



Original Research Article

Microbial Regrowth Assessment of *Moringa oleifera* Seed Protein-Treated Water

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ABSTRACT

This study assessed the microbial regrowth potential of Moringa oleifera seed protein-treated turbid water collected from Ezu River in Amansea, Anambra State, Nigeria. The treated water was preserved for a period of 28 days in two modes: at room temperature of 30 °C and in the refrigerator (-4 °C) and monitored for possible regrowth of the organisms. Three samples from each of the treated water preserved in the two modes were collected every 7 days and the microbial load determined. Steady increase in the THB (regrowth rate of 0.005×10^5 cfu/ml/d to 0.0057×10^5 cfu/ml/d at 30 °C and 0.0015×10^5 cfu/ml/d to 0.0035×10^5 cfu/ml/d at -4 °C) and TCC (regrowth rate of 0.006×10^3 cfu/ml/d - 0.0085×10^3 cfu/ml/d at 30 °C and 0.0014×10^3 cfu/ml/d - 0.0057×10^3 cfu/ml/d at -4 °C) were observed. The faecal coliform disappeared completely in the water preserved in the refrigerator after 7 days and in that of room temperature after 14 days. Analysis of variance showed that there was significant difference between the counts of total heterotrophic bacteria between the two modes of preservation. This work shows that Moringa oleifera seed protein has residual germicidal effect on total and faecal coliform bacteria in the preserved water. Hence Moringa oleifera seed protein can be used for disinfection. The treated water can be preserved for at least 28 days.

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1. INTRODUCTION

The use of plant materials as natural coagulants to clarify turbid waters has been common practice since ancient times. Powdered roasted grains of *Zea mays* were used by soldiers in Peru as a means of settling impurities in the 16th and 17th century. Ancient Sanskrit (Indian) writings made reference to the use of the seeds of the Nirmali tree, *Strychnos potatorum* as a clarifier. Similarly, dried beans (*Vicia faba*) and peach seeds (*Percica vulgaris*) are widely used for this purpose in Bolivia and Peru. Tunaflex and Nirmali seeds have been successfully employed in municipal treatment plants in combination with alum (Rao, 2005).

In recent years, much interest has been focused on developing the use of natural coagulants produced or extracted from microorganisms, animal or plant tissues (Ali *et al.*, 2010; Abidin *et al.*, 2011; Nwaiwu *et al.*, 2012a,b; Shukla, 2016). Coagulants obtained from these natural sources are presumed to be biodegradable and safe for human health (Narasiah *et al.*, 2002). They produce less sludge volume that is only about 20-30% of that produced from the use of alum (Narasiah *et al.*, 2002). One of the sources of natural coagulants that is mostly in use is the *Moringa oleifera* seed extract (Katayon *et al.*, 2006). However, where the crude extract is used for water purification, concentration of organic matter in the treated water increase considerably with the dosage of *Moringa oleifera* solution and might exert a chlorine demand (Ndabigengesere and Narasiah, 1998).

WHO guidelines for the quality of potable water emphasize the over-riding importance of ensuring that drinking water supplies are protected from microbial contamination (Nath *et al.*, 2006). However, it has been reported in literature that water treated with *Moringa oleifera* seed extract cannot be preserved for more than one day (Megersa *et al.*, 2014). This may be true of water treated with crude extract of *Moringa oleifera* seed. It is not known how long water treated with protein isolated from *Moringa oleifera* seed can be preserved. Ogbozige *et al.* (2018), investigated the quality of stored water especially in the arid regions and concluded that most water quality parameters of potable water stored in hygienic condition remain fresh during the first week of storage, but the quality cannot be guaranteed thereafter. Hence this work set out to investigate the microbial regrowth in stored *Moringa oleifera* seed-protein-treated-water.

2. MATERIALS AND METHODS

2.1. Sourcing the Seed

Dried pods of *Moringa oleifera* seeds were harvested from local growers located in Agulu, Anambra State, Nigeria. The seeds were removed from the pods and air-dried in the laboratory until a consistent moisture content value was obtained.

2.2. Identification of Seed

The seeds of *Moringa oleifera* were sent to Herbarium Curator, Dr. Mrs Aziagba, of the Department of Botany, Nnamdi Azikiwe University, Awka. A sample of the seed was kept in Cabinet Number 02, Shelf Number 29 of the Herbarium.

