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Daily Indexes for Predation and Growth of Nematophagous Mushrooms Species of *Hohenbuehelia* (Pleurotaceae) on *Panagrellus redividus*

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Abstract

Biological control is a method of controlling pests through the use of other living organisms. The purposes of this study were to test *Hohenbuehelia* species as biological control agents against *Panagrellus redivivus in vitro*, evaluating nematodes influence on mycelia growth; establishing daily indexes for predation and growth and setting predation percentage. Five species previously identified as 436-*Hohenbuehelia mastrucata* (*Nematoctonus hamatus*), 528-*H. bullulifera* (not described so far), 581-*H. paraguayensis* (*N.* sp.), 582-*H.* sp. (*N.* sp.) and 631-*H. portegna* (*N. campylosporus*) were submitted to anamorphic purification directly from basidioma. Afterwards, 100 nematodes were added to each pure colony for predation test. Evaluation started right after 24 hours of nematode-fungus interaction. Immobilized and/or penetrated nematodes were counted and mycelia growth was measured. Results were subjected to variance analyses. *Hohenbuehelia mastrucata* had the best performance in growth speed, followed by *H. portegna* and *H. paraguayensis*; Nematodes multiplyied much but none specie grew more as an influence of their movement under mycelium, however all species formed trap devices and some of them produced adhesive or repelent substances. Trap devices were formed in control plates also. The plates of *H. paraguayensis* without nematodes grew more than treatments. Cumulative predation of *H. portegna* was the highest at 24 (195.5%) and 48 hours (235%). At the last evaluation day, *H. paraguayensis* preyed the same amount (185.75%) than *H. portegna*, followed by *H. mastrucata* (109.51%). Resulst of predation daily indexes displayed chronological activity for each isolate, where *H. portegna* was very reactive at first 24 hours, *H. mastrucata* raised its predacious activity in 48 hours being constant from this time on and *H. paraguayensis* pointed out itself at 72 hours. Other species presented low predation and growth indexes throughout experiment.

Keywords: biocontrol, adhesive knobs, Basidiomycota, sticky substances, alternative control, endoparasitism

1. Introduction

Phytonematodes attack causes reduction in productivity what can reach alarming levels. They are microscopic worms present in almost all soils, having several plants as hosts, including weeds. Their dissemination occurs by soil and roots transport. Chemical products are usually applied to control them, but a growing concern about human and animal health and environmental care have reduced their use (Ferraz & Brown, 2016). Nematode control is a very difficult task resulting in increasing interest on alternative methods, such as biological control.

Biological control is a natural process occurring in all habitats, nevertheless it can be introduced in agricultural areas by using a living organism (Tranier et al., 2014) to keep low a population level of another organism (Agbenin, 2011). Amongst several potential agents, fungi have been traditionally studied (Vega et al., 2009).

Hohenbuehelia genus contains nematophagous species belonging to Basidiomycota phylum, presenting clamp connections as intrinsic characteristic (Babu et al., 2014). They are filamentous endoparasites that adhere nematodes to a trapping apparatus to immobilize them and penetrate their cuticle, invading the body and digesting its nutrients (Tranier et al., 2014).

Barron (2003) observed ligninases and cellulases production, suggesting that *Hohenbuehelia* (syn. *Nematoctonus*) species decompose woods naturally and nematodes predation is a subset to get nitrogen supplement. It happens due to the high carbon to nitrogen ratio in woods composition maintaining its stability that reduce degradation by fungi (Piskur et al., 2009). Therefore, this genus performs two important functions to ecosystem: wood decomposition and pests control (Mankau, 1980). Further, saprophytic ability turns biological agents into a promising control strategy (Nunes et al., 2010) and nematode trapping fungi present no specificity to host so any nematode in soil can be preyed by them (Askary, 2015).

Koziak et al. (2007) confirm that *Nematoctonus* genus are found in agricultural soils and contains predators and parasitoids species, unlike other nematophagous genus that generally contemplates only one control profile.

Panagrellus redivivus is a free-living nematode visible at naked eye during adult stage that reproduces very fast (Sautter et al., 2007) and for that reason it is frequently used in laboratory trials.

The present study aimed at analyzing the potential of *Hohenbuehelia* species as biological control agents *in vitro* against *Panagrellus redivivus*, identifying a relation between nematode predation and mycelia growth, establishing daily indexes for predation and growth and setting predation percentage. We hypothesize that the more isolates grow the more nematodes are preyed by them.

2. Method

2.1 Fungal Isolates Collection, Identification and Purification

Hohenbuehelia isolates were collected in forest fragments in Palotina city (Latitude 24°17′02″ S, Longitude 53°50′24″ W, Height 333 m asl.) and belong to Fungal Herbarium of Universidade Federal do Paraná, Setor Palotina.

Right after collection, they were submitted to macro and microscopic analysis, being identified as 436-*Hohenbuehelia mastrucata* (*Nematoctonus hamatus*), 528-*H. bullulifera* (not described so far), 581-*H. paraguayensis* (*N.* sp.), 582-*H.* sp. (*N.* sp.) and 631-*H. portegna* (*N. campylosporus*) (Thorn & Barron, 1986; Silva-Filho & Cortez, 2017).

Basidiocarp collected were in a very dirty condition and with low spore formation so they were purified directly from gills and gelatinous context of fresh or dried basidiocarp, right after superficial disinfestation method. The pieces were chosen and cut with needle from areas apparently free from contamination.

First, small pieces of gills were taken using a needle and driven to sterilized laminar flow where got submerged in sodium hypochlorite (1%) during three minutes for disinfestation. In sequence, they were put into a recipient with alcohol (70%) during 30 seconds to remove hypochlorite excess, followed by three washings in sterile water.

