FEDERAL POLYTECHNIC WANNUNE
Sponsored by TETFUND/(TETFUND/DESS/POLY/WANNUNE/ARJ/2022-2025/VOL.1)
WIJSITAR Volume 1 Number 1, 2025
ISSN: 3141-3765 Peer-Reviewed/Open Access/https://wijitars.fedpolywannune.edu.ng/

THE IMPACT OF *MORINGA OLEIFERA* SEED POWDER ON MICROBES IN WATER

[1]**Ibuh Sewuese Gladys**
Department of Science Laboratory Technology,
Federal Polytechnic Wannune,
Benue State, Nigeria

[2]**Ibrahim Basira**
Department of Biological Sciences,
Kaduna State University,
Kaduna, Nigeria

[3]**Saidu Abdulkadir**
Department of Biological Sciences,
Kaduna State University,
Kaduna, Nigeria

Abstract
The study was designed to determine the impact of *Moringa oleifera* seed powder on microbial load in water during dry and wet season. Water samples were collected in sterile plastic bottles for six months, along the Southern axis of river Kaduna(Kakuri). The impact of Moringa seed powder extract on the microbes present in the water was determined by sequential reduction and Agar plate techniques, colony forming units were counted using the Neubauer chamber. Colony forming units per mL (CFU/mL) of each dilution was recorded/calculated using the formula: CFU/mL = CFU*dilution factor 1/aliquot. The experimental design for the treatment was a Complete Randomized Design (CRD), replicated three times. All data collected from this study were subjected to the analysis of variance (ANOVA) using the general linear model of Statistical Analysis System (SAS, 2001). *M.oleifera* seed extract, reduced bacteria load in the dry and wet season, at the highest concentrations of 165.5mg/l and 900.5mg/l (79.2% and 77.4% reduction was recorded). This shows that *M.oleifera* seed powder has antimicrobial properties and can be used in reducing bacteria load in water

Keywords: *M.oleifera (Seed Powder), Water Samples, Dry and Wet Season, & Bacteria Load*

Sponsored by TETFUND/(TETFUND/DESS/POLY/WANNUNE/ARJ/2022-2025/VOL.1)
WIJSITAR Volume 1 Number 1, 2025
ISSN: 3141-3765 Peer-Reviewed/Open Access/https://wijitars.fedpolywannune.edu.ng/

Introduction

Water can support the growth of many types of microorganisms, the growth of some bacteria in water can help digest the poisons from water (Faridi, 2012). However, the presence of other disease causing microbes in water is unhealthy and even life threatening. For example, bacteria that live in the intestinal tracts of humans and other warm blooded animals such as *Escherichia coli, Salmonella, Shigella,* and *Vibrio* can contaminate water if feaces enter the water. One common way to test for water quality, is to determine turbidity. Turbidity gives an indication of the amount of suspended material in water. Materials such as soil when present in water, microorganisms will also be present (Faridi, 2012).

M. oleifera is grown in abundance in India, it's height is about 5-12m with light green fruit pods and its seeds appear to be round when dry with a wood like shell covering. The roots are tuberous in nature and the leaves greenish (Folkard*et al*., 1999). Crushed Moringa seeds clarify and purify water to suit domestic use and lower bacterial concentration in the water making it safe for drinking (Akinwore and Jok, 2006). Though, various sources of water require distinctive amounts of seed powder due to the different particles present.

Raheela *et al.,* (2008) reported the antimicrobial activity of *M. oleifera* has been dose dependent, as the concentrated extracts dose reduced, the microbial activity also reduced. Pritchard *et al*., (2010) recorded 84% decrease in bacterial load at 125mg/L of *M.oleifera* seed. Ghebremichael *et al*., (2005) also recorded decrease in microbes such as *E. coli*. Ugwu *et al*., 2017, recorded 90-98.5% decrease in bacterial load in waste water treated with 50mg/L,100mg/L and 200mg/L of *M.oleifera* seed powder. Nascent findings of Berger *et al* (1984) on the hazards and virulency of *M.oleifera* seed powder in water purification recorded no health hazards .Up to this point, further studies have shown no proof to indicate severe consequences on man, as little doses are been used to treat water (Dishua, 2000). *M. oleifera* leaves, seeds, and seed oil have unsaturated fats that amplify the harmlessness of *Moringa* (Sánchez-Machado *et al*., 2015).

