Evaluation of a nematicide product on parasitic nematodes of the Dominico Harton plantain *(Musa AAB)*

Evaluación de un producto nematicida sobre nematodos fitoparásitos del plátano Dominico Hartón (*Musa* AAB)

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Abstract

To establish the effectiveness of a molecule with nematicida action against parasitic nematode of the plantain, experiments were conducted at the Phytopathology laboratory of the University of Caldas. The effect of concentrations of 0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 16.0, 32.0, 64.0, 96.0 y 240.0 ppm of MCW-2 was evaluated on the mortality of populations of nematodes of the genera *Radopholus*, *Pratylenchus*, *Meloidogyne* y *Helicotylenchus* during 24, 48 and 72 h of exposition. As the relative control Carbofuran was used. The absolute control was the treatment with water. A randomized experimental design was used in a factorial arrangement of two products, 12 concentrations and three times of exposure. The levels of mortality at concentrations above 16 ppm were higher with MCW-2 than with Carbofuran and vice versa; that in all of the concentrations mortality increased with the time of exposition; that *Meloidogyne* was the genus most sensitive to MCW-2 while *Pratylenchus* so was to Carbofuran and that the 50% lethal concentration for MCW-2 for the four genera of nematodes was below 115 ppm and to Carbofuran was between 415 and 715 ppm. It was concluded that MCW-2 constitutes a good option to the control of nematodes in plantain, although it necessary to evaluate it on plants growing in plastic bags.

Key words: Dose-response, *Meloidogyne*, mortality, *Radopholus*.

Resumen

En el Laboratorio de Fitopatología de la Universidad de Caldas, Colombia, se evaluó el efecto de las concentraciones 0.5, 1, 2, 4, 6, 8, 16, 32, 64, 96 y 240 ppm de la molécula MCW-2 sobre la mortalidad de poblaciones de nematodos de los géneros *Radopholus*, *Pratylenchus*, *Meloidogyne* y *Helicotylenchus* durante 24, 48 y 72 h de exposición. Como testigo relativo se empleó Carbofurán y como testigo absoluto se utilizó agua. Se uso un diseño al azar, en un arreglo factorial de dos productos, doce concentraciones y tres tiempos de exposición. Los resultados indicaron que los niveles de mortalidad en las concentraciones superiores a 16 ppm fueron mayores con MCW-2 que con Carbofurán; en todas las concentraciones la mortalidad aumentó a medida que el tiempo de exposición fue mayor; *Meloidogyne* fue el género más sensible a MCW-2, mientras que *Pratylenchus* lo fue a Carbofurán; la concentración letal media de MCW-2 para los cuatro géneros de nematodos fue < de 125 ppm y la de Carbofurán varió entre 415 y 715 ppm. Se concluye que MCW-2 constituye una buena opción para el control de nematodos en plátano, aunque es necesario evaluarlo en plántulas en condiciones de campo.

Palabras clave: Dosis-respuesta, *Meloidogyne,* mortalidad, *Radopholus*.

Introduction

In Colombia the crop of Dominico Harton plantain (*Musa* AAB) is of great social and economic importance, being a traditional sector of the rural economy, with a total of 368392 ha of cultivated land, a production of 2970435 t and an output of 8.1 t/ha. Moreover, it is one of the key products of the family basket, with wide geographical distribution and a source of labor that generates 0.51 jobs/ha per year, equivalent to 177.853 permanent jobs by the year 2011 (Belalcázar *et al.*, 1991; Sanchez and Aranzazu 2000; Martinez *et al.*, 2006; Agronet, 2011). This culture has been primarily used to shade coffee, but participates with 8.1% of the total national agricultural production and ranks fifth in production after coffee, sugar cane, bananas and flowers (Martinez *et al.*, 2006; Agronet, 2011).

