

RESEARCH/INVESTIGACIÓN

MANAGEMENT OF DRY ROT DISEASE OF YAM WITH CASSAVA WASTEWATER

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ABSTRACT

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Dry rot disease caused by the plant-parasitic nematodes *Scutellonema bradys*, *Pratylenchus coffeae*, and *P. brachyurus* limits yam (*Dioscorea* spp.) yield in Brazil. As a result, the development of strategies to reduce agricultural losses caused by these plant-parasitic nematodes is required. The objective of this study was to evaluate the effect of cassava wastewater (manipueira) on the management of dry rot under two growing conditions (without or with supplemental irrigation) and on soil chemical characteristics. The following treatments were considered: manipueira concentrations diluted in water (10, 25, and 50%), volume of applications (3, 5, and 7 L/m on beds), and soil application timing (30 and 120 days after planting); an untreated control was also included. Five months after planting, soil samples from each plot were collected to evaluate soil fertility. At harvest, disease incidence, nematode population densities in soil and crop yield were evaluated. In both experiments, there was no significant interaction between manipueira concentration and volume. An effect only of manipueira volume on the measured variables was observed. In the non-irrigated trial, the application of manipueira to soil reduced disease incidence and nematode population densities and increased fresh weight of yam tubers. In the irrigated trial, a positive effect was observed only at a volume of 5 L/m. In this treatment, total nematode population density was inversely related with soil Ca ($r = -0.67$, $P < 0.017$) and nematode incidence with soil Ca and Mg content ($r = -0.71$, $P < 0.012$). The volume of 5 L/m manipueira might be suitable for nematode management.

Key words: Agroindustrial waste, alternative control, *Dioscorea* spp., *Pratylenchus* spp., *Scutellonema bradys*

RESUMO

Farias, S. P., A. V. D. L. Almeida, S. L. Santos, G. Moura Filho, T. C. A. Gomes, F. S. Rocha e M. F. S. Muniz. 2022. Manejo da casca-preta-do-inhame com manipueira. *Nematropica* 52:45-55.

A casca-preta-do-inhame causada pelos fitonematoides *Scutellonema bradys*, *Pratylenchus coffeae* e *P. brachyurus* tem limitado o rendimento da cultura do inhame (*Dioscorea* spp.) no Brasil. Assim, torna-se necessário o desenvolvimento de estratégias para reduzir as perdas provocadas por estes patógenos. O objetivo deste trabalho foi avaliar o efeito da aplicação de manipueira no manejo da casca-preta, em duas condições de plantio (sem ou com irrigação suplementar) e sobre as características do solo. Os experimentos foram instalados em áreas naturalmente infestadas, em delineamento experimental de blocos casualizados

completos com quatro repetições, em esquema fatorial: concentrações de manipueira diluída em água (10, 25 e 50%), volumes de aplicação (3, 5 e 7 L/m sobre as leiras) aos 30 e 120 dias após o plantio, mais a testemunha. Para o experimento conduzido sob condições de irrigação, aos cinco meses após o plantio foram coletadas amostras de solo por parcela para avaliar a fertilidade do solo. Por ocasião da colheita foram avaliados a incidência da doença, população de nematoides no solo e rizóforos e o rendimento da cultura. Em ambos os experimentos, a análise de variância não apresentou interação significativa entre concentração e volume de manipueira. Observou-se apenas efeito de volume sobre as variáveis avaliadas. Em condições de sequeiro, a aplicação de manipueira ao solo reduziu a incidência da doença, a população de nematoides e também resultou em aumento da massa fresca dos rizóforos. Na área irrigada, o efeito positivo foi observado apenas com a aplicação do volume de 5 L/m. Nesta condição, a população total de nematoides mostrou uma relação inversa com o teor de Ca no solo ($r = -0,67$, $P < 0,017$), e a incidência da doença com a soma dos teores de Ca e Mg ($r = -0,71$, $P < 0,012$). O volume de 5 L/m de manipueira pode ser indicado para o manejo da doença.