2.3. Collection of Raw Water Sample

The raw water sample was collected from Ezu River in Amansea, Awka, the capital of Anambra State, Nigeria.

2.4. Water Disinfection

The microbial load of the raw turbid water was determined. The protein isolated from stored *Moringa oleifera* seed was used to purify the turbid water using jar test apparatus. The standard jar test procedure usually includes rapid mixing for 1 minute at 120 rpm, agitation (slow mixing) for 30 minutes at 30 rpm and 30 minutes settling time (Judith *et al.*, 2014). In this work, however, the settling time was extended to 60 minutes in order to allow for more coagulants contact time with microorganisms in the water. At the end of the settling and clarification period, 25 ml of the supernatant (clarified sample) was pipetted into a sterilized sample bottle for determination of residual microbial loads. Hence an optimal dosage of 50 mg/l was obtained. The clarified water from the 50 mg/l sample was collected in sterilized sample bottles for preservation, both at room temperature and in the refrigerator.

2.5. Preservation of Treated Water

The sterilized sample bottles filled with the *Moringa oleifera* treated water were preserved for a period of 28 days in two ways: (i) at room temperature (an average of 30 °C), (ii) in the refrigerator (at a temperature of -4 °C), and monitored for possible regrowth of the organisms. At the end of every 7 days, three samples were collected from each of the treated water preserved at room temperature and in the refrigerator, and the microbial indicators of water quality were determined.

2.6. Determination of Microbial Water Quality Indicators

2.6.1. Media preparation (Cheesbrough, 2002)

One milliliter (1 ml) of the water sample was serially diluted tenfold in test tubes containing 9 ml of sterile water. One milliliter (1 ml) from dilutions of 10^{-1} for faecal coliforms, 10^{-2} for total coliforms and 10^{-5} for total heterotrophic bacteria counts were plated out in duplicates in petri dishes using the pour plate technique. Eosin Methylene Blue Agar was used for faecal coliform counts which was incubated at 44.5 °C for 48 hours. Mac Conkey Agar was used for total coliform counts while Nutrient Agar was used for total heterotrophic bacteria count (total viable count) and each was incubated at 37 °C for 24 hours.

2.6.2. Determination of colony forming units (CFU)

The colonies that developed after the period of incubation were counted and the colony forming units per milliliter of sample were calculated using Equation 1 (Cheesbrough, 2002).

$$\text{Number of colony forming units (CFU/ml)} = \frac{N}{V \times D} \quad (1)$$

Where N = Number of colonies counted, V = Volume plated out (ml) and D = Dilution factor

2.7. Statistical Analysis

Using the 2016 version of Excel package, two-way analysis of variance tool was used to determine the effect of storage time and place (that is, room temperature and refrigerator) on the bacterial regrowth percentage.

3. RESULTS AND DISCUSSION

3.1. Microbial Status of Raw and Freshly Treated Water

Table 1 shows the observed microbial counts and the percentage reductions after treatment with *Moringa oleifera* seed protein. It can be observed that the Ezu river water has a high concentration of microbial load, above acceptable limits of Nigerian Drinking Water Quality Standards (NDWQS) (Nigerian Industrial Standard, 2007).

Table 1: Microorganism counts in raw water and percentage reductions after treatment

Organism	Raw water (Cfu/l)	NDWQS (Cfu/l)	Treated water (Cfu/l)	% Reduction
Total heterotrophic bacteria	6.1×10^5	0	0.48×10^5	92
Total coliform count	4.0×10^3	10	0.38×10^3	91
Faecal coliform count	3.1×10^3	0	0.36×10^3	88

These values were significantly reduced by treatment with *Moringa oleifera* seed protein. Nwaiwu and Lingmu (2011) also observed a high percentage of microbial population removal alongside turbidity removal using *Moringa oleifera* seed extract. The values of the total heterotrophic bacteria (THB) reduced from 6.1×10^5 cfu/ml to 0.48×10^5 cfu/ml after treatment with *Moringa oleifera* seed extract, representing a reduction of 92%. Total coliform count (TCC) reduced from 4.0×10^3 cfu/ml to 0.38×10^3 cfu/ml, showing a 91% reduction, while faecal coliform count reduced from 3.1×10^3 cfu/ml to 0.36×10^3 cfu/ml (88% reduction). This shows very high water disinfection efficiency of the protein obtained from stored *Moringa oleifera* seed. This agrees with the findings of Abaliwano *et al.* (2008) who reported that *Moringa oleifera* seed protein disinfects water.

