

# Microorganisms in the biological control of root-knot nematode: A metanalytical study

Microrganismos no controle biológico do nematoide das galhas das raízes: um estudo metanalítico

Microorganismos en el control biológico de nematodos de agallas de raíces: un estudio metanalítico

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## Abstract

Nematodes can cause annual losses in the order of 100 billion dollars in crops worldwide. Its control using chemical nematicides proves to be quite aggressive to the environment. For this reason, the management of microorganisms has been promising. However, it is essential to know the control potential of each organism. Thus, the objective of this study was to verify the efficiency of different microorganisms in the biological control of *Meloidogyne* sp. A systematic review of the literature was carried out from 2000 to 2020 with the keywords “*Meloidogyne* and biology control”, resulting in 659 articles, of which 51 were pre-selected and, after the more detailed evaluation, was selected ten published articles. These ten articles generated a total of 83 studies for meta-analyses. Each study included a treatment group using some microorganisms (bacteria, fungus, actinomycetes) for nematode biocontrol, a control group without using biocontrol agents. From this meta-analysis, we can observe that the use of microorganisms decreased the number of galls (42.05%), the number of eggs (57.77%), the gall index (28.58%) and the eggs mass (53.48%). The use of microorganisms was also positive in increasing root mass (832.89%). We can conclude that the use of microorganisms proved to be efficient in controlling nematodes *M. javanica* and *M. incognita*. The fungi *Pleurotus ostreatus* and *Phanerochaete chrysosporium* have more significant potential for biocontrol for these species.

**Keywords:** *Meloidogyne incognita*; *Meloidogyne javanica*; Efficient microorganisms; Meta-analysis.

## Resumo

Os nematóides podem causar perdas anuais da ordem de 100 bilhões de dólares em safras em todo o mundo. O controle do nematoide com nematicidas químicos mostra-se bastante agressivo ao meio ambiente. Por esse motivo, o controle por microrganismos tem se mostrado promissor, porém é importante conhecer o potencial de controle de cada microrganismo. Assim, o objetivo deste estudo foi verificar a eficiência de diferentes microrganismos no controle biológico de *Meloidogyne* sp. Foi realizada uma revisão sistemática da literatura de 2000 a 2020 com as palavras-chave “*Meloidogyne* and biology control” resultando em um total de 659 artigos, dos quais 51 foram pré-selecionados e, após avaliação mais detalhada, foram considerados 10 artigos publicados. Um total de 83 estudos foram considerados para meta-análises, com cada estudo incluindo um grupo de tratamento que consistia no uso de algum microrganismo (bactérias, fungos, actinomicetos) para o biocontrole de nematóides e um grupo controle sem o uso de agentes de biocontrole. A partir dessa meta-análise pode-se verificar que o uso de microrganismos diminuiu o número de galhas (42,05%), o número de ovos (57,77%), o índice de galhas (28,58%) e a massa dos ovos (53,48%). O uso de microrganismos também foi positivo no aumento da massa radicular (832,89%). Pode-se concluir que o uso de

microrganismos se mostrou eficiente no controle dos nematóides *M. javanica* e *M. incognita*. Os fungos *Pleurotus ostreatus* e *Phanerochaete chrysosporium* apresentam maior potencial de biocontrole para essas espécies.

**Palavras-chave:** *Meloidogyne incognita*; *Meloidogyne javanica*; Microrganismos eficientes; Meta-análise.

## Resumen

Los nematodos pueden causar pérdidas anuales del orden de 100 mil millones de dólares en cultivos en todo el mundo. Su control mediante nematocidas químicos resulta bastante agresivo para el medio ambiente. Por esta razón, el control de microorganismos ha sido prometedor, sin embargo, es importante conocer el potencial de control de cada microorganismo. Así, el objetivo de este estudio fue verificar la eficiencia de diferentes microorganismos en el control biológico de *Meloidogyne* sp. Se realizó una revisión sistemática de la literatura desde 2000 hasta 2020 con las palabras clave “*Meloidogyne* y control biológico” dando como resultado un total de 659 artículos, de los cuales 51 fueron preseleccionados y, tras la evaluación más detallada, se consideraron 10 artículos publicados. Se consideraron un total de 83 estudios para metanálisis, y cada estudio incluyó un grupo de tratamiento que consistió en el uso de algún microorganismo (bacterias, hongos, actinomicetos) para el control biológico de nematodos y un grupo de control sin el uso de agentes de control biológico. De este metaanálisis se puede observar que el uso de microorganismos disminuyó el número de agallas (42,05%), el número de huevos (57,77%), el índice de agallas (28,58%) y la masa de huevos (53,48%). El uso de microorganismos también resultó positivo en el aumento de la masa radicular (832,89%). Se puede concluir que el uso de microorganismos resultó ser eficaz en el control de nematodos *M. javanica* y *M. incognita*. Los hongos *Pleurotus ostreatus* y *Phanerochaete chrysosporium* tienen un mayor potencial de biocontrol de estas especies.

**Palabras clave:** *Meloidogyne incognita*; *Meloidogyne javanica*; Microrganismos eficientes; Metaanálisis.

## 1. Introduction

Nematodes consist of soil pathogens that affect diverse cultures worldwide (Coyne et al., 2018; Vos et al., 2012; Wesemael et al., 2011). The losses caused by these organisms in crops reach \$ 100 million per year (Coyne et al., 2018; Fosu-Nyarko & Jones, 2015). The genus *Meloidogyne* sp., known as root-knot nematode, is one of the principal genera of nematodes causing problems in crops, being distributed by the principal producing regions of the world, being the species *Meloidogyne javanica* and *Meloidogyne incognita* as most frequent (Sikora et al., 2008). The reduction in production due to the attack of this pathogen is dependent on several factors, such as climate, soil (Godefroid et al., 2017; Griffiths et al., 2002; McSorley et al., 2008; McSorley & Frederick, 2002) beyond the level of tolerance or resistance of the host plant (Ibrahim et al., 2019; Tsai et al., 2019). However, there are reports that losses due to *Meloidogyne* sp. parasitism can reach up to 100% in cases with no efficient management (Wesemael et al., 2011).

Some control measures are used in the manage these organisms, aiming at maintaining populations at low levels while maintaining the viability of the area. The first option generally used is chemical control with nematicides. However, due to the growing concern with environmental impacts, several effective chemical nematicides in the management of nematode have been restricted worldwide (Hol & Cook, 2005; Schouteden et al., 2015). The search for alternative forms of controls, including the use of biological control organisms, has been gaining prominence in the international scenario in recent decades (Harrier & Watson, 2004; Ruanpanun et al., 2010; Strom et al., 2020; Wesemael et al., 2011).

