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Thermotolerant coliform die-off in water treated with Moringa oleifera seeds

Coliformes termotolerantes em extinção em água tratada com sementes de Moringa oleífera.

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Abstract

The objective of this study was to test the ability of moringa seeds (Moringa oleifera) to eliminate thermotolerant coliforms (TTC) in water samples from a contaminated river in Northeastern Brazil (Rio Acaraú, Sobral). The treatment was administered in two concentrations: 5 g (T1) and 10 g (T2) homogenized seeds/1000 mL water. Twenty water samples were shaken continuously for 60 min and aliquots were drawn at 10, 20, 30 and 60 min to determine the most probable number of TTC. The greatest die-off (99.9%) was observed for T1 at 20 min. Due to the efficiency in eliminating enteric bacteria, the moringa seeds can be considered as a source potentially promising for treatment of water contaminated with fecal matter.

Keywords: *Enterobacteria. Moringa oleifera. Water treatment.*

Resumo

O presente estudo teve como objetivo o uso de sementes de moringa para obtenção do decaimento de coliformes termotolerantes (CT) em amostras de água do Rio Acaraú (Sobral-Ceará). Foram realizados dois tratamentos que consistiram na homogeneização de 5 g (T1) e 10 g (T2) de sementes em 1000 mL da água do rio. As vinte amostras de água submetidas aos tratamentos foram mantidas sob agitação por 60 minutos. Para avaliação da eficiência dos tratamentos, nos períodos de 10, 20, 30 e 60 minutos, foram retiradas alíquotas para a determinação do Número Mais Provável (NMP) de CT. O decaimento de CT mais expressivo ocorreu no período de 20 minutos do T1, com redução de 99,9% do NMP. Devido à eficiência na redução de bactérias entéricas, as sementes de moringa podem ser consideradas um recurso potencialmente promissor para o tratamento de águas com contaminação de origem fecal.

Palavras-chave: *Enterobactéria. Moringa oleifera. Tratamento de água.*

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1. Introduction

The occurrence of thermotolerant coliforms (TTC) in natural water bodies has been studied extensively around the world (PITKÄNEN et al., 2008; COULLIETTE and NOBLE, 2008; JAIANI et al., 2013). TTCs colonize the gastrointestinal tract of warm -blooded animals and include species of the genera Escherichia, Klebsiella and Enterobacter (TÔRRES, 2004). Water bodies are not uncommonly contaminated with TTC through the discharge of untreated domestic sewage (MORESCO et al., 2012).

The relation between public health and water quality was investigated by Musa et al. (1999) who found an association between cases of diarrhea and high TTC concentrations in water supplied for human consumption. On the other hand, Kolsky (1993) points out the complexity of the relation between diarrhea rates and TTC concentrations in view of the many different factors involved in the epidemiology of enteric pathogens.

Much research has been done in search of feasible means of preserving or decontaminating natural water bodies used for human consumption. The use of plant extracts with coagulating and/or antibacterial properties to clarify turbid water has yielded very promising results (SUAREZ et al., 2003; GHEBREMICHAEL et al., 2005).

The moringa tree (Moringa oleifera), a tropical species native to India, has been the object of extensive study due to its remarkable antibacterial qualities. The seeds and leaves of this plant have been shown to contain antibacterial compounds capable of inhibiting the growth of several major pathogens, including Escherichia coli, Vibrio cholerae classic 569B, V. parahaemolyticus, Enterococcus faecalis, Aeromonas caviae (VIEIRA et al., 2010; PEIXOTO et al., 2011). As an additional advantage, treatment with moringa extracts does not affect the pH or taste of drinking water (AMARAL et al., 2006).

The objective of the present study was to evaluate the ability of moringa seed powder to induce thermotolerant coliform die-off in samples of contaminated water.

2. Materials and Methods

2.1 Water sample collection

Twenty water samples were collected manually from an urban stretch of the Acaráu river (Sobral, Ceará, Northeastern Brazil) between February and June (Batch 1) and between July and November (Batch 2), 2007. The samples were placed in previously sterilized 1-L amber vials inside isothermal boxes and sent to the laboratory for analysis within one hour of sampling.

2.2 Origin of moringa seeds

Moringa oleifera seeds were obtained from the Center for Nutrition and Food Production of the Vale do Acaraú State University (NUNPRA/UVA), and specimens were deposited in the herbarium of the same institution under entry numbers 5823, 5824, 5825 and 5826. The seeds were ground into a homogenous powder.

2.3 Water treatment

Two treatments were performed: 5 g (Treatment 1) and 10 g (Treatment 2) moringa seed powder per 1000 mL river water. Samples were homogenized and kept under magnetic stirring. Following the procedures described by Feng et al. (2002), aliquots were drawn at 10, 20, 30 and 60 min and the most probable number (MPN) of TTC was determined in order to evaluate the efficiency of the treatments. Saline solution at 0.85% was used to make serial decimal dilutions (10-1 to 10-5) of all aliquots. An aliquot of 1 mL was taken from each dilution, inoculated in lauryl sulfate broth (Difco) and incubated for 48 hours at 35°C. Inocula were taken from positive tubes, seeded onto EC medium (Difco) and incubated in a warm water bath for 48 hours at 45°C. Based on the table published by Blodgett (2006) MPN values were estimated by multiplying the value of the critical series obtained from positive cultures in EC medium by the average dilution. Untreated river water samples were used as control.