3.2. Quality of Treated Water Preserved at Room Temperature

3.2.1. Microbial counts (room temperature)

Figure 1 shows the counts of microorganisms at the end of every 7 days till the 28 days, in the samples preserved at room temperature of 30 °C.

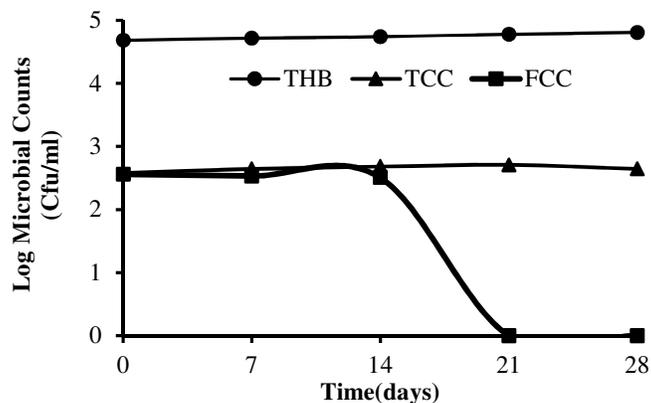


Figure 1: Log microbial count against time (room temperature storage)

At the end of the 7, 14, 21 and 28 days of preservation of the treated water at room temperature, the value of total heterotrophic bacteria increased from 0.48×10^5 cfu/ml in the freshly treated water (zero day) to 0.52×10^5 cfu/ml, 0.55 cfu/ml, 0.60 cfu/ml and 0.64 cfu/ml respectively in the preserved water (Figure 1). Total coliform count increased from 0.38 cfu/ml in the freshly treated water to 0.44 cfu/ml, 0.48 cfu/ml, 0.51 cfu/ml and 0.55 cfu/ml in the preserved water at the end of 7, 14, 21 and 28 days respectively. These increments may be due to the presence of organic matter in the protein isolate. The presence of organic matter in *Moringa oleifera* extract has been reported in literature also reported by (Ndabigengesere and Narasiah, 1998; Megersa *et al.*, 2014). The faecal coliform bacteria, however, showed a different trend from THB and TCC. The values of FC decreased steadily from 0.36 cfu/ml in the freshly treated water to 0.34 cfu/ml and 0.32 cfu/ml at the end of 7 and 14 days of preservation of the treated water. By the end of 21 days of preservation of the treated water, faecal coliform had disappeared. It is likely that there was strong residual disinfection action of the *Moringa oleifera* protein on faecal coliform, which led to the eventual disappearance. This agrees with the report of Delegen *et al.* (2018) that acetone precipitated *Moringa oleifera* seed has a strong antibacterial activity.

3.2.2. Percentage microbial regrowth (room temperature)

The percentage regrowth in THB and TCC, and decrease in FCC with time of preservation of the treated water are presented in Figure 2. Percentage increments and decrease were calculated relative to the freshly treated water.

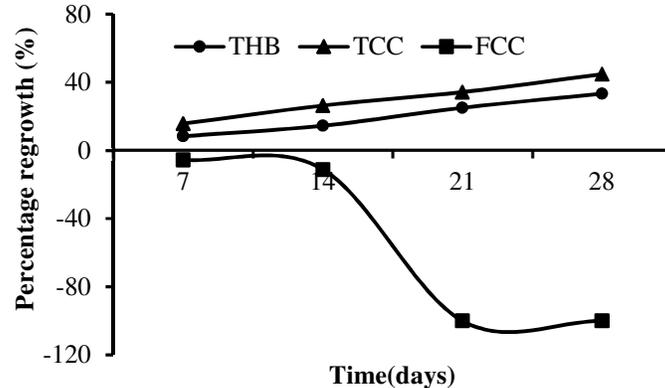


Figure 2: Percentage regrowth against time (room temperature storage)

