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Toxicity of manipueira to *Meloidogyne incognita* in soybean¹

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ABSTRACT

Manipueira, a liquid residue obtained from the cassava industrialization, shows high toxicity to the microbial diversity. This study aimed at evaluating the potential of manipueira applied to the soil to control *Meloidogyne incognita* in soybean. A completely randomized design, in a 2 x 11 factorial scheme, was used, consisting of two application forms of manipueira (single and two applications), in eleven concentrations (0 %, 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % and 100 %), with five replications per treatment. Some agronomic traits and parasitism characteristics were also evaluated. The plants that received a single application of manipueira showed a gain of 100.41 % in root length, while the volume and fresh root mass showed gains of 81.52 % and 28.11 %, respectively, with the two applications. Regarding parasitism, the single application was more effective in reducing the number of juveniles in the soil and roots, where the concentrations of manipueira to kill 50 % of the nematodes were 1.65 % and 4.37 %, respectively. Thus, besides being effective in controlling *M. incognita*, manipueira has a positive effect on the development of soybean and may be recommended as a nematicide and also as an organic fertilizer.

KEYWORDS: *Glycine max*; root-knot nematode; parasitism.

RESUMO

Toxicidade de manipueira sobre
Meloidogyne incognita em soja

A manipueira, resíduo líquido da industrialização da mandioca, apresenta alta toxicidade à diversidade microbiana. Objetivou-se avaliar o potencial de manipueira aplicada ao solo, visando ao controle de *Meloidogyne incognita* na cultura da soja. Utilizou-se delineamento experimental inteiramente casualizado, em esquema fatorial 2 x 11, constituído por duas formas de aplicação de manipueira (única e dividida em duas), em onze concentrações (0 %, 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % e 100%), com cinco repetições por tratamento. Algumas variáveis agrônômicas e características do parasitismo também foram avaliadas. As plantas que receberam aplicação única de manipueira apresentaram ganho no comprimento radicular de 100,41 %, enquanto o volume e a massa fresca do sistema radicular apresentaram ganhos de 81,52 % e 28,11 %, respectivamente, com a aplicação dividida. Quanto ao parasitismo, a aplicação única foi mais eficiente na redução do número de juvenis no solo e na raiz, onde as concentrações de manipueira para matar 50 % dos nematoides foram de 1,65 % e 4,37 %, respectivamente. Assim, a manipueira, além de ser eficiente no controle de *M. incognita*, influencia de forma positiva no desenvolvimento da cultura da soja, podendo ser recomendada como nematicida e, também, como adubo orgânico.

PALAVRAS-CHAVE: *Glycine max*; nematoide das galhas; parasitismo.

INTRODUCTION

Soybean (*Glycine max* L.) is currently distinguished as one of the most expressive crops of the Brazilian economy. It represents more than 58 % of the area cultivated with grains in the country, with an average yield of 3,011 kg ha⁻¹, and is still going through a vigorous process of expansion (Conab 2015). However, depending on the region, several fungi, bacteria, viruses (Santos et al. 2011) and

nematodes may damage the crop and limit its yield (Araujo et al. 2012).

Pratylenchus brachyurus, *Heterodera glycines*, *Meloidogyne incognita*, *M. javanica* and *Rotylenchulus reniformis* are currently the most frequent nematodes in soybean fields. These species are difficult to control and may cause significant damages, thus affecting yield (Chaves et al. 2004). The most aggressive species of *Meloidogyne*, such as *M. incognita*, may reduce the soybean yield in

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30 %, especially when favored by climatic conditions (Araujo et al. 2012). The meloidogynes induce the formation of feeding sites (nurse cells), causing cell changes in vascular elements and losing roots, which reduce the size and efficiency of the root system (Silva et al. 2011).

Phytonematodes are difficult to eradicate, especially in extensive cultivation crops such as soybean, cotton, corn, coffee, etc., due to their survival mechanisms, polyphagia and cosmopolitan distribution (Oliveira et al. 2005, Bruinsma 2013). Because of these characteristics, management strategies, such as crop rotation, use of resistant genotypes and chemical and biological control, should be planned and systematized, in order to integrate cost-effective methods (Santos 2012).

In general, the chemical control brings fast and efficient results (Oliveira et al. 2005). However, the ongoing use of the same active ingredient has caused numerous negative effects, such as high toxicity, risk of environmental contamination, high cost and selection of resistant populations (Dong & Zhang 2006).