The most important phytosanitary problems in banana cultivation are black and yellow Sigatoka (*Mycosphaerella* spp.) that affect the foliage, the black weevil (*Cosmopolites sordidus*) that affects the corm, and plant parasitic nematodes that affect the roots and corm. The latter have forced the development of various methods of handling, from chemical to biological, that help decrease the damage caused (Montiel *et al.*, 1997; Buriticá, 1999; Guzman and Castaño-Zapata, 2004). The nematode problematic is worsened by the lack of adequate cultivation practices, planting susceptible varieties and by the lack of technical knowledge about their agronomic management; but mainly by the use of planting materials of low quality, which favors the propagation and spread of plant parasitic nematodes (Guzman, 2011; Palencia *et al.*, 2006).

The most frequent nematodes in banana plantations are: *Radopholus similis, Pratylenchus* sp., *Meloidogyne* sp. and *Helicotylenchus multicinctus*, which are common and destructive and widely distributed in growing areas worldwide (Guzman and Castaño-Zapata, 2004). In general, the agronomic management of nematodes is done with chemical and biological products; however some studies indicate that the latter are neither effective nor sufficient to substantially reduce populations of plant parasitic nematodes in soil or roots (Gonzalez *et al.*, 2009). The chemical nematode management is more effective than the biological, however, chemical products such as Aldicarb have been removed from the market due to its high toxicity, and others like Carbofuran are highly toxic to humans and to the environment and are not specific against such pathogens (RDS, 2007; EPA, 2010; Wildlife Direct, 2010).

The existence of a molecule was recently reported with nematicidal action on *Meloidogyne*, called MCW-2 (thiosulphene), a systemic non-fumigant product that acts by contact and causes locomotion reduction or paralysis of nematodes, suppresses food consumption an hour after contact, reduces the ability of the nematode to infest, the eggs do not develop, decreases oviposition and hatching or prevents the survival of juveniles (Schiller *et al.*, 2007). This product, however, has not yet been studied against parasitic nematodes of plantain crops, therefore it is necessary to evaluate it as a new molecule with nematicide action.

Materials and methods

The experiment was conducted at the Laboratory of Plant Pathology in the University of Caldas, Colombia, and included the nematode genera: *Radopholus*, which was extracted from roots of Dominico Harton plantain; *Meloidogyne*, which was isolated from fruit tomato roots; *Pratylenchus*, extracted from roots of pineapple; and *Helicotylenchus*, from soil samples where plantain crops existed.

Nematode extraction from affected roots

The extracted crop roots were washed with tap water and after drying them at room temperature a sample of 30 g was taken in an analytical balance (Analytical Plus, Ohaus), cross sectioned into 1 cm pieces for

homogenization before liquefying them with 100 ml of water for three times at the lowest speed for 10 sec with 10 sec intervals. The resulting mixture was passed sequentially through a column of sieves with meshes of 710, 250, 106 and 25 μm. The resulting sample on each sieve was washed with pressurized water to facilitate the release of nematodes, except for the 25 μm sieve, as the final material in it was collected in 28 ml centrifuge tubes. Subsequently, they were subjected to 3800 rpm in a centrifuge (Clay Adams) for 5 min. As a result of the centrifugation sedimentation of heavy particles occurred in the bottom of the tube and supernatant at the top, which was removed. Then the tubes were filled with a sucrose solution (50%) and again centrifuged at 3,800 rpm for 5 min to promote the flotation of nematodes in the sucrose solution and separate the denser particles. The supernatant was placed on 25 μm sieve and washed with tap water at low pressure to remove sucrose and avoid physical deterioration of nematodes. Finally 20 ml of water with nematodes were collected and placed in a Petri dish. Extraction of soil nematodes was done similarly, with omission of the liquefying process (Araya *et al.*, 1995). For counting and identification of individuals a microscope (LW Scientific Revelation III) and the help of the keys of Mai *et al.* (1996) were used.

In vitro evaluation of the nematicidal products

For the toxicity tests active nematodes were used. Suspensions of each genus were adjusted to 50 nematodes/ml of distilled water (Pinkerton and Kitner, 2006). In each Petri dish (60 x 15 mm) 1 ml of each suspension of nematodes was placed and immediately added 10 ml of the appropriate concentration of MCW-2 or carbofuran, at concentrations of 0.5, 1, 2, 4, 6, 8, 16, 32, 64, 96 and 240 ppm of each product.

