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Original research

Natural coagulants from forestry trees to enhance drinking water quality and reduce microbial load

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Abstract

Access to drinking water is a universal right of human beings that has not been achieved globally, so it is necessary to continue working to achieve it. Humans and other living things depend on water for life and health. Forests have long been seen as an important source of clean drinking water. The high cost of treated water makes most rural communities resort to readily available sources that are normally of low quality, exposing them to water-brown diseases. In this light, this research was carried out to confirm the effectiveness of powder extracted from mature-dried seeds of *Moringa oleifera*, *Parkinsonia aculeate*, and *Jatropha curcas* for clarification of water. In the present study, various doses of *Moringa oleifera*, *Parkinsonia aculeate* and, *Jatropha curcas* seeds coagulant were added as 5 ,10 and 20 grams per liter of decanted water. Treated water was kept stable for 0 (control), 12, 24 and 48 hours. With increasing coagulant doses. The measured parameters such as pH, turbidity, TDS, TSS, watercolor, colony-forming unit (CFU), temperature, and electrical conductivity were decreased. Also, the lowest turbidity was achieved by settling time T4, since it displayed 4.29 (NTU), 9.42 (NTU) and 8.72 (NTU) for *M. oleifera*, *P. aculeate* and *J. curcas* seed powder, respectively. Furthermore, the total bacterial count at settling time T1(control) gave the highest CFU 232.48×10^3 cfu⁄ml, 301×10^3 cfu⁄ml and 297.71×10^3 cfu⁄ml for *M. oleifera*, *P. aculeate* and *J. curcas* seed, respectively. On the other hand, the total coliform detected the highest total bacterial count in water samples treated with *Parkinsonia aculeate* tree as compared with the two other trees; it was 391.3×10^2 at the second concentration. Therefore, the main objective of this experiment was to evaluate the efficiency of natural absorbents from *Moronga oleifera*, *Parkinsonia aculeante*, and *Jatropha curcas* in treating drinking water.

Keywords: *Moringa oleifera, Parkinsonia aculeante, Jatropha curcas*, turbidity, natural absorbent, drinking water, total bacterial and coliform count.

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1- INTRODUCTION

Safe water and adequate sanitation are basic to the health of every person on the planet. Many people, especially in Africa and Asia, do not have access to this fundamental need **(Bartram *et al*., 2005)**. About 1 billion people, (15% of the world population) are without safe drinking water worldwide **(UNICEF and WHO 2019)**. The vast majority of these people are located in sub-Saharan Africa, South Asia, and East Asia **(Dungumaro, 2007., UNICEF and WHO 2019)**. Around 5 million lives are lost annually due to drinking and using contaminated water **(WHO and UNICEF 2006)**. Children under five are the most at risk, beside the people who live under unsanitary conditions and the elderly. Four billion cases of diarrhoea are reported every year, causing 1.7 million deaths **(WHO, 2017 and WHO and UNICEF 2006)**.

The population forecast suggests that, an additional 784 million people worldwide will need improved drinking water sources for the Millennium Development Goal (MDG) target to be met **(WHO, 2008)**. From 1990 to 2006, approximately 1.56 billion people gained access to improved drinking-water sources. Improved drinking water coverage in sub-Saharan Africa is still considerably lower than in other regions. Nevertheless, it has increased from 49% in 1990 to 58% in 2006, which means that an additional 207 million Africans are now using safe drinking water **(WHO, 2008)**. The Ghana Demographic and Health Survey DHS (2004) reported that 46.4% of rural households lack access to improved drinking water access to indoor piped water **(Ghana Statistical Service, 2004)**.

Various processes and technologies are being researched to improve the quality of water **(Ullah *et al*., 2020)**. In view of the foregoing, plant-based materials are gaining more attention as substitutes for synthetic chemicals for water and waste purification **(Akhilesh and Nisa, 2018).** Plant-based biomaterials are presumed safe for human health, cheap, locally available and environmentally friendly **(Yin, 2010).** Some rural communities in African countries utilise crude seed extracts to clear turbid river water **(Yin, 2010)** a natural coagulant **(De Paula *et al*., 2018).** Natural coagulants have been recognized for their traditional local water purification **(Choy *et al*., 2014; and Dorea, 2006).** Naturally occurring coagulants are sustainable, environmentally friendly. Natural coagulants have captured the scientific community's attention in the past decades due to their significant health and environmental benefits, and they solves most of the common problems associated with chemical coagulants. In addition, the treated water with chemical processes has been reported to have adverse health impacts **(Amy *et al*., 1988; Dodds *et al*., 1999; Barrett *et al*., 2000 and Westerhoff *et al*., 2004).**

Active coagulants from the seeds of *Vigna unguiculata* and *Parkinsonia aculeate* have been used in Tanzania to purify drinking water in rural communities **(Marobhe *et al*., 2007)**. Application of the low cost *Moringa oleifera* seeds is recommended for ecofriendly, nontoxic, simplified water treatment for rural and peri-urban people living in extreme poverty **(Michael 2010; Areeba and Malika, 2020)**. In another study by **Rodiño-Arguello *et al*., (2015)** it was shown that natural coagulant removal efficiencies prevent achieving the level of residual turbidity requirements of the quality standard for drinking water in Colombia (2 NTU). Also, **Singh *et al*., (2018)** reported that these materials have been used as such, sometimes after some minor treatments, and are widely known as low-cost adsorbents. **Gautam and Saini (2020)** showed the natural coagulants' applications in treating industrial wastewaters and their relative advantages and disadvantages as compared to chemical coagulants.

