Microorganisms and Biological Pest Control: An Analysis Based on a Bibliometric Review

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Abstract: The use of microorganisms for biological pest control as biological control agents (BCAs) and biopesticides was developed worldwide in the 1960s. Despite the abundance of reviews published on this topic, no meta-analysis using bibliometric tools has been published. The objective of this study was to determine patterns of research on microorganisms for the biological control of pests, based on publications available in the SCOPUS® database. The data were obtained from the Elsevier® Scopus meta-database using the search terms “biological pest control” and “microorganism” (title, abstract, and keywords). The main publications were identified, along with the journals, countries, and institutions that have published on the subject. The data were analyzed with VOSviewer to determine the co-occurrence of terms, and four maps were generated. The results show two phases in the scientific research on the subject: The first is the characterization of biological control agents, and the second is focused on the commercial development of biopesticides and biological control agents. The most recent research emphasizes the discovery of new species and strains that have commercial potential, with an emphasis on genetic engineering and biotechnology.

Keywords: biological control agents; biocontrol; biopesticides; Bacillus subtilis; Bacillus thuringiensis; mycoinsecticides; Trichoderma spp.

1. Introduction

The control of pests and weeds in agriculture, as well as insects that cause human diseases, is traditionally managed with chemical insecticides. However, the use of these products has given rise to many problems, such as increased resistance of pathogens and pests to various chemical compounds, as well as environmental pollution and its effects on human health such as cancer and several immune system disorders. As a result of the threat that the chemicals cause by their direct action and residual impact on human health and the environment, consumers increasingly demand pesticide-free foods [1]. In this context, microbial insecticides have arisen as substitutes for chemical products for the control of pests [2,3] and vectors that transmit diseases in humans [4,5] and in animals [6]. Presently, there is a range of microbial insecticides derived from bacteria [7,8], fungi [9], nematodes [10], and viruses [11,12].

Plant growth-promoting agents represent another use of beneficial microorganisms in agriculture [13,14]. Among the most thoroughly-studied species are the rhizobacteria, which are found in diverse environments and are not only characterized by their promotion of plant growth,
but they are also used in order to improve the soil as biofertilizers, root pathogen growth suppressors, inoculants, and pesticides [14–16].

Research on the use of microorganisms for the biological control of pests and diseases in agriculture has been present in the scientific literature since the 1970s. This topic has been widely explored in various areas of knowledge such as characterization of useful species for these purposes [17], genome analysis [18], determination of insecticidal effect [19], genetic manipulation and evaluation [20, 21], insect population resistance [22], their use as biofactories [23] and bioregulatory agents [11], and evaluation of commercial production [24,25].

One of the most studied microorganisms for the biological control of pests and diseases in agriculture is *Metarhizium* spp. The studies regarding this microorganism have addressed diverse topics [26], such as: molecular characterization of hosts isolates [27], pest response to microbial infection [28], mycoinsecticide lethal effects [29], effect of isolate enzymes on hydrolyzing insects cuticles [30], strain selection and formulation [31], application techniques for microbial agents [32], droplet size spectra of oil-based formulation sprayers [33], effects of storage duration and environment on fungal spore survival [34], and mass production of microbial control agents [35], to name some.

Microbial insecticide’s effectiveness has been studied worldwide in a variety of pests of diverse crops, e.g., sugarcane [36], tobacco [37], wheat [38], potato [39], mango [40], tomato [41], and berries [42]. They have been evaluated under diverse conditions: laboratory [43], open fields [44] and greenhouses [45]. They had become a fundamental component for diverse integrated pest management programs [46] and organic production [47]. As presented above, the abundance of published research on the subject makes it relevant to utilize methods based on data meta-analysis such as bibliometrics, which uses statistics and visualization methods in order to explore structures and patterns in scientific production [48], therefore allowing for a larger scientific evolution analysis of the field. This method focuses on a specific research topic; in this case, databases of journals with recognized international prestige were used, which allowed for a global and historical perspective of the phenomenon. Due to the abundance of information regarding the topic, it is impossible to make an exhaustive review, therefore a mini-review remains useful for summarizing the current research. The objective was to establish research patterns in the use of microorganisms for the biological control of pests, based on publications available in the Elsevier® SCOPUS® database.

