TEST Engineering & Management

January - February 2020
ISSN: 0193 - 4120 Page No. 5682 - 5685

Effectiveness of Using Milled Moringa Oleifera Seeds as a Disinfectant in Waste Water Treatment

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Article Info
Volume 82
Page Number: 5682 - 5685
Publication Issue:
January-February 2020

Article History
Article Received*: 18 May 2019*
Revised*: 14 July 2019*
Accepted*: 22 December 2019*
Publication*: 27 January 2020*

Abstract:
Water disinfection is a crucial water treatment process because it deals with the removal of pathogens to make water safe to drink. Moringa oleifera extract was used as a disinfectant and the total coliform count was monitored through microscopic observations. The total coliform bacteria removal at a dosage of 5 ml/100 ml of Moringa Oleifera extract was counted to be 13 colonies from an original count of 200 colonies, translating to about 93.5% removal of the bacterial strains. The results showed that MO extract dosages higher than 5 ml/100 ml remove lesser bacteria due to an increase in organic matter. Moringa oleifera seeds have shown the ability to act as a disinfectant by reducing the total coliform bacteria in waste water by 93.5% at an extract dosage of 5 ml/100 ml proving that it has anti-microbial properties.

Keywords: *Bacteria, Disinfectant, Coliforms, Moringa Extract*

I. INTRODUCTION

Water disinfection generally refers to the removal of pathogens during water treatment and as such, it is very important to ensure that the disinfectants used completely disinfect water to the required standard [1]. Water purification has been accomplished by utilizing chlorine in many water treatment processes. However, it was noticed that the by-products of chlorine such as halogenated organic compounds were related to different ailments observed in a number of people. Moringa oleifera seeds proteins might be an alternative disinfectant compared to synthetic chemicals normally used for water purification, given that the seeds are now used for treating water [2]. The action of antimicrobial peptides contained in MO seeds disturbs the cell membrane by triggering a leakage in the cytoplasm which kills the bacterial cell [3]. An active antimicrobial agent called glucosidal mustard oil flocculates the Gram – positive and Gram – negative bacterial cells and removes them by settling just like in properly flocculated water [4]. The use of Moringa oleifera is expected to help kill the bacterial strains found in waste water that causes antibiotic – resistant human pathogens. The study is therefore conducted to investigate the effectiveness of MO extract in disinfecting waste water.

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II. EXPERIMENTAL METHODS

A. Preparation of the Total coliform bacterial culture

Nutrient broth (130.0 gm) was dissolved in 100 mL distilled water and heated slightly. The mixture was sterilized at 130^0 C for 15 minutes at 15 SPT in an autoclave. The sterilized broth was then cooled to room temperature and used to prepare the total coliform bacterial culture. Total coliform bacterial strain was grown in 10 mL broth at 37^0 C overnight to obtain an exponential growth phase which was used for disinfection studies.

B. Preparation of Moringa Seeds Extract

Dried MO seeds were removed physically from their kernels and were grounded using a pestle and mortar to a fine powder. The powder was then sieved using a 212 – 220 µm sieve for increased solubility and 15 g of the powdered MO seeds was dissolved in a 100 ml of distilled water making a concentration of 150 g/l. The solution was agitated to ensure homogenous mixing using an overhead stirrer at 150 rpm for 1 minute. The mixture was then filtered and the concentrate (MO seeds extract) was used for disinfection studies.

C. Disinfection studies

A pre-determined volume of MO extract was added to 100 mL of the cell cultures and incubated for 0 min, 30 min, 60 min, 90 min, and 120 min without agitation. Cell survival was thereafter assessed by making dilution series of bacterial suspensions obtained after each incubation period, plating on non-selective LB medium agar dishes, and incubating for 48 hours at 37^0 C. Colonies were counted on dishes and the cell survival ratio was estimated by comparing to the control experiment where no Moringa extract was added (0 min incubation time).

