The Evolution of Research on Insect Antimicrobial Peptides: A Bibliometric Perspective

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Abstract. This bibliometric analysis delves into the evolution of research on insect antimicrobial peptides (AMPs), a crucial aspect of entomological studies with significant implications for medicine, agriculture, and biotechnology. The study systematically examines the publication trends, influential research articles, prolific authors, key journals, and collaborative networks that have shaped this research domain over the past decades. Utilizing advanced bibliometric tools and methodologies, we map the progression of scientific inquiry and identify pivotal moments and breakthroughs that have driven the field forward. The results indicate a remarkable growth in the insect AMPs from 1988 until 2024 globally. Our analysis highlights the growing interest and investment in understanding insect AMPs, underscored by an increase in interdisciplinary collaborations and innovative research approaches. By tracing the development and impact of this research area, we provide a comprehensive overview of the knowledge landscape, identify current research gaps, and suggest future directions for enhancing the application of insect AMPs in combating microbial resistance. This study serves as a valuable resource for researchers, policymakers, and practitioners aiming to leverage insect-derived antimicrobial peptides for addressing global health challenges. Nevertheless, the selected literature on published research analysed in the paper is obtained from a single database of Scopus only.

Keywords: Insect, antimicrobial peptide, bibliometric.

INTRODUCTION

The study of insect antimicrobial peptides (AMPs) has gained substantial attention in recent decades due to their potential as alternatives to traditional antibiotics, particularly in the face of rising antimicrobial resistance (AMR) [1], [2]. Insects, which represent the most diverse group of animals, produce a wide array of AMPs as part of their innate immune system, providing a rich source for novel antimicrobial agents [3], [4], [5]. This bibliometric analysis aims to trace the evolution of research on insect AMPs, mapping the trajectory of scientific inquiry and identifying critical issues that have shaped the field.

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The earliest studies laid the groundwork by identifying and characterizing the first insect AMPs, which sparked interest in the potential applications of these peptides. Subsequent research rapidly expanded, exploring the structure, function, and mechanisms of action of insect AMPs across various species. For example, Bulet et al. (1999) provided a comprehensive review of the diverse families of AMPs in insects, underscoring their broad-spectrum activity against bacteria, fungi, and even some viruses. This work has been heavily cited, reflecting its foundational role in consolidating knowledge about insect AMPs and stimulating further research.

Bibliometric analysis is a systematic and impartial method for analyzing literature, designed to assess a scientific field by providing researchers with a comprehensive view of the topic's historical development and key characteristics [6]. This method also enables researchers to delve into the intricate history of a discipline's evolution [7].

In this bibliometric analysis, we developed research questions that will be answered based on the evolutionary data of research related to insect AMPs. Here are the research questions:

RQ1: What is the publication trend of insect AMPs research?
RQ2: Who are the most active contributors to published insect AMPs research?
RQ3: Which insect AMPs papers are the most cited?
RQ4: Which countries are the most productive in insect AMPs publications?
RQ5. Which journals are the most important in insect AMPs publications?
RQ6. What is the pattern of collaboration and co-citation trends in insect AMPs publications?
RQ7. What are the most frequently used author keywords in insect AMPs studies?
RQ8: What is the literature clustering focus in insects AMPs publications?
RQ9: What is the research gap in insects AMPs publications?

Bibliometric analysis is a systematic and impartial method for analyzing literature, designed to assess a scientific field by providing researchers with a comprehensive view of the topic's historical development and key characteristics [6]. This method also enables researchers to delve into the intricate history of a discipline's evolution [7].

SUBJECT AND METHOD

The analysis was conducted by collecting all relevant documents from the Scopus database. The data search was based on article titles related to insect antimicrobial peptides, using the following keywords: "insect" AND "antimicrobial" AND "peptide*" OR "insect" AND "amp*" AND "insect" AND "host" AND "defense AND peptide*" OR "insect" AND "cecropin*" OR "defensin*" OR "attacin*" OR "diptericin*" OR "drosomycin*" OR "gloverin*" OR "moricin*" OR "lebocin*" OR "metchnikowin*" OR "hymenoptaecin*" OR "apidaecin*" OR "proline-rich" AND "peptide*" OR "anoplin*" OR "termicin*" OR "abaecin*" OR "bombinin*".

Data collection was carried out on July 30, 2024, resulting in 296 documents, comprising various types of publications such as books, original research articles, and others.

The dataset was then cleaned to improve accuracy by removing duplicates and articles not relevant to the analysis topic. Irrelevant entries were manually removed by reading the titles, abstracts, and keywords used. The results of the dataset cleaning were recorded using the PRISMA Flow Diagram 2020 [8]. The dataset was then analyzed to answer the research questions of this bibliometric analysis.

Performance analysis related to frequency was conducted using Harzing's Publish or Perish, and mapping analysis was conducted using Bibliometrix, which runs on R Studio software, version 2024.04.2 Build 764. Network analysis was performed using VOSviewer software, version 1.6.20.

