MORINGA OLEIFERA LAM SEEDS AND SOLAR RADIATION IN THE TREATMENT OF DRINKING WATER

(RADIAÇÃO SOLAR E O EXTRATO DE SEMENTES de Moringa oleifera NO TRATAMENTO DE ÁGUA DESTINADA AO CONSUMO HUMANO)

ABSTRACT

AIM: The microbiological quality of water samples in communities that use alternative sources of water for human consumption, using seed extract of *Moringa oleifera* and solar radiation, is evaluated, and provides subsidies for the use of these treatments. METHODS: The multiple tube method is used to determine the most probable number of thermotolerant coliforms and mesophile microorganisms in nine samples of water from alternative sources (wells). These samples were obtained in Cruz das Almas, in the Reconcavo Baiano region, state of Bahia, Brazil. RESULTS: The number of samples of water with moringa seeds and exposed to the sun for two, five and twelve hours showed a reduction in the concentrations of CT / CF of 1,52 log (56,51%) 1,88 log (64,83%) and 2,14 log (71,33%) respectively. The rate reduction for mesophile microorganisms after sun exposure for two, five and twelve hours respectively were 0,24 log (11,60%) 0.18 log (10,11%) and 1,25 log (65,78%). CONCLUSIONS: Although solar radiation was effective in removing bacteria, when used with *Moringa oleifera* seeds extract it was not effective in reducing fecal coliform load to zero. Only mesophile microorganisms reached levels required by legislation.

Keywords: Coliforms. Solar disinfection. *Escherichia coli.* Microbiology. Water quality. Seeds.

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RESUMO

OBJETIVO: Avaliar a qualidade microbiológica de amostras de água em comunidades que utilizam águas de fontes alternativas para o consumo humano, utilizando extrato de sementes de *Moringa oleifera* e radiação solar, além de fornecer subsídios para o uso destes tratamentos. MÉTODOS: Foram analisadas pelo método dos tubos múltiplos para determinar o número mais provável de coliformes totais, termotolerantes e microrganismos mesófilos em nove amostras de água provenientes de fontes alternativas (poços). Tais amostras foram obtidas na cidade de Cruz das Almas, localizada na região do Recôncavo, no estado da Bahia, Brasil. RESULTADOS: O número de amostras da água tratadas com sementes de moringa e expostas ao sol por duas, cinco e doze horas apresentaram redução nas concentrações de CT/CF de 1,52 log (56,51%), 1,88 log (64,83%) e de 2,14 log (71,33%), respectivamente. Já a taxa de redução para os microrganismos mesófilos após exposição ao sol de duas, cinco e doze horas foram respectivamente de 0,24 log (11,60%), 0,18 log (10,11%) e de 1,25 log (65,78%). CONCLUSÕES: A radiação solar foi eficiente na remoção bacteriana, porém usada concomitantemente com o extrato das sementes de *Moringa oleifera* não foi eficiente em reduzir a carga de coliformes termotolerantes a zero. Apenas a redução dos microrganismos mesófilos alcançou os níveis determinados por lei.

Palavras-chave: Coliformes. Desinfecção solar. Escherichia cole. Microbiologia. Qualidade da água. Sementes.

INTRODUCTION

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Drinking water is a highly relevant factor in the development of a country. Unpolluted water guarantees a healthy population and contributes towards the inhabitants' life quality by providing the basic needs for water and sanitary conditions. It is known that 80% of diseases in developing countries are related to water quality and sanitary conditions. In fact, drinkable water warrants public health and economical growth [1].

Although safe access to water consumption is a human right, 83% of human populations do not have access to safe primary water sources in the rural areas of developing countries [2]. The population is thus highly liable to develop diseases from water or water-transmitted diseases.

Waterborne diseases are principally caused by pathogenic microorganisms from animal or human enteral origin, basically transmitted through a fecal-oral route. They are excreted in the feces of infected people and ingested with water or with food made from feces-contaminated water [3].

Water supply sources have a long history associated with a varied spectrum of microbial infections. However, from health perspective, the main aim in water quality management is to guarantee that consumers would not be exposed to disease-determining pathogen doses. The protection of water sources and the treatment of distribution systems have greatly reduced these diseases in developed countries [4, 5].

Moringa oleifera, a native plant from Asia, with seeds characterized by their coagulant and bactericide features, has been widely used in human consumption water treatment processes. Further, the seeds do not change the water's taste or its pH. Recent discoveries in the use of ground *Moringa oleifera* seeds are highly important when the use of

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this coagulant agent is taken into consideration as a low cost alternative to conventional chemical treatment [6].

