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Use and perspective of entomopathogenic fungi biocontrol agents of Asian citrus psyllid (Diaphorina citri): A bibliometric review

Uso e perspectiva de fungos entomopatogênicos como agentes de biocontrole do psilídeoasiático dos citros (Diaphorina citri): Uma revisão bibliométrica

Uso y perspectiva de hongos entomopatógenos como agente de biocontrol del psílido asiático de los cítricos (Diaphorina citri): Una revisión bibliométrica

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Abstract
The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the main insect vector of the bacteria *Candidatus liberibacter* spp., which are the causative agents of Citrus Greening disease, also known as Huanglongbing (HLB). The current lack of treatment for the disease means that intensive work is required to manage psyllid populations, mainly through chemical control methods. Since biological control has become a promising management strategy and entomopathogenic fungi are one of the components of integrated pest management, this review aimed to compare previously published results regarding the most efficient fungi for controlling *D. citri* and to gather information about the best options available. To this end, the bibliography indexed in the Scopus and Web of Science databases was analyzed using bibliometric tools with the R Studio software and the Bibliometrix package. In recent years, *Cordyceps fumosorosea*, *Beauveria bassiana*, and *Hirsutella citriformis* were identified as the most relevant fungus species, showing efficient results. In addition, the countries that publish the most on this subject are also among the largest citrus producers. Studies performed under greenhouse and field conditions are still scarce but necessary to validate the results obtained in the laboratory, as well as those that explore the mechanisms of action involved in the pathogenicity of fungi against *D. citri*, which are matters to consider in future assessments.
Keywords: Entomopathogenic fungi; *Diaphorina citri*; Greening; Biological control; Bibliometric analysis.

Resumo
O psilídeo asiático dos citros, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), é o principal inseto vetor das bactérias *Candidatus liberibacter* spp., que são os agentes causais da doença denominada *greening* ou Huanglongbing (HLB). O fato de não haver tratamento para a doença exige que se realize um trabalho intensivo de manejo das populações do psilídeo, que tem sido realizado principalmente através do controle químico. Considerando que o controle biológico tem se tornado uma estratégia de manejo promissora, e que a utilização de fungos entomopatogênicos é um dos componentes do manejo integrado de pragas, o objetivo desta revisão foi comparar resultados sobre os fungos mais eficientes para o controle de *D. citri* e reunir informações sobre as melhores opções disponíveis. Para tal, foi realizada uma análise da bibliografia indexada nas bases de dados *Scopus* e *Web of Science*, utilizando ferramentas de bibliometria com o software R *Studio* e o pacote Bibliometrix. Foram identificadas as espécies de fungos *Cordyceps fumosorosea*, *Beauveria bassiana* e *Hirsutella citriformis* como as mais relevantes nos últimos anos, apresentando bons resultados de eficiência. Além disso, os países que mais publicam sobre o assunto estão também entre os maiores produtores de citros. Estudos realizados em condições de casa de vegetação e campo ainda são escassos, porém necessários para validação de resultados obtidos em laboratório, assim como aqueles que exploram os mecanismos de ação envolvidos na patogenicidade dos fungos contra *D. citri*, sendo, portanto, um importante foco a se considerar em avaliações futuras.
Palavras-chave: Fungos entomopatogênicos; *Diaphorina citri*; *Greening*; Controle biológico; Análise bibliométrica.

Resumen
El psílido asiático de los cítricos, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), es el principal insecto vector de la bacteria *Candidatus liberibacter* spp., un agente causal de la enfermedad denominada *Greening* o Huanglongbing (HLB). La falta de tratamientos para la enfermedad, resulta en una necesidad de trabajar intensivamente en el manejo de las poblaciones de psílidos, el cual se ha realizado principalmente a través del control químico. Considerando que el control biológico se ha convertido en una estrategia de manejo interesante, y que el uso de hongos entomopatógenos es uno de los componentes del manejo integrado de plagas. El objetivo de esta revisión fue comparar los resultados sobre los hongos más eficientes para el control de *D. citri* y recopilar información sobre las mejores opciones disponibles. Para eso se realizó un análisis de la bibliografía indexada en las bases de datos *Scopus* y *Web of Science*, utilizando herramientas bibliométricas con el software R *Studio* y el paquete Bibliometrix. Las especies de hongos *Cordyceps fumosorosea*, *Beauveria bassiana* e *Hirsutella citriformis* fueron identificadas como las más relevantes en los últimos años, mostrando una buena eficiencia. Además, los países que más publican sobre el tema también se encuentran entre los mayores productores de cítricos. Los estudios realizados en invernaderos y en el campo siguen siendo escasos, aunque son necesarios para validar los resultados obtenidos en el laboratorio. También sería importante estudiar los mecanismos de acción involucrados en la patogenicidad de los hongos contra *D. citri.*
Palabras clave: Hongos entomopatógenos; *Diaphorina citri*; *Greening*; Control biológico; Análisis bibliométrico.

