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BRAZILIAN RESEARCH ON THE TOXICOLOGY OF PESTICIDES IN ZEBRAFISH (Danio *Rerio*): A SCIENTOMETRIC PERSPECTIVE OF GRADUATE STUDIES

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ABSTRACT

The use of pesticides for pest management dates back to ancient times, and with agricultural expansion, their use remains inevitable. Despite this, in recent decades, the toxic effects of pesticides on the environment and non-target organisms have become a significant global concern. In this context, this scientometric review maps the Brazilian scientific production in graduate programs on pesticide toxicology in the zebrafish animal model (*Danio rerio*), using descriptive statistics to identify trends and gaps. Based on works indexed in the Brazilian Digital Library of Theses and Dissertations, resulting from the search with the keywords (Zebrafish OR zebrafish OR *Danio rerio* OR zebrafish) AND (pesticides OR herbicides OR fungicides OR pesticides), 57 studies (2005–2024) were selected. From 2005, the year of the first selected study, to 2024, there has been a growth in scientific production related to the topic. This growth reflects CAPES policies, which aim to strengthen inter-regional research networks and foster collaboration between institutions and researchers. In addition, this study highlights the Brazilian regions and authors with significant contributions in the area. Knowledge about different parameters promotes collaborations and advances in the understanding of the toxic effects of pesticides currently in use.

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Keywords: Bibliometric analysis. Brazilian post-graduation. Environmental contamination. Brazilian research.

INTRODUCTION

The use of substances to protect crops against pests has ancient origins. Initially, these substances were composed of natural products, such as sulfur, vegetable and mineral oils, or ash. Over time, several compounds with different toxicities began to be used, often without concern for their effects. Scientific advances led to the development of the first synthetic pesticides, such as Bordeaux mixture and arsenic-based compounds, followed by organic compounds, such as Dichloro-Diphenyl-Trichloroethane (DDT) (Gerber et al., 2021).

Brazil, as a tropical country with a vast territorial extension, has favorable conditions for large-scale agricultural activities, which led the country to become the world's largest consumer of pesticides in 2008. Currently, the country still accounts for approximately 20% of global pesticide consumption (Sanchez et al., 2017). Only in the last few decades, concern about environmental impacts and toxic effects on health and non-target organisms, such as the animals that make up the surrounding ecosystems, has gained importance in scientific research. Since then, the search for knowledge about the toxicology of pesticides has become essential to prevent environmental impacts and maintain the health of ecosystems (Pathak et al., 2022).

Despite the risks, pesticide use remains essential for agriculture, as it allows for the control of pests, diseases, and weeds that could otherwise significantly devastate crops. With the growth of the global population, the demand for food has also increased. In this context, pesticides enable large-scale production, ensuring global food security—a feat that would not be achievable without their use (Leskovac et al., 2023). However, despite their necessity, pesticides bring several environmental and health complications, especially when used inappropriately (Damalas et al., 2011; Marins et al., 2021). Although research on substances used as pesticides has advanced in recent decades, legislation often does not keep pace with scientific discoveries. Consequently, combined with inappropriate use, the adverse effects on non-target organisms have become increasingly significant, showing, in some cases, the bioaccumulation of pesticides (Clasen et al., 2018; Khoshnood et al., 2023; Kumar et al., 2023).

Given this scenario, research has used bioindicator organisms to evaluate the toxic effects of various substances used as pesticides. Among these organisms, zebrafish (*Danio rerio*) stands out as a model for testing biomarkers and verifying the toxicity of pesticides. The zebrafish genome was fully sequenced in 2013, revealing that approximately 70% of its genes have human equivalents (Howe et al., 2013; Guerra et al., 2021). In addition, zebrafish can bioaccumulate toxic substances even at low concentrations and is

susceptible to mutations. For these reasons, this model has been increasingly used in the development of toxicological studies involving pesticides (Bambino et al., 2017; Guerra et al., 2021).

Thus, this scientometric review aims to map Brazilian graduate studies involving the toxicology of pesticides (fungicides, herbicides and insecticides) and the *Danio rerio* model. This study will provide insights into emerging trends, leading research regions in the field, most studied substances, applications of the zebrafish model, and potential gaps in existing knowledge.

