Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252022v35n414rc

MOTILITY AND MIGRATION OF NEMATODES IN SALINE ENVIRONMENTS[1]

IVIS ANDREI CAMPOS E SILVA[2], ELVIRA MARIA REGIS PEDROSA[2]*, ENIO FARIAS DE FRANÇA E SILVA[2], MARIO MONTEIRO ROLIM[2], THAIS FERNANDA DA SILVA VICENTE[2]

ABSTRACT – Plant parasitic nematodes cause severe agricultural damage in Northeast Brazil. Additionally, soil salinization, especially in the semiarid region of the Northeast, is another factor that limits crop yield. The study evaluated the motility of *Meloidogyne enterolobii* and *Pratylenchus coffeae*, and the vertical migration of *P. coffeae* under saline conditions. Motility was assessed by submitting juveniles of the second stage of *M. enterolobii* and juveniles and adults of *P. coffeae* to saline solutions of NaCl, $CaCl_2$, and $MgCl_2$ at concentrations of 0.00, 0.25, 0.50, 0.75, and 1.00 M, and in a mixed solution (combination of the three salts in a 7:2:1 ratio) at 2, 4, 6, and 8 days of exposure. The migration of *P. coffeae* was studied in segmented columns of 10 cm in length and 4.40 cm in internal diameter, filled with saline soil (mixture of NaCl, $CaCl_2$, and $MgCl_2$) and non-saline, whose evaluations were carried out at 2, 4, and 6 days after soil infestation. The motility and number of active juveniles of both nematodes reduced with increasing saline concentration. From 0.50 M, *M. enterolobii* activity was not observed in any of the exposure periods to NaCl and $CaCl_2$. The increase in the concentrations of NaCl, $CaCl_2$, and $MgCl_2$ exponentially reduced the number of active *P. coffeae*, decreasing its activity from 0.75 M. The vertical migration of *P. coffeae* in the soil was negatively affected by salinity, presenting a more uniform distribution in the non-saline soil.

Keywords: *Meloidogyne enterolobii*. *Pratylenchus coffeae*. Salinity. Segmented soil column.

MOTILIDADE E MIGRAÇÃO DE NEMATOIDES EM AMBIENTES SALINOS

RESUMO – Os nematoides parasitas de plantas causam sérios prejuízos agrícolas no Nordeste brasileiro. Adicionalmente, a salinização do solo, especialmente na região semiárida do Nordeste é outro fator que limita a produtividade das culturas. Avaliou-se nesse estudo, a motilidade de *Meloidogyne enterolobii* e *Pratylenchus coffeae*, e, a migração vertical de *P. coffeae* em um Argissolo Amarelo sob condições salinas. A motilidade foi avaliada pela submissão de juvenis do segundo estádio de *M. enterolobii* e juvenis e adultos de *P. coffeae* em soluções salinas de NaCl, $CaCl_2$, $MgCl_2$ nas concentrações de 0,00, 0,25, 0,50, 0,75 e 1,00 M, e em solução mista (combinação entre os três sais na proporção 7:2:1) aos 2, 4, 6 e 8 dias de exposição. A migração de *P. coffeae* foi estudada em colunas segmentadas de 10 cm de comprimento e 4,40 cm de diâmetro interno, preenchidas com solo salino (mistura de NaCl, $CaCl_2$ e $MgCl_2$) e não salino cujas avaliações foram realizadas aos 2, 4 e 6 dias após a infestação do solo. A motilidade e quantidade de juvenis ativos de ambos nematoides reduziu com o aumento da concentração salina. A partir de 0,50 M não foi observada atividade de *M. enterolobii* em nenhum dos períodos de exposição a NaCl e $CaCl_2$. O aumento das concentrações de NaCl, $CaCl_2$, $MgCl_2$ reduziu exponencialmente o número de *P. coffeae* ativos, sessando sua atividade a partir de 0,75 M. A migração vertical de *P. coffeae* no solo foi negativamente afetada pela salinidade, apresentando distribuição mais uniforme no solo não salino.

Palavras-chave: *Meloidogyne enterolobii*. *Pratylenchus coffeae*. Salinidade. Coluna de solo segmentada.

*Corresponding author
[1]Received for publication in 03/04/2021; accepted in 06/22/2022.
Paper extracted for dissertation of the first author
[2]Department of Agricultural Engineering, Universidade Federal Rural de Pernambuco, Recife, PE, Brazil; ivisandrei@gmail.com - ORCID: 0000-0002-9555-4238, elvira.pedrosa@ufrpe.br - ORCID: 0000-0002-0755-8352, enio.fsilva@ufrpe.br - ORCID: 0000-0002-8652-503X, mario.rolim@ufrpe.br - ORCID: 0000-0003-2111-3875, thais.taty200583@gmail.com - ORCID: 0000-0002-9150-9739.

INTRODUCTION

Phytoparasitic nematodes form an important group of agricultural pathogens that infect plants and lead to reduced yield (IBRAHIM et al., 2016). Estimated losses in world agricultural production due to these pathogens are in the order of US$ 80 billion (SAHAH; MAHAMOOD, 2017). However, the negative impact that plant parasitic nematodes cause on agricultural production is still underestimated (SIKORA et al., 2018).

In recent years, the top ten genera of plant parasitic nematodes that are most scientifically and economically important were ranked in a study conducted by Jones et al. (2013). At the top is the genus *Meloidogyne*, with *M. enterolobii* Yang and Eisenback being one of the most devastating species in agricultural production (POSTNIKOVA et al., 2015). Another highlight is the genus *Pratylenchus*, distributed worldwide, with approximately 70 described species (MAJD TAHERI et al., 2013).

Meloidogyne enterolobii (Syn. *M. mayaguensis*) was originally described by Yang and Eisenback (1983) in China. In Brazil, the first reports were made by Carneiro et al. (2001) in the states of Bahia and Pernambuco in guava orchards (*Psidium guajava* L.) cv. Paluma in the São Francisco Valley. This species has a wide range of hosts and a high reproduction rate, which induces the formation of large galls on the roots of crops (CASTAGNONE-SERENO, 2012).

In the genus *Pratylenchus*, the species *P. coffeae* (Zimmermann) Filipjev and Schuurmans Stekhoven stands out, with the first reports from Indonesia (ZIMMERMANN, 1898). In coffee (*Coffea arabica* L.), this species is considered a highly virulent phytopathogen in several countries worldwide. In Brazil, the first report of this nematode was carried out by Monteiro and Lordello (1974). In coffee in the state of São Paulo. In northeastern Brazil, *P. coffeae* was reported for the first time in yam (*Dioscorea cayennensis* Lam.) by Moura and Monteiro (1995).

