggs, the cephalopharyngeal skeleton, spinous bands on the lateral and ventral surfaces of larval body and posterior spiracles. SEM analysis was done for the morphology based identification of different larval instar stages of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens*. The characterisitc ultra structural details studied were; dorsal organ, terminal organ, ventral organ, labium, mouth hooks, oral cirri, number of spine clusters present dorso-medial to the functional mouth opening, post spiracular discs, tubercles on larval body and types of spines on the anal and thoracic segment.

The average mating time (hrs), pre oviposition period (days), periodicity of egg laying (days), number of eggs laid in a day and during the total life span in *C. megacephala, C. rufifacies*, *C. chani* and *H. ligurriens* were studied. Life cycle related parameters of larval instars like length, weight and duration of life of different larval instars and pupation were studied for all the four species of blow flies. The survival rate (%) in egg, first, second, third instars and pupae were studied and it was found that higher survival rate was seen in the egg and instar I. Effect of temperature and humidity on the pre-oviposition period, eggs laid in a day, periodicity of egg laying (days) and the number of eggs laid by four species of blow flies in its life span have been investigated in this study. The pre-oviposition period was significantly higher in winter in all the four species. The number of eggs laid in a day and total number of eggs laid during the life span by all species were significantly higher in monsoon. The periodicity of egg laying was

significantly higher in winter in all species. The time dependent data corresponding to the length and weight of developmental stages of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* during different seasons were investigated. The length of $I^{st}$, $II^{nd}$ and $III^{rd}$ instar was significantly higher in monsoon in *C. megacephala* and *C. rufifacies,* higher in summer for *C. chani* and in winter for *H. ligurriens.* The weight was significantly higher in monsoon for $II^{nd}$ instar, $III^{rd}$ instar and post feeding stage in *C. megacephala,* $II^{nd}$ instar and post feeding stage in *C. rufifacies.* The weight was significantly higher in summer for $II^{nd}$ instar, $III^{rd}$ instar and post feeding stage of *C. chani*, II instar and post feeding stage in *H. ligurriens.*

The total duration taken by the fly for its development from the egg stage till the emergence of adult fly was shorter in summer, followed by monsoon and winter. When the results of the laboratory rearing of the four species; *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens,* were compared with the outdoor rearing results, it was observed that the developmental duration of different stages in the outdoor rearing were higher than the laboratory results. The variations observed among the life cycle duration in the current study and also other reported works may be well explained by the changing weather patterns and environmental conditions like temperature and humidity in the outdoor rearing sites and the difference in the nature of the decomposing tissue used for rearing. The survival rate of all species were found to be significantly higher in monsoon in comparison to other seasons. The major outcome of this study is that the results of outdoor rearing cannot be simulated in the laboratory. During unexpected variations in climatic variables, there is always a possibility for the formation of microclimatic conditions which directly influence the biology of insects. The present investigation has analyzed the above perspectives by rearing *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* in

different seasons for three consecutive years. The data obtained in this investigation could be used for forensic research purposes in future as a reference data for Kerala, South India.

A high value of coefficient of determination ($R^2$) was obtained for all the four blow fly species for the predicted regression equation which indicated that higher percent of variability in length can be explained by duration. The major advantage of this method is that instars of any length could be applied in the equation without the requirement of the length of the largest instar. This equation was found to give accurate estimate of developmental duration to the level of specific hour corresponding to the length of any particular instar. The regression equation method developed in this study emerged as the best suitable method for the estimation of PMI using life history of the blow flies. The changes observed in the rate of developmental data of the blow flies when compared to the previous works might be due to the changes in humidity, rainfall and temperature prevailing in these geographically different areas and genetic variations of blow flies. This cautions that while performing the assessment of PMI, the investigators should be very careful about the climatic conditions prevailing in the respective study area and signifies the importance of generating location specific data of forensically important species of blow flies. This is the first report on the developmental rate of these species during different seasons from South India and can be used for the PMI assessment of dead bodies under forensic investigations.

Present investigation on the life cycle of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* and the regression equation model constructed for the estimation of PMI has been found to be useful for forensic application in medicolegal investigations in the study region. Further research can be conducted to augment the present study results and for exploring new dimensions in future.

# CHAPTER – 7

# RECOMMENDATIONS

# CHAPTER – 7

## RECOMMENDATIONS

- The findings of the present study provide a significant contribution towards the forensic science research in Central Kerala since the first objective of this study identified the four forensically significant blow flies; *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens* based on the morphological and genetic characteristic features with special inference on their life cycle.
- The ultra structural studies of larval instars using SEM for the morphology based identification of blow flies provide great scope for forensic entomological research.
- The present investigation has provided the applicability of molecular characterization using COI gene for the accurate identification of forensically significant blow flies.
- In this regard, similar kinds of studies on blow flies in the region are encouraged for authenticating the species specific data generation especially for forensic application.
- In forensic investigations, the knowledge of the rate of development of the blow fly in the specific geographical location is very crucial in the accurate estimation of PMI. The present investigation has analyzed the effect of temperature and humidity on the life cycle of blow flies and found a strong influence of these parameters on their life cycle.
- The statistically significant differences observed in the developmental rate of all blow fly species reared in the outdoor during different seasons cautions that while

performing the estimation of PMI, the forensic investigators should be very careful about the climatic conditions prevailing in the respective study area.

- As the outdoor rearing cannot be simulated in the laboratory, data generated in the laboratory cannot be relied for the field application in medico legal cases for the estimation of PMI.
- The data regarding the development rate and life cycle of forensically significant blow flies in monsoon, summer and winter seasons is the first report from the Southern Indian region, especially from Kerala, with special inference on estimation of PMI which may be used for future forensic investigations.
- The regression equation method developed in this study emerged as the best suitable method for the estimation of PMI using life history of the blow flies.
- More and more similar works are encouraged to develop species specific data of forensically significant species from maximum geographical regions.

# REFERENCES

# REFERENCES


Abd Al Galil, F. M., & Zambare, S. (2015). Effect of temperature on the development of calliphorid fly of forensic importance, *Chrysomya rufifacies* (Macquart, 1842). *Int J, 3*, 1099-1103.

Abd Al Galil, F. M., & Zambare, S. (2017). Molecular identification of forensically importantblowflies (Diptera: Calliphoridae) with a record of a new species from Maharashtra India. *Journal of Entomology and Zoology Studies, 5*(1), 13-19.

Abdullah, S. R., Shafie, M. S., & Wahid, S. A. (2022). Forensically important fly larvae on floating corpses in Malaysia: three case reports. *Egyptian Journal of Forensic Sciences, 12*(1), 1-5.

Abou Zied, E. M., Gabre, R. M., & Chi, H. (2003). Life table of the Australian sheep blow fly *Lucilia cuprina* (Wiedemann)(Diptera: Calliphoridae). *Egypt J Zool, 41*, 29-45.

Acosta, X., Centeno, N.D., González-Reyes, A.X., Corronca, J.A., (2021). Contributions to the estimation of the postmortem interval through the length and body weight of two indigenous species of South America: *Lucilia ochricornis* (Diptera: Calliphoridae) and *Lucilia purpurascens*. *Journal of Medical Entomology*, *58*, 548-557.

Acosta, X., Corronca, J. A., González-Reyes, A. X., & Centeno, N. D. (2022). Postmortem Interval Estimation and Validation Through a Comparative Study of South American Flies Reared in the Field Versus Laboratory Conditions. *Journal of Medical Entomology*, *59*(1), 147-161.

Acosta, X., González-Reyes, A. X., Corronca, J. A., & Centeno, N. D. (2021). Estimation of the postmortem interval through the use of development time of two South American Species of forensic importance of the genus Lucilia (Diptera: Calliphoridae). *Journal of Medical Entomology*, *58*(3), 1064-1073.

Adams Z.J.O and Hall M.J.R. (2003). Methods used for the killing and preservation of blowfly larvae and their effect on post-mortem interval length. *Forensic Science International 138*, 50–61.

Aggarwal, A. D. (2005). Estimating the postmortem interval with the help of entomological evidence. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology, 6*(2).

Ahmad, F., Marwi, M., Syamsa, R., Zuha, R., Ikhwan, Z., & Omar, B. (2010). Morphological descriptions of second and third instar larvae of *Hypopygiopsis violacea* Macquart (Diptera: Calliphoridae), a forensically important fly in Malaysia. *Tropical Biomedicine, 27*(1), 134-137.

Ahmad, N. W., Lim, L. H., Dhang, C. C., Chin, H. C., Abdullah, A., Mustaffa, W. N. W., . . . Azirun, S. M. (2011). Comparative insect fauna succession on indoor and outdoor monkey carrions in a semi-forested area in Malaysia. *Asian Pacific Journal of Tropical Biomedicine, 1*(2), S232-S238.

