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BACTERIOPHAGES AS BIOLOGICAL CONTROL AGENTS AND NEW TRENDS IN MODERN AGRICULTURE

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ABSTRACT

From the domestication of several cultivable species and the use of modern techniques such as the internet, automation systems, fertilizers, pesticides, and antibiotics, 21st-century agriculture has been able to supply food continuously to a growing demand of individuals. Problems related to large losses in the field caused by microorganisms such as fungi and bacteria resistant to pesticides and antibiotics, bacterial genera such as *Xanthomonas* spp. and *Pseudomonas* spp. threaten the modern way of life, which can be seen as an advance in relation to the old agricultural systems, characterized by primitive, limited, and less efficient techniques. The objectives of this work were to identify the main microorganisms used in biological control, phage families, and affected cultivars, and evaluate the efficacy of phage-mediated biological control in controlling bacterial diseases by comparing experimental and review articles. This is a systematic literature review article, and 126 articles were included after applying a research protocol. Quantitative and qualitative data on pathovars, cultivars, antibiotics, pesticides, and bacteriophages were extracted. At least five phage families related to the biological control of phytopathogenic bacteria were identified. With the transition of older generations of farmers, combining multiple control techniques, bacteriophages are a little explored potential for biological control of phytopathogenic bacteria.
KEYWORDS: Biological control, IoT, Microorganisms, Phages, Phytopathogens, Pesticides

RESUMO

A partir da domesticação de diversas espécies cultiváveis e do uso de técnicas modernas como internet, sistemas de automação, fertilizantes, pesticidas e antibióticos, a agricultura do século XXI tem conseguido suprir continuamente alimentos para uma demanda crescente de indivíduos. Problemas relacionados as grandes perdas no campo causado por microrganismos como fungos e bactérias resistentes a pesticidas e antibióticos sobretudo, dos gêneros bacterianos *Xanthomonas* spp. e *Pseudomonas* spp. ameaçam o modo de vida moderno, que pode ser visto como um avanço em relação aos antigos sistemas agrícolas, caracterizados por técnicas primitivas, limitadas e menos eficientes. Os objetivos deste trabalho foram identificar os principais microrganismos utilizados no controle biológico, famílias de fagos e cultivares afetadas, e avaliar a eficácia do controle

biológico mediado por fagos no controle de doenças bacterianas, comparando artigos experimentais e de revisão. Este é um artigo de revisão sistemática da literatura, 126 artigos foram incluídos após a aplicação de um protocolo de pesquisa. Dados quantitativos e qualitativos sobre patovares, cultivares, antibióticos, pesticidas e bacteriófagos foram extraídos. Pelo menos cinco famílias de fagos relacionadas ao controle biológico de bactérias fitopatogênicas foram identificadas. Com a transição de gerações mais velhas de agricultores, combinando múltiplas técnicas de controle, os bacteriófagos são um potencial pouco explorado para o controle biológico de bactérias fitopatogênicas.
PALAVRAS-CHAVE: Controle biológico, IoT, Microrganismos ,Fagos, Fitopatógenos, Pesticidas

INTRODUCTION

Since the emergence of agriculture and domestication of various cultivable species, it has been possible to provide a growing food supply and sustain the population growth. Far from the rudimentary techniques and manual planting of grains and seeds used by early farmers, modern agriculture relies on various technologies, such as equipment connectivity and communication with field personnel through the internet, automated irrigation and monitoring systems, pesticides, fertilizers, microorganisms to combat diseases, and antibiotics, always seeking to increase the efficiency of the agricultural sector and combat pathogens (MENDES *et al*., 2022).

According to Xie *et al*. (2021), agricultural sectors are tending to develop ways to serve an environmentally sustainability and the modernization can lead to Modern Era of agriculture, focus in biotechnology. The use of scientific knowledges in the agricultural sector has become even more evident due to the COVID-19 pandemic, leading to a fast way of decision and capital investment for improve those technologies (BOHELJE *et al.*, 2021).

Diseases found in the field can originate from various sources, such as nematodes, insects, Oomycetes, fungi, bacteria, and viruses, and can devastate large areas of cultivation. Phytopathogens, especially phytopathogenic bacteria, can cause critical damage in the field due to their cosmopolitan distribution. The loss estimates at a global level are wheat (21.5%), rice (30.0%), bean (29.3), maize (22.5%), potato (17.2%) and soybean (21.4%), common in food-deficit regions with fast increasing populations, that deal with emerging or re-emerging pests and diseases (Savary *et al*, 2019; Benjamin *et al*, 2021). Pathovars of the genera *Xanthomonas* spp., *Pseudomonas* spp., *Ralstonia* spp., and *Clavibacter* spp. are reported and associated with significant losses in those fields, potentially infecting important commercially crops (WAGEMANS *et al*., 2022).

Cultivars such as tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), rice (*Oryza sativa*), and corn (*Zea mays*) are examples of highly commercially valuable foods (IRIGOYEN *et al.*, 2017), as the global production of these products combined represented approximately 2.5 billion tons in 2020, exerting a significant impact on the economy of various countries, especially in countries like China and India, as a large part of fruits and grains are from Southeast Asia. In Europe and the Americas, Italy, Spain, the United States, and Brazil stand out as global food suppliers (FAOSTAT, 2022).