Palavras-chave: Controle alternativo, *Dioscorea* spp., *Pratylenchus* spp., resíduo agroindustrial, *Scutellonema bradys*

INTRODUCTION

Yam (*Dioscorea* spp.) is an important crop in Brazil as a high-value product for internal and external markets. However, despite its importance, some factors restrict crop yield, such as the damage caused by plant-parasitic nematodes. The dry rot disease caused by *Scutellonema bradys* (Steiner & LeHew) Andrassy, *Pratylenchus coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven, and *P. brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven, is considered the major production constraint for this crop. Both *S. bradys* and *P. coffeae* are the most prevalent nematode species in northeastern Brazil where yam is grown (Muniz *et al.*, 2012; Moura, 2016). For *S. bradys*, population densities in excess of 1,000 nematodes/50 g of tuber peelings produce observable external symptoms of damage (Bridge, 1973). These nematodes cause the breakdown of the cell walls, destruction of cell contents, and necrosis in the tubers (Lebot, 2009). According to Coyne and Affokpon (2018), the most severe symptoms of dry rot are observed in mature tubers, especially during storage. In Brazil, yield losses between 20 and 30% were reported (Pinheiro, 2017).

The management of this disease is based on the exclusion of nematodes. Thus, the use of healthy planting material in areas free of nematodes is the principal management strategy (Moura, 2016). Currently, no synthetic nematicides are registered for use in yam in Brazil (AGROFIT, 2020). Thus, to minimize the impact of plant-

parasitic nematodes, different approaches such as crop rotation, including *Crotalaria* spp. (Silva *et al.*, 2014), or organic amendments with coconut husk powder, castor bean cake, cattle and chicken manure (Morais *et al.*, 2016) have been tested. However, these methods are not entirely effective and farmers have expressed their concerns about their use. In Brazil, by-products such as manipueira (liquid extracted from cassava roots) have shown efficiency in reducing nematode populations, including: *Meloidogyne* spp. on okra (Ponte *et al.*, 1995), tomato (Nasu *et al.*, 2015; Carvalho, 2017), soybean (Fonseca *et al.*, 2016), and guava (Mesquita, 2016); *P. brachyurus* on maize (Roldi *et al.*, 2013) as well as *S. bradys* on yams. Studies have been performed under laboratory, greenhouse, and field conditions but have focused on the treatment of infected yam tubers (Carmo, 2009; Lima *et al.*, 2020). Studies addressing the management of dry rot disease by the use of the manipueira under field conditions are scarce.

The effect of manipueira on plant-parasitic nematodes is attributed to toxic cyanogenic glycosides, particularly linamarin, which releases cyanide via cyanohydrin intermediates when hydrolyzed (Chitwood, 2002). Furthermore, the residue also contains macro and micronutrients (Fayinminnu *et al.*, 2013; Nasu *et al.*, 2015). The objective of this study was to evaluate the effect of manipueira applied to the soil for the management of dry rot disease in two nematode-infested yam fields. The effects on soil chemical characteristics were also determined.

MATERIALS AND METHODS

General information

The experiment was performed during two cropping seasons (March-December) in 2015 and 2018, in the municipality of Taquarana, Alagoas, Brazil, in fields with a previous history of damage by dry rot disease. Experiments were conducted under two irrigation regimes: i) trial I (2015) with

no supplemental irrigation located at 09°39'935''S, 036°25'898''W; and ii) trial II (2018), with supplemental irrigation when needed (drip irrigation), at 09°39'671''S, 036°28'076''W. The sites received 672.2 and 602.9 mm mean rainfall during the experimental periods in fields I and II, respectively. Severe water stress was observed in field I in the first phase of the growth cycle (Fig. 1). (INMET, 2015, 2018).

