RESEARCH/INVESTIGACIÓN

MANAGEMENT OF ROOT-KNOT NEMATODES IN A SUSCEPTIBLE *COFFEA ARABICA* CULTIVAR WITH NEMATICIDES

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ABSTRACT

Gouveia, A.C., S. A. Silva, L. H. Picoli, O. F. Dorigo, J. P. Tomaz, and A. C. Z. Machado. 2024. Management of root-knot nematodes in a susceptible *Coffea arabica* cultivar with nematicides. Nematropica 54:29-40.

Coffee production is of great economic importance in Brazil. Several phytosanitary problems can cause yield losses, including plant-parasitic nematodes. This study aimed to compare the efficacy of nematicides for the control of *Meloidogyne paranaensis* and *M. exigua* in the susceptible coffee cultivar 'IPR 107'. Experiments were conducted under greenhouse conditions. The following treatments were evaluated: the biological nematicides *Bacillus subtilis + B. licheniformis*, *Pochonia chlamydosporia*, *Purpureocillium lilacinum*, *Trichoderma harzianum*, and the chemical nematicide fluensulfone. Inoculated and non-inoculated controls were also included. Evaluation of nematode multiplication was performed 150 days after inoculation (DAI) and expressed as reproduction factor (RF = final population density/initial population density) values. None of the biological nematicide were effective in reducing nematode densities when applied at planting or 30 DAI. Fluensulfone reduced RF values of both nematodes, either when applied at planting and 30 DAI. All the biological nematicides, except *P. chlamydosporia*, improved the development of plants inoculated with *M. paranaensis*. These results reinforce the difficulty of managing plant-parasitic nematodes in perennial crops such as coffee with biological nematicides, especially when the nematode is so aggressive to the plants, such as *M. paranaensis*.

Key words: Arabic coffee, biological control, *Meloidogyne exigua*, *Meloidogyne paranaensis*

RESUMEN

Gouveia, A.C., S. A. Silva, L. H. Picoli, O. F. Dorigo, J. P. Tomaz, and A. C. Z. Machado. 2024. Manejo de nematodos agalladores en una variedad de *Coffea arabica* susceptible con nematicidas. Nematropica 53:29-40.

El café tiene una gran importancia económica en Brasil. Varios problemas fitosanitarios pueden causar pérdidas de rendimiento y los nematodos son uno de los principales organismos responsables de estas pérdidas. Este estudio tuvo como objetivo comparar la eficacia de los nematicidas en el control de *Meloidogyne paranaensis* y *M. exigua* en la variedad de café susceptible IPR 107. Se realizaron experimentos en condiciones de invernadero, con tratamientos compuestos por el control sin nematicidas y sin nematodos, el control inoculado (sin nematicidas), *Bacillus subtilis + B. licheniformis*, *Pochonia chlamydosporia*, *Purpureocillium lilacinum*, *Trichoderma harzianum* y *fluensulfona*. Las evaluaciones de la multiplicación de nematodos se realizaron a los 150 días después de la inoculación (DDI), en función del

factor de reproducción. Ningún nematicida biológico fue efectivo para reducir las poblaciones de nematodos cuando se aplicó al momento de la siembra o a los 30 DDI, solo la fluensulfona redujo los valores de FR de ambos nematodos, ya sea cuando se aplicó al momento de la siembra o a los 30 DDI. Sin embargo, incluso sin control de nematodos, los nematicidas biológicos mejoraron el desarrollo de las plantas. Los resultados obtenidos aquí refuerzan la dificultad de manejar estos patógenos en cultivos perennes como el café con nematicidas biológicos, especialmente cuando el nematodo es tan agresivo para las plantas, como *M. paranaensis*.

Palabras clave: Café arábica, control biológico, *Meloidogyne exigua*, *Meloidogyne paranaensis*

INTRODUCTION

Brazil is the main coffee producing region in the world, responsible for 32% of the global production (ICO, 2018). Despite this notoriety, coffee production is affected by several challenges, such as climate change, demands for product quality, and stresses caused by pests and diseases (Pizetta *et al*., 2016). Among the biotic factors affecting coffee production, plant-parasitic nematodes are considered one of the main limiting factors of coffee production in Brazil (Zambolim and Vale, 2003).

Meloidogyne spp. are the most aggressive nematodes attacking coffee plants in Brazil, reducing plant development and yield (Salgado and Terra, 2022). *Meloidogyne exigua*, *M. incognita*, and *M. paranaensis* are the most important species in Brazilian coffee plantations due to their wide distribution in the country (Oliveira and Rosa, 2018). Among these species, *M. paranaensis* is the most aggressive (Carneiro *et al*., 2008), causing yield losses ranging from 10.7 to 24.5% (Lordello *et al*., 2001). Although *M. exigua* is considered less aggressive than *M. paranaensis* towards coffee plants, the distribution of this nematode in Brazilian coffee plantations is of concern (Zambolim and Vale, 2003).

