



Effect of Storage Containers on the Microbial Load of Domestic Water from Three Sources Treated with *Moringa oleifera* and *Citrullus lanatus* Seed Powders

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Authors' contributions

This work was carried out in collaboration among all authors. Author ON designed the study and wrote the first draft of the manuscript in collaboration with author IJ. Author UGE sourced for the literature materials used in the study and articulated the final write up. Author HCO managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2019/v28i230130

Editor(s):

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Complete Peer review History: <http://www.sdiarticle3.com/review-history/48840>

Original Research Article

Received 30 March 2019

Accepted 13 June 2019

Published 18 June 2019

ABSTRACT

Aim: The effect of storage containers on the microbial load of domestic water from three sources treated with *Moringa oleifera* and *Citrullus lanatus* seed powders in Lekwesi, Abia State was assessed.

Study Design: The jar test method was used for the treatments. One gram (1.0g) each of the plant seed (*Moringa oleifera* and water melon seeds) was weighed and was added separately into 1000 ml of water samples in the different storage containers (clay lined pots, iron/steel tanks and

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polypyrene plastic drums, respectively). The mixture was stirred rapidly for 3 minutes and allowed to stand undisturbed for 1 hour, after which the top water was decanted.

Place and Duration of Study: Advanced Research Laboratory, Department of Microbiology, Gregory University Uтуру, from May to July, 2018.

Methodology: Tenfold serial dilutions was used for processing of all the water samples, after which the volume of exactly 0.5ml of the water sample was planted on the media using the spread plate method and incubated appropriately and other standard microbiological methods were employed to determine microbial loads.

Results: The river water samples had the highest microbial load of 1.2×10^3 - 2.0×10^8 cfu/ml and were reduced to 1.8×10^7 cfu/ml and 1.8×10^8 cfu/ml by *M. oleifera* and *C. lanatus* seed powders after an hour of storage respectively. The well water samples had the least microbial counts of 0.9×10^1 - 1.2×10^4 cfu/ml, and were reduced to 0.5×10^1 cfu/ml and 5.9×10^3 cfu/ml by *M. oleifera* and *C. lanatus* seed powders respectively. The potential pathogenic bacteria (TPPB) were reduced to 3.0×10^2 cfu/ml by *M. oleifera*, while *C. lanatus* was unable to reduce the TPPB after an hour. The microbial load decreased constantly within 24h in the various storage containers (steel, clay and plastic), but increased steadily from 72h to the 336h of post storage. The clay-lined and iron-steel pots maintained the same microbial counts after 4h post storage, but differed significantly after 24h, while the polypyrene plastic drum had the highest microbial count. There was absence of TPPB and Total Faecal Coliform Count (TFCC) in the well water samples after the treatment with *M. oleifera*.

Conclusion: *M. oleifera* was found to be a better water treatment than *C. lanatus*, while the clay-lined pot served as the best domestic water storage container.

Keywords: *Moringa oleifera*; *Citrullus lanatus*; storage container; microbial load water source.

1. INTRODUCTION

Water is one of the most essential sustainers of human life, second after oxygen which the quality is dependent on its source and type, in addition to the anthropogenic or natural occurrences in the concerned area (Nwaugo et al, 2011) [1]. About 70% of the human body weight is made up of water. It therefore plays an important role in the structure and function of the human body (Eze and Anaso, 2014) [2]. The consumption of impure water has resulted in the death of many people, children and adults. Ida, 2013 [3] reported that, the health of a community depends to a large extent on the ample provision of wholesome water supply. Therefore, waterborne diseases (such as cholera, typhoid fever, amoebic dysentery, gastro-enteritis, etc) arising from the consumption of contaminated water exert a high toll of morbidity and mortality worldwide and especially in Nigeria. The difference between river and stream depends solely on the size. While by definition they are the same, in reality, the river is a bigger body of water. A stream is smaller and even allows people to walk across it. The river is a collection of streams, whereas the stream is a single flowing body of water.

The use of traditional natural coagulants of plant origin is a simple, reliable and low cost method of purifying water. There is evidence that the use of

extracts from some plant species possessing both coagulating and antimicrobial properties is safe for human health (Bichi, 2013) [4].