2. Materials and Methods

Bibliometric mapping approach is data driven and relies heavily on computer algorithms and visualization techniques. The map is a visual representation of the topic by showing the relationship between key terms in the field. One of the most popular software used by researchers in the area of scientometrics, which is the study of science [49], is VOSviewer [50], which uses keywords (author and index ones) to create co-occurrence maps. The maps normally guide the analysis, but the expertise in the field is still necessary for a proper interpretation of the map. The bibliometric mapping also generates clusters of keywords that then need to be reviewed by an expert. The expert needs to understand each cluster and give sense to the data. This has an advantage, as it does not allow the expert to be bias in its own expertise [51]. The disadvantage of this kind of study is that many valuable publications might not have been cited due to the quantity of documents being analyzed. Finally, it should be stated that as bibliometric studies do not involve human or animal subjects, therefore, no ethical approval by Institutional Review Board is required.

This literature review followed a quantitative analysis using two methods: performance (analysis of publications based on authors, countries, and institutes) and science mapping (using bibliometric software) [48]. The study of the publications was conducted using keywords. The Elsevier® Scopus library services metabase (www.scopus.com) was chosen to retrieve literature due to its coverage. This database has some advantages over free databases such as Google scholar, which is larger in terms of coverage but less accurate [52].
Originally, the words “biological pest control” were used, searching in the title, abstract, and keywords, which generated 31,130 documents (18 September 2020). However, there are many concepts related to this area of knowledge that are named in different ways, which makes it difficult to gain a clear perspective. Thus, in order to perform a more focused review, we used the same three words in quotation marks and the word “microorganism” was added, since we wanted to focus the review on this particular type of biological control agents, which reduced the number of documents to 1421 (18 September 2020). No time limitations were imposed as it was intended to demonstrate the concept evolution over time. No documents were excluded from the analysis, therefore, journal articles, review chapters, conference articles, short surveys, notes, book chapters, editorials, letters, and errata were included in the analysis. The performance analysis of data was done using Scopus analyze function, which reduces analysis oversights due to reprocessing the original data.

Bibliometric studies have the disadvantage that they do not allow to present all data. Therefore, researchers need to establish cutoff points for the performance analysis of journals, countries, and institutes; 10 was the limit, as it was used in previous publications [52]. Regarding the most cited articles, the cutoff point was 15. Statistical Package for Social Sciences (IBM-SPSS 20) was used to create the graph regarding growth of publications over time.

Content Analysis

VOSviewer software [50] was used for the analysis. A total of 11,012 keywords were retrieved, a minimum number of occurrences of a keyword of 10 was used, 764 meet the threshold, and only 500 were included in order to have an appropriate label visualization. A co-occurrence analysis of keywords and academic terms was performed for the titles and abstracts of the publications, using a co-occurrence method, showing only the elements connected with others, and the normalization method was association strength (AS), with 1.00 resolution, 100% visualization scale, TLS (Total Link Strength) weight, 50% label variation size, and 30% kernel width. The full counting method was used, with a number of records for each term ≥10, and a minimum cluster size of 15 [53]. A network visualization map was created using the retained set of terms. The algorithm was designed so that co-occurring terms are positioned closer to each other, and so that those with greater frequency have larger circles. Terms that were irrelevant to the map were eliminated [54].

3. Results

The number of publications that address issues related to biological control is very high, which makes it difficult to gain a general perspective of the development of research in this area of knowledge. This section provides a bibliometric analysis of the publications related to microorganisms and biological pest control.