Managing *Meloidogyne* spp. in coffee plantations is difficult, and the integration of more than one practice is required, such as the use of resistant cultivars, crop rotation when possible, and the application of chemical and biological nematicides (Arita *et al*., 2020; Sikandar *et al*., 2020;). Biological control of nematodes in Brazil has been gaining market share over time. Biological nematicides represent 35% of the biopesticides used in this country, and in relation to the use of all types of nematicides, biological products accounted for 82% of the Brazilian market in 2019/2020 (Machado, 2022).

Several biological nematicides are available in Brazil (Agrofit, 2021), however, information regarding their efficacy for the control of plant-parasitic nematodes in different crops is sometimes unavailable. In coffee, Arita *et al*. (2020) evaluated two biological nematicides for the management of *M. paranaensis*: *Purpureocillium lilacinum* and *Trichoderma harzianum*. They concluded that neither were effective in reducing *M. paranaensis* population densities in experiments conducted under greenhouse conditions. However, the authors observed an improvement in plant development when *P. lilacinum* was applied, demonstrating a positive effect of this organism on coffee plant health. Nevertheless, Arita *et al.* (2020) only evaluated two biological nematicides, which represent a small portion of the Brazilian market. For this reason, this study aimed to expand the knowledge about the use of biological nematicides in coffee by evaluating the efficacy of *P. lilacinum*, *T. harzianum*, *Bacillus subtilis* + *B. licheniformis*, and *Pochonia chlamydosporia* for the control of *M. paranaensis* and *M. exigua* in a susceptible coffee cultivar, under greenhouse conditions.

MATERIAL AND METHODS

Preparation of nematode inoculum

The *M. paranaensis* population was originally obtained from a coffee plantation in the municipality of Londrina, Paraná State (23º52’26”S 53º54’07”W), and was identified through α-esterase phenotypes (Carneiro *et al*., 2000) as *M. paranaensis* I1 (Carneiro *et al*., 1996). The *M. exigua* population was also obtained from a coffee plantation in the municipality of Lavras, Minas Gerais State (21º14’43”S 44º59’59”W), with a α-esterase phenotype of E1 (Salgado *et al*., 2015). Both populations were maintained under

greenhouse conditions on coffee 'Catuaí Vermelho' or 'Mundo Novo', both being susceptible to these nematodes.

Approximately 60 days before the experiments, *Meloidogyne* spp. eggs were extracted from coffee roots by blending and sieving (Boneti and Ferraz, 1981) and inoculated on to tomato 'Santa Clara' for increase. Eggs extracted from tomato roots were quantified using a Peters slide under a light microscope and used as the inoculum.

Experimental design and logistics

Experiments were conducted at the Institute of Rural Development of Paraná, Londrina, Paraná State, Brazil (23°21'20"S; 51°09'58.2"W). Experiment 1 was conducted from September to January, and Experiment 2 from January to May, with temperatures ranging from 20ºC to 36ºC during the experimental periods.

Coffee 'IPR 107' seedlings, susceptible to both *Meloidogyne* spp., with five pairs of leaves, were transplanted to 945 mL-capacity styrofoam pots filled with a substrate composed of a mixture of soil and sand (12% clay, 1% silt, 87% sand) that had been previously sterilized using dry heat (180ºC for 3 hr). Nematode inoculation was carried out at planting (AP) by pipetting a suspension containing 1,000 eggs of *M. paranaensis* or *M. exigua* per plant, separately, into two holes in the soil near the plant stem. Non-inoculated and inoculated plants without nematicides were used as controls.

The experiments were conducted using a completely randomized design with 12 replicates, with each replicate consisting of one pot containing one coffee plant. Six plants were concurrently inoculated and treated AP with the nematicides according to the treatments described in Table 1; the chemical nematicide fluensulfone (Nimitz®, Adama, Brazil) was also evaluated. The other six plants received the same nematicide treatments but 30 days after inoculation (DAI), in order to assess the effect of each nematicide after nematodes were established on plants (Arita *et al*., 2020). Dosages were calculated based on a density of 5,000 coffee plants per ha. The treatments were diluted according to the manufacturer's instructions and applied using a semi-automatic pipette around the plant stem, following the recommended dosage for coffee.

At 150 DAI, the fresh top weight (FTW; g), dry top weight (DTW; g), and fresh root weight (FRW; g) were measured, and nematode multiplication was assessed. Nematode population densities were determined by extracting eggs and juveniles from the roots using the blender-sieving method (Boneti and Ferraz, 1981), after washing the roots to remove soil. Extracted nematodes were quantified using a Peters slide under a light microscope to determine the final population (FP) of *M. paranaensis* and *M exigua* in each replicate. This value was divided by the initial population density (IP) of 1,000 eggs/plant to calculate the nematode reproduction factor (RF = FP/ IP) in each replicate.

Statistical analyses

The experiment was conducted twice and analyzed separately. The residual normality was verified using the Shapiro-Wilk test at a significance level of 5%. The variance homogeneity was assessed using Bartlett's test at a significance level of 5%. For *M. paranaensis* data, a log transformation (log(y+0.01)) was applied to meet the assumptions of the model, while the data for *M. exigua* remained non-transformed. Mean values were compared using the multiple comparison test (False Discovery Rates), at a significance level of 5% using the matrix contrast. The statistical analyses were conducted using R 2.15.2 (R Core Team, 2020), with the Agricolae

Table 1. Treatments applied to coffee plants.

