Greenhouse and field assessment of biological and chemical agents against guava decline

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ABSTRACT

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Guava decline is a complex disease resulting from the parasitism by *Meloidogyne enterolobii* (*Me*), which predisposes the plant to root rot caused by *Fusarium* sp. Three *in vitro* experiments were carried out with the aim of: (i) selecting the Fluensulfone dosage capable of causing *Me* mortality; (ii) verifying the effect of Fluensulfone on *Pochonia chlamydosporia* and *Trichoderma harzianum*; (iii) evaluating the effect of *Pochonia chlamydosporia* and *Trichoderma harzianum* in pure doses and in association with Fluensulfone on *Me* mortality. Fluensulfone at 2 L.ha⁻¹ had no effect on fungi. The associated and pure doses of treatments had a significant effect on *Me* mortality. A field experiment was also carried out in a guava orchard where decline occurred. The following treatments were tested: Fluensulfone; *P. chlamydosporia*; *P. chlamydosporia* + Fluensulfone; *T. harzianum*; *T. harzianum* + Fluensulfone; Carbofuran, and control. All treatments reduced *Me* population, since relative efficiency was higher than 80%. Fluensulfone used alone or in combination with biological nematicides provided an increase in guava productivity. Maintaining *Me* population low in the field is an important option for increasing productivity and reducing the losses suffered by farmers.

Keywords: *Meloidogyne enterolobii*, Fluensulfone, *Pochonia chlamydosporia, Trichoderma harzianum*

RESUMO

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O declínio de goiaba é uma doença complexa causada pelo parasitismo de *Meloidogyne enterolobii* (Me), que predispõe a planta à podridão radicular causada por *Fusarium* sp. Três experimentos in vitro foram realizados com os seguintes objetivos: (i) selecionar a dosagem de Fluensulfone capaz de causar mortalidade de Me; (ii) verificar o efeito da Fluensulfone sobre *Pochonia chlamydosporia* e *Trichoderma harzianum*; (iii) avaliar o efeito de *Pochonia chlamydosporia* e *Trichoderma harzianum* em doses puras e em associação com Fluensulfone na mortalidade de *M. enterolobii*. Fluensulfone na dose de 2 L.ha⁻¹ não teve efeito sobre os fungos. As doses associadas e puras dos tratamentos tiveram efeito significativo na mortalidade de Me. Um experimento de campo também foi realizado em um pomar de goiaba com ocorrência de declínio. Os seguintes tratamentos foram testados: Fluensulfone*; P. chlamydosporia*; *P. chlamydosporia* + Fluensulfone; *T. harzianum*; *T. harzianum* + Fluensulfone; Carbofurano e o controle. Todos os tratamentos reduziram a população de Me, uma vez que a eficiência relativa foi superior a 80%. O fluensulfone utilizado isoladamente ou em combinação com nematicidas biológicos proporcionou um aumento na produtividade de goiaba. A manutenção da baixa população de Me no campo é uma opção importante para aumentar a produtividade e reduzir as perdas sofridas pelos agricultores.

Palavras-chave: *Meloidogyne enterolobii*, Fluensulfone, *Pochonia chlamydosporia, Trichoderma harzianum*

Guava decline became established in Brazil a few years ago and has been responsible for the eradication of numerous guava orchards (7). According to Gomes et al., the symptoms of decline are attributed to the synergistic action between *Meloidogyne enterolobii* Yang and Eisenback, 1983 and *Fusarium solani* (Mart.) Sacc., and the initial parasitism of the nematode predisposes the plants to intense root degeneration by the fungus, thus making the management of this disease complex.

Management measures that reduce *M. enterolobii* population are essential to keep the plants protected against decline in the field (21). Biological control has been one of the major tools for reducing the use of synthetic nematicides (19). Among fungi with potential for agricultural use in nematode management are *Pochonia chlamydosporia* (Goddard) (24) and *Trichoderma harzianum* Rifai (13).

Another alternative for the root knot nematode management is chemical control; however, numerous synthetic nematicides have already been banned because of their high cost and high toxicity (11). The active principle Fluensulfone, or MCW-2, (5-chloro-2-(3,4,4-trifluorobut-3-phenylsulfonyl) -1,3-thiazole) has nematicidal action and shows low toxicity to non-target organisms, but its effectiveness in guava decline management has not been tested yet.

