World Journal of Microbiology and Biotechnology (2021) 37:180
https://doi.org/10.1007/s11274-021-03151-x

REVIEW

Advances in the biological control of phytoparasitic nematodes via the use of nematophagous fungi

Bianca Guadalupe Flores Francisco[1] · Isabel Méndez Ponce[1] · Miguel Ángel Plascencia Espinosa[1] · Aarón Mendieta Moctezuma[1] · Víctor Eric López y López[1]

Received: 6 July 2021 / Accepted: 15 September 2021 / Published online: 25 September 2021

Abstract

Agricultural production is one of most important activities for food supply and demand, that provides a source of raw materials, and generates commercial opportunities for other industries around the world. It may be both positively and negatively affected by climatic and biological factors. Negative biological factors are those caused by viruses, bacteria, or parasites. Given the serious problems posed by phytoparasitic nematodes for farmers, causing crop losses globally every year, the agrochemical industry has developed compounds with the capacity to inhibit their development; however, they can cause the death of other beneficial organisms and their lixiviation can contaminate the water table. On the other hand, the positive biological factors are found in biotechnology, the scientific discipline that develops products, such as nematophagous fungi (of which *Purpureocillium lilacinum* and *Pochonia chlamydosporia* have the greatest potential), for the control of pests and/or diseases. The present review focuses on the importance of nematophagous fungi, particularly sedentary endoparasitic nematodes, their research on the development of biological control agents, the mass production of fungi *Purpureocillium lilacinum* and *Pochonia chlamydosporia*, and their limited commercialization due to the lack of rigorous methods that enable the anticipation of complex interactions between plant and phytopathogenic agents.

Keywords Biological control · Phytoparasitic nematodes · Nematophagous fungi · Mass production

Introduction

Plant diseases are caused by infectious agents such as fungi, bacteria, nematodes, flagellated protozoa, viruses, viroids, or, even, abiotic factors such as edaphoclimatic alterations and the toxicity generated by pesticides or nutrients (Guzmán et al. 2012). Over the course of millions of years, the association between plants and nematodes has given rise to the evolution of phytoparasitic nematodes, pathogens widely distributed around the world in vascular plants which are often attributed to low crop yields and losses. The Food and Agriculture Organization (FAO) of the United Nations considers a pest any agent causing damage to plants or vegetable products (Armendáriz et al. 2015; Bernard et al. 2017). Phytoparasitic nematodes (PPNs) are a serious global problem for farmers, causing estimated annual crop losses of $118 to $157 billion dollars (Abad et al. 2008; Degenkolb and Vilcinskas 2016; Khan et al. 2020). Two types of PPNs, root-knot nematodes (RKNs) and cyst nematodes (CNs) are obligate parasites of a wide range of agricultural crops. Table 1 presents a summarize of the chemical control used for PPNs, based on previous information of Nicol et al. (2011) and according to Evans et al. (1993), Trudgill and Blok (2001), Luc et al. (2005), Nicol and Rivoal (2008),, Armendáriz et al. (2015), Rehman et al. (2016) and Ebone et al. (2019). Particularly in Mexico, PPNs are a problem for various crops of interest, as presented in Table S1. As a result, farmers have found chemical products (Table 1) as their preferred control method, since their rapid action and solubility in water ensure a uniform distribution on the upper soil layer (Degenkolb and Vilcinskas 2016). However, chemical control methods raise environmental and safety concerns. It has been shown that the application of all the chemical compounds with nematicidal activity potentially risks environmental contamination through mechanisms like

✉ Víctor Eric López y López
vlopezyl@ipn.mx

[1] Centro de Investigación en Biotecnología Aplicada del Instituto Politécnico Nacional. Laboratorio de Bioprocesos, Carretera Estatal Sta. Inés Tecuexcomac-Tepetitla, 90700 Tepetitla de Lardizábal, Tlaxcala, Mexico

Table 1 Chemical control method required for main nematode pest and crops described by Nicol et al. (2011) and according to Evans et al. (1993), Trudgill and Blok (2001), Luc et al. (2005), Nicol and Rivoal (2008), Armendáriz et al. (2015), Rehman et al. (2016) and Ebone et al. (2019)

Chemical control	Main nematode pests	Crops
Phorate, Terbufos, Triazophos, Carbofuran, Carbosulfan, Thiodicarb, Abamectin	*Meloidogyne* spp., *Pratylenchus* spp., *Paratrichodorus* spp., *Longidorus breviannulatus*, *Heterodera* spp., *Punctodera chalcoensis*	Maize
Thiodicarb, Basamid	*Meloidogyne* ssp., *Anguina tritici, Pratylenchus* spp.,*Heterodera avenae*	Barley
Thiodicarb, Basamid	*Belonolaimus longicaudatus, Paratrichodorus* spp., *Pratylenchus* spp., *Criconemella* spp.	Sorghum
Thiodicarb, Basamid	*Meloidogyne* spp., *Ditylenchus dipsaci, Pratylenchus* spp., *Heterodera avenae*	Oat
Basamid	*Anguna tritici, Pratylenchus zeae. Heterodera avenae*	Rye
Carbofuran, Basamid	*Ditylenchus angustus, Aphelenchoides besseyi,., Meloidogyne* spp., *Hirschmanniella* spp., *Pratylenchus* spp., *Heterodera* spp.	Rice
Cadusafos, Ethoproph Phorate, Fosthizate, Fluensulfone, Basamid	*Meloidogyne* spp., *Nacobbus aberrans, Pratylenchus* spp., *Trichodorus* spp., *Globodera* spp	Potato
Basamid	*Pratylenchus brachyurus, Rotylenchus reniformis, Helicotylenchus* spp., *Meloidogyne* spp., *Scutellonema bradys.*	Cassava
Basamid	*Meloidogyne* spp., *Pratylenchus* spp., *Rotylenchus reniformis, Ditylenchus destructor.*	Sweet potato
Phorate, Triazophos, Carbofuran, Carbosulfan, Thiodicarb, Basamid	*Pratylenchus* spp., *Meloidogyne* spp., *Anguina tritici, Ditylenchus dipsaci. Heterodera* spp.	Wheat
Triazophos, Carbosulfan, Thiodicarb, Abamectin, Fluensulfone, Basamid	*Meloidogyne* spp., *Rotylenchuslus reniformis, Hoplolaimus Columbus, Pratylenchus* spp., *Heterodera glycines*	Soybean
Metam sodium	*Heterodera schachitii*	Colza
Thiodicarb, Basamid	*Meloidogyne* spp.	Sunflower seed

direct ingress into waterways, the infiltration of groundwater, surface runoff into rivers, streams, lakes, and reservoirs from neighboring agricultural regions; aerial application and discharge of wastewater from industrial pesticide producers (del Puerto Rodríguez et al. 2014; Zisis 2018). Moreover, these compounds could become toxic to humans when come into contact through possible routes of exposure as: acute oral toxicity, dermal toxicity via absorption through the skin and oxidative stress, inhalation and chronic toxicity causing diseases such as cancer, diabetes mellitus, neurological system disorders, effects on the immune system, endocrine system disturbances and reproductive (sexual/genital) syndromes (Li et al. 2014; Joshi and Sukuraman 2019; Lata et al. 2021). Some of the main nematicides used are basamid, metam sodium, oxamyl, telone, carbofuran, methyl bromide, and thiodicarb (Armendáriz et al. 2015; Ebone et al. 2019).

This has motivated research for the development of environmentally friendly PPN control alternatives to chemcal products currently on the market. Although there are control agents of this type available, not all of them are known to farmers due to the lack of information on both their mechanism of action and benefits. Alternative low impact methods for nematode control, such as genetic and induced resistance or the use of biological control agents, are highly desirable and actively sought (Molinari 2011; Stirling 2011). Within the Integrated Pest Management (IPM), biological control has become an environmentally safe alternative for reducing the use of chemical nematicides. In this context, diverse microbial biological control agents such as *Purpureocillium lilacinum*, *Trichoderma* spp., *Pochonia chlamydosporia* and *Bacillus thuringiensis*, among others, have been evaluated to reduce nematode infestations in susceptible crops (Nordbring-Hertz et al. 2006). *Purpureocillium lilacinum* was known previously as *Paecilomyces lilacinus* (Prasad et al. 2015) and *Pochonia chlamydosporia* var. *chlamydosporia* is also known as *Verticillium chlamydosporium* (Kerry et al. 1984). The present study focuses on the potential of these nematophagous fungi *P. lilacinum* and *P. chlamydosporia*, emphasizing the main challenges for their mass production and formulation.

Phytoparasitic nematodes

Nematodes are pluricellular worm-shaped organisms that generally measure between 0.2 and 2.5 mm in length, depending on the species, and constitute the phylum Nematoda. It is thought that this phylum emerged during the Cambrian explosion (550 million years ago) in marine habitats

(Aguinaldo et al. 1997). Nematodes are found in land systems and marine and freshwater habitats (Bongers and Ferris 1999). Despite their small size, their biological organization is highly complex, wherein circulatory, and respiratory systems are not defined instead of, their body cavity contains a liquid that enables circulation and respiration functions in these organisms. The digestive system comprises a hollow tube extending from the mouth, through the esophagus, intestine, rectum, and anus (Talavera 2003). The large part of these organisms is generally elongated and cylindrical. The adult females of some phytoparasite species change from their cylindrical shape to either a pear or a bag-like appearance. Reproduction can be sexual, hermaphrodite or parthenogenic, namely a form of reproduction based on the development of unfertilized female sex cells (Ben-Ami and Heller 2005). As a result, the control of the species is even more difficult because, once the nematode hatches during stage J_2, it is free-living and search for a host as a source of food. Phytoparasitic nematodes are generally classified according to their location in the vegetable tissue, known as the type of biotrophic relationship established with the host plant (Bello et al. 1994). Their persistence on tissue for long periods indicates the establishment of a very complex host-pathogen relationship, being subject of intense research (Stephen et al. 1998; Sanz-Alférez et al. 2000). Table 2 presents the classification of phytoparasitic nematodes (Guzmán et al. 2012; Armendáriz et al. 2015). Sedentary endoparasitic nematodes that form root knots and cysts currently cause the most damage to agricultural crops and are the most difficult to control causing as well significant crop losses (Trudgill and Blok 2001). The life cycle of these organisms is described in detail below.