Before treatments were applied, four soil subsamples of about 500 cm³ each were randomly

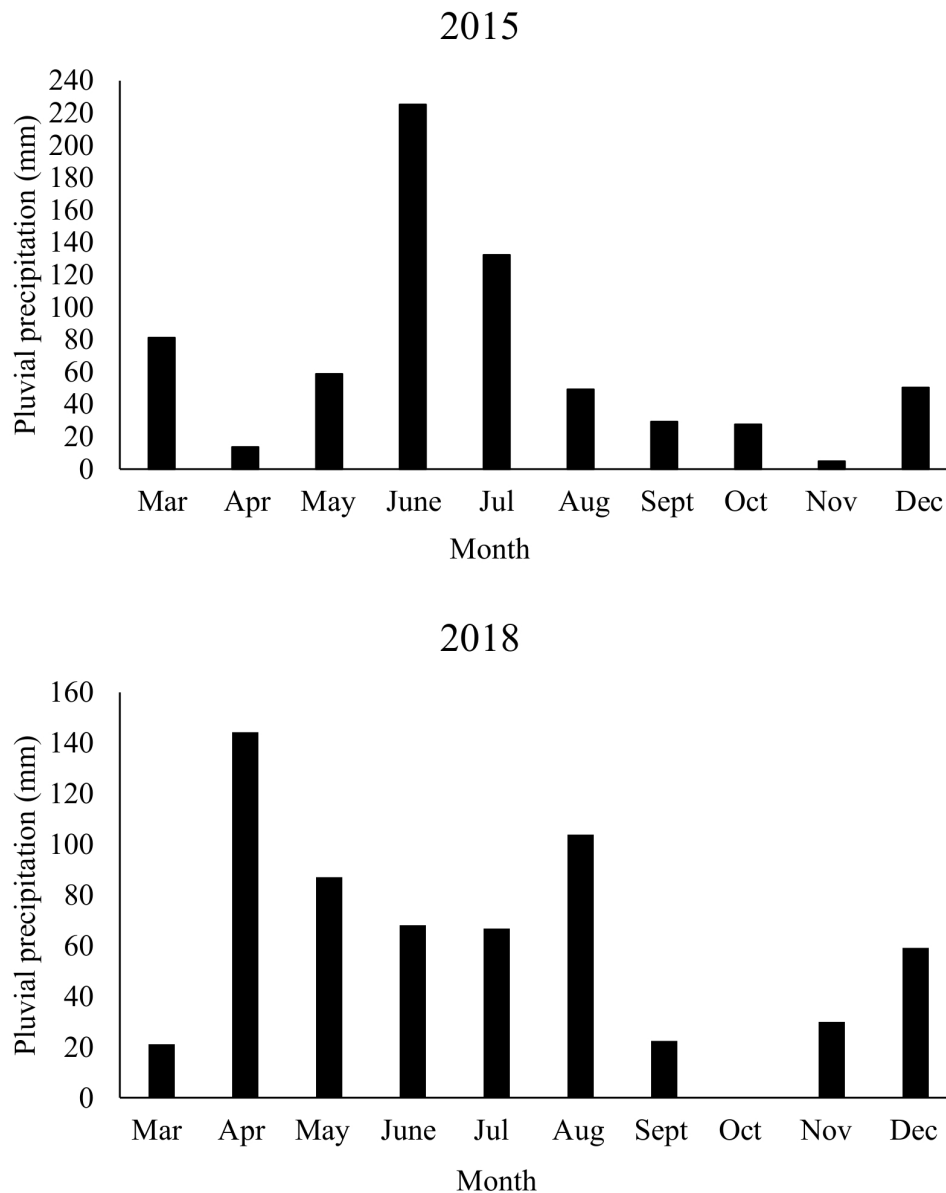


Figure 1. Monthly mean rainfall during the experimental period (March to December) in 2015 and 2018 in Taquarana, Alagoas, Brazil.