Thus, the present study aimed to: i) select the Fluensulfone dosage capable of causing mortality to second-stage juveniles (J2) of *M. enterolobii*; ii) verify the effect of Fluensulfone concentrations on

P. chlamydosporia and *T. harzianum*, and iii) evaluate the efficiency of the associated use of these biological and chemical agents on the management of guava decline in the field.

MATERIAL AND METHODS

Three experiments were carried out *in vitro* and one experiment was conducted *in vivo*.

In vitro experiments
These tests were carried out in the laboratory with the intention of: a) selecting the Fluensulfone dosage capable of causing mortality to second-stage juveniles (J2) of *M. enterolobii*; (b) verifying the effect of Fluensulfone on *P. chlamydosporia* and *T. harzianum* for later association in the field experiment; (c) evaluating the effect of *P. chlamydosporia* and *T. harzianum*, in pure doses and in association with Fluensulfone, on mortality of *M. enterolobii* J2.

Obtaining second-stage juveniles (J2) of *M. enterolobii*
A pure population of *M. enterolobii*, previously identified by isoenzyme esterase electrophoresis (3), was multiplied and maintained in the roots of guava plants (*Psidium guajava* L.), cv. 'Paluma', which were grown in a greenhouse. The substrate used for guava planting was composed of soil and sand at the ratio of 1:1. The soil, collected from an uncultivated site, was sieved and autoclaved for 2 hours at 140ºC, and this process was repeated three times.

Eggs + J2 of *M. enterolobii* were extracted from the roots according to the method of Hussey and Barker, 1973, modified by Bonetti and Ferraz (2). The concentration of the inoculum obtained in aqueous suspension was estimated in a Peters counting chamber under an inverted objective microscope. Subsequently, hatching chambers were installed and kept at 27ºC for five days, until the number of J2 needed for the experiment was reached (4). J2 were collected every 24 hours.

Obtaining biological and chemical nematicides
Fluensulfone® and the *T. harzianum*-based biological nematicide were purchased from Fitoclin Company, situated in Linhares, Espírito Santo State, Brazil. The *P. chlamysodporia*-based nematicide was obtained from Rizoflora Company, situated in Viçosa, Minas Gerais State, Brazil.

Effect of Fluensulfone 40 EC on *M. enterolobii* mortality
To evaluate the effect of Fluensulfone on *M. enterolobii* mortality, doses corresponding to 1 L.ha^{-1}, 1.5 L.ha^{-1}, 2 L.ha^{-1} and 4 L.ha^{-1} were tested, in addition to dose zero (control), as four replicates, totaling 20 sample units. Each sample unit was constituted of a 5-mL test tube, properly identified, containing 100 second-stage juveniles (J2) of *M. enterolobii*. The tubes were maintained at 27°C for 24 hours.

To estimate the number of immobile (inactive) individuals, the contents of each tube were poured through a 0.025mm sieve. The J2 collected after sieving were washed in running water. Subsequently, J2 were again transferred to the tubes containing tap water and allowed to rest for additional 24 hours.

The percentage of mobile and immobile J2 was then visually determined under an inverted objective microscope in a Peters counting chamber. Based on the obtained data, mortality percentage was calculated according to the following equation: PM = NDI x 100/500, where: PM = percentage of mortality; NDI = number of dead individuals.

To obtain normality of error distribution, homogeneity of variances and additive effects of variation factors, data were transformed by $\sqrt{x}$ and submitted to Cochran and Bartlett test (p≥0.05), for homogeneity of variances, and Shapiro-Wilk (p≥0.05), for normality. Data were analyzed by non-linear regression.

Effect of Fluensulfone 40 EC on *P. chlamydosporia* and *T. harzianum*
The effect of Fluensulfone was assessed on *M. entorolobii* mortality and on the fungi *P. chlamydosporia* and *T. harzianum* for subsequently testing them in association (biological treatment + chemical treatment).

The experiment was carried out in a completely randomized design with 5 replicates. The fungi were previously cultured in Petri dishes and subjected to treatment with Fluensulfone, at the concentrations 2 L.ha^{-1} and 4 L.ha^{-1} (0.026 mL and 0.053 mL), diluted in potato-dextrose-agar (PDA) growth medium and incubated at 25 ± 1°C in the dark for 15 days.

After fungal growth, Petri dishes containing PDA growth medium + Fluensulfone aliquots, at 2 L.ha^{-1}, were prepared based on the results of the experiment described above.

