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Biological control of *Meloidogyne javanica* in bean plants by *Hohenbuehelia* spp. and *Trichoderma koningiopsis*

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ABSTRACT

Nematode control strategies are limited and alternative control is demanded. The main aim was to evaluate control potential of *Hohenbuehelia* spp. (*Hohenbuehelia mastrucata*, *Hohenbuehelia barbatula*, *Hohenbuehelia bullulifera*, *Hohenbuehelia portegna*, *Hohenbuehelia petaloides* and *Hohenbuehelia paraguayensis*) and *Trichoderma koningiopsis* against *Meloidogyne javanica* in bean plants in greenhouse. The parameters evaluated were the number of galls and egg masses which were evaluated in three root regions: basal, intermediate and tip; nematode reproduction factor; vegetal growth parameters (plants height on V1, V2, V4, and R5 growth stages, root length, dry weight of root and aerial part dry weight); Treatments were composed by individual interactions of each fungal isolate with 4,000 eggs of *M. javanica*, having a total control (bean) and a partial control (bean + nematode) arranged in completely randomized design with four repetitions. Data were tested by Scott–Knott (5%). For vegetal growth parameters, there was only significance for dry weight of roots. All species were able to control nematodes.

Keywords: nematodes. alternative control. anamorphic. phytopathology.

INTRODUCTION

Meloidogyne genus, known as root gall nematode, covers polyphagous species spread all over the soils and adapted to Brazilian regions (MACHADO, 2014). There are over 90 species, especially *Meloidogyne arenaria*, *Meloidogyne incognita*, *Meloidogyne javanica,* and *Meloidogyne hapla* (FERRAZ, 2018).

Galls are direct symptoms of *Meloidogyne* attack, apart from some cases (FERRAZ; BROWN, 2016). Galls are an expression of a feeding site, the cenocyte, a metabolic drain essential to nematode development (FERRAZ, 2018). Therefore, nematode attack leads to cenocyte formation with eventual, although frequent, galls. Galls may occur early in female maturity (FERRAZ; BROWN, 2016).

As female maturity goes, there is an average deposition of 400 exposed eggs (FERRAZ, 2018), representing the reproductive cycle conclusion. Egg masses counting is studied in many pathosystems for reproduction analysis of *Meloidogyne* spp., as well as to evaluate the reaction of commercial crops (ROSA et al., 2013; CHAVES et al., 2013). No doubt egg masses are much more relevant to consider for epidemiological parameters than galls, besides galls are not an exclusivity of *Meloidogyne* genus (BEDENDO, 2018).

There are several means to control nematodes, classified as chemical, physical, cultural, genetic and biological. Some methods have wider applications while others cannot be applied in some cases. Whenever possible, it is preferred to opt for integrated management (AL-HAZMI; TARIQJAVEED, 2016).

Biological control implies in the use of antagonist microorganisms in the infection site, before or after its occurrence (AGRIOS, 2005), as an important tool to substitute chemical appliance (MORANDI; BETTIOL, 2009).

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Fungi represent the group with the most interesting characteristics to control nematodes, as parasitism (FERRAZ; SANTOS, 1995), enzymes liberation and production of toxic metabolites (MORGAN-JONES; RODRÍGUEZ-KÁBANA, 1985). That means diversified abilities to catch nematodes at any life cycle stage, from eggs to adults (LIU et al., 2009). For that to occur, satisfactory mycelial growth is required to keep fungal in soil (FERRAZ et al., 2010).

Little is known about *Hohenbuehelia* genus in *in vivo* essays, only from PUTZKE et al. (2007). *In vitro* analysis of THORN; BARRON (1984) covered 108 species of *Hohenbuehelia*, from which *Hohenbuehelia atrocaerulea*, *Hohenbuehelia paraguayensis*, *Hohenbuehelia portegna*, *Hohenbuehelia petaloides*, *Hohenbuehelia mastrucata* and *Hohenbuehelia grisea* are endoparasites with nematophagous ability. LUBIAN et al. (2018) verified nematophagous ability for *H. mastrucata*, *H. paraguayensis* and *H. portegna* against *Panagrellus redivivus*.

The main goal was to verify nematophagous potential of *H. mastrucata*, *H. barbatula*, *H. bullulifera*, *H. paraguayensis*, *H. portegna*, *H. petaloides* and *Trichoderma koningiopsis* against *M. javanica*, in bean plants in greenhouse.

MATERIALS AND METHODS

The following isolates were tested: 436 – *H. mastrucata*, 461 – *H. barbatula*, 528 – *H. bullulifera*, 581 – *H. paraguayensis*, 631 – *H. portegna*, *H. petaloides* and TLB17 *T. koningiopsis*, all were recovered from Castellani technique.