Total heterotrophic bacteria increased by 0.04×10^5 cfu/ml (8.3%), 0.07×10^5 cfu/ml (14.4%), 0.12×10^5 cfu/ml (25%), and 0.16×10^5 cfu/ml (33.3%) at the end of 7, 14, 21 and 28 days respectively of preservation of the treated water. The values of TCC after 7, 14, 21 and 28 days of preservation increased by 0.06×10^3 cfu/ml (15.7%), 0.10×10^3 cfu/ml (26.3%), 0.13×10^3 cfu/ml (34.2%) and 0.17×10^3 cfu/ml (44.7%) respectively. These increments may be due to the presence of organic matter in the protein isolate. The presence of organic matter in *Moringa oleifera* extract was also reported by (Ndabigengesere and Narasiah, 1998). The room temperature is thought to play a role in the microbial population. The steady decrease in faecal coliform were 0.02×10^3 cfu/ml (5.5%), 0.04×10^3 cfu/ml (11.1%), 0.36×10^3 cfu/ml (100%) and 0.36×10^3 cfu/ml (100%) at the end of 7, 14, 21 and 28 days respectively of the preservation of the treated water.

3.2.3. Regrowth equations and correlation constants (room temperature)

From Figure 1, which is the chart that showed the microbial values at every 7-day interval in the room-temperature- preserved, water correlation equations and constants were obtained and presented in Table 2. The chart showing percentage microbial increments (Figure 2) yielded correlation equations and constants presented in Table 3.

Table 2: Regrowth equations and correlation constants (room temperature)

Organism	Growth equations	Correlation constant
THB	$Y = 0.0045x + 4.682$	0.9468
TCC	$Y = 0.004x^2 + 0.0136x + 2.5747$	0.9418
FCC	$Y = 0.0006x^3 - 0.0291x^2 + 0.2466x + 2.4497$	0.8966

Table 3. Percentage regrowth equations and correlation constants (room temperature)

Variable	Percentage regrowth equations	Correlation constant
THB	$Y = 8.55x - 1.1$	0.9913
TCC	$Y = 9.49x + 6.5$	0.9969
FCC	$Y = 28.7x^3 - 213.85x^2 + 435.05x - 255.4$	1.0

Table 2 shows that THB had a simple growth equation, TCC had a polynomial of the second order while the equation of the decrease of FCC was a cubic one. The charts showed very high correlation coefficients of 0.9468, 0.9418 and 0.8966 for THB, TCC and FCC respectively. Table 3 shows simple percentage regrowth equations for THB and FCC but a cubic equation for FCC. High correlation coefficients of 0.9913, 0.9969 and 1.0 for THB, TCC and FCC respectively were observed. The high values of the correlation coefficients show that the observed microbial values and percentage increments fitted well into the correlation equations. Hence these equations can well be used to reliably predict microbial values or percentage increments at any given time interval in days.

3.2.4. Daily microbial regrowth (room temperature)

Table 4 presents the daily growth rate of THB, TCC and FCC as the period of preservation of the treated water increased. The daily growth rates of the microorganisms were obtained using Equation 2.

$$\text{Daily growth rate} = \left(\frac{C_0 - C_t}{t} \right) \quad (2)$$

Where: C_0 = Microbial count at time zero, C_t = Microbial count at time t and t = Time in days

From Table 4, it can be seen that total heterotrophic bacteria had a daily growth rate of 0.0057×10^5 cfu/ml/d, 0.005×10^5 cfu/ml /d, 0.0057×10^5 cfu/ml /d and 0.0057×10^5 cfu/ml /d at the end of the 7, 14, 21 and 28 days respectively. This shows a constant daily microorganism growth rate of 0.0057×10^5 cfu/ml /d. It means that there was continued inhibition of growth by the *Moringa oleifera* seed protein because of extended contact time of *Moringa oleifera* seed protein with the residual microorganisms in the water. For total coliform count, growth rate was 0.0085×10^3 cfu/ml/d at the end of the first 7 days. Total coliform growth rates decreased to 0.007×10^3 cfu/ml/d, 0.0061×10^3 cfu/ml/d and 0.006×10^3 cfu/ml/d, at the end of 14, 21 and 28 days, respectively. The decrease in growth rate is an indication of continued germicidal action of the *Moringa oleifera* protein on the microorganisms. Faecal coliform had a consistent daily rate of decrease of 0.0028×10^3 cfu/ml /d during the 14 days of its presence. This showed that the residual *Moringa oleifera* protein had very high action on the faecal coliform and completely eliminated it from the water. A lot of literature reviews have revealed that *Moringa oleifera* seed extracts possess antimicrobial properties (Arun and Rao, 2011).