Studies on the monitoring of coagulation and bacterial decrease with the seed used in the waters of the Nile in Sudan reported a reduction between 80 and 95% in turbidity index and between 1 and 4 log (90 to 99,9%) in bacterial parameters, with microorganisms concentrated in the sediment [7]. A study on the efficiency of seven plant species in the decrease of the number of microorganisms in river water, pH between 6 and 8, showed that the *Moringa oleifera* seed ranked second in efficaciousness [8].

According to Ndabigengesere & Narasiah [9], *M. oleifera* extract increases the rates of organic matter in treated water which, in turn, increases chlorine demand and the formation of trihalomethanes during the disinfection by the above-mentioned chemical agent. This would disapprove the disinfectant in water treated with the plant extract. In fact, other alternatives to chemical treatment for drinkable water are being investigated, among which the widely-used disinfection by ultraviolet radiation is worth mentioning. According to Rincon & Pulgarin [10], solar radiation is capable of inactivating microorganisms due to UV radiation's synergic effect and to water heating by infra-red radiation.

The elimination of turbidity by sedimentation using moringa seeds has a positive effect on the water disinfection process by solar energy. In fact, less than 1% of UV radiation penetrates more than 2cm from the surface in water with high turbidity (>200 UNT), with a high decrease in germicide activities. The inactivation of *E. coli* in water samples with low turbidity was verified after a 7h-solar radiation exposure of water [11]. Similarly, Amaral *et al*. [6] reported a 74,3% decrease in NMP of *E. coli* water treated with moringa seeds and exposed to solar radiation during two hours; a 94,1% decrease in water treated with moringa

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seeds and exposed to solar radiation during five hours; a 100,0% decrease in water treated with moringa seeds and exposed to solar radiation during twelve hours.

According to Wegelin [12], it should be emphasized that disinfection by solar light is efficient only if the water has a less than 30 UNT turbidity rate.

Based on the above and on the scanty information available, current research aimed at (a) evaluating the use of *Moringa oleifer*a seeds extract and solar radiation in the treatment of water from alternative sources in the Reconcavo Baiano region, state of Bahia, Brazil; (b) providing subsidies for treatments in communities that use drinkable water from alternative sources.

MATERIALS AND METHODS

Research was conducted in Cruz das Almas, a town in the Reconcavo Baiano region, state of Bahia, Brazil. Water from wells on farms of the municipality was previously analyzed with regard to microbial load. Water samples from nine wells with the highest concentrations of microorganisms, coliforms and mesophile microorganisms, were subjected to microbiological and physical analyses.

Water was collected in 10 L-bottles with alcohol 70 % and sent to the Laboratory of Animal Microbiology of the Universidade Federal do Recôncavo da Bahia (UFRB), BA Brazil.

Employment of *Moringa oleifera* seeds for the water's physical treatment and disinfection of clarified water by solar radiation

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Sedimentation process consisted in the mixture of the extract from three seeds with well water samples, during one minute, at a fast speed, and then for five minute at a slower rate. Immediately after this stage, samples were left at rest for 24 h for the establishment of sedimentation. It should be enhanced that, prior to the addition of the extract, initial levels of the waters' turbidity and color varied respectively between 0,01 and 1,19 UNT and between 0 and 20 Uhazen.

Samples of supernatant were taken after sedimentation for the analysis of turbidity and color and for the determination of concentrations of total coliforms, thermotolerant coliforms and mesophile microorganisms.

Samples of clarified water were exposed to solar radiation for two, five and ten hours and afterwards analyzed to verify the effects of solar radiation on the researched microorganisms. Samples were conditioned in 2L polyethylene teraphthalate (PET) transparent bottles and placed horizontally on the ground to receive solar radiation from 07:00 to 19:00, with peak between 09:00 and 15:00. Thirty bottles were used, of which 15 were kept in the shade (control); 5 analyzed after a 2h exposure; 5 after a 5h exposure; 5 after a 12h exposure. Thirty bottles were thus filled and controls selected for each exposure time and those which underwent the three exposure times. On the analysis of each sample, its control was also analyzed. Protocol was repeated six times to obtain 30 samples for each time, together with their respective controls.

The temperature for the three exposure times were taken by a Celsius graded mercury thermometer (^{o}C) and conferred by data from the National Meteorological Institute (INMET) from the Cruz das Almas Automatic Station BA Brazil.

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Concentrations of coliforms (total and thermotolerant), mesophile microorganisms, and turbidity and color rates were registered for control samples and for samples from each exposure time.