RESULTS AND DISCUSSION

Descriptive Analysis

A total of 296 documents were obtained from the Scopus database on July 30, 2024. After cleaning the dataset, 117 documents were found to be relevant to the topic of this analysis. The cleaning process is illustrated in the PRISMA Flow Diagram in Figure 1.

The majority of the documents were articles (90; 76.92%) and reviews (19; 16.24%). Other types of documents, such as book chapters, conference papers, errata, and short surveys, accounted for only 8 documents or 6.84%. Detailed key information regarding the selected articles can be found in Table 1.

Table 1. Main information regarding selected articles.

Description	Results
Main Information About The Data	
Timespan	1988 to 2024
Total Sources (Journal, Books, etc.)	88
Documents	117
Citations	8123
Average Citations per Document (All Docs)	68.84
Average Citations per Year (All Docs)	225.64
Average Citations per Author (All Docs)	2206.58
h-index	44
g-index	89
hI, norm	21
hI, annual	0.58
hA-index	15
Document Types	
Article	90
Book Chapter	4
Conference Paper	1
Erratum	2
Review	19

Short Survey	1
Authors	
Total Author Appearances	495
Author of single-authored	3
International Co-Authorship	36.52%
Co-Author per Document	5.32
Language	
English	117

Publication Trend

Publications discussing insect AMPs have been ongoing from 1988 to the present, with at least one publication each year. This analysis can answer research question RQ1: What is the publication trend of insect AMPs research?. The annual publications form a trend that can be seen in Figure 2. This trend reflects the growing interest and increasing number of publications in the field [6], indicating significant developments and shifts in research focus over the past three decades.

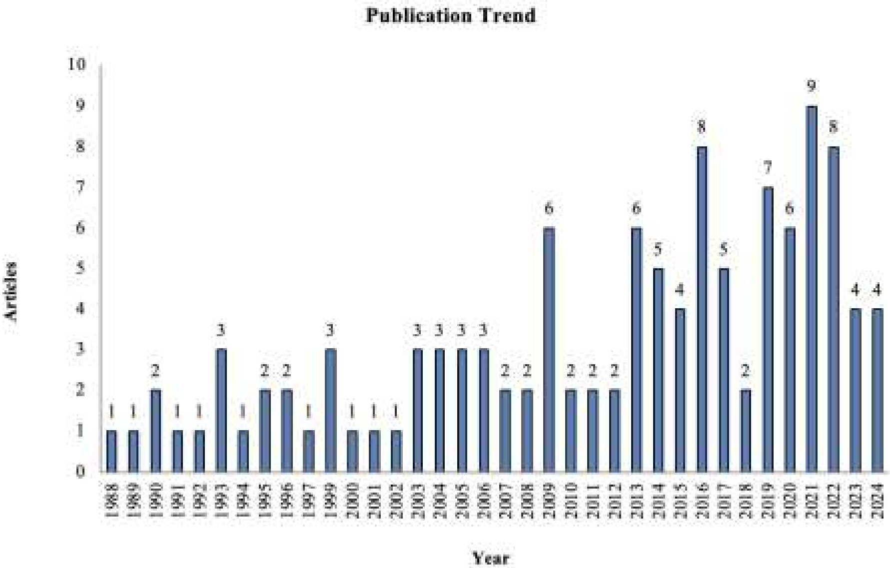

Fig. 1. Publication trend.

The publication activity from 1988 to 2000 shows a relatively low and inconsistent level of interest in the topic. The number of articles per year ranges from 1 to 3, with occasional years (1993 and 1999) showing a slight increase. This suggests that the research field was in its infancy, with limited focus or possibly constrained by the availability of technology or methodologies. This period likely represents the foundational phase of research in this field, where initial discoveries were made, but the scientific community had not yet fully recognized the potential of insect AMPs. The limited number of publications during this time may be attributed to the nascent state of molecular biology techniques and the early stages of interest in insect immune systems.

The period from 2009 to 2019 marks a significant surge in publications, with peaks in 2012 (6 articles), 2016 (8 articles), and 2020 (9 articles). This surge can be linked to several factors, including advancements in molecular biology and genomics, which provided researchers with better tools to study these peptides in detail. Additionally, the growing global concern over antibiotic resistance likely spurred interest in exploring alternative antimicrobial agents, including insect AMPs.

This trend provides a clear visual representation of the growing interest and expansion in the research field of insect antimicrobial peptides over the last few decades.

Most Productive Authors

RQ2: Who are the most active contributors to published insect AMPs research? entails multiple analyses focused on research productivity, including the examination of authors, geographical regions, and organizational affiliations.