Moringa oleifera belongs to a single family of shrubs and trees that is cultivated in the whole of tropical belt. The seeds are eaten green, roasted, powdered and steeped for tea or used in curries. It has found applications in medicinal uses, in cosmetics, in food supplements, and in water treatment (Alo et al, 2012) [5]. Its use for coagulation, co-coagulation, or coagulant aid has been a subject of investigation in many parts of the world and have potential advantage since it is accompanied by very low reduction in alkalinity (Alo et al, 2012) [5].

Citrullus lanatus seeds are known to be highly nutritious and rich sources of proteins, vitamins, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others as well as phytochemical components (Betty et al, 2016) [6]. The seeds of watermelon are known to have economic benefits especially in countries where cultivation is on the increase. The seeds are, for instance, used to prepare snacks, milled into flour and used for sauces. Oil from the seeds are used in cooking and incorporated into the production of cosmetics (Kuma et al, 2014) [7]. In spite of the various potential applications, the

2.5 Preparation of *M. oleifera* and *Citrullus lanatus* Seeds Powder

The fruits of *C. lanatus* were sliced open using a clean stainless steel laboratory knife. The seeds were washed severally with water, air dried at room temperature for one week, sorted to remove bad ones and shelled. The collected *M. oleifera* seed were de-shelled and air dried at room temperature for one week. Direct sunlight was avoided to prevent degradation of some of the plant's phytochemicals or antimicrobial constituents. The dried kernels were pulverized using electric blender to obtain powder. The powders were sieved with a plastic strainer of small pore size to obtain fine powder. The fine powders obtained were stored in sterile air-tight containers in a dark place to prevent oxidation.

2.6 Water Treatment Using *M. oleifera* and *C. lanatus* Seed Powder

The jar test method was used for the analysis. One gram (1.0g) each of the plant seed (*M. oleifera* and water melon seeds) was weighed. This was added to 1000 ml of water samples in their different storage containers (clay lined pots, iron/steel tanks and polypylene plastic drums, respectively). The mixture (water and plant seed powder-1000ml : 1g) was stirred rapidly for 60 seconds and then slowly for 2 minutes. The treated water samples were allowed to stand undisturbed for 1hour, after which the top water was decanted for storage. The stored water was then examined for microbial load at 2, 4, 24, 168 and 336 hour (Edessa et al, 2007) [10].

2.7 Enumeration of Microbial Counts

Ten fold serial dilutions were used for processing of all the water samples. After the dilutions, exactly 0.5ml of the water samples were planted on the media using the spread plate method and incubated appropriately. The colonies formed on the surfaces of the agar were counted with colony counter and was expressed as colony forming unit per ml (cfu/ml) for each of the total viable microorganisms, total coliform, faecal coliform, fungi and possible pathogenic bacteria. On establishment of growth, each culture plate was examined closely for distinct colonies (WHO, 1993; 2003) [11,12]. These counts were done for untreated and treated water samples.

2.8 Statistics

Simple statistical tool was employed to determine the mean among the storage containers and also the mean between untreated and treated water samples.

3. RESULTS

The microbial load of domestic water sources (stream, well and river) from Lekwesi in Umunneochi Local Government Area, Abia State were determined. The river water sample had the highest microbial load of 1.2×10^3 - 2.0×10^8 cfu/ml, while the well water had the least microbial load (0.9×10^1 - 1.2×10^4 cfu/ml) (Table 1).



Fig. 2. stream water source



Fig. 3. River water source



Fig. 4. Well water source

The microbial load of the stream, well and river water samples treated with the *M. oleifera* and *C. lanatus* seed powder stored in clay-lined pot, polypyrene plastic drum and iron-steel pot over days are shown in Tables 2-4. The microbial load decreased constantly after 4h-24hr in the various storage containers, the loads increased steadily from 72h - 336h of the storage. The clay-lined and iron-steel pots maintained the same microbial counts at 1h – 4h of the storage, but differed significantly after 24h, while the polypyrene plastic drum had the highest count. A significant reduction was achieved using the clay-lined pot, followed by iron-steel pot and the least was the polypyrene plastic drum. The *C. lanatus* treated water samples followed the same trend. At 24h onwards, an increase was recorded in all the storage containers, but was significant in the polypyrene plastic drum (Tables 5-7).

