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GLOBAL TRENDS IN ENTOMOTOXICOLOGY FORENSICS: INNOVATIONS, CHALLENGES, AND FUTURE DIRECTIONS

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ABSTRACT

Forensic entomotoxicology is increasingly important in medico-legal investigations, particularly when conventional biological matrices are unavailable due to advanced decomposition. Necrophagous insects can retain xenobiotics and exhibit drug-induced developmental changes that may affect post-mortem interval (PMI) estimation. However, a comprehensive overview of global research trends and methodological progress in this field remains limited. This study supports SDGs 3(Good Health and Well-Being), 16 (Peace, Justice and Strong Institutions), and 9 (Industry, Innovation and Infrastructure) by promoting innovative and standardized forensic entomotoxicology practices to enhance toxicological detection and evidentiary reliability. This study aimed to evaluate global publication trends, summarize analytical and experimental approaches, and identify key challenges in forensic entomotoxicology. A systematic literature search was conducted in Scopus and PubMed for publications published between 1991 and 2025. Records were screened by title, abstract, and full text according to predefined inclusion and exclusion criteria. After removing duplicates and ineligible studies, 87 peer-reviewed articles involving 5,214 insect samples were included for bibliometric and narrative analyses. The results showed a marked increase in publications after 2010, peaking in 2023. Most studies focused on qualitative xenobiotic detection, while several evaluated developmental effects relevant to PMI estimation. Analytical methods evolved from GC-MS and HPLC to advanced LC-MS/MS and spectroscopic techniques. Standardized methodologies and validated reference models are therefore essential to support the routine forensic application of entomotoxicology.

Keywords : *Forensic entomotoxicology; Post-mortem interval estimation; Innovative forensic toxicology.*

INTRODUCTION

Definition and Scope

Forensic entomotoxicology is a specialized branch of forensic medicine that combines entomology and toxicology to investigate poisoning cases using insects as alternative toxicological samples.[1,2,3] Its scope encompasses not only the qualitative

and quantitative detection of xenobiotics (such as drugs, environmental pollutants, or pesticides) within insect tissues, but also the crucial investigation into how these substances influence insect behavior, development, and succession patterns.[1,2,3,4] This field is particularly useful in cases where traditional toxicological matrices, such as blood, urine, or solid organs, are no longer available due to advanced decomposition or skeletonization.[1,2,3,4,5]

Necrophagous insects, especially larvae and pupae, are commonly used as reliable substrates for toxicological analysis, as they can absorb and accumulate toxicants from decomposing tissues.[1,2,3,4,5,6] The modifications in insect development, for instance, can directly impact the accuracy of post-mortem interval (PMI) estimations[6,7,8], making entomotoxicology a vital component in comprehensive death investigations.[2,3,4] These insects can also provide valuable information for post-mortem interval (PMI) estimation.[1,3,4]

Historical Background

The field of forensic entomology, which provides foundational context for entomotoxicology, began gaining modern scientific traction as early as 1970 with seminal discussions on the role of insects in criminal investigations.[9,10] The explicit development of forensic entomotoxicology, however, commenced in the late 1970s, initially driven by the recognition that insects could accumulate metallic elements. Pioneering work by Sohal and Lamb in 1977 and the early 1980s notably demonstrated the bioaccumulation of various metals, including copper, iron, zinc, calcium, and mercury, in houseflies and blowflies fed on contaminated tissues.[1,3,9] This established the principle that xenobiotics could be absorbed and retained within insect matrices.

The first direct application of this concept to drug detection in forensic cases emerged in 1980, when Beyer et al. successfully identified phenobarbital in maggots from a decomposed cadaver, marking a pivotal moment in the field.[1,3,5] Following this breakthrough, research rapidly expanded to encompass a wide array of illicit and pharmaceutical drugs. Studies by Kintz et al. (1990, 1994), Nolte et al. (1992), and Goff et al. (1989, 1991) confirmed the detectability of various substances, including opiates (morphine, codeine), cocaine, benzodiazepines (e.g., diazepam, triazolam, oxazepam), barbiturates, and antidepressants (e.g., amitriptyline, clomipramine, nortriptyline) in different insect stages such as larvae, pupae, and puparia.[1,2,3,11,12,19,20,21,22] These early advancements not only validated the insects's utility as alternative toxicological samples but also highlighted their practical advantages, such as reduced endogenous peaks in chromatograms compared to putrefied human tissues.[5]

A significant advancement in the field involved investigating the impact of these toxicants on insect development, as such modifications can directly affect the accuracy of post-mortem interval (PMI) estimations a cornerstone of forensic entomology.[3.4] Research demonstrated that various substances could accelerate or retard insect growth rates. For instance, morphine, cocaine, and codeine were found to influence the developmental pace of blowflies, introducing a measurable bias in PMI calculations if not accounted for.[6,7,8,12] This intricate relationship between xenobiotic presence and altered insect development underscored the necessity of an entomotoxicological approach in comprehensive death investigations.[2] Thus, forensic entomotoxicology has evolved from a nascent discipline focused merely on detection to a sophisticated field addressing complex interactions between toxicology and entomological parameters, continually refining its methodologies and applications in criminal justice.

Importance and Applications

Forensic entomotoxicology offers several critical advantages in criminal investigations, particularly in scenarios where conventional forensic approaches face limitations:

1. Role in Forensic Investigations

Forensic entomotoxicology is crucial in cases where traditional toxicological samples (e.g., body fluids and tissues) are unavailable due to advanced decomposition or skeletonization. In such compromised states, insects feeding on the corpse become indispensable alternative specimens, capable of providing valuable toxicological evidence that would otherwise be lost.[1,3,13,14] This utility extends to situations where conventional matrices are degraded, making insect analysis a primary investigative tool.[4,5]

2. Estimating Postmortem Interval (PMI)

A primary application lies in refining postmortem interval (PMI) estimations. The presence of drugs or toxins within the decomposed remains can significantly alter the growth and developmental rates of necrophagous insects, such as blowfly larvae.[6,7,8] By understanding these drug-induced modifications, forensic entomologists can adjust traditional PMI models, leading to more accurate reconstructions of timelines in criminal investigations and preventing significant estimation errors if toxicological factors are overlooked.[13,14,15]

3. Detection of Toxins and Drugs

Insects serve as reliable biological matrices for the qualitative detection and, increasingly, quantitative estimation of various toxic substances. Analysis of insect tissues can reveal the presence of illicit drugs, pharmaceutical compounds, or environmental poisons, thereby providing critical insights into the cause of death, even when the human tissues are severely decomposed.[1,3,13,14] This capability is particularly vital for identifying the presence of illegal substances that may have contributed to a fatality.[2]

Research Trends and Progress

The field of forensic entomotoxicology has experienced dynamic growth, marked by distinct trends and advancements:

1. Global Research Trends

There has been a notable increase in publications and research activities worldwide, with significant contributions from key countries. For instance, the United States, Brazil, China, the United Kingdom, and Germany lead in research output, indicating a global commitment to advancing this specialized area.[9] This geographical diversity underscores the international relevance and collaborative nature of forensic entomotoxicology.