Table 4: Daily growth rates of THB, TCC and FCC in treated water preserved at room temperature

Time (days)	Rate/d		
	THB ($\times 10^5$) cfu/ml	TCC ($\times 10^3$) cfu/ml	FCC ($\times 10^3$) cfu/ml
7	0.0057	0.0085	
14	0.005	0.007	0.0028
21	0.0057	0.0061	0.0028
28	0.0057	0.006	

3.3. Quality of Treated Water Preserved in Refrigerator

3.3.1 Microbial counts (refrigerator)

Counts of microorganisms at the end of every 7 days till the 28 days, in the samples preserved in the refrigerator (-4°C) are presented in Figure 3.

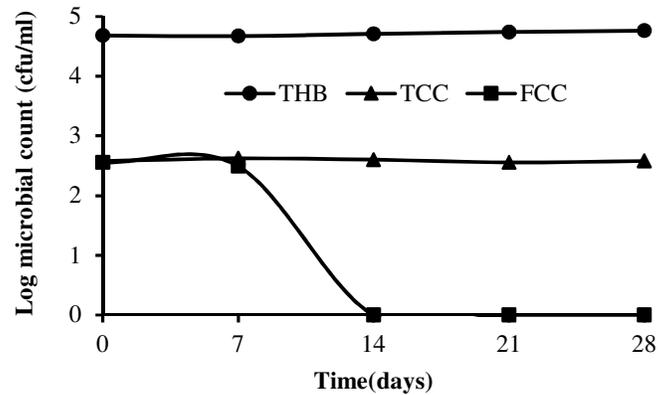


Figure 3: Log microbial count against time (refrigerator storage)

Figure 3 showed an initial decrease in the population of total heterotrophic bacteria from 0.48×10^5 cfu/ml residual in the freshly treated water to 0.47×10^5 cfu/ml at the end of the first seven days. Total heterotrophic bacteria then slowly increased to 0.51×10^5 cfu/ml, 0.55×10^5 cfu/ml and 0.58×10^5 cfu/ml after 14, 21 and 28 days of preservation respectively. The growth pattern of total coliform bacteria was inconsistent. TCC first increased from 0.38×10^5 cfu/ml residual in the freshly treated water to 0.42×10^5 cfu/ml at the end of 7 days. It decreased to 0.40×10^5 cfu/ml and 0.36×10^5 cfu/ml at the end of 14 and 21 days respectively and finally returned to the value of 0.38×10^5 cfu/ml. The utilization of some residual organic matter in the protein isolate may have been responsible for the observed increase in THB and TCC. At the same time the ability of *Moringa oleifera* seed protein to decrease the viability of gram positive and gram positive bacterial cells (Suarez et al., 2003) inhibited rapid regrowth of the microorganisms. Values of faecal coliform count decreased at the end of the first 7 days, after which there was no presence of faecal coliform in subsequent days. This is probably due to the anti-bacterial action of the residual *Moringa oleifera* protein. Abaliwo *et al* (2008) reported that bacteria reduction was experienced when using *Moringa oleifera* coagulant to remove *Escherichia coli* from water.

3.3.2. Percentage microbial regrowth (refrigerator)

Figure 5 shows the percentage increments or decrease of the microbial loads in the refrigerator-preserved treated water at the various storage times(days). The percentages are relative to the freshly treated water.