After superficial purification, basidiocap fragments were carefully placed on Petri plates with PDA (potato, dextrose, agar), medium, sealed with Parafilm and stored into BOD (body oxygen demand) at 25±2 °C.

Purified mycelia colonies were detected visualizing clamp connections on septum of hyphae.

2.2 Predation Test and Mycelia Growth Measuring

For predation test, eight agar discs (8 mm diameter) were removed from borderer mycelia of each purified colony grown in PDA medium and replaced to the center of eight 9-cm-diameter Petri plates containing water-agar (2%) medium.

A pasty mixture of oat flour and distilled water was done to maintain nematodes. Those were collected with a spatula when climbing the boundary of pots, mixed in water and poured under a 400 mesh sieve for cleaning and separation from flour.

Four plates represented the control (without nematodes) and four the treatments (fungus + nematodes). All plates were divided into four quadrants marked on background with marker pen to allow an accurate counting of dead nematodes. In treatments, each quadrant received individually 20 µL of distilled water containing a media of 25 *Panagrellus redivivus*. In sequence, plates were stored into BOD at 25±2 °C throughout evaluation period.

Four measures of radial mycelia growth per plate were done aided by a ruler.

Analysis on predation percentage and mycelia growth started passed 24 hours of nematode-fungus interaction up to third day. Nematode were considered preyed from single hyphae penetration or when glued on hyphae. New borne juveniles were counted together.

Pictures of preyed nematodes and of trap formation were taken using a cell phone camera of 12 Mp coupled to an optical microscope (Nikon, model ECLIPSE E100 LED).

2.3 Statistical Analysis

Obtained data was transformed by $\sqrt{(x + 1)}$ and subjected to analyses of variance (ANOVA). In the case of significant results ($P < 0.05$), Tukey test (5% error probability) was employed to compare averages using SISVAR 5.6® program (Ferreira, 2011).

3. Results

The isolate 582-*H.* sp. could not be identified due to lack of spores, an indispensable structure used for identification.

Most isolates grew in a low rate, so during mycelia purification they were subculture many times to new sterilized PDA medium to conclude the process in order to keep them away from contaminations in early stages.

Each colony took different time to grow on agar before nematodes addition (Table 1). *H. mastrucata* had the fastest colonization profile (relation between time and growth).

Table 1. Growing of *Hohenbuehelia* spp. before nematodes addition

Specie	Growth (cm)	Time (days)	Relation (cm/day)
H. mastrucata	3.61 a	3 c	1.20 a
H. portegna	1.54 b	12 b	0.13 b
H. paraguayensis	1.37 b	33 a	0.04 c
H. bullulifera	0.93 c	32 a	0.03 c
H. sp.	0.27 d	13 b	0.02 c
C.V. (%)	5.57	0	1.81

Note. * Means followed by the same letter in the same column did not differ significantly from each other by Tukey test at 5% of probability.

Panagrellus redivivus did not stimulate mycelia growth to any specie (Table 2).

Table 2. Growth (cm) comparison between treatments (with nematodes) and controls (without nematodes) of *Hohenbuehelia* spp. after 24, 48 and 72 evaluation periods

	Specie	After 24 hours	After 48 hours	After 72 hours
Treatments	*Hohenbuehelia mastrucata*	3.6125 a	4.4625 a	4.500 a
	Hohenbuehelia portegna	1.5375 bc	1.6250 bc	1.8313 b
	Hohenbuehelia paraguayensis	1.3688 c	1.3688 c	1.3938 c
	Hohenbuehelia aff. *bullulifera*	0.9313 d	0.9313 d	0.9313 d
	Hohenbuehelia sp.	0.2750 e	0.275 e	0.3188 e
Control	*Hohenbuehelia mastrucata*	3.5875 a	4.4625 a	4.500 a
	Hohenbuehelia portegna	1.4562 bc	1.5625 bc	1.8250 b
	Hohenbuehelia paraguayensis	1.7375 b	1.7438 b	1.8000 b
	Hohenbuehelia aff. *bullulifera*	0.9313 d	0.9313 d	0.9313 d
	Hohenbuehelia sp.	0.3438 e	0.3438 e	0.3438 e
	C.V. (%)	5.83	5.47	5.89

Note. * Means followed by the same letter in the column did not differ significantly from each other by Tukey test at 5% of probability.

Hohenbuehelia portegna predation stood out from first 24 hours of interaction (Table 3). This isolate released colorless substances with apparent sticky property that attached nematodes to its hyphae precluding any movement out of mycelia, ascribing a great reactiveness.

Table 3. Predation percentage of nematodes by *Hohenbuehelia* spp. after 24, 48 and 72 hours of interaction

Specie	24 hours	48 hours	72 hours
H. mastrucata	11.50 b	63.00 b	109.51 b
H. portegna	117.75 a	195.50 a	235.00 a
H. paraguayensis	27.50 b	70.50 b	183.75 a
H. bullulifera	3.50 b	8.50 c	10.75 c
H. sp.	0.75 b	4.00 c	4.50 c
C.V. (%)	41.52	35.59	28.87

Note. * Means followed by the same letter in the column did not differ significantly from each other by Tukey test at 5% of probability.

The hypothese stabilished was not confirmed shows by the comparison between mycelia growth rate (Table 1) and percentage of controlled nematodes (Table 3). *H. paraguayensis* and *H. bullulifera* had the same growth rate, although mycelia extent differed, but the predation ability was very contrasting since from beginning.

Mycelia extent also did not assured predation success otherwise it would be expected that *H. mastrucata* (3.61 cm) would have preyed more nematodes than *H. portegna* (1.54 cm) or *H. paraguayensis* (1.37 cm), but it did not happened (Table 3).

Nematodes reproduction occurred freely in all treatments multiplying very fast in most of them. It was no possible to select females about to calve, thus some treatments probably received a higher number of them what resulted in lots of newborn juveniles whose movement was not tracked up.