4. DISCUSSION

The microbial load of the domestic water (stream, well and river) sources examined

exceeded the WHO stipulated standard. Total coliforms (TC) ranged from 2.1×10^2 cfu/ml to 1.9×10^5 cfu/ml. The presence of faecal coliforms (FCC) and potential pathogenic bacteria (PPB) were recorded in all the domestic water sources. The level of FCC ranged from 1.3×10^1 to 2.1×10^3 cfu/ml. Sharan (2011) [13] reported similar microbial loads and noted that most rural communities lack access to drinkable water supplies which rely mainly on river, stream, well and pond water sources for their daily water needs. Waters from these sources are faecally contaminated, devoid of treatment and are used directly by the inhabitants [14]. Well water was found to be less contaminated by coliforms when compared to other sources. According to World Health Organization (WHO) guideline [15], total coliform counts must not be detected in 100 ml of drinking water sample. Therefore, the results of the total coliforms obtained showed that all the water sources exceeded the recommended values and may not be safe for drinking, if not treated. However, Thompson et al. (2003) [16] guideline for potable water does not allow the

Table 1. Microbial load of untreated domestic water samples from Lekwesi, Abia State

	THC(Cfu/ml)	TCC(Cfu/ml)	TFCC(Cfu/ml)	TPPB(Cfu/ml)	TFC(Cfu/ml)
River	2.0×10^8	1.9×10^5	2.1×10^3	1.2×10^3	1.1×10^4
Well	1.2×10^4	2.1×10^2	1.3×10^1	0.9×10^1	1.9×10^1
Stream	1.4×10^5	2.5×10^3	5.3×10^1	0.7×10^1	1.5×10^4
WHO standard	1.0×10^2	1.0×10^1	0	0	0

Key: THC = Total heterotrophic count; TCC = Total coliform count; TFCC = Total faecal coliform count; TPPB = Total potential pathogenic bacteria; TFC = Total fungal count

Table 2. Microbial load (cfu/ml) of stream water sample treated with 1.0g of *M. oleifera* seed powder stored in various containers

Time (hour)	Clay-lined pot					Polypyrene plastic drums					Iron / steel pot				
	THC×10 ²	TCC×10 ²	TFCC×10 ¹	TPPB×10 ¹	TFC×10 ¹	THC×10 ²	TCC×10 ²	TFCC×10 ¹	TPPB×10 ¹	TFC×10 ¹	THC×10 ²	TCC×10 ²	TFCC×10 ¹	TPPB×10 ¹	TFC
1	70.0	11	2.3	0.4	9.0	70.0	11	2.3	0.4	9.0	70.0	11	2.3	0.4	0.9×10 ²
2	24.0	3.3	1.5	0.2	5.0	25.0	3.4	1.7	0.2	6.0	24.0	3.3	1.5	0.2	0.5×10 ²
4	7.0	1.2	0.4	NBG	3.1	9.0	1.3	0.5	NBG	3.5	7.0	1.2	0.4	NBG	3.1×10 ¹
24	4.5	0.38	NBG	NBG	0.6	5.1	0.42	NBG	NBG	1.2	4.7	0.4	NBG	NBG	0.8×10 ¹
72	4.9	0.42	NBG	NBG	1.1	5.3	0.55	NBG	NBG	1.5	5.0	0.5	NBG	NBG	1.3×10 ¹
168	5.1	0.63	NBG	NBG	1.9	6.0	0.8	NBG	NBG	2.3	5.4	0.71	NBG	NBG	2.0×10 ¹
336	6.0	0.9	NBG	NBG	2.3	6.3	1.2	NBG	NBG	3.1	6.1	1.1	NBG	NBG	2.6×10 ¹

Key: THC = Total heterotrophic count, TCC = Total coliform count, TFCC = Total faecal coliform count, TPPB = Total potential pathogenic bacteria, TFC = Total fungal count

Table 3. Microbial load (cfu/ml) of well water sample treated with 1.0g of *M. oleifera* seed powder stored in various containers