However, **Zurina *et al.*, (2011)** investigated the capability of *Jatropha curcas* seed and press cake (the residue left after oil extraction) to reduce the turbidity of wastewater through coagulation. The coagulant was prepared by suspending *Jatropha curcas* seed and pressing cake powder into solution. The Jatropha seed was found to be an effective coagulant with more than 96% of turbidity removal at pH 1-3 and pH 11-12. The highest turbidity removal was recorded at pH 3 using a dosage of 120 mg/L. The turbidity removal was high (> 98%) at all turbidities (100 NTU to 8000 NTU), suggesting its suitability for a wide range of industrial waste. Also, Nasrin, *et al.,* (2018) found that the highest turbidity reduction was achieved with the coagulant extracted at a solution pH of 10 and an extraction temperature of 60 °C (pH10/60 °C-Jc press cake). Under these conditions, the coagulant dosage required was reduced by 80%–90%, depending on the coagulation pH degree. At the coagulation pH = 6, the pH10/60 °C- *J. curcas* press cake well reduced the turbidity by 85%. The optimized extraction conditions significantly improved the efficiency of this promising bio-derived coagulant in turbidity reduction. On the other hand, **Onyebuchi *et al.*, (2020)** showed that *Moringa oliefera* seeds, *Parkia biglobosa* (Locust Bean Seed) and *Jatropha Curcas* (Physic Nut) have shown great potential for use in waste water treatment with very high-quality output. Most bio-coagulants have demonstrated efficacy and are advantageous due to factors such as low cost, ease of sourcing, biodegradability, environmental friendliness, and multi-functionality. *Moringa oliefera* seeds could be employed on an industrial scale because of their efficacy in waste water treatment. While **Joel *et al.*, (2021)** observed that rice husk ash, *Jatropha curcas* and *Moringa oleifera* were used and successfully demonstrated their coagulation and flocculation capabilities in turbidity and microbial load removal from domestic wastewater. The main aim of that study was to evaluate the efficiency of natural absorbents from *Moronga oleifera*, *Parkinsonia aculeante*, and *Jatropha curcas* in treating drinking water.

2- MATERIALS AND METHODS

2.1 Water samples

In our study, water samples were collected from The Holding Company of Water and Wastewater, Alexandria Water Company, AL Nozha, Station. The decanted water sample used in the experiment was collected in polyethylene plastic bottles (sterile) and transported to the laboratory using an iced cooler box.

Table (1) Physico-chemical Parameters for water sample before treatments

Trees species / Parameters	*Moringa oleifera*	*Parkinsonia aculeate*	*Jatropha curcas*
Color (Hazen unit)	40.5	43.28	44.77
pH	8.73	8.97	8.91
Nephelometric Turbidity Unit (NTU)	21.18	27.82	24.1
Electrical conductivity (µS⁄cm)	1087.5	1198.4	1189.4
Temperature (°C)	20.10	20.22	21.11
Total dissolved solids mg⁄l (TDS)	892.9	1141.7	1096.7
Total suspended solids (TSS)mg⁄l	222.6	291.2	315.67
Total coliform count (cfu⁄ml)	364.6×10^2	372.4×10^2	361.5×10^2
Total bacterial count (cfu⁄ml)	253.5×10^3	262.4×10^3	267.5×10^3

2.2 Preparation of raw powder materials

Moringa oleifera Parkinsonia aculate and Jatropha curcas seeds were collected. Good matured seeds of dry pods for *Moringa*, *Parkinsonia* and *Jatropha* were selected and used (**Figure1)**. The seeds were washed and shed dried at room temperature, then the clean seed kernels were obtained by removing the seed coat (shell seeds) and drying in a hot air oven for 48 hrs at 35 ^{0} C. Finally, the plant material was converted into a fine powder, which was further utilised for the extraction process. Water treatment was given by directly using seed powder. Suspended for one minute to activate the coagulant properties and form a suspension. Treated water was kept stable for four settling times **(T1=** 0 hours (untreated water), **T2=** 12 hours**, T3=** 24 hour**, T4=** 48 hour**).**

In the present study various doses of *Moringa oleifera*, *Parkinsonia aculeate* and *Jatropha curcas* seed powder like 5 ,10 and 20 g/l were taken from decanted water.

The cleaned water was carefully poured off, when the particles and contaminates had settled to the bottom and then filtered. The resulting suspension was filtered through a 250 µm sieve.

Figure (1). Seeds from right side *Moringa oleifera*, *Jatropha curcas* and *Parkinsonia aculeate.*

2.3 Physico-chemical Parameters of treated water

2.3.1 Determination of water color

Calibrate the 2120IB visual comparison method by the instrumental procedures **(Jones *et al.*, 1973)**.

2.3.2 Determination of pH value

pH determination using procedures adopted by APHA Method 4500-Hl pH Value". The pH is determined in the laboratory using a pH electrode. Recommended sample holding time: 2 hours **(Merriam, 2013)**.

2.3.3 Determination of turbidity

Turbidity was determined by narrowing to the 2130 nephelometric method, which is described in (2130B.) **(James and Dell 1993)**.

2.3.4 Determination of electrical conductivity at 25 °C

The electrical conductivity meter (EC meter) measures the electrical conductivity in a solution. It is commonly used in hydroponics, aquaculture, and fresh water systems to monitor the amount of nutrients, salts, or impurities in the water **(APHA, 1992)**.

2.3.5 Determination of temperature

This analysis is carried out using procedures that use temperature for process use or heat-transmission calculations. For process use or heat-transmission calculations, industrial plants frequently require data on water temperature of 2550 °F **(Goodman, 1964).**

2.3.6 Determination of total dissolved solids at 105 °C (2540lB total solids) and total suspended solids (2540D Total Suspended solids)

Total dissolved solids refer to the total dissolved (filterable) solids determined using the method specified in Title 40 of the Code of Federal Regulations (40 CFR) Part 136 **(Brian Oram, PG 2014)**, whereas total suspended solids refer to the total suspended solids determined in the treatment of the residue according to 2540E **(Degen and Nussberger, 1956).**

2.3.7 Biological parameter by determined the total bacterial count (cfu/ ml) and total coliform of the water samples

The total bacterial count assay was carried out to determine the microbiological quality of domestic water after and before tree coagulant treatments with different doses and at different time points and was performed to observe if there was a positive or negative difference. The pour plate method was used to determine if there was a reduction in the number of colonies in the treated water samples using Nutrient Agar (NA) and Red Violet Broth Agar (RVBA) were used **(Almatar *et al.* ,2014)**. A sequence of four tubes (9 ml) of sterilized distilled water were labelled with 10^{-2} to 10^{-5}. The serial dilution was produced by transferring 1 ml of samples from the flask (which dilution is 10^{-2}) to a 9 ml of sterilized distilled water. One millilitre was sampled and poured onto all the surfaces of the media, and streaks were made using sterile swabs. Calculate the bacteria number (cfu/ml) of a sample as follows:

$$Number\ of\ bacteria/ml = \frac{number\ of\ colonies\ (CFUs)}{dilution\ X\ amount\ plated}$$

The petri dish plates were incubated at 37 °C for 24 h, then the calculation of the total bacterial count as mentioned in the previous equation and the decrease in microorganism number (R) (total) due to three different coagulant treatments were calculated using the equation:

$$\text{The reduction in microorganism number } (R) = \frac{N0 - N}{N0} \text{ x } 100$$

Where: ***N:*** is the number of microorganisms in the sample with different coagulants (CFU/ ml) and ***N0:*** is the number of microorganisms in control samples (CFU/ ml).