Petri dishes with *P. chlamydosporia* and *T. harzianum* discs were also prepared without the addition of Fluensulfone for control. Possible Fluensulfone toxicity on fungi was evaluated when the mycelia of both fungi reached the edge of the dish.

2) *In vivo* experiment
This experiment aimed to evaluate the effect of *P. chlamydosporia* and *T. harzianum* applied alone or in association with Fluensulfone on *M. enterolobii* J2 mortality and was carried out in a commercial guava orchard (cv. 'Paluma') showing natural occurrence of decline in the municipality of Pedro Canário, located in the north of Espírito Santo State, at 39º57'26"W and 18º17'33"S, from March to December 2015. According to the international classification of Köppen, the climate in that region is of subtropical type, presenting dry winter and hot summer (Cwa), and annual average temperature of 24.0°C.

The studied five-year old guava orchard was cultivated at 4x7m spacing between plants, and four planting lines were used for evaluation. Before the application of treatments, chemical and physical soil analyses were performed.

Experimental design was in randomized blocks with four replicates and subdivided plots, which were composed of seven previously tested treatments: *P. chlamydosporia* 2 kg.ha^{-1} (0.026 mL); Fluensulfone 40 EC 2 L.ha^{-1} (0.026 mL); Fluensulfone 40 EC 2 L.ha^{-1} + *P. chlamydosporia* 2 kg.ha^{-1}; *T. harzianum* 1 L.ha^{-1} (0.013 mL); Fluensulfone 40 EC 2 L.ha^{-1} + *T. harzianum* 2 kg.ha^{-1}; Carbofuran 0.4 L.ha^{-1} (0.053 mL), and control (dose zero).

Each experimental unit was composed of five plants, in a 7x4 factorial scheme, in which 7 corresponds to biological and chemical treatments/nematicides, used alone or in combination, at the recommended dosages, according to the description above, and 4 refers to the intervals of evaluation/subplot, i.e., 0, 60, 120 and 180 days.

Treatment dosages were defined according to the manufacturer's recommendation and based on previous tests under laboratory conditions.

Mode of application
Treatments were applied in 1m bands on each planting line after cleaning the mulch present in the planting band.

At the beginning of treatments, the recorded average temperature was 26°C and the wind speed was 3.0 m/s. At the end of application, the average temperature was 25.1°C and the wind speed was 2.9 m/s.

The volume of 6L per treatment was applied with a CO_2 pressurized sprayer equipped with a bar containing two fan-type nozzles, height of approximately 35 cm, forming a 100cm wide application range in the line direction.

To assist in the application, two irrigations were carried out complementary to the drip irrigation system present in the production area. The first irrigation occurred the day before treatment application, using a 10mm blade, and the second irrigation was done right after spraying, at the same amount.

Evaluations of *M. enterolobii* population in roots and soil

For the evaluation of *M. enterolobii* population in the soil, four 0.5L sub-samples were collected from the plant's rhizosphere region in each plot; they were homogenized, totaling samples composed of 2.0 L soil. In the laboratory, *M. enterolobii* population was extracted from the soil according to the methodology proposed by Jenkins (10), and the final nematode population (FP), composed of J2 + eggs of *M. enterolobii*, was quantified in a Peters counting chamber under an inverted objective microscope.

To assess the population in the root system, for each treatment replicate, an average of 50g roots were collected from each of the three central plants. The collected root system was homogenized, totaling 150g samples, which were stored in plastic bags, duly identified and packed in Styrofoam boxes until evaluations. In the laboratory, *M. enterolobii* population was extracted from the root system of plants based on the methodology proposed by Hussey and Barker, 1973, modified by Bonetti and Ferraz (2). The FP was quantified as previously described.

Evaluation of guava fruit production

In December 2016, coinciding with the last evaluation of *M. enterolobii* population, the average productivity of the produced guava fruits was evaluated. Fruits were harvested inside each plot and weighed on an analytical scale of 0.001g accuracy. The average productivity obtained for each treatment was compared with that obtained for the control treatment (dose zero).

Data analysis

Relative Efficiency (RE) was calculated with the adapted formula of Henderson and Tilton (8), $RE = (1 - {}^{P}/_{T})$. 100, where: P = incidence or severity of the disease for each evaluated treatment, T - incidence or severity of the disease for control treatment.

Data on FP from the soil and root were transformed by $log_{10}(x + 1)$ and submitted to Cochran and Bartlett test ($p \geq 0.05$), for homogeneity of variances, and Shapiro-Wilk ($p \geq 0.05$), for normality.

