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Selective Coagulation Mechanism of *Moringa oleifera* Seeds on Gram Positive and Gram Negative Bacteria

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IT MAY BE more practical and reliable to use coagulants such as *Moringa oleifera (M. oleifera)* instead of chemicals such as alum in rural areas where the economic situation, small population densities and affordability are key elements in achieving drinking water quality. This study focused on the coagulation power of *M. oleifera* seed powder water extract concentrations (v/v) (0.5%, 1% and 2%) at different time intervals (1, 2 and 3hrs). The efficacy of coagulation expressed in total viable bacteria (TVB) and total coliform (TC) counts increased with all concentrations as well as contact time, using Cellulose Nitrate Filter, 47mm diameter and pore size 0.45µm. Total microorganisms in the filtrate were enumerated using Nutrient agar and MacConkey agar culture media. *Salmonella* & *Shigella* and Vibrios were also enumerated using the same technique on *Salmonella* & *Shigella* agar (S&S) and thiosulfate-citrate-bile salts-sucrose agar (TCBS) culture media. There is dramatically decrease in all types and groups of bacteria after treatment with *M. oleifera* seed powder. After 1hr and 0.5% *M. oleifera* extract, counts of TVB, TC, S&S and Vibrios decreased by 95.5%, 98.4%, 98.9% and 85.3%, respectively. At 1% the decrease were 96.6%, 98.1%, 97.9% and 98.4%, respectively and at 2% the decreases were 97.3%, 98.2%, 98.4% and 85.5%, respectively. This will directly lead to risky bacterial population imbalance resulting in relatively high incidence of Gram-negative bacteria (usually pathogenic) compared with Gram-positive bacteria.

Keywords: Bacteriostatic/bactericidal ratio, Hemagglutinins-lectins, Selective coagulation, Teichoic & teichuronic acids.

Introduction

Water is used by humans for several purposes, but the purity level of drinking water consumed is very important as it has a direct health effect. At least 2 billion people worldwide may still be exposed to fecal-contaminated sources of drinking water in 2025. Half of the world's population will live in water-stressed areas (WHO, 2018). Drinking water containing disease-causing agents and toxic chemicals require regular monitoring of water quality protocols to control the risks to public health. Turbidity removal is an essential part of conventional and non-conventional treatment processes when surface water is directly used for drinking. This removal is typically obtained with metal salt coagulants such as aluminum sulfate (alum) and calcium hypochlorite that cause particle aggregation by sedimentation and filtration. These two coagulants may be harmful for human health at high levels (WHO, 2007).

The fairly complex and costly systems for traditional purification of drinking water force poor rural communities to use water directly from open streams. Bacterial water- borne diseases then become wide spread throughout the population (Diab & El-Alfay, 2002). The situation is especially critical in low- and middle- income countries where 38% of health care facilities lack an improved water source, 19% does not have improved sanitation, and 35% lack water and soap for hand washing (WHO, 2018). Thus there is a need for safe, simple and inexpensive methods to reduce problems associated with contaminated drinking water. Coagulants present an efficient step in getting rid of more than 90% of turbidity, including diseases causing organisms (Franco et al., 2012; Arantes et

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al., 2014). Almond soaked beans were used in the sixteenth century in Egypt and Sudan to improve water quality (Babu & Chaudhuri, 2005; Libanius, 2008). Synthetic polymers arose later after World War II, but began to be used effectively after the 1960 (Libanius, 2008; Richter, 2009).

M. oleifera is a tropical tree found in Asia, Africa and Latin America. The seeds of this tree contain edible oil and water-soluble substances and have been extensively studied in as a natural coagulant. African rural communities have used their raw seed to clear cloudy water obtained from local rivers. Several studies have shown *M. oleifera* seed powder to be an effective natural coagulant for water treatment (Yin, 2010).

Ndabigengesere et al. (1995) explained the basic coagulation mechanisms inherent in *M. oleifera* seed were due to cationic polyelectrolytes that provide electrostatic interaction, and strong adsorption, neutralizing the particle surface. More important is the specific mechanism of hemagglutinins-lectins or carbohydrate binding proteins found in *Moringa* seeds with the teichoic and teichuronic acids, peptidoglycans and lipopolysaccharides found in Gram positive bacterial cell walls (Ratanapo et al., 2001) leading to cytoskeleton breakdown and even cell death. This mechanism makes the power of coagulation by *M. oleifera* selective as it acts on Gram positive bacteria but not Gram negative bacteria. In addition the coagulated bacteria, if not dead according to the bacteriostatic action will regrow due to the rich organic substrate of *M. oleifera* (Oluduro & Aderiye, 2007). The rich nutritive nature of *Moringa* (Abrams et al., 1993) is illustrated by the fact that each 100g of *Moringa* pods contain 3.7g carbohydrates, 2.5g protein, 0.1g fat, 4.8g fiber, 2g minerals, 24mg potassium, 137mg sulfur and 110mg phosphorus. Also for each 100g of *Moringa* pods there are amino acids at concentrations of 650mg leucine, 390mg theroine, 440mg isoleucine, 80mg tryptophan, 360mg arginine and 150mg lysine.