Table 2 lists top ten most productive authors in insect AMPs field. The table highlights that the majority of the top 10 most productive authors in the field of insect AMPs are affiliated with French institutions, specifically the Institut de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique (IBMC-CNRS). Among the top contributors, P. Bulet leads with 12 papers (10.26%), followed by J.A. Hoffmann (8 papers, 6.84%), C. Hetru (7 papers, 5.98%), D. Hoffmann (6 papers, 5.13%), R. Hoffmann (5 papers, 4.27%), and J.L. Dimarcq (4 papers, 3.42%).

P. Bulet has made significant contributions to the understanding and characterization of insect AMPs. His research has been instrumental in advancing the knowledge of how insects employ AMPs as part of their innate immune system to combat microbial infections [1], [9], [10], [11]. This work has provided foundational insights that have influenced subsequent research in both basic and applied sciences, particularly in the development of novel antimicrobial agents.

A critical observation is that a significant portion of the top authors are affiliated with a single French institution, The Institut de Biologie Moléculaire et Cellulaire, part of the Centre National de la Recherche Scientifique (IBMC-CNRS), suggesting that this institution plays a central role in the research on insect AMPs. As one of the leading institutions in this field, IBMC-CNRS has been at the forefront of discovering and characterizing these peptides, particularly through the work of prominent researchers like P. Bulet and J.A. Hoffmann.

While the French researchers dominate the list, contributions from other countries are significant as well. A. Vilcinskas from Germany, affiliated with the Research Center for Biosystems, Land Use and Nutrition, and the Institute for Phytopathology and Applied Zoology at Justus Liebig University Giessen, ranks second with 10 papers (8.55%).

Another German researcher, M. Rahnamaeian, contributes 4 papers (3.42%) and is affiliated with the Fraunhofer Institute for Molecular Biology and Applied Ecology. M. Yamakawa from Japan also appears in the top 10, with 5 papers (4.27%), affiliated with the Laboratory Biological Defense, National Institute of Sericultural and Entomological Science.

Table 2. Top 10 most productive authors in insect AMPs.

Author	TP	Affiliation	Country
Bulet, P.	12	Institut de Biologie Moleculaire et Cellulaire, CNRS	France
Vilcinskas, A.	10	Research Center for Biosystems, Land Use and Nutrition, Institute for Phytopathology and Applied Zoology, Justus Liebig University Giessen	Germany
Hoffmann, J.A.	8	Institut de Biologie Moleculaire et Cellulaire, CNRS	France
Hetru, C.	7	Institut de Biologie Moleculaire et Cellulaire, CNRS	France
Hoffmann, D.	6	Institut de Biologie Moleculaire et Cellulaire, CNRS	France
Hoffmann, R.	5	Institut de Biologie Moleculaire e Cellulaire, CNRS	France
Yamakawa, M.	5	Laboratory Biological Defense, National Institute of Sericultural and Entomological Science	Japan
Rahnamaeian, M.	4	Department of Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology	Germany
Dimarcq , J.L.	4	Institut de Biologie Moleculaire et Cellulaire, CNRS	France
Cociancich, S.	4	Institut de Biologie Moleculaire et Cellulaire, CNRS	France

1.1 Most Cited Papers

The answer to *RQ3: Which insect AMPs papers are the most cited?* is provided in Table 3, showing that top 10 the most cited insect AMPs paper.

Table 3. Top 10 the most cited insect AMPs.

Source	Title	TC	C/Y
Bulet et al. (1999)	Antimicrobial peptides in insects; structure and function	870	34.8
Lehrer & Ganz (1999)	Antimicrobial peptides in mammalian and insect host defence	680	27.2
Yi et al. (2014)	Insect antimicrobial peptides and their applications	448	44.8
Bulet et al. (2005)	Insect antimicrobial peptides: Structures, properties and gene regulation	416	21.89
Wu et al. (2018)	Insect antimicrobial peptides, a mini review	331	55.17
Cociancich et al. (1993)	Insect defensin, an inducible antibacterial peptide	296	9.55

Fehlbaum et al. (1996)	Structure-activity analysis of thanatin, a 21-residue inducible insect defence peptide with sequence homology to frog skin antimicrobial peptides	274	9.79
Login et al. (2019)	Antimicrobial peptides keep insect endosymbionts under control	271	20.85
Steiner et al. (1988)	Binding and action of cecropin and cecropin analogues: Antibacterial peptides from insects	269	7.47
Hoffmann et al. (1992)	Insect defensins: inducible antibacterial peptides	257	8.03

The most cited paper with 870 citations and 34.80 cited/year, was authored by Bulet et al. and published in 1999 under the title "Antimicrobial peptides in insects". This paper is the most cited in the field, reflecting its seminal role in establishing the importance of AMPs in insects. The high citation count indicates that it has been foundational in the study of insect immunity and has likely influenced a broad range of subsequent research [1].

The second most cited paper is "Antimicrobial peptides in mammalian and insect host defence" by Lehrer and Ganz, published in 1999, which has been cited 680 times, with an average of 27.2 citations per year. This paper, which compares AMPs in both mammalian and insect systems, is highly cited, indicating its relevance to a broader audience beyond just entomologists. The cross-kingdom comparison has likely provided valuable insights into the evolutionary conservation and diversification of AMPs [12].

