RESEARCH NOTE/NOTA DE INVESTIGACIÓN

TREATMENT OF YAM TUBERS INFECTED BY SCUTELLONEMA BRADYS WITH SODIUM HYPOCHLORITE

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ABSTRACT

Almeida, A. V. D. L., M. F. S. Muniz, M. A. Noronha, S. P. Farias, and V. N. Carvalho. 2020. Treatment of yam tubers infected by *Scutellonema bradys* with sodium hypochlorite. Nematropica 50:29-35.

Dry rot caused by *Scutellonema bradys*, *Pratylenchus coffeae*, and *P. brachyurus* is considered the most important disease of yam (*Dioscorea* spp.) in Brazil. The aim of this work was to evaluate the effect of sodium hypochlorite on suppression of *S. bradys* in yam tubers. Greenhouse experiments were performed twice in a completely randomized factorial design. Sodium hypochlorite treatments applied at concentrations of 0, 0.5, and 1.0% with immersion periods of 20, 40, and 60 min were evaluated; all treatment combinations were replicated eight times. Three months after planting, percent emergence of shoots from the seed tubers was evaluated. *Scutellonema bradys* population density in soil, roots and tuber peels, the total nematode population, and the reproduction factor, were evaluated 6 months after planting. In both assays, shoot emergence did not differ among concentrations of sodium hypochlorite and immersion periods. However, *S. bradys* populations were reduced as the sodium hypochlorite at 1.0% for an immersion period of 20 min, could be useful for disinfection of yam tubers infected with *S. bradys*.

Key words: Alternative control, Dioscorea spp., dry rot disease, yam nematode

RESUMO

Almeida, A. V. D. L., M. F. S. Muniz, M. A. Noronha, S. P. Farias e V. N. Carvalho. Tratamento de rizóforos-semente de inhame infectados por *Scutellonema bradys* com hipoclorito de sódio. 2020. Nematropica 50:29-35.

A casca-preta causada por *Scutellonema bradys*, *Pratylenchus coffeae* e *P. brachyurus* é considerada a doença mais importante do inhame (*Dioscorea* spp.) no Brasil. O objetivo deste trabalho foi avaliar o efeito do hipoclorito de sódio no tratamento de rizóforos-semente naturalmente infectados com *S. bradys*. Os experimentos foram conduzidos em telado, em duas épocas, em delineamento inteiramente casualizado, com esquema fatorial ($3 \times 2 + 1$), sendo duas concentrações de hipoclorito de sódio ($0,5 \in 1,0\%$) e três tempos de imersão dos rizóforos ($20, 40 \in 60$ minutos), com oito repetições, além da testemunha (sem uso do hipoclorito). Aos três meses após o plantio foi avaliado o percentual de emergência dos rizóforos-

semente, e aos seis meses foram estimadas as variáveis: população do nematoide no solo, na raiz e na casca dos rizóforos, população final e fator de reprodução. Nos dois ensaios, percentual de emergência não foi afetado pelos tratamentos. Entretanto, observou-se uma redução nas populações do nematoide com o aumento da concentração do produto quando comparado à testemunha. A concentração de 1,0% de hipoclorito de sódio e o tempo de imersão de 20 minutos pode ser empregado para o tratamento de rizóforos-semente de inhame infectados com *S. bradys*.

Palavras-chave: Controle alternativo, Dioscorea spp., casca-preta, nematoide do inhame

Yam (*Dioscorea* spp.) is a very important carbohydrate food crop, especially in Africa. In Brazil, this crop has significant commercial value for both internal and the external markets, producing about 251,489 t on 25,702 ha of cultivated land (Faostat, 2018). More than 90% of Brazilian production is concentrated in the northeastern region, mainly in the states of Bahia, Paraíba, Pernambuco, and Alagoas (Moura, 2016).

Plant-parasitic nematodes are among the organisms affecting yams, mainly *Scutellonema bradys* (Steiner & LeHew) Andrássy, *Pratylenchus coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven, and *P. brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven. These nematodes cause dry rot symptoms of tubers and, consequently, economic losses to farmers. However, *S. bradys* is considered the most important, mainly due to it's widespread distribution and to losses ranging from 20 to 30% (Ferraz and Brown, 2016; Pinheiro, 2017).

