

Ciências da Saúde: Da Teoria à Prática 10

Benedito Rodrigues da Silva Neto
(Organizador)

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(Organizador)

Ciências da Saúde: Da Teoria à Prática 10

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APRESENTAÇÃO

A coleção “Ciências da Saúde: da teoria à prática” é uma obra composta de onze volumes abordará de forma categorizada e interdisciplinar trabalhos, pesquisas, relatos de casos, revisões e inferências sobre esse amplo e vasto contexto do conhecimento relativo à saúde. Além disso, todo o conteúdo reúne atividades de ensino, pesquisa e extensão desenvolvidas em diversas regiões do país, que analisam a saúde em diversos dos seus aspectos, percorrendo o caminho que parte do conhecimento bibliográfico e alcança o conhecimento empírico e prático.

O décimo volume apresenta informações fundamentadas e categorizadas abordando o eixo central da coleção que é da teoria à prática. O leitor poderá encontrar capítulos com explanação teórica geral sobre temas específicos assim como capítulos aplicados e exemplificados por relatos. A progressão exponencial dos avanços tecnológicos tem contribuído de forma especial nos últimos anos com as novas metodologias práticas de estudo das desordens genéticas humanas, microbianas além de oferecer metodologias novas e extremamente sensíveis.

Deste modo, esse volume se destaca por congrega temas atuais e que poderão nortear novas ideias e direcionar o leitor em novos estudos específicos, haja vista que temas como câncer, autoimunidade, ancoramento molecular, tecnologias modernas, leucemia, epigenética, CRISPR, neuropatias, serão amplamente discutidos, além dos diversos relatos de caso, durante todo o livro.

Assim o décimo volume apresenta uma teoria bem fundamentada exemplificada nos resultados práticos obtidos pelos diversos pesquisadores que arduamente desenvolveram seus trabalhos que aqui serão apresentados. Do mesmo modo é de fundamental importância uma estrutura como a Atena Editora capaz de oferecer uma plataforma consolidada e confiável para estes pesquisadores exporem seus resultados. Portanto, nosso profundo desejo é que este contexto possa ser transformado a cada dia, e o trabalho aqui presente pode ser um agente transformador por gerar conhecimento em uma área fundamental do desenvolvimento como a saúde.

Benedito Rodrigues da Silva Neto

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BIOTECHNOLOGY PATENT AS A TOOL FOR PREVENTION AND CONTROL OF THE MOSQUITO *Aedes Aegypti*

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C12P, C07H and C12R. Regarding authorship, the main inventors were Presta Leonard and Avi Ashkenaz, being Yamamoto hide policy, Morishita and Genentech the representatives with the largest number of patent families. The significant growth in the number of biotech patent deposits evidenced in the results demonstrates the interest of companies and research centers. **KEYWORDS:** *Aedes aegypti*, Biotechnology, Patents.

1 | INTRODUCTION

The mosquito *Aedes aegypti* and the arboviruses transmitted by it constitute a major obstacle to public health in Brazil and in the world. The same mosquito that transmits DEN-1, DEN-2, DEN3, DEN-4, responsible for dengue also transmits, Zika virus and CHIKV, responsible for Chikungunya fever.

Identified in 1986, dengue fever is the record of diseases transmitted by the *Aedes aegypti* mosquito with about 1.5 million case reports exceeding more than 800 deaths in 2015 in Brazil (MINISTRY OF HEALTH, 2016). Considering that approximately 2/3 of the world population live in areas infested by potential dengue vectors, combating and monitoring this insect is of the utmost importance since there are no vaccines to treat some of the transmitted

ABSTRACT: This article aimed to analyze the biotechnological domains used to combat the *Aedes aegypti* mosquito under a patent approach. Orbit Intelligence software was used as a patent search database using the term “*Aedes aegypti*” as the key word in the advanced search field, selecting only the patents that used as the dominant technology, Biotechnology. The results showed that the number of biotech patents has grown significantly in the last twenty years, most of which are protected in the United States and the World Intellectual Property Organization (WIPO) via the PCT. The most frequent sections in the patent portfolio are related to the areas of chemistry and human needs represented by subclasses A61K, A61P, A01N, A01P, A01H, C12N, C07K,

diseases (PINHEIRO, CORBER, 1997)

The viral disease called Chikungunya was first documented in Tanzania, reaching Brazil in 2014 and has since attracted the attention of government health agencies. Although not lethal, Chikungunya can cause financial losses in an indirect way, due to the dismay presented by the affected workers (MINISTRY OF HEALTH, 2015).

