



THAMIRIS GABRIELLE BIBIANO

**Sublethal effects of an anxiolytic and two alcoholic substances in
the development of *Chrysomya albiceps* and *Chrysomya
megacephala* (Diptera: Calliphoridae)**

LAVRAS-MG

2024

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, para a obtenção do título de Mestre.

Prof. Dr. Khalid Haddi

Orientador

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**Sublethal effects of an anxiolytic and two alcoholic substances in the development of
Chrysomya albiceps and *Chrysomya megacephala* (Diptera: Calliphoridae)**

**Efeitos subletais de um ansiolítico e duas substâncias alcoólicas no desenvolvimento de
Chrysomya albiceps e *Chrysomya megacephala* (Diptera: Calliphoridae)**

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, para a obtenção do título de Mestre.

APROVADA em 28 de julho de 2024.

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Orientador

LAVRAS-MG

2024

*Aos meus pais, Carlos e Elisângela, e à minha irmã, Giovana, pelo amor incondicional,
apoio e incentivos.*

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“Conheça todas as teorias, domine todas as técnicas, mas ao tocar uma alma humana, seja apenas outra alma humana.”

“Know all theories, master all techniques, but when touching a human soul, be just another human soul.”

“Conozca todas las teorías, domine todas las técnicas, pero al tocar un alma humana, sea solo otra alma humana.”

(Carl G. Jung)

RESUMO

A entomologia forense utiliza-se dos insetos para desvendar crimes. As moscas são os insetos mais encontrados colonizando os cadáveres para o desenvolvimento do seu ciclo. O consumo de diversas substâncias tais como bebidas alcoólicas, fármacos e drogas de abuso tem aumentado nas últimas décadas. Quando ocorre uma morte, tais substâncias permanecem nos tecidos, onde insetos necrófagos irão se alimentar. Através da toxicologia é possível detectar e identificar compostos químicos, porém o estágio de decomposição é um fator que impede esse procedimento. Sabendo-se que os insetos estão presentes no corpo, sua utilização para tais fins é de grande valia. Dessa maneira, o intuito desse trabalho foi avaliar alterações ocorridas no ciclo de desenvolvimento das espécies *Chrysomya albiceps* e *C. megacephala* ao serem expostas a um benzodiazepínico e ao etanol. Larvas de segundo e terceiro instar de *C. megacephala* foram coletadas no setor de compostagem da UFLA e foram transferidas para o Laboratório de Entomologia Molecular e Ecotoxicologia (MEET) para estabelecer a criação. Moscas adultas de *C. albiceps* foram coletadas na Fundação Oswaldo Cruz (FIOCRUZ-RJ), posteriormente identificadas no Laboratório Integrado de Simulídeos e Oncocercoses & Entomologia Médica e Forense (LSOEMF- ICO) onde obteve-se uma geração de juvenis até o estágio de pupa para ser transferido (nº89235-1 SISBio) ao MEET, para estabelecer a criação. Manteve-se as criações em sala climatizada ($27\pm 1^\circ\text{C}$, 12L:12D, UR $60 \pm 10\%$). Esperou-se os indivíduos da F2 eclodidos em condições laboratoriais para a condução dos experimentos de toxicologia. Para o tratamento benzodiazepínico utilizou-se três concentrações: 0,0625; 0,125 e 0,250 $\text{mg}\cdot\text{mL}^{-1}$ do Clonazepam ($2,5\text{mg}\cdot\text{mL}^{-1}$) sendo aplicado 1mL em cada tratamento, e um grupo controle. O etanol foi utilizado de duas maneiras Corote® e etanol puro (97,0%) que foi diluído a etanol (39%), os volumes utilizados: 50, 100, 150, 200 μL , também se utilizou água deionizada e um grupo controle, sendo aplicado 0,2 mL em cada tratamento. Utilizou-se carne moída tratada com as substâncias como substrato de alimentação. Foram dispostas 30 larvas por repetição, sendo quatro repetições por tratamento. Os parâmetros avaliados foram os estádios juvenis e adulto e suas respectivas durações. Para clonazepam os resultados demonstraram a redução nos pesos, em todos estádios e um atraso no tempo de desenvolvimento na menor concentração. Para o Etanol os resultados demonstraram alterações no tempo de desenvolvimento e no peso das pupas. Assim, concluímos que as substâncias utilizadas causam respostas fisiológicas nas moscas durante seu desenvolvimento e tais alterações podem interferir na estimação de IPM.

Palavras-chave: entomotoxicologia; Chrysomyinae; hormese.

GENERAL ABSTRACT

Forensic entomology uses insects to help crimes. Flies are the most commonly found colonizing corpses for their development cycle. In recent decades, the consumption of various substances has increased. These include alcoholic beverages, pharmaceuticals, and drugs of abuse. When a death occurs, such a substance remains in the tissues, where necrophagous insects will feed. Through toxicology, it is possible to detect and identify chemical compounds, but the stage of decomposition is a factor that prevents this procedure. Knowing that insects are present in the body, their use for such purposes is of great value. Thus, this work aimed to evaluate the changes that occurred in the developmental cycle of the species *Chrysomya albiceps* and *C. megacephala* when exposed to benzodiazepine and ethanol. Second and third instar larvae of *C. megacephala* were collected in the composting sector of UFLA and were transferred to the Laboratory of Molecular Entomology and Ecotoxicology (MEET) to establish the creation. Adult flies of *C. albiceps* were collected at the Oswaldo Cruz Foundation (FIOCRUZ-RJ), later identified at the Integrated Laboratory of Simuliids and Onchocerciasis & Medical and Forensic Entomology (LSOEMF- ICO), where a generation of juveniles up to the pupal stage was obtained to be transferred (n°89235-1 SISBio) to M.E.E.T. to establish the creation. The creations were kept in a climatized room ($27\pm 1^\circ\text{C}$, 12L:12D, RH $60\pm 10\%$). The F2 individuals hatched under laboratory conditions were awaited for the performance of toxicological experiments. Three concentrations were used for benzodiazepine treatment: 0.0625; 0.125 and 0.250 $\text{mg}\cdot\text{L}^{-1}$ of clonazepam ($2.5\text{ mg}\cdot\text{mL}^{-1}$), applied in 1mL in each treatment, and a control group. Ethanol was used in two ways Corote[®] and pure ethanol (97.0%) diluted to ethanol (39%), the volumes used were 50, 100, 150 and 200 μL , deionized water was also used and a control group, being applied 0.2 mL in each treatment. Ground meat treated with the substances was used as the food substrate. Thirty larvae were placed per replicate, with four replicates per treatment. The parameters evaluated were the juvenile and adult stages and their respective durations. For clonazepam, the results showed a reduction in weight at all stages and a delay in development time at the lowest concentration. For ethanol, the results showed changes in development time and weight of the pupae. Thus, we conclude that the substances used cause physiological responses in flies during their development and such changes may interfere with the estimation of PMI.

Keywords: entomotoxicology; Chrysomyinae; hormesis.

Impactos sociais, tecnológicos, econômicos e culturais

A entomologia forense, que se dedica ao estudo de insetos em contextos investigativos, apresenta impactos significativos em diversas áreas. Está associada no âmbito da Justiça e Segurança, desempenha um papel crucial na elucidação de crimes, para a determinação do intervalo pós-morte e na análise de cenas de crimes, auxilia na detecção de substâncias presentes em cadáveres. Também permite a extração de material genético para identificação de pessoas. Tais contribuições são essenciais para o sistema de justiça, auxiliando na condenação de culpados e na absolvição de inocentes. Na Saúde Pública as aplicações estendem-se ao controle de surtos de doenças transmitidas por insetos, proporcionando uma compreensão mais aprofundada e estratégias de mitigação para a propagação de enfermidades, além de estar diretamente associada aos crimes de negligência e maus tratos. Ao Meio Ambiente as análises de insetos podem revelar informações valiosas sobre a saúde ambiental e a presença de poluentes ou substâncias tóxicas em determinados ecossistemas, abordando insetos bioindicadores. Além de que na Educação e Pesquisa, as descobertas no campo da entomologia forense impulsionam avanços científicos e tecnológicos, contribuindo significativamente para a educação e a formação de novos profissionais na área. Tais impactos evidenciam a relevância da entomologia forense não apenas para a ciência forense, mas também para a sociedade em geral. Deste modo, este trabalho identificou alterações durante o estágio larval de moscas que são utilizadas para a estimativa do intervalo pós morte (IPM). Tais alterações deram-se devido a utilização do clonazepam, um benzodiazepínico comumente utilizado no país, bem como o etanol que é uma bebida alcoólica mundialmente consumida. Os efeitos ocorridos nos desenvolvimentos dessas larvas indicam que alterações que podem afetar diretamente o cálculo do IPM. Além disso, os resultados obtidos indicam o efeito de hormese, campo ainda não explorado nas ciências forenses, sendo promissor na área da pesquisa e educação.

Social, technological, economic and cultural impacts

Forensic entomology, the study of insects in an investigative context, significantly impacts several areas. It is associated with justice and security and plays a crucial role in solving crimes, determining the post-mortem interval, and analyzing crime scenes, helping to detect substances present in corpses. It also enables the extraction of genetic material for identification purposes.

These contributions are essential to the justice system, helping to convict the guilty and exonerate the innocent. In public health, applications extend to controlling outbreaks of insect-borne diseases, providing a deeper understanding and mitigation strategies for the spread of disease, as well as being directly linked to crimes of neglect and abuse. For the environment, insect analysis can provide valuable information about environmental health and the presence of pollutants or toxic substances in specific ecosystems by studying bioindicator insects. In education and research, discoveries in forensic entomology drive scientific and technological advances and contribute significantly to the education and training of new professionals in the field. These impacts highlight the importance of forensic entomology to forensic science and society in general. For example, this work identified changes in the larval stage of flies used to estimate the post-mortem interval (PMI). These changes were due to the use of clonazepam, a benzodiazepine commonly used in Brazil, and ethanol, an alcoholic beverage consumed worldwide. The effects on the development of these larvae indicate that changes can directly affect the calculation of the MPI. In addition, the results obtained indicate the effect of hormesis, an area not yet explored in forensic science and promising in the field of research and education.

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FIRST PART: THEORETICAL FRAMEWORK

GENERAL INTRODUCTION

Forensic entomology is a science that involves the study of arthropods and insects associated with corpses in criminal investigations (Oliveira-Costa, 2008). Although often associated with crimes against life, this science can also be useful in situations involving living individuals, especially when there is a suspicion of negligent care, as in cases resulting from myiasis (Amendt; Krettek; Zehner, 2004). It is divided into four main areas (Lord; Stevesson, 1986; Neto, 2023):

- Urban Entomology: the insects that are present can cause damage to the furniture / furnishings;
- Stored Product Entomology: the presence of insects causes an infestation or contamination of the products;
- Environmental Entomology: the insects are used as biological indicators that reflect the environmental quality of an area and are used in cases of environmental violations;
- Medico-legal Entomology: the study is concerned with the examination of cases where insects are present in decomposing bodies, as well as instances of mistreatment and neglect of humans or animals,
- Entomogenetic: the genetic material in the tissue or blood present in the insect's digestive system can be analyzed to identify the individual;
- Entomotoxicology: the insects are used to detect possible substances in corpses and the effects of these substances on their development cycle.

Medico-Legal Entomology, based on the insects found on the body and at the crime scene, allows the estimation of the time of death of the victim through the PMI (post-mortem interval) calculation, but various factors can influence this calculation. Thus, in order to detect the presence of substances in a corpse, entomotoxicology is used to detect substances through the insects present in the cadavers (Silva; Reis, 2012; Thé, 2021; Tomberlin *et al.*, 2011; Vairo; Moura, 2021).

Forensic toxicological examinations aim to detect, identify and quantify different substances in different situations, and these substances can be classified as toxic agents, poisons, drugs, narcotics and xenobiotics (Martinis; Dorta; Costa, 2018). In cases, when the body is in an advanced state of decomposition and traditional toxicological tests are not

possible, immature insects can be an alternative to obtain such results, taking into account the bioaccumulation of a substance along a food chain (Andréa, 2008).

Consumption and use of various legal and illegal substances, drugs, abuse of medications, insecticides and other toxins have increased over the years, creating a need for studies focused on entomotoxicology, as exposure to these substances has resulted in many deaths worldwide due to poisoning or overdose (Chopi *et al.*, 2019; Josh; Kumar, 2020). Excessive alcohol consumption and drug abuse have been identified as global problems in recent years (Who, 2022, 2023).

Therefore, the objective of this research was to evaluate the physiological responses of the species *Chrysomya megacephala* and *C. albiceps* when exposed to the benzodiazepine (clonazepam), Corote[®] and pure ethanol, through the ingestion of a treated diet, during the juvenile to adult stages.

1. THEORETICAL FRAMEWORK

1.1 Forensic Entomology

Insects perform a variety of ecological functions, including the decomposition of organic matter. This behavior has been observed by ancient civilizations for thousands of years, and necrophagous insects such as flies and beetles have been found in mummies from ancient Egypt (Greenberg, 1988; Pujol-Luz *et al.*, 2008). However, the first documented report of the use of insects to solve a crime occurred in China in 1235, when a corpse was found in a rice plantation. To solve the case, Tz'u, a lawyer and investigator, asked the workers to place their sickles on the ground. The one that attracted flies due to traces of blood indicated the suspect who later confessed the crime (Oliveira-Costa, 1990 apud Carvalho, 2003; Benecke, 2001).

Despite the facts already mentioned, forensic entomology applied in the post-mortem interval (PMI), as we know it, is attributed to the French doctor Bergeret (1855), who conducted studies of the cadaveric fauna related to the state of decomposition of a corpse, estimating the probable time of death (Pujol-Luz *et al.*, 2008; Santana; Vilas Boas, 2012). For a long time, the work was abandoned, and studies became scarce. It was not until 1894 that the French entomologist Mégnin resumed these studies and published the book “The fauna of corpses” (“La faune des cadavres”), which made the knowledge of this field known worldwide (Oliveira-Costa; Lopes, 1999; Santana; Vilas Boas, 2012).

Even with Mégnin’s resumption, once again put aside until Leclercq resumed and in 1969 published “Entomology and Legal Medicine”. In less than two decades, the studies resumed with publications such as “A Manual of Forensic Entomology” by Smith (1986) (Pujol-Luz *et al.*, 2008). Since then, various research groups focused on forensic entomology have consolidated and it is currently a study of worldwide interest being applied in various countries (Zhu *et al.*, 2007; Lei *et al.*, 2019).

In Brazil, research began only in 1908, with pioneers Oscar Freire in the state of Bahia, who developed work on corpses and carcasses, and Edgard Roquette Pinto in the state of Rio de Janeiro, who studied the carcass fauna (Freire, 1923b; Pujol-Luz *et al.*, 2008; Silva; Reis, 2012). Following this, other researchers such as Herman Lüderwaldt, Samuel Pessôa and Frederico Lane carried out descriptive work on necrophagous beetles in São Paulo between 1911 and 1941 (Pujol-Luz *et al.*, 2008; Santana; Vilas Boas, 2012; Neto, 2023). Within the same period, Freire produced other publications, in 1914 "Some notes for the study of the cadaveric fauna of Bahia" ("Algumas notas para o estudo da fauna cadavérica da Bahia"),

mentored Belfort de Matos (1919), and in 1923, after his death, the work "Brazilian cadaveric fauna" ("Fauna cadavérica brasileira") was published. (Freire, 1923a, 1923b; Pujol-Luz *et al.*, 2008; Neto, 2023).

After the studies of Pessôa & Lane (1941), Brazil went through a scientific void, as the development of research and the publication of works in the field or related to forensic entomology became scarce, until in 1987, in the state of São Paulo, Monteiro-Filho & Penereiro carried out and published a work on entomological succession, marking the return of studies in the field (Pujol-Luz *et al.*, 2008; Neto, 2023). From this resumption, other researchers such as Lopes (1984), Mello (1989), Guimarães (1982) and Papavero (1982), started their work in different lines such as biology, ecology and taxonomy of Diptera. Since then, the following decades have been the stage for the development of more research in different areas considering the diversity of necrophagous insects, and more researchers began to stand out along with their teams, such as Linhares & Carvalho (Pujol-Luz *et al.*, 2008; Neto, 2023).

In the country, research in forensic entomology have progressed. Several works have been developed in the different subfields, thus raising interest in the subject. The book "Forensic Entomology - When Insects are Traces" ("Entomologia Forense - Quando os insetos são vestígios") by Oliveira-Costa (2003), a criminal expert and researcher from the State of Rio de Janeiro, was the first national compilation that brought together global literature, allowing the dissemination and standardization of the language between the criminal expert directly involved in solving cases and the researcher who develops the research, thus expanding knowledge in the field. Shortly after, in 2006, a manual "Collection of insect samples for forensic purposes" ("Coleta de amostras de insetos para fins forenses") was published by Miranda *et al.* (Pujol-Luz *et al.*, 2008; Neto, 2023).

In the last decade, Oliveira-Costa has developed more materials, expanding and collaborating in the dissemination of forensic entomology in the country. In 2013, Oliveira-Costa published her second book "Expert Insects: Forensic Entomology in Brazil" ("Insetos Peritos": A Entomologia Forense no Brasil"). Eight years later, the criminal expert and researcher from the State of Bahia, Thé (2021) published "Forensic Entomology: Insects and Criminal Expertise" ("Entomologia Forense: Os Insetos e a Perícia Criminal") and researchers Vairo and Moura (2021) published "Forensic Entomology in Practice: from Laboratory to Use of Traces" ("Entomologia forense na prática: do laboratório à utilização dos vestígios") (Neto, 2023). The most recent book published by Neto (2023) is "Necrophagous Diptera of Brazil:

contributions to forensic entomology" ("Dípteros necrófagos do Brasil: contribuições à entomologia forense").

1.2 Entomotoxicology

Subfields of forensic entomology, such as urban, environmental, and medical-legal, can be related to toxicology, which is also in the field of forensic sciences. This union consists of Entomotoxicology, which is responsible for covering issues such as the effects of substances related to insects, such as assessing the toxic effects of chemical compounds on the behavior, ecology, and physiology of insects (Silva; Reis, 2012; Thé, 2021; Tomberlin *et al.*, 2011; Vairo; Moura, 2021). It also helps in the detection and identification of xenobiotics that may have caused the death of the individual by overdose, through the larvae exposed to this substance present in the body in tissues and fluids already decomposed. (Amendt; Krettek; Zehner, 2004; Goff; Lord, 1994; Hodecek, 2020; Introna *et al.*, 2001; Salimi *et al.*, 2018).

Several practical applications of entomotoxicology have been used in criminal investigations and research. The detection of substances in scavenging insects provides important answers in cases where there has been exposure to toxic substances, as it can help to establish an IPM with greater precision, as well as helping to reconstruct the events that led to the death. The ease with which toxin concentrations can be analyzed in insects makes it possible to carry out analyses using various materials, such as the insect itself (larvae, pupae, adults) and secondary traces, puparia, exudates and feces (Goff *et al.*, 1991; Candela; Aventaggiato, 2001; Wood *et al.*, 2003).

A variety of techniques can be performed from the collection of insects present at the site, taxonomic identification of the specimens and stage of development. An example of this is toxicological analysis for the detection of substances, such as liquid chromatography, mass spectrometry and immunological assays (Pontes *et al.*, 2022). It is important to identify the chemical compounds because they have a direct effect on the insect cycle, slowing or accelerating the larval development process and altering mass and body length (Josh; Kumar, 2020; Thyssen *et al.*, 2021).

A wide variety of compounds and techniques have been used to understand a wide range of responses. Methods of immunoassays, high performance liquid chromatography (HPLC), liquid chromatography coupled with mass spectrometry (LC-MS), and gas chromatography coupled with mass spectrometry (GC-MS), already widely used in forensic toxicology, are now being applied to entomological samples, consolidating analyses and allowing results to be

obtained in the absence of commonly used traces (Chophi *et al.*, 2019; Istiqomah *et al.*, 2020; Joshi; Kumar, 2020; Tracqui *et al.*, 2004). It is worth noting that the number of studies involving legal and illicit drugs is quite significant due to the increase in their consumption (Who, 2019, 2023).

The first entomotoxicology article was published in 1980 by Beyer and collaborators and was about the use of phenobarbital (Chophi *et al.*, 2019; Istiqomah *et al.*, 2020). The case study, in which larvae of *Cochliomyia macellaria* (Diptera: Calliphoridae) were analyzed to perform a gas chromatography analysis, detected a concentration of phenobarbital present in the larvae collected from a skeletonized corpse suspected of suicide. Since then, several studies have been conducted in this area around the world.

In Brazil, entomotoxicology is still a relatively new field, with just over 20 years of existence in the country, but it has been gaining space in forensic science research over the years. In 2001, Carvalho *et al.*, published a study with the sedative diazepam in blowflies *Chrysomya albiceps* and *C. putoria*, in which this medication caused changes in the development of the species. Since then, several relevant works have been developed by Brazilian researchers, with emphasis on the area (Carvalho *et al.*, 2001; Moura, 2018; Gonçalves, 2022) (Table 1).

In studies, diets have been used as the insects' nutritional source. These can be classified as natural, when meat is used, or artificial diets during the larval stage (Estrada *et al.*, 2009; Moretto, 2011; Ferrari *et al.*, 2008; Thyssen *et al.*, 2011; Pereira, 2011; Silvério, 2008; Lima, 2009; Oliveira *et al.*, 2009; Souza *et al.*, 2011), or even the use of animals such as pigs, rats and rodents that undergo an overdose induction (Martins, 2009). Both techniques allow diets in laboratory conditions reduce the putrefactive odor of the substrate in the environment and also allow control of environmental variation and the concentrations used. In the case of animals exposed to substances while still alive, these are experiments carried out in the field, in places where there is no control of environmental variables, but which allow the substances to be metabolized so that they are subsequently ingested by insects, which is a result close to reality (Santos, 2013; Cipriani, 2020).

The physiological and morphological parameters of the post-embryonic development, time of pupariation and pupation, the duration of each stage, the change in weight and length of the larvae, the analysis of the survival rate of the larvae, the sex ratio of the emerged adults, are the most studied under controlled conditions. The behavioral parameters, such as the attractiveness of the species during the stages of decomposition, are already available in field

studies. However, parameters such as mortality and larval duration can also be analyzed. According to Silva *et al.* (2017) and Chopi *et al.* (2019), it is possible to detect drug concentrations more reliably using insects compared to decomposing tissues, as the substances can accumulate in the digestive tract and cuticle of the larvae while the soft tissue degrades.

The advances and discoveries in this field have been fundamental, as it is a recently explored area. However, some gaps still need to be filled regarding the diversity of substances that can affect the developmental stages and processes of insect physiology. The ingestion, metabolism and excretion of substances, as well as the influence of environmental factors related to these effects, can affect the biodiversity of scavenging insects (Chopi *et al.*, 2019). Researchers also face the difficulty of standardizing protocols related to methodology in the conduction of experiments and analysis of results, which does not affect the comparison of results obtained, but the impact that the study will have (Silva *et al.*, 2017).

Table 1. List of entomotoxicology studies conducted worldwide with their respective substances, species, and researchers.