Akbarzadeh, K., Wallman, J. F., Sulakova, H., & Szpila, K. (2015). Species identification of Middle Eastern blowflies (Diptera: Calliphoridae) of forensic importance. *Parasitology Research, 114*(4), 1463-1472.

Amendt, J., Campobasso. C .P., Gaudry, E., Reiter, C., LeBlanc. H.N., Hall, M.JN (2007). European Association for Forensic Entomology. Best practice in forensic entomology--standards and guidelines. *Int J Legal Med. 121*(2):90-104.

Amendt, J., Krettek, R., & Zehner, R. (2004). Forensic entomology. *Naturwissenschaften, 91*(2), 51-65.

Amendt, J., Richards, C. S., Campobasso, C. P., Zehner, R., & Hall, M. J. (2011). Forensic entomology: applications and limitations. *Forensic science, medicine, and pathology,* 7(4), 379-392.

Amorim, J., & Ribeiro, O. (2001). Distinction among the puparia of three blowfly species (Diptera: Calliphoridae) frequently found on unburied corpses. *Memórias do Instituto Oswaldo Cruz, 96*, 781-784.

Anderson, G. S. (2000). Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). *Journal of Forensic Science, 45*(4), 824-832.

Anderson, G.S., (1999). Wildlife forensic entomology: determining time of death in two illegally killed black bear cubs. Journal of Forensic Science *44*, 856-859.

Andrade-Herrera, K., Núñez-Vázquez, C., & Estrella, E. (2021). Life Cycle of *Chrysomya rufifacies* (Diptera: Calliphoridae) Under Semi-Controlled Laboratory Conditions. *Journal of Medical Entomology, 58*(6), 2138-2145.

Apasrawirote, D., Boonchai, P., Muneesawang, P., Nakhonkam, W., & Bunchu, N. (2022). Assessment of deep convolutional neural network models for species identification of forensically-important fly maggots based on images of posterior spiracles. *Scientific reports, 12*(1), 1-9.

Arce, B. J., Clout, S., Pat, D. L., Bharti, M., Pape, T., & Marshall, S. A. (2020). Viviparity and oviparity in termitophilous Rhiniidae (Diptera: Oestroidea) in the Western Ghats, India. *Oriental insects*, *54*(2), 259-264.

Archer, M. S., Bassed, R. B., Briggs, C. A., & Lynch, M. J. (2005). Social isolation and delayed discovery of bodies in houses: The value of forensic pathology, anthropology, odontology and entomology in the medico-legal investigation. *Forensic Science International, 151*(2-3), 259-265.

Arnott, S., Turner, B. (2008). Post-feeding larval behaviour in the blowfly, *Calliphora vicina*: effects on post-mortem interval estimates. *Forensic Sci Int. 177* (2-3), 162-7.

Ashworth, J. R., & Wall, R. (1994). Responses of the sheep blowflies *Lucilia sericata* and *Lucilia cuprina* to odour and the development of semiochemical baits. *Medical and veterinary entomology, 8*(4), 303-309.

Badenhorst, R., & Villet, M. H. (2018). The uses of *Chrysomya megacephala* (Fabricius, 1794)(Diptera: Calliphoridae) in forensic entomology. *Forensic sciences research, 3*(1), 2-15.

Bala, M., Singh, D., 2015. Development of two forensically important blowfly species (*Chrysomya megacephala* and *Chrysomya rufifacies*) (D iptera: C alliphoridae) at four temperatures in I ndia. Entomological Research *45*, 176-183.

Bansode, S. A., More, V. R., Zambare, S. P., & Fahd, M. (2016). Effect of constant temperature (20 0C, 25 0C, 30 0C, 35 0C, 40 0C) on the development of the Calliphorid fly of forensic importance, *Chrysomya megacephala* (Fabricus, 1794). *Journal of Entomology and Zoology Studies*, *4*(3), 193-197.

Baumgartner, D. L. (1993). Review of *Chrysomya rufifacies* (Diptera: Calliphoridae). *Journal of Medical Entomology, 30*(2), 338-352.

Bernhardt, V., Schomerus, C., Verhoff, M.A.,Amendt,J(2017) Of pigs and men—comparing the development of *Calliphora vicina* (Diptera: Calliphoridae) on human and porcine tissue. *Int J Legal Med***,** *131*, 847–853.

Bharti, M. (2011). An updated checklist of blowflies (Diptera: Calliphoridae) from India. *Halteres, 3*, 34-37.

Bharti, M. (2014). The first record of *Chrysomya chani* Kurahashi, 1979 (Diptera: Calliphoridae) from India, with a key to the known Indian species. *Кавказский энтомологический бюллетень, 10*(2), 305-306.

Bharti, M. (2019). New records of *Chrysomya putoria* and *C. thanomthini* (Diptera: Calliphoridae) from India, with a revised key to the known Indian species. *Journal of Threatened Taxa*.*11*(1), 13188–13190.

Bharti, M., & Kurahashi, H. (2009). Finding of feral derived form (fdf) of *Chrysomya megacephala* (Fabricius) from India with an evolutionary novelity (Diptera, Calliphoridae). *Japanese Journal of Systematic Entomology, 15*(2), 411-413.

Bharti, M., & Singh, B. (2017). DNA-based identification of forensically important blow flies (Diptera: Calliphoridae) from India. *Journal of Medical Entomology, 54*(5), 1151-1156.

Bharti, M., & Singh, D. (2003). Insect faunal succession on decaying rabbit carcasses in Punjab, India. *Journal of Forensic Sciences*, *48*(5), 1133-1143.

Bharti, M., Singh, D., & Sharma, Y. P. (2007). Effect of temperature on the development of forensically important blowfly, *Chrysomya megacephala* (Fabricius)(Diptera: Calliphoridae).*Entomon*, *32*(2), 149.

Brundage, A., Benbow, M. E., & Tomberlin, J. K. (2014). Priority effects on the life-history traits of two carrion blow fly (Diptera, Calliphoridae) species. *Ecological entomology, 39*(5), 539-547.

Brundage, A., Bros, S., & Honda, J. Y. (2011). Seasonal and habitat abundance and distribution of some forensically important blow flies (Diptera: Calliphoridae) in Central California. *Forensic Science International*, *212*(1-3), 115-120.

Bunchu, N., Thaipakdee, C., Vitta, A., Sanit, S., Sukontason, K., & Sukontason, K. L. (2012). Morphology and developmental rate of the blow fly, *Hemipyrellia ligurriens* (Diptera: Calliphoridae): Forensic entomology applications. *Journal of parasitology research, 2012*.

Byrd, J. H., & Butler, J. F. (1997). Effects of temperature on *Chrysomya rufifacies* (Diptera: Calliphoridae) development. *Journal of Medical Entomology, 34*(3), 353-358.

Byrd, J. H., & Tomberlin, J. K. (2019). *Forensic entomology: the utility of arthropods in legal investigations*: CRC press.

CABI. (2001). Data Mining. doi:Avaialable at https://www.cabi.org/isc/datasheet/13286 (Last Accessed on 26-06-2022)

Cammack, J., Cohen, A., Kreitlow, K., Roe, R., & Watson, D. (2016). Decomposition of concealed and exposed porcine remains in the North Carolina Piedmont. *Journal of Medical Entomology, 53*(1), 67-75.

Campobasso, C. P., & Introna, F. (2001). The forensic entomologist in the context of the forensic pathologist's role. *Forensic Science International, 120*(1-2), 132-139.

Catts, E. P., & Goff, M. L. (1992). Forensic entomology in criminal investigations. *Annual review of Entomology, 37*(1), 253-272.

Chakraborty, A., Saha, G. K., & Banerjee, D. (2016). Developmental variation of two different variety of Indian blow flies: *Chrysomya megacephala* (Fabricius, 1794) and *Lucilia cuprina* (Wiedemann, 1830)(Diptera: Calliphoridae) on dead Gallus gallus (Linnaeus, 1758). *J Entomol Zool Stud*, *4*(5), 881-889.

Chen, W., L. Yang, L. Ren, Y. Shang, S. Wang, and Y. Guo. (2019). Impact of constant versus fluctuating temperatures on the development and life history parameters of *Aldrichina grahami* (Diptera: Calliphoridae). *Insects, 10*, 1–15.

Chen, W.-Y., Hung, T.-H., & Shiao, S.-F. (2004). Molecular identification of forensically important blow fly species (Diptera: Calliphoridae) in Taiwan. *Journal of Medical Entomology, 41*(1), 47-57.

Claver, M. A., & Yaqub, A. (2015). Studies on biology of the blowfly *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) under fluctuating temperature in three different seasons. *Int. J. Res. Studies Biosci*, *3*, 107-112.