METHODOLOGY

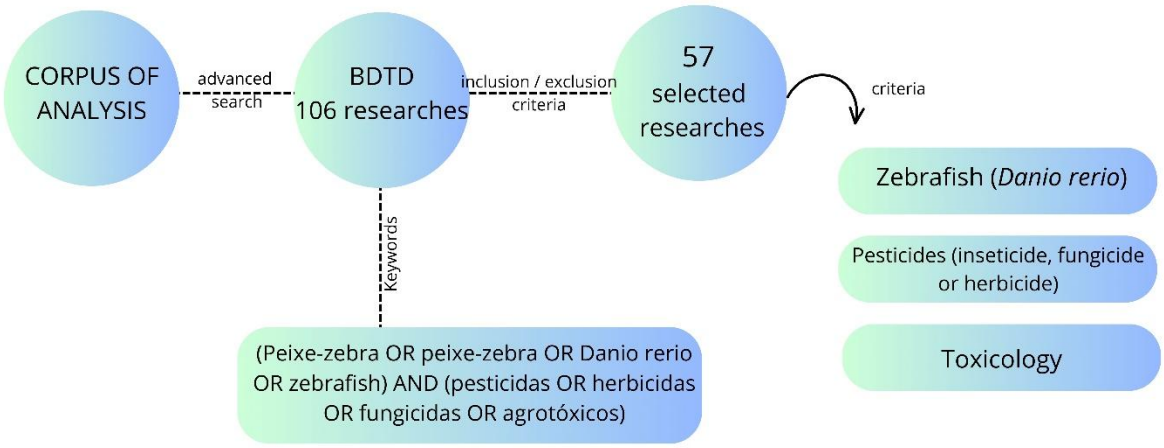
RESEARCH SOURCES, IDENTIFICATION AND INCLUSION CRITERIA OF STUDIES

This study consists of a bibliographic research with a scientometric approach, characterized by a descriptive design and qualitative-quantitative nature. Scientometrics is a branch of science that allows us to understand trends and gaps in scientific production through indicators of production, authorship, among others (Spinak, 1998). In this sense, a search was carried out in the Brazilian Digital Library of Theses and Dissertations (BDTD) to identify studies that investigate the toxicology of pesticides using zebrafish as an animal model. The following search terms were used: (*Zebrafish OR zebrafish OR Danio rerio OR zebrafish) AND (pesticides OR herbicides OR fungicides OR insecticides OR pesticides*).

The search was carried out without a predefined start date, with keywords searched in Portuguese and partially in English. The search was conducted on October 2, 2024, and returned 106 studies. The selection of studies followed the previously established exclusion criteria, which were: 1) Use of pesticides (herbicides, fungicides or insecticides); 2) Zebrafish animal model (*Danio rerio*); 3) Involvement with pesticide toxicology. After analysis, the following studies were excluded: 22 studies because they did not use any type of pesticide; 14 studies for not involving the species *D. rerio*; 3 studies for not using even the species *Danio rerio* nor pesticides; 10 studies for not addressing pesticide toxicology.

Only studies that met the criteria mentioned above and directly addressed pesticide toxicity in zebrafish (*D. rerio*) were selected. After applying the exclusion criteria, the corpus of analysis was established, resulting in 57 studies included in this review, as illustrated in the flowchart below (Figure 1).

Figure 1 - Flowchart of the corpus of the scientometric analysis.



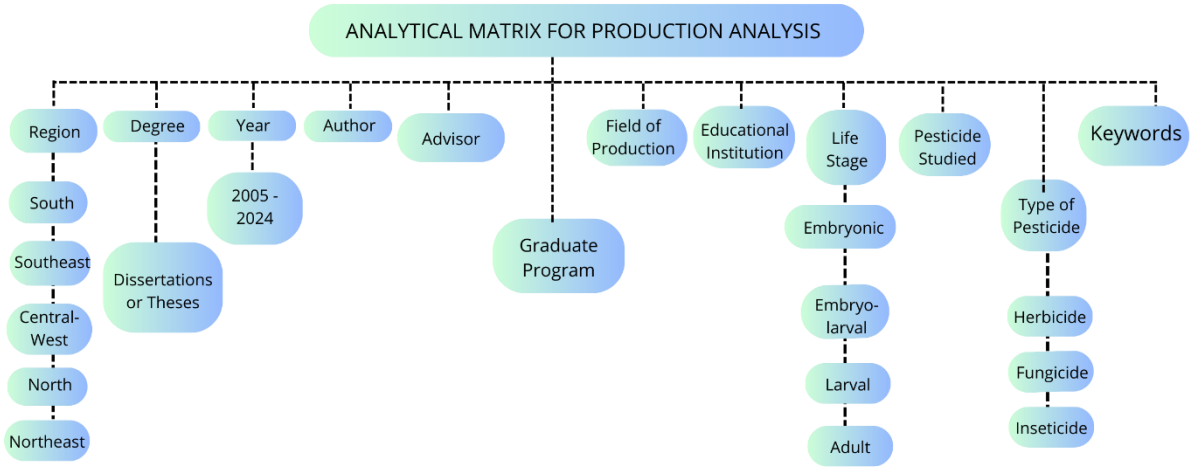
Legend: BDTD (Digital Library of Theses and Dissertations).