collected from 0-20 cm depth within each plot and mixed thoroughly. Nematodes were extracted to estimate the initial population density (Pi) from a composite soil sample of 200 cm³ according to Jenkins (1964). Morphological identification and quantification of the nematodes were done using an inverted light microscope; morphological identification was based on Mai and Mullin (1996). The physicochemical properties of the soils were also assessed. Calcium (Ca), magnesium (Mg), and aluminum (Al) concentrations were determined by KCl 1.0 mol/L extraction; phosphorus (P) and potassium (K) by Mehlich-1 extraction; H + Al, by calcium acetate at pH 7.0 extraction; cation exchange capacity (CEC) at pH 7.0; organic matter (OM) content was obtained according to Walkley-Black method (1934); and saturation coefficient (V) and aluminum saturation (m) were also calculated (Silva, 2009) (Table 1). Yam seed tubers without visible nematode damage were selected for planting.

Treatments and experimental design

The treatments consisted of three manipueira concentrations diluted in water (10, 25, and 50%), and three application volumes (3, 5, and 7 L/m) which were applied to the soil by manual irrigation using watering cans near the base of the yam plants 30 and 120 days after planting. Agricultural procedures such as mineral fertilization, hand weeding in plots, and control of leaf blight (*Curvularia eragrostidis*) were performed

according to the crop requirements.

Both trials were arranged in a completely randomized block design with a factorial arrangement (3 concentrations x 3 volumes) with an additional untreated control, resulting in 10 treatments replicated four times. The plots consisted of four beds 3.5 m in length, spaced 1.2 m apart. The spacing between tuber seeds within a bed was 0.35 m, comprising 11 plants per bed and 44 plants per plot. The plants from each edge of the middle ridgebeds and of the lateral beds were considered as a borders, and the remaining plants (18) were used for sampling.

Collection and chemical composition of manipueira

The manipueira used in in trials I and II was obtained from cassava ‘Sergipana’ and from a mixture of ‘Sergipana’ and ‘Izabelzinha’, respectively, obtained from a cassava flour-producing factory located in the municipality of Taquarana, Alagoas, Brazil. The manipueira was collected directly from the overflow exit of the liquid residue to the settling tanks, after the washing and pressing processes, and immediately utilized. The cyanide content was estimated by the colorimetric test Quantofix® Cyanid (Macherey-Nagel, Düren, Germany). A chemical analysis was also performed for macro- and micronutrients, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (APHA, 1998; Silva, 2009).

Table 1. Soil physicochemical characteristics of the trial sites under non-irrigated (2015) and irrigated conditions (2018).

	Analysis of soil fertility										
	pH	P	K	H+Al	Al	Ca	Mg	CEC effective ^w	V ^x	M ^y	OM ^z
	mgdm ³			-----cmol _c dm ³ -----				-----%-----		g/kg	
Non-irrigated	4.9	20	40	2.97	0.2	0.36	0.11	0.97	21	21	19.3
Irrigated	4.7	214	35	5.67	0.42	1.38	0.57	2.6	28	16	17.7
	Physical analysis (g/kg)										
	Clay		Silt		Fine sand	Coarse sand	Soil texture				
Non-irrigated	120		90		180	610	Sandy loam				
Irrigated	140		30		110	720	Sandy loam				

^wCation exchange capacity.

^xPercentage of soil base saturation.

^yPercentage of soil aluminum saturation.

^zOrganic matter by the Walkley-Black method (1934).

Assessment of soil chemical properties and nematode population densities

To determine the interaction between nematode population densities and the soil environment, data was collected from trial II (under supplemental irrigation). Samples were collected from four locations per plot at 0-20 cm soil depth 150 days after planting (phase of tuber bulking). The variables analyzed were: pH in water, exchangeable bases (Ca, Mg, K), P, Al and CEC, according to Silva (2009).

Nine months after planting, the tubers were harvested and disease incidence (DI), as the percentage of tubers that were visibly diseased relative to the total number assessed in each plot (18 sampling plants), was evaluated (Madden and Hughes, 1999). To obtain yield data, the tubers were weighed and the values extrapolated to kg/ha. Soil and tuber samples were collected from four locations per plot in order to form composite samples and to estimate final nematode population densities according to Jenkins (1964) and Coolen and D'Herde (1972), respectively. For nematode extraction, 50 g of tuber peel and 200 cm³ of soil of each composite sample were used. Extracted nematodes were killed and fixed in heated 4% formaldehyde to preserve the specimens for later examination. Nematode population densities were estimated using an inverted light microscope.