Another highly cited paper is by Yi et al., published in 2014, titled "Insect antimicrobial peptides and their applications." This paper has been cited 448 times, with an average of 44.8 citations per year. With a relatively recent publication date and a high citation rate per year, this paper highlights the practical applications of insect AMPs. Its focus on applications suggests it has been influential not only in academic circles but also in industrial and applied research. The issue that could arise is the risk of overemphasizing applications at the expense of fundamental research, which is essential for long-term innovation [4].

Most Productive Countries

The top 10 most productive countries can be found in Table 4. Based on the analysis, the answer to *RQ4: Which countries are the most productive in insect AMPs publications?* can be determined.

Table 4. Top 10 the most productive countries in insect AMPs.

Country	Articles	SCP	MCP
Germany	17	8	9
China	12	7	5
USA	9	5	4
Korea	8	6	2
France	7	1	6

Japan	6	4	2
India	5	5	0
Italy	4	2	2
Poland	3	2	1
Switzerland	3	2	1

The table provided illustrates the top 10 most productive countries in the field of insect antimicrobial peptides (AMPs), with a distinction between single-country publications (SCP) and multiple-country publications (MCP). This visualization offers insights into both the volume of research output and the level of international collaboration in this specific field of study.

Germany emerges as the most productive country in insect AMPs research, with a total of 17 publications. The nearly equal distribution between SCPs and MCPs (8 and 9, respectively) indicates that German researchers are not only highly productive but also actively engaged in international collaborations. This balanced approach enhances both the depth and breadth of research, incorporating diverse perspectives and methodologies from international partners.

China and the USA follow Germany in terms of productivity, with China slightly ahead. China's strong emphasis on SCPs suggests a robust internal research capacity, while the significant number of MCPs points to China's increasing role in global research networks. The USA shows a similar pattern, with slightly more SCPs, indicating strong domestic research efforts complemented by substantial international collaboration.

The data underscores the importance of international collaboration in advancing research in insect AMPs. Countries with higher numbers of MCPs, such as Germany, France, and China, tend to have a more significant presence in the field, which may be attributed to the diversity of ideas and methodologies that such collaborations foster.

The predominance of SCPs in certain countries, such as Korea and India, suggests a strong domestic research capacity. However, the lack of MCPs might limit the global impact of their research. Increasing international collaboration could be beneficial in integrating these countries more fully into the global research community.

Most Publication Journal

Table 5 lists the top 10 most prolific journals publishing research on insect antimicrobial peptides (AMPs), reflecting the distribution of 117 total papers across various scientific publications. This analysis offers insight into the journals that are most influential in disseminating research in this specific field, as well as the breadth of scientific discourse on insect AMPs. This analysis will answer the question RQ5: Which journals are the most important in insect AMPs publications?.

The distribution of articles across journals with different focus areas ranging from molecular sciences, biochemistry, and microbiology to applied biotechnology and immunology demonstrates the multidisciplinary nature of research on insect AMPs. This diversity reflects the broad applicability and relevance of AMPs across various fields of biological science.

International Journal of Molecular Sciences and Journal of Biological Chemistry are the leading journals, each contributing 5 articles to the research on insect AMPs. Both journals are well-respected in their respective fields. The International Journal of Molecular Sciences is known for its broad coverage of molecular biology, which suggests a strong molecular and biochemical focus in the research being published. The most cited article in this journal is the one discussing the therapeutic applications of insect cecropins, authored by Brady et al in 2019. Cecropins (Cecs) and their synthetic analogs are promising candidates for treating multi-drug resistant (MDR) infections due to their effective antimicrobial activity, low toxicity, and anti-inflammatory properties. Despite challenges such as production costs and the need for advanced delivery systems, these peptides could lead to new drug formulations and coatings for medical devices to combat infections [13]. Other articles published in this journal, such as those by Stączek et al. (2023) and Zhou et al. (2024), are review articles discussing the functions of AMPs in insects, including their broad spectrum of antibacterial, antifungal, and antiparasitic activities, various roles in the host, regulation of brain-controlled processes, maintenance of gut homeostasis, and the evolution of AMPs as antipathogens [5], [14].

The Journal of Biological Chemistry is a long-standing, highly cited journal that covers a wide range of topics in biochemistry, reflecting the biochemical mechanisms underlying AMP activity in insects. The prominence of these journals indicates that research in insect AMPs is deeply rooted in molecular and biochemical studies. The most cited article in this journal is the one authored by Cociancich et al. in 1993, which discusses defensins as antibacterials with a mechanism involving channel formation in the membrane. Insect defensins, cationic peptides found in various insect orders, disrupt the cytoplasmic membrane of Micrococcus luteus by forming channels that cause potassium loss, membrane depolarization, reduced ATP levels, and inhibited respiration. Patch-clamp experiments support the idea that these defensins create channels in the membrane, leading to these detrimental effects [15].