The search for new alternatives to control phytonematodes, in order to reduce those effects and maintain the biodiversity in different agroecosystems, is a global concern (Ferraz & Freitas 2004). Thus, the use of manipueira, a liquid residue obtained from cassava pressing, composed of cyanogenic glycosides, mainly linamarina, that releases cyanide gas, which is toxic to some living beings, including nematodes, may be a viable option (Nasu et al. 2010).

In Brazil, positive tests with manipueira application in the control of nematodes have been successfully performed for different phytonematodes. Examples include *H. glycines* in soybean (Comerlato 2009), *Meloidogyne* spp. in tomato (Nasu et al. 2010), *M. incognita* in carrot (Baldin et al. 2012), *M. incognita* in papaya seedlings (Damasceno et al. 2008) and *Scutellonema bradys* in yam (Almeida et al. 2007). However, there are no researches with application of this residue in root-knot nematode suppressing in soybean. Therefore, this study aimed at evaluating the potential of manipueira applied to the soil for controlling *Meloidogyne incognita* in soybean crops.

MATERIAL AND METHODS

The experiment was performed in a greenhouse, at the Universidade Federal do Piauí, in Bom

Jesus, Piauí State, Brazil, from March to August, 2015.

Plastic pots (4.5 dm³ capacity) were arranged on a bench. The substrate consisted of soil-sand-manure (3:2:1), sterilized in a vertical autoclave at 120 °C and 1.05 kg cm⁻², during 2 h. The substrate (medium coarse texture) presented the following characteristics: pH of 4.3; 15.8 g L⁻¹ of organic matter; 710 g kg⁻¹ of sand; 50 g kg⁻¹ of silt; and 240 g kg⁻¹ of clay.

The nematode inoculum was obtained from a population of *M. incognita*, in soybean crops from Bom Jesus. The eggs and juveniles were extracted according to Coolen & D'Herde (1972), by liquefaction and centrifugation in kaolin with sucrose solution. The inoculum was multiplied in tomato plants (*Solanum lycopersicum* L. 'Santa Clara' cultivar), in pots kept in a greenhouse for 50 days. The species identification was previously performed using temporary (formalin) and permanent slides (glycerin), based on the specific taxonomic keys for the genus (Handoo & Golden 1989). The identification was also made by examining the perineal configuration and confirmed by the isoenzyme electrophoresis technique of a esterase (Carneiro & Almeida 2001).

The experiment was arranged in a completely randomized design, in a 2 x 11 factorial scheme. The manipueira application was performed as it follows: single (application of 100 ml pot⁻¹) and divided in two (two applications of 50 ml with 15 days of interval), in eleven concentrations (0 %, 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % and 100 %), with five replications per treatment. The manipueira was obtained in Redenção do Gurgueia (Piauí State), stored in glass flasks and placed in a refrigerator until the solution was prepared.

Sowing was performed with 5 soybean seeds (Intacta cv. M-Soy 8644 IPRO) per pot, on May 5 (2015), and the emergence occurred within two days. At 14 days after emergence, thinning was performed, leaving 2 plants per pot, which were considered the experimental unit. At 2 days after thinning, a suspension of 3,000 eggs + juveniles pot⁻¹ of *M. incognita* was individually applied with a pipette, adding 10 ml of the inoculum solution over three holes (3.0 cm depth), spaced 2.0 cm from the hypocotyl of soybean plants.

At 7 days after the inoculation, the manipueira was applied separately to each pot, according to the respective form of application and concentrations

tested. The concentrations were determined using manipueira dilutions in distilled water and prepared 2 h before the application of treatments in the soil.

During the experiment, temperature (environment and soil) and relative humidity were monitored. According to the data, it was observed that the average room temperature in the greenhouse ranged 25-35 °C, with soil temperature of 23-32.5 °C and relative humidity of 23-40 % on most days.

The evaluations were performed at 60 days after the first application of manipueira. At first, the following agronomic traits were evaluated: root length - using a graduated ruler; root fresh mass - with a semi-analytical scale; and root volume - calculated by the difference in water volume, after the roots were immersed in a 500 ml test tube.