3.1. Performance Analysis

The analysis period was from 1973 to 2020 (September 18). The documents found in the database analyzed were as follows: 1107 articles, constituting 83% of the total number of documents. Other document types were review chapters (171), conference articles (23), short surveys (23), notes (13), book chapters (8), editorials (2), letters (1), and errata (1). The number of publications on this topic begins in 1973, maintaining constant growth until reaching its peak in 1999. In the next three years, the amount of published research decreases, followed by a resurgence of growth that reaches another peak in 2011 with a subsequent downward trend (Figure 1). For the entire period, the mean was $34.6 \pm 27.2$ articles, with a minimum of 1 (1987) and a maximum of 89 (1999). In 1998, the number of publications per year surpasses 50, which can be considered an inflection point for publications in this area. There are two clearly defined periods, the first of which (1973–1997) has a mean of $6.3 \pm 5.8$ articles, with a minimum of 1 (1987) and a maximum of 21 (1997), while the second period (1998–2019) showed a mean of $56.9 \pm 12$ articles, with a minimum of 38 (2016) and a maximum of 89 (1999).
Of the total number of documents, 1331 were cited, producing a total of 73,915 citations. There are 7 articles with over 1000 citations; 11 had between 500 and 999 citations; 148 had between 100 and 499; 759 had between 11 and 99 citations; and 406 articles had fewer than 10. On average there are 56 citations per document for the period analyzed.

Table 1 shows the 10 main journals, countries, or regions and institutes that are publishing topics related to “biological pest control” and “microorganism.” Out of the 380 journals that had documents on the subject, the 5 journals with the highest number of publications represented 21% of the total, and they are as follows: Applied and Environmental Microbiology, Journal of Invertebrate Pathology, Journal of Applied Microbiology, Applied Microbiology and Biotechnology, and Pest Management Science. The journals with an emphasis on biotechnology and microbiology were notable: The publications in these journals relate to the evaluation of the biological pest control, the use of microorganisms for pest control, as well as the impact of their use on the agri-food sector and the environment.

With regard to the countries or region from 1973 to 2020 (September 18), we observed that the United States was the country with the highest number of contributions (351), which is due to the prolific nature of its institutes and universities that perform research in this area. China showed the second-highest number of contributions, followed by India, France, and the United Kingdom.

There are contributions from authors representing 160 institutions, among which the United States was most prolific, with 6 of the top 15. The three institutions with the highest number of publications were: USDA, ETH Zurich, and the University of Florida.
Table 1. Performance analysis: Journal, Country, and Institute for “biological pest control” and “microorganism” from 1973 to 2020 (18 September).

| Rank | Journal | Pub. | Country | Pub. | Institute | Pub. |
|------|---------|------|---------|------|-----------|------|
| 1    | Applied and Environmental Microbiology Journal of Invertebrate Pathology Journal of Applied Microbiology Applied Microbiology and Biotechnology Pest Management Science Canadian Journal of Microbiology Journal of Economic Entomology Biological Control Frontiers in Microbiology Letters in Applied Microbiology | 97 | United States | 351 | USDA Agricultural Research Service, Washington DC | 65 |
|      |         | 81   | China   | 167  | Department of Agriculture | 45 |
| 3    |         | 47   | India   | 111  | ETH Zurich | 32 |
| 4    |         | 39   | France  | 82   | University of Florida | 22 |
| 5    | Pest Management Science | 36   | United Kingdom | 82   | Wageningen University and Research Centre | 20 |
| 6    | Canadian Journal of Microbiology | 33   | Germany | 76   | Ministry of Education China Chinese Academy of Agricultural Sciences Université de Lausanne UNIL USDA ARS | 19 |
| 7    | Journal of Economic Entomology | 29   | Spain   | 76   | | |
| 8    | Biological Control | 28   | Brazil  | 69   | | |
| 9    | Frontiers in Microbiology | 25   | Canada  | 57   | Beltsville Agricultural Research Center | 18 |
| 10   | Letters in Applied Microbiology | 23   | Switzerland | 53   | Zhejiang University | 18 |

Source: Own elaboration with data from SCOPUS.