Table 5. Top 10 most publication journals.

Sources	Articles
International Journal of Molecular Sciences	17
Journal of Biological Chemistry	12
Antibiotics	9
Peptides	8
Philosophical Transactions of The Royal Society B: Biological Sciences	7
Analytical Biochemistry	6
Antimicrobial Agents and Chemotherapy	5
Applied Microbiology and Biotechnology	4
Developmental and Comparative Immunology	3
Embo Journal	3

Collaboration

The analysis was conducted to answer RQ6: What is the pattern of collaboration and co-citation trends in insect AMPs publications? This is important for understanding the collaboration patterns among authors on the topic of insect AMPs. A map visualization was created using network mapping with VOSViewer, employing the full counting method with a minimum of 1 document per author, resulting in 1093 out of 1093 meeting the threshold (Figure 2).

The network reveals distinct clusters of researchers, each representing groups of co-authors who frequently collaborate. The most prominent nodes in the network are P. Bulet, J.A. Hoffmann, and A. Vilcinskas, which are depicted as central figures within their respective clusters. Their prominence indicates that they are leading contributors to research on insect AMPs and have established extensive collaborative networks.

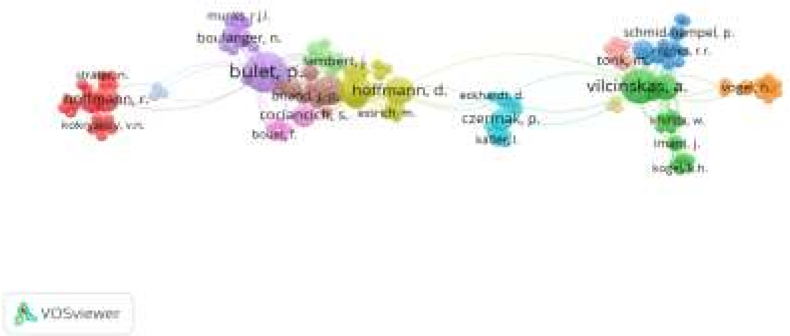

Fig. 2. Network visualization on co-citation based on author.

P. Bulet and J.A. Hoffmann are closely linked within the same cluster (yellow and purple), suggesting frequent collaboration. This is consistent with previous findings where both authors were noted as leading figures in this field, particularly within French research institutions. Both P. Bulet and J.A. Hoffmann are known for their foundational work in identifying and characterizing AMPs in insects, particularly focusing on their role in insect immunity [10]. P. Bulet and J.A. Hoffmann identified many AMPs and their roles such as in Droshopila, Coleopteran, Aeshna cyanea, termite, Heliothis virescens, Pyrrhocoris apterus, and Anopheles gambiae [15], [16], [17].

Vilcinskas appears as the central figure in a separate cluster (green), indicating that while he is a key contributor, his collaborative network is somewhat distinct from the one dominated by Bulet and Hoffmann. This separation might reflect differences in research focus, geographical location, or institutional affiliations. This cluster likely focuses on the applied aspects of insect AMPs and their potential for biotechnological applications [18], [19], [20]. Vilcinskas has published numerous articles discussing the medical applications of AMPs. The potential medical applications of AMPs include their use as alternatives to conventional antibiotics in ectopic therapies, their combined use with antibiotics to restore the susceptibility of multidrug-resistant pathogens, and their use as templates for the rational design of peptidomimetic drugs that overcome the disadvantages of therapeutic peptides [16]. The article written by Vilcinskas also proves that combinations of AMPs could be used therapeutically against Gram-negative bacterial pathogens that have acquired resistance to common antibiotics, in this case abaecin and hymenoptaecin [21].

The network shows some degree of interconnection between clusters, indicating that while certain groups of researchers work closely together, there is also cross-collaboration between these groups.

Most Frequent Keywords

Research Question 7*: "What are the most frequently used author keywords in insect AMP studies?"* was answered to identify the keywords used by authors in their publications related to insect AMPs. The top 10 keywords used can be seen in Table 6.

Table 6. Top 10 most frequently used keywords in insect AMPs studies.

Words	Occurences
Antimicrobial peptides	29
Insect immunity	13
Antimicrobial peptide	11
Insect	8
Insects	7
Cecropin	5
Apidaecin	4
Defensin	4
Defensins	4
Insect defensin	4

The most frequently used keyword is "antimicrobial peptides", which appears 29 times (24.79%). This is followed by related terms such as "antimicrobial peptide" (11 occurrences; 9.40%), indicating a strong emphasis on the general concept of AMPs within the research community. The keyword "insect immunity" appears 13 times (11.11%), making it the second most frequent term after "antimicrobial peptides". The prominence of this keyword suggests that studies often explore how these peptides contribute to the insect's defense mechanisms against pathogens, thus positioning AMPs as critical components of insect immunity.