Brazil has become the center of attention in the last year due to the epidemic caused by the Zika virus. The Zika virus was first identified in Brazil in 2015 by researchers from the Federal University of Bahia after collection of blood samples from patients from the city of Camaçari-Ba. Since then, the Zika virus has caused more than 5,000 cases of microcephaly in newborns, in addition to causing other diseases such as Guillan-Barré syndrome (MINISTRY OF HEALTH, 2017).

Considering the wide geographical dispersion of the *Aedes aegypti* mosquito and the circulation of viral serotypes in most of these areas, it is estimated that 100 million people are infected each year (PINHEIRO, CORBER, 1997). Knowledge of mosquito biology is extremely important since patents may be related to one of the four stages of life of this insect.

Peculiarities of the mosquito *Aedes aegypti*

The mosquito *Aedes aegypti* belongs to the order Diptera, family culicidae, and to the genus *Aedes*, was found and described as being from the northeast region of Africa based on samples found in Egypt (PESSÔA; MARTINS, 1982). This species was introduced in Brazil accidentally in the period of maritime navigation and slave trade (CONSOLI; LOURENÇO-DE-OLIVEIRA, 1994; FORATTINI, 2002).

Aedes aegypti has complete metamorphosis, that is, its life cycle consists of egg, larva (which has four phases), pupa and adult (CHRISTOPHERS, 1960). Adults have a dark color and are marked by white bands in the tarsal segments (Figure 1). However, males can be identified by the presence of feathery antennae and elongated palps. After emergence, adults mate in free flight or on fixed surfaces, requiring a single copulation for fertilization (FUNASA, 2001). Adult males, like females, feed on sugary solutions and other substances found in nature.

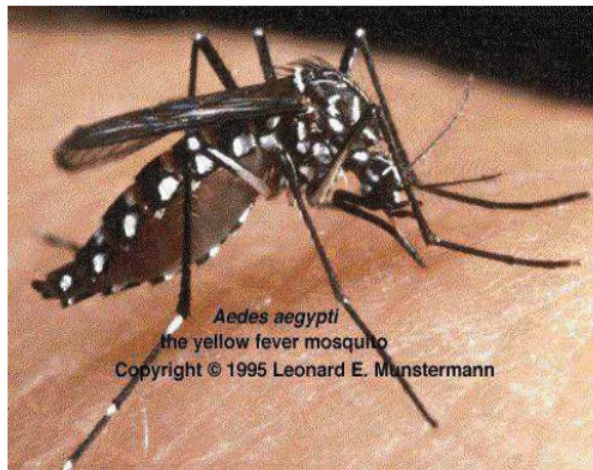


Figure 1 – Adult *Aedes aegypti*

Source: Munstermann (1995)

The females perform the blood supply in vertebrate hosts to accelerate the process of maturation of the eggs, because some proteins are not found in the external environment. These proteins will be metabolized by supplying amino acids to their ovaries and immature eggs (CLEMENTS, 2000). Females use the first hours of the morning and the late hours as preferred times for hematophagy (EIRAS, 2005).

The attraction of females to the blood repellent target may be potentiated by some known chemicals, including carbon dioxide and lactic acid. Carbon dioxide is released through respiration and lactic acid through sweat, and these are some of the stimulators used as a parameter to localize blood food (EDMAN, 1979). With the exception of pregnant females, flight dispersion does not exceed 100 meters if there are hosts. Thus, the best form of dispersion of this species runs through eggs and larvae (FUNASA, 2001). The eggs are deposited by the females on the inner wall of containers found in the external or even internal environment of the residences (FORATTINI, 1962). As an evolutionary mechanism, the *Aedes aegypti* females distribute small batches of eggs in different breeding sites to guarantee the offspring success, hindering control actions (Reiter et al., 1997). For Gubler and Clark (1995) the environmental factors linked to the occupation of the population in the geographic space together with climate, rainfall and temperature, can facilitate the population increase of *Aedes aegypti*.

The choice of sites and oviposition behavior have a significant impact on offspring survival (BENTLEY; DAY, 1989). The females choose the ideal breeding ground that will guarantee the growth and development of the offspring (ZAHIRI; MANFRED, 1998). The eggs are deposited just above the water line on rough or rough surfaces and are resistant to desiccation for long periods (BATES, 1949; REITER, 2007; CHRISTOPHERS, 1960). Depending on the concentration, variables such as intensity of water vaporization, light reflection, larval competition and other chemical elements may act as attractive or repellent at the moment of choice (ALLAN; KLINE, 1995). Therefore, waters with deep dark tones and abundance of food are required by the

females of *Aedes aegypti* (ALLAN; KLINE, 1995; ZAHIRI; MANFRED, 1998).