Clery, J. M. (2001). Stability of prostate specific antigen (PSA), and subsequent Y-STR typing, of *Lucilia* (Phaenicia) *sericata* (Meigen)(Diptera: Calliphoridae) maggots reared from a simulated postmortem sexual assault. *Forensic Science International, 120*(1-2), 72-76.

Courtney G.W., Sinclair B.J. and Meier R. (2000) Morphology and terminology of Diptera larvae.In: Contributions to a manual of palearctic Diptera (with special reference to flies of economic importance) L. Papp and B. Darvas (eds.). Science Herald Press, Budapest, Hungary. pp 85-161.

Das, M. N., & Giri, N. C. (1986*). Design and Analysis of Experiments,* Wiley Eastern Ltd., New Delhi. 295p

Dawson, B. M., Wallman, J. F., Evans, M. J., Butterworth, N. J., & Barton, P. S. (2022). Priority effects and density promote coexistence between the facultative predator *Chrysomya rufifacies* and its competitor *Calliphora stygia*. *Oecologia, 199*(1), 181-191.

Donovan, S. E., Hall, M. J. R., Turner, B. D., & Moncrieff, C. B. (2006). Larval growth rates of the blowfly, *Calliphora vicina*, over a range of temperatures. *Medical and veterinary entomology*, *20*(1), 106-114.

Draper, N. R., & Smith, H. (1998). *Applied regression analysis* (Vol. 326). John Wiley & Sons.327p

Drummond, A. J., Ashton, B., M. B, Buxton S, Cheung M, Cooper A, Heled J, Kearse M, Moir R, Stones-Havas S, Sturrock S, Thierer T and Wilson A (2010). Geneious v5.1, http://www.geneious.com.

Eliza, P., & Zuha, R. M. (2018). Preliminary assessment of cephalopharyngeal skeleton length and body length of *Hemipyrellia ligurriens* (Wiedemann)(Diptera: Calliphoridae) larvae as potential parameters to estimate minimum post mortem interval. *Egyptian Journal of Forensic Sciences*, *8*(1), 1-7.

Erzinclioglu, Z. (1990). The larvae of two closely-related blowfly species of the genus Chrysomya (Diptera, Calliphoridae). *Entomologica Fennica*, *1*(3), 151-153.

Evaldo M, P., Carraro, V. M., & Zanuncio, J. C. (2008). Seasonal abundance of *Chrysomya megacephala* and C. albiceps (Diptera: Calliphoridae) in urban areas. *Revista Colombiana de Entomologia, 34*(2), 197-198.

Faris AM, West WR, Tomberlin JK, Tarone AM.(2020) Field Validation of a Development Data Set for *Cochliomyia macellaria* (Diptera: Calliphoridae): Estimating Insect Age Based on Development Stage. J Med Entomol, 57(1):39-49. doi: 10.1093/jme/tjz156. PMID: 31576404.

Firdaus, A., Marwi, M., Syamsa, R., Zuha, R., Ikhwan, Z., & Omar, B. (2010). Research Note Morphological descriptions of second and third instar larvae of *Hypopygiopsis violacea* Macquart (Diptera: Calliphoridae), a forensically important fly in Malaysia. *Tropical Biomedicine, 27*(1), 134-137.

Gabre, R. M., Adham, F. K., & Chi, H. (2005). Life table of *Chrysomya megacephala* (Fabricius)(Diptera: Calliphoridae). *Acta oecologica*, *27*(3), 179-183.

Gallagher, M. B., Sandhu, S., & Kimsey, R. (2010). Variation in developmental time for geographically distinct populations of the common green bottle fly, *Lucilia sericata* (Meigen). *Journal of Forensic Sciences, 55*(2), 438-442.

Goff, M. L. (2001). *A fly for the prosecution: how insect evidence helps solve crimes*: Harvard University Press.

Goff, M. L., & Flynn, M. M. (1991). Determination of postmortem interval by arthropod succession: a case study from the Hawaiian Islands. *Journal of forensic science*, *36*(2), 607-614.

Goodbrod, J. R., & Goff, M. L. (1990). Effects of larval population density on rates of development and interactions between two species of *Chrysomya* (Diptera: Calliphoridae) in laboratory culture. *Journal of Medical Entomology, 27*(3), 338-343.

Grassberger, M., & Reiter, C. (2002). Effect of temperature on development of the forensically important holarctic blow fly *Protophormia terraenovae* (Robineau-Desvoidy)(Diptera: Calliphoridae). *Forensic Science International, 128*(3), 177-182.

Greenberg, B. (1991). Flies as forensic indicators. *Journal of medical entomology*, *28*(5), 565-577.

Greenberg, B., & Kunich, J. C. (2002). *Entomology and the law: flies as forensic indicators*: Cambridge University Press.

Greenberg, B., & Wells, J. D. (1998). Forensic use of Megaselia abdita and M. scalaris (Phoridae: Diptera): case studies, development rates, and egg structure. *Journal of Medical Entomology*, *35*(3), 205-209.

Gruner, S., Slone, D., Capinera, J., & Turco, M. (2017). Development of the oriental latrine fly, *Chrysomya megacephala* (Diptera: Calliphoridae), at five constant temperatures. *Journal of Medical Entomology, 54*(2), 290-298.

Hadura, A., Sundharavalli, R., Azulia, N. Z., Zairi, J., & Hamdan, A. (2018). Life table of forensically important blow fly, *Chrysomya rufifacies* (Macquart)(Diptera: Calliphoridae). *Trop. Biomed, 35*, 413-422.

Harvey, M., Gaudieri, S., Villet, M. H., & Dadour, I. (2008). A global study of forensically significant calliphorids: implications for identification. *Forensic Science International, 177*(1), 66-76.

Harvey, M., Mansell, M., Villet, M. H., & Dadour, I. (2003). Molecular identification of some forensically important blowflies of southern Africa and Australia. *Medical and Veterinary Entomology, 17*(4), 363-369.

Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* 270(1512), 313-321.

Hu, G., Wang, Y., Sun, Y., Zhang, Y., Wang, M., & Wang, J. (2019). Development of *Chrysomya rufifacies* (Diptera: Calliphoridae) at constant temperatures within its colony range in Yangtze River Delta Region of China. *Journal of Medical Entomology, 56*(5), 1215-1224.

Irish, S., Lindsay, T., & Wyatt, N. (2014). Key to adults of Afrotropical species of the genus *Chrysomya* Robineau-Desvoidy (Diptera: Calliphoridae). *African Entomology, 22*(2), 297-306.

Ishijima, H. (1967). Revision of the third stage larvae of synanthropic flies of Japan (Diptera: Anthomyiidae, Muscidae, Calliphoridae and Sarcophagidae). *Medical Entomology and Zoology, 18*(2-3), 47-100.

James, M. T. (1947). *The flies that cause myiasis in man*: US Department of Agriculture,United States Government Printing Office.

James, M. T. (1971). Genus *Chrysomya* in New Guinea. *Pacific Insects, 13*(2), 361-369.

Jeong, Y., Weidner, L. M., Pergande, S., Gemmellaro, D., Jennings, D. E., & Hans, K. R. (2022). Biodiversity of Forensically Relevant Blowflies (Diptera: Calliphoridae) at the Anthropology Research Facility in Knoxville, Tennessee, USA. *Insects*, *13*(2), 109.

Jordaens, K., Sonet, G., Braet, Y., De Meyer, M., Backeljau, T., Goovaerts, F., Bourguignon, L., Desmyter, S. (2013). DNA barcoding and the differentiation between North American and West European *Phormia regina* (Diptera, Calliphoridae, *Chrysomyinae*). *ZooKeys*, *365*, 149-175

Kashyap, V.K., & Pillay, V.V. (1989).Efficacy of entomological method in estimation of postmortem interval: a comparative analysis. *Forensic science international, 40* ( 3), 245-50.

Kiviste, A., Álvarez González, A., Rojo Alboreca, A., & Ruiz González, A. D. (2002). *Funciones de crecimiento de aplicación en el ámbito forestal*. INIA [España].

Kjer, K., Borowiec, M. L., Frandsen, P. B., Ware, J., & Wiegmann, B. M. (2016). Advances using molecular data in insect systematics. *Current opinion in insect science, 18*, 40-47.

Klong-Klaew, T., Ngoen-Klan, R., Moophayak, K., Sukontason, K., Irvine, K. N., Tomberlin, J. K., Kurahashi H., Chareonviriyaphap T., Somboon P., Sukontason, K. L. (2018). Spatial Distribution of Forensically Significant Blow Flies in Subfamily Luciliinae (Diptera: Calliphoridae), Chiang Mai Province, Northern Thailand: Observations and Modeling Using GIS. *Insects, 9*(4), 181.