The number of galls and egg mass in the root of each treatment were evaluated. For counting eggs, the roots were dyed in a solution (5 mg of acid fuchsin, 250 ml of acetic acid and 750 ml of distilled water), where they were immersed for approximately 2 min (Silva et al. 1988). The egg counting was made with a magnifying glass. The estimated number of juveniles and eggs in the soil of each treatment, extracted from 100 cm³ of soil by centrifugation and flotation (Jenkins 1964), were also evaluated. The number of juveniles and eggs from roots were estimated according to Coolen & D'herde (1972). After the quantification of nematodes, the parasite reproduction factor for each treatment was calculated by the sum of the final soil population and final root population divided by the initially inoculated population (Oostenbrink 1966).

The agronomic and parasitism variables data were submitted to normal analysis by the Shapiro-Wilk test and to analysis of variance (Anova) by the F test ($p < 0.05$). When significant, the averages for manipueira concentrations were used in a regression analysis performed with the aid of the Sigmaplot 10.0 software.

RESULTS AND DISCUSSION

The analysis of variance indicated a significant interaction between the application form and concentration of manipueira, with a significant effect ($p < 0.01$) for all soybean agronomic traits: root length, root volume and root fresh mass (Table 1).

The growth and root development of soybean plants showed a considerable gain after the manipueira

application to the soil, with quadratic responses, depending on the concentrations applied (Figure 1). The highest means for root length were observed in plants that received the single application of manipueira in concentrations from 60 %, reaching a maximum increase (100.41 %) with the manipueira concentration of 100 % (Figure 1A).

Manipueira has a complex chemical composition, including all macro and micronutrients of plants (except molybdenum) (Baldin et al. 2012), what justifies the best root development in plants treated with this solution. Positive results with the use of manipueira on agronomic traits of tomato were also observed by Nasu et al. (2010), with the plants treated with 50 % of manipueira achieving a greater root length.

Regarding root volume (Figure 1B) and root fresh mass (Figure 1C), the best results were obtained with two applications of manipueira in concentrations from 50 %, reaching the maximum increments of 81.53 % and 52.33 %, respectively, with manipueira at 100 %. However, with the single application of manipueira, there was also considerable increase in volume and root fresh mass in concentrations from 50 %, showing respective maximum gains of 50.09 % and 28.11 %, with 100 % of manipueira. Franco et al. (1990) also observed an increase in the shoot and root of tomato, in soil treated with manipueira at 50 %. Baldin et al. (2012), with the application of manipueira at 50 %, obtained an increase in root weight of 101.30 %, which suggests that it is as efficient as the Carbofuran 50 G nematicide.

For the parasitism variables, there was interaction between the application forms and concentrations of manipueira, with a significant effect on the number of juveniles in the root ($p < 0.05$)

Table 1. Summary of the analysis of variance (mean squares and F test) for agronomic traits of soybean plants inoculated with *Meloidogyne incognita*, depending on the application form and concentration of manipueira.

Source/variation	Soybean agronomic trait		
	Root length	Root volume	Root fresh mass
Application form (AF)	334.95**	0.01 ^{ns}	206.91*
Manipueira concentration (MC)	267.02**	215.16**	120.11*
AF x MC	103.72**	71.17**	175.02**
CV (%)	24.11	20.06	25.41

** Significant at 1 %; * significant at 5 %; ^{ns} not significant.

and in the soil ($p < 0.01$) (Table 2). However, other variables were influenced by the individual performance of manipueira concentrations.

The application forms of manipueira influenced the number of juveniles of *M. incognita* in the soil