This study investigated:

- The selective coagulation properties of *M. oleifera* towards bacterial populations
- The non-killing activities (i.e., the bacteriostatic action) of the *M. oleifera* seed powder.
- The potential for the rich nutritive nature of the *M. oleifera* coagulant to provide a suitable environment for bacterial regrowth.

Materials and Methods

Sampling site description

Two water sampling sites were monitored from Ismailia fresh water canal. The first site was at the intake of the Suez Canal Authority water treatment plant (30º 34' 56" N 32º 15' 24" E). The second site was at the final purified water end of the water purification plant. This purification plant uses a conventional process for water treatment described by AWWA (2012).

Plant material

M. oleifera plant from the Egyptian Association of *Moringa*, National Research Center, Dokki-Cairo, Egypt. This is the reference center for *Moringa*. Seeds were de-shelled and air-dried at room temperature before grinding. The white kernel was ground into powder under aseptic conditions and stored in sterile bottles (5g each). Seed powder suspension was prepared by mixing seed powder (5g) into 100ml distilled water and stirring for 30min. using the magnetic stirrer. This mixture was then filtered rapidly through four layers of cotton gauze followed by filtration using Whatman No 0.45µm (Jahn, 1988). This solution considered to be a stock of 100% level, from which 0.5%, 1% and 2% serial concentrations (v/v) were prepared.

Treatment of water sample

Each of the prepared serial concentrations (50ml) was added to (450ml) of the water sample (collected from Ismailia irrigation water channel). The samples were then stirred rapidly for 3min, slowly for 17min and allowed to stand undisturbed for 1hr, 2hrs and 3hrs. The water supernatant (50ml) was collected by careful decantation and subjected to bacteriological analyses. Coagulant mass underwent the same analyses.

Quantitative and Qualitative bacteriological analyses

Inlet and supernatant of treated water samples were bacteriologically analyzed.

Quantitative bacteriological analyses

Total viable counts (TVB, cfu/ml), total coliform (TC, cfu/100ml), *Salmonella* & *Shigella* (S&S, cfu/100ml) and Vibrios (TCBS, cfu/100ml) were obtained by applying the membrane filtration method using recommended culture media (plate count agar and MacConkey agar, S&S agar and TCBS agar). The filtration step used Sartorius

Stedim Biotech Gmbh Cellulose Nitrate Filters, 47mm diameter and with pore size 0.45µm. Bacterial counts were determined after 24hrs to 96hrs of incubation at 35-37ºC (Diab, 1995).

Qualitative bacteriological analyses

Colonial macro-morphology on plates, micro-morphology of stained cell smears in addition to specific biochemical tests was achieved. Microbact™ Identification System 12E Oxoid was applied for the identification of the Gram negative isolates. The Gram positive isolates were identified according to the key of Bergey's Manual of Determinative Bacteriology based on spore formation, catalase test, and sugar fermentation patern.

Bacteriological analyses of the coagulant

After careful decantation of the remaining supernatant, the coagulant was collected using centrifuge (5000rpm/1min). Saline (9ml) was used to dissolute coagulant with vigorous shaking. Both quantitative and qualitative bacteriological analyses of the dispersed coagulant were done as above using the spreading method.

Results and Discussion

Ismailia irrigation water channel (at the TWPP intake point) was treated with *M. oleifera* seed powder concentrations of 0.5, 1 and 2% for 1, 2 and 3hrs. The supernatant and coagulant phases were quantitatively investigated by conducting bacterial counts of TVB, TC, *Salmonella & Shigella* and Vibrios.

Efficacy of M. oleifera coagulation expressed in counts of indicator bacteria

In supernatant counts of the main indicator bacterial groups TVB and TC, as well as counts of *Salmonella* & *Shigella* and Vibrios results were as shown in Figs. 1 (a, b, c), 2 (a, b, c), 3 (a, b, c) and 4 (a, b, c). There were dramatic decreases in the counts of all bacterial groups after 1h. TVB (95.5%, 96.6% and 97.3%); TC (98.4%, 98.1% and 98.2%); S&S (98.9%, 97.9% and 98.4) and Vibrios (85.3, 98.4 and 85.5) were observed at concentrations of *M. oleifera* extracts at 0.5, 1 and 2%, respectively, compared to counts in the original water samples.

TVB counts after treatment with *M. oleifera* seed powder extract gradually decreased with time and were 0.5% treatment level (22.2, 17.86 and 13.42 cfu/ml), 1% (16.86, 12.32 and 6.66) and 2% (13.22, 8.46 and 10.54 cfu/ml) after 1, 2 and 3hrs, respectively. Omodamiro et al. (2014) also reported that the microbial load in the water sample reduced drastically as the concentration of the *Moringa* solution increased from 1 to 5% (w/v). Similar to previous results we found the TVB are the most affected group. This result is consistent with why *Moringa* tends to be more effective against Gram positive than Gram negative bacteria (Ali et al., 2001; Palombo & Semple, 2001).

