Investigation note

Biological effectiveness of Nemmax nematicide in the cultivation of coffee (*Coffea arabica* L.)

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Abstract

Phytoparasitic nematodes are one of the main pathogens that affect the cultivation of coffee trees (*Coffea arabica* L.), reducing the yield between 10 and 70%. The objective of this study was to evaluate the biological effectiveness of the nematicide of biological origin Nemmax, for the control of *Meloidogyne incognita*, *Pratylenchus* spp. and *Dorylaimus* spp., associated to this crop in Motozintla, Chiapas, in 2014. Two population samplings were carried out, one prior to the application of treatments, and another one to the 120 days later, obtaining the number of nematodes in 100 g of floor. The Nemmax treatments (at doses of 2, 4 and 6 L ha with 3 applications), Nemacur 400 CE (Fenamiphos) (3 L has one application) and an absolute control. In the control of *Meloidogyne incognita*, a significant difference was observed in treatment 5 (133.2), which represents the control, since an increase in the population of the nematodes was observed, compared with the other treatments, which were statistically equal, presenting the same differences for *Pratylenchus* spp. With the nematode *Dorylaimus* spp., statistical differences were presented, with treatment 5 (249.6) with the greatest difference, 4 (18), 1 (8.2), 2 (6) and 3 (3.4). Treatment 3, of Nemmax at a dose of 6 L/ha was the one that presented the lowest population of the filiform nematodes of the genus *Meloidogyne incognita*, *Pratylenchus* spp. and *Dorylaimus* spp.

Keywords: biological control, coffee, phytopathogenic nematodes.

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Coffee *Coffea* spp. (*Gentianales*: *Rubbiaceae*) is native to Ethiopia, the introduction in Mexico was about 1790 Medina *et al*. (2016). The main coffee producing countries in the world are Brazil with a production of 3 019 051 t per year, Vietnam with 1 460 800 t, Colombia with 745 084 t, Indonesia with 639 305 t, Ethiopia with 469 091 t, Honduras 362 367 t, India with 348 000 t, Peru with 277 760, Guatemala with 236 145, Uganda with 203 535 and Mexico with a production of 151 714 t year^{-1} (FAO, 2016).

In Mexico, the cultivation area is 700 000 ha, Chiapas, Veracruz, Puebla and Oaxaca, the main producing states, which together contribute nearly 90% of production (SIAP, 2016)

In our country, *Coffea arabica* L. occupies a little more than 97% of the coffee area, while the species *C. canephora* Pïerre is located in the rest of the area (AMECAFE, 2012).

Phytoparasitic nematodes constitute one of the main phytosanitary problems that affect the cultivation of coffee (*Coffea arabica* L.), cause yield reductions ranging between 10 and 70% (Gomez and Rivera, 1987). Numerous species of nematodes have been reported in association with this crop; however, the species of the genera *Meloidogyne* and *Pratylenchus* are the most widely distributed and those that cause the greatest losses in the crop (Inomoto *et al*., 1998; Herrera and Marban, 1999; Oliveira *et al*., 1999; Campos and Villain, 2005).

Nematodes represent a phytosanitary problem that is increasing and unfortunately few products are available for their control, according to the above the objective of the research was to evaluate the biological effectiveness of nematicide of biological origin Nemmax, for the control of nematodes *Meloidogyne incognita*, *Pratylenchus* spp. and *Dorylaimus* spp., associated with the cultivation of coffee.

Location of the experiment: the study was carried out in coffee plants of the Catuai commercial variety with a history of nematodes, located in Soconusco, municipality of Motozintla, Chiapas. The laboratory part was carried out at the Antonio Narro Autonomous Agrarian University (UAAAN), in Saltillo, Coahuila.