Many microorganisms are known to influence the life cycle of nematodes in the soil (Schouteden et al., 2015; Sikora et al., 2008; Vos et al., 2012; Watson et al., 2020). Among these microorganisms, we have bacteria (Chiellini et al., 2019; Fernandes et al., 2013), fungi (Du et al., 2020; Sohrabi et al., 2020; Wei et al., 2009) and actinomycetes (Nimnoi & Ruanpanun, 2020) that have the ability to prey or parasite species of nematodes. These microorganisms are common in the soil and can be antagonistic to nematodes in several ways, for example, through specialized structures for the capture of nematodes, such as constricting rings, three-dimensional networks of hyphae and adhesive structures (Lopes et al., 2007). Another form of nematode biocontrol is through the release of toxic compounds in the soil solution with nematostatic potential (Huang et al., 2020; Liu et al., 2020) or employing aqueous extracts of substrates for the cultivation of fungi which produce substances with toxic potential during mycelial growth (Hu et al., 2019; Roberts et al., 2005). The toxin-saturated substrate is also an alternative for nematode control (Moazezikho et al., 2020).

Several scientific studies have been conducted to determine the potential of microorganisms in controlling different species of nematodes (Alvarado-Herrejón et al., 2019; Harrier et al., 2012; Vos et al., 2012). To expose the state of the art of scientific research related to the biological control of *Meloidogyne* spp. by soil microorganisms acting as biocontrol agents, a survey of the international and national panorama of publications regarding the management of *Meloidogyne* sp. by microorganisms, which include antagonistic, predatory, and parasitic organisms. Among the various species found in the literature, we can mention *Trichoderma* sp. (Affokpon et al., 2011), *Pochonia* sp. (Hu et al., 2019; Wesemael et al., 2011), *Bacillus* sp. (Beeman et al., 2019; Fernandes et al., 2013; Mazzuchelli et al., 2020; Zhao et al., 2018), *Paecilomyce* sp. (Eapen et al., 2005; Fernandes et al., 2014; Huang et al., 2020), *Pseudomonas* sp. (Moazezikho et al., 2020; Sohrabi et al., 2018), among others. We hypothesize that we can use efficient microorganisms as biocontrol agents for *Meloidogyne* sp.

Using the meta-analysis tool, we seek to identify in the world literature evidence of microorganisms in the biological control of root-knot nematodes. This knowledge becomes essential because it presents itself as an economically viable and ecologically sustainable alternative to control this soil-borne pathogen.

This work aimed to verify, through the meta-analysis tool, different microorganisms with potential for the biocontrol of *Meloidogyne* sp.

## 2. Methodology

### *Classification of research*

We classified the research as descriptive-exploratory. Using data of literature was described the effect of microorganisms such as control agents of root-knot nematode. We used scientific articles as a source of information. It works is classified as a bibliographic as for technical procedures (Silveira & Córdova, 2009).

### *Systematic review and data collection*

The systematic review consisted of searching scientific articles in Science Direct and Scopus databases during May 2020. The words "*Meloidogyne* AND biological control" was used in the databases. A total of 659 publications were found between the years 2000 and 2020. The initial selection was made based on the title and summary and was selected 51 publications. Subsequently, we have carried out a more detailed analysis of the works using the following criteria: (i) articles written in English or Portuguese; (ii) studies that presented the mean and a dispersion measure as a coefficient of variation, standard error, or standard deviation; (iii) the studies should have been conducted in a greenhouse; (iv) only biological control treatments; (v) studies should report results on the control of *Meloidogyne* sp. The response variables considered in this work were: number of galls, number of eggs, gall index, egg mass and root mass. The final number of articles was reduced to 10, resulting in 83 studies (Table 1).

We excluded publications characterized as bibliographic reviews or articles that did not relate the direct interaction of the microorganism with the nematode. Publications that presented data from the combined application of two microorganism species were considered a study for the combined treatment. There was no restriction on the host plant considered at the time of the selection of works. For the cases of microorganism surveys, we considered a study for each species of microorganism associated with a species of nematode (Figure 1).

**Table 1** - List of selected articles for the meta-analysis.

Author	Periodical
Affokpon et al. (2011)	Soil Biology e Biochemistry 43, 600-608
Du et al. (2020)	Plos One 13, 1-14
Fernandes et al. (2013)	Trópica – Ciências Agrárias e Biológicas 7(1), 76-81
Kiwnick & Sikora (2009)	Biological Control 38, 179–187
Moazezikho et al. (2020)	Egyptian Journal of Biological Pest Control 30(15), 1-8
Nimnoi e Ruanpanun (2020)	Biological Control 145, 1-8
Soharabi et al. (2020)	Indian Phytopathology

Source: Authors.

### Statistical analysis

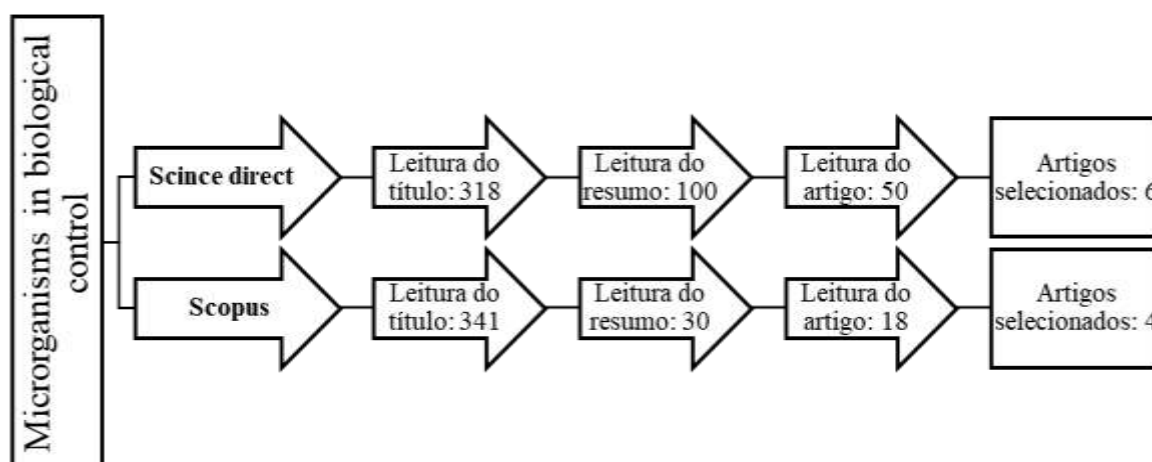
To estimate the effects of microorganisms on the root-knot nematode was used the natural logarithm (ln) of the ratio between the mean of the treatment group and the mean of the control group as an effective measure for analysis (Hedges et al., 1999) and calculated according to the equation:

$$\ln R = \ln\left(\frac{X_t}{X_c}\right)$$

Where:  $X_t$  is the mean of the treatment group, and  $X_c$  is the control group's mean. We used logarithmic transformation to balance the positive and negative effects and maintain symmetry in the analysis, especially when the data show discrepancies (Rosenberg et al., 2000).