Petri dishes with nematodes were incubated at 28 °C in the dark. The mobility evaluation of nematodes was done according to the product exposure times, which were 24, 48 and 72 hours. To do this, before each assessment, the Petri plates were shaken gently to disperse individuals; later observations at the stereoscope (Leica Zoom 2000) were made, with 25 to 30x magnification, placing the boxes on a circle of paper the same diameter (60 mm), divided into triangular sections. In each sector, the number of nematodes exhibiting own movement was recorded; those which showed no movement were touched gently with a filament and if they reacted were then recorded as active nematodes; those who showed no mobility were individually extracted from this solution and left in sterile distilled water in Petri dishes for 24 h in the dark. After this time, the mobility assessment was performed again as described above; finally nematodes that showed no mobility were recorded as dead individuals (Pinkerton and Kitner, 2006).

Experimental design

A complete randomized experimental design in a factorial arrangement with two nematicidal products, 11 concentrations for each and three exposure times of nematodes at each concentration, plus an absolute control, for a total of 69 treatments was used, with four replications and fifty nematodes per repetition (Table 1). Each nematode genus was evaluated separately, and records for mobility and mortality rate for the four genera studied were made. The data obtained were subjected to analysis of variance and for separating main effects Tukey tests at a probability level of 5% were performed by using the program Statistical Analysis System (SAS, 2009). With the average mortality data in each of the concentrations for both products and by simple regression technique, the median lethal concentration for each kind of nematode studied was calculated.

Results and discussion

The analysis of variance for nematicide effect (number of dead individuals) showed significant differences ($P < 0.05$) among the products tested, concentrations and

exposure times for each of the nematode genus studied. The interactions product x concentration, product x time of exposure and concentration x time of exposure also showed significant effects, indicating that the nematicide action of the products used depends on the concentration and exposure time (Table 1), which is confirmed by high coefficients of determination (R^2 > 0.9) found.

Nematicidal effect of MCW-2 and Carbofuran

At concentrations > 64 ppm the action of MCW-2 was higher than that of Carbofuran (P < 0.05); by contrast, in concentrations of 32 ppm or less the response was reversed except for *Meloidogyne*, since in this genus mortality with MCW-2 was equal to or greater than that obtained with Carbofuran (Figure 1B). The highest concentration of MCW-2 (240 ppm) caused 100% mortality (Figure 1A, C and D) except for *Meloidogyne* which experienced the same level of mortality at a concentration of 64 ppm. This indicates that this group of nematodes is more sensitive to the action of the product. To achieve rates of mortality above 50% in the nematode genera *Radopholus, Pratylenchus* and *Helicotylenchus* was necessary to use concentrations above 64 ppm.

The concentrations of carbofuran caused low levels of mortality in the nematode genera evaluated, reaching maximum values of 30% mortality with the highest product concentration (240 ppm), when applied to *Pratylenchus* and *Helicotylenchus* genera (Figure 1C and D). However, when the product is used in high concentrations it increased its nematicide action but, as we know, it also increases its toxic action for humans and the environment. This product has one of the highest acute toxicity to humans, only Parathion and Aldicarb are more toxic. A minimum amount (1 ml) of carbofuran can be fatal, since its toxic effects are due to its activity as cholinesterase inhibitor for which it is considered a neurotoxic pesticide (Bayer, 2003; FMC, 1995).

Table 1. Analysis of variance (mean squares) for nematicide effect (dead individuals) of two chemical products in different concentrations and exposure times in four genera of plant parasitic nematodes.