Products: the Nemmax product, of biological origin with nematicidal activity contains a mixture of extracts of vegetable origin 40% (40 g L^{-1}), rhizogenic bacteria *Bacillus* spp. and *Pseudomonas* spp. $1x10^8$ UFC mL^{-1} 10% (10 g L^{-1}), available phosphorus 2% (2 g L^{-1}), zinc 1% (1 g L^{-1}), chelating agents 1.5% (1.5 g L^{-1}), fulvic acids 500 ppm (0.5 g L^{-1}), diluents and conditioners 45 (45 g L^{-1}) and the commercial product Nemacur 400 CE (Fenamiphos) with an equivalent to 35 g of IA L^{-1} of product.

Experimental design: a randomized block design was used, with 5 treatments and 5 repetitions. Each experimental unit consisted of two 2-year-old coffee bushes, with a planting distance of 1 m in length, the distance between blocks was 2 m and the useful plot was 2 bushes. An analysis of variance (Anova) and a mean comparison test by Tukey (p= 0.05) were performed.

Initial sampling: a previous sampling was carried out in two coffee bushes per experimental unit, in order to know the population of nematodes present. A sample of 1 kg of soil of the silty migajon type was taken at the north cardinal point at a depth of 40 cm and was transferred to the UAAAN

Nematology laboratory. From each sample of 1 kg of soil, a subsample of 100 g of soil was taken to be processed by the Baerman funnel methodology (Kleynhans, 1999). The nematodes obtained were identified by means of stereoscopic and compound microscope observations and taxonomic keys.

Field trials: prior to the application of the product Nemmax and Nemacur® 400 CE, a trench was opened around the drip zone of each experimental coffee, in order to bring the product into contact with the root system. The products were applied with a manual sprayer, with capacity of 15 L brand Solo® later covered with soil.

Table 1. Evaluated treatments used to control phytoparasitic nematodes of coffee trees.

Treatment	Nematicide	Dose (product ha^{-1})
T1	Nemmax	2 L
T2	Nemmax	4 L
T3	Nemmax	6 L
T4	Nemacur® 400 CE (fenamiphos)	3 L
T5	Absolute witness	-

During the experiment, the same agronomic management was carried out, such as the control of weeds and foliar diseases. The Nemacur® 400 CE nematicide was applied once in the mooring phase of the coffee cherry, at the beginning of the establishment of the experiment and the Nemmax nematicide was applied three times, the first in the fruit mooring stage and the others with intervals of 15 days between each application.

Final sampling of the nematode population: at 120 days after the last application of the products, in each of the experimental units (two trees), a sample of 1 kg of soil was taken from each coffee bush, at the point North cardinal, in the drip zone to a depth of 40 cm. the two sub-samples were mixed and only a representative sample of 1 kg of soil was taken. The 25 samples were sent to the UAAAN Nematology Laboratory, 100 g of soil of each one were analyzed, by the Baerman funnel technique (Kleynhans, 1999). Nematodes present with emphasis on the genera *Meloidogyne incognita*, *Pratylenchus* spp. and *Dorylaimus* spp. they were identified under the stereoscopic and compound microscopes and the taxonomic keys.

Study results

Initial population of phytoparasitic nematodes associated with coffee

The results of the initial population of phytoparasites are shown in Table 2. The largest population corresponded to *Dorylaimus* spp. that fluctuated between 78.59 to 118.40, followed by *M. incognita* that fluctuated between 42.6 to 77.4 and finally *Pratylenchus* spp. from 18.2 to 24.6, being the lowest compared to the other genres.

Table 2. Initial population of the phytoparasitic nematodes *M. incognita*, *Pratylenchus* spp. and *Dorylaimus* spp. in each of the treatments.

Treatment	Numbers of nematodes in 100 g of soil		
	M. incognita	*Pratylenchus* spp.	*Dorylaimus* spp.
T1	77.4 a	18.2 a	118.4 a
T4	60 b	18.79 a	108.4 a
T3	54 c	24.6 a	110.19 a
T2	46.8 c	22.79 a	98.8 a
T5	42.6 c	20.6 a	78.59 a

The values with the same letter between columns do not show statistical difference according to the Tukey test (p= 0.05).

