Article

Analysis of research on *Metarhizium anisopliae* in the last 40 years

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Abstract

Biological control is used for the regulation of pest insect populations and to avoid the induction of insect resistance due to the excessive use of agrochemicals, as well as to solve waste problems for the organic production of food. There are more than 750 species of entomopathogenic fungi that can be used for the biological control of pests, being *Metarhizium anisopliae*, one of the most used commercially. The objective of this review was to determine the most relevant issues regarding research concerning *M. anisopliae* during the period from 1976 to 2018. A revision was made in the meta search Scopus®, using as keyword *Metarhizium anisopliae* only in title for October 9, 2018. The main journals, countries and institutions that have published on the subject were identified. The data was analyzed using VOSviewer® software under the principle of co-occurrence of terms. The results show that this microorganism has been widely studied at an international level, especially in the period from 2008 to 2014. The investigations are concentrated in three clusters: production-activity, *Beauveria bassiana* and termites. It is concluded that this microorganism continues to be analyzed from a commercial perspective and because biological control agents tend to be local, their investigations will continue to concentrate on the development and characterization at the molecular level of local (native) strains with commercial potential.

Keywords: *Beauveria bassiana*, biological control, entomopathogens, pests, termites.

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Introduction

The excessive use of chemical products in agricultural crops is a problem, since some insects have developed resistance to them (Lezama-Gutiérrez *et al*., 2012). Therefore, it has become necessary to look for alternative means for the biological control of pests in order to minimize the use of these chemicals (Mirhaghparast *et al*., 2013). Biological control provides additional tools for the management of pests that break the resistance cycles of insects, as well as solve waste problems for the production of organic food (Fravel, 2005). There are more than 750 species of entomopathogenic fungi disseminated in the environment that cause fungal infections to certain insects, which can regulate up to 80% of their populations (Lobo *et al*., 2016). Among the most important genera are: *Metarhizium*, *Beauveria*, *Aschersonia*, *Entomophthora*, *Zoophathora*, *Hirsutella*, *Fusarium* and *Verticillium* (Acuña-Jiménez *et al*., 2015).

In this context *Metarhizium anisopliae* is a filamentous fungus characterized and used for the control of pests, in addition the use of its enzymes is used as a biological catalyst in the industrial sector (Alonso-Díaz *et al*., 2007). It is one of the most common species that has been found, studied and used throughout the world mainly as a biological control agent (ACB) (Curran *et al*., 1994). This fungus has been successfully applied since the 1970s in Brazil to control pests in sugarcane (Rangel *et al*., 2006). *M. anisopliae* has also been used as a biological control agent or ACB for pests of various insects: termites (Isoptera), lobsters (*Locusta migratoria* L.), spittlebugs (*Aeneolamia* spp., *Prosapia* spp., *Nilaparvata lugens* Stål), beetles (*Adoryphorus couloni* B., *Antitrogus parvulus* Britton, *Aphodius tasmaniae* Hope, *Oryctes rhinocerus* L.), screwworm (*Diatraea saccharalis* F.) and for the control of mosquito vectors of malaria, including their larval stages (Buti *et al*., 1994).

Research on the use of microorganisms for the biological control of pests in agriculture, is present in the scientific literature since the seventies, being a subject widely explored from various lines of knowledge (Camargo *et al*., 2016); however, to date there is no holistic review to understand what research has focused on this fungus. The objective of this review was to determine the most relevant issues regarding research concerning *M. anisopliae* during the period from 1976 to 2018.

Materials and methods

The analyzed publications were obtained with the search engine Scopus® Elsevier (www.scopus.com) for the period from 1976 to 2018 (October 9). Initially the word *Metarhizium* was used, only in the title, which generated 1 708 documents. Publications were found regarding several species of the genus *Metarhizium* such as: *M. anisopliae* (1 272 publications), *M. acridum* (107), *M. robertsii* (173), *M. brunneum* (121), *M. rileyi* (16), *M. majus* (7), *M. guizhouhense* (6), *M. pingshaense* (6), the species *M. anisopliae* was the most studied, by which only the publications of *M. anisopliae* were retained or used for the analysis. Of the total of documents analyzed, 1 216 were articles, occupying 95.6% of the total. Other documents were: book chapters (7), reviews (14), articles in the press (4), conference documents (14), letters (2), notes

(3), surveys (3) and *erratum* (9). With these data a bibliometric analysis was elaborated using the terms of the keywords, titles and summaries of the publications to show only the elements connected to each other.