The larva is formed by head, thorax, abdomen and consists of four evolutionary stages that are dependent on temperature variations, presence of organic matter and density of larvae for development in the breeding place (Figure 2). Inside the containers the larvae move similarly to the serpents with displacement in 'S' format in the direction of the surface, making it possible to distinguish larvae of *Aedes aegypti* from other mosquitoes. The transformation of the larva into an adult mosquito lasts on average three days in a process called pupation, in which pupae do not feed on any type of food (FUNASA, 2001).

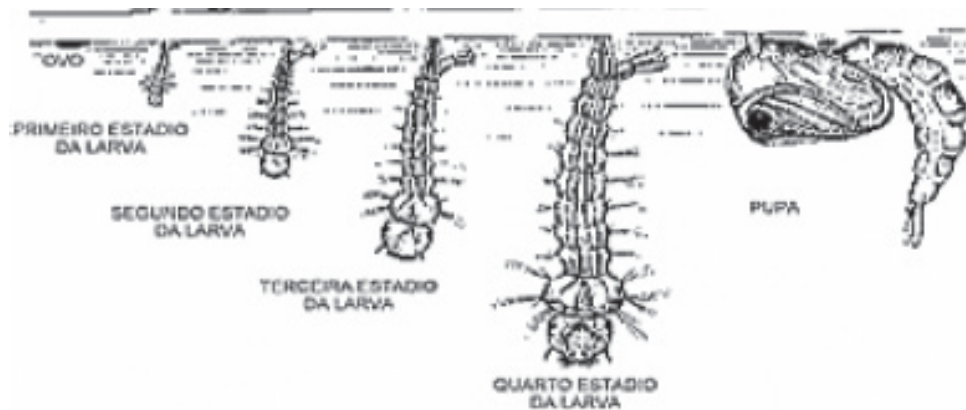


Figure 2 – Pupa and larvae composed of four stages (pupation)

Source: Funasa (2001)

Vector control is essential to prevent outbreaks and epidemics as there are no vaccines to treat some of the mosquito-borne diseases. Patents related to forms of control helped to improve the traditional techniques used by endemic agents.

Control Strategies

Some of the mosquito control and combat strategies currently used were established by the National Program to Combat Dengue (PNCD). The forms of chemical and mechanical control are the most widespread because they are often used by agents of endemics in public residences or places. In the mechanical control, the health agents perform routine visits as a form of prevention, eliminating possible breeding grounds or larval focus in order to interrupt the life cycle. On the other hand, chemical control has been represented by insecticides and larvicides, used in the population reduction of adult mosquitoes and larvae (ZARA et al., 2016).

Given this reality, the new trends advocate the substitution of chemical control for new techniques with low environmental impact, such as the use of natural compounds and biological control. Biotechnology has played an important role in supplying new technologies to combat the *Aedes aegypti* mosquito. This science encompasses

different areas of knowledge that includes basic science applied among other technologies providing growth in the areas of research and product development. Within applied sciences, genetic engineering has been prominent as it provides sophisticated mosquito control techniques through insertion of lethal genes and genes capable of rendering adult mosquitoes sterile.

The technique that inserts lethal genes into mosquitoes was called RIDL and was known to have been used to obtain the transgenic strain OX513A of the mosquito *Aedes aegypti* already available for commercialization in Brazil. In general, the RIDL method is based on the insertion of a gene encoding a transcription factor termed tetracycline-repressible transcription activator (tTA). When the coding gene is expressed, this factor acts on the activation of the expression of a second gene inserted in the transformed lineage, which produces a product lethal to the mosquito. However, the functionality of this expressed transcription factor can be suppressed by the presence of tetracycline, thus providing conditional regulation of lethal gene activation (ALPHEY, 2002). The transgenic *Aedes* technique is only one of the hundreds of contributions that biotechnology has been making in the fight against the mosquito that transmits the four most worrying viral diseases today.

