



The use of *Moringa Oleifera* and *Salvia Hispanica* as auxiliaries in the solar disinfection water treatment process by Sodis

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ABSTRACT

Solar Water Disinfection, known as SODIS, is a treatment method involving the exposure of water to solar radiation (UVA light and heat) to eliminate pathogenic microorganisms. Organic coagulants extracted from *Moringa oleifera* (T1) and *Salvia hispanica* (T2) were used in conjunction with the filtration process as auxiliaries. This process included sand filters with particle sizes ranging from 0.25 mm to 0.850 mm and a non-woven geotextile placed at its base. After undergoing the coagulation/flocculation/sedimentation/filtration process, the samples were exposed to sunlight in a solar concentrator using bottles painted on half of their surface (PR) and unpainted bottles (BR). The analyzed parameters included physicochemical properties such as pH, electrical conductivity, turbidity, and apparent color, as well as microbiological parameters, including *E. coli* and total coliforms. The results of physicochemical analyses demonstrated the superiority of T1 in terms of turbidity and apparent color. Regarding microbiological analyses, they showed the effective removal of *E. coli* and total coliforms, with 100% inactivation observed in the samples. It was observed that the applied treatment sequence significantly reduced the microbiological load of the samples; however, it cannot be stated with certainty whether the coagulants exerted any influence on this result.

Keywords: coagulants, coliforms, filtration, microorganisms.

O uso de *Moringa Oleifera* e *Salvia Hispanica* como auxiliares no processo de desinfecção solar pelo Sodis no tratamento de água

RESUMO

O sistema de desinfecção solar, conhecido como SODIS (Solar Water Disinfection), é um método que envolve a exposição da água à radiação solar (luz UVA e calor) para eliminar microrganismos patogênicos. Coagulantes orgânicos extraídos da *Moringa oleifera* (T1) e *Salvia hispanica* (T2) foram usados em conjunto com o processo de filtração como auxiliares.



Esse processo incluiu filtros de areia com tamanhos de partícula variando de 0,25 mm a 0,850 mm e uma manta geotêxtil não tecida colocada em sua base. Após passarem pelo processo de coagulação/floculação/sedimentação/filtração, as amostras foram expostas ao sol em um concentrador solar usando garrafas pintadas na metade de sua superfície (PR) e garrafas não pintadas (BR). Os parâmetros analisados incluíram propriedades físico-químicas como pH, condutividade elétrica, turbidez e cor aparente, bem como parâmetros microbiológicos, incluindo *E. coli* e coliformes totais. Os resultados das análises físico-químicas demonstraram a superioridade de T1 em termos de turbidez e cor aparente. Em relação às análises microbiológicas, as análises demonstraram a remoção eficaz de *E. coli* e coliformes totais, com 100% de inativação observada nas amostras. Portanto, foi observado que a sequência de tratamento aplicada reduziu significativamente a carga microbiológica das amostras; contudo, não se pode afirmar com certeza se os coagulantes exerceram alguma influência nesse resultado.

Palavras-chave: coagulantes, coliformes, filtração, microrganismos.

1. INTRODUCTION

Most of the coagulants used in water treatments are inorganic coagulants (aluminum sulfate and ferric chloride) and synthetic coagulants produced from polymers (Baptista *et al.*, 2017; Janna, 2016) which are mostly associated with diseases such as Alzheimer's, in addition to increasing environmental impacts through of toxic and non-biodegradable substances (Ang and Mohammad, 2020; Yin, 2010).

According to Paterniani (2005), gastrointestinal illnesses transmitted through the consumption of contaminated water stand out among the most lethal diseases in developing countries. Furthermore, on a global stage, it is estimated that 2 billion people in the world consume water infected with pathogenic microorganisms (Kurniawan *et al.*, 2023; Zare *et al.*, 2022).

Organic coagulants extracted from tree seeds and bark such as *Moringa oleifera* and *Salvia hispanica* seeds possess cation capacity, enabling them to coagulate colloidal particles in suspension by destabilizing charges and achieving water clarification. Furthermore, these organic coagulants are non-toxic-and-biodegradable (Tawakkol *et al.* 2019; Okuda *et al.* 2001).

Currently, the main method for disinfecting water is chlorination, used throughout the world, presenting excellent results (Spasiano *et al.*, 2015); however, this method contains by-products that are harmful to human health. In addition to being potentially carcinogenic (Liu *et al.*, 2020), other disinfection methods use UV radiation, but require high amounts of energy from producing sources and are costly. The use of the solar disinfection system (SODIS) is an opportunity for a possible solution to this problem (Byrdin *et al.*, 2018; Oelgemöller *et al.*, 2006).