DATA EXTRACTION

Microsoft Excel 365® was used to compile the database of the theses and dissertations included, as well as to create tables and figures. All search results considered were tabulated in a digital spreadsheet.

The dataset was structured to include the following information: Title, Year, Author, Type of Work, Advisor, Graduate Program, Area of Study, Regional Distribution, Institution, Keywords, Zebrafish Life Stage, Type of Pesticide Used, and Pesticide Name, as illustrated in Figure 2.

Figure 2 - Categorization of the content of academic production: Analytical matrix of productions.



DATA ANALYSIS

The data were obtained, collected, organized and analyzed based on descriptive statistics, which aim to infer the behavior of the population through the construction of tables, graphs and numerical indicators (Farias et al., 2006). The results were quantified and discussed with the aid of visual representations.

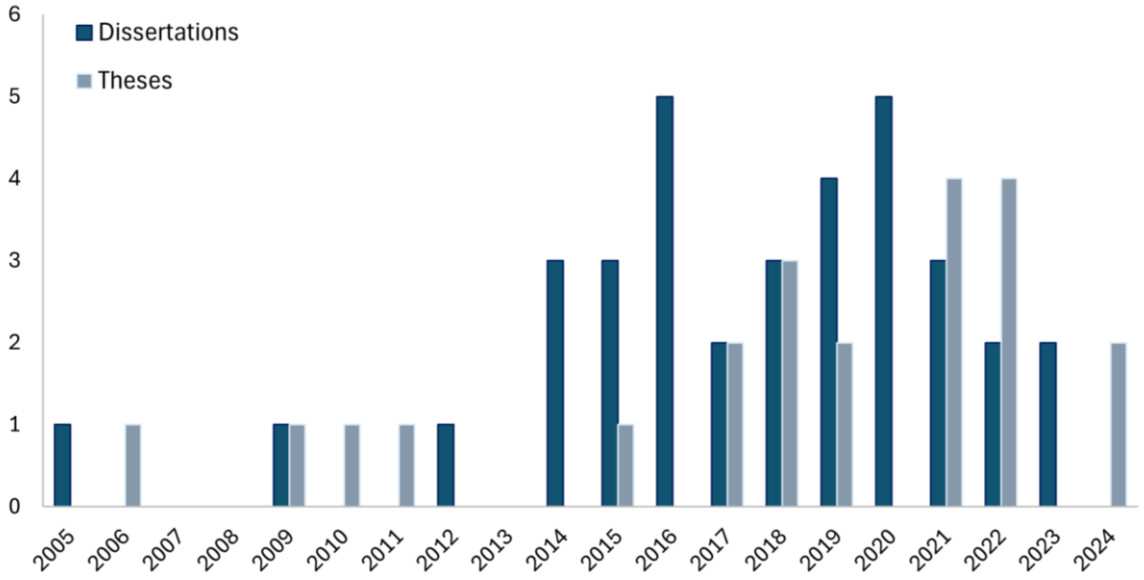
RESULTS AND DISCUSSIONS

Scientometric analysis allows you to understand how research advances in a specific area. This study focuses on the field of pesticide toxicology, with an emphasis on herbicides, insecticides and fungicides that affect the species D. rerio. Based on the previously established quali-quantitative criteria, the results of the analysis are presented below.

After the relevance analysis, 57 studies were selected. Among these, most of the productions correspond to dissertations, representing 61% (35) of the total, while 39% (22) are theses. As expected, the number of theses is lower than that of dissertations, since the time and depth required for doctoral research (48 months) are significantly higher than for master's research (24 months).

Based on this initial analysis, it is also possible to establish an overview of the growth in the number of dissertations and theses on the topic analyzed over the years, as illustrated in Figure 3.

Figure 3 - Distribution of Dissertations and Theses by Year.



Source: derived from the theses and dissertations selected in this review.

As observed in Figure 3, the first research on the subject of this review dates from 2005 in the BDTD database. Although no specific time limit has been established, the technology allows access to relatively recent studies on this search platform. This is because many dissertations and theses prior to the year 2000 may not have been included in the platform due to technological limitations at the time.

