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Antibacterial Efficacy of *Moringa oleifera* Seeds for Water Purification

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ABSTRACT

This study investigated the antibacterial efficacy of *Moringa oleifera (M.oleifera*) seeds as a natural coagulant for water purification, addressing the critical need for clean water due to its significant impact on human health and disease prevalence linked to contaminated water. The research was carried out to observe the effect of *M. oleifera* on the microbial load of Raw Water (RW) and treated water (TW) sourced from the Ibn Ziad wastewater treatment facility in Constantine. Results of bacteriological analyses show that the raw water is heavily polluted with high levels of the targeted bacteria: 4.4×10^9 CFU/ml in total germs, 2.4×10^4 CFU/100 ml in total coliforms and total streptococci, and 1.1×10^4 CFU/100ml in fecal coliforms, *Escherichia coli*, and fecal streptococci, with no decrease observed for sulfite-reducing clostridia. However, the water treated with *M. oleifera,* at a dose of 20 $g \cdot L^{-1}$, demonstrates a significant reduction of 99% in total germs, 96% in total coliforms, and 98% in fecal coliforms, with a complete absence of *Escherichia coli*, streptococci, as well as a complete elimination of total sulfite-reducing clostridia, which falls with the standard guidelines for using this water in irrigation. The obtained results confirmed that *M. oleifera* seeds positively impact the reduction of pathogenic microorganisms found in wastewater.

Keywords: antibacterial activity, microbes, *Moringa oleifera,* seeds, water purification.

INTRODUCTION

Water plays a crucial role in all aspects of human life, industrial processes, and agriculture, all of which are increasingly strained by socio-economic development and a growing global population. The World Health Organization estimates that up to 80% of diseases and illnesses worldwide are linked to inadequate sanitation, unsafe water, or water scarcity (Delelegn et al., 2018; Kenea et al., 2023). Contaminated water contains various water-borne diseases like diarrhoea, typhoid, cholera, amoebic dysentery, giardiasis and certain neurological disorders. It has been estimated that about 6 million children die from diarrhoea every year in developing countries. This alarming situation is essentially due to the lack of effective processes (WHO, 2015).

Coagulation-flocculation is an essential technique for removing turbidity from water, which consists of suspended particles (both inorganic and organic), dissolved organic materials, and pathogenic microorganisms, including bacteria, viruses, and parasites. This critical process is the first step in conventional water treatment and typically uses aluminum sulfate as the coagulant (Degremont, 2005). In addition to aluminum sulfate, inorganic coagulants such as ferric salts and polyaluminum chloride are frequently employed in wastewater treatment plants for coagulation. However, these synthetic coagulants have notable drawbacks, including the production of inorganic sludge that necessitates complex and costly management, as well as significant variations in pH following treatment (Andrade et al., 2021). The use of natural coagulants, such as *Moringa oleifera* seeds, emerges as a promising solution for treating wastewater before its reuse for irrigation. This plant, originally from Asia, has now spread widely across Africa (Yamaguchi et al., 2021), attributed to its drought resistance, rapid growth, and nutritional value, making Moringa increasingly cultivated. *M. oleifera* roots, leaves, seed, fruit, fowers, bark and immature pods are used as

cardiac and circulatory stimulants, have antipyretic, antiepileptic, antitumor, antiinfammatory, antiulcer, diuretic, antihypertensive, cholesterol lowering, antispasmodic, antidiabetic, hepatoprotective, antioxidant, antibacterial and antifungal properties, and are being used for the treatment of various ailments in the indigenous system of medicine (Anwar et al., 2007). Several studies have investigated the efficacy of *M. oleifera* seeds in wastewater treatment. Research has demonstrated that the seed extracts can significantly reduce bacterial load in wastewater, thereby improving the overall water quality (Ghebremichael et al., 2005; Anwar et al., 2007; Shebek et al., 2015, Al-Kindi and Al-Haidri 2021; Freire et al., 2015; Yamaguchi et al., 2021; Shah et al., 2023). Therefore, the aim of this study was to evaluate the antibacterial efficacy of *M.oleifera* seeds in wastewater treatment, focusing on various bacteriological indicators.