3. Results

Table 1 show the data related to treatment 1 (5g/100 mL) in the water sample from the Batch 1. It was observed that the untreated water showed higher levels of MPN to the treated samples. At all times (10, 20, 30 and 60 minutes) of treatment 1 was detected TTC reduction of more than 90%, especially the time 20 minutes - with reduction of 99.9%.

Table 1 – Most Probable Number of thermotolerant coliforms/100 mL (TTC) in samples of contaminated water subjected to Treatment 1 with moringa seeds (5g/1000mL).

For the Treatment 2 were used the water samples from the Batch 2, and there was a TTC reduction of more than 75% at all times (10, 20, 30 and 60 minutes). The highest TTC die-off rates (87.4%) were observed for aliquots drawn at 30 minutes (Table 2).

Table 1 – Most Probable Number of thermotolerant coliforms/100 mL (TTC) in samples of contaminated water subjected to Treatment 1 with moringa seeds (5g/1000mL).

Samples (Batch 1)	Most Probable Number of thermotolerant coliforms/100 mL (TTC)				
	Control	Treatment time (minutes)			
		10	20	30	60
1	16.000.000	1.400	1.300	35.000	1.200.000
2	1.400	450	450	780	680
3	7.800	450	780	780	1.100
4	78.000	1.400	1.400	14.000	14.000
5	1.100	450	450	680	1.100
6	9.300	2.100	920	2.300	920
7	680	200	200	450	450
8	21.000	2.300	4.300	4.300	9.300
9	24.000	9.300	4.300	150	110
10	9.300	4.300	930	210	930
Average	1.615.258	2.235	1.503	2.365	122.859
Die-off	-	99,8%	99,9%	99,8%	92,4%

Table 2 – Most Probable Number of thermotolerant coliforms/100 mL (TTC) in samples of contaminated water subjected to Treatment 2 with moringa seeds (10g/1000mL).

Samples (Batch 2)	Most Probable Number of thermotolerant coliforms/100 mL				
	Control	Treatment time (minutes)			
		10	20	30	60
1	2.100	920	920	920	360
2	2.300	360	360	360	360
3	2.300	920	920	<3,0	360
4	920	360	920	<3,0	360
5	2.300	360	360	<3,0	360
6	4.300	2.300	1.100	360	920
7	9.300	920	2.300	360	2.300
8	210	<3,0	94	110	200
9	380	110	110	360	210
10	7.500	210	200	1.500	1.500
Average	3.161	646,3	728,4	397,9	693
Die-off	-	79,5%	76,9%	87,4%	78%

4. Discussion

The large difference in control TTC levels between the two batches of river water may be attributed to seasonal variation. Batch 1 was collected during the rainy season (February to June) while Batch 2 was collected during the dry season (July to November). A similar pattern was observed by Musa et al. (1999), who reported significantly higher TTC levels in water collected during the rainy season.

The high levels of TTC detected in samples of untreated water from the Acaraú river match findings by Vasconcellos et al. (2006), who reported MPN values for river water samples in the range of <3 to 2.1 x 104. The authors indicated domestic sewage discharge as a possible source of the coliforms detected in their samples and drew attention to the health risks posed by direct contact with river water containing microorganisms associated with outbreaks of diarrhea and urinary infection.

In our study the highest TTC die-off rates – over 90% - were observed for water from Batch 1 treated with moringa seed extract at 5 g/1000 mL (T1) for 20 minutes. Likewise, the Treatment 2 reduced the coliform load up to 87,4% in water samples from Batch 2. Our die-off rates agree with the results of studies evaluating the antibacterial action of extracts prepared with different parts of the moringa plant, especially the seeds (CÁCERES et al., 1991; MAYAER and STELZE, 1993). According to Jahn et al. (1986), moringa seeds contain the bactericidal substances pterygospermin, 4-(α-L-rhamnosyloxy)-benzylisothiocyanate and 4-(α-L-rhamnosyloxy)-phenylacetonitrile. However, in addition to bacterial growth inhibitors (ANWAR et al., 2007), moringa seeds also contain a recombinant protein capable of flocculating Gram -negative and Gram-positive bacteria (BROIN et al., 2002), making moringa seed extracts an even more attractive alternative to conventional water purifiers.

Kumar and Gopal (1999) also tested extracts of M. oleifera on river water samples contaminated with TTC and reported die-off rates (89.72%) compatible with ours. The authors concluded that moringa seed extracts can be used to purify water bodies contaminated with TTC and E. coli and thereby potentially help prevent a number of water-borne diseases.

Likewise, Olayemi and Alabi (1994) reduced by 65% TTC levels in water treated with moringa extraxts for 24 hours and, in a similar study using samples of surface water, Oluduro & Aderiye (2007) observed 97.5% TTC die-off after 24 hours of treatment.

Amagloh and Benang (2009) purified water with powdered moringa seed extract and observed significant reductions in MPN values. According to the authors, since bacteria are normally associated with solid particles, TTC die-off may have been due to the action of flocculating agents in the moringa extract.

In both our treatments the fall in MPN values during the first 30 minutes was followed by a new increase. Possibly, the increased availability of organic matter contributed to the proliferation of microorganisms resistant to treatment. In fact, according to Ndabigengesere and Narasiah (1998), by increasing the amount of organic matter in the water, M. oleifera actually stimulates bacterial growth. This is echoed by Amaral et al. (2006) who believe the large amount of biodegradable organic matter added to the water as a result of treatment with moringa seed extracts may serve as bacterial substrate.

Our results support the conclusion that the Moringa seed powder can be feasible alternative for the control of enteric bacteria in water bodies in domestic regions without sanitation or running water.

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