University of São Paulo
"Luiz de Queiroz" College of Agriculture

Production of *Purpureocillium lilacinum* and *Pochonia chlamydosporia* by submerged fermentation, and their effects on *Tetranychus urticae* and *Heterodera glycines* through seed inoculation of common beans plants

Daniela Milanez Silva

Dissertation presented to obtain the degree of Master in Science. Area: Entomology

Piracicaba
2020

Daniela Milanez Silva
Bachelor in Biological Science

Production of *Purpureocillium lilacinum* and *Pochonia chlamydosporia* by submerged fermentation, and their effects on *Tetranychus urticae* and *Heterodera glycines* through seed inoculation of common beans plants

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **ITALO DELALIBERA JUNIOR**

Dissertation presented to obtain the Master in Science. Area: Entomology

Piracicaba
2020

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Silva, Daniela Milanez

Production of *Purpureocillium lilacinum* and *Pochonia chlamydosporia* by submerged fermentation, and their effects on *Tetranychus urticae* and *Heterodera glycines* through seed inoculation of common beans / Daniela Milanez Silva. - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2020.

75 p.

Dissertação (Mestrado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Fungos 2. Estrutura de resistência 3. Fermentação líquida 4. Fitonematoide 5. Ácaro rajado I. Título

ACKNOWLEDGMENTS

I want to thank my supervisor Prof. Dr. Italo Delalibera Junior for the trust and for providing outstanding opportunities that I had during my academic journey in the lab. I also want to thank Dr. Gabriel Moura Mascarin for the guidance and for always being present and ready to help with all questions that I had.

I want to thank all my friends from the Laboratory of Pathology and Microbial Control of Insects – ESALQ/USP, who helped me a lot, especially Dr. Cassiara Gonçalves and Ph.D. Natasha Iwanicki for helping and teaching me about molecular analyses. A special thanks to Prof. Dr. Mario Massayuki Inomoto and his team for taking the time to assist me during all my experiments with nematodes. Dr. Victor Moura for sharing his knowledge and helping me during the experimental setup. I also thank Prof. Dr. Wesley for providing space on the greenhouse for all my experiments.

I thank all the professors of the Department of Entomology and Acarology – ESALQ/USP for the knowledge acquired during these two years.

As for my friends: Amanda Bortolotti, Bianca Corrêa, Carla Marassato, Leticia Parra, Sara Moreno, Taisa Pavani, and Vitor Isaias thank for being there when I needed them most, even just for a hug or a senseless talk. Thank you for helping with all my experiments! I love you guys a lot!!

I want to thank the Go Winners family, they helped me when I needed it most, I will carry you all in my heart. “Só acaba se a gente deixar”.

Most importantly, I am truly grateful to my family for being always supportive and for their relentless motivation to me.

To the National Council for Scientific and Technological Development (CNPq – project: 830701/1999-0) thanks for the financial support.

EPIGRAPH

É muito melhor lançar-se em busca de conquista grandiosas, mesmo expondo-se ao fracasso, do que alinhar-se com os pobres de espíritos, porque vivem numa penumbra cinzenta, onde não conhecem nem vitória, nem derrota (Theodore Roosevelt).

SUMMARY

RESUMO

Produção de *Purpureocillium lilacinum* e *Pochonia chlamydosporia* por fermentação submersa, e seus efeitos em *Tetranychus urticae* e *Heterodera glycines* pela inoculação em sementes de feijoeiro

Os fungos *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae) e *Purpureocillium lilacinum* (Hypocreales: Ophiocordycipitaceae) são normalmente encontrados no solo e nas plantas. Os microescleródios são estruturas de resistência com potencial para serem usadas no tratamento de sementes, com a finalidade de controlar pragas e patógenos veiculados pelo solo, assim como auxiliar na promoção do crescimento de plantas. Neste estudo foi avaliada a capacidade de *P. lilacinum* e *P. chlamydosporia* promoverem crescimento de plantas de feijão (*Phaseolus vulgaris*) e de reduzirem as populações do ácaro rajado, *Tetranychus urticae,* e do nematoide de cistos da soja, *Heterodera glycines*, através do tratamento de sementes. Inicialmente, um *screening* com 37 isolados de *P. lilacinum* e 2 isolados de *P. chlamydosporia* foi conduzido, avaliando-se a produção de microescleródios em três meios de cultura. Todos os isolados de *P. lilacinum* e *P. chlamydosporia* produziram microescleródios e propágulos submersos, sendo que os isolados mais produtivos atingiram > 10^4 microescleródios ml^{-1}. Além disso, observou-se a formação de elevadas quantidades de propágulos submerssos para a maioria dos isolados (> 10^9 propágulos ml^{-1}). Os isolados *P. lilacinum* ESALQ1744, ESALQ2482 e ESALQ2593, e *P. chlamydosporia* ESALQ5405 e ESALQ5406 foram selecionados para os estudos em plantas. A inoculação com microescleródios de *P. lilacinum* e *P. chlamydosporia* não incrementou os parametros vegetativos dos feijoeiros, exceto pelo peso seco de raizes no segundo experimento, onde todos os isolados foram superiores ao controle não inoculado. A densidade de nematoide de cisto foi semelhante entre os tratamentos com fungos e as plantas não inoculadas, exceto no terceiro experimento, onde as plantas inoculadas com o isolado ESALQ5406 apresentaram menores densidades totais do que o controle e no segundo experimento onde a densidade de nematoides por grama de raíz, foi menor nos tratamentos com ESALQ5406 e ESALQ2593. As plantas tratadas com todos os isolados apresentaram menores populações do ácaro-rajado. Embora conídios aéreos de *P. lilacinum* e *P. chlamydosporia* sejam usado para o controle de nematóides, neste estudo, demonstrou-se que esses também apresentam potencial para o manejo de pragas da parte aérea através do tratamento de sementes de feijão com microescleródios.

Palavras-chave: Fungos, Estruturas de resistência, Fermentação líquida, Fitonematoide, Ácaro-rajado

ABSTRACT

Production of *Purpureocillium lilacinum* and *Pochonia chlamydosporia* by submerged fermentation, and their effects on *Tetranychus urticae* and *Heterodera glycines* through seed inoculation of common beans plants

The filamentous fungi *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae) and *Purpureocillium lilacinum* (Hypocreales: Ophiocordycipitaceae) are commonly found in soils and vegetations. Microesclerotia is resistant structures with potential for use in seed treatments aiming to control aboveground diseases and pests, as well as improving plant growth. Thus, this study aimed to investigate the ability of *P. lilacinum* and *P. chlamydosporia* to promote common bean (*Phaseolus vulgaris*) growth and to suppress populations of *Tetranychus urticae* and *Heterodera glycines*, through seed treatment. Initially, a screening with 37 Brazilian isolates of *P. lilacinum* and 2 isolates of *P. chlamydosporia* was carried outto evaluate the microsclerotia production in three culture media. All isolates produced microsclerotia and submerged propagules. The best isolates produced > 10^4 microsclerotia ml^{-1}. In addition, a high concentration of submerged propagules (conidia and blastospores) was observed for most isolates (above 10^9 propagules ml^{-1}). The *P. lilacinum* isolates ESALQ1744, ESALQ2482, and ESALQ2593, and *P. chlamydosporia* ESALQ5405 and ESALQ5406 were selected for the studies on plants. Overall, common bean plants inoculated with *P. lilacinum* and *P. chlamydosporia* did not differ from non-inoculated plants regarding the vegetative parameters, except for the dry weight of roots in the second experiments, where all the isolates were superior to the non-inoculated control. The population density of cystoid nematodes was similar among the treated plants and the uninoculated ones, except for the third experiment in which the plants inoculated with isolate ESALQ5406 presented lower densities than the control. However, for nematodes per root weight, it was observed lower population densities in fungal treated plants ESALQ5406 and ESALQ2593 at the second experiment. A reduction in populations of the two-spotted mite in fungi-treated plants was observed for all isolates. Although aerial conidia of *P. lilacinum* and *P. chlamydosporia* has been used for the control of nematodes, in this study it was demonstrated that these two fungal species also have the potential for the management of pests of the aerial part through bean seed treatment with microsclerotia.

Keywords: Fungi, Resistant structure, Liquid fermentation, Plant nematode, Two-spotted spider mite

1. INTRODUCTION

Biological control aims the reduction of populations of agricultural pests and diseases through the use of natural enemies, and it presents a lower risk to humans since it is based on the study of the relationships between living beings in the environment (EILENBERG et al., 2001; PARRA et al., 2002; PARRA, 2014). In 2019, the brazillian biopesticide market showed a 70% increase in the use of biological products for the control of important agricultural pests and diseases, a significant result that is a milestone in the history of the sector in Brazil (BRITO, 2019).

The use of microbial control of insects is advantageous due to the specificity and selectivity. Many pathogens, such as fungi, viruses, and bacteria, are specific to insects and mites. Thus, they are able to decrease the risk of biological imbalance in the agroecosystem by not affecting non-target organisms (BORGES & HUSSEY, 1971; ALVES, 1998).

Fungi of the order Hypocreales are potential control agents of important pests. Some, such as *Purpureocillium lilacinum* (Hypocreales: Ophiocordycipitaceae) and *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae), is already marketed as biocontrol agents. Currently, there are three products registered for use only against plant nematodes (two of *P. lilacinum* and one of *P. chlamydosporia*) in Brazil (AGROLINK, 2019; DÍAZ-SILVEIRA & HERRERA, 1998). The potential of these fungi against insects and mites has also been demonstrated (ANGELO et al. 2012; MEDEIROS et al. 2018).

The use of fungi for agricultural pest management is an old method, however, new researches and new fungal isolates with potential to control pests of high economic importance are presented every year, showing that the microbial control is still an emerging area. Overall, the use of fungi for the control of important pests, such as mites of the family Tetranychidae and plant nematodes of the genus *Heterodera*, has several advantages, including specificity and selectivity. In addition, when applied, these biocontrol agents have low risk to the environment, as they do not affect non-target organisms, a common fact when broad-spectrum insecticides and acaricides are used (ALVES, 1998; CARVALHO, 2006; GLARE et al., 2012; LACEY et al., 2015).

Phytophagous mites are important pests found in many crops around the world. Spider mites are important pests worldwide, especially the two-spotted spider mite, *Tetranychus urticae*, which is polyphagous, attacking various crops (MORAES & FLECHTMANN, 2008; GRBIC et al., 2011). The species feeds on the cellular content of

plants, which justifies studies of possible resistance induced response mediated by the endophytic fungi (GARRIDO et al., 2015).

In addition to their use as direct biocontrol agents, some fungi often promote several benefits for crops, as they colonize plants as endophytes. This interaction with the plants results in positive effects, such as growth improvement and induced resistance against pests and diseases. Recently, it has been observed that fungi of the order Hypocreales are able to colonize several crops, promoting many benefits, as aforementioned. However, there is still much to be explored in this association; the results available so far show the importance of this relation for yield gain and lower use of high toxicity and broad-spectrum insecticides (RUSSO et al., 2018; RONDOT & REINEKE, 2018; KRELL et al., 2018).

The filamentous fungi *P. chlamydosporia* and *P. lilacinum* (order Hypocreales), occur in various environments and are commonly found in soils and plants, and infecting phytonematodes, insects and mites. They are mainly studied aiming the control of plant nematodes due to the production of enzymes that aid in the penetration in cysts and females of *Heterodera* spp. and *Meloidogyne* spp. The use of *P. chlamydosporia* and *P. lilacinum* as endophytes can have great advantages against soil-borne pests that attack below-ground parts, as these fungi modulate the plant defense response. They can also increase the growth rates of plants and compete with soil microbiota (MACIÁ-VICENTE et al., 2009b; KIRIGA et al., 2018).

The production of fungal propagules can be accomplished through two main forms, solid and submerged fermentation. In the first one, a nutrient-poor substrate is used, no advanced technology is required for the production, and the final propagule of interest is conidia. The challenge of this type of production is mainly the time to produce propagules, a process that takes ten to fifteen days to achieve a high concentration. In production via submerged fermentation, it is possible to produce different types of fungal propagules in a shorter time; reaching high concentrations requires only a few days of production. The structures produced depend on the fungus species and may consist of microsclerotia, chlamydospores, blastospores, or even submerged conidia (LACEY et al., 2015; JARONSKI & MASCARIN, 2017).

Microesclerotium is a resistance structure that can be produced by manipulating the liquid culture medium (MASCARIN et al., 2015). This propagule is an option to plant inoculation, aiming the endophytic colonization, as it is very stable under field conditions and may remain viable until conditions become suitable for their growth (JACKSON &

JARONSKI, 2009). Blastospores are yeast-like cells produced by budding, septation, and fragmentation of the hyphae after the host penetration (BIDOCHKA et al., 1987; HUMPHREYS et al., 1989; FENG et al., 1994). Submerged conidia (i.e., microcycle conidia) differ from aerial conidia only in superficial morphology; this propagule may be originated from both mycelial growth and blastospores and shows hydrophobic and hydrophilic physicochemical characteristics, an adaptation to a nutritionally limited environment (THOMAS, 1987; HEGEDUS et al., 1990; CHO et al., 2006).

So far, there are no published studies relating to the use of bean seed treatments with microsclerotia (MS) of *P. chlamydosporia* and *P. lilacinum* and their effect on the population reduction of *T. urticae*. Tamai et al. (1999), Fregonesi et al. (2016), Martins (2016), Galzer & Filho (2016), and Barbosa et al. (2018) presented important results using biological fungal agents and bacteria to control the two-spotted spider mite. However, *P. chlamydosporia* and *P. lilacinum* is usually not explored for the control of mites.

Hence, the goal of this research was to select strains of *P. chlamydosporia* and *P. lilacinum* based on production by submerged fermentation to evaluate their endophytic capability on *Phaseolus vulgaris* and their effects in *T. urticae* and *Heterodera glycines*. At first, (i) molecular characterization of the isolates of *P. chlamydosporia* and *P. lilacinum* was conducted, then their (ii) production potential via submerged fermentation was evaluated. Some isolates were selected to (iii) investigate their endophytic capacity in *P. vulgaris*, and finally, it was determined the (iv) effect of fungal inoculation in beans against *T. urticae* and *H. glycines*.

2. BIBLIOGRAPHIC REVIEW

2.1. Plant growth-promoting fungi

Plant growth-promoting fungi (PGPF) have different roles in natural and agricultural ecosystems. Not only they can act as pathogens of many species of insects and nematodes, but they can also act indirectly by endophytically colonizing important plant crops and, as a result of this interaction, promote interesting secondary effects. Among these benefits, PGPF can increase the absorption of minerals and nutrients from the soil, promote growth, control pests, and diseases, and even increase plant sensitivity against external effects (AZCÓN-AGUILAR & BAREA, 1997; PARNISKE, 2008; AZCÓN-BONFANTE & GENRE, 2010; JABER & OWNLEY, 2018; RONDOT & REINEKE, 2018) The symbioses promoted by endophytic fungi with plants demonstrate that benefits can be also multifarious (SCHARDL et al., 2004).

The term “endophytic” was proposed by DeBary (1866), which described any organism that can colonize living tissues of autotrophs without causing disease (BEHIE & BIDOCHKA, 2014). Although this concept was crafted more than 150 years ago, a novel trend among researchers is the use of some biological control agents, such as entomopathogenic fungi, as endophytes of plants, something whose importance is being demonstrated with each newly published work. Vega (2018) reported thirty-eight papers showing negative effects on insects due to the colonization of fungi in plants, and these effects were observed for thirty-three insect species, seventeen families, and eight orders. This shows that this emerging research area has been improving over time.

Several genera of entomopathogenic fungi show endophytic potential. Fungi of the genera *Metarhizium* and *Beauveria* are able to endophytically colonize various parts of plants. It was already demonstrated that these fungi can translocate nitrogen from insects to the plant in exchange for carbon (BEHIE & BIDOCHKA, 2014). This association promotes plant survival in nitrogen-limited soil and increases the weight and the length of leaves and root (GARCÍA et al., 2011; BEHIE et al., 2012; BEHIE & BIDOCHKA, 2014; JABER & ENKERLI, 2017; RUSSO et al., 2018).

Similar to these, other groups of fungi have been studied as endophytic agents. Lopez & Sword (2015) presented in their study the first report of growth promotion on cotton plants by the endophytic association of *Purpureocillium lilacinum*, pointing out

the lower attractiveness to *Helicoverpa zea*, an herbivorous insect. Other PGPF recently studied are *Pochonia* spp. The advantage of this fungus is that it can endophytically colonize a wide variety of plant species, such as *Hordeum vulgare*, *Triticum aestivum*, *Solanum lycopersicum*, *Lactuca sativa* and *Pistacia vera* (MONFORT et al., 2005; EBADI et al., 2009; DIAS-ARIEIRA et al., 2011; ESCUDERO & LOPEZ-LLORCA, 2012; MUKHTAR et al., 2013).

Colonization of *Pochonia* causes a modification in the transcriptome of the plant, inducing the modulation of several stress responses, such as the biosynthesis of phenolic compounds and papillae formation; these described compounds have a structural hole and performance an important role in plant defense responses (BORDALLO et al., 2002; MACIÁ-VICENTE et al., 2009a; ESCUDERO & LOPEZ-LLORCA, 2012; LARRIBA et al., 2015).

Facing new agricultural challenges, such as higher human food consumption and chemicals reduction, endophytism can help to achieve a new and sustainable pest management model. With a holistic vision on control of agricultural pests, adding direct and indirect effects aforementioned, it can provide to farmers, healthy crops with good quality. The implementation of endophytic fungi in roots is interesting to establish a competition with nematodes and microorganisms that could cause damage to the plant (STIRLING, 2011).

2.2. *Purpureocillium lilacinum*

Purpureocillium lilacinum (= Sin. *Paecilomyces lilacinus*) (Thom) Samson (Hypocreales: Ophiocordycipitaceae) is a filamentous fungus, saprophytic, and ubiquitous in soil, vegetationand insects.Prasad et al (2015) analyzed the genome of *P*. *lilacinum* and was capable off identify the proteins that explain the multi-trophic characteristic of this fungus (i.e., saprophytic, egg-parasite, and plant-endophytic). Additionally, only a few isolates are capable to induce several infections on vertebrates, including humans (LUANGSA-ARD et al., 2011). This species is highly investigated to control plant-parasitic nematodes (ESSER & EL-GHOLL, 1993; ATKINS et al., 2005). Commercially, this fungus is currently registered and formulated to control nematodes.

In 1910, fungi that belonged to the genus *Purpureocillium* were classified by Thom as *Penicillium* (RAPER & THOM, 1949), although, three years earlier, Bainier

(1907) had already isolated and described *Paecilomyces lilacinus* (BROWN & SMITH, 1957). A few years after, Samson reclassified some species that belonged to the genus *Penicillium* as *Paecilomyces* (RAPER & THOM, 1949). Luangsa-ard et al. (2011) suggested, upon molecular tools, the division of the genus *Paecilomyces* to create a new genus, *Purpureocillium*, to accommodate species of *Paecilomyces lilacinus* and related species. Moreira et al. (2018) and Luangsa-ard et al. (2011), through genetic sequencing of strains of *P*. *lilacinum*, verified that it was not possible to discriminate between species harmful and helpful to humans. It was suggested that genetic differences exist and could be detected using other techniques (STIRLING, 2015).

The fungus *P. lilacinum* is well known to control nematodes. Many reports are available and show the ability to suppress different plant-parasitic nematodes. Siddiqui et al. (2000) noticed that using an ethyl acetate extract of *P. lilacinum*, it was possible to kill *Meloidogyne javanica* (e.g. root-knot) *in vitro*, and, in field conditions, the fungus was able to suppress soil-borne root-infecting fungi and also the root-knot nematode. Kiewnick & Sikora (2006) showed that the inoculation of propagules of *P. lilacinum* in the soil before tomatoes transplantation resulted in a higher reduction of *Meloidogyne incognita* population and resulted in lower damage in infested plants.

In accordance with Kiewnick & Sikora (2006), there are several mechanisms of action of *P. lilacinum* against nematodes, e.g., the infection in sedentary stages, especially in eggs and females. It is known as well the production of secondary metabolites and enzymes that help in the nematogenic action of this fungus, such as leukinotoxins, chitinases, and proteases (KHAN et al., 2003; PARK et al., 2004; KIEWNICK & SIKORA, 2006).

Purpureocillium spp. have the capacity to control other important plant-parasitic nematodes, such as *Heterodera* spp., *Globodera* spp., *Meloidogyne* spp., *Radopholus* spp. and *Pratylenchus* spp. (JATALA et al., 1979; SIDDIQUI et al., 2000, KIEWNICK et al., 2004; KEPENEKCI et al., 2018a, b). In a recent study, conducted by Hajji et al. (2017), *P. lilacinum* was capable of reducing the infection of *M. javanica* in potatoes up to 65%, and for the populations in soil, it was observed a rate of reduction of 46% to the same nematode. This was also shown on *M. incognita* control (KEPENEKCI et al., 2018a).