The Solar Water Disinfection system, or SODIS, consists of exposing water to the sun in containers that allow the passage of solar radiation (Beattie *et al.*, 2019; Morse *et al.*, 2020), with the sun as an energy source, thus representing an accessible and safe system at low cost (McGuigan *et al.*, 2012). This method was first presented in a brochure published by UNICEF in 1984 with good results and has been used as a solution in emergencies to replace conventional water treatments in low- and middle-incomes (Samoili *et al.*, 2022; Wegelin and Meierhofer, 2002). The inactivation of microorganisms occurs due to the high load of UV rays or even the increase in temperature (reaching the level of pasteurization); the microorganisms undergo denaturation of proteins or oxidation in specific conditions of sun exposure, as they contain enzymes and absorbent components that are easily altered by radiation from the sun (García-Gil *et al.*, 2022; Marques *et al.*, 2013).

Given the proposal, the research evaluates the combination of organic coagulants

associated with the solar disinfection system (SODIS) in obtaining treated water for human supply.

2. MATERIAL AND METHODS

To carry out the tests, water supply from the Jacutinga River (Class 3 according to CONAMA No. 357. Conama, 2005), in the State of Paraná, Brazil was collected in 50 L containers and then sent to the Water Resources Laboratory of the Federal Technological University of Paraná, Londrina campus (Ricci and Wiecheteck, 2021). Two natural organic coagulants extracted from seeds of *Moringa oleifera* (T1) and *Salvia hispanica* (T2) were considered for the research (Figure 1 and 2, respectively); both were used to reduce water turbidity to enable greater efficiency of the disinfection system (Parsa *et al.*, 2020).



Figure 1. *Moringa oleifera* coagulant solution - A: seeds and B: saline solution.



Figure 2. *Salvia hispanica* coagulant solution – A: seeds in the mortar and B: solution in the magnetic stirrer.

The *Moringa oleifera* peeled seed-based coagulant solution was obtained by grinding 10 g of seeds in a blender and then adding 1 L of distilled water and 1 M NaCl. The saline solution was added to increase the extraction of the active coagulation component (Okuda *et al.*, 2001; Schmitt *et al.*, 2014; Vizibelli *et al.*, 2019). A fabric sieve was subsequently used to remove the organic matter residue, ultimately leaving only the liquid from the mixture.

To produce the coagulant derived from *Salvia hispanica*, 10 g of seeds were macerated with the aid of a mortar and pestle (Figure 2A), then 1 L of HCl solution at 0.001 mol L^{-1} was

added to adjust the solution's pH and enhance the extraction efficiency and the solution was kept under magnetic stirring for 1 hour (Figure 2B). After stirring, the solution remained at rest for 24 hours and was then strained through a fabric sieve, allowing the separation of the peels and seeds, according to Ahmad *et al.* (2022).

A pre-test defined the optimal coagulant concentrations as 2 mL L⁻¹ for T1 and 6 mL L⁻¹ for T2. After that, the test was carried out on the Jar-test equipment (218 – LDB/06, Nova Ética, Brazil). The equipment was first activated for 3 minutes at 150 rpm for the coagulation process to take place, then the rotation was reduced to 15 rpm for 10 minutes for the flocculation process to occur (Terechova *et al.*, 2014). After this, the device was turned off and after a 30-minute interval, the water was directed to a filter system.

The filters were built by adapting graduated cylinders with taps at the bottom so that water could be collected. The filtering medium was constructed with a 15 cm layer of sand with particle sizes ranging from 0.425 mm to 0.850 mm, and three layers of geotextile blanket at the base (Vizibelli *et al.*, 2019). The entire system can be seen in Figure 3.



Figure 3. Jar-test equipment associated with filters.

The water drained through the filters was collected in 1 L PET bottles, where for each treatment two bottles painted with black paint on half of their surface (PR) and two unpainted bottles (BR) were arranged so that it was possible to obtain high temperatures and to observe the disinfection efficiency of the increase in temperature (Cazu *et al.*, 2022).

For the solar disinfection test (SODIS), solar concentrators were used (Figure 4A), built as proposed by Instituto Mexicano de Tecnología del Agua - IMTA (Castillo *et al.*, 2016). The concentrator was placed in the North position where the bottles were exposed to the sun for 4 hours, between 10 am and 2 pm (the time of highest sun intensity) on a clear day (Figure 4B) (Paterniani *et al.*, 2005).