2.4 Statistical Analysis

Steel and Torrie (1980) used the split plot system in randomized complete block design (RCBD) in analysing this experimental data. The main plot was for different doses of seed powder, and the sub was settling times with three replicates. Each type of plant has its own stats.Statistical analysis was done by ANOVA, F-test, and LSD.

3- RESULTS

3.1. Physicochemical Parameters

3.1.1. Water color and pH for water samples

The color was measured for water samples from decanted water. Water treated with three different doses of *M. oleifera, P. aculeate* and *J. curcas* seeds powder under four settling time were presented in **Table (2)**.

A statistical analysis showed that there were highly significant settling time and doses. It was found the color were 40.25 Hazen unit, 20.88 Hazen unit, 9.63 Hazen unit and 5.22 Hazen unit for treatments T1, T2, T3 and T4, respectively for *M. oleifera* seeds powder, however they were 45.11 Hazen unit, 33.4 Hazen unit, 22.71 Hazen unit and 15.5 Hazen unit for treatments T1, T2, T3 and T4, respectively for *P. aculeate* seeds powder, **Tables (2 and 3)** it was clear that the dose 5 g/l (C1) recorded the highest color compare with dose 10 g/l (C3) of *M. oleifera* seeds powder. As for the other two species, they gave the same direction.

Table (2) The effect of treatment settling time of *Moringa oleifera*, *Parkinsonia aculeate* and *Jatropha curcas* seeds powder on some quality parameters for water samples

Settling time	T1(o time)	T2 (12 hrs)	T3(24 hrs)	T4 (48 hrs)	
Water color					**LSD**
Moringa oleifera	40.25	20.88	9.63	5.22	3.031
Parkinsonia aculeate	45.11	33.40	22.71	15.5	4.011
Jatropha curcas	43.51	40.45	35.71	20.54	4.021
pH					**LSD**
Moringa oleifera	8.65	7.21	7.28	7.8	0.3301
Parkinsonia aculeate	8.56	7.94	7.37	7.41	0.4101
Jatropha curcas	8.71	7.45	7.51	7.32	0.4231
The Electrical Conductivity (µS⁄cm)					**LSD**
Moringa oleifera	23.69	11.80	8.15	4.29	3.9669
Parkinsonia aculeate	29.44	21.92	15.85	9.42	4.4211
Jatropha curcas	31.29	20.19	12.18	8.72	4.51
Temperature (°C)					**LSD**
Moringa oleifera	19.63	19.10	19.58	19.58	0.3925
Parkinsonia aculeate	19.51	19.25	19.51	18.99	0.3825
Jatropha curcas	19.65	19.54	19.22	19.25	0.4011
The Total Dissolved Solids mg⁄l (TDS)					**LSD**
Moringa oleifera	963.15	448.78	251.74	142.41	111.1
Parkinsonia aculeate	892.51	501.91	390.34	250.52	112.5
Jatropha curcas	903.6	496.21	359.91	201.52	111.9
The Total Suspended Solids mg⁄l (TSS)					**LSD**
Moringa oleifera	273.70	127.55	66.74	50.59	12.29
Parkinsonia aculeate	297.7	259.21	117.61	96.51	14.69
Jatropha curcas	270.1	233.02	159.62	112.1	15.01
The Turbidity Nephelometric Turbidity Unit (NTU)					**LSD**
Moringa oleifera	23.69	11.80	8.15	4.29	3.9669
Parkinsonia aculeate	29.44	21.92	15.85	9.42	4.4211
Jatropha curcas	31.29	20.19	12.18	8.72	4.51

**Mean values for three replicates, (T1: time 0-hour control, T2: time 12 hrs, T3: time 24 hrs and T4: 48 hrs).*

The results of the analysis of variance revealed a highly significant difference between treatments. The data presented in **Table (4)** showed that the settling time T1 (control) with 5g/l of powder processed from *M. oleifera* (C1) gave the highest color 40.5 Hazen units. On the other hand, the lowest color with the highest dose (20 g/l) at settling time T4 (24 hrs), was 4.51 Hazen unit. It was noticed that the highest dose of *P. aculeate* seeds powder at settling time T4 (24 hrs) gave the lowest color 8.15 Hazen unit. The results showed that the power from *J. curcas* gave the highest color at settling time T1 (control) with dose (C1), it was 44.77 Hazen unit, Moreover, the

lowest color was achieved by settling time T4 (48 hrs) with a dose of 20 g/l (C3) with value of 9.8 Hazen units **(Table 4)**.

pH was measured for decanted water samples of clarification water treated with three different doses of *M. oleifera*, *P. aculeate* and *J. curcas* seed powder under four settling times, which are presented in **Tables (2 and 4)**. ANOVA revealed that the differences between treatments for pH were not significant. Also, the interaction was not significant.

Table (3) The effect of coagulant concentrations of *Moringa oleifera*, *Parkinsonia aculeate* and *Jatropha curcas* (C1:5 g/L, C2: 10 g/L, C3: 20 g/L of seeds powder) on some quality parameters for water samples

Settling time	C1	C2	C3	
Water color				**LSD**
Moringa oleifera	21.75	19.05	16.19	2.4208
Parkinsonia aculeate	31.51	29.05	20.54	3.4210
Jatropha curcas	29.96	20.15	15.96	3.0125
pH				**LSD**
Moringa oleifera	7.63	7.59	7.52	0.2789
Parkinsonia aculeate	7.77	7.96	7.54	0.2981
Jatropha curcas	7.94	7.52	7.43	0.28011
The Turbidity Nephelometric Turbidity Unit (NTU)				**LSD**
Moringa oleifera	12.75	11.67	11.54	1.7405
Parkinsonia aculeate	15.51	13.79	10.01	1.8961
Jatropha curcas	13.34	12.7	9.69	1.7961
The Electrical Conductivity (µS⁄cm)				**LSD**
Moringa oleifera	675.69	674.03	653.08	47.611
Parkinsonia aculeate	759.12	694.31	667.72	49.1160
Jatropha curcas	695.24	672.11	644.09	48.2921
Temperature (^{o}C)				**LSD**
Moringa oleifera	19.65	19.30	19.02	0.3849
Parkinsonia aculeate	19.96	19.42	19.59	0.3941
Jatropha curcas	19.92	19.51	19.32	0.3822
The Total Dissolved Solids mg⁄l (TDS)				**LSD**
Moringa oleifera	468.31	474.81	411.44	49.929
Parkinsonia aculeate	50.01	496.82	440.22	50.292
Jatropha curcas	496.31	475.62	430.50	49.999
The Total Suspended Solids mg⁄l (TSS)				**LSD**
Moringa oleifera	136.11	132.55	119.77	20.683
Parkinsonia aculeate	154.21	150.00	132.9	21.036
Jatropha curcas	196.31	165.45	15.40	23.101