2. Analytical Techniques

Progress in analytical methodologies has been pivotal in enhancing the capabilities of entomotoxicology. Advances in techniques such as gas chromatography (GC) coupled with mass spectrometry (MS), high-performance liquid chromatography (HPLC), and various electrochemical methods have significantly improved the sensitivity and specificity for detecting and quantifying toxicants in insect tissues.[1,13,14,16] These sophisticated techniques enable the identification of a broad spectrum of xenobiotics, even at trace levels, thereby overcoming limitations posed by degraded traditional samples.

3. Standardization and Protocols

A critical ongoing trend is the push towards standardization of research protocols. To ensure consistency and comparability of results across different studies and laboratories globally, there is a recognized need for agreed-upon guidelines. These guidelines encompass aspects such as the selection of common model organisms and toxicants, standardized procedures for sample collection and preservation, appropriate sample sizes, and validated analytical methods.[2,4,13,17] Such standardization is crucial for translating research findings into reliable forensic casework.

Challenges and Future Directions

Despite significant progress, forensic entomotoxicology continues to face several challenges that shape its future trajectory:

1. Inconsistencies and Limitations: A major challenge remains the inconsistencies observed across studies, particularly concerning the quantitative correlation

between drug concentrations in insects and the decomposed tissues they feed upon.[2,11,13,14] The complex pharmacokinetics of drugs within insect physiology, compounded by environmental variables, often makes quantitative extrapolation unreliable. Addressing these inconsistencies necessitates more sophisticated analytical techniques and controlled experimental designs to reduce variability and improve precision.

2. Interdisciplinary Collaboration: Advancing the discipline and addressing its inherent limitations requires intensified interdisciplinary collaboration. Enhanced cooperation between forensic entomologists, toxicologists, analytical chemists, and other related fields (e.g., microbiologists, statisticians, geneticists) is essential. Such synergistic efforts can lead to a more comprehensive understanding of complex biological interactions and facilitate the development of robust methodologies applicable to real-world forensic cases.[2,9]
3. Emerging Technologies: The future of forensic entomotoxicology is promising, with the integration of new technologies holding immense potential. Advances in DNA analysis, including genetic barcoding for species identification and human DNA detection from insect gut contents, are enhancing accuracy and reliability.[2,4,9] Furthermore, the application of molecular genetics, such as gene expression studies to refine insect age estimation, and the use of advanced spectroscopic techniques and statistical modeling (e.g., machine learning) for data interpretation, are emerging areas that promise to revolutionize the field.[9,13,14,18]

METHODS

This study applied an integrated bibliometric analysis and narrative review to comprehensively map global research trends, methodological developments, and scientific advances in forensic entomotoxicology. A two-stage approach was implemented: (i) quantitative bibliometric mapping to analyze publication outputs, authorship and country collaborations, journal contributions, and keyword co-occurrence networks; and (ii) qualitative narrative synthesis to critically evaluate analytical techniques, experimental designs, and emerging thematic directions. As this research was based exclusively on published literature, no ethical approval was required. This study supports SDG 3 (Good Health and Well-Being), SDG 16 (Peace, Justice and Strong Institutions), and SDG 9 (Industry, Innovation and Infrastructure) by advancing innovative and standardized forensic entomotoxicology methods that improve toxicological detection, post-mortem interval estimation, and the reliability of scientific evidence in medico-legal investigations.

A combined bibliometric analysis and narrative review was conducted using Scopus and PubMed databases, covering publications from 1991 to 2025. Peer-reviewed English-language articles addressing xenobiotic detection in insects, drug- or toxin-induced effects on insect development, or forensic applications of entomotoxicology were included. Following systematic screening, 87 studies involving 5,214 insect samples across various developmental stages were analyzed. Data extracted included publication characteristics, insect matrices and taxa, xenobiotic classes, analytical platforms (e.g., GC-MS, LC-MS/MS, HPLC), and reported forensic outcomes. Bibliometric visualization was performed using VOSviewer to generate keyword, authorship, and country collaboration networks, as well as temporal

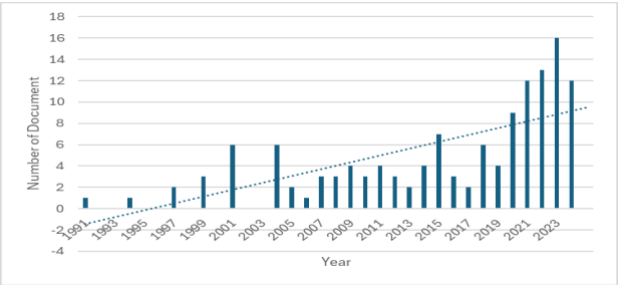

Figure1. Research Trends in Entomotoxicology worldwide

and density maps. Methodological rigor was assessed based on protocol standardization, reporting completeness, and reproducibility. Quantitative bibliometric results were integrated with narrative synthesis to elucidate methodological evolution, toxicological impacts on insect development, analytical challenges, and future research gaps in forensic entomotoxicology**.**

RESULTS

The Application of Forensic Entomotoxicology in Determining the Postmortem Interval: Past and Present Perspectives