Since subplots were repeated measures over time (days), Mauchly's test (15) was applied to verify sphericity, i.e., to verify whether the covariance matrix was similar to an identity matrix, according to that recommended by Huynh and Feldt (9), for repeated measures over time.

While data were verified for sphericity, analysis of variance was performed for the subdivided plot arrangement; the qualitative factors were submitted to means comparison test, and the quantitative factor (evaluation interval) underwent regression analysis ($p \leq 0.05$).

Significant qualitative factors were analyzed according to Tukey's test ($p \leq 0.05$). For the analysis, R (17) computational application was used.

RESULTS AND DISCUSSION

1) *In vitro* experiments

In the *in vitro* experiment, the higher the applied concentration of Fluensulfone 40 EC, the higher the mortality rate of *M. enterolobii* (Figure 1). Data were fit to the nonlinear exponential model.
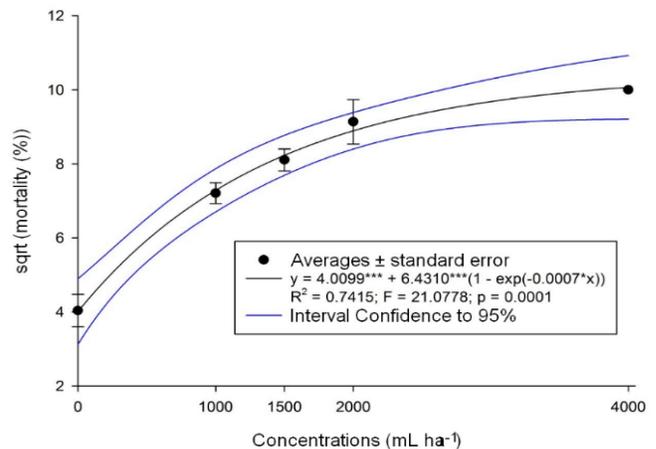

Figure 1. Average mortality of *M. enterolobii* J2 treated with different dosages of the synthetic nematicide Fluensulfone 40 EC under laboratory conditions. *Data transformed by $\sqrt{x}$.

The concentrations 2 L.ha-1 and 4 L.ha-1 were equally efficient.

Fluensulfone 40 EC at 1 L.ha-1, 1.5 L.ha-1, 2 L.ha-1 and 4 L.ha-1 caused 36%, 69%, 85% and 100% *M. enterolobii* J2 mortality, respectively, while 16% mortality was observed for the control. These results enabled the selection of the two doses that led to the highest nematode mortality (2 L.ha-1 and 4 L.ha-1) for the two subsequent assays.

Effect of Fluensulfone 40 EC on *P. chlamydosporia* and *T. harzianum*

Fluensulfone showed no toxicity to *P. chlamydosporia* and *T. harzianum*, at 2 L.ha-1, since the mycelial growth of both fungi in dishes with nematicide treatments was the same, compared to control.

Application of 4 L.ha-1 caused fungal death. Thus, 2 L.ha-1 was understood as the most suitable concentration for subsequent testing both *in vitro* and *in vivo*.

Effect of *P. chlamydosporia* and *T. harzianum* applied alone or in combination with Fluensulfone on *M. enterolobii* J2 mortality

After 24 hours of application, all treatments provided a high level of mortality of *M. enterolobii* J2, compared to control. Except for Fluensulfone + *T. harzianum*, the treatments led to an average mortality of *M. enterolobii* J2 that did not differ statistically from that caused by Carbofuran (positive control) (Table 1).

2) *In vivo* experiment

The Relative Efficiency (RE) of the analyzed treatments indicated satisfactory levels of *M. enterolobii* management, greater than 80% after 120 days (Figure 2).

Treatments based on Fluensulfone, *P. chlamydosporia*, Fluensulfone + *P. chlamydosporia* and *T. harzianum* showed RE superior to 90% at 120 days after application, while treatments based on Fluensulfone

Table 1. Average mortality of second-stage juveniles (J2) of *M. enterolobii* treated with biological nematicides based on *P. chlamydosporia* and *T. harzianum* and the synthetic nematicide Fluensulfone 40 EC, applied alone or in association.