Klong-Klaew, T., Ngoen-Klan, R., Moophayak, K., Sukontason, K., Irvine, K. N., Tomberlin, J. K., Somboon P., Chareonviriyaphap T., Kurahashi H.,Sukontason, K. L. (2018). Predicting geographic distribution of forensically significant blow flies of subfamily Chrysomyinae (Diptera: Calliphoridae) in Northern Thailand. *Insects, 9*(3), 106.

Klong-Klaew, T., Sukontason, K., Sribanditmongkol, P., Moophayak, K., Sanit, S., & Sukontason, K. L. (2012). Observations on morphology of immature Lucilia porphyrina (Diptera: Calliphoridae), a fly species of forensic importance. *Parasitology research*, *111*(5), 1965-1975.

Kulshrestha, P., & Chandra, H. (1987). Time since death: an entomological study on corpses. *The American journal of forensic medicine and pathology*, *8*(3), 233-238.

Kurahashi, H and Chowanadisai, L. 2001. Blow flies (Insecta: Diptera: Calliphoridae) from Indochina. *Species Diversity*. 6:185–242

Kurahashi, H. (1979). A new species of Chrysomya from Singapore, with notes on C. defixa (Diptera: Calliphoridae). *Journal of Medical Entomology*, *16*(4), 286-290.

Kurahashi, H. (1982). Probable origin of a synanthropic fly *Chrysomya megacephala*, in New Guinea (Diptera: Calliphoridae). In *Biogeography and ecology of New Guinea* (pp. 689-698). Springer, Dordrecht.

Kurahashi, H. (1991). Blow flies from Samoa with description of a new species of *Chrysomya* (Diptera, Calliphoridae). *Japanese Journal of Entomology, 59*(3), 627-636.

Kurahashi, H. (1997). Blow flies (Insecta: Diptera: Calliphoridae) of Malaysia and Singapore. *Raffles Bulletin of Zoology, Suppl., 5*, 1-88.

Lee, H.L., Krishnasamy, M., Abdullah, A.G. and Jeffery, J. (2004). Review of forensically important entomological specimens in the period of 1972–2002. *Trop. Biomed*,21, 69–75

Lertthamnongtham, S., Sukontason, K., Sukontason, K., Piangjai, S., Choochote, W., Vogtsberger, R., & Olson, J. (2003). Seasonal fluctuations in populations of the two most forensically important fly species in northern Thailand. *Annals of Tropical Medicine & Parasitology, 97*(1), 87-91.

Li, L., Wang, Y., Wang, J., Ma, M., & Lai, Y. (2016). Temperature-dependent development and the significance for estimating postmortem interval of *Chrysomya nigripes* Aubertin, a new forensically important species in China. *International journal of legal medicine, 130*(5), 1363-1370.

Li, X., Yang, Y., Li, G., Li, H., Wang, Q., & Wan, L. (2014). The effect of dietary fat levels on the size and development of *Chrysomya megacephala* (Diptera: Calliphoridae). *Journal of Insect Science, 14*(1).

Liu, D. and Greenberg, B. (1989). Immature Stages of Some Flies of Forensic Importance. *Annals of the Entomological Society of America, 82*, 80-93.

Lutz, L., Zehner, R., Verhoff, M. A., Bratzke, H., & Amendt, J. (2021). It is all about the insects: a retrospective on 20 years of forensic entomology highlights the importance of insects in legal investigations. *International journal of legal medicine, 135*(6), 2637-2651.

Ma, Y., Hu, C., & Min, J. (1998). Effects of temperature on the growth and development of four common necrophagous flies and their significance in forensic medicine. *Chin. J. Forensic Med, 13*(8).

Madeira, N. (2001). Would *Chrysomya albiceps* (Diptera: Calliphoridae) be a beneficial species? *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 53*, 1-5.

Marchetti, D., Arena, E., Boschi, I., & Vanin, S. (2013). Human DNA extraction from empty puparia. *Forensic Science International, 229* (1-3), e26-e29.

Mashaly, A., & Ibrahim, A. (2022). Forensic entomology research in Egypt: a review article. *Egyptian Journal of Forensic Sciences, 12*(1), 1-15.

Matuszewski, S., Bajerlein, D., Konwerski, S., & Szpila, K. (2010). Insect succession and carrion decomposition in selected forests of Central Europe. Part 1: Pattern and rate of decomposition. *Forensic Science International, 194*(1-3), 85-93.

Matuszewski, S., Bajerlein, D., Konwerski, S., & Szpila, K. (2011). Insect succession and carrion decomposition in selected forests of Central Europe. Part 3: Succession of carrion fauna. *Forensic Science International, 207*(1-3), 150-163.

Mello, R. d. S., Queiroz, M., & Aguiar-Coelho, V. M. (2007). Population fluctuations of calliphorid species (Diptera, Calliphoridae) in the Biological Reserve of Tinguá, state of Rio de Janeiro, Brazil. *Iheringia. Série Zoologia, 97*, 481-485.

Mendonça, P. M., Dos Santos-Mallet, J. R., & De Carvalho Queiroz, M. M. (2012). Ultrastructure of larvae and puparia of the blowfly *Chrysomya megacephala* (Diptera: Calliphoridae). *Microscopy Research and Technique, 75*(7), 935-939.

Mendonça, P.M., Santos-Mallet, J.R.D., Queiroz, M.M.D.C. (2010). Ultra morphological characteristics of immature stages of *Chrysomya albiceps* (Wiedemann 1819) (Diptera: Calliphoridae), a fly specie of forensic importance. *Microscopy Research and Technique* 73, 779-784.

Moophayak, K., Klong-Klaew, T., Sukontason, K., Kurahashi, H., Tomberlin, J. K., & Sukontason, K. L. (2014). Species composition of carrion blow flies in northern Thailand: altitude appraisal. *Revista do Instituto de Medicina Tropical de São Paulo, 56*, 179-182.

Muskan, H.D., Singh, G.K., Chauhan, V., Shweta, J.S., Shukla, S., 2022. Small Size, Big Impact: Insects for Cadaver Examination. Crime Scene Management within Forensic Science: *Forensic Techniques for Criminal Investigations*, 75.

Nandi, B. (2004). *Checklist of Calliphoridae (Diptera) of India*, Rec. zool. Surv. India*, Dcc.231* : 1-47.

Niederegger, S., J. Pastuschek, and G. Mall. (2010). Preliminary studies of theinfluence of fluctuating temperatures on the development of various forensically relevant flies. *Forensic Sci. Int*. *199*, 72–78.

Nigoghosian, G., Weidner, L. M., & Stamper, T. I. (2021). A technique to mount Sarcophagidae and Calliphoridae (Diptera) larvae for forensic identification using geometric morphometrics. *Forensic Science International: Synergy*, *3*, 100135.

Nordin, N.H., Ahmad, U.K., Rabbit, A., Kamaluddin, M.R., Ismail, D., Muda, N.W., Wahab, A.R. and Mahat, N.A.(2020). Development patterns of necrophagous flies infesting rabbit carcasses decomposing in Mount Kapur Cave and its surrounding primary forest in Kuching, Sarawak. *Tropical Biomedicine*, *37*(2): 333–356

Olea, M. S., Juri, M. J. D., & Centeno, N. (2011). First report of *Chrysomya megacephala* (Diptera: Calliphoridae) in northwestern Argentina. *Florida Entomologist, 94*(2), 345-346.

Omar, B. (2002). Key to third instar larvae of flies of forensic importance in Malaysia. Entomology and the law: flies as forensic indicators. Cambridge University Press, Cambridge, 120-127.

Owings, C. G., Spiegelman, C., Tarone, A. M., & Tomberlin, J. K. (2014). Developmental variation among *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) populations from three ecoregions of Texas, USA. *International journal of legal medicine, 128*(4), 709-717.

Patton, W. S. (1931). Insects, Ticks, Mites and Venomous Animals of Medical and Veterinary Importance. Part II. Public Health. *Insects, Ticks, Mites and Venomous Animals of Medical and Veterinary Importance. Part II. Public Health.*

Pelletti, G., Martini, D., Ingrà, L., Mazzotti, M.C., Giorgetti, A., Falconi, M., Fais, P., 2022. Morphological characterization using scanning electron microscopy of fly artifacts deposited by *Calliphora vomitoria* (Diptera: Calliphoridae) on household materials. *International Journal of Legal Medicine 136*, 357-364.

Pittner, S., Bugelli, V., Weitgasser, K., Zissler, A., Sanit, S., Lutz, L., Monticelli F., Campobasso CP., Steinbacher P.,Amendt, J. (2020). A field study to evaluate PMI estimation methods for advanced decomposition stages. *International journal of legal medicine, 134*(4), 1361-1373.