TC counts decreased with time 1h and 3h, but increased at 2h at the 0.5% treatment level (5.54, 6.06 and 5.28 cfu/100ml), but decreased gradually at the concentration of 1% (6.56, 6.06 and 4.54 cfu/100ml) and 2% (6.2, 4.72 and 3.74 cfu/100ml). Omodamiro et al., (2014) reported reduction in the total coliform bacterial count in water at the 1% (w/v) concentration level of *M. oleifera* extract. Daniyan et al. (2011) reported lower counts occur of total coliform bacterial count after using *M. oleifera* extract than when using potash alum as determined using the most probable number technique. Alo et al. (2012) reported that at 2% (w/v) concentration of *M. oleifera* extract, the total number of mesophilic bacteria and the total numbers of coliform bacteria were decreased to 5 x 10^{-2} cfu/ml and 2 x 10^{-2} MPN/ml.

Salmonella & *Shigella* counts on S&S agar increased gradually with time of 1, 2 and 3hrs at all *M. oleifera* extract concentrations of 0.5% (2.28, 4.28 and 5.02 cfu/100ml), 1% (4.38, 5.3 and 5.68 cfu/100ml) and 2% (3.42, 3.76 and 4.36 cfu/100ml), respectively. Oluduro & Aderiye (2007) reported similar results that *Salmonella* & *Shigella* counts increased with time. This suggests *M. oleifera* exerted bacteriostatic impacts on these organisms because regrowth occurred due to the presence of bacterial cells that were not totally inactivated.

Vibrios count on TCBS agar decreased with time at 1hr and 3hrs but increased at 2hrs at both concentrations of 0.5% (0.86, 2.5 and 1.36 cfu/100ml) and 1% (0.96, 1.06 and 0.62 cfu/100ml) respectively. Vibrios counts decreased at 2hrs at 2% concentration (0.72, 0.52 and 0.76 cfu/100ml) after 1, 2 and 3hrs, respectively. The bioactivity of *M. oleifera* extracts against Vibrios has not been widely researched, although Peixoto et al. (2011) did report bioactivity of *M. oleifera* extracts against Vibrios.

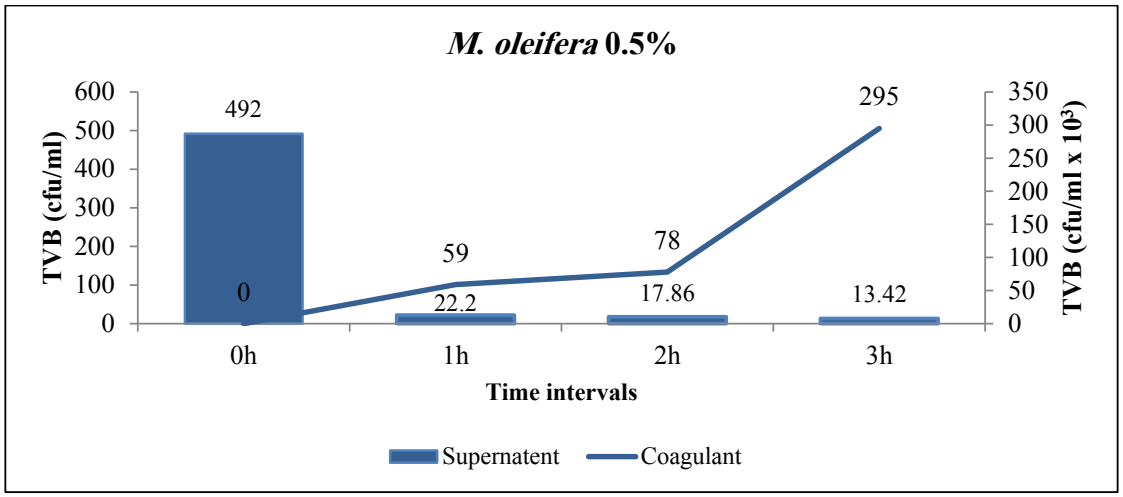

Fig. 1a. TVB mean counts of the supernatant (cfu/ml) and the coagulant (cfu/ml x 10³) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentration of 0.5% at 1, 2 and 3hrs.