Life cycle of sedentary endoparasitic nematodes

The lifecycle of endoparasitic nematodes comprises five stages (Mandal et al. 2021) (Fig. 1). The first is the juvenile stage J_1, established with the molting or cuticle change that occurs inside the egg, wherein the female RKNs lay their eggs directly on the roots, while the CNs eggs remain inside the body of gravid females, forming a protective cyst (Perry 1989). The presence of root exudates of a host plant stimulates egg eclosion and emerges during the second juvenile stage J_2, that is the free-living stage in the soil where nematode infects its host, and once J_2-stage individuals have penetrated the root, they remain fixed to hosts and subterranean stems (Talavera 2003). The infection process occurs when the nematode reaches the host plant and perforates its cell walls, enzymatically softening them with its oral stylet. Cyst nematodes migrate intracellularly, via the cortex, directed to the vascular cylinder, where they induce specialized feeding structures (Fig. 1a), while J_2-stage RKNs migrate intercellularly and feed on the giant cells that are formed via repeated cycles of mitosis without cytokinesis (Fig. 1b) (Jones et al. 2011; Davies et al. 2015; Rehman et al. 2016). Growth toward the interior of the cell wall occurs together with elements of xylem, which facilitates the absorption of nutrients in the developing syncytium (Fosu-Nyarko et al. 2016). Nematodes grow rapidly in size and undergo molting while being transformed into juvenile stages J_3 and J_4, while the last stage of the life cycle occurs when the nematodes enter the adult phase, becoming sexually dimorphic, with the females swelling and remaining sessile throughout the parasitic life cycle. On the contrary, adult males recover motility and are attracted by the females in order to inseminate and fertilize the eggs (Perry 1989). Life cycle, from egg to adult stage, may comprise three or four weeks under optimal environmental conditions, especially temperature, but may take longer in cold temperatures (Agrios 2005).

Hence, to establish an effective biological control of PPNs, in particular cyst-forming and root-knot nematodes, it should be undertaken prior to the infection of the plant, when nematodes are in J_2 stage, where they are free-living in the soil and searching for a host to feed. *Meloidogyne* spp. an RKN, is highly pathogenic and induces hypertrophy and hyperplasia in the root cells, forming root knots and damaging the plant, thus reducing its yield and predisposing it to infection via other pathogens such as bacteria and fungi (del Cid Prado et al. 2018). The CN of maize, *Punctodera chalcoensis*, is the second most important in Mexico after the golden nematode, given the impact of the damage it causes to maize crops, reducing maize quality and yield by up to 90% (Sosa-Moss 1987). The seriousness of this damage depends on the susceptibility of the crop, density of the nematode population, and the adequate levels of soil humidity (Nicol et al. 2011). The foliar symptoms consist of delayed growth, chlorosis, occasional leaf necrosis, and flaccidity in young plants, resulting in reduced yield and even, death (Van Gundy et al. 1974). Juvenile nematodes of the first J_1 stage remain inactive in the soil for at least one year, until the eggs hatch in search for a host (Perry 1989). Microbial agents have been reported in the literature as methods for the biological control of nematodes. Below, we focus on the nematicidal potential of the nematophagous fungi *Purpureocillium lilacinum* and *Pochonia chamydosporia*.

Agents for the biological control of phytoparasitic nematodes

Biological control can be an environmentally friendly pest preventer option compared to that of synthetic chemical nematicides, since it harnesses the power of natural control provided by live organisms and/or their metabolites or subproducts. Biological control involves the use of non-native species derived from animals, plants, bacteria, viruses, and fungi to prevent, eliminate, or, even, reduce the damage

Table 2 Classification of phytoparasitic nematodes and their characteristics

Classification	General description	Characteristics	Genus and/or representative species.
Phytoparasitic nematodes of the root system			
Endoparasites	Completely penetrating the roots, they feed, grow, and lay their eggs either in the interior of the roots or adhered to their exterior	Sedentary: By means of their small delicate stylet, juvenile females enter the tissue of the host, develop a fixed feeding site, and induce the formation of a trophic system known as the syncytium, which are cells for their care and breeding. They become immobile and bulge-shaped in order to form and deposit their eggs. The males lack a functional digestive apparatus Migratory: They retain their mobility and are not fixed in one feeding site within the tissue of the plant. Settling on and migrating via tissue, they do not form egg sacs and all stages of their development are parasitic. Their eggs develop inside the host. Solely juvenile individuals are found on the exterior of the plant	*Meloidogyne, Globodera, Heterodera, Nacobbus, Punctodera* and *Cactodera* *Hirschmanniella, Radopholus* and *Pratylenchus*
Semi-endoparasites	Solely the upper part of the nematode penetrates the roots, while the lower part remains in contact with the soil. The lower part of the females is projected from the roots and takes on a bulge shape Juveniles and immature females rarely penetrate the roots of the host	Sedentary: Irregularly bulge-shaped, the females feed with the body partially absorbed by the roots, develop an egg sac, and feed on modified cells Migratory: Females introduce part of their body into the tissue, conserving their vermiform appearance and depositing their eggs liberally in the soil. Feeding on non-modified cells, all of their stages of development are parasitic	*Tylenchulus semipenetrans, Rotylenchulus reniformis*, *Sphaeronema* and *Tylenchulus Hoplolaimus* and *Helicotylenchus*
Ectoparasites	Introducing their head into the host, they feed without penetrating the roots. These large ectoparasites have a larger stylet than the endoparasites They solely detach for reproduction and lay their eggs directly in the soil	Migratory: With a long stylet, they feed keeping their body outside the tissue and lay their eggs individually in the soil or in the rhizosphere. Feeding on cortical cells, they form, in some cases, specialized parasitic relationships, while all of their stages of development are parasitic Sedentary: Presenting a generally thick sausage-shaped body, they feed for long periods on one cell, laying their eggs in a dispersed pattern in the soil. All of their stages of development are parasitic	*Paratylenchus, Xiphinema, Criconemella, Belonolaimus, Paratrichodorus, Hemicriconemoides, Longidorus, Ttrichodorus.* *Criconemella, Criconema*
Phytoparasitic nematodes of shoot system tissue			
Ectoparasites	They remain above the surface of the tissue of the host and feed by inserting the stylet into the cells	They mainly feed on the epidermis of the cells of young leaves, stems, or flower primordia The majority of these nematodes can be found attacking the foliage	*Anguina, Aphelenchoides* and *Ditylenchus*
Migratory endoparasites	They are able to completely penetrate the tissue of the plant, moving freely via stem, leaves, flower primordia, or seeds	They remain vermiform when moving and feed via tissue Frequently migrating between the soil and the roots, they have a moderately strong stylet	*Aphelenchoides, Bursaphelenchus* and *Ditylenchus*