Methodology

The study was carried out at Kakuri drain, which is part of the Southern axis of river Kaduna during the dry and wet seasons with the sampling points indicated. Regarding the materials for the study, *M. oleifera* seeds were bought at Kasuwan Barchi (Kaduna) and identified at the Herbarium center of Biological Science Department, Kaduna State University for identification. Samples were collected for six months in duplicate from the Kakuri drain of river Kaduna, for jar test and microbial load test in plastic bottles with screw caps and taken to the laboratory in an ice chest for analysis as used by Zakky*et al*., (2016), Zakky and Auta, (2017).

The impact of *M. oleifera* concentrations on microbial load was carried out at 24 diagnostic laboratories Kaduna. Seed powder concentrations of *M.oleifera* were prepared, One hundred grammes (100 gm) of air dried seeds of *M.oleifera* were peeled and milled to fine powder and sieved to a mesh size of 150µm using an electric miller. 25 g of the powdered seeds were separately extracted in 500ml conical flasks with 50% ethanol (Ethanolic extraction). The conical flasks were plugged with rubber corks, then shaken at 120 rpm for 30 min and allowed to stand at room temperature for 5 days with occasional manual agitation of the flask using a sterile glass rod at every 24hour.The extracts were separately filtered using sterile Whatman no. 1 filter paper. Filtrate were concentrated and kept in an air tight container and different doses used to determine the impact of the concentration on microbes in water (Edeoga*et al*.,2005).

Sponsored by **TETFUND**/(TETFUND/DESS/POLY/WANNUNE/ARJ/2022-2025/VOL.1)
WIJSITAR Volume 1 Number 1, 2025
ISSN: 3141-3765 Peer-Reviewed/Open Access/https://wijitars.fedpolywannune.edu.ng/

A Nutrient Agar (NA) culture medium was prepared before the experiment. According to manufacturer's guide (APHA,2005), twenty-three gramme (23g) of Powdered Agar was weighed and suspended in 1 liter of distill water heated with regular stirring and allowed to boil for 1 minute to dissolve the powder. The solution was autoclaved at 121°C for 15 minutes and left to cool to 45-50^{0}C. The ready media was poured 15 ml into sterile petri dishes and left standing for thirty minutes to solidify. Thereafter, serially diluted samples of the untreated and treated water with Moringa were plated onto the Nutrient Agar medium. The pH of the medium was 7.2-7.6 at room temperature.

Untreated water samples from the Southern axis of river Kaduna (Control) and the treated water with varying concentrations from 0.5-5.0 up to 180mg/l in the dry season and 200-1000mg/l in the wet season was used to carry out a quantitative test for microbial load using sequential reduction and plating technique. Ten clean test tubes were labelled 1/10 to 1/100; all ten test tubes contained 9m1 of 0.1% peptone water preparation. 1 ml of water sample from river Kaduna was obtained through a pipette and transferred into the first test tube labelled 1/10 and thoroughly mixed, 1ml from the 1/10 dilution was transferred into the next dilution 1/20 and thoroughly mixed and 1ml from the test tube labelled 1/20 dilution was also transferred into 1/30 and the same procedure continued up to 1/100. Used Pipette tips were discarded into a beaker containing 70% ethanol. Using pipette tip, 0.2 ml diluted sample was drowning from the last tube with 1/100 in both the control and the water treated with varying concentrations of Moringa (0.5-1000mg/l) was carefully transferred into different petri dishes containing 15ml of Nutrient Agar media in solidified form and labelled appropriately. A colon wire loop was heated on a Bunsen burner for a few minutes and used to spread the sample on the Nutrient Agar media. Afterwards, all the petri dishes were incubated in an inverted position for 24 hours at 37ºC and observations were made the following day (Jaime *et al.*,1986). Colony forming units per mL (CFU/mL) of each dilution was recorded/calculated using the formula:

CFU/mL = CFU*dilution factor*1/aliquot

Regarding Smear Slide Preparation and Microscopy, a drop of Normal saline (emulsifier) was placed on a clean slide, ensuring there was no spill, **s**amples were picked from each of the colony forming units on the different petri dishes labelled with different concentrations of treatment with Moringa (0.5-1000mg/l) using a wire loop, a loop full of each of the sample was picked and placed on the slide and smeared. Smeared slides were placed on a microscope and viewed under x10 magnification and the cell colony forming units for each of the concentrations were counted using a Neubauer counting chamber (Jaime *et al.*,1986). The experimental design for the treatment was a Complete Randomized Design (CRD), replicated three times. All data collected from this study were subjected to the analysis of variance (ANOVA) using the general linear model of Statistical Analysis System (SAS, 2001) to analyze the differences among group means in the treated sample. Significant differences among means were separated using the Duncan multiple range test (Duncan, 1955) in the SAS package.

Result

Table 1: *Impact of M.oleifera Seed Powder Concentrations on Microbes in Water During the Dry Season*

CONCENTRATIONS(MG/L)	VIABLECOUNT(VC) CFU/ML X 10	TOTAL VIABLE COUNT (TVC) CFU/ML X 10^{6L}
CONTROL(0)	6.10 ± 0.12^{a}	1525.00 ± 1.16^{a}
15.5	4.74 ± 0.14^{b}	1184.09 ± 35.76^{b}
45.5	3.17 ± 0.12^{c}	791.67 ± 30.05^{c}
75.5	2.73 ± 0.12^{cd}	683.33 ± 30.05^{cd}
105.5	2.20 ± 0.06^{de}	550.00 ± 14.43^{de}
135.5	1.80 ± 0.12^{ef}	450.00 ± 28.87^{ef}
165.5	1.27 ± 0.09^{f}	316.67 ± 22.04^{f}
P-VALUE	0.000	0.000

All mean values with different superscript in a roll are significantly different ($P< 0.05$)

a > b > c > d > e > f and all mean values with the same superscript in a roll are closely related and have no significant difference ($P > 0.05$), cd, de, ef.

Table 2: *Impact of M. oleifera Seed Powder Concentrations on Microbes in Water During the Wet Season*

CONCENTRATIONS(MG/L)	VIABLE COUNT (VC) CFU/ML X 10	TOTAL VIABLE COUNT (TVC) CFU/ML X 10^{6L}
CONTROL(0)	7.00 ± 0.80^{a}	1750.00 ± 200.00^{a}
190.5	3.93 ± 0.26^{b}	949.00 ± 56.88^{b}
300.5	3.48 ± 0.29^{bc}	868.75 ± 73.15^{bc}
500.5	2.98 ± 0.23^{bc}	743.75 ± 57.17^{bc}
700.5	2.60 ± 0.28^{cd}	650.00 ± 69.97^{c}
900.5	1.58 ± 0.38^{d}	393.75 ± 95.95^{d}
P-VALUE	0.000	0.000

All mean values with different superscript are considered to be significantly different ($P< 0.05$), a>b >c> d while, mean values with the same superscript are closely related and have no significant differences ($P>0.05$), bc, cd.

Table 3: *Viable Count in the Dry and Wet Season of the Bacteria Isolated (VC) CFU/ML x 10)*

	SEASON		
	Dry	Wet	P-value
CONTROL	6.10 ± 0.12	7.00 ± 0.80	
KLEBSIELLA SP.	5.47 ± 0.11	2.77 ± 0.44	0.000
ESCHERICHIA COLI	3.56 ± 0.10	3.64 ± 0.54	0.865

Key: sp-Species

Sponsored by TETFUND/(TETFUND/DESS/POLY/WANNUNE/ARJ/2022-2025/VOL.1)
WIJSITAR Volume 1 Number 1, 2025
ISSN: 3141-3765 Peer-Reviewed/Open Access/https://wijitars.fedpolywannune.edu.ng/

Table 4: *Total viable count in the dry and wet season of Bacteria Isolated (TVC) CFU/ML x 10^{6L})*