Assuming that the methods and characteristics of the sample are different and can introduce variability between the real effects, we used a random-effects model, as suggested by (Viechtbauer, 2010). An analysis of the mixed-effects model (variables or moderators at the study level) was considered based on the assumption of random variation in effect size between studies. Thus, it may be responsible for at least part of the heterogeneity of the real effects (Viechtbauer, 2010).

**Figure 1** - Methodology for searching and selecting articles for the meta-analysis.



Source: Authors.

Negative effects indicate an increase in the control index of the treatment with the microorganism compared to the control. Positive values indicate that there is no control by the treatment compared to the control. If a 95% confidence interval

does not overlap with zero, it will be considered a significant response to treatments. All analyses it was performed using the metafor package version 2.4 in RStudio version 1.2.5033 (Higgins &Thompson, 2002).

For a more practical interpretation of the result, we transformed the lnRR value into a response percentage (%) (Dai et al., 2020; Hou et al., 2020; Zeffa et al., 2018):

$$R = (e^{\ln RR} \times 100) - 100 \quad \text{Equation 2}$$

### ***Heterogeneity and moderating variables***

The heterogeneity of the data was quantified using  $I^2$ . This index describes the percentage of the total variability of the studies and compares the meta-analysis of different types and sizes of studies with different results and effect measures (Higgins &Thompson, 2002). The  $I^2$  confidence interval and its significance at 95% probability were also verified. The percentages of 25, 50 and 75% indicate low, moderate, and high heterogeneity, respectively (Higgins & Thompson, 2002). If the heterogeneity test is significant, we assess the variance and contribution of each study to decide whether to continue the meta-analysis.

In cases where the heterogeneity was high, it performed a meta-analysis of the subgroups to incorporate one or more moderating variables responsible for at least part of the heterogeneity between the effect size. It considered three effect moderators: (i) type of microorganism used in biological control (for example, bacteria or fungi); (ii) species of nematode used in the study (*Meloidogyne javanica* or *M. incognita*); and (iii) the species of microorganism used in biological control (for example, *Bacillus* sp. or *Pleurotus ostreatus*).

It analyzed the estimates produced by the meta-analysis and their respective confidence intervals (95% CI) in forest plot graphs. It considered the effect size significant when the confidence interval did not overlap to zero. The metafor (Viechtbauer, 2010) and metaviz (Kossmeier, et al., 2020) packages were used for the analysis and visualization of results, respectively, using the RStudio version software (R Core Team, 2020).

### ***Publication bias and sensitivity analysis***

The data were analyzed through a funnel plot to verify whether the literature review was subject to publication bias (Egger et al. 1997). The trim and fill method (Duval &Tweedie, 2000a; Duval & Tweedie, 2000 b; Duval, 2005) was used to estimate the number of studies potentially missing from a meta-analysis to the suppression of the most extreme studies on one side of the funnel graph. This method demonstrates how the total size of the summary effect would change if the apparent bias removed.

We carried out a sensitivity analysis to evaluate the variance and the contribution of each study to the total size of the effect (Duval, 2005). The studies that showed significant variance and low contribution compared to others in the data set were removed one at a time, and the meta-analysis redone. This analysis shows how much the effect size changes in the absence of the removed study.

## **3. Results and Discussion**

Initially, the study raised 51 continuous articles. After the exclusion criteria, it was considered ten articles for the meta-analysis. The articles were generated in 6 countries, with five species of host plants (Table 2) between 2000 and 2020. A total of 83 studies (n) were analyzed. Each study included a treatment group with a microorganism (bacteria, fungus, actinomycete or a mixed treatment composed of bacteria and fungi) and a control group. The variables presented were the number of galls (n = 29), galls index (n = 51), number of eggs (n = 61), eggs mass (n = 43) and root mass (n = 43).

**Table 2** - Number of studies and type of host plant by country.

Country	N. of article	N. of studies	Host plant
Germany	1	10	Tomato
Belgium	2	36	Tomato
Brazil	3	8	beans, tomato, lettuce
China	1	5	Tomato
Iran	2	21	potato, tomato
Thailand	1	3	pepper
Total	10	83	5

Source: Authors.

The inoculation period of the microorganisms did in a single application in pre-planting. The cultivation time of the plants in the greenhouse varied from 30 to 112 days.

### 3.1 General meta-analysis

The control of the root-knot nematode from the inoculation of efficient microorganisms such as bacteria and fungi proved to be positive for all variables. We can observe that the results (estimate  $\pm$  standard error) for the number of galls and eggs, galls index and eggs mass are significant. These results showed an effect of the treatment compares to the control, reducing the damage of the pathogen. As for the mass variable, we can see that the presence of microorganisms contributed to the increase in the root mass of the host plant. In an epilogue, we can observe the effect of the treatments in all the variables analyzed.

We observed that the heterogeneity was highly significant for the complete set of data by the Cochran test (Cochran, 1954) for all variables analyzed (Table 3). Quantifying the extent of heterogeneity between studies is extremely important, as it can influence the conclusions of the meta-analysis (Higgins & Thompson, 2002). According to Borenstein et al. (2009), whenever this heterogeneity is identified, it can be incorporated into the statistical model through a meta-analysis of the effect or explained, at least partially, by the subgroup method.

**Table 3** - Heterogeneity of the complete data set, assessed by the Cochran test.

Variable	Heterogeneity test			
	Q	df	P	I <sup>2</sup> %
Galls	267,46	28	<01	89,53
Eggs	173250,56	60	<01	99,97
Galls index	243,36	50	<01	79,45
Eggs mass	2104,6	42	<01	98
Root mass	77,59	42	<0,1	45,87

Source: Authors.