1. Introduction

The Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) is one of the most economically significant citrus pests worldwide. It is the main vector of the phloem-restricted bacteria *Candidatus liberibacter* spp., which are the causative agents of the illness known as Huanglongbing (HLB) or Citrus Greening disease, one of the most severe crop diseases in the world (Bové, 2014). Population management of *D. citri* is currently the most important basic strategy available to prevent HLB spread, as no cure exists for it (Maluta et al., 2022).

Management of the insect vector predominantly focuses on chemical control methods. However, this strategy has several negative effects, including on the environment, and it leads to food contamination, poses a risk of contamination to workers, and may lead to pest resistance to insecticides (Chen *et al*., 2018; Vanaclocha *et al*., 2019). Given these undesirable effects, biological control methods are becoming increasingly attractive and more common for managing citrus crop pests and diseases. These biological methods have mainly involved insecticides based on microorganisms, and they can be applied via spraying, interspersed, or in conjunction with other types of management (Wendel *et al*., 2022).

Entomopathogenic fungi play a critical role in biological control methods. In addition to their effectiveness and affordability, several other advantages are associated with using these microorganisms, such as their broad-spectrum insecticidal activity, diverse species range, complex metabolic types, and adequate safety levels for humans and other non-target organisms. They can also be mass-produced easily, and host resistance against these entomopathogens is currently unlikely to occur (Ou *et al*., 2019).

Studies carried out in laboratories, greenhouses, and field conditions with isolates of entomopathogenic fungi have demonstrated the potential of these microorganisms to control *D. citri*. These have demonstrated high pathogenicity levels against the insect, mainly nymphs, and that adding agricultural adjuvants in the application of fungus suspension can improve the control efficiency of adults (Kumar *et al*., 2017; Arnosti *et al*., 2019; Avery *et al*., 2021). The need to investigate the most welcoming and adverse environmental conditions for each fungus, including temperature, relative humidity, ultraviolet radiation, and other adopted agricultural practices was also highlighted by these studies, as was the importance of understanding the mechanisms of action involved in the metabolic processes of entomopathogenic fungi that lead to insect control (Aguila *et al*., 2022; Pérez-González et al., 2022).

This review therefore aimed to support and provide a theoretical framework on biological alternatives for the control of the Asian citrus psyllid. For this purpose, we compared studies and compiled results on the most efficient entomopathogenic fungi for psyllid control, using bibliometric tools to identify more sustainable options for pest management.

2. Methodology

For the bibliometric analysis, a search was conducted on scientific studies focused on the biological control of the Asian citrus psyllid by entomopathogenic fungi. This information was obtained from February to April 2023 through the Web of Science and Scopus databases, accessed from the “Periódicos da Capes” platform. The first step was to define the period to be reviewed. We decided to use research published in the last 10 years. Other inclusion criteria were then defined, and they included studies published in English with a Qualis classification of A1 and A2, using the following types of documents: articles, review articles, and book chapters.

Terms in English were used as descriptors along with the Boolean operators “AND” and “OR.” Therefore, the search criteria were as follows:

- *Web of Science*: TS= ("*Diaphorina citri*" AND "*Isaria*" OR "*Cordyceps*" OR "*Metarhizium*" OR "*Beauveria*" OR "*Hirsutella*" OR "*entomopathogenic*")
- *Scopus*: TITLE-ABS-KEY ("*Diaphorina citri*" AND "*Isaria*" OR "*Cordyceps*" OR "*Metarhizium*" OR "*Beauveria*" OR "*Hirsutella*" OR "*entomopathogenic*")

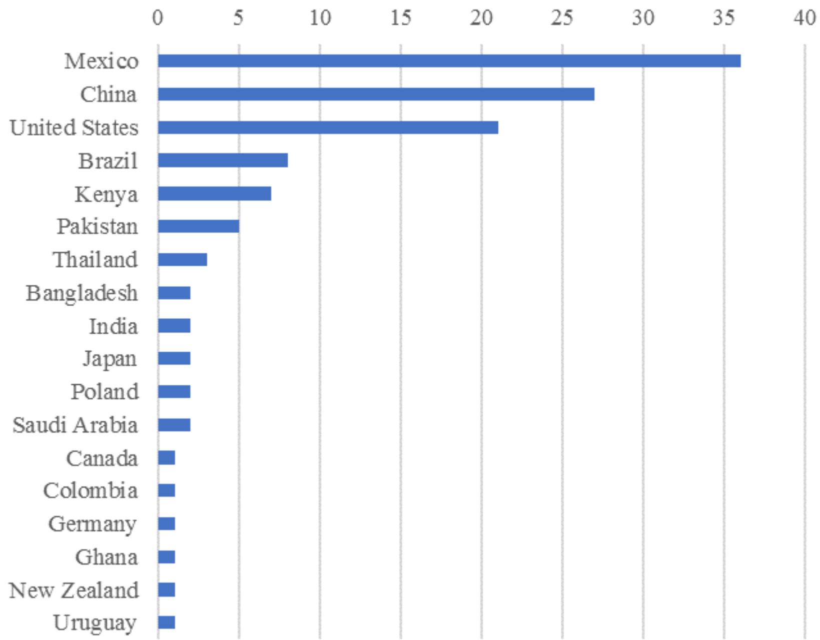
Publication data were then exported from the platform and processed using Excel, through data distribution in a spreadsheet containing the following information: publication title, entomopathogenic fungi species studied, authors, database, journal and Qualis, publication year, publication type and publication DOI. The selected studies were retrieved in full text and those that met the inclusion criteria were fully read. Subsequently, publication data was processed using R version 4.2.2, R studio software, as well as the Bibliometrix package through the Biblioshiny interface for tabulation and discussion, as per Allaire (2012), Derviş (2019) and Jalal (2019).