Hohenbuehelia bullulifera produced dark metabolites that leaded nematodes to escape from agar to the plate side resulting in low predation, but this specie had a noticeable ability to repel them. *H.* paraguayensis, *H.* sp. and *H. mastrucata* allowed nematodes to move freely.

Hohenbuehelia mastrucata penetrated nematodes body aggressively (Figure 1B) what explains reduction superior to 100% of population initially added only at 72 hours. During reproduction, females stopped to save energy and this halt enabled massive predation (Figure 1A).

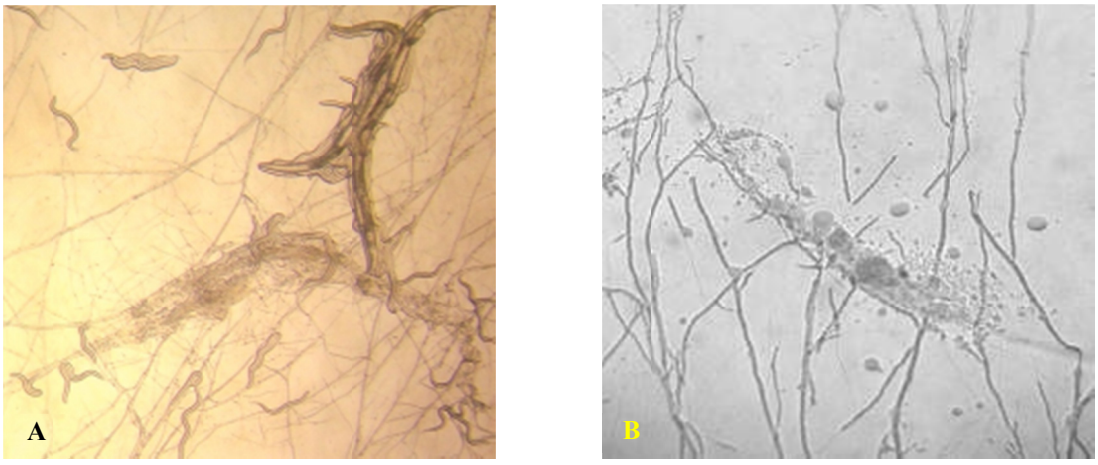

Figure 1. Massive predation of females and juveniles by *H. mastrucata*. Image taken by optical microscope using 40× and 100× magnifying objectives, respectively

On the other hand, the first reaction of *H. portegna* was to release sticky substances through hourglass shaped adhesives knobs (Figure 2A) that glued nematodes on hyphae system even though they could reproduce normally (Figure 2B). This chemical property gave an advantage over other species ending up in higher predation levels.

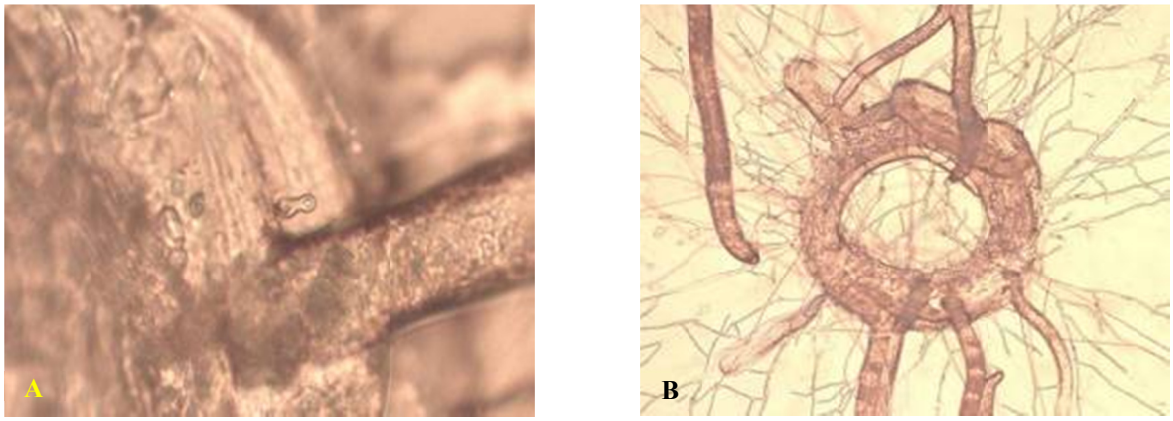

Figure 2. Hourglass shaped adhesive knobs (A) and penetration (B) by *Hohenbuehelia portegna*. Images taken by optical microscope using 400× and 100× magnifying objectives, respectively

Traps were formed the most by *Hohenbuehelia bullulifera* (Figure 3).

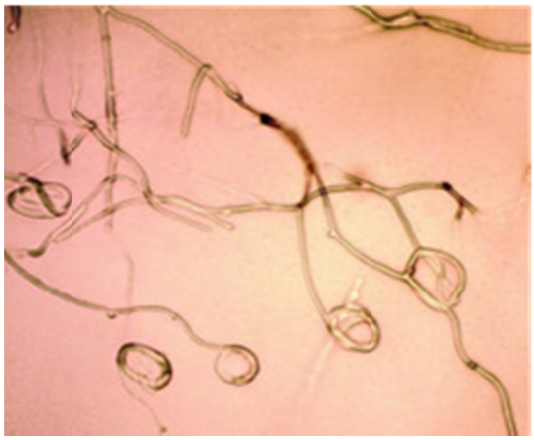

Figure 3. Non-constricting rings (traps) developed by *H. bullulifera*. Images taken by optical microscope using 400× magnifying objective

Isolates had different chemical reactions in front of *P. redivivus* presence. Some species naturally released dark substances previously to mycelia growth as *H. paraguayensis* (Figures 4A and 4B) and *H. bullulifera*. Such substances derived from *H. paraguayensis* had no appearent effect on nematodes.