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Antidepressants					
Amitriptyline	<i>Parasarcophaga ruficornis</i> ²	L, P	Delay in larval and pupal stages. Significant pupal mortality. Detection of substance.	United States	GOFF <i>et al.</i> , 1993
	<i>Calliphora vicina</i> ¹	L	No detection of substance	Scotland	WILSON; HUBBARD; POUNDER, 1993
	Coleoptera: Dermestidae Diptera: Phoridae	Pp, Ex	Detection of substance	United States	MILLER <i>et al.</i> , 1994
	<i>Calliphora vicina</i> ¹	L, P	Delay in the stages and all cycle durations	United Kingdom	SADLER <i>et al.</i> , 1995
	<i>Calliphora vicina</i> ¹	L	Significant effect in evaluated parameter weight. Detection of substance	United Kingdom	SADLER <i>et al.</i> , 1997
	<i>Calliphora vicina</i> ¹	A	Accelerated the attraction	Iraq	KADHIM, HILAL, OKAILY, 2016
	<i>Chrysomya albiceps</i> ¹	L	Accelerated the development	Turkey	AÇIKGÖZ, 2018

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Citalopram	<i>Chrysomya megacephala</i> ¹	L	Effect on development rate and duration of larval stage	Brazil	MORETTO, 2011
	<i>Chrysomya albiceps</i> ¹	L	Accelerated the development	Turkey	AÇIKGÖZ, 2018
Clomipramine	Diptera <i>n.s.</i>	L	Case study	France	KINTZ <i>et al.</i> , 1990
	<i>Lucilia sericata</i> ¹	L	Detection of substance	Italy	CAMPOBASSO <i>et al.</i> , 2004
Fluoxetine	<i>Chrysomya megacephala</i> ¹	L	Effect in development of the larvae	Brazil	ALONSO, 2015
	<i>Chrysomya putoria</i> ¹				
	<i>Dermestes maculatus</i> ³	L, P, A	Detection and quantitation of substance	Argentina	ZANETTI <i>et al.</i> , 2016
	<i>Lucilia sericata</i> ¹				ZANETTI;
	<i>Sarcophaga crassipalpis</i> ²	L, P, Pp	Detection and quantitation of substance	Argentina	FERRERO; CENTENO, 2019
Nortriptyline	Coleoptera: Dermestidae	Pp, Ex	Detection of substance	United States	MILLER <i>et al.</i> , 1994
	Diptera: Phoridae				
	<i>Calliphora vicina</i> ¹	L	Significant effect in evaluated parameter weight. Detection of substance	United Kingdom	SADLER <i>et al.</i> , 1997

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Nortriptyline	<i>Lucilia sericata</i> ¹	L	Detection of substance	Italy	CAMPOBASSO <i>et al.</i> , 2004
Temazepam	<i>Calliphora vicina</i> ¹	L, P	Delay in the stages and all cycle	United Kingdom	SADLER <i>et al.</i> , 1995
Trazodone + Trimipramine	<i>Calliphora vicina</i> ¹	L, P	Delay in the stages and all cycle	United Kingdom	SADLER <i>et al.</i> , 1995
Antibiotics					
Ampicillin	<i>Phaenicia sericata</i> ¹	L, P, A	No significant impact	United States	SHERMAN; WYLE; THRUPP, 1995
Cefazolin	<i>Phaenicia sericata</i> ¹	L, P, A	Reduction in larval survival. Significant mortality	United States	SHERMAN; WYLE; THRUPP, 1995
Ceftizoxime	<i>Phaenicia sericata</i> ¹	L, P, A	No significant impact	United States	SHERMAN; WYLE; THRUPP, 1995
Ceftriaxone	<i>Calliphora vomitoria</i> ¹	L	No significant impact	Germany	PREUßER <i>et al.</i> , 2021
	<i>Lucilia sericata</i> ¹	L	Significant mortality	Germany	PREUßER; FISCHER; JURETZEK, 2023
Ciprofloxacin	<i>Phaenicia sericata</i> ¹	L, P, A	No significant impact	United States	SHERMAN; WYLE; THRUPP, 1995

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Ciprofloxacin	<i>Chrysomya putoria</i> ¹		No significant impact	Brazil	FERRAZ <i>et al.</i> , 2014
Clindamycin	<i>Phaenicia sericata</i> ¹	L, P, A	No significant impact	United States	SHERMAN; WYLE; THRUPP, 1995
Gentamicin	<i>Phaenicia sericata</i> ¹	L, P, A	Reduction in larval survival. Maturation of the surviving pupae to adults	United States	SHERMAN; WYLE; THRUPP, 1995
	<i>Chrysomya putoria</i> ¹	L	Significant mortality	Brazil	FERRAZ <i>et al.</i> , 2016
Levofloxacin	<i>Calliphora vomitória</i> ¹	L	No significant impact	Germany	PREUßER <i>et al.</i> , 2021
	<i>Lucilia sericata</i> ¹	L	Significant mortality	Germany	PREUßER; FISCHER; JURETZEK, 2023
Mezlocillin	<i>Phaenicia sericata</i> ¹	L, P, A	No significant impact	United States	SHERMAN; WYLE; THRUPP, 1995
Vancomycin	<i>Phaenicia sericata</i> ¹	L, P, A	No significant impact	United States	SHERMAN; WYLE; THRUPP, 1995

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Hard Drugs					
Cocaine	<i>Boettcheriska peregrina</i> ²	L	Accelerated the development	United States	GOFF; OMORI; GOODBROD, 1989
	Coleoptera <i>n.s.</i>	L	Case study	United States	MANHOFF <i>et al.</i> , 1991
	Calliphoridae <i>n.s.</i>	L	Case study	Mexico	NOLTE; PINDER; LORD, 1992
	<i>Lucilia sericata</i> ¹	L	Detection of substance	Italy	CAMPOBASSO <i>et al.</i> , 2004
	<i>Ornidia obesa</i> ¹	-	Change in insect attractiveness in succession	Brazil	MARTINS <i>et al.</i> , 2010;
	<i>Chrysomya albiceps</i> ¹ <i>Chrysomya putoria</i> ¹	L	Accelerated the development	Brazil	CARVALHO; LINHARES; PALHARES, 2012
	<i>Chrysomya megacephala</i> ¹	L	Detection of substance	Brazil	DA SILVA ANTONIO, 2013

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Cocaine	<i>Calliphora vomitoria</i> ¹	L	Fed larvae developed less in length and weight. Increased the length of the second and third instar stages, but led to the shortening of pupation and accelerated eclosion	United Kingdom	WOOD; PYPER; CASALI, 2022
Ecstasy (MDMA+MDA)	<i>Parasarcophaga ruficornis</i> ²	L, P, Pp	Accelerated the development. Significant mortality. Detection of substance	United States	GOFF <i>et al.</i> , 1997
Ecstasy	<i>Chrysomya albiceps</i> ¹ <i>Chrysomya megacephala</i> ¹ <i>Chrysomya putoria</i> ¹	L	Accelerated the development rate of <i>C. megacephala</i>	Brazil	LIMA, 2009
Heroin	<i>Sarcophaga (Boettcherisca) peregrina</i> ²	L, P	Accelerated stage larval, but delay in the pupariation	United States	GOFF <i>et al.</i> , 1991

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Heroin	Diptera: Calliphoridae, Muscidae, Sepsidae, Milichiidae; Coleoptera: Melyridae, Carabidae, Coccinellidae. Hymenoptera: Formicidae	L, P, A	No effects on insect succession patterns, but did influence carcass decomposition stages	Saudi Arabia	AL-QAHTNI <i>et al.</i> , 2021
	<i>Chrysomya albiceps</i> ¹	-	Impact on succession pattern	Saudi Arabia	MASHALY; AL-KHALIFA, AL-QAHTNI, 2020
	<i>Calliphora vomitoria</i> ¹	L	Change in weight and size. Longest pupation stage	United Kingdom	WOOD; PYPYER; CASALI, 2022
	<i>Boettcherisca peregrina</i> ²		Accelerated the development	United States	GOFF <i>et al.</i> , 1991
Methamphetamine	<i>Parasarcophaga ruficornis</i> ²	L, P	Accelerated the development	United States	GOFF <i>et al.</i> , 1992

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Methamphetamine	<i>Calliphora vomitória</i> ¹	L	Accelerated the development	United States	GOFF; BROWN; OMORI, 1992
	<i>Chrysomya albiceps</i> ¹				
	<i>Chrysomya megacephala</i> ¹	L	Delayed the development rate of <i>C. putoria</i>	Brazil	LIMA, 2009
	<i>Chrysomya putoria</i> ¹				
	<i>Calliphora vomitoria</i> ¹	L	Delay in the time from egg to adult. Mortality in during the pupation period. Lengths of larvae and pupae were significant	Australia	MAGNI <i>et al.</i> , 2014
	<i>Calliphora stygia</i> ¹	L, P, A	Accelerated the development	Australia	MULLANY <i>et al.</i> , 2014
	Diptera, Coleoptera		Detection of substance	Indonesia	NOFIRA, 2023
Alcohol					
Ethanol	<i>Phormia regina</i> ¹	L	Decrease of growth	United States	TABOR <i>et al.</i> , 2005
	<i>Phormia regina</i> ¹	L	Accelerated of growth	United States	MONTHEI, 2009
Thiopental	<i>Calliphora vicina</i> ¹	L, P	Significant effect in evaluated parameter weight. Defection of substance	United Kingdom	SADLER <i>et al.</i> , 1997

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Thiopental	<i>Chrysomya albiceps</i> ¹				
	<i>Chrysomya megacephala</i> ¹	L	Delay in development and high mortality	Brazil	SOTO, 2008
Alcohol n.e.	<i>Chrysomya putoria</i> ¹				
	Coleoptera				
	Diptera Hymenoptera	-	Impact on the attractiveness of insects present in the succession	Saudi Arabia	AL-KHALIFA, MASHALY; AL-QAHTNI, 2021
Insecticides/Pesticides					
Allout	<i>Sarcophaga haemorrhoidalis</i> ²	L, A	Accelerated the growth of larvae	India	ZEHRA; MISHRA, 2020
Cypermethrin	<i>Chrysomya albiceps</i> ¹	L	Delay in the development	Nigeria	EKRAKENE; ODO, 2017

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Diazinon	<i>Chloroprocta</i>				
	<i>idioidea</i> ¹				
	<i>Chrysomya albiceps</i> ¹				
	<i>Chrysomya</i>				
	<i>megacephala</i> ¹				
	<i>Chrysomya putoria</i> ¹				
	<i>Cochliomyia</i>	-	Influence of insect attractiveness on colonization	Brazil	CAVALCANTE, 2017
	<i>hominivorax</i> ¹				
	<i>Cochliomyia macellaria</i> ¹				
<i>Lucilia eximia</i> ¹					
<i>Paralucilia</i>					
<i>paraensis</i> ¹					
Dimethoate	<i>Chrysomya indiana</i> ¹				
	<i>Chrysomya</i>				
	<i>megacephala</i> ¹	L	Delay in the development	India	ABD AL-GALIL <i>et al.</i> , 2021
	<i>Chrysomya rufifacies</i> ¹				
<i>Chrysomya suffranae</i> ¹					

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Paraquat Dichloride	Diptera <i>n.s.</i>	L	Detection of substance in larvae tissue	Malaysia	LAWAI; ABDUL; NGAINI, 2015;
	<i>Chrysomya rufifacies</i> ¹	-	Detection in tissue with impact on succession pattern	Malaysia	MAHMOOD <i>et al.</i> , 2015
Malathion	<i>Chrysomya megacephala</i> ¹	L	Case study	United States	GUNATILAKE; GOFF, 1989;
	<i>Chrysomya rufifacies</i> ¹				
	<i>Chrysomya megacephala</i> ¹	L	Delay in stage larval. Mortality significant. Detection of substance in larvae tissue	Malaysia	RASHID <i>et al.</i> , 2008
	<i>Chrysomya megacephala</i> ¹	L, P	Insect succession and the development of carrion flies	Chine	YAN-WEI <i>et al.</i> , 2010
	Coleoptera <i>n.s.</i>				
	<i>Chrysomya megacephala</i> ¹	L	Altered decomposition rates and species diversity in colonization	Chine	SHI <i>et al.</i> , 2010;
	<i>Chrysomya rufifacies</i> ¹				
<i>Chrysomya megacephala</i> ¹	L	Significant alterations in damage to the larvae midgut and retard development	Egypt	BAKR <i>et al.</i> , 2012	

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Maxo	<i>Sarcophaga haemorrhoidalis</i> ²	L	Delay in the growth	India	ZEHRA; MISHRA, 2020
Permethrin	<i>Lucilia sericata</i> ¹	L	No significant impact	Nigeria	EKRAKENE; ODO, 2017
Terbufos	Diptera <i>n.s.</i>	-	Accelerated carcass decomposition. Affective the species richness and abundance of necrophagous flies. Delay in the succession pattern. Mortality significative of the dipterafaune	Brazil	JALES <i>et al.</i> , 2020
Hormones					
Corticoids	<i>Sarcophaga tibialis</i> ²	L	Delay in the stage	South Africa	MUSVASVA <i>et al.</i> , 2001
Estrogenic	<i>Chrysomya megacephala</i> ¹	-	Acceleration in the growth process, shortening pupation time. Slowed the growth	Brazil	SANTOS, 2013
Nandrolone decanoate	<i>Chrysomya albiceps</i> ¹ <i>Chrysomya megacephala</i> ¹ <i>Chrysomya putoria</i> ¹	L	Accelerated the development	Brazil	SOUZA <i>et al.</i> , 2011

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Levonorgestrel	<i>Chrysomya chloropyga</i> ¹	L	Increase in larval size, but with no significant effect on development	Brazil	GUIMARÃES, 2019
Methohexital sodium (hydrocortisone sodium succinate)	<i>Sarcophaga tibialis</i> ²	L	Accelerated stage	South Africa	MUSVASVA <i>et al.</i> , 2001
Progesterone	<i>Chrysomya chloropyga</i> ¹	L	Mortality significant, but the developmental rate not affected	South Africa	DA SILVA; VILLET, 2006
	<i>Chrysomya megacephala</i> ¹		Acceleration in the growth process, reduced pupation time, and slowed growth	Brazil	SANTOS, 2013
Testosterone	<i>Chrysomya albiceps</i> ¹	L	No significant impact	Brazil	FERRARI <i>et al.</i> , 2008
Opioids/Opiates					
Alimemazine	Diptera <i>n.s.</i>	L	Case study	France	KINTZ <i>et al.</i> , 1990
Bromazepam	<i>Lucilia sericata</i> ¹	L	Detection of substance	Italy	CAMPOBASSO <i>et al.</i> , 2004
Butylscopolamine bromide (Buscopam [®])	<i>Chrysomya megacephala</i> ¹	L	Delay in the development	Brazil	OLIVEIRA <i>et al.</i> , 2009
	<i>Chrysomya putoria</i> ¹	L	Mass gain. Delay in the development	Brazil	THYSSEN; GRELLA, 2011

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Carbamazepine	<i>Chrysomya megacephala</i> ¹	L	Delay in the time of emergence	Brazil	MARQUES, 2017
Codeine	Calliphoridae	Pp, A	Enzymatic cuticle extraction	France	BOUREL <i>et al.</i> , 2001a
	Dermestidae	P			
	<i>Lucilia sericata</i> ¹	L, P	Accelerated stage larval. Delay in the stage pupal	France	BOUREL <i>et al.</i> , 2001a
	<i>Chrysomya albiceps</i> ¹	L, P	Accelerated in the stage larval	Egypt	FATHY <i>et al.</i> , 2008
	<i>Lucilia sericata</i> ¹	L	Delay in the development	Switzerland	KHARBOUCHE <i>et al.</i> , 2008
Phenobarbital	<i>Cochliomyia macellaria</i> ¹	L	Detection substance of case study	United States	BEYER; ENOS; STAJIC, 1980
	Diptera <i>n.s.</i>	L	Case study	France	KINTZ <i>et al.</i> , 1990
	<i>Chrysomya albiceps</i> ¹				
	<i>Chrysomya megacephala</i> ¹	L	Delay in development and high mortality	Brazil	SOTO, 2008
	<i>Chrysomya putoria</i> ¹				
Flunitrazepam	<i>Chrysomya megacephala</i> ¹	L, Pp, A	Detection of substance	Brazil	OLIVEIRA <i>et al.</i> , 2014
	<i>Chrysomya megacephala</i> ¹	L, P, A	No significant impact	Brazil	BAIA <i>et al.</i> , 2016

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Levomepromazine	<i>Lucilia sericata</i> ¹	L	Detection of substance	Italy	CAMPOBASSO <i>et al.</i> , 2004
Methadone	<i>Lucilia sericata</i> ¹	L	Detection of substance	Belgium	GOSSELIN <i>et al.</i> , 2010
Morphine	<i>Calliphora vicina</i> ¹	L	Delay in the development	Italy	INTRONA <i>et al.</i> , 1990
	<i>Lucilia sericata</i> ¹	L, P, A	Delay in the stage larval	France	HEDOUIN <i>et al.</i> , 1999a
	<i>Lucilia sericata</i> ¹	L	Detection of substance	France	HEDOUIN <i>et al.</i> , 1999b
	<i>Dermestes frisci</i> ³	L, P, A	Delay in the development	France	BOUREL <i>et al.</i> , 2001c
	<i>Thanatophilus cinuatus</i> ⁴				
	<i>Lucilia sericata</i> ¹	L	Delay in the development	France	BOUREL <i>et al.</i> , 2001a
	<i>Calliphora vicina</i> ¹	L, P	Detection of substance	France	HEDOUIN <i>et al.</i> , 2001
<i>Protophormia terraenovae</i> ¹					

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Morphine	<i>Calliphora vomitoria</i> ¹	L	Detection of substance	France	CAILLIEZ <i>et al.</i> , 2001
	<i>Calliphora stygia</i> ¹	L	Detection of substance	Australia	GUNN <i>et al.</i> , 2006
	<i>Calliphora stygia</i> ¹	L, P	No significant impact	Australia	GEORGE <i>et al.</i> , 2009
	<i>Calliphora stygia</i> ¹	L	Physiological response	Australia	PARRY <i>et al.</i> , 2011
Oxycodone (Oxycontin®)	<i>Phormia regina</i> ¹	L	Significantly longer in length	United States	MONTHEI, 2009
Paracetamol	<i>Calliphora vicina</i> ¹	L	No significant impact	Scotland	WILSON; HUBBARD; POUNDER, 1993;
Tramadol	<i>Lucilia sericata</i> ¹	L	Maximum sizes were reached earlier in treated larvae. The development period increased significantly with increasing initial dose administered	Egypt	EL-SAMAD; EL-MOATY; MAKEMER, 2011
	<i>Chrysomya albiceps</i> ¹	L	Delay in the development	Nigeria	EKRAKENE; ODO, 2017

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Paracetamol (Tylenol®)	<i>Calliphora vicina</i> ¹	L	Accelerated the development	United Kingdom	O'BRIEN; TURNER, 2004
	<i>Chrysomya rufifacies</i> ¹	L	Detection of substance	Malaysia	KHAN <i>et al.</i> , 2015
Sodium pentothal	<i>Calliphora vicina</i> ¹	L	Death of larvae	United Kingdom	SADLER <i>et al.</i> , 1997
Propoxyphene	<i>Calliphora vicina</i> ¹	L	No significant impact	Scotland	WILSON; HUBBARD; POUNDER, 1993
Salicylates	<i>Calliphora vicina</i> ¹	L	Effect significative in parameters evaluated. Defection of substance	United Kingdom	SADLER <i>et al.</i> , 1995
Topiramate (Amato®)	<i>Chrysomya megacephala</i> ¹	L	Mass gain	Brazil	PEREIRA, 2011
Chemotherapeutics					
Cyclophosphamide	<i>Chrysomya megacephala</i> ¹	L	No significant impact	Brazil	ALMEIDA, 2015
	<i>Chrysomya megacephala</i> ¹	L, P, A	Delay in the development	Brazil	TRIVIA; PINTO, 2018

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Methotrexate	<i>Chrysomya megacephala</i> ¹	L, P, A	Decreased the sizes of larvae and adults. Altered the survival rate. Changed the sex ratio	Brazil	TRIVIA; PINTO, 2018
Sedatives					
Alprazolam	<i>Chrysomya megacephala</i> ¹	L	Delay in the stages of larval and pupal. Pupation was delayed	India	PAWAR; DESHMUKH, 2022
Diazepam	<i>Chrysomya albiceps</i> ¹	L, P, A	Accelerated the development	Brazil	CARVALHO <i>et al.</i> , 2001
	<i>Chrysomya putoria</i> ¹				
	<i>Wohlfahrtia nuba</i> ²	L	Accelerated the cycle. Detection substance	Egypt	KHEDRE, 2005
	<i>Chrysomya albiceps</i> ¹	L	Accelerated the development	Turkey	AÇIKGÖZ, 2018
	Calliphoridae <i>n.s.</i>		Detection of substance	Brazil	MARINHEIRO, 2019
Phencyclidine	<i>Parasarcophaga ruficornis</i> ²	L, P	No significant impact for larvae, but the pupation was slowed	United States	GOFF <i>et al.</i> , 1994
Ketamine	<i>Lucilia sericata</i> ¹	L	Delay the early development. Impact in body length	Chine	ZOU <i>et al.</i> , 2013
	<i>Chrysomya megacephala</i> ¹	L	Prolongation of the pupation period	Chine	LÜ <i>et al.</i> , 2014

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Ketamine	<i>Chrysomya megacephala</i> ¹	L	Delay in the development	India	SINGH; HEER; WADHAWAN, 2016
Lorazepam	<i>Chrysomya rufifacies</i> ¹	L	Delay in the development. Length, weight and width decreased.	India	ANNASAHEB BANSODE; RAMRAO MORE, 2023
Methylphenidate	Calliphoridae <i>n.s.</i>	L	Detection of substance	New Zealand	BUSHBY <i>et al.</i> , 2012
	<i>Chrysomya chloropyga</i> ¹	L	Prolongation of the stage larval. Delay in the stage pupae	South Africa	VISSER, 2016
Methylphenidate hydrochloride + Phenobarbital	<i>Chrysomya albiceps</i> ¹				
	<i>Chrysomya megacephala</i> ¹	L	Delay in the development	Brazil	REZENDE, 2012
	<i>Chrysomya putoria</i> ¹				
	<i>Chrysomya albiceps</i> ¹	L	Delay in the development. Alteration in larval and pupal viabilities	Brazil	REZENDE <i>et al.</i> , 2014
Nordiazepam	<i>Calliphora vicina</i> ¹	L, P	Detection of substance	United Kingdom	WOOD <i>et al.</i> , 2003
	<i>Calliphora vicina</i> ¹	L, P	No significant impact	Belgium	PIEN <i>et al.</i> , 2004

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Oxazepam	<i>Calliphora vicina</i> ¹	L, P	Detection of substance	United Kingdom	WOOD <i>et al.</i> , 2003
Triazolam	Diptera <i>n.s</i>	L	Case study	France	KINTZ <i>et al.</i> , 1990
Zolpidem	<i>Chrysomya megacephala</i> ¹	L	Accelerated the development and effect in the weight dependent of concentration	Saudi Arabia	AL-SHURAYM <i>et al.</i> , 2021
	<i>Chrysomya saffrana</i> ¹		Delay in that the developmental durations of different instars, and effect in width and weight dependent of concentration	India	AL-KERIDIS <i>et al.</i> , 2022
Others					
Antifreeze (ethylene glycol)	<i>Lucilia sericata</i> ¹ <i>Lucilia cuprina</i> ¹	L, P, A	Mortality significative. Effect significative in eclose and width. Delay in the developmental.	United State	ESSARRAS <i>et al.</i> , 2017

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Cadmium	<i>Chrysomya albiceps</i> ¹	L, P, A	All stages were affected: Larval development time of both sexes. Male total development time was longer than that of females. Mortality significant. Adult weight of females was higher than that of males. The larval stage had higher mean pupal and adult mass, but also reduced adult longevity and fecundity. Adult longevity and fecundity were also reduced by cadmium exposure in the adult stage.	Saudi Arabia	AL-MISNED, 2001
	<i>Lucilia sericata</i> ¹	L, A		United Kingdom	MOE; STENSETH; SMITH, 2001
Lead	<i>Lucilia cuprina</i> ¹		Influence in the survival rate and development time	Brazil	BESSA <i>et al.</i> , 2021
Aqueous extract of <i>Ipomoea carnea</i>	<i>Chrysomya albiceps</i> ¹		Delay in the development	Brazil	CRUZ JUNIOR <i>et al.</i> , 2020
Zinc phosphide	Coleoptera Diptera Hemiptera Hymenoptera	-	Delay in the colonization	Egypt	ABD EL-BAR <i>et al.</i> , 2016