Source	d.f	Genera			
		Radopholus	***Meloidogyne*** [a]	***Pratylenchus***	***Helicotylenchus***
Model	44	653.1**	328.2**	653.1**	615.7**
Product (P)	2	1225.7**	635.5**	896.3**	841.9**
Concentration (C)	9	1734.1**	74.4**	1950.3**	1815.9**
Time (T)	2	275.8**	130.3**	130.6**	190.2**
P x C	9	1041.9**	519.7**	962.2**	908.2**
P x T	4	42.9**	106.4**	15.6*	24.6**
C x T	18	32.2**	72.2 **	22.6**	22.8**
Error	21	6.7	6.9	4.9	5.0
R^2		0.951	0.905	0.9	0.961
V.C. (5%)		31.2	95.9	27.9	30.7

* P < 0.05, ** P < 0.01.

a. The degrees of freedom (d.f.) for this genus are seven given that eight concentrations of the products were obtained.

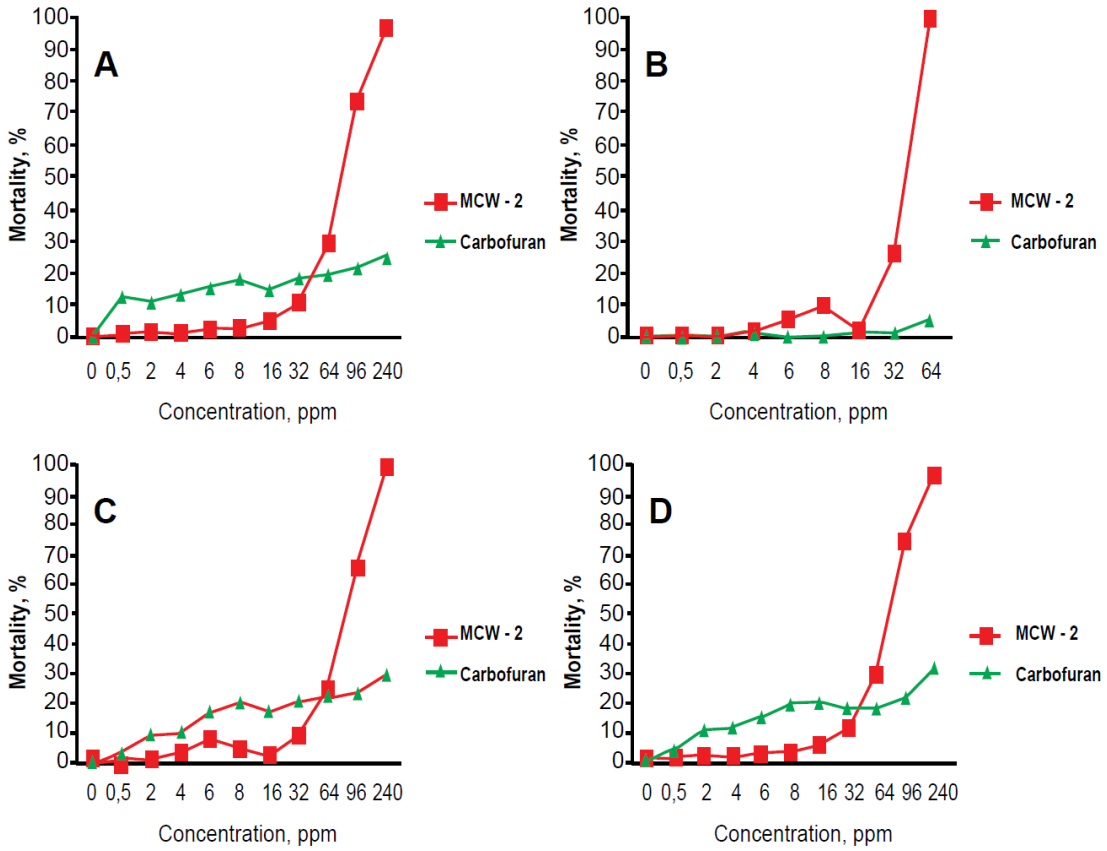

Figure 1. *In vitro* effect of different concentrations of MCW-2 and Carbofuran on mortality in populations of plant parasitic nematodes. (A) *Radopholus*, (B) *Meloidogyne*, (C) *Pratylenchus* and (D) *Helicotylenchus*.