In order to mitigate losses in the field caused by phytopathogens, strategies for combat using chemical pesticides and antibiotics have been developed, resulting in increasing resistance of microorganisms to antimicrobials and others environmental issues involving chemical pesticides (ROUSIS *et al*., 2021). Nevertheless, the combat have been developed over time, such as the use of microorganisms with antagonistic capacity through competition or biomolecule production, plant growth promoters, and bacteriophages are examples of approaches against fungi and bacteria, less harmful to the environment (VURUKONDA *et al*., 2018).

Bacteriophages are viruses that attack bacteria, they are among the most abundant microorganisms in nature, and they are self-replicating and specific to their bacterial hosts, making biological control using phages an ecologically viable therapeutic approach (GOODRIDGE; ABEDON, 2003), considering that diseases such as bacterial wilt, and vegetables from the solanaceae family can be hard to treat by traditional treatment methods (SINGH; KESHARWANI, 2021). Furthermore, according to the reports of future market insight (FMI company), the world demand of Bacteriophages has a significant growth of 4,2% CAGR (compound annual growth rate) that can lead into US$62.72 in 2030, giving a expansion close to 1.5x, giving a great opportunity for investments.

Despite their efficacy, biological control-based products are underutilized compared to copper bactericides and antibiotics (BALOGH *et al*., 2008). Bacteriophages, in particular, have largely unexplored potential for use in agricultural pest management. The aim of this study is to identify the main microorganisms used in biological control, phage families, and affected cultivars, and to evaluate the effectiveness of phage-mediated biological control in managing microbial diseases caused by phytopathogens. We compare the use of phages to that of pesticides and antibiotics by analyzing both experimental and review articles. Our objective is to provide current data on the utilization and efficacy of phages in the field as an ecologically sound and effective alternative to traditional chemical interventions.

MATERIAL AND METHODS

This is a systematic review study of indexed articles and newspapers. The strategic approach adopted consisted of data screening by stages, as outlined by MOHER and COLS. (2015). Briefly, a set of selected keywords was used, forming a search string, consisting of Bolean operators “and” and “or”. The string "Bacteriophages" and "Biological Control" and "Agriculture" and "Phytobacteria" or "Plant Pathogens" and "Antibiotics" and "Pesticide" and "Chemical Control" was subjected to selected databases, PubMed / Medline, ScienceDirect, Web of Science and Google Scholar.

SCREENING OF THE WORKS

After the protocol was applied, a total of 1,057 articles were returned, of which 832 were eliminated after the preliminary analysis of the data. The 225 remaining articles underwent title and abstract analysis, where 94 studies did not fit the scope of this review, as well as the exclusion of duplicate inputs. One hundred and twenty-six articles were selected for complete text analysis and to compose this work.

APPLICATION OF ELIGIBILITY CRITERIA AND DATA ORGANIZATION

The data survey included published studies in 3 languages (English, Portuguese, and Spanish). Publications that reported quantitative and qualitative data on the use of microorganisms as a form of biological control, pesticide use, antibiotics, different phytopathogen control approaches, literature reviews, and experimental articles were selected. Book chapters, theses, dissertations, short announcements, guidelines, posters, and publications that presented inconclusive evaluations were excluded. The survey period took place between February and September 2022, with no date limit for publications.

The screening data were divided into 2 clusters, called C1 and C2, where the information of the studies of C1 had as focused on review articles, the information extracted was; 1) quantitative and qualitative of the microorganisms, pesticides, and antibiotics used.; 2) quantitative and qualitative data of reported disease; 3) and affected cultivation. For C2, data were obtained only from experimental articles, addressing data on; 1) the type of control used (chemical and/or biological); 2) quantitative and qualitative data of the microorganisms used in biological control; 3) qualitative of pesticides and antibiotics used in the field; 4) qualitative of reported pathogens; 5) Families of bacteriophages reported in the studies analyzed; 6) Origin of isolates when available.

EXTRACTION AND EVALUATION OF THE COLLECTED DATA

The search was performed in all previously selected articles, being collected the following information; Type of chemical compound used, genera and species of phytopathogens, diseases caused and affected cultivation. After the collection phase from the previous step, the preliminary data were processed through the LibreOffice Calc Electronic spreadsheet (Ver. 7.4.0-3) (The Document Foundation) for Linux, and subsequently performed the *quantitative and qualitative of information obtained through Logical testing* using Pdfgrep (Ver. 2.1.2.) Software for Linux, Poppler Libraries (Ver. 22.09.0) and Libpcre (Ver. 8.45). *For graphic representations, software*, Obsidian (Ver. 0.15.9) and Filelight (Ver. 22.06.0) were used for Linux.