In Brazil, *M. enterolobii* and *P. coffeae* are harmful to the main crops of economic importance or subsistence because they have a wide range of hosts and high aggressiveness and are adapted to local soil and climate conditions (CASTRO; LIMA; CARNEIRO, 2003). In the Brazilian Northeast, this scenario is no different. Losses in the production of sugarcane (*Saccharum* spp.), yam, coffee, banana (*Musa* sp.), guava, and tomato (*Lycopersicum esculentum* L.) are common due to the presence of these other species of *Meloidogyne* and *Pratylenchus* (ALMEIDA et al., 2018; MOURA et al., 2012; SILVA et al., 2016; SILVA; SANTOS, 2017).

Another factor limiting agricultural yield in the Brazilian semiarid region is salinity, considered the main abiotic factor present in this region (RIBEIRO; RIBEIRO FILHO; JACOMINE, 2016). Salinity affects crops due to the increase in soil osmotic potential, causing physiological disorders in plants (MACHADO; SERRALHEIRO, 2017). Furthermore, the structure and composition of the soil-resident micro-invertebrate community can be affected by the sensitivity of these organisms to salinity (NIELSEN et al., 2011).

Salinization occurs in about 7% of soils worldwide (PEDROTTI et al., 2015). In Brazil, saline and/or sodic soils are reported mainly in the semiarid region of the Northeast and some regions of Rio Grande do Sul and the Pantanal Mato-Grossense. In these areas, the main soluble salts found are constituents of chlorides, sulfates, and bicarbonates of sodium, calcium, and magnesium (RIBEIRO; RIBEIRO FILHO; JACOMINE, 2016).

Although it is widely reported that plant parasitic nematodes can adapt to the most varied environmental conditions, information on the behavior of these worms in saline environments is scarce in the literature. Thus, this study aimed to study the motility and migration of plant parasitic nematodes in saline environments as a function of time.

MATERIAL AND METHODS

Two trials were carried out at the Laboratory of Phytonematology of the Federal Rural University of Pernambuco (UFRPE). In the first one, the motility of *P. coffeae* and *M. enterolobii* was evaluated in different saline concentrations of different types of salts at 2, 4, 6, and 8 days of exposure to salinity; and, in the second, the vertical migration of *P. coffeae* in saline and non-saline soils was investigated.

Obtaining the nematodes

The population of *P. coffeae* was obtained from yam tubers from the coast infected by the nematode, collected at the Pernambuco Supply and Logistics Center (CEASA), Recife - PE. The extraction of nematodes was based on the funnel technique of Baermann (1917), whose aqueous suspension containing *P. coffeae* was poured over 200 mesh sieves superimposed on another 500 mesh to obtain a greater number of adults retained on the 200 mesh sieve; thus, a greater number of juveniles in the sieve of 500 mesh. To identify this species, semi-permanent slides were prepared with 20 adult females, whose specimens were evaluated for morphological structures and measured with an optical microscope (400-1,000x), using the keys and descriptions of Mai and Mullin (1996) for diagnosis and Castillo and Vovlas (2007).

The population of *M. enterolobii* was obtained from guava roots infected by the nematode in the Senador Nilo Coelho Irrigation District,

Núcleo 6, Petrolina – PE. After the collection, in the Laboratory of Phytonematology of the UFRPE, the eggs and juveniles of the second stage (J2) were extracted from the methodology of Hussey and Barker (1973), using for the study the J2 hatched after 48 and 72 hours after incubation, which were then quantified (number of nematodes/mL) on a Peters slide.

Trial 1 – Motility of *P. coffeae* and *M. enterolobii* in saline solutions

To study the motility of *P. coffeae* and *M. enterolobii* in saline media, two experiments were carried out separately for each nematode species. 1200 ± 120 juveniles contained in 2 mL of distilled water were deposited in plastic containers (5.0 cm in diameter x 4.5 cm in height), with 18 mL of sodium chloride (NaCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$) or mixed solution (combination between the salts in the proportion 7:2:1) in concentrations of 0.00 (control with distilled water); 0.25; 0.50; 0.75 and 1.00 M. The salt solutions were kept at room temperature in the laboratory (22 ± 3ºC). Nematode motility was determined after two, four, six, and eight days of exposure to salinity by counting the number of active nematodes and the total number of nematodes.

For the preparation of saline solutions, the equation was used: m = V × MM × M, where m is the mass of the salts (g) to be used to reach the required molar level; V (L), the volume of the solution; MM, the molar mass of the salt in question, and M, the required molarity (mol/L).

Next, the nematodes were quantified on a Peters slide, with the aid of an optical microscope at 100× magnification, considering active nematodes, those that moved at the time of counting, regardless of intensity; and, as the total number of nematodes, the sum of all individuals found during the count.

Trial 2 - Vertical migration of *P. coffeae*

The migration of *P. coffeae* was studied in polyvinyl chloride (PVC) columns adapted according to the methodology proposed by Pinkerton et al. (1987). The columns were composed of four rings of 2.00 cm in height, superimposed on a ring of 2.00 cm in height, which was perforated 1.00 cm above its base for nematode infestation in the columns (Figure 1).

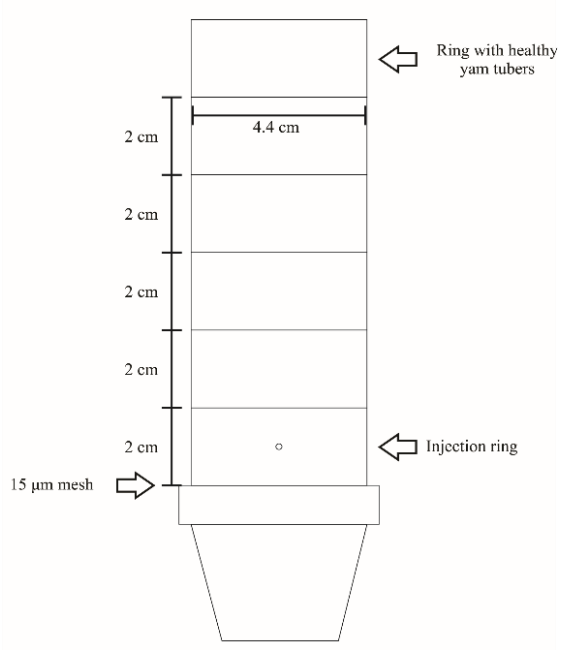

Figure 1. Experimental set-up used to assess migration of *Pratylenchus coffeae*.