**Mean values for three replicates, seeds coagulant concentrations (C1:5 g/L, C2: 10 g/L, C3: 20 g/L of seeds powder)*

3.1.2. Water turbidity and electrical conductivity

The turbidity measured for water samples from the decanted water stage of clarification water treated with three different coagulants of *M. oleifera*, *P. aculeate* and *J. curcas* seed powder under four settling times were presented in **Tables (2 and 5)**, which indicated that the settling time T1 (control) gave the highest turbidity (23.63 Nephelometric Turbidity Unit (NTU), 29.79 NTU and 31.29 NTU for *M. oleifera*, *P. aculeate* and *J. curcas* seed powder, respectively).

On the other hand, the lowest turbidity was achieved by settling time T4 (48 hrs), since it displayed 4.29 (NTU), 9.42 (NTU) and 8.72 (NTU) for *M. oleifera*, *P. aculeate* and *J. curcas* seed powder, respectively. **Table (5)** revealed the highly significant difference between doses at four settling time. The lowest turbidity at settling time T4 (48 hrs) with 20 g/l of powder processed from *M. oleifera*, *P. aculeate* and *J. curcas* seed powder (C3) was 2.92 (NTU), 5.52 (NTU), and 4.93 (NTU), respectively.

Table (4) A Two-way ANOVA (LSD $_{0.05}$) showed the effect of the interaction between the two factors (treatment times and coagulants concentrations) on the Color (Hazen unit) and pH.

Settling time	Color (Hazen unit)								
	Moringa oleifera			*Parkinsonia aculeate*			*Jatropha curcas*		
	C1	C2	C3	C1	C2	C3	C1	C2	C3
T1	40.5	43.2	37.9	43.76	42.38	40.31	44.77	43.52	40.98
T2	30.12	19.23	14.24	33.91	25.79	19.8	35.42	26.11	20.92
T3	12.51	9.81	8.74	15.92	10.98	9.73	16.76	11.71	9.98
T4	6.97	5.92	4.51	10.79	9.59	8.15	11.71	10.47	9.81
$LSD_{0.05}$		4.193			4.915			5.013	
	pH								
T1	8.73	8.79	8.81	8.97	8.71	8.82	8.92	8.91	8.74
T2	7.39	7.15	6.81	7.93	7.54	7.01	7.99	7.74	7.37
T3	7.44	7.27	7.14	7.74	7.52	7.30	7.84	7.71	7.39
T4	7.36	7.15	7.32	7.51	7.30	7.33	7.63	7.52	7.33
$LSD_{0.05}$		0.5579			0.5699			0.5797	

***T1**:0-hour control(immediately), **T2**:12 hrs, **T3**: 24, and **T4**: 48 hrs of settling time and **C1**=5 g/l, **C2**= 10 g/l, and **C3** = 20 g/l plants seed powder.* $LSD_{0.05}$ mentioned as interaction between time and concentration

The electrical conductivity was measured for decanted water samples of clarification water treated with three different coagulants of *M. oleifera*, *P. aculeate* and *J. curcas* seed powder under four settling times. **Table (5)** showed the ANOVA, which revealed that the differences between treatments for electrical conductivity were significant. The lowest electrical conductivity was 432.93 µS⁄ cm, 443.95 µS⁄ cm and 440.11 µS⁄ cm with treatment T4 of the seed powder processed from *M. oleifera*, *P. aculeate* and *J. curcas*. From **Table (3**), it is clear that the dose of 5 g/l (C1) recorded the highest electrical conductivity compared with the dose (C3) of *M. oleifera* seed powder. As for the other two species, they gave the same direction. ANONA revealed that there were no significant differences between doses and settling time, **Table (5).**

3.1.3. Temperature and total dissolved solids (TDS) of water

The temperature was measured for water samples from decanted water. **Tables (2 and 6)** show the water treated with three different coagulants of *M. oleifera*, *P. aculeate* and *J. curcas* seed powder under four settling times. The results showed that the temperature was 19.51 oC, 19.24 oC, 19.50 oC and 18.99 oC for T1, T2, T3 and T4, respectively, for *P. aculeate* seeds powder. Like for the other two species, it gave the same direction **(Table 2)**. From **Tables (3 and 6),** it was clear that the dose of 0.5 g/l (C1) recorded the highest temperature compared with the dose (C3) of *M. oleifera* seed powder. As for the other two species, they gave the same direction.

The data presented in **Table (6)** showed that the settling time T1 (0 hrs) with 5g/l of powder processed from *M. oleifera* (C1) gave the highest temperature 20.10 ^{o}C, on the other hand, the lowest temperature with the highest dose (20 g/l) at settling time T4 (48 hrs) was 19.7 ^{o}C. Also, it was noticed that the highest dose of *P. aculeate* seeds powder at settling time T4 (48 hrs) gave the lowest temperature was 19.45 ^{o}C, While the highest temperature was achieved by settling time T1 (control) with dose (C1) with a value of 20.22 ^{o}C. The results showed that the seed power from *J. curcas* gave the highest temperature at settling time T1 (0 control) with dose (C1), it was 21.11 ^{o}C. However settling time (12 hrs) at dose 10 g/l (C2) was 19.79 ^{o}C. On the other hand, the lowest temperature was achieved by settling time T4 (48 hrs) with a dose of 20 g/l (C3) with a value of 19.41 ^{o}C **(Table 6)**.