Figure 4. SODIS test (A- Arrangement of the solar concentrator; B- Exposure of the concentrator to the open air).

The test was conducted in duplicate. The parameters analyzed were the physical-chemical pH, electrical conductivity, turbidity, apparent color, microbiological *E. coli*, and total coliforms at the SODIS outlet (APHA *et al.*, 2012). Statistical analyses were performed using ANOVA variance using a block design, with the aid of the statistical program R.

3. RESULTS AND DISCUSSION

Initially, the parameters observed for water in its raw state were: pH 7.38; electrical conductivity 72.3 $\mu\text{S cm}^{-1}$; turbidity 558 NTU, apparent color 1650 uH, and testing positive for *E. coli* and total coliforms. Figure 5 represents the pH and electrical conductivity behavior during the test.

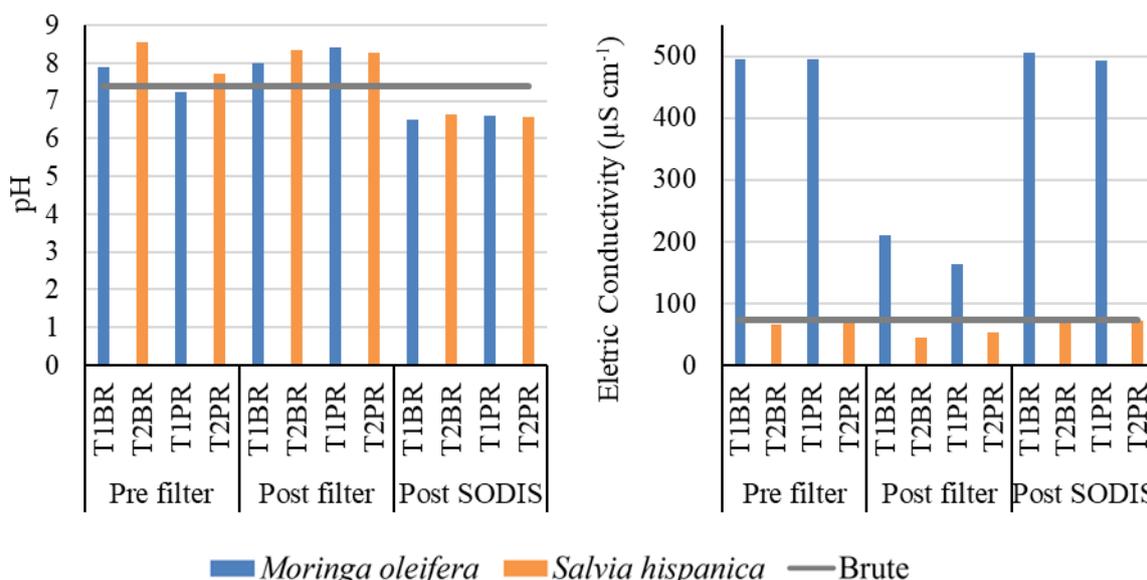


Figure 5. pH and electrical conductivity behavior during the test. Note: T1BR: *Moringa oleifera* with an unpainted bottle; T1PR: *Moringa oleifera* with bottle painted on 50% of its surface; T2BR: *Salvia hispanica* with an unpainted bottle; T2PR: *Salvia hispanica* with bottle painted on 50% of its surface.

When analyzing Figure 5, it is observed that the pH values decreased in the analyses carried

out after sun exposure, the T1 and T2 treatments presented values lower than the gross result of 7.38, with the T1 treatment obtaining a value of 6.46, giving the lowest result in the entire study. By increasing the water temperature, there is also increased molecular activity, consequently leading to greater dissociation of ionic compounds, resulting in a higher concentration of H^+ ions (Cunha *et al.*, 2010). It is also noted that during the jar-test test regarding the pre- and post-filter, the T2 values increased significantly with the raw value, and during the pre-filter inspection in T1BR2 the treatment presented 8.71. Furthermore, the samples did not show significant differences between the two treatments conducted.

The significant change in electrical conductivity levels from the coagulant extracted from *Moringa oleifera* seeds is due to the presence of NaCl, since the salt releases Na^+ ions, resulting in a considerable increase in this parameter, also observed in studies by Asmatulu *et al.* (2013).