The management of dry rot disease is based on methods of exclusion, including the use of nematode-free seed tubers planted in areas free of nematodes (Moura, 2016). To date, there are no nematicides registered for use on yam in Brazil (Agrofit, 2019). Thus, aiming for an alternative option, products such as sodium hypochlorite (NaOCl) have been evaluated. In Jamaica, NaOCl used for the treatment of infected yam tubers reduced the development of dry rot caused by P. coffeae (Hutton, 1998). In Brazil, NaOCl was evaluated for the treatment of other propagative positive results material. with in some pathosystems (Lordello et al., 1994; Garcia and Tenente, 2001).

Considering that infected seed tubers are the main way the causal agents of dry rot are disseminated, the treatment of this propagative material should avoid the introduction of the pathogens into new cropping areas. In view of this, the objective of this study was to evaluate the effect of NaOCl in the treatment of yam seed tubers naturally infected by *S. bradys*.

The study was carried out at the Center for Agricultural Sciences, Federal University of Alagoas (09°28'02'' S; 35°49'43''W, altitude 127 m), Rio Largo, State of Alagoas, Brazil. The study was conducted over two consecutive years (Experiment 1: March to September 2016 and Experiment 2: March to September 2017).

Yam seed tubers (D. cavenensis cv. Da Costa) at the beginning of the sprouting stage with an average weight between 320-430 g, without soil adhering to the surface, were used in the experiments. To determine the initial population (Pi) of the nematodes, 1 g of tuber peel was taken from each individual seed tuber head (portion where dry rot was more pronounced and the nematodes were concentrated) and processed in a blender, followed by centrifugation in sucrose solution and kaolin (Coolen and D'Herde, 1972). Only 1 g was removed to minimize wounds on tubers and to avoid secondary infection by microorganisms. Nematode identification was performed based upon morphological characters according to descriptions by Mekete et al. (2012) using an inverted light microscope. The estimate of nematode population density was based on the average of two counts of 1 mL in a Peters slide (Astel, Botucatu, SP, Brazil). Microscopic observations confirmed the presence of S. bradys in both experiments. No Pratylenchus spp. were detected.

The tubers were placed in polyethylene mesh bags and, other than the untreated control, subjected to one of six treatments: immersion in either 0.5 or 1.0% concentrations of NaOCl (based on 12% NaOCl) for one of three immersion periods (20, 40, and 60 min). Subsequently, the seed tubers were planted in 8-L capacity plastic pots, filled with soil sterilized in a hot air oven $(100^{\circ}C/24 \text{ hr})$, and maintained under greenhouse conditions until evaluated. The experimental design was a completely randomized factorial arrangement (3 x 2 + 1) with eight replications.

Three months after yam planting, the percentage of tubers sprouting was evaluated, and after six months, the final population of S. bradys was estimated. Due the migratory behavior of the nematode, the final population was based on individuals from roots, tubers, and soil. The aerial part of the plant was removed, and the root system and tuber peel were washed in tap water, weighed and processed according to Coolen and D'Herde (1972). Extraction of nematodes from soil samples (100 cm³) followed the protocol of Jenkins (1964). After extraction, the nematodes were killed and fixed in a heated 4% formaldehyde solution. The identification and quantification of the final population density of S. bradys in the soil (NPS), tuber peel (NPT), and roots (NPR) were performed as previously described. These values were added to determine the total nematode population density (TNP). The reproduction factor (RF) of the nematode [RF = Final population (roots + tubers +soil)/Initial population in the tubers] was calculated for each treatment and replicate.

Results were subjected to analysis of variance (F test) and nematode population data were transformed to $\sqrt{x + 1}$. Means were compared by Tukey's test at 5% probability, using the statistical program SISVAR version 5.6 (Ferreira, 2011).

In both experiments, there were no significant differences ($P \le 0.05$) in the interaction between NaOCl concentrations and immersion periods, for the evaluated variables (Table 1). However, significant differences were observed when the factors were analyzed separately. The percentage of tuber emergence was not affected by the treatments in both experiments (Table 2).

No significant differences ($P \le 0.05$) were observed in initial population densities of *S. bradys* among the treatments, indicating uniformity of the nematode population in the propagative material tested. However, in both experiments, compared with the control, the two concentrations of NaOCl significantly reduced ($P \le 0.05$) NPR, NPT, TNP, and RF (Table 3). The population density of *S. bradys* in the soil was significantly different between the treatments and the control only in the second experiment (Table 3).