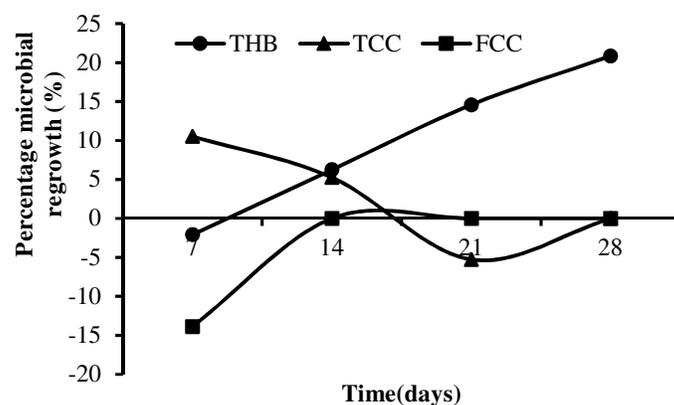


Figure 4: Percentage microbial growth against time (Refrigerator storage)

At the end of the first 7 days of preservation of the treated water in the refrigerator, the value of THB decreased by 0.01×10^5 cfu/ml (-2.8%). Thereafter its total increments were 0.03×10^5 cfu/ml (6.25%), 0.07×10^5 cfu/ml (14.58%) and 0.10×10^5 cfu/ml (20.83%) after 14, 21 and 28 days of preservation respectively. TCC was observed to have increased by 0.04×10^3 cfu/ml (10.52%) at the end of the first 7 days. It however had a total increase of 0.02×10^3 cfu/ml (5.26%) at the end of 14 days. At end of 21 days TCC value was decreased by 0.02×10^3 cfu/ml (0.56%). TCC had returned to the original value of 0.38×10^3 cfu/ml (increase of 0%) at the end of 28 days. Faecal coliform showed a different trend from THB and TCC, the same as in the case of the treated sample preserved at room temperature. The values of FC reduced by 0.05×10^3 cfu/ml (14%), at the end of the first 7 days and then completely disappeared by the end of 14 days of preservation of the treated water. It is opined that the residual action of the *Moringa oleifera* protein was sufficient to completely eliminate the Faecal coliform by the 14 days of storage of the treated water. This so because Nwaiwu *et al* (2012a) found that *Moringa oleifera* seed exhibited good antimicrobial properties, the highest among other natural coagulants studied. It was recommended that their use in water treatment should be encouraged.

3.3.3. Regrowth equations and correlation constants (refrigerator)

Table 5: Regrowth equations and correlation constants (room temperature)

Variable	Growth equations	Correlation constant
THB	$Y = -1E-05x^3 + 0.0006x^2 - 0.0048x + 4.6807$	0.9961
TCC	$Y = 3E-05x^3 - 0.0015x^2 + 0.0154x + 2.579$	0.984
FCC	$Y = 0.0006x^3 - 0.0209x^2 + 0.0328x + 2.6621$	0.8974

Table 6: Percentage regrowth equations and correlation constants (room temperature)

Variable	Percentage regrowth equations	Correlation constant
THB	$Y = 7.706x - 9.37$	0.9956
TCC	$Y = 3.5067x^3 - 23.67x^2 + 41.203x - 10.52$	1.0
FCC	$Y = 2.3133x^3 - 20.82x^2 + 60.147x - 55.52$	1.0

Table 5 shows that THB, TCC and FCC had cubic polynomial equations. The charts showed very high correlation coefficients of 0.9961, 0.984 and 0.8974 for THB, TCC and FCC respectively. Table 6 shows simple percentage regrowth equations for THB, while TCC and FCC showed cubic equations. High correlation coefficients of 0.9956, 1.0 and 1.0 for THB, TCC and FCC respectively were observed. The higher values of the correlation coefficients show that the observed microbial values and percentage increments fitted better into the correlation equations in the refrigerator-preserved water than those in the room-temperature-preserved water. These equations can be used to reliably predict microbial values or percentage increments at any given time interval in days.

3.3.4. Daily microbial regrowth (refrigerator)

Table 7 presents the daily growth rate of THB, TCC and FCC as the period of preservation of the treated water in the refrigerator increased. The daily growth rates of the microorganisms were obtained using Equation 2.