The electrical conductivity analysis of the T2 treatment did not show a significant difference in the raw value, also observed by Di Marsico *et al.* (2018), not exceeding values of $76 \mu S cm^{-1}$. It is notable from Figure 5 that there was also a decrease in electrical conductivity values for the treatment obtained through the coagulants produced from *Moringa oleifera* and *Salvia hispanica* seeds, proving the efficiency of the filtering system in retaining ions together with the efficiency of the coagulant in aggregating the particles present in the water (Libânio, 2010).

After sun exposure, it is notable that treatment T1 demonstrates elevated levels of electrical conductivity. This occurs due to the increase in molecular activity resulting from the temperature rise, where electrical conductivity is influenced by the number of dissolved ions, particularly in the case of a saline solution, such as the coagulant obtained from *Moringa oleifera* seeds (Ribeiro *et al.*, 2009).

Current legislation does not present limit values for electrical, but it indicates limits for sodium and chloride concentrations, potentially suggesting that the treatment may not align with the proposed standards. Figure 6 represents the results and percentage of turbidity and apparent color removal efficiency.

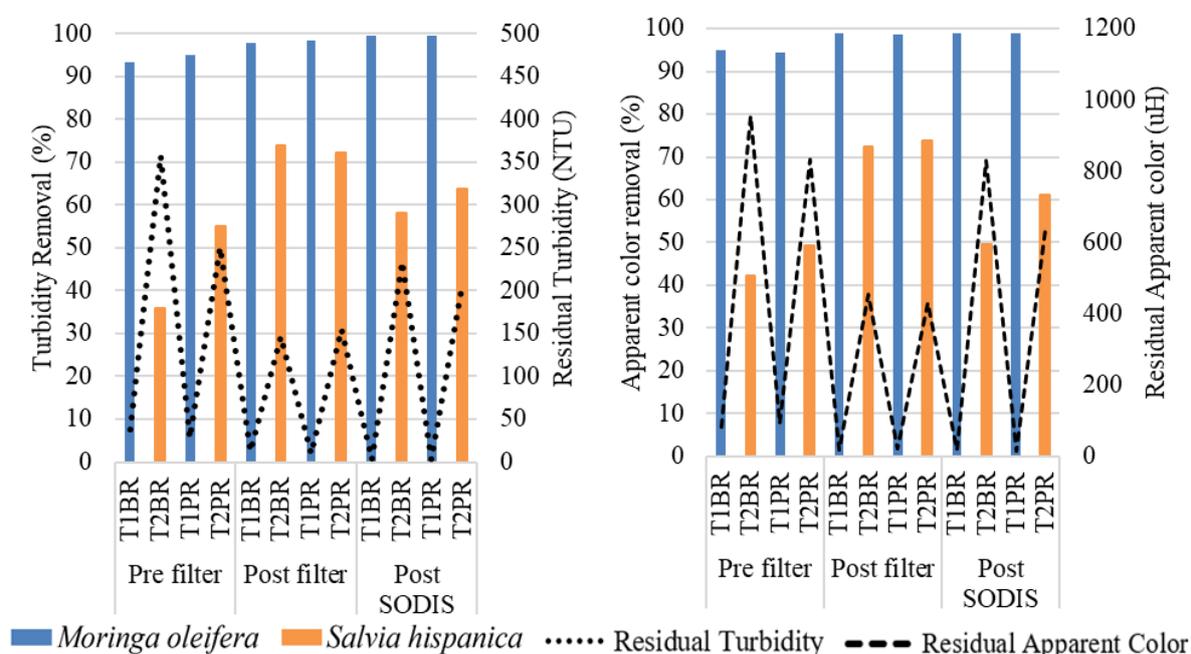


Figure 6. Removal efficiency of (a) turbidity and (b) apparent color during the test and their residual values. Note: T1BR: *Moringa oleifera* with an unpainted bottle; T1PR: *Moringa oleifera* with bottle painted on 50% of its surface; T2BR: *Salvia hispanica* with an unpainted bottle; T2PR: *Salvia hispanica* with bottle painted on 50% of the surface.

As shown in Figure 6, treatment T2 showed low turbidity removal efficiency during the pre-filter phase analyses, compared to T1, which at this stage of the experiment demonstrated results greater than 90% of turbidity removal. The T2 treatment only achieved considerable results when the water passed through the filter system, which began to present a standard of values above 65%.