The concentration of 1.0% NaOCl was the most efficient, when compared with the control and the 0.5% concentration ($P \le 0.05$) for NPR, NPT, TNP, and RF. However, in the first and second

experiments, no significant differences ($P \le 0.05$) were observed between the concentrations of NaOCl for RF and NPR, respectively (Table 3). Reductions of 72.0 and 84.0% were observed in NPT and of 77.0 and 80.5% in RF, when NaOCl was applied at 1.0%, in the first and second experiments, respectively.

There were no significant differences ($P \le 0.05$) between the immersion periods of yam tubers in NaOCl solution, suggesting that the higher exposure of the propagative material to the product did not interfere in nematode reproduction. However, in both experiments, the three immersion periods tested significantly reduced the population density of *S. bradys* for NPR, NPT, TNP, and RF, when compared with the control (Table 4).

In a study evaluating the efficiency of NaOCl at 0.12% for 40 min for the suppression of P. coffeae in yam tubers (D. cayenensis), a significant reduction of nematode population density in tuber peel was observed without interfering with the emergence of the propagative material (Hutton, 1998). The effectiveness of NaOCl has also been observed in pathosystems such as Globodera spp. in potato (Solanum tuberosum) (Wood and Foot, 1977), Radopholus similis in Musa spp. (Lordello et al., 1994), and Aphelenchoides besseyi in guinea grass (Panicum maximum) (Garcia and Tenente, 2001). In the latter studies, NaOCl did not affect tuber rhizome sprouting or seed germination and vigor. On the other hand, Silva et al. (2003) did not obtain good control of Meloidogyne sp. when 0.5% NaOCl was used with immersion periods of 15, 30, and 45 min in yacon (Smallanthus sonchifolius syn. Polymnia sonchifolia).

Sodium hypochlorite is an effective oxidizing agent, and the mode of action on plant-parasitic nematodes has been attributed to the destruction of survival structures such as cysts (Wood and Foot, 1977), interference in pathogen attraction (Forrest et al., 1988), and reduction in hatch, motility, and infectivity of the nematode (Stanton and O'Donnell, 1994).

The suppressive effect of NaOCl on *S. bradys* population densities in yam tubers is of great importance because this propagative material represents the main dissemination form of the nematode. Although eradication of the nematode was not obtained, the treatment was valid and did reduce *S. bradys* population densities. As a consequence of the physicochemical properties of NaOCl, it must be used in conditions of low-light

Table 1. Analysis of variance of th Experiment I	he effect	of sodium hypoc	hlorite (NaOCI)	and immersion pe	sriods on Scutellon	he effect of sodium hypochlorite (NaOCI) and immersion periods on <i>Scutellonema bradys</i> population density.	ation density.
Course of wariation				Mean-square residue	re residue		
	DF	Pi ^u	NPS ^v	NPR ^w	NPT ^x	TNP ^y	RF^{z}
NaOCI	2	23452.4^{ns}	419.1^{ns}	16395.7^{**}	50551.9**	139238.0^{**}	1.0^{**}
Immersion period	с	73898.8^{ns}	232.1^{ns}	9355.6**	27835.4**	76248.4**	0.6^{**}
NaOCl x Immersion period	9	59970.2^{ns}	363.1^{ns}	2654.7^{ns}	$80404.8^{ m ns}$	22571.1^{ns}	1.8^{ns}
Error	50	431428.6	200.0	783.0	2621.7	4559.9	0.09
Mean		864.3	14.3	42.6	86.4	143.3	0.3
CV (%)		76	69.0	65.7	59.3	47.1	56.5
Experiment II							
J				Mean-square residue	re residue		
Source of variation	DF	Pi	NPS	NPR	NPT	TNP	RF
NaOCI	7	57924.7 ^{ns}	1548.2**	9312.3**	286785.3**	447751.7**	1.0^{**}
Immersion period	ю	78114.4^{ns}	1069.6^{**}	5786.8**	174342.3**	276930.7^{**}	0.6^{**}
NaOCl x Immersion period	9	61327.1^{ns}	3083.9^{ns}	17051.1^{ns}	505793.7^{ns}	806181.6^{ns}	$1.7^{\rm ns}$
Error	50	7907.3	57.1	533.0	7183.5	6935.4	0.0
Mean		716.3	11.8	45.5	135.1	192.4	0.3
CV (%)		13.1	64.1	50.7	62.7	43.3	47.0
^u Pi: Initial population. ^v NPS: Nematode population in soil. ^w NPR: Nematode population in root. ^x NPT: Nematode population in tuber peel. ^y TNP: Total nematode population. ^z RF: Reproduction factor. **, ^{ns} Significant at 1% probability by F-test, and not significant, respectively.	vil. oot. her peel. h	st, and not signif	icant, respective	, in the second s			