Symptomatic okra roots were submitted to the nematode extraction process, according to JENKINS (1964). The specie was analyzed and identified as *M. javanica* (CHITWOOD, 1949). Nematodes number was calibrated in Peter Chamber at 4,000 eggs/J2 for inoculation. Eggs were added in soil five days after seedling transplant.

In vitro colonization of *Hohenbuehelia* spp. (preliminary test)

The matrix colony of each isolate, 436, 461, 528, 581, 631 and *H. petaloides* was growing on malt extract agar (MEA) from which 10 agar plugs (3 mm diameter) were removed and transferred to the center of new Petri plates containing MEA, for recolonization and purification. The plates were then sealed with parafilm and stored into bio-oxygen demand (BOD) chamber at 23.3 °C, without light. Each treatment had five repetitions disposed in a completely random design.

The new colonies were evaluated for mycelial growth efficiency (speed and extent). Two measures (cm) were taken from diametrically opposite directions. Evaluations occurred every other five days during six weeks. Data were run by Tukey's test at 5% error probability using SISVAR 5.6 program (FERREIRA, 2011).

The inocula preparation process considered the mycelia grown in MEA from which 10 plugs (8 mm diameter) were taken and added into a beaker containing 42 g of sterile rice (2h, 120 °C, 1 atm) for the individual colonization of all above-mentioned isolates. The beaker was sealed with aluminum paper and kept into BOD at 25 °C.

Bean seed germination test and seedling colonization

Bean seeds (cultivar IPR Uirapuru), kindly given by IAPAR Institute, were submitted to germination test in accordance to the Seed Analysis Rules (BRAZIL, 2009).

To guarantee root colonization, each bean seed was sowed 2 cm deep together with three rice grains fully colonized (quantity set from preliminary test) in trays containing commercial substrate.

Meloidogyne javanica control by fungi isolates and growth promotion in bean plants

The species *H. mastrucata*, *H. portegna*, *H. petaloides*, *H. paraguayensis* and *T. koningiopsis* previously purified on MEA and grown in sterile rice grains were added in trays in the first place, being separated from bean seeds by a thin layer of substrate. Passed 13 days from emergence, seedlings were transplanted to 3 L vases (one seedling per vase). Vases were covered with hay autoclaved for two hours (120 °C, 1 atm).

The vases were filled with eutroferric red latosol soil (SANTOS et al., 2018) and sand (2:1, respectively), besides 45 g of sawdust. Around 10 soil samples were randomly taken from vases and turned into a composed soil sample which was submitted to chemical analysis and interpretation, being fertilized accordingly.

The experiment was set in a completely random design with five repetitions conducted in a greenhouse. The treatments were labeled as: T1 – *M. javanica* + *H. mastrucata*; T2 – *M. javanica* + *H. paraguayensis*; T3 – *M. javanica* + *H. portegna*; T4 – *M. javanica* +

H. petaloides; T5 – *M. javanica* + T. *koningiopsis*; T6 – partial control with only *M. javanica* and T7 – absolute control without treatment and pathogen.

For growth promotion, plants height at V1, V3, V4, and R7 stages were measured. Root evaluations were done 82 days after nematodes inoculation, at R8 stage, considering egg masses and galls counting, dry weight of roots, dry weight of the aerial part and root length.

Roots were detached from the aerial part in the greenhouse, packed and identified. In laboratory, they were submitted to triple washing in water, dried in absorbent paper and placed into dye solution (1 mL fuchsine + 30 mL distilled water) for 60 min to get egg masses exposition. Regarding to the counting, each root was separated into three different parts: basal, intermediate and the extremity. Hence, fragments of the respective parts were analyzed using magnifying eyes (4 ×). Roots were entirely analyzed. Afterwards, dry roots were weighted and submitted to nematode extraction (JENKINS, 1964). Eggs were counted in Peters chamber and used to set the reproduction factor of *M. javanica*, in accordance to the methodology of FERRAZ (1996).

Temperature and humidity readings were taken daily using a thermohygrometer.

For statistics, data were analyzed by Scott–Knott test at 5% of error probability through SISVAR 5.6 program (FERREIRA, 2011).

RESULTS

The best mycelial growth was set for *H. barbatula* and *H. mastrucata* which, in seven days, reached 9.0 cm (full plate), followed by *H. petaloides* with 3.07 cm. The other species did not reach over 1.0 cm of growth in seven days (Table 1).

Table 1. Diameter of mycelial growth (cm) of *Hohenbuehelia* species cultivated in Malt Extract Agar stored into BOD at 25 °C for 42 days.