It is also observed that the T1 treatment throughout the entire period of the experiment showed significant results for the analyzed parameter, with the highest value being observed after the SODIS treatment, where it was possible to verify 99.03% of turbidity removal, corroborating with studies of Paterniani *et al.* (2005), where an efficiency of 80% was obtained for removing turbidity using SODIS.

It can be observed that the treatment based on the *Moringa oleifera* coagulant once again showed high efficiency, making it possible to identify that T1 demonstrated more than 90% apparent color removal during all stages of the experiment. In treatments where T2 was applied, it was noted that after applying the filter, a result greater than 80% was obtained; however, this efficiency was reduced after the application of SODIS.

The increase in post-SODIS apparent color observed for T2 is inherent to the fact that the long resting time of the water was decisive for the destabilization of the colloidal particles and the reaction of the organic coagulant in the water, increasing the apparent color levels. Cristóvão *et al.* (2015), using industrial organic coagulant, also observed a considerable increase in apparent color.

Table 1 presents the results of the statistical analysis of variance ANOVA for the turbidity parameter considering a completely randomized design (DIC) in a factorial scheme.

Table 1. Statistical analysis of variance ANOVA for the turbidity parameter.

Source of variation	Degree of freedom	Sum of Squares	Medium Square	Value-f	Value - p
Treatment	1	9058,3	5	195,034	0
Filter	2	800,8	3	8,621	0,002366
Treatment x Filter	2	366,1	2	3,941	0,038060
Residue	18	836,0	4		
Total	23	11061,1	1		

From Table 1, it is notable from the statistical analysis that there was a significant difference in interaction for the treatments considering the pre- and post-filter analyses. Therefore, using the Tukey test presented in Table 2, a summary of the average measurements for the two treatments carried out by organic coagulants is shown.

Table 2. Tukey test for mean values of the turbidity parameter.

Treatment	Pre-filter	Post-filter
T1	94,07	98,02
T2	45,52	68,60

The results obtained in the Tukey test in Table 2 demonstrate the superiority of the T1 treatment over the T2 treatment in removing turbidity when compared in all stages of the experiment. Table 3 shows the analyses carried out for the post-SODIS stage, in which temperature interference was considered for the BR and PR bottles.

Analyzing Table 3, a statistical difference can be seen again between the interactions of the treatments (T1 and T2), but the statistical analysis shows that there is also a difference in the different paint arrangements on the bottles exposed in the sun in the SODIS stage. Applying the Tukey test to the post-SODIS stage, Table 4 was obtained.

Table 3. Statistical analysis of variance ANOVA for the turbidity parameter within the post-SODIS block.

Font of Variance	DG	SS	MS	Value-f	Value - p
Treatment	1	2977,99	5	5364,9	0,0000002
Pinter	1	16,62	4	29,9	0,0054290
Treatment x Pinter	1	15,26	3	27,5	0,0063244
Residue	4	2,22	2		
Total	7	3012,09	1		

Table 4. Tukey test for the mean values of the post-SODIS block of the turbidity parameter.

Bottles	Treatment T1	Treatment T2
BR	99,41 a	58,07 a
PR	99,53 a	53,71 b

Observing Table 4, the different painting arrangements influenced the turbidity removal for the T2 treatment. It is worth mentioning that for T1 the paintings did not influence the analysis of this parameter, being considered statistically equal.

According to Wegelin and Meierhofer (2002), bottles painted on 50% of their surface allow a circulatory movement of water, whereas in treatment T2, due to the presence of a considerable amount of organic matter dissolved by the treatment's low efficiency, directly impacts turbidity.

Table 5 presents the results of the ANOVA analysis of variance with the results obtained for the apparent color removal values, as well as in the case of turbidity removal data, pre- and post-filter analyses were considered.

Table 5. Statistical analysis of variance ANOVA for the apparent color parameter.

Font of variance	DG	SS	MS	Value-f	Value - p
Treatment	1	9295,3	5	195,034	0
Filter	2	997,6	4	8,621	0,00011158
Treatment x Filter	2	595,9	2	3,941	0,00160037
Residue	18	570,4	3		
Total	23	11459,2	1		

The statistical analysis obtained in Table 5 with the apparent color removal values due to the action of coagulants demonstrates that there was indeed a significant difference between the different treatments; therefore, Table 6 represents the Tukey test with a summary of the mean comparisons of the analyzed parameter.

Table 6. Tukey test for mean values of the apparent color parameter.