Each column was 10.00 cm long, 4.40 cm in internal diameter, and 153.00 cm³ in internal volume. The columns were filled with soil collected at the Carpina Sugarcane Experimental Station (EECAC-UFRPE) at a depth of 0.00 to 0.40 m, according to the attributes presented in Table 1. The soil was maintained with a density of 1.40 $g.cm^{-3}$ and humidity = 15%, similar to field conditions. The base of the infestation ring was covered with a 15.00 µm mesh to keep the nematodes in the system, and the rings were arranged over a pot. A PVC ring containing 3.00 g of healthy chopped yam tubers was placed on top of the columns to serve as a stimulus to nematodes (BARROS et al., 2019). After the columns were completely assembled, a parafilm was placed on top to prevent water loss through evaporation and maintain moisture during the experiment.

Table 1. Soil attributes used in the migration test of *Pratylenchus coffeae*.

Soil attributes	Depth
	0.0 – 0.4 m
Sand (%)	75.6
Sílt (%)	10.4
Clay (%)	14.0
Pot capacity (U %)	15.0
D_s (g.cm^{-3})	1.38
CE_{es} (dS.m^{-1})	0.59

D_s: soil density; CE_{es}: electric conductivity of the saturation extract.

To fill the columns, the soil was initially passed through a sieve with a 2.0 mm mesh and autoclaved at 120 °C with a pressure of one atmosphere for four hours. Subsequently, the soil was dried in an oven at 105 °C for 24 h. Then, the soil was artificially salinized with the salts NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$, diluted in distilled water, in the proportion 7:2:1. The soil was then moistened at 15% to reproduce the corresponding "saline soil" and then used to fill the columns. The electrical conductivities of the saturation extract of the soils used were 0.59 dS.m^{-1} (soil considered not saline) and 15.0 dS.m^{-1} (soil artificially salinized).

Approximately 1200 ± 120 juveniles and adults of *P. coffeae* were placed in each column through the infestation hole, which was sealed with adhesive tape after inoculation. To maintain soil moisture, the columns were weighed daily on an analytical balance to replace the evaporated water when necessary (PUDASAINI; VIAENE; MOENS, 2007).

The columns were dismantled two, four, and six days after the soil infestation (DAI) with nematodes, which were extracted from the soil of each ring using the centrifugal flotation technique in sucrose solution (JENKINS, 1964). Soon after, counting was performed under an optical microscope with 100× magnification to determine the total number of nematodes (live and dead) per ring.

The ambient temperature throughout the experiment remained between 20 and 25 °C, as indicated by a temperature sensor connected to the HOBO® data logger.

Statistical analysis

In both experiments, a randomized block design was used, with treatments arranged in a split-plot scheme, as indicated by the Mauchly sphericity test. Analysis of variance was performed with the F test, unfolding the analyzes whenever the interaction was significant through regressions, the Tukey test, and the X^2 test. Data were analyzed using the R software (R CORE TEAM, 2018).

In the first experiment, the treatment arrangement consisted of a factorial scheme of 4 (periods of salinity exposure: two, four, six, and eight days) × 4 (salt sources: NaCl, $CaCl_2$, $MgCl_2$, and mixed solution) × 5 (salt concentrations: salts: 0.00, 0.25, 0.50, 0.75, and 1.00 M), with four replications, totaling 320 experimental units. In the second experiment, the treatments were arranged in a factorial scheme of 3 (periods after inoculation: two, four, and six DAI) × 2 (soil salinity levels: 0.59 dS.m^{-1}, 15.0 dS.m^{-1}) × 5 (counting rings), with four replications, totaling 120 experimental units.

RESULTS AND DISCUSSION

Trial 1 – Motility of *P. coffeae* and *M. enterolobii* in saline solutions

The number of active *M. enterolobii* juveniles was significantly influenced ($p < 0.0001$) by all isolated factors, except for different sources of salts ($p > 0.05$). There was an interaction between the evaluation days, salt sources, and salinity levels ($p < 0.0001$). For the total number of *M. enterolobii*, there was a significant effect for the sources of salts and salinity levels ($p < 0.0001$); there was no significance for the exposure time ($p > 0.05$), but there was a significant interaction between the salinity level and the source of the salts ($p < 0.05$, Table 2).

The number of active J_2 of *M. enterolobii* decreased with increasing saline concentration and time of exposure to salts (Figure 2). The reduction in the number of active J_2 of *M. enterolobii* occurred exponentially in all salts, and the highest number of active J2 was found at 0.00 M concentration after 2 days of exposure to salts NaCl (1090), $CaCl_2$ (1205), $MgCl_2$ (1175) and mixed solution (1185). From the concentration of 0.50 M, the activity of J_2 of *M. enterolobii* was not observed in any of the periods of exposure to NaCl and $CaCl_2$ salts. For $MgCl_2$ and mixed solution, nematode activity was recorded up to 0.50 M; however, nematode motility ceased from 0.75 M.

Table 2. Summary of the analysis of variance for the effects of salinity exposure days, salt types (ST) and salinity levels (SL) on the number of active (AJ) and total (TJ) juveniles of *Meloidogyne enterolobii*.

Source	*M. enterolobii* – AJ				
	DF	SS	MS	F	P (> F)
Block	3	10655	3552	1.0657	0.41
Day	3	270985	90328	27.103	< 0.0001
Error (a)	9	29995	3333		
ST	3	10465	3488	2.4725	0.077
Day:Salt	9	84185	9354	6.63	< 0.0001
Error (b)	36	50790	1411		
SL	4	57628108	14407027	6383.03	< 0.0001
SL:Day	12	446202	37184	16.4742	< 0.0001
SL:ST	12	211622	17635	7.8133	< 0.0001
SL:Day:ST	36	237028	6584	2.9171	< 0.0001
Error (c)	192	433360	2257		
	M. enterolobii – TJ				
Block	3	42025	14008	1.5352	0.2713
Day	3	24845	8282	0.9076	0.4748
Error (a)	9	82125	9125		
ST	3	194615	64872	10.7676	< 0.0001
Day:Salt	9	90535	10059	1.6697	0.1328
Error (b)	36	216890	6025		
SL	4	192493	48123	7.1219	< 0.0001
SL:Day	12	68567	5714	0.8456	0.6022
SL:ST	12	163648	13637	2.0182	0.0245
SL:Day:ST	36	202253	5618	0.8314	0.7398
Error (c)	192	1297360	6757		

DF: Degree of freedom; MS: Mean square; SS: Sum of square; P>F: F-test significance level.