Research trend in entomotoxicology in every year is increasing. The first research published in 1991 written about drugs identified in maggots, fly larvae, and beetles feeding through it.[5] The highest trend of entomotoxicology research was coming in 2023, which were 16 articles. In such year, the highest citated article was telling about the entomotoxicological analysis using ATR-FTIR spectroscopy combined with chemometrics.[23] The second highest citated article was telling about another alternative of analysis method in entomological samples, reflected on some cases.[24] The following article was written about an effect of ceftriaxone and levofloxacin, antibiotics which affect the development of *Lucilia sericata*.[25] For a lower citated article was written about a biochemical and physiological component, aluminum phosphide and cypermethrin to carry the flies as an essential role in forensic entomotoxicology.[26] The similar citated number article was published about the detection and validation of the opioids and metabolites in larvae of *Lucilia sericata* by UHPLC-TF-MS.[27] Another similar citated article in that year was written about a relation of Lorazepam with the growth of maggot.[28] The only article citated in twice was written about an effect of another neurotoxicant, Terbufos, on larval activity.[29] The number of article citated in once were variously written about some methods of entomotoxicology analysis, such as a detection of Ethyl glucuronide in larvae[30], diazinon effect in poisionings[31], preliminary analysis of Methamphetamine in *Lucilia sericata*[32], a death investigation in forensic entomology[33], analysis of poisons and drugs[34], forensic veterinary analysis in *Lucilia sericata*[35], and effect analysis of thiamethoxam on the flies and beetle.[36] The other two articles with no citation were written about a review of forensic entomology recent condition in India[37] and the utilization of solid-phase extraction and gas chromatography-mass spectroscopy to determine an Asenapine Maleate.[38] The increasing trend of that research in 2023 was related with some alternative methods to analyze in entomotoxicology research, one of them is a Mass Spectroscopy.[23,27,38] The lowest trend was published one article in each year, which were 1991, 1995, and 2006. In 1991 and 1995, the entomotoxicology research were firstly explained in basic relation with drugs and a continuous theory in related with insects and other arthropods.[5,12] In 2006, the only article was published a method to detect and quantify insects.[16]

Some years had zero publication in entomotoxicology research, which were 1993 and 2003. In the range years of 1991 to 1995, there was only a basic theory and explanation about entomotoxicology. In 2001, few of articles were started to talk about a relation of drug and toxic substances in entomological specimens.[1,7] Some articles in 2004 were developed to write for a new analysis method in entomotoxicology, such as a toxicological data and growth characteristics in *Calliphora vicina* and gunshot residues extraction from the larvae.[39,40] There is a gap of finding and knowledges of entomotoxicology research in between of such years.

Table 1. The most cited articles on forensic entomotoxicology

SCR	Article Title	Author	Journal	Vol (No)	Year	Citation
1	Forensic entomology: Applications and limitations	Amendt, J., Richards, C.S., Campobasso, C.P., Zehner, R., Hall, M.J.R.	Forensic Science, Medicine, and Pathology	7(4), pp. 379–392	2011	403
2	Entomotoxicology	Introna, F., Campobasso, C.P., Goff, M.L.	Forensic Science International	120(1-2), pp. 42–47	2001	162
3	Entomotoxicology: A new area for forensic investigation	Goff, M.L., Lord, W.D.	American Journal of Forensic Medicine and Pathology	15(1), pp. 51–57	1994	100
4	Determination of drug levels and the effect of diazepam on the growth of necrophagous flies of forensic importance in southeastern Brazil	Carvalho, L.M.L., Linhares, A.X., Trigo, J.R.	Forensic Science International	120(1-2), pp. 140–144	2001	98
5	The detection of toxic substances in entomological specimens	Gagliano-Candela, R., Aventaggiato, L.	International Journal of Legal Medicine	114(4-5), pp. 197–203	2001	83
6	Drug analysis in blowfly larvae and in human tissues: A comparative study	Campobasso, C.P., Gherardi, M., Caligara, M., Sironi, L., Introna, F.	International Journal of Legal Medicine	118(4), pp. 210–214	2004	82
7	Entomotoxicology, experimental set-up and interpretation for forensic toxicologists	Gosselin, M., Wille, S.M.R., Fernandez, M.D.M.R., ... De Boeck, G., Bourel, B.	Forensic Science International	208(1-3), pp. 1–9	2011	79
8	Effects of morphine in decomposing bodies on the development of	Bourel, B., Hédouin, V., Martin-Bouyer, L., ...	Journal of Forensic Sciences	44(2), pp. 354–358	1999	64

	Lucilia sericata (Diptera: Calliphoridae)	Deveaux, M., Gosset, D.				
9	Codeine accumulation and elimination in larvae, pupae, and imago of the blowfly *Lucilia sericata* and effects on its development	Kharbouche, H., Augsburger, M., Cherix, D., ... Champod, C., Mangin, P.	International Journal of Legal Medicine	122(3), pp. 205–211	2008	56
10	Forensic entomo-toxicology	Pounder, D.J.	Journal of the Forensic Science Society	31(4), pp. 469–472	1991	55

Table 1, "The most cited articles on forensic entomotoxicology," reveals a series of seminal publications that have shaped and guided research trends in forensic entomotoxicology over the past few decades. Analysis of the ten most cited articles in forensic entomotoxicology (Table 1) reveals a publication pattern centered on legal medicine and forensic science journals. These journals serve as primary platforms for the dissemination of fundamental research, new methodologies, and discussions concerning the limitations and challenges in this field. A prominent finding is the dominance of *Forensic Science International* as a premier journal. This journal published articles by Introna et al. (2001), Carvalho et al. (2001), and Gosselin et al. (2011).[2,3,7] This dominant presence indicates its role as a core journal for cutting-edge research in forensic science, including entomotoxicology. Another journal that stands out as the most productive contributors is *International Journal of Legal Medicine*. This journal published articles by Gagliano-Candela & Aventaggiato (2001), Campobasso et al. (2004), and Kharbouche et al. (2008).[5,6,8]. Its focus on legal and medical aspects confirms the importance of entomotoxicology within the context of legal investigations.

Foundational Discipline and Conceptual Evolution

The work of Pounder (1991) marked a foundational milestone in defining entomotoxicology as a promising tool in forensic science. In his article "Forensic Entomo-Toxicology," Pounder introduced the central premise that drugs present in decomposing bodies can be detected through the analysis of maggots that have fed on the remains. He also highlighted the potential of puparia to extend the toxicological analysis timeline by several years.[5] This fundamental contribution paved the way for subsequent research in the field.

