Effectiveness of Moringa Oleifera Pod Extracts in Bacterial Removal from Grey Water for Non Portable Use

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Abstract:- Water is very useful to human beings, plants and animals. Apart from consumption in the case of portable water, the non- portable water is also useful in some instances, however, sometimes it contain some bacteria which need to be disinfected. This research work was carried out to disinfect gray water obtained from students' hostel at main campus of Bayero University, Kano. Crude extract of this plants' pod was injected into water treatment sequence. The result indicates a considerable reduction in the concentration of bacteria present in the grey-water samples. It revealed that addition of 100 mg/L of 0.5% (w/v) and 1.0% (w/v) M. oleifera pod extract were only effective in removing bacteria up to 6.9% and 12.4% respectively.

This study has shown that the Moringa Oleifera (M.O) pod has little effect in the removal of bacteria from grey-water.

Keywords:- Moringa oleifera (M.O), Water soluble, Coagulants, Coagulation, Waste water, Crude extracts, Concentration, Bacteria, Grey-water.

I. INTRODUCTION

The world population is increasing day-by-day. This is associated with increase in the demand of water for domestic, industrial, irrigational and other useful purposes. As the demand increases, there is mostly shortage in supply of water especially in the urban cities all over the world. Hence the need to balance this increase in demand of water with the corresponding supplies. According to (Redwood 2007), the recycle of waste water and reuse is one of the strategies that can be employed to minimize the shortage of waste water especially in the areas faced with water shortage and high demand of water for human and animal consumption, Industrial and other useful purposes.

Treatment/disinfection of grey-water is one of the techniques that were successfully applied in the U.S, Australia and other parts of the world so as to make the water useful. This grey-water is obtained from waste water sources such as toilets, showers, bath and other sources which are too numerous to be mentioned.

Untreated waste water (grey-water) is often used as a source of water for irrigational farming to grow crops especially vegetables in most rural and urban areas (Obuobie et al., 2006). The waste water is obtained from urban streams, shallow ponds and drains etc. (Al-Jayyousi, 2003) which mostly served as sources of irrigation to vegetable farmers. The use of this waste water in vegetable farming leads to the transmission of pollution-related diseases affecting human health.

Gray-water mostly contains bacteria which when used to grow fruits and vegetables that are eaten raw can affect human/animal health, hence the need to disinfect grey-water before using it in vegetable farming and other useful applications.

The word Grey-water is used to refer to all forms of waste water discharged from houses with the exception of black-water. Figure 1.0 below is an illustration of some grey-water sources.

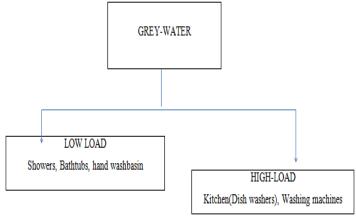


FIG. 1.0: Major Grey-water Sources

Any fruit/vegetable grown with Grey-water is considered harmful when eaten raw unless it was treated prior to its application to the plant.

II. MATERIALS AND METHODS

MATERIALS

The materials used in this project work are distilled water, nutrients, Agar, grey-water sample, M.O. Pods, Cotton wool, and filter paper (Whatman no.1)

EQUIPMENTS

The equipment used in this research work are: Autoclave, Conical flask, Beakers, Test tubes, Test tubes

ISSN No:-2456-2165

racks, measuring cylinder, wire loops Petri dishes, Magnetic stirrer, Weighing balance, Disposable syringe (5ml and 2ml), Jar test apparatus, Incubator, and Colony counter.

METHODS

The procedures that were used in the project are described below:

Original Grey-water Sample

Sterilizing the distilled water

For many of the short term experiments, it is possible to "sterile" the distilled water by boiling so that the water will be free from contamination of any form of bacteria. The procedure is as follows:

The distilled water was placed in a loosely covered container. The container was then placed in an autoclave where the water was heated (Sterilized) at a temperature of 126^{0} C for 15 minutes. After it cools, the lid on the water container was the tightened and kept for safe used.

Preparation of media (Reagent)

2.8g of nutrients agar was dissolved in 100ml of the sterile distilled water and shaken vigorously for 15 minutes until it becomes clear and yellow in color. The solution was then allowed to cool to a temperature of about $47^{0}C - 50^{0}C$ and became in a semi-solid state. The solution was then transferred into petri dishes and allowed to solidify completely.