Treatment	Pre-filter	Post filter
T1	94,71 a	98,80 a
T2	45,76 b	73,18 b

Based on the data shown in Table 6, the prevalence of T1 treatment about T2 is notable. Considering the statistical analyses, T2 treatment presents statistically inferior results in all weighted stages. Table 7 presents statistical analysis data for the post-SODIS stage considering

the apparent color removal parameter and its interactions with the different treatments.

Table 7. Statistical analysis of variance ANOVA for the apparent color parameter within the post-SODIS block.

Font of variance	DG	SS	MS	Value-f	Value - p
Treatment	1	3784,5	3	2249,13	0,0000012
Pinter	1	72,7	5	43,22	0,0027709
Treatment x Pinter	1	64,5	4	38,35	0,0034571
Residue	4	6,7	2		
Total	7	3928,5	1		

Applying the statistical test demonstrated in Table 7, considering at this stage the interference of temperature rise, it is possible to observe again the statistical difference between the treatments; in addition to this, a difference in the painting arrangement is also observed. The Tukey test was applied, as shown in Table 8.

Table 8. Tukey test for the average values of the post-SODIS block of the apparent color parameter.

Bottles	Treatment T1	Treatment T2
BR	98,68 a	49,50 b
PR	99,03 a	61,21 a

As shown in Table 8, the temperature interference is notable for the apparent color parameter, and for the T2 treatment, a considerable improvement in the removal of apparent color from the bottles that presented the PR paint was noticed. Furthermore, there is no relevance in the interaction between the arrangements of the bottles for the T1 treatment, with no significant difference between them. In their studies, Scalize *et al.* (2012) proved that temperature influences the efficiency of several coagulants, causing inadequate treatments depending on the properties of the compound applied. Figure 7 represents the temperature behavior in the SODIS test.

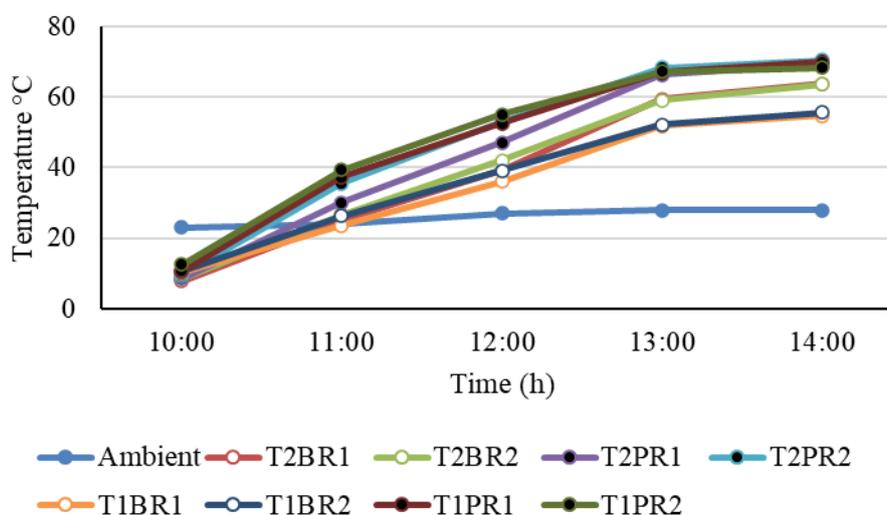


Figure 7. Graph of water temperature behavior during SODIS. Note: T1BR: *Moringa oleifera* with an unpainted bottle; T1PR: *Moringa oleifera* with bottle painted on 50% of its surface; T2BR: *Salvia hispanica* with an unpainted bottle; T2PR: *Salvia hispanica* with bottle painted on 50% of its surface.

As can be seen in Figure 7, the temperature of bottles that were arranged with black paint achieved higher results compared to bottles that were not painted. Also noteworthy is the time it took PR bottles to reach the pasteurization temperature for microorganisms. Temperature analyses conducted at noon showed temperatures above 50°C, while the BR bottles only reached this temperature an hour later.

When comparing the temperatures of treatments T1 and T2, a considerable difference was observed with the arrangement of the BR bottles, in which at the end of the analysis T2 presented 63°C, a higher temperature compared to T1, but no discrepancy was observed between T1 and T2. T2 with PR layout. However, at the end of the analysis at 14h00, the two treatments and bottle arrangements showed temperatures above 55°C. Figure 8 represents the analysis of total coliforms and *E. coli* of the samples after passing through the filter system.