Over time, the understanding of the roles and applications of entomotoxicology has become increasingly sophisticated. A pivotal contribution to this development was made by Introna et al. (2001) through their article *"Entomotoxicology,"* published in *Forensic Science International*, which has been cited 162 times to date. This publication stands as one of the earliest and most influential works that explicitly discusses the use of insects particularly larvae and puparia in forensic toxicological analysis. It highlights the potential of these biological specimens to detect drugs and toxins in decomposing tissues and underscores their relevance in estimating the postmortem interval (PMI).

The bibliometric prominence of this article reflects its foundational role in shaping the field, especially as drug-related deaths have continued to rise, further emphasizing the practical utility of entomotoxicology in forensic investigations. Introna et al. reinforced the dual function of this approach both in substance detection and time of death estimation while also candidly acknowledging the methodological limitations that remain inherent in its application.

Innovation in the Detection and Quantification of Toxic Substances

A foundational contribution to the advancement of entomotoxicology was presented by Gagliano-Candela and Aventaggiato (2001) in their highly cited article *"The Detection of Toxic Substances in Entomological Specimens,"* published in the *International Journal of Legal Medicine* and cited 83 times. The study identifies insect-derived specimens particularly larvae, pupae, and puparial cases as reliable matrices for detecting a wide range of toxic substances, including drugs, metals, and pesticides, especially in cases of advanced decomposition where human tissues are no longer viable. One of the article's key findings is that drug concentrations are often higher in puparial cases than in other developmental stages, which may be attributed to bioaccumulation.

Additionally, the study highlights how specific toxicants, such as mercury and heroin, can influence larval growth and development, thereby altering postmortem interval (PMI) estimations. This underlines the dual role of entomotoxicology in both toxic substance detection and PMI estimation. The authors also emphasize the necessity of using highly sensitive and specific analytical techniques namely gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography-mass spectrometry (HPLC-MS) to achieve accurate identification and quantification. However, the study also acknowledges certain limitations, such as the inconsistent presence of drugs in later insect stages like pupae or adult flies. Overall, their work solidifies entomotoxicology as a promising and practical alternative for forensic toxicological analysis when conventional biological evidence is compromised.

A pivotal study by Campobasso et al. (2004), titled *"Drug Analysis in Blowfly Larvae and in Human Tissues: A Comparative Study"* and published in the *International Journal of Legal Medicine* (cited 82 times), provides critical insights into the relationship between drug concentrations in human tissues and in blowfly larvae feeding on those tissues. The study confirmed that all examined drugs including opiates, cocaine, barbiturates, and antidepressants present in human tissues were also detected in blowfly larvae, thereby validating their reliability for qualitative toxicological analysis in forensic contexts. However, the researchers observed that drug concentrations in larvae were consistently lower than in corresponding human tissues, with notable variation depending on larval developmental stage and the chemical properties of the drugs. Actively feeding larvae exhibited higher concentrations than post-feeding larvae, due to ongoing metabolic and elimination processes during development.

Drug-specific patterns further illustrated these complexities: opiates and cocaine showed greater accumulation in liver tissue and feeding larvae than in blood; clomipramine, an antidepressant, was found in higher concentrations in larvae than in human tissue, suggesting potential bioaccumulation; and phenobarbital demonstrated significant retention in post-feeding larvae due to its lipophilic nature. These findings underscore the importance of selecting appropriate larval stages preferably active feeders for accurate toxicological analysis. While the study affirms the utility of blowfly larvae in detecting drugs when human tissues are decomposed or absent, it also highlights the unresolved challenge of achieving reliable quantitative extrapolation,

given the distinct pharmacokinetics between insects and humans. Collectively, these results reinforce entomotoxicology's value in forensic investigations, particularly for qualitative drug detection in advanced decomposition cases.

The Impact of Toxic Substances on Insect Development: Implications for PMI Estimates

A critical aspect of entomotoxicology lies in understanding how the presence of drugs or toxins within decomposing tissues affects the developmental rate of necrophagous insects. This phenomenon has direct implications for the accuracy of postmortem interval (PMI) estimations one of the principal applications of forensic entomology. Carvalho et al. (2001), in a highly cited study (*Forensic Science International*, cited 98 times), investigated the effects of diazepam on the development of necrophagous flies *Chrysomya albiceps* and *Chrysomya putoria*, revealing that larvae fed on diazepam-treated tissues exhibited accelerated development during the early larval stage (18–54 hours), but experienced delayed pupariation and adult emergence. Using GC-MS, diazepam was detected in both rabbit tissues particularly the heart and liver and in insect samples, with notable bioaccumulation in *C. putoria* puparia. The drug also influenced adult mortality patterns, with *C. albiceps* showing earlier onset of mortality compared to *C. putoria*. These findings underscore the potential for significant errors in postmortem interval (PMI) estimation when drug presence is not accounted for, and highlight the need for further research on how specific substances affect insect development in forensic contexts.

Kharbouche et al. (2008), in their study *"Codeine Accumulation and Elimination in Larvae, Pupae, and Imago of the Blowfly Lucilia sericata and Effects on Its Development"* (*International Journal of Legal Medicine*, cited 56 times), investigated the pharmacokinetics and developmental impact of codeine and its metabolites norcodeine and morphine across different life stages of *Lucilia sericata*. The study confirmed that codeine could be reliably detected in larvae reared on pig liver spiked with therapeutic to lethal doses, with larval concentrations showing a linear correlation with substrate levels during the active feeding stage. However, larvae consistently underestimated tissue concentrations, limiting their value for quantitative analysis. Codeine exposure accelerated larval growth, shortening the larval stage by up to 29 hours and the total development time by 21 hours, potentially introducing substantial bias in postmortem interval (PMI) estimations.

Notably, norcodeine persisted longer and at higher concentrations than morphine, suggesting differential metabolism or elimination, while the detection of codeine in adult (imago) stages pointed to possible resumed ingestion after emergence. These findings provide critical insights into the toxicological reliability and developmental influence of codeine, emphasizing the utility of *L. sericata* for qualitative forensic toxicology while cautioning against its use for precise PMI estimation due to xenobiotic-induced developmental shifts.