MATERIALS AND METHODS

Sample preparation

The Ibn Ziad wastewater treatment plant is located 12 km from the city of Constantine, in the Hamma Bouziane district, along the road to Mila; it covers an area of 12 hectares (Figure 1). Tests were conducted on two types of water: raw wastewater and treated wastewater. These samples were sourced from the Iben Ziad wastewater treatment plant in the Constantine province (Algeria). Water samples were collected from both the inlet and outlet of the plant using sterile 1-liter glass bottles with stoppers. The most commonly employed sampling method involves completely filling the bottles without shaking them, while minimizing contact with air as much as possible. This process adheres to the guidelines outlined in ISO 5667-1:2006, which provides international recommendations for the microbiological analysis of wastewater.

Plant material

The mature seeds of *M.oleifera* obtained from southern Algeria (Ghardaia nursery) were deshelled and dried at ambient temperatures (25 °C) for 5 days before milling. The white kernels were ground into using a mortar and pestle and then passed through a 2 mm sieve to obtain the fine powder. Two grams of this fine seed powder was added to 100 mL of distilled water. The obtained mixture was agitated for 1 hour to extract the coagulant used to treat the waters and filtered using Whatman N°1 before using. The filtrate was shaken again (Ndabigengesere et al., 1995; Shahzad et al., 2014).

Decimal dilutions

The selection of the number of dilutions largely depends on the microbial load of the water being analyzed. According to Dellaras (2010), the procedure for preparing decimal dilutions is as follows: 10 ml of the water sample is aseptically introduced into a glass bottle containing 90

Figure 1. Study site and location of sampling station

ml of sterile distilled water, using a graduated pipette. Once the bottle is closed, it is shaken to ensure thorough mixing, resulting in a 1/10 dilution. Then, 10 ml from this 10^1 dilution is taken and transferred to a second bottle to produce a 10^2 dilution. This process should be repeated until the desired dilution is achieved.

Bacteriological analyses

Bacteriological quality is one of the important parameters of water potability. It is measured by the presence of a pollution indicator of organisms, in particular, total germs and fecal coliforms (*Escherichia coli*). The bacteriological analyses of raw wastewater, treated wastewater, and wastewater treated with *M.oleifera* were conducted using the most probable number method, which aims to detect and quantify the following germs: total germs (TG), total coliforms (TC), fecal coliforms (FC) and *Escherichia Coli*, total streptococci (TS), fecal streptococci (FS) and sulfite-reducing clostridia (SRC). The method used to isolate and determine these bacteria is the NPP liquid count technique in line with the AFNOR NF T90-413 standard (1985).

Total germs

This measure provides an overall assessment of the harmful nature of the water, without identifying the specific sources of contamination. *E. coli* is a key bacterium used to indicate fecal contamination (Edberg et al., 2000). Both total bacteria and *E. coli* serve as indicators to assess pollution levels and water quality (Adamou et al., 2020).

To assess total bacterial counts from the water sample, 1 ml was transferred into two prepared and numbered Petri dishes. Each dish was then filled with approximately 15 ml of nutrient agar and gently mixed by rotating before allowing it to solidify. The prepared dishes were incubated under two different conditions: one at 37 °C for 24 to 48 hours and the other at 22 °C for 72 hours. The results are expressed as the number of germs per milliliter (ISO 6222:1999) (ISO 1999).

Total coliforms

Total coliforms are quantified according to ISO 9308 (2012). From the water sample to be analyzed (mother solution or the three successive dilutions), the following are aseptically transferred:

- 10 ml into three tubes containing 10 ml of BCPL D/C medium.
- 1 ml into three tubes containing 10 ml of BCPL S/C medium.
- 0.1 ml into three tubes containing 10 ml of BCPL S/C medium.