OTHER SOURCES CONSULTED

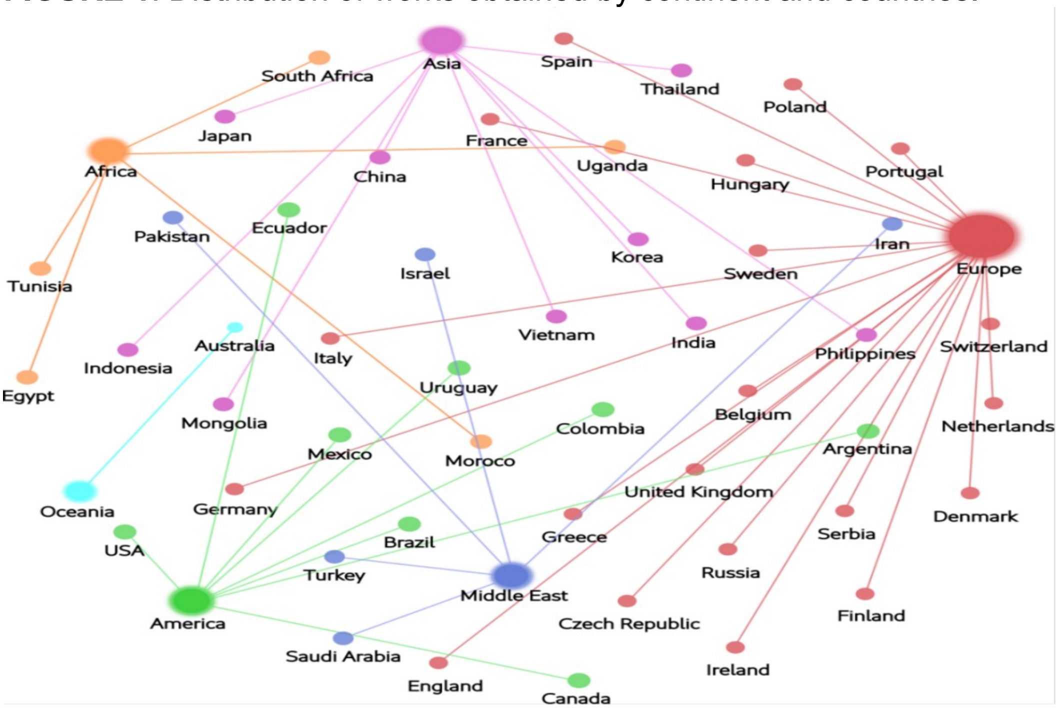
The following additional database was used; World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), European Food Safety Authority (EFSA), National Agency and Sanitary Agency (Anvisa), Phytosanitary Pesticide System (Agrofit), National Cancer Institute (Inca), as they provide data on regulation, impact on health, and use of chemicals and biological ones was consulted.

RESULTS AND DISCUSSION

DENSITY OF ARTICLES, GROUPED BY REGION

The data obtained from the 126 manuscripts analyzed by this review article provide insight into the source of studies (Figure 1), as well as the pathovar-related dispersion, and chemical and biological control methods adopted by almost 50 countries. The Asian continent presented the highest density of work recovered in the analyzed databases, with 46 articles, followed by the European and American continents with 40 and 37 publications respectively.

FIGURE 1: Distribution of works obtained by continent and countries.



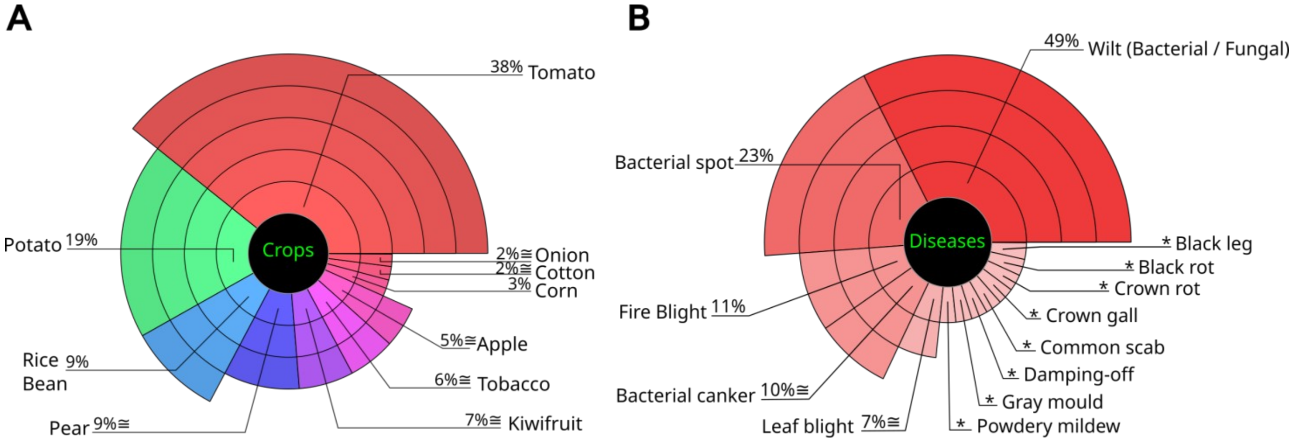
Source: The Authors (2023)

The density of scientific papers provided from the Asian continent, especially from China, is a reflection of Chinese economic activity since the production of much of fruits, vegetables, and cereals oscillates between 70 to 90% Compared to European and American products (FAOSTAT, 2022).