Table 2 shows the most-cited articles on microorganisms and biological pest control. The most notable topics were antagonists, or microorganisms for control of phytopathogens, as well as growth-promoting microorganisms (which stimulate root growth, fix nitrogen, and solubilize phosphorus or microelements). These articles allowed us to determine the most relevant topics in this area of knowledge: *Trichoderma*, rhizobacteria, *Pseudomonas*, probiotic bacteria, endophytic bacteria, *Wolbachia pipientis*, non-pathogenic interactions, entomopathogenic bacteria (*Bacillus thuringiensis/Bacillus subtilis*), *Azospirillum*, *Aspergillus flavus*. Some reviews regarding biological control agents and biocontrol were also included.

Table 2. The 15 most-cited articles for “biological pest control” and “microorganism” from 1973 to 2020 (18 September).

| Rank | Author (year) | Title | Journal | Citations |
|------|---------------|-------|---------|-----------|
| 1    | Harman, et al. [55] | *Trichoderma* species—Opportunistic, avirulent plant symbionts | Nature Reviews Microbiology | 1806 |
| 2    | Lugtenberg and Kamilova [56] | Plant-growth-promoting rhizobacteria | Annual Review of Microbiology | 1626 |
| 3    | Haas and Défago [57] | Biological control of soil-borne pathogens by fluorescent pseudomonads | Nature Reviews Microbiology | 1337 |
| 4    | Compant, Duffy, Nowak, Clément and Barka [1] | Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects | Applied and Environmental Microbiology | 1258 |
Table 2. Cont.

| Rank | Author (year) | Title | Journal | Citations |
|------|---------------|-------|---------|-----------|
| 5    | Verschuere, et al. [58] | Probiotic bacteria as biological control agents in aquaculture | Microbiology and Molecular Biology Reviews Journal of Experimental Botany | 1206 |
| 6    | Whipps [59] | Microbial interactions and biocontrol in the rhizosphere | Journal of Experimental Botany | 1068 |
| 7    | Hallmann, et al. [60] | Bacterial endophytes in agricultural crops Wolbachia pipientis: Microbial manipulator of arthropod reproduction | Canadian Journal of Microbiology | 1039 |
| 8    | Stouthamer, et al. [61] | Nonpathogenic interactions of bacteria in soil | Annual Review of Microbiology | 906 |
| 9    | Dillon and Dillon [18] | The gut bacteria of insects: Nonpathogenic interactions | Annual Review of Entomology | 804 |
| 10   | Gatesoupe [62]. | The use of probiotics in aquaculture | Aquaculture | 791 |
| 11   | Berg [63] | Plant-microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture | Applied Microbiology and Biotechnology | 705 |
| 12   | Ferré and Van Rie [22] | Biochemistry and genetics of insect resistance to Bacillus thuringiensis Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control | Annual Review of Entomology | 683 |
| 13   | Bravo, et al. [64] | Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon | Toxicon | 679 |
| 14   | Hoitink and Boehm [65] | Bacillus (insecticidal activity, metabolism, and molecular aspects); genetics (RNA, DNA, genetic analysis); and the nematode Meloidogyne | Annual Review of Phytopathology | 607 |
| 15   | Vorholt [66] | Microbial life in the phyllosphere | Nature Reviews Microbiology | 600 |

Source: Own elaboration with data from SCOPUS.

3.2. Science Mapping

Term co-occurrence analysis provides an overview of research trends by reflecting the topics addressed. The analysis was performed using VOSviewer software. The VOSviewer results showed 11,012 keywords, of which those that had occurrences greater than 10 were retained, and generic terms related to the research process (e.g., “problem,” “research”) were eliminated. Thus, 495 terms were retained, organized into 5 clusters with 66,471 links: bacteria and fungi (enzymes, genome, growth, genes); biological control (virulence, spores, infection); Bacillus (insecticidal activity, metabolism, and molecular aspects); genetics (RNA, DNA, genetic analysis); and the nematode Meloidogyne. Figure 2 shows the evolution of topics over time. Those that appear in blue are topics present at the beginning of the year 2000, while red topics were more common in the years after 2015. The most recent topics are related to genomics, while initial topics centered on prevention and control of insects.
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Figure 2. VOSviewer overlay visualization for “biological pest control” and “microorganism” from 1973 to 2020 (18 September).