Considering the identification of the most relevant entomopathogenic fungi, R studio software and the Bibliometrix package were used to analyze the occurrence of terms, allowing us to identify keywords related to entomopathogenic fungi species, as well as to quantify the frequency in which these words appeared. This granted us to assess the relevance of each fungus used for psyllid control, as per Aria and Cuccurullo (2017) and Derviş (2019).

3. Results and Discussion

Through the Web of Science database, we found 109 published studies on the biological control of the citrus psyllid through microorganisms. As only studies published in the last 10 years were used, 95 studies were retrieved (excluding two “correction-type” publications). Regarding the distribution by country, we noted that the four countries with the highest number of publications were Mexico, with 36 publications; China, with 27; the United States, with 21; and Brazil, with eight publications (Graph 1).

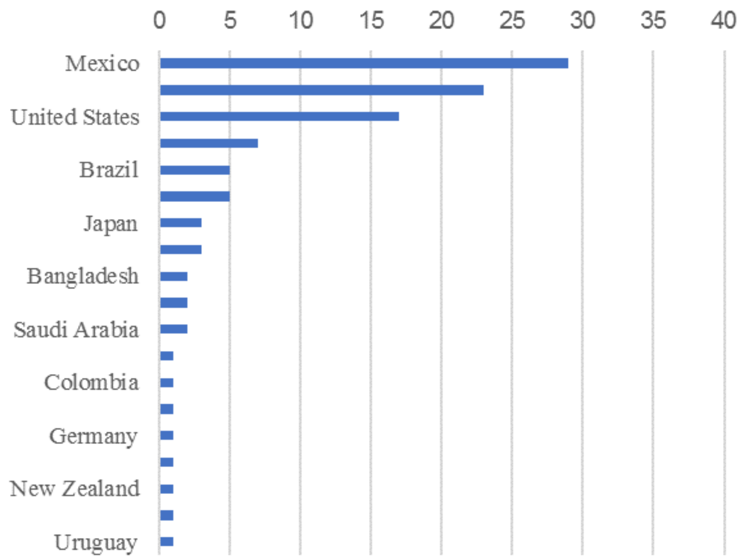
Graph 1 - Distribution of publications by country in the Web of Science database.



Source: Authors (2023).

We found 92 studies in the Scopus database, 77 of which were published within the last 10 years (excluding two “correction-type” publications). Regarding distribution by country, we found that the four countries with the highest number of publications were Mexico, with 29 publications; China, with 23; the United States, with 17; and Kenya, with seven publications (Graph 2). It is important to note that some results were obtained in both databases (these are the same studies), and some were different between the databases.

Graph 2 - Distribution of publications by country in the Scopus database.



Source: Authors (2023).

Among the studies obtained using bibliometrics, those that met the publication criteria in journals with Qualis A1 and A2 classification were analyzed. Duplicated studies and those that did not specifically deal with psyllid control through entomopathogenic fungi were excluded; therefore, a total of 53 studies were used for the qualitative synthesis of this review. Table 1 shows the most relevant species used to control *D. citri*, based on the frequency with which they appeared in the analyzed studies.

Table 1 - Number of occurrences of entomopathogenic fungi species used to control *D. citri*.

Fungi species	Occurrence (number)
Cordyceps (Isaria) fumosorosea	23
Beauveria bassiana	19
Hirsutella citriformis	11
Metarhizium anisopliae	9
Cordyceps javanica	5
Lecanicillium sp.	2
Aspergillus fijiensis	1
Clonostachys rosea	1
Metarhizium sp.	2

Source: Authors (2023).

Based on the data obtained through bibliometric analysis, we observed that the entomopathogenic fungal species most used for biological control were *Cordyceps fumosorosea*, *Beauveria bassiana*, and *Hirsutella citrifrormis*. Of these, *C. fumosorosea* and *B. bassiana* have already been consolidated and commercially formulated for the control of several types of harmful arthropods and disease vectors, such as mites, aphids, mealybugs, whiteflies, thrips, leaf miners, psyllids, and caterpillars (Quiroz *et al*., 2019). In addition, other biocontrol agents were indicated, demonstrating the importance of research on this subject for increasing agricultural pest control measures by seeking to identify the potential species' pathogenicity and efficiency under field conditions using the most viable formulations.