We performed the meta-analysis by the random effect model to estimate the means and their respective 95% confidence intervals for each effect measure (Borenstein et al. 2009; Gurevitch & Hedges 1999). Subgroup analyzes are models that incorporate one or more moderators (study-specific categorical or continuous covariates) that, in turn, may explain part of the heterogeneity found between the effect of the actual measure (Borenstein et al., 2009). These models aim was to verify the influence of different groups of microorganisms and different species of nematodes in explaining the possible

heterogeneity between the measures of effect. It observed a significantly high heterogeneity for the moderating groups (Table 4).

**Table 4 -** Heterogeneity of biological control microorganisms of root-knot nematodes according to the groups of moderators of the effect.

Moderator / Variable	Galls				Eggs				Eggs mass				Root mass				
	Qe	df	p	I <sup>2</sup>	Qe	df	p	I <sup>2</sup>	Qe	df	p	I <sup>2</sup>	Qe	df	p	I <sup>2</sup>	
Agent	<i>Bacteria</i>	246,8	17	<0,01		231,35	17	<0,01	95.28	102,26	13	<0,01	88.23	18,87	17	<0,01	14.13
	Fungus	1,47	3	0,69		134,48	35	<0,01	0	1286,3	18	<0,01	98.05	7,05	14	<0,01	0
	Actinomycetes	-	-	-		-	-	-	-	182,7	2	<0,01	99.02	15,36	2	<0,01	85.93
	Fungus + bacteria	0	7	10		172409	17	<0,01	0	1,85	6	0,93	0	2,69	6	<0,01	0
Nemat.	<i>M. javanica</i>	251,66	24	<0,01	89.93	173069	24	<0,01	100	105,53	20	<0,01	80.82	31,71	24	<0,01	26.86
	<i>M. incognita</i>	1,47	3	0,69	0	134,48	35	<0,01	76.30	1516	21	<0,01	98.99	37,94	17	<0,01	58.76
Microorganism	<i>Bacillus sp.</i>	53,22	3	<0,01	94.27	141,3	3	<0,01	98.29	-	-	-	-	0,61	3	0,89	0
	<i>Trichoderma sp.</i>	-	-	-		134,48	35	<0,01	76.30	-	-	-	-				
	<i>P.lilacinus</i>	-	-	-		-	-	-	-	16,81	9	0,05	46.81	0,55	9	1	0
	<i>P.ostreatus</i>	1,47	3	0,69	0	-	-	-	-	0,48	3	0,9	0	-	-	-	
	<i>P.fluorescens</i>	19,8	6	<0,01	70	24,25	6	<0,01	75.85	16,07	6	0,01	62.34	6,27	6	0,39	11.60
	<i>P. chrysosporium</i>	-	-	-		-	-	-	-	-	-	-	-	0,2	4	1	0
	<i>P.fluorescens</i>	59,94	6	0,95	90.34	22,84	6	<0,01	79.04	21,85	6	<0,01	74.88	6,21	6	0,4	14.05
	<i>Streptomyces sp.</i>	-	-	-		-	-	-	-	182,65	2	<0,01	99.73	15,36	2	<0,01	85.93
	<i>G.mosseae;B.subtilis;</i>																
<i>T.harzianum</i>	1,57	6	<0,01	0	172409	6	<0,01	100	1,85	6	0,93	99.02	2,59	6	0,86	0	

Source: Authors.

The meta-analysis showed that inoculation of plants with efficient microorganisms generated an estimate (estimate  $\pm$  standard error) of significant decrease ( $p < 0.01$ ) in the number of galls in the root system. The average was 42% ( $L_n = -0.51 \pm 0.06$ ,  $n = 29$ ) in inoculated treatments compared to non-inoculated treatments. The use of microorganisms also showed a positive effect on the number of eggs of the nematode. There was an average reduction in the number of eggs in the root system of 57.77% ( $L_n = -0.80 \pm 0.02$ ,  $n = 61$ ). For gall index, we only found researches with fungi as a biological agent. For this variable, the average reduction was 28.58% ( $L_n = -0.40 \pm 0.05$ ,  $n = 51$ ). When the complete set of data for the egg mass variable was analyzed, the mean reduction was 53.48% ( $L_n = -0.80 \pm 0.08$ ,  $n = 43$ ). For the root mass, the action of the microorganisms was also efficient, generating an average increase in the order of 832.89% ( $L_n = 0.33 \pm 0.05$ ,  $n = 43$ ) (Table 5).

**Table 5** - Effect (%) of the use of microorganisms on the number of galls, eggs, gall index, eggs mass and root mass of the root-knot nematode.

Variable	Effect (%)	Agent			
		Bacteria	Fungi	Fungus + bacteria	Actinomycetes
Galls	- 42,05	- 42,49	- 81,31	- 40,69	
Eggs	- 57,77	- 57,57	- 66,73	- 59,3	
Galls index	- 28,58		- 28,58		
Eggs mass	- 53,48	- 37,64	- 54,05	- 40,54	- 77,47
Root mass	832,89	599,36	456,13	2743,75	65,4

Source: Authors.