Analysis of the results

VOSviewer® software (Center for Science and Technology Studies, 2018) was used for the analysis. An analysis of co-occurrence of key words and academic terms in the titles and abstracts of the publications was made, the method was normalization-force of association (FA), resolution of 1.00, scale of visualization to 100%, weight TLS, tag variation size of 50% and core width of 30%. The complete counting method was established, with a number of records of each term ≥10, and a minimum cluster size of 15 (van Eck and Waltman, 2010). With the terms retained the map was created for the visualization of the network, those terms with more frequency are presented in larger bubbles, the irrelevant terms were eliminated (Heersmink *et al.*, 2011).

Results

The number of publications dealing with aspects related to *M. anisopliae* is abundant, then the bibliometric indicators related to this microorganism are analyzed.

Performance analysis

The distribution of the publications referring to this research topic is presented in Figure 1. The number of publications on this topic began in 1976 maintaining an irregular growth, for the year of 1998 it reaches its first peak in 2008, year in which that the greatest number of publications is presented (75), followed by 2014 (74). After 2015, the number of publications has been decreasing. Of the total of documents, 1 121 have been cited accumulating a total of 24 504 citations. There are 24 articles that have more than 100 citations, 93 have between 50 and 90 appointments and 623 articles have less than 10 citations.

With regard to the countries, it is observed that Brazil is the country with the highest number of contributions (241). The United States of America has second place with 228 publications, followed by the United Kingdom (139), China (97) and India (82). In Latin America, Mexico stands out with 54 publications, followed by Colombia with 22 and Argentina with 10 publications. The contributions come from authors from 160 institutions: 29 Brazilian, 21 American, 16 belonging to the United Kingdom, 13 Chinese and 11 Mexican. Within the 11 Mexican institutions are, the State University of Colima (12), UNAM (9), the University of Guanajuato (8), the College of Postgraduates (6) and the Autonomous University of Yucatan (4).

Table 1 shows the 10 journals with the highest number of publications, ordered by country and institution. The five journals with the highest number of publications are: Journal of Invertebrate Pathology, Biocontrol Science and Technology, Mycological Research, Biological Control and Biocontrol. They emphasize journals with emphasis on entomology, pathology and mycology, as well as biological control.

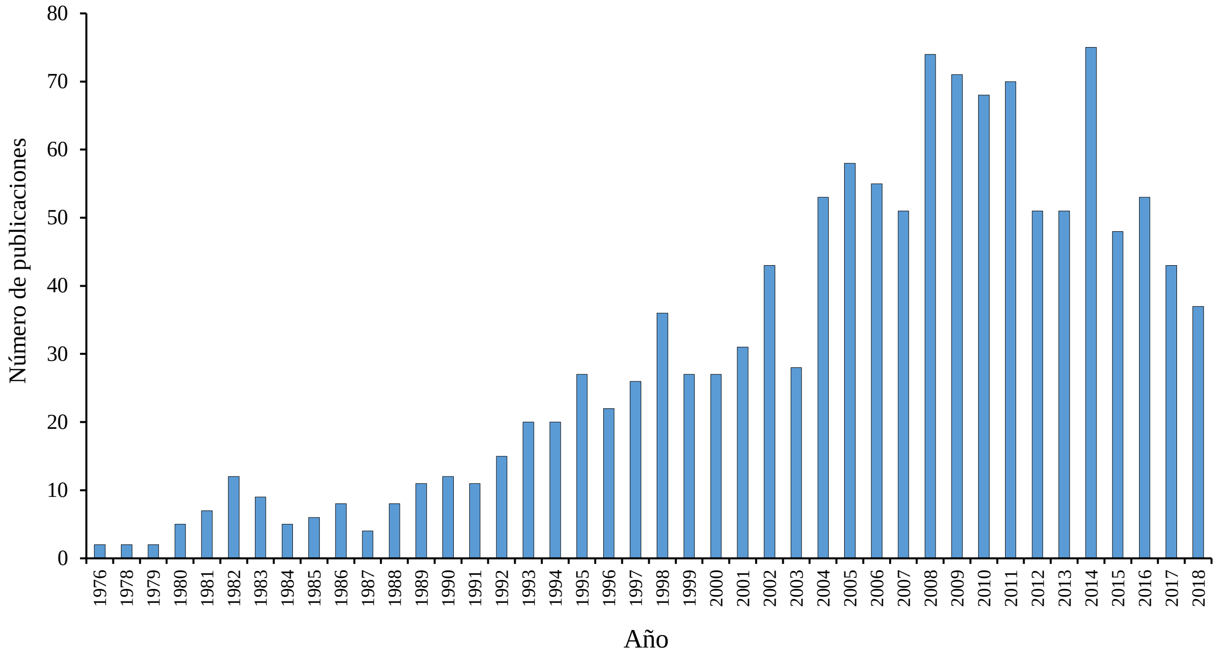

Figure 1. Distribution of publications on *M. anisopliae* by year from 1976 to 2018 (October 9, 2018).