Species	7 days	14 days	21 days	28 days	35 days	42 days
H. barbatula	9.00e	9.00e	9.00d	9.00d	9.00d	9.00e
H. bullulifera	9.00a	0.84a	1.48a	2.16a	2.67a	3.47b
H. mastrucata	9.00e	9.00e	9.00d	9.00d	9.00d	9.00e
H. petaloides	3.07d	4.71d	5.54c	5.89c	6.08c	6.7d
H. portegna	0.91c	1.34c	2.35b	9.09b	3.85b	4.44c
H. paraguayensis	0.74b	1.08b	1.58a	1.95a	2.24a	2.41a
C.V. (%)	3.27	4.84	6.07	7.68	10.14	12.8
General Media	4.54	4.33	4.83	6.18	5.47	5.84

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

As soon the vegetative development of the species was known the experiment schedule was properly set, having the inocula in the required quantity in young age according to the condition of each specie, such as growth speed.

The species *H. mastrucata*, *H. paraguayensis* and *H. portegna* had higher values for root expansion, although did not differ from the treatment inoculated with *M. javanica* (Table 2), this last due to the excess of galls formation (Table 3).

Table 2. Influence of the treatments on dry weight of bean roots (DMR).

Treatments	DMR
Absolute control	0.35a
H. mastrucata	0.55b
H. paraguayensis	0.59b
H. petaloides	0.26a
H. portegna	0.45b
T. koningiopsis	0.25a
Partial control	0.54b
C.V. (%)	38.35
General Media	0.43

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

Table 3. Counting of galls from the base (GB), intermediate (GI), extremity part of root (GE) and total galls (TG) for each treatment with the respective reduction indexes for each part of the root, compared to the partial control (nematode).

Treatments	GB	Reduction (%)	GI	Reduction (%)	GE	TG	Reduction (%)
Absolute control	0.00a	*	0.00a	*	0.00a	0.00a	*
H. mastrucata	51.33a	↓ 86.72	23.33a	↓ 71.07	10.00a	84.66a	↓ 81.42
H. paraguayensis	54.00a	↓ 86.03	26.33a	↓ 67.36	19.00a	99.33a	↓ 78.20
H. petaloides	97.00a	↓ 74.91	27.00a	↓ 66.53	6.66a	130.66a	↓ 71.32
H. portegna	79.33a	↓ 79.48	34.00a	↓ 57.84	19.66a	126.33a	↓ 72.27
T. koningiopsis	287.00b	↓ 25.77	72.66b		11.66a	309.00b	↓ 32.18
Partial control	386.67c		80.66b		29.66a	455.66c	
C.V. (%)	26.38		81.07		79.72	41.51	
General Media	136.48		37.71		13.81	172.23	

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

Plants heights measured at V1, V3, V4, and R5 stages did not differ among treatments, it is to say that none of them promoted plant growth. Also, there was no difference for the dry weight of the aerial part and root length.

Treatments differed for the counting of basal galls, intermediate galls and total galls (Table 3). Analyses of galls in the extremity of the roots ended up with no difference, suggesting that fungal colonization varied significantly, possibly due to the lower aeration as depth goes or due to the shorter time fungi had to get established in roots before nematode inoculation.

Data showed great ability of *Hohenbuehelia* species to reduce galls formation of *M. javanica* in bean roots, followed by *T. koningiopsis*. This last was only efficient to the basal roots.

Fungal activity over egg masses reduction had a similar tendency with relevance only for egg masses on basal part and total egg masses (Table 4). The best reduction percentuals in the basal part of the root were reached by *H. mastrucata*, *H. portegna* and *H. petaloides* at rates of 74.80, 70.68 and 65.41%, respectively.

Table 4. Counting of egg masses from the base (EMB), intermediate (EMI), extremity part of root (EME) and total egg masses (TEM) for each treatment with the respective reduction indexes for each part of the root compared to the partial control (nematode).

Treatments	EMB	Reduction (%)	EMI	EME	TEM	Reduction (%)	Reduction (%)
Absolute control	0.00a	*	0.00a	0.00a	0.00a	*	*
H. mastrucata	137.50b	↓ 74.80	61.75a	36.00a	235.25b	↓ 66.39	↓ 81.42
H. paraguayensis	329.75c	↓ 39.56	81.25a	39.75a	450.75c	↓ 35.60	↓ 78.20
H. petaloides	188.75b	↓ 65.41	77.50a	14.75a	281.00b	↓ 59.85	↓ 71.32
H. portegna	160.00b	↓ 70.68	68.25a	48.50a	276.75b	↓ 60.46	↓ 72.27
T. koningiopsis	278.50c	↓ 48.96	66.00a	14.75a	359.25b	↓ 48.68	↓ 32.18
Partial control	545.75d		110.75a	43.50a	700.00d		
C.V. (%)	44.06		90.92	109.01	45.91		
General Media	234.32		66.50	28.18	329.00		

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

Figure 1 indicates that galls quantity is not proportional to egg masses quantity once there were galls without egg mass formation (A), one egg mass per gall as well as four egg masses per gall (B) and finally a huge gall from basal part containing over 10 galls (C).