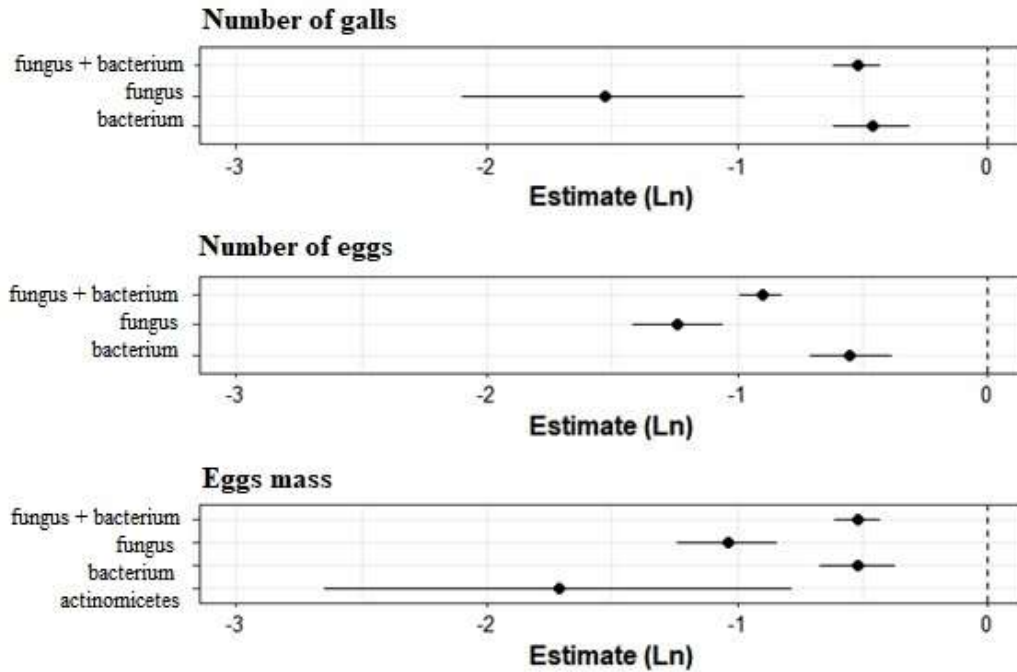
### 3.2 Biocontrol Agent

The magnitude of the responses varied among the microorganisms studied. As there were different species of microorganisms within each group of agents analyzed, the data set was divided into subgroups (moderators) to explain the heterogeneity between the studies. The first moderator group studied was the agent used in biocontrol. We analyzed studies where the agents used in biocontrol were bacteria, fungi, actinomycetes and the mixture between bacteria and fungi.

It observed that the use of bacteria decreased the number of galls by 42.49%, while with fungi, the decrease was 81.31%, with the greatest effect obtained for this variable ( $L_n = -1.53 \pm 0.29$ ,  $n = 4$ ). When was used a mixture of groups (fungi + bacteria), the reduction was 40.69%. The greatest reduction in the number of eggs occurred with fungi as control agents. The average for this group was 66.73% ( $L_n = -1.24 \pm 0.09$ ,  $n = 36$ ). The control from bacteria and the mixture of fungi + bacteria was 57.57% and 59.30%. As for the mass of eggs in the root system, actinomycetes provided a reduction of 77.47% ( $L_n = -1.71 \pm 0.48$ ,  $n = 3$ ). For bacteria, the decrease was 37.64%, fungi 54.05%, and the mixture of fungi and bacteria decreased the egg mass by 40.54%. For the root mass, the agent that showed the highest efficiency was the mixture of fungi + bacteria 2743.75% ( $L_n = 0.17 \pm 0.06$ ,  $n = 7$ ). For this variable, the group of fungi showed no statistical difference with respect to the control ( $L_n = -0.01 \pm 0.14$ ,  $n = 15$ ) (Figure 2). The group was not subdivided into moderator for the gall index because the studies were performed only with fungi as a control agent.



**Figure 2** - Effect measures for the moderating group formed by the biocontrol agent for the number of galls, number of eggs and eggs mass of root-knot nematode. The graph shows the estimates of the effect measure (ln), with the bars representing the 95% confidence interval.



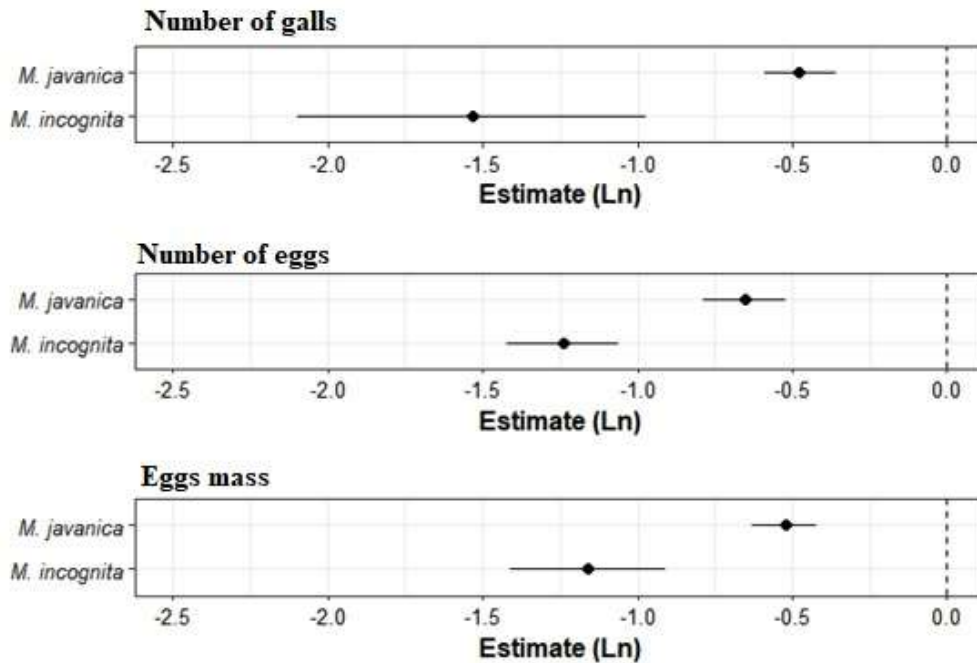
Source: Authors.

### 3.3 Nematode species

We evaluate the species of nematode used in each study in one moderating subgroup. For this subgroup, heterogeneity varied among nematode species within the analyzed variables. For the nematode *M. javanica*, the heterogeneities were high for the variables number of galls (89.93%), the number of eggs (100%), and for the egg mass (80.82%). For the variable root mass, the heterogeneity was low (26.86 %). For the species *M. incognita*, heterogeneity was null for the variable number of galls, high for the number of eggs (76.30%) and egg mass (98.99%) and moderate for root mass (58.76%).

We evaluated each nematode species to observe whether the different species would result in responses with different magnitudes. It observed that the magnitude of the response was higher for the species *M. incognita* compared to *M. javanica* in all the variables analyzed (Figure 3).