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Treatment	% of emergence				
Treatment	Experiment I	Experiment II			
Control	100 a ^z	100 a			
0.5%; 20 min	100 a	100 a			
0.5%; 40 min	90 a	100 a			
0.5%; 60 min	90 a	90 a			
1.0%; 20 min	80 a	80 a			
1.0%; 40 min	80 a	80 a			
1.0%; 60 min	60 a	70 a			

Table 2. Percentage of emergence of yam seed tubers three months after the treatment with different concentration of aqueous sodium hypochlorite (%) for different immersion periods (min).

²Means followed by the same letter within a column do not significantly differ by the Tukey test at 5% probability.

Table 3. Initial Scutellonema bradys population density (Pi) in 1 g of yam tuber peel, nematode population densities in 100 cm³ soil (NPS), 1 g roots (NPR) or tuber peel (NPT), total nematode population density (TNP), and reproduction factor (RF) evaluated 6 months after the treatment of yam seed tubers in different concentration of aqueous sodium hypochlorite (NaOCl).

Experiment I						
$N_{a}OC1(0/)$	S. bradys population density					
NaOCl (%)	Pi	NPS	NPR	NPT	TNP	RF
Control (water)	862.5 a	22.5 a	97.1 a	179.7 a	299.3 a	0.7 a
0.5	833.3 a	15.0 a	43.7 b	91.3 b	150.0 b	0.3 b
1.0	895.8 a	10.8 a	23.3 c	50.5 c	84.6 c	0.2 b
CV (%)	41.8	64.0	32.1	29.6	22.8	10.2
Experiment II						
$N_{-}OC1(0/)$	S. bradys population density					
NaOCl (%)	Pi	NPS	NFR	NPT	TNP	RF
Control (water)	700.0 a	30.0 a	88.3 a	368.2 a	486.6 a	0.7 a
0.5	722.6 a	8.8 b	44.1 b	133. 4 b	186.2 b	0.3 b
1.0	726.3 a	8.8 b	32.7 b	59.1 c	100.6 c	0.1 c
CV (%)	46.7	42.2	23.1	29.6	20.3	5.0

²Means followed by the same letter on columns do not significantly differ, according to Tukey test at 5% probability.

and moderate temperatures to avoid the degradation of the product. Based on the current results, we recommended a concentration of 1.0% NaOCl and the immersion period of 20 min for the treatment of yam propagative material infected with S. bradys.

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Experiment I						
Immersion			S. bradys pop	pulation density	τ	
period (min.)	Pi	NPS	NPR	NPT	TNP	RF
Control	862.5 a ^z	22.5 a	97.1 a	179.7 a	299.3 a	0.7 a
20	956.3 a	11.3 a	37.0 b	62.4 b	110.6 b	0.3 b
40	843.8 a	13.8 a	32.1 b	70.9 b	116.7 b	0.2 b
60	793.8 a	13.8 a	31.4 b	79.4 b	124.5 b	0.2 b
CV (%)	41.8	64.0	32.1	29.6	22.8	10.2
Experiment II						
Immersion			S. bradys pop	pulation density	7	
period (min.)	Pi	NPS	NPR	NPT	TNP	RF
Control	700.0 a	30.0 a	88.3 a	368.2 a	486.6 a	0.7 a
20	723.8 a	10.6 b	41.5 b	119.2 b	171.3 b	0.2 b
40	711.3 a	8.8 b	38.2 b	94.5 b	141.5 b	0.2 b
60	726.9 a	6.8 b	35.5 b	75.0 b	117.4 b	0.2 b
CV (%)	46.7	42.2	23.1	29.6	20.3	5.0
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Table 4. Initial *Scutellonema bradys* population density (Pi) in 1 g of yam tuber peel, nematode population densities in 100 cm³ soil (NPS), 1 g roots (NPR) or tuber peel (NPT), total nematode population density (TNP), and reproduction factor (RF) evaluated 6 months after the treatment of yam seed tubers for different immersion periods in aqueous sodium hypochlorite solution.

^zMeans followed by the same letter on columns do not significantly differ, according to Tukey test at 5% probability.

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