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Zinc phosphide	<i>Chrysomya albiceps</i> ¹				
	<i>Chrysomya chloropyga</i> ¹	-	No significant impact in activate	Nigeria	ABAJUE; EWUIM, 2020
	<i>Chrysomya regalis</i> ¹				
Ivermectin	<i>n.s.</i>	-	Effect in the colonizing fauna, in the total time of colonization and the time of the decomposition stages. In the physical patterns and behavior of larval masses	Brazil	BRANCOLI, 2013
Losartan Potassic	<i>Chrysomya megacephala</i> ¹		No significant impact in the development but there was the reduced size of the adults	Brazil	BARBOSA, 2020
Mercury	<i>Creophilus maxillosus</i> ⁵	L	No significant impact	Finland	NUORTEVA; NUORTEVA, 1982
Nicotine	<i>Calliphora vomitoria</i> ¹	L, P	No significant impact in developmental time, but affected survival during the pupation period and larval length.	Australia	MAGNI <i>et al.</i> , 2016
	<i>n.s.</i>	-	Interference in the insect colonization and oviposition	Brazil	GUERRA <i>et al.</i> , 2016

Substances	Specie/Family	Stages/structures	Effects	Country	Author/Year
Gunshot residue (Lead - Pb, Barium - Ba, Antimony - Sb)	<i>Calliphora dubia</i> ¹	L	Detection of substances	Australia	ROETERDINK; DADOUR; WATLING, 2004
Ephedrine sulphate	<i>Chrysomya albiceps</i> ¹	L, P, A	Detection of substance. Delay in the developmental time	Egypt	FOUDA <i>et al.</i> , 2017
Warfarin	Diptera <i>n.s.</i>	L	Detection of substance.	Egypt	EL-GAWAD <i>et al.</i> , 2020
<i>n.s.</i>	<i>Calliphora vicina</i> ¹ <i>Lucilia sericata</i> ¹ <i>Sarcophaga crassipalpis</i> ²	-	Case study	India	SAMNO <i>et al.</i> , 2020

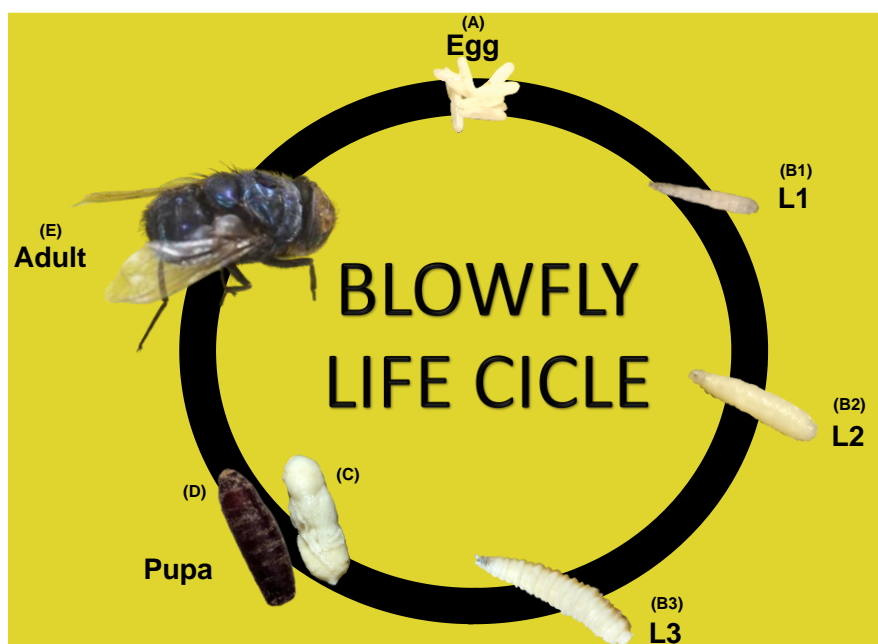
Legend: *n.s.* Not specified; 1 Diptera: Calliphoridae; 2 Diptera: Sarcophagidae; 3 Coleoptera: Dermestidae; 4 Coleoptera: Silphidae; 5 Coleoptera: Staphylinidae; 6 Diptera: Syrphidae; A- Adult, Ex - Exuviae, L- Larva, P- Pupa, Pp- Puparium.

1.3 Calliphoridae

Calliphoridae, also known as "blowflies" or "screwflies", are a family of flies in the order Diptera. They are necrophagous and play an important role in forensic entomology, being found colonizing corpses in large quantities even at the beginning of the decomposition process. The corpse releases putrefactive odor volatiles that attract the females that lay eggs in the orifices, whether natural or caused (Gullan; Cranston, 2017; Silva; Reis, 2012), where the larvae will develop, as in their immature stage they feed on dead tissue (Catts; Goff, 1992; Carvalho *et al.*, 2000; Mise; Almeida; Moura, 2007; Oliveira; Vasconcelos, 2010).

Blowflies have a life cycle with holometabolous metamorphosis, that is, they go through the stages of egg, larva, pupa and adult (Gullan; Cranston, 2017) (Figure 1). As adults, these flies are medium to large size, 5-15mm, and have bristles all over the body, especially on the thorax, which is an important feature for species identification (Vargas; Wood, 2010). The first pair of wings is of the membranous type, with the presence of a well-developed calypter, and the second pair is modified, called halteres. They have compound eyes that cover a large area of the head and the antennae are short of the aristate type (Carvalho; Mello-Patiu, 2008; Leite; De Sá, 2010). Sexual dimorphism can also be observed in most species, with morphological differences such as eye position, body size: males tend to be smaller than females (Hu; Yuan; Lei, 2011; Byrd; Tomberlin, 2020) (Figure 2).

Figure 1. The life cycle of *Chrysomya megacephala*: (A) egg, (B) larvae - (B1) instar 1, (B2) instar 2, (B3) instar 3, (C) pupa, (D) puparium or case pupa and (E) adult.



(Bibiano; Frazão, 2024)

Figure 2. Sexual dimorphism, *Chrysomya megacephala*, in the eye position: Males with eyes holoptic eyes (left) and Females with dichoptic (right).



(Bibiano, 2024)

The subfamily Chrysomyinae (Calliphoridae) has some features that distinguish it from other subfamilies. The wings have a specific bristle pattern on the radial vein, which is present in the dorsal view and absent in the ventral view. They have a metallic color pattern on the abdomen and thorax, which can be blue, green or bright metallic bronze (Carvalho; Mello-Patiu; Almeida, 2021). It comprises two tribes that include their respective genera: Chrysomyini - *Chloroprocta* spp., *Chrysomya* spp., *Cochliomyia* spp., *Compsomyiops* spp., *Hemilucilia* spp. and *Paralucilia* spp., and Phormiini -, *Phormia* spp. and *Protophormia* spp. (Amat, 2009; Carvalho; Mello-Patiu, 2008; Dear, 1985; Singh; Wells, 2011; Szpila *et al.*, 2013; Whitworth, 2010, 2019). In Brazil, the representatives of Chrysomyini are *Cochliomyia* spp. (2 species), *Chrysomya* spp. (4 species), *Compsomyiops fulvicrura*, *Chloroprocta idiodea*, *Paralucilia xanthogeneiates* and *Hemilucilia* spp. (4 species) (De Carvalho; Ribeiro, 2000; Madeira-Ott, 2023).

Several species of blowflies previously restricted to Europe, Asia, and Africa migrated to the Americas during the voyages of migrated, allowing them to adapt and become established throughout the continent (Baumgartner; Greenberg, 1984; Richard; Ahrens 1983; Wells; Byrd; Tantawi, 1999; Carvalho; Mello-Patiu; Almeida, 2021; Neto, 2023). Researchers such as Wells, Byrd and Tantawi (1999) stated that Brazil was the gateway for *Chrysomya* Robineau-Desvoidy flies to spread throughout the Americas.

The genus *Chrysomya* is a word from the Greek, where *chrysós*= gold and *miya*= fly (Oliveira-Costa, 2013) and it includes 12 species (Gagné, 1981; Guimarães; Papavero 1999; Silva *et al.*, 2012), among which four have been present in Brazil, just over 50 years, introduced in the last years of the 1970s, reported in the south and southeast of the country (Corrêa *et al.*, 2010). *Chrysomya albiceps* (Wiedemann) and *C. megacephala* (Fabricius) in the state of São Paulo (Guimarães; Prado; Linhares, 1978), *C. putoria* (Wiedemann) in the states of Paraná (Imbiriba; Izutani; Milhoreto, 1977) and São Paulo (Guimarães; Prado; Linhares, 1978). Initially, the species *C. rufifacies* (Macquart) was reported in northeastern Brazil just over a decade ago, in 2012, but more detailed taxonomic studies showed that the reported species was *C. albiceps* (Silva *et al.*, 2012; De Castro Ribeiro *et al.*, 2013).

The flies of this genus are vectors of different pathogens and cause myiasis, which is important for the medical-veterinary field (Baumgartner; Greenberg, 1984; Monzon *et al.*, 1991; Guimarães; Papavero 1999; Ferraz *et al.*, 2014; Ribeiro *et al.*, 2011), but also for forensic studies, as it is associated with cadavers (Oliveira-Costa, 2008; Barros-Souza, 2012). The most abundant species is *C. albiceps* (Wiedemann), which, in addition to being necrophagous, is predatory to other larvae in competition for food (Baumgartner; Greenberg, 1984; Queiroz, 1997; Faria *et al.*, 1999).

Although *C. albiceps* and *C. megacephala* share several similarities, some features are very striking for each species. *Chrysomya albiceps* has a whitish anterior spiracle, silver-white parafacialia, and tegument coloration with a greenish sheen (Mello-Patiu; Almeida, 2021). On the other hand, *C. megacephala* has a more robust body with blue and purple placement, presents a brown/chestnut anterior spiracle, and the head is relatively large compared to the body and the other species, making it easy to recognize and distinguish (González-Mora; Peris, 1988; Whitworth, 2010; Mello-Patiu; Byrd; Tomberlin, 2020; Almeida, 2021).

Other differences can be seen in the larval stages of both species. The larvae of *C. albiceps* differ from other Calliphoridae in that they have very visible projections in their tegument. This feature is also present in the species *C. rufifacies* (Macquart), but it is not known to occur in Brazil (De Carvalho-Queiroz *et al.*, 1997; Tomberlin, 2020; Wells *et al.*, 1999). The larvae of *C. megacephala* do not have tubercles or fine bristles, they have a partially pigmented oral sclerite that is very visible as a spot near the buccal hooks (González-Mora; Peris, 1988; Wells *et al.*, 1999).

REFERENCES

- ABAJUE, M. C.; EWUIM, S. C. Evaluation of activities of dipteran maggots on a poisoned pig cadaver at Nnamdi Azikiwe University Awka, Nigeria. **Egyptian Journal of Forensic Sciences**, v. 10, n. 1, p. 1-8, 2020. doi.org/10.1186/s41935-020-00208-0
- ABD AL-GALIL, F. M.; ZAMBARE, S. P.; AL-MEKHLAFI, F. A.; AL-KERIDIS, L. A. Effect of dimethoate on the developmental rate of forensic importance Calliphoridae flies. **Saudi Journal of Biological Sciences**, v. 28, n. 2, p. 1267-1271, 2021. doi.org/10.1016/j.sjbs.2020.12.022
- ABD EL-BAR, M. M., SAWABY, R. F.; EL-HAMOULY, H.; HAMDY, R. A. preliminary identification of insect successive wave in Egypt on control and zinc phosphide-intoxicated animals in different seasons. **Egyptian Journal of Forensic Sciences**, v. 6, n. 3, p. 223-234, 2016. doi.org/10.1016/j.ejfs.2016.05.004
- AÇIKGÖZ, H. N. Multiple drug analysis of *Chrysomya albiceps* larvae provides important forensic insights to unravel drug-associated mortalities. **Entomological News**, v. 128, n. 1, p. 99-107, 2018. doi.org/10.3157/021.128.0116
- AL-KHALIFA, M.; MASHALY, A.; AL-QAHTNI, A. Impacts of antemortem ingestion of alcoholic beverages on insect successional patterns. **Saudi journal of biological sciences**, v. 28, n. 1, p. 685-692, 2021. doi.org/10.1016/j.sjbs.2020.10.060
- AL-KERIDIS, L. A.; AL-MEKHLAFI, F. A.; ABD AL-GALIL, F. M.; MOHAMED, R. A. E. H.; AL-SHURAYM, L. A.; ALHAG, S. K.; WADAAN, M. A.; AL-KHALIFA M. S.; ZAMBARE, S. P. Effect of the sedative drug zolpidem tartrate on the immature and mature stages of carrion flies *Chrysomya rufifacies* and *Chrysomya indiana*. **Journal of King Saud University-Science**, v. 34, n. 1, p. 101676. 2022. doi.org/10.1016/j.jksus.2021.101676
- ALMEIDA, C. D. S. Análise do efeito do quimioterápico ciclofosfamida no desenvolvimento larval de *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae). 2015.
- AL-MISNED, F. A. M. Biological effects of cadmium on life cycle parameters of *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae). **Kuwait Journal of Science & Engineerin**, v. 28, n. 1, p.179–188. 2001.
- ALONSO, M. A. Desenvolvimento de imaturos de espécies de importância forense, *Chrysomya megacephala* (F.) e *Chrysomya putoria* (W.) (Diptera: Calliphoridae), sob influência de diferentes temperaturas e/ou cloridrato de fluoxetina. 2015.
- AL-QAHTNI, A.; MASHALY, A.; HADDADI, R.; AL-KHALIFA, M. Seasonal impact of heroin on rabbit carcass decomposition and insect succession. **Journal of Medical Entomology**, v. 58, n. 2, p. 567-575. 2021. doi.org/10.1093/jme/tjaa237
- AL-SHURAYM, L. A.; AL-MEKHLAFI, F. A.; AL GALIL, F. M. A.; ALHAG, S. K.; AL-KERIDIS, L. A.; MOHAMED, R. A. E.H.; WADAAN, M. A.; AL-KHALIFA, M. S. Effect of zolpidem tartrate on the developmental rate of forensically important flies *Chrysomya megacephala* (Diptera: Calliphoridae) and *Chrysomya saffrana*. **Journal of Medical Entomology**, v. 58, n. 6, p. 2101-2106, 2021. doi.org/10.1093/jme/tjab071
- ALVES, A. C. F.; DOS SANTOS, W. E.; CREÃO-DUARTE, A. J. Diptera (Insecta) de importância forense da região Neotropical. **Entomotropica**, v. 29, n. 2, p. 77–94, 2014.
- AMAT GARCÍA, E. C. Contribución al conocimiento de las Chrysomyinae y Toxotarsinae (Diptera: Calliphoridae) de Colombia. **Revista Mexicana de Biodiversidad**, v. 80, n. 1, p. 693- 708, 2009.
- AMENDT, J; KRETTEK, R.; ZEHNER, R. Entomologia forense. **Naturwissenschaften**, v. 91, p. 51–65, 2004.
- ANDRÉA, M. M. de. Bioindicadores ecotoxicológicos de agrotóxicos. 2008. Disponível em: <http://www.infobibos.com/Artigos/2008_4/Bioindicadores/index.htm>.

- ANNASAHEB BANSODE, S.; RAMRAO MORE, V. Effect of Lorazepam on the Development of the Hairy Maggot Blow Fly, *Chrysomya rufifacies* (Macquart): Implication for Forensic Entomology. **Journal of Toxicology**, v. 2023. 2023. doi.org/10.1155/2023/1051736
- BAIA, T. C.; CAMPOS, A.; WANDERLEY, B. M. S.; GAMA, R. A. The Effect of Flunitrazepam (Rohypnol®) on the Development of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) and its Implications for Forensic Entomology. **Journal of forensic sciences**, v. 61, n. 4, p. 1112-1115, 2016. doi.org/10.1111/1556-4029.13104
- BAKR, R.; RAMADAN, R.; EL-SAWY, S.; HUSSINI, S. Ultrastructure of the midgut of the third larval instar of *Chrysomya megacephala* (Diptera: Calliphoridae) fed on malathion treated diet. **Academic Journal of Biological Sciences, D. Histology & Histochemistry**, v. 3, n. 1, p. 13–26, 2012. doi: 10.21608/eajbsd.2012.14141
- BARBOSA, F. A. Análise do efeito do anti-hipertensivo Losartana Potássica no desenvolvimento de *Chrysomya megacephala* (Diptera: calliphoridae). 2020.
- BARBOSA, R.R.; MELLO-PATIU, C.A.; MELLO, R.P.; QUEIROZ, M. M. C. New records of calyptrate dipterans (Fanniidae, Muscidae and Sarcophagidae) associated with the decomposition of domestic pigs in Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 104, n. 6, p. 923–926, 2009. doi.org/10.1590/S0074-02762009000600018
- BARBOSA, R.R.; MELLO-PATIU, C.A.; URURAHY-RODRIGUES, A.; BARBOSA, C.G.; QUEIROZ, M. M. C. Temporal distribution of ten calyptrate dipteran species of medicolegal importance in Rio de Janeiro, Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 105, n. 2, p. 191–198, 2010. doi.org/10.1590/S0074-02762010000200014
- BARROS-SOUZA, A.S.; FERREIRA-KEPPLER, R.L.; AGRA, D.B. Development Period of Forensic Importance Calliphoridae (Diptera: Brachycera) in Urban Area Under Natural Conditions in Manaus, Amazonas, Brazil. **EntomoBrasilis**, v. 5, p. 99-105, 2012. doi.org/10.12741/ebrasilis.v5i2.201
- BAUMGARTNER, D. L.; GREENBERG, B. The genus *Chrysomya* (Diptera: Calliphoridae) in the New World, **Journal of Medical Entomology**, v. 21, p.105–113, 1984. doi.org/10.1093/jmedent/21.1.105
- BENECKE, M. A brief history of forensic entomology. **Forensic Science International**, v. 120, p. 2–114, 2001.
- BESSA, B. G. D. O.; SILVA-NETO, H. D. A.; COLTRO, W. K.; ROCHA, T. L.; LOPES, W. R. Lead toxicity in *Lucilia cuprina* and electrochemical analysis: a simple and low-cost alternative for forensic investigation. **Analytical and bioanalytical chemistry**, v. 413, p. 3201-3208, 2021. doi.org/10.1007/s00216-021-03257-z
- BEYER, J. C.; ENOS, W. F.; STAJIC, M. Drug identification through analysis of maggots. **Journal of Forensic Sciences**, v. 25, n. 2, p. 411–412, 1980. doi.org/10.1520/JFS12147J
- BOUREL, B.; TOURNEL, G.; HEDOU, V.; DEVEAUX, M.; GOFF, M.L.; GOSSET, D. Morphine extraction in necrophagous insects remains for determining ante-mortem opiate intoxication. **Forensic Science International**, v. 120, n. 1-2, p.127–131, 2001a. doi.org/10.1016/S0379-0738(01)00428-5
- BOUREL, B.; TOURNEL, G.; HEDOU, V.; GOFF, M. L.; GOSSET, D. Determination of drug levels in two species of necrophagous Coleoptera reared on substrates containing morphine. **Jornal Science Forensic**, v.46, p.600–603, 2001c.
- BRANCOLI, D. L. O efeito da ivermectina na duração das fases de decomposição, sobre os insetos necrófagos e interpretação termográfica da distribuição espacial da massa larval de dípteros em carcaças de cabras (*Capra aegragrus hircus* L., 1758) (Doctoral dissertation, [sn]). 2013. doi.org/10.47749/T/UNICAMP.2013.912265
- BUSHBY, S. K.; THOMAS, N.; PRIEMEL, P. A.; COULTER, C. V.; RADES, T.; KIESER, J. A. Determination of methylphenidate in Calliphorid larvae by liquid-liquid extraction and liquid chromatography mass spectrometry - forensic entomotoxicology using an in vivo rat brain model. **Journal of pharmaceutical and biomedical analysis**, v. 70, p. 456–461. 2012. doi.org/10.1016/j.jpba.2012.06.024

- BYRD, J. H.; BRUNDAGE, A. Forensic entomology. In: **Veterinary Forensic Medicine and Forensic Sciences**. CRC press, 2020. p. 67-111.
- CAILLIEZ, J. C.; BOUREL, B.; HÉDOUIN, V.; GOSSET, D.; FLEURISSE, L.; CREUSY, C.; GOFF, M. L. Immunohistochemical contribution to the study of morphine metabolism in Calliphoridae larvae and implications in forensic entomotoxicology. **Journal of forensic sciences**, v. 46, n. 3, p. 596-599. 2001. doi: 10.1520/JFS15009J
- CAMPOBASSO, C. P.; GHERARDI, M.; CALIGARA, M.; SIRONI, L.; INTRONA, F. Drug analysis in blowfly larvae and in human tissues: a comparative study. **Internacional Journal Legal Medical**, v.118, p. 210-214, 2004. doi.org/10.1007/s00414-004-0448-1
- CANDELA R.G.; AVENTAGGIATO, L. The detection of toxic substances in entomological specimens. **Internacional Jornal Legal Medical.**, v.114, p.197-203, 2001. doi.org/10.1007/s004140000181
- CARVALHO, C. J. B.; MELLO-PATIU, C. A. de. Key to the adults of the most common forensic species of Diptera in South America. **Revista Brasileira de Entomologia**, v. 52, p. 390-406, 2008. doi.org/10.1590/S0085-56262008000300012
- CARVALHO, C. J. B.; MELLO-PATIU, C. A.; ALMEIDA, L. M. O papel da taxonomia na entomologia forense. In: Entomologia forense na prática: do laboratório à utilização dos vestígios. 1 ed. Campinas, SP; **Millennium Editora**, p. 225-273, 2021.
- CARVALHO, C. J. B.; RIBEIRO, P. B. Chave de identificação das espécies de Calliphoridae (Diptera) do Sul do Brasil. **Revista Brasileira de Parasitologia Veterinária**, v. 9, n. 2, p. 169-173, 2000.
- CARVALHO, L. M. L. D. A Toxicologia e a Entomologia Forense. In: CARVALHO, L. M. L. D. Entomologia Forense: Quando os insetos são vestígios. Rio de Janeiro: **Millennium Editora**, 2003.
- CARVALHO, L. M. L. D.; THYSSEN, P. J.; LINHARES, A. X.; PALHARES, F. A. B. A checklist of arthropods associated with pig carrion and human corpses in Southeastern Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 95, p. 135-138, 2000. doi.org/10.1590/S0074-02762000000100023
- CARVALHO, L. M.; LINHARES, A. X.; PALHARES, F. A. B. The effect of cocaine on the development rate of immatures and adults of *Chrysomya albiceps* and *Chrysomya putoria* (Diptera: calliphoridae) and its importance to postmortem interval estimate. **Forensic Science International**; v. 220, n. 1-3, p. 27-32, 2012.
- CARVALHO, L. M.; LINHARES, A. X.; TRIGO, J. R. Determination of drugs levels and the effect of diazepam on the growth of necrophagous flies of forensic importance in southeastern Brazil. In: **Forensic Science Internation**. v.12, p. 140-144. 2001.
- CASTRO RIBEIRO, A.; CARDOSO, D.; DOS SANTOS LESSA, C. S.; MOYA-BORJA, G. E.; AGUIAR, V. M. First Record of *Chrysomya rufifacies* (Macquart)(Diptera, Calliphoridae) in Southeastern Brazil. **EntomoBrasilis**, v. 6, n. 3, p. 245-247, 2013.
- CATTS, E.P.; GOFF, M.L. Forensic entomology in criminal investigations, **Annual Review of Entomology**, v. 37, p. 253-272, 1992.
- CAVALCANTE, K. D. S. Avaliação do efeito do Diazinon na composição e sucessão de assembleias de calliphoridae em carcaças de coelhos domésticos. Dissertação (Mestrado em Biodiversidade Tropical) – Departamento de Pós-Graduação, Universidade Federal do Amapá, Macapá. 2017.
- CORRÊA, E. C.; KOLLER, W. W.; BARROS, A. T. M. Abundância relativa e sazonalidade de espécies de *Chrysomya* (Diptera: Calliphoridae) no Pantanal sul-mato-grossense, Brasil. **Revista Brasileira de Parasitologia Veterinária**, v. 19, p. 85-88, 2010.
- CRUZ JUNIOR, O. F. S.; de JESUS CORRÊA-NETO, J.; GOMES, L. Efeitos do extrato aquoso de *Ipomoea carnea* subsp. fistulosa sobre o desenvolvimento de larvas de *Chrysomya albiceps* (Wiedemann, 1819) (Calliphoridae: Diptera). **Biotemas**, v. 33, n. 1, p. 1-7, 2020. doi.org/10.5007/2175-7925.2020.e67136