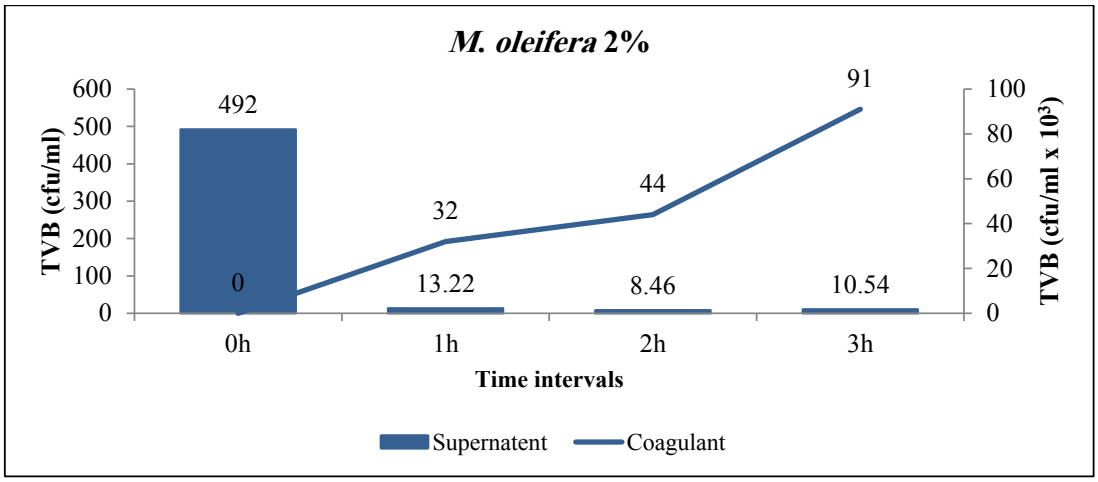

Fig. 1b. TVB mean counts of the supernatant (cfu/ml) and the coagulant (cfu/ml x 10³) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentration of 1% at 1, 2 and 3hrs.

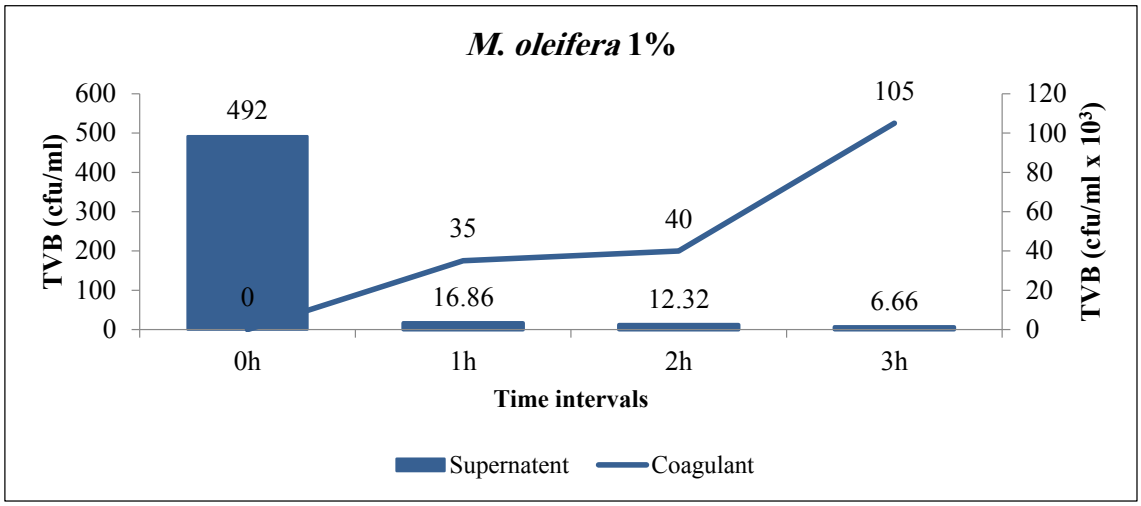

Fig. 1c. TVB mean counts of the supernatant (cfu/ml) and the coagulant (cfu/ml x 10³) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentration of 2% at 1, 2 and 3hrs.

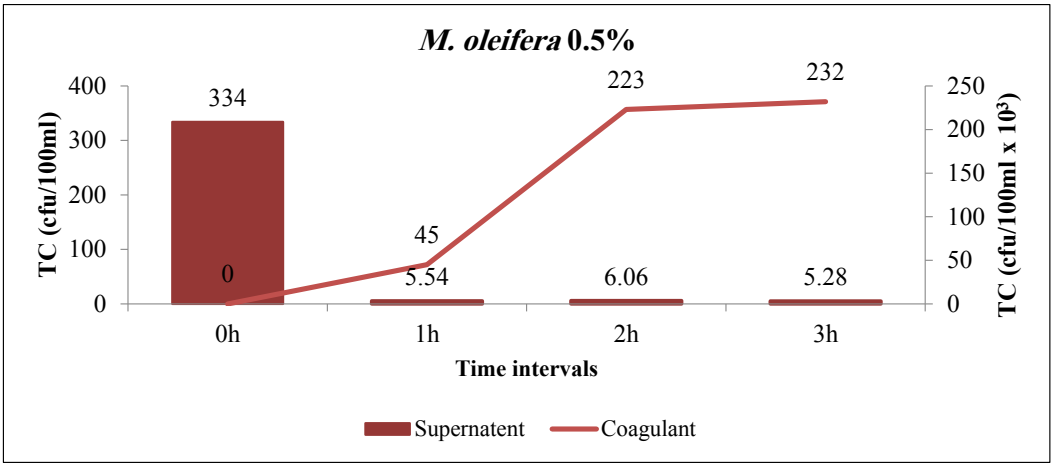

Fig. 2a. TC mean counts of the supernatant (cfu/100ml) and the coagulant (cfu/100ml x 10^3) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentration of 0.5% at 1, 2 and 3hrs.