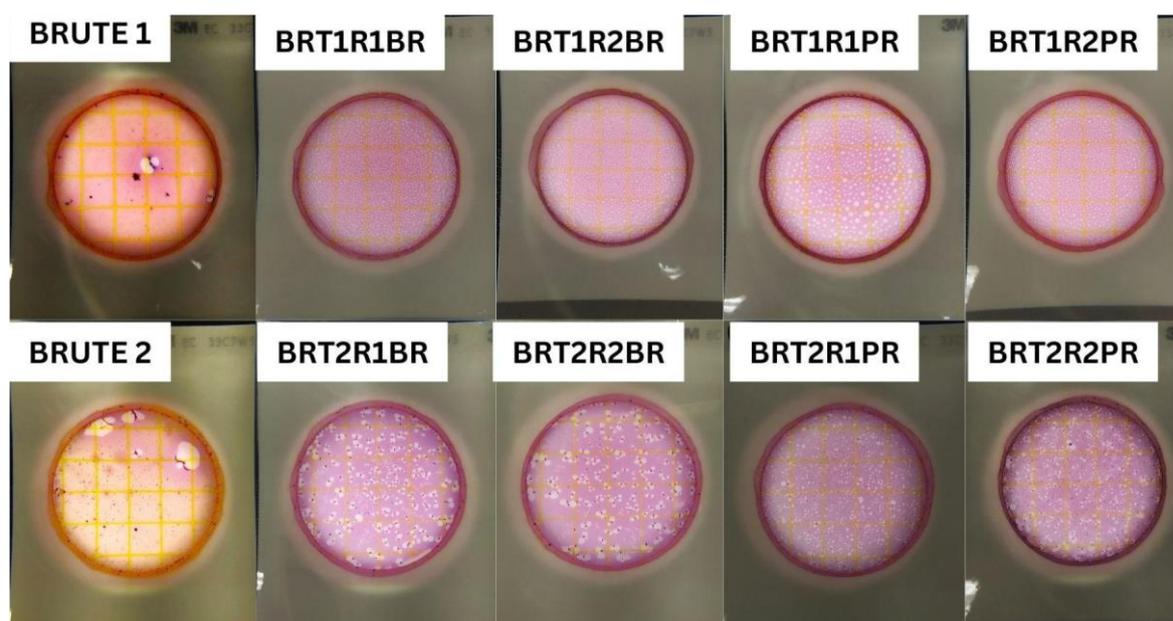


Figure 8. shows the total coliform and *E. coli* analyses of the raw sample and the water collected after passing through the filter. Note: BRT1BR: *Moringa oleifera* with an unpainted bottle; BRT1PR: *Moringa oleifera* with bottle painted on 50% of its surface; BRT2BR: *Salvia hispanica* with an unpainted bottle; BRT2PR: *Salvia hispanica* with bottle painted on 50% of its surface.

Observing Figure 8 and Table 9, it is possible to notice the presence of 1 CFU mL⁻¹ of *E. coli*, indicating fecal contamination in the raw sample. Furthermore, the second analysis of the raw sample indicated the presence of 4 CFU mL⁻¹ of coliform totals.

After the application of the T1 treatment, it was not possible to detect the presence of *E. coli* and total coliforms, indicating the potential inhibitory capacity of the coagulant extracted from *Moringa oleifera* for these microorganisms, particularly due to the high saline concentration of the solution. Ribeiro (2021) demonstrated that flour produced from *Moringa oleifera* seeds has potential properties against pathogenic microorganisms present in contaminated water. Figure 9 represents the analysis of total coliforms and *E. coli* in the samples after the application of the Solar Disinfection System (SODIS) on a clear-sky day.

Table 9. Total coliform and *E. coli* count of the raw sample and the water collected after passing through the filter.

Sample	<i>E. coli</i> (CFU mL ⁻¹)	Total coliform (CFU mL ⁻¹)
BRUTE 1	1	2
BRUTE 2	0	4
BRT1R1BR	0	0
BRT1R2BR	0	0
BRT1R1PR	0	0
BRT1R2PR	0	0
BRT2R1BR	0	143
BRT2R2BR	0	130
BRT2R1PR	0	133
BRT2R2PR	0	137

Note: BRT1BR: *Moringa oleifera* with an unpainted bottle; BRT1PR: *Moringa oleifera* with bottle painted on 50% of its surface; BRT2BR: *Salvia hispanica* with an unpainted bottle; BRT2PR: *Salvia hispanica* with bottle painted on 50% of its surface.