Standardization and Future Direction

Recent global developments in forensic entomotoxicology have increasingly emphasized the need for methodological standardization and a deeper understanding of the biological mechanisms that influence toxicant dynamics in necrophagous insects. Gosselin et al. (2011), in their article *"Entomotoxicology, Experimental Set-Up and Interpretation for Forensic Toxicologists"* published in *Forensic Science International*, provided a comprehensive synthesis of current challenges and methodological advancements in the field. Their work underscores the importance of establishing international consensus on insect euthanasia procedures, sample preservation methods, and validated analytical protocols to

minimize variability and enhance the reliability of toxicological interpretations.

A notable contribution of the study is its emphasis on the adoption of high-sensitivity analytical platforms, such as LC-MS/MS, which enable the detection of xenobiotics from single insect specimens particularly puparia, which offer chemical stability and prolonged drug retention compared to decomposed biological tissues. Furthermore, Gosselin et al. introduced refined sampling recommendations, including allowing larvae to purge their gut contents prior to analysis and standardizing the selection of developmental stages to reduce detection bias and improve reproducibility across laboratories.

Complementing recent advancements in entomotoxicological methodologies, Amendt et al. (2011), in their influential article *"Forensic Entomology: Applications and Limitations"* published in *Forensic Science, Medicine, and Pathology* (cited 403 times), offered a comprehensive set of best practices that remain foundational to forensic entomology. Their review emphasized the critical role of accurate insect sampling and climatic data particularly ambient temperature measurements and integration of local weather station records to improve the precision of post-mortem interval (PMI) estimations using thermal summation models. They also highlighted the transformative potential of molecular techniques, including DNA barcoding and gene expression profiling, which enable early-stage species identification and, in some cases, detection of human DNA from larval gut contents, thereby linking maggots to specific victims or crime scenes. Furthermore, they addressed key challenges such as drug-induced alterations in insect development, larval-generated heat, and geographical variability in growth rates all of which can affect PMI estimations. Their work reinforces the necessity of localized reference data, statistical validation, and interdisciplinary approaches to enhance both the scientific accuracy and legal admissibility of entomological evidence in forensic casework.

Despite notable advancements, Gosselin et al. (2011) emphasized that the accurate interpretation of entomotoxicological data in real forensic cases remains largely aspirational, describing it as a "utopia" due to limited understanding of drug pharmacokinetics in insects and the significant influence of environmental factors such as temperature and humidity. To address these challenges, the authors advocate for future research to prioritize the investigation of physiological processes during larval feeding, drug absorption, metabolism, and excretion across different developmental stages, alongside studies on postmortem drug redistribution and stability. They also call for the harmonization of experimental protocols that better replicate human decomposition conditions. These efforts are deemed essential to enhance the reliability and quantitative interpretability of entomotoxicological evidence. Collectively, these insights affirm the field's growing forensic value particularly in cases involving skeletonized remains and postmortem interval (PMI) estimation while highlighting critical gaps that must be bridged for entomotoxicology to become a standard and validated component of forensic toxicology practice.

Table 2. The top ten authors on forensic entomotoxicology research

SCR	Author	Document Number
1	Bourel, B.	8
2	Hédouin, V.	6
3	Zanetti, N.I.	5
4	Magni, P.A.	5
5	Gosset, D.	5
6	Gama, R.A.	5
7	Dadour, I.R.	5
8	Centeno, N.D.	5
9	Al-Mekhlafi, F.A.	5
10	Al-Khalifa, M.S.	5

Overall, the bibliometric trend indicates that forensic entomotoxicology has evolved from an exploratory field into a more

structured scientific discipline, with a strong emphasis on the integration of modern analytical technologies, methodological validation, and practical relevance in forensic investigations involving highly decomposed or skeletonized bodies.

In the field of forensic entomotoxicology, Bourel, B. stands out as the most prolific contributor, with a total of eight publications, reflecting a significant and sustained impact on the development of this discipline. Based on publication affiliations and collaboration patterns, Bourel is most likely associated with research institutions in France, such as Université de Lille, which are known for their active roles in forensic entomology and toxicology. Other prominent French researchers include Hédouin, V. (six publications) and Gosset, D. (five publications), further highlighting France's leading role in entomotoxicology research. In addition to French contributors, authors such as Zanetti, N.I. and Magni, P.A. suggest active involvement from researchers based in Italy and South America, demonstrating that the field is international in scope, though still marked by strong regional hubs of expertise.

When cross-referenced with Table 1, which presents the most cited articles in forensic entomotoxicology, Bourel is listed as one of the key authors in the influential study *"Effects of morphine in decomposing bodies on the development of Lucilia sericata"*, published in the *Journal of Forensic Sciences* in 1999, with 64 citations. This indicates that Bourel's contributions are not only quantitative in terms of publication count but also qualitative, as evidenced by citation metrics and the continued relevance of his work in shaping the field. This particular article investigated the impact of morphine on fly development, providing critical evidence on how drug presence can alter larval growth rates and thus affect postmortem interval (PMI) estimations one of the central concerns in forensic entomotoxicology.

Moreover, while not all authors listed in Table 2 appear explicitly in the list of most cited articles (Table 1), their frequent publication suggests a broader ongoing effort to expand methodological and empirical knowledge in the field. Their work likely supports developments in standardizing sampling protocols, refining analytical methods, and exploring the pharmacokinetics of xenobiotics in necrophagous insects.

Table 3. The most contributed journal on forensic entomotoxicology research

SCR	Journal	Document Number
1	Journal of Forensic Sciences	14
2	International Journal of Legal Medicine	10
3	Forensic Science International	10
4	Journal of Medical Entomology	7
5	Egyptian Journal of Forensic Sciences	5
6	Science and Justice	4
7	Journal of Forensic and Legal Medicine	4
8	Saudi Journal of Biological Sciences	3
9	International Journal of Medical Toxicology and Legal Medicine	3
10	American Journal of Forensic Medicine and Pathology	3

Taken together, these bibliometric findings suggest that forensic entomotoxicology has progressed from an exploratory subdiscipline into a more structured and methodologically robust scientific field. The thematic evolution evident through the citation impact of foundational works by Amendt et al. (2011), Introna et al. (2001), and Goff and Lord (1994) highlights an increasing emphasis on the integration of molecular techniques, advanced analytical technologies such as LC-MS/MS, and rigorously validated experimental protocols. At the same time, the

sustained publication productivity of leading researchers reflects the field's dynamic trajectory, with recent studies increasingly focusing on environmental influences, xenobiotic metabolism within insect systems, and the forensic utility of entomotoxicological evidence in cases involving advanced decomposition or skeletonization.