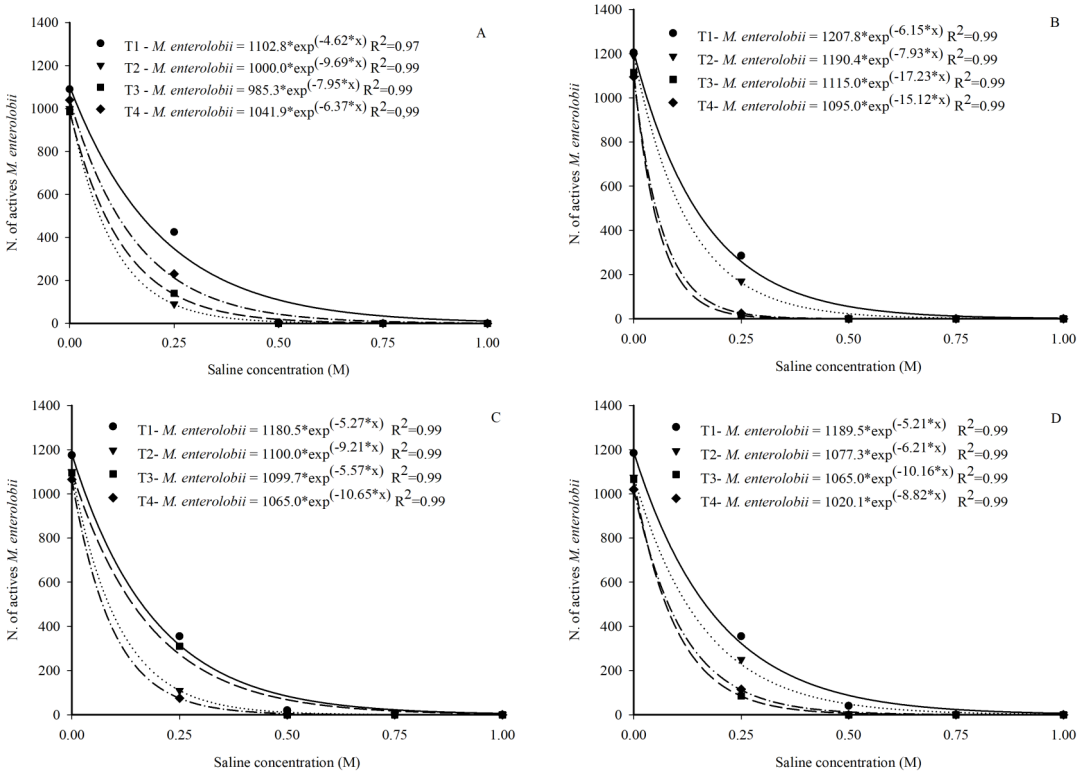

Figure 2. Effect of increasing concentrations of NaCl (A), $CaCl_2$ (B), $MgCl_2$ (C) and mixed solution (D) at two (T1), four (T2), six (T3) and eight (T4) days of exposure on the number of active second stage juveniles of *Meloidogyne enterolobii*.

About the salt types within the saline concentrations, significant results were observed for NaCl and $CaCl_2$, fitting to a linear and quadratic regression model, respectively (Figure 3). For $MgCl_2$ and the mixed solution, the total number of *M. enterolobii* had no significant effect regardless of the saline concentrations used. The increase in saline concentration caused a linear increase in the total number of J_2 of *M. enterolobii* in NaCl solutions, resulting in a rise of 7.2% in the total number of J_2 in distilled water for the solution with the highest molarity. In $CaCl_2$ solutions, the estimated saline concentration with the highest number of J_2 of *M. enterolobii* was 0.38 M, corresponding to 1255 nematodes, which then decreased with increasing concentrations.

The number of active specimens of *P. coffeae* was significantly influenced by all isolated factors ($p < 0.0001$), with an interaction between the exposure time factors, types of salts, and salt concentration levels ($p < 0.0001$). For the total number of *P. coffeae*, there was a significant effect ($p < 0.05$) only for the types of salts, with exposure time and saline concentration levels not significant ($p > 0.05$). However, the interaction between exposure time and types of salts was significant ($p < 0.05$) (Table 3).

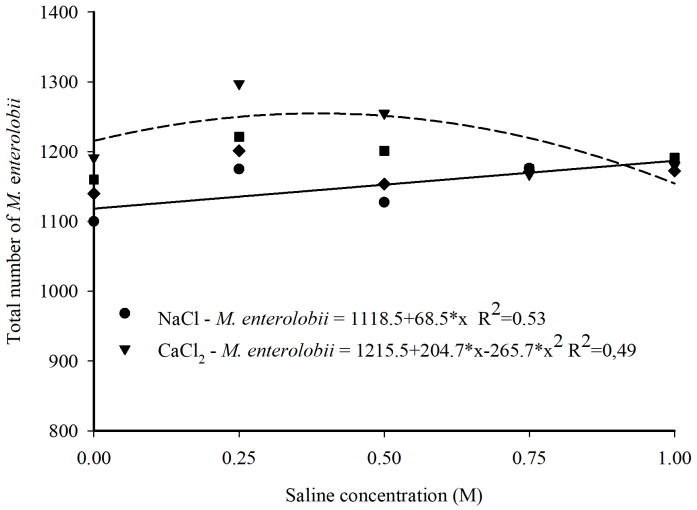

Figure 3. Salt type within salt concentration levels, corresponding to the total number of second stage juveniles of *Meloidogyne enterolobii*.

Table 3. Summary of the analysis of variance for the effects of salinity exposure days, salt types (ST) and salinity levels (SL) on the number of active (AJ) and total (TJ) juveniles of *Pratylenchus coffeae*.

Source	*P. coffeae* – AJ				
	DF	SS	MS	F	P (> F)
Block	3	14365	4788	0.7565	0.5459
Day	3	272265	90755	14.3385	0.0008
Error (a)	9	56965	6329		
ST	3	140535	46845	20.1556	< 0.0001
Day:Salt	9	79475	8831	3.7995	0.0018
Error (b)	36	83670	2324		
SL	4	45652982	11413246	3733.1229	< 0.0001
SL:Day	12	485347	40446	13.2292	< 0.0001
SL:ST	12	679827	56652	18.5302	< 0.0001
SL:Day:ST	36	371162	10310	3.3723	< 0.0001
Error (c)	192	587000	3057		
	P. coffeae – TJ				
Block	3	30314	10105	0.9771	0.0445
Day	3	97724	32575	3.1500	0.0791
Error (a)	9	93071	10341		
ST	3	74604	24868	4.0947	0.0134
Day:Salt	9	139781	15531	2.5573	0.0219
Error (b)	36	218635	6073		
SL	4	64593	16148	2.2626	0.0639
SL:Day	12	121107	10092	1.4141	0.1620
SL:ST	12	39478	3290	0.4610	0.9351
SL:Day:ST	36	280863	7802	1.0932	0.3416
Error (c)	192	1370280	7137		

DF: Degree of freedom; MS: Mean square; SS: Sum of square; P>F: F-test significance level.