Preparation of serial dilution

8 test tubes were cleaned with water, rinsed with water and allowed to dry. The test tubes were sterilized in an autoclave at 126°C for 15minutes and allowed to cool. The test tubes were numbered 01-08 and arranged serially in a test tube racks. 9.9 ml of the diluting fluid (sterile distilled water) was transferred into each of the test tube using a disposable 5ml syringe. 0.1ml of the original water sample(grey-water) was then transferred into test tube 01 containing 9.9ml of the diluting fluid(distilled water) using a 2 ml disposable syringe; making 10ml and mixed thoroughly by blowing lots of bubbles with the syringe for couple of seconds. Test tube 01 now contains 1/100 concentration of bacteria in the original sample because 0.1ml is 1/100 of 10ml. Using another new 2ml syringe, 0.1ml was removed from test tube 07. Blowing was continued for seconds and good mixing was obtained. Using another new 2ml syringe, 0.1ml was removed from test tube 07. The syringe was wiped and the content of the syringe was emptied into test tube 08. Blowing was continued for seconds and good mixing was obtained, hence 0.1ml from test tube 08 was plated.

Plating and Incubation

After preparing a serial dilution of the water sample as described above using sterile distilled water of 1:100. An appropriate aliquot (portion) of the dilution (0.1ml) was transferred in duplicates from test 08 into petri dishes that contain a solidified nutrient agar for 24 hours. Colonies formed in each plate after 24hours incubation were counted using a colony counter and the number of bacteria per ml of the water sample was calculated as colony forming unit per ml(cfu/ml).

Treatment of the water sample with M.O Pod extracts (0.5% and 1%) w/v

Preparation of stock solution and water sample (Greywater)

Dried, matured M.O. Pods was obtained from Shinkafi Local Government Zamfara State as the sufficient quantity could not be gotten in kano during the period of this study. The procedure of preparation used includes:

The dried M.O. Pod was grounded to a size of 425µm to achieve proper solubilization of active ingredients in the pod. Different concentrations of M.O. pod solution were made by dissolving 0.5g and 1g of the M.O. Pod powder weighed on a triple beam balance into 100ml of sterile distilled water each contained in a conical flask and 0.5% and 1% concentration of solution was obtained. The solution was shaken properly for 5 minutes using a magnetic stirrer to extract and activate the coagulant and antimicrobial proteins in the pod powder and then filtered through filter paper (whatman no. 1). Fresh solution were prepared daily and kept refrigerated to prevent any ageing effects (such as change in pH, viscosity and coagulation activity). Solutions were shaken vigorously before use.

The raw water samples used in this study were collected from Aliko Dangote male hostel laundry of Bayero University Kano. Water samples were collected approximately every second to third day, and stored in a plastic tank in the lab.

Sample Treatment

Samples were treated first with 0.5% weight by volume MOPC with 5 different dosages (20, 40, 60, 80, and 100) mg per liter of the water sample in duplicate.

The same process was repeated for 1% w/v MOPC with the same volume and also in duplicate.

Coagulation process (jar test)

The equipment used in this study was jar test apparatus with 5 beakers (Figure 2.0).



Figure 2.0: Jar test Apparatus.

The procedure used in carrying out the coagulation process includes:

Each of the 5 beakers was filled with 250ml of the water sample with identical bacterial level. Different volumes (20, 40, 60, 80, and 100) mg/L of the chosen coagulant (0.5%) were added to 5 of the jars (number 1-5, Figure 3.1) respectively. The mixture was then stirred at 50 rpm for 20 minutes. Then the propellers of the apparatus were stopped completely. After treating the sample with 0.5% concentration of MOPC, serial dilution was carried out as described above on each beaker 1-5 and 0.1ml from test tube 8 with dilution factor of 10^{-9} was plated and incubated at 35° c for 24 hours, hence the number of bacteria (CFU/ml) was calculated and the % removal efficiency of the bacteria due to the effect of the MOPC was determined in each case using the below expressions.

No. of bacteria (CFU/ml) = $\frac{No.of \ colonis \div Amount \ plated}{Dilution \ Factor}$

Removal efficiency (%), $R_e = \left(\frac{Ci-Cf}{Ci}\right) \times 100$

Where; R_e is the removal efficiency (%),

C_i is the initial No. of bacteria counted before treatment of the sample

C_fis the final No. of bacteria counted after treatment of the sample

The same experiment was carried on by the other category of concentration (1%) w/v each in duplicate.

III. RESULT AND DISCUSSION

RESULTS

The results obtained in this study are presented in the following tables. The indices of the concentration of bacteria in grey water before treatment in Table 3.0 show that grey water was highly contaminated with high concentration of bacteria.