Treatment	Trademark	Dosage per hectare[z]
Non-inoculated check	-	-
Inoculated check	-	-
Fluensulfone	Nimitz®	2,000 ml
Purpureocillium lilacinum	Nemat®	200 g
Trichoderma harzianum	Ecotrich®	250 g
Bacillus subtilis + B. licheniformis	Quartzo®	300 g
Pochonia chlamydosporia	Rizotec®	1,000 g

[z]According to manufacturers recommendation.

package (Mendiburu, 2015).

RESULTS

In Experiment 1, plants inoculated with *M. exigua* had significantly higher FRW in the controls and in the treatment with *P. chlamydosporia* applied AP (Fig. 1A). Conversely, treatment with fluensulfone AP had the lowest values of FRW (Fig. 1A). In Experiment 2, the FRW of plants inoculated with *M. exigua* was highest in the inoculated control and lowest in the treatment with fluensulfone AP compared to the other treatments (Fig. 1B). When *M. paranaensis* was inoculated on coffee plants in Experiment 1, higher values of FRW were obtained in the non-inoculated control. The application of *T. harzianum*, *P. chlamydosporia*, and *Bacillus* spp. AP or 30 DAI, or *P. lilacinum* applied 30 DAI, had the lowest values of FRW (Fig. 1C). In Experiment 2, the non-inoculated control had the highest FRW, while the application of *T. harzianum*, *P. chlamydosporia*, and *Bacillus* spp. applied AP resulted in the lowest FRW (Fig. 1D).

No significant differences were observed for FTW between the treatments in Experiments 1 and 2 for plants inoculated with *M. exigua* (Fig. 2A, B). Conversely, the FTW of coffee plants inoculated with *M. paranaensis* in Experiment 1 was reduced by the application of *P. chlamydosporia* AP. All of the other treatments applied AP or 30 DAI, along with both controls, had the highest values of FTW (Fig. 2C). In Experiment 2, the application of *P. chlamydosporia* AP again reduced the FTW of coffee plants, and only the inoculated control had significantly higher values of FTW (Fig. 2D).

The DTW of plants inoculated with *M. exigua* was lower in the treatments with *T. harazianum*, *P. lilacinum*, and *Bacillus* spp. applied 30 DAI, while the non-inoculated control had the highest value of DTW in Experiment 1 (Fig. 3A). In Experiment 2, only the treatment with *T. harzianum* AP reduced the DTW of plants, and the non-inoculated control again had the highest value of DTW (Fig. 3B). The DTW of plants inoculated with *M. paranaensis* in Experiment 1 was higher in the non-inoculated control, while the treatment with *P. chlamydosporia* AP had the lowest value of DTW (Fig. 3C). In Experiment 2, the DTW was higher in the non-inoculated control and in the treatment with fluensulfone applied AP, while the treatment with *P. chlamydosporia* AP again showed the lowest value of DTW (Fig. 3D).

The RF values for *M. paranaensis* and *M. exigua* in coffee under different biological treatments are shown in Table 2 and Fig. 4. In general, the RF values of *M. paranaensis* and *M. exigua* were higher in Experiment 1 compared to Experiment 2 (Table 2). *Meloidogyne exigua* multiplied adequately on the inoculated control in both experiments, with RF values of 227.7 and 9.4 in Experiments 1 and 2, respectively (Fig. 4). In Experiment 1 (Fig. 4A), the application of *P. chlamydosporia* 30 DAI resulted in higher *M. exigua* multiplication compared to other treatments, while the application of fluensulfone AP resulted in the lowest RF values. Fluensulfone also reduced the RF values of *M. exigua* in Experiment 2 (Fig. 4B), both AP and 30 DAI. However, in Experiment 2, the application of *T. harzianum* AP or *P. lilacinum* 30 DAI resulted in higher *M. exigua* multiplication compared to other treatments (Fig. 4B).

The multiplication of *M. paranaensis* in the inoculated controls was also adequate in both experiments (RF = 25.4 and 12.9 in Experiments 1 and 2, respectively). The matrix contrast analysis showed that for *M. paranaensis*, only the application of fluensulfone AP resulted in a significant reduction in the RF values in both experiments (Fig. 4). None of the biological nematicides, whether applied AP or 30 DAI, reduced the multiplication of *M. paranaensis* on coffee in Experiments 1 and 2 (Table 2). Additionally, the application of *P. lilacinum* (Experiment 1) or *Bacillus* spp. (Experiment 2) applied AP resulted in higher multiplication of *M. paranaensis* (Fig. 4C, D).

The coffee roots were more damaged by *M. paranaensis* than by *M. exigua*, and typical symptoms of nematode parasitism were observed in all treatments (except in the non-inoculated control). These symptoms included severe splitting and cracking of the cortical root tissue in *M. paranaensis*-infected plants and galled roots in *M. exigua*-infected plants (data not shown).