Statistical analyses

Data were transformed to $\sqrt{(x+1)}$ and subjected to analysis of variance. Linear or polynomial regressions were performed in order to evaluate the effect of manipueira concentration and volume on *S. bradys* and/or *Pratylenchus* spp. population densities in tubers (NPT), final

population densities (Pf; soil + tuber peels), DI, and fresh weight of healthy tubers (FWT). Regression analyses were also performed to evaluate the effect of concentration and volume on soil characteristics in the irrigated trial. The analyses were performed using the Software SAEG 9.1 (2007). Pearson's correlation analysis ($P < 0.05$) between K or Ca and Pf and Di was also performed.

RESULTS

The Pi in plots before treatments were applied was: i) 20-40 nematodes/200 cm³ of soil (field 1 - without supplemental irrigation) with the presence of only *S. bradys* and; ii) 20-25 specimens /200 cm³ of soil (field 2 - with supplemental irrigation), with a mixed nematode population consisting of *S. bradys* and *Pratylenchus* spp. The analyses of variance were not statistically significant for this variable ($P > 0.05$).

The chemical composition of the manipueira showed contrasting values concerning some nutrients (Table 2). In trial I (non-irrigated), the concentrations of K and Ca were approximately 75% (3,456 mg/L) and 72% (727 mg/L) higher, respectively, than the manipueira used in trial II (K - 860 and Ca - 203.8 mg/L). However, in trial II (irrigated), concentrations of Mg and N were 73% (2,436 mg/L) and 58% (3,066 mg/L) higher, respectively, compared to non-irrigated conditions (Mg - 670 mg/L and N - 1,289 mg/L). In both experiments, the concentration of cyanide was approximately 3.0 mg/L in pure manipueira.

Manipueira had little effect on soil chemical concentrations (Table 3). The analysis of variance showed a significant effect of the concentration of manipueira only on K content. Moreover, the effect of the interaction between concentration and volume on K was also observed only with the

Table 2. Chemical concentrations of the manipueira used in the experiments in non-irrigated (2015) and irrigated conditions (2018).

	Macronutrients					Micronutrients				Oxygen demand	
	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn	BOD ^y	COD ^z
Non-irrigated	1,289	402	3,456	727	670	1.09	80.62	1.21	37.43	133,100	203,200
Irrigated	3,066	441	860	203.8	2,436	3.61	32.5	0.54	2.48	58,500	92,000

^yBOD – biochemical oxygen demand.

^zCOD – chemical oxygen demand

Table 3. Chemical concentrations in soil (0-20 cm) in an irrigated (trial I) yam area in relation to manipueira concentrations in water (%) and application volumes (L), in Taquarana, Alagoas, Brazil (March to December of 2018).

Manipueira	pH	P	K	Al	Ca	Mg	CEC ^w _{pH 7}	V ^x	M ^y
		----mgdm ³ ----		-----cmol _c dm ³ -----				-----%-----	
Concentration									
0 %	4.53	375.75	34.50	1.01	1.07	0.41	6.59	24.00	39.25
10 %	4.52	318.67	38.00	0.74	1.08	0.48	6.72	24.92	31.33
25 %	4.47	341.92	52.83	0.78	1.02	0.59	6.87	25.50	30.83
50 %	4.52	380.42	73.50	0.70	1.06	0.56	6.69	27.50	27.75
<i>P</i> ^z	>0.05	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Application volume									
0 L	4.53	375.75	34.50	1.01	1.07	0.41	6.59	24.00	39.25
3 L	4.48	324.67	54.33	0.73	1.07	0.52	6.81	25.42	29.75
5 L	4.56	364.92	58.00	0.72	1.10	0.59	6.70	27.83	27.92
7 L	4.47	351.42	52.00	0.78	1.00	0.52	6.77	24.67	32.25
<i>P</i> ^z	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
CV (%)	5.08	36.08	61.53	24.46	26.61	32.98	5.16	18.00	26.92

^wCation exchange capacity.