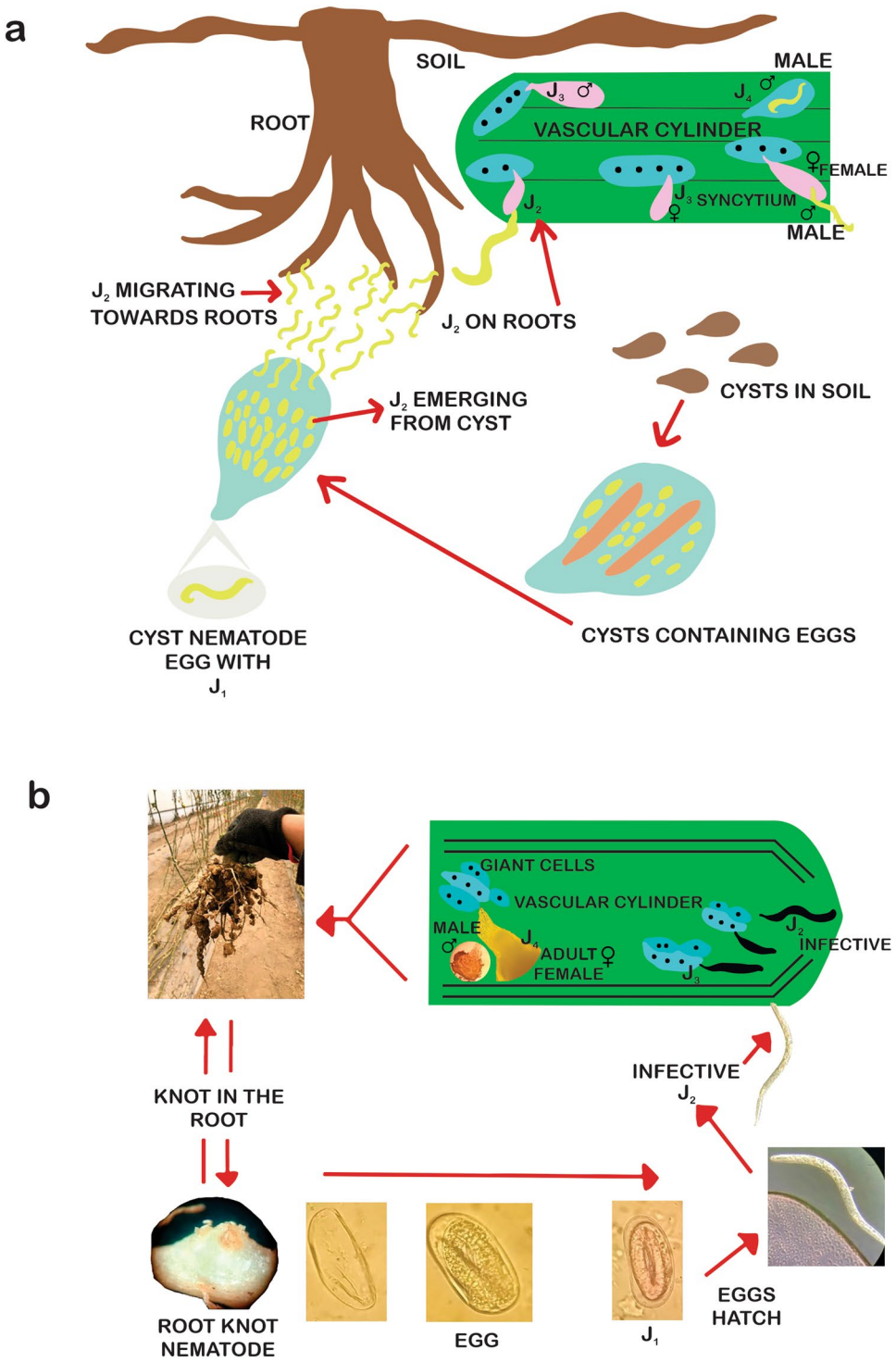

Fig. 1 Life cycle of **a** a cyst-forming nematode and **b** a root-knot nematode at different stages of development

to plants or their products (Lopez-y-Lopez et al. 2000; Armendáriz et al. 2015; Khan et al. 2020). Predatory organisms, such as parasitic fungi, nematodes, microarthropods, and pathogens, such as viruses and bacteria, are natural enemies of PPNs and reduce the size of their populations. While the control exerted by these organisms has been found to be effective under laboratory conditions, effective results have not been completely obtained via their application in the field, due to environmental circumstances (Galindo et al. 2013). Nematophagous bacteria and fungi are among the main microbial groups with potential as biological agents for the control of RKNs and CNs (Khan et al. 2020), given the need for environmentally beneficial and low-cost alternatives to chemical measures for the control of PPNs, without

affecting vertebrates, crops, and other non-target organisms. Therefore, highly specific antagonists, preferably soil-borne, are the most appropriate for the above-described objective. Diverse entomopathogenic, nematophagous, and fungicides have been reported for the control of soil-transmitted insect pests and plant pathogens; from which nematophagous fungi may fulfil an analogous function for the biocontrol of PPNs (Degenkolb and Vilcinskas 2016). Table 3 shows the main characteristics of nematophagous fungi, which can be grouped as scavengers (predators), endoparasites, parasites of eggs and females or toxin producers, according to their mode of infecting nematodes.

Although nematophagous fungi show a variable specificity regarding to the nematode species they infect, in general, they infect vermiform and/or nematode eggs. *Purpureocillium lilacinum* presents superior attributes for infecting nematode eggs, using its conidia to penetrate inside (Sagüés et al. 2011), while *P. chlamydosporia* var. *chlamydosporia* infects nematode eggs and J_2-stage juveniles by means of its ability to produce secondary metabolites such as aurovertins and pochonins, among others (Shinonaga et al. 2009; Zhou et al. 2010). The mechanism of interaction between fungi and nematode begins during the immobile stage J_1, when the nematode is yet to leave the egg. *P. chlamydosporia* hyphae grow towards the eggs, forming appressoria with its hyphae tips that penetrate the eggshell, then digesting the content of both immature and mature (containing juveniles) eggs (Nordbring-Hertz et al. 2006). Larriba et al. (2014) indicate that this parasitic mechanism comprises three stages: (1) adhesion, wherein the hyphae recognizes the surface and components of the chorion and secretes glycoproteins for egg adhesion; (2) an appressorium in the hyphae tip, via the secretion of proteases (P32, VCP1, and SCP1) which expose the chitin layer of the egg (in turn degraded by chitinases) that enables the penetration phase and finally, (3) the fungus colonization inside the egg affecting the first developmental stage of embryos as well as J_1 to J_2 juveniles actively developing. Subsequently, the fungus absorbs nutrients such as trehalose, a carbohydrate essential for the development and survival of the PPN and also for physiological processes, such as the hatching of the egg, development and growth during the different nematode developmental stages, sugar transport, energy accumulation and protection of somatic cells (Behm 1997; Sellito et al. 2016; Avelar et al. 2017; Silva-Valenzuela et al. 2020).

Figure 2 depicts some structural chemical forms related to a variety of metabolites produced by nematophagous fungi, for example, there are 139 compounds reported for *P. chlamydosporia* (Niu 2017; Silva-Valenzuela et al. 2020), from which aurovertins D, E, F, I and the phomalactones present nematicide activity against PPNs (Hellwing et al. 2003; Shinonaga et al. 2009; Niu et al. 2010; Zhou et al. 2010; Kumar et al. 2013; Wang et al. 2015; Bogner et al. 2017; Niu 2017). Similarly, Fig. 3 shows the main compounds of *P. lilacinum* with nematicide activity, among which 2-ethyl butyric acid, phenyl ethyl alcohol, benzoic acid, benzene acetic acid,

Table 3 Classification of nematophagous fungi

Fungi	Characteristics	Examples	References
Scavengers (predators)	Capture vermiform nematodes via specialized organs formed in the hypha Use constricting rings, adhesive networks, and traps to capture said prey	*Arthrobotrys, Dactylaria, Dactylella* *Drechslerella* *Monacrosporium.* *Gamsylella*	Sagüés et al. (2011), Li et al. (2015), Zhang et al. (2020)
Endoparasites	Use conidia to infect nematodes, growing within their interior. Spores may be either mobile or immobile and adhesive They have a more restricted range of hosts than the nemotode scavengers and spend their vegetative life within the infected nematodes	*Ophiocordycipitaceae* *Catenaria anguillulae* (mobile spores) (mobile spores) *Drechmeria coniospora* *Hirsutella rhossiliensis, Haptoglossa* (mobile or adhesive spores) (mobile or adhesive spores)	Moosavi and Zare (2012)
Parasites of eggs and females	Use appressorium to carry out the infection Present a variable specificity with regard to the nematode species they infect	*Pochonia chlamydosporia* *Purpureocillium lilacinum*	Sagüés et al. (2011)
Toxin producers	Immobilize the nematodes prior to penetration, using substances such as alkaloids, peptides, terpenoids, sterols, aliphatic compounds, and quinones. These are produced in specialized hypha strands located in the tips of the hyphae that grow chemotropically in the mouth of their prey and digest the content	*Pochonia chlamydosporia* *Purpureocillium lilacinum*	Hellwig et al. (2003), Shinonaga et al. (2009), Zhou et al. (2010), Li and Zhang (2014)