Table 1. Performance analysis: institute, country and magazine.

Rank	Institute	Pub.	Country	Pub.	Journal	Pub.
1	International Centre of Insect Physiology and Ecology Nairobi	55	Brazil	241	Journal of Invertebrate Pathology	127
2	Universidade Federal do Rio Grande do Sul	47	USA	228	Biocontrol Science and Technology	70
3	Universidade de Sao Paulo - USP	43	UK	139	Mycological Research	37
4	University of Bath	42	China	97	Biological Control	35
5	Cornell University	41	India	82	Biocontrol	21
6	Brazilian Agricultural Research Company-Embrapa	39	Australia	57	Journal of Economic Entomology	21
7	Boyce Thompson Institute for Plant Research	35	Kenya	55	Veterinary Parasitology	21
8	Swansea University	29	Mexico	54	FEMS Microbiology Letters	20
9	University of Maryland	28	Canada	46	Neotropical Entomology	19
10	Universidade Federal de Goias	28	Iran	33	Journal of Applied Entomology	16

In Table 2, the 10 most cited articles are presented. These articles allow us to rescue the most relevant topics in this area of knowledge, such as: Genomic sequencing, biological control, genetic groups of *Beauveria bassiana* fungi, combinations of mycoinsecticides, pathogenicity, among other topics.

Table 2. The 10 most cited articles on control and *M. anisopliae*.

Rank	Authors	Title	Year	Journal	Quotes
1	Gao, Q.; Jin, K.; Ying, S.-H.; Zhang, Y.; Xiao, G., Shang, Y.; Duan, Z.; Hu, X.; Xie, X.-Q.; Zhou, G. and Peng, G.	Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*	2011	PLoS Genetics	307
2	Bischoff, J. F.; Rehner, S. A. and Humber, R. A.	A multilocus phylogeny of the *Metarhizium anisopliae* lineage	2009	Mycologia	264
3	Meyling, N. V. and Eilenberg, J.	Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control	2007	Biological Control	200
4	Zimmermann, G.	Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*	2007	Biocontrol Science and Technology	186
5	Hu, G. and St. Leger, R. J.	Field studies using a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that it is rhizosphere competent	2002	Applied and Environmental Microbiology	169
6	Wang, C. and St. Leger, R. J.	A collagenous protective coat enables *Metarhizium anisopliae* to evade insect immune responses	2006	Proceedings of the National Academy of Sciences of the United States of America	152
7	Bidochka, M. J.; Kamp, A. M.; Lavender, T. M.; Dekoning, J. and De Croos, J. N. A.	Habitat association in two genetic groups of the insect-pathogenic fungus *Metarhizium anisopliae*: Uncovering cryptic species?	2001	Applied and Environmental Microbiology	148
8	Leger, R. J. St.; Charnley, A. K. and Cooper, R. M.	Characterization of cuticle-degrading proteases produced by the entomopathogen *Metarhizium anisopliae*	1987	Archives of Biochemistry and Biophysics	147
9	Kershaw, M. J.; Moorhouse, E. R.; Bateman, R.; Reynolds, S. E.; Charnley, A. K.;	The Role of destruxins in the pathogenicity of *Metarhizium anisopliae* for three species of insect	1999	Journal of Invertebrate Pathology	142
10	St. Leger, R. J.; Frank, D. C.; Roberts, D. W. and Staples, R. C.	Molecular cloning and regulatory analysis of the cuticle degrading protease structural gene from the entomopathogenic fungus *Metarhizium anisopliae*	1992	European Journal of Biochemistry	142

Mapping of science

An analysis of the co-occurrence of terms was carried out, this provides an overview of the trends of the research, reflecting the topics addressed. The results establish 649 terms, of which those with co-occurrences greater than 10 were conserved, eliminating generic terms related to the research process, 389 terms were retained, organized in 3 clusters with 27 104 relationships. In Figure 2, the three clusters are observed: one referring to the activity/production of *Metarhizium anisopliae* (green cluster), another referring to the relationship and interaction with *Beauveria bassiana* (red cluster) and a third related to termites (blue cluster).