DISCUSSION

As a perennial crop, coffee plants remain in the field for many years, allowing for multiple generations of plant-parasitic nematodes. Management using biological nematicides could be an option to reduce nematode damage and increase

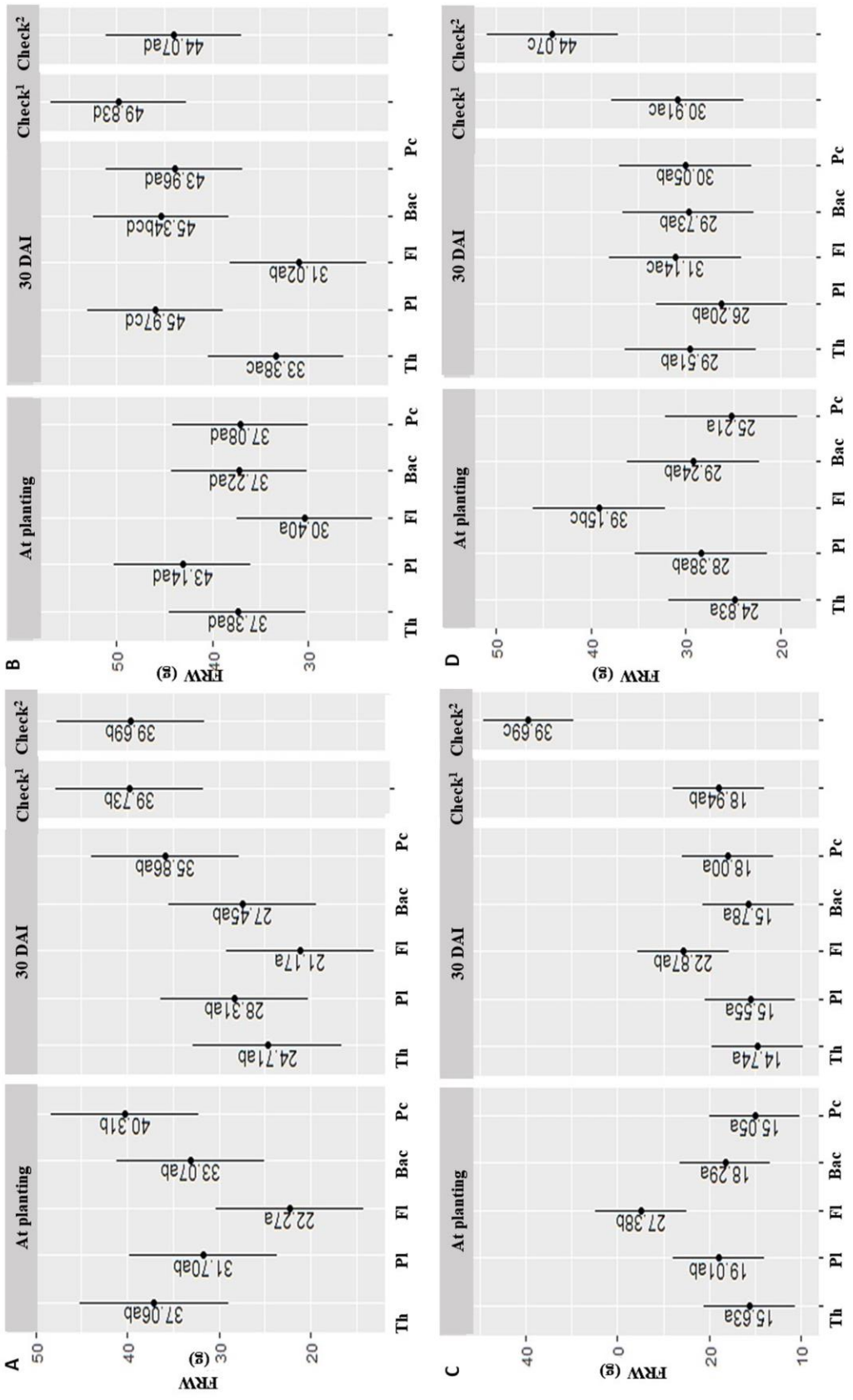

Figure 1. Fresh root weight (FRW) of coffee 'IPR 107' in Experiments 1 (A, C) and 2 (B, D), inoculated with *Meloidogyne exigua* (A, B) or *M. paranaensis* (C, D) and with the application of different nematicides at planting or 30 days after inoculation (DAI) and in the inoculated (Check1) and non-inoculated (Check2) checks. Data were not transformed (*M. exigua*: A, B) or log(FRW+0.01) transformed (*M. paranaensis*: C, D). Th = *Trichoderma harzianum*, Pl = *Purpureocillium lilacinum*, Fl = fluensulfone, Bac = *Bacillus* spp., Pc = *Pochonia chlamydosporia*.