Keywords such as "cecropin" (5 occurrences; 4.27%), "apidaecin" (4 occurrences; 3.418%), and "defensin" (4 occurrences; 3.418%) indicate that specific types of AMPs are frequently studied and discussed. The mention of these specific peptides points to a focused interest in certain AMPs that may be particularly effective or widely studied. For instance, cecropins and defensins are well-known AMPs with strong antimicrobial properties, making them popular subjects for research.

Clustering Focus

Clustering analysis was performed using the full counting method, with a minimum occurrence threshold of 5 terms, resulting in 157 documents that met the criteria. This analysis answers *RQ8: "What are the main clustering focuses in insect AMP literature?"*

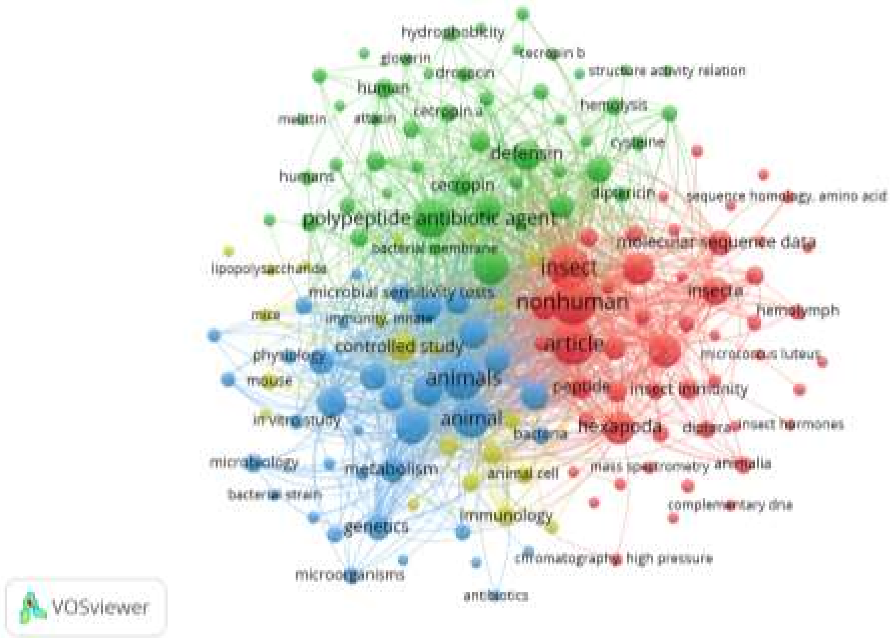

Fig. 3. Network visualization on themes in insect AMPs.

Figure 3 shows a visualization map of the analysis results. The network is divided into four distinct clusters, each representing a different thematic focus within the research landscape.

The red cluster is genetic and molecular insights into insect AMPs cluster. This cluster is heavily focused on the genetic and molecular aspects of insect AMPs. It includes keywords such as "insect," "nonhuman," "molecular sequence data," and "insect immunity." This cluster indicates a strong emphasis on understanding the genetic foundations and molecular mechanisms that underlie the production and function of AMPs in insects. Studies here likely explore how AMPs are encoded within insect genomes, how they are regulated, and how they interact with pathogens at a molecular level. The research in this cluster is fundamental for identifying new AMPs and understanding their evolutionary development across different insect species.

The green is functional characterization of AMPs cluster. focuses on the functional properties of AMPs, with key terms such as "polypeptide antibiotic agent," "defensin," "cecropin," and "structure activity relation." This cluster is concerned with the detailed biochemical and biophysical characterization of AMPs. It highlights how specific AMPs, such as defensins and cecropins, function as antimicrobial agents by interacting with bacterial membranes and disrupting microbial integrity. Studies in this cluster are crucial for understanding how AMPs work at a structural level, which is essential for designing synthetic peptides or improving natural ones for therapeutic use.

The blue is comparative and cross-species studies cluster. It represents research that places AMPs within a broader biological context, incorporating terms like "animals," "animal," "metabolism," "genetics," and "microbiology."

The yellow is experimental approaches and applications. The cluster is associated with experimental and methodological aspects of AMP research, featuring terms like "controlled study," "in vitro study," "microbial sensitivity tests," and "mice." This cluster underscores the importance of rigorous experimental designs and methodologies in studying AMPs. It includes both in vitro and in vivo studies that test the efficacy of AMPs against different microbial strains, using various model organisms, including mammals. Research in this cluster is critical for translating fundamental discoveries about AMPs into practical applications, such as developing new antimicrobial drugs or treatments for infectious diseases.

Evolution of Research Topic

The co-occurrence network analysis image generated using VOSviewer provides a detailed visualization of the evolution of research topics related to insect AMPs from 1988 to 2024. The color gradient, ranging from blue (older research) to yellow (more recent research), illustrates how the focus of research has shifted over time (Figure 4).