In the environmental part, some natural compounds such as clove, citronella and lemon grass essential oils were investigated for larvicidal and insecticidal activity against dengue mosquito, zika and chikungunya. Costa et al. (2005) evaluated clove essential oil in immatures of *Aedes aegypti*, demonstrating mortality of up to 100% of the larvae tested. On the other hand, the use of natural enemies or pathogens as predators is one of the most ecological and safe practices used in mosquito control. Several species of fish, invertebrates, bacteria, fungi and parasites were used efficiently against the immature *Aedes aegypti* in laboratory and field tests, but satisfactory results have not yet been demonstrated in the reduction of mosquito-borne diseases when submitted to this method of control (ZARA et al., 2016).

Other advances in the area of elaboration of new products such as attractions, traps, repellents, biocides, biorepellents, transgenic mosquitoes, drugs and irradiated mosquitoes can be found through technological prospecting.

Technological prospecting

Caruzo and Tigre (2004) defined technological prospecting as a method capable of detecting long-term technical-scientific advances that can significantly influence important sectors of society such as industry and the economy. For Amparo (2012), technological prospecting contributes to the process of innovation and technological management allowing companies and research and development centers to survive the environment of so much competitiveness. Technological prospecting involves a number of methods and techniques in which researchers choose qualitative or quantitative tools with no precepts for choosing one. In general, the researcher will

define the technique that is most appropriate to his / her need, and the definition depends on some conditions such as time and available cost, scope of the study and mainly application of the technology in context (INOVA PAULA SOUZA, 2015).

Patents are privileges granted by the government to inventors or representatives of technologies in a transitional and territorial validity. Some requirements such as novelty, inventive characteristic or industrial application are required to meet the patentability process. Thus, the inventors undertake to disclose all technical-scientific content used in the development of prototypes (INPI, 2017). For Borém and Santos (2008), there is a very great difference between scientific discovery and invention. Scientific discovery is characterized by the revelation of something that already exists in nature. Therefore, they are not considered inventions: scientific theories, living beings, biological material or genome.

The principle of territoriality proposes that patents be valid only in the geographical places where they were granted. Therefore, for these technologies to be protected in other countries it is necessary for inventors to deposit patents in each country or through the Patent Cooperation Treaty (PCT). The Patent Cooperation Treaty (PCT) consists of 145 contracting states and made it possible for inventions to be protected in several countries at the same time through the international patent application replacing part of the requests made through the intellectual property offices (BORÉM; SANTOS, 2008).

Patent families are a set of applications filed or granted in more than one country to protect the same invention developed by common inventors and can be subdivided into two types. Patent applications which have no claim of priority are entitled as First Patent Application or Document of Origin. Only such documents are expedient as priority documents for filing the patent application at another national patent office or international organization. That is, only these documents are capable of originating a family of patents. The patent application which, on the other hand, at the time of filing, claim as a priority the First Patent Application, are entitled Second Patent Application. Upon filing a Second Patent Application, the First Order shall be entitled Priority Document (INPI, 2016).

The laws governing the fruits of biotechnology are not the same as those governing technologies in other areas. The patent system was initially developed to protect products from the mechanical, electrical and chemical areas, and later included techniques and processes. In the 1980s, American researchers applied for a genetically modified bacterium used to degrade oil, setting the precedent for intellectual protection of biotech patents. Biotech patents are based on living organisms, so some laws were developed to guide researchers and companies interested in protecting their inventions. (BORÉM; SANTOS, 2008). In the field of biotechnology certain materials are patentable as compositions containing amino acid fragments, viruses or genetic material once they are characterized as compositions, extraction/isolation processes, engineered plant production, mutant microorganisms, monoclonal antibodies, hybridomas and others (BOREM; SANTOS, 2008).

Biotechnology goes through a period of expansion in the field of research. However, new inventions concerning living organisms generate discussions about their patentability. This article has as main objective to analyze the biotechnological domains used in patents to combat the mosquito *Aedes aegypti*, under a quantitative patent approach, with the following specific objectives:

- Analyze the number of biotechnology patents deposited;
- Quantify the number of patent families deposited in countries and organizations;
- Identify international patent codes;
- Point the main representatives.

2 | METHODOLOGY

Orbit Intelligence was the tool used for patent research. This software was chosen due to its wide coverage of publications in national and international offices, having publications grouped in patent families with inclusion of deposits after the deadline of 12 months. Orbit Intelligence is a system for searching, selecting and processing data extracted from the FAMPAT (Patent Family) and FULLPAT (Individual Requests) bases, produced by the weak American company Questel Orbit (AXONAL, 2017). In this system, professionals can filter and select documents of interest by technological area of knowledge enabling a better search strategy.