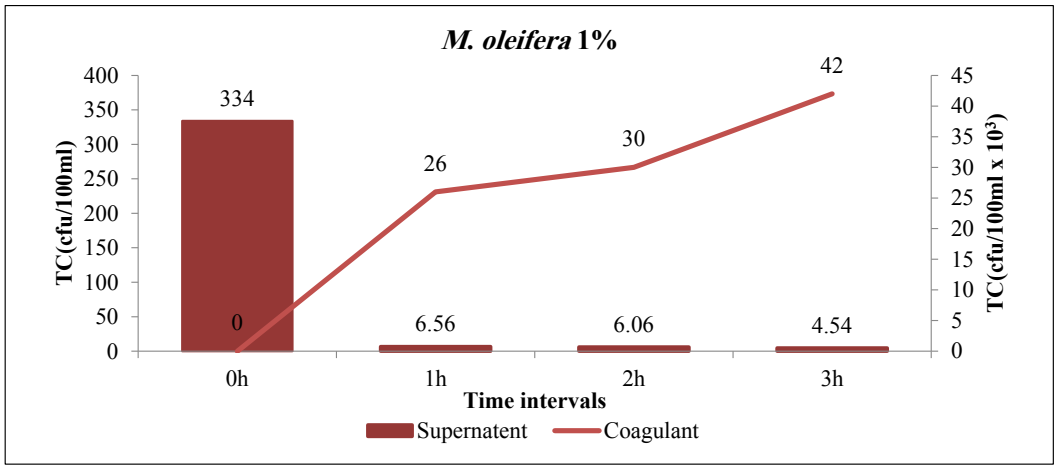

Fig. 2b. TC mean counts of the supernatant (cfu/100ml) and the coagulant (cfu/100ml x 10^3) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentration of 1% at 1, 2 and 3hrs.

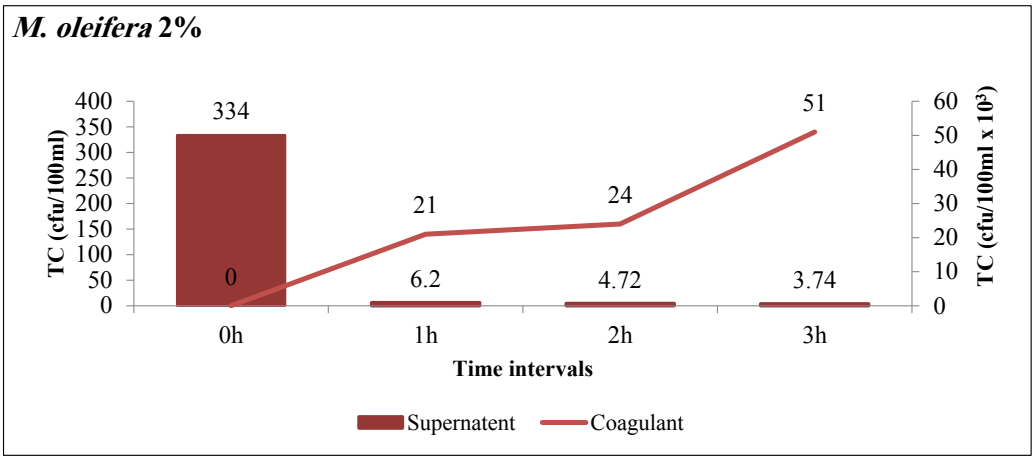

Fig. 2c. TC mean counts of the supernatant (cfu/100ml) and the coagulant (cfu/100ml x 10^3) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentration of 2% at 1, 2 and 3hrs.