- DA SILVA ANTONIO, A. Análise qualitativa de cocaína em imaturos de *Hemilucilia segmentaria* (Fabricius)(Diptera: Calliphoridae) criados em tecidos de coelhos intoxicados por cromatografia em camada delgada (CCD). 2013.
- DA SILVA, C.; VILLET, M. H. Effects of prophylactic progesterone in decomposing tissues on the development of *Chrysomya chloropyga* (Wiedeman) (Diptera: Calliphoridae). **African Entomology**, v. 14, n. 1, p. 199-202, 2006.
- DEAR, J. P. A revision of the new world Chrysomyini (Diptera: Calliphoridae). **Revista Brasileira de Zoologia**, v. 3, p. 109-169, 1985.
- EKRAKENE, T.; ODO, P. E. Comparative developmental effects of tramadol hydrochloride and cypermethrin on *Chrysomya albiceps* (Weid.) (Diptera: Calliphoridae) reared on rabbit carrions. **Science World Journal**, v. 12, n. 1, p. 28-32, 2017.
- EL-GAWAD, A.; KENAWY, M. A.; BADAWY, R. M.; EL-BAR, A.; MARAH, M. Fly maggots as bio-indicators of warfarin poisoning in rabbits as an animal model. **Egyptian Academic Journal of Biological Sciences, E. Medical Entomology & Parasitology**, v. 12, n. 2, p. 43-48, 2020. doi:10.21608/eajbse.2020.111132
- EL-SAMAD L. M.; EL-MOATY, Z. A.; MAKEMER, H. M. Effects of tramadol on the development of *Lucilia sericata* (Diptera: calliphoridae) and detection of the drug concentration in postmortem rabbit tissues and larvae. **Journal of Entomology**, v. 8, n. 4, p. 353-364, 2011. doi: 10.3923/je.2011.353.364
- ESSARRAS, A.; PAZZI, M.; DADOUR, I. R.; MAGNI, P. A. The effect of antifreeze (ethylene glycol) on the survival and the life cycle of two species of necrophagous blowflies (Diptera: Calliphoridae). **Science & Justice**, v. 58, n. 2, p. 85-89, 2018. doi.org/10.1016/j.scijus.2017.12.008
- FARIA, L. D. B.; ORSI, L.; TRINCA, L. A.; GODOY, W. A. C. Larval predation by *Chrysomya albiceps* on *Cochliomyia macellaria*, *Chrysomya megacephala* and *Chrysomya putoria*. **Entomologia experimentalis et applicata**, v. 90, n. 2, p. 149-155. 1999.
- FATHY, H. M.; ATTIA, R. A.; YONES, D. A.; ELDEEK, H. E.; TOLBA, M. E.; SHAHEEN, M. S. Effect of Codeine Phosphate on developmental Stages of Forensically Important Calliphoride Fly: *Chrysomya albiceps*. **Mansoura Journal of Forensic Medicine and Clinical Toxicology**, v. 16, n. 1, p. 41-59, 2008.
- FERRARI, A. C.; SOARES, A. T.; GUIMARÃES, M. A.; THYSSEN, P. J. Efeito da testosterona no desenvolvimento de *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae). **Medicina (Ribeirão Preto)**, v. 41, n. 1, p. 30-34, 2008.
- FERRAZ, A. C.; DALLAVECCHIA, D. L.; SILVA, D. C. D.; de CARVALHO, R. P.; SILVA FILHO, R. G. D.; AGUIAR-COELHO, V. M. Evaluation of the influence of the antibiotic ciprofloxacin in the development of an Old-World screwworm fly, *Chrysomya putoria*. **Journal of Insect Science**, v. 14, n. 1, p. 3, 2016. doi.org/10.1093/jis/14.1.3
- FERRAZ, A. C.; DALLAVECCHIA, D. L.; SILVA, D. C.; FIGUEIREDO, A. L.; PROENÇA, B.; SILVA-FILHO, R. G.; AGUIAR, V. M. Effects of the antibiotics Gentamicin on the postembryonic development of *Chrysomya putoria* (Diptera: Calliphoridae). **Journal of Insect Science**, v. 14, n. 1, p. 279, 2014. doi.org/10.1093/jisesa/ieu141
- FOUDA, MOHAMAD & DALI, ALAA & HAMMAD, KOTB & KABADAIA, MOHAMAD. Detection and effect of ephedrine sulphate on the development rate of the forensic blow fly larvae *Chrysomya albiceps* (Diptera: Calliphoridae) colonize a dog carcass. **International Journal of Advanced Research in Biological Sciences**. v. 4, p. 118-126, 2017. doi:10.22192/ijarbs
- FREIRE, O. Fauna cadaverica brasileira (continuação e fim). **Revista de Medicina**, v. 4, n. 24, p. 27-41, 1923.
- FREIRE, O. Fauna cadaverica brasileira (continuação). **Revista de Medicina**, v. 2, n. 23, p. 14-40, 1923.
- GAGNÉ, Raymond J. *Chrysomya* spp., Old World blow flies (Diptera: Calliphoridae), recently established in the

Americas. Bulletin of the ESA, v. 27, n. 1, p. 21-22, 1981. doi.org/10.1093/besa/27.1.21

GEORGE, K. A.; ARCHER, M. S.; GREEN, L. M.; CONLAN, X. A.; TOOP, T. Effect of morphine on the growth rate of *Calliphora stygia* (Fabricius) (Diptera: Calliphoridae) and possible implications for forensic entomology. **Forensic science international**, v. 193, n. 1-3, p. 21-25. 2009. doi.org/10.1016/j.forsciint.2009.08.013

GOFF, M. L.; BROWN, W. A.; HEWADIKARAM, K. A.; OMORI, A. I. Effect of heroin in decomposing tissues on the development rate of *Boettcherisca peregrina* (Diptera, Sarcophagidae) and implications of this effect on estimation of postmortem intervals using arthropod development patterns. **Journal of Forensic Science**. v. 36, p. 537–542. 1991.

GOFF, M. L.; BROWN, W. A.; OMORI, A. I. Preliminary observations of the effect of methamphetamine in decomposing tissues on the development rate of Parasar- (Diptera: Sarcophagidae) and the implications of this effect on the estimations of postmortem intervals. **Journal of Forensic Science**, v. 37, n.3, p. 867–872, 1992.

GOFF, M. L.; BROWN, W. A.; OMORI, A. I.; LAPOINTE, D. A. Preliminary observations of the effects of amitriptyline in decomposing tissues on the development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and implications of this effect to estimation of postmortem interval. **Journal of Forensic Science**. v. 38, p. 316–322, 1993. doi.org/10.1520/JFS13410J

GOFF, M. L.; MILLER, M. L.; PAULSON, J. D.; LORD, W. D.; RICHARDS, E.; OMORI, A. I. Effects of 3,4-methylenedioxyamphetamine in decomposing tissues on the development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and detection of the drug in postmortem blood, liver tissue, larvae, and puparia. **Journal of Forensic Science**, v. 42, n. 2, p.276–280, 1997. doi:10.1520/JFS14110J

GOFF, M. L.; OMORI, A. I.; GOODBROD, J. R. Effect of cocaine in tissues on the development rate of *Boettcherisca peregrina* (Diptera: Sarcophagidae). **Journal of Medical Entomology**, v. 26, n. 2, p. 91–93, 1989. doi.org/10.1093/jmedent/26.2.91

GOMES, G. Processos Auto-Organizados: Efeitos de substâncias químicas que agem no sistema nervoso sobre o desenvolvimento e padrão de dispersão larval pós-alimentar de dípteros (Calliphoridae e Muscidae). Dissertação (mestrado) - Universidade Estadual Paulista, Instituto de Biociências de Rio Claro, 2006.

GOMES, L. Entomologia Forense: Novas tendências e tecnologias nas ciências criminais. **Technical Books**, 2010.

GONÇALVES, J. R. Entomotoxicologia no Brasil: avanços, limitações e perspectivas. 2022.

GONZÁLEZ-MORA, D.; PERIS, S. V. Los calliphoridae de España: 1: Rhiniinae y chrysomyinae (díptera). 1988.

GOSSELIN, M.; FERNANDEZ, M. D. M. R.; WILLE, S. M.; SAMYN, N.; DE BOECK, G.; BOUREL, B. Quantification of methadone and its metabolite 2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine in third instar larvae of *Lucilia sericata* (Diptera: Calliphoridae) using liquid chromatography-tandem mass spectrometry. **Journal of analytical toxicology**, v. 34, n. 7, p. 374-380, 2010. doi.org/10.1093/jat/34.7.374

GREENBERG, B. Flies as forensic indicators. **Journal of Medical Entomology**, v. 28, p. 565–577, 1991.

GREENBERG, B.; KUNICH, J.C. Entomology and the Law: Flies as Forensic Indicators, first ed., **Cambridge University Press**, 2002.

GUERRA, T. S.; BARNABE, A. S.; FERRAZ, R. N.; GODOY, M. P. Avaliação de possível interferência do tabagismo na ovoposição de larvas de Calliphoridae (Díptera) em carcaça de *Mus musculus* L. (Rodentia: Muriade) em São Paulo, SP. **Revista Brasileira de Criminalística**, v. 5, n. 1, p. 18-26, 2016. dx.doi.org/10.15260/rbc.v5i1.108

GUIMARÃES, G. P. Influência de levonorgestrel no desenvolvimento pós-embriônico de *Chrysomya megacephala* (FABRICIUS), 1794 (DIPTERA: CALLIPHORIDAE). 2019.

GUIMARÃES, J. H.; A. P. PRADO; A. X. LINHARES. Tree newly introduced blowfly species in southern Brazil (Diptera, Calliphoridae). **Revista Brasileira de Entomologia**, v. 22, p. 53–60, 1978.

GUIMARÃES, J. H.; PAPAVERO, N. Myiasis in man and animals in the Neotropical region. Bibliographic database. São Paulo, **Editora Plêiade/FAPESP**. 308 p. 1999.

GULLAN, P. J.; CRANSTON, P. S. Insetos: fundamentos da entomologia. [Tradução e Revisão Técnica Eduardo da Silva Alves dos Santos, Sonia Maria Marques Hoenen – 5. ed. – Rio de Janeiro: **Roca**. 2017

GUNATILAKE, K.; GOFF, M. L. Detection of organophosphate poisoning in a putrefying body by analyzing arthropod larvae. **Journal of Forensic Science**, v. 34, n. 3, p.714–716, 1989. doi.org/10.1520/JFS12698J

GUNN, J. A.; SHELLEY, C.; LEWIS, S.W.; TOOP, T.; ARCHER, M. The determination of morphine in the larvae of *Calliphora stygia* using flow injection analysis and HPLC with chemiluminescence detection. **Journal of Analytical Toxicology**, v. 30, n.8. p.519–523, 2006. doi.org/10.1093/jat/30.8.519

HEDOUIN, V.; BECART, A.; TOURNEL, G.; DEVEAUX, M.; GOSSET, D.; BOUREL, B.; MARTIN-BOUYER, L. Effects of Morphine in Decomposing Bodies on the Development of (Diptera: Calliphoridae). **Journal of Forensic Sciences**, v. 44, n. 2, p. 354-358, 1999a. doi: 10.1520/JFS14463J

HEDOUIN, V.; BÉCART, A.; TOURNEL, G.; DEVEAUX, M.; GOSSET, D.; BOUREL, B.; MARTIN-BOUYER, L. Determination of drug levels in larvae of *Lucilia sericata* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine. **Journal of Forensic Science**, v. 44, p. 351– 353, 1999b. doi:10.1520/JFS14462J

HEDOUIN, V.; BOUREL, B.; BÉCART, A.; TOURNEL, G.; DEVEAUX, M.; GOFF, M. L.; GOSSET, D. Determination of drug levels in larvae of *Protophormia terraenovae* and *Calliphora vicina* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine. **Journal of Forensic Science**, v. 46, n. 1, p.12–14, 2001.

HODECEK, J. Revisiting the concept of entomotoxicology. **Forensic Science International: Synergy**, v. 2, p. 282-286, 2020. doi.org/10.1016/j.fsisyn.2020.09.003

HU, Y.; YUAN, X; LEI, C. Sexual size dimorphism decreases with temperature in a blowfly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). **Ecological Entomology**, v. 36, n. 1, p. 111-115, 2011. doi: 10.1111/j.1365-2311.2010.01251.x

INTRONA, F.; CAMPOBASSO, C. P.; GOFF, M. L. Entomotoxicology. **Forensic Science International**, v. 120, n. 1–2, p. 42–47, 2001. doi.org/10.1016/S0379-0738(01)00418-2

INTRONA, F.; LO DICO, C.; CAPLAN, Y. H.; SMIALEK, J. E. Opiate analysis in cadaveric blowfly larvae as an indicator of narcotic intoxication. **Journal of Forensic Science**, v. 35, n.1, p. 118–122, 1990. doi:10.1520/JFS12808J

JALES, J. T.; de MOURA BARBOSA, T.; dos SANTOS, L. C.; RACHETTI, V. D. P. S.; GAMA, R. A. Carrion decomposition and assemblage of necrophagous dipterans associated with Terbufos (Organophosphate) intoxicated rat carcasses. **Acta tropica**, v. 212, p. 105652, 2020. doi.org/10.1016/j.actatropica.2020.105652

JOSHI, N.; KUMAR, R. Utility of Entomotoxicology in Medico-Legal Investigations. **International Journal of Innovative Research in Science, Engineering and Technology (IJIRSET)**, v.9, n 6, 2020.

KADHIM, A.I.; HILAL, S. M.; OKAILY, R.A. Effect of imipramine on structures and shapes of wings of the blow fly *Calliphora vicina* (Diptera: Calliphoridae). **Al-Kufa University Journal for Biology**, v. 8, n. 2, 2016.

KHAN, A. A. M.; MAHMOOD, W. M. A. W.; SHAMSUDDIN, S. A.; ZAINI, N. S. A.; MOHAMED, K.; ABD RASHID, R. U. M. I. Z. A. Analysis of paracetamol in forensic blowfly samples from intoxicated-paracetamol carcass. **Malaysian Applied Biology Journal**, v. 44, n. 1, p. 31-35. 2015.

KHARBOUCHE, H.; AUGSBURGER, M.; CHERIX, D.; SPORKERT, F.; GIROUD, G.; WYSS, C.; CHAMPOD, C.; MANGIN, P. Codeine accumulation and elimination in larvae, pupae, and imago of the blowfly *Lucilia sericata* and effects on its development. **International Journal Legal Medical**, v. 122, p. 205–211,

2008. doi.org/10.1007/s00414-007-0217-z

KHEDRE, A. M. Effect of diazepam on the development of *Wohlfahrtia nuba* (diptera: sarcophagidae) and determination of drug levels in postmortem tissue, larvae and puparia. **Egyptian Journal of Zoology**, v. 45, p. 349-360, 2005.

KINTZ, P.; GODELAR, B.; TRACQUI, A.; MANGIN, P.; LUGNIER, A. A.; CHAUMONT, A. J. Fly larvae: a new toxicological method of investigation in forensic medicine. **Journal of Forensic Science**, v. 35, n.1, p. 204-207, 1990. doi: 10.1520/JFS12821J

LAWAI, V.; ABDUL RAHIM, N. A.; NGAINI, Z. Blowfly larval tissues as a secondary detector for determining Paraquat-related death in rabbit carcass. **Journal of Forensic Science**, v. 60, n. 6, p. 1620–1624, 2015. doi.org/10.1111/1556-4029.12852

LEI, G., LIU, F., LIU, P., ZHOU, Y., JIAO, T., DANG, Y. A bibliometric analysis of forensic entomology trends and perspectives worldwide over the last two decades (1998-2017). **Forensic Science International**. v. 295, p. 72-82. 2019. doi.org/10.1016/j.forsciint.2018.12.002

LEITE, G. L. D.; DE SÁ, V. G. M. Apostila: Taxonomia, Nomenclatura e Identificação de Espécies. Universidade Federal de Minas Gerais, Instituto de Ciências Agrárias, v. 50, 2010.

LEVINE, B.; GOLLE, M.; SMIALEK, J. E. An unusual drug death involving maggots. **The American journal of forensic medicine and pathology**, v. 21, n. 1, p. 59–61, 2000.

LIMA, C. G. P. D. Detecção e estudo sobre o efeito da metanfetamina e do ecstasy no desenvolvimento de imaturos de três espécies de *Chrysomya* (Diptera: Calliphoridae) de importância forense. Dissertação (mestrado) - Universidade Estadual Paulista, Instituto de Biociências de Botucatu. 2009.

LORD, W. D.; STEVENSON, J. R. Directory of forensic entomologists. 2 ed. Misc. Publ. **Armed Forces Pest Mgt. Board**, Washington, D.C, 42 p. 1986.

LÜ, Z.; ZHAI, X.; ZHOU, H.; LI, P.; MA, J.; GUAN, L.; MO, Y. Effects of Ketamine on the Development of forensically important Blowfly *Chrysomya megacephala* (F.) (Diptera: Calliphoridae) and its Forensic Relevance. **Journal of Forensic Sciences**, v. 59, n. 4, p. 991-996. 2014. doi.org/10.1111/1556-4029.12430

MADEIRA-OTT, T. Filogenia, biogeografia e revisão de espécies Neotropicalais de *Paralucilia* Brauer & Bergenstamm, 1891 (Diptera, Calliphoridae)= Phylogeny, biogeography and revision of Neotropical species of *Paralucilia* (Brauer & Bergenstamm, 1891 (Diptera, Calliphoridae)). Tese de Doutorado. 2023.

MAGNI, P. A.; PAZZI, M.; VINCENZI, M.; ALLADIO, E.; BRANDIMARTE, M.; DADOUR, I. R. Development and validation of a GC–MS method for nicotine detection in *Calliphora vomitoria* (L.) (Diptera: Calliphoridae). **Forensic Science International**, v. 261, p. 53-60. 2016. doi: 10.1016/j.forsciint.2015.11.014

MAGNI, P.A.; PACINI, T.; PAZZI, M.; VINCENZI, M.; DADOUR, I. R. Development of a GC–MS method for methamphetamine detection in *Calliphora vomitoria* L. (Diptera: Calliphoridae). **Forensic Science International**, v. 241, p. 96–101, 2014. doi: 10.1016/j.forsciint.2014.05.004.

MAHMOOD, W. M. A. W.; KHAN, A. A. M.; SHAMSUDDIN, S. A.; ZAINI, N. S. A.; MOHAMED, K.; RASHID, R. A. Paraquat dichloride detection from forensic blowfly samples. **Malays Appl Biol.**, v. 44, n. 1, p. 133–138. 2015.

MANHOFF, D. T.; HOOD, I.; CAPUTO, F.; PERRY, J.; ROSEN, S.; MIRCHANDANI, H. G. Cocaine in decomposed human remains. **Journal of Forensic Science**, v. 36, n. 6, p. 1732–1735. 1991. doi.org/10.1520/JFS13196J

MARINHEIRO, B. C. D. O. Detecção de diazepam em larvas de moscas calliphoridae através de HPLC MS/MS. 2019.

MARQUES, J. D. S. M. O efeito da carbamazepina sobre o desenvolvimento larval de *Chrysomya megacephala* (diptera: calliphoridae). 2017.

- MARTINIS, B. S. D.; DORTA, D. J.; COSTA, J. **Toxicologia forense**. [s.l: s.n.]. 2018
- MARTINS, E. Análise dos processos de decomposição e sucessão ecológica em carcaças de suínos (*Sus scrofa* L.) mortos por disparo de arma de fogo e overdose de cocaína e protocolo de procedimento diante de corpo de delito. Dissertação (mestrado) - Universidade Estadual Paulista, Instituto de Biociências de Botucatu, 2009.
- MARTINS, E.; NEVES, J. A.; MORETTI, T. C.; GODOY, W. A. C.; THYSSEN, P. J. Breeding of *Ornidia obesa* (Diptera: Syrphidae: Eristalinae) on pig carcasses in Brazil. **Journal of Medical Entomology**, v. 47, n. 4, p. 690-694. 2010. doi.org/10.1093/jmedent/47.4.690
- MASHALY, A.; AL-KHALIFA, M.; AL-QAHTNI, A. *Chrysomya albiceps* Wiedemann (Diptera: Calliphoridae) colonising poisoned rabbit carcasses. **Entomological Research**, v. 50, n. 11, p. 552-560. 2020. doi.org/10.1111/1748-5967.12483
- MILLER, M. L.; LORD, W.D.; GOFF, M. L.; DONNELLY, B.; MCDONOUGH, E. T.; ALEXIS, J. C. Isolation of amitriptyline and nortriptyline from fly puparia (Phoridae) and beetle exuviae (Dermestidae) associated with mummified human remains. **Journal of Forensic Science**, v. 39, n. 5, p.1305–1313. 1994. doi: 10.1520/JFS13717J
- MISE, K. M.; ALMEIDA, L. M.; MOURA, M. O. Levantamento da fauna de Coleoptera que habita a carcaça de. **Revista Brasileira de Entomologia**, v. 51, n. 3, p. 358–368, 2007. doi.org/10.1590/S0085-56262007000300014
- MOE, S. J.; STENSETH, N.C.; SMITH, R.H. Effects of a toxicant on population growth rates: sublethal and delayed responses in blowfly populations. **Functional Ecology**, v. 15, n. 6, p.712–721. 2001.
- MONTHEI, D. R. Entomotoxicological and thermal factors affecting the development of forensically important flies. Doctoral dissertation. Virginia Tech. 2009.
- MONZON, R. B.; SANCHEZ, A. R.; TADIAMAN, B. M.; NAJOS, O. A.; VALENCIA, E. G.; RUEDA, R. R.; VENTURA, J. V. M. A comparison of the role of *Musca domestica* (Linnaeus) and *Chrysomya megacephala* as mechanical vectors of helminthic parasites in a typical slum area of metropolitan Manila. **Southeast Asian Journal of Tropical Medicine Public Health**, v. 22, n. 2, p. 222-228, 1991.
- MORETTO, R. E. Efeito de citalopram no desenvolvimento larval de *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) para uso forense. Trabalho de conclusão de curso (bacharelado - Ciências Biológicas) - Universidade Estadual Paulista, Instituto de Biociências de Rio Claro. 2011.
- MOURA, F. T. Entomotoxicologia: perspectivas no Brasil e Rio Grande do Norte. (Bachelor's thesis, Universidade Federal do Rio Grande do Norte). 2018
- MULLANY, C.; KELLER, P. A.; NUGRAHA, A. S.; WALLMAN, J. F. Effects of methamphetamine and its primary human metabolite, p-hydroxymethamphetamine, on the development of the Australian blowfly *Calliphora stygia*. **Forensic science international**, v. 241, p. 102-111, 2014. doi.org/10.1016/j.forsciint.2014.05.003
- MUSVASVA, E.; WILLIAMS, K. A.; MULLER, W. J.; VILLET, M. H. Preliminary observations on the effects of hydrocortisone and sodium methohexital on development of *Sarcophaga (Curranella) tibialis* Macquart (Diptera: sarcophagidae), and implications for estimating post mortem interval. **Forensic Science International**, v. 120, n. 1–2, p. 37–41. 2001. doi.org/10.1016/S0379-0738(01)00415-7
- NETO, C. H. G. Dípteros necrófagos do Brasil: contribuições à entomologia forense. **Editora Autografia**. 2023.
- NOFIRA, Y. Analisis Metamfetamin pada Maggot Serangga Necrophagous Menggunakan Gas Chromatography-Mass Spectrometry (GCMS) sebagai Aplikasi Entomotoksikologi Forensik. Tese de Doutorado. Universitas Sumatera Utara. 2023.
- NOLTE, K. B.; PINDER, R. D.; LORD, W. D. Insect larvae used to detect cocaine poisoning in a decomposed body. **Journal of Forensic Science**, v. 37, n. 4, p. 1179–1185. 1992. doi:10.1520/JFS13304J

NUORTEVA, P.; NUORTEVA, S. L. The fate of Mercury in sarcosaprophagous flies and in insects eating them. **Ambio**, v. 11, n. 1, p. 34–37. 1982.