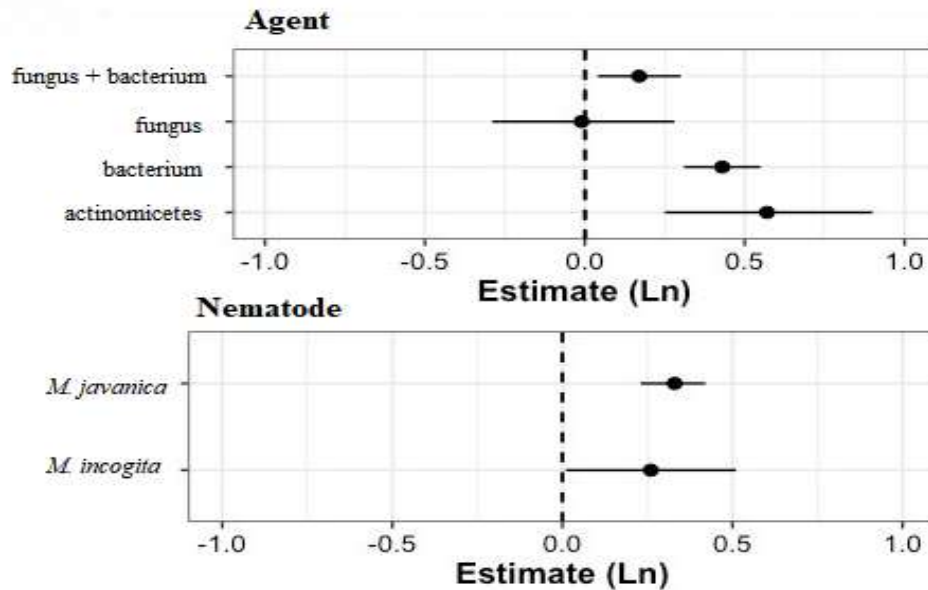
**Figure 3** - Effect measures for the moderating group formed by the nematode species for the number of galls, number of eggs and eggs mass of root-knot nematode. The graph shows the estimates of the effect measure (Ln), with the bars representing the 95% confidence interval.



Source: Authors.

The use of efficient microorganisms reduced the number of galls by 55.42% in the studies with *M. incognita* and 31.41% in *M. javanica*. When we analyzed the effect of treatments on the number of eggs, we observed that the effect was also more significant for *M. incognita* than *M. javanica*, 63.80% and 38.65%, respectively. We found the same effect for egg mass, 63.13% for *M. incognita* and 32.79% for *M. javanica*. Only *M. incognita* was utilized in studies in which the gall index was evaluated. The reduction in this variable was approximately 26.17%. When microorganisms in the mass root action, we observed positive results, with the most significant increase in mass occurring when the associated nematode was *M. incognita*, 313.42%. When the associated nematode was *M. javanica*, this increase was 45.65% (Figure 4).

**Figure 4** - Effect measures of moderating groups, biocontrol agent and nematode species for the root mass variable of root-knot nematodes. The graph shows the estimates of the effect measure (Ln), with the bars representing the 95% confidence interval.



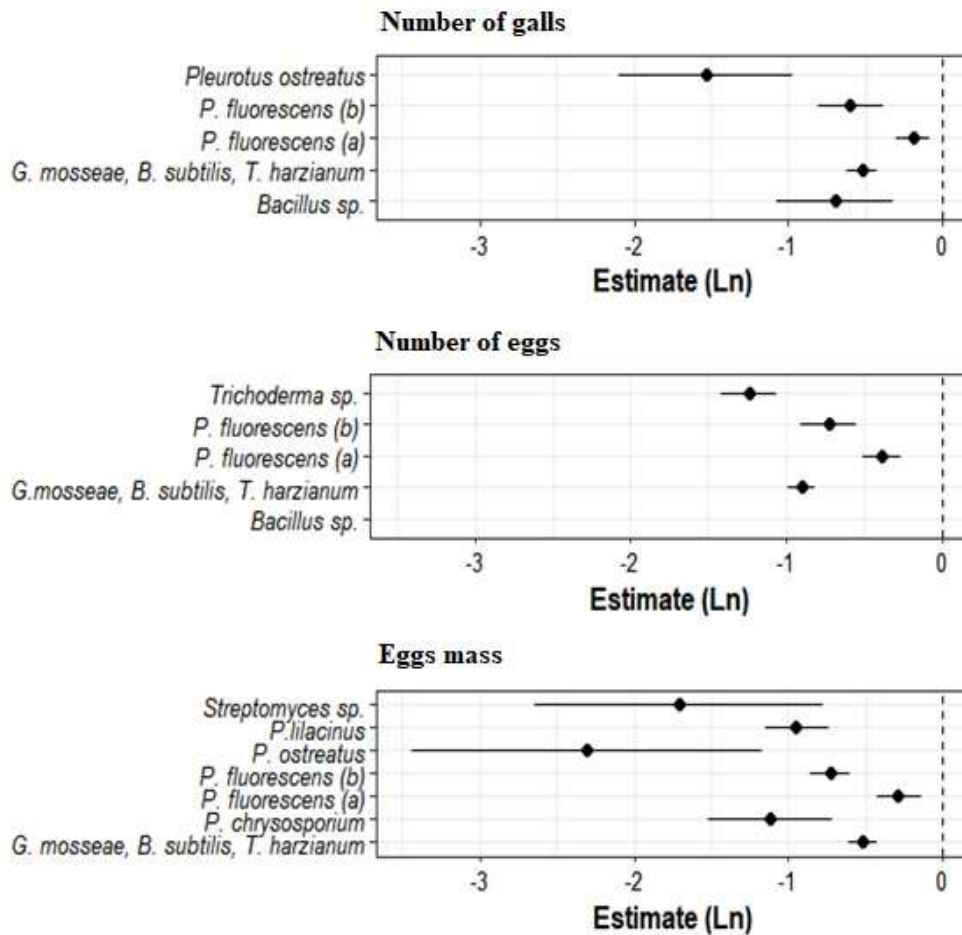
Source: Authors.

### 3.4 Species of microorganism used in biocontrol

We found a wide variety of microorganismspecies used as biocontrol agents. As the heterogeneity within the groups was relatively high, these species were separated into a moderating subgroup to assess whether this heterogeneity would be reduced. This effect only occurred for the root mass in which the heterogeneity went from moderate to low. In the other variables, the heterogeneity remained high (Table 3).