An important factor to be considered is the interaction between *D. citri* infection by entomopathogenic fungi and the presence of *Candidatus liberibacter* in the insect's body. Orduño-Cruz *et al*. (2015) demonstrated, under laboratory conditions, that individuals of *D. citri* carrying *Candidatus liberibacter asiaticus* were more susceptible to fungal infection by isolates of *B. bassiana*, *C. fumosorosea*, and *Metarhizium anisopliae* than CLas-free individuals. This result was consistent for the three isolates tested despite having different virulence levels against *D. citri* adults. The authors considered that the extraction of nutrients from the insect by the bacteria impairs and interrupts the insect's general physiology; consequently, the insect may allocate less energy to defend itself against the fungal infection.

According to the bibliometric analysis, *C. fumosorosea* was the most frequently cited species, having been named in 23 of the evaluated studies. This may be due to the fact that this fungus has shown greater pathogenicity and viability over time. Luo *et al*. (2022) evaluated the efficiency of *C. fumosorosea* under laboratory and greenhouse conditions. Their results demonstrated high pathogenicity against *D. citri*, with nymphs as more susceptible to attack by the fungus, with a mortality rate of 100% after three days of application of $1x10^7$ conidia/mL. They also observed a reduction in the emergence of adults and the rate of female longevity. These findings were similar to those by Morales-Reyes *et al*. (2018) under laboratory conditions, in which insects exposed to 10^7 propagules/mL of conidia and blastospores died in six days at a temperature of 25°C. In a greenhouse, treatment with blastospores reduced *D. citri* populations by about 60% after 21 days. Corrêa *et al*. (2020) selected *C. fumosorosea* isolates for blastospore production and evaluation of desiccation tolerance, obtaining blastospore concentrations above $1x10^9$ spores/mL, with viability rates above 75% after drying.

In terms of the effects on the feeding activity of *D. citri*, Maluta Castro and Lopes (2022) observed, through the electrical penetration graph technique, suction activities of the psyllid between 30 and 96 hours after application of *C. fumosorosea*, using a formulated product distributed commercially in Brazil, named "*Challenger.*" The authors found a significant interruption in the activities of the psyllid style, mainly in the phloem vessels. Qasim *et al*. (2021) focused on the enzymatic activity and expression of genes related to detoxification in citrus psyllid adults and nymphs under *C. fumosorosea* stress. Overall, the activity of four enzymes significantly decreased, while the catalase enzyme activity increased at different times. Likewise, the expression of different genes related to insecticide resistance decreased substantially. This regulation of genes and enzymes caused a deterioration in the defense system of treated psyllid populations. Similarly, Keppanan *et al*. (2018) evaluated a toxin produced by *C. fumosorosea* and the toxicity level of this substance to *D. citri*, tested at concentrations of 1, 2, and 3%. They found that the highest mortality rate was achieved at 3% mg /L 120 hours after application, reducing over 80% of the insect population.

Since adult psyllids have more active movements than nymphs, greater application efficiency is needed to reach them. Furthermore, as infection by entomopathogenic fungi requires cuticular contact with the pest, their effectiveness can be enhanced using other pesticides, such as adjuvants and other oils, to ensure better homogeneity in suspension and propagation dispersion. Based on this, Arnosti *et al*. (2019) evaluated the effects of adjuvants on the adhesion of *C. fumosorosea* to psyllids in the laboratory. Using scanning electron microscopy, the authors observed that the fungus adhered predominantly in the posterior ventral region (abdomen) compared to the anterior dorsal region (thorax). Kumar *et al*. (2017) performed a laboratory efficiency test in which mixed treatments of *C. fumosorosea* with petroleum oils performed better than the fungus alone against *D. citri*, with the highest mean survival time of *D. citri* being an average of 12 days. Previously, Avery *et al*. (2013) assessed the compatibility of *C. fumosorosea* blastospores with a variety of oils and copper-based fungicides, obtaining reduced *in vitro* growth of *C. fumosorosea*. Petroleum-based materials resulted in reduced growth, and botanical oils, borax, and some of the copper-based fungicides led to increased growth, suggesting that tank mixing of the fungus with these latter products should be avoided.

Another challenge related to controlling citrus psyllids and greening concerns residential areas, where pesticide application is impossible, and abandoned orchards, where no management is carried out. In this regard, Patt *et al*. (2015) developed an auto disseminator ("dispenser") to inoculate entomopathogens in these areas with the aim of inducing epizootics. In a greenhouse test with *C. fumosorosea*, they found that an average of 55% of *D. citri* adults developed mycosis (sporulation), and when sporulated adult corpses with mature conidia were placed next to immature ones in potted plants, more than 90% of immature psyllids had mycosis. Corroborating these results, Chow *et al*. (2018), using dispensers with *C. fumosorosea*, showed a reduction in the average reproduction of *D. citri* by 90% and the average intensity of attack by adults by 76% and by nymphs by 82%. Furthermore, with regard to the persistence of the fungus in the area, Pick *et al*. (2022) verified that *C. fumosorosea* propagules can be transferred between plants by biotic factors (through other insects, for example), also considering the psyllid when the fungal hyphae grow through the tarsi and attach to the plant leaves. In their fungus persistence test in the field, they found that *C. fumosorosea* persisted for at least 35 days and caused up to 40% mortality of *D. citri* adults.