O'BRIEN, C.; TURNER, B. Impact of paracetamol on *Calliphora vicina* larval development. **International journal of legal medicine**, v. 118, p. 188-189, 2004. doi.org/10.1007/s00414-004-0440-9

OLIVEIRA, H. G.; GOMES, G.; MORLIN JR, J. J.; VON ZUBEN, C. J.; LINHARES, A. X. The effect of Buscopan® on the development of the blow fly *Chrysomya megacephala* (F.) (Diptera: Calliphoridae). **Journal of forensic sciences**, v. 54, n. 1, p. 202-206, 2009. doi.org/10.1111/j.1556-4029.2008.00926.x

OLIVEIRA, J.S.; BAIA, T. C.; GAMA, R. A.; LIMA, K. M. G. Development of a novel non-destructive method based on spectral fingerprint for determination of abused drug in insects: an alternative entomotoxicology approach. **Microchemical Journal**, v. 115, p. 39–46. 2014. doi: 10.1016/j.microc.2014.02.009

OLIVEIRA, T. C.; VASCONCELOS, S. D. Insects (Diptera) associated with cadavers at the Institute of Legal Medicine in Pernambuco, Brazil: Implications for forensic entomology. **Forensic Science International**, v. 198, n. 1–3, p. 97–102, 2010. dx.doi.org/10.1016/j.forsciint.2010.01.011.

OLIVEIRA-COSTA, J. "Insetos Peritos": A Entomologia Forense no Brasil. 1º ed. Campinas, SP: **Millennium Editora**, 2013.

OLIVEIRA-COSTA, J. Entomologia Forense: Quando os insetos são vestígios. 2º ed. Campinas, SP: **Millennium Editora**, 2008.

OLIVEIRA-COSTA, J.; LOPES, S. A relevância da entomologia forense para a perícia criminal na elucidação de um caso de suicídio. **Entomologia y Vectores**, v. 7, n.2, p. 203–209. 1999.

PARRY, S.; LINTON, S. M.; FRANCIS, P. S.; O'DONNELL, M. J.; TOOP, T. Accumulation and excretion of morphine by *Calliphora stygia*, an Australian blow fly species of forensic importance. **Journal of insect physiology**, v. 57, n. 1, p. 62-73, 2011. Doi.org/10.1016/j.jinsphys.2010.09.005

PAWAR, H. M.; DESHMUKH, G. M. Effect of alprazolam on the developmental stages of forensically important blow fly *Chrysomya megacephala* (Diptera: Calliphoridae). **Journal of Entomology and Zoology Studies**, v. 10, n. 3, p. 101-104. 2022. doi.org/10.22271/j.ento.2022.v10.i3b.8996

PEREIRA, M. C. Ação de topiramato sobre o desenvolvimento larval de *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). Trabalho de conclusão de curso (bacharelado - Ciências Biológicas) - Universidade Estadual Paulista, Instituto de Biociências de Rio Claro, 2011.

PIEN, K.; LALOUP, M.; PIPELEERS-MARICHAL, M.; GROOTAERT, P.; DE BOECK, G.; SAMYN, N.; BOONEN, T.; VITS, K.; WOOD, M. Toxicological data and growth characteristics of single post-feeding larvae and puparia of *Calliphora vicina* (Diptera: Calliphoridae) obtained from a controlled nordiazepam study. **International journal of legal medicine**, v. 118, p. 190-193, 2004. doi.org/10.1007/s00414-004-0441-8

PONTES, L. P. P.; DA COSTA FRANCES, P. A.; DE MAGALHÃES, L. K. A. Agentes tóxicos e o desenvolvimento de insetos: uma revisão bibliográfica e sua aplicabilidade em entomotoxicologia. **Revista Brasileira de Criminalística**, v. 11, n. 2, p. 30-37, 2022. doi.org/10.15260/rbc.v11i2.502

PREUBER, D.; BRÖRING, U.; FISCHER, T.; JURETZEK, T. Effects of antibiotics ceftriaxone and levofloxacin on the growth of *Calliphora vomitoria* L. (Diptera: Calliphoridae) and effects on the determination of the post-mortem interval. **Journal of Forensic and Legal Medicine**, v. 81, p. 102207. 2021. doi.org/10.1016/j.jflm.2021.102207

PREUBER, D.; FISCHER, T.; JURETZEK, T. Effects of antibiotics ceftriaxone and levofloxacin on the growth of *Lucilia sericata* (Diptera: Calliphoridae). **Medical and Veterinary Entomology**, v. 37, n. 4, p. 805-815. 2023. doi.org/10.1111/mve.12685

PUJOL-LUZ, J. R.; ARANTES, L. C.; CONSTANTINO, R. Cem anos da entomologia forense no Brasil (1908-2008). **Revista Brasileira de Entomologia**, v. 52, p. 485-492, 2008. doi.org/10.1590/S0085-56262008000400001

- QUEIROZ, M. M. C. Aspectos da bioecologia de *Chrysomya albiceps* (Wiedemann, 1819) (Diptera, Calliphoridae), em condições de laboratório. Dissertação (Mestrado em Ciências Veterinárias) - Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, Seropédica - RJ, 1991.
- QUEIROZ, M. M. D. C.; MELLO, R. P. D.; LIMA, M. M. Morphological aspects of the larval instars of *Chrysomya albiceps* (Diptera, Calliphoridae) reared in the laboratory. **Memórias do Instituto Oswaldo Cruz**, 92, 187-196. 1997. doi.org/10.1590/S0074-02761997000200010
- RASHID, R. A.; OSMAN, K.; ISMAIL, M. I.; ZUHA, R. M.; HASSAN, R. A. Determination of malathion levels and the effect of malathion on the growth of *Chrysomya megacephala* (Fabricius) in malathion-exposed rat carcass. **Tropical Biomedicine**, v. 25, n. 3, p. 184–190. 2008.
- REZENDE, F. Avaliação do desenvolvimento de imaturos de três espécies de *Chrysomya* (Diptera: Calliphoridae) de importância forense, sob o efeito de cloridrato de metilfenidato isolado e associado a Fenobarbital (Doctoral dissertation, Universidade Estadual de Campinas (UNICAMP). Instituto de Biologia). 2012.
- REZENDE, F.; ALONSO, M. A.; SOUZA, C. M.; THYSSEN, P. J.; LINHARES, A. X. Developmental rates of immatures of three *Chrysomya* species (Diptera: calliphoridae) under the effect of methylphenidate hydrochloride, phenobarbital, and methylphenidate hydrochloride associated with phenobarbital. **Parasitology research**, v. 113, p. 1897-1907, 2014. doi.org/10.1007/s00436-014-3837-5
- RICHARD, R. D.; AHRENS, E. H. New distribution record for the recently introduced blowfly *Chrysomya rufifacies* (Macquart) in North America, **Southwestern Entomologist**, v.8, n. 3, p. 216–218. 1983.
- ROETERDINK, E. M.; DADOUR, I. R.; WATLING, R. J. Extraction of gunshot residues from the larvae of the forensically important blowfly *Calliphora dubia* (Macquart) (Diptera: Calliphoridae). **International journal of legal medicine**, v. 118, p. 63-70, 2004. doi: 10.1007/s00414-003-0408-1
- ROQUETTE-PINTO, E. Nota sobre a fauna cadavérica do Rio de Janeiro. **A Tribuna Médica**, v. 21, n. 1-11, p. 413-417, 1908.
- SADLER, D. W.; FUKU, C.; COURT, F.; POUNDER, D. J. Drug accumulation and elimination in *Calliphora vicina* larvae. **Forensic science international**, v. 71, n. 3, p. 191-197, 1995. doi.org/10.1016/0379-0738(94)01663-1
- SADLER, D. W.; RICHARDSON, J.; HAIGH, S.; BRUCE, G.; POUNDER, D. J. Amitriptyline accumulation and elimination in *Calliphora vicina* larvae. **The American Journal Forensic Medicine and Pathology**, v. 18, n. 4, p. 397-403, 1997.
- SALIMI, M.; RASSI, Y.; CHATRBAGOUN, O.; KAMALI, A.; OSHAGHI, M. A.; SHIRI-GHALEH, V.; PARKHIDEH, S. Z. Toxicological analysis of insects on the corpse: a valuable source of information in forensic investigations. **Journal of Arthropod-Borne Diseases**, v. 12, n. 3, p. 219, 2018.
- SAMNOL, A.; KUMAR, R.; MAHIPAL SINGH, S.; PARIHAR, K. Study of insect larva used to detect toxic substance through decomposed bodies. **International Journal Forensic Science and Pathology** v. 7, n. 1, p. 420-422, 2020.
- SANTANA, C. S.; BOAS, D. S. V. Entomologia forense: insetos auxiliando a lei. **Revista Ceciliansa**, v. 4, n. 2, p. 31-34, 2012.
- SANTOS, F. V. D. Efeito de anticoncepcional humano no desenvolvimento larval de *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) para uso forense. Trabalho de conclusão de curso (bacharelado - Ciências Biológicas) - Universidade Estadual Paulista, Instituto de Biociências de Rio Claro, 2013.
- SHERMAN, R. A.; WYLE, F.A.; THRUPP, L. Effects of seven antibiotics on the growth and development of *Phaenicia sericata* (Diptera: Calliphoridae) larvae. **Journal Medical Entomology**, v. 32, p.646–649. 1995. doi.org/10.1093/jmedent/32.5.646

- SHI, Y. W.; LIU, X. S.; WANG, H. Y.; ZHANG, R. Z. Effects of Malathion on the insect succession and the development of *Chrysomya megacephala* (Diptera: calliphoridae) in the field and implications for estimating postmortem interval. **The American Journal Forensic Medicine and Pathology.**, v. 31, n. 1, p. 46–51. 2010. doi: 10.1097/PAF.0b013e3181c215b4
- SILVA, A. R.; REIS, B. S. A importância da entomologia forense nas investigações criminais. In: **VII CONNEPI-Congresso Norte Nordeste de Pesquisa e Inovação.** 2012.
- SILVA, E. I.; WILHELM, B.; VILLET, M. H. Forensic entomotoxicology revisited—towards professional standardisation of study designs. **International Journal of Legal Medicine**, v. 131, p. 1399-1412. 2017. doi.org/10.1007/s00414-017-1603-9
- SILVA, J. O.; CARVALHO-FILHO, F. D. S.; ESPOSITO, M. C.; REIS, G. A. First record of *Chrysomya rufifacies* (Macquart) (Diptera, Calliphoridae) from Brazil. **Revista Brasileira de Entomologia**, v. 56, p. 115-118. 2012. doi.org/10.1590/S0085-56262012000100019
- SINGH, B.; WELLS, J. D. Chrysomyinae (Diptera: Calliphoridae) is monophyletic: a molecular systematic analysis. **Systematic Entomology**, v. 36, n. 3, p. 415-420, 2011. doi.org/10.1111/j.1365-3113.2011.00568.x
- SOTO, D. A. E. The effect of two barbiturates on the development of three calliphoridae species (Diptera) of forensic importance. (Doctoral dissertation). 2008.
- SOUZA, C. M.; THYSSEN, P. J.; LINHARES, A. X. Effect of nandrolone decanoate on the development of three species of *Chrysomya* (Diptera: Calliphoridae), flies of forensic importance in Brazil. **Journal of medical entomology**, v. 48, n. 1, p.111-117. 2014. doi.org/10.1603/ME09291
- TABOR, K. L.; FELL, R. D.; BREWSTER, C. C.; PELZER, K.; BEHONICK, G.S. Effects of antemortem ingestion of ethanol on insect successional patterns and development of *Phormia regina* (Diptera: Calliphoridae). **Journal of Medical Entomology**, v. 42, p. 481-489. 2005. doi.org/10.1093/jmedent/42.3.481
- THÉ, T. S. **Entomologia Forense: Os Insetos e a Perícia Criminal**, 2021.
- THYSSEN, P. J.; GRELLA, M. D. Efeito da escopolamina sobre o desenvolvimento de *Chrysomya putoria* (Diptera: Calliphoridae) e sua importância para a estimativa do intervalo pós-morte. **Revista Brasileira de Criminalística** v. 1, n. 1, p. 39-42. 2011.
- TOMBERLIN, J. K.; MOHR, R.; BENBOW, M. E.; TARONE, A. M.; VANLAERHOVEN, S. A roadmap for bridging basic and applied research in forensic entomology. **Annual Review of Entomology**, v. 56, p. 401–421, 2011. doi.org/10.1146/annurev-ento-051710-103143
- TRIVIA, A. L.; DE CARVALHO PINTO, C. J. Analysis of the effect of cyclophosphamide and methotrexate on *Chrysomya megacephala* (Diptera: Calliphoridae). **Journal of forensic sciences**, v. 63, n. 5, p. 1413-1418, 2018. doi.org/10.1111/1556-4029.13740
- VISSER, H. The influence of Methylphenidate on the development of the forensically significant blow fly *Chrysomya chloropyga* (Diptera: Calliphoridae) in the Western Cape Province. University of Cape Town. **Master Thesis.** 2016.
- VAIRO, K. P.; MOURA, M. O. Entomologia forense na prática: do laboratório à utilização dos vestígios. 1 ed. Campinas, SP; **Millennium Editora**, 2021.
- VARGAS, J.; WOOD, D. M. Calliphoridae (Blow flies). In Brown, B. V.; A. Borkent; J. M. Cumming; D. M. Wood; N. E. Woodley & M. A. Zumbado. Manual of Central American Diptera, volume 2. Ottawa, **NRC Research Press**, 728 p. 2010.
- WELLS, J. D.; BYRD, J. H.; TANTAWI, T. I. Key to third-instar Chrysomyinae (Diptera: Calliphoridae) from carrion in the continental United States. **Journal of Medical Entomology**, v. 36, n. 5, p. 638-641. 1999. doi.org/10.1093/jmedent/36.5.638
- WHITWORTH, T. Keys to the genera and species of blow flies (Diptera: Calliphoridae) of the West Indies and

description of a new species of *Lucilia* Robineau-Desvoidy. **Zootaxa**, v. 2663, n. 1, p. 1-35, 2010.

WHITWORTH, T. Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America, North of Mexico. In: **Forensic Entomology**. CRC Press, p. 413-443. 2019.

WILLIAMS, K. A.; VILLET, M. H. Effects of hydrocortisone and sodium methohexital on growth rate of *Chrysomya chloropyga* Wiedemann (Diptera: Calliphoridae): developmental and behavioural indications of presence of drugs. **Durban Museum Novitates**, v. 37, p. 25–29. 2014.

WILSON, Z.; HUBBARD, S.; POUNDER, D. J. Drug analysis in fly larvae. **The American Journal Forensic Medicine and Pathology**, v. 14, n.2, p. 118–120. 1993.

WOOD, M.; LALOUP, M.; PIEN, K.; SAMYN, N.; MORRIS, M.; MAES, R. A. A.; de BRUIJN, E. A.; MAES, V.; DE BOECK, G. Development of a rapid and sensitive method for the quantitation of benzodiazepines in *Calliphora vicina* larvae and puparia by LC-MS-MS. **Journal of analytical toxicology**, v. 27, n.7, p. 505-512. 2003. doi.org/10.1093/jat/27.7.505

WOOD, T.; PYPER, K.; CASALI, F. Effects of cocaine and heroin, and their combination, on the development rate of *Calliphora vomitoria* (Diptera: Calliphoridae). **Science & Justice**, v. 62, n. 4, p. 471-475, 2022.

WORLD HEALTH ORGANIZATION (WHO). Alcohol. 2022. Disponível em: <https://www.who.int/es/news-room/fact-sheets/detail/opioid-overdose>

WORLD HEALTH ORGANIZATION (WHO). Drugs (psychoactive). 2023. Disponível em: https://www.who.int/health-topics/drugs-psychoactive#tab=tab_1

WORLD HEALTH ORGANIZATION (WHO). Opioid overdose. 2023. Disponível em: <https://www.who.int/es/news-room/fact-sheets/detail/opioid-overdose>

WORLD HEALTH ORGANIZATION (WHO). The public health dimension of the world drug problem: how WHO works to prevent drug misuse, reduce harm and improve safe access to medicine. 2019. Disponível em: <https://www.who.int/publications/i/item/WHO-MVP-EMP-2019.02>

YAN-WEI, S.; XIAO-SHAN, L.; HAI-YANG, W.; RUN-JIE, Z. Effects of malathion on the insect succession and the development of *Chrysomya megacephala* (Diptera: Calliphoridae) in the field and implications for estimating postmortem interval. **The American Journal of Forensic Medicine and Pathology**, v. 31, n. 1, p. 46-51, 2010. doi:10.1097/PAF.0b013e3181c215b4

ZANETTI, N. I.; FERRERO, A. A.; CENTENO, N. D. Determination of fluoxetine in *Dermestes maculatus* (Coleoptera: dermestidae) by a spectrophotometric method. **Science Justice**, v. 56, n.6, p. 464–467. 2016. doi.org/10.1016/j.scijus.2016.07.005

ZANETTI, N. I.; FERRERO, A. A.; CENTENO, N. D. The use of two fly species to detect the anti-depressant Fluoxetine post-mortem (Diptera: Calliphoridae: *Lucilia sericata* Meigen, Sarcophagidae: *Sarcophaga crassipalpis* Macquart). **Entomologica Americana**, v. 125, n. 1-4, p. 4-9, 2019. doi.org/10.1664/1947-5136-125.1.9

ZEHRA, N.; MISHRA, V. An entomotoxicological study on the influence of house hold toxins on the colonization of carrion by *Sarcophaga haemorrhoidalis*. **IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)**, v.14, p. 34-40. 2020.

ZHU, G.H.; XU, X.H.; YU, X.J.; ZHANG, Y; WANG, J.F. Puparial case hydrocarbons of *Chrysomya megacephala* as an indicator of the postmortem interval. **Forensic Science International**, v. 169, n. 1, p. 1–5. 2007. doi.org/10.1016/j.forsciint.2006.06.078

ZOU Y, HUANG M, HUANG R.; WU, X.; YOU, Z.; LIN, J.; HUANG, X.; QIU, X.; ZHANG, S. Effect of ketamine on the development of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) and preliminary pathological observation of larvae. **Forensic Science International**, v. 226, n. 1-3, p.273–281. 2013. doi: 10.1016/j.forsciint.2013.01.042

SECOND PART: ARTICLES

ARTICLE I

Sublethal effects of clonazepam on the development of blowflies of forensic importance

Journal of Medical Entomology standards (preliminary version)

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Abstract

The presence of fly larvae in cadavers is used for estimating calculating the post-mortem interval (PMI). Anxiolytics have been increasingly consumed in the world, and in Brazil there is a problem of improper consumption. The presence of some substances in the food source of larvae influences the development of insects. Therefore, the objective of the present study was to evaluate the effects on the biology of *Chrysomya albiceps* and *C. megacephala* of different concentrations of the benzodiazepine clonazepam. New larvae (> 1h years old) were exposed to three sublethal concentrations (0.0625, 0.125 and 0.250 mg mL⁻¹) of the clonazepam. Parameters such as larval, pupal, puparium and adult weights and the developmental periods of each stage were observed. The results obtained showed a reduction in weights and a delay in developmental time, the larvae and pupae, at the lowest concentration for *C. albiceps* (F=124.719; df=3; p<0.001) and *C. megacephala* (F=61.817; df=4; p<0.001). Therefore, we

concluded that clonazepam, even at very low concentrations, affects parameters that are directly used in the determination of post-mortem interval.

Keywords: Entomotoxicology; Diptera; benzodiazepines; hormesis.

1 INTRODUCTION

Forensic entomology, as a study related to criminal investigations, uses insects and other arthropods (Oliveira-Costa 2008, 2013). The medico-legal subfield is particularly focused on cases of crimes against life in which the presence of insects in decomposing corpses (Gomes 2010) and this approach is fundamental to estimate the time of death of the victim through the calculation of the Post Mortem Interval (PMI). For this, it is necessary to know, through identification, the species of entomofauna found in carcasses or cadavers at that stage (Paseto 2014). The group of blowflies or screw worm flies (Diptera: Calliphoridae) deserves attention, not only for being the group with the highest occurrence in carcasses and corpses, but also for being associated with health in medical and veterinary issues (Neto, 2023; Carvalho, Mello-Patiu and Almeida, 2021). They are characterized by having a bright blue, green or metallic bronze color in the thorax and abdomen (Carvalho, Mello-Patiu 2008; Leite and De Sá, 2010).

In post-mortem situations, when the corpse is in an advanced stage of decomposition, making it impossible to obtain the substrate (organs, blood, urine, gastric content) used for toxicological studies, insects in their immature stage are a valuable alternative for obtaining the presence of substances in corpses (Andréa 2008). In this context, entomotoxicology, a recent branch of forensic entomology, plays a crucial role in the detection and identification of substances present in tissues and fluids that have already decomposed. Such substances may have caused death through misuse, poisoning, or even overdose (Goff and Lord 1994; Introna, Campobasso, and Goff, 2001; Amendt, Krettek, and Zehner 2004; Salimi *et al.* 2018; Hodecek 2020). Performing entomotoxicological analyses on larvae can indicate the presence of

substances, as the influence of different substances on larval development rates can lead to an inaccurate estimate of the PMI (Goff 1992; Hedouin *et al.* 1999; Introna, Campobasso and Goff 2001).

Benzodiazepines are a class of psychotropic drugs known for their anxiolytic and sedative effects. They play an important role in the treatment of disorders related to anxiety and insomnia. However, there are growing concerns about safety and the potential for abuse, such as dependence (Boulkenafet *et al.* 2020; Stillion 2023). In Brazil, benzodiazepines are among the classes of drugs most used by the population. However, this use is still inappropriate, since many people do not follow the prescription correctly, making it a substance commonly associated with deaths, such as overdoses (Oliveira-Costa, 2013; Basto *et al.* 2017).

Therefore, this work aimed to evaluate the effects of different concentrations of the benzodiazepine clonazepam on the biology of *C. albiceps* and *C. megacephala*. This work also aimed to test the following hypotheses: 1) The clonazepam present in the substrate does affect the development of the larvae and pupae of *C. albiceps* and *C. megacephala*; 2) The species *C. albiceps* and *C. megacephala* do present any alteration in their biological cycle when exposed to different concentrations of Clonazepam; 3) There are differences between the physiological responses during the development of the species *C. albiceps* and *C. megacephala*.