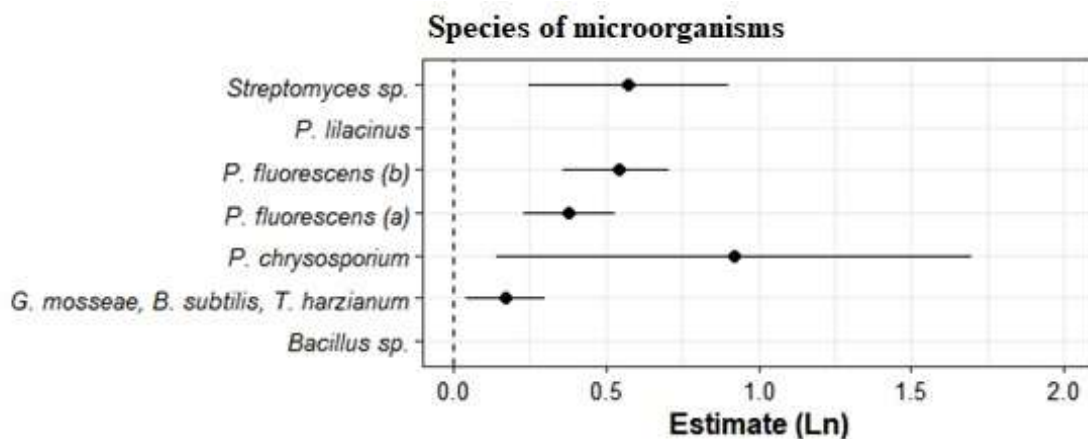
Except for *Bacillus* sp. which did not affect decreasing the number of eggs, the other microorganisms showed positive control levels in all analyzed variables. Using *Pleurotus ostreatus* was obtained the most significant decrease in the number of galls, 81.31% of control. For the number of eggs, the most significant reduction occurred with the use of species of *Trichoderma* sp., 66.73%. The eggs mass also showed a higher reduction with *P. ostreatus*, 68.42%, followed by actinomycetes, which also showed a good level of control for this variable, 77.47% (Figure 5). The biocontrol agent that presented the most considerable effect in increasing root mass was *Phanerochaete chrysosporium*, 165% (Figure 6). The use of *P. chrysosporium* also generated the most significant effect in the gall index, 55.90 % (Figure 7).

**Figure 5** - Effect measures for the moderating group formed by the microorganism species for the number of galls, number of eggs and eggs mass of root-knot nematode. The graph shows the estimates of the effect measure (ln), with the bars representing the 95% confidence interval.



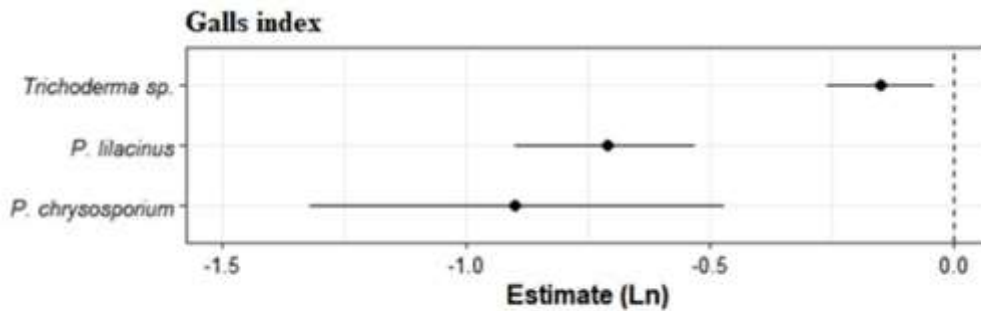
Source: Authors.

**Figure 6** - Effect measures of the moderating group species of microorganism for the root mass variable of root-knot nematodes. The graph shows the estimates of the effect measure (ln), with the bars representing the 95% confidence interval.



Source: Authors.

**Figure 7** - Effect measures for the moderating group formed by the microorganism species for the gall index to root-knot nematode. The graph shows the estimates of the effect measure (ln), with the bars representing the 95% confidence interval.



Source: Authors.

Production in tropical and subtropical areas is highly dependent on adequate control of nematodes (Cannayane & Rajendran, 2001), especially those of the genus *Meloidogyne* sp. known as root-knot nematodes, which are generally the most harmful. Considering the difficulty of handling these nematodes, the adoption of several combined control tactics can present better results. This idea also applies to the combination of microorganisms used in biological control, which is a tactic that can increase the potential for nematode control (Hallman et al., 2009). Research on the use of antagonistic microorganisms is receiving increasing attention (Choi et al., 2020; Harrier & Watson, 2004; Liu et al., 2020; Luambano et al., 2019; Schouteden et al., 2015; Strom et al., 2020; Whipps, 2004). Different genera of microorganisms are being used to promote nematode control. The studies suggest several mechanisms as possibly responsible for this biocontrol activity, including the production of root metabolites, competition for space and nutrients, mycoparasitism, promoting plant growth and inducing defense responses in plants (Melo & Azevedo, 2000; Nimnoi & Ruanpanun, 2020; Sohrabi et al., 2020).

The principal genera frequently associated with nematode biocontrol are *Pseudomonas* spp. and *Bacillus* spp. (Carneiro et al., 1998). Several researchers have reported the nematicidal action of species of the genus *Bacillus* in the control of different nematodes (Chen & Dickson, 2004; Kempster et al., 2001; Vaz et al., 2001) for example, *Bacillus thuringiensis*, *B. laterosporus*, *B. circulans*, *B. subtilis*, *B. pumilis*, *B. cereus*, *B. sphaericus* and *B. licheniformis*. In this meta-analysis, these genera showed satisfactory levels of control for the number of galls (*Bacillus* sp. and *P. fluorescens*). For the number of eggs, only the genus *P. fluorescens* showed effectiveness in control. The genus *Bacillus* sp. had no significant effect on root mass (Figures 4 and 6). This result agrees with other authors who also did not observe a significant difference in root mass using *Bacillus* (Fernandes et al., 2014, 2013; Lazaretti & Bettioli, 1997).

The promotion of plant growth due to the treatment with *Bacillus* species depends mainly on the ability of the microbial isolate to interact with the host (Carneiro et al., 1988; Kerry & Bourne, 2002; Machado et al., 2010), which may not happen for all hosts. Similar results were obtained by other authors when using *Bacillus* (Fabry et al., 2007; Fernandes et al., 2013; Machado et al., 2012). Possibly such microorganisms are not growth promoters in the host plants used in these studies. However, although potential biocontrol agents have not generated beneficial effect of increasing plant biomass for these plants (Araújo & Marchesi, 2009; Dallemole-Giaretta et al., 2010; Lopes et al., 2007; Siddiqui et al., 2006), there was also no undesirable phytotoxic action (Fernandes et al., 2014). On the other hand, the action of these genera in decreasing the number of galls on the roots was quite effective, which agrees with other authors who report an excellent control or inhibitory effect on nematodes by microorganisms of the genus *Bacillus* and *Pseudomonas* (Dallemole-Giaretta et al., 2010; Nagesh et al., 2013; Huang et al., 2020).