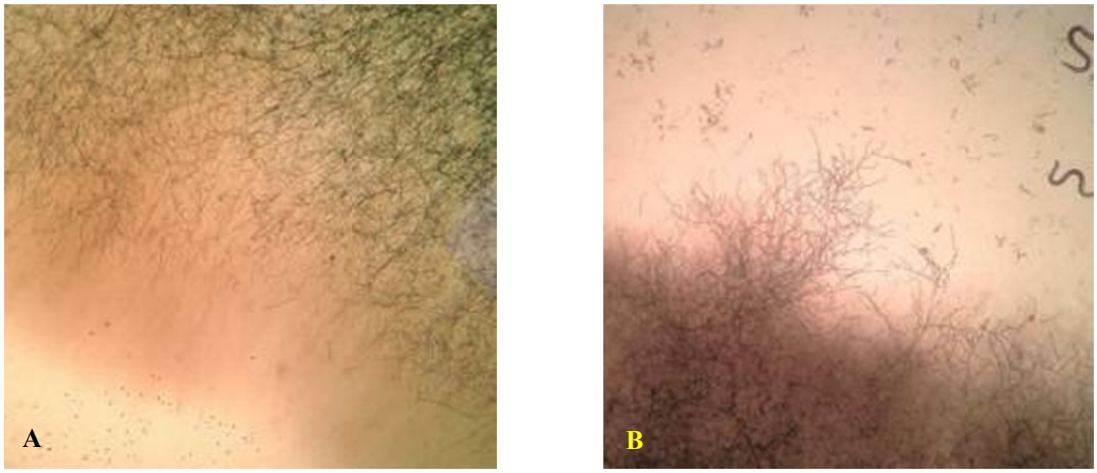

Figure 4. Chemical compounds released by *H. paraguayensis*. Images taken by optical microscope using 40× magnifying objective

Analysis of daily indexes allowed identifying behavior reaction on time. Daily predation indexes are important to set activity peak for each specie. Growth and predation daily indexes are represented by lowercase letters (Table 4) while capital letters are referent to cumulative growth and predation.

Table 4. Daily indexes for growth and predation of *Hohenbuehelia* spp. after 24, 48 and 72 hours

Specie	Growth (cm)			Predation		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
H. mastrucata	3.61 aA	0.85 bA	0.04 cA	2.87 bB	12.94 aB	11.56 aB
H. portegna	1.54 aBC	0.09 bBC	0.21 bB	29.44 aA	19.44 bA	9.87 cA
H. paraguayensis	1.37 aC	0.00 bC	0.02 bC	6.87 bB	10.75 bB	28.31 aA
H. bullulifera	0.93 aD	0.00 bD	0.00 bD	0.87 aB	1.37 aC	0.56 aC
H. sp.	0.27 aE	0.00 bE	0.05 aE	0.19 aB	0.81 aC	0.25 aC
C.V. (%)	20.72			40.07		

Note. * Means followed by the same lowercase letter within the line and by the same capital letter within the column did not differ significantly from each other by Tukey test at 5% of probability.

Daily indexes reveal that *H. portegna* was very reactive at first 24 hours whereas *H. mastrucata* raised its predation in 48 hours being constant from this time on and *H. paraguayensis* pointed out itself at 72 hours. Other species presented low indexes throughout experiment.

Daily growth indexes hint at declining ability for *H. mastrucata* (Table 4). In fact, it happened because this specie had grown very fast, colonizing almost all substrate surface in 48 hours. Cumulative growth displays how superior *H. mastrucata* was during all experiment, followed by *H. portegna* and *H. paraguayensis*.

Apparently, increasing predation indexes suggest better performance to *H. mastrucata* and *H. paraguayensis*, but cumulative predation reveals better control of nematodes by *H. portegna* and *H. paraguayensis*, followed by *H. mastrucata*.

4. Discussion

Third column (Table 1) reveals how fast each specie grew. Fast growing is an evident desirable characteristic related to successful appliance of biological agents in fields and their subsequent ability to colonize soil, but it is important to alert that in vitro growth pattern does not indicate similar behaviour in nature (Szabó et al., 2012).

Substrate is one cause of growth variance, but colony growth varies within genus and isolate also (Safavi et al., 2007; Wiriya et al., 2014) what explains Table 1.

According to Ren and Yao (2013), biomass fungi production decreases in poor nutritional substrate. However, plates containing nematodes as a nutritional source presented the same growth than control ones. Each treatment received about 100 specimens that moved freely above hyphae of most species enabling an easy capture. This amount possibly satisfied fungi nutritional requirement readily (Soto-Barrientos et al., 2011), not being necessary further growth, so as to *H. paraguayensis* that grew more in nematodes absence at all evaluation times (Table 2).

The low growth rate of *H. paraguayensis* and *H. bullulifera* (Table 1) could turn difficult their mixture with other nematophagous fungi presenting additional modes of action and chemical compatibility to other nematodes species to improve biocontrol technique, because these species would be probably overgrown by fungal as *Trichoderma* spp. or be antagonized by them (Szabó et al., 2012).

The presence of *P. redivivus* by itself did not stimuli fungi growth. Andersson et al. (2014), studied genes expression during infection process and proved chemical specificity between fungal-nematode interactions. However, nematode-trap fungi do not appear to have specificity to nematode host (Kerry, 2000). Our results suggest that trap formation does not imply in higher predation since the specie that formed more traps (*H. bullulifera*) was one that less preyed nematodes (Table 3). In addition, as fungi developed traps even in plates without nematodes, modifications on mycelia pattern and on growth were definitively not influenced by nematodes movement.

Thorn and Barron (1986) observed that *N. brevisporus* did not formed adhesive knobs from conidia in the presence of nematodes while *N. angustatus* could not adhere its spore to nematode body. Their conclusion shows that even both species are predators they were not stimulated by nematodes.