Treatments	Mortality (%)
P. chlamydosporia	97.3 a
T. harzianum	91.0 a
Fluensulfone 2L.ha^{-1}	85.0 a
Fluensulfone 2L.ha^{-1} + *P. chlamydosporia*	81.3 a
Fluensulfone 2L.ha^{-1} + *T. harzianum*	66.0 ab
Carbofuran	100.0 a
Control	22.7 b
CV (%)	18.7
F	4.5201
P	0.0094363

Means followed by the same letter do not differ according to Tukey's test at 5% probability. Data transformed by $\sqrt{x}$ and presented in original form.

Table 2. Summary of the analysis of variance for the final population (FP) of roots obtained by the sum of eggs + second-stage juveniles (J2) and number of galls (NG) induced by *M. enterolobii* in roots of guava cv. 'Paluma' in a commercial area showing natural occurrence of decline in Pedro Canário, Espírito Santo State, Brazil.

Sources of Variation	FP	NG
	F	F
Treatments (T)	1.2626	0.6319
Block	1.5404	0.7342
Time (t)	15.9335	20.6545
Interaction (Txt)	1.7883	0.9542
CV1 (%)[1]	22.72	60.75
CV2 (%)[2]	18.36	35.56

[1]Coefficient of variation for the plot; [2]Coefficient of variation for the subplot.

+ *T. harzianum* and Carbofuran showed 90% RE at 180 days after application. At 60 days, all treatments showed RE lower than 74% (Figure 2).

The transformed data for the FP of *M. enterolobii* presented homogeneity of variances, according to Cochran and Bartlett test (p≥0.05), and normality, according to Shapiro-Wilk test (p≥0.05). Analysis of sphericity using Mauchly's test showed that both FP and number of galls (NG) were not significant at 1% and 5%, respectively ($W_{M.\ enterolobii}$=0.57349, p=0.052334; W_{gall}=0.9373, p=0.93735, respectively), thus indicating data sphericity.

For the variable FP in the soil, data sphericity was also found (W_{soil}=0.6699, p=0.16056). Thus, the data were analyzed by using subplots divided in time.

Based on the analysis of variance, considering the variable FP, there was an interaction between treatments and intervals of evaluations. However, for the variable NG, the interaction was not significant (Table 2).

Unfolding of the factor treatment within the levels of the factor time indicated that the treatments only had an effect on the FP of *M. enterolobii* in guava roots from 120 days (Table 3), suggesting a residual action of the systemic products on the nematoid.

At 120 days, Fluensulfone applied alone or in association with *P. chlamydosporia* reduced FP compared to control and, at 180 days, Fluensulfone and Carbofuran were the treatments that reduced this variable (Table 3).

Unfolding of the factor time within the levels of the factor treatment indicated that the data fit best to the polynomial model. All treatments reduced the FP of *M. enterolobii* from 60 days on, and the best result was observed at 120 days (Figure 3).

Except for Carbofuran, the remaining treatments allowed a slight increase in *M. enterolobii* population after 120 days (Figure 3).

Evaluation of guava productivity

Fluensulfone alone or in combination with *T. harzianum* and *P. chlamydosporia* provided increased productivity, but there was no difference between each other (Figure 4).

When applied alone, Fluensulfone 40 EC showed efficiency in the management of *M. enterolobii*. This result corroborates studies
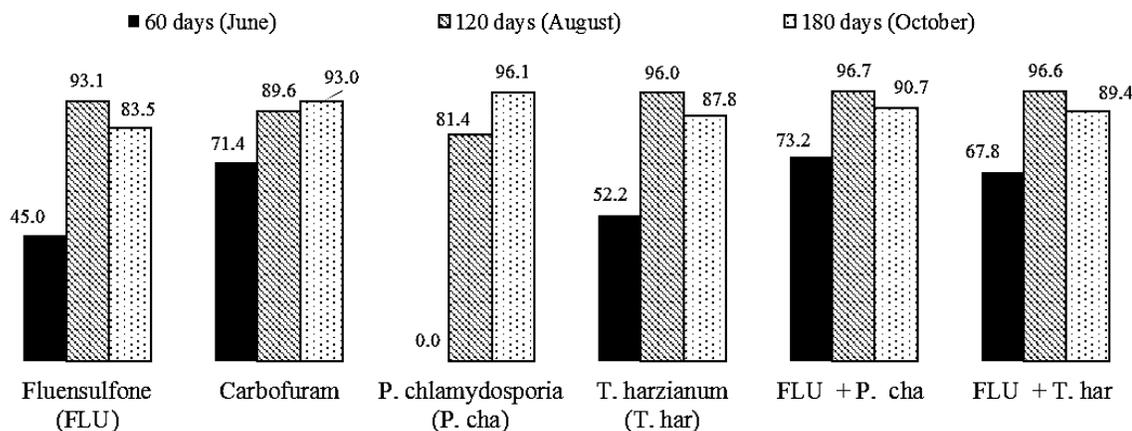

Figure 2. Relative Efficiency (RE; %) of different treatments in the management of *M. enterolobii* in a field cultivated with guava cv. 'Paluma' presenting symptoms of decline in Pedro Canário, Espírito Santo State, Brazil.