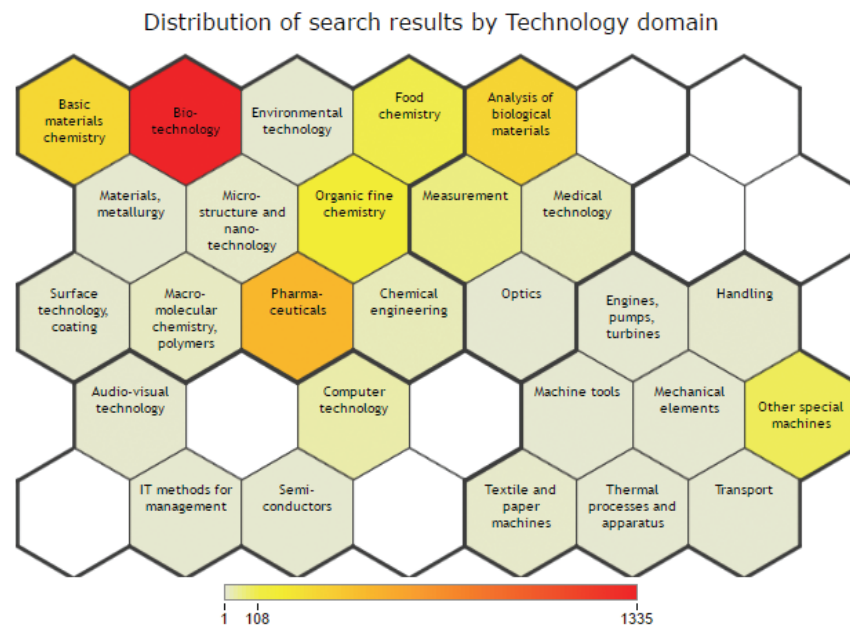
FAMPAT has a geographical coverage and contains 83 national authorities, including INPI and six regional patent authorities (EPO, WIPO, OAPI, ARIPO, EAPO and CGC). The intelligent Orbit tools include the databases available worldwide, and the results can be filtered by database.

The term “*Aedes aegypti*” was initially used as a keyword and used as the main keyword filter contained in the title, description, summary and claim to design the first analysis made by the program. The selection of these search fields was based on more accurately identifying documents with relevant technologies. Subsequently, Biotechnology science was chosen as the dominant technology and, in possession of the required patent documents, a second analysis was carried out to identify: a) the number of patents deposited in the last 20 years (01/1997 - 03/2017); b) number of patent families deposited in countries and organizations between 01/1997 and 03/2017; c) more frequent international subclasses in the patent portfolio; d) representatives of the analyzed patents; e) Inventors with the highest number of patent families.

3 | RESULTS AND DISCUSSION

As a result of the first analysis described in the above methodology, a “hive” chart was generated with the distribution of the dominant technologies used in the patents

(Graph 1). This graph model shows the family number of patents by the color intensity of each hexagon, that is, the more intense the color the greater the number of patent families per hexagon. The first analysis made by the software using the term *Aedes aegypti* as a keyword showed that the most dominant technologies were biotechnology, then pharmaceutical, biological materials and analysis, basic materials chemistry, organic fine chemicals and special machines.