Gronvold et al. (1996) observed trap formation of *Duddingtonia flagrans* being influenced by hyphae aging, with better activity at 30 ^{o}C than 20 ^{o}C, enduring for two or three weeks, period from what on trap inductility reduced. The authors also guarantee that larvae migration (*Ostertagia ostertagi*) was indispensable for trap formation and as their movement reduces at 10 ^{o}C it explicates low trap formation at this temperature, though their work did not considered control plates to conclude whether trap would be induced only in plates with nematodes.

Despite fungi did not increase their growth in response to nematodes movement under mycelium, some of them produced metabolites, attracting or repulsing them (Figure 4). Mankau (1980) points out that several nematophagous fungal species release substances from infection pegs (Figure 2A) that may intoxicate nematodes by indirect contact and immobilize them promptly for predation.

Drechsler (1946) reveals a common 8-shaped structure (Figure 2A) formed at the hyphae tip as an active glandular cell responsible for producing adhesive substances in *Nematoctonus* genus with a possible occurrence of a second or third adhesive knob. During microscopic observations, this tiny apparatus was only found twice for *H. portegna* under a cadaver, one time for *H. bullulifera* and none time for other species. Thorn & Barron (1986) observed nematodes predation by *N. cylindrosporus* even no adsehive knobs was reported for this specie, similarly to *H. paraguayensis*. According to them, *H. mastrucata* may be predatory as well as endoparasite, which adhesive knobs are few or very tiny. We did not find adhesive knobs in *H. mastrucata* mycelia.

Babu et al. (2014) studied predation pattern of *N. robustus* (*H. grisea*) and observed nematodes attached to hyphae by hourglass shaped adhesives knobs, being penetrated later, just as noticed for *H. portegna* (Figures 2A and 2B).

These structures hold nematodes before body penetration. A single knob would be attached one juvenile avoiding its movement Drechsler (1946). However, we rarely saw such structures what makes us to believe that each glandular cell may produce large amounts of or very toxic adhesive substance(s), since more than 100 nematodes got sticked on hyphae in 24 hours. Interestingly low or no motility was noticed to nematodes attached by *H. portegna*. In fact, the presence of adhesive knobs enables a single hyphae system to capture large numbers of nematodes (Thorn & Barron, 1986).

The presence and activity of adhesives knobs classify a parasitic relationship. Misleading classification related to the mode of action, allows a fungal that presents adhesive knobs to be positionated in at least two different categories: predacious and alternative parasite, beyond being an antibiotic producer (Chen & Dickson, 2004; Lopez-Llorca et al., 2008). Thorn and Barron (1986) performed an identification key for *Nematoctonus* species and related the presence of adhesive knobs to classify predacious species while the absence of this structure determine endoparasitic species.

Nematodes are sttoped by adhesive spores or hyphae of *Nematoctonus* species (Thorn & Barron, 1986). High sporulation levels are expected in locals where groups of nematodes are preyed Drechsler (1946), but not a

single spore were seen to none isolate colony at any evaluation time, even in area with massive predation (Figure 1A). Herewith, predation success does not rely exclusively on spore formation.

Most field trials on biological control of nematodes using trapping-fungi as a manageable tool utilize spores as potential structures to vehicular them into soil (Giuma & Cooke, 1974; Sahebani & Hadavi, 2008; Noweer & Al-Shalaby, 2014) and keep longer their permanency in it. In this study, *Hohenbuehelia* species did not present spores formation in asexual state (*Nematoctonus*).

Not only hypha penetration and spores are known to be involved in nematode paralysis, also chemical substances play an important role. Singh and Mathur (2010) evaluated culture filtrates of 14 fungal species and noticed that all of them presented antagonistic activity against *Meloidogyne incognita*, in different levels, after 24 and 72 hours of interaction. Likewise, predation of *H. bullulifera*, *H. portegna* and *H. paraguayensis* may have been influenced by chemical compounds releasing. It is known that *Hohenbuehelia* genus contains endoparasites species that produce toxins (Moosavi1 & Askary, 2015). Probably toxin production differs among species and isolates what ends up in varied control ability.

Degenkolb and Vilcinskas (2016) proceed a fermentation protocol to identify secondary metabolites biosynthesized by *Nematoctonus robustus* (*H. grisea*). Among them, dihydropleurotinic acid, pleurotin and leucopleurotin showed weak activity against fungal and bacteria, but no nematicidal effect on *Caenorhabditis elegans*. However, tests against others nematode species were not driven by these authors.

In this study, substances were released on agar not only in plates containing *P. redivivus*, but also in control plates, indicating physiological stress or natural defense (Girotto et al., 2008). In this last case, these substances probably have properties against others organisms too, increasing the potential of *Hohenbuehelia* species as biological control agents, since many species from Basidiomycota are expected to produce antibiotic molecules (Robins et al., 1945).

Facultative parasites usually form trap structures in hyphae in presence of nematodes, specially the motile ones (Moosavi1 & Askary, 2015). *Hohenbuehelia* spp. produced traps in plates with and without nematodes (Figure 4), but none nematode were seen immobilized by traps.

Askary (2015) reported many opinions about how trap are formed, most of them related to varied specific substances released by organs of nematode body as a possible stimuli to trap formation. There was one particular consideration on nutritional status, regarding to the confirmed influence caused by transference of mycelia from a richer agar medium to a poorer one, such as performed in the present study.

Aparecido et al. (2008) noticed morphological changes induced by substrate changing within the same fungal isolate. It was unknown whether *Hohenbuehelia* species changed their mycelia pattern growth exclusively to capture nematodes so far, although our results show such changes also as consequence of mycelia transference from PDA to water-agar (2%) medium.