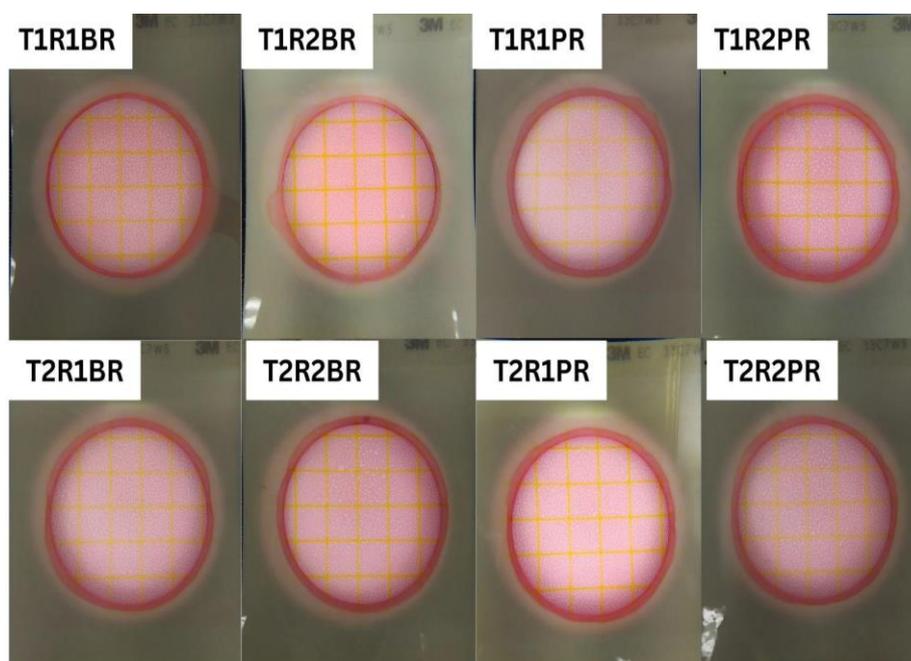


Figure 9. Microbiological analysis of *E. coli* and total coliforms of post-SODIS test samples. Note: T1BR: *Moringa oleifera* with an unpainted bottle; T1PR: *Moringa oleifera* with bottle painted on 50% of its surface; T2BR: *Salvia hispanica* with an unpainted bottle; T2PR: *Salvia hispanica* with bottle painted on 50% of its surface.

Through Figure 9 and Table 10, it can be seen that the sun exposure treatment was effective for both scenarios and the two different arrangements of PET bottles, achieving 100% inactivation of *E. coli* and total coliforms. Furthermore, it is possible to observe that despite the difference in the increase in the temperature of the bottles, there was no interference in the temperature parameter for the inhibition of the analyzed microorganisms. According to Ordinance GM/MS No. 888 (Brasil, 2021), for public consumption, water must be free of 100 mL of *Escherichia coli* and total coliforms at the treatment exit; therefore, both T1 and T2 complied with current legislation.

Table 10. Total coliform and *E. coli* count of post-SODIS test samples.

Sample	<i>E. coli</i> (CFU mL ⁻¹)	Total coliform (CFU mL ⁻¹)
BRT1R1BR	0	0
BRT1R2BR	0	0
BRT1R1PR	0	0
BRT1R2PR	0	0
BRT2R1BR	0	0
BRT2R2BR	0	0
BRT2R1PR	0	0
BRT2R2PR	0	0

Note: BRT1BR: *Moringa oleifera* with an unpainted bottle; BRT1PR: *Moringa oleifera* with bottle painted on 50% of its surface; BRT2BR: *Salvia hispanica* with an unpainted bottle; BRT2PR: *Salvia hispanica* with bottle painted on 50% of its surface.

4. CONCLUSIONS

According to the results of the experiment, the coagulant extracted from *Moringa oleifera* seeds stands out above the coagulant based on *Salvia hispanica* removing up to 99% of the parameters turbidity and apparent color. The possibility of the association of organic coagulants in the inactivation of *E. coli* and total coliforms through the solar disinfection system is also demonstrated, given that both treatments showed 100% inhibition of the growth of these microorganisms. Furthermore, it was possible to observe that the different arrangements of PET bottles (BR and PR) did not influence disinfection. However, the use of painted PET bottles along with the use of a solar concentrator is recommended, as it can optimize sun exposure time by up to 1 hour due to the rapid increase in temperature.

5. ACKNOWLEDGEMENTS

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