Increased saline concentrations of NaCl, $CaCl_2$, $MgCl_2$, and the mixed solution resulted in an exponential reduction in the number of active *P. coffeae* at two, four, six, and eight DAI. At concentrations of 0.75 and 1.00 M, no activity of *P. coffeae* was observed at all times and salts. The highest values of active specimens of *P. coffeae* recovered were observed on two days of treatment exposure, at a concentration of 0.00 M, 1055 for NaCl, 1010 for $CaCl_2$, 1075 for $MgCl_2$, and 1035 for mixed solution (Figure 4).

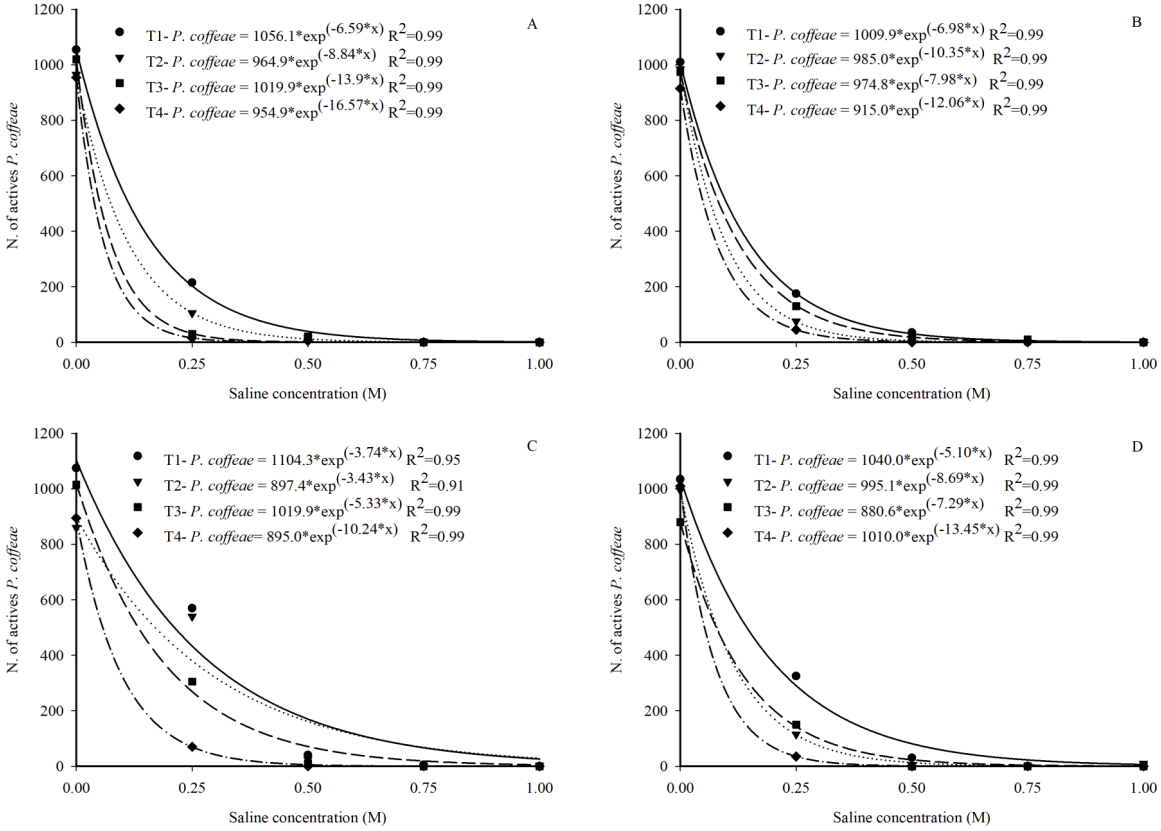

Figure 4. Effect of increasing concentrations of NaCl (A), $CaCl_2$ (B), $MgCl_2$ (C) and mixed solution (D) at two (T1), four (T2), six (T3) and eight (T4) days of exposure on the number of active specimens of *Pratylenchus coffeae*.

There was no significant difference in the total number of juveniles and adults of *P. coffeae* during the two days of exposure to salinity in the different salts used in the study (Figure 5). At four days, there was a significant difference in the total number of *P. coffeae* between NaCl (1189) and $MgCl_2$ (1145). After six days of exposure to the salts, a total number of 1244 *P. coffeae* was observed in the mixed solution, which was significantly higher than that obtained in $MgCl_2$ and $CaCl_2$ (Figure 5). In the last evaluation, performed at eight days of exposure to salts, in the $MgCl_2$ solution, the highest values were observed in the total number of *P. coffeae* ($P < 0.05$); and, in NaCl and $CaCl_2$, the lowest averages were found for the same variable compared to the other treatments (Figure 5).

In the motility tests for both species, it was found that the wave movement of the nematodes was more evident when exposed to distilled water, which was reduced as the saline concentration and the time of exposure to the salts increased until they became apparently inactive. The survival of nematodes can be influenced by various salts and ions, with different effects at different concentrations, in which the survival rate is low at higher concentrations (JAIRAJPURI; AZMI; BAJAJ, 1974).

In a study with different levels of saline concentrations of NaCl, $MgCl_2$, $CaCl_2$, Na_2SO_4, and $MgSO_4$, Maggenti and Hardman (1973) reported that increasing salinity to 4 mmho cm^{-1}, the population level of *M. javanica* was reduced half. Edongali and Ferris (1982) identified a reduction in the density of *M. incognita* at salinity levels greater than 2.5 mmho cm^{-1} for NaCl and $CaCl_2$. In a study carried out in India, Ray and Das (1980) reported that *Pratylenchus* species were tolerant to soil conditions with electrical conductivity up to 4 mmho cm^{-1}; however, there is a report of a negative correlation between soil salinity and the occurrence of *Pratylenchus* species (CHALANSKA; LABANOWSKI; SAS, 2016).