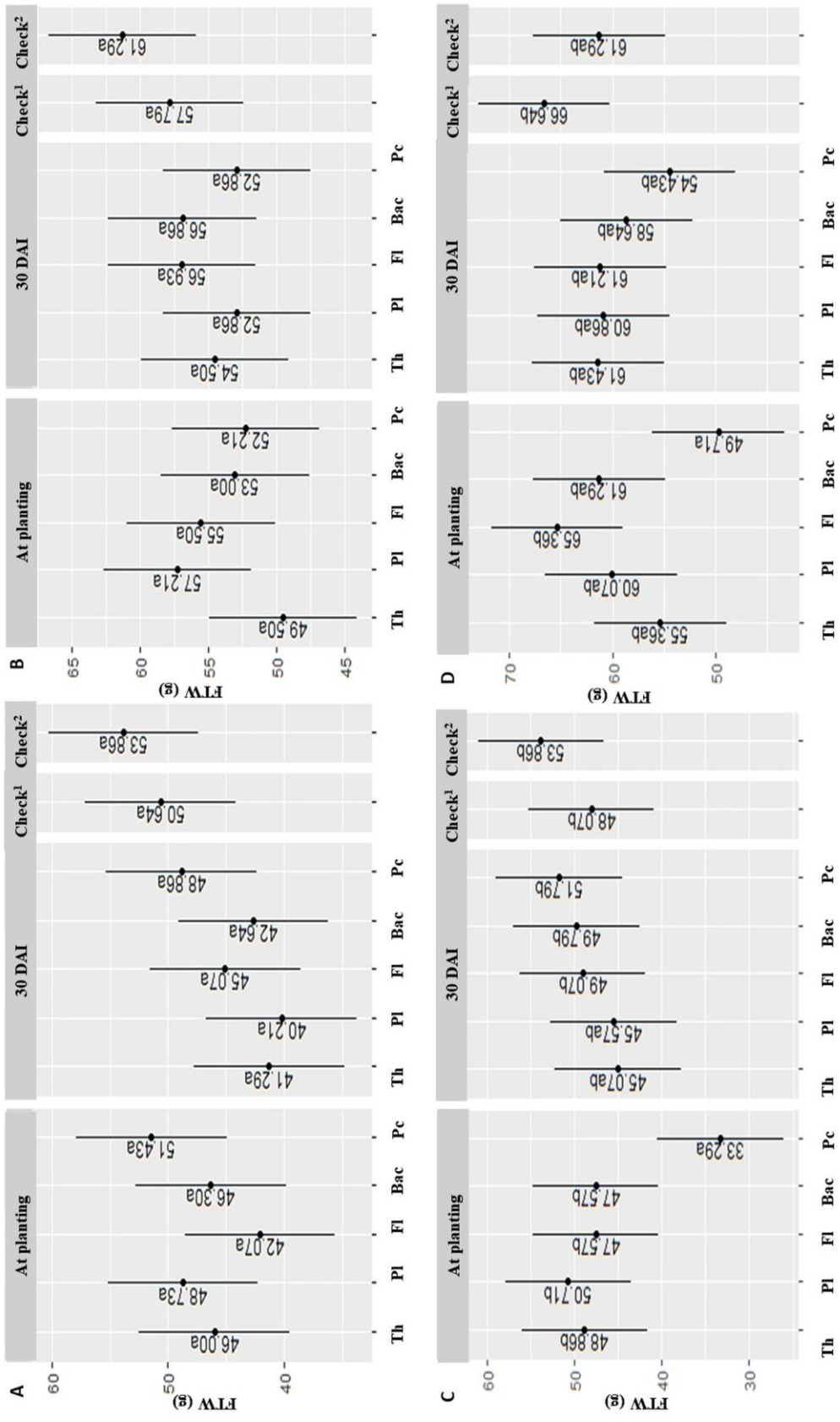

Figure 2. Fresh top weight (FTW) of coffee cultivar IPR 107 in Experiments 1 (A, C) and 2 (B, D), inoculated with *Meloidogyne exigua* (A, B) or *M. paranaensis* (C, D) and with the application of different biological nematicides at planting or 30 days after inoculation (DAI) and in the inoculated (Check1) and non-inoculated (Check2) checks. Data were not transformed (*M. exigua*: A, B) or log(FRW+0.01) transformed (*M. paranaensis*: C, D). Th = *Trichoderma harzianum*, Pl = *Purpureocillium lilacinum*, Fl = fluensulfone, Bac = *Bacillus* spp., Pc = *Pochonia chlamydosporia*.