Pitts, K. M., & Wall, R. (2005). Winter survival of larvae and pupae of the blowfly, *Lucilia sericata* (Diptera: Calliphoridae). *Bulletin of entomological research*, *95*(3), 179-186.

Potapov AM., Beaulieu F., Birkhofer K., Bluhm SL., Degtyarev MI., Devetter M., Goncharov AA., Gongalsky KB., Klarner B., Korobushkin DI., Liebke DF., Maraun M., Mc Donnell RJ., Pollierer MM., Schaefer I., Shrubovych J., Semenyuk II., Sendra A, Tuma J.,…….. Tůmová M.(2022). Feeding habits and multifunctional classification of soil-associated consumers from protists to vertebrates. *Biol Rev Camb Philos Soc.* 97(3),1057-1117.

Priya Bhaskaran K. P., & Sebastian, C. D. (2015). Molecular barcoding of green bottle fly, Lucilia sericata (Diptera: Calliphoridae) using COI gene sequences. *J Entomol Zool Stud*, *3*, 10-12.

Qiu, D., Cook, C. E., Yue, Q., Hu, J., Wei, X., Chen, J., ... & Wu, K. (2017). Species-level identification of the blowfly *Chrysomya megacephala* and other Diptera in China by DNA barcoding. *Genome*, *60*(2), 158-168.

Queiroz, M. M. d. C. (1996). Temperature requirements of *Chrysomya albiceps* (Wiedemann, 1819)(Diptera, Calliphoridae) under laboratory conditions. *Memórias do Instituto Oswaldo Cruz, 91*, 785-788.

Radhakrishnan, S., Gopalan, A. K. K., Ravindran, R., Rajagopal, K., Sooryadas, S., & Promod, K. (2012). First record of *Chrysomya albiceps* Wiedemann, 1819 (Diptera: Calliphoridae) maggots from a sambar deer (Rusa unicolor) in Kerala, South India. *Journal of parasitic diseases, 36*(2), 280-282.

Rajagopal, K. (2013). Application of Forensic Entomology in Crime Scene Investigations in Malaysia, Univ. of Malaya, Kuala Lumpur. Ph.D. Thesis.

Ramaraj, M. P., Selvakumar, C., Ganesh, M. A., & Janarthanan, S. (2014). Report on the occurrence of synanthropic derived form of *Chrysomya megacephala* (Diptera: Calliphoridae) from Royapuram fishing harbour, Chennai, Tamil Nadu, India. *Biodiversity Data Journal*,26(2):e1111. doi: 10.3897/BDJ.2.e1111.

Reibe-Pal, S., & Madea, B. (2015). Calculating time since death in a mock crime case comparing a new computational method (ExLAC) with the ADH method. *Forensic Science International, 248*, 78-81.

Reject Paul, M. P., & Binoy, C.F (2021). Ultrastructure of second instar larva of *Hemipyrellia ligurriens* (Wiedemann) (Diptera: Calliphoridae), a forensically important blow fly species from India. *Entomon*, *46*(1), 47–52.

Reject Paul, M.P., & Binoy, C.F (2021). Life cycle and development rate of *Hemipyrellia ligurriens* (Wiedemann)(Diptera: Calliphoridae) during monsoon season in South India: applications in estimation of postmortem interval. *Journal of Veterinary and Animal Sciences*, *52* (3): 292 – 297.

Reject Paul, M.P., & Binoy, C.F (2022). Forensic implications of the seasonal changes in the rate of development of the blowfly, Chrysomya megacephala (Fabricius) (Diptera, Calliphoridae). .*Entomon*, *47*(4), 375-382.

Richards, C. S., & Villet, M. H. (2009). Data quality in thermal summation development models for forensically important blowflies. *Medical and Veterinary Entomology, 23*(3), 269-276.

Rosati, J. Y., Pacheco, V. A., Vankosky, M. A., & VanLaerhoven, S. L. (2015). Estimating the number of eggs in blow fly (Diptera: Calliphoridae) egg masses using photographic analysis. *Journal of medical entomology*, *52*(4), 658-662.

Roy, P., & Dasgupta, B. (1975). Seasonal occurrence of muscid, Calliphorid and Sarcophagid flies in Siliguri, West Bengal, with a note on the identity of *Musca domestica* L. *Oriental Insects, 9*(3), 351-374.

Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution *4*,406-425.

Salimi, M., Rassi, Y., Oshaghi, M., Chatrabgoun, O., Limoee, M., Rafizadeh, S., 2018. Temperature requirements for the growth of immature stages of blowflies species, *Chrysomya albiceps* and *Calliphora vicina*, (Diptera: Calliphoridae) under laboratory conditions. Egyptian Journal of Forensic Sciences *8*, 1-6.

Sanit, S., Sukontason, K. L., Sribanditmongkol, P., Klong-Klaew, T., Samerjai, C., Sontigun, N., ... & Sukontason, K. (2012). Surface ultrastrucure of larva and puparia of blow fly

*Hypopygiopsis tumrasvini* Kurahashi (Diptera: Calliphoridae). *Parasitology research*, *111*(6), 2235-2240.

Senior-White, R., Aubertin, D. and Smart, J.1940. *The fauna of British India including remainder of the oriental region:* Diptera Vol VI family Calliphoridae Vol.VI. Taylor and Francis, London, United Kingdom.41-43

Shiao, S.-F., & Yeh, T.-C. (2008). Larval competition of *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae): behavior and ecological studies of two blow fly species of forensic significance. *Journal of Medical Entomology, 45*(4), 785-799.

Siddiki, S., & Zambare, S. (2017). Studies on time duration of life stages of *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae) during different seasons. *Journal of Forensic. Research, 8*, 379.

Silahuddin S A., Latif B., Kurahashi., Walter E., Heo C C (2015). The Importance of Habitat in the Ecology of Decomposition on Rabbit Carcasses in Malaysia: Implications in Forensic Entomology, *Journal of Medical Entomology*, *52*(1), 9–23.

Singh, B., Kurahashi, H., & Wells, J. (2011). Molecular phylogeny of the blowfly genus *Chrysomya*. *Medical and Veterinary Entomology, 25*(2), 126-134.

Singh, D., & Bharti, M. (2001). Further observations on the nocturnal oviposition behaviour of blow flies (Diptera: Calliphoridae). *Forensic Science International, 120*(1-2), 124-126.

Singh, D., & Sidhu, I. S. (2004). A check list of blow flies (Diptera: Calliphoridae) from North-west of India. *Uttar Pradesh Journal of Zoology*, 63-71.

Singh, D., Bharti, M., & Singh, T. (1999). Forensic entomology in the Indian perspective. *Journal of Punjab Academy of Sciences, 1*, 217-220.

Singh, R., Kumawat, R., Singh, G., Jangir, S. S., Kushwaha, P., & Rana, M. (2022). Forensic entomology: A novel approach in crime investigation. *GSC Biological and Pharmaceutical Sciences, 19*(2), 165-174.

Sinha, S. K., & Nandi, B. C. (2007). Studies on Life History of *Hemipyrellia ligurriens* (Wiedemann)(Diptera: Calliphoridae) in Sundarbans Biosphere Reserve, West Bengal, India. *Records of the Zoological Survey of India*, *107*(1), 63-70.

Sinha, S., & Nandi, B. (2004). Notes on calliphorid flies (Diptera: Calliphoridae) from Sunderbans Biosphere Reserve and their impact on man and animals. *Journal of Bombay Natural History Society, 101*, 415-420.

Siriwattanarungsee, S., Sukontason, K. L., Kuntalue, B., Piangjai, S., Olson, J. K., & Sukontason, K. (2005). Morphology of the puparia of the housefly, *Musca domestica* (Diptera: Muscidae) and blowfly, *Chrysomya megacephala* (Diptera: Calliphoridae). *Parasitology Research, 96*(3), 166-170.

Smith, K.G., 1986. *A manual of forensic entomology.* Cornell University Press, Ithaca, NewYork. 103 pp.

Sontigun, N., Sukontason, K. L., Klong-Klaew, T., Sanit, S., Samerjai, C., Somboon, P., Thanapornpoonpong, S.C., Klong-Klaew, T.,Amendt.J., Sukontason, K. (2018). Bionomics of the oriental latrine fly *Chrysomya megacephala* (Fabricius)(Diptera: Calliphoridae): temporal fluctuation and reproductive potential. *Parasites & vectors, 11*(1), 1-12.

Speight, M. R., Hunter, M. D., & Watt, A. D. (1999). *Ecology of insects: concepts and applications*. Blackwell Science Ltd.

Stevens, J., West, H., & Wall, R. (2008). Mitochondrial genomes of the sheep blowfly, *Lucilia sericata*, and the secondary blowfly, *Chrysomya megacephala*. *Medical and veterinary entomology, 22*(1), 89-91.