The action of this fungus is not restricted to plant growth promotion or population decrease as an indirect effect (plant endophytic). It has also a direct effect on pest control, such as nematodes and arthropods in general. A recent study isolated

P. lilacinum from a cadaver of a citrus blackfly nymph, *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) (MEDEIROS et al. 2018). Furthermore, Angelo et al. (2012) reported the ability of *P. lilacinum* to control larvae of *Rhipicephalus microplus* through *in vitro* trials. *P. lilacinum* was also able to suppress ants of *Acromyrmex* spp., at the concentration of 10^6 conidia ml^{-1}, with a TL_{50} of just 6 days (GOFFRÉ & FOLGARAIT, 2015).

It was also noticed the effect of *P. lilacinum* against larvae of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and in larvae of *Phthorimaea operculella* (Lepidoptera: Gelechiidae), both important worldwide pests of potato crops (KEPENEKCI et al., 2013), and to the flour beetle *Tribolium confusum* (BARRA et al., 2015). Hotaka et al. (2015), performing *in vitro* experiments, noticed 70% mortality of adult females of *Thrips palmi* Karny (Thysanoptera: Thripidae), caused by *P. lilacinum*. The same authors tested the virulence of this fungus on field conditions, applying in *Dendrobium* sp. orchid plants, and the number of individuals/orchids of *T. palmi* decreased after the first inoculation, whereas in the fourth inoculation the number of individuals reached zero.

P. lilacinum it is not pathogenic against all insect orders but has the ability to modulate the behavior of some species. Studies conducted by Eberhard et al. (2017) demonstrated that *P. lilacinum* induced *Edessa rugomarginata* (Hemiptera: Pentatomidae) to perch certain parts of the plant, grabbing them with the legs, dispersing most effectively the aerial conidia. This same behavioral manipulation is observed on Zygomycetes fungi.

The pathogenicity of *P. lilacinum* to mites, including *T. urticae* has already been reported, Fiedler & Sosnowka (2007) achieved 50% of control of the two-spotted mite with only 10^6 conidia ml^{-1} within two days after inoculation, and Mustu et al. (2016), using 10^8 conidia ml^{-1}, achieved 85% of *T. urticae* control. On the other hand, Yesilayer (2018), with the same concentration, controlled only 45% of two-spotted mites within seven days after inoculation. Similarly, to *P. chlamydosporia*, *P. lilacinum* presents the ability to effectively control helminths that cause gastrointestinal disease, such as *Fasciola hepatica* (NAJAFI et al., 2017).

As a downside, *P. lilacinum* can have negative effects on insect predators, Yoder et al. (2018) noticed the mortality of predator mite *Balaustium murorum* (Parasitengona: Erythraeidae) in the field and in laboratory conditions. Mustu et al.

(2016), through *in vitro* tests, demonstrated the action of this fungus against the predator mite *Neoseiulus californicus* (Acari: Phytoseiidae).

2.3. *Pochonia chlamydosporia*

The fungus *Pochonia chlamydosporia* (Goddard) (Hypocreales: Clavicipitaceae) was described by Zare & Gams. It was previously classified as *Verticillium chlamydosporium*, with the teleomorphic phase being *Metacordyceps chlamydosporia* (ZARE et al., 2001; SUNG et al., 2007). This fungus is well known by the formation of chlamydospores, which are multicellular resistance structures produced during its life cycle. *P. chlamydosporia* differs also by varieties, such as *chlamydosporia*, *catenulate,* and *catenulate* biotype A variety (ATKINS et al., 2003).

The genus *Verticillium* holds approximately 190 species described that shares several morphological structures well characterized (ZARE et al., 2004). With the advance of biotechnology, several fungi that belonged to the genus *Verticillium* were reclassified, based on their differences in ecology and molecular features (REHNER & SAMUELS, 1995; GAMS & ZARE, 2002). Thereby, fungi like *Lecanicillium* spp. and *Pochonia* spp. were separated and characterized as new genera (ZARE & GAMS, 2001a; ZARE et al., 2001). The species are widespread worldwide, belonging to the family Clavicipitaceae (Ascomycota: Pezizomycotina: Sordariomycetes: Hypocreales) (LIN et al., 2015)

Therefore, in this new classification, the anamorph stage of *Pochonia* is included in the clade A of the family Clavicipitaceae, which also comprises *Aschersonia* spp. (Mont.), *Metarhizium* spp., *Nomuraea* spp. (now *Metarhizium*) (Maublanc) and *Paecilomyces* spp. (s.l.) genus (SUNG et al., 2007).

Larriba et al. (2014) identified the portion of the genome of *P. chlamydosporia* related to pathogenicity against their hosts (e.g., nematodes). It was observed that 81 genes encode proteins related to relations between pathogen or symbiont organisms, 47 of which have already been identified as pathogenicity genes.

P. chlamydosporia acts mainly against important phytonematodes, notably *Heterodera* spp., *Meloidogyne* spp., *Globodera* spp., *Nacobbus* spp. and *Rotylenchulus* spp., acting as a nematodes suppressor, and as a natural antagonist of soil microbiota (LARRIBA et al., 2014).

Pochonia is saprophytic, facultative parasites of invertebrates (DALLEMOLE-GIARETTA et al., 2015) and are well known for the parasitism of cysts and mature nematode females due to the capacity of this fungus to produce chitinases and other proteolytic enzymes during the infection process, which degrades the chitin layers, increasing the pathogenicity against nematodes (BATISTA & FONSECA, 1965; LOPEZ-LLORCA & ROBERTSON, 1992; TIKHONOV et al., 2002; ESCUDERO & LOPEZ-LLORCA, 2012; ROSSO et al., 2011; MANZANILLA-LÓPEZ et al., 2013; ARANDA-MARTINEZ et al., 2018; LIN et al., 2018). According to Lopez-Llorca et al. (2002), the infection of these fungi in eggs of nematodes occurs by adhesion, appressoria formation, and finally the penetration. Escudero & Lopez-Llorca (2012) reported that the penetration in eggs of *M. javanica* is held early by appressoria and later by penetration of the hyphae.

Apart from the action against nematodes, *P. chlamydosporia* shows potential to control gastrointestinal parasites, due to direct action in eggs, with chitinases secretion. Braga et al. (2007, 2009 and 2010), Araújo et al. (2009), Fereira et al. (2011), and Dias et al. (2012) have already demonstrated the capacity of this fungus to control helminths, such as *Taenia* spp., *Ascaris lumbricoides*, *Enterobius vermicularis,* and *Fasciola* spp. It was also reported infections by *Pochonia* spp. in eggs of snails and Coleoptera (ZARE & GAMS, 2004).

2.4. Submerged fermentation

Submerged fermentation is an efficient technique to produce fungi. It can be classified into two categories: (i) submerged liquid fermentation, where the fungus is constantly agitated and the propagules produced are blastospores, submerged conidia and microsclerotia; and (ii) stationary liquid fermentation, where there is no agitation and the only propagule is aerial conidia (JARONSKI & MASCARIN, 2017).

Many fungi require specific culture media to be cultivated that provides the nutrients necessary to grow and to develop and, consequently, to produce the propagule for future use. This production is most commonly carried out by using solid media (grains) since no advanced production technology is required and it is a simple method using a relatively poor nutrient medium, whereas the conidia are the main propagule formed in this method (ALVES, 1998; JARONSKI & JACKSON, 2012; JARONSKI & MASCARIN, 2017).

Submerged fermentation allows the production of several fungal propagules, e.g., blastospores, submerged conidia, and microsclerotia (ALVES, 1998; JARONSKI & JACKSON, 2012; JARONSKI & MASCARIN, 2017). The production of different propagules is only achieved by the manipulation of the media, which, differently from solid media, requires a diversity of nutrients, salts, and vitamins, making the culture medium extremely rich (JARONSKI & MASCARIN, 2017). According to Jaronski & Jackson (2012), the utilization of these liquid media confers new insights on the use of entomopathogenic fungi to promote control of different targets in different parts of plants, such as aerial and soil portion.

Although the liquid media present several salts and vitamins that perform an important role in the development or even in the productivity of the fungi, the presence of carbon, nitrogen, and oxygen is fundamental to obtain the propagule at high concentrations. These three compounds are the most abundant in the cell and provide the fungal culture with energy supply, mainly used for growth. The carbon: nitrogen ratio is very important, if it is too low it will result in lower development. However, at a higher rate, it will result in conidiogenesis, i.e., induce conidia production (JACKSON &SCHISLER, 1995; MIRANDA, 2006; JACKSON &JARONSKI, 2009; MASCARIN et al., 2015).

From an economic point of view, it is necessary to understand that the costs of the submerged fermentation are higher than those of a solid fermentation, the main reasons being the cost of equipment. The cost-benefit of liquid fermentation should be evaluated since this method allows the production in a shorter time and high concentration, making it in the long term cheaper than solid fermentation (JACKSON, 1997; JARONSKI & JACKSON, 2012; MASCARIN et al., 2010).

2.5. Bean crop

Phaseolus vulgaris L. (Fabaceae) or common bean is an annual herbaceous plant. It grows and is cultivated worldwide with Brazil, India, United States, and Mexico is the largest producers (FAOSTAT, 2020). The common bean is the third-largest grain crop in Brazil, lower only to soybean and corn crops (CONAB, 2019). According to the Food and Agriculture Organization of the United Nations (FAOSTAT, 2020), in 2017, Brazil was responsible for the production of 3,000 tons of beans, which represents 80% of the South American production and 9% of the world production.

Several soil pests are major threats to common bean crops, notably *Delia platura* (seeds larvae), *Heterodera glycines* and *Elasmopalpus lignosellus*; regarding pests from the aerial portion, the major ones are *Diabrotica speciosa*, *Tetranychus urticae* (two-spotted spider mite), *Bemisia tabaci* biotypes A and B (whitefly), *Euschistus heros* (brown stink bug), *Heliothis* spp. and *Thecla jebus* (QUINTELA, 2001; OLIVEIRA et al., 2018).

2.6. *Tetranychus urticae* (Koch, 1836)

Tetranychus urticae adults' colors range from yellow-green to dark green, with dark stains in the back (BOTTON & NAVA, 2019). This mite is cosmopolitan and polyphagous, causing damage in diverse crops, e.g., strawberry, tomato, cotton, peach, pineapple, avocado, cocoa, soybean. Due to the feeding, when an intense infestation occurs, it can be noted speckling bronzed or silvery marks in the leaves, a sign of decreased photosynthetic ability, leading to their death (LOURENÇÃO et al., 2000; AGUIAR-MENEZES et al., 2007; MORAES & FLECHTMANN, 2008; AGROLINK; ROGGIA & SOSA-GOMEZ, 2012).

The economic damages caused by this pest are alarming. According to Suekane et al. (2012), the damages caused by the two-spotted mite in soybean crop result in an increase in the final costs of the production, with 15.8% applied just to control this pest. When not controlled, *T. urticae* reduced 80% of strawberry production, and in higher densities, it has the capacity to shorten the cycle of the crop (RIBEIRO et al., 2019).

The main control strategy used in crops of *P. vulgaris* is still through the use of chemical pesticides, being pyrethroids and phenylthiourea the active ingredients most used in Brazil (MEYER, 2003; WHALON et al., 2008; AGROFIT, 2020). According to reports of the Insecticide Resistance Action Committee (IRAC) and other researchers, *T. urticae* has shown resistance to 95 active ingredients (TANG et al., 2014; MONTEIRO et al., 2015; SATO et al., 2016). It is well known that the intensive use of chemicals leads to the selection of resistant pest populations, causes impacts on populations of natural enemies, leaves residues on fruits, and also contaminates the environment (ATTIA et al., 2013).

In Brazil, the biocontrol agents commercially available are two predatory mites (*Neoseiulus californicus* and *Phytoseiulus macropilis* [Acari: Phytoseiidae]) and one

entomopathogenic fungus (*Beauveria bassiana* [Hypocreales: Cordycipitaceae]) (GARRIDO et al., 2015; AGROLINK, 2020). Biological control is the key to protect biodiversity and to preserve local natural enemies (WAJNBERG et al., 1999). According to Strong & Pemberton (1999), biological control provides maintenance and preservation of the ecosystem.

2.7. *Heterodera glycines*

Heterodera glycines belong to the Family Hoplolaimidae, where the genera *Helicotylenchus*, *Rotylenchulus*, *Scutellonema,* and *Globodera* are also placed (LUC et al., 2005; AGRIOS, 2005; FERRAZ& BROWN, 2016). The life cycle of nematodes includes the egg, four juvenile states (J1 remains in the egg and hatch occurs with J2) and adults, separated into male and female (young females are small and white, older females are larger and yellow or brown). Unlike females, which feed in sexual maturity and oviposit (each female produces 300 to 600 eggs), males have a shorter life cycle and a minor role in the disease process, as they do not parasitize plants; instead, they contribute only to the genetic diversity of the population. *H. glycines* juveniles (females in third and fourth stages) alter their body shape and become sedentary. The optimal life cycle generally lasts three to four weeks (SCHMITT & RIGGS, 1989; YOUNG, 1992; LUC et al., 2005; AGRIOS, 2005; FERRAZ & BROWN, 2016).

After the egg hatches, the second-stage juvenile (J2) penetrates the roots and *Heterodera* spp. initiate their cycle, by penetrating the tips of young primary roots or the apical meristems of secondary roots of the host plant, piercing with the stylets into feeding off cells of the cortex, causing the enlargement of these cells (e.g. syncytia, nutritious cells for the nematode), thus hindering the absorption of water and nutrients at first, and then reducing the size of the plants. Hence, the infection symptoms can appear at patches, though if the soil is in good condition the infection may become asymptomatic in the aerial part (GRUNDLER et al., 1998; DIAS et al., 2010). When heavily infested the yield could be reduced in 30 to 75% (AGRIOS, 2005).

In Brazil, two important events are reported: in 1970, there was the first report of *Heterodera fici* in fig trees in the Rio Grande do Sul, and in the 1990s, *H. glycines*, the most feared phytonematode species by farmers was reported in the country (LIMA et al., 1992; LORDELLO et al., 1992; MONTEIRO & MORAIS, 1992; FERRAZ & BROWN, 2016). The dissemination of *H. glycines* in the country took place so rapidly

due to several biotic and abiotic factors acting together, such as irrigation, agricultural machinery/tools, and animals that helped it to spread over short and long distances (AGRIOS, 2005; EMBRAPA, 2010).

The cyst nematode has the ability to parasitize several hosts, the main one being soybeans (RIGGS, 1992). However, *H. glycines* are considered a major threat to *P. vulgaris* if it is rotated with soybeans (LUC et al. 2005). The main control strategy is based on soil fumigation, although it is not economically viable. Another control tactic that is showing its potential is the use of biological control agents, such as fungi and predator nematodes (AGRIOS, 2005).

3. MATERIAL AND METHODS

3.1. Reactivation of isolates

The *P. chlamydosporia* and *P. lilacinum* isolates were grown inside Petri dishes, containing PDA medium (Difco®), kept in chambers (25°C±1 °C and 12:12h L:D) for 10 days. After growth, conidial suspensions were prepared, serial diluted to $1x10^3$ conidia ml^{-1}, then aliquots of 100 µl of each dilution were transferred to Petri dishes containing PDA and, with a Drigalsky spatula, they were spread across the plate to homogenize them.

The plates were stored in a growth chamber (at 25 ± 1°C, 12:12h L:D photoperiod) for three days or until initial colony development was achieved. Subsequently, one colony plug (classified as one conidium) was removed from the culture medium, characterizing the culture or monosporic colony. The isolates were preserved in cryogenic tubes containing 10% glycerol and stored in a -80°C freezer (JARONSKI & JACKSON, 2012). All experiments were performed by using the preservation obtained for each isolate.

3.2. Characterization of *Pochonia chlamydosporia* and *Purpureocillium lilacinum* isolates

The isolates used (Table 1) in this study belong to the Collection of Entomopathogenic Microorganisms “Prof. Sérgio Batista Alves” of the Laboratory of Pathology and Microbial Control of Insects, at the Department of Entomology and Acarology of the University of São Paulo (ESALQ-USP). Species identification of the *Purpurecillium lilacinum* and *Pochonia chlamydosporia* isolates were confirmed morphologically and molecularly in this study.

Table 1. Isolate code, a substrate of origin, and sample location of the selected isolates of *Purpureocillium lilacinum* and *Pochonia chlamydosporia* screened.

	Isolate code	Species	Origin
1	ESALQ489	*P. lilacinum*	Hemileucidae
2	ESALQ668	*P. lilacinum*	*Solenopsis* sp
3	ESALQ774	*P. lilacinum*	Soil

4	ESALQ1744	*P. lilacinum*	Soil
5	ESALQ1749	*P. lilacinum*	Soil
6	ESALQ1766	*P. lilacinum*	Soil
7	ESALQ1771	*P. lilacinum*	Soil
8	ESALQ1906	*P. lilacinum*	*Galleriamellonella*
9	ESALQ1907	*P. lilacinum*	*G. mellonella*
10	ESALQ1908	*P. lilacinum*	*G. mellonella*
11	ESALQ1909	*P. lilacinum*	*G. mellonella*
12	ESALQ1994	*P. lilacinum*	Soil
13	ESALQ1995	*P. lilacinum*	*Tenebrio molitor*
14	ESALQ1996	*P. lilacinum*	*T. molitor*
15	ESALQ2077	*P. lilacinum*	*G. mellonella*
16	ESALQ2078	*P. lilacinum*	*G. mellonella*
17	ESALQ2164	*P. lilacinum*	Soil
18	ESALQ2165	*P. lilacinum*	Soil
19	ESALQ2166	*P. lilacinum*	Soil
20	ESALQ2167	*P. lilacinum*	Soil
21	ESALQ2482	*P. lilacinum*	*T. molitor*
22	ESALQ2509	*P. lilacinum*	*T. molitor*
23	ESALQ2593	*P. lilacinum*	Soil
24	ESALQ2599	*P. lilacinum*	Soil
25	ESALQ2645	*P. lilacinum*	Soil
26	ESALQ2715	*P. lilacinum*	Soil
27	ESALQ2716	*P. lilacinum*	Soil
28	ESALQ2718	*P. lilacinum*	Soil
29	ESALQ2765	*P. lilacinum*	Soil
30	ESALQ2774	*P. lilacinum*	Soil
31	ESALQ2776	*P. lilacinum*	Soil
32	ESALQ2780	*P. lilacinum*	Soil
33	ESALQ2832	*P. lilacinum*	Soil
34	ESALQ2833	*P. lilacinum*	Soil
35	ESALQ2847	*P. lilacinum*	Soil
36	ESALQ3599	*P. lilacinum*	*Toxoptera citricida*
37	ESALQ3600	*P. lilacinum*	Soil
38	ESALQ3602	*P. lilacinum*	Soil
39	ESALQ3603	*P. lilacinum*	Soil
40	ESALQ3605	*P. lilacinum*	Soil
41	ESALQ5405	*P. chlamydosporia*	Soil
42	ESALQ5406	*P. chlamydosporia*	Soil

3.2.1. Morphological analyses

The isolates of *P. chlamydosporia* and *P. lilacinum* were cultivated in Petri dishes (diameter = 9 cm) with potato dextrose agar medium – PDA (Difco®). The fungi were maintained in an incubation growth chamber (25°C±1 °C, 12:12h L:D) until the beginning of sporulation. Glass slides were prepared, with the aid of a platinum handle, it was removed a small amount of mycelium from the plate, added in glass slides and drops of the blue lactic dye (Lactophenol Blue), for the observation of conidiophores under the microscope (DM4000B, Leica® Microsystems, Wetzlar, Germany), and the morphological identification was carried out based upon the classification key of Humber (2012).

3.2.2. Molecular identification

DNA extraction and PCR analyses

The DNA from the isolates was extracted according to a protocol adapted from Dellaporta et al. (1983). Fungal samples were harvested and kept in Eppendorf tubes (1.5 ml), in which 300 µl of extraction buffer was added to each sample. The isolates were macerated using L-Beader 24 (Loccus Biotechnology), with three cycles of the 30s at 4,000 rpm, with a pause of 10 seconds between each cycle.

Subsequently, 200 µl of extraction buffer and 35 µl of SDS 20% solution were added, the samples were stirred for homogenization and heated in a water bath at 65°C for 10 minutes. Then, it was added 160 µl of potassium acetate (CH_3CO_2K) to each tube, followed by agitation and centrifugation for 10 minutes at 14,000 rpm. After the pellet formation, the supernatant of each sample was transferred to new sterilized tubes contained 500 µl of isopropanol, and they were centrifuged again for 10 minutes at 14,000 rpm.

The supernatants were discarded and 500 µl of ethanol 70% were added. Once again, the tubes were centrifuged for 5 minutes at 14,000 rpm. The supernatants were disposed and 50 µl of sterile Milli-Q® water was added. The DNA concentration values were measured with the aid of the BioDrop® apparatus.