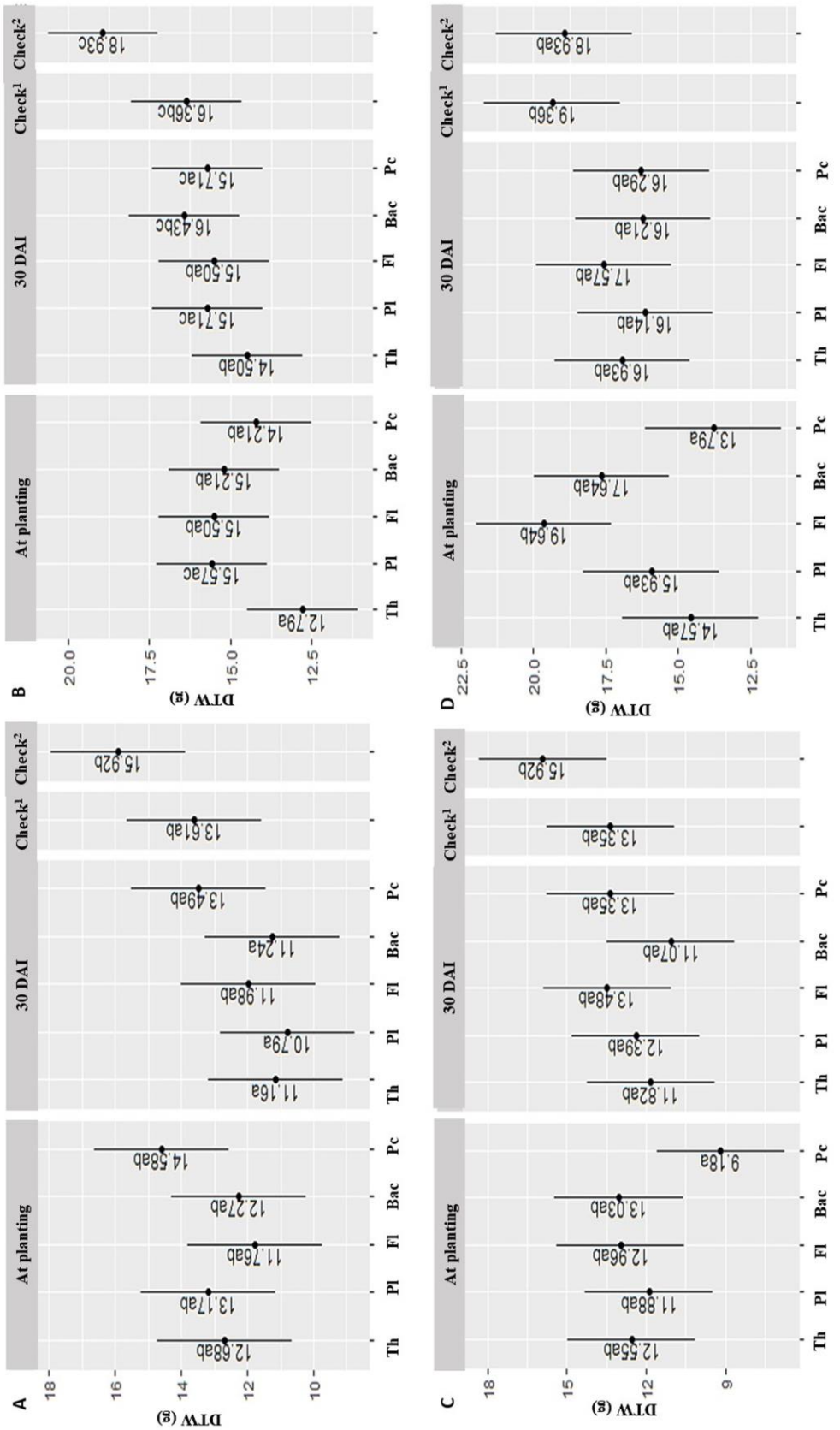

Figure 3. Dry top weight (DTW) of coffee 'IPR 107' in Experiments 1 (A, C) and 2 (B, D) inoculated with *Meloidogyne exigua* (A, B) and *M. paranaensis* (C, D) and then treated with biological nematicides at planting or 30 days after inoculation (DAI) and in the inoculated (Check1) and non-inoculated (Check2) checks. Data were not transformed (*M. exigua*: A, B) or log(FRW+0.01) transformed (*M. paranaensis*: C, D). Th = *Trichoderma harzianum*, Pl = *Purpureocillium lilacinum*, Fl = fluensulfone, Bac = *Bacillus* spp., Pc = *Pochonia chlamydosporia*.

Table 2. Reproduction factor (RF) values[Z] of *Meloidogyne paranaensis* and *M. exigua* in coffee 'IPR 107' after the application of biological nematicides at planting (AP) or 30 days after inoculation (30) in two experiments.

Treatment	*Meloidogyne paranaensis*				*Meloidogyne exigua*			
	Experiment 1		Experiment 2		Experiment 1		Experiment 2	
	AP	30	AP	30	AP	30	AP	30
Fluensulfone	0.58	5.08	0.01	0.36	0.17	24.35	0.27	1.90
Purpureocillium lilacinum	33.32	12.79	1.53	7.60	160.77	283.45	15.74	46.17
Trichoderma harzianum	23.12	23.47	7.35	2.59	185.10	200.27	40.89	30.48
Bacillus subtilis + B. licheniformis	20.57	12.89	18.47	15.22	157.10	275.18	4.77	20.84
Pochonia chlamydosporia	9.34	26.15	10.33	10.61	237.28	386.96	15.87	18.39
Control	25.42		12.87		227.69		9.41	

[Z]Values are means of six replicates.

the longevity of plants as some of them are considered plant growth-promoting agents (Arita *et al*., 2020; Machado, 2022). Moreover, when applied at planting, some biological nematicides can protect the root system against nematode penetration (Machado, 2022), enabling better establishment of seedlings and reducing costs associated with replanting seedlings that succumb to nematode parasitism in the early stages of development.