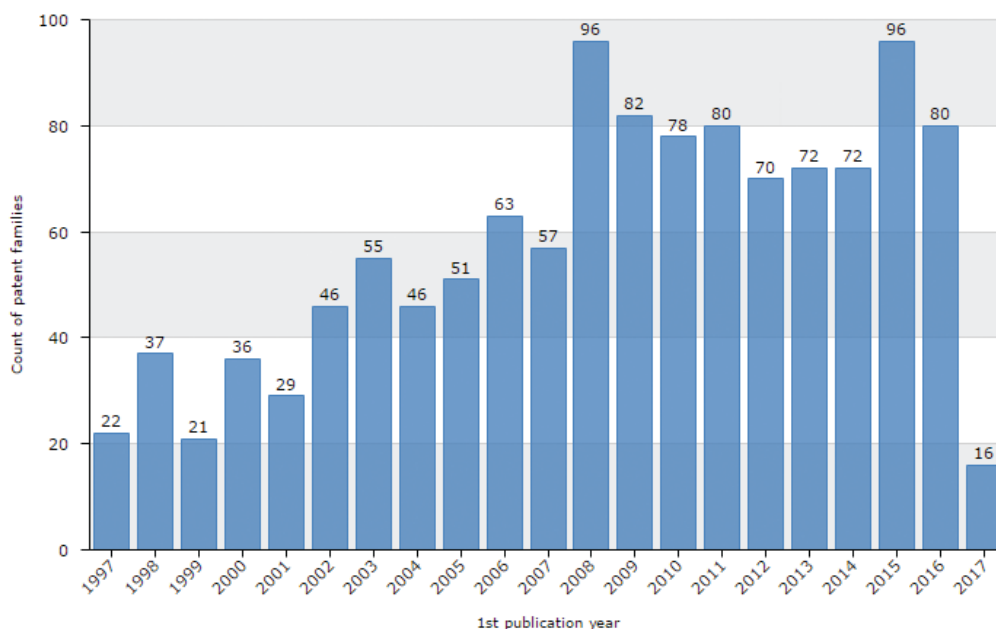


Graph 1 – Distribution of search results by dominant technology

Source: Prepared by the author with the help of software Orbit Intelligence (2017)

Considering that the objective of this study was to analyze the biotechnological contributions used to combat the *Aedes aegypti* mosquito and under a patent approach, we selected all patents of the hexagon biotechnology as shown in Graph 1. The definition of biotechnology as a dominant technology associated with the analysis tool “documents” for the first year of publication provided a total of 1335 patents deposited in the last 20 years (Figure 2). Orbit Intelligence pointed out that of these, only 930 have commercial validity and the other 405 are no longer in force. Graph 2 clearly shows the significant growth since 1997 reaching its highest peak in 2008, repeating the same in 2015. Despite the increase in the number of patents, we distinguish a clear balance in the graph evidenced between the years 2008 and 2016 highlighting the of this important sector in the last ten years.

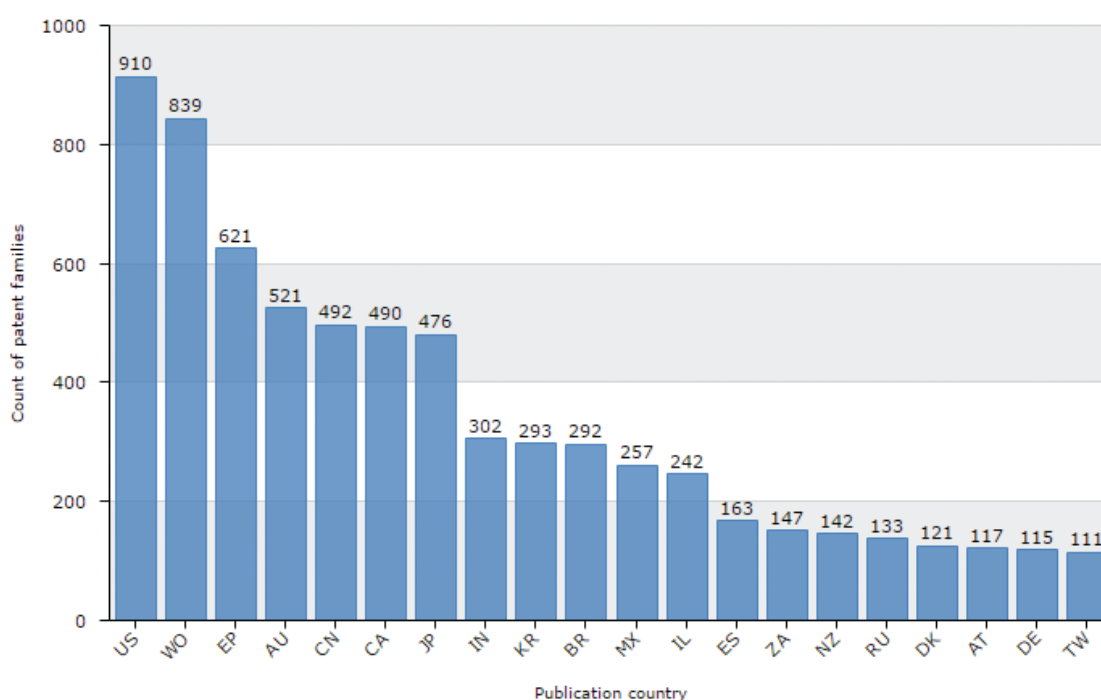
The genome of the *Aedes aegypti* mosquito was sequenced and published in 2007 stimulating the increase of genetic and molecular techniques in the United States and Europe. The steady increase in the number of patents shows that biotechnology has been a major weapon for research and development of products that are likely to consolidate in the coming years. We know that representatives or companies that own the technologies can protect them in more than one country.