Amplification reactions were performed with the selected primers: ITS5 (5'- G AAG AAC CGT AGT AAA AGT GGA) and ITS4 (5'- GC TAT TGA TAT GCT TCC TCC) (O'DONNELL et al., 1998; DODD et al., 2002; MORTON et al., 2003; MEDINA-

CANALES et al., 2014; SEQUEIRA et al., 2017). Polymerase chain reactions (PCRs) were conducted with 25 µl of total volume, which contained milli-Q®water (9.5 µl), 1X Taq Mix® Reaction Buffer (12.5 µl), diluted primers at 25 pmol/µl (1 µl each) and DNA (1 µl). The samples were amplified using a thermocycler (Equipment MyCycle™ Thermal Cycler Bio-Rad, USA) with an initial cycle of denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 45s, annealing of 56°C for the 30s and polymerization at 72°C for 60s, and ending with a final extension at 72°C for 5 min and cooling at 70°C for 2 min (modified from MEDINA-CANALES et al., 2014).

The quality and weight of the PCR products were analyzed on agarose gel (1% w/v), with 0.5X TBE. For the sequencing reactions, PCR products were purified using the EasyPure® PCR Purification Kit (Trans Gen Biotech), according to the manufacturer's instructions. Only the primer ITS5 was used for sequencing. Sequences were edited and aligned using the ClustalW tool from BioEdit (version 7.0.4.1) to obtain a consensus for each species (HALL, 1999).

Phylogenetic tree

Data from sequencing were analyzed through Maximum Likelihood Tree (ML) on MEGA (version 10.1.5), implementing the evolutive model Kimura 2-parameter with invariant sites (KIMURA, 1980). The analysis was conducted considering the gaps as complete deletions, and performing 1000 bootstrap replicates. In addition to the sequences obtained by the DNA sequencing, fourteen taxonomically authenticated reference sequences were included. The phylogenic trees were created and edited on MEGA (TAMURA et al., 2013; KUMAR et al., 2018). The obtained data were condensed when the bootstrap values were above 70% (BISCHOFF et al., 2006).

3.3. Submerged fermentation of *Pochonia chlamydosporia* and *Purpureocillium lilacinum*

Conidial suspensions were obtained by harvesting the plates containing sporulating fungi. Then, a 10 ml 0.004% aqueous solution of sorbitan monooleate (Tween® 80) was added, homogenized and the conidia counted. Then five ml of the suspensions standardized at 5 x 10^6 conidia ml^{-1} were added into baffled flasks (250 ml) (Glass, Bellco®, Vineland, NJ, USA) containing 45 ml of the liquid media used in

submerged fermentation, totalizing 50 ml per vial. The flasks were placed on an orbital shaker table at 300 rpm and 28 ± 2°C, 12h L:D (MARCONI®, Model: MA 830) and shaken manually daily to prevent mycelial growth on the walls.

Two liquid media used were an adaptation by IWANICKI et al (2018) of the media developed by Jackson & Jaronski (2012) and by Adámek (1965). The first medium used had a C: N ration of 10:1 (named Jackson 4), the carbon source used was glucose (45 g l^{-1} 20% w/v) with yeast extract as the nitrogen source (45 g l^{-1}); the second medium used had a C: N ratio 50:1 (Jackson 6) (glucose – 81 g l^{-1} and yeast extract – 9 g l^{-1}). Both media contained salts, metals, and vitamins, which were composed of KH_2PO_4, 4.0 g l^{-1}; $CaCl_2•2H_2O$, 0.8 g l^{-1}; $MgSO_4•7H_2O$, 0.6 g l^{-1}; $FeSO_4•7H_2O$, 0.1 g l^{-1}; $CoCl_2•6H_2O$, 0.1 g l^{-1}; $MnSO_4•H_2O$, 0.016 g l^{-1}; $ZnSO_4•7H_2O$, 0.014 g l^{-1}; thiamine, riboflavin, pantothenate, niacin, pyrodoxamine, thioctic acid, 500 µg l^{-1} each; and folic acid, biotin, vitamin B12, 50 µg l^{-1} each. The adapted Adamek medium had as carbon source glucose (40 g l^{-1}) and yeast extract as a nitrogen source (80 g l^{-1}) and corn steep liquor (40 g l^{-1}). The source of salts with trace metals and vitamins was the same as the aforementioned.

Two, three and four days after fungal transfer to the culture media, 1 mL samples were taken under aseptic conditions and diluted with distilled water + Tween® 80 (1:10) to evaluate the concentration of microsclerotia produced under glass slides (25 x 75 mm) (using 50 µl of culture diluted samples) and analyzing the culture morphology. Only pigmented microsclerotia larger than 50 µm diameter were considered (JACKSON & JARONSKI, 2009). For the adapted Adamek medium, the concentration of blastospores and submerged conidia were also evaluated for all isolates.

3.4. Experimental set up for the *in vivo* bioassays

3.4.1. Slow drying of selected isolates

Selected isolates were submitted to submerged fermentation for four days and then mixed with Diatomaceous earth (5% w/v) (Sigma-Aldrich, Co., St. Louis, MO). This mixture was filtrated in filter papers (70 mm, Unfil, Brazil) using a vacuum pump connected to a Büchner funnel and a Kitasato flask (Figure 1). The final material was transferred to Petri dishes (90x15 mm) and it was submitted to slow drying in a drying

chamber for 20 hours at 50-60% of relative humidity (RH) and 1-2 hours at 15-20% RH until the samples reached the low water activity. After reaching water activity ≤ 0.2 measured in the LabMaster-aw equipment (Novasina®, Lachen SZ, Switzerland), samples of 25 mg were obtained to evaluate the viability, and the remainder was stored inside of vacuum package in polyethylene bags at 5°C.

Figure 1. Filtration of the mycelium mat obtained + Diatomaceous earth 5% w/v using a vacuum pump connected into a Büchner funnel and a Kitassato flask.

3.4.2. Microesclerotia viability

Twenty-five milligrams of microsclerotia + Diatomaceous earth (MS+TD) were sprinkled on Petri dishes with agar + water (2% w/v), which were incubated inside growth chambers at 28°C in the dark. After 24h, microsclerotia germination was evaluated, by counting the formation of hyphae outside the granules, with the aid of a phase-contrast microscope (DM4000B, Leica® Microsystems, Wetzlar, Germany) (JACKSON & JARONSKI, 2009; KOBORI et al., 2015). The plates were kept once again inside growth chambers (12h photophase) for seven days. To determine the production, of conidia spores were harvested and diluted in water + 0.04% Tween 80®. And the concentrations (conidia g^{-1} of MS+TD) were measured by counting in a Neubauer chamber.

3.4.3. Seed treatment of common beans with *P. lilacinum* and *P. chlamydosporia*

The *P. vulgaris* cultivar used in all experiments was “IAC Milênio”. Before the MS+TD inoculation, the seeds were surface-sterilized for 45 seconds in 70% ethanol, followed by 1 minute in 1.5% of commercial sodium hypochlorite, 45 seconds on 70% ethanol, and washed three times with distilled water (adapted from AKUTSE et al., 2013).

The seeds of bean plants were covered by the combination of MS+TD of the selected isolates (*P. lilacinum* ESALQ1744, ESALQ2482, ESALQ2593, *P. chlamydosporia* ESALQ5405 and ESALQ5406). The MS concentrations were standardized by estimating the amount of microsclerotia required for the production of 10^{12} conidia ha^{-1}, according to the viabilities obtained for each sample. 0.5% w/v Gum arabic was added to the MS+TD to provide the adhesion on the seed surface. The control was based on common bean seeds treated only with gum arabic. The seeds were maintained in filter papers (70mm, Whatman®) in order to dry out the excess of suspension, then individually planted inside plastic pots (10 per treatment) filled with 40% sand and 60% soil. The pots were kept in a greenhouse with daily irrigation and natural photophase and temperature.

3.5. Effects of *P. lilacinum* and *P. chlamydosporia* on *T. urticae* fecundity

One transparent plastic clip cage (4.5 cm high x 3.8 cm diameter) with a mesh at the top (0.09 mm mesh size) (Figure 2) was placed on each bean plant leafs in the V4 stage, approximately 21 days after seeding (CANASSA et al., 2019). Subsequently, one *T. urticae* female was individually transferred to each clip cage.

The spider mite fecundity was estimated at the 7^{th} day of infestation by counting the number of eggs and eventually, any emerged larvae. The treatments were subjected to a randomized block design with ten plants (replicates) per treatment. The experiments were repeated three times.

Figure 2. Experimental set up using clip cages to hold the spider mites.

3.6. Effect of *P. lilacinum* and *P. chlamydosporia* in common bean

Common bean seeds were treated with four *P. lilacinum* isolates, two *P. chlamydosporia* isolates, and control 0.5% w/v of Gum arabic. At the end of the study, after plant growth, the plants were removed from the pots and their roots washed to remove the soil. Measurements of the length of the aerial part (cm), the root (cm), and their fresh and dry weight (g) were determined.

Three fragments of the roots, the stem, and the leaves were cut from each plant to assess fungal colonization. The samples were sterilized by the following steps: 70% ethanol for 30 seconds, 1.25% commercial sodium hypochlorite for 60 seconds, 70% ethanol for 30 seconds, rinsed in distilled water three times. Afterward, aliquots of 50 µL of the last rinsing were collected in the beginning and at the end of the sterilization process and plated into the PDA medium to confirm the success of the sterilization (BEHIE et al., 2015).

Subsequently, the samples were maintained in filter papers to remove the excess of water. The fragments were plated in PDA medium with 0.5 g l^{-1} of cycloheximide, 0.2 g l^{-1} of chloramphenicol, 0.5 g l^{-1} of Dodine (65%), and 0.01 g l^{-1} of crystal violet (BEHIE et al., 2015). The plates were incubated in growth chambers for 20 days.

Fungal colonization was visually analyzed based on colony morphology and microscopical observations. For the soil analysis, suspensions with distilled water + 0.05% Tween® 80 were prepared and 50 µl of the 10^{0}, 10^{-1}, 10^{-2} and 10^{-3} dilutions

were plated in the same medium at 25 ± 1°C, 12:12h L:D. The colonies were visually observed in an optical microscope.

3.7. Effects of *P. lilacinum* and *P. chlamydosporia* on *H. glycines*

The nematode populations used in the experiments were provided by the Laboratory of Nematology located at the Department of Plant Pathology and Nematology from ESALQ/USP. The common bean plants, previously seed inoculated with the fungal isolates (ESALQ1744, ESALQ2482, ESALQ2593, ESALQ5405 and ESALQ5406), were inoculated with eggs and J2 of *H. glycines* race 3 on plants at the V4 stage (approximately 15 days after planting). By using a spatula, two holes were made close to the plant stem (2 and 4 cm of depth). The inoculum was homogenized by using a magnetic stirrer, and 1,000 eggs plus J2 individuals (initial population) were inoculated in each plant, and the holes were closed with vermiculite to avoid heat stress and humidity loss. The evaluations occurred 45 days after the inoculation, according to the nematode life cycle in the plant (NIBLACK, 2005).

The nematodes were extracted following the methodology described by Coolen & D'herde (1972). Roots were removed from in the pots, washed and cut in 1 cm pieces, followed by blending with 1% commercial sodium hypochlorite, added to dissolve the egg masses present in the roots. The obtained suspension was poured on top of two sieves, the first of 60 "mesh" (0.260 mm – to retain the coarse particles) and the second of 500 "mesh (0.025 mm – to retain fine particles).

The nematodes were collected in a beaker and centrifuged twice. First at 1,800 rpm for 5 min, with kaolin. In the second, the supernatant was discarded, a sucrose solution (1.15 g ml^{-1}) was added and centrifugation occurred at 1,800 rpm for 1 min. The nematodes were collected using a 500 "mesh" sieve. In order to maintain the nematodes integrity, 300 µl of formaldehyde was added.

The number of nematodes was estimated based upon two 0.5 ml counts on Peters' slide, under the microscope. Afterward, it was estimated the final population (FP), the number of nematodes per gram (Nem g^{-1}) of root and the percentage of control, based on the population obtained in control plants. Measurements of the fresh and dry weight of aerial and root parts (g) and the weight of the pods (g) of *P. vulgaris* plants were taken to observe if the treatment had any effect on plant growth.

3.8. Statistical analyses

The microsclerotia (MS) production of *P. lilacinum* and *P. chlamydosporia* were analyzed by generalized linear models (GLM) with Poisson distribution for errors and log-link function, in which fixed effects included experiment, isolate, day, medium and the interaction between isolate, day and medium, while random effects were attributed to the observational level and day per experimental unit, medium and isolate. A linear mixed model was fitted to the logarithm of the submerged propagules yield, including the effects of the experiment, isolate, day, and the interaction between isolate and day as fixed and random day effects per experimental unit in the linear predictor. In this case, we also modeled the variance as a function of isolate, to account for variance heterogeneity. The significance of the effects was assessed using likelihood-ratio (LR) tests at $P < 0.05$. The quality and adjustment of the selected models (goodness-of-fit) were assessed using half-normal plots with simulation envelopes with the package 'hnp' (MORAL et al., 2017) in conjunction with the Akaike information criterion (AIC), in which the lowest AIC indicates the best adjustment. In another approach, we carried out an analysis that allowed to look at the marginal performance of fungal isolates, regardless of medium and day of fermentation, in which we were able to establish a ranking order from best to poorest producers of microsclerotia and submerged propagules.

The cluster analysis was performed to MS or submerged propagules data for multiple fungal isolates. Predicted means derived from model fits were normalized prior to turning into distances based on the Euclidean method, a measure of similarity. Subsequently, Euclidean distances were subjected to a hierarchical clustering tree (in rows and columns) taking into account measurements for liquid media and fungal isolates using Ward's method. The heat map was computed and described using a function of heatmap.2 in the 'gplots' package (WARNES et al., 2020).

The female fecundity (number of eggs laid by each spider mite female up to seven days upon feeding on bean leaves) data were analyzed by fitting the Poisson model with the log link function. The additive effects of experiment and treatment were included in the linear predictor. The significance of the effects was assessed using Chi-squared tests. Multiple comparisons were performed by obtaining the 95% confidence intervals for the estimated treatment effects and separated by the Tukey HSD method using the package 'emmeans' (LENTH, 2020). The number of nematodes as final

population size and the average number of nematodes per gram of root mass were analyzed using Negative Binomial (or overdispersed Poisson) and Gaussian models, respectively, and included the isolate in the linear predictor. The number of nematodes per gram was transformed using the square root transformation prior to analysis. Samples that exhibited value “zero” due to egg diapause in *H. glycines* were dropped from the analysis. When the treatment effect scored significantly, then multiple pairwise comparisons of means were performed with the Tukey HSD method at $P < 0.05$.

All plant trait variables (plant responses to fungal isolates) were analyzed using linear models with Gaussian distribution for errors, including isolate, experiment, and their interaction term as fixed effects and replicate plants as a random effect in the linear predictor. Prior to analysis in order to meet normality assumptions, the response variables recorded after 45 days of bean plant growth in the greenhouse and encompassing aerial part length, root length, plant height, and root dry weight were all logarithm-transformed. The aerial part and whole plant dry weights did not require data transformation. We included isolate as a fixed effect and replicate plants as a random effect in the linear predictor, as the analysis was conducted within each experimental dataset. The significance of the effects was assessed by using *F* tests. Goodness-of-fit was assessed using half-normal plots with simulation envelopes (MORAL et al., 2017). Significant differences between treatments were determined by multiple comparisons based on contrast using the Tukey HSD method with 5% of significance with the package ‘emmeans’. All statistical analyses were performed in R (R CORE TEAM, 2020).

4. RESULTS

4.1. Morphological and molecular analyses of *Purpureocillium* and *Pochonia* isolates

The morphological analysis led to the identification of the selected isolates belonging to the genera *Purpureocillium* and *Pochonia*, according to the characteristic from the classification key by Humber (2012).

The molecular data confirmed that the presumed *P. lilacinum* isolates indeed belonged to this species, except for isolate ESALQ2726, that was phylogenetically closer to *Cordyceps javanica*. This isolate was then not included in the following studies. As to *P. chlamydosporia*, ESALQ5406 was confirmed as belonging to this species; however, ESALQ5405 aligned with *P. chlamydosporia* var. *catenulate* and *P. gonioides*, and even though its classification remains unclear, from this point on it will be referred to as *P. chlamydosporia* (Figure 3).

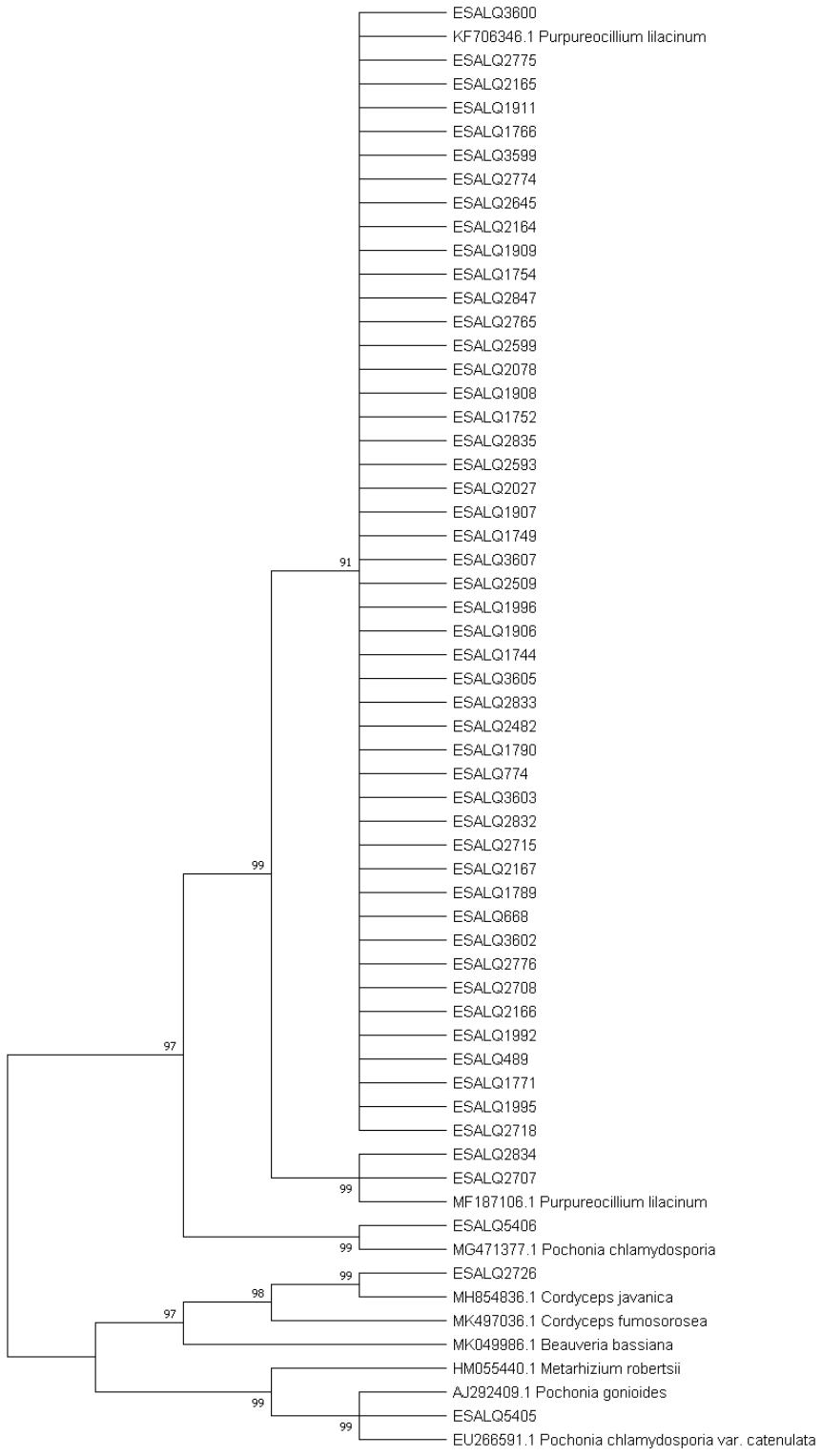

Figure 3. Evolutionary analysis by Maximum Likelihood method. Phylogenetic tree Kimura 2-parameter model with invariants sites. Performing 1000 bootstrap replicates.

4.2. Microsclerotia production

All *P. lilacinum* and *P. chlamydosporia* isolate produced MS on the three different liquid culture media, modified Adamek, Jackson 6 (C: N ratio 50:1) and Jackson 4 (C: N 10:1), with exception of ESALQ1907 (did not produce on Jackson 4 and 6), ESALQ1908 (did not produce on modified Adamek) and ESALQ2509 (did not produce on modified Adamek and Jackson 4) (Figures 4 and 5). The MS exhibited darker pigmentation when produced in modified Adamek medium, which is richer in nitrogen and iron phosphate concentration.