Table (5) A Two-way ANOVA (LSD $_{0.05}$) showed the effect of the interaction between the two factors (treatment times and coagulants concentrations) on the Nephelometric Turbidity Unit (NTU) and Electrical Conductivity (µS⁄cm)**.**

Settling time	Nephelometric Turbidity Unit (NTU)								
	Moringa oleifera			***Parkinsonia aculeate***			***Jatropha curcas***		
	C1	**C2**	**C3**	**C1**	**C2**	**C3**	**C1**	**C2**	**C3**
T1	21.18	22.7	27.17	27.82	24.77	29.14	24.1	22.2	21.41
T2	14.43	11.38	9.59	19.34	15.94	12.92	20.21	15.44	13.11
T3	9.36	3.64	6.46	14.66	10.94	7.71	15.6	11.19	8.38
T4	6.01	3.95	2.92	10.01	7.33	5.52	9.91	6.92	4.93
$LSD_{0.05}$		**0.7697**			**0.7922**			**0.7969**	
	Electrical conductivity (µS⁄cm)								
T1	1087.5	1129.7	1154.8	1198.4	1179.2	1152.1	1189.4	1172.5	1140.3
T2	659.32	615.78	550.1	869.21	815.92	791.1	894.00	842.50	807.72
T3	521.3	515.6	497.22	626.59	604.71	574.34	634.6	569.41	579.75
T4	443.6	436.89	418.21	596.74	579.99	549.11	571.43	549.92	509.71
$LSD_{0.05}$		**95.22**			**96.66**			**96.97**	

****T1**:0-hour control(immediately), **T2**:12 hrs, **T3**: 24, and **T4**: 48 hrs of settling time and **C1**=5 g/l, **C2**= 10 g/l, and **C3** = 20 g/l plants seed powder. $LSD_{0.05}$ mentioned as interaction between time and concentration*

The TDS was measured for water samples from decanted water. Water treated with three different coagulants of *M. oleifera*, *P. aculeate* and *J. curcas* seeds powder under four settling times were presented in **Tables (2 and 6)**. The results of the analysis of variance revealed a highly significant difference between treatments.

It was found the TDS were 963.15 mg/l, 448.78 mg/l, 251.74 mg/l and 142.41 mg/l for treatments T1, T2, T3, and T4, respectively, for *M. oleifera* seed powder **(Table 2)**. From **Table (3)**, it was clear that the dose of 5 g/l (C1) recorded the highest TDS compared with the dose (C3) of *M. oleifera* seed powder.

The data presented in **Table (6)** showed that the settling time T1 (0 hrs) with 5 g/l of powder processed from *M. oleifera* (C1) gave the highest TDS of 892.9 mg/l. On the other hand, the lowest TDS with the highest dose (20 g/l) at settling time T4 (48 hr) was 130.5 mg/l. It was noticed that the highest dose of *P. aculeate* seeds powder at settling time T4 (48 hrs) gave the

lowest TDS was 195.7 mg/l. While the highest TDS was achieved by settling time T1 (control) with dose (C1) with a value of 1141.7 mg/l. The results showed that the seed power from *J. curcas* gave the highest TDS at settling time T1 (0 hr) with a dose (C1). It was 1096.71 mg/l. On the other hand, the lowest TDS was achieved by settling time T4 (48 hrs) with a dose 20 g/l (C3) with a value of 179.54 mg/l **(Table 6).**

Table (6) A Two-way ANOVA (LSD $_{0.05}$) showed the effect of the interaction between the two factors (treatment times and coagulants concentrations) on the temperature (^{o}C) and total dissolved solids mg/l (TDS).

Settling time	Temperature (°C)								
	Moringa oleifera			***Parkinsonia aculeate***			***Jatropha curcas***		
	C1	**C2**	**C3**	**C1**	**C2**	**C3**	**C1**	**C2**	**C3**
T1	20.10	19.97	19.00	20.22	20.4	19.79	21.11	20.97	19.99
T2	19.15	19.13	19.01	19.54	19.72	19.54.	19.77	19.79	19.54
T3	19.81	19.65	19.30	19.94	19.75	19.51	19.71	19.42	19.33
T4	19.53	19.73	19.7	19.91	19.74	19.45	19.71	19.55	19.41
$LSD_{0.05}$		**0.7697**			**0.7922**			**0.7969**	
	Total Dissolved solids mg/l (TDS)								
T1	892.9	1052.9	974.9	1141.7	992.13	977.4	1096.7	1007.41.9	979.9
T2	519.9	485.1	356.7	696.42	572.71	488.1	689.33	654.31	492.92
T3	307.77	239.9	193.7	513.15	409.92	354.92	533.9	429.2	370.14
T4	176.5	132.5	130.4	301.21	229.3	195.7	298.7	218.4	179.54
$LSD_{0.05}$		**99.86**			**99.98**			**101.74**	

***T1**:0-hour control(immediately), **T2**:12 hrs, **T3**: 24, and **T4**: 48 hrs of settling time and **C1**=5 g/l, **C2**= 10 g/l, and **C3** = 20 g/l plants seeds powder. $LSD_{0.05}$ mentioned as interaction between time and concentration*

3.1.4. Total suspended solids (TSS)

The TSS measured for water samples from the decanted water stage of clarification, the water treated with three different coagulants of *M. oleifera*, *P. aculeate* and *J. curcas* seed powder under four settling time was presented in **Tables (2)** and **(7)**. Mean values presented in **Table (2)** indicated that the settling time T1 (control) gave the highest TSS (273.70 mg/l, 297.7 mg/l and 270.1 mg/l for *M. oleifera*, *P. aculeate* and *J. curcas* seeds powder, respectively). On the other hand, the lowest TSS was achieved by settling time T4 (48 hrs). The highest TSS was obtained from 5 g/l of powder processed from *M. oleifera*, *P. aculeate* and *J. curcas* (C1) at 136.11 mg/l, 154.21 mg/l, and 196.31 mg/l, respectively **(Table 3)**. Furthermore, **Table (7)** revealed that the highly significant interaction between doses occurred at four settling times. The highest TSS was obtained from 5 g/l of powder processed from *M. oleifera*, *P. aculeate* and *J. curcas* (C1) at settling time T1 (control), and they were 222.9 mg/l, 291.21 mg/l, and 315.67 mg/l, respectively. The lowest TSS at settling time T4 (48 hr) with 20 g/l of powder processed from *M. oleifera*, *P. aculeate*, and *J. curcas* seed powder (C3) were 32.9 mg/l, 44.1 mg/l, and 49.72 mg/l, respectively.

3.1.5. Water total bacterial count and total coliform count (CFU)

The CFU measured for water samples from the decanted water stage of clarification water treated with three different doses of *M. oleifera*, *P. aculeate* and *J. curcas* seed powder under four settling times was presented in **(Table 8)**. Moreover, the data illustrated that the settling time

T1 (control) gave the highest CFU of 232.48×10^3 cfu⁄ ml, 301×10^3 cfu⁄ ml and 297.71×10^3 cfu⁄ ml for *M. oleifera*, *P. aculeate* and *J. curcas* seed powder, respectively. On the other hand, the lowest CFU was achieved by settling time T4 (48 hrs), since it displayed 18.6×10^2 cfu⁄ ml, 69.41×10^2 cfu⁄ ml and 50.43×10^2 cfu⁄ ml for *M. oleifera*, *P. aculeate* and *J. curcas* seeds powder, respectively. The highest CFU was obtained from 5 g/l of powder processed from *M. oleifera*, *P. aculeate* and *J. curcas* (C1), which were 141.52×10^3 cfu⁄ ml, 155.59×10^3 cfu⁄ ml and 149.97×10^3 cfu⁄ ml, respectively.