^xPercentage of soil base saturation.

^yPercentage of soil aluminum saturation.

^zProbability

volume of 7 L/m ($P \leq 0.01$). For the other interactions the effects were not significant ($P > 0.05$) (Fig. 2). The levels of exchangeable K in the soil due to increasing concentration of manipueira applied at 7 L/m, increased linearly (Fig. 2A). Thus, the higher the concentration of manipueira applied to the soil, the greater the content of exchangeable K. However, Pearson's correlation did not show significant differences between K and the variables evaluated. There was an inverse relationship between Pf and Ca concentration ($r = -0.67$, $P < 0.017$) and DI with the sum of Ca and Mg concentrations in soil ($r = -0.71$, $P < 0.012$).

In both trials, there was not a significant interaction between concentration and volume of manipueira. An effect only of volume on the variables was observed. In trial I that did not receive irrigation, a reduction in NPT, Pf, DI, and an increase in FWT were observed (Fig. 3). When supplemental irrigation was applied (trial II), the best results were obtained with the volume of 5 L, represented by a quadratic model (Fig. 4). At a volume of 7 L there was an increase in DI, NPT, Pf and a decline in FWT (4.45 kg) in relation to

application volumes of 3 L and 5 L. In trial I, yield of yam was 4,000 kg/ha, while in trial II, yam yield was 27,000 kg/ha.

DISCUSSION

The lack of differences among concentrations of manipueira (10, 25 and 50%) applied to soil on the variables DI, NPT, Pf, and FWT is consistent with previous data (Nasu *et al.*, 2015). In this study the same concentrations were evaluated to control *M. incognita* on tomato under field conditions using an application volume of 4 L/m. This treatment did not have an impact on final number of galls and eggs.

Concerning the application volume, under non-irrigated conditions, there was a reduction in nematode population densities and an increase in FWT with increasing application volumes of manipueira. Under irrigated conditions, a decrease in FWT and an increase in DI and nematode population densities were observed at an application volume of 7 L. This discrepancy may be due to the low concentration of cyanide (3.0