Graph 2 – Number of patents deposited in the last 20 years

Source: Prepared by the author with the help of software Orbit Intelligence (2017)

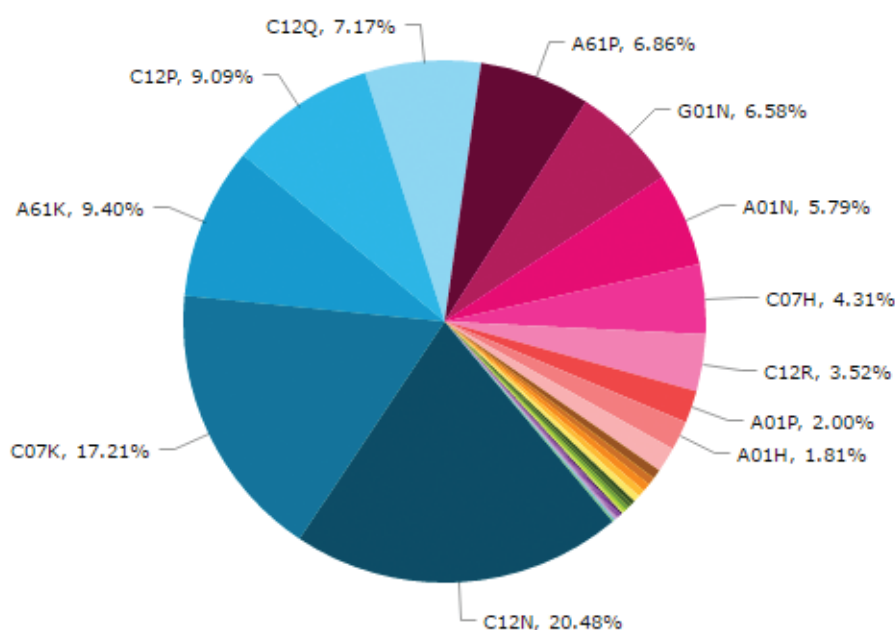
With this, we did a second analysis with the ‘documents’ tool, to ascertain the number of patent families linked to biotechnology that were deposited in countries and organizations (Graph 3). The United States (US) is the country with the largest number of patent deposits in biotechnology, exhibiting a number of 910 patent families, followed by patents filed with the World Intellectual Property Organization (WIPO) via PCT with 839, European Office with 621, China with 492, Canada with 490, Japan with 476, India with 302 and Republic of Korea with 293. Despite facing serious problems with the triple Brazil occupies only the tenth place in the ranking with 292 deposits.



Graph 3 – Number of patents family deposited in countries and organizations between 1997 and

Source: Prepared by the author with the help of software Orbit Intelligence (2017)

Orbit Intelligence software identified the major international subclasses in the portfolio of patents requested by CPC classification. Among the divisions mentioned above, we find technologies pertinent to section G, corresponding to Physics, represented by subclass G01N. However, the main subclasses found belong to sections A and C, corresponding to the Human Needs and Chemistry areas, being represented by the subclasses A61K, A61P, A01N, A01P, A01H, C12N, C07K, C12P, C07H, C12R (Graph 4).



Graph 4 – Most frequent international subclasses in the portfolio of patents deposited by CPC classification

Source: Prepared by the author with the help of software Orbit Intelligence (2017)