Among the evaluated studies, another entomopathogen widely used in research was *B. bassiana*, discussed in 19 evaluated studies. This species rendered results to *C. fumosorosea* for pathogenicity and control efficiency against *D. citri*. In a study performed under laboratory conditions in the United States, Wendel *et al*. (2022) tested different formulations of entomopathogenic fungi, selecting an isolate from *C. fumosorosea* and *B. bassiana* due to their efficiency in controlling *D. citri.*, availability, commercial use of the product, registration (or possibility of registration) of the formula in the country, and market prospects. Ausique *et al*. (2017) also evaluated an isolate of *C. fumosoroesa* and another of *B. bassiana*, which showed citrus psyllid mortality rates of 77.8 and 78.4%, respectively, under laboratory and 80.6 and 83.5%, respectively, in semi-field (plants grown in the soil under semi-controlled conditions) conditions. In the field, monthly applications over a year in a commercial citrus orchard resulted in mortality rates ranging from 57.8% to 96.1%. This mortality rate tended to increase with increasing maximum relative humidity, and the percentage of sporulated corpses was negatively associated with maximum temperatures.

Conceschi *et al*. (2016) analyzed the horizontal transmission of *B. bassiana* and *C. fumosorosea* between cadavers of *D. citri* and uninfected adults of *D. citri*, as well as between cadavers of *Toxoptera citricida* (citrus black aphid) and uninfected adults of *D. citri*, under laboratory and semi-field conditions. In the laboratory, cadavers of *D. citri* infected with *B. bassiana* and *C. fumosorosea* on citrus plants resulted in mortality rates of *D. citri* adults ranging from 51.2 to 81.9% and 36.2 to 68%, respectively. When *T. citricida* cadavers were used, mortality rates of uninfected *D. citri* adults ranged from 35.4 to 87.7% with *B. bassiana* and from 41.7 to 80.4% with *C. fumosorosea*. Under semi-field conditions, horizontal transmission was also confirmed, although the mortality rates of *D. citri* adults were lower than those in the laboratory. This may have been due to the larger size of the experimental units used in the field, environmental conditions and higher temperatures, humidity variation, wind, and rain. In the study by Gandarilla-Pacheco *et al*. (2013), nymphs of *D. citri* sprayed with a suspension of *C. fumosorosea* showed higher mortality (84.2%), while nymphs sprayed with *B. bassiana* showed greater development of mycosis.

More recently, a study performed by Cisneros *et al*. (2022) under laboratory conditions identified and evaluated two isolates of *B. bassiana* through two application forms: direct insect spraying and exposure of psyllid adults on sprayed foliage. The isolates showed a mortality rate of approximately 70% with application via direct spraying of $1x10^8$ conidia/mL and mortality of 60 to 73% through exposure of adults to sprayed foliage of $3x10^8$ conidia/mL. This is higher than the level achieved by the experiment's control, an isolate of *C. fomosorosea* used commercially in the United States. Still, the *B. bassiana* isolates showed higher growth rates at higher temperatures compared to the control isolate when checking the thermal profile. In the study by Cruz-Cruz *et al*. (2020), the most pathogenic isolate of *B. bassiana* had a mortality rate against *D. citri* of 58% under laboratory conditions.

Considering the persistence of the fungus in the area, Bamisile *et al*. (2019) developed a test in a greenhouse in which citrus seedlings were inoculated for four months after planting with a *B. bassiana* isolate through foliar spraying. Seedling colonization was maintained for up to 12 weeks. The application resulted in a psyllid survival rate of only 2% after eight days of treatment. Furthermore, *D. citri* females feeding on these plants laid fewer eggs than those feeding on endophyte-free seedlings.

Another entomopathogenic fungus that stood out in the bibliometric results was *Hirsutella citriformis*, as, according to our analysis, it was one of the three most frequently used fungi for controlling *D. citri*. It has not been detected in Brazil and is mainly associated with the citrus psyllid in Mexico. According to Pérez-González *et al.* (2022), it is a fungus that needs a longer infection period to cause the insect’s death than other entomopathogenic fungi. Additionally, the mode of action of its secondary metabolites on *D. citri* is still unknown. Despite this, it presented with similar control levels as those of *B. bassiana*. This panorama can be supported by the study by Cortez-Madrigal *et al*. (2014), from which the authors assessed the pathogenicity and enzymatic activity against *D. citri* of isolates of *B. bassiana* and *H. citriformis*. The authors observed wide variability in the production of enzymes, mainly proteases, which are most commonly associated with virulent isolates of entomopathogenic fungi. Based on pathogenicity, they verified greater potential for managing *D. citri* through *B. bassiana*, and in relation to enzyme production, the greatest potential was verified through *H. citriformis*.