2. MATERIAL AND METHODS

2.1 Collection sites

2.1.1 Collection of the *C. albiceps*

Adult specimens of *C. albiceps* were collected with the help of an entomological network at the Oswaldo Cruz Foundation (FIOCRUZ) in the city of Rio de Janeiro/RJ, and later identified under a stereoscopic microscope (Zeiss Stemi sv6) at the Integrated Laboratory of Simuliids and Onchocerciasis & Medical and Forensic Entomology (LSOEMF- ICO). A cage

was set up with the collected adults, where minced meat was offered as oviposition substrate. The newly obtained individuals were followed until the pupal stage, at which point they were prepared for transport (n°89235-1 SISBio) to the Laboratory of Molecular Entomology and Ecotoxicology (M.E.E.T.) of the Department of Entomology of the Federal University of Lavras (DEN-UFLA), for the development of toxicological experiments.

2.1.2 Collection of the *C. megacephala*

Larvae specimens of *C. megacephala* were collected with entomological forceps in compost material at the Composting Sector of the Federal University of Lavras (G-OLEO/UFLA). The specimens were taken to the Laboratory of Molecular Entomology and Ecotoxicology (M.E.E.T.) of the Department of Entomology - UFLA, where they were transferred to a minced meat substrate, as this substrate allows a better development (Rabêlo *et al.* 2011). The specimens were followed until emergence, when they were properly identified under a stereoscope (Trinocular Stereoscope Microscope ZOOM DI-152T) using the dichotomous key for adult specimens of Carvalho & Mello-Patiu (2008). These adults were placed in plastic cages (21.5x21.5x25cm³) with voil sleeves (adapted from Estrada *et al.*, 2008) to establish rearing.

2.2 Description of obtaining the clonazepam

Since clonazepam is a controlled medication, it was obtained through a medical prescription for research purposes and authorized by a specialist physician, psychiatrist. The medication was picked up at the municipal public pharmacy with the presence of the prescription.

2.3 Conditions laboratorial of rearing

All developmental stages were maintained in a climate-controlled room at $27 \pm 1^\circ\text{C}$, 12L:12D and a relative humidity of $60 \pm 10\%$ (Estrada *et al.*, 2008).

2.3.1 Rearing of adult individuals

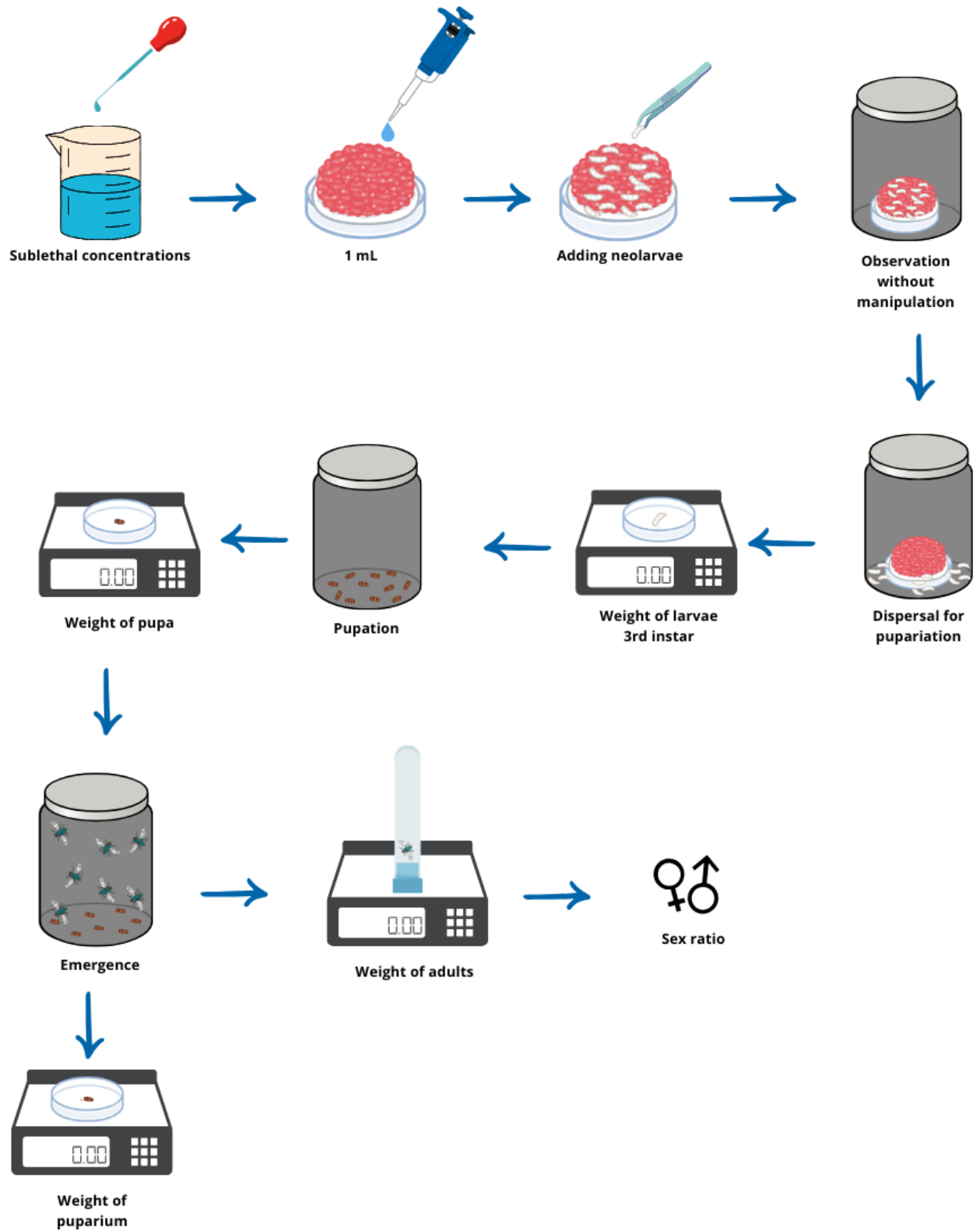
For adult insects, 80% sucrose solution and water were offered as food. For the newly emerged females, 20 g of minced meat, changed daily, was offered for 4 days for the maturation of the ovarian follicles. After 11 days, a substrate was offered to lay and collect eggs.

2.3.2 Rearing of immatures individuals

Eggs obtained from oviposition were removed from the substrate with a fine, soft-tipped brush and transferred to a fresh substrate containing approximately 1.5g of 24-hour decayed minced meat per larva. The larvae were placed above the food (100mL disposable pots) in a rearing pot (300mL plastic pots) with a cut-out lid and voile to allow air in, with vermiculite at the bottom. When they reached the pupal stage, they were removed from the vermiculite and transferred to the cages until they emerged.

2.4 Toxicology Bioassays

Figure 1. Stages of the toxicology bioassays procedure



(Bibiano, 2024)

2.4.1 Bioassays with *C. albiceps*

First, a bioassay was performed with *C. albiceps* to obtain the sublethal concentrations. Two concentrations were prepared from clonazepam ($2.5 \text{ mg}\cdot\text{mL}^{-1}$): 0.125 and $0.250 \text{ mg}\cdot\text{mL}^{-1}$, as well as deionized water and the control without the addition of any liquid. In the treatments where the liquids were added, 1 mL was used for each replicate. Subsequently, the substrate was subjected to mechanical homogenization, after which the 30 neonate larvae were placed on it, with each larva representing a repetition. Four repetitions were conducted for each concentration. The substrate was not manipulated until the larvae began to leave it, to avoid stressing the individuals. The migrating larvae were observed for dispersal behavior and the onset of pupation, followed by the pupation stage and the emergence of adults. During these developmental stages, the individuals were weighed using an analytical balance (BEL Engineering M214Ai).

The biological parameters evaluated were: duration of the larval stage, duration of the pupal stage, egg-pupa, total cycle duration (egg-adult), weight of the pupa, weight of the puparium, weight of the adult and sex ratio of the emerged adults. To test the effect of the substance on the above parameters, the data were subjected to one-way ANOVA and Dunn's multiple comparison test to compare the treatments with the control. For the egg-adult parameter, only individuals that were able to complete the entire cycle were considered. Larval weight (L3) was not evaluated because the specimens pupated in the substrate. The factor of sex ratio of emerged adults was not analyzed, because this species presents the behavior of generations with only one sex (Queiroz 1991).

2.4.2 Bioassays with *C. megacephala*

The experiments with the species *C. megacephala* were performed after the cohort reached the first generation (F2) of flies hatched in the laboratory, to avoid bioaccumulation of

substances that might be present in the substrate where they were collected and adapted to laboratory conditions. The experimental design was performed in blocks of two replicates each with 30 neonate larvae (<1h old) per replicate. Each replicate contained 40 g of minced meat in an initial state of putrefaction of 24 h in disposable plastic containers of 100 ml, which were placed in larger plastic containers (item 2.3.2). The experiment was carried out under the same conditions as in the rearing room.

Three concentrations were prepared: 0.0625, 0.125, and 0.250 mg.mL⁻¹, of commercially available clonazepam (2.5 mg.mL⁻¹), deionized water, and the control without addition of any liquid. In treatments where liquid was added, 1 mL was used for each replicate. The substrate was then mechanically homogenized and the larvae were placed on the substrate. There was no manipulation of the substrate until the larvae began to leave it, to avoid any stress to the individuals. The migrating larvae were monitored as they exhibited dispersal behavior, began to pupate, reached the pupation stage, and subsequently emerged as adults. At these developmental stages, the individuals were weighed using an analytical balance (BEL Engineering M214Ai).

The biological parameters evaluated were: larval stage duration, pupal stage duration, egg-pupa, total cycle duration (egg-adult), larval weight (L3), pupal weight, puparium weight, adult weight, and sex ratio of emerged adults. To test the effect of the substance on the parameters, the data were subjected to one-way ANOVA and Dunn's mean comparison test to compare the treatments with the control. For the adult egg parameter, only individuals that were able to complete the entire cycle were considered. For the adult weight parameter, data were subjected to ANOVA Two-Way with Holm-Sidak's means comparison test for the factors sex ratio of emerged adults and concentrations. All statistical tests were performed using the GLM package in the RStudio program software for students.

3. RESULTS

3.1 Effects of different concentrations of clonazepam on the juvenile stages of *C. albiceps*

Analysis of variance showed significant differences in all the parameters evaluated. The duration of the larval stage ($F=128.237$; $df=3$; $p<0.001$) was higher in the water treatment and lower in the 0.125 mg.mL^{-1} concentration (Table 1). For the duration of the pupal stage ($F=38.579$; $df=4$; $p<0.001$), there was an increase and a decrease in the 0.125 and 0.250 mg.mL^{-1} concentrations, respectively. For the duration of the egg-pupa stage ($F=128.237$; $df=3$; $p<0.001$) and the duration of the total cycle (egg-adult) ($F=124.719$; $df=3$; $p<0.001$), there was a difference in all treatments, with the longest duration in the water treatment and a reduction of this time in the 0.125 mg.mL^{-1} concentration (Table 1).

For morphological parameters related to weight, pupa weight ($F=176.828$; $df=3$; $p<0.001$) and puparium weight ($F=68.005$; $df=3$; $p<0.001$) were highest in the water treatment and decreased in all the clonazepam concentrations, with the lowest weight in the 0.250 concentration (Table 2) (Figure 2). For adult weight ($F=109.428$; $df=3$; $p<0.001$), only the 0.125 and 0.250 mg.mL^{-1} concentrations showed a reduction compared to controls (Table 2) (Figure 2a).

3.2 Effects of different concentrations of clonazepam on the juvenile stages of the *C. megacephala*

megacephala

Analysis of variance showed significant differences for the concentration of $0.0625 \text{ mg.mL}^{-1}$ in all evaluated parameters: larval stage duration ($F=61.817$; $df=4$; $p<0.001$), pupal stage duration ($F=170.337$; $df=4$; $p<0.001$), egg-pupa ($F=61.817$; $df=4$; $p<0.001$) and egg-adult stage duration ($F=189.853$; $df=4$; $p<0.001$) (Table 1), larval weight ($F=63.849$; $df=4$; $p<0.001$) and pupa weight ($F=72.435$; $df=4$; $p<0.001$) (Figure 3). On the other hand, puparium weight ($F=7.409$; $df=4$; $p=0.116$) was the only parameter where the differences were not significant for all treatments (Figure 1b) (Table 2).

For adult weight, analysis of variance showed significant differences ($F=63.132$; $df=4$; $p<0.001$). The factors treatment ($F=14.158$; $df=4$; $p<0.001$) and sex ($F=15.659$; $df=1$; $p<0.001$) did not show a significant effect when evaluated separately. However, when the interaction "treatment x sex" was analyzed, the difference was significant ($F=3.236$; $df=4$; $p=0.012$) at the concentration of $0.0625 \text{ mg}\cdot\text{mL}^{-1}$ compared to the control (Table 3). For the sex ratio, the analysis was significant ($t=3.957$; $df=1$; $p<0.001$) in the water treatment and at the concentration of 0.125 mg mL^{-1} (Figure 2b).

Table 1. Effect of different concentrations of clonazepam on the duration (in days) of developmental stages of de *C. albiceps* and *C. megacephala*.

Specie	Treatment(mg/ml)	Duration in days of stages			
		Larva	Pupal	Egg-pupae	Egg-adult
<i>C. albiceps</i>	Control	9.11±0.055	4.49±0.047	10.11±0.055	13.59±0.078
	Water	9.85±0.072***	4.62±0.052	10.85±0.072***	1465±0.076***
	0.125	8.21±0.049***	4.74±0.044***	9.21±0.049***	12.96±0.058***
	0.250	9.55±0.434	3.97±0.103***	10.55±0.434***	13.52±0.516***
<i>C. megacephala</i>	Control	5.50±0.047	5.54±0.069	6.50±0.047	10.02±0.079
	Water	5.40±0.052	5.17±0.031	6.40±0.052	9.57±0.055
	0.0652	6.04±0.009***	6.96±0.009***	7.04±0.009***	12.0±0.000***
	0.125	5.60±0.050	5.33±0.046	6.60±0.050	9.93±0.015
	0.250	5.70±0.044	5.38± 0.050	6.70±0.044	10.08±0.015

*** $p<0.001$ (Teste de Holm-Sidak Significative). The stage durations are expressed as Mean \pm SE

Table 2. Effect of different concentrations of clonazepam in weight (mg) of larvae, pupae, puparium and adults of *C. albiceps* and *C. megacephala*.

Specie	Treatment _(mg/ml)	Weight (mg)			
		Larva	Pupa	Puparium	Adult
<i>C. albiceps</i>	Control	-	67.99±0.49	9.85±0.12	41.01±0.55
	Water	-	70.86±0.50 ^{***}	10.86±0.19 ^{***}	41.69±0.78
	0.125	-	59.82±0.36 ^{***}	9.11±0.12 ^{***}	33.65±0.12 ^{***}
	0.25	-	50.92±0.18 ^{***}	7.36±0.32 ^{***}	27.24±0.16 ^{***}
<i>C. megacephala</i>	Control	72.84±0.45	56.14±0.37	4.87±0.82	40.93±0.38
	Water	74.69±0.65	58.09±0.49	4.79±0.76	41.79±0.42
	0.0652	82.93±0.94 ^{***}	64.94±0.85 ^{***}	5.36±0.16	35.14±0.67 ^{***}
	0.125	75.02±0.52	59.84±0.43	4.97±0.86	42.02±0.41
	0.25	75.64±0.46	63.50±0.45	4.77±0.68	42.10±0.45

^{***}p<0.001 (Significant Holm-Sidak Test). The stage durations are expressed as Mean ± SE

- 1 Figure 2. Average weight (mg) of the different development stages: pupa, puparium and adult of (A) *C. albiceps*; and larva, pupa, puparium e
 2 adult of (B) *C. megacephala*, in the different concentrations of clonazepam.

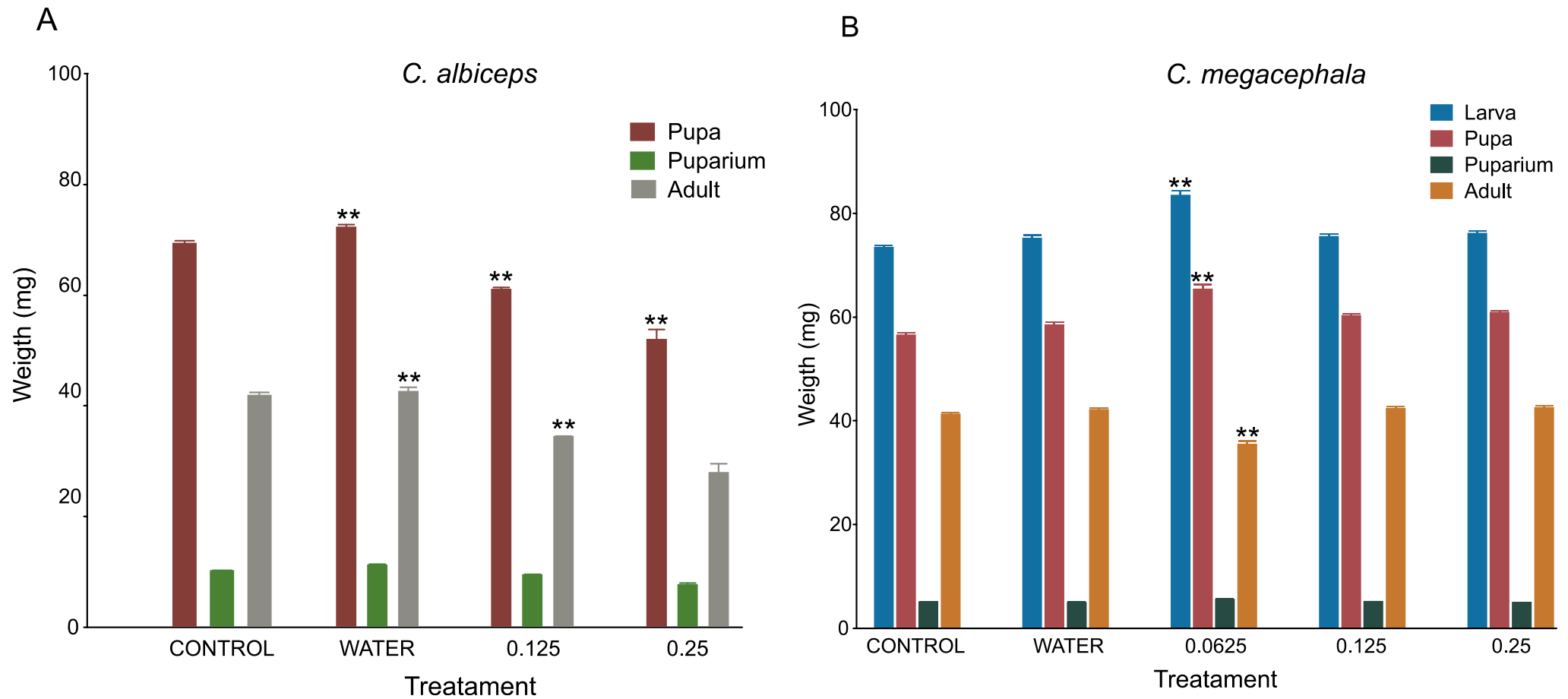
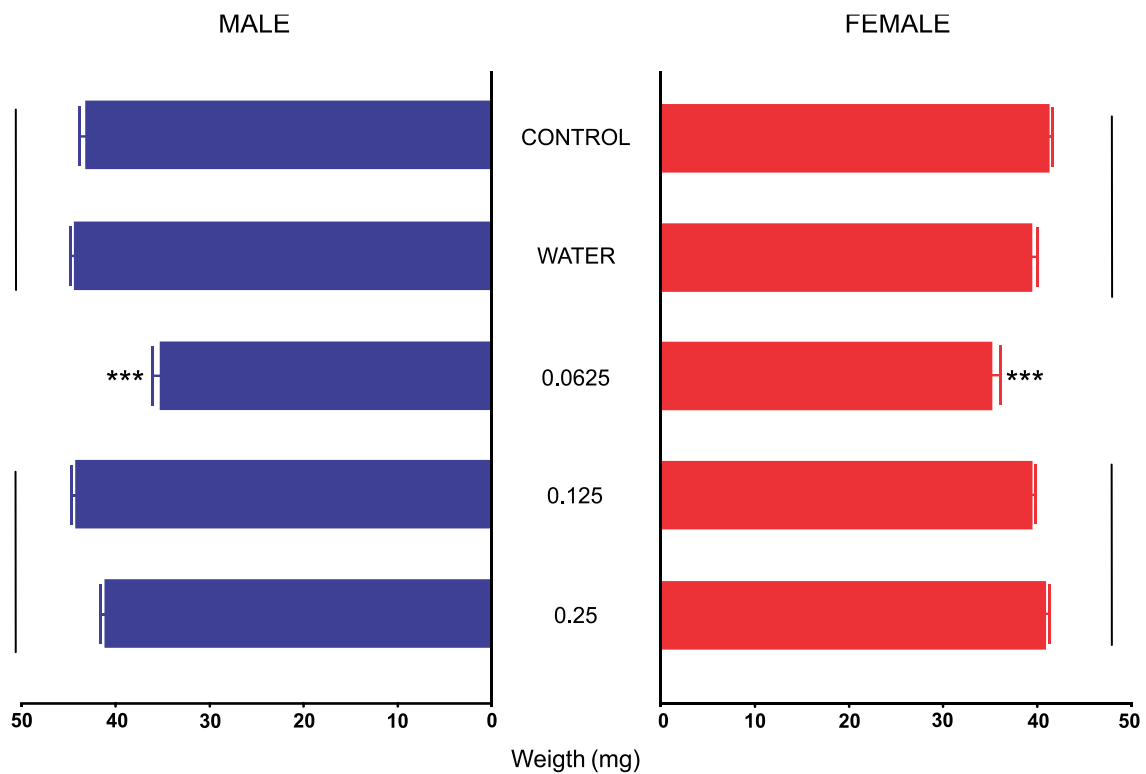


Table 3. Effect of clonazepam in weights (mg) of adults females and males, of *C. megacephala*.

Treatment _(mg/ml)	Weights	
	Female	Male
Control	40.81 ± 0.47	41.03 ± 0.59
Water	39.37 ± 0.43	44.15 ± 0.54
0.0652	35.11 ^{***} ± 0.98	35.17 ^{***} ± 0.91
0.125	39.35 ± 0.62	44.29 ± 0.54
0.25	41.18 ± 0.50	43.08 ± 0.71

***p<0.001 (Significant Holm-Sidak Test). The stage durations are expressed as Mean ± SE.

Figure 3. Average weight of emerged adults of *C. megacephala*, at different clonazepam concentrations.



Legend: ***p<0.001 (Significant Holm-Sidak Test). The vertical bars indicate No significant difference between the treatments.

4. DISCUSSION

No studies were identified in the literature that evaluated the effects of sublethal concentrations of clonazepam on the development of *C. megacephala* and *C. albiceps*. The articles found in this context, using benzodiazepines, investigated the effects at doses considered lethal in *C. albiceps* (Carvalho, Linhares and Trigo 2001; Singh, Heer and Wadhawan 2014; Al-Shareef, Alazwari and Al-Ghamdi 2021). Studies using low doses have been found, but these are studies of substances such as antibiotics, psychoactive substances, organophosphate insecticides, and others (Chopi 2019; Stillion 2023). Understanding these modifications is of great importance as they are directly related to the calculation of the PMI.

The duration of the cycle of these flies is fundamental for forensic studies, as the developmental stages of necrophagous insects allow the analysis of the time from death to the location of the corpse, in addition to contributing to the understanding of the events that occurred during this period. Thus, the results showed that clonazepam, at a concentration of 0.125 mg.mL^{-1} , caused an acceleration in the duration of the larval stage of *C. albiceps*. This behavior was also found by Al-Shareef, Alazwari & Al-Ghamdi (2021) utilized diazepam, who reported that the duration of the larval stage of the treated group was 84h while the control group was 96h. Carvalho, Linhares & Trigo (2001) found the fastest development in the first 18h of *C. albiceps* and *C. putoria* larvae exposed to diazepam. El-shehaby *et al.* (2019) also tested diazepam. However, they saw that it delayed the average time of larval development in three generations of *C. albiceps*.