Studies have confirmed that the biocontrol agents, *Bacillus* and *Pseudomonas* sp. are effective in reducing the number of juveniles in the second stage (J2) of root-knot nematode (Huang et al., 2020) with reduced incidence rates to 64.58%, 50.00%, respectively, which corroborates the results obtained in this meta-analysis (Figures 5). Seo et al. (2012) also found similar results and concluded in their studies that these agents reduce the disease caused by root-knot nematodes. Freitas et al. (2005) tested *B. cereus* isolates to control *M. javanica* observed reduce the number of galls by about 55% when applied via tomato seeds. Fabry et al. (2007) reported that the microbiolization of tomato seeds with *Citrobacter freundii* and *Pseudomonas putida* reduced the number of *M. javanica* galls in a greenhouse, thus showing that the use of these organisms can be a viable alternative for the control of these pathogens (Affokpon et al., 2011; Moazezikho et al., 2020).

Between the different microorganisms with the potential to control root-knot nematodes, fungi are particularly attractive and have great potential (Benítez et al., 2004). Several possible mechanisms, including the production of antifungal metabolites, competition for space and nutrients, mycoparasitism, promotion of plant growth and induction of defense responses in plants, have been suggested as mechanisms by their biocontrol activity (Sohrabi et al., 2020). As mentioned in the literature, in this meta-analyze, we can also observe the high potential for controlling the different genera of fungi. The most significant control effect was obtained when fungi were used as a control microorganism for all the variables analyzed. For the number of galls, we observed the best effect with the use of *P. ostreatus*. For eggs mass, *P. ostreatus* followed by *Phanerochaete chrysosporium*. For gall index, the most significant effect was also obtained with *P. chrysosporium* followed by *Paecilomyces lilacinus*. For the number of eggs, the most considerable effect was obtained by using *Trichoderma* sp. For root mass *P. chrysosporium*. *P. chrysosporium* is a white root fungus that has been identified as nematophagous (Du et al., 2020).

When *M. incognita* eggs are treated with this species of fungus, the same parasites the eggs producing hyphae, which first surround the shell and then destroy the egg. Some studies show that egg-parasitic fungi such as *P. lilacinus*, *Pochonia* sp. and *T. harzianum* can secrete protease and chitinase. These enzymes degrade specific cyst nematode proteins that effectively destroy the eggshell and then parasitize and kill the eggs (Wei et al., 2009). Protein and chitin are the principal chemical constituents of the cuticle and eggshell of the nematode. Infection of *M. incognita* eggs by strains of *P. chrysosporium* may also be involved in the decomposition of the eggshell by producing protease and chitinase (Du et al., 2020). However, it is unclear whether all gall nematodes species can be digested by these fungus species, as the host variety of nematophagous species is specific. *P. ostreatus*, for example, can capture and consume *Aphelenchus avenae* and *Tylencholaimus parvus*, while *Oscheius tipulae* are resistant to *P. Ostreatus* (Marlin et al., 2019).

Fungi of the genus *Trichoderma* sp. are also cited as potential agents in the biocontrol of diseases caused by soil pathogens, including nematodes (Affokpon et al., 2011; Benítez et al., 2004; Sandoval et al., 2020). In the studies that used the genus *Trichoderma* as a potential biocontrol agent, we observed an effective action in both variables presented (number of eggs and gall index). Fungi of this genus can be considered excellent candidates for the biological control of the root-knot nematode (Du et al., 2020).

Besides that, another essential point to be considered is the effect of the mixture of microorganisms. Several factors influence the activity of biological control microorganisms. It is not surprising that a single biocontrol agent grown under appropriate physical and chemical conditions, when introduced into a new complex environment such as soil, can lose its function (Melo & Azevedo, 2000). However, it is likely that, in places where biological control occurs naturally, such an event is the result of a mixture of antagonists, much more than a high population of just one antagonist (Raj et al., 2017; Roberts et al., 2005). An antagonist mixture could increase the effectiveness and reliability of the control due to the expansion of the spectrum of action mechanisms against the target nematode. In the case of this meta-analysis, we obtained studies in which the microorganism used in biocontrol was the mixture of three different agents, two fungi (*Glomus mosseae* and *Trichoderma harzianum*) and a species of bacteria (*Bacillus subtilis*). This treatment demonstrated efficient control for all the variables

presented (Figures 4 and 5). Similar studies, with the combined application of *G. mosseae* and *P. polymyxa*, significantly reduced nematode pathogenicity indices so that the nematode J2 population decreased by 59% (Abbasi & Sharf, 2011; Melo & Azevedo, 2000; Sohrabi et al., 2015).

Other authors show that *G. mosseae* reduced the J2 root penetration of *M. incognita* in tomato plants compared to control plants (Bais et al., 2006; Sohrabi et al., 2018). Two factors could explain these observations: the radius of action of the root exudates (Ku & Leuven, 2006) that could affect the behavior of soil nematodes when close to mycorrhizal roots (Vos et al., 2012) or that the nematode penetration could be hampered for the mycorrhiza presence in roots (Ferraz et al., 2010). Other authors also state the efficiency in reducing the number of galls of *Meloidogyne* sp. by combined treatments of *P. chlamydosporia* and *B. subtilis* (Inomoto, 2016; Lopes et al., 2007; Machado et al., 2012). According to the literature, this effect is directly related to the colonization of the soil nematode eggs by the fungus and the alteration of the plant's root exudates or induction of systemic resistance of the plant by the bacteria transmitted via seeds (Inomoto, 2016; Lopes et al., 2007; Machado et al., 2012;). The synergism observed in these studies corroborates the hypothesis that the mixture of antagonists may represent an efficient measure of nematode management, as it brings together several mechanisms of action (Abbasi et al., 2011; Inomoto, 2016; Melo & Azevedo, 2000; Raj et al., 2017).

Regarding the effect of the nematode species, we found that, for all variables evaluated, the effect obtained by *M. incognita* was more significant when compared to *M. javanica*. This effect is expected since it is known that *M. incognita* is more aggressive to the roots of host plants compared to *M. javanica*, which may lead to its higher ability to infect root tissue (Carraro-Lemes et al., 2020; Inomoto, 2016).

#### 4. Conclusion

We conclude from this meta-analysis that i) the use of biological control was effective for the nematode species, *M. incognita* and *M. javanica*; ii) the use of fungi proved to be more efficient in the biological control for the species *M. incognita* and *M. javanica* when compared to bacteria; iii) the combination of different genera of microorganisms can be a good management option in the control of these *Meloidogyne* species; iv) biological control is presented as an alternative to chemical control for these species of *Meloidogyne* sp.

However, further studies are needed to improve our understanding of the mechanisms used by each microorganism group to control or suppress the pathogen.

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