Under laboratory conditions, Pérez-González *et al*. (2015) evaluated the growth and conidiation of *H. citriformis* isolates, as well as the virulence of conidia in psyllid adults and nymphs. The authors observed insect mortality from six days after application, with the mortality rate of adult insects for the most pathogenic isolates being 85.6 to 88.1%, while that of nymphs was 50.5 to 81.7%, in addition to sporulation of the isolates in insects being observed from 10 days after application. Ibarra-Cortés *et al*. (2017) tested the susceptibility of *D. citri* nymphs and adults to *H. citriformis* infection in the laboratory.

The authors found that the nymphs were more susceptible than adults and that adults contaminated with fungal conidia were not able to transmit the infection to nymphs. Under semi-field conditions, the conidial infectivity of Mexican isolates of *H. citriformis* was evaluated against adult *D. citri*, and the most pathogenic isolates showed mortality rates of 50.6 to 51.05% (Pérez-González et al., 2016).

In a field test comparing pathogenicity between *H. citriformis* isolates, the infectivity of conidia against *D. citri* adults by contact was assessed by Pérez-González *et al*. (2020). The treatments applied to the aerial part of citrus remained bagged in the field 21 days after application, and all isolates showed pathogenicity for the psyllid. The isolate with the highest mortality rate (74.1%) also had the highest percentage of sporulation (71.1%) in the insect.

It must be considered that differences in the mortality rate may be caused by the genetic variability of the isolates evaluated. Under laboratory conditions, Cruz-Juárez *et al*. (2018) exposed an isolate of *H. citriformis* to different concentrations of ethyl methane sulfonate to obtain mutants with better production and germination of conidia, aiming to maintain virulence against *D. citri*. The mortality rate achieved by mutant and wild-type isolates was 100 and 94.4%, respectively, within 12 days. Sporulation in psyllid adults was 60.5 and 73.4% for wild-type and mutant isolates, respectively. Subsequently, under laboratory conditions, the viability and pathogenicity of *H. citriformis* conidia were analyzed by Pérez-González *et al*. (2019), considering cultivation in PDA culture medium (potato-dextrose agar) or rice. The conidia cultivated in both media were pathogenic to adult psyllids, which were killed six days after exposure to the conidia. Cultivation in rice made it possible to increase the production of conidia with a shorter total processing time.

According to our bibliometric analysis, the fourth entomopathogenic fungus used for the management of citrus psyllid was *M. anisopliae*. Despite being an entomopathogen already consolidated for the biological control of pests in general, it showed less expressiveness than *H. citriformis* for the pest in recent years. In some studies comparing the pathogenicity of different fungi, the efficiency of *M. anisopliae* was surpassed by others, such as *Cordyceps* fungi. However, in other studies, the entomopathogen displayed better efficiency compared to the other fungi discussed previously.

Rosas-García *et al*. (2018a) tested the biological activity of *M. anisopliae* isolates together with essential oils sprayed on *D. citri* adults fed with orange tree branches in laboratory tests. They found that the most pathogenic isolate achieved a mortality rate of 77%. In the field, combination of this *M. anisopliae* isolate with cypress essential oil resulted in a mortality rate of 56.73%. In addition to pathogenicity testing, Rosas-García *et al*. (2018b) analyzed the activation of isoforms of the Pr1 protein gene from *M. anisopliae* during the pathogenic process in psyllid adults. This protein is probably the main enzyme involved in the cuticle's degradation and the fungus's penetration into the host. The insects were infected with a conidia suspension and kept on *Murraya paniculata* branches for seven days. The activity of the isoforms was detected by RT-PCR, and the results indicated that some isoforms are probably activated by the infectious process.

In addition to *M. anisopliae*, other species of *Metarhizium* appeared in the bibliometric results. In the pathogenicity test against *D. citri* by Gandarilla-Pacheco *et al*. (2013), there were two isolates of *Metarhizium brunneum*, which presented mortality rates of 49.6 and 74.6%, with little to no sporulation in the nymphs. The efficiency of these isolates was surpassed by other fungi used in the research (*C. fumosorosea* and *B. bassiana*). Wendel *et al*. (2022) used an isolate of *M. robertsii* in their laboratory-developed efficiency test, which was the main candidate in a mycoinsecticide development program in the United States and which showed good efficiency, having been selected by the authors for carrying out other researches with *D. citri*.

It is necessary to consider that abiotic factors such as light, humidity, and temperature affect the stability and persistence of entomopathogenic fungi. Specifically, temperature can affect germination and growth. Orduño-Cruz *et al*. (2015a) evaluated in the laboratory fungal isolates of *B. bassiana*, *M. anisopliae*, *C. fumosorosea*, and *H. citriformis* regarding *in vitro* growth, germination (after different incubation times) and sporulation, at four temperatures: 20, 25, 30 and 35 ºC. The authors observed that *H. citriformis* isolates had lower growth, germination, and sporulation rates than other fungi and were not considered competitive in the *in vitro* evaluation. From the *in vivo* evaluation, using conidial suspensions, selected isolates of all fungi caused mortalities above 80% in *D. citri* adults, except *H. citriformis*, which caused a maximum of 40% mortality. However, mortality caused by *H. citriformis* blastospore suspensions was 60%, and through dried conidia, *H. citriformis* and *M. anisopliae* caused 100% mortality (Orduño-Cruz *et al*., 2015b). These results may suggest that using a combination of biological control approaches, both inundative (using isolates in suspension) and inoculative (using isolates as dried conidia in autoinoculation devices), could present good control potential.