Stork, N. E. (2008). Insect diversity: facts, fiction and speculation. *Biological journal of the Linnean Society, 35*(4), 321-337.

Subramanian, H., & Mohan, K. R. (1980). Biology of the blowflies *Chrysomyia megacephala*, *Chrysomyia rufifacies* and *Lucilia cuprina*. *Kerala Journal of Veterinary Science, 11*(2), 252-261.

Sukontason, K. L., Bhoopat, T., Wannasan, A., Sontigun, N., Sanit, S., Amendt, J., Samerjai, C., Sukontason, K. (2018). *Chrysomya chani* Kurahashi (Diptera: Calliphoridae), a blow fly species of forensic importance: Morphological characters of the third larval instar and a case report from Thailand. *Forensic sciences research, 3*(1), 83-93.

Sukontason, K. L., Bunchoo, M., Khantawa, B., Piangjai, S., Rongsriyam, Y., & Sukontason, K. (2007). Comparison between *Musca domestica* and *Chrysomya megacephala* as carriers of bacteria in northern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health, 38*(1), 38.

Sukontason, K. L., Chaiwong, T., Chaisri, U., Kurahashi, H., Sanford, M., & Sukontason, K. (2011). Reproductive organ of blow fly, *Chrysomya megacephala* (Diptera: Calliphoridae): ultrastructural of testis. *Journal of parasitology research, 2011*.

Sukontason, K. L., Narongchai, P., Kanchai, C., Vichairat, K., Piangjai, S., Boonsriwong, W., ... & Sukontason, K. (2006). Morphological comparison between *Chrysomya rufifacies* (Macquart) and *Chrysomya villeneuvi* Patton (Diptera: Calliphoridae) puparia, forensically important blow flies. Forensic science international, *164*(2-3), 230-234.

Sukontason, K. L., Sanit, S., Limsopatham, K., Wannasan, A., Somboon, P., & Sukontason, K. (2022). *Chrysomya pinguis* (Walker)(Diptera: Calliphoridae), blow fly of forensic importance: A review of bionomics and forensic entomology appraisal. *Acta Tropica*, 106506.

Sukontason, K. L., Sukontason, K., Piangjai, S., Boonchu, N., Chaiwong, T., Vogtsberger, R. C., Kuntalue B., Thijuk N.,Olson, J. K. (2003). Larval morphology of *Chrysomya megacephala* (Fabricius)(Diptera: Calliphoridae) using scanning electron microscopy. *Journal of Vector Ecology: Journal of the Society for Vector Ecology, 28*(1), 47-52.

Sukontason, K., Narongchai, P., Kanchai, C., Vichairat, K., Sribanditmongkol, P., Bhoopat, T., Kurahashi, H., Chockjamsai, M., Piangjai, S., Bunchu, N., Vongvivach, S., Samai, W., Chaiwong,T., Methanitikorn, R., Ngern-klun, R., Sripakdee, D., Boonsriwong, W., Siriwattanarungsee, S., Srimuangwong, C.,….. Hanterdsith, B., (2010). Differentiation between *Lucilia cuprina* and *Hemipyrellia ligurriens* (Diptera: Calliphoridae) larvae for use in forensic entomology applications. *Parasitol Res*. *106*, 641–646

Sukontason, K., Methanitikorn, R., Sukontason, K. L., Piangjai, S., & Olson, J. K. (2004). Clearing technique to examine the cephalopharyngeal skeletons of blow fly larvae. *Journal of vector ecology: journal of the Society for Vector Ecology, 29*(1), 192-195.

Sukontason, K., Narongchai, P., Kanchai, C., Vichairat, K., Sribanditmongkol, P., Bhoopat, T., Kurahashi, H., Chockjamsai, M., Piangjai, S., Bunchu, N., Vongvivach, S. (2007). Forensic entomology cases in Thailand: a review of cases from 2000 to 2006. *Parasitology Research 101*, 1417-1423.

Sukontason, K., Piangjai, S., Siriwattanarungsee, S., & Sukontason, K. L. (2008). Morphology and developmental rate of blowflies *Chrysomya megacephala* and *Chrysomya rufifacies* in Thailand: application in forensic entomology. *Parasitology Research, 102*(6), 1207-1216.

Sukontason, K., Sribanditmongkol, P., Chaiwong, T., Vogtsberger, R.and Piangjai, S. (2008).Morphology of immature stages of *Hemipyrellia ligurriens* (Weidemann) (Diptera: Calliphoridae) for use in forensic entomology applications. *Parasitology research*.*103*,877-887

Szpila K. and Villet M.H. (2011). Morphology and Identification of first instars of African blowflies (Diptera: Calliphoridae) Commonly of Forensic Significance. Journal of Medical Entomology, *48*,738 -752.

Szpila, K., Hall, M. J. R., Pape, T., & Grzywacz, A. (2013). Morphology and identification of first instars of the European and Mediterranean blowflies of forensic importance. Part II. Luciliinae. *Medical and Veterinary Entomology*, *27*(4), 349-366.

Szpila, K., Hall, M., Sukontason, K., & Tantawi, T. (2013). Morphology and identification of first instars of the European and Mediterranean blowflies of forensic importance. Part I: Chrysomyinae. *Medical and Veterinary Entomology, 27*(2), 181-193.

Tachibana, S. I., & Numata, H. (2006). Seasonal prevalence of blowflies and flesh flies in Osaka City. *Entomological Science*, *9*(4), 341-345.

Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.

Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120.

Tan, S. H., Aris, E. M., Surin, J., Omar, B., Kurahashi, H., & Mohamed, Z. (2009). Sequence variation in the cytochrome oxidase subunit I and II genes of two commonly found blow fly species, *Chrysomya megacephala* (Fabricius) and *Chrysomya rufifacies* (Macquart)(Diptera: Calliphoridae) in Malaysia. *Trop. Biomed, 26*(2), 173-181.

Tantawi, T. I., & Greenberg, B. (1993). The effect of killing and preservative solutions on estimates of maggot age in forensic cases. *Journal of Forensic Science*, *38*(3), 702-707.

Tomberlin, J. K., Benbow, M. E., Tarone, A. M., & Mohr, R. M. (2011). Basic research in evolution and ecology enhances forensics. *Trends in Ecology & Evolution, 26*(2), 53-55.

Tourle R., Downie D and Villet M. (2009) Flies in the ointment: a morphological and molecular comparison of *Lucilia cuprina and Lucilia sericata* (Diptera: Calliphoridae) in South Africa. *Medical and Veterinary Entomology. 23*(1), 6–14.

Tullis, K., & Goff, M. L. (1987). Arthropod succession in exposed carrion in a tropical rainforest on O'ahu Island, Hawai'i. *Journal of Medical Entomology, 24*(3), 332-339.

Tumrasvin, W., Kurahashi, H., & Kano, R. (1979). Studies on medically important flies in Thailand VII report on 42 species of Calliphorid flies, including the taxonomic keys (Diptera: Calliphoridae). *The Bulletin of Tokyo Medical and Dental University, 26*(4), 243-272.

Turner, B., & Howard, T. (1992). Metabolic heat generation in dipteran larval aggregations: a consideration for forensic entomology. *Medical and Veterinary Entomology, 6*(2), 179-181.

Verma, K., & Reject Paul, M. P. (2016). *Lucilia sericata* (Meigen) and *Chrysomya megacephala* (Fabricius)(Diptera: Calliphoridae) development rate and its implications for forensic entomology. *Journal of Forensic Science and Medicine, 2*(3), 146.

Viero, A., Montisci, M., Pelletti, G., & Vanin, S. (2019). Crime scene and body alterations caused by arthropods: implications in death investigation. *International journal of legal medicine, 133*(1), 307-316.

Von Aesh, L., Pellet, J., & Cherix, D. (2003). Activity and behaviour of blowflies on pig liver baits in spring. *Bulletin de la Société entomologique Suisse*, *76*, 201-206.

Wall, R., Howard, J., Bindu, J., 2001. The seasonal abundance of blowflies infesting drying fish in south-west India. Journal of Applied Ecology, 339-348.

Wang, J., Li, Z., Chen, Y., Chen, Q., & Yin, X. (2008). The succession and development of insects on pig carcasses and their significances in estimating PMI in south China. *Forensic Science International, 179*(1), 11-18.

Wang, Y., Ma, My., Jiang, X.Y., Wang, J.F., Li, L.L., Yin, X.Y., Wang, M., Lai, Y., Tao, L.Y. (2017). Insect succession on remains of human and animals in Shenzhen, China. *Forensic Science International, 271*, 75-86.

Wells, J. D., & Kurahashi, H. (1994). *Chrysomya megacephala* (Fabricius)(Diptera: Calliphoridae) development: rate, variation and the implications for forensic entomology. *Medical Entomology and Zoology, 45*(4), 303-309.