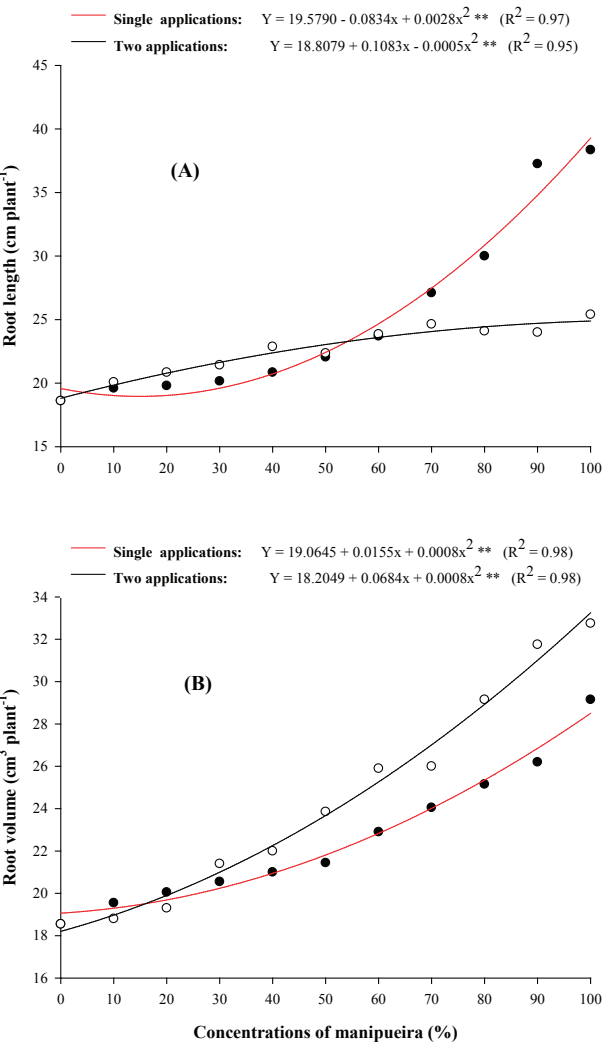


Figure 1. Root length (A), root volume (B) and root fresh mass (C) of soybean plants, depending on the application form and concentration of manipueira, in the management of *Meloidogyne incognita*. ** Significant at 1 %.

Table 2. Summary of the analysis of variance (mean squares and F test) for *Meloidogyne incognita* parasitism variables in soybean plants, depending on the application form (AF) and concentration (MC) of manipueira.

Variation source	Parasitism variable of <i>M. incognita</i>						
	NJS	NJR	NG	EM	NER	NES	RF
AF	3,505.82 ^{ns}	254,138.27**	6,833.53 ^{ns}	2.47 ^{ns}	41,279.22 ^{ns}	90.90 ^{ns}	0.94 ^{ns}
MC	13,934,066.24**	275,016.02**	77,436.16**	576.21**	331,873.99**	12,420.54**	17.07**
AF x MC	111,286.42**	46,693.72*	2,499.85 ^{ns}	15.67 ^{ns}	56,777.42 ^{ns}	316.90 ^{ns}	0.84 ^{ns}
CV (%)	11.69	12.48	8.57	14.82	8.47	18.96	31.76

** Significant at 1 %; * significant at 5 %; ^{ns} not significant. NJS - number of juveniles in the soil; NJR - number of juveniles in the root; NG - number of galls; EM - egg mass; NES - number of eggs in the soil; NER - number of eggs in the root; RF - reproduction factor.

with exponential reduction, in response to the concentrations applied (Figure 2A). The lower averages of this variable have been observed with the single application and, according to the regression equation, the lethal manipueira concentration (LC_{50}) was 1.65. The most significant reduction of juveniles in the soil was observed from the concentration of 10 %, with a reduction of 94.14 %, if compared to the control. For the two applications treatment, a greater lethal concentration was determined ($LC_{50} = 4.44$), reaching the most significant reduction (94.95 %) at the concentration of 30 %.

The reduction in the population of *M. incognita* in this study may be explained by the presence of toxic compounds in manipueira, which acted as a nematicide substance. The main toxic compounds of manipueira are hydrocyanic acid (HCN) and free cyanide (CN⁻), which have antimicrobial action, with different action routes. The HCN interferes in the nematode enzymatic activity (Fioretto & Brinholi

1985) and the CN- acts on nematode nerve cells, forming cianohemoglobina, which causes paralysis in the nervous system, including the respiratory chain

(Nasu 2008). This explains the best results with the single application via soil, which provided higher nematodes mortality rates.