According to bibliometric analysis, among the entomopathogenic fungi with less expressiveness for the management of *D. citri* are *Cordyceps javanica*, *Lecanicillium* sp., *Aspergillus fijiensis*, and *Clonostachys rosea*. The *C. javanica* fungus had its efficiency evaluated in five studies. Hussain *et al*. (2021), using scanning electron microscopy, found that the stages of the *C. javanica* infection process include spore adherence and germ tube formation within 24 hours after inoculation, penetration pins and mycelium growth on the wings of psyllids after 72 hours, cuticle rupture after 96 hours, and mycelial mass colonizing the host's body after 144 hours. Under laboratory conditions, carrying out treatment with blastospores, the mortality rate reached 100% within seven days after exposure to a concentration of $1x10^7$ spores/mL. Mellin-Rosas *et al*. (2016), using a suspension of conidia $1x10^7$ conidia/mL, found that an isolate of *C. javanica* achieved a mortality rate of up to 95% of psyllid nymphs and adults after 13 days of treatment.

Also under laboratory conditions, Awan *et al*. (2021a) found that an isolate of *C. javanica* was effective in terms of virulence, presenting a mortality rate between 70 and 75% $1x10^7$ conidia/mL after seven days of exposure. Furthermore, they tested a new formulation of conidia with sesame oil, which increased the shelf life (percentage of viability) by 16 weeks. The formulation also improved virulence, achieving a psyllid mortality rate of 100% after seven days of exposure at the same concentration. In this sense, aiming to increase the viability of isolates, Awan *et al*. (2021b), this time using another entomopathogenic fungus (*B. bassiana*), found that the conidia formulation prepared with sesame oil again resulted in improved shelf life, virulence, and tolerance to thermal stress, compared to unformulated conidia. Under field conditions, Avery *et al*. (2021) compared the efficiency of treatments with *C. javanica* (alone), *C. javanica* mixed with mineral oil, and pesticides already marketed to control *D. citri* mixed with the same oil. The results showed that *C. javanica*, alone and with oil, was able to suppress the psyllid population by 60 to 83% up to 14 days after treatment and was compatible with natural enemies found at the site, mainly ladybugs.

Regarding the genus *Lecanicillium*, evaluating different species, Lu *et al*. (2015) carried out tests in the laboratory and in a greenhouse, in which the mortality rates of *D. citri* adults reached 100% seven days after inoculation of the suspension of *L. attenuatum* and an unidentified species, while for *L. psalliotae*, complete mortality occurred six days after inoculation, with all suspensions of $1,0x10^8$ conidia/mL, under conditions of 25º C of temperature and 90% of relative humidity, in the laboratory. Under the same conditions in a greenhouse with myrtle seedlings (*Murraya paniculata*), mortality rates caused by the same isolates were 92.55, 100, and 100%, respectively, nine days after inoculation. Keppanan *et al*. (2019) found that a secondary metabolite produced by *Lecanicillium lecanii*, which is toxic to *D. citri*, may be involved in the external process of appressorium formation in the insect’s cuticle and in the process of fungal penetration into the host. The metabolite toxicity test (3%) showed the highest mortality rate (85%) 120 hours after treatment application.

Concerning the use of *Aspergillus fijiensis*, only one study was found. In the laboratory, for all instars of psyllid nymphs, the fungus showed mortality greater than 70% after seven days of application of $1x10^8$ conidia/mL. Under greenhouse conditions, the efficiency was lower than that obtained in the laboratory, probably due to environmental conditions (Yan *et al*., 2022). Some secondary metabolites produced by this fungus, such as aflatoxins, are known to be toxic and carcinogenic, potential risks that must be considered when studying the possibility of using this fungus for biological pest control. The isolate evaluated by the study was identified as not producing aflatoxins. However, the safety in relation to humans, animals, and other non-target organisms is unknown, making it necessary to investigate these issues and check the possible impacts on the environment before studying the control efficiency of *D. citri* in the field.

Only one search was also found for *Clonostachys rosea*. Yang *et al*. (2021) tested the pathogenicity of this species against *D. citri* adults in the laboratory, and the highest mortality rate was 46.67% when the insects were treated with a spore suspension of 1×10^8 conidia/mL. The gene expression of the psyllids was analyzed and identified five days after inoculation, and genes involved in the immune response and apoptosis, in addition to genes encoding cuticle proteins, were among the differentially expressed genes. The results indicated that the pathogenicity of *C. rosea* against *D. citri* adults probably occurred through penetration of the cuticle, suppressing the host's immune response and initiating apoptosis.