Arboleda *et al.* (2010), when evaluating the effect in vitro of aqueous extracts of roots, stems, leaves and fruits of castor (*Ricinus communis* L.) in concentrations of 25%, 50% and 100% vs. carbofuran at a dosage of 330 ppm on the burrowing nematode *R. similis*, found after 48 h, a better effect of the former at a concentration of 100% with control values ranging between 67% and 73%.

The results in this study show that MCW-2 is more efficient than Carbofuran to reduce populations of plant parasitic nematodes, especially when employed at relative high concentrations; moreover, its use in concentrations <240 ppm is not efficient as nematicide and in higher doses is toxic to humans and polluting the environment.

Effects on *Radopholus*

For both Carbofuran and MCW-2, increasing the exposure time at each concentration of the product meant increases in the mortality percentage of *Radopholus*. This tendency was particularly sustained when the concentration of both products was 16 ppm or greater. At no exposure time the mortality with Carbofuran was > 30% in the concentrations used. With MCW-2 mortality greater than 50% was reached when nematodes were exposed for 48 hours at a concentration of 96 ppm. The maximum mortality achieved with this product was 99% with an exposure of 72 hours at a concentration of 240 ppm, whereas with Carbofuran the maximum mortality was 28% with the same concentration and exposure time (Figure 2A).