Table 3. Final population obtained by the sum of eggs + J2 of *M. enterolobii* extracted from roots of guava cv. 'Paluma' treated with synthetic and biological nematicides alone or in association, at different periods after application in a commercial area showing natural occurrence of decline in Pedro Canário, Espírito Santo State, Brazil.

Treatments	Intervals (days)			
	0^{ns}	60^{ns}	120	180
Fluensulfone*	13,250	2,945	460 b	873 b
P. chlamydosporia*	9,750	1,657.5	510 ab	2,453.3 ab
T. harzianum*	8,750	1,115	442.5 ab	1,592.5 ab
Fluensulfone+*P. chlamydosporia**	5,000	930	432.5 b	1,403.8 ab
Fluensulfone+*T. harzianum**	12,750	990	1,350 ab	1,047.5 ab
Carbofuran*	11,750	4,695	2,425 ab	582.5 b
Control*	3,215	3,465	13,008.5 a	15,060.7 a

Means followed by the same letter in the column do not differ according to Tukey's test at 5% probability; [ns] Not significant according to the F test in the analysis of variance; *Data transformed by $log_{10}(x+1)$ and presented in original form.

Control
$$y = 450.56x^2 + 2255.3x - 330.06$$
$$R^2 = 0.8791$$

Fluensulfone (2000)
$$y = 2624.9x^2 - 17152x + 27520$$
$$R^2 = 0.988$$

Corbofuran
$$y = 1303.1x^2 - 10093x + 20322$$
$$R^2 = 0.9868$$

P. chlamydosporia
$$y = 2355.6x^2 - 14266x + 21437$$
$$R^2 = 0.9816$$

T. hrazianum
$$y = 2196.3x^2 - 13196x + 19493$$
$$R^2 = 0.9707$$

Fluensulfone (2000) + P. chlamydosporia
$$y = 1260.3x^2 - 7430.2x + 11065$$
$$R^2 = 0.9829$$