The production of MS differed among the culture media, and the concentration varied among isolates. For the Jackson 4 medium, the production varied from $2x10^3$ to $1.7x10^4$ MS ml^{-1}; the most productive isolates in this medium were ESALQ1906, ESALQ1909, ESALQ2164, ESALQ2166, ESALQ2482, ESALQ2593, ESALQ2599 and ESALQ2765 (1.0 – 1.70 x 10^4 MS ml^{-1}), and the ones with the lowest MS ml^{-1} concentration being ESALQ668 and ESALQ2832 (2 x 10^3 MS ml^{-1}).

For the Jackson 6 medium, production ranged between 3 x 10^2 and 6 x 10^4 MS ml^{-1}, with the most productive isolates being ESALQ1744, ESALQ1771, ESALQ2164, ESALQ2599, ESALQ2645 (1.0 – 1.96 x 10^4 MS ml^{-1}), ESALQ2482 (6.1 x 10^4 MS ml^{-1}) and ESALQ2593 (2.2 x 10^4 MS ml^{-1}), and ESALQ5406 as the less productive (3.3 x 10^2 MS ml^{-1}).

In the modified Adamek medium, the MS concentration varied from 1 x 10^3 to 2 x 10^4 MS ml^{-1} in the last day, with five isolates standing out: ESALQ2078, ESALQ2164, ESALQ 2509, ESALQ2593 and ESALQ2718 (1 – 1.16 x 10^4 MS ml^{-1}). The lowest productions were from isolates ESALQ668, ESALQ1996, and ESALQ2776 (1.66 x 10^3 MS ml^{-1}). In addition, isolate ESALQ5406 did not produce any MS on the last day.

Based on the estimated general mean of the ranking graph (Figure 4) for fungal isolates based on all media and time the three isolates that stood out were: ESALQ2482, ESALQ2164, and ESALQ2593. On the other hand, the three isolates considered the least productive were ESALQ5406, ESALQ5405 e ESALQ2776. All highlighted isolates were of *P. lilacinum. P. chlamydosporia* isolates did not stand out in any culture media, with productions ranging from 3.3 x 10^2 to 7 x 10^3 MS ml^{-1}.

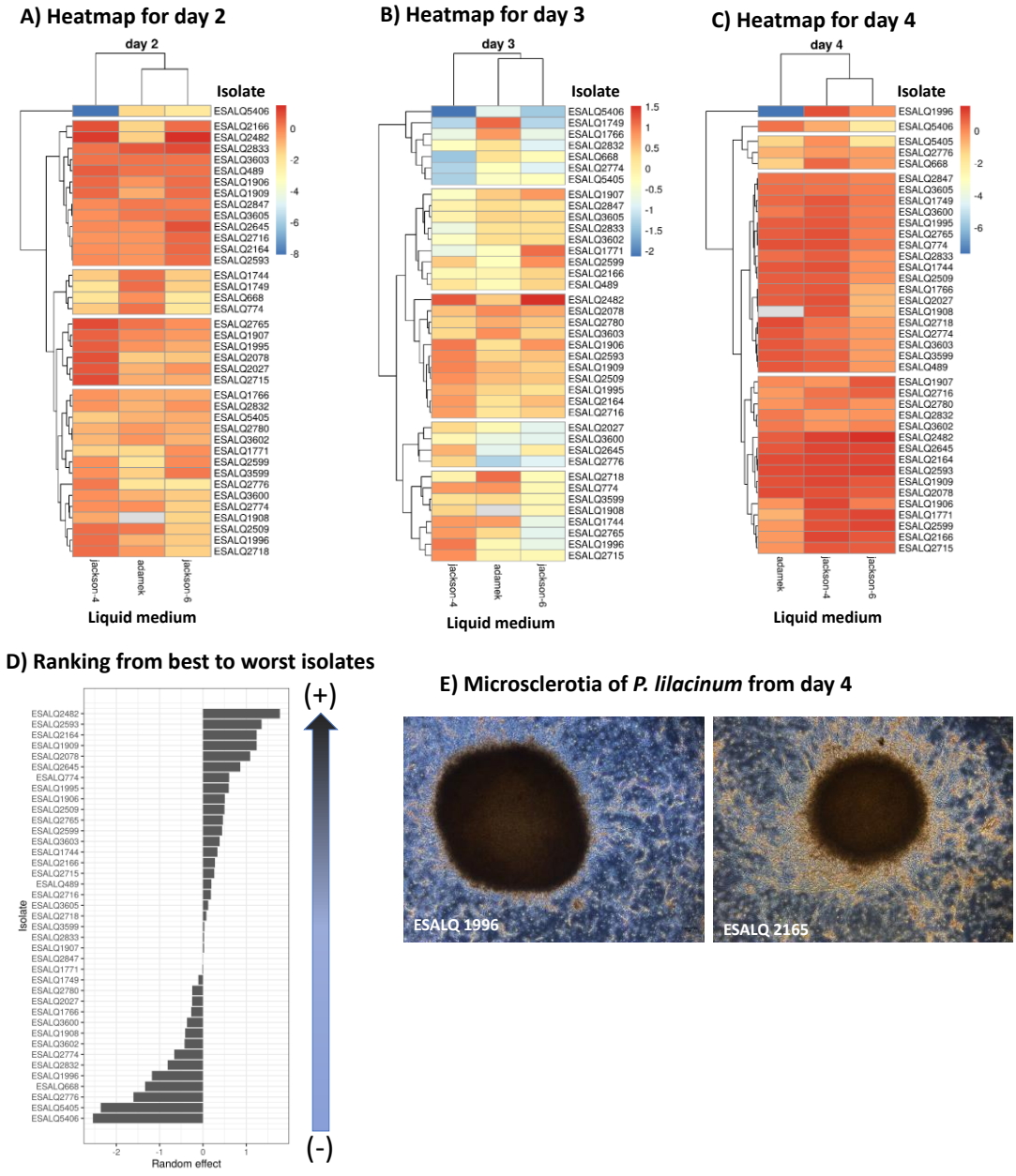

Figure 4. Heatmaps from day 2 to 4 representing the estimated means of microsclerotia production across the 39 fungal isolates within *Purpureocillium lilacinum* and *Pochonia chlamydosporia* grown in three different liquid media (modified media: Adamek [A], Jackson-4 [B], and Jackson-6 [C]), as well as the ranking graph for fungal isolates based on the estimated general mean of the population (D). Scale from blue to red means that high MS are highlighted in red color, whereas low MS yields are assigned with blue color.

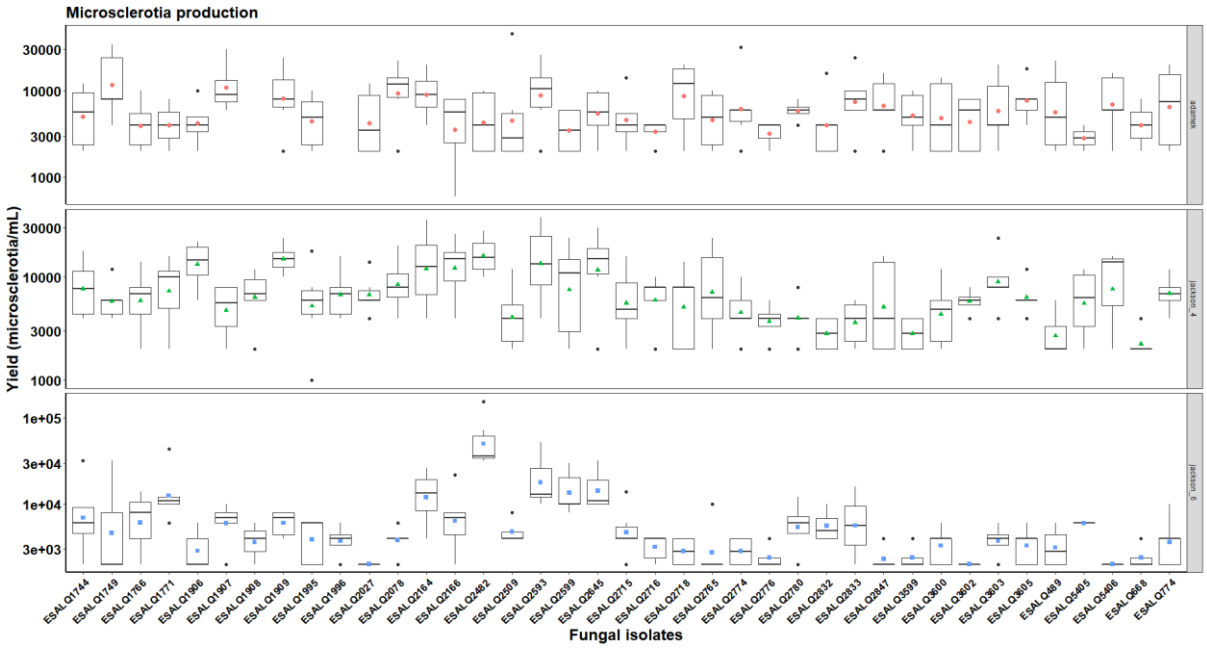

Figure 5. Microsclerotia production by the 39 fungal isolates of *Purpureocillium lilacinum* and *Pochonia chlamydosporia* grown in three different liquid media (Adamek, Jackson-4, and Jackson-6) recorded for day 4 of growth. Y-axis is shown in the log10-scale. Different symbols with distinct colors represent the mean values along with boxplots for each fungal isolate.

4.3. Submerged propagules production

In the modified Adamek medium, it was observed that other submerged propagules, similar to blastospores and submerged conidia, were produced in high concentration. However, the morphological and physicochemical evaluations of these structures were not performed in this study, therefore it was not possible to differentiate them, and consequently, the evaluation considered them all together, here referred to as submerged propagules.

Looking at the marginal performance of isolates corrected by experiment and fermentation day (Figures 6 and 7), it was possible to rank from best to worst producers of submerged propagules among the isolates tested. In this regard, the two *P. chlamydosporia*, isolate ESALQ5405 followed by ESALQ5406, showed the overall highest production yields of submerged propagules, whilst the worst production was obtained with isolate ESALQ1909 of *P. lilacinum*. These results also corroborate with the heatmap output and facilitate the selection of best fungal isolates.

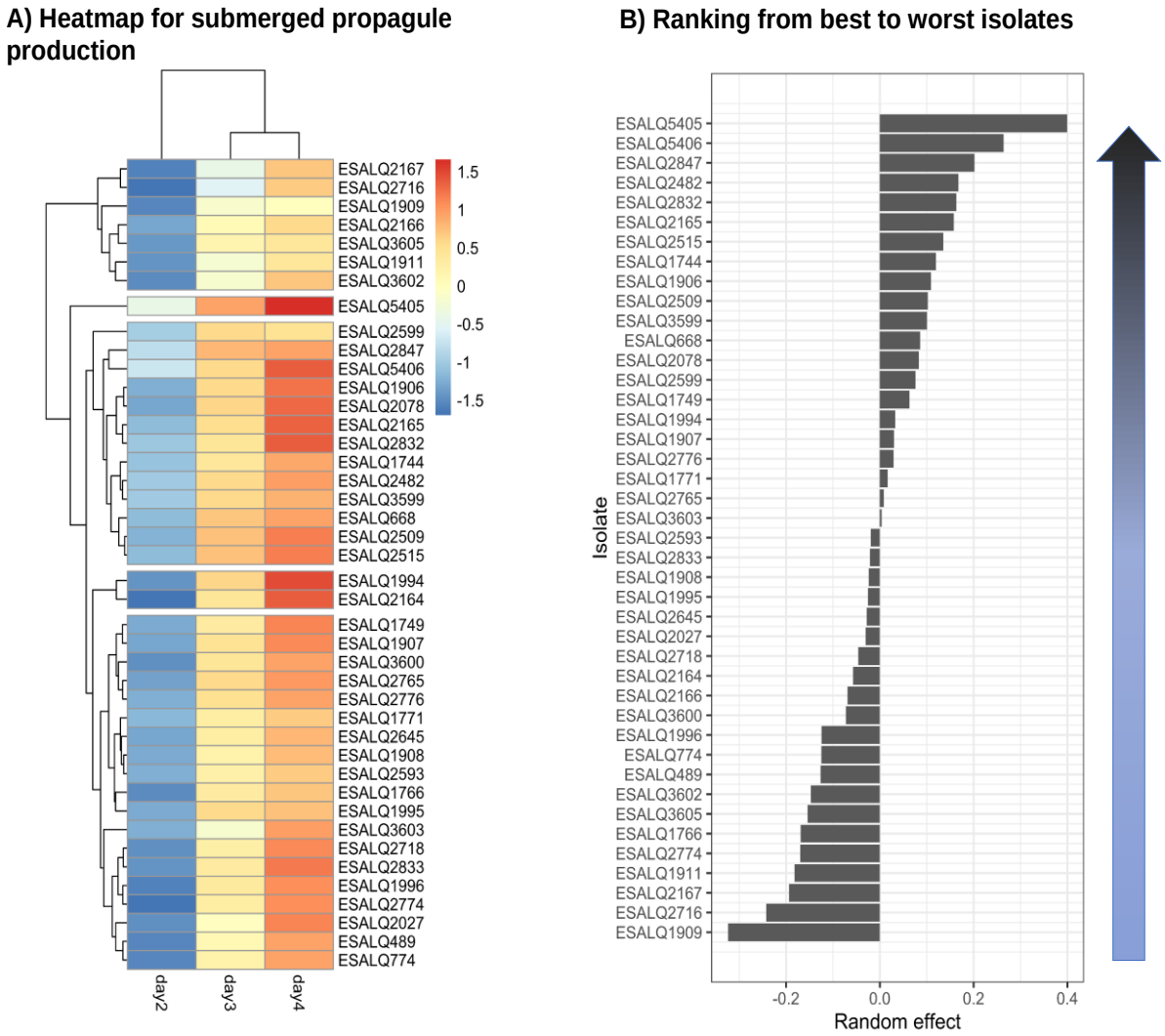

Figure 6. Heat map (A) and rank from best to worst isolates for predicted mean concentration of submerged propagules (submerged conidia + blastospores) of *P. lilacinum* and *P. chlamydosporia* on 4th day of culture growth on modified Adamek medium. Scale from blue to red means that high submerged propagules are highlighted in red color, whereas low submerged propagules yields are assigned with blue color.

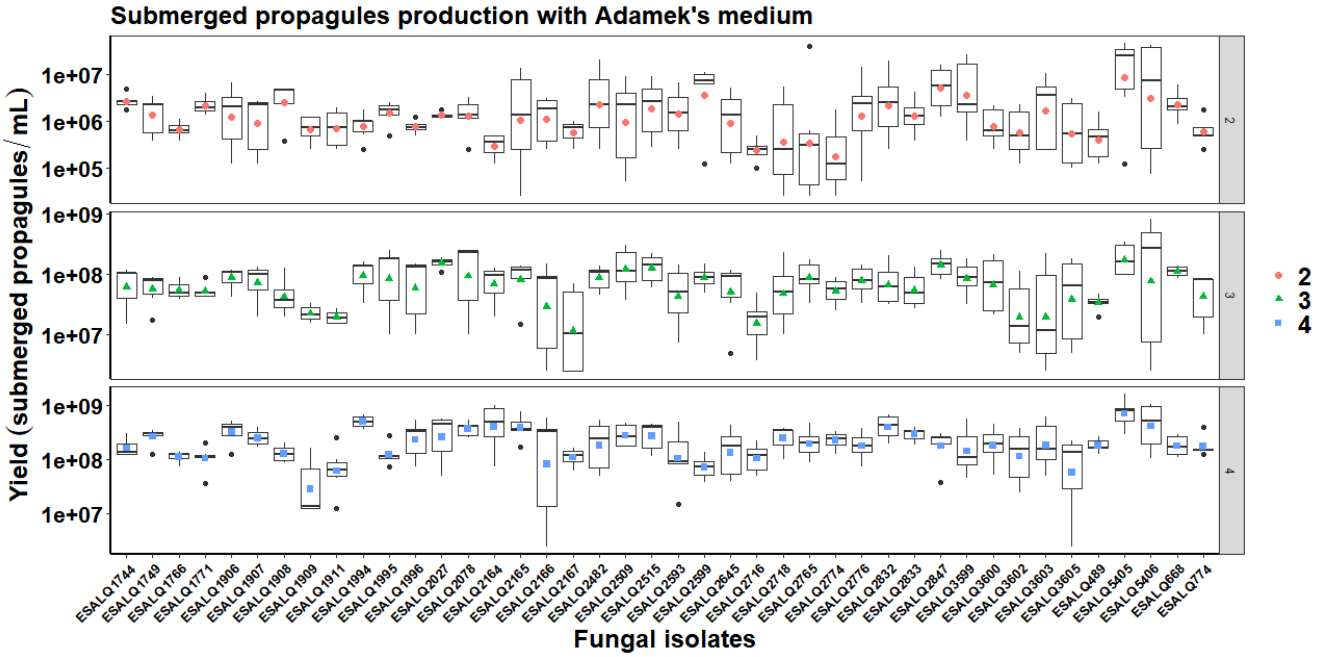

Figure 7. Mean concentration of submerged propagules (submerged conidia + blastospores) *Purpureocillium lilacinum* and *Pochonia chlamydosporia* during four days (2th, 3th and 4th day) of culture growth on modified Adamek medium. Y-axis is presented in the log10-scale. Different symbols with distinct colors represent the mean values along with boxplots for each fungal isolate.

4.4. Endophytic colonization and plant growth stimulation of *P. lilacinum* and *P. chlamydosporia* in *P. vulgaris*

The colonization of bean plants by treatment of seeds with MS of ESALQ1744, ESALQ2482, ESALQ2593 (*P. lilacinum*), ESALQ5405, and ESALQ5406 (*P. chlamydosporia*) was lower than expected (Table 2). Fragments of roots, stems, and leaves were maintained on selective medium for 15 days, after which few *P. lilacinum* and *P. chlamydosporia* colonies were observed.

Forty-five days after seeding (DAS), the only vegetative parameter that differed from the control group was the length of the aerial part, whereas the plants treated with the fungal isolates ESALQ2482, ESALQ5405 and ESALQ5406 exhibited shorter length of the aerial part (Table 2). While the root dry weight decreased with seed-inoculated bean plants with ESALQ2593 and ESALQ5405. Nonetheless, there were no differences between treatments for the variables root length and dry weight of the aerial part of the plants.

Table 2. Common bean plant traits (*Phaseolus vulgaris*) after seed treatment with different isolates of *Purpureocillium lilacinum* and *Pochonia chlamydosporia* after 45 days of growth under greenhouse conditions.

Treatment	**Length of the aerial part (cm)**	**Root length (cm)**	**Dry weight of the aerial part (g)**	**Root dry weight (g)**
Control (no fungus)	20.6 ± 1.49 ab	23.0 ± 0.76 ns	0.46 ± 0.043 ns	0.17 ± 0.03 ab
ESALQ1744	20.8 ± 1.42 ab	25.2 ± 0.99	0.49 ± 0.05	0.20 ± 0.02 a
ESALQ2482	19.4 ± 1.55 bc	25.0 ± 0.97	0.41 ± 0.04	0.18 ± 0.02 ab
ESALQ2593	21.2 ± 1.20 a	25.2 ± 1.18	0.37 ± 0.04	0.13 ± 0.02 b
ESALQ5405	17.7 ± 1.27 c	25.6 ± 1.41	0.36 ± 0.05	0.13 ± 0.02 b
ESALQ5406	19.2 ± 1.51 bc	26.7 ± 1.10	0.39 ± 0.06	0.16 ± 0.01 ab
Treatment effect (linear model with Gaussian distribution)	F = 5.47, df = 5, 153, P = 0.0001	F = 1.27, df = 5, 153, P = 0.278	F = 2.199, df = 5, 153, P = 0. 06	F = 3.63, df = 5, 153, P = 0.0039

Means (± SE, n = 10 plants per treatment) of 3 independent experiments) of significant contrasts between means at $P < 0.05$, while no significance was indicated by “ns”.

4.5. Effects of *P. lilacinum* and *P. chlamydosporia* on *T. urticae* fecundity

The effect of fungal isolates through seed coating in grown common bean plants exposed to two-spotted spider mites was measured by the production of eggs and nymphs per leaf. As shown in Figure 8, the indirect fungal effect was significant to egg density as all fungal isolates tested, within *P. chlamydosporia* and *P. lilacinum*, induced a great reduction in egg numbers laid by spider mite females (χ^2 = 257.97, df = 5, 113, $P < 0.0001$). These results indicate that the indirect effect modulated by fungal

treatment in common bean plants was pronounced against eggs as they may have negatively affected the fecundity of spider mite females.