Related to the eggs number extracted from the roots, it is seen that the best treatment was *H. mastrucata* alike the absolute control outcome (Table 5). In sequence, the species *H. paraguayensis*, *H. petaloides* and *H. portegna* stood out. A third level was formed by *T. koningiopsis*. Finally, the treatment containing only nematodes, showed the highest eggs number and, consequently, the highest reproduction factor, as expected.

Root dry weight results did not have relation with the performance of the treatments to control nematodes, especially for *H. petaloides* (Table 2).

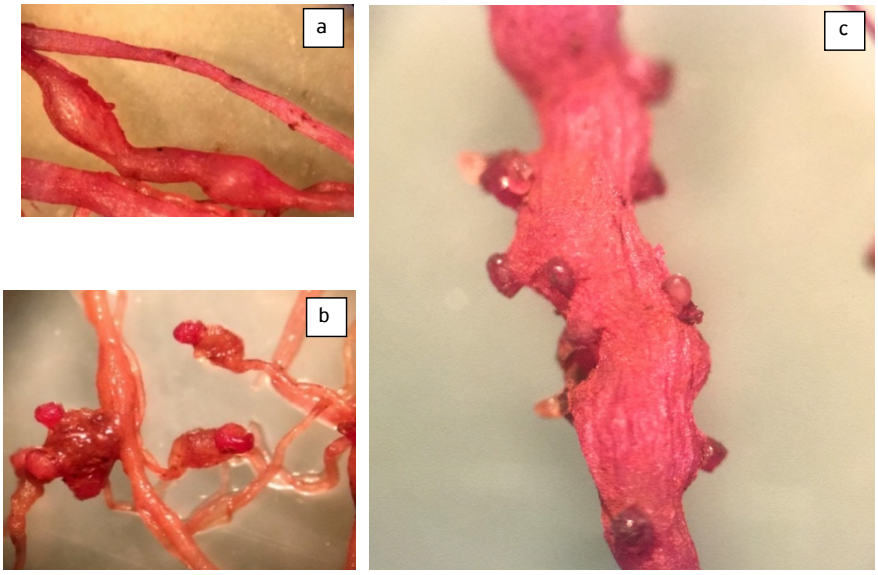

Figure 1. Indirect relation between galls and egg masses of *M. javanica* in bean roots. a = galls without egg masses. b = two galls with one egg mass each and one gall containing four egg masses. c = one big gall with 10 visible egg masses.

Table 5. Eggs number, reproduction factor (RF) of *M. javanica* and reduction percentual of eggs by each treatment compared to the partial control (nematode).

Treatments	Eggs number	RF	Reduction (%)
Absolute control	0.00a	*	
H. mastrucata	1644.50a	0.41	↓84.86
H. paraguayensis	3845.36b	0.96	↓64.60
H. petaloides	4103.46b	1.03	↓62.22
H. portegna	3021.63b	0.76	↓72.18
T. koningiopsis	5900.99c	1.48	↓45.67
Partial control	10862.20d	2.72	
C.V. (%)	39.64	0.66	
General Media	4896.36	1.23	

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

DISCUSSION

According to FERRAZ et al. (2010), an abundant mycelial growth is required for fungal introduction in soil for biological control to succeed. However, *in vitro* analysis showed no relation between mycelial growth of *Hohenbuehelia* species and their control over nematodes (THORN; BARRON, 1986; LUBIAN et al., 2018). The present work also confirms this nonrelation once the general effect of the treatments (Tables 3 and 4) had no direct relation to the mycelial growth, having *H. portegna* as an example (Table 1).

Dry weight of the aerial part was not affected by any treatment (data not shown). However, some stimuli on bean was expected, especially due to reports related to *Trichoderma* species. In the same way, there was no promotion on bean growth (SANTIN, 2008; AGUIAR et al., 2014). PUTZKE et al. (2007) verified reduction on fresh matter of tomatoes treated with *H. paraguayensis* compared to *H. portegna*.