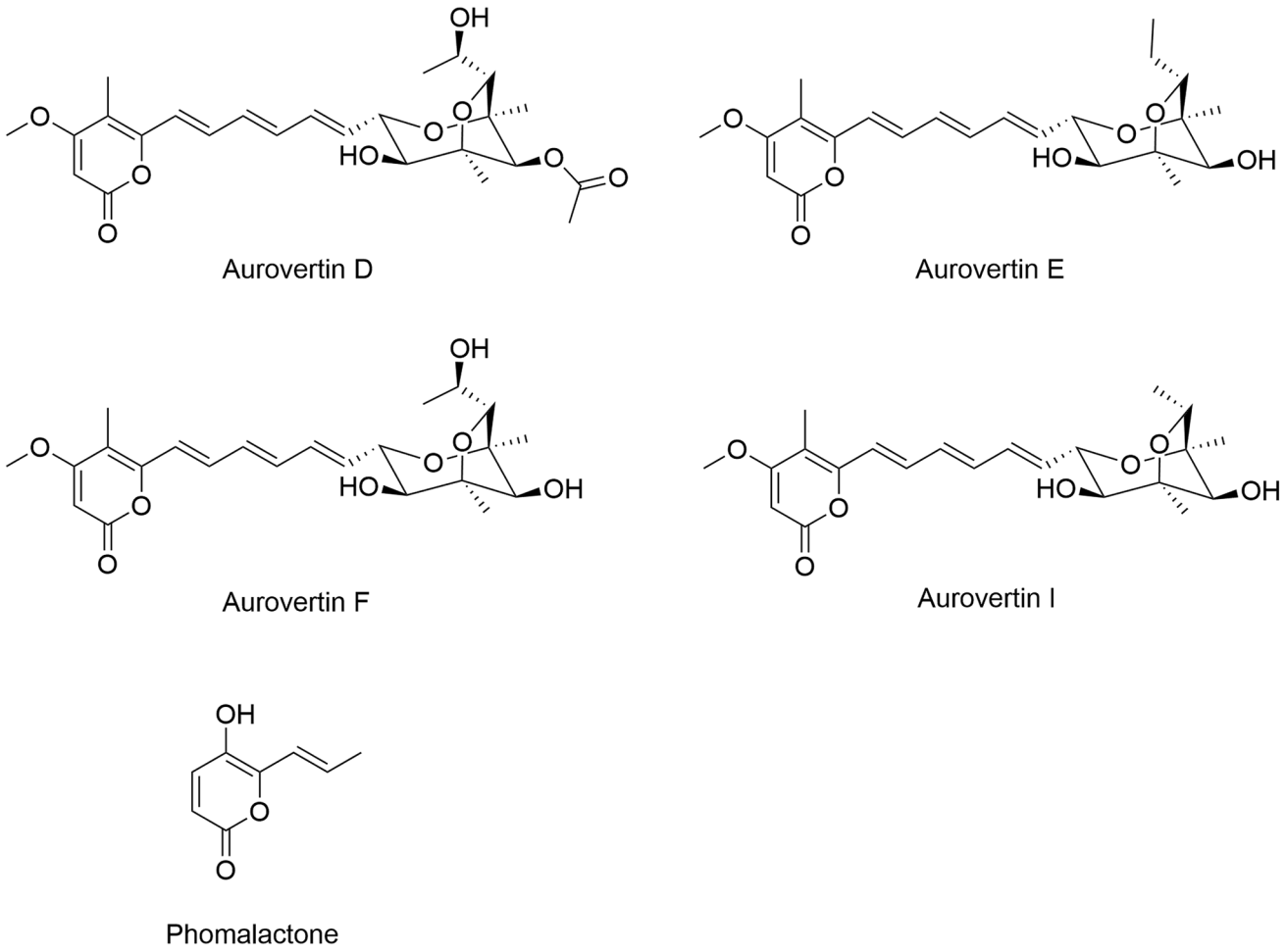

Fig. 2 Structure of compounds that show nematicidal activity produced by *P. chlamydosporia*

3,5-Di-t-butylphenol (Sharma et al. 2020) and ethyl acetate (Sharma et al. 2014) present an effective nematicide action against *M. incognita*, inhibiting the eclosion of the egg mass and the growth of juveniles during stage J_2.

Challenges for the production of the nematophagous fungi *Pochonia chlamydosporia* and *Purpureocillium lilacinum*

According to the information described before about how nematophagous fungi exert their effect on PPNs, it is important to preserve their attributes of action, either in terms of their spores, chlamydospores or produced metabolites. However, one of the main challenges is to reduce the fermentation times reported, since the evidence shows that, metabolites of interest are produced in more than ten days, in specific culture media. This could complicate industrial production, due to the fact that longer times and greater scales for production implies higher energy costs of maintaining temperature, aeration, agitation and higher labor costs, given the duration of the process. Studies conducted mainly on submerged fermentation are presented below, along with a description of production conditions that could be applied to develop robust processes for mass production of nematophagous fungi.

Dube and Smart (1987) conducted experiments of *P. lilacinum* (Thorn) Samson at flask level (500 mL) with periodical agitation for 10 days at 25–30 °C in soaked and drained sterile wheat seeds, adding 4 g of wheat seed inoculated with 4×10^7 conidia. It was observed a suppression of the RKNs of *M. incognita*, at their egg mass, and hatching in greenhouse experiments conducted on tomato (*Lycopersicon esculentum*), tobacco (*Nicotiana tabacum*), and pepper (*Capsicum annuum*). Cabanillas and Barker (1989) produced *P. lilacinum* in plastic bags containing wheat kernels inoculated with a spore suspension (3.5×10^7 spores/mL), incubated for 21 days at 25 °C, obtaining 3.5×10^9 spores/g of wheat kernels. In the other hand, Cabanillas et al. (1989) tested the growth temperature (12, 16, 20, 24, 28, 30, 32, 34, and 36 °C) of 13 *P. lilacinum* isolates in a 125 Erlenmeyer flask containing 20 mL of potato dextrose broth inoculated

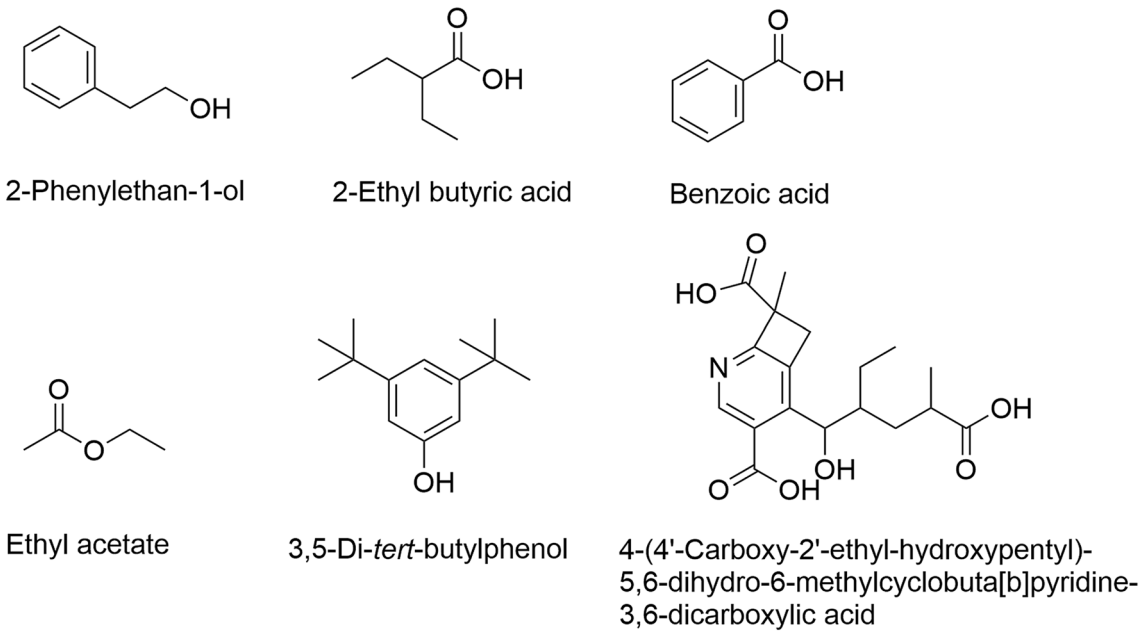

Fig. 3 Structure of compounds that show nematicidal activity produced by *P. lilacinum*

with a 5 mm disc containing a 7-day-old PDA culture, agitated manually and then incubated in the dark. They found that optimum temperatures for *P. lilacinum* ranged from 24 to 30 °C, although they also observed similar growth patterns obtained varying levels of mycelium production. Interestingly, the best results for the control of *M. incognita* in tomato plants were obtained using an isolate from Peru and a mixture of *P. lilacinum* isolates, with the Peruvian strain PL 84 − 1 achieving moderate growth (3.23 mg/mL), lower than the 7.1 mg/mL obtained using one of the strains tested before.

Kerry et al. (1986) evaluated the conidia production in Czapek broth with the addition of trace elements from different *P. chlamydosporia* strains, using a 150 mL conical flask with 75 mL of medium incubated at 19 °C, 180 rpm for 28 days, obtaining large numbers of conidia (up to 8.4×10^7/mL) but not chlamydospores. Stirling and Smith (1998) developed granular formulations containing either *P. chlamydosporia* or *Arthrobotrys dactyloides* produced in glucose-peptone yeast or glucose-corn steep, respectively, in a two-liter Erlenmeyer flask. They harvested 10 g of dry mycelia from 1-liter liquid medium, using the biomass obtained to produce 1 kg of granules. They also reported that *A. dactyloides* was more effective in controlling RKNs in field trials conducted on a tomato plantation in Queensland, Australia. It was also reported the evaluation of media containing yeast extract, peptone, soybean meal, cotton seed meal, crushed maize meal, neopeptone, or malt extract in combination with 40 g/L glucose, which was used for the mass production of *P. chlamydosporia* at flask level, with 30 mL of medium incubated in 125 mL Erlenmeyer flasks at 25 °C and 200 rpm for 5 days (Stirling et al. 1998). They found that media containing either cottonseed meal or soybean flour were the most suitable for biomass production, obtaining 18 g/L and 15 g/L respectively, while conidia concentrations of approximately 6×10^8 conidia/mL were obtained for both media. Interestingly, the same study conducted fermentation with YPD medium in a 20-L bioreactor under initial conditions of 0.6 vvm, 200 rpm and 25 °C, making it one of the few studies conducted at bioreactor scale, producing 8–11 g/L biomass over four production runs. The authors describe that chlamydospores were not produced in this submerged culture (Stilring and Smith 1998).

Mo et al. (2005) evaluated the effect of 21 carbon sources and 15 nitrogen sources on the mycelial growth and sporulation of *P. chlamydosporia*, finding that sweet potato and L-tyrosine are the optimal carbon and nitrogen sources, respectively, for mycelial growth (5.1 g/L), whereas sweet potato and casein peptone were suitable for sporulation (1.71×10^8 conidia/mL). The authors also found different nutritional requirements for sporulation and growth, obtaining maximum conidia production (4.7×10^7 conidia/mL) with a carbon:nitrogen ratio (C:N) of 10 and initial pH of 3.7, while 9.25 g/L of biomass were produced at initial pH of 6.8 and C:N ratio of 40. The incubation conditions were 100 mL medium in a 250 mL conical flask at 170 rpm and 28 °C for seven days. Hernández and Hidalgo (2008) proposed a production method involving solid state fermentation in KlamiC® polypropylene bags using the *P. chlamydosporia* var. *catenulata* strain IMI SD 187, selected for its properties

as a bioregulator for mass production, since it is able to grow in a pH range of 4–9 at soil temperatures among 9–38 °C. This method is recommended for the management of the *Meloidogyne* spp. populations that infect tomato, cucumber, capsicum, carrot, beet, and lettuce crops, among others.