After transferring the samples, the tubes were gently shaken to homogenize the contents without introducing air into the Durham tube. All prepared tubes were then incubated at 37 °C for 24 to 48 hours. Following incubation, the tubes that are considered positive showed turbidity, with a color change from violet to yellow, and gas production in the Durham tube (ISO 2012).

Fecal coliforms and Escherichia coli

Fecal coliforms and *E. coli* were quantified following the ISO 9308 (2012) standard. Positive BCPL tubes identified during coliform enumeration were subcultured by transferring a loopful into a tube containing Schubert medium with a Durham tube. The gas in the Durham tube was expelled, and the medium was thoroughly mixed. The tubes were then incubated at 44 °C for 24 to 48 hours. Tubes showing both bacterial growth and gas production were deemed positive. The presence of *Escherichia coli* was confirmed by the formation of a red ring at the surface after adding 2 to 3 drops of Kovacs reagent (ISO 2012).

Total streptococci

To assess total streptococci counts from the water sample, 10 ml was aseptically transferred into three tubes containing 10 ml of Rothe D/C medium. Additionally, 1 ml was transferred into three tubes containing 10 ml of Rothe S/C medium, and 0.1 ml was also transferred into three tubes containing 10 ml of Rothe S/C medium. The tubes were then shaken to ensure homogenization while avoiding the introduction of air into the Durham tube. Following this, the prepared tubes were incubated at 37°C for 24 to 48 hours. After incubation, the tubes that exhibited microbial turbidity were considered positive 4 (ISO 7899-2 (2012)).

Fecal streptococci

Fecal streptococci were counted following the ISO 7899 (2012) standard: The positive Rothe tubes identified during the streptococci enumeration were subcultured using a loop into a

tube containing LITSKY EVA medium. Any gas present in the Durham tube should be expelled, and the medium should be mixed thoroughly. The tubes were then incubated at 37 °C for 24 hours. Tubes were considered positive if they displayed both microbial turbidity and a violet or whitish disc at the bottom (ISO 2012)

Sulfite reducing clostridia

The enumeration of sulfate-reducing anaerobic bacteria follows a specific procedure as outlined in the NFT 90-415 standard (1985). First, approximately 250 ml of the water sample (whether the original solution or three successive dilutions) were placed into a sterile tube and heated at 75 °C for 15 minutes to destroy any vegetative forms of sulfate-reducing anaerobic bacteria. After heating, the tube was rapidly cooled under running water. The sample was then divided into four sterile tubes, with 5 ml per tube, and 18 to 24 ml of meat-peptone agar is added to each. The tubes were gently mixed to prevent the introduction of air bubbles and oxygen, then allowed to solidify for 30 minutes before being incubated at 37 °C for 48 hours. Observations are made at 24 and 48 hours. Finally, all black colonies were counted, and the total number of colonies from the four tubes was reported per 20 ml of the analyzed water.

Statistical analysis

The data were analyzed using MINITAB 18 software. The analysis was carried out using analysis of variance (ANOVA). For each parameter three experimental replicates were performed, followed by a Fisher comparison of means test at 5% levels. The homogeneous groups were separated using the Newman-Keuls test at 5% levels.

RESULTS

Total germs

The results shown in Figure 2 indicate a high concentration of total germs in the untreated wastewater, with an average of 4.4×10^9 CFU/ml at 22 °C and 2.4×10^8 CFU/ml at 37 °C. Following treatment, this concentration significantly decreased, achieving a removal rate of 99.96% at 22 °C and 99.95% at 37 °C. Treatment with Moringa seeds led to an even greater reduction ($p < 0.001$), with removal rates of 99.99% at 22 °C and 99.97% at 37 °C.

Total coliforms

The results shown in Figure 3 indicate that the total coliform concentrations in the raw water are 2.4×10^4 CFU/100 ml. Following treatment, the effluent showed a highly significant reduction, with coliform levels decreasing to 1.1×10^4 CFU/100 ml, reflecting a 54.16% removal rate. On the other hand, treatment with *M. oleifera* seeds significantly improved removal rates, achieving 80.83% for raw wastewater and 96.35% for treated water.