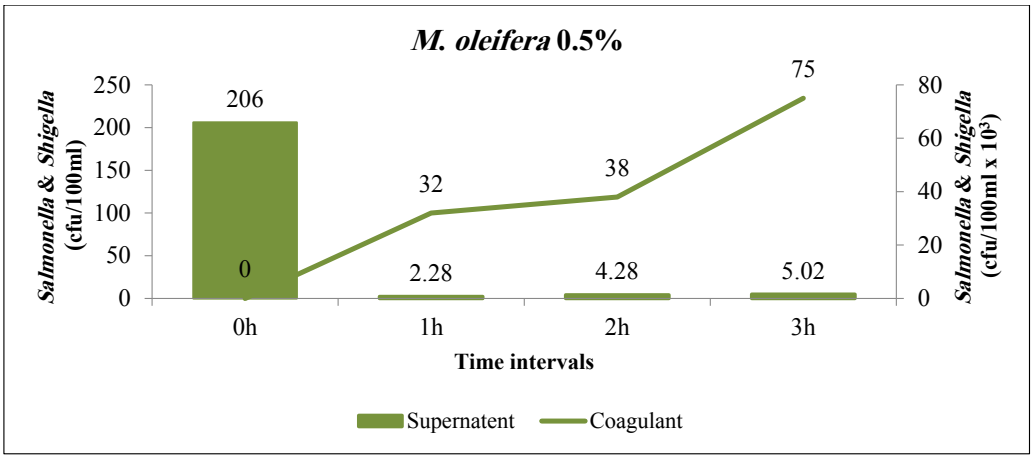

Fig. 3a. Salmonella & Shigella mean counts of the supernatant (cfu/100ml) and the coagulant (cfu/100ml x 10^3) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentration of 0.5% at 1, 2 and 3hrs.

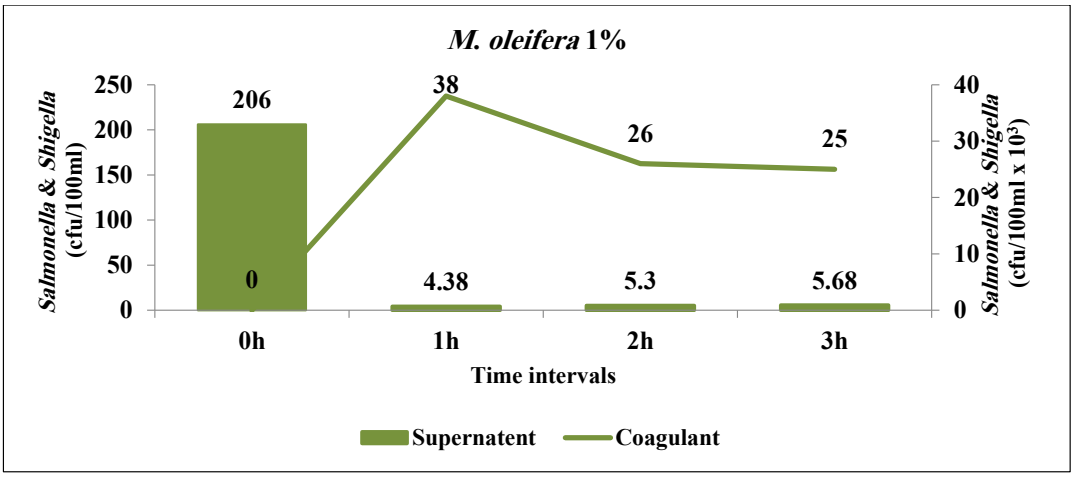

Fig. 3b. Salmonella & Shigella mean counts of the supernatant (cfu/100ml) and the coagulant (cfu/100ml x 10^3) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentration of 1% at 1, 2 and 3hrs.

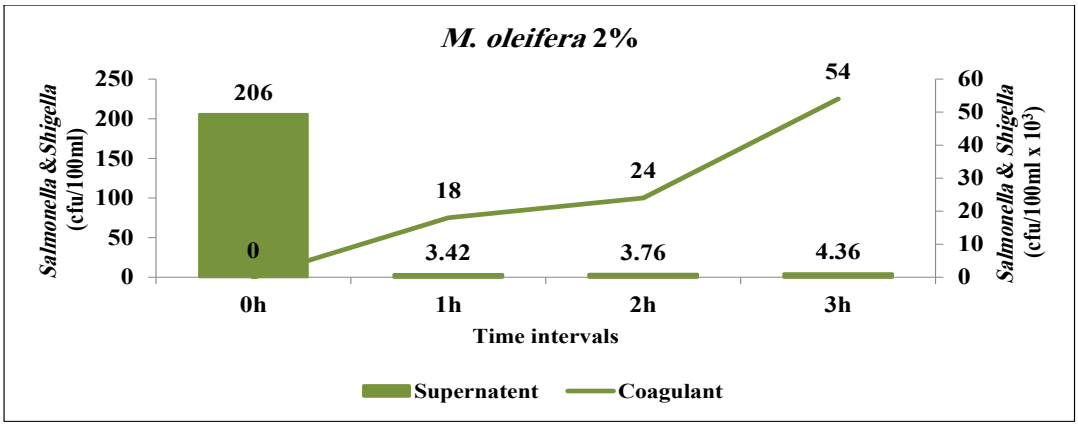

Fig. 3c. Salmonella & Shigella mean counts of the supernatant (cfu/100ml) and the coagulant (cfu/100ml x 10^3) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentration of 2% at 1, 2 and 3hrs.