Panagrellus redivivus are readly consumed by both *H. portegna* and *H. mastrucata* (Thorn & Barron, 1986). *Hohenbuehelia paraguayensis* and *H. portegna* preyed 34.96 and 17.17% of *Panagrellus* sp. at third day, respectively (Putzke et al., 2007) referent to an initial population of 500 nematodes, what means to say that both fungi preyed 174 and 85 individuals, respectively. However, in this study, *H. portegna* preyed 235 nematodes at third day, followed by *H. paraguayensis* and *H. mastrucata* with 183 and 109 nematodes preyed, respectively. These results matches to an affirmation of Chen and Dickson (2004) that pathogenicity variance among isolates is pretty normal all the more among different species.

Thorn and Barron (1986) proved that all *Hohenbuehelia* species correspond to *Nematoctonus* nematophagous species, however, they did not present a predation estimative by them. All isolates tested here controlled nematodes at some degree with direct hyphae penetration.

Panagrellus redivivus reproduces in fast rate, duplicating individuals in few days once females reach sexual maturity in 3 days, bearing approximately 10 to 40 juveniles at once (Sautter et al., 2007). Moreover, there was uneven reproduction in some quadrants causing high variability coefficient (Table 4). This reproduction could not be tracked up or marked on plate because nematodes moved a lot.

During control process, body penetration took some time to occur what classify the isolate as parasitic (Chen & Dickson, 2004) and as nematodes multiplied much, the predation score got high (Table 3) with predation surpassing 100%, since the more promising species preyed new borne juveniles too.

Capture leads to hypha penetration with progressive parasitism, but *Nematoctonus* genus does not form an infection bulb, specific structure of digestion process (Chen & Dickson, 2004). Anyways, body content was absorbed and total or partially disintegrated in 48 hours by all isolates.

According to Nordbring-Hertz et al. (2006), hyphae attachment on nematode cuticle depends on glycoproteins compatibility. Based on this affirmation, it is plausible to think that *H. paraguayensis* and *H. mastrucata* had higher compatibility, since their physical action over nematodes was more intense than for *H. portegna*.

Fungi predatory potential may be related to chemical specificity to nematode species and also among life cycle stages of them. This phenomenon happens to *Paecilomyces lilacinus* and *Pochonia chlamydosporia*, specialized in *Meloidogyne* spp. eggs parasitism, but with low predation on juveniles (Aït Hamza el al., 2017). Furthermore, *Hohenbuehelia* sp. had preference on *P. redivivus* than on *Rhabditis terricola* (Thorn & Barron, 1986).

Gene expression determines the activity of some fungal species as *Trichoderma* sp. that produces chitin degrading enzyme while parasites nematodes eggs (Szabó et al., 2012). Therefore, even *H. bullulifera* and *H.* sp. did not showed high activity against juveniles of *P. redivivus* they could display another result against plant parasitic nematodes. Putzke et al. (2007) proved ability of *H. paraguayensis* and *H. portegna* on reducing gall formation of *M. javanica* in tomato roots in greenhouses trials, as well as, predation over *Panagrellus* spp.

5. Conclusions

None specie grew more influenced by nematodes movement under mycelium. Contrariwise, *H. paraguayensis* grew more in plates without nematodes.

The best predation performance was set by *H. portegna* and *H. paraguayensis*, followed by *H. mastrucata*, while *H.* sp. and *H. bullulifera* showed low potential as endoparasites agents. *H. mastrucata* had the best performance in growth speed, followed by *H. portegna* and *H. paraguayensis*.

Daily predation indexes revealed higher activity to *H. portegna* at 24 hours, *H. mastrucata* at 48 hours on and *H. paraguayensis* at 72 hours.

We did not report proportional relation between growth area and predation percentage to any specie, diclining our hyphotese.

This research indicates that *Hohenbuehelia* species produce substances with nematicidal properties that deserve future investigations.