MICROBIAL PROFILE AND DISEASES RELATED TO CULTIVARS OF COMMERCIAL INTEREST

From the data originating from C1, almost 40% of publications cite tomato as one of the main affected crops, followed by potato at 19% and rice at 9%. The main reported diseases are wilt caused by bacteria and fungi, representing approximately 50% of the citations, followed by bacterial spot at 23%, fire blight at 11%, bacterial canker at 10%, and leaf blight, representing about 7% of the publications (Figure 2).

FIGURE 2: Distribution of citation density found in C1. A) Qualitative of the main affected crops. B) qualitative of the most cited diseases.
≅ : approximately or equal to.
*: <1%.



Source: The Authors (2023)

The Solanaceae family is one of the most commercial interest vegetable families (TONI *et al*., 2020). Among the solanaceous species, the tomato stands out because it has great commercial value, ranging from, in-natura consumption with several cultivars to various processed products such as sauces, extracts, juices, dried tomatoes, and ketchup, making this a market estimated at around 1 billion dollars (BARRAZA *et al*., 2020).

Losses accumulated in the field caused by phytopathogen diseases, especially the blackleg and bacterial spot, have been attracting more and more attention, because great demands such as tomatoes, potatoes, and rice, among others, are affected, coinciding with great economic damage. (MAJEED; MUHAMMAD, 2019).

Diseases caused by other microorganisms and parasites such as viruses, viroids, oomycetes, actinomycetes, insects, and nematodes represented less than 15% of the quantitative analyzed. Although diseases caused by viruses can seriously compromise the affected crop, resulting in the loss of a large part of the planting (PANNO *et al*., 2021), they had a citation density of less than 10% compared to bacteria at 46% and fungi 39%.

Among microorganisms found in the analyzed studies, the main cited bacterial genera are *Pseudomonas* spp., *Xanthomonas* spp., *Pectobacterium* spp., *Ralstonia* spp., and *Clavibacter* spp. The main pathovars among the cited genera were *Pseudomonas syringae* pv. *syringae*, *Xanthomonas campestris*, X. *oryzae*, X. *vesicatoria*, X. *euvesicatoria*, and X. *perforans*, *Pectobacterium cavotovorum* subsp. *cavotovorum*, *Ralstonia solanacearum*, and *Clavibacter michiganensis* subsp. *michiganensis*. The genera *Pseudomonas* spp. and *Xanthomonas* spp. made up more than 50% of the phytopathogens. Only the genus *Clavibacter* spp. belong to the group of Gram-positive bacteria, and both groups have bacillary morphology and cosmopolitan distribution (CARMO *et al*., 2021).

Biotic and abiotic factors such as heavy rain, high relative humidity, can contribute to the development of the disease, since leaf tissue lesions caused by strong winds can serve as the initial point of infection. Other sources such as contaminated water, infected vegetable remnants, and contaminated tools can also assist in the long-term propagation and persistence of pathogens in the environment (RYAN *et al*., 2011).

MAIN PESTICIDES AND ANTIBIOTIC CLASSES USED IN AGRICULTURE

In an attempt to contain, elaborate or execute pest control techniques, it is fundamental the prior knowledge of the genera and species of installed microorganisms, given that the first line strategy against microorganisms (bacteria, viruses and fungi) or other pests (insects and nematodes) is the application of chemical and antibiotic pesticides (BUTTIMER *et al*., 2017).

Copper oxychloride, Mancozeb, or the combination of both and dichloro-diphenyl-Trichloroethane (DDT) constituted about 80% of the pesticides found in the analyzed works, all cited pesticides are classified as extremely toxic (AGROFIT, 2022), others with high carcinogenic potential in particular DDT (INCA, 2022). Another problem encountered is that some products are reported as counterfeit or poor quality (BALAYIANNIS *et al*., 2009).

These approaches need to be used with caution or updated, because in addition to contamination of water and soil by pesticide waste, the use of banned market products aggravates environmental pollution and raise the risks of disease development in humans and animals (LOPES-FERREIRA *et al*., 2022), contributing with increased public health expenses and the emergence of resistant bacterial isolates (SONG *et al.*, 2021).

The use of antibiotics used in the field is another approach frequently used, nevertheless, it is not as comprehensive as clinical practice (KARIM; HOSSAIN, 2018), streptomycin and oxytetracycline were the main antibiotics reported in the studies analyzed (98%). Although classes such as; Penicillin, ampicillin, tetracycline, and gentamicin were reported, they represented less than 2% of the data analyzed.

Sturdy strains for antimicrobials mentioned are often reported (SONG *et al*., 2021). Since its introduction to the US market in 1955 streptomycin was the first-choice antibiotic for the protection of crops, its constant use, led to resistant isolates and was later discouraged for use in the European Union.

Like the other antibiotic classes mentioned, antimicrobials have been losing space over time due to the emergence of super bacteria, making treatment alternatives even more scarce (McMANUS *et al.*, 2002).