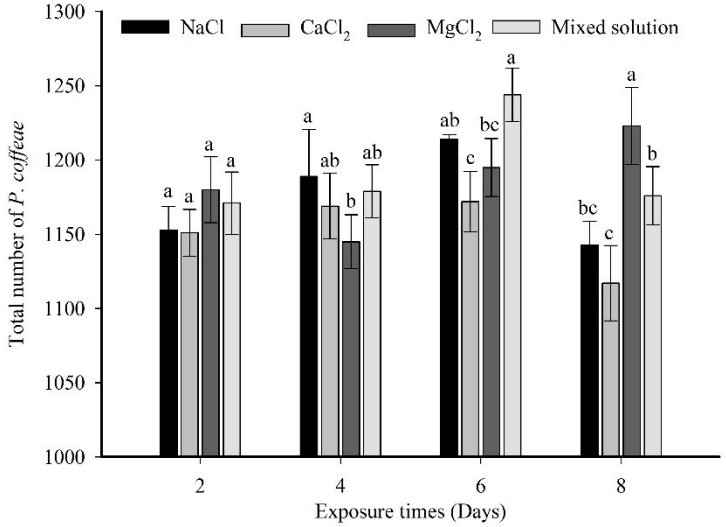

Figure 5. Interaction between exposure time to salinity and different types of salts on the total number of specimens of *Pratylenchus coffeae*.

Due to the water deficit, high evaporation rate, and physical and chemical characteristics of the soils, there are large areas with salinization problems in the semiarid region of the Brazilian Northeast, with greater intensity when irrigated (SILVA et al., 2011). Holanda et al. (2016) listed surveys in reservoirs in Northeast Brazil to assess the quality of water for irrigation, finding variations in the electrical conductivity of these waters from 0.07 to 5.97 $dS.m^{-1}$, values that are within limits evaluated in this research and the main ions were Ca, Mg, Na, and Cl.

Trial 2 - Vertical migration of *P. coffeae*

The migration of *P. coffeae* specimens was influenced ($p < 0.0001$) by distance, however, there was no significant effect of time and different soils (non-salted and salinized) on nematode migration. The interaction between time, migration distance, and soil types occurred significantly for the total number of *P. coffeae* ($p < 0.0001$, Table 4).

Table 4. Summary of the analysis of variance for the effects of time (Days), distance migrated (Section), and soil types (Soil) on total number of *Pratylenchus coffeae* juveniles (TJ).

Source	*P. coffeae* – TJ				
	DF	SS	MS	F	P (> F)
Block	3	6133	2044	4.0798	0.0675
Day	2	2940	1470	2.9335	0.1292
Error (a)	6	3007	501		
Section	4	1866813	466703	359.3097	< 0.0001
Day:Section	8	223627	27953	21.5210	< 0.0001
Error (b)	36	46760	1299		
Soil	1	53	53	0.0491	0.8256
Soil:Day	2	3927	1963	1.8067	0.1758
Soil:Section	4	1084813	271203	249.5736	< 0.0001
Soil:Day:Section	8	179907	22488	20.6948	< 0.0001
Error (c)	45	48900	1087		

DF: Degree of freedom; MS: Mean square; SS: Sum of square; P>F: F-test significance level.

In the non-saline soil (Figure 6A), at two DAI, the total number (juveniles and adults) of *P. coffeae* specimens was concentrated in the three rings corresponding to the range from 0 to 6 cm in height, with a higher percentage in the ring corresponding to 4-6 cm, indicating that 37.90% of the nematodes migrated 4-6 cm in two days. At four DAI, despite the ring corresponding to 4-6 cm continuing to harbor a higher percentage of *P. coffeae*, 3.70% of the nematodes had already migrated 8 to 10 cm, reaching the top of the column. Over time, the percentages of nematodes began to

decrease in the 0-2 and 2-4 cm rings and to increase in the 6-8 and 8-10 cm rings; although the 4-6 cm ring still contained more nematodes since at six DAI 17.90% of the nematodes had migrated 8 to 10 cm.

The migration of juveniles and adults of *P. coffeae* along the columns filled with saline soil (Figure 6B) occurred more slowly than in the non-saline soil. No juveniles or adults of *P. coffeae* reached the top of the columns at six DAI. The highest percentages of *P. coffeae* at two and four DAI were found in the inoculation ring, corresponding to 64.30 and 51.50%, respectively. Most nematodes (49.40%) at six DAI were concentrated between 2 and 4 cm of migration distance.

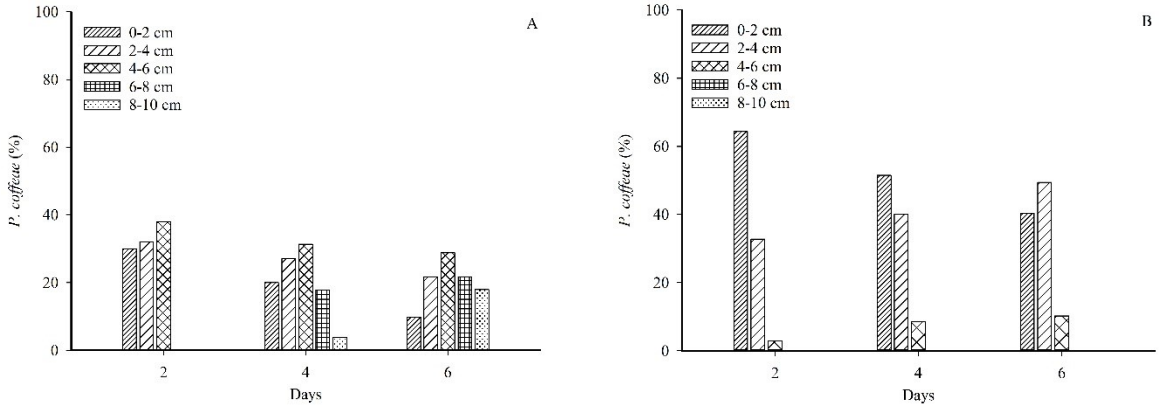

Figure 6. Distribution of juveniles and adults of *Pratylenchus coffeae* in columns filled with non-saline (A) and saline (B) soil as a function of time.

Juveniles and adults of *P. coffeae* moved in a well-distributed way along the columns with non-saline soil, with the presence of nematodes in all sections, a fact that was not observed in the columns filled with saline soil.