Time (hour)	Clay-lined pot					Polypyrene plastic drums					Iron / steel pot				
	THC×10 ²	TCC×10 ¹	TFCC×10 ¹	TPPB×10 ¹	TFC×10 ¹	THC×10 ²	TCC×10 ¹	TFCC×10 ¹	TPPB×10 ¹	TFC×10 ¹	THC×10 ²	TCC×10 ¹	TFCC×10 ¹	TPPB×10 ¹	TFC×10 ¹
1	60.0	10.0	0.5	0.6	0.8	60.0	10.	0.5	0.6	0.8	60.0	10.0	0.5	0.6	0.8
2	25.0	4.3	NBG	0.1	0.2	27.0	4.5	NBG	0.1	0.2	25.0	4.3	NBG	0.1	0.2
4	7.5	1.9	NBG	NBG	NFG	8.3	2.2	NBG	NBG	NFG	7.5	1.9	NBG	NBG	NFG
24	0.98	0.2	NBG	NBG	NFG	2.05	0.8	NBG	NBG	NFG	1.3	0.8	NBG	NBG	NFG
72	1.01	0.5	NBG	NBG	NFG	2.26	0.7	NBG	NBG	NFG	1.40	0.6	NBG	NBG	NFG
168	1.5	1.0	NBG	NBG	NFG	2.5	1.2	NBG	NBG	NFG	1.7	1.1	NBG	NBG	NFG
336	2.1	1.7	NBG	NBG	NFG	3.2	1.9	NBG	NBG	NFG	2.9	2.3	NBG	NBG	NFG

Key: THC = Total heterotrophic count, TCC = Total coliform count, TFCC = Total faecal coliform count, TPPB = Total potential pathogenic bacteria, TFC = Total fungal count

Table 4. Microbial load (cfu/ml) of river water sample treated with 1.0g of *M. oleifera* seed powder stored in various containers

Time (hour)	Clay-lined pot					Polypylene plastic drums					Iron / steel pot				
	THC	TCC×10 ²	TFCC×10 ²	TPPB×10 ¹	TFC×10 ¹	THC	TCC×10 ²	TFCC×10 ¹	TPPB×10 ¹	TFC×10 ¹	THC	TCC×10 ²	TFCC×10 ²	TPPB×10 ¹	TFC×10 ¹
1	7.0×10 ⁷	54.0	4.8	39.0	12.0	7.0×10 ⁷	54.0	48.0	39.0	12.0	7.0×10 ⁷	54.0	4.8	39.0	12.0
2	1.8×10 ⁷	23.0	2.0	19.0	9.0	1.9×10 ⁷	24.0	21.0	20.0	10.0	1.8×10 ⁷	23.0	2.0	19.0	9.0
4	5.1×10 ⁶	7.0	0.6	4.5	7.0	5.4×10 ⁶	9.0	7.0	4.7	0.8	5.1×10 ⁶	7.0	0.6	4.5	7.0
24	0.8×10 ⁴	1.2	NBG	NBG	0.4	1.8×10 ⁴	1.7	1.3	0.2	0.6	1.0×10 ⁴	1.5	NBG	NBG	0.5
72	0.9×10 ⁴	1.4	NBG	NBG	0.8	2.0×10 ⁴	2.0	1.5	0.3	1.2	1.1×10 ⁴	1.8	NBG	NBG	1.0
168	1.2×10 ⁴	1.8	NBG	NBG	1.4	1.5×10 ⁴	2.3	1.8	0.5	2.3	1.4×10 ⁴	2.0	NBG	NBG	1.8
336	1.5×10 ⁴	2.0	NBG	NBG	2.1	1.9×10 ⁴	2.5	2.5	1.0	3.7	1.7×10 ⁴	2.3	NBG	NBG	3.3

Key: THC = Total heterotrophic count, TCC = Total coliform count, TFCC = Total faecal coliform count, TPPB = Total potential pathogenic bacteria, TFC = Total fungal count

Table 5. Microbial load (cfu/ml) of well water sample treated with 1.0g of *C. lanatus* seed powder stored in various containers