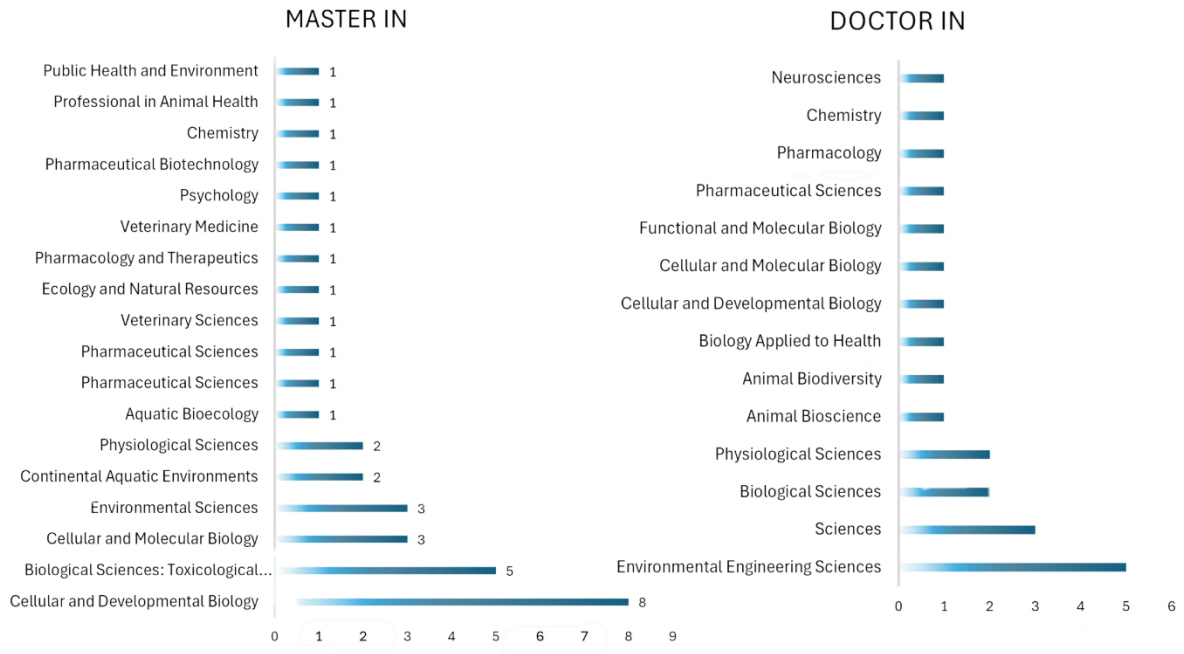
Despite the growth in the number of dissertations and theses, there is a more significant increase in the number of theses. This growth reflects the maturation of scientific research in Brazil, encouraged by CAPES through its various programs. The emphasis on

doctoral research is relevant as it is expected to produce discoveries of greater impact, due to the requirement for originality, driving scientific advancement.

There is a clear trend of growth in this area of research, especially in the last decade (Chelinho et al., 2019; Brühl et al., 2019; Cezarette et al., 2024; EEA, 2023). In 2021, the year with the highest number of studies in the area, seven graduate studies were developed on this topic. With the growth and incentives for graduate studies, it is evident that the theme of this review stands out and is expanding rapidly. This increase in research is critical to understanding how pesticide use affects non-target organisms and to establishing safer limits for their application. In this way, these studies make it possible to improve the current legislation, which is often inadequate, contributing to the prevention of damage to non-target species and to the strengthening of environmental preservation related to the use of these toxic compounds.

With the growing relevance of pesticide toxicology in *D. rerio*, more graduate programs (PG) have started to incorporate this theme in their research. In addition, the areas that develop studies on the subject have expanded, resulting in a greater number of degrees associated with this line of research. Being a multidisciplinary topic, it can be integrated into different scientific approaches. Thus, a qualitative-quantitative analysis of the types of titrations offered by PGs was performed, as illustrated in Figure 4.

Figure 4 – Quantification of the titles awarded by the Graduate Programs in research on the toxicology of pesticides in zebrafish (*Danio rerio*).

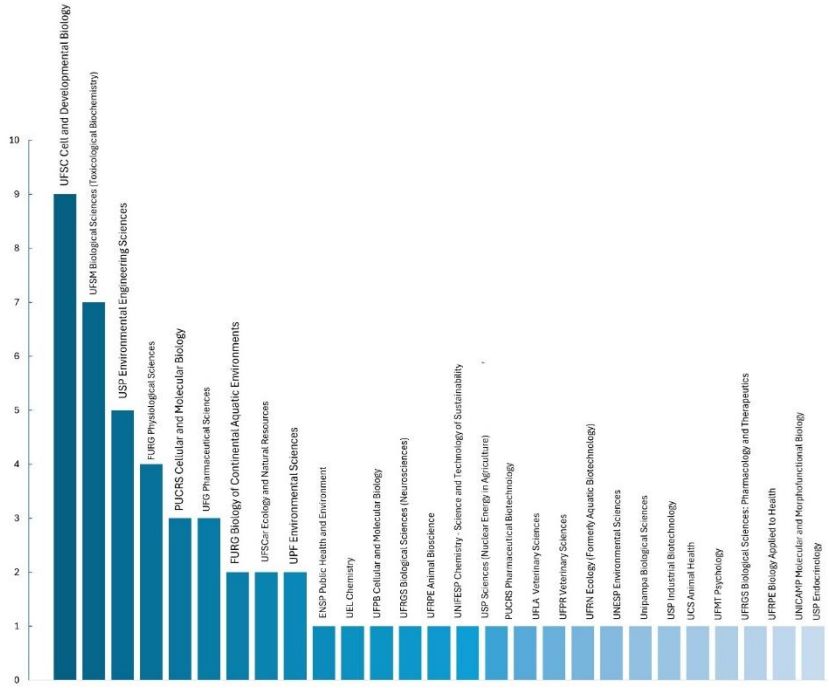


Source: derived from the theses and dissertations selected in this review.