Wells, J., & LaMotte, L. (2017). The role of a PMI-prediction model in evaluating forensic entomology experimental design, the importance of covariates, and the utility of response variables for estimating time since death. *Insects*, *8*(2), 47.

Whitaker, A. (2014). *Development of blowflies (Diptera: Calliphoridae) on pig and human cadavers: implications for forensic entomology casework* (Doctoral dissertation, King's College London (University of London).

Williams, K. A., Wallman, J. F., Lessard, B. D., Kavazos, C. R., Mazungula, D. N., & Villet, M. H. (2017). Nocturnal oviposition behavior of blowflies (Diptera: Calliphoridae) in the southern hemisphere (South Africa and Australia) and its forensic implications. *Forensic science, medicine, and pathology, 13*(2), 123-134.

Xu, W., Wang, Y., Wang, Y.-h., Zhang, Y.-n., & Wang, J.-f. (2022). Diversity and dynamics of bacteria at the *Chrysomya megacephala* pupal stage revealed by third-generation sequencing. *Scientific reports, 12*(1), 1-9.

Yang, S.-T., & Shiao, S.-F. (2012). Oviposition preferences of two forensically important blow fly species, *Chrysomya megacephala* and *C. rufifacies* (Diptera: Calliphoridae), and implications for postmortem interval estimation. *Journal of Medical Entomology, 49*(2), 424-435.

Yang, Y., Liu, Z., Li, X., Li, K., Yao, L. and Wan, L. 2015.Technical note: Development of *Hemipyrellia ligurriens* (Wiedemann) (Diptera: Calliphoridae) at constant temperatures: Applications in estimating postmortem interval .*Forensic Science International*, 253, 48-54

Yang, Y.Q., Li, X.B., Shao, R.Y., Lyu, Z., Li, H.W., Li, G.P., Xu, L.Z.,Wan, L.H., (2016). Developmental times of *Chrysomya megacephala* (Fabricius)(Diptera: Calliphoridae) at constant temperatures and applications in forensic entomology. *Journal of forensic sciences, 61*(5), 1278-1284.

Yanmanee, S., Husemann, M., Benbow, M. E., & Suwannapong, G. (2016). Larval development rates of *Chrysomya rufifacies* Macquart, 1842 (Diptera: Calliphoridae) within its native range in South-East Asia. *Forensic Science International, 266*, 63-67.

Yusseff-Vanegas, S.Z., Agnarsson, I., 2017. DNA-barcoding of forensically important blow flies (Diptera: Calliphoridae) in the Caribbean Region. *PeerJ* , *5*, e3516.

Zajac, B. K., Sontigun, N., Wannasan, A., Verhoff, M. A., Sukontason, K., Amendt, J., & Zehner, R. (2016). Application of DNA barcoding for identifying forensically relevant Diptera from northern Thailand. *Parasitology Research, 115*(6), 2307-2320.

Zhang, Y., Wang, Y., Sun, J., Hu, G., Wang, M., Amendt, J., & Wang, J. (2019). Temperature-dependent development of the blow fly *Chrysomya pinguis* and its significance in estimating postmortem interval. *Royal Society open science, 6*(9), 190003.

Zhang, Y., Wang, Y., Yang, L., Tao, L., & Wang, J. (2018). Development of *Chrysomya megacephala* at constant temperatures within its colony range in Yangtze River Delta region of China. *Forensic sciences research, 3*(1), 74-82.

Zumpt, F. (1965). *Myiasis in Man and Animals in the Old World. A Textbook for Physicians, Veterinarians and Zoologists*., Butterworth & Co. XV , pp 267.

# APPENDIX

# I. PUBLICATIONS

**1.**

**https://doi.org/10.33307/entomon.v47i4.790**
**ENTOMON 47(4): 375-382 (2022)**
**Article No. ent. 47403**

## Forensic implications of the seasonal changes in the rate of development of the blowfly, *Chrysomya megacephala* (Fabricius) (Diptera, Calliphoridae)

**M.P. Reject Paul and C.F. Binoy***

*Division of Applied and Forensic Entomology, Research & Post Graduate Department of Zoology, St. Thomas' College (Autonomous), Thrissur 680001, Kerala, India.*
*Email: rpaulmp@stthomas.ac.in; binoycf@stthomas.ac.in*

**ABSTRACT:** Studies on the development rate of *Chrysomya megacephala* (Fabricius) suggested that the blowfly as a significant candidate for forensic investigations. Under natural ambient conditions development rate of *C. megacephala* in monsoon, winter and summer seasons indicated significant differences among seasons. The larvae began pupation at 92$^{nd}$ h in summer, 157$^{th}$ h in the monsoon season and 191$^{st}$ h in winter. Rapid larval growth in terms of length was observed in summer. During summer, the length of the larvae increased to a maximum of 13.9 mm at 54$^{th}$ h. Time taken for the emergence of the adult fly was 164, 249 and 311h in summer, monsoon and winter seasons respectively. Life table studies were conducted to assess the percentage survival and mortality by recording the survival rate of different development stages. Molecular diagnosis of species was done using COI gene. The analysis included molecular sequences of other samples of the same species from different regions of India. The neighbor-joining method allowed us to identify the species at molecular level with precision and accuracy.


**KEYWORDS:** Larval growth, pupation, adult emergence, seasonal differences, life table, molecular diagnosis

### INTRODUCTION

The blowfly, *Chrysomya megacephala* (Fabricius) (Diptera, Calliphoridae), a synanthropic fly, commonly known as oriental latrine fly inhabiting human settlements and commonly seen on decomposing cadavers, fish, carrion, human feces and sweet materials; indicating its medical, veterinary and forensic significance. Due to their cosmopolitan distribution, ubiquity and abundance, *C. megacepha* is recognized as the one of the most important species of insects in forensic entomology (Badenhorst and Villet, 2018). The larvae feed and grow on soft tissues of living and dead vertebrates especially mammals, birds and fish (Yang and Shiao, 2012). The adult flies were usually attracted to decaying cadavers and reach within a few hours of death of the animal (Zumpt, 1965), and it has been considered as an important fly for the determination of minimum postmortem interval (Wang *et al.,* 2008). Medico legal cases world over have reported the forensic relevance of *C. megacephal* (Gruner *et al*., 2017; Richards and Villet, 2009; Amendt *et al*., 2004; Goff and Flynn, 1991). For the determination of minimum postmortem intervals, age of larvae will be helpful (Gruner *et al*., 2017). Studies focusing the

* Author for correspondence

2.

**RESEARCH ARTICLE**
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2021.52.3.292-297

# Life cycle and development rate of *Hemipyrellia ligurriens* (Wiedemann) (Diptera: Calliphoridae) during monsoon season in South India: applications in estimation of postmortem interval

**M. P. Reject Paul[1] and C. F. Binoy[2*]**
Research & Post Graduate Department of Zoology,
St. Thomas' College (Autonomous), Thrissur-680001, Kerala,

Citation: Reject Paul, M. P. and C. F. Binoy. 2021. Life cycle and development rate of *Hemipyrellia ligurriens* (Wiedemann) (Diptera:Calliphoridae) during monsoon season in South India: applications in estimation of postmortem interval. *J. Vet. Anim. Sci*. **52** (3): 292 – 297.
DOI: https://doi.org/10.51966/jvas.2021.52.3.292-297

*Received: 27.05.2021* *Accepted: 12.07.2021* *Published: 30.09.2021*

**Abstract**

*Hemipyrellia ligurriens, considered as one of the forensically important blow fly species, has a wide distribution in many countries including India. To conduct forensic entomological investigations involving deaths of livestock, human beings and wild animals, standard life cycle data should be prepared for the local blow fly species under various weather conditions. Reliable forensic entomological data specific to geographic locations in India are not available presently to assist the post mortem interval assessment. In this study, life cycle and the rate of development of H.ligurriens was determined during monsoon season in Kerala, South India. Survival rate observed from egg to adult emergence was 44.68 %. Total duration of development of the species from oviposition till adult emergence was 462.57 h. Growth curves based on the age, specific length parameter and time taken for development of each larval stage was constructed. This development model would be helpful for the medical, veterinary and law enforcement officials in forensic estimation of post mortem interval by analyzing the length parameters of larvae collected from decomposed dead bodies of humans, cadavers of wild animals and livestock.*

**Keywords:** *Hemipyrellia ligurriens, development stages, postmortem interval, veterinary forensics*

The *Hemipyrellia* genus is represented by four species in the Oriental region. In India, it is represented by two species; *H.pulchra* and *H.ligurriens* (Senior-White *et al*.,1940;Nandi, 2004; Bharti,2011), the latter being reported on decomposing human cadavers in Malaysia (Rajagopal, 2013), Thailand (Moophayak *et al*., 2014)and other regions (Chen *et al*., 2004;Lee *et al*., 2004; Sukontason, 2007) and has significant forensic importance. These speciesare widely distributed