Fecal coliforms and *Escherichia coli*

The results presented in Figure 3 indicate an average concentration of fecal coliforms of 1.1×10^4 CFU/100 ml in the untreated wastewater. Following

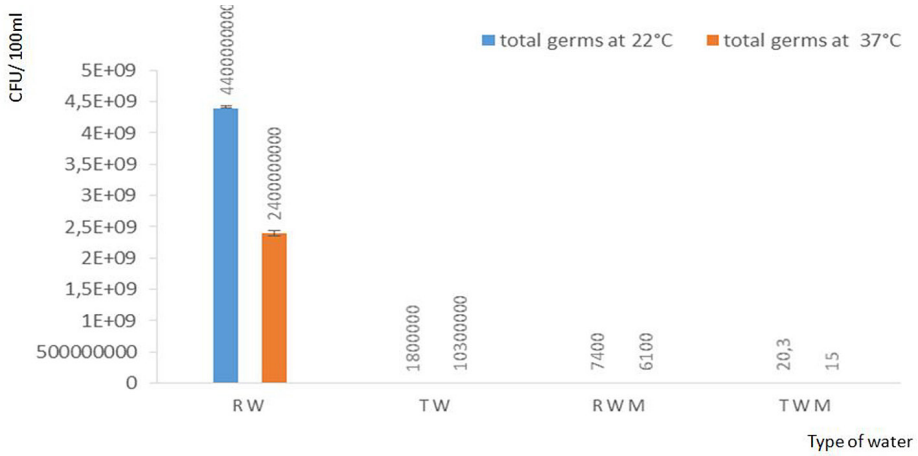

Figure 2. Estimation of total germ counts at 22 °C and 37 °C in raw wastewater (RW) as well as treated wastewater (TW) before and after the addition of *M. oleifera* seeds.

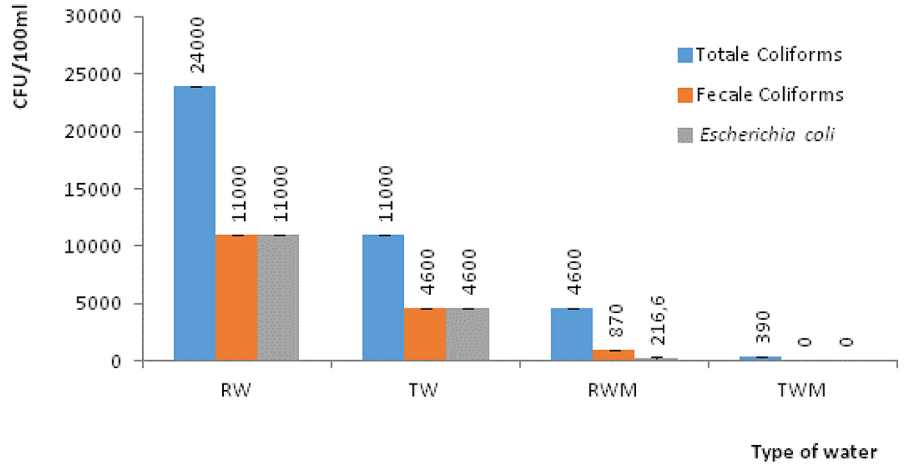

Figure 3. Estimation of total coliforms (TC), fecal coliforms (FC), and *Escherichia coli* in raw wastewater (RW) and treated wastewater (TW) before and after the addition of *M.oleifera* seeds

treatment, there was a significant reduction, achieving a removal rate of 58.18%. In contrast, the water treated with *M. oleifera* seeds demonstrated significantly higher fecal coliform removal rates ($p < 0.001$); the removal rate in raw water was 92.09%, while in treated water, it increased to 97.10%.