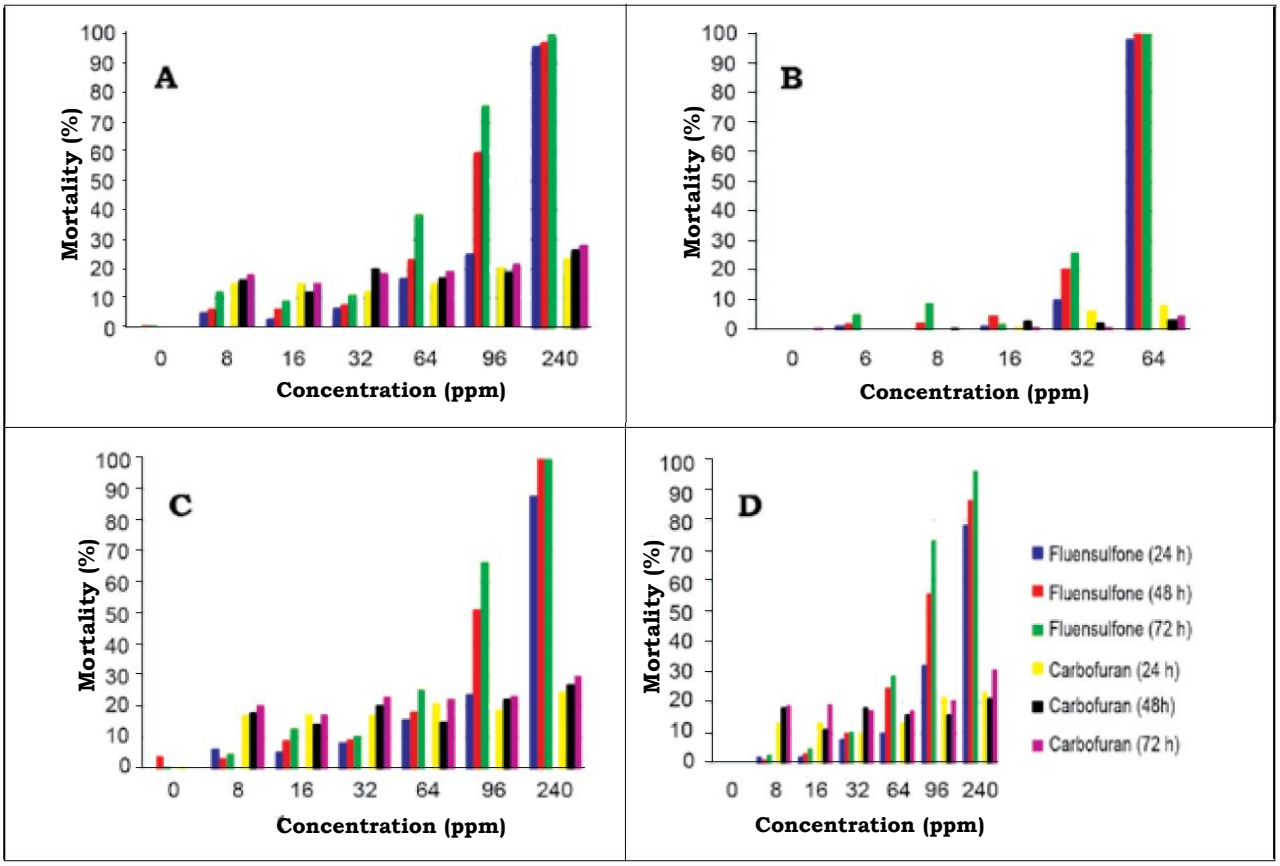

Figure 2. Effect of time of exposure to different concentrations of nematicides on the mortality of nematodes of the genera *Radopholus* (A), *Meloidogyne* (B), *Pratylenchus* (C) and *Helicotylenchus* (D).

Effects on *Meloidogyne*

Unlike the other nematode genera, in this genus a mortality of 100% was reached when nematodes were exposed to a concentration of 64 ppm for 48 or 72 h. For the other concentrations and at the same times of exposure, mortality was always lower than 30%. When nematodes were treated with Carbofuran, mortality did not exceed 5%, even with the highest concentration of the product. For both products, the increase in the exposure time of nematodes at different concentrations did not cause sustained increases in mortality percentage of the populations (Figure 2B).

Oka *et al.* (2009) found irreversible nematicide activities of MCW-2 against second stage juveniles of *M. javanica* under *in vitro* conditions, after exposure for 48 hours at concentrations as low as 0.5 mg/l, in contrast to Fenamiphos or Cadusafos. When the nematode was exposed to MCW-2 for shorter periods, the mobile juveniles were paralyzed, but eventually recovered their movement after being washed with sterile distilled water. Similarly, Driver and Louws (2003) found that MCW-2 provides similar levels of control of *Meloidogyne* spp. in pumpkin, compared to Vydate and Telone.

Effects on *Pratylenchus*

Starting from the concentration of 64 ppm and 48 h of exposure, mortality caused by MCW-2 was consistently higher than that produced by Carbofuran. The increase in the mortality rate due to longer exposure time to the product was sustained from the concentration 16 ppm for MCW-2 and 32 ppm for Carbofuran. To achieve a 50% mortality with MCW-2 was necessary a minimum exposure of 48 hours at a concentration of 96 ppm; however, this did not happen with Carbofuran whose

maximum mortality (30%) was achieved with the highest concentration and longer exposure time (Figure 2C).

Pinkerton and Kitner (2006) evaluated the effect of biological products on the mobility in vitro and reproduction in Totem strawberry plants of individuals of *P. penetrans*, exposed to a range of concentrations of the product for 24, 48, and 72 h and found that exposure for 72 h to the product DiTera at a concentration of 1380 mg/l, resulted in the immobilization of 90% of the nematodes without recovery of mobility; while Fenamiphos in solution with 70 µl/l immobilized about 75% of the nematodes, although 17% of them regained mobility during subsequent incubation in water.

Effects on *Helicotylenchus*

As with previous nematodes genera, the increased exposure time to both products caused an increase in mortality of the populations of *Helicotylenchus*; however, this trend was only sustained from concentrations 16 ppm for MCW-2 and 32 ppm for Carbofuran. Starting from the concentration of 64 ppm and at all exposure times, mortality caused by MCW-2 was consistently superior to that produced by Carbofuran. With MCW-2 mortality was greater than 50%, when the nematodes were treated with a solution of 64 ppm during 48 h of exposure, reaching the highest mortality with the most concentrated product solution and the maximum exposure time. With Carbofuran, the maximum mortality achieved did not exceed 31% (Figure 2D).