1. *Research Scholar: Email Id : rpaulmp@stthomas.ac.in , Ph:9656370352*
2. **Assistant Professor and corresponding author: email: binoycf@stthomas.ac.in, Ph: 8921645795*

3. https://doi.org/10.33307/entomon.v46i1.586
ENTOMON 46(1): 47-52 (2021)
Short Communication No. ent. 46106

# Ultra structure of second instar larva of *Hemipyrellia ligurriens* (Wiedemann) (Diptera: Calliphoridae), a forensically important blow fly species from India

**M.P. Reject Paul and C.F. Binoy***

*Research & Post Graduate Department of Zoology, St. Thomas' College (Autonomous), Thrissur 680001, Kerala, India. Email: rpaulmp@stthomas.ac.in; binoycf@stthomas.ac.in*

**ABSTRACT:** Ultra structural characters of second instar larvae of *Hemipyrellia ligurriens* are elucidated through micrographs (Scanning Electron Microscope). Morphological details of maxillary palpi, antennae, oral cirri, facial mask, labial lobe, spinulations, and papillae of anal segment are described. Oral cirri are ten in number, arranged bilaterally on each side of the functional mouth opening and gently curved medially. The labial lobes are distinctively demarcated with fleshy projections antero–ventrally and have a characteristic shape. Thoracic spines have a bulbous base, slender sharp tips and are directed backwards. Prominent dorsal and ventral anal papillae with projected tips and broad conical base were present surrounded by microtrichia. The ultrastructure details of *H. ligurriens* would help in the rapid and accurate identification of the species in forensic investigations and to estimate time since death in medico legal cases. This is the first report on the ultra-structural features of *H. ligurriens.* © 2021 Association for Advancement of Entomology

**KEYWORDS:** *Hemipyrellia ligurriens,* identification, micrograph, scanning electron microscope

Forensic examinations involving decomposed dead bodies need a careful scrutiny of the entomological evidence as the latter being very significant in calculating time of death when the natural postmortem signs of body hold no significance beyond certain level of putrefaction. Studies on insects of forensic significance is very much rudimentary in India except for a few reports on selective species (Bala and Singh, 2015; Bharti and Singh, 2003; Kulshrestha and Chandra, 1987; Rao *et al.,* 1984). *Hemipyrellia ligurriens* (Calliphoridae: Luciliinae) seems to be a synanthrope found in close association with human habitats, garbage dumps, decaying animal bodies and cadavers. The adult flies are generally considered as the vectors of many enteric pathogens (Sinha and Nandi, 2007). Kano and Sato (1952) reared this species on raw fish in Japan and described the larval stages. Ishijima (1967) described the third instar larvae of *H. ligurriens* while Bunchu *et al.* (2012) studied the morphological characters of larval stages of the species using light and stereo microscopy.

The oldest descriptions about all the three larval instars of *H. ligurriens* were provided by Tao (1927) and Knipling (1939). The keys provided in these works were of limited application owing to lack of species specific details. In recent works, blowfly species were identified based on the

* Author for correspondence

**II. NCBI GenBank submission details.**

| Sl.No | Species | Gene | Accession Numer |
|---|---|---|---|
| 1 | *C. megacephala* | Mitochondrial COI | MW522614.1 |
| 2 | *C. rufifacies* | Mitochondrial COI | OM019083.1 |
| 3 | *C. chani* | Mitochondrial COI | MW600494.1 |
| 4 | *H. ligurriens* | Mitochondrial COI | MN831480.1 |

**III.**

**Seasonal life cycle data of *C. megacephala, C. rufifacies, C. chani* and *H. ligurriens***

| Species | Season | Pre-oviposition period (days) | Eggs laid in a day (nos.) | Periodicity of egg laying (days) | Eggs laid during life span (nos.) | Duration of different life cycle stages (hrs) | | | | | | Total life cycle period from egg till the emergence of adult fly |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Incubation period of eggs (hrs) | Instar I | Instar II | Instar III | Post feeding Stage | Pupation stage | |
| *Chrysomya megacephala* | Monsoon | 9.33 ± 1.00 | 374.67 ± 8.53 | 4.33 ± 0.35 | 2796.33 ± 114.39 | 15.67 ± 2.52 | 16.67 ± 1.53 | 22.33 ± 2.08 | 45.33 ± 13.32 | 32.00 ± 5.29 | 95.00 ± 3.61 | 227.00 ± 22.52 |
| | Summer | 8.44 ± 0.88 | 344.78 ± 14.73 | 4.17 ± 0.25 | 2442.89 ± 80.12 | 12.00 ± 2.00 | 12.00 ± 0 | 17.00 ± 1.00 | 27.00 ± 4.36 | 23.00 ± 4.58 | 77.00 ± 5.57 | 168.00 ± 5.29 |
| | Winter | 10.33 ± 0.71 | 317.00 ± 7.16 | 4.67 ± 0.35 | 2217.11 ± 69.91 | 24.00 ± 2.65 | 23.00 ± 6.08 | 28.00 ± 5.29 | 49.00 ± 6.25 | 36.33 ± 10.69 | 125.67 ± 7.37 | 286.00 ± 23.26 |
| *Chrysomya ruffifacies* | Monsoon | 7.56 ± 0.53 | 281.00 ± 5.45 | 4.50 ± 0.35 | 1953.89 ± 22.70 | 17.00 ± 3.00 | 17.67 ± 2.08 | 20.00 ± 4.58 | 50.00 ± 4.58 | 29.33 ± 4.04b | 89.00 ± 1.00 | 223.00 ± 13.45 |
| | Summer | 7.67 ± 0.71 | 246.89 ± 11.68 | 4.17 ± 0.25 | 1849.11 ± 26.05 | 12.00 ± 1.73 | 16.67 ± 2.52 | 15.67 ± 3.51 | 15.00 ± 5.00 | 16.00 ± 4.00 | 73.00 ± 2.65 | 148.33 ± 6.43 |
| | Winter | 9.22 ± 0.67 | 215.33 ± 5.87 | 4.67 ± 0.35 | 1723.78 ± 12.18 | 18.33 ± 5.03 | 21.33 ± 3.51 | 32.67 ± 3.51 | 46.67 ± 7.02 | 36.67 ± 1.53 | 111.33 ± 3.79 | 267.00 ± 18.68 |
| *Chrysomya chani* | Monsoon | 7.78 ± 0.67 | 265.00 ± 6.27 | 4.56 ± 0.33 | 1735.11 ± 12.62 | 21.67 ± 4.51 | 20.33 ± 4.04 | 23.67 ± 5.13 | 42.00 ± 11.14 | 30.33 ± 10.21 | 108.00 ± 3.61 | 246 ± 20.08 |
| | Summer | 8.33 ± 1.00 | 234.44 ± 5.75 | 4.44 ± 0.33 | 1640.22 ± 5.40 | 15.67 ± 3.51 | 15.33 ± 2.89 | 16.67 ± 4.51 | 22.67 ± 4.51 | 25.33 ± 11.93 | 96.67 ± 1.53 | 192.33 ± 8.15 |
| | Winter | 10.11 ± 0.60 | 221.00 ± 5.72 | 4.78 ± 0.26 | 1627.22 ± 7.82 | 27.00 ± 5.57 | 18.67 ± 2.52 | 27.33 ± 2.52 | 42.00 ± 6.56 | 52.00 ± 10.58 | 153.33 ± 5.51 | 320.33 ± 24.03 |
| *Hemipyrelia ligurriens* | Monsoon | 9.44 ± 0.53 | 238.78 ± 3.96 | 3.67 ± 0.35 | 1531.56 ± 16.01a | 27.33 ± 4.04 | 18.00 ± 2.00 | 29.33 ± 5.51 | 62.00 ± 1.73 | 139.33 ± 14.64 | 154.33 ± 10.6 | 430.33 ± 33.5 |
| | Summer | 8.89 ± 0.78 | 221.33 ± 4.98 | 3.78 ± 0.36 | 1481.67 ± 9.99 | 17.67 ± 2.31 | 16.00 ± 1.00 | 16.33 ± 1.53 | 55.33 ± 5.69 | 81.00 ± 9.85 | 113.67 ± 8.15 | 300.00 ± 23.07 |
| | Winter | 10.44 ± 0.53 | 187.11 ± 4.46 | 3.56 ± 0.39 | 1340.56 ± 18.3 | 35.00 ± 5.29 | 16.67 ± 1.53 | 28.67 ± 4.51 | 59.67 ± 3.51 | 125 ± 46.18 | 192.33 ± 9.61 | 457.33 ± 54.31 |