Detection of microbiological concentrations

The detection of the Most Probable Numbers (MPN) of total (TC) and thermotolerant (CF) coliforms was undertaken by the multiple tube technique, according to Apha [13], with a series of 10 tubes and dilutions at 10^0. Specific detection of TC was undertaken with positive tubes of Lauryl Sulfate Tryptose Broth. Tubes with Brilliant Green Bile Broth 2% with gas and the MPN of total coliforms were registered. Further, 100 mL^{-1} was determined by a MPN table specifically prepared for inoculated dilutions.

MPN of CF was also done from the positive tubes of Lauryl Sulfate Tryptose Broth. Tubes with EC broth with gas and MPN of thermotolerant coliforms were registered. Further, 100 mL^{-1} was determined by a MPN table specifically prepared for inoculated dilutions.

Dilutions from10^0 to 10^2 of samples were performed to count the strictly aerobic mesophile and viable facultative microorganisms by peptonated water 0,1% as diluent. Further, 1 mL of samples and/or dilutions was poured on petri plates and the previously melted and cooled plate count agar (PCA) was introduced on the plates. When the medium solidified, the plates were inverted and incubated at 35 ± 2°C, for 24 - 48 h. Counting of colonies was undertaken by a colony counter. Average number of colonies in the plates was multiplied by the corresponding dilution factor and the result expressed in colony-forming units per mL of sample (UFC. mL-1) [13].

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Determination of turbidity and color rates

Turbidity rates were calculated by a bench turbidity meter ADAMO, TB 1000, and rates were given in UNT. Color rates were obtained by color meter Del Lab, DLNH-100, and results given in Uhazen.

Analysis of results

The efficiency of microbial removal was based by determining logarithmic units (UL) to avoid super evaluation of total and thermotolerant coliforms and of mesophile microorganisms by numbers such as 90 and 99,0% [14].

RESULTS AND DISCUSSION

Figure 1 shows the decrease of total (CT) and thermotolerant (CF) coliforms in the water exposed to solar radiation. It should be underscored that all detected TC concentrations were characterized as CF.

Water with the moringa seeds extract and exposed to solar radiation for 2 hours had a 1,52 log (56,51%) decrease in CT/CF concentrations (Figure 1), whereas microbial reductions for water exposed for 5 hours and 12 hours were 1,88 log (64,83%) and 2,14 log (71,33%) respectively. Consequently, high reduction rate occurred after a 12-hour exposure, even though water quality was not suitable according to drinkability standards set by the

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Ministry of Health [15], in spite of the above high removal indexes. According to this normatization, drinkable water is equivalent to water without *Escherichia coli* or thermotolerant coliforms per 100 mL of the sample.

Reduction rates for mesophile microorganisms (Figure 2) after a 2-h exposure to solar radiation were 0,24 log (11,60%); 0,18 log (10,11%) after 5 hours of exposure and 1,25 log (65,78%) after 12 hours of exposure. Contrastingly to the removal of *E. coli*, exposure to sun radiation during 12 hours was enough to meet the legal drinkability stance of the Decree 518 of the Ministry of Health [15]. Maximum rate of 500 UFC. mL^{-1} is allowed for this group of microorganisms.

Results of current research do not coincide with those by Amaral *et al.* [6]. When these authors investigated the reaction of *Moringa oleifera* seeds and of solar radiation in water treatment from dams, they reported removal index of 99,99% for *E. coli*. Water was, therefore, drinkable.

As Table 1 shows, the water analyzed in current research had low initial color and turbidity and after the introduction of the moringa seed extract, their levels increased greatly. It may be surmised, following Ndabigengesere & Narasiah [9], that the introduction of moringa seeds extract may have greatly increased the load of organic matter and nutrients, as the increase in color and turbidity rates demonstrated. The new organic load may have caused the multiplication of total and thermotolerant coliforms of the saprophyte microbiota even after 12 hours of solar exposure.

When Madsen *et al.* [7] used the moringa seeds extract in the turbid waters of the river Nile, Sudan, they reported that microorganisms concentrated in the sediment and that the

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number of *S. typhimurium* and *S. soney* and, in some cases, *E. coli*, increased in the following 24 h.

An increase in *E. coli* related to nutrient increase in the water owing to the addition of *Moringa oleifera* extract may be related to the finding of Byappanahalli & Fujioka [16]. These researchers reported that *E. coli* may multiply itself and increase its number by 2 logs when nutrients are added to the soil. The same researchers also verified that *E. coli* increases its number by 2 logs when the least quantity of waste water is added to the sterilized soil.