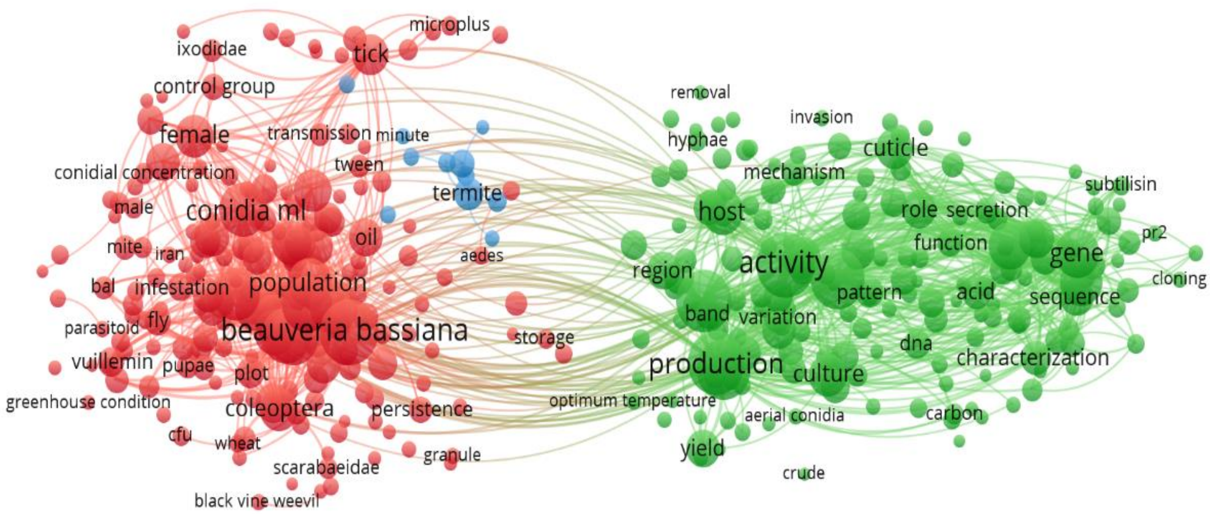

Figure 2. Visualization of the co-occurrence network using the VOSviewer® program.

Discussion

This study dealt with bibliometric methods to know the behavior of scientific publications related to *M. anisopliae*. Based on these results, the three clusters related to this microorganism will be discussed separately below.

Production-activity

Chemical insecticides have been commonly used to control agricultural pests, termites and biological vectors, such as mosquitoes and ticks, these chemicals have generated harmful effects on the environment and resistance in pests and vectors. Due to the growing public concern, research has increased on alternatives, especially biological control agents such as fungi and entomopathogenic bacteria, for their control (Aw and Hue, 2017). The use of these fungi has evolved to become commercial products (bioinsecticides), so variables such as conidia production, sporulation and virulence (Zhang *et al*., 2018), are strategic in research that focuses on in evaluating culture media or methods of reproduction or propagation of spores or conidia of *M. anisopliae* (Shah *et al*., 2005).

The microbial activity refers to the changes that are expressed in the activity of the microorganisms, in the case of *M. anisopliae*, the investigations have included the biological activity of this microorganism when it is combined with essential oils (Rosas-Garcia *et al*., 2018), the analysis of the enzymatic activity of proteases obtained from *M. anisopliae* (St Leger *et al.*, 1999; Shah *et al.*, 200), their antifungal and fungal activity (Wang *et al.*, 2005), insecticide (Charnley, 1991) and biological on various pests such as the white worm (*Premnotrypes vorax* Hustache) (Villamil *et al*., 2016), the red spider (*Tetranychus evansi* Baker & Pritchard) (Azandeme-Hounmalon *et al*., 2018), the potato moth (*Phthorimaea operculella* Zeller) (Khorrami *et al*., 2018), or the rhizosphere (Meyling and Eilenberg, 2007), just to mention a few examples.

Another sub-area of research relevant to this microorganism is the molecular aspect. Several investigations have analyzed the role of several enzymes (Dextruxins, MaNAG1, MaNAG2, MaNAG3 and MaNAG4) during the life cycle of *Metarhizium anisopliae* (de Oliveira *et al*., 2018), characterized the toxic ribonucleases of this microorganism as potential insecticides against ribosomes. for the control of disease vectors (Olombrada *et al*., 2017), as well as the function of some genes on the adaptation to the environment of this microorganism (Zeng *et al*., 2018) and its level of virulence (Santos *et al.*, 2017).