Fluensulfone (2000) + T. harzianum
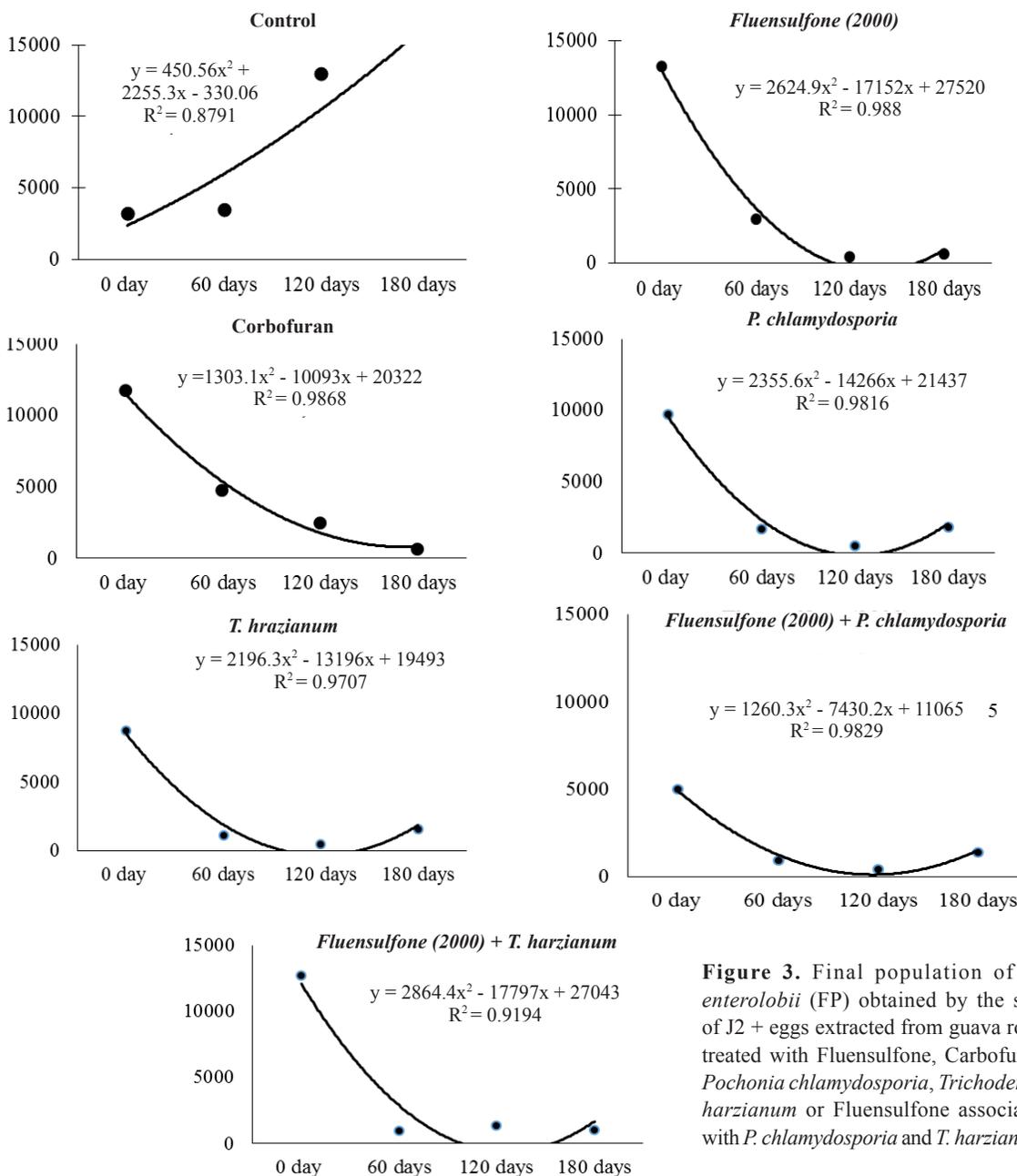$$y = 2864.4x^2 - 17797x + 27043$$
$$R^2 = 0.9194$$

Figure 3. Final population of *M. enterolobii* (FP) obtained by the sum of J2 + eggs extracted from guava roots treated with Fluensulfone, Carbofuran, *Pochonia chlamydosporia*, *Trichoderma harzianum* or Fluensulfone associated with *P. chlamydosporia* and *T. harzianum*.
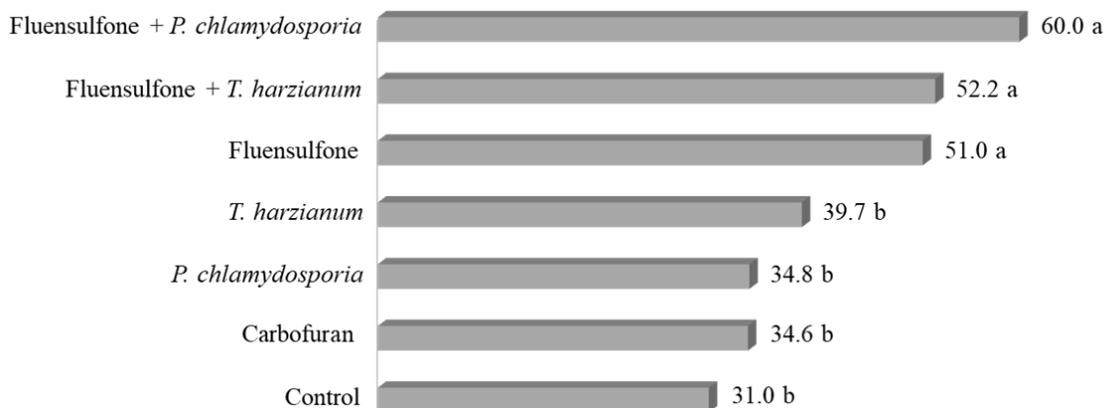
Figure 4. Average productivity, per treatment, of fruits (kg) of guava cv. 'Paluma' manifesting symptoms of decline and treated with Fluensulfone, biological nematicides based on *P. chlamydosporia* and *T. harzianum*, Carbofuran, Fluensulfone + *P. chlamydosporia*, Fluensulfone + *T. harzianum*.

demonstrating that Fluensulfone has nematicidal action and affects the nematode reproduction, development, feeding and motility (11). Fluensulfone 40 EC has also been reported as a new and promising chemical tool for the management of plant nematodes.