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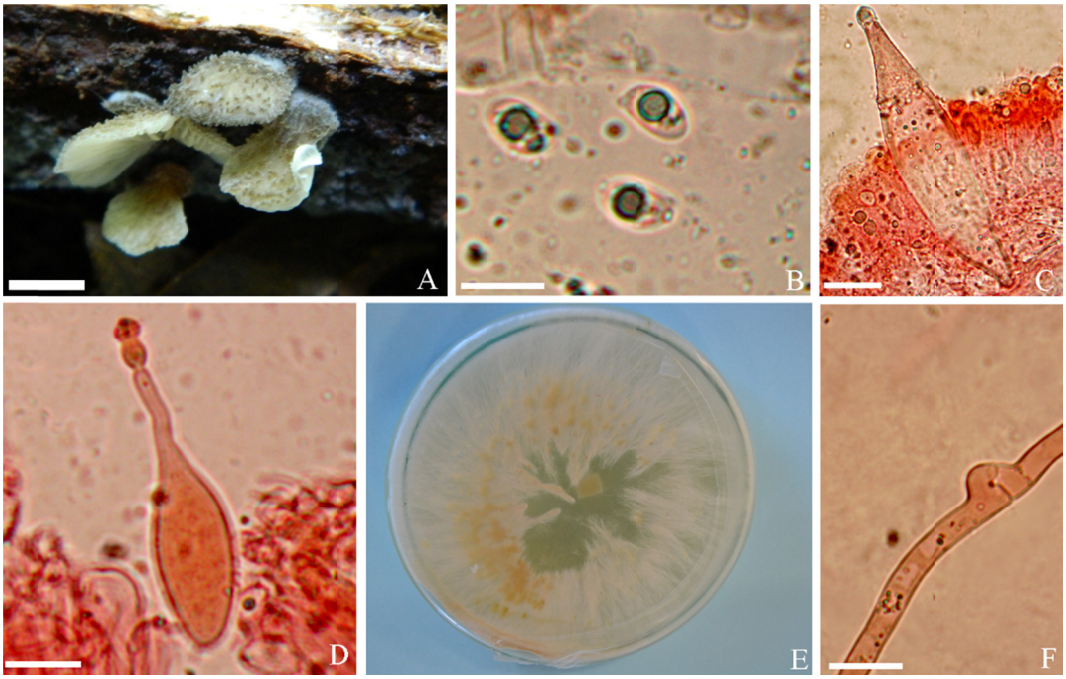
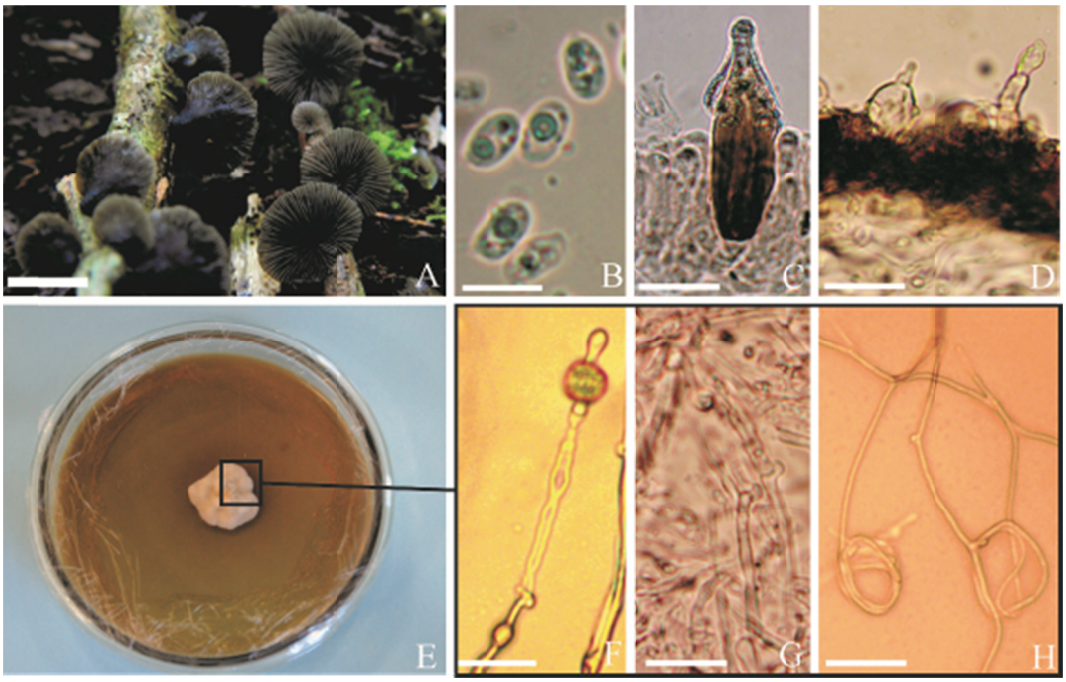
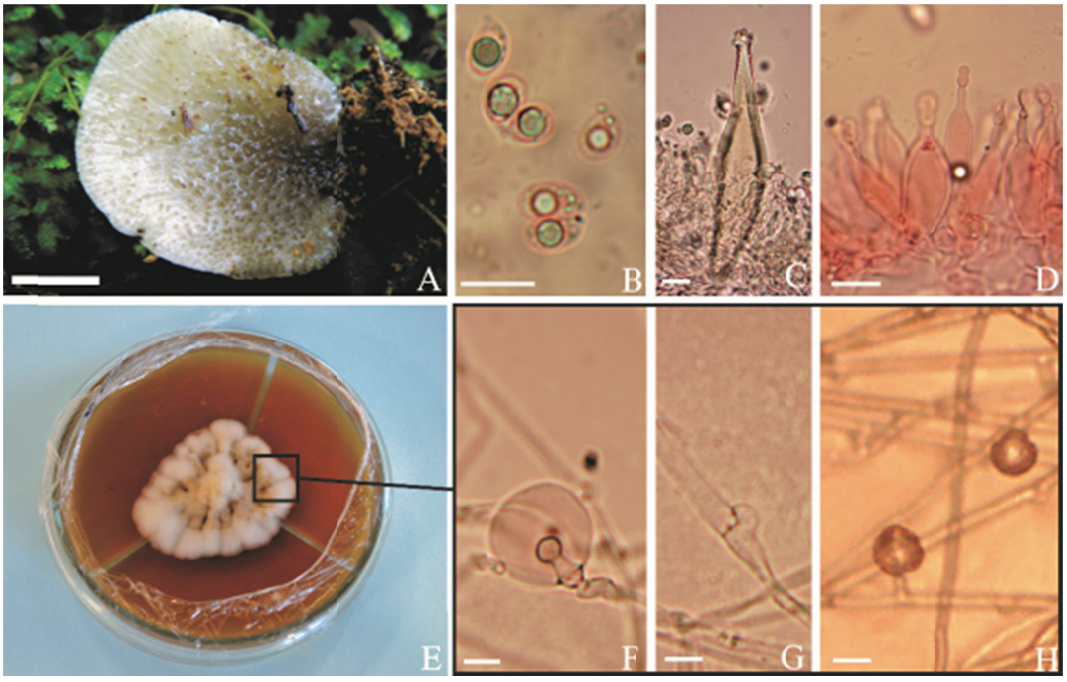
Appendix