Table (7) A Two-way ANOVA ($LSD_{0.05}$) showed the effect of the interaction between the two factors (treatment time and coagulant concentrations) on the total suspended solids (TSS) mg⁄ l.

Settling time	Total Suspended Solids (TSS) mg⁄ l								
	Moringa oleifera			***Parkinsonia aculeate***			***Jatropha curcas***		
	C1	**C2**	**C3**	**C1**	**C2**	**C3**	**C1**	**C2**	**C3**
T1	222.9	278.9	300.7	291.21	311.91	274.99	315.67	294.11	284.9
T2	187.4	118.6	85.9	254.11	205.61	197.8	261.11	210.74	174.33
T3	76.9	65.11	60.00	99.16	83.11	79.71	102.74	79.11	64.21
T4	59.1	60.5	32.9	79.71	54.33	44.1	84.71	57.44	49.72
$LSD_{0.05}$		**40.17**			**41.97**			**42.02**	

***T1**:0-hour control(immediately), **T2**:12 hrs, **T3**: 24, and **T4**: 48 hrs of settling time and **C1**=5 g/l, **C2**= 10 g/l, and **C3** = 20 g/l plants seeds powder. $LSD_{0.05}$ mentioned as interaction between time and concentration*

Table (8) A Two-way ANOVA ($LSD_{0.05}$) showed the effect of the interaction between the two factors (treatment time and coagulant concentrations) on total coliform count (cfu⁄ ml) and total bacterial count (cfu⁄ ml) with the two different media.

Settling time	Total coliform count (cfu⁄ ml) with 10^{-2} dilution								
	Moringa oleifera			***Parkinsonia aculeate***			***Jatropha curcas***		
	C1	**C2**	**C3**	**C1**	**C2**	**C3**	**C1**	**C2**	**C3**
T1	364.6×10^2	334.1×10^2	384.3×10^2	372.4×10^2	391.3×10^2	296.7×10^2	361.5×10^2	334.1×10^2	356.7×10^2
T2	213.7×10^2	265.1×10^2	236.4×10^2	351.7×10^2	324.1×10^2	287.9×10^2	200.1×10^2	208.1×10^2	311.9×10^2
T3	220.8×10^2	87.5×10^2	70.2×10^2	258.7×10^2	221.1×10^2	101.7×10^2	203.7×10^2	245.3×10^2	214.4×10^2
T4	37.8×10^2	28.3×10^2	21.1×10^2	88.2×10^2	43.7×10^2	36.1×10^2	55.1×10^2	45.1×10^2	32.4×10^2
LSl		**23.54**			**13.87**			**14.36**	
	Total bacterial count (cfu⁄ml) with 10^{-3} dilution								
T1	253.5×10^3	223.0×10^3	273.0×10^3	262.4×10^3	294.3×10^3	256.7×10^3	267.5×10^3	243.1×10^3	269.7×10^3
T2	192.1×10^3	154.0×10^3	125.6×10^3	241.7×10^3	242.1×10^3	205.9×10^3	214.1×10^3	197.1×10^3	201.9×10^3
T3	109.8×10^3	76.5×10^3	59.11×10^3	149.7×10^3	112.1×10^3	96.7×10^3	196.7×10^3	154.3×10^3	119.4×10^3
T4	26.9×10^3	17.8×10^3	10.5×10^3	57.2×10^3	33.7×10^3	28.1×10^3	48.1×10^3	34.1×10^3	21.4×10^3
$LSD_{0.05}$		**11.36**			**12.79**			**12.94**	

***T1**:0-hour control(immediately), **T2**:12 hrs, **T3**: 24, and **T4**: 48 hrs of settling time and **C1**=5 g/l, **C2**= 10 g/l, and **C3** = 20 g/l plants seeds powder. $LSD_{0.05}$ mentioned as interaction between time and concentration*

Table (8) revealed the highly significant difference between doses at four settling times. The highest CFU was obtained from 5 g/l of powder processed from *M. oleifera*, *P. aculeate* and *J. curcas* (C1) at settling time T1(control), and were 253.5×10^3 cfu⁄ ml, 273.00×10^3 cfu⁄ ml and

267.15×10^3 cfu⁄ ml, respectively. The lowest CFU at settling time T4 (48hr) with 20 g/l of powder processed from *M. oleifera*, *P. aculeate* and *J. curcas* seed powder (C3) were 10.52×10^3 cfu⁄ ml, 55.15×10^3 cfu⁄ ml and 49.17×10^3 cfu⁄ ml, respectively. Furthermore the total coliform detected the highest total bacterial count in water samples treated with *Parkinsonia aculeate* tree as compared with the two other trees; it was 391.3×10^2 at the second concentration. The reductions of total coliforms and total bacterial count in both media in raw samples at zero-time treatment coagulants, the results are shown in **Figures (2, 3, 4, 5**). Very significant removal of total bacterial count and coliforms in the two different media was found after treatment with natural coagulants. The best reduction percentage occurred in the third concentration (20 g/l plant seed powder) for the three-tree coagulant and was increased by the increasement of the sitting time in **Figures (2 and 3)**. Furthermore, the *Jatropha curcas* coagulant had the most effective bacterial inhibition **(Table 8)**.