Since *E. coli*, *Klebsiella* sp and *Enterobacter cloacae* may multiply in river water with 3,2 mg/L of dissolved organic carbon and in treated water with concentrations 0,4 and 0,8 mg/L [17], this fact may explain the presence of total and thermotolerant coliforms in waters treated with *M. oleifera* seeds extract *and exposed to the sun*, as in current research. Corroborating findings by Ndabigengesere & Narasiah [9], an increase in nutrients and dissolved organic carbon occurs in the water when treatment with moringa seeds extract is undertaken

Contrastingly to findings by Amaral *et al.* [6], who registered total elimination of *E. coli* in water treated with *M. oleifera* seeds and exposed to solar radiation for 12 hours, current research (Figures 1 and 2) demonstrates that the addition of moringa seeds extract to water with initial low levels of turbidity and color may have decreased the disinfectant action of solar radiations.

However, current research showed (Figures 1 and 2) that solar radiation in water samples in colorless PET bottles may be a tool in the improvement of the microbiological quality of drinking water by humans in regions with restriction to water quantity and quality, such as in the Brazilian northeastern semi-arid region and in Africa.

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Solar radiation reduces the number of mesophile microorganisms and of *E. coli*, a microorganism of the fecal coliform group and higher than the other traditional indicators of fecal pollution since it survives for a smaller interval in the environment, very much like the intestine-originating pathogens. During the hot periods total coliforms may multiply in the water and provide false positive results [18].

The positive activity of solar radiation as water disinfectant has been notified by Conroy *et al.* [19] in studies with 349 children of the Maasai community in Africa. The authors underscored that the consumption of water exposed to solar radiation reduced significantly cases of diarrhea by water when compared to those who consumed water which was not exposed to solar radiation.

Pinfold [20] examined the relationship between bacterial indicators of water quality and children's diarrhea in the Philippines and verified that children who drank highly polluted water (>1,000 *E. coli* / 100 mL) had significantly high diarrhea occurrence (p<0,01) that those who consumed less polluted water.

Although there is no report of total inactivation of thermotolerant coliforms, results demonstrate that simple low cost solutions may avoid water-caused diseases with high mortality rates, especially in children from developing countries. In the semi-arid regions of Brazil where the use of water from alternative sources, such as wells, springs and dams, is common, this type of water presents a high contamination risk for the environment and for animals. Since most of the developing countries, including Brazil, lies within high solar radiation regions, between 35°N and 35°S, they have the benefit of water disinfection by solar radiation [12].

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Although in the urban areas sanitary policies in Brazil are widespread to provide quality water to the population, this concern is practically non-existent for the rural populations. In terms of health policy, the consumer should not have the control of water quality and it is highly important that a joint venture by different professionals guarantees the health and the prevention of waterborne diseases [21].

Immense work should be undertaken for the effective control of the quality of water consumed in the rural areas and educational activities should be programmed for consumers.

CONCLUSIONS

Solar radiation has been efficient in the removal of bacteria. However, it was not efficient to decrease thermotolerant coliform load to zero when used concomitantly with *M. oleifera* seeds extract. Only the reduction of mesophile microorganisms reached levels meeting Brazilian legislation. In current research, moringa seeds extract possibly decreased the disinfecting power of solar radiation since its addition increased the organic load.

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Table 1. Arithmetic means of color and turbidity rates in water samples from nine wells in the rural region of the Recôncavo Baiano BA Brazil, and in water samples exposed to solar radiation at three intervals.

	without seeds	**addition of extract from 3 seeds**
Turbidity (UNT)	1.19	18.50
Color (UHazen)	9.58	37.80