	SEASON		
	Dry	Wet	P-value
CONTROL	1525.00±1.16	1750.00±200.00	
KLEBSIELLA SP.	1367.50±26.89	692.50±110.75	0.000
ESCHERICHIA COLI	890.00±25.33	910.00±135.97	0.865

Discussion

The result in table 1 showed significant difference (P<0.05) between the treated group and the control in the dry season. Both Viable Count (VC) and Total Viable Count (TVC) values decreased with increase in the concentrations of Moringa. The lowest concentrations of VC and TVC (1.27±0.09 and 316.67±22.04) were observed at the highest concentrations of Moringa (165.5mg/l). Similar trends were seen during the wet season (table 2), as the highest value of the Bacteria viable count (VC) (7.00±0.80CFU/ML x 10) and Total Bacteria viable count (TVC) (1750.00±200.00CFU/ML x 10^{6L}) were observed in the control and the lowest VC (1.58±0.38) and TVC (393.75±95.95) were observed in the treated group at concentrations 900.5mg/l.

Viable count of bacteria isolated in table 3 *Klebsiella* sp. (2.77±0.44) and *E. coli* (3.64±0.54) were lower in the wet season than in the dry season. There was significant difference (P<0.05) of *Klebsiella* sp. in the dry season compared to that of the wet season. On the other hand for *E.coli,* there were no significant differences (P>0.05) in the dry and wet season.

In table 4, *Klebsiella* species in the water were higher (1367.50±26.89) in the dry season than in the wet season (692.50±110.75). There was no significant difference in the concentration of *E.coli* in both seasons(P>0.05). *E.coli* was higher in the wet season (910.00±135.97) than in the dry (890.00±25.33).The total viable count in the water were only reduced even after treatment with Moringa. *M.oleifera* inhibited the growth of microbes with increasing doses of the concentration, 79.2% reduction in bacteria load was achieved in the dry season at the highest concentration (165.5mg/l).

This report is similar to the findings of Ordonez *et al.,* (2006), this is due to susceptibility of the species towards concentration of the extracts of *M.oleifera* seed powder, that destroys the specie that is not tolerant to it. Similarly, Raheela*et al.,* (2008) reported that, the antimicrobial activity of *M. oleifera* is dependent on doses of the extracts, as the concentration of the extracts decreased the activity also decreased, different minimum inhibitory concentrations (MIC) values were observed against different species of microbes.

In the wet season, 77.4% reduction in bacteria load was achieved after treatment with concentrations 900.5mg/l of *M oleifera.* Moringa seed powder extract exhibited antimicrobial activities related to that of the phytochemicals of some secondary metabolites like Cardiac glycosides, saponins, terpenoids, alkaloids, flavonoids and tannins (Ramawat, 2007; Carson and Hammer, 2010; Savoia, 2012).

The findings on the bacteria viable count (VC) isolated and the TVC in the dry and wet season agrees with that of Alo *et al.,* (2012) and Arafat & Mohamed (2013), the seed extracts of *M.oleifera* had shown their effectiveness in the reduction of total viable count, total coliform and fecal

Sponsored by **TETFUND**/(TETFUND/DESS/POLY/WANNUNE/ARJ/2022-2025/VOL.1)
WIJSITAR Volume 1 Number 1, 2025
ISSN: 3141-3765 Peer-Reviewed/Open Access/https://wijitars.fedpolywannune.edu.ng/

coliform microbial activity on coliform and its contribution to purify and improve water quality, although some bacteria were present in the water after treatment. The presence of *Escherichia coli* bacterium in water is therefore indicative of recent fecal pollution (Faridi, 2012).

Conclusion

M.oleifera seed powder was more effective in the dry season at concentrations 165.5mg/l,79.2% reduction in the amount of bacteria in the water were recorded while in the wet season,77.4% reduction in the amount of bacteria were observed at concentrations 900.5mg/l. The seed powder of Moringa exhibited antibacterial activity on all the organisms that were identified (*Klebsiella* sp. and *Escherichia coli*) as the bacteria concentrations in the water decreased with increasing concentrations of *M. oleifera* seed powder.