Among the *Meloidogyne* spp. that parasitize coffee in Brazil, *M. paranaensis* and *M. exigua* are considered the most important either due to the aggressiveness of the former species or the widespread distribution of the latter in coffee plantations (Zambolim and Vale, 2003; Arita *et al*., 2020). Several studies have demonstrated the efficacy of nematicides applied to coffee plants, but control of *M. paranaensis* is not always achieved, primarily due to the extent of root damage caused by the nematode (Arita *et al*., 2020). The majority of studies have reported successful control of *M. exigua* through the application of biological nematicides, while limited information is available regarding the control of *M. paranaensis*.

One of the most extensively studied organisms for controlling *Meloidogyne* spp. in coffee is *Pasteuria penetrans*, a bacterium capable of parasitizing *M. exigua* and reducing its population densities in infested Brazilian coffee plantations (Maximiano *et al*., 2001; Campos and Silva, 2008). However, there are currently no biological nematicides available in the Brazilian market containing *Pasteuria* spp. for the control of *Meloidogyne* spp. (Agrofit, 2021; Machado, 2022).

The fungi, *P. chlamydosporia* and *P. lilacinum*, have also been studied as potential biological control agents for *M. exigua* in coffee (Campos and Silva, 2008). However, in our study, these fungi did not demonstrate the same potential for reducing *M. exigua* population densities when applied at planting and 30 DAI. For *M. paranaensis*, none of the fungi were able to reduce population densities at planting or 30 DAI. Both *P. chlamydosporia* and *P. lilacinum* are chitinolytic fungi that parasitize nematode eggs (Morton *et al*., 2004). As the inoculum used in our experiments primarily consisted of *Meloidogyne* spp. eggs, the observed differences in plant development when *M. exigua* was inoculated with the application of *P. chlamydosporia* at planting, leading to improved root development in Experiment 1, could be attributed to this mode-of-action. However, this effect was not observed for *M. paranaensis*, which aligns with the findings reported by Arita *et al*. (2020), where no control of *M. paranaensis* was observed with the application of *P. lilacinum* at planting. It is worth noting that, in the study by Arita *et al*. (2020), some effect on nematode population density was observed when *P. lilacinum* was applied 60 DAI, although this effect was not observed in our study.

Another fungus used in our study, *T. harzianum*, although not registered by the Brazilian Ministry of Agriculture for the control of *Meloidogyne* spp. (AGROFIT, 2021), has demonstrated efficacy, especially due to its ability to induce plant resistance (Martínez-Medina *et al*., 2013). However, we did not observe a reduction in nematode population densities with the application of *T. harzianum* at planting or 30 DAI, which is consistent with the findings of Arita *et al*. (2020) who applied *T. harzianum* in coffee 'Mundo Novo' inoculated with *M. paranaensis* under greenhouse conditions. There is no available information regarding the control of *M. exigua* using *T. harzianum*.