Bacteriological analyses of *Escherichia coli* indicated elevated levels at the entrance of the wastewater treatment plant, with an average concentration of 1.1×10^4 CFU/100 ml. The reduction in fecal coliforms was statistically significant at the 5% level, showing a decrease of 58.18%, with concentrations around 4.6×10^3 CFU/100 ml, which still exceeded acceptable limits for safe reuse (Blumenthal et al. 2000). In contrast, the treatment with *M.oleifera* seeds exhibited exceptionally high reduction rates, achieving a remarkable 98.03% reduction (2.16×10^2 CFU/100 ml) at the inlet and a complete elimination (100%) at the outlet.

Streptococci

The analyses indicate relatively high concentrations of total streptococci at the entrance of the wastewater treatment plant, measuring 2.4×10^4 CFU/100 ml. In contrast, fecal streptococci concentrations are lower, approximately 1.1×10^4 CFU/100 ml in the raw water. Following treatment, these concentrations decrease, with a reduction rate of 54.16% for total streptococci and 58.18% for fecal streptococci (Figure 4). Notably, the treatment with *M.oleifera* seeds results in the complete elimination of these microorganisms, achieving a 100% reduction rate.

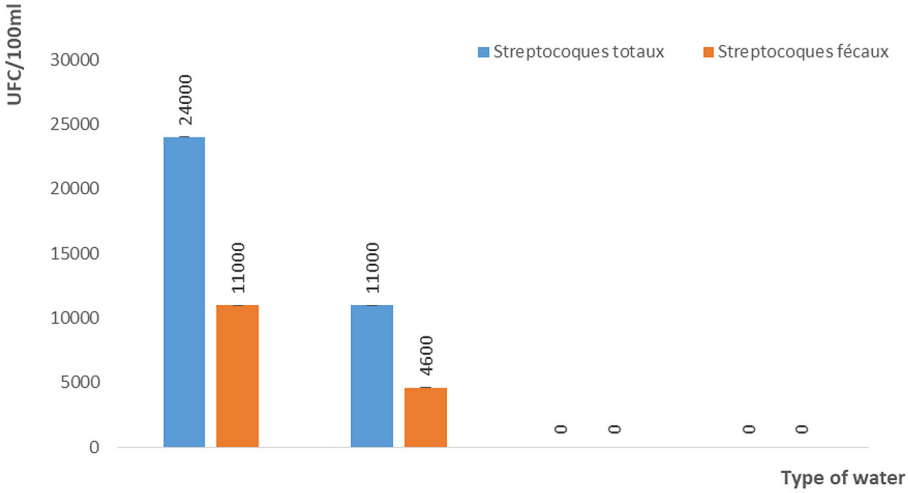

Figure 4. Estimation of total streptococci (TS) and fecal streptococci (FS) in raw wastewater (RW) and treated wastewater (TW) before and after the addition of *M.oleifera* seeds

Sulfite-reducing clostridia

The enumeration results (Table 1) indicate that sulfite-reducing clostridia concentrations are significantly elevated at both the inlet and outlet of the wastewater treatment facility. Interestingly, the results from both raw and treated wastewater samples treated with *M.oleifera* seeds demonstrated complete elimination of these spores, achieving a 100% reduction rate.

DISCUSSION

Results of bacteriological analyses indicate that raw wastewater is heavily polluted with high levels of various indicator bacteria, such: total germs, total and fecal coliforms, *Escherichia coli*, total streptococci, and sulfite-reducing clostridia (Figure 5) signaling significant contamination. The very high concentrations of these indicators are due to the abundance of nutrients, sufficient dissolved oxygen, alkaline pH, and moderate temperatures, all of which create ideal conditions for bacterial growth and proliferation (Smith et al., 2022).

After conventional treatment, all measured parameters showed significant reductions; however, they still remained above the acceptable limits for safe reuse in irrigation (Blumenthal et al., 2000). In contrast, the water treated with *M.oleifera* seeds exhibited even greater reductions and in accordance with standard norms (Blumenthal

Table 1. Enumeration of sulfite-reducing clostridia

Parameter	Types of water			
	RW	TW	RWM	TWM
SRC (spores/20 ml)	Innumerable	Innumerable	0	0

Note: RW – raw water, TW – treated water, RWM – raw water treated with MO seeds, TWM – treated water treated with MO seeds, SRC – sulfite-reducing clostridia.