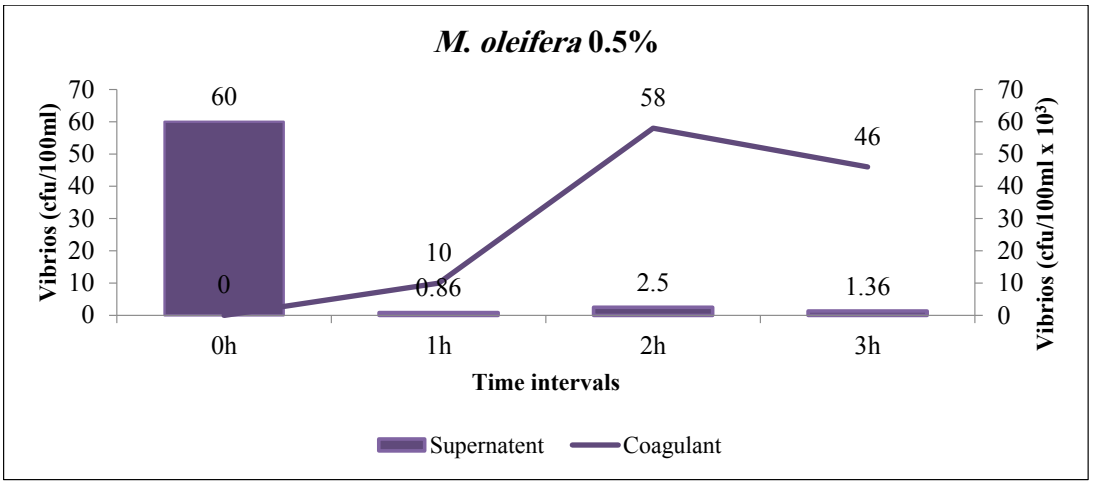

Fig. 4a. Vibrios mean counts of the supernatant (cfu/100ml) and the coagulant (cfu/100ml x 10³) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentrations of 0.5% at 1, 2 and 3hrs.

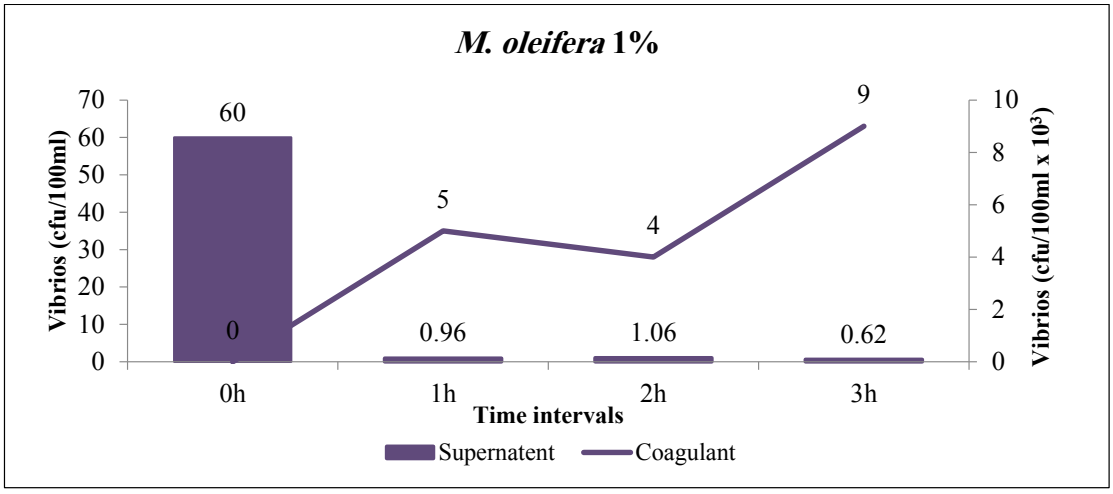

Fig. 4b. Vibrios mean counts of the supernatant (cfu/100ml) and the coagulant (cfu/100ml x 10³) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentrations of 1% at 1, 2 and 3hrs.