Some authors have already stated that the antagonists *T. harzianum* and *P. chlamydosporia* cause reduction in the population of *Meloidogyne* spp. (1, 5, 25), which may explain the present results. Meyer et al. (2000) reported that *Trichoderma* spp. is a notorious biological control agent for producing substances capable of inhibiting the hatching and mobility of *M. incognita* juveniles.

Compared to control, Fluensulfone applied alone or associated with *P. chlamydosporia* reduced the FP at 120 days. At 180 days, Fluensulfone and Carbofuran were most effective in reducing the FP. Reductions in the populations of *M. incognita* and *M. exigua* on coffee plants were observed at the end of 270 days after the application of synthetic nematicides (14). Meher et al. (16) demonstrated that Cadusafos persisted in the soil for around 120 days; however, this period may vary depending on the nematicide and even on the soil conditions.

Fluensulfone has low toxicity to non-target organisms and strong lethal action on eggs and adult nematodes (11), which explains the results obtained in the present experiment. Carbofuran is a systemic insecticide and nematicide used to control nematodes.

Freitas et al. (6) stated that non-fumigant nematicides, such as Carbofuran, are inhibitors of acetylcholinesterase, responsible for stimulus transmission; thus, the affected nematodes do not feed or move when under their effect (two to three months), dying from starvation. On the other hand, the action of Fluensulfone on nematodes is not accompanied by hypercontraction, which is common in nematodes that come into contact with organophosphates and carbamates (11).

All treatments, except Carbofuran, allowed a small increase in the FP after 120 days. Based on the obtained results, reapplication of these treatments is recommended, which would not cause problems to human health and the environment, since Fluensulfone presents low toxicity, and biocontrol agents are innocuous to humans and animals (25).

Fluensulfone, alone or in combination with the antagonists, caused a decrease in *M. enterolobii* population, which reflected in increased guava productivity. Siddiqui and Mahmood (20) reported that some chemical nematicides have the effect of paralyzing nematodes and exposing them to antagonists, leading these biocontrol agents to increase their efficacy, which may explain the present results.

According to several authors, some nematode antagonistic organisms are not affected by nematicides such as Aldicarb, Carbofuran,

Mirol, Forate and Oxamyl, which may enable the combined use of biological and chemical nematicides (23). Khan et al. (12) reported that the combination of *Bacillus* sp. with the nematicide Fertinemakil was the most effective treatment in reducing the population of *M. javanica* and improving eggplant growth.

In another study, Radwan et al. (18) noticed that several antagonist-based bioproducts, including *Trichoderma* sp., had their efficiency increased, due to association with the nematicide Oxamyl, in reducing both J2 and galls of *M. incognita* for the vegetative growth of tomato.

The positive result of *T. harzianum* in reducing nematode FP over time is justifiable since this antagonist inhibits and blocks the development of soil pathogens due to its antibiosis, parasitism and competition (1). However, the main characteristic that makes this organism a good biological control agent for *Meloidogyne* spp. is the production of toxic compounds (22).

Thus, *T. harzianum* is another option for the management of *Meloidogyne* spp. according to the results obtained in the present study and the characteristics that make it a good nematode control agent, which include acting in the preventive protection of plants, in the restoration of the microbial community and in the recovery of the soil structure weakened by intensive agricultural practice, besides releasing secondary metabolites that stimulate plant growth (22).

P. chlamydosporia, applied alone or in association with Fluensulfone, also showed a significant reduction in the FP of *M. enterolobii* during the evaluation periods, which led to increased productivity. In other studies, *P. chlamydosporia* has also been identified as an important biocontrol agent of nematodes with significant potential for different cultures. Dallemole-Giaretta et al. (5) observed that the antagonist significantly reduced the number of eggs of *M. javanica*.

This antagonist can colonize eggs in the cell multiplication and embryonic development phases more easily than juveniles; however, the effect of this antagonist may act on the suppression of females, juveniles, eggs and galls of *Meloidogyne* (5).

Fluensulfone 40 EC applied alone also provided increased guava productivity. Kearn et al. (11) found a positive effect of Fluensulfone on *M. incognita* and *M. javanica* population reduction and suggested that this nematicide molecule is a promising new strategy for plant nematode management.

Maintaining a low population of *M. enterolobii* in the field, through the management alternatives presented here, is an important option for increasing the productivity and reducing the losses suffered by farmers.

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