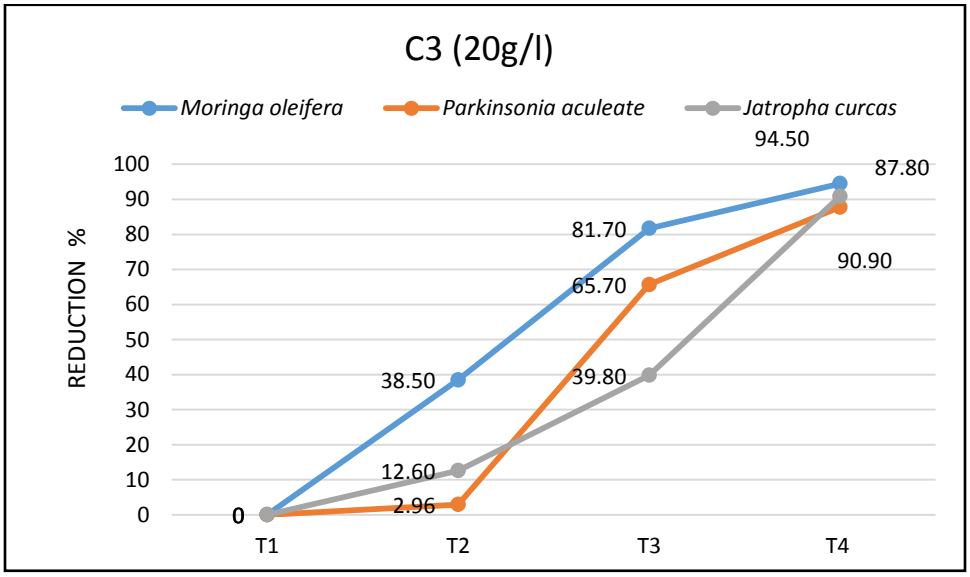

Figure (2) For the three materials, the reduction percentage (%) for total coliform on Red Violet Broth Agar (RVBA media) within time at treatment was 20 g/l plant seed powder

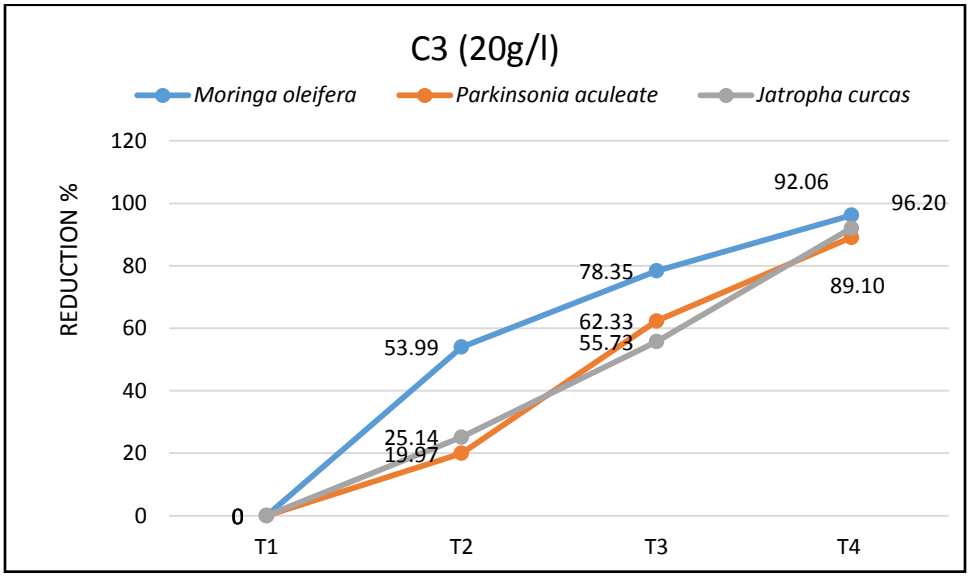

Figure (3) For the three materials, the reduction percentage (%) for total coliform on Red Violet Broth Agar (RVBA media) within time at treatment was 20 g/l plant seed powder

A **B**

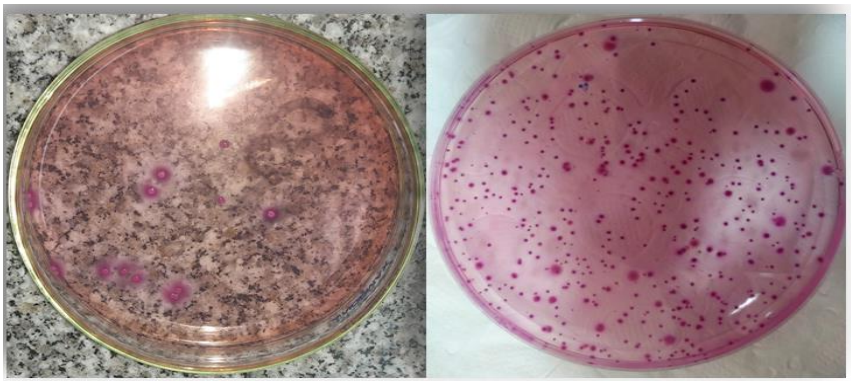

Figure (4) The total coliforms count(cfu/ml) in Red Violet Broth Agar (RVBA) **(A:** plats after treatment and**, B:** control**)** at treatment 20 g/l *Moringa oleifer*a seeds powder **(C3)**

A **B**

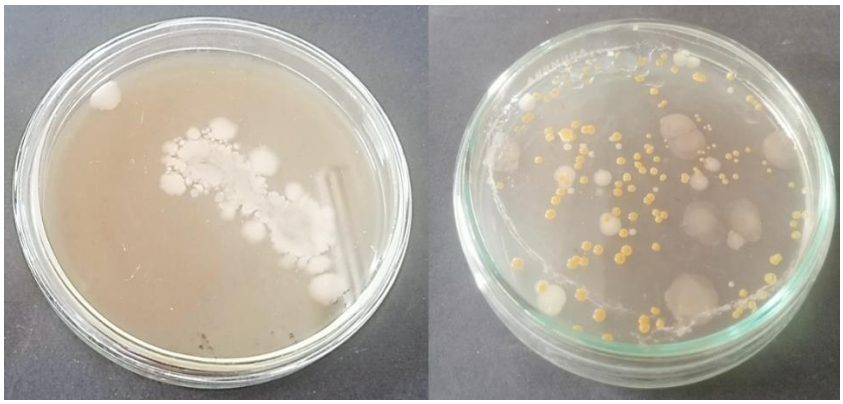

Figure (5) The total bacterial count (cfu/ml) in nutrient agar plants **(A:** plats after treatment and**, B:** control**)** at treatment 20 g/l *Moringa oleifera* seeds powder **(C3)**