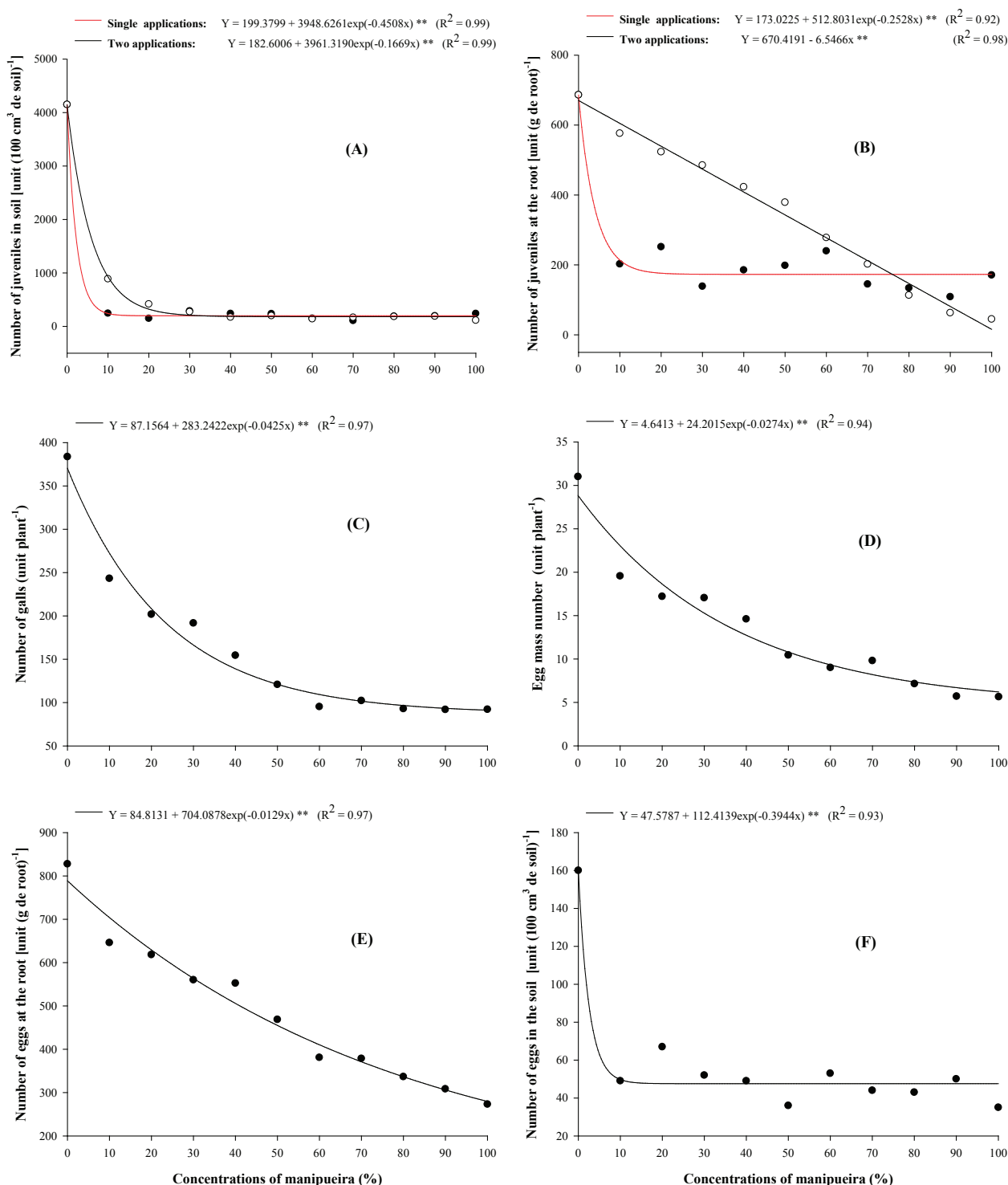


Figure 2. Number of *Meloidogyne incognita* juveniles in the soil (A), roots (B) and galls (C), egg mass (D), eggs in the root (E) and eggs in the soil (F) in soybean plants, depending on the form of application and concentration of manipueira.
 ** Significant at 1 %.

Nasu et al. (2007) tested manipueira in the control of *M. incognita* *in vitro*, with concentrations of 10 %, 25 %, 50 %, 75 % and 100 %, reaching 100 % of mortality in all the treatments. In another study about the effect of manipueira on *M. incognita* in tomato plants, using treatments with manipueira up to 10 % of dilution from 40 mg L⁻¹ of CN⁻ in pure manipueira, the control was effective, causing the death of 100 % of the juveniles (Nasu et al. 2010).

For the number of *M. incognita* juveniles in the root, there was a significant difference between the application forms of manipueira. Using the exponential regression equation, a LC₅₀ = 4.37 % of manipueira was estimated for the single application treatment (Figure 2B). Maximum reductions were found at concentrations from 20 % of manipueira, with a percentage of 68.80. For the treatment with two applications, a greater LC₅₀ of 58.84 % was estimated by the linear polynomial regression equation. With this split application treatment, a greater reduction than with the single application was only observed with concentrations greater than 80 %, reaching a maximum decrease (97.65 %) in the concentration of 100 %. Once again, these results indicate the potential of manipueira as a nematicide, providing higher concentrations of HCN and CN⁻, when the single dose is applied via soil.