In *C. megacephala*, the presence of clonazepam at the lowest concentration used increased the development time. This result highlights a difference in relation to the species *C. albiceps* and also to previous studies, such as those by Al-Shuraym *et al.* (2021) using *C.*

megacephala and *C. saffrana*, and Pawar & Deshmukh (2022) using *C. megacephala*, which observed the same behavior depending on the concentrations the alprazolam used, i.e. the higher the concentration, the longer the development time. Despite the use of high doses, Singh, Heer and Wadhawan (2014) found a similar result, where the half-lethal dose, the smallest used in that study, affected the larval and pupal development, which was longer compared to the control and other treatments. For the pupal stage, Lü *et al.* (2014) found an increase in the duration of pupation in the association of ketamine with temperature changes.

Among the morphological parameters used to analyse the effects of substances on insect development, weight gain stands out because it can be used for entomotoxicological analyses. (Thyssen *et al.* 2021). In this way, the weights of larvae, pupae and adults were also discussed by Pawar & Deshmukh (2022) and Al-Shuraym *et al.* (2021), and they observed that the weights decreased with increasing concentration of zolpidem. For *C. megacephala*, there was a significant increase in weight in the larval and pupal stages, a result also found by Baia *et al.* (2016) with flunitrazepam. However, our results showed a significant decrease in weight only in the adult stage, which differs from the results obtained by the authors, suggesting that this result is due to the bioaccumulation of the substance by the insects (Baia *et al.* 2016).

Pupal duration of *C. albiceps* was increased and decreased at concentrations of 0.125 mg.mL⁻¹ and 0.250 mg.mL⁻¹, respectively. Carvalho *et al.* (2001) showed that the time for pupation and adult emergence was delayed in the treated group with diazepam. Elshehaby *et al.* (2019) observed in three generations that the time to reach the pupal stage was longer after benzodiazepine exposure. Similarly, the duration of the egg-pupa and complete cycle was different for all treatments, with the greatest reduction in time at the 0.125 mg.mL⁻¹ concentration. This result differs from the results found in the literature, such as Elshehaby *et*

al. (2019) and Al-Shareef *et al.* (2021), who found a delay in the treated group compared to the control.

In pupa and puparium weights, the highest weights were found in the treatment with water and decreasing concentrations, with the lowest weight at concentration 0.250 mg mL^{-1} . Reigada & Godoy (2006) emphasize that humidity has a great influence on the weight gain of individuals, and Rezende (2018) adds that a low concentration of humidity in the substrate can induce non-feeding. Such statements explain the highest weight found in the water treatment. Carvalho *et al.* (2001) found a great difference in the treated larvae, which had a weight almost double that of the control group.

The results obtained can be explained based on studies evaluating hormesis in insect development. This effect is based on physiological responses in the presence of low doses of substances in the insect's body, which are triggered in insects when they are exposed to stress factors such as low doses of a substance, a behavior that cannot be observed at higher concentrations, where toxic effects predominate (Calabreses 2005, 2010). The observed results are well explained by this effect, since the alteration of the cycle or the gain or loss of weight are ways to compensate for the effects that the substance has on the organism. Therefore, it is necessary to understand the effects of sublethal concentrations and hormesis. This phenomenon has been widely studied in agricultural applications for pest control. However, little has been done to understand it in forensic entomology.

Insects respond to chemical compounds in different ways depending on the concentrations to which they are exposed. Thus, we conclude that clonazepam, even in sublethal concentrations, could alter the development of *C. albiceps* in different ways. And even at very low doses, it could delay the development time of *C. megacephala*, in addition to affecting

parameters such as weight at different stages. These changes may directly affect PMI estimation. However, such effects still need to be studied and explored, as it is necessary to understand above all the relationship with hormesis and its contribution within entomotoxicology, making it necessary to evaluate this substance at other low dosages, sublethal dosages already metabolized, as well as in association with temperature variations, for a better understanding of the observed effects, bringing it closer to the reality that occurs in humans.

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REFERENCES

- Al-Shareef, LO, Alazwari, OM, Al-Ghamdi, SS. 2021. Effect of diazepam on the growth of *Chrysomya albiceps* (Wiedemann 1819) (Diptera: Calliphoridae) in rabbit carcass. *Entomology and Applied Science Letters*, 8(2-2021), 100-108. <https://doi.org/10.51847/I71rmSia0>
- Al-Shuraym, LA, Al-Mekhlafi, FA, Abd Al Galil, FM, et al. 2021. Effect of zolpidem tartrate on the developmental rate of forensically important flies *Chrysomya megacephala* (Diptera: Calliphoridae) and *Chrysomya saffranaea*. *Journal of Medical Entomology*, 58(6), 2101-2106. <https://doi.org/10.1093/jme/tjab071>
- Alves, ACF, dos Santos, WE, Creão-Duarte, AJ. 2014. Diptera (Insecta) de importância forense da região Neotropical. *Entomotropica*, 29(2), 77-94.
- Amendt, J, Krettek, R, Zehner, R. 2004. Forensic entomology. *Naturwissenschaften*, 91, 51-65. <https://doi.org/10.1007/s00114-003-0493-5>
- ANDRÉA, MM. de. Bioindicadores ecotoxicológicos de agrotóxicos. 2008. Available from: <http://www.infobibos.com/Artigos/2008_4/Bioindicadores/index.htm>.

Baia, TC, Campos, A, Wanderley, BMS, Gama, RA. 2016. The effect of Flunitrazepam (Rohypnol®) on the development of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) and its implications for forensic entomology. *Journal of forensic sciences*, 61(4), 1112-1115. <https://doi.org/10.1111/1556-4029.13104>

Bastos, FIPM, Vasoncellos, MTL, De Boni, RB, Reis, NBD, Coutinho, CFDS. 2017. III Levantamento Nacional sobre o uso de drogas pela população brasileira. Available from:
<<https://www.arca.fiocruz.br/handle/icict/34614>>

Boulkenafet, F, Dob, Y, Karroui, R, Al-Khalifa, M, Boumrah, Y, Toumi, M, Mashaly, A. 2020. Detection of benzodiazepines in decomposing rabbit tissues and certain necrophagic dipteran species of forensic importance. *Saudi journal of biological sciences*, 27(7), 1691-1698.

Calabrese, EJ. 2010. Hormesis is central to toxicology, pharmacology and risk assessment. *Human & experimental toxicology*, 29(4), 249-261. <https://doi.org/10.1177/0960327109363973>

Calabrese, EJ. 2005. Toxicological awakenings: the rebirth of hormesis as a central pillar of toxicology. *Toxicology and applied pharmacology*, 204(1), 1-8. <https://doi.org/10.1016/j.taap.2004.11.015>

Caleffe, RR, Oliveira, SR, Satiko, N, Conte, H. 2015. Calliphoridae (Diptera) de interesse forense com ocorrência em Maringá-Pr-Brasil. *Revista Uningá*, 43, 10-15. Available from:
<<http://www.mastereditora.com.br/uninga>>.

Carvalho, CJBD, Mello-Patiu, CAD. 2008. Key to the adults of the most common forensic species of Diptera in South America. *Revista Brasileira de Entomologia*, 52, 390-406. <https://doi.org/10.1590/S0085-56262008000300012>

Carvalho, CJB, Mello-Patiu, CA, Almeida, LM. 2021. O papel da taxonomia na entomologia forense. In: Vairo, KP, Moura, MO. Entomologia forense na prática: do laboratório à utilização dos vestígios. 1ª ed. Campinas, SP: Millennium Editora, p. 225-273.

Carvalho, LMLD, Thyssen, PJ, Linhares, AX, Palhares, FAB. 2000. A checklist of arthropods associated with pig carrion and human corpses in Southeastern Brazil. *Memórias do Instituto Oswaldo Cruz*, 95, 135-138. <https://doi.org/10.1590/S0074-02762000000100023>

- Carvalho, LM, Linhares, AX, Trigo, JR. 2001. Determination of drug levels and the effect of diazepam on the growth of necrophagous flies of forensic importance in southeastern Brazil. *Forensic Science International*, 120(1-2), 140-144. [https://doi.org/10.1016/S0379-0738\(01\)00421-2](https://doi.org/10.1016/S0379-0738(01)00421-2)
- Chophi, R, Sharma, S, Sharma, S, Singh, R. 2019. Forensic entomotoxicology: current concepts, trends and challenges. *Journal of forensic and legal medicine*, 67, 28-36. <https://doi.org/10.1016/j.jflm.2019.07.010>
- Elshehaby, M, Tony, M, Abdellah, NZ, Abd Elreheem, AEM. 2019. Effects of sildenafil citrate and diazepam on developmental stages of forensically important fly: *Chrysomya albiceps* (Diptera: Calliphoridae). *International Journal of Entomology Research*, 4(6), 82-89.
- Estrada, DA, Grella, MD, Thyssen, PJ, Linhares, AX. 2009. *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) developmental rate on artificial diet with animal tissues for forensic purpose. *Neotropical Entomology*, 38, 203-207. <https://doi.org/10.1590/S1519-566X2009000200006>
- Goff, M. L., & Lord, W. D. (1994). Entomotoxicology: a new area for forensic investigation. *The American journal of forensic medicine and pathology*, 15(1), 51-57.
- Gomes, L. 2010. Entomologia Forense: Novas tendências e tecnologias nas ciências criminais. Rio de Janeiro, RJ: *Technical Books*, 526 p.
- Hanski, I. 1987. Nutritional ecology of dund-and-carrion-feeding insects.
- Hodecek, J. 2020. Revisiting the concept of entomotoxicology. *Forensic Science International: Synergy*, 2, 282-286. <https://doi.org/10.1016/j.fsisyn.2020.09.003>
- Introna, F, Campobasso, CP, Goff, ML. 2001. Entomotoxicology. *Forensic science international*, 120(1-2), 42-47. [https://doi.org/10.1016/S0379-0738\(01\)00418-2](https://doi.org/10.1016/S0379-0738(01)00418-2)
- Leite, GLD, SÁ, VGM. 2010. Apostila: Taxonomia, Nomenclatura e identificação de espécies. *Universidade Federal de Minas Gerais, Instituto de Ciências Agrárias*, 50.
- Lü, Z., Zhai, X., Zhou, H., et al. 2014. Effects of Ketamine on the development of forensically important blowfly *Chrysomya megacephala* (F.)(Diptera: Calliphoridae) and its forensic relevance. *Journal of forensic sciences*, 59(4), 991-996. <https://doi.org/10.1111/1556-4029.12430>

Neto, CHG. 2023. Dípteros necrófagos do Brasil: contribuições à entomologia forense. Rio de Janeiro, RJ: *Autografia*. 298p.

Oliveira-Costa, J, Mello-Patiu, CA, Lopes, SM. 2001. Dípteros muscóides associados com cadáveres humanos na cena da morte no estado do Rio de Janeiro, Brasil. *Boletim do Museu Nacional*, 464, 1-6.

Oliveira, TC, Vasconcelos, SD. 2010. Insects (Diptera) associated with cadavers at the Institute of Legal Medicine in Pernambuco, Brazil: implications for forensic entomology. *Forensic Science International*, 198(1-3), 97-102. <https://doi.org/10.1016/j.forsciint.2010.01.011>

Oliveira-Costa, J. 2013. "Insetos Peritos": A Entomologia Forense no Brasil. 1º ed. Campinas, SP: *Millennium Editora*. 487p.

Oliveira-Costa, J. 2008. Entomologia Forense: Quando os insetos são vestígios. 2º ed. Campinas, SP: *Millennium Editora*. 420p.

Paseto, ML. 2014. Sarcophagidae (Diptera) e Lepidoptera associados a carcaças de suínos (*Sus scrofa L.*) em área agropastoril de Uberlândia, Minas Gerais. (Master's dissertation) - Universidade Estadual de Campinas, Instituto de Biologia, Campinas, SP. <https://hdl.handle.net/20.500.12733/1622437>
<https://doi.org/10.47749/T/UNICAMP.2014.926004>

Pawar, HM, Deshmukh, GM. 2022. Effect of alprazolam on the developmental stages of forensically important blow fly *Chrysomya megacephala* (Diptera: Calliphoridae). *Journal of Entomology and Zoology Studies*, 10(3), 101-104. <https://doi.org/10.22271/j.ento.2022.v10.i3b.8996>

Rabêlo, KCN, Thyssen, PJ, Salgado, RL, et al. 2011. Bionomics of two forensically important blowfly species *Chrysomya megacephala* and *Chrysomya putoria* (Diptera: Calliphoridae) reared on four types of diet. *Forensic Science International*, 210(1-3), 257-262.

Reigada, C, Godoy, WAC. 2006. Larval density, temperature and biological aspects of *Chrysomya megacephala* (Diptera: Calliphoridae). *Arquivo Brasileiro de Medicina Veterinário Zootécnico*, 58(4), 562-566.

Rezende, F. 2018. Thermal requirements of immatures of two species of *Chrysomya* (Diptera: Calliphoridae) created under different temperatures and food substrates (Doctoral dissertation). Campinas (Brazil):

UNIVERSIDADE ESTADUAL DE CAMPINAS Instituto de Biologia.

Salimi, M, Rassi, Y, Chatrabgoun, O, et al. 2018. Toxicological analysis of insects on the corpse: a valuable source of information in forensic investigations. *Journal of arthropod-borne diseases*, 12(3), 219.

Singh, D, Heer, BK, Wadhawan, B. 2016. Detection of Ketamine hydrochloride and its effect on the development of immature stages of a forensically important blow fly *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). *Journal of Entomology and Zoology Studies*, 4(2), 91-97.

Singh, D, Heer, BK, Wadhawan, B. 2014. Effect of ketamine hydrochloride in combination with Xylazine on the development of *Chrysomya megacephala* fab. *Indian Journal of Forensic Medicine & Toxicology*, 8(1), 1140.

Stillion, K. 2023. Forensic Entomotoxicology: The effects of drugs on the life cycle and succession of forensically important insects.

Thyssen, PJ, Mira, CT, Grella, MD, Linhares, AX. 2021. Experimentação em Entomotoxicologia. In: VAIRO, K. P.; MOURA, M. O. Entomologia forense na prática: do laboratório à utilização dos vestígios. 1ª ed. Campinas, SP: *Millennium Editora*.87-104.

Vairo, KP, Moura, MO. 2021. Entomologia forense na prática: do laboratório à utilização dos vestígios. 1ª ed. Campinas, SP: *Millennium Editora*, 332p.

ARTICLE II

Sublethal effects of two alcoholic substances on biological parameters of blowflies of forensic importance

Journal of Forensic Science International standards (preliminary version)

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Abstract

Flies are often found on corpses and the dynamics of development of their larvae are used to calculate the post-mortem interval (PMI). Alcohol is the most widely consumed beverage in the world, but its use can cause various collateral damage. Similarly, the presence of the substances in the body of insects can cause some physiological responses. The aim of this work was to evaluate the effects of ethanol and Corote[®] at different concentrations on the biology of *C. megacephala*. Newly hatched larvae were exposed by ingestion of substrate treated with five volumes of alcohols. Parameters of the juvenile and adult stages and the duration of each stage were evaluated. The results showed changes in developmental time of larval stage ($F=17.803$; $df=5$; $p=0.003$) and ($F=17.508$; $df=5$; $p=0.004$), and pupal stage ($F=36.175$; $df=5$; $p<0.001$) and ($F=19.838$; $df=5$; $p=0.001$) for ethanol and Corote, respectively. We conclude that ethanol and Corote[®] alter the life parameters of *C. megacephala* used to estimate PMI.

Keywords: Entomotoxicology; Calliphoridae; ethanol; hormesis.

1 INTRODUCTION

The presence of insects on corpses often occurs in criminal investigation scenarios, and these insects are used to infer the post-mortem interval (PMI), such an analysis falls within the field of forensic entomology [11,19,20]. Blowflies have frequently a higher abundance in decomposing bodies and are of great importance for obtaining information about some events before the death occurrence [7,18].

Through entomotoxicology, it is possible to detect and identify substances in decomposed tissues that are the cause of death, such as poisoning or overdose [1,9,15, 23,24]. The use of larvae for entomotoxicological studies provides a more accurate estimate of PMI, as the substances affect larval development [10,12,16].

Alcohol is the most widely consumed legal substance in the world. According to the World Health Organization, more than half of the world's population consumes alcohol [28]. In Brazil, 66.4% of the population consumes alcohol during their lifetime [2]. This consumption can lead to various health problems, such as mental and behavioral disorders [29], and previous studies has shown a strong relation between alcohol consumption and some suicide cases due to abuse or overdose [3]. Corote[®] is a popular beverage in Brazil, largely due to its affordability and relatively low alcohol content (13.5% vol.).

Therefore, the aim of this study was to evaluate the effects of different concentrations of ethanol and Corote[®] on the developmental cycle of *C. megacephala* and to test the following hypotheses: 1) The Corote[®] present in the substrate does not affect the development of the larvae and pupae of *C. megacephala*; 2) *Chrysomya megacephala* does not show any alteration in the biological cycle when exposed to different concentrations of Corote[®]; 3) The pure ethanol present in the substrate does not affect the development of the larvae and pupae of *C. megacephala*; and 4) *Chrysomya megacephala* does not show any alteration in the biological cycle when exposed to different concentrations of ethanol; 6) There is difference in the development the blowfly the Corote[®] and ethanol treatments.

2. MATERIAL AND METHODS

2.1 Collection sites

Chrysomya megacephala larvae were collected with entomological tweezers at the Composting Sector of the Federal University of Lavras (G-OLEO/UFLA). The specimens were transported to the Molecular Entomology and Ecotoxicology Laboratory (M.E.E.T.) of the Department of Entomology - UFLA, where they were placed on a ground meat substrate for feeding. The development of the larvae up to the emergence of the adults was observed under a stereoscope for identification (Trinocular Stereoscope Microscope ZOOM DI-152T) using the dichotomous key for adult specimens by Carvalho & Mello-Patiu [6]. From these adults, plastic cages (21.5x21.5x25cm³) with voile sleeves (adapted from Estrada [8]) were set up to establish the reading.

2.2 Description of obtaining the chemicals used: Corote and Ethanol

Corote is an alcoholic beverage, with alcohol content 13,5%, sold commercially in the country. Pure ethanol put the exact (97% i guess) of alcohol in the ethanol used, was obtained from the SIGMA-ALDRICH company.

2.3 Laboratory rearing conditions

The rearing at all stages took place in a climate-controlled room at $27 \pm 1^\circ \text{C}$, 12L:12D and relative humidity of $60 \pm 10\%$ [8].

2.3.1 Rearing of adult individuals

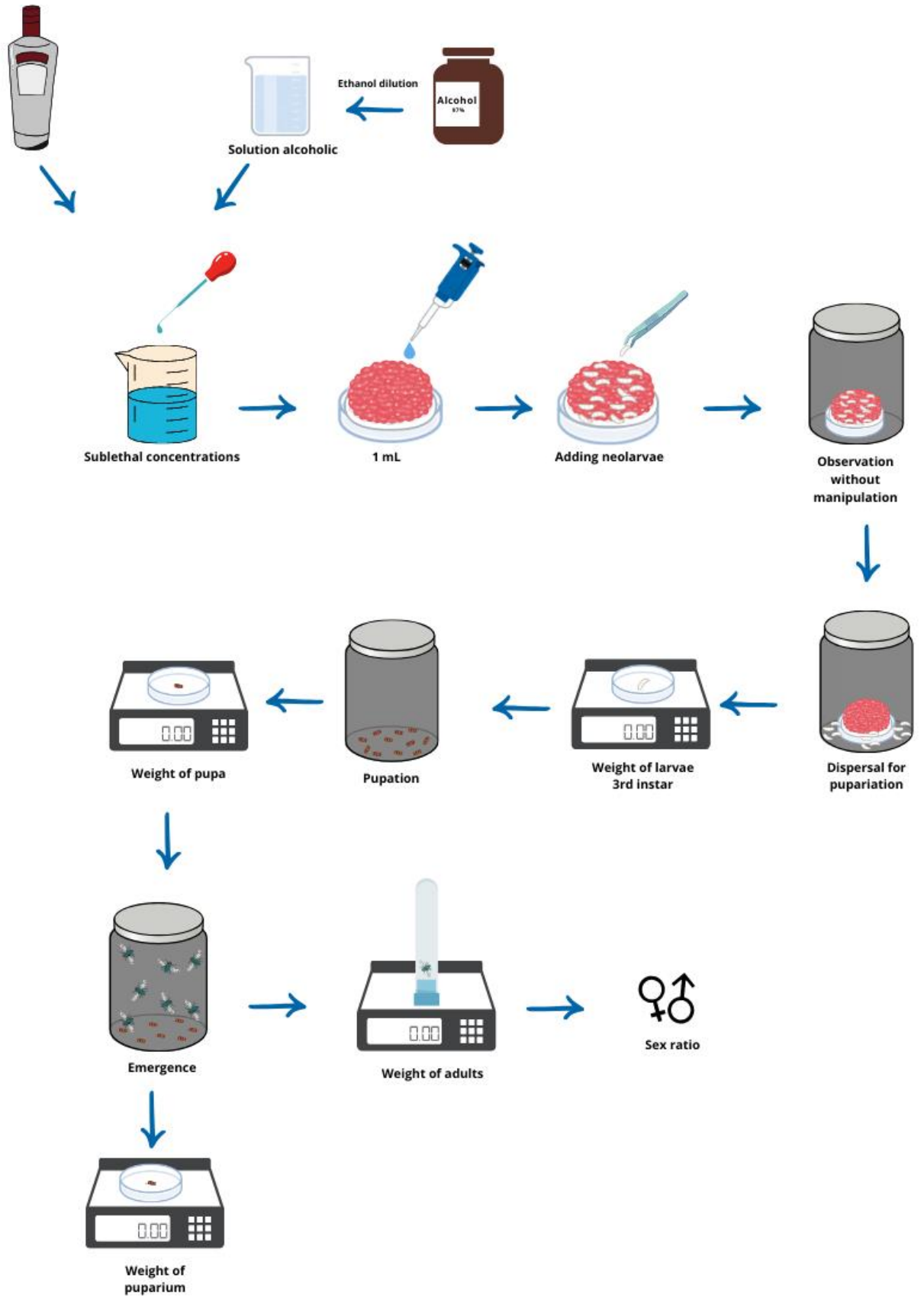
Adults were fed an 80% sucrose solution and water. Over a period of 4 days, 20 g of minced ground meat was offered to mature the ovarian follicles. After 11 days, minced meat was offered again to obtain eggs.

2.3.2 Rearing of immatures individuals

The eggs were transferred to a substrate containing approximately 1.5g of 24-hour minced ground meat per larva. The larvae were placed above the food in a breeding pot with a cut-out lid and voile, with vermiculite at the bottom to allow the migrating larvae to pupate. At the pupal stage, they were transferred to the birdcages until they emerged.

2.4 Toxicology Bioassays

Figure 1. Stages of the toxicology bioassays procedure



(Bibiano, 2024)

Experiments were conducted with first generation (F2) laboratory hatched *C. megacephala* flies. The experimental design was organized in four replicates with 30 new larvae (<1h old) per replicate, each replicate consisting of 40g of minced meat at the initial stage of decomposition, and each replicate was stored in disposable plastic containers of 100mL, which in turn were placed in larger plastic containers, 300mL plastic pots with vermiculite. The experiment was conducted under the same environmental conditions as the breeding room.