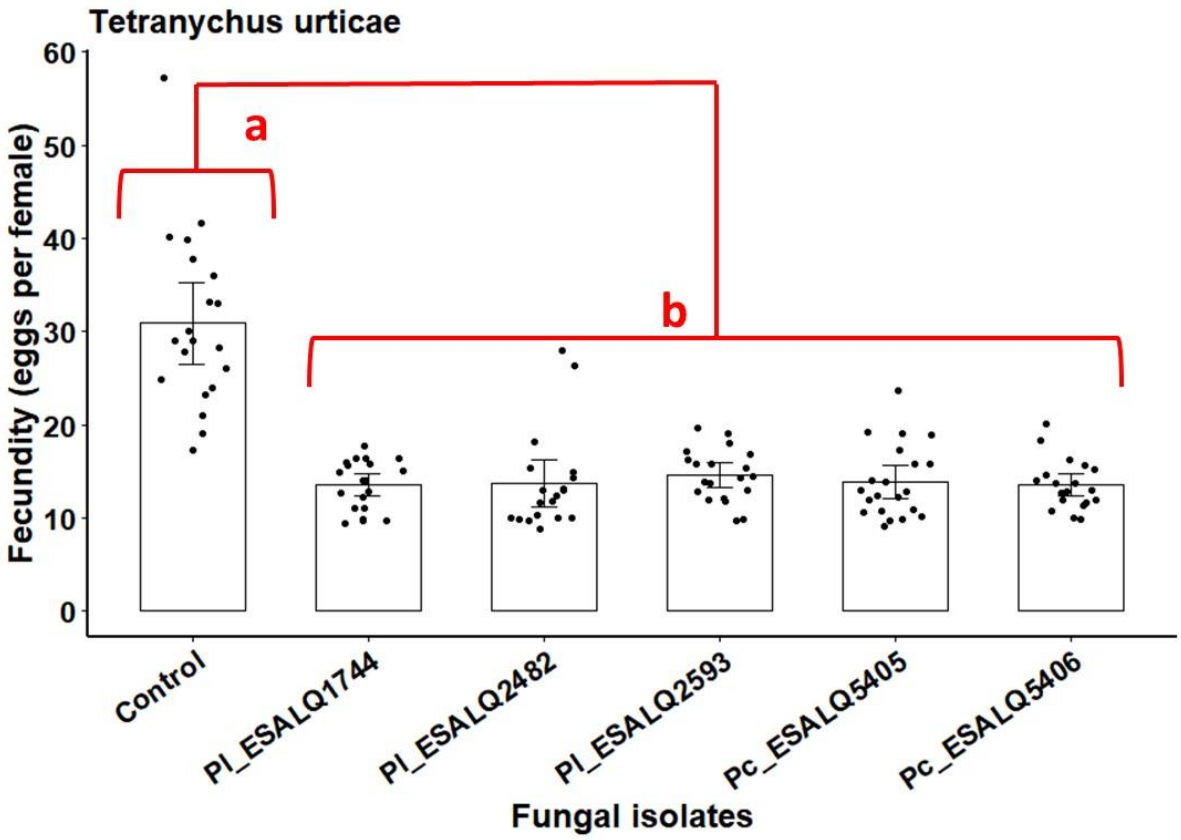

Figure 8. The number of eggs laid (plus emerged larvae) by *Tetranychus urticae* after seven days of infestation in bean plants seed-inoculated with six treatments: control (0.5% w/v of Gum arabic), microsclerotia (10^{12} conidia ha^{-1}) of the isolates ESALQ1744, ESALQ2482 and ESALQ2593 of *Purpureocillium lilacinum*, and isolates ESALQ5405 and ESALQ5406 of *Pochonia chlamydosporia*. Means (± 95% confidence interval, n = 20 biological replicates from 2 independent experiments) of spider mite counts of eggs and nymphs per leaf. Significant contrasts between means at $P < 0.05$".

4.6. Effects of *P. lilacinum* and *P. chlamdydosporia* on *H. glycines*

The results of the three experiments carried out over time are presented separated as the analysis showed a significant effect of experimental replicates. For the first and second biological replicate (Figure 9), there was no significant difference among all the treatments ($\chi^2 = 5.9909$, df = 5, $P > 0.0001$). For the last replicate, the isolate *P. chlamydosporia* ESALQ5406 was able to decrease the final population of *H. glycines* in relation to the uninoculated control ($\chi^2 = 13.643$, df = 5, $P < 0.0001$).

According to the analysis the *P. lilacinum* ESALQ1744 had a higher nematode population than ESALQ5406 and ESALQ2593 only (*P* = 0.0223).

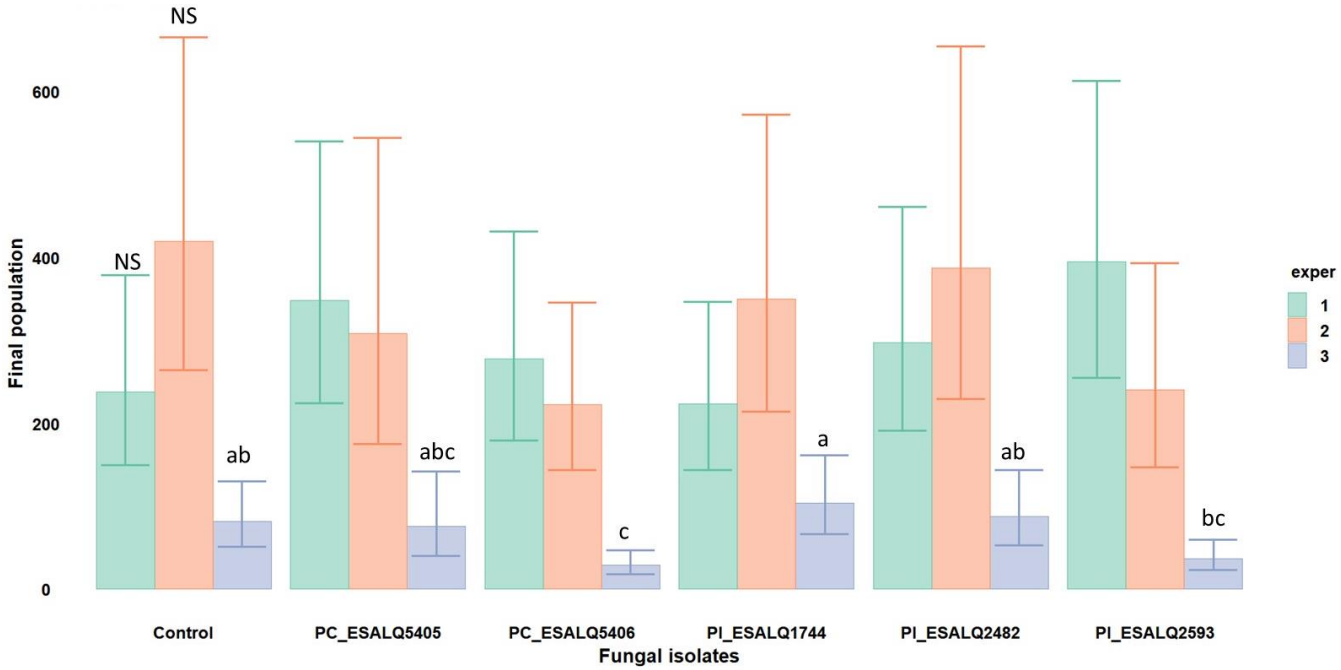

Figure 9. The final population of the soybean cyst nematode (*Heterodera glycines*) challenged with seed-treated common bean plants with isolates of *Purpureocillium lilacinum* (P.l.) and *Pochonia chlamydosporia* (P.c.), after 45 days of growth under greenhouse conditions. Means with 95% confidence intervals (n = 10 plants per treatment and 3 independent experiments) followed by irrespective letters are significantly different from each other according to the Tukey HSD test (*P* < 0.05), while "NS" means no significance to the treatment effect within each experiment. Eggs in diapause were considered unviable and then dropped from the analysis.

Meanwhile, regarding the data on nematodes per root gram (nem g^{-1}), there was a significant effect of treatments on nematode density (*F* = 2.46, df = 5, 132, *P* = 0.0364) (Figure 10). This result revealed that the fungal treatments *P. chlamydosporia* ESALQ5406 and *P. lilacinum* ESALQ2593 resulted in a lower nematode population per gram of roots in comparison with the control plants, only for the second greenhouse trial.

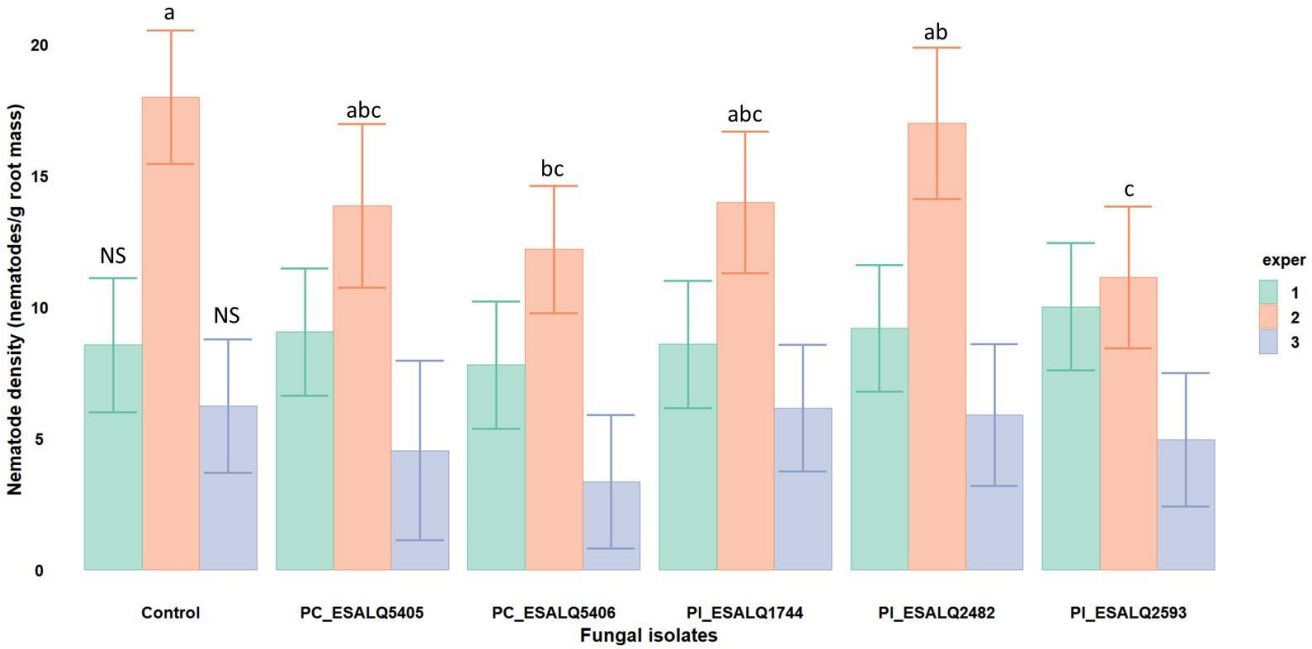

Figure 10. Soybean cyst nematode (*Heterodera glycines*) per root gram (based on fresh weight) challenged with seed-treated common bean plants with isolates of *Purpureocillium lilacinum* (P.l.) and *Pochonia chlamydosporia* (P.c.). After 45 days of growth under greenhouse conditions. Means with 95% confidence intervals (n = 10 plants per treatment and 3 independent experiments) followed by irrespective letters are significantly different from each other according to the Tukey HSD test ($P < 0.05$), while “NS” means no significance to the treatment effect within each experiment. Eggs in diapause were considered unviable and then dropped from the analysis.

5. DISCUSSION

This study showed for the first time the high production of submerged propagules of *P. lilacinum* and *P. chlamydosporia*. Thus, this opens a new opportunity to explore these propagules in plant protection and growth promotion and perhaps replace the traditional aerial conidia as the active ingredient of fungal biopesticides. Nevertheless, further studies should be conducted to discriminate against those propagules and to evaluate their virulence against pests. Microesclerotia is pigmented resistance structures that are produced when submitted to stress conditions (JARONSKI & JACKSON, 2008). The formation of these structures has been intensively studied, especially with *Beauveria* and *Metarhizium* being the focus (JARONSKI, 2013; ZHOU et al., 2015; HUARTE-BONNER et al., 2019). However, these structures have never been observed *in vivo* conditions, only *in vitro*, in a liquid medium capable to induce the production (JARONSKI & JACKSON, 2009). Microesclerotia is an important structure for field application of fungi since they can resist low humidity (GARCIA et al., 2011), more resistant to ultraviolet radiation (FARGUES et al., 1997) and higher temperatures (MEYLING & EILENBERG, 2007). In the current study, the production of microsclerotia by *Purpureocillium lilacinum* and *Pochonia chlamydosporia* was explored by submerging fermentation. The data obtained in the experiments indicate that the highest MS production occurred under a high concentration of carbon, with the C: N ratio of 50:1 (Jackson 6 medium). It is known from other studies that media with a high concentration of carbon (> 25 g l^{-1}) and appropriate amounts of nitrogen induce the production of MS, which leads to an increased concentration of aerial conidia (JACKSON, 1997; KOBORI et al., 2015). Jackson & Schisler (1995) and Jaronski & Jackson (2009) observed that the depletion of nitrogen regulates the aggregation of hyphae for the formation of MS, while the consumption of carbon induces the melanization process, confirming the results obtained in this work, wherein the medium with a higher C: N ratio, the production of MS was increased. In the media where the C: N ratio was lower, the MS had more melanized aspects (culture media Jackson 4 – 10:1 – and modified Adamek), although a lower concentration was obtained. Song et al. (2014) observed that, when submitting *Metarhizium rileyi* to a liquid culture media with a low (or non-existing) concentration of carbon and high of nitrogen, there was no formation of MS, suggesting that the

combination of the two nutrients is necessary, and it promotes nutritional stress in the fungus, thus inducing the formation of MS.

Blastospores are yeast-like cells that can be produced from hyphae in the arthropods hemolymph (FENG et al., 1994), and submerged conidia are similar to aerial conidia except for the physicochemical characteristic (CHO et al., 2006). Both propagules can be produced in high concentrations in rich liquid medium. These types of cells are interesting because they germinate faster (CHO et al., 2006; JARONSKI & MASCARIN, 2017) than aerial conidia, shortening the time of infection and mortality of pest arthropods. The production of these structures has already been reported in several genera of fungi, e.g. *Metarhizium* (ISSALY et al., 2005; YPSILOS & MAGAN, 2005; INGLIS et al., 2006; BERNARDO et al., 2018; CAROLINO et al., 2019), *Beauveria* (MASCARIN et al., 2015a, 2015b; BERNARDO et al., 2018), *Cordyceps* (VIDAL et al., 1998; ASAFF et al., 2006; MASCARIN et al., 2010; MASCARIN et al., 2015b), *Hirsutella* (ROMERO-RANGEL et al., 2012) and *Trichoderma* (JAKUBÍAKOVÁ et al., 2006; SIMKOVIC et al., 2008; KOBORI et al., 2015). However, the production of these structures by *P. lilacinum* and *P. chlamydosporia* has yet to be reported. In this study, it was observed the production of blastospores-like and submerged conidia-like cells in the modified Adamek medium for the first time. This liquid medium promotes the formation of submerged propagules and intense biomass formation (KASSA et al., 2004). The production of submerged conidia and blastospores can be related to the available concentrations of carbon and nitrogen (BIDOCHKA et al, 1987; HEGEDUS et al., 1990), characteristics present in high concentrations in the medium used. Twenty-two of the forty isolates yielded blastospores-like and submerged conidia-like above 10^9 submerged propagules ml^{-1} with only four days of fermentation, meaning high concentrations in a short time, compared to other studies: Romero-Rangel et al. (2012) obtained $3.8x10^7$ blastospores ml^{-1} of *Hirsutella* sp. with a long incubation period (seven days); Jackson et al. (2003) reported the production of $1x10^9$ blastospores ml^{-1} for *Cordyceps fumosorosea* with four days of growth, changing only the nitrogen source; and study of Jackson et al. (1997), the maximum concentration of blastospores of *C. fumosorosea* was $6.8x10^8$ blastospores ml^{-1}, also with four days of growth. The manipulation of the liquid medium is an essential factor to obtain high concentrations of the desired propagule, in a short time and with the ability to resist the stabilization process (JARONSKI & MASCARIN, 2017). In just two days of growth,

Iwanicki et al. (2018) obtained a concentration of 5.8 x 10^8 blastospores ml^{-1} for an isolate of *M. robertsii*, which remained with high viability after the drying process.

As for the production of submerged conidia, Kobori et al. (2015) obtained a maximum concentration of 9.5 x 10^8 submerged conidia ml^{-1} for *Trichoderma harzianum* after seven days of growth. The production of this type of propagules is highly related to the limitation or depletion of nitrogen sources present in the environment, and although the carbon source is not an extremely essential factor for the formation of these structures, the presence of glucose helps in the swelling, accelerating the germination process and resulting in the production of submerged conidia (JUNG et al., 2014). Even though, in the present study it was not possible to discriminate between submerged conidia and blastospores, both propagules are produced by *P. lilacinum* and *P. chlamydosporia* in the modified Adamek medium. Further studies, involving physicochemical and morphological analyses, are required to quantify each of these propagules.

The low results observed for endophytic colonization of *P. lilacinum* and *P. chlamydosporia* in *P. vulgaris* may have been due to the effect of abiotic (low temperature) and biotic factors. The use of more efficient detection methods such as Green fluorescent protein (GFP) or PCR methodologies could improve the recovery rate. Similarly, Mutune et al. (2019) were unsuccessful in detecting *M. anisopliae* and *B. bassiana* in bean plants (seeds treated with fungal conidia), though, they observed a high rate of reduction in the population of *Ophiomyia phaseoli*. Akutse et al. (2013) failed to detect the colonization of *Metarhizium* spp. on *Vicia faba* and *P. vulgaris*. The rate of recovery of fungi in host plants, whether by treating seed or soil, is closely related to the fungal species inoculated and the host, and it decreases over time (BROWNBRIDGE et al., 2012; AKUTSE et al., 2013; RUSSO et al., 2015; PARSA et al., 2016). Although the observation of the colonization failed, a successful reduction of populations of the two-spotted mite and cyst nematode was noticed, thus a hypothesis could be proposed: *P. lilacinum* and *P. chlamydosporia* could be inducing the plant to invest more in defense than promoting its growth, a process named ‘trade-off’ (COLEY & BARONE, 1996). The trade-off strategy could be explained as the plant allocates more resources to a specific function, in detriment of others (BRIGGS & SCHILTZ, 1990; HERMS & MATTSON, 1992; WEINER et al., 2009). A trade-off was reported by Richmond et al. (2003) when *Neotyphodium lolli* colonized *Lolium perenne*

and *Digitaria sanquinalis*: the authors showes that the latter species allocated a higher proportion of resources for growth when competing with the former.

In this study, common bean plants inoculated with *P. lilacinum* ESALQ1744, ESALQ2482 and ESALQ2593, and *P. chlamydosporia* ESALQ5405 and ESALQ5406 significantly reduced *T. urticae* population fecundity. In a recent study, Canassa et al. (2019) observed negative effects on *T. urticae* population growth when *P. vulgaris* seeds were inoculated with *M. robertsii*, *B. bassiana* and a combination of both entomopathogenic fungi; it was reported significant population reduction, although there were no differences between fungal treatments, applied either individually or combined. After inoculating common bean seeds with *B. bassiana*, *Lecanicillium lecanii,* and *C. fumosorosea*, Dash et al. (2018) reported a decrease in population growth and reproduction of *T. urticae*. Endophytic fungi colonized *Vicia faba* (*Beauveria* spp., *Hypocrea* spp., *Gibberella* spp., *Metarhizium* spp., *Trichoderma* spp. and *Fusarium* spp.) and were capable of causing 100% mortality in adults of *Liriomyza huidobrensis*, and the isolates of *Beauveria* spp. and *Hypocrea* spp. stood out as the most virulent against this insect pest (AKSTUSE et al., 2013). Seed treatment of *P. vulgaris* with *B. bassiana*, *Hypocrea lixii*, *M. anisopliae*, *T. asperellum,* and *T. atroviride* negatively affected the oviposition of *O. phaseoli* (MUTUNE et al., 2016). Treatment of cotton seeds with *P. lilacinum* and *B. bassiana* revealed negative effects on the survival of *Helicoverpa zea* through the suppression of leaf-feeding by the caterpillars (LOPEZ & SWORD, 2015).

For nematode bioassays, in this study, the plants treated with *P. lilacinum* and *P. chlamydosporia* showed variable results between the replicates. Despite these variable results, it was possible to observe a reduction in the population density of *H. glycines* only for some isolates in two out of three replicates*. P. lilacinum* was capable to suppress *Pratylenchus thornei* and further improved dry weights of shoots and roots of *Triticum* spp. (KEPENEKCI et al., 2018b). In *Abelmoschus* spp., the bacterium *Pasteuria penetrans* and *P. lilacinum* were effective in reducing the number of galls, egg masses, and nematode fecundity of *M. incognita*, resulting in a pronounced antagonism against the root-knot nematode (MUKHTAR et al., 2013). Tomato plants colonized by *P. chlamydosporia* presented the colonization of the fungi into galls and egg masses of *M. javanica* by the endophytic pathways (ESCUDERO & LOPEZ-LLORCA, 2012). Similar results were recently obtained by Ghahremani et al. (2019), in which *P. chlamydosporia* was able to reduce the reproduction, female fecundity, and

the infection by *M. incognita* on tomato plants. These authors also observed systemic resistance against the root-knot nematode triggered by the fungus through an induced expression of salicylic acid.