Approximately 18% of the juveniles and adults of *P. coffeae* placed in the columns with non-saline soil migrated 10 cm and reached the top at six DAI. This confirmed the attraction of nematodes to yams and adequate soil conditions for migration. This result corroborates that found by Francilino et al. (2017), who reported that at five DAI, 14.60% of *P. coffeae* specimens were found in yam husks more than 5 cm away from the infestation access. In the columns with saline soil, the distance covered by the nematodes reduced to the range from4 to 6 cm, where approximately 10.20% of the nematodes were observed. This fact indicated that the migration of nematodes in saline soil was negatively affected.

Studies to evaluate the migration of *Pratylenchus* species in saline environments are not mentioned in the literature. However, in studies with populations of *M. javanica* (Treub) Chitwood and *M. incognita* (Kofoid and White) Chitwood, Prot (1978a, 1979) reported that these nematodes, when exposed to different concentration gradients of sodium chloride, potassium, calcium nitrate, and magnesium sulfate, were repelled and migrated in the opposite direction to the highest concentration of salts. Additionally, Prot (1978b) observed that the J2 of *M. javanica* moved preferentially towards regions with lower concentrations of various salts.

Despite the direct effects that high concentrations of salts may impose on the nematode and possible mechanisms by the population to reduce the effects of this stress, such as osmoregulation (MAGGENTI; HARDAN, 1973); It is plausible to infer that the damage caused to plants by saline stress, such as reduced growth and alteration of root metabolism, indirectly affect the nematode, restricting the potential of plants to meet the nutritional demand of parasites. The penetration of J_2 of *M. incognita* into roots of okra (*Abelmoschus esculentus* L.) cv. Pusa Sawni and cucumber (*Cucumis sativus* L.) cv. Point Sett was delayed, and nematode infectivity, development, and reproduction were decreased in saline soil (KHAN; KHAN; KHAN, 1997).

The results obtained in the present study highlight the importance of the degree of salinity and the source of the salts in the motility of *M. enterolobii* and the increase of the saline concentration and the time of exposure to the salts in the reduction and inhibition of the migration of the two nematode species studied. Understanding how soil salinity affects nematode physiology, especially plant parasites and knowing sources and concentrations of salts that limit mobility, migration, and host plant recognition by the nematode may contribute to the joint management of soil and these parasites in environments agricultural.

Inadequate soil and irrigation management in the northeastern semiarid region due to the indiscriminate use of fertilizers, pesticides, excess

water, or water with a high level of salts contributes substantially to the expansion of areas of degraded soils (CASTRO; ARAÚJO; SANTOS, 2019; FREIRE et al., 2020). The migration of plant parasitic nematodes can be influenced by the physical and chemical attributes of the soil, but due to the lack of information about the movement of Pratylenchus and other nematodes in saline environments and, in particular, in Brazilian saline soils, it is necessary the development of new research, both in the laboratory and in the field. Determining the type and degree of salinity in the soil is essential for determining the salinity and nematode management techniques that will be adopted.

CONCLUSIONS

The increase in saline concentrations of NaCl, $CaCl_2$, $MgCl_2$, and mixed solution decreased the motility of juveniles and adults of *M. enterolobii* and *P. coffeae*.

Juveniles and adults of *P. coffeae* can migrate 10 cm in non-saline soils in six days.

The migration of *P. coffeae* is negatively affected by the increase in soil salinity.

ACKNOWLEDGMENTS

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Fundação de Amparo à Pesquisa do Estado de Pernambuco (Facepe) for granting scholarships. To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for granting scholarships and financial support.

REFERENCES

ALMEIDA, N. O. et al. Occurrence and correlations of nematodes, *Fusarium oxysporum* and edaphic factors on banana plantations. **Journal of Phytopathology**, 166: 265-272, 2018.

BAERMANN, G. Eine einfache Methode zur Auffindung von Anklyostomum (Nematoden) Larven in Erdproben. **Geneesk Tijdschr Nederlandsch-Indie**, 57: 131-137, 1917.

BARROS, B. E. A. et al. Mobility of *Pratylenchus coffeae* in segmented soil columns submitted to water flows and plant stimuli. **Semina: Ciências Agrárias**, 40: 2189-2200, 2019.

CARNEIRO, R. M. D. G. et al. Primeiro registro de *Meloidogyne mayaguensis* em goiabeira no Brasil. **Nematologia Brasileira**, 25: 223-228, 2001.

CASTAGNONE-SERENO, P. *Meloidognye enterolobii* (= *M. mayaguensis*): profile of an emerging, highly pathogenic, root-knot nematode species. **Nematology**, 14: 133-138, 2012.

CASTILLO, P.; VOVLAS, N. *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, biology, pathogenicity and management. **Nematology Monographs and Perspectives**, v. 6, p. 528, 2007.

CASTRO, F. C.; ARAÚJO, J. F.; SANTOS, A. M. Susceptibility to soil salinization in the quilombola community of Cupira - Santa Maria da Boa Vista - Pernambuco – Brazil. **Catena**, 179: 175-183, 2019.

CASTRO, J. M. C.; LIMA, R. D.; CARNEIRO, R. M. D. G. Variabilidade isoenzimática de populações de *Meloidogyne* spp. provenientes de regiões brasileiras produtoras de soja. **Nematologia Brasileira**, 27: 1-12, 2003.

CHALANSKA, A.; LABANOWSKI, G.; SAS, D. Root-lesion nematodes (*Pratylenchus* spp.) in ornamental plant nurseries–influence of soil texture, acidity, salinity and organic matter content. **Communications in Biometry and Crop Science**, 11: 98-104, 2016.

EDONGALI, E. A.; FERRIS, H. Varietal response of tomato to the interaction of salinity and *Meloidogyne incognita* infection. **Journal of Nematology**, 14: 57-62, 1982.

FRANCILINO, A. H. et al. Efeito do fluxo de água, isca vegetal e volume de poros do solo na mobilidade de *Pratylenchus coffeae*. **Nematropica**, 47: 63-73, 2017.

FREIRE, M. B. G. S. et al. Salt Affected Soils in the Brazilian Semiarid and hytoremediation as a Reclamation Alternative. In: TALEISNIK, E.; LAVADO, R S. (Orgs.) **Saline and Alkaline Soils in Latin America**. Springer, 2020, p. 119-139.

HOLANDA, J. S. et al. Qualidade da água para irrigação. In: GHEYI, H. R. et al. (Eds) **Manejo da salinide na agricultura: Estudos básicos e aplicados**. Fortaleza, CE: Instituto Nacional de Ciência e Tecnologia em Salinidade, 2016, s/v, cap 4, p. 35-50.

HUSSEY, R. S.; BARKER, K. R. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. **Plant Disease Report**, 57: 1025-1028, 1973.