Figure 5. Demonstrations of some enumerations of total germs (A), sulfite-reducing Clostridium (B), total coliforms (C), fecal coliforms and *E. coli* (D), and total and fecal streptococci (E) in raw water. MO (+) presence of *M. oleifera*, MO (-) absence of *M. oleifera*

et al., 2000, WHO, 2006). The findings clearly indicate that Moringa seed powder can effectively influence a variety of bacterial species, including total germ, total coliforms, fecal coliforms, *E. coli*, total streptococci, fecal streptococci, and sulfite-reducing clostridia.

While total coliforms are a broad group that includes bacteria from various sources, fecal coliforms are a more specific subset that is better suited as an indicator of recent fecal pollution from warm-blooded animals (Li et al., 2021). These results align with the findings of Asrafuzzaman et al. (2011), who reported a fecal coliform removal rate of up to 96% using Moringa seeds. The obtained result aligns also with the findings of Sutherland et al. (2022) who found that the addition of *M. oleifera* seeds to raw water resulted in significant microbial reductions, achieving removal rates of 85–95% for fecal coliforms and up to 100% for *Escherichia coli*.

The findings of this study also indicate the complete elimination of streptococci following the addition of *M. oleifera* seeds. Similar results were documented by Aitmelloul et al. (2020) and Vunain et al. (2019), who reported a 100% reduction in fecal streptococci. Contamination of water supplies by streptococci is indicative of fecal pollution, typically arising from animal waste, and can also occur in human as well as plant sources (Sinton et al., 2007)

Sulfite-reducing clostridia are frequently used as indicators of historical or intermittent fecal contamination in water sources. Their persistence suggests a failure at a specific point in the natural filtration process, potentially allowing contaminants to pass through (Robles et al., 2000). Studies by Wéry et al. (2009) and Wen et al. (2010) highlight the significant environmental dispersal of *Clostridiaceae* via wastewater treatment plant effluents. The persistence of these bacteria in the environment can be attributed to their extraordinary resilience to produce spores that can endure challenging environmental conditions, allowing them to persist in water sources. While there may not be a direct citation confirming a 100% reduction in sulfite-reducing clostridia specifically, the existing research highlights the significant antibacterial properties of *M. oleifera* seeds and their effectiveness in reducing bacterial populations, including clostridia.

The strong antibacterial activity observed can be linked to the high concentration of natural compounds in the *M. oleifera* seed extract, including pterygospermin, benzyl isothiocyanate, and 4-(L-rhamnopyranosyloxy) benzylglucosinolate (Xiao et al., 2020). Adji et al. (2020) reported that the seed and leaf extract of *M. oleifera* played a role in preventing some of the effects of the pathogenesis of diarrhea due to bacterial infection. Methanol, N-hexane, ethyl acetate, flavonoids, phenols, saponins, alkaloids, tannins, and steroids from the seed and leaf extract of *M. oleifera* have antibacterial effects.

The antibacterial properties of *M. oleifera* can also be ascribed to the presence of a natural cationic protein (MOCP) that acts as a flocculant, decreasing the turbidity and removing negatively charged particles, including bacteria (Ndabigengesere et al., 1995). The cationic proteins found in *M. oleifera* seeds are initially attracted to the bacteria through electrostatic forces. Once adhered, the protruding hydrophobic loop on the protein may penetrate the bacterial cell wall and destroy the bacteria (Suarez et al. 2005; Jerri et al. 2012; Shebek et al., 2015).

CONCLUSIONS

The investigation into the antibacterial efficacy of the *M. oleifera* seeds for water purification has demonstrated promising results, highlighting the potential of these seeds as an effective natural coagulant for wastewater treatment. The study demonstrates the superior antibacterial properties of *M. oleifera* seeds compared to conventional wastewater treatment methods. However, further research is necessary to evaluate the effectiveness of this plant in the wastewater purification process, with additional analyses focused on improving the treatment technique utilizing *M. oleifera* seed powder.

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