The number of galls (Figure 2C) and egg mass (Figure 2D) of *M. incognita* in soybean plants reduced exponentially with the manipueira concentrations. The reduction of 50 % in the averages of these variables was observed with manipueira concentrations at 24.94 % and 33.07 %. The maximum reduction of these variables occurred at a concentration of 100 % of manipueira, with respective percentages of 75.38 % and 78.49 %. According to Nasu et al. (2010), studying tomato plants previously inoculated with *M. incognita*, the treatments with manipueira at 10 % and 25 % led to a smaller number of galls, with respective reductions of 76.6 % and 69.90 %. Damasceno et al. (2008) showed a reduction in the number of galls and egg mass of *M. incognita* only when manipueira was diluted at 50 % in papaya seedlings.

The number of *M. incognita* eggs in the root (Figure 2E) and soil (Figure 2F) decreased after the application of manipueira, with an exponential decrease as the concentration increased. The reduction of 50 % in these variables averages was possible after the manipueira application at concentrations

of 63.68 % and 3.15 %, respectively. The maximum reduction in the number of eggs in the root (64.68 %) and soil (68.90 %) were observed at concentrations of 100 % and 10 % of manipueira, respectively. These results corroborate those obtained by Nasu et al. (2010), who used manipueira at 10 % and had a lower number of galls and eggs per root system of tomato (84.62 %). Nasu (2008) demonstrated that tomato plants inoculated with *M. incognita* under manipueira treatment at 25 % concentration reduced by 85 % the number of eggs, when compared to the control.

The reproduction factor of *M. incognita* in the soybean plants decreased after the application of manipueira. The exponential regression equation indicated a LC₅₀ of 5.22 % (Figure 3). A more significant reduction was observed with the application of 20 % of manipueira, which reduced the reproduction factor by 72.0 %, if compared to the control (5.62), reaching 1.58. The best results with concentrations from 20 % occur probably due to the lower viscosity of manipueira and the greater ease of penetrating into the soil, with a faster and more effective action. These results corroborate those by Nasu et al. (2010), who found a lower reproduction factor at concentrations of manipueira lower than 50 %.

In the present study, it is observed that soybean, in the absence of management (control), showed a high reproduction factor of 5.62 (Figure 3). Therefore, this soybean cultivar (Intacta cv. M-Soy 8644 IPRO) may be considered susceptible to

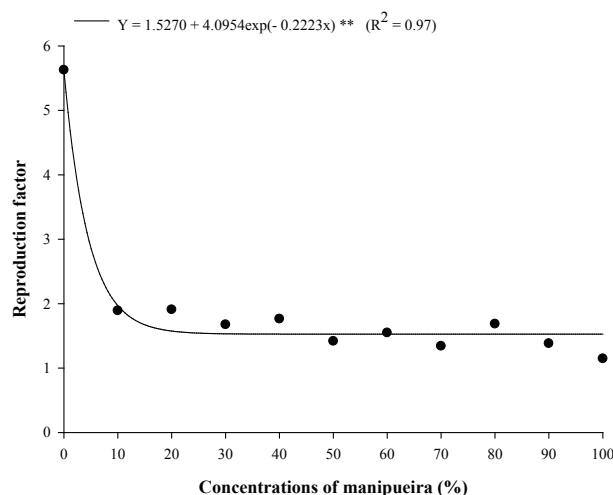


Figure 3. *Meloidogyne incognita* reproduction factor in soybean, depending on the concentration of manipueira. ** Significant at 1 %.

M. incognita (Alves et al. 2011), what implies a growth of the nematode population in the area at the end of the crop production cycle. A reproduction factor below 1.0 is desirable to lead to a population reduction in the area (Alves et al. 2011). Thus, when manipueira was applied to the substrate, this residue acted lethally on the nematodes, reducing the number of individuals and their reproduction. However, it is still necessary to combine the use of this alternative to other methods, in order to reduce even more the nematode reproduction factor.

It is fundamental to perform future studies with manipueira under field conditions to enable the use of this substance in soybean crops. In this case, manipueira could also be used as an alternative fertilizer, in addition to a chemical defense. Its fertirrigation could be used similarly to what is performed with sugarcane, where vinasse is used in the main producing regions.

CONCLUSIONS

1. Manipueira presents toxicity to *Meloidogyne incognita* and influences positively the development of soybean;
2. The single application of manipueira (unique dose) is effective in *M. incognita* suppressiveness;
3. Manipueira concentrations from 20 % inhibit the parasitic action of *M. incognita* in soybean.

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