QUANTITATIVE AND QUALITATIVE DATA ON BIOLOGICAL CONTROL AGENTS, ANTIBIOTICS, PESTICIDES, AND PATHOVARS ASSOCIATED WITH CULTIVATIONS OF INTEREST

In C2 profile, which represents more than 70% of the analyzed works, there was a predominance of the combination of non-pathogenic bacteria and fungi as biological control agents (BCA), antibiotics, pesticides, and secondary metabolites, tested alone or in combinations, representing 52 % of publications. The use of bacteriophages as a therapeutic approach accounted for 40% while isolated pesticide use or its combination with other formulations represented approximately 8% of the data analyzed.

Among the phytopathogens, there was a similarity to the group of pathogens reported in C1, although the genus Xanthomonas spp. has predominated in C2. This change of focus with a bias toward Patovars of the genus Xanthomonas spp. It may indicate that these bacteria have a greater economic impact, compared to the genera Pseudomonas spp. Erwinia spp. Ralstonia spp. and Clavibacter spp. However, the high density of publications of the genres cited (21%, 11%, 11%, and 7% respectively) make these pathovars, a constant target of research due to the potential damage caused by the various fruit, vegetables, and cereal cultivars.

Like the data analyzed in C1, the profile of fruits, vegetables, and cereals was similar to the data found in C2, presenting subtle changes in relation to crops such as potatoes, rice, and kiwifruit. Tomato cultivation still remained in focus as well as C1. This may indicate the importance of analyzed crops, and light changes may also suggest that approaches using BCA and bacteriophages may be migrating to other cultivars of interest.

The qualitative data from bacteria and fungi strains used as a form of biological control most prevalent in publications were; *Pseudomonas Fluorescent*, *Bacillus Subtilis*, B. *Velezensis*, B. *Amyloliquefaciens*, B. *thuringiensis*, and *Trichoderma* spp. Cupric bactericides and acibenzolar-s-Methyl (ASM) were the most used compounds, oxytetracycline, and streptomycin were the most frequent antibiotics.

METABOLITES PRODUCED BY BCA

The adoption of approaches including BCA is not new, the *pseudomonas fluorescent* bacteria was one of the most employed species in an attempt to suppress phytopathogens in the field among the data analyzed in C2. Among the metabolites produced by this species, one of the main inhibition factors and bactericidal activity to pathogens besides the production of bacteriocin, and other substances, is the production of siderophore, pseudobacin, and pioverdine.

The main mechanism of action of siderophores is that these molecules act by chelating the available iron in the surrounding environment, making it difficult or impossible for the pathogen to survive, since iron is used in some enzymatic reactions or as cofactors, thus iron deficiency can cause inhibition of microorganism growth through the reduction of DNA molecule synthesis (KUMAR *et al*., 2012).

Other microorganisms that are candidates for biological control are strains of the Bacillus genus. The main characteristics of this BCA genus in the control of diseases caused by various pathovars are the promotion of plant growth through the increase of root contact surface, acting to increase nutrient absorption by the plant and also in the production of antibiotic substances (ELANCHEZHIYAN *et al*., 2018).

Since their first reports around 1900, Bacillus spp. strains have been employed in the control of insect, fungal, and phytopathogenic bacterial species. The production of insecticidal and antibiotic proteins is the main characteristic of biocontrol through antagonistic activity (PÉREZ-GARCÍA *et al*., 2011).

During the sporulation process, crystalline inclusions containing proteins called δ-endotoxins or Cry proteins and Cyt cytolytic toxin are produced, which are highly specific to insects and larvae but harmless to humans. Several natural products have been developed and successfully used in the field based on proteins produced by Bacillus thuringiensis (PÉREZ-GARCÍA *et al*., 2011).

Of the antibiotic products produced by Bacillus spp. strains, lipopeptides are the most frequently cited molecules with potential biological control activity. These are amphiphilic molecules, where one part of the molecule is hydrophilic, characterized by promoting interaction or affinity with water molecules, while the hydrophobic part exhibits aversion to water due to opposite polarity. Lipopeptides can be classified according to the sequence of amino acids, and can be surfactins, iturins, fengycins, or bacillomycins (BRAVO *et al*., 2007).

Surfactins are a class of compounds that act as biosurfactants, their ability to reduce surface water tension and form micelles, makes this molecule a candidate for use as a natural antimicrobial, as well as the potential synergistic effect with iturine A, fengicins, and bacilomine D (AMBRICO; TRUPO, 2017).

The combined action of surfactin and the class of Iturins occurs with Iturin A promoting the removal of calcium (Ca) and magnesium (Mg) cations from the cell wall of microorganisms, especially fungi. In the membrane, it acts by inducing the formation of ion channels, promoting electrochemical instability of K+ ions, and can act alone or create space for the action of surfactin. The latter acts on the pathogen's cell membrane due to its surfactant properties, destabilizing the cell membrane by forming micelles, creating pores, and causing cell lysis. This makes use of Bacillus spp. strains and other microorganisms an increasingly desirable approach (AMBRICO; TRUPO, 2017).