Concentration – mortality relation

To establish the relationship between product concentrations and mortality caused by each of them after 72 h of exposure in the populations of the four genera of parasitic plant nematodes, a simple linear regression analysis was performed, whose results are included in Table 2. Figure 2 shows the results for MCW-2. In the four nematode genera studied the effect of MCW-2 product concentrations was more marked than that produced by Carbofuran, since the slopes of the equations were always higher in the first than the second. In general, the genus *Meloidogyne* was the most sensitive to MCW-2 when compared with the other genera studied; in turn, these showed similar sensitivity to the product, since the slopes of the regression equations were alike (Figure 2). Wirtano *et al.* (2009) found an LC50 of 25.3% and 19 ppm for the nematicides Clorpyfiros and Carbofulsan respectively, after 24 h of exposure of *Melodogyne* individuals to such products.

The results indicate that an increase of 10 ppm in the concentration of MCW-2 would at least cause 4% increase mortality in populations of *Radopholus, Pratylenchus* and *Helicotylenchus*, while in *Meloidogyne*

Table 2. Components of regression for the variables concentration - mortality and median lethal concentration (LC50%) for four nematode genera treated with two nematicide products.

Product	Nematode genus	Intercept	Slope	R^2	$LC_{50\%}$ (ppm)
MCW – 2	*Radopholus*	1.6	0.44	0.906	110.0
	Meloidogyne	5.5	1.47	0.916	31.8
	Pratylenchus	1.8	0.43	0.940	112.1
	Helicotylenchus	1.5	0.40	0.906	121.5
Carbofuran	*Radopholus*	12.8	0.06	0.460	620.0
	Meloidogyne	0.17	0.07	0.87	711.8
	Pratylenchus	12.3	0.09	0.508	418.9
	Helicotylenchus	11.0	0.09	0.582	433.3

populations this mortality increments would be of 14.7%. *Helicotylenchus* was the genus that presented a higher LC50% with MCW-2. For the case of Carbofuran, the same analysis established that it is necessary to increase its concentration in 100 ppm to raise *Radopholus'* mortality by 6%, *Meloidogyne* by 7% and *Pratylenchus* and *Helicotylenchus* by 9% (Table 2). The same regression equations were used to calculate the concentration of each product that produces 50% mortality (LC50%) in the populations of the four nematode genera studied. The results indicate that genus *Meloidogyne* is the most susceptible to MCW-2 since it presented the lowest LC 50%; while the other nematode genera exhibited a similar sensitivity to the product. In the nematode genera studied the LC50% was higher with Carbofuran, being the most sensitive gender to the product *Pratylenchus*, since its LC50% was the lowest, although slightly lower than that shown by *Helicotylenchus* (Table 2). Importantly, the coefficients of determination for the regression equations obtained when the nematodes were treated with MCW-2 are significantly higher than those achieved with Carbofuran; which gives them a greater probabilistic reliability to the results obtained with the first.

Conclusions

- In this study, MCW-2 was a more efficient product than Carbofuran in its nematicide action.
- The non-fumigant nematicide MCW-2 must be employed at relative high concentrations (> 110 ppm) to cause mortalities greater than 50%, except for nematodes of the genus *Meloidogyne*, with which its nematicidal action is achieved at lower concentrations.
- Carbofuran presented a higher nematicidal action than MCW-2 at low concentrations of both products, but its effects on mortality were significantly lower. At relative high concentrations, the nematicidal action of MCW-2 is superior to that obtained with Carbofuran.
- Increases in the nematode exposure time to both products determine an increase in the mortality values; particularly in relatively high concentrations.
- *Meloidogyne* was the most sensitive genus to MCW-2 while *Pratylenchus* was it to Carbofuran. The average lethal concentration of the former MCW-2 for the four genera of nematodes is less than for the latter.
- MCW-2 has nematicide action and constitutes a good choice for the control of nematodes in bananas, although it must be evaluated under field conditions.

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