References

Akinwore, A.O & Jok, I.I (2006). An Investigation of the potentials of *Moringa oleifera* seed as a coagulation for suspended solids in Fish Culture Waste Water. *The Nigerian journal of forestry 31st Annual Conference,* pp26-30.

Alo, M.N., Anyim, C. & Elom, M. (2012). Coagulation and Antimicrobial activities of *Moringa oleifera* Seed storage at 3°C temperature in turbid Water. *Journal of Advance and Applied Science Research* 3,887-894.

American Public Health Association (APHA) (2005). *Standard Methods for the Examination of Water and Wastewater*, 20thed. American Public Health Association, Washington, D.C

Arafat, M.G., O. Mohammed (2013). Preliminary Study on Efficacy of Leaves,Seeds and Bark Extracts of *Moringa oleifera* in Reducing Bacterial Load in Water.*International Journal of Advanced Research*4:124-130.

Berger, M.R. Habs, M. Jahan S.A and Schmah, S. (1984) Toxicological assessment of seed from stenopetala, two highly efficient primary coagulants for domestic water treatment of tropical raw waters. *East African Medical Journal*, 61:712-716.

Carson, C.F. & Hammer, K.A. (2010).*Chemistry and Bioactivity of Essential Oils in Lipid and Essential Oils as Antimicrobial Agents*. pp 5-6

Dishua S. (2000). Water clarification using *Moringa oleifera* Gate Information Service, Germany. Retrieved from http://www.gtz.de/gate/gate/ideafp(Accessed 10th May, 2017).

Donatus, B., Francis, J. O. & Umar, A.A. (2018). Water Quality Assessment and Mapping Using Inverse Distance Weightel Interpolation: A Case of River Kaduna, Nigeria. *Nigerian Journal of Technology* 37(1), 249-261

Duncan D.B (1955). *Duncan Multiple Range and Multiple F- Test*. Biometrics, 11:1-5

Edeoga, H.O, D.E Okwuand B.O Mbaebie,(2005).Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4(7), 685-688.

Faridi, A.A (2012). *A Text Book of Environmental Microbiology;* pp.190-262. New Delhi: Random Publications

Folkard, G., Sutherland, J.,and Shaw, R.(1999)Water clarification using *Moringa oleifera* seed coagulant: technical brief No: 60.*Waterlines*,17(4),15-18.

Ghebremichael K.A, Gunaratna K.R, Henriksson H, (2005). A simple purification activity assay of the coagulant protein from *Moringa oleifera* seed. *Water Resources* 39:2338–2344

Jaime S.Colome, Raul.J.Cano, Mark A.Kubinski,DavidV.Grady(1986).*Laboratory Exercises in Microbiology*. California Polytechnic State University San Luis Obispo, pp 63-76

Ordonez RM, Ordonez AA, SayagoJ.E **(2006).** *Fruit Peptides*, Nieva MMI, Isla MI, , 27, 1187-1191.

Pritchard M, Makandawire T, Edmondson A, O'Neill J.G, Kululanga G (2010).Potential of Using Plant Extracts for Purification of Shallow well Water in Malawi. *Journal of Physics and Chemistry of the Earth*. 34:799-805

Savoia D, (2012). Plant –derived antimicrobial compounds: Alternatives to antibiotics. *Future Microbiology,* 7:979-990

Ugwu S.N, Umuokoro, A.F, Echiegu E.A, Ugwuishiwu ,B.O. & Enweremadu C.C (201**7):**Comparative Study of the Use of Natural and Artificial Coagulants for the Treatment of Sullage(Domestic waste water).*Journal of Cogent Engineering* 4(1) pp 108-112

Zakky S.K, & Auta I.K, (2017). Physicochemical and Hydrobiological Studies of Algae Diversity and Distribution in an Outdoor Polluted Water of River Romi.*Katsina Journal of Natural and Applied Sciences* 6 (2) ISSN 2141-0755

Zakky S.K, Ezra A.G, Abdul Hameed A, NayayaA, J. (2016). Study of Heavy Metals and Physicochemical Attribute of Romi River, Kaduna, Nigeria. *International Journal of Fisheries and Aquatic Studies*; 4(1).