Results of the final population of phytoparasitic nematodes associated with coffee

The results obtained after the application of the nematicides are observed in Table 3, the population of *M. incognita* decreased significantly compared to the initial population in the treatments (1, 2, 3, and 4) except in the absolute control (T5). Treatment 3 was the one with the highest nematicidal effect on *M. incognita*. Okeniyi *et al*. (2010) reported nematicidal effects with plant extracts up to 100% on *M. incognita*. The population of *Pratylenchus* spp. it also decreased significantly, with treatment 3 causing the greatest control effects on this nematode.

Table 3. Final population of the phytoparasitic nematodes M. incognita, *Pratylenchus* spp. and *Dorylaimus* spp. in each of the treatments.

Treatment	Numbers of nematodes in 100 g of soil		
	M. incognita	*Pratylenchus* spp.	*Dorylaimus* spp.
T1	4 bc	4 bc	6 bc
T2	3.4 bc	2.2 bc	4 c
T3	0.8 c	1.4 c	2.4 c
T4	5.6 b	6.6 b	13.4 b
T5	28.4 a	38 a	132.8 a

The values with the same letter between columns do not show statistical difference according to the Tukey test (p= 0.05).

Kepenekci *et al*. (2016) evaluated, in vitro, the extracts of the indigenous plants *H. niger*, *M. azedarah* and *X. strumarium* on *Pratylenchus thornei*, showing a mortality rate of 100% after 72 h of exposure time. It can be seen that in the absolute control T5 there is a significant difference compared to the other treatments showing an increase in the initial population, the commercial control T4 behaved similar to the treatments T1 and T2 respectively. In *Dorylaimus* spp. A significant decrease in its final population was observed in all treatments except the absolute control T5. Commercial control 4 showed the lowest control levels on this nematode compared to the other treatments. Treatment 2 and 3 had the highest nematicidal effects.

The results obtained agree with Salazar *et al*. (2014) who evaluated plant extracts of *Quassia amara* and *Brugmansia suaveolens* on *Meloidogyne* sp. reaching up to 89% mortality. Vinueza *et al*. (2006) evaluated 15 different plant extracts, achieving up to 100% mortality on *M. incognita*. These high levels of mortality have been attributed by Rice and Coats (1994) to secondary metabolites present as amino acids and alkaloids. Carneiro *et al*. (1998) tested 21 strains of Bacillus spp. against *Meloidogyne javanica* using the supernatant, where the *B. brasiliensis* strain was effective in killing the juveniles and the strains *B. aizawai* and *B. morrisoni* were effective in immobilizing them. In the same way Leyns *et al*. (1995) tested the nematicidal activity of mixtures of spores and crystals of three Bt isolates against juvenile and adult stages of *Caenorhabditis elegans*.

When analyzing the samples obtained from the root system, after the application with nematicides, no nodulations were found in the root that are usually caused by adult females of *M. incognita*, in none of the samples. Parada and Guzmán (1997), mention that the use of papaya extract (*Carica papaya*) provides protection to the roots of the infection by juveniles of *M. incognita*, on the other hand, mention that garlic extract (*Allium sativum*), also reduces the degree of infection in the roots of bean plants (*Phaseolus vulgaris*).

Conclusions

The biological nematicides evaluated significantly reduced populations of phytoparasitic nematodes, proving to be an efficient alternative for the control of filiform nematodes of the genera *Meloidogyne incognita*, *Pratylenchus* spp. and *Dorylaimus* spp. The 6 L ha^{-1} dose of Nemaxx was found to be the most effective without showing phytotoxicity. The absolute witness, showed an increase in the population of the nematodes under study, at the same time the fruit trees of coffee tree showed symptoms of chlorosis in the leaves and little development in the outbreaks of the year.

In the root system of each of the experimental units, no root nodules were found, which usually caused by *Meloidogyne incognita*.

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