Researches on biocontrol of nematodes generally consider the introduction of the antagonist agent before transplanting the plant host (DALLEMOLE-GIARETTA et al., 2008; AL-HAZMI; TARIQJAVEED, 2016), resulting in a longer interaction to reduce nematode inoculum. It is an advantage for ovicidal antagonists, such as *Trichoderma* (SZABÓ et al., 2012) and *Pochonia chlamydosporia* (NUNES et al., 2010). In this study, fungi were added during sowing, having 19 days to establish in the substrate and roots before the addition of *M. javanica*.

About the galls, SANTIN (2008) reported less expression in bean treated with *Trichoderma harzianum*, while SHARON et al. (2001) verified an index of 0.5 galls in tomato treated with a savage isolate of *T. harzianum*. Also, *T. koningiopsis* presented the ability to reduce galls formation (Table 3). Related to this, VINALE et al. (2008) cites the existence of *Trichoderma* strains with distinct effectiveness. Likewise, AL-HAZMI; TARIQJAVEED (2016) checked different performances among species, justified by pathogenic ability of the isolates or their origin.

In respect of galls formation and the occurrence of eggs and juveniles in bean roots, BORGES et al. (2013) checked similar results for the control treatment inoculated with *M. incognita* only and for plants treated with *Trichoderma* sp. In fact, *T. koningiopsis* did not present the best role to reduce galls formation in bean roots (Table 3) but it differed from the treatment inoculated with *M. javanica*, at least.

By analyzing the reduction percentual of galls and egg masses of *M. javanica* (Tables 3 and 4), it was verified that *H. mastrucata*, *H. portegna* and *H. petaloides* presented control potential superior to many commercial products of antagonists, such as Rizotec in cucumber (VIGGIANO et al., 2014), Rizotec and Onix SC + Quality WG in tomato (CARVALHO, 2017), Nemix TS in soybean (NUNES et al., 2010), Trichodermil SC 1306 and Quality in bean (AGUIAR et al., 2014), revealing the potential for commercial use, especially remembering that these results were obtained with low dosage of inocula.

In the pathosystem *M. incognita* × lettuce, MARINO; SILVA (2013) confirmed the efficiency of four isolates of *Pleurotus osteatrus*, a genus belonging to the same family as *Hohenbuehelia*. According to them, all isolates reduced galls and egg masses indexes compared to the control, ranging from 74 to 89% for galls number and from 87 to 98% for egg masses formation. Their results are very similar to *Hohenbuehelia* spp. performance, which varied from 74.9 to 86.72% for galls and from 39 to 74.8% for egg masses, except for *H. paraguayensis* for both parameters.

Table 5 reveals that a high reduction in galls does not imply necessarily in the same reduction for egg masses, having *H. paraguayensis* as an example. Egg masses have an undeniable impact on epidemiology once they lead to higher nematode population for the next cycles (MARINO; SILVA, 2013). PIRES et al. (2016) also testified the same situation expressed in Figure 1 in bean plants infected by *M javanica*. According to them, the cultivars which presented higher galls number were not the same that expressed higher number of egg masses.

On the other hand, no matter what crop or biocontrol agent it is, there is a strong relation between egg masses number and galls expression (LOPES et al., 2007; PUTZKE et al., 2007; SANTIN, 2008; DALLEMOLE-GIARETTA et al., 2008; NUNES et al., 2010; MACHADO et al., 2013; PINHEIRO et al., 2013).

Reproduction factor (RF) is about the relation of final and initial population of nematodes, indicating their reproduction index throughout the growing stages of a given plant specie. FERRAZ (1996) adapted a scale of RF indexes to set reductions varying from 0 to 25% which classifies a cultivar as highly susceptible, from 26 to 50% as susceptible, from 51 to 95% as quite resistant and over 95% as resistant. In this context, the bean treated with *T. koningiopsis* ended up in a susceptible reaction, while the bean plants treated with *Hohenbuehelia* species were quite resistant. The treatment of *M. javanica* expressed the highest index of nematode reproduction (Table 5).

Results suggest that *Hohenbuehelia* species have a quick action over nematodes whose fast control has been already identified by LUBIAN et al. (2018).

CONCLUSION

All species tested presented biological control potential against *Meloidogyne javanica* in bean plants at some extent.

AUTHORS' CONTRIBUTIONS

Conceptualization: Stangarlin, J.R. **Data curation:** Lubian, C. **Formal analysis:** Agustinha, A.M. **Investigation:** Lubian, C. **Methodology:** Portz, R.L. **Supervision:** Kuhn, O.J. **Writing – original draft and Writing – review & editing:** Lubian, C.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this published article.

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CONFLICTS OF INTEREST

The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

ETHICAL APPROVAL

Not applicable.

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