Time (hour)	Clay-lined pot					Polypylene plastic drums					Iron / steel pot				
	THC×10 ³	TCC×10 ¹	TFCC×10 ¹	TPPB×10 ¹	TFC×10 ¹	THC×10 ³	TCC×10 ¹	TFCC×10 ¹	TPPB×10 ¹	TFC×10 ¹	THC×10 ³	TCC×10 ¹	TFCC×10 ¹	TPPB×10 ¹	TFC×10 ¹
1	9.0	18.3	1.0	0.9	1.5	9.0	18.3	1.0	0.9	1.5	9.0	18.3	1.0	0.9	1.5
2	7.8	13.2	0.8	0.9	1.2	8.3	15.4	0.8	0.9	1.3	7.8	13.2	0.8	0.9	1.2
4	7.0	11.0	0.6	0.9	0.9	7.3	13.1	0.7	0.9	1.0	7.0	11.0	0.6	0.9	0.9
24	7.3	11.9	0.7	1.0	1.1	7.8	13.8	0.9	1.1	1.3	7.4	12.1	0.8	1.1	1.2
72	8.0	13.1	1.0	1.2	1.2	8.3	14.2	1.2	1.4	1.5	8.1	13.8	1.1	1.3	1.4
168	8.8	14.0	1.8	1.7	1.8	9.1	15.9	2.1	2.2	2.0	8.9	14.5	1.9	2.0	1.9
336	9.1	14.8	2.2	2.0	2.1	9.7	17.1	3.0	3.1	2.9	9.3	15.6	2.5	2.3	2.1

Key: THC = Total heterotrophic count, TCC = Total coliform count, TFCC = Total faecal coliform count, TPPB = Total potential pathogenic bacteria, TFC = Total fungal count

Table 6. Microbial load (cfu/ml) of stream water sample treated with 1.0g of *C. lanatus* seed powder stored in various containers

Time (hour)	Clay-lined pot					Polypylene plastic drums					Iron / steel pot				
	THC×10 ⁵	TCC×10 ³	TFCC×10 ²	TPPB×10 ¹	TFC×10 ¹	THC×10 ⁵	TCC×10 ³	TFCC×10 ²	TPPB×10 ¹	TFC×10 ¹	THC×10 ⁵	TCC×10 ³	TFCC×10 ²	TPPB×10 ¹	TFC×10 ¹
1	1.30	1.9	0.48	0.7	12.0	1.30	1.9	48.0	0.7	12.0	1.30	1.9	0.48	0.7	12.0
2	1.22	1.82	0.44	0.7	1.0	1.25	1.83	45.0	0.7	1.1	1.22	1.82	0.44	0.7	1.0
4	1.2	1.8	0.42	0.7	0.8	1.25	1.83	45.0	0.7	1.1	1.2	1.8	0.42	0.7	0.8
24	1.22	1.84	0.5	0.9	1.3	1.26	1.88	0.6	1.1	1.7	1.23	1.85	0.5	1.0	1.5
72	2.9	2.1	0.9	1.4	1.9	3.2	2.5	1.2	1.7	2.4	3.0	2.2	1.0	1.5	2.1
168	3.2	2.5	1.1	1.8	2.1	3.7	2.8	1.6	2.2	3.0	3.3	2.6	1.4	2.0	2.7
336	3.9	3.0	1.5	2.3	3.0	4.2	3.5	2.2	2.9	3.7	4.0	3.1	1.9	2.5	3.3

Key: THC = Total heterotrophic count, TCC = Total coliform count, TFCC = Total faecal coliform count, TPPB = Total potential pathogenic bacteria, TFC = Total fungal count

Table 7. Microbial load (cfu/ml) of river water sample treated with 1.0g of *C. lanatus* seed powder stored in various containers

Time (hour)	Clay-lined pot					Polypylene plastic drums					Iron / steel pot				
	THC×10 ⁸	TCC×10 ⁵	TFCC×10 ³	TPPB×10 ³	TFC×10 ³	THC×10 ⁸	TCC×10 ⁵	TFCC×10 ³	TPPB×10 ³	TFC×10 ³	THC×10 ⁸	TCC×10 ⁵	TFCC×10 ³	TPPB×10 ³	TFC×10 ³
1	1.83	1.70	1.8	1.2	6.5	1.83	1.70	1.8	1.2	6.5	1.83	1.70	1.8	1.2	6.5
2	1.79	1.65	1.6	1.0	6.3	1.81	1.68	1.7	1.1	6.4	1.79	1.65	1.6	1.0	6.3
4	1.46	1.35	1.27	0.8	4.4	1.61	1.42	1.54	0.9	5.2	1.46	1.35	1.27	0.8	4.4
24	1.49	1.38	1.29	0.79	4.5	1.68	1.49	1.58	0.94	5.3	1.50	1.40	1.31	0.82	4.8
72	1.53	1.45	1.32	0.81	5.0	1.74	1.50	1.67	1.0	5.8	1.55	1.5	1.38	0.89	5.3
168	1.6	1.5	1.4	0.89	5.9	1.8	1.6	1.7	1.3	6.1	1.64	1.54	1.47	0.9	6.0
336	1.68	1.59	1.47	0.9	6.1	1.9	1.7	1.8	1.4	6.9	1.7	1.6	1.5	1.1	6.4