The diversity of titles involving the research theme of this review is remarkable, as shown in Figure 4. These adaptations by higher education institutions reflect the demands associated with the scientific evolution of the area, leading to an increase in the production of research (Sposito et al., 2018; Gonçalves et al., 2020; Guerra et al., 2021). Another explanation lies in the diversity of programs that incorporate this theme, since it is highly relevant today and covers aspects of several areas in its development. Interdisciplinarity not only enriches academic training, but also contributes to the search for more integrated and effective solutions. As areas such as Public Health and Environmental Engineering become more involved with pesticide research, innovation and application of the results are expanded.

Likewise, a qualitative-quantitative analysis of the institutions and graduate programs (PPGs) responsible for these studies was carried out, as illustrated in Figure 5. The institution with the PPG that contributed the most to studies in the area of pesticide toxicology in *D. rerio* was the Federal University of Santa Catarina (UFSC). The quantification revealed that UFSC produced a total of nine studies at the graduate level through its Graduate Program in Cell and Developmental Biology. Another notable contributor is the PPG in Biological Sciences (Toxicological Biochemistry) at the Federal University of Santa Maria (UFSM), which produced a total of seven studies. It is evident that other graduate programs began to be inserted in research on pesticide toxicology in *D. rerio*.

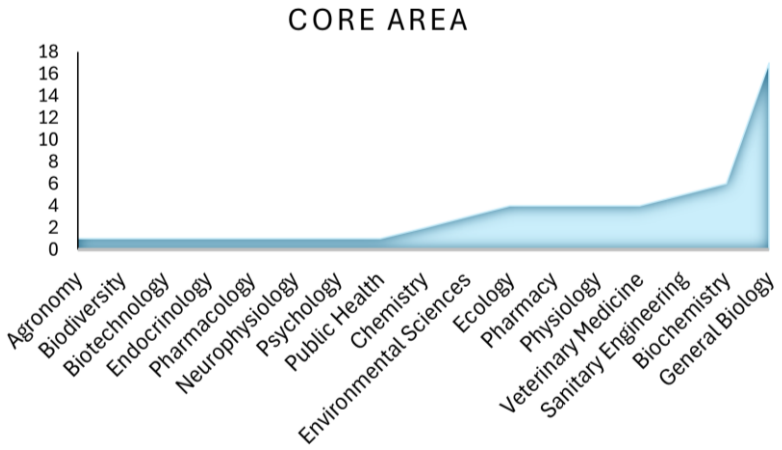
Figure 5 - Quantification of Graduate Programs and their respective institutions.



Source: *Sucupira* Platform - *CAPES.*

In addition, the areas of knowledge of each PPG listed in the Sucupira Platform, which hosts the dissertations and theses reviewed in this study, were analyzed. This analysis provided a more specific view of these areas to understand the interests of the scientific community in relation to pesticide toxicology in *Danio rerio*. The quantified data are presented in Figure 6.

Figure 6 - Distribution of the main areas of theses and dissertations.