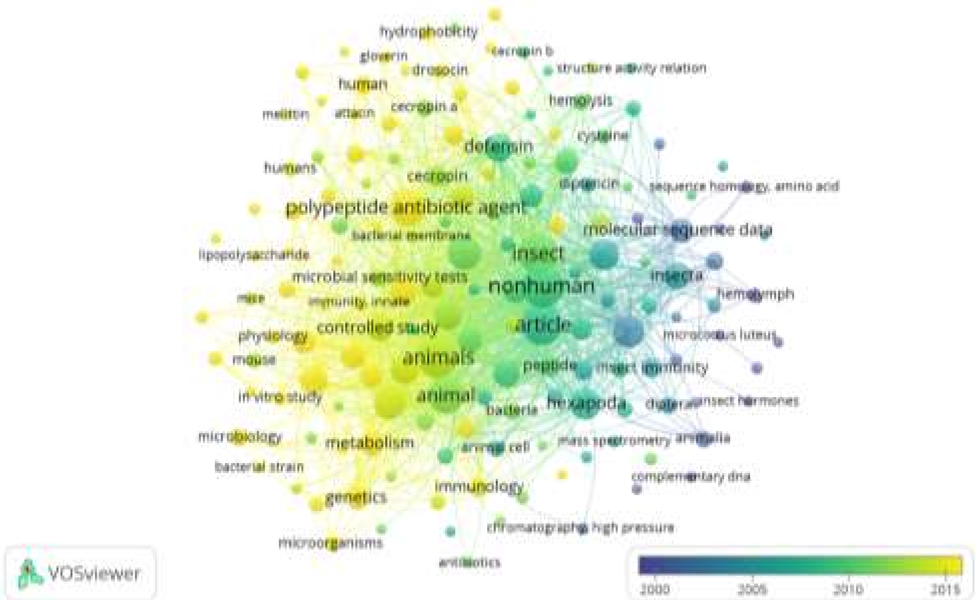

Fig. 4. Overlay visualization on themes in insect AMPs.

Initially, from 1988 to around 2000, the research was predominantly centered on the discovery and basic characterization of AMPs. During this period, foundational studies aimed at identifying these peptides, understanding their molecular sequences, and exploring their basic immune functions in insects [1], [12], [15], [16], [22], [23]. Techniques such as high-pressure chromatography and complementary DNA sequencing were crucial in these early efforts, helping to lay the groundwork for understanding how AMPs contribute to insect immunity.

As research progressed into the early 2000s through 2010, there was a noticeable shift toward more detailed mechanistic and structural studies. Researchers began to focus on how specific structural features of AMPs, such as hydrophobicity and amphipathicity, influenced their ability to disrupt microbial membranes. This period saw an emphasis on structure-activity relationships, where studies linked the physical characteristics of AMPs to their biological functions [24], [25]. Concurrently, there was a growing interest in microbial sensitivity tests, which were used to assess the efficacy of AMPs against various pathogens [3], [21], [26], [27], [28], [29], [30], providing insights critical for potential therapeutic applications.

From 2010 onward, the research began to adopt a more application-oriented and interdisciplinary approach. Recent studies have increasingly explored the use of AMPs as polypeptide antibiotic agents, highlighting their potential in medicine, agriculture, and biotechnology [2], [14], [31], [32], [33], [34]. There has also been a growing interest in the interplay between AMPs and host physiology, including metabolism and immune regulation. This reflects a broader understanding of how these peptides function within complex biological systems, rather than merely as isolated antimicrobial agents. Additionally, the integration of genetics into AMP research has opened up new avenues for exploring how these peptides are regulated and how their production can be enhanced or manipulated.

Looking forward, emerging trends suggest that future research might expand beyond traditional antimicrobial roles for AMPs. Potential areas of growth include immune modulation, where AMPs could be used to influence host immune responses, and biotechnological applications in non-medical fields, such as pest control or environmental management. Overall, the evolution of research on insect AMPs reflects a trajectory from basic discovery to sophisticated applications, with an increasing emphasis on understanding these peptides within broader biological and practical contexts. This trend suggests that AMPs will continue to be a vital area of research with significant implications for various scientific and industrial fields.

Research Gap and Future Direction

The density analysis of insect AMPs was conducted to identifying research gaps by highlighting areas that are well-explored and those that may require further investigation. This is crucial for developing a comprehensive understanding of the topic of insect AMPs. This analysis answered RQ9: "What is the research gap in insect AMP publications?"

Figure 5 is a heatmap-style co-occurrence network analysis generated using VOSviewer. Terms located on the periphery might represent underdeveloped or niche topics that could be significant areas for future investigation.