The *P. chlamydosporia* strain YMF 1.00613, isolated from tobacco root infected with *M. incognita*, was used to isolate and identify four aurovertin-type metabolites, including a new compound, aurovertin I (A1), and three known metabolites, aurovertins E, F and D (shown in Fig. 2) (Niu et al. 2010). The authors obtained a production level of 20 L in 500 mL Erlenmeyer flasks containing 200 mL of production medium, 20% unpeeled potato, and 2% glucose, at a pH of 7, incubated at 28 °C, 180 rpm for 12 days. Both the mycelium and the fermentation broth were filtered and concentrated for characterization. After 48 h, aurovertins D and F presented a nematicide effect on the free-living nematode *Panagrellus redivivus*, with determined LC_{50} values of 41.7 and 88.6 µg/mL, respectively. However, the four aurovertins did not present inhibitory effects on the eclosion of *M. incognita* eggs (Niu et al. 2010). These results are important and reveal the need of producing not only mycelium and spores (conidia or chlamydospores) but also the metabolites and enzymes involved in PPNs inhibition. Wang et al. (2015) cultured different *P. chlamydosporia* strains, including the YMF 1.00613 strain isolated from tobacco nodules infected with *M. incognita*, in 250 mL PDB medium contained in 500-mL flasks incubated at 28 °C, 250 rpm for 12 days. The *P. chlamydosporia* strains that presented nematicide activity produced a distinctive yellow fermentation broth. Chemical studies have shown that yellow metabolites consist of polyketides from the aurovertins D, E, E, and I, with aurovertin D achieving the highest mortality for the RKN *M. incognita*, with a LC_{50} value of 16 µg/mL at their stage J_2 and 33.50 µg/mL for *C. elegans*. It was also observed that aurovertin was produced from the fifth day of fermentation until the growth of the fungus concluded.

On the other hand, the addition of chitosan to the culture medium improved sporulation, as seen for the production of extracellular enzymes of *P. chlamydosporia*, and the parasitic effect on RKN eggs, observed in 50 mL culture medium containing chitosan concentrations of 0.1 mg/mL, 1 mg/mL, and 2 mg/mL as the nutrient source, mineral salts, yeast extract and 1% (w/v) glass wool (Escudero et al. 2016). Compared to the control (without chitosan), it was observed a 2-fold (0.1 mg/mL) to 4-fold (1 to 2 mg/mL) increase in proteolytic activity. The combinations were incubated in 250 mL flasks for 30 days, in darkness. It was also found that chitosan, at a concentration of 0.1 mg/mL, does not affect the viability and germination of chlamydospores and improves mycelial growth compared to cultures without chitosan (Escudero et al. 2017). Silva et al. (2017) grew *P. chlamydosporia* (var. *catenulata* and *chlamydosporia*) and *P. lilacinum* fungal strains in PDA medium + streptomycin (0.5 g/L) for a period of 18–21 days at 26 °C ± 0.5 °C in darkness. The cells (spores and mycelia) obtained from these cultures were suspended in distilled water + Tween 80 (0.05% v/v). The suspensions were then homogenized and filtered to facilitate the assays development with *M. enterolobii* nematode eggs, to evaluate their efficacy in reducing root infection and nematode reproduction in potted plants.

Another study cultured *P. chlamydosporia* in 250 mL Erlenmeyer flasks with dextrose and potato broth, incubated at 25 °C for ten days in a rotary shaker, with the medium then filtered at the end of the incubation. The filtered culture was used to simulate the effect of mortality during *M. incognita* egg eclosion and at their J_2-stage, at 20, 40, 60, 80, and 100% filtrate concentrations diluted with water and taken from six isolates. The impact on mortality was within a range of 11.3–76.3% after 72 h of filtering and increased in line with the concentration. The fungus PC-6 inhibited nematode egg eclosion by 58.17%, with PC-1 achieving the second highest level of inhibition (Uddin et al. 2019).

Shirazi et al. (2019) cultured the fungi *P. chlamydosporia* and *P. lilacinum* on solid substrates for two months, using wheat, barley, rice husks, and rice bran, finding that *P. chlamydosporia* produced spores in wheat, barley, and rice bran after 30 days and after 60 days in rice husks, with an average of 1.5×10^8 and 7×10^7 spores/g, respectively. *P. lilacinum* colonized all the substrates, with higher spore yields obtained in barley grains and rice husks, with an average of 2.7×10^8 and 1.5×10^8 spores/g, respectively. The viability of both fungi decreased after 60 days of storage at 25 °C.

The production of *P. lilacinum* KU8 used five different agro-residues, such as wheat bran fine particles, beer waste, sugarcane bagasse, coffee husks and spent tea waste, with 10 g of each substrate placed in a 250 mL Erlenmeyer flask along with 4 mL of a mineral salt medium. The fermentation was carried out at a pH of 4.4 and a temperature of 30 °C for up to 12 h in order to obtain maximum biomass. The isolate produced a biomass of 107.46 mg/gdfs (mg of biomass per gram of dried fermented substrate) in rice bran. The experiment was conducted to evaluate the production of a bionematicide for the management of PPNs (Mousumi Das et al. 2020).

In other study, *P. lilacinum* 6029 spores were used in a medium based on karanja (a species of tree from the pea family *Millettia pinnata*) cake to inoculate Erlenmeyer flasks containing 100 mL Czapek-Dox broth and a karanja-cake based broth at a C:N ratio of 35.88 and a pH of 5.9. The fungal culture was incubated in darkness for 7 and 15 days at 27 °C, with the fermentation then filtered and used for *in vitro* bioassays to evaluate a possible nematicide effect. It was found that the karanja-cake-based culture medium killed 100% of J_2 *M. incognita* nematodes, while a 78.28% mortality was observed for the filtered Czapek-Dox broth

12 h post exposure (Sharma et al. 2014). A recent study conducted by the same authors reported the identification of nematicide metabolites based on a directed fractionation produced using the fungus *P. lilacinum* grown in a defatted karanja cake-based liquid medium. The mortality rate of *M. incognita* during egg mass eclosion was 94.6% by the fifth day, while the maximum nematicide effect observed for J_2 nematodes was 62% after 48 h of exposure (Sharma et al. 2020). The results reported by Ferreira et al. (2020) show that the application of ketamine, both *in vitro* and *in vivo*, confirms the nematicide potential of this molecule in the fungus *P. chlamydosporia*, which was cultured in a 250 mL Erlenmeyer flask containing a dextrose and potato liquid medium, incubated at 28 °C, 120 rpm for 20 days, filtrating after to separate the extract from the mycelial mass. Separated mass was used for the extraction process via maceration, while the ketamine compound was identified by nuclear magnetic resonance spectroscopy.

According to the information above mentioned, the conditions evaluated for cultures of nematophagous fungi at flask level were oriented mainly to the application against phytoparasitic nematodes instead of their massive production. However, for the case of submerged fermentation, such conditions and raw materials are useful for the process engineering in order to stablish the next approach in bioreactor studies at the laboratory scale and the subsequent steps for scaling-up the production process. In such approach, the effect of parameters such as agitation, aeration, dissolved oxygen tension, mixing, etc., on morphology, spores, conidia, and metabolite production must be elucidated.

Market opportunity

Pesticide market is predicted to reach USD $70.89 billion by 2025 (Industry ARC 2020). The biopesticides represent only 3% with a market of USD $2.2 billion globally and it was estimated to grow over 5% between 2020 and 2026 (Global Markets Insights 2019). For the case of bionematicides market, in 2015 it was valued over USD $143 million with an increment of 4% annual (Global Markets Insights 2015), whereas nematicide market in 2020 was valuated at USD $1.3 billion (Market and Market 2020). Hence, there is a huge market opportunity to replace synthetic pesticides with bionematicides. Table 4 presents the cost comparison between the principal raw materials reported in fermentation process for nematophagous fungi production, the price of biobased nematicides with nematophagous fungi, the cost of synthetic products commercialized in Mexico (local providers), and doses of application. Given these prices, the bionematicides can be competitive compared to synthetic products. It seems logical that agro-based low-cost raw materials can be used for large scale production of nematophagous fungi. However, there are intrinsic steps of pre-treatments for such raw materials and local availability that increases the total process cost. Additionally, the time required for many processes described for cultures of nematophagous fungi comprises several days, and some efforts must be made to reduce such time. A system for the production and formulation of the biological control agent should be developed to obtain a product with a suitable long shelf life, providing it certain competitive advantages in the environment in which it will be applied. Subsequently, the process should be escalated to pilot level to obtain enough product that allows to evaluate its activity in both the greenhouse and the field. Finally, if the product achieves the required attributes and its production is viable at both technical and economic terms, it will be registered and commercialized (Janisiewicz and Korsten 2002). Placing more biobased products with a high activity on the market will not only be an economic benefit but also a huge profit for the environment and human well-being.