Thus, the current trajectory of forensic entomotoxicology is defined by interdisciplinary collaboration, international scholarly engagement, and a strong push toward forensic standardization, all of which are necessary to enhance the field's reliability and admissibility in legal contexts. Continued attention to bibliometric trends can help identify research gaps, strengthen global research networks, and support the development of best practices in forensic science.

A bibliometric assessment of the journals contributing to forensic entomotoxicology research reveals a focused yet interdisciplinary pattern of scholarly dissemination. As shown in Table 3, the *Journal of Forensic Sciences* emerges as the most prolific outlet, publishing 14 relevant articles. This is closely followed by the *International Journal of Legal Medicine* and *Forensic Science International*, each contributing 10 documents. The prominence of these three journals indicates a strong institutional recognition of forensic entomotoxicology within the broader framework of forensic science and legal medicine. Their consistent publication of entomotoxicology-related work reflects a sustained academic interest and a demand for methodological rigor in forensic practice.

Complementing this core group are journals such as the *Journal of Medical Entomology* (7 documents) and the *Journal of Forensic and Legal Medicine* (4 documents), which highlight the inherently interdisciplinary nature of the field. These journals attract contributions from researchers in entomology, toxicology, pathology, and criminal investigation, reflecting the integrative approach required to understand insect-drug interactions in post-mortem contexts.

Notably, regionally based journals like the *Egyptian Journal of Forensic Sciences*, the *Saudi Journal of Biological Sciences*, and the *International Journal of Medical Toxicology and Legal Medicine* also appear among the top ten contributors. Although these journals have a lower volume of publications (3–5 documents each), their presence signals a growing global engagement with entomotoxicological research, particularly from underrepresented regions. This geographic diversification may foster unique perspectives on climate, species variation, and regional forensic challenges, enriching the field's knowledge base.

The journal publication trend aligns well with the findings from the citation analysis of the most influential articles. Highly cited works such as Amendt et al. (2011) and Goff & Lord (1994) were published in high-impact forensic journals, reinforcing the notion that these outlets serve as both incubators and amplifiers of critical research in forensic entomotoxicology. Moreover, several of the most cited articles identified earlier, such as those by Bourel et al. (1999), Campobasso et al. (2004), and Carvalho et al. (2001), were also published in these leading journals, confirming their central role in shaping discourse and advancing methodologies in the discipline.

A critical examination of the relationship between Table 3 and Table 1 reveals a strong and significant correlation. The journals that are most productive in publishing entomotoxicology research are also the ones that publish the most influential, highly cited articles. This synergy confirms their status as the intellectual core of the discipline. Forensic Science International (FSI), ranked third for publication volume, has published three of the ten most-cited articles [Table 1. No. 2, 4, 7]. This disproportionate impact establishes FSI as arguably the most influential single venue for foundational research in entomotoxicology.

The International Journal of Legal Medicine, second in volume, has published three of the top ten cited articles [Table 1. No. 5, 6, 9], solidifying its importance in the field.

This analysis demonstrates that a journal's influence is not solely a function of publication volume. While the *Journal of Forensic Sciences* is the most frequent outlet, *Forensic Science International* emerges as the central hub for the seminal works that have most profoundly shaped the discourse and direction of forensic entomotoxicology.

Given its exceptional impact, *Forensic Science International* warrants further discussion. FSI has cemented its reputation as a premier global journal by consistently publishing high-impact, field-advanced research. It is published by Elsevier, a leading academic publisher renowned for its rigorous peer-review standards and broad international reach through platforms like ScienceDirect.

The journal's academic prestige is further enhanced by its official affiliations. FSI is the official journal for numerous leading professional organizations, including the International Association of Forensic Sciences (IAFS) and the European Network of Forensic Science Institutes (ENFSI). This affiliation is significant because it ensures a steady stream of high-quality, relevant submissions from the world's leading practitioners and researchers. It also signifies that the journal operates at the heart of the global forensic science community, making it the natural venue for research intended to set standards, address critical challenges, and define the future of the discipline.

Overall, this journal-level bibliometric analysis supports the view that forensic entomotoxicology is no longer a peripheral or exploratory topic, but a recognized subfield within forensic science. The concentration of publications in core forensic and medico-legal journals, paired with contributions from entomological and biological outlets, underscores the field's evolution toward a methodologically mature and globally relevant discipline. The increasing distribution of publications across diverse journals and regions suggests a promising future for interdisciplinary collaboration and knowledge integration in forensic entomotoxicology.

Table 4. The top ten institutions on forensic entomotoxicology research

SCR	Institution	Document Number	Country
1	College of Sciences	8	United States
2	King Saud University	8	Saudi Arabia
3	Universidad Nacional de Quilmes	5	United States
4	Universite Catholique de Lille	5	France
5	Universidade Federal do Rio Grande do Norte	5	Brazil
6	Thamar University	5	Saudi Arabia
7	Universite de Lille	5	France
8	University of Bisha	5	Saudi Arabia
9	Faculte Libre des Sciences	4	France
10	Inst. Med. Legale et de Med. Social	4	United States

In Table 4, the College of Sciences in the United States appears as the institution with the highest number of publications in entomotoxicology research. Correspondingly, Table 5 shows that the United States ranks sixth among the most productive countries, with a total of ten documents. One of the institution's notable contributions includes a seminal publication from 2001, recognized as one of the earliest comprehensive explanations of entomotoxicology.[12] Researchers affiliated with the College of Sciences have produced a diverse body of

work, including studies on forensic practices and guidelines,[41] the effects of various drugs,[42-44] and the impact of multiple chemical compounds.[45-48]

King Saud University in Saudi Arabia occupies the second position, producing an equivalent number of publications. Consistently, Saudi Arabia also ranks sixth among the most productive countries (Table 5), with one of its widely cited studies focusing on the growth rate of Calliphoridae flies.[46]

The subsequent six institutions in the ranking each contributed a comparable number of publications. These include Universidad Nacional de Quilmes (United States), Université Catholique de Lille (France), Universidade Federal do Rio Grande do Norte (Brazil), Thamar University (Saudi Arabia), Université de Lille (France), and the University of Bisha (Saudi Arabia. The geographic distribution of these institutions reflects the broader country-level productivity shown in Table 5, where Brazil occupies the second position, followed by France in fourth place, below India.