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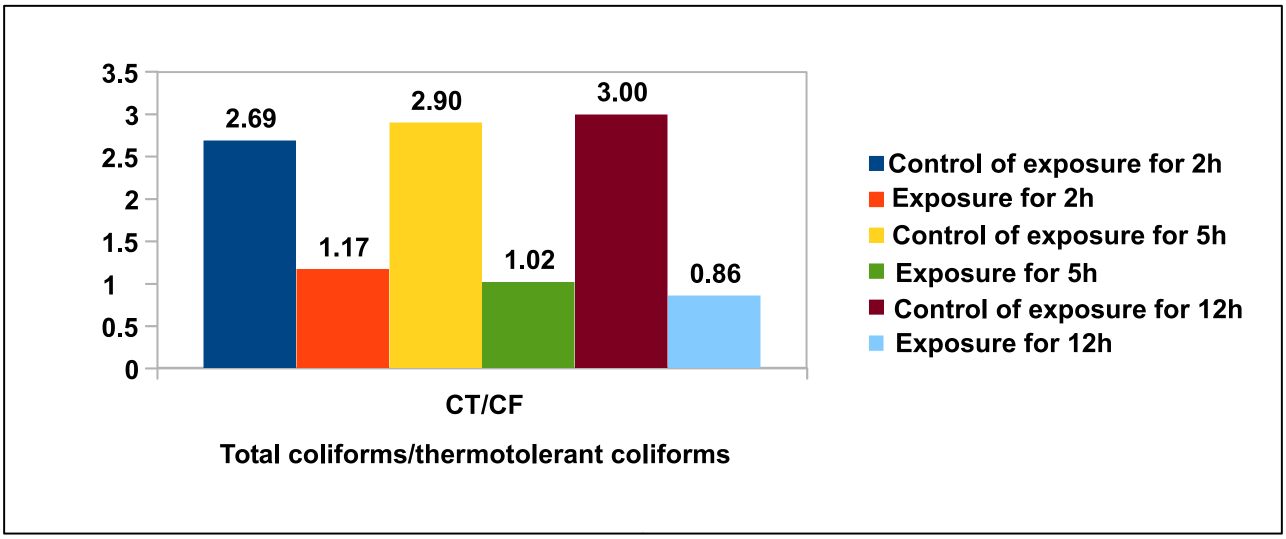

Figure 1. Arithmetical means of logarithmic units (LU), total coliform (CT) and thermotolerant coliforms (CF) in water samples from nine wells in the rural region of the Recôncavo Baiano BA Brazil, with *M. oleifera* seed extract and exposed to sun radiation for 2, 5 and 10 hours.

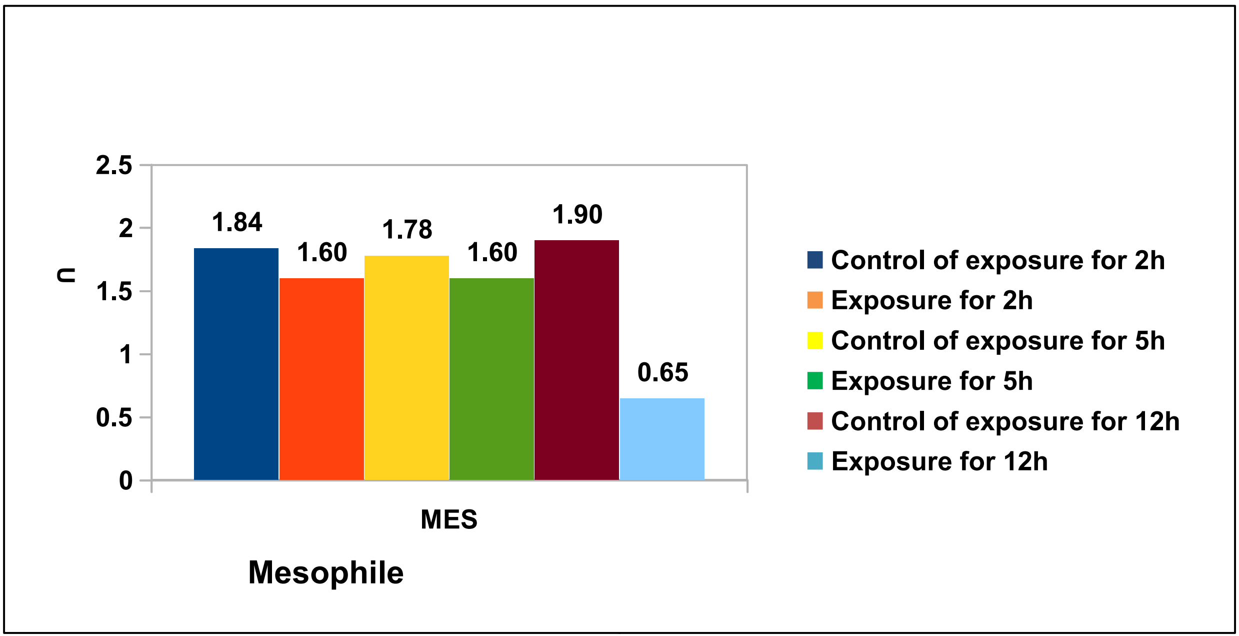

Figure 2. Arithmetic means of logarithmic units (LU) of mesophile microorganisms (MES) in water samples of water from nine wells in the rural region of the Recôncavo Baiano BA Brazil, with *M. oleifera* seeds extract and exposed to solar radiation for 2, 5 and 12 hours.