Table 7: Daily growth rates of THB, TCC and FCC in treated water preserved in refrigerator

Time (days)	Rate/d		
	THB ($\times 10^5$) cfu/ml	TCC ($\times 10^3$) cfu/ml	FCC ($\times 10^3$) cfu/ml
7	-0.0014	0.0057	
14	0.002	0.0014	
21	0.0033	-0.0009	-0.007
28	0.0035	0.00	

Table 7 shows that total heterotrophic bacteria in the treated samples preserved in the refrigerator had daily regrowth rates of -0.0014×10^5 cfu/ml/d, 0.002×10^5 cfu/ml/d, 0.0033×10^5 cfu/ml/d and 0.0035×10^5 cfu/ml/d at the end of the 7, 14, 21 and 28 days of preservation respectively. The initial decrease, negative regrowth (that is decay rate), and the subsequent low regrowth rates, showed the action of residual *Moringa oleifera* seed protein as a disinfectant against the microorganisms due to the extended contact time. Total coliform count in the refrigerator-preserved water had lower regrowth rates (than the room temperature samples) of 0.0057×10^5 cfu/ml/d, 0.0014×10^5 cfu/ml/d, and -0.0009×10^5 cfu/ml/d (decay rate) at the end of the 7, 14, and 21 days, respectively. Faecal coliform had a daily rate of decrease of -0.007×10^5 cfu/ml/d (decay rate) during the 7 days of its presence. The residual *Moringa oleifera* protein was active on the faecal coliform till complete elimination of the microbes.

3.4. Comparisons of the Effect of Storage Time and Place (Room Temperature and Refrigerator)

The analysis of the population regrowth of total heterotrophic bacteria showed that there were significant differences ($F_{\text{observed}}=21.50909 > F_{\text{critical}} = 6.388233$ and $P\text{-value} = 0.005746 < 0.05$) between the two modes of storage over the period of preservation (Table 8).

Table 8: Comparison between storage at room temperature and refrigerator

Variable	F_{observed}	F_{critical}	P-value
THB	21.50909	6.388233	0.005746
TCC	0.671902	6.388233	0.645292
FCC	4.092063	6.388233	0.100552

The regrowth rates of THB were lower in the refrigerator (maximum regrowth rate of 0.0035 cfu/ml /d). This shows that, in terms of THB, it is better to preserve water treated with *Moringa oleifera* seed protein in the refrigerator (-4 °C) than at room temperature (30 °C). The analysis of the population regrowth of TCC showed that there were no significant differences ($F_{\text{observed}} = 0.671902 < F_{\text{critical}} = 6.388233$ and $P\text{-value} = 0.645292 > 0.05$) between the two modes of storage over the period of preservation (Table 8). This shows that, in terms of regrowth of TCC, water treated with *Moringa oleifera* seed protein can be preserved in any of the two modes of preservation. Faecal coliform count regrowth analysis showed that there were no significant differences ($F_{\text{observed}} = 4.092063 < F_{\text{critical}} = 6.388233$ and $P\text{-value} = 0.100552 > 0.05$) between the two modes of storage over the period of preservation (Table 8). In terms of regrowth of FCC, therefore, water treated with *Moringa oleifera* seed protein can be preserved in any of the two modes of preservation.

4. CONCLUSION

Water treated with *Moringa oleifera* seed protein was preserved for a period of 28 days. There was some form of increase in total heterotrophic bacteria and total coliform counts as the period of preservation increased. On the other hand, faecal coliform decreased with time of preservation. Comparison of the two modes of preservation using two-way analysis of variance showed that there were significant differences between THB counts in the samples stored at room temperature and those stored in the refrigerator, but no significant differences in the TCC and FC counts stored under the two conditions. In terms of THB regrowth, preservation in the refrigerator proved better than room temperature preservation because the refrigerator-stored sample had an average daily regrowth rate of 0.0035×10^5 cfu/ml/d which was lower than 0.0057×10^5 cfu/ml/d at room temperature. The regrowth may be due to the presence of organic matter in the isolated protein. However, it must be stated that the rate of microorganism regrowth is low. Understandably, the organic content of the protein isolate is lower than that of the crude extract. The total annihilation of faecal coliform at room temperature and in the refrigerator, point to the fact that *Moringa oleifera* protein isolate may be specifically toxic to bacteria of fecal origin. This further supports its recommendation for water treatment. This study has also shown that water treated with *Moringa oleifera* seed protein can be preserved

for a period of, at least, 28 days either in the refrigerator or at room temperature without serious reduction in microbial quality. Further studies should aim at eliminating the organic content of the protein isolate.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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