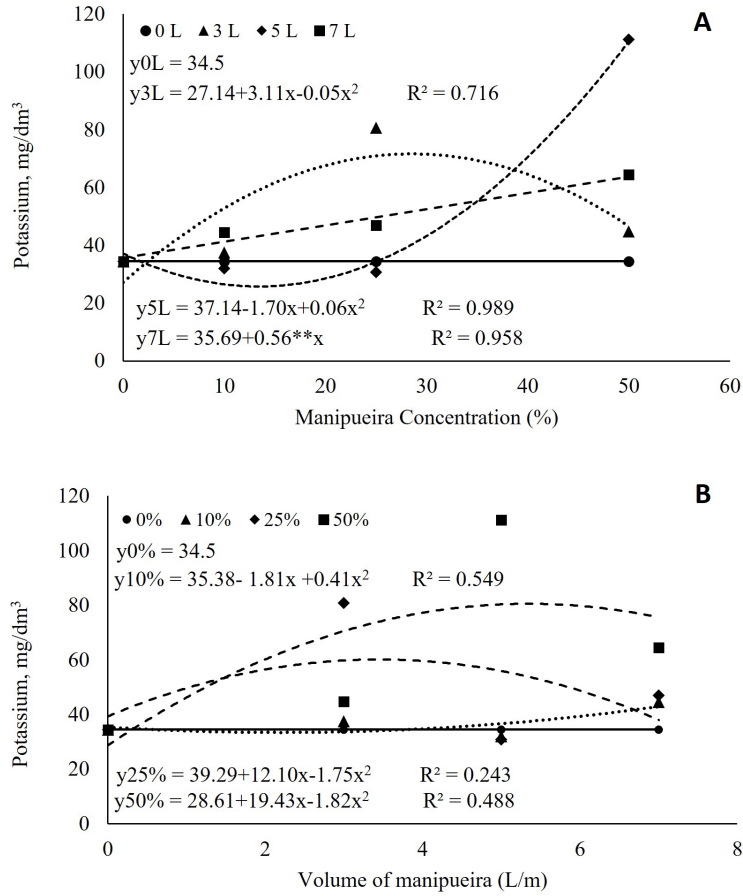


Figure 2. Potassium concentration in the soil in an irrigated condition, in relation to manipueira concentrations in water (A) and application volumes (B), in Taquarana, Alagoas, Brazil (March to December, 2018).

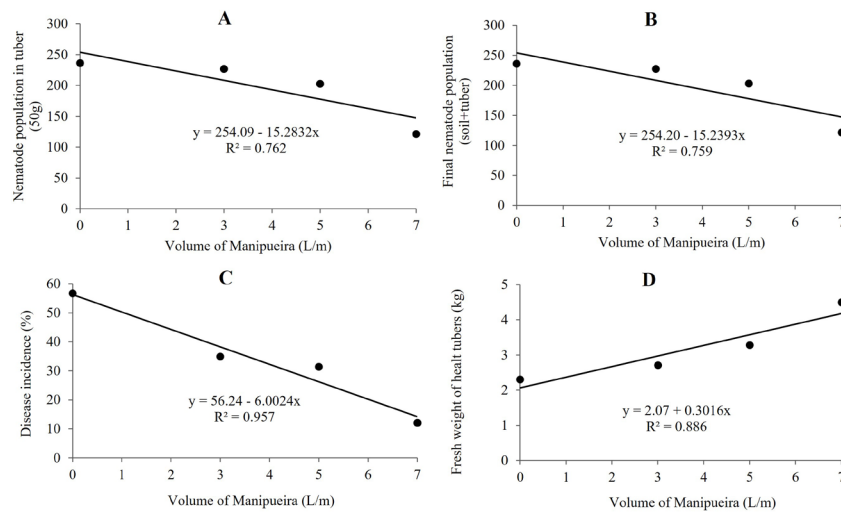


Figure 3. Effect of application volume of manipueira (L/m) under a nonirrigated condition (trial I) on variables: A – nematode population in tuber (50 g); B – final nematode population (tuber+soil); C – disease incidence (%); D – fresh weight of health tubers (kg).

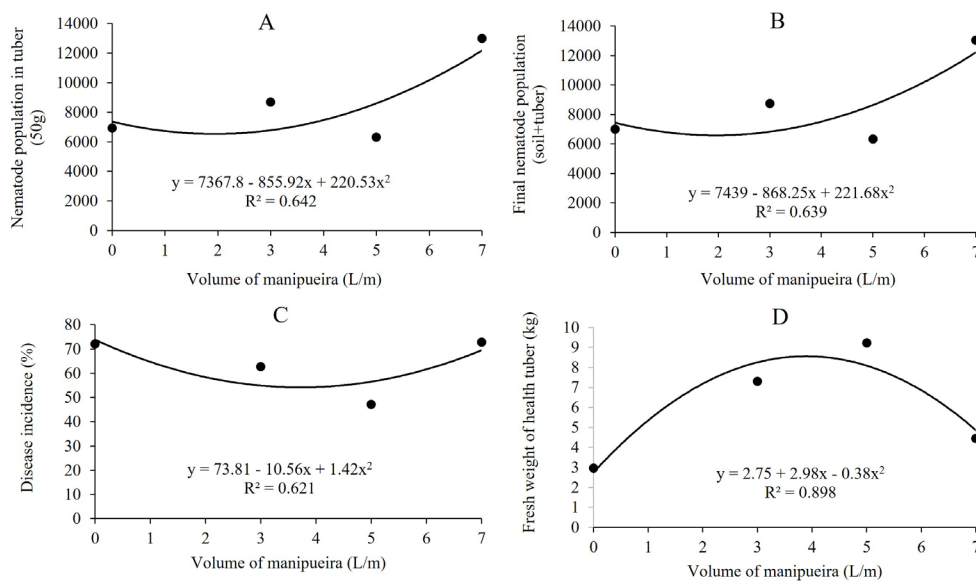


Figure 4. Effect of application volume of manipueira (L/m) under an irrigated condition (trial II) on variables: A – nematode population in tuber (50 g); B – final nematode population (tuber+soil); C – disease incidence (%); D – fresh weight of health tubers (kg).