Table 4 Cost comparison between some examples of raw materials reported for nematophagous fungi production, biobased products, and synthetic control agents

Presentation	Strain	Biological control product	Synthetic chemical product	Raw material/active ingredient	Quantity	Dose	Price USD
				Potato Dextrose Agar	500 g		102.49
				Potato Dextrose Broth	450 g		77.75
				Yeast extract	1 kg		16.47
				Casein peptone	300 g		46.40
				Dextrose	1 kg		4.2
				Wheat	1 kg		0.45
				Rice husk	1 kg		0.23
				Barley	1 kg		0.45
Liquid suspension	*Paecilomyces lilacinus* LAL P832	Pacilomic®			1 L	1 L/ha	33.08
	Paecilomyces lilacinus	SPECTRUM Pae L®			1 L	4 L/ha	35
	Paecilomyces fumosoroseus y *Paecilomyces lilacinus*	Bio Delta Plus ®			1 L	2–4 L/ha	14.80
Powder	*Pochonia chlamydosporia* IMI SD 187	KlamiC®			1 L	0.5 kg/ha	70
	Paecilomyces lilacinus	Nematrof®			1 kg	2 kg/ha	17.33
	Paecilomyces lilacinus y *Pochonia chlamydosporia*	IMPERIUM®			1 kg	1–2 kg/ha	44.20
Emulsifiable concentrate			NEMACUR® 400 CE	Fenamiphos	1 L	10–15 L/ha	49.95
			MOCAP® CE	Ethoprophos	1 L	5.5-7 L/ha	42.50
			Verango Prime®	Fluopyram	1 L	1 L/ha	325
Powder			Coster 5G®	Terbufos	5 kg	20–25 kg/ha	21.5
Granulate			Velfuran 5.G®	Carbofuran	20 kg	40–50 kg/ha	75.45

Conclusions

Given the growing concerns on the environment, pollution, and health risks caused by many conventional agrochemicals, the demand for natural biological products is constantly increasing in all markets. It is expected that the use of chemical nematicides could be eliminated completely and substituted for biological alternatives, such as the use of fungi and bacteria which, further to killing nematodes, they promote plant growth without causing environmental damage. The aim is to raise awareness of bionematicides on the market in order to increase sustainable agriculture. The development of any biological control agent should take into consideration the life cycle of the target organism to develop the best formulation and application strategies. In order to achieve mass production, biomass, conidia or chlamydospore development is necessary, as it is the production of enzymes and metabolites from nematophagous fungi to evaluate which of them better inhibit PPNs in the short term to prevent the infection of the plant. The development of media for both submerged fermentation and solid-state strategies for production may be a significant step for achieving a robust production process. However, there is also a lack of research on the operating conditions for fermenters at a scale that could satisfy the needs for the application of this technology, which, as we have reported here, depends on the strains, the isolation source, and their ability to maintain its nematicidal activity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11274-021-03151-x.

Acknowledgements The authors acknowledge the financial supported provided by BIOKRONE S. A de C. V. and the received fellowship from Instituto Politécnico Nacional.

Declarations

Conflict of interest The authors have no conflict of interest.

References

Abad P, Gouzy J, Aury JM, Castagnone-Sereno P, Danchin EGJ et al (2008) Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nat Biotechnol 26:909–915. https://doi.org/10.1038/nbt.1482

Agrios GN (2005) Plant Pathology, 5th edn. Elsevier Academic Press, Amsterdam

Aguinaldo AM, Turbeville JM, Linford LS, Rivera MC, Garey JR, Raff RA, Lake JA (1997) Evidence for a clade of nematodes, arthropods and other moulting animals. Nat 387:489–493. https://doi.org/10.1038/387489a0

Armendáriz GI, Quiña CD, Rios SM, Landázuri AP (2015) Plant pathogenic nematodes and their control strategies. University of the Armed Forces-ESPE. https://doi.org/10.13140/RG.2.1.1599.9446

Avelar Monteiro TS, Lopes EA, Evans HC, Grassi de Freitas L (2017) Interactions between *Pochonia chlamydosporia* and nematodes. In: Manzanilla-López R, Lopez-Llorca L (eds) Perspectives in sustainable nematode management through Pochonia chlamydosporia applications for root and rhizosphere health. Sustainability in plant and crop protection. Springer, Cham, pp 77–96. https://doi.org/10.1007/978-3-319-59224-4_4

Behm CA (1997) The role of trehalose in the physiology of nematodes. Int J Parasitol 27:215–229. https://doi.org/10.1016/s0020-7519(96)00151-8

Bello A, Escuer M, Arias M (1994) Nematological problems, production systems and Mediterranean environments. EPPO Bull 24:383–391. https://doi.org/10.1111/j.1365-2338.1994.tb01394.x

Ben-Ami F, Heller J (2005) Spatial and temporal patterns of parthenogenesis and parasitism in the freshwater snail *Melanoides tuberculata*. J Evol Biol 18:138–146. https://doi.org/10.1111/j.1420-9101.2004.00791.x

Bernard GC, Egnin M, Bonsi C (2017) The Impact of plant-parasitic nematodes on agriculture and methods of control. Nematol Concepts Diagn Control. https://doi.org/10.5772/intechopen.68958

Bogner CW, Kamdem RS, Sichtermann G, Matthäus C, Hölscher D, Popp J, Proksch P, Grundler FM, Schouten A (2017) Bioactive secondary metabolites with multiple activities from a fungal endophyte. Microbiol Biotechnol 10:175–188. https://doi.org/10.1111/1751-7915.12467

Bongers T, Ferris H (1999) Nematode community structure as a bioindicator in environmental monitoring. Trends Ecol Evol 14:224–228. https://doi.org/10.1016/s0169-5347(98)01583-3

Brodie BB (1998) Potato cyst nematodes (*Globodera* species) in Central and North America. In: Marks JR, Brodie BB (eds) Potato cyst nematodes: biology, distribution and control. CAB International, Wallingford, pp 317–331

Cabanillas E, Barker KR (1989) Impact of *Paecilomyces lilacinus* inoculum level and application time on control of *Meloidogyne incognita* on tomato. J Nematol 21:115–120

Cabanillas E, Barker KR, Nelson LA (1989) Growth of isolates of *Paecilomyces lilacinus* and their efficacy in biocontrol of *Meloidogyne incognita* on tomato. J Nematol 21:164–172

Cabrera-Hidalgo AJ, Valdovinos-Ponce G, Mora-Aguilera G, Rebollar-Alviter A, Marban-Mendoza N (2014) Occurrence of *Nacobbus aberrans* in horticultural crops in northwestern Michoacán, Mexico. Nematropica 44:107–117

Davies LJ, Brown CR, Elling AA (2015) Calcium is involved in the $R_{Mc1(blb)}$–mediated hypersensitive response against *Meloidogyne chitwoodi* in potato. Plant Cell Rep 34:167–177. https://doi.org/10.1007/s00299-014-1697-1

Degenkolb T, Vilcinskas A (2016) Metabolites from nematophagous fungi and nematicidal natural products from fungi as an alternative for biological control. Part I: metabolites from nematophagous ascomycetes. Appl Microbiol Biotechnol 100:3799–3812. https://doi.org/10.1007/s00253-015-7233-6

del Cid Prado VI, Tovar SA, Hernández JA (2001) Distribution of species and races of *Meloidogyne* in Mexico. Rev Mex Fitopatol 19:32–39

del Cid Prado VI, Franco-Navarro F, Godinez-Vidal D (2018) Plant parasitic nematodes and management strategies of major crops in Mexico. In: Subbotin S, Chitambar J (eds) Plant parasitic nematodes in sustainable agriculture of North America. Sustainability in plant and crop protection. Springer, Cham, pp 31–68. https://doi.org/10.1007/978-3-319-99585-4_2

del Puerto-Rodríguez AM, Suárez TS, Palacio EDE (2014) Effects of pesticides on health and the environment. Rev Cubana Hig Epidemiol 52:372–387

Dube B, Smart GC (1987) Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. J Nematol 19:222–227

Ebone LA, Kovaleski M, Cardoso DC (2019) Nematicides: history, mode, and mechanism action. Plant Sci *Today* 6:91–97. https://doi.org/10.14719/pst.2019.6.2.468

Escobar-Avila IM, Medina-Canales MG, Tovar-Soto A (2017) Distribution, life cycle and histological changes by *Heterodera* sp. in carrot in Puebla. Rev Mex Fitopatol 35:304–313. https://doi.org/10.18781/r.mex.fit.1606-7

Escobar-Avila IM, López-Villegas E, Subbotin SA, Tovar-Soto A (2018) First report of carrot cyst nematode *Heterodera carotae* in Mexico: morphological, molecular characterization, and host range study. J Nematol 50:229–242. https://doi.org/10.21307/jofnem-2018-021

Escudero N, Sebastião R, Lopez-Moya F, Naranjo-Ortiz Miguel A, Marin-Ortiz AI, Thornton Christopher R, Lopez-Llorca LV (2016) Chitosan enhances parasitism of *Meloidogyne javanica* eggs by the nematophagous fungus *Pochonia chlamydosporia*. Fungal Biol 120:572–585. https://doi.org/10.1016/j.funbio.2015.12.005

Escudero N, Lopez-Moya F, Ghahremani Z, Zavala-Gonzalez EA, Alaguero-Cordovilla A, Ros-Ibañez C, Lacasa A, Sorribas FJ, Lopez-Llorca LV (2017) Chitosan increases tomato root colonization by *Pochonia chlamydosporia* and their combination reduces toot-knot nematode damage. Front Plant Sci 8:1415. https://doi.org/10.3389/fpls.2017.01415