Pretests were carried out to determine the volumes used: 50, 100, 150, 200 μ L of pure ethanol and Corote[®], deionized water, and the control without addition of any liquid. Ethanol (97%) was diluted to ethanol (39%), the same concentration as Corote[®]. In treatments where liquid was added, 0.2 mL was applied for each replicate, the substrate was mechanically homogenized, and the neon larvae were placed on the substrate. The substrate was not manipulated until the larvae left it, thus avoiding stress to the individuals. The migrating larvae were monitored until they reached the pupal stage and the adults emerged. At these stages, individuals were individually weighed using an analytical balance (BEL Engineering M214Ai).

Several biological parameters were analyzed, including the duration of the larval and pupal stages, the egg-pupa period, the duration of the complete cycle (egg-adult), larval weight (L3), pupal weight, puparium weight, adult weight, and the sex ratio of emerged adults.

2.4.1 Statistical analyses

To evaluate the effect of the substance on the mentioned parameters, the data were subjected to one-way ANOVA and the means comparison test using the "glht" and "cld" functions of the R multcomp package, with the aim of comparing the treatments with the control. For the egg-adult parameter, only individuals that completed the entire cycle were considered. As for the adult weight, the data were submitted to two-way ANOVA with the Holm-Sidak means comparison test, taking into account the factors of sex ratio of emerged adults and concentrations. All statistical tests were performed using GLM in the RStudio program, software for student use.

3. RESULTS

3.1 Effects of different concentrations of ethanol on the juvenile stages of *C. megacephala*

The differences of variance were significant for the following parameters: duration of the larval stage ($F=17.803$; $df=5$; $p=0.003$) and egg-pupa stage ($F=17.864$; $df=5$; $p=0.003$) for 50 and 100 μL showed a longer duration compared to the control; duration of the pupal stage ($F=36.175$; $df=5$; $p<0.001$) for 150 and 200 μL showed a longer duration compared to the control; the duration of the egg-adult stage ($F=26.344$; $df=5$; $p<0.001$) increased with increasing volumes (Table 1).

For the parameters related to the body mass in the stages (larvae, pupae and adults), the analysis was significant only for the weight of the pupa ($F=14.423$; $df=5$; $p<0.001$) in all treatments compared to the control. For larval weight ($F=9.753$; $df=5$; $p=0.083$) and puparium weight ($F=2.571$; $df=5$; $p=0.766$), the analyses were not significant (Table 2) (Figure 2a).

For the relevant parameters, the analysis of variance for the adult phase showed significant differences for weight ($F=145.720$; $df=5$; $p<0.001$) (Table 2) (Figure 2a).

3.2 Effects of different doses of Corote on the juvenile stages of *C. megacephala*

Parameters related to the duration of the stages had significant results. The duration of the larval stage ($F=17.508$; $df=5$; $p=0.004$), for 50 and 100 μL , which was longer compared to the control; the duration of the pupal stage ($F=19.838$; $df=5$; $p=0.001$); the duration of the egg-pupa ($F=17.618$; $df=5$; $p=0.003$) was higher in volume 200 μL , being greater than all treatments and the control; the duration of the complete cycle ($F=18.289$; $df=5$; $p=0.003$) was greater in volumes 150 and 200 μL compared to the control (Table 1).

The analysis of variance was significant for the parameter pupal weight ($F=11.364$; $df=5$; $p=0.045$) for 50 μL compared to the control. On the other hand, the analyses were not significant for the parameters larval weight ($F=6.241$; $df=5$; $p=0.284$) and puparium weight ($F=2.148$; $df=5$; $p=0.828$) (Table 2) (Figure 2b).

For adult weight ($F=7.160$; $df=5$; $p=0.209$), sex ($F=0.441$; $df=1$; $p=0.199$), and the interaction 'treatment x sex' ($F=4.271$; $df=5$; $p=0.511$), analyses of variance showed no significant differences (Table 2) (Figure 2b).

4. DISCUSSION

Despite the fact that alcohol is a legal substance consumed worldwide, studies on ethanol in the development of necrophagous flies are scarce in the literature, especially if we look for works that have tried to investigate the effects of sublethal doses. To date, no work has been found using the species *Chrysomya megacephala*, which is of great importance for conclusions in forensic entomology.

The larval development time is of utmost importance as it helps in the calculation of IPM and the presence of ethanol affects its duration. The results showed that the presence of a small amount of pure ethanol and Corote[®] promoted a delay in the larval stage, causing the larvae to stay longer in this stage. These data differ from those found in studies such as Osorio-Arenas & Murgas [22], where the time spent by *Lucilia eximia* larvae reduced the time to reach the pupal stage. Monthei [17], found *Phormia regina*, which when exposed to substrate containing alcohol, showed a significant change in its development.

The total development time was similar in both treatments, with the time in ethanol increasing with increasing volume, and for Corote, volumes 150 and 200 μL were higher compared to the control. These results are different from the studies of Monthei [17] with *P. regina*, where the time was shorter in the treatments.

One of the morphological parameters used to analyze the effects of substances on insect development is weight gain [25]. For *L. eximia*, Osorio-Arenas & Murgas [22] found a decrease in larval growth at a concentration of 10% ethanol. Tabor *et al.* [24] and Monthei [17] showed in their studies, both conducted with *P. regina*, that higher concentrations of ethanol induce a decrease in growth. As for the weight of the adults, the data show that the weight decreased compared to the control, but among the treatments, the result was proportional to the volumes applied, that is, the weight increased as the applied volume increased, a behavior also found in *P. regina*, by Monthei [17].

The observed behaviors can be explained based on studies investigating hormesis in insect development. This effect is based on physiological responses triggered when insects are exposed to stressors, such as a substance, even at low doses [4,5], from which improved performance or resistance can be inferred, since exposure to lethal doses can be harmful. According to Monthei [17], the presence of alcohol prevents feeding until it has evaporated,

allowing the larvae to feed again and influencing the behavior of staying longer on the substrate to feed.

With regard to alcoholic substances, when the weight parameter was compared and a discrepancy in the responses was observed, it can be postulated that Corote®, a product suitable for consumption, contains additive substances in lower concentrations, which inhibited the response obtained from the pure compound.

Understanding hormesis is essential to assess the effects of substances on insect behavior, as blowflies are crucial for estimating PMI. Thus, we can conclude that ethanol, pure or in Corote®, even in sublethal doses, affects the developmental time of *C. megacephala*, delaying its duration and other parameters such as weight at different stages.

REFERENCES

- [1] AMENDT, J.; KRETTEK, R.; ZEHNER, R. Entomologia forense. Naturwissenschaften, v. 91, p. 51–65. 2004
- [2] BASTOS, F. I. P. M.; VASCONCELLOS, M. T. L.; DE BONI, R. B.; REIS, N. B.; COUTINHO, C. F.S. (Org.). III Levantamento Nacional sobre o uso de drogas pela população brasileira. Rio de Janeiro: FIOCRUZ/ICICT, 528 p. 2017.
- [3] BRASIL, Ministerio da Saúde. ÁLCOOL NO BRASIL: CONSUMO EM NÚMEROS. 2020. Disponível em: < <https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/svsa/promocao-da-saude/folder-alcool-no-brasil-consumo-em-numeros> > Acesso em 02 jan de 2024.
- [4] CALABRESE, E. J. Toxicological awakenings: the rebirth of hormesis as a central pillar of toxicology. Toxicology and Applied Pharmacology, v. 204, n. 1, p. 1–8. 2005. doi: 10.1016/j.taap.2004.11.015
- [5] CALABRESE, E. J. Hormesis is central to toxicology, pharmacology and risk assessment. Human & Experimental Toxicology. v. 29, n. 4, p. 249–261. 2010. doi:10.1177/0960327109363973
- [6] CARVALHO, C. J. B.; MELLO-PATIU, C. A. de. Key to the adults of the most common forensic species of Diptera in South America. Revista Brasileira de Entomologia, v. 52, p. 390–406. 2008.
- [7] CARVALHO, C. J. B.; MELLO-PATIU, C. A.; ALMEIDA, L. M. O papel da taxonomia na entomologia forense. In. VAIRO, K. P.; MOURA, M. O. Entomologia forense na prática: do laboratório à utilização dos vestígios. 1ª ed. Campinas, SP: Millennium Editora, p. 225-273. 2021.
- [8] ESTRADA, D. A.; GRELLA, M. D.; THYSSEN, P. J.; LINHARES, A. X. “*Chrysomya albiceps* (Wiedemann)(Diptera: Calliphoridae) developmental rate on artificial diet with animal tissues for forensic purpose.” Neotropical Entomology, v. 38, n. 2, p. 203–207. 2009.
- [9] GOFF, M. LEE; LORD, W. D. Entomotoxicology. A new area for forensic investigation. The American Journal of Forensic Medicine and Pathology, v. 15, n. 1, p. 51–57. 1994.
- [10] GOFF, M. L.; BROWN, W. A.; OMORI, A. I. Preliminary observations of the effect of methamphetamine in decomposing tissues on the development rate of Parasar- (Diptera: Sarcophagidae) and the implications of this effect on the estimations of postmortem intervals. **Journal of Forensic Science**, v. 37, n.3, p. 867–872, 1992.
- [11] GOMES, L. Entomologia Forense: Novas tendências e tecnologias nas ciências criminais. Technical Books, 2010.
- [12] HEDOUIN, V.; BECART, A.; TOURNEL, G.; DEVEAUX, M.; GOSSET, D.; BOUREL, B.; MARTIN-BOUYER, L. Effects of morphine in decomposing bodies on the development of *Lucilia sericata* (Diptera: Calliphoridae). **Journal of Forensic Sciences**, v. 44, n. 2, p. 354-358, 1999. doi: 10.1520/JFS14463J
- [13] HEDOUIN, V.; BÉCART, A.; TOURNEL, G.; DEVEAUX, M.; GOSSET, D.; BOUREL, B.; MARTIN-BOUYER, L. Determination of drug levels in larvae of *Lucilia sericata* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine. **Journal of Forensic Science**, v. 44, p. 351– 353, 1999. doi:10.1520/JFS14462J
- [14] HEDOUIN, V.; BOUREL, B.; BÉCART, A.; TOURNEL, G.; DEVEAUX, M.; GOFF, M. L.; GOSSET, D. Determination of drug levels in larvae of *Protophormia terraenovae* and *Calliphora vicina* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine. **Journal of Forensic Science**, v. 46, n. 1, p.12–14, 2001.
- [15] HODECEK, J. Revisiting the concept of entomotoxicology. Forensic Science International: Synergy, 2020.
- [16] INTRONA, F.; CAMPOBASSO, C. P.; GOFF, M. L. Entomotoxicology. Forensic Science International, v. 120, n. 1–2, p. 42–47, 2001. doi:10.1520/JFS12808J

- [17] MONTHEI, D. R. Entomotoxicological and thermal factors affecting the development of forensically important flies. Doctoral Thesis. Faculty of Virginia Polytechnic Institute and State University. 2009.
- [18] NETO, C. H. G. Dípteros necrófagos do Brasil: contribuições à entomologia forense. Editora Autografia. 2023.
- [19] OLIVEIRA-COSTA, J. "Insetos Peritos": A Entomologia Forense no Brasil. 1º ed. Campinas, SP: Millennium Editora, 2013.
- [20] OLIVEIRA-COSTA, J. Entomologia Forense: Quando os insetos são vestígios. 2º ed. Campinas, SP: Millennium Editora, 2008.
- [21] OLIVEIRA, C. J.; MELLO, P. C. A.; LOPES, S. M. Dípteros muscóides associados com cadáveres humanos no local da morte, no estado do Rio de Janeiro, Brasil. Boletim Museu Nacional, v. 464, p. 1–7. 2001.
- [22] OSORIO-ARENAS, M. A.; MURGAS, A. S. Efecto del porcentaje de etanol en el crecimiento Larval de *Lucilia eximia* (Wiedemann, 1819) (Diptera: Calliphoridae). Revista Científica Centros, v. 4, n. 1, p. 34-45, 2015.
- [23] SALIMI, M.; RASSI, Y.; CHATRABGOUN, O.; KAMALI, A.; OSHAGHI, M. A.; SHIRI-GHALEH, V.; PARKHIDEH, S. Z. Toxicological analysis of insects on the corpse: a valuable source of information in forensic investigations. **Journal of Arthropod-Borne Diseases**, v. 12, n. 3, p. 219, 2018.
- [24] TABOR, K. L.; FELL, R. D.; BREWSTER, C. C.; PELZER, K.; BEHONICK, G.S. Effects of antemortem ingestion of ethanol on insect successional patterns and development of *Phormia regina* (Diptera: Calliphoridae). Journal of Medical Entomology, v. 42, p. 481-489. 2005. doi.org/10.1093/jmedent/42.3.481
- [25] THYSSEN, P. J.; MIRA, C. T.; GRELLA, M. D.; LINHARES, A. X.; Experimentação em Entomotoxicologia. In: VAIRO, K. P.; MOURA, M. O. Entomologia forense na prática: do laboratório à utilização dos vestígios. 1ª ed. Campinas, SP: **Millennium Editora**. 87-104. 2021.
- [26] VAIRO, K. P.; MOURA, M. O. Entomologia forense na prática: do laboratório à utilização dos vestígios. 1ª ed. Campinas, SP: Millennium Editora, 2021. 332p.
- [27] WORLD HEALTH ORGANIZATION (WHO). Harmful use of alcohol kills more than 3 million people each year, most of them men. 2018. Available from: < <https://www.who.int/news/item/21-09-2018-harmful-use-of-alcohol-kills-more-than-3-million-people-each-year--most-of-them-men>> Acess em 02 jan de 2024.
- [28] WORLD HEALTH ORGANIZATION (WHO). Alcohol. 2022 Available from: <<https://www.who.int/es/news-room/fact-sheets/detail/alcohol>> Acess em 02 jan de 2024.

Table 1. Effect of Ethanol and Corote[®] on the durations (in days) of the stages evaluated of *C. megacephala*.

Substance	Treatment _(μl/ml)	Duration in days of stages			
		Larval	Pupal	Egg-pupae	Egg-adult
Ethanol	Control	5.35 ± 0.11	5.18 ± 0.09	6.35 ± 0.11	9.53 ± 0.15
	Water	5.50 ± 0.12	5.56 ± 0.12	6.50 ± 0.12	10.06 ± 0.15 ^{***}
	50	5.84 ± 0.06 ^{**}	5.44 ± 0.08	6.84 ± 0.06 ^{**}	10.29 ± 0.08 ^{***}
	100	5.92 ± 0.05 ^{**}	5.39 ± 0.07	6.92 ± 0.05 ^{**}	10.31 ± 0.09 ^{***}
	150	5.75 ± 0.06	5.69 ± 0.06 ^{***}	6.75 ± 0.06	10.41 ± 0.08 ^{***}
	200	5.75 ± 0.06	5.84 ± 0.04 ^{***}	6.75 ± 0.06	10.57 ± 0.08
Corote	Control	5.39 ± 0.11	5.22 ± 0.10	6.39 ± 0.11	9.61 ± 0.15
	Water	5.56 ± 0.12	5.50 ± 0.13	6.56 ± 0.12	10.06 ± 0.18
	50	5.91 ± 0.05 ^{**}	5.48 ± 0.08	6.80 ± 0.06	10.25 ± 0.07
	100	5.90 ± 0.05 ^{**}	5.39 ± 0.07	6.90 ± 0.05	10.31 ± 0.10
	150	5.81 ± 0.05	5.67 ± 0.07	6.84 ± 0.05	10.45 ± 0.09 ^{**}
	200	5.73 ± 0.06	5.73 ± 0.05 ^{***}	7.15 ± 0.09 ^{**}	10.45 ± 0.10 ^{**}

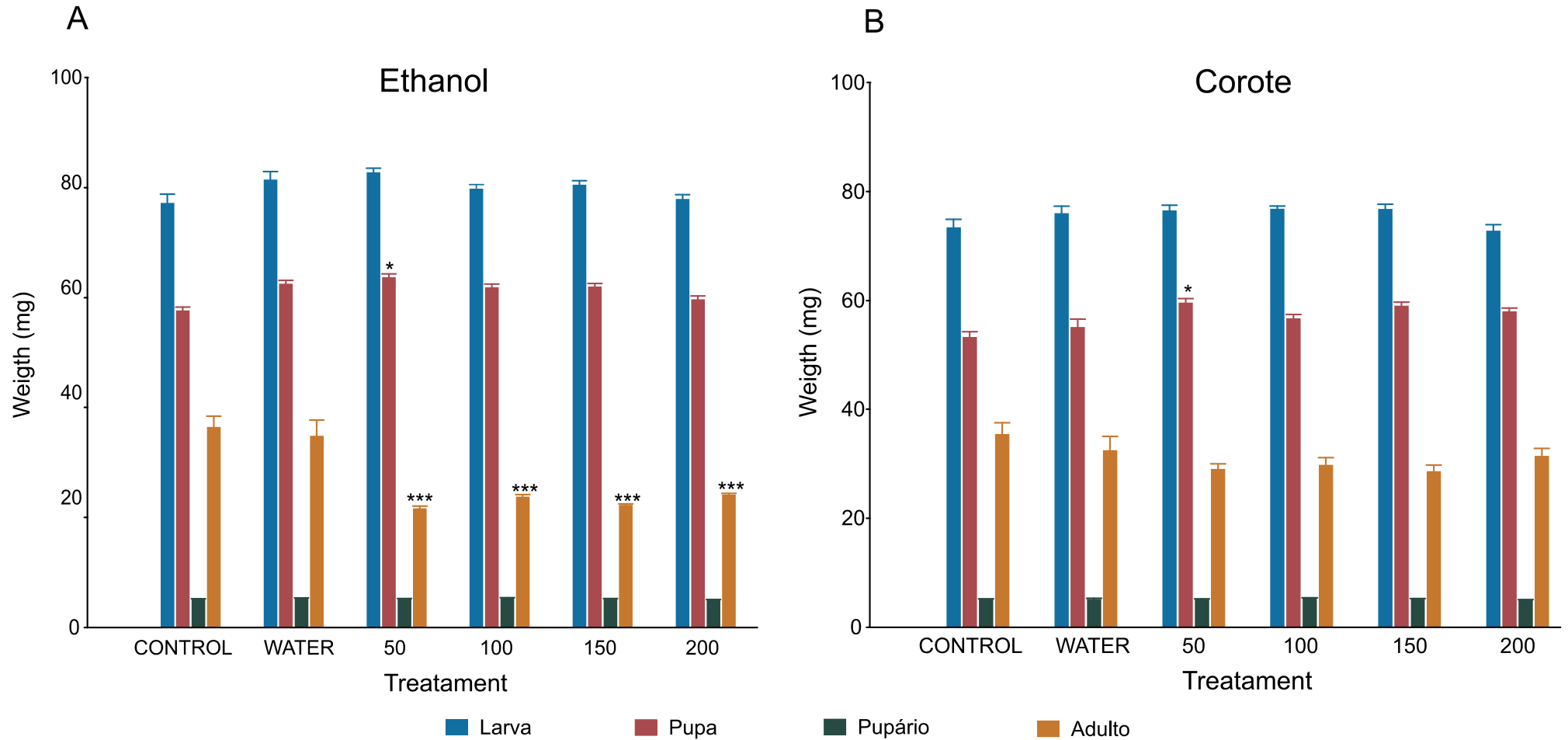
p= '***' 0.001; '**' 0.01; '*' 0.05 (Teste de Holm-Sidak Significative). The stage durations are expressed as Mean ± SE

Table 2. Effect of Ethanol and Corote[®] in the weight (mg) of the evaluated morphological parameters of the juvenile stages, adults, females, and males of *C. megacephala*.

Substance	Treatment _(μl/ml)	Weight					
		Larva	Pupa	Puparium	Adult	Female	Male
Ethanol	Control	77.11 \pm 2.19	57.51 \pm 1.05	5.09 \pm 1.06	36.28 \pm 2.16	35.28 \pm 4.88	36.88 \pm 2.09
	Water	81.33 \pm 1.99	62.39 \pm 1.03	5.09 \pm 0.35	34.71 \pm 3.11	34.74 \pm 4.16	35.65 \pm 4.94
	50	82.62 \pm 1.11	63.56 \pm 0.88*	5.06 \pm 0.23	21.53 \pm 0.67***	19.65 \pm 0.62	22.89 \pm 0.73
	100	79.71 \pm 1.40	61.74 \pm 0.99	5.29 \pm 0.13	23.60 \pm 0.74***	21.19 \pm 0.77	24.93 \pm 0.67
	150	80.39 \pm 1.19	61.87 \pm 0.91	5.19 \pm 0.01	22.10 \pm 0.49***	21.21 \pm 0.57	22.90 \pm 0.38
	200	77.76 \pm 1.31	59.56 \pm 1.06	4.94 \pm 0.17	23.97 \pm 0.47***	23.31 \pm 0.72	24.33 \pm 0.44
Corote	Control	77.18 \pm 2.07	57.43 \pm 0.99	5.09 \pm 0.11	35.29 \pm 2.64	33.03 \pm 4.60	36.88 \pm 2.09
	Water	79.09 \pm 1.69	61.29 \pm 1.15	5.01 \pm 0.40	32.32 \pm 2.99	30.96 \pm 3.30	33.66 \pm 4.24
	50	80.03 \pm 1.56	62.77 \pm 0.99*	5.05 \pm 0.21	28.89 \pm 1.23	27.38 \pm 1.78	29.99 \pm 1.35
	100	79.56 \pm 1.09	61.35 \pm 0.96	5.28 \pm 0.13	29.66 \pm 1.72	30.67 \pm 2.71	29.01 \pm 1.65
	150	79.98 \pm 1.11	61.23 \pm 0.81	5.13 \pm 0.12	28.48 \pm 1.53	30.18 \pm 2.14	27.09 \pm 1.46
	200	75.96 \pm 1.65	59.58 \pm 1.04	4.95 \pm 0.17	31.31 \pm 1.71	33.10 \pm 2.60	29.76 \pm 1.66

p= '***' 0.001; '**' 0.01; '*' 0.05 (Teste de Holm-Sidak Significative). The stage durations are expressed as Mean \pm SE

Figure 2: Average weight (mg) of different developmental stages of the *Chrysomya megacephala* fly. The stages include larva, pupa, puparium, and adult. The data are presented for Ethanol (A) and Corote[®] (B).



Legend: p= '****' 0.001; '***' 0.01; '*' 0.05 (Significant Tukey Test)

FINAL CONSIDERATIONS

Forensic entomology has gained prominence in research because understanding the dynamics and presence of insects at crime scenes has proven to be important in solving some cases. Thus, it is essential to study the main insects involved in this dynamic, such as beetles and flies, and to understand their behavioral, physiological, and biological responses to different situations when attracted to a body or carcass.

It has been shown that the increased consumption of various substances by people, and their presence in their bodies, has a significant impact on the behavior of insects. In light of this, it has become essential to monitor the changes that may occur, which is crucial for assessing the effects of these substances on insects. Although entomotoxicology is a relatively new and underexplored field of study compared to other disciplines, it has already yielded fascinating discoveries. Countries with tropical climates, such as Brazil, have contributed promising results in this field.

To this end, understanding the responses of “blowflies” is of great importance in the field of forensic dynamics, and the presence of substances can affect this process. The results obtained from this study demonstrated that both substances tested, clonazepam, Corote and ethanol, had a significant effect on the development time of the evaluated species. The pupa weight was the most impacted parameter in the present study, as both substances exhibited an increase in weight at the lowest concentration used, indicating a hormesis effect.

These studies are promising, as the analyses conducted through insects yield valuable qualitative results that contribute to the elucidation of the facts. Such findings contribute expanding the studies of toxicology associated with forensic entomology and diversifying the area of applicability of studies related to hormesis in insects. Furthermore, these data permit criminal experts to contemplate potential alterations in PMI calculations when composing reports. Nevertheless, it is essential to take into account various factors, including species, geographical regions, climatic conditions, and other variables, as these factors can affect insects and cause them to behave differently.