mg/L) in the manipueira used in this study. Under irrigated conditions, soil moisture may have diluted cyanide in the soil, diminishing the effectiveness of the manipueira for nematode suppression. The efficacy of manipueira to control *Meloidogyne* spp. with a cyanide concentration of 25 to 40 mg/L applied to the soil has been reported (Nasu *et al.*, 2015; Carvalho, 2017). In cassava plants cyanide concentration varied depending on cultivar (Borges *et al.*, 2002; Guédé *et al.*, 2013), where it was grown (Ubwa *et al.*, 2015), harvest period (Cohen *et al.*, 2007; Oliveira *et al.*, 2012), and processing stage (Chisté and Cohen, 2006; Chisté *et al.*, 2010; Guédé *et al.*, 2013). No information was found on the lethal dose of cyanide necessary to kill 50% of the population of plant-parasitic nematodes.

The values of BOD found in the present study are considered high according to Ferreira *et al.* (2001). The average value of BOD from manipueira ranges from 14,000 – 34,000 mg/L. Excess organic matter consumes oxygen during decomposition resulting in environmental problems in water reservoirs (Scandolera *et al.*, 2001). The use of manipueira for nematicidal purposes, as in the present study, is important since the disposal of this residue has environmental risks of contamination (Santos *et al.*, 2012; Ribeiro *et al.*, 2015).

As previously demonstrated (Ponte, 2001;

Nasu *et al.*, 2015), significant levels of macro and micronutrients were also found in manipueira. In the present study, the concentrations of K and Ca in the manipueira applied under the non-irrigated condition were higher than those of the manipueira applied with irrigation. However, the concentration of Mg in the manipueira applied under irrigation was higher compared to that under non-irrigated conditions. The lower FWT and the increment of NPT of approximately 13,000 nematodes/50 g of tuber peel, and DI of 73% observed at an application volume of 7 L with irrigation, may have occurred due to the deficiency of some nutrients in the soil. An excess of Mg may cause a decrease of K and Ca in the soil (Malavolta, 1979).

The effect K has on plant-parasitic nematodes has been documented. Barbosa *et al.* (2010) demonstrated a decrease in the reproduction of *Heterodera glycines* on soybean by fertilization with K. However, despite the effect of manipueira on K content in the soil, no correlation was observed between this nutrient and the variables considered in this study. There was an inverse relationship between Pf and Ca concentration and DI with the sum of the Ca and Mg concentrations of the soil. Calcium is of great importance to plants; it is related to cell wall and plasma membrane integrity, and its deficiency makes plants more susceptible to nematodes (Hurchanik *et al.*, 2003).

Regarding Mg, there is little information on its role on plant pathogens.

The low tuber yield (approximately 4,000 kg/ha) observed in the non-irrigated condition was attributed to the low levels of rainfall, especially at the beginning of the yam crop cycle, that resulted in delayed sprouting of tubers seed. Soil moisture affects the survival and distribution of plant-parasitic nematodes, exerts a considerable influence on nematode behavior, and consequently on the potential damage to crops. Moreover, plant-parasitic nematodes exhibit a preference for water potentials slightly below field capacity (Ferraz and Brown, 2002). This feature may have influenced the Pf found in the current study.

Even though positive findings have been observed with manipueira for the management of the dry rot disease on yam, additional studies should be performed under field conditions to evaluate the application of manipueira with higher cyanide contents, in order to provide more accurate information to farmers.

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