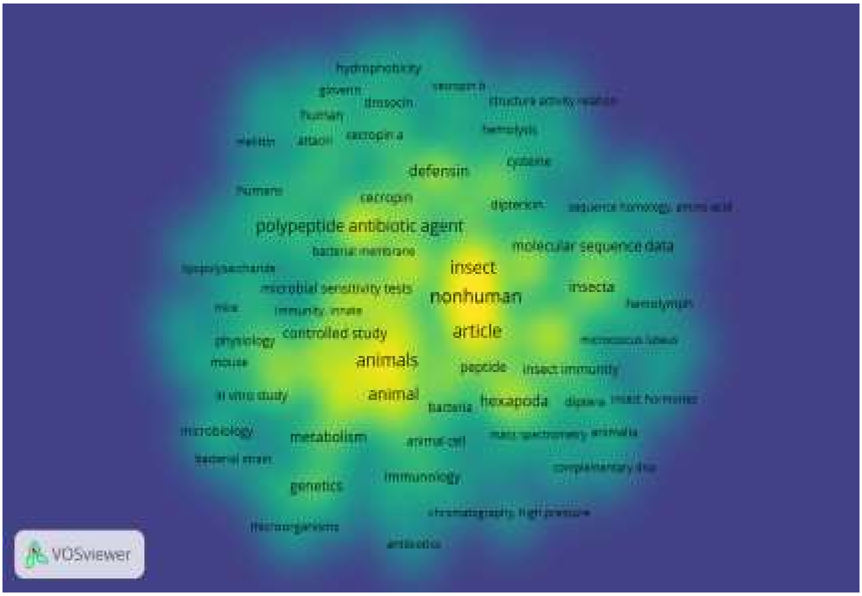

Fig. 5. Density visualization on themes in insect AMPs.

Terms related to "structure activity relation" and "hydrophobicity" are present but less central in the network. This may indicate that while structural studies are conducted, they might not be as integrated into the broader research narrative. There may be a need for more detailed structural biology studies that link specific AMP structures to their functional activities. Understanding how the structure of AMPs determines their mechanism of action, stability, and specificity could be critical for designing synthetic analogs or enhancing their efficacy in applied settings. Additionally, investigating the hemolytic potential of AMPs to ensure their safety for therapeutic use, and studying cysteine's role in the stability and function of AMPs.

Specific AMPs such as “gloverin” and “drosocin”, which are not as widely studied as more common peptides like “defensins” and “cecropins”, also represent potential areas for deeper investigation. Research into these lesser-known peptides could uncover unique properties or applications that have been overlooked.

Interactions between AMPs and microbial components like lipopolysaccharides (LPS) are essential for understanding their role in targeting Gram-negative bacteria, but this aspect may be underrepresented compared to general studies on AMPs. There could be an opportunity to explore these interactions in greater detail, particularly in how AMPs contribute to the recognition and neutralization of pathogens during the innate immune response.

The term "insect hormones" represents a potentially rich but underexplored area in the study of insect AMPs. By examining how insect hormones influence AMP expression and function, researchers could uncover new layers of complexity in insect immunity and potentially develop innovative applications. This area of research could significantly enhance our understanding of the physiological integration of immune responses in insects and expand the potential uses of AMPs in both scientific and practical contexts.

There may be insufficient focus on how AMPs influence or are influenced by the host's overall physiology and metabolic state. Investigating how different physiological conditions or metabolic disorders affect AMP production and activity could reveal important insights into the conditions under which AMPs are most effective or how they might be leveraged in therapeutic contexts.

The use of advanced analytical techniques like high-pressure chromatography may not be as central in the current literature. However, it could play a crucial role in the detailed characterization of AMPs, particularly in identifying new peptides, understanding their purity and stability, and optimizing their production for research and therapeutic use.

While microbial sensitivity tests are a key part of applied AMP research, there is room to expand these studies to include a wider range of pathogens, particularly those that are resistant to traditional antibiotics. Addressing these gaps could significantly advance our understanding of AMPs, leading to more effective applications in medicine, agriculture, and biotechnology.

CONCLUSIONS

In this bibliometric analysis, we have traced the evolution of research on insect antimicrobial peptides (AMPs) from its inception in the late 1980s to the present day, providing a detailed perspective on how the field has developed and where it is heading. The study reveals a clear progression from early foundational work focused on the discovery and characterization of these peptides, to more sophisticated mechanistic and structural analyses, and finally to application-oriented research with broad interdisciplinary implications.

As the field matured, researchers began to explore the specific interactions between AMPs and microbial membranes, delving into structure-activity relationships that linked physical properties of AMPs to their biological functions. This phase of research was essential for advancing our understanding of how these peptides can be harnessed for therapeutic purposes. In recent years, the focus has shifted toward application-driven studies, with an increasing emphasis on the use of AMPs in medicine, agriculture, and biotechnology. The integration of genetic and immunological insights has further enriched our understanding of AMP regulation and their broader biological roles.

Looking ahead, the field of insect AMPs is poised for further expansion, with potential applications extending beyond traditional antimicrobial uses. Future research is likely to explore innovative roles for AMPs in immune modulation and biotechnological advancements, while also addressing challenges such as AMP resistance and optimizing their use in various settings. This analysis underscores the dynamic nature of AMP research and highlights its growing significance in both scientific inquiry and practical applications.

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