COPPER-BASED PESTICIDES AND THEIR RELATIONSHIP WITH THE ENVIRONMENT

Synthetic compounds, especially cupric bactericides, as well as other chemical pesticides, have been losing efficacy as resistant bacterial isolates arise due to the excessive spraying of these compounds. Copper can act both in the interruption of enzymatic activity of various metabolic pathways of target pathogen and protein cleavage by the formation of intracellular complexes, making this pesticide widely used in various crops (PEREIRA *et al*., 2021), being one of the compounds most cited in the data in C2.

However, the same method of control may not be so efficient against all phytopathogens, for example, pectobacterium carotovorum subsp. carotovorum bacteria causing soft rot is reported by approximately 17% of the studies analyzed in C2, but there are no chemical pesticides recorded in Brazil against this phytopathogen (CARMO *et al*., 2021).

The lack of registration in certain countries, added to the poor quality of certain pesticides, as well as in C1 data, lead to overdose or the acquisition of low-quality products, banned or counterfeit, especially in border areas, where an estimated approximately 20% of pesticides that supply Greece and up to 10% of products marketed throughout Europe are illegal (BALAYIANNIS *et al*., 2009).

Another problem originating from this practice is environmental contamination, different pesticide concentrations; Aldrin, Chlordane, Dieldrin, Endrin, Heptachlor Epoxide, Lindane, 4.4'-DDT, Endosulfan, Chlorphrifos, Diazinon, Methyl-Pathion, and Malation, were detected in water and solid particles at almost 10 sampling points in the Uruguay River Basin, all above the values advocated by international authorities (WILLIMAN *et al*., 2017).

In addition to environmental contamination, pesticides may exposure in organic agriculture, through residual amounts of impurities from the active ingredient of pesticides detected in sulfur packaging and other components used by farmers who use or prioritize only natural products, lighting the alert of how much In fact organic products are free of pesticide residues (BALAYIANNIS *et al*., 2009).

SYSTEMIC RESISTANCE INDUCER AND FORMULATIONS BASED ON BACTERIOPHAGES

Presenting another approach, Acibenzolar-S-Methyl (ASM) is a systemic resistance inducer, representing approximately 10% of the data in C2. It performs a similar function to salicylic acid and methyl jasmonate, stimulating plant defense responses rather than directly acting on the pathogen. ASM application typically involves spraying in combination with other products, such as copper pesticides, antibiotics, or BCA, presenting good results in synergy (CAVALCANTI *et al*., 2006). Nevertheless, it is worth noting that problems related to phytotoxicity and reduced productivity should be observed (LANG *et al*., 2007).

Strategies based on biological control such as phage spraying or a combination of phages, copper-based products, or ASM show good efficacy, usually better than just one of the strategies (OBRADOVIC *et al*., 2005). It is necessary to emphasize that copper-based products and other pesticides can inactivate phage formulations, reducing their efficacy (BUTTIMER *et al*., 2017). Phages associated with ASM can provide efficacy greater than 85% in certain cases (IBRAHIM *et al*., 2017). This synergy occurs due to the Phages action on the pathogen population, consequently reducing the hypersensitivity reactions (HR) promoted by ASM (OBRADOVIC *et al*., 2005).

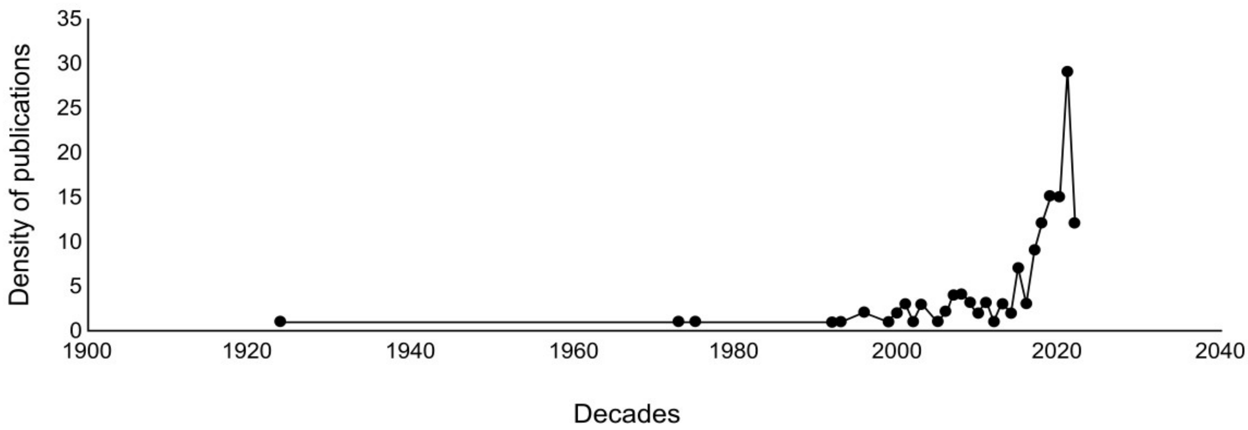
START OF PHAGOTHERAPY AND TRENDS

Around 40% of therapeutic approaches analyzed in C2, the use of bacteriophages as a therapeutic alternative to chemical pesticides and antibiotics has been gaining prominence over time, although the use of phages in the field is almost as old as bacteria of the genus Bacillus spp. (PÉREZ-GARCÍA *et al*., 2011). This strategy was overshadowed by the use of the first antibiotics, which exhibited better short-term efficacy (LIU *et al*., 2021).