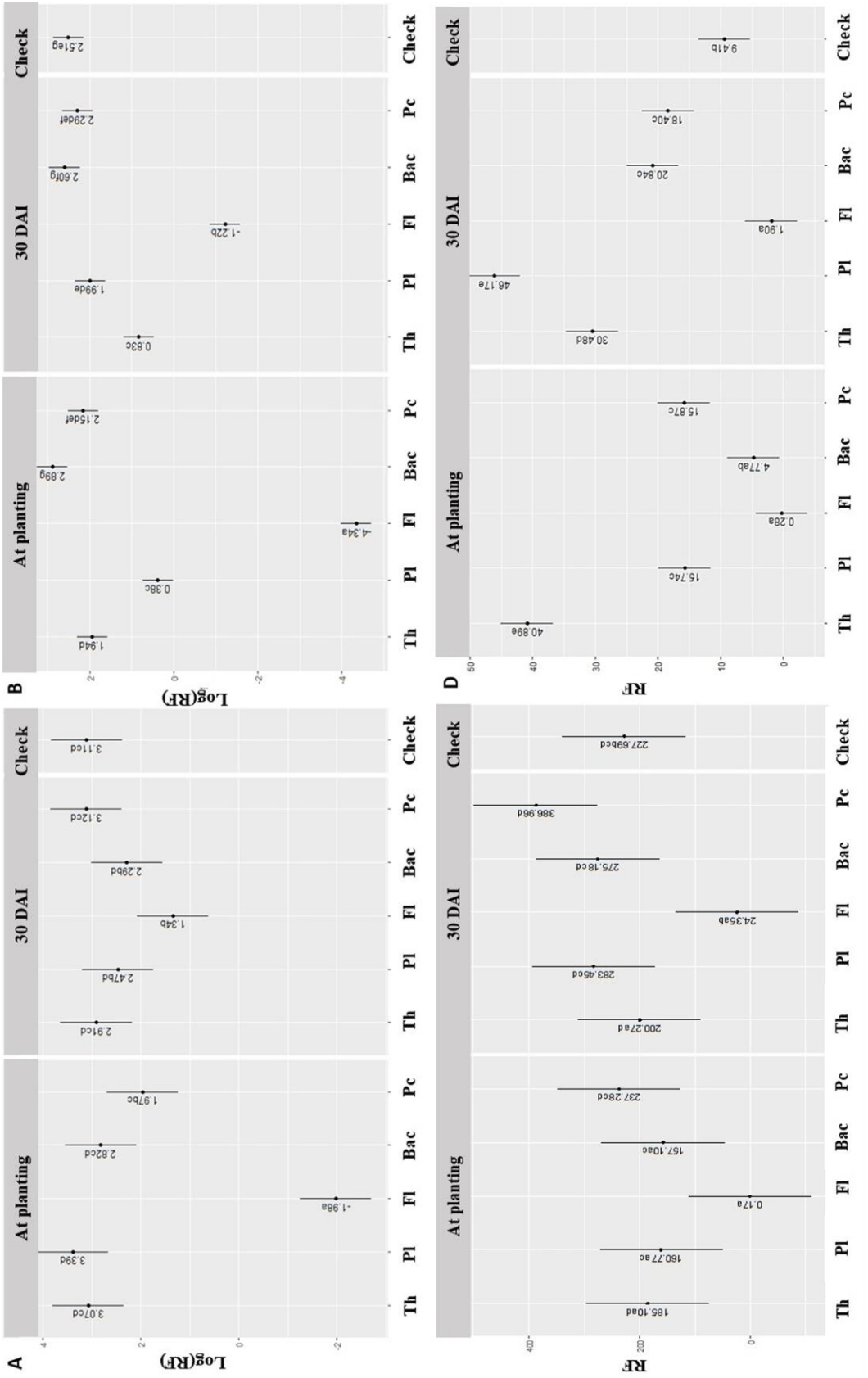

Figure 4. Reproduction factor (RF) of *Meloidogyne exigua* (A, B) and *M. paranaensis* (C, D) on coffee 'IPR 107' in Experiments 1 (A, C) and 2 (B, D), with the application of biological nematicides at planting or 30 days after inoculation (DAI). Data were not transformed (*M. exigua*: A, B) or log(FTW+0.01) transformed (*M. paranaensis*; C, D). Th = *Trichoderma harzianum*, Pl = *Purpureocillium lilacinum*, Fl = fluensulfone, Bac = *Bacillus* spp., Pc = *Pochonia chlamydosporia*.

Arpini *et al*. (2018) evaluated the same commercial *Bacillus* spp. biological nematicide used in our study for the control of *M. exigua* under field conditions and observed that rates from 300 g/ha resulted in a reduction in nematode population densities similar to that obtained with the chemical nematicide, cadusafos. *Bacillus* spp. primarily protect the roots against nematode penetration (Hashem *et al*., 2019). In our study, *Bacillus* spp. applied at a dosage of 300 g/ha showed some efficacy in the control of *M. exigua* when applied at planting, but this effect was not observed for *M. paranaensis*.

The chemical fluensulfone was the only nematicide that effectively reduced population densities of both nematodes when applied at planting or 30 DAI. Arita *et al*. (2020) also observed a high level of control of *M. paranaensis* in coffee with fluensulfone under greenhouse conditions. Under field conditions, in nematode-infested coffee plantations in Brazil, Corte *et al*. (2015) also demonstrated the efficacy of fluensulfone in reducing *M. exigua* densites.

In addition to nematode control, biological nematicides have been reported to improve plant development (Arita *et al*., 2020; Machado, 2022). Both of these studies observed that the application of *P. lilacinum* resulted in increased development of coffee inoculated with *M. paranaensis*, even in the non-inoculated control. In our study, the application of *P. lilacinum*, *T. harzianum*, and *Bacillus* spp. at planting or 30 DAI improved the FTW of plants inoculated with *M. paranaensis* in Experiment 1, supporting these previous results.

The results obtained in this study regarding the biological control of *Meloidogyne* spp. in coffee plants reinforces the difficulty in managing these pathogens in perennial crops like coffee, especially when dealing with a highly aggressive nematode such as *M. paranaensis*. Our findings also demonstrated that the highest FRW, FTW, and DTW were achieved in non-inoculated plants, particularly in the case of *M. paranaensis*. For *M. exigua*, the inoculated control plants also exhibited higher values of FRW due to galling incited by the parasitism of this nematode in coffee plants. Thus, due to the high aggressiveness of *M. paranaensis* towards coffee, the primary strategy for its management is exclusion, based on avoiding or evading the nematode in non-infested fields (Agrios, 2005). The presence of *M. paranaensis* severely hampers the development of coffee plants, and none of the tested nematicides allowed the plants to grow as if they were not infested by the nematode.

Combined with the use of resistant cultivars against these nematodes, biological control can provide additional benefits to the crop by potentially improving plant development. In addition to reducing nematode population densities through genetic resistance, this combined effect could result in yield improvements. Chemical control of *Meloidogyne* spp. in coffee is also promising and integrating it with resistant cultivars or biological control agents could further enhance plant development. Further studies should be conducted to evaluate the feasibility and effectiveness of integrating these management tools for controlling *Meloidogyne* spp. in coffee fields.

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