Evans E, Trudgill DL, Webster JM (1993) Plant parasitic nematodes in temperature agriculture. CABI Publishing, Wallingford

Ferreira SR, Machado ART, Furtado LF et al (2020) Ketamine can be produced by *Pochonia chlamydosporia*: an old molecule and a new anthelmintic? Parasit Vectors 13:527. https://doi.org/10.1186/s13071-020-04402-w

Fosu-Nyarko J, Nicol P, Naz F, Gill R, Jones MGK (2016) Analysis of the transcriptome of the infective stage of the beet cyst nematode, *H. schachtii*. PLoS ONE 11(1):47511. https://doi.org/10.1371/journal.pone.0147511

Galindo E, Serrano-Carreon L, Gutierrez CR, Allende R, Balderas K, Patino M, Trejo M, Wong MA, Rayo E, Isauro D, Jurado C (2013) The challenges of introducing a new biofungicide to the market: a case study. Electron J Biotechnol 16:5. https://doi.org/10.2225/vol16-issue3-fulltext-6

Global Market Insights (2015) https://www.gminsights.com/industry-analysis/bionematicides-market. Accessed 02 July 2021

Global Market Insights (2019) https://www.gminsights.com/industry-analysis/biopesticides-market. Accessed 02 July 2021

Guzmán POA, Castaño ZJ, Villegas EB (2012) Main plant parasitic nematodes and symptoms caused in economically important crops. Agron 20:38–50

Hellwing V, Mayer-Bartschmid A, Muller H, Greif G, Kleymann G, Zitzmann W, Tichy HV, Stadler M (2003) Pochonins A – F, new antiviral and antiparasitic resorcylic acid lactones from

Pochonia chlamydosporia var. *catenulata*. J Nat Prod 66:829–837. https://doi.org/10.1021/np020556v

Hernández MA, Hidalgo DL (2008) KlamiC®: Agricultural bionematicida from the fungus *Pochonia chlamydosporia* var. *catenulata*. Rev Protección Veg 23:131–134

Industry ARC (2020) https://www.industryarc.com/Report/18229/pesticides-market-research-report-analysis.html. Accessed 02 July 2021

Janisiewicz W, Korsten L (2002) Biological control of postharvest diseases of fruits. Ann Rev Phytopathol 40:411–441. https://doi.org/10.1146/annurev.phyto.40.120401.130158

Jones L, Giorgi C, Urwin P (2011) *C. elegans* as a resource for studies on plant parasitic nematodes. In Jones J, Gheysen G, Fenoll C (eds) Genomics and molecular genetics of plant-nematode interactions, Springer Netherlands, pp 175–220. https://doi.org/10.1007/978-94-007-0434-3_10

Joshi AKR, Sukumaran BO (2019) Metabolic dyshomeostasis by organophosphate insecticides: insights from experimental and human studies. EXCLI J 18:479–484. https://doi.org/10.17179/excli2019-1492

Kerry BR, Simon A, Rovira AD (1984) Observations on the introduction of *Verticillium chlamydosporium* and other parasitic fungi into soil for control of the cereal cyst-nematode *Heterodera avenue*. Ann Appl Biol 105:509–516. https://doi.org/10.1111/j.1744-7348.1984.tb03077.x

Kerry BR, Irving F, Hornsey JC (1986) Variation between strains of the nematophagous fungus, *Verticillium Chlamydosporium* Goddard. I. Factors affecting growth *in vitro*. Nematologica 32:461–473. https://doi.org/10.1163/187529286X00336

Khan RAA, Najeeb S, Mao Z, Ling J, Yang Y, Li Y, Xie B (2020) Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic bacteria and root-knot nematode. Microorganisms 8:401. https://doi.org/10.3390/microorganisms8030401

Kumar V, Ashok S, Park S (2013) Recent advances in biological production of 3-hydroxypropionic acid. Biotechnol Adv 31:945–961. https://doi.org/10.1016/j.biotechadv.2013.02.008

Lara-Posadas AV, Núñez-Sánchez AE, López-Lima D (2016) Plant parasitic nematodes associated to banana roots (*Musa acuminata* AA) in central Veracruz, México. Rev Mex Fitopalol 34:116–130. https://doi.org/10.18781/R.MEX.FIT.1507-7

Larriba E, Jaime MD, Carbonell-Caballero J, Conesa A, Dopazo J, Nislow C, Martín-Nieto J, Lopez-Llorca LV (2014) Sequencing and functional analysis of the genome of a nematode egg-parasitic fungus. Fungal Genet Biol 65:69–80. https://doi.org/10.1016/j.fgb.2014.02.002

Lata R, Komal T, Neha K, Neelam S, Sukhbir S, Ajmer SG, Arun LS, Jyotsna K (2021) An extensive review on the consequences of chemical pesticides on human health and environment. J Clean Prod 283:124657. https://doi.org/10.1016/j.jclepro.2020.124657

Li GH, Zhang KQ (2014) Nematode-toxic fungi and their nematicidal metabolites. In: Zhang KQ, Hyde K (eds) Nematode-trapping fungi. Fungal Diversity Research Series. Springer, Dordrecht, pp 313–337. https://doi.org/10.1007/978-94-017-8730-7_7

Li Y, Zhang C, Yin Y, Cui F, Cai J, Chen Z, Jin Y, Robson MG, Li M, Ren Y, Huang X, Hu R (2014) Neurological effects of pesticide use among farmers in China. Int J Environ Res Publ Health 11:3995–4006. https://doi.org/10.3390/ijerph110403995

Li J, Zou C, Xu J, Ji X, Niu X, Yang J, Huang X, Zhang KQ (2015) Molecular mechanisms of nematode-nematophagous microbe interactions: basis for biological control of plant-parasitic nematodes. Annu Rev Phytopathol 53:67–95. https://doi.org/10.1146/annurev-phyto-080614-120336

Lopez-y-Lopez EV, Chavarria-Hernandez N, Fernandez-Sumano P, de la Torre M (2000) Fermentation processes for bioinsecticide production. An overview. Recent Res Devel Biotec Bioeng 3:1–20

Luc M, Sikora RA, Bride J (2005) Plant parasitic nematodes in subtropical agriculture, 2nd edn. CABI Bioscience, Egham

Mandal HR, Katel S, Subedi S, Shrestha J (2021) Plant parasitic nematodes and their management in crop production: a review. J Agric Nat Resour 4:327–338. https://doi.org/10.3126/janr.v4i2.33950

Market and Markets (2020) https://www.marketsandmarkets.com/Market-Reports/nematicides-market-193252005.html. Accessed 02 July 2021

Martínez GJ, Díaz VT, Allende MR, Ortiz MJA, García ERS, Carrillo FJA (2014) Phytoparasitic nematodes associated with the cultivation of papaya (*Carica papaya* L.) in Colima, México. Rev Mexicana Cienc Agric 5:317–323. https://doi.org/10.29312/remexca.v5i2.969

Martínez GJ, Díaz VT, Allende MR, García ERS, Carrillo FJA (2015) First report of *Meloidogyne enterolobii* infesting tomato in Culiacan, Sinaloa, Mexico. Rev *Mexicana* Cienc Agric 6:2165–2168. https://doi.org/10.29312/remexca.v0i11.786

Martínez GJ, Valdés T, Molar R, Manjarrez JE, Carrillo FJA (2019) Identification and distribution of *Meloidogyne* spp. in tomato in Sinaloa Mexico. Rev *Mexicana* Cienc Agric 10:453–459. https://doi.org/10.29312/remexca.v10i2.392

Medina-Molina CO, Medina-Canales MG, Torres-Coronel R, Carvajal-Sandoval A, Tovar-Soto A (2018) Histological changes for root-knot nematode *Meloidogyne incognita* in beetroot roots (*Beta vulgaris*). Polibotánica 46:193–202. https://doi.org/10.18387/polibotanica.46.12

Mo M, Xu C, Zhang K (2005) Effects of carbon and nitrogen sources, carbon-to-nitrogen ratio, and initial pH on the growth of nematophagous fungus *Pochonia chlamydosporia* in liquid culture. Mycopathologia 159:381–387. https://doi.org/10.1007/s11046-004-5816-3

Molinari S (2011) Natural genetic and induced plant resistance, as a control strategy to plant-parasitic nematodes alternative to pesticides. Plant Cell Rep 30:311–323. https://doi.org/10.1007/s00299-010-0972-z

Moosavi MR, Zare R (2012) Fungi as biological control agents of plant-parasitic nematodes. In: Mérillon J, Ramawat K (eds) Plant defense: Biological control. Progress in Biological Control, vol 12. Springer, Dordrecht, pp 67–107. https://doi.org/10.1007/978-94-007-1933-0_4

Mousumi Das M, Haridas M, Sabu A (2020) Process development for the enhanced production of bio-nematicide *Purpureocillium lilacinum* KU8 under solid-state fermentation. Bioresour Technol 308:0960–8524. https://doi.org/10.1016/j.biortech.2020.123328

Nicol JM, Rivoal R (2008) Global Knowledge And Its Application For The Integrated Control And Management Of Nematodes On Wheat. In: Ciancio A, Mukerji KG (eds) Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes. Integrated Management of Plant Pests and Diseases, vol 2. Springer, Dordrecht, pp 251–294. https://doi.org/10.1007/978-1-4020-6063-2_13