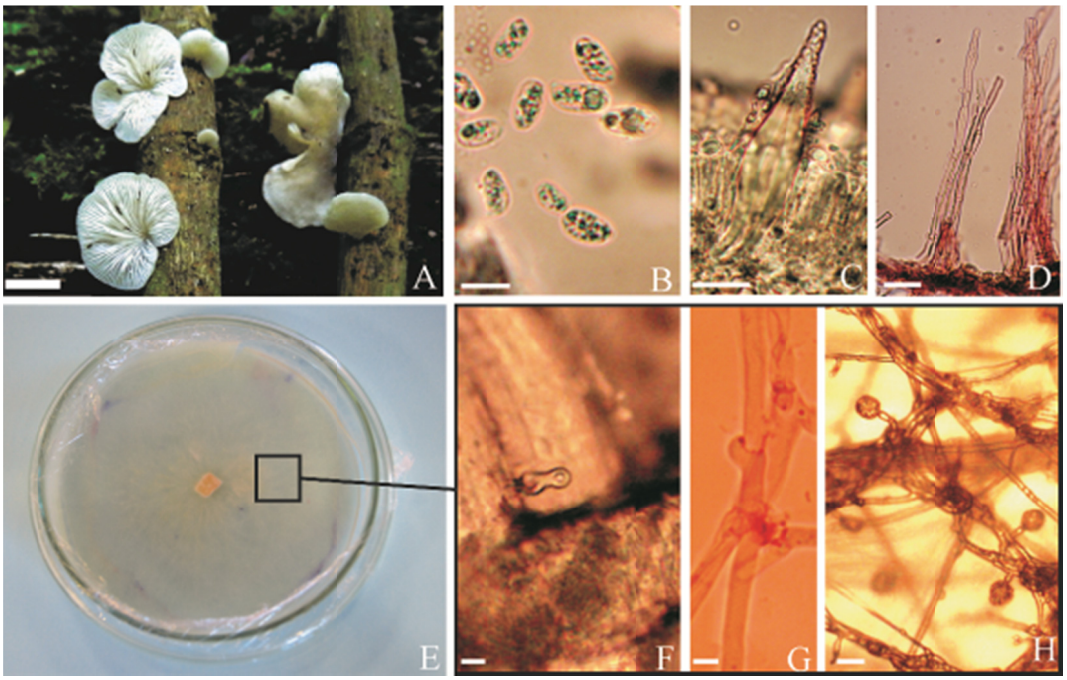
Appendix 1. 436-*H. mastrucata*; A: basidioma; B: spores S; C: metuloids; D: queilocistidios; E: mycelium; F: fibula. Escale: A: 5 mm; B, C, D, F: 10 µm



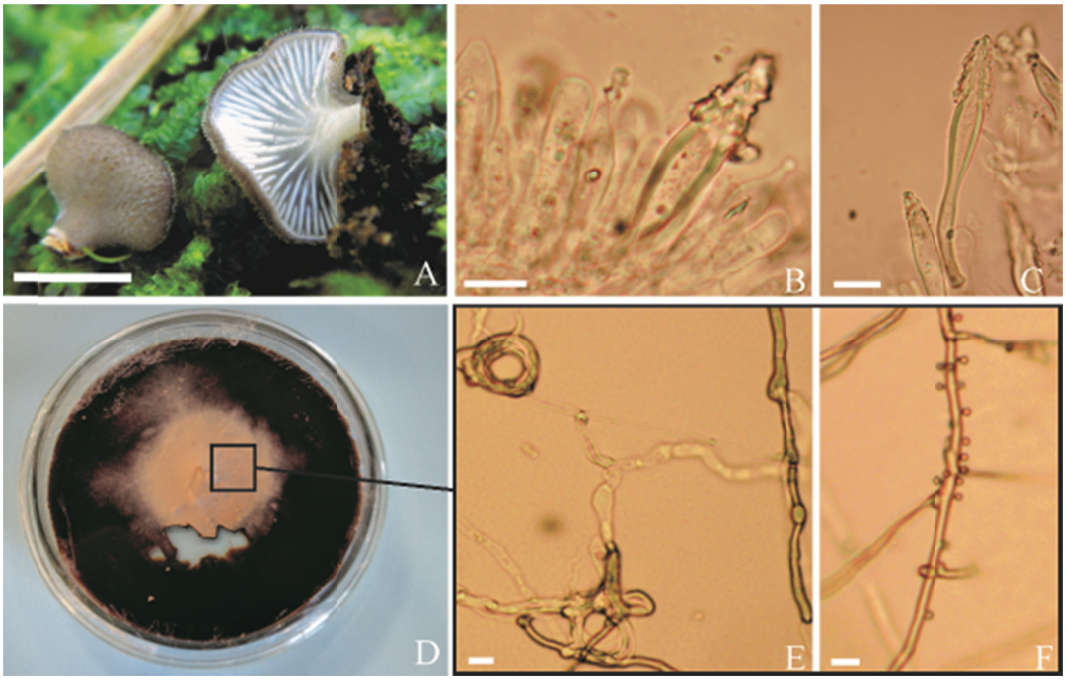
Appendix 2. 528-*H. bullulifera*; A: basidioma; B: spores; C: metuloids; D: queilocistidios; E: mycelium; F: chlamydosporo; G: fibula; H: traps. Escale: A: 5 mm; B, C, D, F, G, H: 10 µm



Appendix 3. 581-*H. paraguayensis*; A: basidioma; B: spores; C: metuloids; D: queilocistidios; E: mycelium; F: adhesive cell; G: fibula; H: not identified structures. Escale: A: 5 mm; B, C, D, F, G, H: 10 µm



Appendix 4. 631-*H. portegna*; A: basidioma; B: spores; C: metuloid; D: contexto trama; E: mycelium; F: adhesive cell; G: fibula; H: not identified structures. Escale: A: 5 mm; B, C, D, F, G, H: 10 µm



Appendix 5. 582-*H*. sp.; A: basidioma; B: metuliod; C: queilocistidios; D: mycelium; E: trap, chlamydosporo and fibula; F: not identified structures. Escale: A: 5 mm; B, C, E, F: 10 µm