The first associations of bacteriophages with phytopathogenic bacteria were observed by Hallmann and Hemstreet, where the use of decomposed cabbage filtrate was employed in the control of *Xanthomonas campestris* pv. *campestris*, opening the way for phages to be tested against different pathovars in various crops (MALLMANN; HEMSTREET, 1924).

According to the data analyzed only in the studies selected for this review, it was possible to draw a parallel between the emergence of the first products based on biological control agents (BCA and phages) with the density of publications (Figure 3) and the increasing market interest in alternatives to antibiotics and pesticides since the 2000s, given that in the early nineteenth century, the issue of bacterial resistance was not yet a problem, as it coincides with the emergence of the first reports of antibiotics (PEREIRA; PITA, 2005).

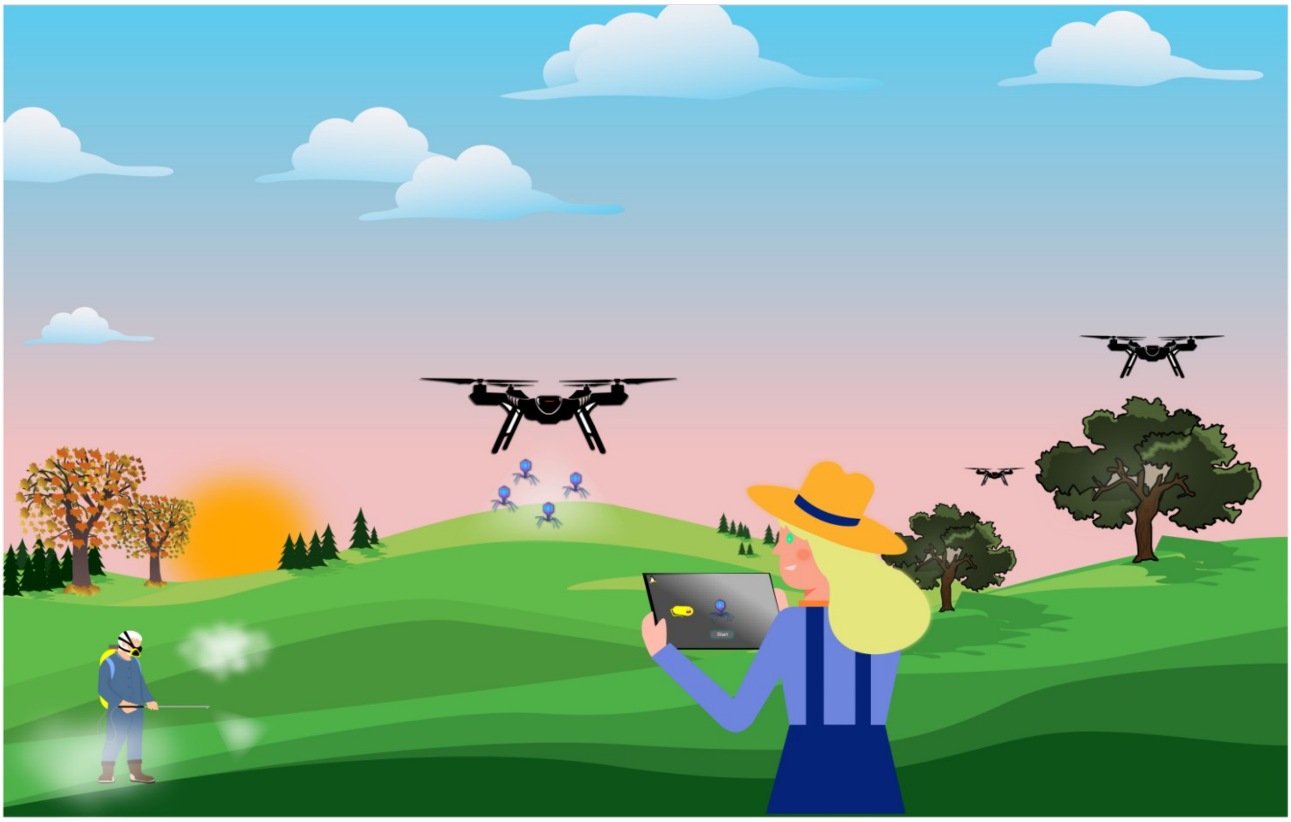
FIGURE 3: Increase in the number of publications over time, related to microorganisms such as bacteria, fungi, and viruses used as biological control.



Source: The Authors (2023)

Another point is that, in addition to the pressure exerted by bacterial resistance, the second or third generation of early nineteenth-century farmers, having more affinity, skill, and greater interest in the Internet of things, may have influenced the search for other approaches (figure 4).