The result of the symbiotic relationship that endophytic fungi establish with their hosts can be demonstrated in several ways, such as growth promotion (PARSA et al., 2016; RUSSO et al., 2018), nitrogen and carbon exchange (PARNISKE, 2008) and even repellency to pests, such as herbivorous insects, nematodes and phytopathogenic fungi (SCHARDL et al., 2004; JABER & OWNLEY, 2018), i.e., the fungi modify the plant fitness to increase stress tolerance, disease resistance and decrease herbivory (WHITHE et al., 2010; VIDAL & JABER, 2015). In *Hordeum vulgare* plants, *P. chlamydosporia* modifies the plant transcriptome, as it can induce the encoding of genes in the heat shock proteins (HSP) family, which are responsible to turn on various stress responses in the roots (LARRIBA et al., 2015). Proteins belonging to the HSP family are expressed when the plant senses unfavorable biotic and abiotic conditions, such as temperature changes, presence of pathogens or pests (SCHÖFFL et al., 1999; WANG et al., 2003; AL-WHAIBI, 2011). The colonization of *P. chlamydosporia* in barley (*H. vulgare*) and tomato plants (*Solanum lycopersicum*) induced the metabolism of jasmonic acid (JA), salicylic acid and the activation of effector-triggered immunity (ETI) and pattern-triggered immunity (PTI), increasing the defense response and the resistance to plant diseases (BOLLER & FELIX, 2009; DODDS & RATHJEN, 2010; LARRIBA et al., 2015; GHAHREMANI et al. 2019). The jasmonic acid and salicylic acid are known to mediate pathways that are related to plant resistance, inducing plant responses against external damage and pathogen infection (BARROS et al., 2010; RUAN et al., 2019). Escudero & Lopez-llorca (2012) suggested that the defence system manipulation by fungi (*Pochonia* or *Purpureocillium*) suppresses the development and reproduction of nematodes, although no studies proved the systemic resistance (GHAHREMANI et al., 2019).

The mechanisms that induce these negative effects of endophytic fungi against pests are still unclear (WHITHE et al., 2010). However, studies indicate that this relationship leads to the production of secondary metabolites, production of superoxides (reactive oxygen radicals), change of the phytosterol profile *in planta*, or even an indirect effect translated by an induced systemic defense in plants. Alkaloids produced can affect neurotropic activities and feeding (being deterrent) of insects, and reduce parasitism of nematodes, while having an anti-nematode activity (SCHARDL et

al., 2004, 2007). The data obtained in this study raised the hypothesis that the production of secondary compounds could be affecting *T. urticae* females during oviposition and hatching of eggs, which resulted in lower eggs laid on the fungal treatments compared to the control.

Common bean seeds coated with *P. lilacinum* and *P. chlamydosporia* resulted in the reduction of oviposition of the two-spotted spider mite *T. urticae*. The use of endophytic fungi could be a new approach to integrated pest management due to their potential in improving crop protection and production through different mechanisms (MORAGA, 2020). Although it was not possible to determine the colonization on *P. vulgaris* using selective culture medium, the use of other techniques, such as PCR and GLP, could be useful to identify the fungi on plants. These results bring a new perspective for the use of nematophagous fungi in seed treatment that might enhance plant protection and sanity.

REFERENCES

ADÁMEK, L. Submerse cultivation of the fungus *Metarhizium anisopliae* (Metsch.). **Folia Microbiologica,** v. 10, n. 4, p. 255-257, 1965.

AGRIOS, G. N. **Plant Pathology**. Academic Press, 5th Edition. 952p, 2005.

AGROFIT. Ministério da Agricultura, Pecuária e Abastecimento. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed 17 January 2020), 2020.

AGROLINK. **Ácaro – rajado.** https://www.agrolink.com.br/problemas/acaro-rajado_126.html. (Accessed 02 January 2020), 2020.

AGUIAR-MENEZES, E. L., AQUINO, A. M., CORREIA, M. E. F., MENEZES, E. B. **Ácaros: taxonomia, bioecologia e sua importância agrícola.** Embrapa Agrobiologia, 1ª Edição, 24p, 2007.

AKTUSE, K. S., MANIANIA, N. K., FIABOE, K. K. M., VAN DEN BERG, J., EKESI, S. Endophytic colonization of *Vicia faba* and *Phaseolus vulgaris* (Fabaceae) by fungal pathogens and their effects on the life-history parameters of *Liriomyza huidobrensis* (Diptera: Agromyzidae). **Fungal Ecology**, v. 6, p. 293-301, 2013.

ALVES, S. B. (Ed.). **Controle microbiano de insetos.** 2° edição. FEALQ, Piracicaba. 1163 p. 1998.

AL-WHAIBI, M. H. Plant heat-shock proteins: A mini-review. **Journal of King Saud University – Science**, v. 23, p. 139-150, 2011.

ANGELO, I. C., FERNANDES, E. K. K., BAHIENSE, T. C., PERINOTTO, W. M. S., GOLO, P. S., MORAES, A. P. R., BITTENCOURT, V. R. E. Virulence of *Isaria* sp. and *Purpureocillium lilacinum* to *Rhipicephalus microplus* tick under laboratory conditions. **Parasito Research,** v. 111, p. 1473-1480, 2012.

ARANDA-MARTINEZ, A., GRIFOLL-ROMERO, L., ARAGUNDE, H., SANCHO-VAELLO, E., BIARNÉS, X., LOPEZ-LLORCA, L. V., PLANAS, A. Expression and specificity of chitin deacetylase from the nematophagous fungus *Pochonia chlamydosporia* potentially involved in pathogenicity. **Scientific Reports**, v. 8, n. 2170, p. 1-12, 2012.

ARAÚJO, J. M., ARAÚJO, J. V., BRAGA, F. R., CARVALHO, R. O., SILVA, A. R., CAMPOS, A. K. Interaction and ovicidal activity of nematophagous fungus *Pochonia chlamydosporia* on *Taenia saginata* eggs. **Experimental Parasitology**, v. 121, p. 338–341, 2009.

ASAFF, A., CERDA-GARCÍA-ROJAS, C. M., VINIEGRA-GONZÁLEZ, G., TORRE, M. Carbon distribution and redirection of metabolism in *Paecilomyces fumosoroseus* during solid-state and liquid fermentations. **Process Biochemistry**, v. 41, p. 1303-1310, 2006.

ATIKINS, S. D., HIDALGO-DIAZ, L., KALISZ, H., MAUCHLINE, T. H., HIRSCH, P. R., KERRY, B. R. Development of a new management strategy for the control of root-knot nematodes (*Meloidogyne* spp.) in organic vegetable production. **Pest Management Science**, v. 59, p. 183-189, 2003.

ATKINS, S. D. CLARK, I. M., PANDE, S., HIRSCH, P. R., KERRY, B. R. The use of real-time PCR and species-specific primers for the identification and monitoring of *Paecilomyces lilacinus*. **FEMS Microbiology Ecology**, v. 51, p. 257-264, 2005.

ATTIA, S., GRISSA, K. L., LOGNAY, G., BITUME, E., HANCE, T., MAILLEUX, A. C. A review of the major biological approaches to control the worldwide pest *Tetranychus urticae* (Acari: Tetranychidae) with special reference to natural pesticides. **Journal of Pest Science**, v. 86, p. 361-386, 2013.

AZCÓN-AGUILAR, C., BAREA, J. M. Applying mycorrhiza biotechnology to horticulture: significance and potentials. **Scientia Horticulturae**, v. 68, p. 1-24, 1997.

BAINIER, G. Mycothéque de l'École de Pharmacie. XI. *Paecilomyces*, genre nouveau de Mucédinées. **Bulletin de la Societé Mycologique de France.**, v.23, p. 26-27, 1907.

BARBOSA, T. dos S., ANDRADE, D. J. de., POLANCZYK, R. A., DUARTE, R. T. Susceptibility of *Tetranychus ogmophallos* (Acari: Tetranychidae) to *Beauveria bassiana* and *Metarhizium anisopliae*. **Florida Entomologist,** v. 101, n. 2, p. 249-253, 2018.

BARRA, P., ETCHEVERRY, M., NESCI, A. Efficacy of 2, 6-di (t-butyl)-p-cresol (BHT) and the entomopathogenic fungus *Purpureocillium lilacinum*, to control *Tribolium confusum* and to reduce aflatoxin B1 in store maize. **Journal of stored products research**, v. 64, p. 72-79, 2015.

BARROS, F., SAGATA, E., FERREIRA, L. C. C., JULIATTI, F. C. Indução de resistência em plantas contra fitopatógenos. **Biosci. Journal**, v. 26, n. 2, p.231-239, 2010.

BATISTA, A. C., FONSECA, O. M. *Pochonia humicola* n. gen. E n. sp., uma ‘curiosa entidade fúngica dos solos do Nordeste do Brasil. **Publ. Inst. Micol**, Recife, v. 462, n. 5, p. 1-11. 1965.

BEHIE, S. W., BIDOCHKA, M. J. Ubiquity of insect-derived nitrogen transfer to plants by endophytic insect-pathogenic fungi: an additional branch of the soil nitrogen cycle. **Applied and Environmental Microbiology**, v. 80, n. 5, p. 1553-1560, 2014.

BEHIE, S. W., JONES, S. J., BIDOCHKA, M. J. Plant tissue localization of the endophytic insect pathogenic fungi *Metarhizium* and *Beauveria*. **Fungal Ecology** 13, 112-119, 2015.

BEHIE, S. W., ZELISKO, P. M., BIDOCHKA, M. J. Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. **Science**, v. 336, p. 1576-1577, 2012.

BERNARDO, C. C., BARRETO, L. P., SILVA, C. S. R., LUZ, C., ARRUDA, W., FERNANDES, E. K. K. Conidia and blastopores of *Metarhizium* spp. and *Beauveria bassiana* s.l.: Their development during the infection process and virulence against the tick *Rhipicephalus microplus*. **Ticks and Tick-borne Disease**, v. 9, p. 1334-1342, 2018.

BIDOCHKA, M. J., PFEIFER, T. A., KHACHATOURIANS, G. G. Development of the entomopathogenic fungus *Beauveria bassiana* in liquid cultures. **Mycopathologia**, v.99, p. 77-83, 1987.

BISCHOFF, J. F., REHNER, S. A., HUMBER, R. A. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. **Mycologia**, v. 101, v. 4, p. 512-530, 2009.

BOLLER, T., FELIZ, G. A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. **Annual Review of Plant Biology**, v. 60, p. 379-406, 2009.

BONFANTE, P., GENRE, A. Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. **Nature communications,** p. 1-48, 2010.

BORDALLO, J. J., LOPEZ-LLORCA, L. V., JANSSON, H. B., SALINAS, J., PERSMARK, L., ASENSIO, L. Colonization of plant roots by egg-parasitic and nematode-trapping fungi. **New Phytologist**, v. 154, p. 491-499, 2002.

BORGES, H. D., HUSSEY, N. W. **Microbial control of insects and mites.** New York, London: Academic Press, 861p, 1971.

BORSARI, A. P., CLAUDINO, M. Mercado e Percepção do produtor brasileiro. ABCBio – Associação Brasileira das Empresas de Controle Biológico, Agroanalysis. https://www.abcbio.org.br/informativos/publicacoes (Accessed 10 October 2018), 2018.

BOTTON, M., NAVA, D. E. AGEITEC – Agência Embrapa de Informação Tecnológica. www.agencia.cnptia.embrapa.br/gestor/morango/arvore/CONT000fmxotm4c02wyiv8065610drz016oe.html#. (accessed 16 July 2019). 2019.

BRAGA, F. R., ARAÚJO, J. V., CAMPOS, A. K., CARVALHO, R. O., SILVA, A. R., TAVELA, A. O., MACIEL, A. S. Observação *in vitro* da ação dos isolados fúngicos *Duddingtonia flagrans, Monacrosporium thaumasium* e *Verticillium chlamydosporium* sobre ovos de *Ascaris lumbricoides* (Linnaeus, 1758). **Revista da Sociedade Brasileira de Medicina Tropical**, v. 40, n. 3, p. 356-358, 2007.

BRAGA, F. R., ARAÚJO, J. V., CARVALHO, R. O., SILVA, A. R., ARAUJO, J. M., TAVELA, A. O., CAMPOS, A. K. Ovicidal effect to nematophagous fungi on *Taenia taeniaeformis* eggs. **World Journal of Microbiology and Biotechnology**, v. 25, p. 533–535, 2009.

BRAGA, F. R., ARAÚJO, J. V., SILVA, A. R., ARAUJO, J. M., CARVALHO, R. O., CAMPOS, A. K., ALVES, C. D. F. *Duddingtonia flagrans*, *Monacrosporium thaumasium* and *Pochonia chlamydosporia* as possible biological control agents of *Oxyuris equi* and *Austroxyuris finlaysoni*. **Journal of Helminthology**, v. 84, p. 21–25, 2010.

BRITO, D. Mercado de biodefensivos cresce mais de 70% no Brasil em um ano. **Ministério da Agricultura, Pecuária e Abastecimento.** www.agricultura.gov.br/noticias/feffmercado-de-biodefensivos-cresce-em-mais-de-50-no-brasil (Accessed 03 January 2020), 2019.

BRIGGS, M. A., SCHULTZ, J. C. Chemical defense production in *Lotus corniculatus* L. II. Trade-offs among growth, reproduction and defense. **Oecologia**, v. 83, p. 32-37, 1990.

BROWN, A. H. S., SMITH, G. The genus *Paecilomyces* Bainier and its perfect stage *Byssochlamys* westling. **Transactions of the British mycological Society**, v. 40, p. 17-89, 1957.

BROWNBRIDGE, M., REAY, S. D., NELSON, T. L., GLARE, T. R. Persistence of *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte following inoculation of radiata pine seed and seedlings. **Biological Control**, v. 61, p. 194-200, 2012.

CANASSA, F., TALL, S., MORAL, R. A., LARA, I. A. R., DELALIBERA, I., MEYLING, N. V. Effects of bean seed treatment by the entomopathogenic fungi *Metarhizium robertsii* and *Beauveria bassiana* on plant growth, spider mite population and behavior of predatory mites. **Biological Control**, v. 132, p. 199-208, 2019.

CAROLINO, A. T., GOMES, S. A., TEODORO, T. B. P., MATTOSO, T. C., SAMUELS, R. I. **Journal of Pure and Applied Microbiology**, v. 13, n. 3, p. 1629-1634, 2019.

CARVALHO, R. da S. Biocontrole de moscas-das-frutas: histórico, conceitos e estratégias. **Cruz das Almas: Embrapa Mandioca e Fruticultura Tropical**, v. 7, n. 3, p. 14-18, 2006.

CASTILLO-LOPEZ, D., SWORD, G. A. The endophytic fungal entomopathogens *Beauveria bassiana* and *Purpureocillium lilacinum* enhance the growth of cultivated cotton (*Gossypium hirsutum*) and negatively affect survival of the cotton bollworm (*Helicoverpa zea*). **Biological Control**, v. 89, p. 53-60, 2015.

CHO, E. M., LIU, L., FARMERIE, W., KEYHANI, N. O. EST analysis of cDNA libraries from entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana*. I. Evidence for stage-specific gene expression in aerial conidia, *in vitro* blastospores and submerged conidia. **Microbiology**, v. 152, p. 2843-2854, 2006.

CONAB - Companhia Nacional de Abastecimento. https://www.conab.gov.br/ (accessed 03 January 2020), 2020.

COOLEN, W. A., D'HERDE, C. J. **A method for the quantitative extraction of nematodes from plant tissue**. State Agriculture Research Center – GHENT, Belgium., p. 77, 1972.

COLEY, P. D., BARONE, J. A. Herbivory and Plant defenses in tropical forests. **Annul. Rev. Ecol. Syst.** v. 27, p. 305-335, 1996.

KASUYA, M. C. M., FERRAZ, S. *Pochonia chlamydosporia* promotes the growth of tomato and lettuce plants. **Acta Scientiarum Agronomy**, v. 37, N. 4, p. 417-423, 2015.

DASH, C. K., BAMISILE, B. S., RAVINDRAN, K., QASIM, M., LIN, Y., ISLAM, S. U., HUSSAIN, M., WANG, L. Endophytic entomopathogenic fungi enhance the growth of *Phaseolus vulgaris* L. (Fabaceae) and negatively affect the development and reproduction of *Tetranychus urticae* Koch (Acari: Tetranychidae). **Microbial Pathogenesis**, v. 125, p. 385-392, 2018.

DE BARY, A. **Morphologie und Physiologie der Pilze, Flechten und Myxomyceten**. Hofmeister's Handbook of physiological Botany, Vol. 2, Leipzig: W.Engelmann, 1866.

DELLAPORTA, S. L., WOOD, J., HICKS, J. B. A plant DNA mini preparation: Version II. **Plant Molecular Biology Reporter,** v. 1, I. 4, pp. 19-21, 1983.

DIAS, A. S., ARAÚJO, J. V., BRAGA, F. R., ARAUJO, J. M., PUPPIN, A. C., FERNANDES, F. M., PERBONI, W. R. Biological control of *Fasciola hepatica* eggs with the *Pochonia chlamydosporia* fungus after passing through the cattle gastrointestinal tract. **Parasitology Research**, v. 110, p. 663–667, 2012.

DIAS, W. P., GARCIA, A., SILVA, J. F. V., CARNEIRO, G. E. S. **Nematoides em soja: identificação e controle.** Circular Técnica 76, Embrapa Soja, 8p, 2010.

DIAS-ARIEIRA, C. R., SANTANA, S. M., DE FREITAS, L. G., DA CUNHA, T. P. L., BIELA, F., PUERARI, H. H., CHIAMOLERA, F. M. Efficiency of *Pochonia chlamydosporia* in *Meloidogyne incognita* control in lettuce crop (*Lactuca sativa* L.). J. **Food Agric. Environ.** v. 9, p. 561–563, 2011.

DIAS-SILVEIRA, M. F., HERRERA, J. O. An overview of nematological problems in Cuba. **Nematologica,** v. 28, p. 151-164, 1998.

DODD, S. L., LIECKFELDT, E., CHAVERRI, P., OVERTON, B. E., SAMUELS, G. J. Taxonomy and phylogenetic relationships of two species of Taxonomy and phylogenetic relationships of two species of *Hypocreales* with *Trichoderma* anamorphs. **Mycological Progress,** v. 1, n. 4, p. 409-428, 2002.

DOODS, P. N., RATHJEN, J. P. Plant immunity: towards an integrated view of plant-pathogen interactions. **Nature Reviews: Genetics**, v. 11, p. 539-540, 2010.

EBADI, M., FATEMY, S., RIAHI, H. Evaluation of *Pochonia chlamydosporia var. chlamydosporia* as a control agent of *Meloidogyne javanica* on pistachio. **Biocontrol Sci. Technol**. V. 19, p. 689–700, 2009.

EBERHARD, W., PACHECO-ESQUIVEL, J., CARRASCO-RUEDA, F., CHRISTOPHER, Y., GONZALES, C., RAMOS, D., URBINA, H., BLACKWELL, M. Zombie bugs? The fungus *Purpureocillium Lilacinum* may manipulate the behaviour of its host *Edessa rufomarginata.* **Mycologia,** v. 106, n. 6, p. 1065-1072, 2017.

EILENBERG, J., HAJEK, A., LOMER, C. Suggestions for unifying the terminology in biological control. **BioControl**, v. 46, p. 387-400, 2001.

ESCUDERO, N., LOPEZ-LLORCA, L. V., Effects on plant growth and root-knot nematode infection of an endophytic GFP transformant of the nematophagous fungus *Pochonia chlamydosporia*. **Symbiosis,** v. 57, p. 33–42, 2012.

ESSER, R. P., EL-GHOLL, N. E. *Paecilomyces lilacinus,* a fungus that parasitizes nematode eggs. **Florida Department of Agriculture,** Division of Plant Industry Nematology Circular 203, 1993.

FAOSTAT - Food and Agriculture Organization of the United Nations Statistics. http://faostat.org/ (accessed 17 January 2020), 2020.

FARGUES, J., ROUGIER, M., GOUJET, R., SMITS, N., COUSTERE, C., ITIER, B. Inactivation of conidia of *Paecilomyces fumosoroseus* by near-ultraviolet (UVB and UVA) and visible radiation. **Journal of Invertebrate Pathology**, v. 69, n. 1, p. 70-78, 1997.

FENG, M. G., POPRAWSKI, T. J., KHACHATOURIANS, G. G. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control worldwide. Biocontrol Science and Technology, Oxford, v. 4, n.3, p. 34. 1994.

FERRAZ, L. C. C. B., BROWN, D. J. F. **Nematologia de plantas: fundamentos e importância**. Manaus: Norma Editora, 251p, 2016.