Key: THC = Total heterotrophic count, TCC = Total coliform count, TFCC = Total faecal coliform count, TPPB = Total potential pathogenic bacteria, TFC = Total fungal count

presence of faecal coliforms. The faecal coliforms were found highest in river water sample, while the least was in the well water. The contamination of these water sources is possibly due to poor protections and exposure to human contamination and domestic wastes. The behavioral and unhygienic practices of the users might also be contributing to the high microbial load. It was observed that the users of these water sources (stream and river) defaecated, bathed and washed around the same water sources used for their domestic activities. Furthermore, faecal matter produced by the cattle inevitably increases the contamination of the water sources.

The water samples treated with the *M. oleifera* and *C. lanatus* seed powders were stored in clay-lined pot, polypyrene plastic drum and iron-steel pot over days (14 days) in conformity with the NAFDAC stipulated period. The microbial loads decreased continuously within 24hr in the various storage containers; even the FCC and potential pathogenic bacteria (PPB) were reduced. Orji et al. (2006) [17] have demonstrated the potential of using *M. oleifera* for purification of water, while Sule et al. (2011) [18] reported similar findings using *C. lanatus* (melon seed). The reduction in the microbial loads as the days of storage increased is similar to the observation of [19]. Eniola et al. (2007); Sule et al. (2011); Radha et al. (2015) [15,18,20] affirms that microbiological quality of treated water stored in proper containers reduces diarrhoeal and other waterborne diseases. After then, the microbial loads increased steadily starting at 72 hr to 14th day of the storage for *M. oleifera*, while *C. lanatus* increased at 24hr onwards. The clay-lined and iron-steel pots maintained the same microbial counts at 1hr – 4hr of storage, but differed significantly after 24hr, while the polypyrene plastic drum had the highest count. Mud pot gradually increased the cooling effects to the stored water thereby also reduced the microbial population [21]. A significant reduction was achieved using the clay-lined pot, followed by iron-steel pot and the polypyrene plastic drum had the highest microbial counts. Packiyam et al. (2016) ; Zand and Hoveidi (2015) [22,23] found that the bacteriological qualities of water could be improved by storage, which depends on the treatment the water source has undergone. This corroborate with the study of [24] and [20]. However, Eniola et al. (2007) [15] indicated that pot coated with brass, copper, silver, zinc or aluminum is a low cost microbial safety drinking

water storage container. Tunggolou and Payus (2017) [24] affirms copper to be more poisonous to bacteria than other metals such as stainless steel or aluminum. Sarsan (2013) [21] ascertained that plastic drums had lesser effect on coliforms and microorganisms. Another reason that makes the purification possible might be due to the functional groups in the amino acids of the plants seed proteins (Sotheeswaran et al, 2012; Onwuchekwa et al, 2019) [25,26]. Alo et al. (2012) [5] stated that most plants seed proteins are positively charged, they bind to the contrarily charged particles in the water through the mechanism of adsorption and neutralization especially when the pH is below 10.

5. CONCLUSION

The presence of pathogens render the water samples unfit for drinking and were reduced with 1.0g of the plants seed powder. More reduction was achieved using the clay-lined pot, followed by iron-steel pot, while the polypyrene plastic drum had the highest microbial counts as a storage container. This showed that *M. oleifera* and *C. lanatus* seed powders as a natural microbial coagulants was effective when used at a loading dose of 1.0g in 1L of water over time. Also, *M. oleifera* was found to be a better coagulant compared to *C. lanatus*, while the clay-lined pot served as the best domestic water storage container.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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