FIGURE 4: Meeting of different generations of farmers. On the left, the man representing the first generations of farmers, still linked to old customs, such as the use of pesticide spraying manually. On the right, the young agriculture riding a drone, spraying microorganisms in an extensive area in the field with the help of connectivity and the internet of things.



Source: The Authors (2023)

MAIN FAMILIES OF BACTERIOPHAGES, RELATIONSHIP WITH NON-TARGET BACTERIA, INDUSTRIAL PROFILE, AND PROTECTION AGAINST UV RADIATION

In the qualitative data analyzed in C2, 5 Phages families were found; Podoviridae, Myoviridae, Inoviridae, Siphoviridae, and Cystoviridae were the most prevalent, representing 22%, 21%, 7%, 5%, and 2% respectively. 42% of the studies did not mention the family of the phages used, and only 1 study cited a phage with different characteristics, suggesting its classification in a new genus (LIU *et al*., 2021).

The origin of the phages analyzed in C2 did not follow a pattern, with phages obtained from various sources reported, including vegetable, and fruit remains obtained from different sampling points, soil samples from different depths and surrounding particulate matter, fragments of fruit lesions, leaves, stems, runoff water from rivers, lakes and streams, surface waters, water from fruit and vegetable processing, and river deltas. This indicates that phage screening is relatively simple given their wide dispersion in nature due to their contact with their host.

Although it is possible to obtain phages from virtually any soil, water, or organic matter sample, caution is needed when using phages for biological control with a broad spectrum of action. While a phage that has multiple bacterial hosts as targets are desirable, avoiding or reducing the need for phage cocktails, rare but not impossible elimination of non-target bacteria in the field and enterobacteria related to humans may occur when lytic phages are isolated from places like sewage (BUTTIMER *et al*., 2017).

Phages are subject to two main life cycles, where, after adsorption and incorporation of genetic material into the bacterial host, some phages develop the lytic cycle while others develop the lysogenic cycle (CLOKIE *et al*., 2011). In the virulent or lytic cycle, phage proteins hijack the cellular machinery of the target cell, where viral proteins are produced, resulting in the production and formation of viral particles, ultimately lysing the bacterial cell and releasing phages in the process. In the lysogenic or temperate cycle, phages integrate their genome with that of the host, potentially remaining dormant for several generations and incorporated into the bacterial genetic material until the lytic cycle is induced (HOCKENBERRY; WILKE, 2021).

Although the use of copper-based pesticides still accounts for a significant portion of products used in the field (GRIFFIN *et al*., 2017), the adoption of phage-based products is gaining momentum, with companies such as the American OmniLytics, which developed one of the first phage-based products, the biopesticide AgriPhage, in the fight against strains of the Xanthomonas spp and Pseudomonas spp genera. Otsuka Pharmaceutica developed the product XylPhi-PD, a phage mixture used in the protection of grape crops. In Europe, the Hungarian company Environivest and APS Biocontrol in Scotland developed the products ErwinPhage® Plus and Biolyse® PB in the fight against diseases such as fire blight and soft rot, respectively (STEFANI *et al*., 2021), making biopesticides or bioprotectors the subject of intense studies (COLLINGE *et al*., 2022).

The most reported phage formulations in C2 are cocktails, which may contain several phages with affinity to various hosts, which may be from the same family or not (ZACZEK-MOCZYDŁOWSKA *et al*., 2020). It is also suggested to use products such as corn flour, skim milk, casein, sucrose, and lignin, as they contribute to the persistence of the phage in the field, avoiding the inactivation of viral particles due to direct UV radiation exposure from continuous sunlight (VU; OH, 2020).

The lyophilization of phages with 0.5 M Trehalose allowed the product to remain viable for at least 90 days, avoiding the incidence of the disease and maintaining the product stable in comparison to non-formulated viral particles in the study by ÁLVAREZ *et al*. (2022). Phages that receive these products tend to persist in the environment, as the formulations act to minimize the impact of UV radiation directly on the phage DNA, in addition to helping to steady the virions due to the contact of the product with the surface of the plant (BALOGH *et al*., 2018). Another strategy adopted to avoid inactivation by UV radiation is applications in the late afternoon or early morning (BALOGH *et al*., 2018). Cocktails with multiple phages are usually used to prevent phytopathogens from acquiring resistance to a single phage, allowing pathogenic microorganisms to be eliminated before resistance arises, but eventually, a phage-resistant strain may emerge; however, the emergence of phage resistance results in decreased or absent pathogenicity, causing the pathogen to evolve into a weakened resistant phenotype (WAGEMANS *et al*., 2022).

The traditional control methods for diseases caused by phytopathogens (antibiotics and pesticides) have faced some resistance from consumers, especially in Europe, raising an alert about possible health and environmental damage, demanding other therapeutic approaches for pest control (RODRÍGUEZ *et al*., 2019).

CONCLUSION

Phytopathogenic diseases in commercial crops result in considerable economic losses, highlighting the need for continuous research on the subject. Understanding the microbial profile of microorganisms installed in crops is crucial for the development of effective pesticides, less polluting and offering less risk to human health. Thus, the use of bacteriophages has gained prominence as a therapeutic approach to replace chemical pesticides.

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