When the analysis was divided into two periods, one spanning from 1973 to 1997 (Figure 3) and the other from 1998 to 2019, variations were observed with respect to the topics addressed in the two periods. In the earlier period, the relevant topics were (1112 keywords, 5 occurrences): *Bacillus thuringiensis* (red cluster); insects (green cluster); and microorganisms (blue cluster). The contributions in this time span were focused on determination of the viability and characterization of the growth and fungicidal action of microorganisms used for the biological control of insects.

Figure 3. VOSviewer overlay visualization for “biological pest control” and “microorganism” from 1973 to 1997.
In the mapping that covers the years 1998 to 2020 (18 September), there are many concepts that are not related to the rest (concepts without lines); however, the emphasis was on the use of the concepts “biocontrol” and “pest control” (Figure 4). The green cluster encompasses aspects related to different bacteria used for pest control (e.g., hemiptera, arthropods) and aspects associated with their viability (e.g., virulence, sporulation, toxicity, proteins, mortality, ecology). The blue cluster focuses on genomics, sequencing, gene expression, DNA, ribosomes, etc. The red cluster brings together concepts related to fungi that affect both plants and soil, as well as beneficial microorganisms such as rhizobacteria. There is a fourth, almost imperceptible, yellow cluster, which encompasses nematodes, and a fifth, even smaller, pink cluster, which includes aspects related to plant extracts.

![Figure 4. VOSviewer overlay visualization for “biological pest control” and “microorganism” from 1998 to 2020 (18 September).](image_url)

4. Discussion

4.1. Biological Control

There is no single definition of biological control, or biocontrol. The term refers to the use of organisms and microorganisms, including bacteria, viruses, fungi, and nematodes, in order to improve the management of diseases, pests, and weeds or other vermin with an emphasis on commercial products or biopesticides [24]. The objective of biological control is to reduce the populations of pests and diseases (pathogens) to levels that do not cause damage to crops, in order to produce higher-quality food and better nutritional content [67]. There are three mechanisms by which microorganisms act in biological control: (a) competition, (b) antibiosis, (c) parasitism/predation, and (d) induced systemic resistance [65].

Despite advances in the characterization and evaluation of biological control microorganisms, their use remains limited by factors such as the specificity of some microorganisms, which control only one pest and not all those that can occur in a crop; they are not capable of having a significant effect on the control of the pest, or they only provide partial control [65] or their efficacy is variable due to different climatic conditions [68]; as well as high production costs [69]. However, some biological
control agents have been developed for commercial purposes, using fermentation, preservation, and storage techniques [3]; for example, the genus \textit{Trichoderma} and several species of \textit{Bacillus} [70]. For 2009, microbial biopesticide sales are estimated to have reached $1.6 billion dollars, 3.5% of the agrochemical market [69].

Biological control by microorganisms has been widely used for: the control of Citrus \textit{Diaprepes abbreviatus} with \textit{Steinernema riobrave}; \textit{Pasteuria penetrans}, \textit{Arthrobotrys anchonia}, \textit{Alcaligenes faecalis} strain MOR02 for the control of plant parasitic nematodes [67,71]; the use of \textit{Leconicillium} entomopathogens ZJLS07, ZJLA08, and ZJLP09 for the control of the vector (\textit{Diaphorina citri}) of HLB (Huanglongbing) in citrus [72].

4.2. Biopesticides

Within this category are mycoinsecticides; there are several products based on various species and subspecies of fungi that have been used as active ingredients of this type of product developed during the last five decades [73,74]. Some examples are \textit{Beauveria bassiana}, \textit{Metarhizium anisopliae}, \textit{Isaria fumosorosea}, and \textit{B. brongniartii}, which are used in order to control 48 families of insects from the following orders: Hemiptera, Coleoptera, Lepidoptera, Thysanoptera, and Orthoptera. However, the most relevant microbiological control program is \textit{Metarhizium anisopliae} for the control of spittlebug in sugar cane and grasslands in South America [75], as well as \textit{Metarhizium flavoviride} for the control of locust and grasshoppers in Africa [28,30,32]. Research on this topic has centered around formulations and concentrations [76], with a commercial focus [32].