 Table 3.0: Initial concentration of bacteria in the water

samples							
Plated Plate No.	Amount Plated (ml)	Dilution Factor			No. of colonies counted (cfu)		
					Test 1	Test 2	
P-GW 08	0.1	10-9			213	221	

DISCUSSION

Effect of MOPC on bacterial removal

The collected water sample was analyzed for total bacteria present before and after the treatment with 0.5% and 1% w/v MOPC at various doses (20, 40, 60, 80, and 100) ml respectively in each case. This study was carried out to get preliminary information on the coagulant activity of Moringa oleifera pod in bacterial removal from grey water and the result was shown in appendix A and B. In addition, the plot of the result of the treated grey water with 0.5% and 1% w/v of MOPC, as shown in figure 3.0, has a reasonable reduction in the concentration of the bacteria when compared with the original water sample before treatment, implying the effect of the MOPC solution on the water sample in bacterial removal. Mangale et al., 2012 was reported that the application of this low cost Moringa oleifera coagulant is recommended for eco-friendly, nontoxic, simplified water treatment.

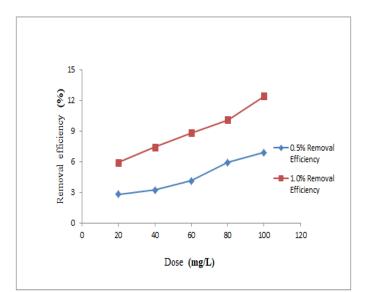


Figure 3.0: Variation of bacterial removal efficiency with dose of 0.5% and 1.0% w/v *M. Oleifera* pod extract.

Figure 3.0 shows the effect of *Moringa oleifera* pod coagulant (MOPC) on bacteria present in the water sample when treated with 0.5% and 1.0% w/v respectively. From the graph MOPC concentration of 0.5% w/v reduced bacterial levels between 2.8% to 6.9% at 20mg/L to 100mg/L doses. Thus MOPC of concentrations of 1.0% w/v also resulted in decreased in the bacterial levels from 5.9% to 12.4% at 20mg/L to 100mg/L doses respectively. From

these results, it is evident that effective reduction will be achieved at a dosage of 100 mg/L of 14% to 15% w/v concentration of MOPC.

Based on the results obtained, concentrations of 0.5 - 1.0% (w/v) gave (2.80%-12.4%) reduction of bacteria. Ghebre michael, (2004) documented an average of 1.1-4.0 log reductions of several microorganisms including E. *coli*. Broin *et al.*, 2002 reported that a recombinant MO protein was able to flocculate gram-positive and gram-negative bacteria cells. On the other hand, MO may also directly act upon microorganisms and result in growth inhibition.

IV. CONCLUSION AND RECOMMENDATION

CONCLUSION

The concentrations of MOPC used in this study 0.5-1.0% (w/v) can reduce bacterial levels up to 12%. *Moringa* is found to be a sustainable, cheap solution for coagulation in water treatment. *Moringa oleifera* pod can be produced locally at low cost therefore the use of *Moringa oleifera pod* would have several technical benefits, especially in tropical developing countries and rural communities. It will help them treat water for small scale vegetable farming. The possibility of using *Moringa oleifera* pod at farm level is good, and provides a realistic alternative to conventional methods, presuming that an adequate amount of 100 plantations are established. It is a method that certainly can be considered as a good, sustainable and cheap solution for farmers, if the supply of *Moringa oleifera* pods can be guaranteed.

RECOMMENDATION

Adequate measures should be taken so that, vegetable farmers and crops/vegetables consumers should be aware of the present of bacteria in grey-water and how to disinfect the grey-water before using in irrigation farming.

Other method of disinfection can be combined with M.O to completely remove disinfect the grey-water as only 12% of disinfection was achieved using this method.

The use of waste water especially grey water with low microbial quality for non-portable use is common in rural and most urban areas in Nigeria, as also in other low-income countries worldwide. Hence, this project should be carried on pilot scale by local authorities and farmers as a risk reduction measures to reduce health risks from irrigated rural and urban vegetable farming. Moringa oleifera is widely grown in Nigeria. While the other plant parts like leaves, barks and roots may be used for medicinal purposes, the pods and seeds are equally efficient for coagulation. Therefore more people should be encouraged to engage in the plantation of Moringa oleifera business so that the people of the community especially farmers can obtain the pod and seed at very cheap cost or at no cost for wastewater treatment. Once plantations are established and the supply of pods and seeds secured, Moringa provides a good, cheap and sustainable coagulant in treating waste water for nonportable use especially for rural and urban vegetable farming. Guidelines for people of the community such as

farmers on the best practices for extraction and use of *Moringa oleifera* for efficient water treatment should be researched into on-farm, with farmer's participation in order to promote its adoption by farmers.

Finally, this research was only performed for a short period; hence further studies should be carried out to draw definite conclusions on this project using different stock solutions of MOPC.

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