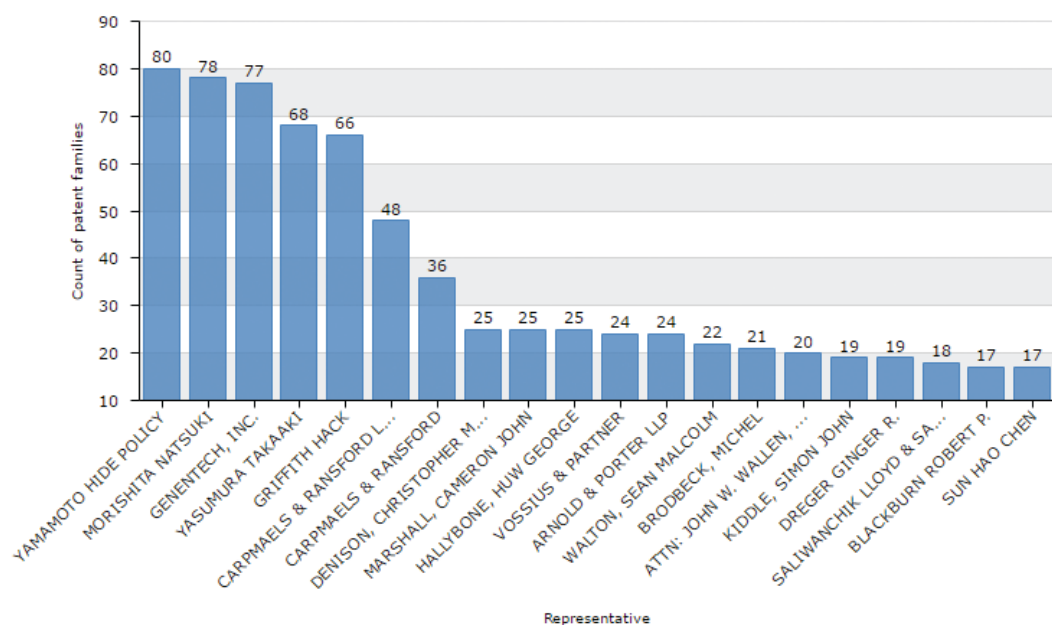
CPC Classification	Technology	Applications
C12N	Genetic engineering or mutations. Use of microorganisms or enzymes	Biocides, repellents or attractants, production of microbial fungi
C07K	Peptides	Fragmentation or modification of peptides by the removal or addition of amino acids
A61K	Preparation for Medical Purposes	Medications
C12P	Process fermentation	Production of organic compounds
G01N	Investigation or analysis for chemical or physical properties	Testing/measuring process involving microorganisms or enzymes

Table 1 – Description of technologies and applications of international subclasses

Source: Prepared by the author with the help of software Orbit Intelligence (2017)

We can see from Table 1 that the technologies used have adherence to traditional biotechnological techniques evidenced by the C12P and A61K classifications. The C12P classification uses fermentative process technology or processes that use enzymes to synthesize desired chemical compounds. However, more sophisticated technologies are represented by the C12N and C07K classifications, which use genetic engineering, microorganism and enzymes to produce biocides, repellents, attractants or microbial fungi. The development of biocides and natural repellents for mosquito control has grown significantly in recent years due to new trends that advocate the substitution of organophosphate insecticides harmful to human and environmental health.

The institutions or representatives who deposit the patents are considered holders of the patents. The percentage of documents represents the interest of companies in the area of biotechnology, characterized by the expansion of research and development in the private sector. Through Graph 5, we show the twenty principal representatives of the deposited patents. This analysis shows that the vast majority of patents, which used biotechnology as the dominant technology, were deposited by researchers and companies. The list is led by Yamamoto with 80 patent families, followed by Morishita with 78, Genentech with 77, Yasumura Takaaki with 68, Griffithhack with 66, Carpmaels and Ransford with 36, making up the six largest representatives. The analysis of depositor representatives provides data on previous cooperations between companies, as institutions develop technologies in sync and as a result, the document is deposited in co-ownership.



Graph 5 – Representatives of patents reviewed

Source: Prepared by the author with the help of software Orbit Intelligence (2017)

4 | CONCLUSION

Based on the data obtained in this study on biotech domains used to combat the *Aedes aegypti* mosquito under a patent approach, it was found that 70% of the 1335 patents found have commercial validity.

Patent families are granted in more than one country to protect a same invention developed by common inventors. Thus, the United States was the country with the largest number of patent deposits in biotechnology, exhibiting a number of 910 patent families, followed by patents deposited by PCT in the World Intellectual Property Organization (WIPO) with 839 and European Office of patents (EP) with 621. On the other hand, Brazil appeared only in tenth place in the ranking of the countries that most deposit biotechnological patents associated to the combat of the mosquito *Aedes aegypti*. The international subclasses C12N, C07K, C12P and G01N deposited by CPC classification attest to the prevalence of patents using biotechnological techniques for the development of protected documents. Yamamoto hide policy, Morishita and Genentech appeared as the largest depositors of the technologies found. Although they are not the owners of the inventions themselves, the American researchers Presta Leonard and Avi Ashkenazi were the greatest inventors of technologies in the biotechnology area, evidencing that creators are not always the representatives or owners of the technologies they develop.

From the data obtained, the impacts of biotechnology are inevitable and the inquiries are even more relevant. The significant growth in the number of patent deposits evidenced in the above results demonstrates the interest of companies and research centers. But new advances in the intellectual property system related to technologies derived from living organisms are required so that biotechnology can continue to evolve.

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