IBRAHIM, S. K. et al. Plant-parasitic nematodes on stone fruits and citrus in Lebanon. **Lebanese Science**

Journal, 17: 9-24, 2016.

JAIRAJPURI, M. S.; AZMI, M. I.; BAJAJ, H. K. Studies on nematode behaviour I. Effect of pH and salt concentrations on the survival of *Hoplolaimus indicus*, *Helicotylenchus indicus*, *Xiphinema basiri* and *Mylonchulus minor*. **Indian Journal of Nematology**, 4: 171-181, 1974.

JENKINS, W. R. A rapid centrifugal-flotation technique for separating nematodes from soil. **Plant Disease Reporter**, 48: 692-692, 1964.

JONES, J. T. et al. Top 10 plant-parasitic nematodes in molecular plant pathology. **Molecular Plant Pathology**, 14: 946-961, 2013.

KHAN, M. W.; KHAN, A. A.; KHAN, M. R. Effect of soil salinity on penetration, development and pathogenicity of *Meloidogyne incognita* on okra and cucumber. **Indian Journal of Nematology**, 27: 194-208, 1997.

MACHADO, R. M. A.; SERRALHEIRO, R. P. Soil salinity: Effect on vegetable crop growth: Management practices to prevent and mitigate soil salinization. **Horticulturae**, 30: 1-13, 2017.

MAGGENTI, A. R.; HARDAN, A. The effects of soil salinity and *Meloidogyne javanica* on tomato. **Journal of Nematology**, 5: 231-234, 1973.

MAI, W. F.; MULLIN, P. G. **Plant-parasitic nematodes**: a pictorial key to genera. Ithaca: Cornell University Press, 1996. 277 p.

MAJD TAHERI, Z. M. et al. Molecular and phylogenetic studies on Pratylenchidae from Iran with additional data on *Pratylenchus delattrei, Pratylenchoides alkani* and two unknown species of *Hirschmanniella* and *Pratylenchus*. **Nematology**, 15: 633-651, 2013.

MONTEIRO, A. R.; LORDELLO, L. G. E. Encontro do nematoide *Pratylenchus coffeae* atacando cafeeiro em São Paulo. **Revista de Agricultura**, 49: 164-164, 1974.

MOURA, R. M. et al. Espécies do fitonematóide do gênero *Meloidogyne* (Nematoda–Heteroderidae) encontradas associadas à cultura da cana–de–açúcar no estado de Pernambuco. **Anais da Academia Pernambucana de Ciência Agronômica**, 8: 193-204, 2012.

MOURA, R. M.; MONTEIRO, A. R. *Pratylenchus coffeae* on yams in Brazil. **Fitopatologia Brasileira**, 20: 256-256, 1995.

NIELSEN, A. L. et al. Effect of soil salinity on entomopathogenic nematode survival and behaviour. **Nematology**, 13: 859-867, 2011.

PEDROTTI, A. et al. Causas e consequências do processo de salinização dos solos. **Revista Eletrônica em Gestão, Educação e Tecnologia Ambiental**, 19: 1308-1324, 2015.

PINKERTON, J. N. et al. Vertical migration of *Meloidogyne chitwoodi* and *M. hapla* under controlled temperature. **Journal of Nematology**, 19: 152-157, 1987.

POSTNIKOVA, O. A. et al. Transcriptome analysis of resistant and susceptible alfalfa cultivars infected with root-knot nematode *Meloidogyne incognita*. **Plos One**, 10: 1-17, 2015.

PROT, J. C. Behaviour of juveniles of *Meloidogyne javanica* in salt gradients. **Revue de Nématologie**, 1: 135-142, 1978a.

PROT, J. C. Influence of concentration gradients of salts on the movement of second stage juveniles of *Meloidogyne javanica*. **Revue de Nématologie**, 1: 21-26, 1978b.

PROT, J. C. Influence of concentration gradients of salts on the behaviour of four plant parasitic nematodes. **Revue de Nématologie**, 2: 11-16, 1979.

PUDASAINI, M. P.; VIAENE, N.; MOENS, M. The influence of host and temperature on the vertical migration of *Pratylenchus penetrans*. **Nematology**, 9: 437-447, 2007.

R CORE TEAM (2018). R: **A language and environment for statistical computing**. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

RAY, S.; DAS, S. N. Nematodes of saline soils in Orissa, India. **Indian Journal of Nematology**, 10: 231-235, 1980.

RIBEIRO, M. R.; RIBEIRO FILHO, M. R.; JACOMINE, P. K. T. Origem e classificação dos solos afetados por sais. In: GHEYI, H. R et al. (Eds.). **Manejo da Salinidade na Agricultura: Estudos Básicos e Aplicados**. Fortaleza, CE: Instituto Nacional de Ciência e Tecnologia em Salinidade, 2016. s/v, cap. 2, p. 9-16.

SAHAH, M. M.; MAHAMOOD, M. Nematodes-a lesser known group of organisms. In: SHAH, M. M.; MAHAMOOD, M. (Eds.). **Nematology-concepts, diagnosis and control**. Rijeka, Croácia: Intech, 2017. s/v, cap. 1, p. 3-18.

SILVA, J. L. A. et al. Evolução da salinidade em

solos representativos do Agropólo Mossoró-Assu cultivado com meloeiro com água de deferentes salinidades. **Agropecuária Científica no Semiárido**, 11: 26-31, 2011.

SILVA, M. S. et al. Comportamento de genótipos de RB de cana-de-açúcar ao parasitismo dos nematoides das galhas. **Revista Brasileira de Ciências Agrárias**, 11: 73-79, 2016.

SILVA, M. C. L.; SANTOS, C. D. G. Distribution of *Meloidogyne enterolobii* in guava orchards in the state of Ceará, Brazil. **Revista Caatinga**, 30: 335-342, 2017.

SIKORA, R. A. et al. Reflections and challenges: nematology in subtropical and tropical agriculture. In: SIKORA, R. A. et al. (Eds.) **Plant parasitic nematodes in subtropical and tropical agriculture**. Wallingford, UK: Cabi, 2018, s/v, cap 1, p. 1-19.

YANG, B; EISENBACK, J. D. *Meloidogyne enterolobii* n. sp. (Meloidogynidae), a root-knot nematode parasitizing pacara earpot tree in China. **Journal of Nematology**, 15: 381-391, 1983.

ZIMMERMANN, A. W. P. De nematoden der koffie wortels. Deel I. **Mededeel´s Lands Plantentuin**, 27: 1-64, 1898.