D. Effect of Moringa Extract on Total Coliform Bacteria Count

Fig. 1; Membrane Filtration Analysis equipment used for analysing the effect of MO extract on Total coliform bacteria

The bacterial cell morphological changes were observed under the microscope after the water was treated with MO extract. MO extract was added to a 24 hr total coliform bacterial culture and incubated for 0, 60, 90 and 120 minutes. A few drops of crystal violet were added for 60 seconds to a slur of culture on a slide at the different stages. The slide was washed off with water and Gram Iodine was added for 60 seconds. Alcohol was used to decolourise the slide until no colour was appearing and it was then washed immediately with water. It was counter-stained with safranin for 60 seconds, washed with water and blotted dry with a filter paper.

III. RESULTS AND DISCUSSION

Fig. 2 (a) – (f) shows the pictorial representation of the results obtained after 24 hours of incubation time while Table 1 shows the number of counted colonies. MO seeds are known to contain an antibiotic compound called pterygospermin that prevents the growth of bacteria including the antibiotic – resistant human pathogens [5] therefore different doses of MO

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extract were used to destroy the bacterial strains. The total number of the coliform bacteria present in the water sample used was counted to be more than 200 colonies at the beginning of the experiment. The results show that a higher bacterial removal was achieved at a dose of 5 ml/100 ml with a total coliform count of 13 from the initial 200 representing a bacterial removal of 93.5%. Dosages of 10, 15, 20 and 25 ml/100 ml gave a total coliform count of 24, 65, 74 and 100 respectively showing a decline in the removal of bacteria with an increase in MO extract dosage. The decline in removal of bacterial strains is caused by an increase in the organic matter within the higher doses MO extract thereby reducing its effectiveness. This principle was further remarked by [6] during the disinfection studies using Moringa Oleifera that despite the fact that Moringa oleifera seeds disinfects, it can lead to less bacterial strain removal in its application on water disinfection if administered at high dosages, hence leading to higher dosages introducing more organic matter to water.

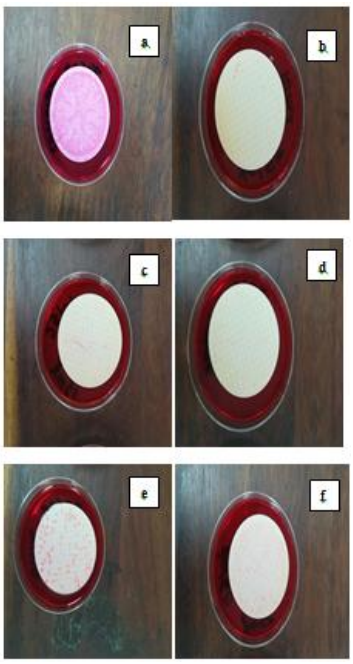

Fig. 2: Membrane Filter Papers where different doses of MO extract were added (a) Control sample, (b) colony count after adding 5 ml/100 ml, (c) colony count after adding 10 ml/100 ml, (d) colony count after adding 15 ml/100 ml, (e) colony count after adding 20 ml/100 ml and (f) colony count after adding 25 ml/100 ml

The total coliform bacteria were counted using colony counter and Table 1 shows the total coliform bacteria that was counted.

Table 1; Total coliform bacteria counted

Figure	Moringa Dosage (ml/ 100 ml)	Total number of Colonies counted
(a)	0	More Than 200
(b)	5	**13**
(c)	10	24
(d)	15	65
(e)	20	74
(f)	25	100

CONCLUSION

Moringa Oleifera seeds managed to remove the total coliform bacterial significantly in waste water treatment with a total removal of 93.5% at a dosage of 5 ml/100 ml. Its ability to kill the bacteria was decreasing with an increase in MO extract dosage due to increased organic matter introduced to the water. Moringa oleifera may substitute the commonly used disinfecting agents due to its environmental friendliness.

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