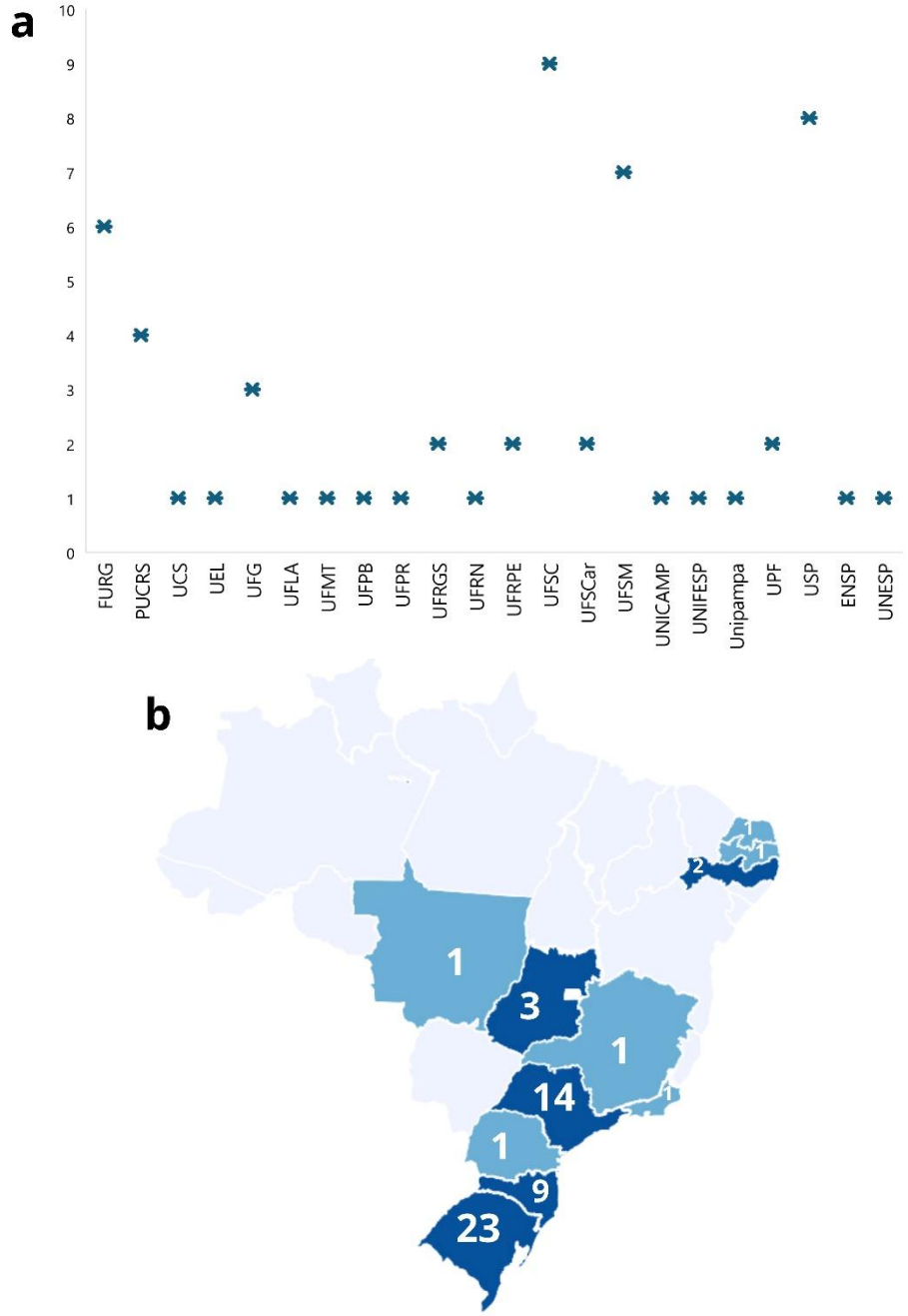


Source: *Sucupira* Platform - *CAPES.*

The main area that predominates is General Biology. Although the topic of pesticide toxicology in *D. rerio* can encompass several fields of study for its development (biology, chemistry, agronomy, environmental engineering, among others), its basis remains Biology in terms of content. However, other areas have also started to incorporate it, such as Biochemistry (6 papers) and Sanitary Engineering (5 papers), due to the development opportunities that the theme provides.

Based on the data, an analysis was carried out to identify which institutions are most active in research on pesticide toxicology in *D. rerio* and which regions are conducting more studies in this area. This allows mapping research niches in the country and emerging areas. The mapped institutions and regions can be seen in Figures 7a and 7b.

Figure 7 - a. Institutions of origin of the dissertations and theses; **b.** Distribution of research by state in Brazil.



Source: data obtained from the theses and dissertations selected in this review.

CAPES has invested in the decentralization of research in Brazil, aiming to balance investments to promote scientific development. As a result, the trend is for regions historically underrepresented in the survey, such as the Northeast, North and Midwest, to show growth. As shown in Figure 7.B, the Northeast and Midwest regions are increasingly promoting research involving the toxicology of pesticides in *D. rerio* within their graduate programs. Although most research in this area is still concentrated in the South and Southeast regions of Brazil, the growth in other regions is evident through the inclusion of institutions in the Northeast and Midwest.