Completing the top ten institutions are Faculté Libre des Sciences (France) and Institut Médical Légal et de Médecine Sociale (United States), both of which are located in countries ranked among the ten most productive contributors to entomotoxicology research.

Table 5. The top ten countries on forensic entomotoxicology research

SCR	Country	Document Number
1	India	22
2	Brazil	15
3	Italia	13
4	France	11
5	Egypt	11
6	United States	10
7	United Kingdom	10
8	Saudi Arabia	9
9	Australia	9
10	Germany	8

Among countries in the world, the largest number published document belongs to India. Surrounded of Asia countries, the country with the highest number of articles published similarly goes to India. Since 2004, several writers coming from India productively wrote and published articles related with entomotoxicology up to recent year. Started in 2004, the article was explained of a basic theory of past, present, and future of forensic entomology.[49] In recent year, 2025, the updated article published about the identification of the cypermethrin effect on the growth of *Lucilia sericata* (Diptera: Calliphoridae).[50]

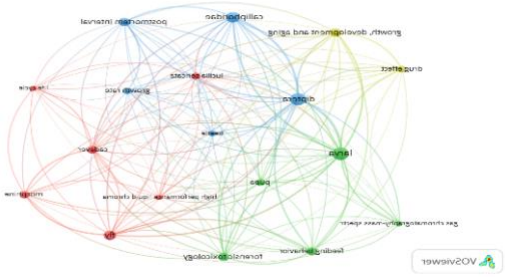

Figure 2A Network visualization

The network visualization from VOSviewer analysis showed a set of scientific publications which several keywords used in relationship with highly frequent occurrence about entomotoxicology forensics. Representing from each node or circles on some keyword prominently appears were “larva”, “diptera”, and “fly”. These words indicate to be the main topic of this study field. In addition, “Beetle” and “Pupa” were observed to be in connection. From the lines connecting the node or circles visualized on the Figure 2A, dividing by thickness of the line as thicker as highly appeared among other keywords. For instance, “larva” and “diptera” were connected by thicker green to blue line, as well as happened in “calliphoridae” and “diptera” with a blue strong line. In the other way, colors represent the cluster of keywords into distinct group by a different color, shown in blue, red, green, and yellow. Blue cluster has the focus in entomological with calliphoridae and postmortem interval which specifically emphasizes the strong connection

of postmortem interval with the insects, calliphoridae, life cycle to further estimate the time since death. This is in a relationship with the biological processes. However, this blue cluster has a clear relationship with green group by connecting the line with "diptera", while weak line with red cluster by a line with "*Lucilia sericata*" and "growth rate". The red cluster shows a focus in toxicological and life cycle aspects of forensic insects by utilizing several keywords, such as "fly", "morphine", "cadaver", "life cycle", "growth rate", and "*Lucilia sericata*". This group seems to be focused on the interaction of drugs, which is "morphine" with insects, such as "fly" "*Lucilia sericata*" found in "cadaver". This connection applies an investigation about substance like morphine that could affect the growth of forensic insects by connecting with the blue cluster by "*Lucilia sericata*" and "calliphoridae". The green color shows a connection with entomological development and methodology, including several keywords as biological aspects of insect development, such as larva, pupa, diptera and their feeding behaviour. The forensic toxicology roles as a bridge to link the methodologies applied in forensic entomology, such as gas chromatography-mass spectrophotometry and high-performance liquid chroma applied to detect toxins in entomological samples. This group is connected with the blue cluster by "diptera" and "calliphoridae", similarly with the red cluster by "fly" and "morphine".

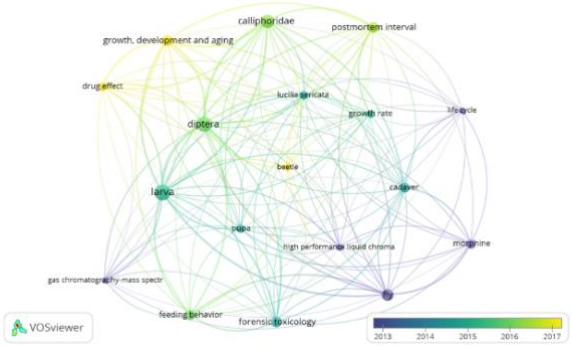

Figure 2B Overlay Visualization

Figure 2B presents a visualization of several keywords used in several publication year related with forensic entomotoxicology in 2013 to 2017. The color of node shows in three different color group, which are dark blue or purple, green or light green, and yellow. However, the line indicates the strength of each relationship with the occurances. The early developing themes are grouped in blue or purple color, which in 2013 to 2015 were commonly used "fly", "morphine", and "cadaver" in the initial research. The analytical methods, such as a high performance liquid chroma and gas chromatography-mass spectrophotometry lean on the middle period. In the middle period, 2014 to 2015, "larva" and "pupa" showed commonly used later. In last 10 years, 2015 to 2017, the green to yellow color represents several keywords like "calliphoridae", "postmortem interval", "growth, development, and aging", and "drug effect". It means that the use of specific insect families, like "calliphoridae" are developed in recent research.

A density visualization shows in Figure 2C represents the concentration of keywords used in Entomotoxicology Forensic study based on the occurrence differed in the density of each clusters. The visible node of each keywords shows an indicate frequency, the heatmap effect of each density appears for underlying connections, and the color intensity means a crucial element of density. As the highest density areas coming from red color, "fly" has a strong focus on entomological evidence in published literatures. In relationship with it, "high