FERREIRA, S. R., ARAÚJO, J. V., BRAGA, F. R., ARAUJO, J. M., CARVALHO, R. O., SILVA, A. R., FREITAS, L. G. Ovicidal activity of seven *Pochonia chlamydosporia* fungal isolates on *Ascaris suum* eggs. **Tropical Animal Health and Production**, 43, 639–642, 2011.

FIEDLER, Z., SOSNOWSKA, D. Nematophagous fungus *Paecilomyces lilacinus* (Thom) Samsonis also a biological agent for control of greenhouse insects and mite pests. **Biocontrol,** v.52**,** p. 547–558, 2007.

FREGONESI, A. F., MOCHI, D. A., MONTEIRO, A. C. Compatibilidade de isolados de *Beauveria bassiana* a inseticidas, herbicidas e maturadores em condições de laboratório. **Agricultural Entomology/ Scientificarticle,** v. 83, p. 1-8, 2016.

GAMS, W., ZARE, R. New generic concepts in *Verticillium* sect. Prostrata. **Mycol Res** v. 106, p.130–131, 2002.

GARCIA, J. E., POSADAS, J. B., PERTICARI, A., LECUONA, R. E. *Metarhizium anisopliae* (Metschnikoff) Sorokin promotes growth and has endophytic activity in tomato plants. **Advances in Biological Research**, v. 5, n. 1, p. 22-27, 2011.

GARRIDO, L. R., HOFFMANN, A., SILVEIRA, S. V. **Manejo de pragas e doenças.** Embrapa Uva e Vinho, 1ª Edição, 2015.

GBBIC, M., VAN LEEUWEN, T., CLARK, R. M., et al., 2011. The genome of *Tetranychus urticae* reveals herbivorous pest adaptions. **Nature**, v. 479, p. 487-492.

GHAHREMANI, Z., ESCUDERO, N., SAUS, E., GABALDÓN, T., SORRIBAS, F. J. *Pochonia chlamydosporia* induces plant-dependent systemic resistance to *Meloidogyne incognita*. **Front. Plan. Science**, v. 10, p. 1-8, 2019.

GLARE, T., CARADUS, J., GELERNTER, W., JACKSON, T., KEYHANI, N., KÖHL, J., MARRONE, P., MORIN, L., STEWART, A. Have biopesticides come of age? **Trends in Biotechnology,** v. 30, n. 5, p.250-258, 2012.

GLAZER, E. C. W., FILHO, W. S. de A., Utilização do *Bacillus thuringiensis* no controle biológico de pragas. **Revista Interdisciplinar de Ciências Aplicada – RICA,** v. 1, n. 1, p. 1-4, 2016.

GOFFRÉ, D., FOLGARAIT, P. J. *Purpureocillium lilacinum, a* potential agent for biological control of the leaf-cutting ant *Acromyrmex lundii.* **Journal of Invertebrate Pathology**, v. 130, p. 107-115, 2015.

GRAHAM, P. H., RANALLI, P. Common bean (*Phaseolus vulgaris* L.). **Field Crops Research**, v. 53, p. 131-146, 1997.

GRUNDLER, F. M. W., SOBCZAK, M., GOLINOWSKI, W. Formation of wall openings in root cell of *Arabidopsis thaliana* following infection by plant-parasitic nematode *Heterodera schachtii*. **European Journal of Plant Pathology**, v. 104, p. 545-551, 1998.

HAJJI, L., HLAOUA, W., REGAIEG, H., HORRIGUE-RAOUANI, N. Biocontrol potential of *Verticillium leptobactrum* and *Purpureocillium lilacinum* against *Meloidogyne javanica* and *Globodera pallid* on potato (*Solanum tuberosum*). **Am. J. Potato Res.** v. 94, p.178-183, 2017.

HALL, T. A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. **Nucl. Acids. Symp. Ser**. v.41, p.95-98, 1999.

HEGEDUS, D. D., BIDOCHKA, M. J., KHACHATOURIAN, G. G. *Beauveria bassiana* submerged conidia production in a defined medium containing chitin, two hexosamines or glucose. **Applied Microbiology and Biotechnology**, v. 33, p. 641-647, 1990.

HERMS, D. A., MATTSON, W. J. The Dilemma of Plants: To Grow or Defend. **The Quarterly Review of Biology**, v. 67, n. 3, p. 283-335, 1992.

HOTAKA, D., AMNUAYKANJANASIN, A., MAKETON, C., SIRITUTSOONTORN, S., MAKETON, M. Efficacy of *Purpureocillium lilacinum* CKPL-053 in controlling *Thrips palmi* (Thysanoptera: Thripidae) in orchid farms in Thailand. **Appl. Entomol. Zool.,** v. 50, p. 317-329, 2015.

HUARTE-BONNET, C., PAIXÃO, F. R. S., MASCARIN, G. M., SANTANA, M., FERNANDES, E. K. K., PEDRINI, N. The entomopathogenic fungus *Beauveria bassiana* produces microsclerotia-like pellets mediated by oxidative stress and peroxisome biogenesis. **Environmental Microbiology Reports**, 2019.

HUMBER, R. A. **Identification of entomopathogenic fungi.** In: Manual of Techniques in Invertebrate Pathology, USDA-ARS, 2012.

HUMPHREYS, A. M., MATEWELE, P., TRINCI, A. P., GILLESPIE, A. T. Effects of water activity on morphology, growth and blastospore production of *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces farinosus* in batch and fed-batch culture. **Mycological Research**, Cambridge, v. 92, n. 3, p. 257-264, 1989.

INGLIS, P. W., SARMENTO, R. B. C., GAVIÃO, C. F. C., FRAZÃO, H., VALADARES-INGLIS, C. A mutant of *Metarhizium anisopliae* var. *acridum* with enhanced submerged conidiation. **World Journal of Microbiology & Biotechnology**, v. 22, p. 1083-1085, 2006.

ISSALY, N., CHAUVEAU, H., AGLEVOR, F., FARGUES, J., DURAND, A. Influence of nutrient, pH and dissolved oxygen on the production of *Metarhizium flavoviride* Mf189 blastospores in submerged batch culture. **Process Biochemistry**, v. 40, p. 1425-1431, 2005.

IWANICKI, N. S., FERREIRA, B. O., MASCARIN, G. M., DELALIBERA, I. Modified Adamek's medium renders high yields of *Metarhizium robertsii* blastospores that are desiccation tolerant and infective to cattle-tick larvae. **Fungal Biology**, v. 122, p. 883-890, 2018.

JABA, L. R. ENKERLI, J. Effect of seed treatment duration on growth and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. **Biological control**, v. 103, p. 189-195, 2016.

JABER, L. R., OWNLEY, B. H. Can we use entomopathogenic fungi as endophytes for dual biological control of insects pests and plant pathogens? **Biological Control**, v. 116, p. 36-45, 2018.

JACKSON, M. A. Optimizing nutritional conditions for the liquid culture production of effective fungal biological control agents. **Journal of Industrial Microbiology Biotechnology**, v. 19, p. 180-187, 1997.

JACKSON, M. A., CLIQUET, S., ITEN, L. B. Media and Fermentation process for the rapid production of high concentrations of stable blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus*. **Biocontrol Science and Technology**, v. 13, n. 1, p. 23-33, 2003.

JACKSON, M. A., JARONSKI, S. T. Production of microsclerotia of the fungal entomopathogen *Metarhizium anisopliae* and their potential for use as a biocontrol agent for soil-inhabiting insects. **Mycological Research**, v. 113, p. 842-850, 2009.

JACKSON, M. A., SCHISLER, D. A. Liquid culture production of microsclerotia of *Colletotrichum truncatum* for use as bioherbicidal propagules. **Mycological Research**, v. 99, n.7, p. 879-884, 1995.

JACKSON, M. A., MCGUIRE, M. R., LACEY, L. A., WRAIGHT, S. P. 1997. Liquid culture production of desiccation-tolerant blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus*. **Mycol. Res.** v. 101, n. 1, p. 35-41, 1997.

JAKUBÍAKOVÁ, L., FARKAS, V., KOLAROVA, N., NEMCOVIC, M. Conidiation of *Trichoderma atroviride* isolate during submerge cultivation in a laboratory stirred-tank fermenter. **Folia Microbiol**, v. 51, p. 209-2013, 2006.

JARONSKI, S. T. Mass production of entomopathogenic fungi: state of the art. In: MORALES-RAMOS, J. A., ROJAS, M. G., SHAPIRO-ILAN, D. J. (Eds.). **Mass Production of Beneficial Organisms.** Elsevier Inc., Amsterdam, p. 357-415, 2013.

JARONSKI, S. T., JACKSON, M. A. Efficacy of *Metarhizium anisopliae* microesclerotial granules. **Biological Science and Technology**, v. 18, n. 8, p. 849-863, 2008.

JARONSKI, S. T., JACKSON, M. A. Mass production of entomopathogenic Hypocreales. In: Manual of techniques in insect pathology. Lacey L. **Academic Press,** London. 2° ed, p. 317-328. 2012.

JARONSKI, S. T., MASCARIN, G. M. Chapter 9: Mass production of fungal entomopathogens, 2017. IN: JARINSKI, S. T. **Mass Production of Entomopathogenic Fungi: State of the Art.** Elsevier Inc.

JATALA, P., KALTENBACH R., BOCANGEL, M. Biological control of *Meloidogyne incognita acrita* and *Globodera pallida* on potato. **Journal of Nematology**, v. 11, 1979.

JUNG, B., KIM, S., LEE, J. Mycrocycle conidiation in filamentous fungi. **Mycobiology**, v. 42, n. 1, p. 1-5, 2014.

KASSA, A., STEPHAN, D., VIDAL, S., ZIMMERMANN, G. Production and processing of *Metarhizium anisopliae* var. *acridum* submerged conidia for locust and grasshopper control. **Mycol. Res.** v. 108, n. 1, p. 93-100, 2004.

KEPENECKI, I., TULEK, A., ALKAN, M., HAZIR, S. Biological control potential of native entomopathogenic nematodes against the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) in Tukey. **Pakistan Journal of Zoology**, v. 45, n. 5, p. 1415-1422, 2013.

KEPENEKCI, I., HAZIR, S., OKSAL, E., LEWIS, E. E. Application methods of *Steinernema feltiae, Xenorhabdus bovienii* and *Purpureocillium lilacinum* to control root-knot nematodes in greenhouse tomato systems. **Crop Protection**, v. 108, p. 31-38, 2018a.

KEPENEKCI, I., OKSAL, E., SAGLAM, H. D., ATAY, T., TULEK, A., EVLICE, E. Identification of Turkish isolate of the Entomopathogenic fungi, *Purpureocillium lilacinum* (syn: *Paecilomyces lilacinus*) and its effect on potato pests, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) and *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) **Egyptian Journal of Biological Pest Control,** v. 25, n. 1, p. 121-127, 2015.

KEPENEKCI, I., TOKTAY, H., OKSAL, E., BOZBUGA, R., IMREN, M. Effect of *Purpureocillium lilacinum* on root lesion nematode, *Pratylenchus thornei*. **Journal of Agricultural Sciences**, v. 24, p. 323-328, 2018b.

KHAN, A., WILLIAMS, K. L., MOLLOY, M. P., NEVALAINEN, H. K. M. Purification and characterization of a serine protease and chitinases from *Paecilomyces lilacinus* and detection of chitinase activity on 2D gels. **Protein Expr. Purif**. V. 32, p. 210–220, 2003.

KIEWNICK, S., MENDOZA, A., SIKORA, R. A. Efficacy of *Paecilomyces lilacinus* strain 251 for biological control of the burrowing nematode *Radopholus similis*. **J. Nematol**. V. 36, p. 326–327, 2004.

KIEWNICK, S., SIKORA, R. A. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. **Biological Control,** v. 38, p. 179-187, 2006.

KIMURA, M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotides sequences. **Journal of Molecular Evolution**, v. 16, p. 11-120, 1980

KUMAR, S., STECHER, G., LI, M., KNYAZ, C., TAMURA, K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. **Molecular Biology and Evolution**, v. 35, p. 1547-1549, 2018.

KIRIGA, A. W., HAUKELAND, S., KARIUKI, G. M., COYNE, D. L., BEEK, N. V. Effect of *Trichoderma* spp. and *Purpureocillium lilacinum* on *Meloidogyne javanica* in commercial pineapple production in Kenya. **Biological Control**, v. 118, p. 27-32, 2018.

KOBORI, N. N., MASCARIN, G. M., JACKSON, M. A., SCHILSER, D. A. Liquid culture production of microsclerotia and submerged conidia by *Trichoderma harzianum* active against damping-off disease caused by *Rhizoctonia solani*. **Fungal Biology**, v. 119, p. 179-190, 2015.

KRELL, V., UNGER, S., JAKOBS-SCHOENWANDT, D., PATEL, A. V. Endophytic *Metarhizium brunneum* mitigates nutrient deficits in potato and improves plant productivity and vitality. **Fungal Ecology**, v. 34, p. 43-49, 2018.

LACEY, L. A., GRZYWACZ, D., SHAPIRO-ILAN, D. I., FRUTOS, R., BROWNBRIDGE, M., GOETTEL, M. S. Insect pathogens as biological control agents: Back to the future. **Journal of Invertebrate Pathology**, v. 132, p. 1-41, 2015.

LARRIBA, E., JAIME, M. D. L. A., CARBONELL-CABALLERO, J., CONESA, A., DOPAZO, J., NISLOW, C., MARTÍN-NIETO, J., LOPEZ-LLORCA, L. V. Sequencing and functional analysis of the genome of a nematode egg-parasitic fungus, *Pochonia chlamydosporia.* **Fungal Genetics and Biology**, v. 65, p. 680, 2014.

LARRIBA, E., JAIME, M. D. L. A., NISLOW, C., MARTÍN-NIETO, J., LOPEZ-LLORCA, L. V. Endophytic colonization of barley (*Hordeum vulgare*) roots by nematophagous fungus *Pochonia chlamydosporia* reveals plant growth promotion and a general defense and stress transcriptomic response. **Journal Plant Research**, v. 128, p. 665-678, 2015.

LENTH, R. V. Emmeans: Estimated marginal means, aka least-squares means [Computer software manual]. http://cran.project.org/package=emmeans (R package version 1.4.1), 2020.

LIMA, R. D., FERRAZ, S., SANTOS, J. M. Ocorrência de *Heterodera* sp. em soja no Triângulo Mineiro. **NematologiaBrasileira**, v. 16, p. 101:102, 1992.

LIN, R., LIU, C., SHEN, B., BAI, M., LING, J., CHEN, G., MAO, Z., CHENG, X., XIE, B. Analysis of the complete mitochondrial genome of *Pochonia chlamydosporia* suggests a close relationship to the invertebrate-pathogenic fungi in Hypocreales. **BMC Microbiology**, v. 15, n. 5, p. 2-15, 2015.

LIN, R., QIN, F., SHEN, B., et al. Genome and secretome analysis of *Pochonia chlamydosporia* into egg-parasitic mechanisms. **Scientific Reports**, v. 8, n. 1123, p. 1-16, 2018.

LOPEZ, D. C., SWORD, G. A. The endophytic fungal entomopathogens *Beauveria bassiana* and *Purpureocillium lilacinum* enhance the growth of cultivated cotton (*Gossypium hirsutum*) and negatively affect survival of the cotton bollworm (*Helicoverpa zea).* **Biological control,** v. 89, p. 53-60, 2015.

LOPEZ-LLORCA L. V., BOAG B. Biological properties of a red pigment produced by the nematophagous fungus *Verticillium suchlasporium*. **Nematol medit**, v. 21, p. 143–149, 1993.

LOPEZ-LLORCA, L. V., ROBERTSON, W. M., Immunocytochemical localization of a 32-kDa protease from the nematophagous fungus *Verticillium suchlasporium* infected nematode eggs. **Exp. Mycol**. V. 16, p. 261–267, 1992.

LOPEZ-LLORCA, L.V., OLIVARES-BERNABEU, C., SALINAS, J., JANSSON, H. B., KOLATTUKUDY, P. E. Pre-penetration events in fungal parasitism of nematode eggs. **Mycol. Res**. v. 106, p. 499–506, 2002.

LORDELLO, A. I. L., LORDELLO, R. R. A., QUAGGIO, J. A. *Heterodera* sp. reduz a produção de soja no Brasil. **Nematologia Brasileira**, v. 16, p. 101, 1992.

LOURENÇÃO, A. L., MORAES, G. J., PASSOS, F. A., AMBROSANO, G. M. B., SILVA, L. V. F. Resistência de morangueiros a *Tetranychus urticae* Koch (Acari: Tetranychidae). **Anais da Sociedade Entomológica do Brasil,** v. 29, n. 2, 2000.

LUANGSA-ARD, J., HOUBRAKEN, J., van DOORN, T., HONG, S. B., BORMAN, A. M., HYWEL-JONES, N. L., SAMSON, R. A. *Purpureocillium,* a new genus for the medically important *Paecilomyces lilacinus*. **Federation of Europe microbiological societies,** v. 321, p. 141-149, 2011.

LUC, M., SIKORA, R. A., BRIDGE, J. **Plant parasitic nematodes in subtropical and tropical agriculture**, second ed. CABI Publishing, Cambridge, 2000.

MACIÁ-VICENTE, J. G., JANSSON, H. B., TALBOT, N. J., LOPEZ-LLORCA, L. V. Real-time PCR quantification and live-cell imaging of endophytic colonization of barley (*Hordeum vulgare*) roots by *Fusarium equiseti* and *Pochonia chlamydosporia*. **New Phytologist**, v. 182, p. 213-228, 2009b.

MACIÁ-VICENTE, J. G., ROSSO, L. C., CIANCIO, A., JANSSON, H. B., LOPEZ-LLORCA, L. V. Colonization of barley roots by endophytic *Fusarium equiseti* and *Pochonia chlamydosporia*: effects on plant growth and disease. **Annual Appl. Biology**, v. 155, p. 391-401, 2009a.

MANTZOUKAS, S., CHONDROGIANNIS, C., GRAMMATIKOPOULOS, G. Effects of three endophytic entomopathogens on sweet sorghum and on the larvae of the stalk borer *Sesamia nonagrioides.* **Entomologia Experimentaliser Applicata**, v. 154, p. 78-87, 2015.

MANZANILLA-LÓPEZ, R. H., ESTEVES, I., FINETTI-SIALER, M. M., HIRSCH, P. R., WARD, E., DEVONSHIRE, J., HIDALGO-DÍAZ, L. *Pochonia chlamydosporia*: Advances and Challenges to improve its performance as a biological control agent of sedentary ento-parasitic nematodes. **Journal of Nematology**, v. 45, n. 1, p. 1-7, 2013.

MANZANILLA-LÓPEZ, R., ESTEVES, I., DEVONSHIRE, J. Biology and Management of *Pochonia chlamydosporia* and Plant-Parasitic nematodes. In: **Perspectives in Sustainable nematode management through *Pochonia chlamydosporia* applications for root and rhizosphere health**. Springer International Publishing, p. 47-76, 2017.

MARTINS, F. A. A. ***Beauveria bassiana* e acaricidas sintéticos avaliados em condições *in vitro* recomendados para a cultura do mamoeiro no controle de *Tetranychus urticae* Koch.** 2016, 98 f. Dissertação (Mestrado) – Programa de Pós-Graduação em Horticultura Tropical, do Centro de Ciências e Tecnologia Agroalimentar, Pombal, 2016.

MASCARIN, G. M., ALVES, S. B., LOPES, R. B. Culture media selection for mass production of *Isaria fumosorosea* and *Isaria farinosa*. **Brazilian Archives of Biology and Technology**, v. 53, n. 4, p. 753-761, 2010.

MASCARIN, G. M., JACKSON, M. A., KOBORI, N. N., BEHLE, R. W., DELALIBERA, I. Liquid culture fermentation for rapid production of desiccation tolerant blastospores of *Beauveria bassina* and *Isaria fumosorosea* strains. **Journal of Invertebrate Pathology**, v. 127, p. 11-20, 2015.

MASCARIN, G. M., JACKSON, M. A., KOBORI, N. N., BEHLE, R. W., DUNLAP, C. A., DELALIBERA, I. Jr. Glucose concentration alters dissolved oxygen levels in liquid cultures of *Beauveria bassiana* and effects formation and bioefficacy of blastospores. **Applied Microbiology and Biotechnology**, Berlin, v.99, n. 16, p. 6653-6665, 2015b.

MASCARIN, G. M., KOBORI, N. N., VITAL, R. C. J., JACKSON, M. A., QUINTELA, E. D. Production of microsclerotia by Brazilian strains of *Metarhizium* spp. Using submerged liquid culture fermentation. **World Journal of Microbiology and Biotechnology**, v.30, p.1583-1590, 2014.

MEDEIROS, F. R., LEMOS, R. N. S. de., RODRIGUES, A. A. C., FILHO, A. B., OLIVEIRA, L. de J. M. G. de., ARAÚJO, J. R. G. Occurrence of *Purpureocillium lilacinum* in citrus Black flynymphs. **Revista Brasileira de Fruticultura,** v.40, n. 2, 2018.