Considering other strategies for biological control of the citrus psyllid, it is essential to consider the interaction of entomopathogenic fungi with other natural enemies, such as the parasitoid *Tamarixia radiata*, which is widely used in orchards where chemical control is reduced or non-existent. In this regard, Ibarra-Cortés *et al*. (2017) studied the interaction effect between *B. bassiana* and *M. anisopliae* and the parasitoid *T. radiata* on *D. citri* nymphs under laboratory conditions. They found that the greatest emergence of parasitoid adults was obtained when the fungi were applied to *D. citri* nymphs carrying parasitoid pupae, compared to nymphs carrying eggs or larvae. The hypothesis is that when nymphs-carrying larvae were inoculated with fungi, this process of energy accumulation in the parasitoid larvae was negatively affected, perhaps due to the need to allocate more energy to overcoming the infection than to establishing reserves for pupation, which may have negatively affected overall development and led to *T. radiata* adults having reduced longevity.

The same was previously found by Chow *et al*. (2016) in their study with *C. fumosorosea*, in which the emergence of the parasitoid was not affected when nymphs containing *T. radiata* pupae (nine days after parasitism) were covered with a formulation and colonized by fungal hyphae. The authors also observed that females of the parasitoid oviposited in hosts already infected with the fungus without visible hyphae but not in hosts with visible hyphae. More recently, Aguila *et al*. (2021) also observed this negative interaction between *T. radiata* and *B. bassiana* when the fungus suspension was applied up to 48 hours after exposing the nymphs to the parasitoid. The results show that the development times of entomopathogenic fungi are generally shorter than those of parasitoids, so by releasing parasitoids, allowing parasitism to occur for a longer period before applying the fungus, *T. radiata* could be used in combination with entomopathogenic fungi to control *D. citri*, without affecting the emergence of the parasitoids.

Investigating the results found through bibliometric analysis and the data presented in each research, we found that some of them were developed through the isolation of fungi native to the region where the studies were carried out, obtained by collecting naturally infected psyllid cadavers in the field, subsequently performing the morphological and genetic characterization, as well as the identification of the isolated fungi. Among eight researches initiated in this way, six were carried out in China, with the other two coming from the United States and Mexico (Lu *et al*., 2015; Awan *et al*., 2021a; Awan *et al*., 2021b; Qasim *et al*., 2021; Cisneros *et al*., 2022; Luo *et al*., 2022; González, Flores & Guerra, 2022; Yan *et al*., 2022). This procedure reinforces the importance of considering the specificity of each isolate, in addition to the species of entomopathogenic fungus recommended for control and the geographic region from which the isolate originates, depending on the environmental conditions favorable for the fungus development.

The pathogenicity of isolates can be easily affected by several abiotic factors, such as temperature, relative humidity, and ultraviolet radiation. Therefore, it is essential to validate results obtained in the laboratory also under greenhouse and field conditions, allowing for verification, including whether the isolate studied has the ability to develop well in other regions and guarantee the same performance. Among the studies found by the bibliometric analysis, only five were developed under field conditions, three of which were carried out in the United States, one in Brazil, and one in Mexico (Ausique *et al*., 2017; Chow *et al*., 2018; Pérez-González *et al*., 2020; Avery *et al*., 2021; Pick *et al*., 2022). Furthermore, few studies have been developed to explore and explain the mechanisms of action involved in the control of psyllids by fungi, with seven studies having been found with this objective, four of which were carried out in China, one in Brazil, and one in Mexico (Keppanan *et al*., 2019; Quiroz *et al*., 2019; Qasim *et al*., 2021; Yang *et al*., 2021; Aguila *et al*., 2022; González et al., 2022; Maluta et al., 2022).

Finally, we found that the largest number of publications is distributed among countries that are among the world's largest producers of citrus, mainly orange, selling fresh fruit or juice (USDA, 2023). Despite occupying a prominent position mainly due to the production and export of orange juice, Brazil still presents few studies on the biological control of the psyllid through entomopathogenic fungi compared to the other countries included in the bibliometric analysis.

4. Conclusion

Based on the information in this review, we were able to verify which aspects have been the focus of studies related to the use of entomopathogenic fungi to control *D. citri* in recent years. Considering the importance of alternative practices for more sustainable management of the Asian citrus psyllid, the identification of new isolates of these fungi based on morphology and genetics and the estimation of efficiency in relation to insect mortality, paying attention to the virulence and viability of the fungi over time, help promote the development of bioinsecticides for the biological control of *D. citri*.

Bibliometrics enabled a comprehensive analysis to be performed. The fungi *C. fumosorosea*, *B. bassiana*, and *H. citriformis* were identified as the most intensely researched for effects against the psyllid, achieving promising results. Furthermore, it made it possible to observe that despite the growing interest in the adoption of biological control through these and other entomopathogens, publications on the use of this tool for managing citrus psyllids are still rare, especially with regard to studies investigating the mechanisms of action and those performed in greenhouse and field conditions. These would allow us to explore the influence of abiotic factors on the effectiveness and persistence of fungal propagules and to validate the results found in laboratory studies.

This review provided a theoretical basis to check available options for controlling *D. citri*. Future studies and field trials in different regions are encouraged to observe fungi behavior under various environmental conditions and when faced with different natural enemies. These entomopathogens provide a promising option for controlling the pest, with a high specificity, a low probability of resistance development, and compatibility with other control strategies.

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