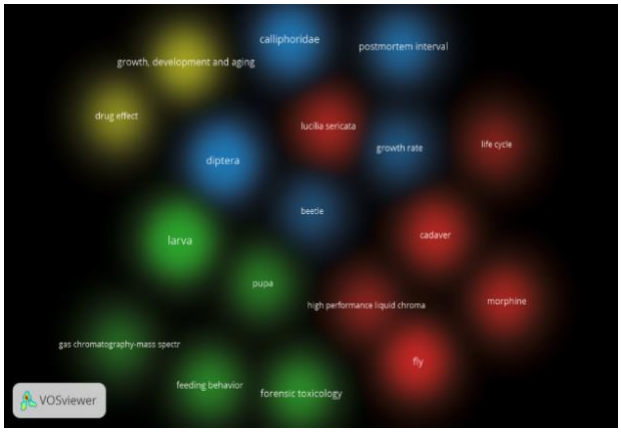

Figure 2C Cluster density visualization

performance liquid chroma" as a method used and "morphine" as a toxicological analysis of

substances found on cadavers show strongly related with the entomotoxicological framework. The high-density area in green color shows a significance visualize in "larva" with some relationships with "pupa" and "diptera" as a biological foundation of insect analysis in forensic context. The following emerging area in blue color was shown in "diptera", "calliphoridae", and "postmortem interval" which are more specialized topics in the relationship of toxicological and fly interaction studies. The "drug effect" as appeared in yellow color with lower density with "growth, development, and aging" showed a broader theme from "forensic toxicology", while closer relationship with "larva" and "calliphoridae".

Figure 3A shows a relationship visualization of co-authorship's countries in international research collaboration based on shared publications. Each node represents a country which the size depending on the number of publications. In this context, India and Germany appears to be the country with the highest number of publications. The links connecting each node seems to be a collaborative relationship which the thickness means the strong collaboration. It intends to a great collaboration between Western European (Germany, Italy, Belgium, France) and Brazil resulted in some productive publications. The collaboration between India with Egypt and Algeria shows a strong collaboration. The colors clustered are representing in some different group of countries.

Figure 3A Network visualization

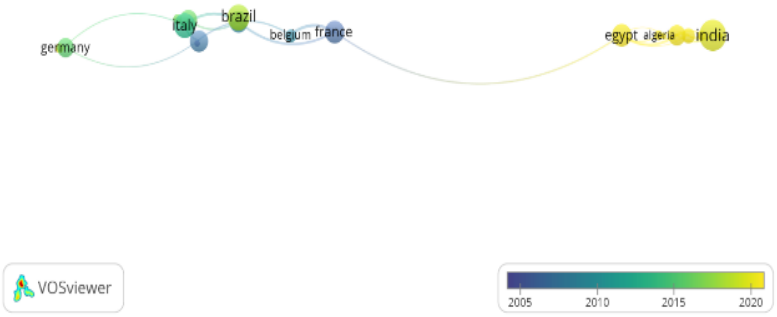

Figure 3B Overlay visualization

Figure 3. Country mapping with VOS viewer (A) Network visualization; (B) Overlay visualization. The length of connection indicates the level of linkage, with a minimum number of documents per author is five documents.

Figure 3B shows an international research collaboration between countries in several years, from 2005 to 2020, as shown in color legend. In early to middle period, 2005 to 2015, a well-established and possibly mature collaborative network was highly active in between Germany, Italy, Brazil, Belgium, and France. For the North African or Middle Eastern and India cluster, several publications were recently published significantly in last 5 years (2015-2020). In this term, India emerges as a significant and relatively recent global collaborator as the greatly node visualized which similarly happened in Brazil in previous years.

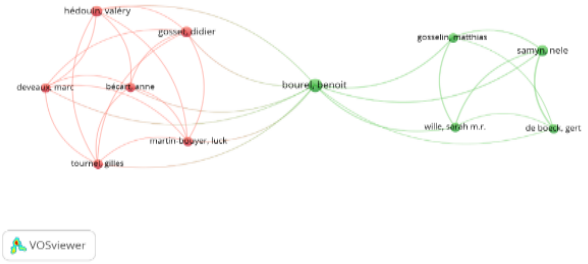

Figure 4A Network visualization

A connecting visualization in Figure 4A presents about a collaboration among individual researchers in each node and color which in different groups. Those researchers relate to lines representing as thicker as more frequent collaboration. The red cluster is intending to a french or closely collaborating related group as written as their name, which are “hedouin, valery”, “gosset, didier”, “deveaux, marc”, “becart, anne”, “martin-bouyer, luck”, and “tournel, gilles.” in numerous and strong connections. Another group collaboration in light green cluster which are strongly indicates Belgian or Dutch as representing from their name. Moreover, “Bourel, Benoit” shows a central node of others which suggests that collaboratively acts as a main of an extensive several research in both different groups from different countries.

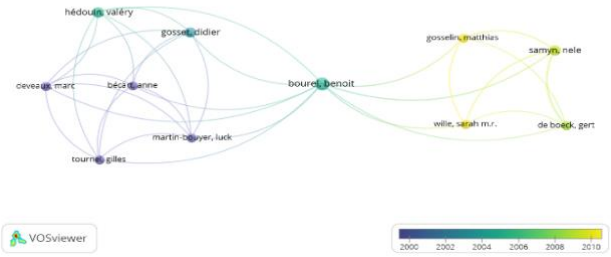

Figure 4B Overlay visualization

Figure. 4. Author distribution and collaboration with VOSviewer. (A) Network visualization; (B) Overlay visualization. The length of the connection indicates the level of linkage, with a minimum of five documents

Figure 4B indicates an evolution of research collaborations between individual authors over 10 years, from 2000 until 2010. The dark blue or purple color shows several authors whose was published in early years, 2000 to 2004. Each author had a contribution in the early establishment of entomotoxicology forensic study. The light blue color happened in the middle period, while green or yellow in the recent years. The middle era showed in green to yellow color is indicating a strong suggestion of an emerging collaborative network. In addition, “Bourel, Benoit” still, as a central bridge which linking several authors in initial to recent years were consistently active to contribute a significant portion in those periods for this study.

CONCLUSION

This study provides a comprehensive overview of global research trends in forensic entomotoxicology from 1991 to 2025, demonstrating substantial growth in scientific publications and highlighting the increasing importance of necrophagous insects as alternative toxicological matrices in medico-legal investigations. Bibliometric and narrative analyses revealed significant advances in analytical technologies, particularly LC-MS/MS, alongside growing

international collaboration and methodological development. However, challenges remain, including the lack of standardized protocols, inconsistent quantitative correlations between insect and human tissue drug concentrations. Future research should focus on international standardization, multicenter validation studies, and the integration of emerging technologies such as artificial intelligence, machine learning, and omics approaches to improve the reliability, reproducibility, and forensic applicability of entomotoxicological evidence.

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