4.3. Rhizobacteria

The rhizosphere is the layer of the soil that is influenced by the root, and it is richer in bacteria than the rest of the soil. The microorganisms of the rhizosphere promote the secretion of metabolites that can be used by the plant as nutrients through various mechanisms such as biofertilizers, rhizoremediators, phytostimulants, and stress controllers. Some of these bacteria can be used in the biocontrol of bacteria, since they reduce the damage caused by pathogens through several mechanisms: antagonism; signal interference; predation and parasitism; induced systemic resistance; competition for the use of nutrients; and interference in the activity, survival, germination, and sporulation of pathogens [56].

4.4. Trichoderma

Another genus that has been studied is the \textit{Trichoderma} fungus, which has been used commercially for several years [68] for the control of soil, leaf, or vascular pathogens [3,77], although it is also known for various effects such as promotion of root growth and development, crop productivity, resistance to abiotic stress, and better assimilation of nutrients [55].

There are various products that have been developed commercially for use in protective agriculture, one of the most successful of which is \textit{Trichoderma harzianum} strain T-22. It is sold under various brand names worldwide. \textit{Trichoderma harzianum} strain T-39 competes for nutrients and interferes with the production of lytic enzymes, which slows the germination of the conidia of the pathogen \textit{Botrytis cinerea} [78]. It also exists in a commercial presentation [77].

4.5. \textit{Bacillus subtilis}

\textit{Bacillus subtilis} is a widely-studied microorganism [79], with few publications as a biological control agent, although it is available as a commercial product, e.g., \textit{Bacillus subtilis} strain QST713 [77]. It promotes plant growth, protects against attacks by pathogenic fungi, and degrades organic polymers in the soil [80]. It has been estimated to control more than 40 plant diseases [77], and its use has been reported in seeds for cotton, peanuts, soybeans, wheat, beans, and barley for the control of \textit{Rhizoctonia}, \textit{Fusarium}, \textit{Aspergillus}, among others [24]. The most thoroughly-studied topics have been: resistance, evaluation, plant growth, impact, and characterization.
4.6. *Bacillus thuringiensis*

Various species of *Bacillus* have achieved market penetration due to their ability to kill a range of invertebrate pests [69]. The bacteria *Bacillus thuringiensis*, for example, has the ability to produce proteins with insecticidal properties during its sporulation phase [81], and it has been used in order to control insects of the orders Lepidoptera, Diptera, and Coleoptera [2], and other invertebrates such as nematodes [64] and mites, generally in spray formulations and in transgenic crops [22]. The most recurrent themes related to this topic were related to mortality, concentration rate, exposure, action, pathogenicity, and production. This is evidence that the approach tends towards the commercial production of this microorganism.

5. Conclusions

A bibliometric review of microorganisms and biological pest control was conducted. The work covered various perspectives: structure of publications, most influential countries and institutes, analysis of term co-occurrence, and discussion of the most important terms in the most-cited articles. The conclusions follows:

The most cited topics refer to some species and genera in particular, which have proven to be broad spectrum, environmentally friendly, and not harmful to humans: *Trichoderma*, Rhizobacteria, *Bacillus thuringiensis*, *Bacillus subtilis*, nematodes, and several species of mycoinsecticides.

It should be noted that this particular topic demonstrates the advancement from basic science research to applied science and finally to the development of technological solutions, which explains the decrease in the number of publications, since researchers tend to focus on basic science, new discoveries, or hot topics.

Future contributions in this area of knowledge will focus on the discovery of new species and strains that have commercial potential with an emphasis on genetic engineering and biotechnology.

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