4. DISCUSSION

The results obtained from the effect of coagulant concentration and settling time of clarification water treated with *M. oleifera P. aculeate* and *J. curcas* seeds powder on parameters of color, pH, turbidity, electrical conductivity, temperature, total dissolved solids (TDS), total suspended solids (TSS), and total coliform count are generally in agreement with the results of **Olayemi *et al.*, (1994),** who examined the efficacy of *M. oleifera Lam* seeds paste for water purification. According to chemical analysis, seeds contain 34.1% protein, 15% carbohydrates, and 15.5% lipids. Physiochemical tests and spectral studies led to the elucidation of asteroidal glycoside-strophantidin as a bioactive agent in the clarification and sedimentation of inorganic and glycoside-strophantidin as a bioactive agent in the seed. It reduced the total microbial and coliform counts by 55% and 65%, respectively, after 24 hours. Also**, Ndabigengesere and Narasiah (1998)** showed that the seeds contain water soluble positively charged proteins that act as an effective coagulant. However, the crude *M. oleifera* extract (though efficient in the removal of turbidity) increased the organic load in the treated water. While, **Moramudaii *et al.*, (2001)** showed that mature seed powder at 50 mg/20 ml reduced the turbidity (NTU) by 95% within 2 hrs. It was also found that extracts of mature *M. oleifera* seeds have the ability to clarify textile

dye solutions. Studies on pH and conductivity showed that pH is slightly reduced and electrical conductivity is rapidly increased when they were treated with *M. oleifera* seeds, whilst electrical conductivity was found to be dependent on treatment time and temperature. Mature seed extracts of *M. oleifera* significantly reduced the bacterial growth in polluted waters. An anti-microbial activity was found in crude aqueous extracts of *M. oleifera* seeds. **Broin *et al.*, (2002)** reported that the bacteria count in the sludge was reduced significantly with increased *M. oleifera* coagulant dosage. Also, **Oluduro *et al.*, (2007)** showed the efficacy of *M. oleifera* seeds (LAM) in reducing total bacteria and coliforms in raw water. Its antibacterial activity on some selected enteric bacterial pathogens was also investigated. About 88 and 97.5% of the total bacteria and coliforms, respectively, were reduced in the surface water after 24 hrs of treatment. **Pritchard *et al.*, (2009)** showed that the addition of *M. oleifera*, *J. curcas* and Guar gum can considerably improve the quality of shallow well water. Turbidity reduction was higher for more turbid water. A reduction efficiency exceeding 90% was achieved by all three extracts on shallow well water that had a turbidity of 49 NTU. The reduction in coliforms was about 80% for all extracts. The pH of the water samples increased with dosage, but remained within acceptable levels for drinking water for all the extracts. **Bodlund et *al.*, (2014)** evaluated different varieties of mustard seed extracts and cake for their coagulation properties against synthetic clay solution and turbid water from a pond in Chennai, India. The protein content, molecular mass of the protein, and thermo-stability between different mustard types of seed extracts were evaluated. Mass spectrometric analysis was performed to identify the active coagulant protein from mustard seed extract and compared with Moringa coagulant protein. While, **Sivaranjani and Rakshit (2016)** reported that seeds are also used as a primary coagulant in drinking water clarification and wastewater treatment due to the presence of a water-soluble cationic coagulant protein, which is able to reduce the turbidity of the treated water. There are many other species like *Vigna unguiculata*, *Voandzeia subterranea*, *Arachis hypogaea*, *Vicia faba* and *Parkinsonia aculeata*, that are also used for the purification of water for drinking purposes. On the other hand, the results agree with **García *et al.*, (2018)** who reported that natural coagulants as an alternative to chemical coagulants, conducting a comprehensive review of the most researched and their potential application in the treatment of drinking water. Also**, Vunain and Biswick (2019)** studied *M. oleifera* seeds, *J. curcas* seeds, *Cassava peels*, potato peels, and rice husks as agro-wastes were selected as coagulants in water clarification. *Jatropha curcas* extract was mainly made up of proteins with molecular weights of between 20 and 35 kDa. The optimized extraction conditions significantly improved the efficiency of this promising bio-derived coagulant in turbidity reduction. This study demonstrates the potential employability of these enhanced bio-coagulants **(Khodapanah *et al.*, 2018)**. According to **Onyebuchi *et al.*, (2020),** the coagulant aids or primary coagulants in the treatment of drinking water. Inorganic polymers possess high molecular weight. They are usually known as polyelectrolytes, in aqueous suspension, they absorb small particles easily. Organic polymers are generally classified as anionic, cationic, and nonionic. However, **Joel *et al.*, (2021)** investigated *J. curcas* and *M. oleifera* seeds as plant-based coagulants for turbidity and microbial load removal from sewage wastewater. In contrast to *Moringa oleifera*, which increased microbial load content, their findings showed that the microbiological load in wastewater cleared with *Jatropha curcas* and rice husk ashes was completely reduced. Our results aren't in harmony with that study because we recoded the lowest bacterial load with *Moringa oleifera* water treatment. The results of this study have demonstrated that plant-based materials used performed effectively in turbidity and bacteria removal from sewage wastewater.The reduction of total bacteria count on NA media in this experiment ranged from 96.4% to 92.06% and 89.1% using *Moringa oleifera*, *Jatropha curcas*, and *Parkinsonia*

aculeate, respectively **(Figure 3 and 5)**. Also, the highest reduction percentage (%) for the total coliform on Red Violet Broth Agar (RVBA media) within time at treatment of 20 g/l *Moringa oleifera* seed powder was represented in **Figures (2 and 4).** A previous study by **Suarez *et al.*, (2003)** demonstrated the ability of a recombinant *Moringa oleifera* protein to decrease the viability of Gram negative or Gram-positive bacterial cells. A study conducted by **Michael, (2010)** showed that efficient reduction (80% to 99.5%) of high-turbidity pathogenic surface water may be an indication of the bactericidal activity of these natural coagulants. Moreover, **Asrafuzzaman *et al.*, (2011)** recorded about 89 to 96% total coliform reduction with natural products such as *Moringa oleifera, Cicer arietinum, and Dolichos lablab* coagulant treatment of turbid water. Also, according to the **(WHO, 2011)**, *E. coli* is the preferred criteria for assessing the quality of drinking water. Also, drinking water should not contain any bacteria indicative of pollution caused by *Pseudomonas* sp. The total bacterial count should not exceed 500 CFU (colony-forming unit) per mL.

5-Conclusion

The high cost of chemical-treated water makes most people in rural communities resort to any readily available sources, which are normally of low quality, exposing them to water-borne diseases. Therefore, the main objective of this experiment is to improve drinking water quality and decrease harmful bacteria by using safe materials such as *Moronga oleifera*, *Parkinsonia aculeante*, and *Jatropha curcas*. In light of that research, the recorded data confirmed that the high doses of the natural powder have effectiveness for water clarification.

Authors' contributions

This work was carried out in collaboration between both authors. Authors Pousy Ali Salaheldin and Mayada Ali Sabra helped in data curation, method preparation, investigation, literature searches and statistical interpretation. Author Pousy Ali Salaheldin supervised the study. Authors Pousy Ali Salaheldin and Mayada Ali Sabra wrote the original draft of the manuscript. Both authors read and approved the final manuscript.

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