Nicol JM, Turner SJ, Coyne DL, Den Nijs L, Hockland S, Maafi ZT (2011) Current nematode threats to world agriculture. In: Jones J, Gheysen G, Fenoll C (eds) Genomics and molecular genetics of plant-nematode interactions. Springer, Dordrecht, pp 21–43. https://doi.org/10.1007/978-94-007-0434-3_2

Niu XM (2017) Secondary Metabolites from *Pochonia chlamydosporia* and Other Species of *Pochonia*. In: Manzanilla-López R, Lopez-Llorca L (eds) Perspectives in sustainable nematode management through *Pochonia chlamydosporia* spplications for root and rhizosphere health. Sustainability in Plant and Crop Protection. Springer, Cham, pp 131–168. https://doi.org/10.1007/978-3-319-59224-4_7

Niu XM, Wang YL, Chu YS, Xue HX, Li N, Wei LX, Mo MH, Zhang KQ (2010) Nematodetoxic aurovertin-type metabolites from a

root-knot nematode parasitic fungus *Pochonia chlamydosporia*. J Agric Food Chem 58:828–834. https://doi.org/10.1021/jf903259n
Nordbring-Hertz B, Hans-Börje J, Tunlid A (2006) Nematophagous Fungi. ELS. https://doi.org/10.1038/npg.els.0004293
Perry RN (1989) Dormancy and hatching of nematode eggs. Parasitol Today 5:377–383. https://doi.org/10.1016/0169-4758(89)90299-8
Prasad P, Varshney D, Adholeya A (2015) Whole genome annotation and comparative genomic analyses of bio-control fungus *Purpureocillium lilacinum*. BMC Genom 16:1004. https://doi.org/10.1186/s12864-015-2229-2
Rehman S, Gupta VK, Goyal AK (2016) Identification and functional analysis of secreted effectors from phytoparasitic nematodes. BMC Microbiol 16:48
Romero BM, Macías CMG, Carrillo FA, Rojas CM, Hernández RJS, Duarte OJD (2019) Identification and distribution of *Meloidogyne* species in Baja California Sur, Mexico. Rev *Mexicana* Cienc Agric 10:337–349. https://doi.org/10.29312/remexca.v10i2.1603
Sagüés MF, Purslow P, Fernández S, Fusé L, Iglesias L, Saumell C (2011) Nematophagous fungi used for the biological control of gastrointestinal nematodes in cattle and their forms of administration. Rev Iberoam Micol 28:143–147. https://doi.org/10.1016/j.riam.2011.06.009
Sánchez PJF, Lugo GGA, Mundo OM, Reyes O, Ley Tandingan ID, Ole BJ (2016) Search and isolation of nematophagous fungi vs *Meloidogyne* spp. in northern Sinaloa, México. Rev *Mexicana* Cienc Agric 7:1829–1839. https://doi.org/10.29312/remexca.v7i8.95
Santacruz-Ulibarri H, Pedroza MAA (1983) Preliminary study of the nematode fauna in the state of Michoacan. Nematropica 13:120–121
Sanz-Alférez S, Sanz-Alférez S, Uribe X, Aristizábal FA, Herreros E, del Campo FF, Fenoll C (2000) Cis-elements nematode-responsive promoters In: de Vries GE, Metzlaff K (eds) Developments in plant genetics and breeding, Elservier, pp 177–182. https://doi.org/10.1016/S0168-7972(00)80119-X
Sellito VM, Curto G, Dallavalle E, Ciancio A, Colagiero M, Pietrantonio L, Bireescu G, Stoleru V, Storari M (2016) Effect of *Pochonia chlamydosporia*-based formulates on the regulation of root-knot nematodes and plant growth respose. Front Life Sci 9:177–181. https://doi.org/10.1080/21553769.2016.1193827
SENASICA (2013) Stem and bulb nematode (*Ditylenchus dipsaci* Kühn, 1857). General Directorate of Plant Health-National Phytosanitary Epidemiological Surveillance System. Mexico DF. Technical information No. 18, p 24
Sharma A, Sharma S, Dalela M (2014) Nematicidal activity of *Paecilomyces lilacinus* 6029 cultured on karanja cake medium. J Microb Pathog 75:16–20. https://doi.org/10.1016/j.micpath.2014.08.007
Sharma A, Gupta A, Dalela M, Sharma S, Sayyed RZ, Enshasy HAE, Elsayed EA (2020) Linking organic metabolites as produced by *Purpureocillium lilacinum* 6029 cultured on karanja deoiled cake medium for the sustainable management of root-knot nematodes. Sustainability 12:8276. https://doi.org/10.3390/su12198276
Shinonaga H, Kawamura Y, Ikeda A, Aoki M, Sakai N, Fujimoto N, Kawashima A (2009) The search for a hair-growth stimulant: new radicicol analogues as WNT5A expression inhibitors from *Pochonia chlamydosporia* var. *chlamydosporia*. Tetrahedron Lett 50:108–110. https://doi.org/10.1016/j.tetlet.2008.10.099
Shirazi R, Fatemy S, Naeimi S (2019) Solid-state fermentation and viability of *Pochonia chlamydosporia* and *Purpureocillium lilacinum* on some organic substrates. *BioControl in* Plant Protection 6:1–14. https://doi.org/10.22092/bcpp.2019.119398
Silva SD, Carneiro R, Faria M, Souza DA, Monnerat RG, Lopes RB (2017) Evaluation of *Pochonia chlamydosporia* and *Purpureocillium lilacinum* for Suppression of *Meloidogyne enterolobii* on tomato and banana. J Nematol 49:77 85. https://doi.org/10.21307/jofnem-2017-047
Silva-Valenzuela M, Rojas-Martínez RI, Manzanilla-López RH, Macías-Ruvalcaba ML, Aranda-Ocampo S, Zavaleta-Mejía E (2020) Endophytic fungi: a biological alternative for the management of phytoparasite nematodes. Nematropica 50:101–117
Sosa-Moss C (1986) Cyst Nematodes in Mexico, Central and South America. In: Lamberti F, Taylor CE (eds) Cyst Nematodes. Nato ASI Series (A: Life Sciences). Springer, Boston, pp 397–398. https://doi.org/10.1007/978-1-4613-2251-1_34
Sosa-Moss C (1987) Cyst nematodes in Mexico, Central and South America. Nematol Mediterr 15:1–12
Stephen BM, John B, Jafar Y, Isgouhi K, Pim Z, Valerie MW (1998) The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, the nucleotide binding, leucine rich repeat family of plant genes. Plant Cell 10:1307–1319. https://doi.org/10.1105/tpc.10.8.1307
Stirling GR (2011) Biological control of plant-parasitic nematodes: an ecological perspective, a review of progress and opportunities for further research. In: Davies K, Spiegel Y (eds) Biological control of plant-parasitic nematodes: progress in biological control, vol 11. Springer, Dordrecht, pp 1–38. https://doi.org/10.1007/978-1-4020-9648-8_1
Stirling GR, Smith LJ (1998) Field test of formulated products containing either *Verticillium chlamydosporium* or *Arthrobotrys dactyloides* for biological control of root-knot nematodes. Biol Control 11:231–239. https://doi.org/10.1006/bcon.1997.0604
Stirling GR, Licastro KA, West LM, Smith LJ (1998) Development of commercially acceptable formulations of the nematophagous fungus *Verticillium chlamydosporium*. Biol Control 11:217–223. https://doi.org/10.1006/bcon.1997.0602
Talavera RM (2003) Manual de Nematologia agrícola. Introducción al análisis y al control nematológico para agricultores y técnicos de agrupaciones de defensa vegetal. Instituto de reserva y formación agraria y pesquera. Consejería de agricultura y pesca de los Illes Balears
Trudgill DL, Blok VC (2001) Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. Annu Rev Phytopathol 39:53–77. https://doi.org/10.1146/annurev.phyto.39.1.53
Uddin MN, Saifullah, Ahmad M, Khan W, Khan B (2019) Evaluation of *Pochonia chlamydosporia* (Goddard) isolates for suppression of *Meloidogyne incognita*, root-Knot nematode of tomato. J Agric Sci 11:70. https://doi.org/10.5539/jas.v11n5p70
Van Gundy SD, Perez BJG, Stolzy LH, Thomson IJ (1974) A pest management approach to the control of *Pratylenchus thornei* on wheat in Mexico. J Nematol 6:107–116
Wang YL, Li LF, Li DX, Wang B, Zhang K, Niu X (2015) Yellow pigment aurovertins mediate interactions between the pathogenic fungus *Pochonia chlamydosporia* and its nematode host. J Agric Food Chem 63:6577–6587. https://doi.org/10.1021/acs.jafc.5b02595
Zhang Y, Yang Guangzhu FM, Deng C, Zhang KQ, Yu Z, Xu J (2020) Comparative analyses of mitochondrial genomes provide evolutionary insights into nematode-trapping fungi. Front Microbiol 11:617. https://doi.org/10.3389/fmicb.2020.00617
Zhou H, Qiao K, Gao Z, Vederas JC, Tang Y (2010) Insights into radicicol biosynthesis via heterologous synthesis of intermediates and analogs. J Biol Chem 285:41412–41421. https://doi.org/10.1074/jbc.M110.183574
Zisis V (2018) Pesticide fate in soil-sediment-water environment in relation to contamination preventing actions. Curr Opin Environ Sci Health 4:5–9. https://doi.org/10.1016/j.coesh.2018.03.001