MEDINA-CANALES, M. G., RODRÍGUEZ-TOVAR, A. V., MANZANILLA-LÓPEZ, R. H., ZUNIGA, G., TOVAR-SOTO, A. Identification and molecular characterisation of new Mexican isolates of *Pochonia chlamydosporia* for the management of *Meloidogyne* spp. **Biocontrol Science and Techonology,** v. 24, n. 1, p. 1-21, 2014.

MEYER, J. R. **Resistance to pesticides.** https://projects.ncsu.edu/cals/course/ent425/library/tutorials/applied_entomology/resistance.html (Accessed 16 July 2019), 2003.

MEYLING, N. V., EILENBERG, J. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. **Biological Control**, v. 43, n. 2, p. 145-155, 2007.

MIRANDA, M. Z. de. **Trigo: germinação e posterior extrusão para obtenção de farinha integral extrusada de trigo germinado**. Passo Fundo: EmbrapaTrigo, 12 p, 2006.

MONFORT E., LOPEZ-LLORCA L. V., JANSSON H. B., SALINAS J., PARK J. O., SIVASITHAMPARAM K. Colonization of seminal roots of wheat and barley by egg-parasitic nematophagous fungi and their effects on *Gaeumannomyces graminis* var. *tritici* and development of root-rot. **Soil Biology and Biochemistry**, v. 37, p. 1229– 1235, 2005.

MONTEIRO, A. R., MORAIS, S. R. A. C. Ocorrência do nematóide de cisto da soja *Heterodera glycines* Ichinohe, 1952, prejudicando a cultura em Mato Grosso do Sul. **Nematologia Brasileira**, v. 16, p. 101, 1992.

MONTEIRO, V. B., GONDIM Jr, M. G. C., OLIVEIRA, J. E. M., SIQUEIRA, H. A. A., SOUSA, J. M. Monitoring *Tetranychus urticae* Koch (Acari: Tetranychidae) resistance to abamectin in vineyards in the lower Middle São Francisco valley. **Crop Protection**, v. 69, p. 9096, 2015.

MORAES, G. J., FLECHTMANN, C. H. W. 2008. **Manual de Acarologia, Acarologia Básica e Ácaros de Plantas Cultivadas no Brasil**. Holos Editora, Ribeirão Preto. 308 p.

MORAL, R., HINDE, J., DEMÉTRIO, C. Half-Normal plots and overdispersed models in R: The hnp package. **Journal of Statistical Software**, v. 81, p. 1-23, 2017.

MORAGA, E. Q. Entomopathogenic fungi as endophytes: their broader contribution to IPM and crop production. **Biocontrol Science and Technology**, 2020.

MOREIRA, D. C., OLIVEIRA, M. M. E., BORBA, C. M. Human pathogenic *Paecilomyces* from food. **Microorganisms**, v. 6, n. 64, p. 1-8, 2018.

MORTON, C. O., MAUCHLINE, T. H., KERRY, R., HIRSCH, P. R. PCR-based DNA fingerprinting indicates host-related genetic variation in the nematophagous fungus *Pochonia chlamydosporia*. **Mycol. Res.** V. 107, n. 2, p. 198-205, 2003.

MUTUNE, B., EKESI, S., NIASSY, S., MATIRU, V., BII, C., MANIANIA, N. K. Fungal endophytes as promising tools for the management of bean stem maggot *Ophiomyia phaseoli* on beans *Phaseolus vulgaris*. **Journal of Pest Science**, v. 89, n. 4, p. 993-1001, 2016.

MUKHTAR, T., HUSSAIN, M. A., KAYANI, M. Z. Biocontrol potential of *Pasteuria penetrans, Pochonia chlamydosporia, Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra. **Phytopathologia Mediterranea,** v. 52, p. 66-76, 2013.

MUSTU, M., DEMIRCI, F., KÖKSAL, M., SERBES, C., ARMAGAN, B. Mortality effects of *Isaria farinosa* and *Purpureocillium lilacinum* (Sordariomycetes: Hypocreales) on the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae) and its predator *Neoseiulus californicus* (Acari: Phytoseiidae) under controlled conditions. **EntomologiaGeneralis**, v. 35, i. 4, p. 243-252, 2016.

NAJAFI, F., REZAIE, S., KIA, E. B., MAHMOUDI, M., KHODAVAISY, S., MOHEBALI, M., GHARAGOZLOU, M. J., ROKNI, M. B., MOWLAVI, G. In vitro assay of *Paecilomyces lilacinus* biocontrol effects on *Faciola hepatica* eggs illustrated in scanning electron micrographs. **Iran J. Parasitology**, v. 12, n. 1, p. 22-28, 2017.

NIU, X. M., WANG, Y. L., CHU, Y. S., XUE, H. X., LI, N., WEI, L. X., MO, M. H., ZHANG, K. Q. Nematode toxicaurovertin-type metabolites from a rook-knot nematode parasitic fungus *Pochonia chlamydosporia*. **Journal Agric Food Chem**, v. 58, p. 828–834, 2010.

NIBLACK, T. L. Soybean cyst nematode management reconsidered. **Plant Disease**, v. 89, n. 10, p. 1020-1026, 2005.

O'DONNELL, K., CIGELNIK, E., NIRENBERG, H. Molecular systematic and phylogeography of the *Gibberella fujikuroi* species complex. **Mycologia**, v. 90, n. 3, p. 465-493, 1998.

OLIVEIRA, L. F. C., OLIVEIRA, M. G. C., WENDLAND, A., HEINEMANN, A. B., GUIMARÃES, C. M., FERREIRA, E. P. B., QUINTELA, E. D., BARBOSA, F. R., CARVALHO, M. C. S., LOBO-JUNIOR, M., SILVEIRA, P. M., SILVA, S. C. **Conhecendo a fenologia do feijoeiro e seus aspectos fitotécnicos.** Embrapa Arroz e Feijão, 64p, 2018.

PARK, J. O., HARGREAVES, J. R., MCCONVILLE, E. J., STIRLING, G. R., GHISALBERTI, E. L., SIVASITHAMPARAM, K. Production of leucinostatins and nematicidal activity of Australian isolates of *Paecilomyces lilacinus* (Thom) Samson. **Lett. Appl. Microbiol**. V. 38, p. 271–276, 2004.

PARNISKE, M. Arbuscular mycorrhiza: the mother of the plant root endosymbioses. **Nature Reviews,** v. 6, p. 763-775, 2008.

PARRA, J. R. P. Biological control in Brazil: An overview. **Scientia Agricola**, v. 71, n. 5, p. 345-355, 2014.

PARRA, J. R. P., BOTELHO, P. S. M., CORREA-FERREIRA, B. S., BENTO, J. M. S. **Controle biológico no Brasil: parasitóides e predadores.** Editora Manole, São Paulo. 609p, 2002.

PARSA, S., GARCÍA-LEMOS, A. M., CASTILLO, K., ORTIZ, V., LÓPEZ-LAVALLE, L. A. B., BRAUN, J., VEGA, F E. Fungal endophytes in germinated seeds of the common bean, *Phaseolus vulgaris*. **Fungal Biology**, v. 120, p. 783-790, 2016.

PASQUA, S.D., DALLEMOLE-GIARETTA, R., SANTOS, I., REINER, D. A., LOPES, E. A. Combined application of *Pochonia chlamydosporia* and solid by-product of the wine industry for the control of *Meloidogyne javanica*. **Applied Soil Ecology**, v. 147, 2020.

PORTZ, R. L., GARTNER, M., VIGO, S., FRANZENER, G., STANGARLIN, J. R., FURLANETTO, C. Ocorrência do nematoide do cisto da soja (*Heterodera glycines*) no oeste do Paraná. **FitopatologiaBrasileira,** v. 26, 2001.

PRASAD, P.; VARSHNEY, D.; ADHOLEYA, A. **2015.** Whole genome annotation and comparative genomic analyses of bio-control fungus *Purpureciollium lilacinum*. **BMC Genomics**, v. 16, p. 1-14.

QUINTELA, E. D. Production of microsclerotia by Brazilian strains of *Metarhizium* spp. using submerged liquid culture fermentation. **World J Microbiol Biotechnol**, v. 30, p. 1583–1590, 2014.

R CORE TEAM R. C. R: A Language and Environment for Statistical Computing. R Found Stat Comput, 2020.

RAPER, K. B., THOM, C. **A manual of the *Penicillia.*** Williams & Wilkins, Baltimore, 916p, 1949.

REHNER S. A., SAMUELS G. J. Molecular systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorphs. **Can J Bot,** v. 73 (Suppl. 1), p.S816–S823, 1995.

RIBEIRO, M. G. P. de. M., FILHO, M. M., GUEDES, I. M. R., MALDONADE, I. R. **Prejuízos causados pelo ácaro rajado no morango e manejo desta praga.** https://www.grupocultivar.com.br/artigos/prejuizos-causados-pelo-acaro-rajado-e-manejo (Acessed 24 July 2019), 2019.

RIGGS, R. D. Host range, In: RIGGS, R.D.; WRATHER, J.A. (Ed.). **Biology and management of the soybean cyst nematode.** St. Paul: The AmericalPhytopathological Society, p. 107-114, 1992.

RICHMOND, D. S., GREWAL, P. S., CARDINA, J. Competition between *Lolium perenne* and *Digitaria sanguinalis*: Ecological consequences for harboring an endosymbiotic fungus. **Journal of Vegetation Science**, v. 14, p. 835-840, 2003.

RODRÍGUEZ-KÁBANA, R., MORGAN-JONES, G., GODOY, G., GINTIS, B. O. Effectiveness of species of *Gliocladium, Paecilomyces* and *Verticillium* for control of *Meloidogyne arenaria*in field soil. **Nematropica,** v. 14, n. 2, p.155-170, 1984.

ROGGIA, S., SOSA-GOMEZ, D. R. Manejo de ácaros-praga em soja. **Embrapa soja**, 2ªimpressão, 2012.

ROMERO-RANGEL, O., MALDONADO-BLANCO, M. G., AGUILAR-LÓPEZ, C. C., ELIAS-SANTOS, M., RODRÍGUEZ-GUERRA, R., LÓPEZ-ARROYO, J. I. Production of mycelium and blastosporesof *Hirsutella* sp. in submerged culture. **Afr. J. Biotechnol.** v. 11, p. 15336-15340, 2012.

RONDOT, Y., REINEKE, A. Endophytic *Beauveria bassiana* in grapevine *Vitis vinifera* (L.) reduce infestation with piercing-sucking insects. **Biological control**, v. 116, p. 82-89, 2018.

ROSSO, L. C., FINETTI-SIALER, M. M., HIRSCH, P. R., CIANCIO, A., KERRY, B. R., CLARK, I. M. Transcriptome analysis shows differential gene expression in the saprotrophic to parasitic transition of *Pochonia chlamydosporia*. **Appl. Microbiol. Biotechnol**. v. 90, p. 1981–1994, 2011.

RUAN, J., ZHOU, Y., ZHOU, M., YAN, J., KHURSHID, M., WENG, W., CHENG, J., ZHANG, K. Jasmonic acid signaling pathway in plants. **Internation Journal of Molecular Sciences**, v. 20, 2019.

RUSSO, M. L., PELIZZA, S. A., VIANNA, M. F., ALLEGRUCCI, N., CABELLO, M. N., TOLEDO, A. V., MOURELOS, C., SCORSETTI, A. C. Effect of endophytic entomopathogenic fungi on soybean *Glycine max* (L.) Merr. Growth and yield. **Journal of King Saud University – Science**, 2018.

SATO, M. E., VERONEZ, B., STOCCO, R. S. M., QUEIROZ, M. C. V., GALLEGO, R. Spiromesifen resistance in *Tetranychus urticae* (Acari: Tetranychidae): Selection, stability and monitoring. **Crop Protection**, v. 89, p. 278-283, 2016.

SCHARDL, C. L., GROSSMAN, R. B., NAGABHYRU, P., FAULKNER, J. R., MALLIK, U. P. Loline alkaloids: Currencies of mutualism. **Phytochemistry**, v. 68, p. 980-996, 2007.

SCHARDL, C. L., LEUCHTMANN, A., SPIERING, M. J. Symbioses of grasses with seedborne fungal endophytes. **Ann. Rev. Plant. Biol.**, v. 55, p. 315-350, 2004.

SCHMITT, R. D., RIGGS, R. D. Populations dynamics of *Heterodera glycines* in the southeastern United State. **The Texas A & M University System**, p. 1-7, Series Bulletin 336, 1989.

SCHÖFFL, F., PRÄNDL, R., REINDL, A. Molecular responses to heat stress. In: SCHINOZAKI, K., YAMAGUCHI-SHINOZAKI, K (Eds). **Molecular responses to cold, drought, heat and salt stress in higher plants.** Landes Co., Austin, pp. 81-98, 1999.

SEQUEIRA, D. C. M., MENEZES, R. C., OLIVEIRA, M. M. E., ANTAS, P. R. Z., LUCA, P. M., OLIVEIRA-FERREIRA, J., BORBA, C. M. *Purpureocillium lilacinum*: Outcome of the infection in C57BL/6 MurineModels. **Frontiers in Microbiology**, v. 8, 2014.

SIDDIQUI, I. A., QURESHI, S. A., SULTANA, V., EHTESHAMUL-HAQUE, S., GHAFFAR, A. Biological control of root rot-knot disease complex of tomato. **Plant and Soil,** v. 227, p. 163-169, 2000.

SIMKOVIC, M., DITTE, P., KURUCOVÁ, A., LAKATOS, B., VARECKA, L. Ca^{2+}-dependent induction of conidiation in submerged cultures of *Trichoderma viride*. **Can. J. Microbiol**, v. 54, p. 291-298, 2008.

SONG, Z., SHEN, L., ZHONG, Q., YIN, Y., WANG, Z. Liquid culture production of microsclerotia of *Purpureocillium lilacinum* for use as bionematicide. **Nematology**, v. 18, p. 719-726, 2016.

SONG, Z., YIN, Y., JIANG, S., LIU, J., WANG, Z. Optimization of culture medium for microsclerotia production by *Nomuraea rileyi* and analysis of their viability for use as a mycoinsecticide. **BioControl**, v. 59, p. 597-605, 2014.

STIRLING, G. R. **Biological control of plant-parasitic nematodes: Soil Ecosystem management in sustainable agriculture.** CABI, 2nd edition. 536p, 2015.

STIRLING, G. R. Biological control of plant-parasitic nematode: 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**. Ed. Springer, v. 11, Dordrecht, p. 1-38, 2011.

STRONG, D. R., PEMBERTON, R. W. Food Webs, Risks of alien enemies and reform of biological control, IN: WANJNBERG, E., SCOTT, J. K.; QUIMBY, P. C. 1999. **Evaluating indirect ecological effects of biological control.** CABI Publishing, 261p, 1999.

SUEKANE, R., DEGRANDE, P. E., MELO, E. P., BERTONCELLO, T. F., JUNIOR, I. S. L., KODAMA, C. Damage level of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) in soybeans. **Revista Ceres**, v. 59, n. 1, p. 77-81, 2012.

SUNG, G. H., HYWEL-JONES, N. L., SUNG, J. M., LUANGSA-ARD, J. J., SHRESTHA, B., SPATAFORA, J. W. Phylogenetic classification of Cordyceps and the clavicipitaceous fungi. **Stud. Mycol**. 57, 5–59, 2007.

TAMAI, M. A., ALVES, S. B., NEVES, P. J. Patogenicidade de *Beauveria bassiana* (BALS.) VUILL ao ácaro *Tetranychus urticae* Koch. **Sci. Agric.** V. 56, n. 2, p. 285-288, 1999.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A., KUMAR, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. **Molecular Biology and Evolution**, v. 30, n. 12, p. 2725-2729, 2013.

TANG, X., ZHANG, Y., WU, Q., XIE, W., WANG, S. Stage-specific expression of resistance to different acaricides in four field population of *Tetranychus urticae* (Acari: Tetranychidae). **Journal of Economic Entomology**, v. 107, n. 7, p. 1900-1907, 2014.

THOMAS, K. C., KHACHATOURIAN, G. G., INGLEDEW, W. M. Production and properties of *Beauveria bassiana* conidia cultivated in submerged culture. **Can. J. Microbiol**, v. 33, p. 12-20, 1987.

TIKHONOV, V. E., LOPEZ-LLORCA, L. V., SALINAS, J., JANSSON, H. B. Purification and characterization of chitinases from the nematophagous fungi *Verticillium chlamydosporium* and *V. suchlasporium*. **Fungal Genet. Biol.** V. 35, p. 67–78, 2002.

TORRE, M de la., CÀRDENAS-COTA, H. M. Production of *Paecilomyces fumosoroseus* conidia in submerged culture. **Entomophaga**, Paris, v.41, p. 443-453. 1996.

VEGA, F. E. The use of fungal entomopathogens as endophytes in biological control: a review. **Mycologia**, v. 110, v. 1, p. 4-30, 2018.

VEGA, F. E., JACKSON, M. A., MECADIER, G., POPRAWSKI, T. The impact of nutrition on spore yields for various fungal entomopathogens in liquid culture. **World Journal of Microbiology and Biotechnology**, v. 19, p. 363-368, 2003.

VIDAL, C., FARGUES, J., LACEY, L. A., JACKSON, M. A. Effect of various liquid culture media on morphology, growth, propagule production, and pathogenic activity to *Bemisia argentifolii* of the entomopathogenic Hyphomycete, *Paecilomyces fumosoroseus*. **Mycopathologia**, v. 143, p. 36-46, 1998.

VIDAL, S., JABER, L. R. Entomopathogenic fungi as endophytes: Plant-endophyte-herbivore interactions and prospects for use in biological control. **Current Science**, v. 109, n. 1, 2015.

WAJNBERG, E., SCOTT, J. K., QUIMBY, P. C. **Evaluating indirect ecological effects of biological control.** CABI Publishing, 261p, 1999.

WARNES, G. R., BOLKER, B., BONEBAKKER, L., GENTLEMAN, R., LIAW, W. H. A., LUMLEY, T., MAECHLER, M., MAGNUSSON, A., MOELLER, S., SCHWARTZ, M., VENABLES, B., gplots: Various R Programming Tools for Plotting Data. **R package version 3.0.1.2** https://CRAN.R-project.org/package=gplots. 2020.

WANG, W., VINOCUR, B., ALTMAN, A. Plant response to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. **Planta**, v. 218, p. 1-4, 2003.

WEINER, J., CAMPBELL, L. G., PINO, J., ECHARTE, L. The allometry of reproduction within plant population. **Journal of Ecology**, v. 97, p. 1220-1233, p. 2009.

WHALON, A. E., MOTA-SANCHEZ, R. M., HOLLINGWORTH, R. M., DUYNSLAGER, L. **Arthropods resistant to pesticides database (ARPD).** https://www.pesticideresistance.org/ (Accessed 10 January 2020), 2008.

WHITHE, J. F., TORRES, M. S. Is plant endophyte-mediated defensive mutualism the result of oxidative stress protection? **Physiologia Plantarum**, v. 138, p. 440-446, 2010.

YESILAYER, A. Efficiency of two different entomopathogen fungi *Beauveria bassiana* and *Purpureocillium lilacinum*TRI against *Tetranychus urticae*. **Applied ecology and environmental research**, v. 16, n. 5, p. 6077-6086, 2018.

YODER, J. A., FISHER, K. A., DOBROTKA, C. J. A report on *Purpureocillium lilacinum* found naturally infecting the predatory mite, *Balaustium murorum* (Parasitengona: Erythraeidae). **International Journal of Acarology**, v. 44, n. 4-5, p. 139-145, 2018.

YOUNG, L. D. Cropping sequence effects on soybean and *Heterodera glycines*. **Plant Disease**, v. 76, n.1, p.76-81, 1992.

YPSILOS, I. K., MAGAN, N. Characterisation of optimum cultural environmental conditions for the production of high numbers of *Metarhizium anisopliae* blastospores with enhanced ecological fitness. **Biocontrol Science and Technology**, v. 15, n. 7, p. 683-699, 2005.

ZARE, R., GAMS, W. A revision of Verticillium section Prostrata. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov.**Nova Hedwigia**, v.73, p. 1–50, 2001a.

ZARE, R., GAMS, W., EVANS, H. C. A revision of Verticillium section Prostrata V. The genus *Pochonia*, with notes on Rotiferophthora. **Nova Hedwigia** v. 73, p. 51–86, 2001.

ZARE, R., GAMS, W., SCHROERS, H. J. The type species of *Verticillium* is not congeneric with the plant-pathogenic species placed in *Verticillium* and it is not the anamorph of ‘Nectria’ inventa. **Mycol Res** v. 108, p. 576–582, 2004.

ZARE, R., GAMS. W. **A monograph of *Verticillium* section Prostrata.** Rostaniha (Botanical Journal of Iran). Supplement, 3, 188p, 2004.

ZHOU, G. et al., Involvement of an alternative oxidase in the regulation of hyphal growth and microesclerotial formation in *Nomuraea rileyi* CQNr01. **World Journal of Microbiology and Biotechnology**, v. 31, n. 9, p. 1343-1352, 2015.