Beauveria bassiana

B. bassiana is an entomopathogenic fungus (Abdu-Allah *et al*., 2015). Its mode of action is by contact (Alcala-Gomez *et al*., 2017), contaminating the microflora of the cuticle of the insect, germinating the spore on the insect pest and the hypha is introduced to the integument (tissue that forms the outer wall of the cuticle of the body of the insects) and enter the hemocoel and then spread by the hemolymph throughout the insect, causing its death by toxins (by toxemia) that this same secret (Petlamul and Prasertsan, 2012). It presents a great genetic diversity and usually develops in agricultural habitats (Bidochka *et al.*, 1998; Rustiguel *et al.*, 2018). Some of the pests it controls are: Whitefly (*Trialeurodes vaporariorum* Westwood), thrips (*Thrips* spp), blind hen (*Phyllophaga* spp. Bates), black donut (*Spodoptera littoralis* Boisduval), among others (Polanczyk *et al.*, 2010; Mirhaghparast *et al.*, 2013), as well as arthropod pests in poultry production (de Oliveira *et al*., 2014). It is currently used as a commercial biological control product in combination with *M. anisopliae*.

B. bassiana and *M. anisopliae* are combined due to the strategies that each follows, both their mode of action is contact, only that the former has a toxic strategy using oosporeins and invades the host while the latter has a growth strategy with the formation of appressoria and invasion of the host, this makes the effect for the biological control of pests greater (Rustiguel *et al*., 2018). Although other studies have focused on establishing which of these two fungi has better fungicidal effect (Barbosa *et al*., 2018), as well as their combinations with some insecticides (Rivero-Borja *et al*., 2018), with various chemical compounds, such as thymol, and application methods (Sinia and Guzmán-Novoa, 2018), for pest control. In general, the research topics deal with formulations, mortality and virulence (Oliveira *et al*., 2018), laboratory bioassays, field evaluation, and their evaluation for the control of various pests.

Termites

Termites (*Coptotermes curvignathus* (Holmgren) Isoptera: Rhinotermitidae, *Coptotermes heimi*) are a threat to plants and agricultural crops, although they are excellent decomposers of dead wood and other sources of cellulose, they become a serious problem when they attack homes and crops (Hussain *et al*., 2011). Due to significant losses in annual and perennial crops and damage, different control methods have been adopted such as physical, chemical and biological control (Wright *et al*., 2005). Chemical control has been a successful method to prevent termite attack, but biological methods are suitable alternatives (Verma *et al*., 2009), especially when seeking to reduce the use of chemicals that harm the environment and human health (Yii *et al*., 2016).

M. anisopliae and *B. bassiana* positively infect termites (Rath, 2000), by invading their host through the integument and causing death by depletion of host metabolites, due to a destruction of vital tissues or a combination of both (Wang and Powell, 2004). Previous research found that *M. anisopliae* causes a percentage of 71-84% of infections in termites after 15 days of treatment (Kin *et al*., 2017). Other studies have focused on topics such as: The mortality rate according to different concentrations of conidia per ml (Samsuddin *et al*., 2015; Riaz *et al*., 2017; Keppanan *et al*., 2018), its compatibility with some pesticides (Yii *et al*., 2016) or its effect on other species when used for termite control (Abonyo *et al*., 2016).

Conclusions

The objective of this work was to determine the most relevant issues regarding research concerning *M. anisopliae* during the period from 1976 to 2018, for which a bibliometric review of the concept *Metarhizium anisopliae* was carried out. In addition to establishing the most influential countries and institutes on the subject. The analysis of co-occurrence of terms and the discussion on the most important terms within the most cited articles was carried out. Derived from this analysis some important conclusions are obtained as it is that the topic has been relevant for the scientific community, with more intense periods in scientific productivity than others (2004-2014). The country with the highest number of publications is Brazil and Mexico ranks 8th worldwide in terms of the number of publications. The investigation could be grouped in that dedicated exclusively to *M. anisopliae*, the one where this fungus is combined with another (*B. bassiana*) and the use of *M. anisopliae* for the control of termites. Finally, it should be noted that this particular topic demonstrates the progress of research from basic science to applied science and finally to the development of technological solutions. Future contributions on this fungus will be about the discovery of new species and strains that have commercial potential with an emphasis on genetic engineering and biotechnology.

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