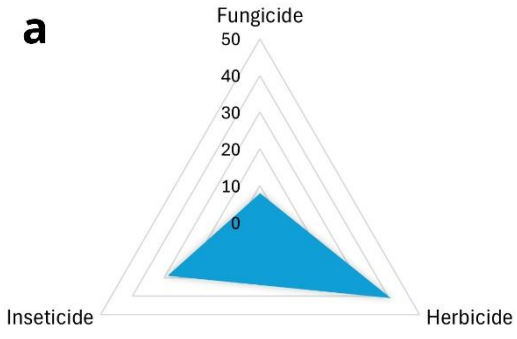
The progress highlighted in this review reflects CAPES' investments in stimulating

research hubs across the country and strengthening graduate programs in areas that previously had less infrastructure and financial support (Schwartzman, S., 2022). In addition, CAPES and other development agencies have invested in specific programs to support regions with fewer resources, such as the Graduate Development Program (PDPG), which seeks to improve training and academic production in these regions. These initiatives are helping to build a solid scientific base in the Northeast and Midwest, enabling the expansion of multidisciplinary topics such as pesticide toxicology, which can range from regional ecology to public health.

The growth of research in several regions of Brazil fosters the emergence of collaborative networks between universities in different locations. In this way, institutions in the North and Northeast could collaborate with universities in the South and Southeast, which have more advanced infrastructure, to carry out studies on the toxicity of pesticides in Amazonian or semi-arid environments, strengthening scientific production with different regional perspectives.

Continuing the discussions, another relevant data to understand the panorama of scientific production on the subject of this review is the qualitative and quantitative analysis of the pesticides researched. From the analysis of the included studies, the types (fungicide, insecticide or herbicide) and the specific names of the pesticides investigated were mapped. This analysis is illustrated in Figure 8 a; b.

Figure 8 - a. Quantification of the type of pesticide (fungicide, insecticide or herbicide) used in the theses and dissertations; **b.** Mapping of pesticides used in theses and dissertations. Legend: HB (herbicide-based); IN (insecticide-based); FB (fungicide-based).



Source: data obtained from the theses and dissertations selected in this review.

The analysis of the types and names of pesticides investigated in the studies indicates a predominance of herbicides, particularly glyphosate and 2,4-D. This prevalence is due to their extensive use in Brazilian agriculture and the growing concern about the environmental and toxicological impacts of these compounds. However, this concentration of research on herbicides also reveals gaps that may guide future studies. Although herbicides predominate, other types of pesticides, such as insecticides and fungicides, have been studied on a smaller scale, especially in aquatic organisms such as zebrafish. In the Brazilian context, where the use of insecticides is significant in agriculture, more in-depth studies on their toxic effects on non-target organisms could be valuable.

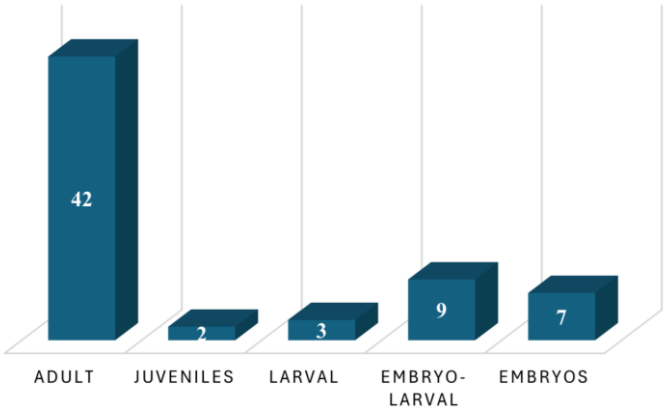
Another relevant aspect is the lack of studies on the combined effects of different types of pesticides, such as mixtures of herbicides and insecticides, which can interact synergistically and result in more pronounced toxic effects than those caused by each substance alone. This issue is particularly relevant in Brazilian agricultural environments, where the simultaneous application of multiple pesticides in the same area is common. Studies evaluating the toxicity of these mixtures in zebrafish or other model organisms could broaden the understanding of the ecotoxicological risks involved, providing critical data for better regulation and management of pesticides.

Based on the data in Figure 8.b, it was observed that the toxicological effects of glyphosate-based herbicides, its pure compound, and 2,4-dichlorophenoxyacetic acid are the most studied in graduate research. Despite their benefits in weed control, concerns have arisen regarding the toxicity of these compounds to the environment and non-target

species (Fiorino et al., 2018; Oliveira et al., 2024). As a result, these compounds have been highlighted in research with zebrafish, as they are an indirect target of their toxic effects and serve as a model with significant genomic similarity with humans (Lopes et al., 2018). From the results, it can be inferred that research on the chronic and long-term effects of compounds such as glyphosate and 2,4-D represent an area that could be further explored. Most studies focus on acute effects, but there is a need for studies examining prolonged exposure in aquatic organisms, which would better reflect scenarios of continued contamination in rivers and lakes near agricultural areas.

Another parameter of interest for quantification involving pesticide toxicology is the life stage of the model used, in this case, zebrafish. This model is notable for its rapid development and transparency during the embryo-larval stage, allowing the monitoring of morphological development. Thus, the studies were quantified based on the life stages used for toxicity tests: embryo, embryolarval, larval, juvenile, and adult, as shown in Figure 9. It can be inferred that most research is conducted using adult zebrafish. However, the use of embryos is expected to grow due to their advantages.