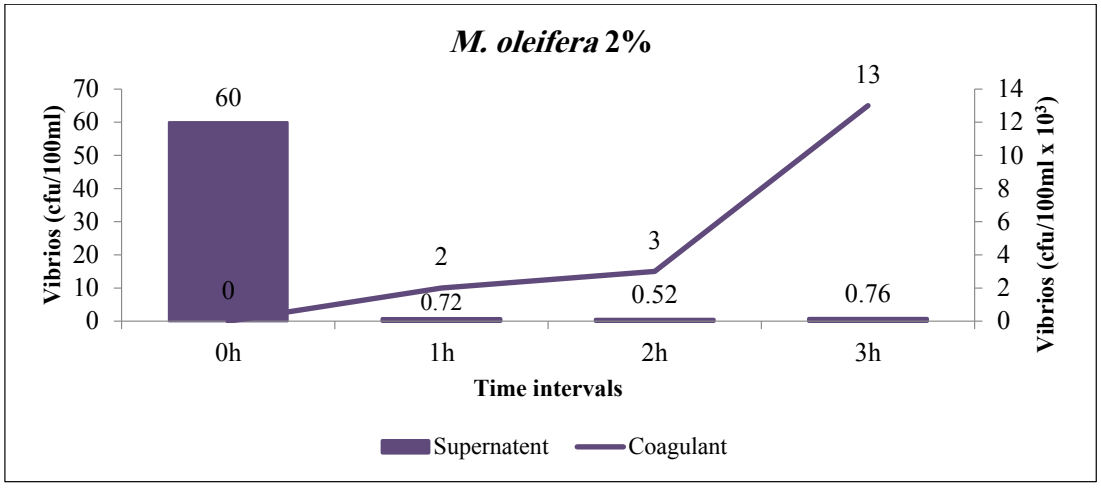

Fig. 4c. Vibrios mean counts of the supernatant (cfu/100ml) and the coagulant (cfu/100ml x 10³) of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentrations of 2% at 1, 2 and 3hrs.

Bacteriological regrowth in the coagulation material

In coagulant counting results of main indicator bacterial group TVB and TC in addition to *Salmonella* & *Shigella* and Vibrios were as shown in Figs. 1 (a, b, c), 2 (a, b, c), 3 (a, b, c) and 4 (a, b, c) there were dramatically increased after 1hr to all concentrations.

TVB and TC counts of all concentrations of *M. oleifera* seed powder water extract gradually increased with time after 1, 2 and 3hrs. *Salmonella* & *Shigella* counts (S&S) showed the same behavior except at 1% concentration, where gradual decrease was observed with time. Counts of Vibrios at 0.5% concentration increased with time 1, 2 and reversed at 3hrs. Vibrios counts at 1% increased with time at 1 and 3h but decreased at 2hrs (Table 1).

All the bacterial counts showed gradual increases with time whatever the concentration of *M. oleifera*. This is due to regrowth of bacteria. Madsen et al. (1988) reported that in the first 1hr to 2hrs of treatment, a bacterial reduction of 1^{-4} log units was obtained due to regrowth of the bacteria being concentrated in the coagulated sediment. For *Salmonella typhimurium* and *Shigella sonnei*, and also in some cases for *Escherichia coli* but not for *Vibrio cholera*, a secondary bacterial increase due to regrowth in the supernatant water was consistently observed during the 24hrs observation period.

It is worth noting that treated water when left to stand may deteriorate in quality than even untreated water due to bacterial regrowth (Oluduro & Aderiye, 2007). Water should be quickly separated from coagulant by filtering after coagulation, as the coagulant obtained by *M. oleifera* treatment is not 100% free from pathogenic bacteria (Alo et al., 2011).

Conclusion

Bacterial TVB and TC counts were negatively correlated with an increase in both concentrations of *M. oleifera* seed powder and time. This was not the case for S&S and Vibrios. The coagulant should be removed immediately after the recommended time for treatment (1hr) to prevent regrowth of the bacteria; especially Gram negative, in the treated water. *M. oleifera* seed powder power to eliminate water turbidity was expressed as reduction in counts of the mean bacterial pollution indicators. The obtained irregular count reductions made it difficult to rely on *M. oleifera* seed powder in water purification for drinking.

The coagulation role of *M. oleifera* seems to be mainly physical, leaving the coagulated bacteria viable, so it does not have any obvious disinfection role. The direct bactericidal action on Gram positive bacteria, bacteriostatic action on Gram negative bacteria and the rich nutritive nature of the coagulant material give chance for the regrowth of Gram negative bacteria in the purified water. Regrowth of Gram negative pathogenic bacteria such as *Salmonella*, *Shigella* and Vibrios due to the rich nutrients in the *M. oleifera* extract must be controlled.

TABLE 1. Mean counts of bacterial indicators in the coagulant material of Ismailia irrigation water channel treated with *M. oleifera* seed powder concentrations of 0.5%, 1% and 2% at 1, 2 and 3hrs.

Bacterial indicators	0.5% (v/v)			1% (v/v)			2% (v/v)		
	1hr	2hrs	3hrs	1hr	2hrs	3hrs	1hr	2hrs	3hrs
TVB (cfu/ml x 10^3)	5.9	7.80	29.5	3.5	4.0	10.5	3.2	4.4	9.1
TC (cfu/100ml x 10^3)	4.5	22.3	23.2	2.6	3.0	4.20	2.1	2.4	5.1
S&S (cfu/100ml x 10^3)	3.2	3.80	7.50	3.8	2.6	2.50	1.8	2.4	5.4
Vibrios (cfu/100ml x 10^3)	1.0	5.80	4.60	0.5	0.4	0.90	0.2	0.3	1.3

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