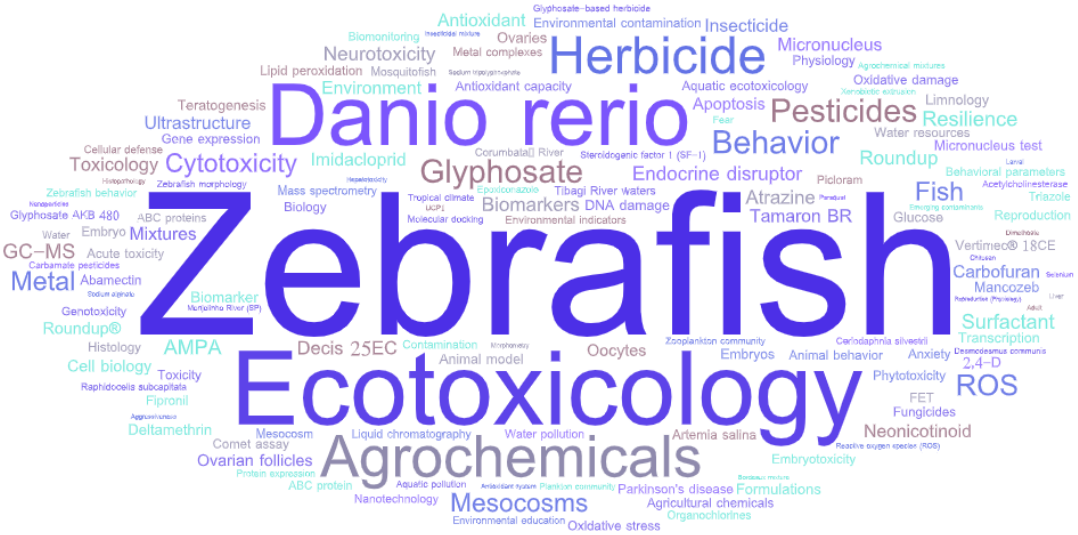
Figure 9 - Life stages of zebrafish (*Danio rerio*) used in research involving pesticide toxicology.



Source: data obtained from the theses and dissertations selected in this review.

One of the most important indicators present in the studies is keywords. A qualitative analysis of the keywords and their co-occurrence provides insights into the research topics and highlights trends (Zuanazzi et al., 2020). In this way, the keywords of the studies were analyzed, and a word cloud was created using the WordArt® program. Word clouds provide an overview of the key topics mentioned in studies, making it easier to understand large volumes of data. The word cloud can be seen in Figure 10.

Figure 10 - Word clouds of the keywords extracted from the dissertations and theses included in this review.



From the generated word cloud, it is possible to quickly visualize and identify the main thematic focuses of the studies. The terms *Zebrafish*, *Danio rerio,* and *Ecotoxicology* were frequently cited, along with pesticide names, allowing inferences about the topic covered in this review through word cloud visualization. Ecotoxicological studies seem to stand out within pesticide toxicology. The emphasis on terms associated with ecotoxicology also indicates an approach that goes beyond the direct impact of pesticides, addressing the fate and effects of pollutants on the environment and on non-target organisms, such as zebrafish. This overview suggests that researchers are increasingly interested in parameters that reflect environmental realities, aligning with the sustainability and conservation guidelines promoted by funding agencies, such as CAPES. Thus, the word cloud serves as a valuable tool to provide an overview of studies and identify research gaps.

CONCLUSION

This scientometric review provides insights into emerging trends and challenges related to pesticide toxicity in zebrafish (*Danio rerio*) and identifies existing gaps in research. The results highlight the growth of ecotoxicological studies, reflecting a global concern for environmental health and the impact of chemical compounds on aquatic ecosystems. The prevalence of studies focused on the toxicity of herbicides, particularly compounds such as glyphosate and 2,4-D, reinforces the relevance of these pesticides due to their widespread use in Brazilian agriculture and their potential environmental implications. In addition, the regional analysis indicates a gradual increase in research in areas such as the Midwest and Northeast, suggesting an expansion of research capacities

in these regions. The data provided also facilitate the formation of cooperative networks between institutions and their respective researchers.

By offering an overview of the most frequently used keywords, the word cloud highlights the predominance of essential terms, reflecting the central focus of the studies and suggesting a consolidated approach to relevant topics. However, it also identifies areas that deserve further exploration, such as specific biomarkers and bioaccumulation processes. Thus, this review not only contributes to visualize the scientific progress in pesticide toxicology, but also outlines strategic directions for future investigations and incentive policies, aiming at the sustainable and integrated development of scientific research in Brazil.

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